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Tree growth and water use efficiency during the twentieth century: from global trends to local drivers

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**Tree growth and water use efficiency during the 20th century: from
global trends to local drivers**

Justin M. Mathias

**Dissertation submitted to the
Eberly College of Arts and Sciences
at West Virginia University**

in partial fulfillment of the requirements for the degree of

**Doctor of Philosophy
in
Biology**

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Abstract

Tree growth and intrinsic water use efficiency during the 20th century: from global trends to local drivers

Justin M. Mathias

The frequency and severity of extreme environmental conditions will continue to increase under global environmental change. How terrestrial plants respond to prolonged, and often novel environmental stressors, will have profound impacts on, and feedbacks with, the Earth climate system at local to continental scales. Central to these feedbacks are plant stomata, actively regulated pores on the leaves of plants that act as a control valve over the fluxes of carbon dioxide (CO₂) into the leaf during photosynthesis and water vapor (H₂O) out of the leaf during transpiration. Importantly, changes in stomatal aperture do not affect the fluxes of CO₂ and H₂O equally, tipping the balance between CO₂ uptake and H₂O loss, or water use efficiency (WUE), from the leaf to the canopy scale. Understanding the environmental factors driving changes in leaf physiology is of paramount concern under climate change as small changes in tree WUE can have major effects on biogeochemical processes over large geographical areas. In this dissertation, I use a dendroisotopic approach to investigate the drivers of tree growth and intrinsic water use efficiency (iWUE) over the twentieth century, a period of rapid environmental change. I begin by synthesizing tree ring carbon and oxygen isotope data from published literature to examine how changes in the underlying component parts, photosynthesis and stomatal conductance to water, have driven changes in iWUE since the early twentieth century. I then focus my analysis to the Central Appalachian Mountains in the eastern United States, where I investigate how moderate climate change, along with reductions in acidic air pollution, have affected the growth and physiology of red spruce, a tree species that has been historically sensitive to acidic pollution. Finally, I expand my analyses of the environmental drivers of growth and physiology to two of the most common broadleaf deciduous tree species in the eastern United States, northern red oak and tulip poplar. This research suggests at the global scale, most tree species have shown increasing iWUE since the early 1900s, which positively accelerated in 1963, and has largely been a result of stimulated photosynthesis. At a more local scale, I found reductions in acidic pollution, alongside increases in atmospheric CO₂ concentrations, led to increased growth and iWUE of red spruce trees since *ca.* 1989. Lastly, I found the tree species studied exhibit a range of sensitivity to acidic pollution—while red spruce trees were highly sensitive, the two studied broadleaf deciduous trees were less so, highlighting the nuance and complexity behind tree responses to environmental change. Overall, these results showcase the active physiological response of trees to environmental change consistent with physiological theory and will help guide decisions regarding the importance of environmental factors in future model development and parameterization.

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Chapter 1. General Introduction.

1.1. Research Overview.

Since the onset of the industrial revolution there has been significant global change—concentrations of atmospheric CO₂ have risen by *ca.* 34% (Keeling, Piper, Bollenbacher, & Walker, 2016), global temperatures have increased by *ca.* 0.8°C (of which, more than 60% occurred during the past four decades) (Hansen *et al.*, 2010), acidic air pollution has become increasingly pervasive, particularly in developing countries (WHO, 2016), and the frequency and severity of extreme weather events such as drought is increasing (Trenberth *et al.*, 2014). How terrestrial plants respond to these prolonged, and often novel environmental stressors will have profound impacts on, and feedbacks with, the Earth climate system at local to continental scales (Swann *et al.*, 2016; Kooperman *et al.*, 2018). As such, there has been substantial progress in understanding the role of forest ecosystems in this rapidly changing environment through experimental manipulation of environmental conditions (Hendrey *et al.*, 1999; Dickson *et al.*, 2000; Miglietta *et al.*, 2001; Norby *et al.*, 2001; Hanson *et al.*, 2017), development and benchmarking of coupled atmosphere-biosphere process-based models (Zaehle *et al.*, 2014; De Kauwe *et al.*, 2014; Walker *et al.*, 2015), and assessment of data generated from Forest Inventory and Analysis (FIA) plots (Jenkins *et al.*, 2001; Thomas *et al.*, 2010).

Groundbreaking studies investigating the effects of elevated atmospheric CO₂ on trees in unmanaged forest stands used a Free-Air CO₂ Enrichment (FACE) approach whereby ambient CO₂ concentrations were maintained at levels predicted by the end of the 21st century (Hendrey *et al.*, 1999; Dickson *et al.*, 2000; Miglietta *et al.*, 2001; Norby *et al.*, 2001). After roughly a decade of forest exposure to elevated CO₂, major FACE results revealed plants, on average, responded by increasing rates of maximum and diurnal leaf photosynthesis, decreasing stomatal conductance, and increasing leaf level water use efficiency (iWUE) (Ainsworth & Long, 2004; Ainsworth & Rogers, 2007). A recent synthesis of data generated across multiple FACE experiments has also highlighted the stimulatory effect of increased atmospheric CO₂ on long-term plant growth, showing a nearly 30% increase in woody biomass over a decade of treatment (Walker *et al.*, 2019). Importantly, these results from FACE studies have provided a testable framework for guiding hypotheses regarding expected changes in plant physiology under increasing atmospheric CO₂ in the future.

More recent large-scale experiments have expanded upon FACE studies by incorporating a warming component, such as the Spruce and Peatland Responses Under Changing

Environments (SPRUCE) in northern Minnesota (Hanson *et al.*, 2017), as increases in atmospheric CO₂ ultimately lead to warmer temperatures at the Earth's surface (IPCC, 2014). SPRUCE and similar experiments have shown that warming may increase rates of litter decomposition (Butenschoten *et al.*, 2011) and emissions of trace gases such as CO₂ and CH₄ (Gill *et al.*, 2017), as well as reduce the size of soil carbon pools through increased respiratory losses (Wilson *et al.*, 2016; Melillo *et al.*, 2017). Moreover, these experiments have provided critical information on aboveground plant processes, showing that warming and elevated CO₂ can lead to a longer growing season for vegetation, but also result in a greater vulnerability to frost damage (Richardson *et al.*, 2018).

In addition to releasing CO₂, fossil fuel burning contributes to the emissions of oxidized forms of nitrogen and sulfur, which undergo various reactions and return to the biosphere as strong acids (i.e. sulfuric acid, nitric acid) (Driscoll *et al.*, 2001; Elliott *et al.*, 2007). Though much progress has been made in reducing acidic pollution in the United States due to the Clean Air Act and subsequent amendments (EPA, 2015), acid deposition remains a pervasive problem in many areas globally (WHO, 2016). Whole watershed acidification experiments, such as in the Fernow Experimental Forest in Parsons, West Virginia (Lanning *et al.*, 2019), offer a unique opportunity to examine how consistently high levels of simulated acidic deposition may influence ecosystem processes. For example, under prolonged exposure to high acid deposition rates, soils experience reductions in buffering capacity (Bowman *et al.*, 2008), leach essential base cations such as calcium and magnesium (DeHayes *et al.*, 1999; Driscoll *et al.*, 2001), and mobilize toxic metals such as aluminum (Driscoll *et al.*, 2001), which have the potential to reduce forest growth and productivity (Engel *et al.*, 2016). The effects of acid deposition may also extend to the water cycle through the effects on tree physiological responses, as has been documented in the Fernow Experimental Forest where trees have been shown to use more water under acidic conditions (Lanning *et al.*, 2019).

Importantly, precipitation is expected to become more variable under future environmental change, with droughts becoming more extreme (Trenberth *et al.*, 2014) and rain events increasing in both frequency and severity (Zhang *et al.*, 2013; Stott, 2016). Drought experiments, especially when coupled with modelling techniques are invaluable for understanding forest responses to environmental change from the plant to global scale (McDowell *et al.*, 2013) and have guided the development of demography based vegetation

models, such as the Ecosystem Demography model Version 2 (Medvigy *et al.*, 2009), that have been used to examine the importance of water availability in constraining estimates of various fluxes in the global carbon cycle (Trugman *et al.*, 2017).

Though each of the aforementioned experiments and techniques have been invaluable for understanding the effects of a given environmental factor on tree growth and physiology, it is critical to consider the integrated effect of many factors under ambient conditions. For example, Forest Inventory and Analysis (FIA) plots, established in the 20th century, undergo periodic resampling of standing biomass, tree mortality, and recruitment and, over time, have been used to examine forest responses to nitrogen and sulfur deposition (Thomas *et al.*, 2010; Horn *et al.*, 2018) and drought (Shaw *et al.*, 2005), among many other factors. Additionally, process-based models that incorporate fundamental physiological relationships of plants can simulate plant responses to a variety of ecosystem perturbations (Aber & Federer, 1992; Parton *et al.*, 1992) and aid in hypothesis development and testing, particularly when model results do not match observed ecosystem responses (Zaehle *et al.*, 2014; De Kauwe *et al.*, 2014; Walker *et al.*, 2015). Like FACE, warming, acid deposition, and precipitation/drought experiments, FIA plots and modeling studies have led to valuable insights regarding ecosystem functioning, but critically, often lack the ability to evaluate historical ecosystem changes beyond a few decades, largely due to time span or model parameterization constraints.

Dendroecology fills this knowledge gap and provides a powerful lens to examine the factors driving tree growth and physiology over time, as the integrated response of trees to environmental change is archived in woody biomass increment each year. A dendroecological approach can be particularly useful in remote areas where it is difficult to establish long-term monitoring plots or eddy-covariance towers but are representative of larger geographical areas that play major roles in the global carbon and water cycles (Levesque *et al.*, 2019). Importantly, changes in growth (i.e. tree ring widths) can be used to estimate annual aboveground biomass increment through the use of species-specific allometric relationships, providing an assessment of annual changes in carbon sequestration (Dye *et al.*, 2016). Further, when coupled with relative basal area and relative abundance of each tree species, ecosystem-scale carbon cycling dynamics can be resolved at fine-scale temporal resolution (Teets *et al.*, 2018), allowing a more accurate overview of the role of a given forest in the context of environmental change.

Extending dendroecological work to the analysis of tree ring stable isotopes has become increasingly common, providing information on processes related to photosynthesis (A) (Mathias & Thomas, 2018; Thomas, Spal, Smith, & Nippert, 2013), stomatal conductance (g_s) (Grams, Kozovits, Häberle, Matyssek, & Dawson, 2007; Rossella Guerrieri et al., 2019; Mathias & Thomas, 2018; Scheidegger, Saurer, Bahn, & Siegwolf, 2000; Thomas et al., 2013), and tree intrinsic water use efficiency (iWUE) (Frank et al., 2015; Rossella Guerrieri et al., 2019; Thomas et al., 2013), as well as nitrogen cycling and availability (Evans, 2001; Smith *et al.*, 2016; Burnham *et al.*, 2016; Sabo *et al.*, 2020). As such, changes in the tree ring stable isotope signature of carbon ($\delta^{13}\text{C}$), oxygen ($\delta^{18}\text{O}$), and nitrogen ($\delta^{15}\text{N}$) are most widely, and often simultaneously, used in studies examining tree responses to environmental change. Briefly, the $\delta^{13}\text{C}$ signature of tree rings serves as a record of the diffusional and biochemical properties of photosynthesis, whereby increasing $\delta^{13}\text{C}$ is indicative of a reduction in the concentration of leaf intercellular CO_2 (C_i), which can occur through increased photosynthetic rates or reduced stomatal conductance (Farquhar *et al.*, 1982, 1989). On the other hand, tree ring $\delta^{18}\text{O}$ signatures are not related to changes in leaf photosynthesis, but are sensitive to changes in stomatal conductance (Barbour, 2007; Ubierna and Farquhar, 2014), such that coupling tree ring $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ can aid in partitioning changes in iWUE between its component parts, photosynthesis and stomatal conductance (Scheidegger *et al.*, 2000; Grams *et al.*, 2007; Bögelein *et al.*, 2012; Guerrieri *et al.*, 2019). Lastly, tree ring $\delta^{15}\text{N}$ may serve as an index of ecosystem nitrogen (N) availability, where through various mechanisms, enrichment in tree ring ^{15}N (more positive $\delta^{15}\text{N}$) is indicative of a more open ecosystem N cycle (i.e. $N_{\text{supply}} > N_{\text{demand}}$) (Aber *et al.*, 1998; Evans, 2001, 2007; Pardo *et al.*, 2002; Craine *et al.*, 2009; Lovett & Goodale, 2011; Gerhart & McLauchlan, 2014). Given the central role of trees in regulating the global cycles of carbon and water (Pan *et al.*, 2011; Schlesinger & Jasechko, 2014) in a period of rapid environmental change, the overall aim of this research is to use a multiproxy dendroecological approach to identify the long-term drivers of tree growth and physiology in the context of environmental change.

1.2. Study Area and Plot Descriptions.

This research project was conducted under two broad themes—the first theme represents a meta-analysis of tree ring carbon and oxygen isotope chronologies from 84 sites globally where I examine the underlying component parts driving global iWUE. The sites included in the meta-analysis span 10 biomes and include tree species growing in 1) temperate broadleaf and mixed forests, 2) temperate conifer forests, 3) montane grasslands and shrublands, 4) Mediterranean forests, woodlands and scrub, 5) tundra, 6) temperate grasslands, savannas, and shrublands, 7) boreal forests and taiga, 8) deserts and xeric shrublands, 9) tropical and subtropical moist broadleaf forests, and 10) tropical and subtropical coniferous forests. As such, large differences in soil, vegetation, and parent material exist among sites. Mean growing season temperatures ranged from 2.84 °C to 32.70 °C, while total growing season precipitation ranged from 0.62 mm to 1693.02 mm across all 84 sites. The large range in temperature and precipitation among sites resulted in large differences in growing season vapor pressure deficit, which ranged from 71.8 Pa to 3,213.6 Pa. The sampled trees included in this analysis range from *ca.* 70 to *ca.* 550 years old and represent 36 unique tree species across a spectrum of leaf and wood types.

The second theme of this research project was field-based and was conducted in the Monongahela National Forest throughout the state of West Virginia. I selected five study locations, of which three were previously established in 2011 as part of a carbon balance and soil nitrogen cycling study (Smith *et al.*, 2016) and contain red spruce (*Picea rubens* Sarg.) as co-dominant canopy trees. The remaining two sites are reference watersheds located within the Fernow Experimental Forest in Parsons, West Virginia and are largely dominated by broadleaf deciduous trees including the two study species, red oak (*Quercus rubra*) and tulip poplar (*Liriodendron tulipifera*).

The three red spruce stands are located along a 100-km long north-south transect in the Central Appalachian Mountains. The northernmost site is located on McGowan Mountain (MCG; 38°58' N, 79°41' W) and is positioned within the Appalachian Plateau physiographic province, while Span Oak Run (SOR; 38°37' N, 79°46' W), and Cranberry Glades (CGL; 38°12' N, 80°17' W) are located in the Allegheny Mountain Section physiographic province of West Virginia (USGS, 1946). Each site is ≥ 900 meters in elevation and has a southwest aspect with slopes ranging from 0-10%. The mean annual temperature for this region in West Virginia was 9.5°C over the last 75 years (WV Climate Division 4, NOAA, 2017). The annual total

precipitation over the last 75 years was 1229.6 mm yr⁻¹, with 47% of the precipitation during the growing season from May to September (WV Climate Division 4, NOAA, 2017). Soils at each of the three red spruce dominated sites consist of a thick organic layer containing litter from by red spruce, eastern hemlock (*Tsuga canadensis*), yellow birch (*Betula alleghaniensis*), and red maple (*Acer rubrum*) which are the dominant canopy species. These sites are mature closed canopy stands with no evidence of anthropogenic or natural disturbance, such as logging or fire, since logging events in the early 1900's.

The two additional study locations containing broadleaf deciduous trees are located in the Fernow Experimental Forest, Parsons, WV. Both Watershed 10 (WS10; 39.0566°N, -79.6789°W) and Watershed 13 (WS13; 39.0617°N, -79.6805°W) are located within the Appalachian Plateau physiographic province (USGS 1946) and are considered reference watersheds for the Fernow, since they have remained free from disturbance since the early 1900s. In addition to the two study species, red oak and tulip poplar, tree species including American beech (*Fagus grandifolia*), red maple (*Acer rubrum*), and sugar maple (*Acer saccharum*) are commonly found in each watershed. The mean elevation of WS10 and WS13 is ~725 and ~694 meters above sea level, respectively. The topography at WS10 and WS13 is relatively steep, with 26% and 25% slopes, respectively, and while WS10 has a southerly aspect, WS13 has a northerly aspect. Soils at WS10 and WS13 consist of a relatively thin organic layer with litter primarily contributed from the genera *Quercus* and *Liriodendron*, as well as *Acer* and *Fagus*. Over the past 75 years mean annual temperature (MAT) at WS10 and WS13 was 9.0°C and 9.2°C, respectively, ranged on average from 2.6°C to 20°C across the year, and increased by 0.75°C at both watersheds. Mean annual precipitation (MAP) at WS10 and WS13 was 1314 mm and 1310 mm, respectively, with the majority of precipitation occurring in July (10.2 ± 0.2% of total annual precipitation) and the least amount of precipitation occurring in October (6.8 ± 0.2% of total annual precipitation) (PRISM Climate Group, 2004). Mean annual precipitation increased during the study period and was driven largely by precipitation during June and July (+ 0.75 mm yr⁻¹) (PRISM Climate Group, 2004).

1.3. Study Objectives.

This research project that included a meta-analysis of previously published research as well as a field-based component across five forested study locations in the Central Appalachian Mountains had three main aims:

In *Chapter 2*, I synthesized tree ring carbon and oxygen isotope data from previously published articles in a new, physiologically meaningful way to examine the trends and drivers of tree intrinsic water use efficiency (iWUE) over the twentieth century. To do this, I performed a literature search on Web of Science using the phrase “tree ring, carbon, oxygen, isotope”. From articles that met strict selection criteria, I digitally extracted dendroisotopic data from high-quality figures published in each article, after which I developed species-level chronologies of iWUE and leaf water oxygen isotope enrichment above the source water ($\Delta^{18}\text{O}_{\text{lw}}$) for each site. I then used linear mixed effects models to identify those factors (i.e. atmospheric CO_2 , temperature, etc.) most important in driving iWUE over the twentieth century at the global scale, after which I coupled iWUE and $\Delta^{18}\text{O}_{\text{lw}}$ chronologies to examine whether changes in iWUE were primarily driven by leaf photosynthesis or stomatal conductance.

In *Chapter 3*, I focused my analysis of the factors driving changes in iWUE to red spruce trees at three study locations in the Central Appalachians. Each study location is downwind of the highly industrialized Ohio River Valley and experienced some of the highest, most pervasive, levels of acid deposition in the eastern United States before the Amendments to the Clean Air Act in 1990. I hypothesized that reductions in acidic deposition, along with increasing atmospheric CO_2 concentrations would positively affect the growth of red spruce trees. To test this hypothesis, I collected increment cores from at least 20 randomly sampled red spruce trees for growth and carbon isotope analysis at each study location. I also measured the response of leaf photosynthesis to increasing atmospheric CO_2 (i.e. A- C_i curve) to reconstruct leaf physiology through time using C_i derived from tree ring carbon isotope signatures. I then used linear mixed effects models to determine the factors driving growth and iWUE, in addition to the specific temporal contribution and sensitivity to growth of each factor since 1940.

In *Chapter 4*, I expanded my analysis of the drivers of tree growth and physiology to two of the most ecologically important broadleaf deciduous tree species in the eastern United States, northern red oak and tulip poplar. Similar to *Chapter 3*, I collected increment cores from at least 20 trees at two study locations in the Fernow Experimental Forest in Parsons, West Virginia. I

also developed A-C_i curves for each tree species for upper and lower canopy leaves from at least five trees of each species at each study location. I hypothesized broadleaf deciduous trees would be sensitive to acidic air pollution, but also sensitive to climate change that has occurred in this region. I used linear mixed effects models to test this hypothesis and identify those factors important in driving growth and iWUE over time, as well as to predict potential changes in iWUE under future climate scenarios.

1.4. Literature Cited.

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Chapter 2. A global meta-analysis of historical changes in intrinsic water use efficiency of trees using the dual isotope method.

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2.1. Abstract.

Multiple lines of evidence point to increasing tree intrinsic water use efficiency (iWUE), or the ratio of net photosynthesis (A_{net}) to stomatal conductance (g_s), over the last century. Rising atmospheric CO₂ (C_a) is often directly linked to increasing iWUE, though whether this predominantly occurs through stimulated A_{net} or reduced g_s has remained elusive. Moreover, whether C_a interacts with climate, and if tree physiological responses are different among functional types remains an open question. Synthesizing data from 113 unique tree ring chronologies across the globe, we show a widespread *ca.* 40% increase in iWUE over the twentieth century. A dual carbon-oxygen isotope approach revealed that stimulated A_{net} dominated iWUE in *ca.* 90% of examined cases, whereas reductions in g_s occurred in the remaining *ca.* 10%, of which the overwhelming majority (>90%) were broadleaf deciduous trees. Our study showcases the active physiological response of trees to environmental change is remarkably consistent with physiological theory and will help guide decisions regarding the importance of factors in model parameterization.

2.2. Introduction.

The frequency and severity of extreme environmental conditions will continue to increase under global environmental change (Zhang *et al.*, 2013; Cai *et al.*, 2015; Stott, 2016; Cohen *et al.*, 2018). How terrestrial plants respond to prolonged, and often novel environmental stressors, will have profound impacts on, and feedbacks with, the Earth climate system at local to continental scales (Swann *et al.*, 2016; Kooperman *et al.*, 2018). Central to these feedbacks are plant stomata, actively regulated pores on the leaves of plants that act as a control valve over the fluxes of carbon dioxide (CO₂) into the leaf during photosynthesis and water vapor (H₂O) out of the leaf during transpiration. Importantly, changes in stomatal aperture do not affect the fluxes of CO₂ and H₂O equally, as the sum of resistances for the diffusion of CO₂ from the atmosphere to Rubisco are much greater than those for H₂O from the surface of leaf mesophyll cells to the atmosphere (Nobel, 1991). Indeed, as stomatal aperture changes, so does water use efficiency (WUE), or the ratio of CO₂ uptake to H₂O released from the leaf to canopy scale (Keenan *et al.*, 2013). Consequently, understanding the environmental factors driving changes in leaf physiology is of paramount concern under climate change as small changes in tree WUE can have major effects on biogeochemical processes such as continental runoff, over large geographical areas (Lemordant *et al.*, 2018; Kooperman *et al.*, 2018).

Approaches using tree ring carbon isotopes (Peñuelas *et al.*, 2011; van der Sleen *et al.*, 2015; Frank *et al.*, 2015; Guerrieri *et al.*, 2019), eddy-flux measurement analysis (Keenan *et al.*, 2013; Knauer *et al.*, 2016; Guerrieri *et al.*, 2019), atmospheric composition analysis (Keeling *et al.*, 2017), and Earth system modeling techniques (Ito & Inatomi, 2012; Knauer *et al.*, 2016; Keeling *et al.*, 2017; Cernusak *et al.*, 2019; Guerrieri *et al.*, 2019) have shown trends of recently increasing WUE. These increases can occur by stimulation of leaf photosynthetic rates (A_{net}) (Ainsworth & Long, 2004; Ainsworth & Rogers, 2007), reduced stomatal conductance to water (g_s) (Ainsworth & Long, 2004; Ainsworth & Rogers, 2007), or some combination of the two. A fundamental physiological response found in numerous CO₂ enrichment experiments is that WUE of many plants is improved as a result of increasing CO₂ (C_a) stimulating photosynthesis and causing partial stomatal closure (Ainsworth & Rogers, 2007; Franks *et al.*, 2013). Yet, environmental factors distinct from C_a have independent effects on A_{net} and g_s and, therefore, may modulate the response of WUE to rising CO₂, especially across functionally distinct plant groups with differences in wood (Guerrieri *et al.*, 2019) and leaf morphology (Rumman *et al.*,

2018). Despite this, few studies have thoroughly examined the effects of multiple factors over controls of WUE, and even fewer have considered the underlying component parts, A_{net} and g_s (Cernusak et al., 2019; Rossella Guerrieri et al., 2019; Mathias & Thomas, 2018; Rumman et al., 2018; Thomas et al., 2013; G. Xu, Liu, Belmecheri, et al., 2018). This has, in part, been due to the complexity of partitioning H₂O gas fluxes at ecosystem scales (Scanlon *et al.*, 2019), in addition to the difficulty in attributing changes in isotopically derived intrinsic water use efficiency (iWUE; the ratio of A_{net} to stomatal conductance to water, g_s) to A_{net} or g_s without the accompaniment of physiological measurements (Mathias & Thomas, 2018; Thomas et al., 2013).

An emerging technique of coupling isotopically derived estimates of iWUE with oxygen isotope leaf water enrichment above source water ($\Delta^{18}\text{O}_{lw}$; derived from tree ring $\delta^{18}\text{O}$) allows a qualitative attribution of changes in iWUE to underlying A_{net} and g_s (Boettger, Haupt, Friedrich, & Waterhouse, 2014a; Bögelein et al., 2012; Grams et al., 2007; Rossella Guerrieri et al., 2019; Scheidegger et al., 2000; G. Xu, Liu, Belmecheri, et al., 2018). As $\Delta^{18}\text{O}_{lw}$ is inversely related to g_s (Farquhar *et al.*, 2007), increasing iWUE accompanied by constant or decreasing $\Delta^{18}\text{O}_{lw}$ is necessarily driven by increases in A_{net} , while increasing $\Delta^{18}\text{O}_{lw}$ coinciding with increasing iWUE results from a reduced g_s or a combination of reduced g_s and increased A_{net} (Bögelein et al., 2012; Graham D. Farquhar et al., 2007; Rossella Guerrieri et al., 2019). As such, there currently exists a wealth of previously untapped long-term records of tree physiological responses to environmental change within numerous dendrochronological studies from around the world, providing a historical view of how iWUE has changed globally over the last century. In this analysis we 1) synthesize published data from tree ring carbon and oxygen isotope chronologies to examine global trends in tree-ring derived intrinsic water use efficiency, 2) identify those environmental factors, and their interactions, that best explain multi-decadal to centurial trends in iWUE, and 3) investigate the potential underlying changes in A_{net} and g_s through an analysis of coupled tree ring derived iWUE and $\Delta^{18}\text{O}_{lw}$ over time (Bögelein et al., 2012; Grams et al., 2007; Rossella Guerrieri et al., 2019; Scheidegger et al., 2000; G. Xu, Liu, Belmecheri, et al., 2018).

2.3. Methods.

Data Collection and Extraction

We compiled a dataset comprised of 113 unique tree ring stable isotope chronologies from 36 tree species at 84 sites across the globe. Data were collected from published articles resulting from a literature search on Web of Science using the search phrase “tree ring, carbon, oxygen, isotope.” Articles published prior the search date (7/15/19) were identified for potential use in our global analysis, and of the 378 candidate articles resulting from the search, 49 met all selection criteria for inclusion, which were: 1) present raw, uncorrected $\delta^{13}\text{C}$ or $\delta^{13}\text{C}$ -derived variables (i.e. $\Delta^{13}\text{C}$, iWUE) and $\delta^{18}\text{O}$ stable isotope signatures from absolutely dated tree ring chronologies, 2) provide identification information regarding tree species sampled and the respective tree ages, 3) include geographic coordinates of each study location, 4) contain high quality figures from which the isotope data could be extracted *or* be accompanied by a publicly available supplemental data file, 5) contain meta-data regarding the type of tree ring sample used (i.e. α -cellulose, bulk wood), and 6) be sampled from mature trees (>50 years) that were not subject to experimental treatments.

We used WebPlotDigitizer, a semi-automated data extraction application with built-in tools to extract data from a variety of plot types, with the ability to save files in TAR or JSON format (Rohatgi, 2019), to retrieve $\delta^{13}\text{C}$ -derived and $\delta^{18}\text{O}$ isotope data from published figures. Briefly, from each article that met the selection criteria, we first captured a high-quality image file for each relevant figure in TIFF format and assigned numerical values to the x-axis and y-axis, respectively. This was accomplished by assigning values to the known endpoints of each axis, in turn, and linearly interpolating values between the points. We then identified each unique data point on a given figure, after which the abscissa and ordinate were automatically calculated. This was performed for each tree- or species-specific chronology within a given site. Typically, species-averaged chronologies for $\delta^{13}\text{C}$ -derived and $\delta^{18}\text{O}$ isotope data of all sampled trees were presented in published literature; however, in cases where individual tree chronologies were presented, we first extracted data for each year at the tree level before calculating a species-level mean for a given species within a given site, for direct comparison among observations across all studies.

Environmental factors examined as potential drivers of tree physiology in this study include atmospheric CO_2 (C_a , ppm), as well as climate factors temperature (TMP, $^{\circ}\text{C}$),

precipitation (PPT, mm), and vapor pressure deficit (VPD, kPa) given their relevance to large scale controls over the carbon and hydrologic cycles (Knapp *et al.*, 2004; Xu *et al.*, 2019; Zhang *et al.*, 2019; Cernusak *et al.*, 2019). Annual C_a concentrations were obtained from Scripps Institute of Oceanography ice-core merged data product (Keeling *et al.*, 2015; MacFarling Meure *et al.*, 2006), while climate data were obtained from the Climatic Research Unit (CRU TS4.03) global gridded data product (Harris & Jones, 2019). Climate data from the CRU TS4.03 data product are provided at a $0.5^\circ \times 0.5^\circ$ spatial resolution at monthly time-step from 1901 – 2018 and are validated using local observations from ground-based meteorological stations. From the CRU TS4.03, we extracted TMP, PPT, and vapor pressure (VAP, in hPa) for each of the 84 unique study locations, and calculated VPD from VAP and TMP using methods from Allen (Allen *et al.*, 1998). We calculated annual (x_{ann}) and growing season (x_{grw}) values for each climate variable over the period 1901 – 2015 using means for TMP and VPD, while using sums for PPT. The range of months over which the growing season occurs at each site was determined based on information provided in each published article.

Lastly, we compiled characteristics unique to each site and chronology included in this analysis such as biome type, tree ring type, and tree phenology type (Table S2.1). Biome type was determined based on GPS coordinates provided for each unique site coupled with a global geographical biome classification database outlined in Dinerstein *et al.* (2017), while tree ring type was determined based upon known ring morphology confirmed using the The Xylem Database (Schweingruber & Landolt, 2010).

Carbon Isotope Calculations

The extracted $\delta^{13}C$ -derived chronologies fell into four distinct categories—raw tree ring $\delta^{13}C$ ($\delta^{13}C_{raw}$, $N = 45$), atmospheric CO_2 -corrected $\delta^{13}C$ ($\delta^{13}C_{cor}$, $N = 36$), carbon isotope discrimination ($\Delta^{13}C$, $N = 25$), and intrinsic water use efficiency (iWUE, $N = 7$). While there is a consistent post-photosynthetic fractionation ($\sim 2\text{‰}$) between leaf organic matter and carbon assimilated into woody biomass (Badeck *et al.*, 2005; Gessler *et al.*, 2014; Frank *et al.*, 2015), in addition to fractionation related to photorespiration (Ubierna & Farquhar, 2014), none of the published studies identified in our analysis took this into account when presenting values of $\delta^{13}C_{cor}$, $\Delta^{13}C$, or iWUE. As such, all published values of $\delta^{13}C_{cor}$, $\Delta^{13}C$, and iWUE were first converted to $\delta^{13}C_{raw}$ before calculation of $\delta^{13}C$ -derived chronologies that included these effects

following Laverne *et al.* (2019). We calculated $\delta^{13}\text{C}_{\text{raw}}$ when values of atmospheric $\delta^{13}\text{CO}_2$ -corrected $\delta^{13}\text{C}$ were given using

$$\delta^{13}\text{C}_{\text{raw}} = \delta^{13}\text{C}_{\text{cor}} + (\delta^{13}\text{C}_{\text{atm}} + 6.4), \quad (1)$$

and when discrimination, $\Delta^{13}\text{C}$, was presented using

$$\delta^{13}\text{C}_{\text{raw}} = -\frac{1000 \cdot \Delta^{13}\text{C} - 1000 \cdot \delta^{13}\text{C}_{\text{atm}}}{1000 + \Delta^{13}\text{C}}, \quad (2)$$

where $\delta^{13}\text{C}_{\text{atm}}$ is the carbon isotopic signature of atmospheric CO_2 on the year of ring formation (-6.4 ‰ being the $\delta^{13}\text{C}_{\text{atm}}$ signature prior to the industrial revolution) (Farquhar *et al.*, 1982; McCarroll & Loader, 2004). In cases where intrinsic water use efficiency was presented, we first calculated $\Delta^{13}\text{C}$ using

$$\Delta^{13}\text{C} = \frac{\frac{-i\text{WUE}}{0.625} \cdot (b-a) + C_a \cdot (b-a)}{C_a} + a, \quad (3)$$

where a (4.4 ‰) is the fractionation associated with the diffusion of CO_2 (Craig, 1953; Farquhar *et al.*, 1982), b (28 ‰) is the mean fractionation related to the preferential utilization of $^{12}\text{CO}_2$ over $^{13}\text{CO}_2$ during carboxylation by Rubisco (G. Farquhar *et al.*, 1982; Ubierna & Farquhar, 2014), and C_a is the atmospheric CO_2 concentration during the year of ring formation (Keeling *et al.*, 2015; MacFarling Meure *et al.*, 2006). We then used Equation 2 to calculate $\delta^{13}\text{C}_{\text{raw}}$ from $\Delta^{13}\text{C}$ (Farquhar *et al.*, 1982, 1989). To calculate $i\text{WUE}$ from $\delta^{13}\text{C}_{\text{raw}}$ including photorespiration and post-photosynthetic fractionation effects, we first calculated leaf internal CO_2 (C_i) using

$$C_i = \frac{\frac{(\delta^{13}\text{C}_{\text{atm}} - (\delta^{13}\text{C}_p - d))}{\left(1 + \frac{(\delta^{13}\text{C}_p - d)}{1000}\right)} - a - f \cdot \left(\frac{\Gamma^*}{pC_a}\right)}{b-a} * C_a, \quad (4)$$

where $\delta^{13}\text{C}_p$ is the isotopic signature of the tree ring, d accounts for the carbon isotope fractionation from leaf to wood (bulk wood = 1.9 ‰, cellulose = 2.1 ‰) (Badeck *et al.*, 2005; Frank *et al.*, 2015), f is the isotopic fractionation associated with photorespiration (12 ‰) (Ubierna & Farquhar, 2014), pC_a is the partial pressure of atmospheric CO_2 (Pa), and Γ^* is the CO_2 compensation point in the absence of dark respiration (Pa) (Laverne *et al.*, 2019b). We calculated Γ^* according to

$$\Gamma^* = \Gamma_{25}^* * \left(\frac{P_{\text{atm}}}{P_0}\right) * e^{\left(\frac{\Delta H_a \cdot (T - 298)}{RT \cdot 298}\right)}, \quad (5)$$

where ΔH_a is the energy of activation (37830 J mol⁻¹) (Bernacchi *et al.*, 2001), T is the leaf temperature (°C), R is the molar gas constant (8.314 J mol⁻¹ K), P_{atm} is the ambient atmospheric pressure (Pa), P_0 is the atmospheric pressure at sea level (101325 Pa), and Γ_{25}^* is the CO_2

compensation point at 25°C (4.332 Pa) (Bernacchi *et al.*, 2001; Stocker *et al.*, 2019). We assumed T reflects the mean air temperature over the growing season. We then calculated iWUE (mmol CO₂ mol⁻¹ H₂O) as

$$iWUE = (C_a - C_i) * 0.625. \quad (6)$$

For each of the preceding calculations we assumed leaf structure, mesophyll conductance (g_m), and mesophyll CO₂ fractionation remained unchanged throughout the length of each of the chronologies (Seibt *et al.*, 2008); however, we do realize minor species differences in leaf morphology or small changes over time due to environmental influences could have minor influences over g_m , and thusly $\delta^{13}\text{C}$ -derived chronologies (Flexas *et al.*, 2008). Although it could increase uncertainty around our estimates of C_i , and thus iWUE, this assumption is nevertheless standard across dendroisotopic studies reconstructing iWUE from tree ring carbon isotopes (Frank *et al.*, 2015; Rossella Guerrieri *et al.*, 2019; Laverigne, Voelker, *et al.*, 2019; Mathias & Thomas, 2018; Peñuelas *et al.*, 2011; Thomas *et al.*, 2013).

Oxygen Isotope Calculations

We calculated tree ring ^{18}O enrichment above the source water ($\Delta^{18}\text{O}_{\text{tr}}$) from raw tree ring $\delta^{18}\text{O}$ ($\delta^{18}\text{O}_{\text{tr}}$) using

$$\Delta^{18}\text{O}_{\text{tr}} = \frac{\delta^{18}\text{O}_{\text{tr}} - \delta^{18}\text{O}_p}{1 + \left(\frac{\delta^{18}\text{O}_p}{1000}\right)}, \quad (6)$$

where $\delta^{18}\text{O}_p$ is the oxygen isotopic composition of precipitation during ring formation (Cheesman & Cernusak, 2016). Given the lack of long-term records of $\delta^{18}\text{O}_p$ at the study locations, we estimated site-specific $\delta^{18}\text{O}_p$ over the length of each respective chronology following methods from Guerrieri *et al.* (2019) using

$$\delta^{18}\text{O}_p = 0.52 * T_a - 0.006 * T_a^2 + 2.42 * P_a - 1.43 * P_a^2 - 0.046 * \sqrt{E} - 13, \quad (7)$$

where T_a is the mean annual temperature (°C), P_a is total annual precipitation (m), and E is the elevation (m) of the study location above sea level (Barbour *et al.*, 2001). Finally, from $\Delta^{18}\text{O}_{\text{tr}}$ we calculated the enrichment of leaf water ^{18}O above source water ($\Delta^{18}\text{O}_{\text{lw}}$) using

$$\Delta^{18}\text{O}_{\text{lw}} = \frac{\Delta^{18}\text{O}_{\text{tr}} - \varepsilon_{\text{wc}}}{1 - p_x p_{\text{ex}}}, \quad (8)$$

where ε_{wc} (27 ‰) is the fractionation associated with carbonyl oxygen atoms exchanging with water during wood (i.e. cellulose) synthesis (Sternberg *et al.*, 1986; Cheesman & Cernusak,

2016) and p_x is the fraction of stem water at the site of cellulose synthesis, of which a given proportion of oxygen atoms, p_{ex} , are exchanged. While $p_x \approx 1$, a value in the range of 0.20 – 0.42 is typically used for p_{ex} (Cernusak *et al.*, 2005; Sternberg & Ellsworth, 2011; Belmecheri *et al.*, 2018), with higher values of p_{ex} often observed in more arid sites (Cheesman & Cernusak, 2016). For this study, we used a conservative value (0.40) for $p_x p_{ex}$ (Sternberg & Ellsworth, 2011).

Statistical analyses

To examine long-term trends in iWUE, we fit a linear mixed effects (LME) model using the R package *nlme* (Pinheiro *et al.*, 2018) including *year* as the sole fixed effect with *tree species* nested within *site* as random factors. In this analysis, we examined trends in iWUE across the entire chronology for which climate data were available (full: 1901 – 2015), in addition to two unique periods (pre: 1901 – 1963, post: 1963 – 2015) after we identified a breakpoint in the full iWUE chronology (across all sites and species) using the *segmented* package in R (Muggeo, 2008). Additionally, we used generalized least squares (GLS) regression to examine trends in isotope-derived response variables (iWUE, $\Delta^{18}O_{lw}$, etc.) separately for each species within a given site, as well as for environmental factors (PPT, TMP, etc.) at a given site for each time period (i.e. full, pre, post) using the R package *nlme* (Pinheiro *et al.*, 2018). Each LME and GLS model used to determine temporal trends were fit via restricted maximum likelihood and included a first-order autocorrelation structure to account for temporal autocorrelation in the chronologies.

We further used LME models to identify which environmental factors were most responsible in driving the observed changes in iWUE over the last 115 years using the R package *nlme* (Pinheiro *et al.*, 2018). We used the *dredge()* function from the *MuMIn* R package (Bartoń, 2017) to examine all possible model LME model combinations ($N = 113$) that included the environmental factors C_a , TMP_{grw} , PPT_{grw} , VPD_{grw} , wood type, and leaf type as independent variables, as well as interactions up to order 2. We identified the top candidate model as having the lowest corrected Akaike information criterion (AIC_c) for subsequent parameter inference. Prior to this analysis continuous environmental factors were grouped by each unique chronology and centered to their mean to reduce multicollinearity (Schielzeth, 2010; Guerrieri *et al.*, 2019). Each LME model examined included a temporal autocorrelation structure of order 1, as well as species nested within site as a random factor, and were fit via maximum likelihood for

comparison of models with different fixed effects. The top candidate model was then refit via restricted maximum likelihood. We used the R package *visreg* (Breheny & Burchett, 2017) to visualize the nature of significant interactions between environmental factors on iWUE over the range experienced by each factor.

As a complement to LME model parameter inference, we examined the independent effects of each environmental factor included as a fixed effect in the final model on iWUE through hierarchical partitioning using the R package *hier.part* (Mac Nally & Walsh, 2004). Hierarchical partitioning does not consider potential interactions between factors, but instead determines the contribution of a given environmental factor to iWUE through an examination of every possible model structure containing a given predictor variable. By doing so, hierarchical partitioning avoids enhancing or diminishing the variance explained by any given predictor variable, and circumvents issues related to multicollinearity among predictor variables (Murray & Conner, 2009). To do this, we used the *rand.hp()* function, which randomizes the matrices containing response and predictor variables and computes independent effects, set at 1,000 repetitions to determine variable contributions to iWUE.

We performed ANCOVA to examine differences in the rate of change of iWUE and $\Delta^{18}\text{O}_{\text{lw}}$ over time depended upon leaf type, wood type, or biome, and performed ANOVA to compare iWUE means between leaf type, wood type, and biome. Further, we used the R package *emmeans* (Lenth, 2019) to test for pairwise differences among groups from ANCOVA model fits, and used the R package *multcomp* (Hothorn *et al.*, 2008) to generate connecting letters reports from Tukey's HSD *post hoc* test for multiple comparisons of means from ANOVA model fits. We have made all data and code supporting this analysis publicly available on github.com/justinmathias.

2.4. Results and Discussion.

Using 113 unique tree ring carbon and oxygen isotope chronologies comprising 36 different species across 84 sites globally (Fig. 2.1a), we show tree-level iWUE increased, on average, by *ca.* 40% ($iWUE = 0.23 \cdot \text{year} - 369.16$) over the last century (1901 – 2015) (Fig. 2.1b). We identified a breakpoint in the combined iWUE chronology at 1963, after which iWUE increased linearly at a rate of $0.39 \pm 0.01 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O year}^{-1}$ (1.67% year^{-1}), or approximately 3.9 times faster than the previous 63 years ($F = 207.14$, $p < 0.0001$) (Fig. 2.1b). When considering individual chronologies, the increases in iWUE were widespread, with 93% (105 of 113 chronologies) of those examined having positive trends over 1901 – 2015 and 84% (95 of 113) of those examined having positive trends over 1963 – 2015 (Table S2.1). These data reinforce reports of increasing iWUE in the United States (Guerrieri *et al.*, 2019) and Europe (Peñuelas *et al.*, 2011; Frank *et al.*, 2015), and showcase the active long-term regulation of tree physiology during the Anthropocene from trees spanning 10 biomes in six continents that represent a spectrum of leaf and wood types (Table S2.1).

Globally, differences in vegetation physiognomy may have large impacts on iWUE, and despite much work examining plant physiological processes within biomes, there have been few largescale comparisons of historical tree iWUE across biomes at a global scale (Peñuelas *et al.*, 2011; Ito & Inatomi, 2012; Frank *et al.*, 2015; Guerrieri *et al.*, 2019). In this study, we found the rate of increase of iWUE from 1963 to 2015 differed among the 10 biomes represented ($F = 7.21$, $p < 0.001$) and ranged between $0.101 \pm 0.07 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O year}^{-1}$ for trees growing in deserts and xeric shrublands to $0.611 \pm 0.07 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O year}^{-1}$ for Mediterranean forests, woodlands and scrub (Fig. S2.1a, Table S2.2). Like deserts and xeric shrublands, the rate of iWUE increase since 1963 for trees growing in the tundra and in temperate grasslands, savannas, and shrublands was low, with a mean across the three biomes of $0.131 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O year}^{-1}$. All other biomes exhibit mean rates of iWUE increase after 1963 greater than $0.409 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O year}^{-1}$ (Table S2.2). Further, mean iWUE over 1963 – 2015 ranged from a low of $59.9 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ in tropical and subtropical moist broadleaf forests to a high of $91.5 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ in temperate conifer forests (Fig. S2.1a). However, we found no relationship between the rate of iWUE increase and mean iWUE in each biome ($p = 0.67$).

When considering changes in iWUE since 1963 with respect to plant functional traits, the rate of iWUE increase was *ca.* 21% higher for those tree species with 100% tracheids relative to

diffuse porous trees ($p = 0.022$), but we found no difference in the rate of iWUE increase between trees with 100% tracheids and ring porous trees ($p = 0.61$) or between diffuse porous trees and ring porous trees ($p = 0.27$) (Fig. S2.2a). Our results are similar to iWUE trends from tree rings presented by Frank *et al.* (2015) in European forests, but are in contrast to work by Saurer *et al.* (2014) and Wang *et al.* (2018) using tree rings and flux tower measurements, respectively, who showed broadleaf deciduous trees (i.e. ring porous and diffuse porous) in the Northern hemisphere having greater rates of increase in iWUE than trees with 100% tracheids over the last century. Additionally, we found mean iWUE during 1963 – 2015 was different among all wood types ($F = 782.96, p < 0.001$), being the highest in those species with 100% tracheids and lowest in ring porous species (Fig. S2.2b), consistent with recent findings from 12 tree species at 8 forested sites in the United States (Guerrieri *et al.*, 2019). Further, patterns in mean iWUE for each wood type fall intuitively along a gradient of hydraulic conductivity, with those species with 100% tracheids being more similar to valves, while ring porous trees are akin to pipes (Sperry, 2003). There were no differences in the rate of iWUE increase among trees with different leaf functional types since 1963 ($F = 1.05, p = 0.37$) (Fig. S2.2c). However, mean iWUE was different among trees with different leaf types, with needleleaf evergreen trees being the highest, followed by needleleaf deciduous trees, broadleaf evergreen trees, and finally broadleaf deciduous trees (Fig. S2.2d).

Changes in climate and C_a have strong effects on vegetation function from the leaf (Ainsworth & Long, 2004; Ainsworth & Rogers, 2007) to global scale (Cornwell *et al.*, 2018; Cernusak *et al.*, 2019; Haverd *et al.*, 2020), yet how environmental change has influenced iWUE across large spatial scales over the last century is not fully resolved. Guerrieri *et al.* (2019) recently showed increasing C_a and climate change led to increased iWUE in tree species across the United States, but whether this response is conserved across biomes experiencing a much larger range in climate is still unknown. To address this knowledge gap, we used linear mixed effects (LME) models to examine the importance of environmental factors in driving leaf physiology. Across all species and study locations, LME model results indicated that iWUE from 1963 – 2015 was strongly, positively related to C_a and growing season vapor pressure deficit (VPD_{grw}), and was negatively related to growing season precipitation (PPT_{grw}), although a large proportion of the total variance was attributed to differences among sites (Table 2.1). Further, when extending this analysis to changes in iWUE over the last 115 years (1901 - 2015), LME

model main effects were remarkably consistent with data from 1963 - 2015, with the exception of growing season temperature (TMP_{grw}) having a marginally positive effect on iWUE across the 115-year chronology (Table S2.4). As a complement to our analysis of the effects of C_a , PPT_{grw} , TMP_{grw} , and VPD_{grw} on tree iWUE, we used hierarchical partitioning (HP), which alleviates potential problems due to multicollinearity, to estimate the individual contribution of a given environmental parameter to tree iWUE. For the period 1901 – 2015, HP indicated that of the 42% variance in iWUE explained by our model, C_a accounted for 59.6% ($z = 1,375.49$, $p < 0.05$), more than any other factor considered. We found VPD_{grw} , TMP_{grw} , and PPT_{grw} contributed 23.6% ($z = 501.33$, $p < 0.05$), 16.2% ($z = 340.07$, $p < 0.05$), and 0.6% ($z = 12.64$, $p < 0.05$), respectively. Of the 31% variance in iWUE explained in our model over the period 1963 – 2015, the influence of C_a declined to 45.2% ($z = 455.58$, $p < 0.05$) and the influence of TMP_{grw} increased to 28.0% ($z = 357.20$, $p < 0.05$), whereas the contributions of VPD_{grw} and PPT_{grw} were similar to the 1963 – 2015 chronology with 26.1% ($z = 269.66$, $p < 0.05$) and 0.7% ($z = 6.42$, $p < 0.05$), respectively.

Vapor pressure deficit, or dryness of the atmosphere, regulates stomatal conductance, and therefore, both photosynthesis and transpiration (Grossiord *et al.*, 2020). Air temperature drives VPD (Grossiord *et al.*, 2020), but also affects leaf metabolism, including the ratio of photosynthesis to photorespiration (Dusenge *et al.*, 2019). Precipitation is a proxy for soil moisture content and, thus, the amount of water that can be taken up by plant roots. Increasing atmospheric CO_2 may affect both stomatal conductance and photosynthesis (Ainsworth & Long, 2004; Ainsworth & Rogers, 2007). Therefore, the individual effects of VPD, precipitation, air temperature, and atmospheric CO_2 on our observations of iWUE over the last 115-years are grounded in well-established plant physiology. Here, we show that these environmental drivers interact to regulate tree iWUE and are dependent on leaf and wood functional types, demonstrating the complexity and nuance of tree responses to environmental change. Across all chronologies, the response of tree iWUE to VPD_{grw} after 1963 was dependent upon both C_a and TMP_{grw} (Table 2.1), such that the effect of VPD_{grw} on iWUE was diminished under higher C_a (Fig. 2.2a) and was amplified under higher TMP_{grw} (Fig. 2.2b). When considering the entire 115-year period, increasing C_a not only ameliorated the effect of VPD_{grw} (Fig. S2.3a), but also the effect of warming TMP_{grw} (Table S2.4), suggesting the effect of temperature becomes increasingly important after 1963 (Fig. S2.3b). Furthermore, we found increasing C_a led to larger

increases in iWUE in those tree species that had 100% tracheids, with respect to trees that were diffuse porous or ring porous, and the effect of C_a was diminished in broadleaf deciduous trees relative needleleaf evergreen trees (Table S2.3). When considering leaf type, we found interactions between C_a and PPT_{grw} where the effects of increasing C_a on iWUE were greatest with low PPT_{grw} and diminished at high PPT_{grw} for needleleaf evergreen (Fig. 2.3a), and needleleaf deciduous (Fig. 2.3b), in contrast to broadleaf deciduous trees where the effect of increasing C_a offset the negative effects of high PPT_{grw} on iWUE (Fig. 2.3c). Interestingly, we found no interaction between C_a and PPT_{grw} on iWUE of broadleaf evergreen trees, which instead showed an interaction between C_a and VPD_{grw} on iWUE (Fig. 2.3d). When considering functional wood types, we found more complex interactions, where there were interactions between TMP_{grw} and PPT_{grw} on iWUE of tree species with 100 % tracheids and diffuse porous trees, where the greatest iWUE in trees with 100% tracheids was at low TMP_{grw} and low PPT_{grw} (Fig. S2.4a) and the greatest iWUE in diffuse porous trees was at high TMP_{grw} and low PPT_{grw} (Fig. S2.4b). On the other hand, ring porous trees showed an interaction between VPD_{grw} and PPT_{grw} , where iWUE was greatest at high VPD_{grw} and low PPT_{grw} (Fig. S2.4c). These results underscore the intricate interplay between C_a , temperature, precipitation and evaporative water demand on tree physiology, highlight important differences in tree responses to environmental change across tree functional traits, and may have important implications for tree iWUE as climate changes and C_a continues to rise (Zhang *et al.*, 2019; Grossiord *et al.*, 2020).

Annually resolved, canopy-integrated iWUE chronologies reconstructed from tree ring carbon isotope signatures clearly show a positive trend over the twentieth century explained, in part, by interactions between C_a , PPT_{grw} , VPD_{grw} , and TMP_{grw} . Yet, whether the increases in iWUE were due to underlying stimulated A_{net} , reduced g_s , or some combination thereof, cannot be explained using carbon isotope signatures alone. Therefore, we combined analyses of the chronologies of iWUE with those of $\Delta^{18}O_{lw}$ to qualitatively partition the increases in iWUE between independent changes in A_{net} and g_s . Of all chronologies showing an increase in iWUE since 1963, we found 18.6% (N = 21) of the studies showed decreasing $\Delta^{18}O_{lw}$ (Fig. 2.4a), indicating increased g_s across the 53-year period, 71.7% (N = 81) showed constant $\Delta^{18}O_{lw}$ (Fig. 2.4b), indicating no change in g_s , and 9.7% (N = 11) showed increasing $\Delta^{18}O_{lw}$ (Fig. 2.4c), reflecting a decrease in g_s despite consistent increases in iWUE (Fig. 2.4d,e,f). Trends in $\Delta^{18}O_{lw}$ over time were not different among wood types ($F = 1.81$, $p = 0.16$) (Fig. S2.5a), although there

were differences due to leaf type ($F = 2.97, p = 0.03$), with broadleaf deciduous trees showing slightly increasing $\Delta^{18}\text{O}_{\text{lw}}$, and all other leaf types being no different from each other and having either a nonsignificant slope or a negative slope (Fig. S2.5b). There were no relationships between the trends in $\Delta^{18}\text{O}_{\text{lw}}$ and mean TMP_{grw} , mean PPT_{grw} , or mean VPD_{grw} across all chronologies since 1963 (Fig. S2.6a,b,c), nor was there a relationship between the trend in $\Delta^{18}\text{O}_{\text{lw}}$ and the trend in TMP_{grw} (Fig. S2.6d). $\Delta^{18}\text{O}_{\text{lw}}$ trends did, however, become more negative (corresponding to increased g_s) in sites becoming wetter since 1963 (Fig. S2.6e) and more positive (corresponding to reduced g_s) in sites where VPD was increasing (Fig. S2.6f), although the variability in $\Delta^{18}\text{O}_{\text{lw}}$ trends explained by PPT_{grw} trends and VPD_{grw} trends was only 15% and 6%, respectively. These findings are similar to Guerrieri *et al.* (2019) who showed negative or constant $\Delta^{18}\text{O}_{\text{lw}}$ trends in wetter sites.

Stimulated A_{net} driving increased iWUE may have large effects on the carbon cycle through increased carbon sequestration, while increasing iWUE due to reduced g_s may have large effects on the hydrologic cycle, through reductions in transpiration. Where iWUE increased from 1963 to 2015 (Fig. 2.4a,b,c), the temporal analysis of $\Delta^{18}\text{O}_{\text{lw}}$ suggests g_s increased in 18.6 % of all examined chronologies (Fig. 2.4d), whereas g_s remained constant in 71.7 % (Fig. 2.4e) and decreased in 9.7 % (Fig. 2.4f), as $\Delta^{18}\text{O}_{\text{lw}}$ is inversely related to g_s (Barbour, 2007; Bögelein *et al.*, 2012; Graham D. Farquhar *et al.*, 2007; Grams *et al.*, 2007; Scheidegger *et al.*, 2000). This points to enhanced A_{net} overwhelming increases in g_s where $\Delta^{18}\text{O}_{\text{lw}}$ declined (Fig. 2.4a,d), as well as driving increasing iWUE under constant g_s where $\Delta^{18}\text{O}_{\text{lw}}$ remained constant (Fig. 2.4b,e), such that increasing iWUE was driven by stimulated A_{net} in *ca.* 90% of chronologies examined. As such, this suggests reductions in g_s alone where $\Delta^{18}\text{O}_{\text{lw}}$ increased, or in combination with increasing A_{net} , occurred in less than 10% those chronologies examined (Fig. 2.4c,f). The widespread increase in A_{net} underscored by the combined analysis of iWUE and $\Delta^{18}\text{O}_{\text{lw}}$ is in line with global observations of increasing vegetation productivity as C_a has been increasing (Cernusak *et al.*, 2019; Haverd *et al.*, 2020), and builds upon recent observations showing widespread stimulated A_{net} resulting in increased iWUE in the United States (Guerrieri *et al.*, 2019). Moreover, the rate at which iWUE increased was highest in those chronologies with increasing $\Delta^{18}\text{O}_{\text{lw}}$ (reduced g_s) (Fig. 2.4c), followed by those with constant $\Delta^{18}\text{O}_{\text{lw}}$ (constant g_s) (Fig. 2.4b), and finally those with decreasing $\Delta^{18}\text{O}_{\text{lw}}$ (increased g_s) (Fig. 2.4a). This again highlights the importance of stimulated A_{net} in driving increasing iWUE in all cases and supports

reductions in g_s , inferred through increasing $\Delta^{18}\text{O}_{\text{lw}}$, exacerbating realized increases in iWUE. Finally, those chronologies that showed no trend in iWUE since 1963 ($N = 18$) had constant ($N = 14$) or decreasing $\Delta^{18}\text{O}_{\text{lw}}$ ($N = 4$), suggesting any potential increases in A_{net} were not sufficient to offset the increase or constant g_s .

2.5. Conclusion.

Our coupled analysis of iWUE and $\Delta^{18}\text{O}_{\text{lw}}$ highlights the importance of stimulated A_{net} as the main driver behind increasing iWUE over the last 115 years, although some proportion of iWUE is still unexplained by factors not included in this study. Through the analysis of tree rings, we cannot exclude potential effects of changes in leaf area index (Frank *et al.*, 2015), tree level photosynthesis or hydraulic conductivity with age which may reduce both A_{net} and g_s (Ewers *et al.*, 2005; Ryan *et al.*, 2000), or height or size effects on carbon isotope discrimination (Brienen *et al.*, 2017). Further, we cannot examine air pollution effects over the study period due to the paucity of available data for the study locations, which could influence iWUE (Boettger *et al.*, 2014a; Holmes, 2014; Mathias & Thomas, 2018). Nevertheless, these data represent a variety of tree ages and types with unique overlap among different time periods throughout the analysis and present a strong case for C_a , PPT_{grw} , and VPD_{grw} as drivers of the consistently observed global trends in iWUE.

This study using tree ring chronologies representing 34 species across 10 biomes presents clear evidence of a ~40% increase in tree iWUE over the 20th century (Fig. 2.1), similar in magnitude to the ~34% increase in C_a which occurred over the same time (Keeling *et al.*, 2015). Importantly, coupling iWUE with $\Delta^{18}\text{O}_{\text{lw}}$ revealed stimulated A_{net} as a consistent driver behind increasing iWUE across ~90% of the examined chronologies, while reduced g_s , or a combination of increasing A_{net} and reduced g_s , was responsible for increases in iWUE in the remaining ~10% (Fig 2.4). The rate at which iWUE increased more than tripled after 1963 and occurred within 5 years of a similar breakpoint in C_a (Fig. S2.7). Furthermore, we identified C_a as a main factor in driving increases in iWUE, which may simultaneously stimulate A_{net} and reduce g_s (Ainsworth & Long, 2004; Ainsworth & Rogers, 2007), although generally C_a stimulated iWUE to a greater degree on those trees containing 100 % tracheids. The widespread patterns of stimulated A_{net} from this study are in line with recent findings of a 31% increase in global photosynthetic carbon gain over the 20th century (Haverd *et al.*, 2020), and directly track increasing C_a (Cernusak *et al.*, 2019; Haverd *et al.*, 2020). However, these data also show the dependency of iWUE on climate factors and suggest trees in areas that experience future increases in PPT_{grw} , or lower VPD_{grw} , may have lower realized iWUE than those areas experiencing drier conditions, or lower evaporative demand, in turn (Table 2.1). Nevertheless, these data showing increased iWUE over the 20th century encompass a spectrum of tree functional types across a broad geographic area,

highlight the complexity of tree responses to environmental change, and reinforce the importance of stimulated photosynthesis, and not reductions in leaf g_s , as the primary driver in global increases in iWUE.

2.6. Tables and Figures.

Table 2.1. Linear mixed effect model results and parameters for the best model (lowest AICc) examining the drivers of tree ring derived iWUE for the period 1963 – 2015 when only considering environmental factors climate and CO₂ (2nd column), including wood type as a fixed effect with no interactions in the model (3rd column), and when including leaf type as a fixed effect with no interactions in the model (4th column). Intercept values for each model represent the value of iWUE when each numerical environmental factor included in the model is at its mean value during the study period. Leaf and wood type parameter estimates indicate the difference from the original intercept value for each respective variable, with comparisons made with “100% tracheids” at the base level for wood type and “needleleaf evergreen” as the base level for leaf type. The marginal R² describes the goodness of model fit given fixed effects only, while the conditional R² describes the goodness of model fit including fixed and random effects (tree species nested within site). Model parameter significance is denoted by an asterisk where ~ denotes p < 0.06, *denotes p < 0.05, **denotes p < 0.01, and ***denotes p < 0.001, while ^{ns} denotes not significant.

Parameter	1963 – 2015	1963 – 2015 _{wood}	1963 – 2015 _{leaf}
Intercept (μmol mol ⁻¹)	83.078 ± 1.401***	87.297 ± 1.292***	87.592 ± 1.326***
C _a (ppm)	0.238 ± 0.007***	0.238 ± 0.007***	0.238 ± 0.007***
PPT _{grw} (mm)	-0.010 ± 0.001***	-0.010 ± 0.001***	-0.010 ± 0.001***
TMP _{grw} (°C)	0.246 ± 0.135 ^{ns}	0.245 ± 0.135 ^{ns}	0.245 ± 0.135 ^{ns}
VPD _{grw} (kPa)	13.141 ± 1.476***	13.131 ± 1.477***	13.131 ± 1.477***
CO ₂ :TMP _{grw}	0.012 ± 0.007 ^{ns}	0.012 ± 0.007 ^{ns}	0.012 ± 0.007 ^{ns}
CO ₂ :VPD _{grw}	-0.175 ± 0.073*	-0.172 ± 0.073*	-0.172 ± 0.073*
TMP _{grw} :VPD _{grw}	1.919 ± 0.915*	-	1.936 ± 0.915*
Diffuse Porous	-	-17.641 ± 2.851***	-
Ring Porous	-	-18.321 ± 2.727***	-
Needleleaf Deciduous	-	-	-3.064 ± 2.667 ^{ns}
Broadleaf Evergreen	-	-	-10.720 ± 7.954 ^{ns}
Broadleaf Deciduous	-	-	-18.952 ± 2.285***
Marginal R ²	0.12	0.38	0.39
Conditional R ²	0.88	0.89	0.89

Figure 2.1. Tree locations from which chronologies of $iWUE$ and $\Delta^{18}O_{lw}$ were developed (a) and group mean-centered $iWUE$ by species within site over the period 1901 – 2015. The color of data points in (a) and (b) correspond to the biome from which trees were growing, while the size of the circle in (a) corresponds to the number of trees used in the development of each carbon and oxygen isotope-derived chronology, respectively. The vertical dashed line in (b) occurs at the year 1963 where the rate of change in $iWUE$ increases. The solid lines in (b) denote the average trend in $iWUE$ for the period before (1901 – 1963) and after (1963 – 2015) the identified breakpoint.

Figure 2.2. Visualization of the nature of the interaction between growing season vapor pressure deficit and atmospheric CO₂ (a) and growing season vapor pressure deficit and CO₂ (b) on iWUE during the period 1963 – 2015. The interactions shown represent a given predicted value of iWUE throughout the range experienced by each group mean-centered environmental factor since 1963. Values listed for each environmental factor represent its value with respect to the mean during the study period. Parameter estimates for each interaction are listed in Table 2.1.

Figure 2.3. Visualization of the nature of the interaction between atmospheric CO₂ and growing season precipitation (PPT_{growing}) on iWUE for needleleaf evergreen (a), needleleaf deciduous (b), and broadleaf deciduous (c) tree species, and between atmospheric CO₂ and growing season VPD (VPD_{grw}) for broadleaf evergreen trees (d). The interactions shown represent a given predicted value of iWUE throughout the range experienced by each group mean-centered environmental factor since 1963. Values listed for each environmental factor represent its value with respect to the mean during the study period.

Figure 2.4. Standardized chronologies of iWUE (a, b, c) and $\Delta^{18}\text{O}_{\text{lw}}$ (d, e, f) binned by the individual site $\Delta^{18}\text{O}_{\text{lw}}$ trend for the period 1963 – 2015. Red data points (a,d) contain individual chronologies with decreasing $\Delta^{18}\text{O}_{\text{lw}}$, blue data points (b,e) contain individual chronologies with constant $\Delta^{18}\text{O}_{\text{lw}}$, and green data points (c,f) contain individual chronologies with increasing $\Delta^{18}\text{O}_{\text{lw}}$. The respective slope for iWUE and $\Delta^{18}\text{O}_{\text{lw}}$ for each category (decreasing, constant, increasing $\Delta^{18}\text{O}_{\text{lw}}$) is listed in each respective panel, along with the p value with the corresponding LME model fit where species chronology is nested within site as a random factor. The solid black line in each panel represent the average trend across all data points.

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Chapter 3. Disentangling the effects of acidic air pollution, atmospheric CO₂, and climate change on recent growth of red spruce trees in the Central Appalachian Mountains.

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3.1. Abstract.

In the 45 years after legislation of the Clean Air Act, there has been tremendous progress on reducing acidic air pollutants in the Eastern U.S., yet limited evidence exists that cleaner air has improved forest health. Here, we investigate the influence of recent environmental changes on the growth and physiology of red spruce (*Picea rubens* Sarg.) trees, a key indicator species of forest health, spanning three locations along a 100 km transect in the Central Appalachian Mountains. We incorporated a multi-proxy approach using 75-year tree ring chronologies of basal tree growth, carbon isotope discrimination ($\Delta^{13}\text{C}$, a proxy for leaf gas exchange) and $\delta^{15}\text{N}$ (a proxy for ecosystem N status) to examine tree and ecosystem level responses to environmental change. Results reveal the two most important factors driving increased tree growth since *ca.* 1989 are reductions in acidic sulfur pollution and increases in atmospheric CO_2 , while reductions in pollutant emissions of NO_x and warmer springs played smaller, but significant roles. Tree ring $\Delta^{13}\text{C}$ signatures increased significantly since 1989, concurrently with significant declines in tree ring $\delta^{15}\text{N}$ signatures. These isotope chronologies provide strong evidence that simultaneous changes in C and N cycling, including greater photosynthesis and stomatal conductance of trees and increases in ecosystem N retention, were related to recent increases in red spruce tree growth and are consequential to ecosystem recovery from acidic pollution. Intrinsic water use efficiency (*i*WUE) of the red spruce trees increased by ~51% across the 75-year chronology, and was driven by changes in atmospheric CO_2 and acid pollution, but *i*WUE was not linked to recent increases in tree growth. This study documents the complex environmental interactions that have contributed to the recovery of red spruce forest ecosystems from pervasive acidic air pollution beginning in 1989, about 15 years after acidic pollutants started to decline in the U.S.

3.2. Introduction.

Recent observations of enhanced tree growth in temperate forest ecosystems of the northern hemisphere have raised questions about the underlying causal mechanisms (Boisvenue & Running, 2006; Engel *et al.*, 2016; Fang *et al.*, 2014; S. E. Johnson & Abrams, 2009; McMahon, Parker, & Miller, 2010; Thomas *et al.*, 2013). Is this increased productivity a consequence of forest regrowth following disturbance, or is tree growth being enhanced by environmental changes, such as CO₂ fertilization and climate change (Schimel *et al.*, 2000; Joos *et al.*, 2002)? These are critical questions because forest ecosystems are a significant part of the terrestrial carbon (C) sink that removes nearly 30% of anthropogenic C emissions each year, providing a negative feedback that moderates climate change (Le Quéré *et al.*, 2017). Yet, a large amount of uncertainty exists about the continued capacity of forests to sequester C emissions (Friedlingstein *et al.*, 2006). Initially, forest regrowth after disturbance provides a large C sink, but this sink may not persist as forest productivity slows during the late stages of stand development, thereby limiting the capacity of the C sink (Caspersen *et al.*, 2000; Fang *et al.*, 2014). The persistence of the effects of increased CO₂ and climate change on forest C sequestration are harder to predict and are likely to interact with the dynamics of forest recovery, possibly resulting in either positive or negative feedbacks on the C sink (Anderson-Teixeira *et al.*, 2013). In order to partition the relative contributions by these sets of drivers, and how their complex interactions affect forest C sinks, we need a deeper understanding of how simultaneous changes in key environmental variables affect long-term C cycling in forest ecosystems.

For decades, temperate forests in the eastern U.S. have experienced anthropogenic disturbance, including pervasive levels of acidic air pollution from fossil fuel combustion (Greaver *et al.*, 2012). Before environmental legislation designed to curb pollutant emissions became effective, national levels of SO₂ and NO_x emissions were as high as 29.6 and 25.6 million metric tons annually (Lefohn *et al.*, 1999; EPA, 2015) (Figure S3.1a,b). Since the Clean Air Act and subsequent amendments, SO₂ and NO_x emissions have fallen from their highest levels by 84% and 56%, respectively (Lefohn *et al.*, 1999; EPA, 2015). Although significant progress has been made in understanding C cycling following many types of disturbance in forest ecosystems (Liu *et al.*, 2011), mechanisms that govern recovery from acid deposition following reductions in acidic air pollution are still poorly understood. It has been projected that the recovery of terrestrial ecosystems following long-term acid deposition takes decades (Likens

et al., 1996; Driscoll *et al.*, 2001), and now over four decades after the Clean Air Act of 1970, there remains scant evidence of forest ecosystem recovery from the long history of disturbance by acid deposition in the Eastern U.S. (Engel *et al.*, 2016; Lawrence *et al.*, 2015; Li *et al.*, 2010; Thomas *et al.*, 2013).

In addition to the declines in acidic air pollution since the Clean Air Act, there have been substantial increases in atmospheric CO₂ concentrations (Figure S1c), along with significant changes in climate (Figure S3.1d). Increasing atmospheric CO₂ concentrations stimulate forest productivity, thereby enhancing carbon storage (Norby *et al.*, 2005; Fernández-Martínez *et al.*, 2017); however, these responses are often modulated by changes in temperature or precipitation (Granda *et al.*, 2014; Jennings *et al.*, 2016), in addition to acid pollutants (Fernández-Martínez *et al.*, 2017). It is therefore critical to understand how each of these factors contribute to the recovery of trees from acid pollution and the extent, and nature, of any interactive effects between tree recovery and environmental change.

Tree ring growth and isotope chronologies have become important proxies used to examine historical changes in productivity, plant physiology, and ecosystem processes in a rapidly changing environment (Dye *et al.*, 2016; McLauchlan *et al.*, 2017; Thomas *et al.*, 2013). While tree ring chronologies provide an opportunity to examine changes in forest productivity (Dye *et al.*, 2016), these datasets are strengthened by linking observed changes in growth to underlying physiology (Belmecheri *et al.*, 2014; Thomas *et al.*, 2013). In particular, carbon isotope signatures of tree rings provide information regarding the diffusive and biochemical processes of photosynthesis (Farquhar *et al.*, 1982, 1989). Nitrogen isotope signatures ($\delta^{15}\text{N}$) of tree rings record changes in ecosystem N status, although the exact mechanisms have yet to be fully reconciled (Evans, 2001; Gerhart & McLauchlan, 2014). It has been postulated that tree ring $\delta^{15}\text{N}$ reflects pollutant emissions and deposition of oxidized N compounds (Elliott *et al.*, 2007; Hietz *et al.*, 2010; Gurmesa *et al.*, 2017); however, there is also abundant evidence that $\delta^{15}\text{N}$ reflects ecosystem N cycling dynamics (Nadelhoffer & Fry, 1994; Evans, 2001, 2007; Craine *et al.*, 2009, 2015; Hobbie & Högberg, 2012; Burnham *et al.*, 2016; McLauchlan *et al.*, 2017). These proxies become more informative when an integrative approach is taken, combining multiple indicators of ecosystem status in order to track long term changes in

ecosystem function (Choi *et al.*, 2005; Kwak *et al.*, 2011; Leonelli *et al.*, 2012; Zeng *et al.*, 2014).

For this study, we incorporated a multi-proxy approach, using tree ring basal area increment (BAI), $\Delta^{13}\text{C}$ and $\delta^{15}\text{N}$, to explore the drivers of recent growth and physiological changes of red spruce trees (*Picea rubens* Sarg.), an economically and ecologically important conifer species in temperate forests of the eastern U.S. and a key indicator species of forest disturbance by acidic air pollution (Siccama *et al.*, 1982; Johnson & Siccama, 1983; Hamburg & Cogbill, 1988). Our objectives were to (i) identify those factors most related to proximate increases in red spruce growth in the Central Appalachian Mountains; (ii) examine the relationships between tree growth, reconstructed seasonally-integrated leaf physiology, including $i\text{WUE}$, and environmental factors; and (iii) explore the use of tree ring $\delta^{15}\text{N}$ as an integrator of historical ecosystem N cycling. To meet these objectives, we examined red spruce tree growth using tree rings at three forest locations spanning a 100 km transect in the Central Appalachian Mountains where we have previously examined drivers of ecosystem N availability (Smith *et al.*, 2016). We used Kendall's rank correlation analyses and generalized linear mixed models (GLMMs) to disentangle the multiple environmental effects and their sensitivities on red spruce tree growth into discrete components.

3.3. Methods.

Study locations and red spruce tree growth analyses

Three red spruce forest stands were chosen along a north-south transect of 100 km along the Central Appalachian Mountains. McGowan Mountain (MCG; 38°58' N, 79°41' W) is positioned within the Appalachian Plateau physiographic province, while Span Oak Run (SOR; 38°37' N, 79°46' W), and Cranberry Glades (CGL; 38°12' N, 80°17' W) are located in the Allegheny Mountain Section physiographic province of West Virginia (USGS, 1946). Each site is ≥ 900 meters in elevation and has a southwest aspect with slopes ranging from 0-10%. The mean annual temperature for this region in West Virginia was 9.5°C over the period of 1940-2014 (WV Climate Division 4, NOAA, 2017). The annual total precipitation across this time period was 1229.6 mm yr⁻¹, with 47% of the precipitation during the growing season from May to September (WV Climate Division 4, NOAA, 2017). Soils at each of the three sites consist of a thick organic layer with litter contributed by red spruce (*Picea rubens* Sarg.), eastern hemlock (*Tsuga canadensis*), yellow birch (*Betula alleghaniensis*), and red maple (*Acer rubrum*) as dominant canopy species. These sites are mature closed canopy stands with no evidence of anthropogenic or natural disturbance, such as logging or fire, since logging events in the early 1900's. Overall tree density at MCG, SOR, and CGL was 534, 617, and 675 trees ha⁻¹, with red spruce contributing *ca.* 23%, 25%, and 48% of the trees, respectively. Soils at MCG are classified as earnest and are moderately well to poorly drained, very deep, and have parent material that is colluvium derived from acid shale, siltstone, and sandstone (USDA, 2015). SOR and CGL are snowdog series soils and are very deep, moderately well drained, and colluvium derived from acid sandstone, siltstone, and shale (USDA, 2015). The underlying geology at MCG and CGL consists of Pennsylvanian Pottsville sandstone, while SOR is underlain by Devonian Chemung Shale (WVGES, 1968). Thus, the soil buffering capacities are different at each site with MCG having the lowest buffering capacity and CGL having the greatest buffering capacity.

At each site, 20-25 red spruce trees, greater than 10 cm diameter at breast height (1.4 m aboveground), were cored using a 5.15 mm increment borer (Haglöf Inc., Madison, MS). Trees were randomly selected to avoid any potential biases during growth analyses, and cores were taken perpendicular to the slope to avoid compression and expansion wood. Twenty cores from MCG were harvested at the end of the 2013 growing season, with five additional cores harvested

after the 2014 growing season. At SOR and CGL, all tree cores were collected at the end of the 2014 growing season. Cores were returned to lab where they were processed according to standard dendrochronological techniques, as described by Stokes and Smiley (Stokes & Smiley, 1996). Chronologies were crossdated using WinDENDRO (Regent Instruments, Inc.), and statistically confirmed using dplR (Bunn *et al.*, 2015), after which an individual calendar year was assigned to each growth ring. The average age of sampled red spruce trees from the 3 locations was 109 ± 27 years (mean $\pm$ s.d.). The early portion of the tree ring chronologies represented growth release of saplings after logging in the early 1900's and therefore, the focus of this study was the time period after juvenile growth, 1940 to 2014. The expressed population signals (EPS), a measure of the common variance in a chronology, for each red spruce site across years 1940-2014 were all greater than 0.85, indicating that our sample size adequately represents the tree population (Wigley *et al.*, 1984). Raw ring widths of each year were converted to basal area increment (BAI) to better characterize tree growth and to minimize the effects of tree size and age on annual growth trends using

$$BAI = \pi(R_n^2 - R_{n-1}^2), \quad (1)$$

where R is the radius of the tree at a given year of ring formation, n . At each site, BAI was averaged for each year using the 20-25 red spruce trees at each location to develop site-level chronologies, which we treated as replicates ($N = 3$) in this study.

Isotopic analysis of tree rings

A subset of 5 randomly chosen tree cores were selected from each location to develop C and N isotope chronologies for the red spruce trees. As with BAI, C and N isotopes were averaged for similar years and forest site was a replicate ($N = 3$). Samples were analyzed for their stable C or N isotope composition with a ThermoFisher Delta V+ isotope ratio mass spectrometer interfaced with a Carlo Erba NC 2500 Elemental Analyzer. For these analyses, tree cores were dissected under microscope by scalpel into individual growth rings at the boundary of earlywood and latewood for years 1940-2014. Tree ring whole wood from each individual year was then finely chopped to homogenize the sample, and 1 ± 0.2 mg was packed into tin capsules for C isotopic analysis. Carbon isotope composition was measured as $\delta^{13}\text{C}$ (‰) using

$$\delta^{13}\text{C} (\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) * 1000, \quad (2)$$

where R_{sample} is the ratio of $^{13}\text{C}:^{12}\text{C}$ in the wood sample and R_{standard} is the ratio of $^{13}\text{C}:^{12}\text{C}$ in the standard Pee Dee belemnite (PDB) from the Pee Dee River Formation in Hemingway, South

Carolina. The conversion of leaf carbohydrate to wood was then accounted for by subtracting 3‰ from the wood $\delta^{13}\text{C}$ (Leavitt & Long, 1982; Thomas et al., 2013). Leaf-corrected $\delta^{13}\text{C}$ values were converted to isotope discrimination ($\Delta^{13}\text{C}$) to account for the progressive depletion in atmospheric $\delta^{13}\text{CO}_2$ due to fossil fuel burning using

$$\Delta^{13}\text{C} = \left(\frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{plant}}}{1 + \frac{\delta^{13}\text{C}_{\text{plant}}}{1000}} \right), \quad (3)$$

where $\delta^{13}\text{C}_{\text{air}}$ and $\delta^{13}\text{C}_{\text{plant}}$ are the stable carbon isotope compositions of the atmosphere and plant, respectively (Farquhar *et al.*, 1982). Atmospheric $\delta^{13}\text{C}$ values for a given year up to 2004 used to calculate $\Delta^{13}\text{C}$ were interpolated using the high precision records of atmospheric $\delta^{13}\text{C}$ obtained from Antarctic ice cores (Francey *et al.*, 1999) by McCarroll and Loader (2004). After 2004, atmospheric $\delta^{13}\text{C}$ was calculated as the average change per year (-0.0199 ‰) during the 1940 - 2004 period (Keeling, Piper, Bollenbacher, & Walker, 2010). For this study, needle structure, mesophyll conductance and mesophyll CO_2 fractionation of red spruce trees were assumed to have not changed across the 75-year tree ring chronology (Seibt *et al.*, 2008).

For N isotope samples, whole wood for three consecutive years was combined (e.g. 1941, 1942, and 1943 comprise 1942) to ensure an adequate sample mass for analysis (~10 mg) given the low N concentration in wood and, therefore, represent a wood N isotope signal integrated over 3 years. Wood nitrogen isotope composition was measured as $\delta^{15}\text{N}$ (‰) using

$$\delta^{15}\text{N} (\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) * 1000, \quad (4)$$

where R_{sample} is the ratio of $^{15}\text{N}:^{14}\text{N}$ in the wood sample and R_{standard} is the ratio of $^{15}\text{N}:^{14}\text{N}$ in atmospheric N_2 .

Reconstruction of photosynthetic parameters across the chronology

In order to simulate changes in seasonally-integrated photosynthesis (A), stomatal conductance (g_c), and intrinsic water use efficiency ($i\text{WUE}$) across the tree ring chronology from 1940-2014, field measurements of the relationship between A and leaf intercellular CO_2 (C_i) ($A-C_i$ curves) were coupled with the $\Delta^{13}\text{C}$ chronology, following the methods of (Thomas et al., 2013). First, $\Delta^{13}\text{C}$ was converted to C_i/C_a , the ratio of leaf intercellular CO_2 to atmospheric CO_2 , using

$$\frac{C_i}{C_a} = \frac{\Delta^{13}\text{C} - a}{b - a}, \quad (5)$$

where a is the diffusional fractionation of CO_2 through the stomata (4.4‰) and b is the

biochemical fractionation of CO₂ by Rubisco (27‰) (Farquhar *et al.*, 1982). For each individual year of the chronology, the respective C_i/C_a was multiplied by the atmospheric CO₂ concentration (Keeling *et al.*, 2015) to determine C_i . Values of isotopically-derived C_i were then used to predict the seasonally integrated A using $A-C_i$ relationships derived from measurements at each of our three sites. Stomatal conductance was calculated using methods from (Farquhar & Sharkey, 1982) using

$$g_c = \left| \frac{0-A}{C_a-C_i} \right|, \quad (6)$$

where A is seasonally-integrated photosynthesis, C_a is the atmospheric CO₂ concentration and C_i is the leaf intercellular CO₂ concentration.

For the calculation of A and g_c , we measured $A-C_i$ response curves from at least three red spruce trees per site during June, July, and August 2014. Between 0900 and 1600 EST, we harvested small branches containing intact sun needles from the upper canopy and immediately placed them into water picks to maintain the transpiration stream. $A-C_i$ curves were measured using an open-flow gas exchange system with red/blue LED lights (LI6400XT, Li-Cor, Inc., Lincoln, NE) by placing intact needles into the cuvette at saturating light (1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). After *ca.* 5 min equilibration in the cuvette, rates of photosynthesis were measured at nine ambient CO₂ concentrations between 50-1200 $\mu\text{L CO}_2 \text{ L}^{-1}$ air beginning at ambient CO₂ (400 ppm) at the time of the study. After gas-exchange analyses, all needles were taken to the laboratory and scanned to determine projected leaf area, and gas exchange of red spruce was expressed on a total leaf area basis.

Intrinsic water use efficiency was calculated using C_i and C_a where

$$i\text{WUE} = \frac{A}{g_s} = (C_a - C_i) * 0.625, \quad (7)$$

whereby Fick's law of diffusion of CO₂ into the leaf is solved for the quantity $\frac{A}{g_c}$ and g_c is converted to stomatal conductance to water (g_s) by accounting for the different diffusivities of H₂O and CO₂ (Farquhar *et al.*, 1989).

Statistical analyses

Breakpoint analyses were performed using the R package 'segmented' (Muggeo, 2008) with the red spruce chronologies of BAI, $\Delta^{13}\text{C}$, and $\delta^{15}\text{N}$ to determine specific critical years where significant directional shifts in the 1940-2014 chronologies of BAI, $\Delta^{13}\text{C}$, and $\delta^{15}\text{N}$ occurred. For all further analyses used to examine environmental effects on these proxies of tree

growth, photosynthetic physiology and ecosystem N status, we used 1989 as the critical year where this directional shift occurred, which was determined as the average breakpoint of BAI, $\Delta^{13}\text{C}$, and $\delta^{15}\text{N}$ chronologies (Table 1). We used linear mixed effects (LME) models using the ‘nlme’ package in R (Pinheiro *et al.*, 2018) with year as the single predictor variable, accounting for temporal autocorrelation with an AR(1,0) structure, and including site as a random factor, to examine temporal trends in BAI, A , g_c , and $\delta^{15}\text{N}$ for before (1940-1989) and after (1989-2014) the critical year. We also used breakpoint analysis to identify a critical directional shift that occurred in the $i\text{WUE}$ chronology and used the same LME model structure to determine changes in $i\text{WUE}$ before and after this year.

Initially, we performed exploratory bootstrapped Pearson’s correlations using the R package ‘treeclim’ (Zang & Biondi, 2015) to examine the relationships between annual, growing season (May – September), and monthly mean, maximum, and minimum temperatures, precipitation, and Palmer Drought Severity Index (PDSI) and red spruce tree-ring chronologies from 1940-2014 (WV Climate Division 4, NOAA, 2017). All chronologies were prewhitened prior to these analyses to remove first order autocorrelation.

Kendall’s rank correlation, a nonparametric correlation analysis (Sokal & Rohlf, 2011), was used to examine the relationships between potential explanatory environmental parameters and the dependent variables BAI, $\Delta^{13}\text{C}$, A , and g_c over the 1940-2014 chronology, as well as the period 1989-2014. For these analyses, environmental parameters included U.S. emissions of SO_2 and NO_x (Lefohn *et al.*, 1999; EPA, 2015), atmospheric CO_2 concentrations (Keeling *et al.*, 2015), and climate variables (annual, growing season, and monthly mean temperatures, and precipitation) (WV Climate Division 4, NOAA, 2017). We also examined the potential effects of wet deposition of SO_4^{2-} and NO_3^- in precipitation (WV18; NADP, 2015); however, since NADP measurements only partially covered our 1940-2014 chronology, and because the trends of pollutant emissions and trends of atmospheric wet deposition were strongly related (Figure S1a,b), we only used pollutant emissions in these analyses. For $\delta^{15}\text{N}$, we used Pearson product-moment correlation to examine the relationships between wood $\delta^{15}\text{N}$, U.S. NO_x emissions (EPA, 2015), and NO_3^- deposition (NADP, 2015), as well as the relationships between $\delta^{15}\text{N}$ and $\Delta^{13}\text{C}$, seasonally integrated A , g_c , and BAI of the red spruce trees.

Whereas trends in pollutant emissions and CO_2 were clear across the 75-year chronology, changes in climate variables were less clear due to the high interannual variability of temperature

and precipitation that occurs in this region of the Central Appalachian Mountains. Thus, to carefully examine the potential influences of temperature and precipitation on the chronologies of BAI, $\Delta^{13}\text{C}$, A , and g_c , we first examined the linear trends of annual, growing season and monthly climate variables to determine if significant changes across 1940 to 2014 had occurred. Second, we used analysis of covariance (ANCOVA) to determine whether trends in temperature or precipitation were different before and after 1989. Third, we used analysis of variance (ANOVA) to determine if climate variables during the period 1940-1989 were different from 1989-2014. Finally, ANCOVA was used to determine whether changes in the responses of BAI, A , and g_c to temperature or precipitation were different before and after 1989.

To examine the influence and interactions of environmental parameters on red spruce BAI from 1940-2014 and 1989-2014, we utilized generalized linear mixed models (GLMMs) and model averaging using the R packages ‘nlme’ and ‘MuMIn’ (Bartoń, 2017; Pinheiro *et al.*, 2018). Models differing by less than four AICc units, with respect to the best model, were averaged to identify the nature of the relationship between predictor variables and BAI (Fernández-Martínez *et al.*, 2017). The environmental parameters included in this analysis were selected *a priori* based on their known importance to tree growth and physiology and informed by the results of the initial Kendall’s rank correlation and bootstrapped Pearson’s correlations. For this analysis, we included atmospheric CO_2 , national emissions of SO_2 , national emissions of NO_x , and mean April temperatures as predictor variables, exploring all possible combinations of models, including interactions. We also used combinations of other environmental factors in the GLMM averaging, but the four factors listed above consistently provided the best model fit, capturing the greatest variation in BAI while minimizing the unidentified biotic or abiotic contributions to the temporal variation in BAI, determined as an ‘unknown’ residual effect from the consensus model. In the GLMMs, we accounted for temporal autocorrelation using an AR(1,0) structure, and included environmental parameters as fixed effects, while site was included as a random effect.

The average model was used to identify the relative effect each parameter had on BAI for the 1989-2014 period (Fernández-Martínez *et al.*, 2017). To do this, we first used the average model to predict BAI for a given year. We then ran the model to predict BAI holding a given environmental parameter constant at its respective median value for the region over 1989-2014 (atmospheric CO_2 : 371.5 ppm, NO_x emissions: 20.52 million metric tons, SO_2 emissions: 14.04

million metric tons, mean April temperature: 10.08 °C), while letting all other parameters change as observed. The difference in slope between BAI predicted from the original model and BAI predicted when holding a particular environmental parameter constant was the contribution of that parameter to predicted BAI. The sensitivity of BAI to each parameter was then determined by dividing the contribution of given parameter to BAI by its own trend over 1989-2014. Finally, we determined the total contribution each environmental parameter had on the total change in BAI over 1989-2014 by multiplying the sensitivity of BAI to each respective environmental parameter by that parameter's total change over 1989-2014 (atmospheric CO₂: +46.8 ppm, NO_x emissions: -13.78 million metric tons, SO₂ emissions: -19.50 million metric tons, mean April temperature: +1.82 °C). Standard errors were propagated throughout all calculations using the R package 'propagate' (Spiess, 2017).

Significance was assigned at the $\alpha = 0.05$ level for all analyses. All statistical analyses were performed using JMP software for Macintosh v.12.0 (SAS Institute, Cary NC) or R Version 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria).

3.4. Results.

Tree ring chronology breakpoints and trends in BAI, $\Delta^{13}\text{C}$ and $\delta^{15}\text{N}$

Breakpoint analysis of the 1900-2014 red spruce BAI chronology revealed two distinct breakpoints, with the first at 1941 ± 2.1 years, marking the end of the juvenile growth phase of the red spruce trees (Figure 3.1). The second breakpoint in BAI was 1988 ± 2.4 years, where a directional shift from declining BAI to increasing BAI was observed (Figure 3.1, Table 3.1). Breakpoints in 1940-2014 chronologies of $\Delta^{13}\text{C}$ and $\delta^{15}\text{N}$ exhibit directional changes in the late 20th century that were contemporaneous with those in BAI. Breakpoint analysis for tree ring $\Delta^{13}\text{C}$ indicates a directional shift in tree ring $\Delta^{13}\text{C}$ signatures at 1991 ± 1.5 years (Figure 3.2a, Table 3.1). For tree ring $\delta^{15}\text{N}$, breakpoint analysis shows a directional shift in tree ring $\delta^{15}\text{N}$ signatures at 1987 ± 3.8 years when averaged over the three sites (Figure 3.2b, Table 3.1). One-way ANOVA indicated breakpoint years were not significantly different for a given response variable (BAI, $\Delta^{13}\text{C}$, or $\delta^{15}\text{N}$) across sites ($F = 0.74$, $p = 0.52$), nor for a given site (MCG, SOR, or CGL) across response variable ($F = 0.83$, $p = 0.53$). Thus, the overall average year where directional changes were observed in the 1940-2014 chronologies of BAI, $\Delta^{13}\text{C}$, and $\delta^{15}\text{N}$ at all 3 sites was 1989 ± 1.5 years.

Using 1989 as the overall breakpoint year, red spruce BAI showed a $0.16 \text{ cm}^2 \text{ yr}^{-1}$ decline, or a total reduction of 48.9%, between 1940-1989 ($p < 0.0001$), but a $0.34 \text{ cm}^2 \text{ yr}^{-1}$ increase, or a total increase of 105.8%, from 1989-2014, ($p < 0.0001$) (Figure 1). Tree ring $\Delta^{13}\text{C}$ signatures declined by 17% between 1940-1989 from 20.7 ‰ to 17.7 ‰ ($p < 0.0001$), after which $\Delta^{13}\text{C}$ signatures increased steadily by 0.070 ‰ each year from 1989-2014 ($p < 0.0001$) (Figure 3.2a). Growth rates following the 1989 breakpoint were not different than growth rates during the juvenile period from 1908 to 1941 (ANCOVA, $F = 2.92$, $p = 0.09$; Figure 3.1). In addition, tree ring $\Delta^{13}\text{C}$ signatures for the year 2013 were not different from those for the year 1941 (ANOVA, $F = 0.652$, $p = 0.47$; Figure 3.2a).

Analysis of the nitrogen isotope composition in whole wood of red spruce tree rings for the 48 years prior to 1989 revealed a mean $\delta^{15}\text{N}$ signature of 0.404 ‰, but no trend between 1940-1989 ($p = 0.23$; Figure 3.2b). After 1989, however, the $\delta^{15}\text{N}$ chronology shows an abrupt, significant depletion in the heavier N isotope in all three red spruce locations as $\delta^{15}\text{N}$ progressively declined, on average, by 0.083‰ each year from the mean $\delta^{15}\text{N}$ signatures of 0.404 ‰ before 1989 ($p < 0.0001$; Figure 3.2b). The average N concentrations in red spruce tree

rings were 32% greater after 1989 (0.059%) compared to wood N concentrations before 1989 (0.045%) ($F = 47.37$, $p < 0.0001$).

Changes in environmental factors across the 1940-2014 tree ring chronology

We examined whether there were changes that occurred near 1989 for one or more environmental factors that could possibly explain the observed near simultaneous changes in BAI, tree ring $\Delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures. From 1940-1989, SO_2 emissions in the U.S. averaged 23.13 million metric tons yr^{-1} , but declined by 0.73 million metric tons yr^{-1} from 1989-2014 (Figure S3.1a; ANCOVA, $F = 129.7$, $p < 0.001$) (Lefohn *et al.*, 1999; EPA, 2015). From 1940-1989, NO_x emissions in the U.S. increased by 0.92 million metric tons yr^{-1} ($R^2 = 0.92$, $p < 0.01$), but declined by 0.56 million metric tons yr^{-1} from 1989-2014 (Figure S3.1b; $R^2 = 0.91$, $p < 0.001$) (EPA, 2015). On average, atmospheric CO_2 concentration increased from 311 ppm in 1940 to 397 ppm in 2014, or 1.2 ppm yr^{-1} (Figure S1c) (Keeling *et al.*, 2015). Moreover, the rate at which atmospheric CO_2 concentration increased was 1.0 ppm yr^{-1} greater during the 1989-2014 period than during 1940-1989 (ANCOVA, $F = 136.5$, $p < 0.01$) (Keeling *et al.*, 2015).

Mean annual temperatures in this region have increased significantly over the past 75 years by 0.50°C (WV Climate Division 4, NOAA, 2017) (Figure S3.2a; $R^2 = 0.04$, $p = 0.04$). In contrast, there were no significant changes in mean annual precipitation (Figure S3.2b), growing season temperature (Figure S3.2c), or growing season precipitation from 1940-2014 (Figure S3.2d) (WV Climate Division 4, NOAA, 2017). With the exception of April mean temperatures, temperature and precipitation in this region have not changed significantly at or around 1989 in a way that could affect BAI, $\Delta^{13}\text{C}$, and $\delta^{15}\text{N}$. Mean April temperatures increased by 0.06°C yr^{-1} from 1989-2014, but showed no trend prior to 1989 (Figure S3.1d; ANCOVA, $F = 3.90$, $p = 0.05$), and therefore, mean April temperatures were 0.72°C warmer during 1989-2014 than during 1940-1989 (ANOVA, $F = 4.10$, $p = 0.05$).

Assessing environmental influences on seasonally integrated leaf physiology

Chronologies of seasonally integrated leaf physiology modeled using $\Delta^{13}\text{C}$ revealed an 11% reduction in A ($p < 0.0001$; Figure 3.3a) in red spruce and a 47% reduction in g_c ($p < 0.0001$; Figure 3.3b) from 1940-1989. From 1989-2014, there was a 43% increase in A ($p < 0.0001$; Figure 3.3a) and a 67% increase in g_c ($p < 0.0001$; Figure 3.3b). Using historical monthly, seasonal, and annual climate variables (WV Climate Division 4, NOAA, 2017), atmospheric CO_2 concentrations (Keeling *et al.*, 2015), and U.S. SO_2 and NO_x emissions (Lefohn

et al., 1999; EPA, 2015) to examine environmental influences on A and g_c of red spruce trees, Kendall's rank correlation revealed that A across the entire 1940-2014 chronology was negatively correlated with SO_2 ($\tau = -0.39$, $p < 0.0001$) and NO_x ($\tau = -0.27$, $p < 0.0001$) and positively correlated with atmospheric CO_2 ($\tau = 0.20$, $p < 0.0001$), mean annual temperatures ($\tau = 0.22$, $p < 0.0001$), and mean April temperatures ($\tau = 0.17$, $p = 0.0002$). Across the entire chronology, g_c was negatively correlated with NO_x emissions ($\tau = -0.66$, $p < 0.0001$) and atmospheric CO_2 ($\tau = -0.43$, $p < 0.0001$) and positively correlated with May temperatures ($\tau = 0.13$, $p < 0.01$), June temperatures ($\tau = 0.15$, $p < 0.01$), and June precipitation ($\tau = 0.14$, $p < 0.01$). Increases in A from 1989-2014 are correlated with increases in atmospheric CO_2 ($\tau = 0.73$, $p < 0.0001$), increases in April temperatures ($\tau = 0.23$, $p < 0.01$) and reductions in pollutant emissions of SO_2 ($\tau = -0.72$, $p < 0.0001$) and NO_x ($\tau = -0.69$, $p < 0.0001$). Furthermore, the temperature response of A was higher for a given April temperature during 1989-2014 than during 1940-1989 (ANCOVA; $F = 8.94$, $p = 0.003$) (Figure 3.4a). Increases in g_c from 1989-2014 are correlated with increases in atmospheric CO_2 ($\tau = 0.63$, $p < 0.0001$), increases in April temperatures ($\tau = 0.21$, $p < 0.01$) and May temperatures ($\tau = 0.21$, $p < 0.01$), as well as reductions in pollutant emissions of SO_2 ($\tau = -0.62$, $p < 0.0001$) and NO_x ($\tau = -0.59$, $p < 0.0001$). These environmental factors were the most important influences on A and g_c and complete Kendall's rank correlation analyses are shown in in Table S3.1 for 1940-2014 and in Table 3.2 for 1989-2014.

In this study, there was a 58% increase in seasonally integrated $iWUE$ across the 75-year red spruce chronology ($p < 0.0001$; Figure 3.3c), where $iWUE$ was positively correlated with A ($r = 0.25$, $p = 0.0002$) and negatively correlated with g_c ($r = -0.83$, $p < 0.0001$). There was, however, a breakpoint in $iWUE$ averaged across the 3 sites at 1995 ± 2.5 years, where $iWUE$ declined 13% after that year ($p < 0.0001$; Figure 3.3c). Across the 75-year chronology, BAI of red spruce trees was not correlated with $iWUE$ ($p = 0.10$), but BAI was positively correlated with A ($r = 0.52$, $p < 0.0001$) and g_c ($r = 0.39$, $p < 0.0001$). Kendall's rank correlation revealed that $iWUE$ during 1940-1995 was positively correlated with atmospheric CO_2 ($\tau = 0.71$, $p < 0.0001$), NO_x ($\tau = 0.61$, $p < 0.0001$), mean March temperatures ($\tau = 0.14$, $p = 0.006$), and mean November temperatures ($\tau = 0.13$, $p = 0.013$). There was a negative correlation between $iWUE$ during 1940-1995 with June precipitation ($\tau = -0.16$, $p = 0.002$), mean May temperatures ($\tau = -0.13$, $p = 0.015$), and mean June temperatures ($\tau = -0.15$, $p = 0.006$), mean October temperatures ($\tau = -$

0.13, $p = 0.015$), and mean growing season temperatures ($\tau = -0.10$, $p = 0.05$). After the 1995 breakpoint, after which $iWUE$ declined, Kendall's rank correlation indicated that $iWUE$ was not significantly correlated with any of the environmental factors that we examined.

Tree ring nitrogen isotope composition

Reductions in tree ring $\delta^{15}N$ after 1989 were synchronous with increases in tree ring $\Delta^{13}C$ (Figure 5a; MCG, $r = -0.98$, $p = 0.005$; SOR, $r = -0.96$, $p = 0.01$; CGL, $r = -0.99$, $p < 0.001$), and strongly correlated with increases in A (Figure 3.5b; MCG, $r = -0.99$, $p = 0.002$; SOR, $r = -0.97$, $p = 0.007$; CGL, $r = -0.99$, $p = 0.002$) and g_c (Figure 3.5c; MCG, $r = -0.98$, $p = 0.004$; SOR, $r = -0.95$, $p = 0.01$; CGL, $r = -0.99$, $p < 0.001$) at each red spruce site, although the correlations between tree ring $\delta^{15}N$ and red spruce growth were more variable (Figure 3.5d; MCG $r = -0.87$, $p = 0.056$; SOR $r = -0.93$, $p = 0.02$; CGL $r = -0.60$, $p = 0.28$). The proximate reduction of $\delta^{15}N$ after 1989 was positively correlated with the decline in national emissions of NO_x ($r = 0.92$, $p < 0.0001$; Figure S3.3a) and local wet deposition of NO_3^- ($r = 0.86$, $p < 0.0001$; Figure S3.3b).

Assessing environmental influences on BAI

We first used bootstrapped Pearson's correlations to explore the relationships between current year ring width indices at each red spruce location and monthly mean temperature and precipitation of the current and previous year (Figure S3.4, Table S3.2) (WV Climate Division 4, NOAA, 2017). We found no significant correlations between radial growth and these climate variables, except for a weak positive correlation with the previous year's July precipitation at one red spruce location (SOR) ($r = 0.18$, $p < 0.05$). There were no significant correlations between tree radial growth and maximum monthly temperature, minimum monthly temperature, or PDSI at our red spruce study locations (Table S3.2).

Kendall's rank correlation revealed that BAI of red spruce trees from 1940-2014 declined as atmospheric CO_2 increased ($\tau = -0.14$, $p = 0.002$), as pollutant emissions of NO_x ($\tau = -0.34$, $p < 0.0001$) and SO_2 increased ($\tau = -0.12$, $p = 0.01$) (Table S3.1). GLMMs identified only a negative relationship between NO_x emissions and BAI over 1940-2014 ($p < 0.001$, -0.353 ± 0.05 slope estimate $\pm$ standard error), with no other models with a $\Delta AICc < 4$. Kendall's rank correlation showed increases in BAI from 1989-2014 were correlated with increases in atmospheric CO_2 ($\tau = 0.41$, $p < 0.0001$), increases in April temperatures ($\tau = 0.26$, $p < 0.001$) and reductions in pollutant emissions of SO_2 ($\tau = -0.42$, $p < 0.0001$) and NO_x ($\tau = -0.36$, $p < 0.0001$). Additionally, the temperature response of BAI to April temperatures was greater during 1989-

2014 than during 1940-1989 (ANCOVA; $F = 6.96$, $p = 0.009$) (Figure 3.4b).

In order to specifically examine the recent increases in BAI from 1989-2014, model averaging of the best models ($\Delta AICc < 4$ from the best model; hereafter referred to as ‘average model’) determined by GLMMs indicated the importance of mean April temperatures, a negative interaction between SO_2 and NO_x emissions, a positive interaction between atmospheric CO_2 and SO_2 emissions, and a positive interaction between atmospheric CO_2 and NO_x emissions (Table S3.3). We found that over the 1989-2014 period the average model predicts an increase in BAI of $0.29 \pm 0.02 \text{ cm}^2 \text{ yr}^{-1}$ ($t = 15.60$, $p < 0.001$) (Figure 3.6a), in close agreement with observed BAI ($R^2 = 0.85$, $p < 0.001$) (Figure S3.5). Of the $0.29 \text{ cm}^2 \text{ yr}^{-1}$ increase in BAI, increasing atmospheric CO_2 contributed to a $0.17 \pm 0.02 \text{ cm}^2 \text{ yr}^{-1}$ increase of BAI ($t = 96.05$, $p < 0.01$). Reductions in national emissions of SO_2 contributed to a $0.11 \pm 0.02 \text{ cm}^2 \text{ yr}^{-1}$ increase of BAI ($t = 17.18$, $p < 0.01$), while decreasing emissions of NO_x reduced BAI by $0.06 \pm 0.02 \text{ cm}^2 \text{ yr}^{-1}$ ($t = -7.68$, $p < 0.01$). Higher April temperatures contributed to a small increase in BAI by $0.04 \pm 0.03 \text{ cm}^2 \text{ yr}^{-1}$ ($t = 3.65$, $p < 0.01$) (Figure 3.6a). Unknown contributions due to unidentified biotic or abiotic factors were calculated as the difference between the observed change and all known contributions and accounted for $0.04 \pm 0.05 \text{ cm}^2 \text{ yr}^{-1}$ ($t = 1.08$, $p = 0.28$). Sensitivity analysis of GLMM results revealed that over 1989-2014 average BAI was the most sensitive to increases in April temperatures and reductions in SO_2 emissions, followed by reductions in NO_x , and increases in atmospheric CO_2 (Table 3.3).

These results indicate that increasing atmospheric CO_2 was responsible for the largest change in basal area of the red spruce trees, producing a $4.38 \pm 0.6 \text{ cm}^2$ increase between 1989-2014. Reductions in national SO_2 emissions were the second most important factor, contributing to a $2.96 \pm 0.52 \text{ cm}^2$ increase in basal area (Figure 3.6b). Lastly, reductions in national emissions of NO_x negatively impacted basal area by $-1.70 \pm 0.52 \text{ cm}^2$, while increases in mean April temperatures contributed positively to a $1.01 \pm 0.80 \text{ cm}^2$ increase in basal area (Figure 3.6b).

3.5. Discussion.

A synchronous change in three tree ring proxy indicators, BAI, $\Delta^{13}\text{C}$ and $\delta^{15}\text{N}$, identified 1989 as the average year that three forest stands of red spruce trees, spanning 100 km in the Central Appalachian Mountains, began a 25-year period of accelerated tree growth (Table 3.1). Prior to 1989, there was a 49-year period of declining tree growth, characteristic of reduced growth of red spruce trees exposed to high levels of acidic air pollution and deposition in the eastern U.S. (Cook *et al.*, 1987; Johnson *et al.*, 1988; Eager & Adams, 1992). Our observations of recent increases in growth of red spruce trees are similar to observations of increased growth of red spruce in the northeastern U.S. (Engel *et al.*, 2016) and other tree species in the eastern U.S. (Johnson & Abrams, 2009; McMahon *et al.*, 2010; Thomas *et al.*, 2013; Fang *et al.*, 2014). In our study, GLMM model averaging used to examine the environmental influences on growth of red spruce from 1989-2014 indicated recovery from reduced acidic air pollution, a fertilization effect of increased atmospheric CO_2 , and a positive influence of early spring temperatures, thus highlighting the importance of multiple environmental factors on the proximate increases in red spruce tree growth in the Central Appalachian Mountains.

Since the Clean Air Act of 1970 and subsequent amendments, pollutant emissions in the U.S. have dropped considerably. From 1940-1989, SO_2 emissions averaged 23.13 million metric tons yr^{-1} , but declined by 0.73 million metric tons yr^{-1} from 1989-2014 (Figure S3.1a) (Lefohn *et al.*, 1999; EPA, 2015). Kendall's rank correlation placed the decline in SO_2 emissions as the topmost environmental factor contributing to the observed growth increases in red spruce trees since 1989 (Table 3.2). GLMM model averaging also pointed to a strong effect of decreasing SO_2 emissions on tree growth across 1989-2014, as well as a high sensitivity to these pollutant reductions (Figure 3.6, Table 3.3). The changes in the red spruce $\Delta^{13}\text{C}$ chronology, a proxy indicator for photosynthetic physiology, further support recovery of these trees from acid pollution. The decrease in $\Delta^{13}\text{C}$ in the red spruce tree rings from 1940 to 1989, corresponding to reductions in A and g_c , has been shown in many studies examining the effects of acidic sulfur pollution on the C isotope signatures of trees (Savard *et al.*, 2004; Santruckova *et al.*, 2007; Rinne *et al.*, 2010; Boettger *et al.*, 2014b; Kwak *et al.*, 2016), including species from the eastern U.S. (Li *et al.*, 2010; Thomas *et al.*, 2013). The increase in $\Delta^{13}\text{C}$, corresponding to increases in A and g_c , that we observed after 1989 as SO_2 emissions declined has also been found in studies as a function of decreasing pollution (Boettger *et al.*, 2014b; Li *et al.*, 2010; Thomas *et al.*, 2013), and

is opposite from observations of reduced tree ring $\Delta^{13}\text{C}$ with increased tree height or as trees get older (Mcdowell *et al.*, 2011; Brien *et al.*, 2017). Thus, our data are consistent with the hypothesis that reductions in acidic air pollution and deposition from the historically high totals in the 1970s reached a critical level around 1989 that red spruce trees were better able to tolerate, thereby beginning the recovery of tree growth and photosynthetic physiology of red spruce trees in the Central Appalachian Mountains that has persisted for 25 years as pollution levels continued to decline.

Our study suggests that increases in growth and A after 1989 were likely enhanced by increases in atmospheric CO_2 concentrations, which have increased substantially over the last 75 years and have increased by *ca.* 13% from 1989-2014 (Keeling *et al.*, 2015). Although numerous experiments using large square-wave increases in CO_2 , such as FACE experiments, often show increased tree growth (Norby *et al.*, 2005), an effect of increasing CO_2 on growth is rarely observed in tree ring studies, possibly because the annual step changes in CO_2 are small and any changes in growth are masked by other environmental factors positively or negatively contributing to growth. In our study, both Kendall's rank correlation and GLMM model averaging indicate strong effects of increasing CO_2 on red spruce growth from 1989-2014 (Figure 3.6). In addition, if A is considered a diffusive process, where $A = (C_a - C_i) \cdot g_c$, then the 43% increase in A of red spruce trees after 1989 is likely produced by the combination of the 13% increase in C_a and the 67% increase in g_c as acidic pollution declines. Our data suggesting a CO_2 enhancement of growth and A of red spruce trees since 1989 are consistent with flux tower observations of increased forest productivity from 23 forest sites in the U.S. and Europe from 1995 to 2011, where increases in atmospheric CO_2 were shown to stimulate GPP and NEP (Fernández-Martínez *et al.*, 2017).

Both SO_4^{2-} deposition and elevated CO_2 reduce g_c experimentally (Borer *et al.*, 2005; Ainsworth & Rogers, 2007) and, thus, the combined effects of acid deposition and increasing atmospheric CO_2 are likely responsible for the 47% decline in g_c of red spruce trees from 1940-1989. If we assume that the increase in g_c after 1989 represents a complete recovery from acid deposition, then the 15% reduction in g_c from 1940-2014 reflects only the effect of increasing CO_2 on g_c of the red spruce trees (Figure S3.6a) and is more in line with observed reductions in g_c per ppm CO_2 increase from elevated CO_2 experiments using trees (Ainsworth & Rogers, 2007). Under this assumption, our data indicate that acidic air pollution is a 3.7x greater forcing

factor on g_c of red spruce trees than increasing CO₂ (Figure S3.6a). Numerous experiments, as well as dendroisotopic studies, have shown that $iWUE$, the ratio of A to stomatal conductance of water, of many plants is improved as a result of a combination of increasing CO₂ stimulating A and causing partial stomatal closure (Ainsworth & Rogers, 2007; Franks *et al.*, 2013; Keenan *et al.*, 2013; Knauer *et al.*, 2016), and this has been hypothesized to be an important mechanism leading to increased forest productivity. Over the 1940-2014 tree ring chronology, $iWUE$ of red spruce trees increased 51%, or +0.68% yr⁻¹, as A increased by 27% and g_c declined by 15% from 1940-2014. If we use the Ball-Berry model to predict the theoretical change in $iWUE$ if impacted by changes in CO₂ alone, using a constant C_i/C_a ratio of 0.72 (mean at 1940), then $iWUE$ increases by 30% over the tree ring chronology (Figure S6b), tracking the increase in atmospheric CO₂ (Knauer *et al.*, 2016). If we model $iWUE$ using the decline in g_c attributed to increasing CO₂ for red spruce trees in this study (-15%, Figure S3.6a) and an increase in A that is predicted from CO₂ enrichment studies using trees (Ainsworth & Rogers, 2007), scaled over the range of CO₂ during our study (9.3% increase in A from 1940-1989), then $iWUE$ increases by 41% over the tree ring chronology (Figure S3.6b). Both of these examples, point to the much greater sensitivity of g_c to acidic sulfur pollution than to CO₂ and the subsequent role of pollution in the observed increases of $iWUE$. In addition, several analyses have observed that increases in $iWUE$ do not always translate into greater tree growth (Silva *et al.*, 2010; Peñuelas *et al.*, 2011; Lévesque *et al.*, 2014). In our study, BAI declined 48.9% from 1940-1989 as $iWUE$ increased 67.2%; From 1989-1995, BAI increased 63.6% as $iWUE$ increased 0.6%; And, from 1995-2014, BAI increased 37.4% as $iWUE$ decreased 9.8%. Thus, our study agrees with these analyses that $iWUE$ is a poor predictor of tree growth, especially in mesic environments, such as our red spruce forest locations in the Central Appalachian Mountains.

From 1940-1989, NO_x emissions increased by 0.92 million metric tons yr⁻¹, but declined by 0.56 million metric tons yr⁻¹ from 1989-2014 (EPA, 2015). GLMM model averaging indicated that tree growth of red spruce across 1989-2014 was negatively impacted by reductions in NO_x emissions after 1989 (Figure 3.6). This result was surprising given that Kendall's rank correlation identified strong negative correlations between NO_x emissions and BAI, $\Delta^{13}C$, A and g_c after 1989 (Table 2), as well as across the entire 1940-2014 chronology (Table S3.1). These contradictory results highlight the uncertainty associated with the effects of N deposition on tree growth since N deposition may act as a source of soil acidity and negatively impact tree growth

or may act as a fertilizer source of N and positively impact tree growth (Thomas *et al.*, 2010; Jennings *et al.*, 2016).

The negative correlations between tree ring $\delta^{15}\text{N}$ and BAI, $\Delta^{13}\text{C}$, A and g_c after 1989 add to the difficulty of interpreting the NO_x emissions results from our GLMM model averaging, as our $\delta^{15}\text{N}$ chronology would suggest red spruce tree growth and photosynthesis increased as ecosystem N availability declined. However, even with the dramatic declines in N deposition in this region, an examination of N cycling at these three red spruce forest sites suggests that these ecosystems are not strongly N-limited (Smith *et al.*, 2016). Given the close relationship between tree ring $\delta^{15}\text{N}$ and declines in national emissions of NO_x and local wet deposition of NO_3^- (Figure S3.3), one may hypothesize that the $\delta^{15}\text{N}$ signatures in the red spruce tree ring chronologies reflect changes in the $\delta^{15}\text{N}$ signatures of pollutant sources, since NO_3^- produced from fossil fuel combustion is enriched in ^{15}N relative to biogenic sources (Felix *et al.*, 2012). However, the evidence that tree ring $\delta^{15}\text{N}$ reflects $\delta^{15}\text{N}$ from fossil fuel sources is limited (Gerhart & McLauchlan, 2014), and although the source $\delta^{15}\text{N}$ signature of N deposition over the tree-ring chronology is unknown at our locations, our data do not support this hypothesis given the constant wood $\delta^{15}\text{N}$ prior to 1989 when NO_x emissions were increasing dramatically (Figure 3.2b, Figure S3.1).

An alternative hypothesis is that $\delta^{15}\text{N}$ in plant tissues, including tree rings, reflects ecosystem N supply relative to demand, where a more open N cycle, defined as a scenario where N supply exceeds plant demand, typically results in greater rates of net nitrate production (a process that strongly fractionates against ^{15}N) followed by the loss of isotopically light nitrate by leaching and/or denitrification (Gerhart & McLauchlan, 2014). The loss of ^{15}N -depleted nitrate enriches the residual pool of plant available N that, in turn, leads to elevated values of $\delta^{15}\text{N}$ in plant tissue (Pardo *et al.*, 2002). Following this line of reasoning, the red spruce tree-ring $\delta^{15}\text{N}$ chronology between 1940 and 1989 may reflect a more open N cycle resulting from declining tree growth rates and, therefore, a reduced plant demand for N by red spruce trees. Likewise, the decrease in wood $\delta^{15}\text{N}$ after 1989 may have resulted from a tighter, more closed N cycle, as increased tree growth created a greater demand for N during a time of reductions in N deposition (Figure 3.1, Figure S3.1b). In addition, N concentrations in wood were 32% greater after 1989 compared to before 1989, and this, along with greater tree growth, further suggests that plant N uptake and retention increased after 1989. Our $\delta^{15}\text{N}$ data reflect observations of declining tree

ring $\delta^{15}\text{N}$ signatures across the continental U.S. in recent decades (Elmore *et al.* 2016; McLauchlan *et al.*, 2017) and support the patterns found in the European NITREX studies where experimental removal of N deposition from throughfall led to rapid ecosystem recovery and a more closed N cycle as losses of gaseous N through denitrification and NO_3^- leaching declined (Bredemeier *et al.*, 1998; Corre & Lamersdorf, 2004). Thus, the $\delta^{15}\text{N}$ chronology found in these red spruce trees has broad implications for regional forest ecosystem recovery to pollution, as well as increased water quality and fewer occurrences of eutrophication, since it is likely that less N is lost into stream runoff as N inputs from pollution decline, as has been shown in this region by Eshleman *et al.* (2013).

Changes in historical climate were the least influential environmental variables affecting BAI, $\Delta^{13}\text{C}$, and seasonally integrated A and g_c of red spruce trees after 1989, possibly due to the high interannual variability of temperature and precipitation in the Central Appalachian region (WV Climate Division 4, NOAA, 2017). Precipitation at our study locations did not significantly change over the study period. Precipitation was not different before and after 1989 (Figure S2b,d), and thus, was not identified by either Kendall's rank correlation or GLMMs as having an influence on BAI, $\Delta^{13}\text{C}$, A or g_c from 1989 to 2014. Our results are contrary to Levesque *et al.* (2017), who concluded that increasing water availability after 1984, and not increases in CO_2 or reductions in acid pollution, has been the primary factor driving increases in BAI, A and g_c of two broadleaf species, *Quercus rubra* and *Liriodendron tulipifera*, at Black Rock, NY, where annual precipitation increased 4% and growing season precipitation increased 19% during 1984-2014 compared to 1950-1983 (PRISM Climate Group, 2017).

On the other hand, mean annual temperatures in this Central Appalachian region have significantly increased by 0.50°C over the past 75 years, that was largely contributed to by a 0.72°C increase in mean April temperatures during 1989-2014 compared to 1940-1989 (WV Climate Division 4, NOAA, 2017). Our tree ring data point to changes in early spring phenology of red spruce trees due to these warmer temperatures after 1989 that are manifested as greater tree growth. First, Kendall's rank correlation identified a strong positive correlation between mean April temperatures and BAI, A and g_c of red spruce trees during 1989-2014. Second, the GLMM average model indicated a main effect of April temperatures on BAI of red spruce during 1989-2014 that contributed to tree growth increases, and showed a very high sensitivity, suggesting that even small changes in spring temperatures can affect tree growth. Finally,

ANCOVA indicated that greater mean April temperatures after 1989 translated to greater seasonally integrated *A* and, a subtle, but significant, increase in *BAI* of red spruce trees compared to 1940-1989 (Figure 3.4). Our observations of an accelerated early spring phenology of red spruce trees due to higher spring temperatures are similar to observations of other tree species globally (Black *et al.*, 2000; Piao *et al.*, 2007), as well as in the eastern U.S (Richardson *et al.*, 2009; Dragoni *et al.*, 2011; Elmore *et al.*, 2016).

A large number of elevated CO₂ experiments have generated critical information on how the C cycle is changing in the world's ecosystems (Long *et al.*, 2004; Norby *et al.*, 2005). However, isolating the effects of increasing CO₂ on tree growth in forest ecosystems has been difficult to confirm due to the small annual step-changes in CO₂ and the myriad of interacting environmental factors known to affect whole-tree growth over multi-decadal time spans (Luo & Reynolds, 1999; Hyvönen *et al.*, 2007a). This study provides evidence for increasing CO₂ having a positive effect on tree growth, but also indicates a broad range of complexity as red spruce forest ecosystems recover from decades of acidic air pollution and the realization that none of the environmental factors that are examined here act in isolation. Although declines in acidic air pollution and increases in atmospheric CO₂ have contributed to increases in red spruce tree growth after 1989, climate change during the early spring also has occurred, which in turn, positively affected the growth of the red spruce trees. It remains unclear how long the increases in growth of these red spruce stands will be sustained. Plant available N will likely be important to maintain this increase in productivity (Luo *et al.*, 2004) and, despite evidence of increased N uptake and retention by the red spruce trees, N inputs from anthropogenic sources are clearly declining. Although the purpose of our study was to examine the timing and physiological basis of recent growth by red spruce trees in the Central Appalachian Mountains, a simple scaling exercise using our tree growth data, red spruce allometry (Whittaker *et al.*, 1974; Siccama *et al.*, 1994), and forest inventory biomass data (Kelldorfer *et al.*, 2012) coupled with forest land cover estimates (Homer *et al.*, 2015) (Figure S3.7) suggests the carbon sink in the Northeastern temperate forest could have been reduced by as much as 0.79 Pg C since 1940 due to acid deposition (Table S4), or roughly equivalent to 12% of anthropogenic growth in global atmospheric C in 2016 (Le Quéré *et al.*, 2017), if all species responded the same as red spruce trees. While this back of the envelope estimate clearly needs refinement using a range of species sensitivities to acidic pollution, our assessment suggests that a significant portion of the increases

in the temperate forest carbon sink in the U.S. observed by Pan *et al.* (2011) could be explained by recovery of trees from decades of acid deposition. This highlights the necessity to better quantify the influence of acidic air pollution on forest ecosystems globally in order to accurately resolve the contribution of forests to the global carbon cycle.

3.6. Tables and Figures.

Table 3.1. Estimated breakpoints ($\pm$ standard errors) where a directional change in the 1940-2014 chronologies of red spruce basal area increment (BAI), $\Delta^{13}\text{C}$, and $\delta^{15}\text{N}$ occurred for McGowan Mountain (MCG), Span Oak Run (SOR) and Cranberry Glades (CGL). At each site, $N = 20\text{-}25$ red spruce trees. The overall breakpoint, defined as the average breakpoint at all 3 sites and all 3 proxies, was the year 1989 ± 1.5 years.

Site	BAI	$\Delta^{13}\text{C}$	$\delta^{15}\text{N}$
MCG	1985 ± 1.8	1990 ± 0.8	1985 ± 2.4
SOR	1993 ± 2.3	1992 ± 0.9	1981 ± 1.8
CGL	1987 ± 2.2	1993 ± 1.0	1994 ± 7.4

Table 3.2. Kendall's rank correlation coefficients (τ) between environmental parameters and the regional chronologies from 1989 to 2014 of basal area increment (BAI), $\Delta^{13}\text{C}$, seasonally integrated net photosynthesis (A), and seasonally integrated stomatal conductance (g_c) of red spruce trees from three locations in the central Appalachian Mountains. Environmental parameters included national SO_2 and NO_x emissions (Lefohn *et al.*, 1999; EPA, 2015), atmospheric CO_2 concentrations (Keeling *et al.*, 2015), and monthly, growing season, and annual precipitation and temperatures (WV Climate Division 4, NOAA, 2017). τ is shown for significant correlations only, and * denotes $p < 0.01$, ** denotes $p < 0.001$, while *** denotes $p < 0.0001$.

Environmental Parameter	BAI	$\Delta^{13}\text{C}$	A	g_c
NO_x	-0.364***	-0.326***	-0.689***	-0.594***
SO_2	-0.422***	-0.338***	-0.718***	-0.618***
Atmospheric CO_2	0.414***	0.343***	0.727***	0.625***
MGT			0.207	0.193
January temperature			-0.203	-0.215*
February temperature			-0.175	-0.171
April temperature	0.263**		0.233*	0.210*
May temperature			0.190	0.211*
February precipitation	-0.154		-0.186	
March precipitation			-0.165	
December precipitation			0.167	0.157

Table 3.3. Mean sensitivity ($\pm$ standard errors) of BAI to each environmental parameter determined to be significant by the average model for the 1989 to 2014 period. Sensitivities are defined as the change in BAI (cm^2) to a one-unit change for each of the environmental factors listed and were determined by dividing the contribution of each factor to BAI by the respective trend of each factor over 1989-2014. Units for environmental factors are in parentheses.

Factor	Sensitivity
Atmospheric CO ₂ (ppm)	0.093 ± 0.013
SO ₂ emissions (10 ⁶ metric tons)	-0.152 ± 0.029
NO _x emissions (10 ⁶ metric tons)	0.123 ± 0.040
April temperature (°C)	0.556 ± 0.521

Figure 3.1. Average basal area increment (BAI) of red spruce trees from three forest sites in the Central Appalachian Mountains from 1900-2014 (N = 20-25 trees for each site). Open circles indicate the juvenile growth phase after logging in the early 20th Century, whereas closed circles represent the mature growth phase.

Figure 3.2. Chronology of carbon isotope discrimination, $\Delta^{13}\text{C}$, (a) and (b) wood $\delta^{15}\text{N}$ for red spruce trees ($N = 5$ per site) from three forest sites in the central Appalachian Mountains. The $\Delta^{13}\text{C}$ chronology in (a) includes 1940-2014, while the $\delta^{15}\text{N}$ chronology in (b) includes 1940-2013. The vertical dashed lines in (a) and (b) are at 1989, the average critical year for the region determined via breakpoint analysis where a directional shift in BAI, $\Delta^{13}\text{C}$, and $\delta^{15}\text{N}$ occurred. The three red spruce forest sites are McGowan Mountain (MCG), Span Oak Run (SOR), and Cranberry Glades (CGL).

Figure 3.3. Chronology of seasonally integrated photosynthesis modeled using $\Delta^{13}\text{C}$ for red spruce trees ($N = 5$ per site) from three forest sites in the central Appalachian Mountains from 1940-2014 (a), seasonally integrated stomatal conductance modeled using $\Delta^{13}\text{C}$ (b), and intrinsic water use efficiency ($i\text{WUE}$) derived from the chronologies of $\Delta^{13}\text{C}$ (c). The vertical dashed lines in (a) and (b) are at 1989, the average critical year for the region determined via breakpoint analysis where a directional shift in BAI, $\Delta^{13}\text{C}$, and $\delta^{15}\text{N}$ occurred. The vertical dashed line in (c) is at 1995, the critical year where a directional shift in $i\text{WUE}$ occurred. The three red spruce forest sites are McGowan Mountain (MCG), Span Oak Run (SOR), and Cranberry Glades (CGL).

Figure 3.4. Relationships between seasonally integrated photosynthesis (a) and basal area increment with mean April temperatures (b) before and after 1989. ANCOVA was used to determine if the response of seasonally integrated photosynthesis or basal area increment to April temperatures were different before and after 1989, the average critical year for the region determined via breakpoint analysis where a directional shift in BAI, $\Delta^{13}\text{C}$, and $\delta^{15}\text{N}$ occurred. Climate data are from West Virginia Division 4, which is the region that contains the three red spruce stands in this study (WV Climate Division 4, NOAA, 2017).

Figure 3.5. Relationships between carbon isotope discrimination (a), seasonally integrated photosynthesis (b), seasonally integrated stomatal conductance (c), and basal area increment with wood $\delta^{15}\text{N}$ (d) at each red spruce site (N = 5 trees per site) from 1989-2013. In each panel, correlation coefficients are from top to bottom, McGowan Mountain (MCG), Span Oak Run (SOR), and Cranberry Glades (CGL).

Figure 3.6. Contribution of atmospheric CO₂, NO_x emissions, SO₂ emissions, and mean April temperatures to the change in BAI each year predicted by the GLMM average model for 1989-2014 (a), and the contribution of CO₂, NO_x emissions, SO₂ emissions, and mean April temperatures to the total change in BAI over 1989-2014 (b). Numbers in brackets indicate the direction and magnitude of changes in environmental parameters. Numbers in brackets in panel (a) represent the trend in each respective environmental parameter over 1989-2014, while numbers in brackets in panel (b) represent the total change in each respective environmental parameter over 1989-2014. Units for CO₂ and April T_{mean} are ppm and °C, respectively, while units for NO_x and SO₂ are 10⁶ metric tons. Unknown contributions in (a) were calculated as the difference between the observed change and all known contributions. Models with $\Delta AIC_c < 4$ were included in the average model (see Table S3 for model structure). Asterisks (*) indicate significance at the $\alpha = 0.05$ level.

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Chapter 4. Air pollution and climate change interact to influence the growth and water use efficiency of *Quercus rubra* and *Liriodendron tulipifera* in the Central Appalachian Mountains.

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4.1. Abstract.

Many environmental factors, including climate change, increasing atmospheric CO₂, and reductions in acidic air pollution, may alter the balance between forest carbon uptake and water loss and will likely have cascading effects on forest ecosystem function. Here, we identify the individual effects of changing environmental factors, and their interactions, on the long-term growth and physiology of two broadleaf deciduous tree species growing in the Central Appalachian Mountains. We collected 84 increment cores from *Quercus rubra* (N = 41) and *Liriodendron tulipifera* (N = 43) from two adjacent study locations in the Fernow Experimental Forest, Parsons, WV, USA, from which we developed chronologies of basal area increment (BAI) and intrinsic water use efficiency (iWUE). Sustained increases in BAI of both mature tree species since 1960 were largely driven by reduced acidic sulfur pollution and increasing summer precipitation, while declining acidic nitrogen pollution negatively impacted tree BAI. Increasing atmospheric CO₂ largely contributed to increasing iWUE but increases in iWUE were attenuated by acidic nitrogen pollution. Interactive effects between environmental factors reveal that responses to environmental drivers of growth and physiology are complex and do not operate in isolation. Our results underscore the importance of considering the interactive effects of air pollution and climate change and on tree growth and physiology over multi-decadal time scales, and this study highlights the need to include acidic air pollution and acid deposition in Earth System Models.

4.2. Introduction.

Forest ecosystems represent 97% of the land carbon sink in the conterminous United States, currently sequestering 16% of national carbon emissions from fossil fuels into trees and soil (Woodbury *et al.*, 2007; Lu *et al.*, 2015). Forests also play a central role in the hydrologic cycle by regulating large fluxes of water through transpiration (Schlesinger & Jasechko, 2014). Thus, environmental influences over leaf physiology of trees have cascading effects over ecosystem function. Yet, predicting responses to environmental change has been difficult due to the complex, and sometimes counteracting nature of environmental drivers on forest productivity (Fernández-Martínez *et al.*, 2017, 2019; Mathias & Thomas, 2018). Improving our predictions of forest dynamics in the face of environmental change requires the ability to disentangle the influence of the many factors that affect driving tree growth and physiology.

Invaluable work has documented forest responses to elevated atmospheric CO₂ (eCO₂). Generally, eCO₂ results in increased photosynthesis and reduced stomatal conductance (Ainsworth & Rogers, 2007) that translates to greater productivity (Norby *et al.*, 2005; Fernández-Martínez *et al.*, 2019; Walker *et al.*, 2019) and increased water use efficiency, the ratio of carbon fixed to the amount of water transpired, at the leaf and ecosystem level (Peñuelas *et al.*, 2011; Knauer *et al.*, 2016; Guerrieri *et al.*, 2019). Importantly, environmental factors such as temperature (Way & Oren, 2010; Wu *et al.*, 2011), precipitation (Beer *et al.*, 2010; Wu *et al.*, 2011), and nitrogen availability (Thomas *et al.*, 2010; Horn *et al.*, 2018) impact these processes and may modify the response to eCO₂ (Fernández-Martínez *et al.*, 2019). Often overlooked, however, acidic air pollution and ozone can exert additional controls over leaf physiology, reducing photosynthetic rates (Choi *et al.*, 2014; Lombardozzi *et al.*, 2015) and stomatal conductance (Borer *et al.*, 2005), negatively impacting tree growth (Horn *et al.*, 2018), and offsetting positive effects of eCO₂ on productivity (Fernández-Martínez *et al.*, 2017; Mathias & Thomas, 2018).

Dendroecology has gained substantial traction as an approach to examine the multi-decadal response of forest stands to the myriad of changing environmental factors, including in locations experiencing high acid pollutant loads (Boettger, Haupt, Friedrich, & Waterhouse, 2014; Engel *et al.*, 2016; Kosiba, Schaberg, Rayback, & Hawley, 2018; Mathias & Thomas, 2018; Savard, Bégin, & Marion, 2014; Thomas *et al.* 2013). For example, tree ring width and stable carbon isotope chronologies provide annual resolution to estimate plot-level biomass (Dye

et al., 2016) and track ecosystem productivity across large spatial scales (Babst *et al.*, 2014; Belmecheri *et al.*, 2014; Levesque *et al.*, 2019). Furthermore, carbon isotope signatures ($\delta^{13}\text{C}$) are directly related to leaf physiological properties during carbon fixation (Farquhar, O'Leary, & Berry, 1982) and can be used to model reconstructed leaf photosynthesis, stomatal conductance, and intrinsic water use efficiency over time (Battipaglia *et al.*, 2013; Lavergne *et al.*, 2019; Mathias & Thomas, 2018; Thomas *et al.*, 2013). As such, dendroecology provides an important lens to examine the growth and physiological responses of trees to environmental change over multi-decadal to centurial timescales.

The northeastern United States is one of the most productive, heavily forested areas of the U.S. (Pan *et al.*, 2013). Importantly, this region of the U.S. has experienced significant climate change over the past century as both temperature and precipitation have changed (PRISM Climate Group, 2004). It has also experienced some of the highest levels of acidic air pollution since the rise of industrialization (Driscoll *et al.*, 2001). Dendroecological studies have pointed to the recovery of evergreen conifers following reductions in pollutant emissions after adoption of the Clean Air Act of 1970 as a primary driver of recent tree growth in this region (Kosiba *et al.*, 2018; Mathias & Thomas, 2018; Thomas *et al.*, 2013). However, the extent to which reductions in acid pollutants affect the growth and physiology of broadleaf deciduous trees is not as clear (Sullivan *et al.*, 2013; Bishop *et al.*, 2015; Levesque *et al.*, 2017).

In this study, we tease apart the combined effects of changing atmospheric CO_2 , temperature, precipitation, and sulfur (S) and nitrogen (N) acid deposition on the long-term growth and intrinsic water use efficiency (iWUE) of two of the most ecologically important broadleaf tree species in the eastern U.S., northern red oak (*Quercus rubra*) and tulip poplar (*Liriodendron tulipifera*). We focus our analysis on 110-year old forest stands located in two small, adjacent watersheds in the Central Appalachian Mountains. These watersheds are downwind from a large number of coal-fired power plants along the Ohio River basin and have experienced moderate changes in climate over the last century. Consistent with experimental observations, one may hypothesize eCO_2 will stimulate tree growth and iWUE (Peñuelas *et al.*, 2011; Walker *et al.*, 2019). Alternatively, climate may be a more important driver of tree growth and physiology, as has been shown in the northeastern U.S. (Levesque *et al.*, 2017). Lastly, given the proximity to coal-fired power plants in the Ohio River basin, high acid pollutant loads may moderate, and potentially overwhelm, any positive effects from eCO_2 or changing climate as has

been seen in conifers in this region (Mathias & Thomas, 2018; Thomas et al., 2013), as well as for other tree species globally (Fernández-Martínez *et al.*, 2017). In this study, we use linear mixed effects models to determine which environmental factors, and their interactive effects, are the most important drivers of the growth and physiology of northern red oak and tulip poplar in the Central Appalachian Mountains since the mid-20th century.

4.3. Methods.

Site descriptions and increment core collection and processing

Two broadleaf deciduous forest stands in the Fernow Experimental Forest, Parsons, WV were selected for this study. Both Watershed 10 (WS10; 39.0566°N, -79.6789°W) and Watershed 13 (WS13; 39.0617°N, -79.6805°W) are located within the Appalachian Plateau physiographic province (USGS 1946) and are considered reference watersheds for the Fernow; they have remained free from disturbance since the early 1900s. Both watersheds contain the study species northern red oak (hereafter *Quru*) and tulip poplar (hereafter *Litu*) as canopy tree species. In addition to the two study species, American beech (*Fagus grandifolia*), red maple (*Acer rubrum*), and sugar maple (*Acer saccharum*) are found between the two locations. Mean elevation of WS10 and WS13 is ~725 and ~694 meters above sea level, respectively. The topography at WS10 and WS13 is relatively steep, with 26% and 25% slopes, respectively, and while WS10 has a southerly aspect, WS13 has a northerly aspect. Soils at WS10 and WS13 consist of a relatively thin organic layer with litter primarily contributed from the genera *Quercus* and *Liriodendron*, as well as *Acer* and *Fagus*. Soils at the study locations are classified as Calvin channery silt loam derived from acidic sandstone and shale, being well-drained, and moderately deep (USDA Soil Survey Geographic (SSURGO), 2015). During the study period (1960 – 2015), mean annual temperature (MAT) at WS10 and WS13 was 9.0°C and 9.2°C, respectively, ranged on average from 2.6°C to 20°C across the year, and increased by 0.75°C at both watersheds. Mean annual precipitation (MAP) at WS10 and WS13 was 1314 mm and 1310 mm, respectively, with the majority of precipitation occurring in July ($10.2 \pm 0.2\%$ of total annual precipitation) and the least amount of precipitation occurring in October ($6.8 \pm 0.2\%$ of total annual precipitation) (PRISM Climate Group, 2004). Mean annual precipitation increased during the study period and was driven largely by precipitation during June and July ($+ 0.75 \text{ mm yr}^{-1}$) (PRISM Climate Group, 2004).

During the 2016 growing season, ≥ 20 trees of each species larger than 10 cm diameter at breast height (1.4 m aboveground) and older than ~80 years were cored at each study location using a 5.15 mm increment borer (Haglöf Inc., Madison, MS). Cores were taken perpendicular to the slope to avoid compression and expansion wood. Following collection, increment cores were returned to the lab where they were air dried and processed according to standard dendrochronological techniques (Stokes & Smiley, 1996). Increment cores were scanned at 1000

dpi to create a digital image, after which each growth ring was measured to the nearest 0.001 mm and assigned at the boundary between earlywood and latewood using WinDENDRO (Regent Instruments, Inc.). Following identification of individual growth rings, chronologies for each respective species within a given watershed were separately crossdated and statistically confirmed using dplR (Bunn *et al.*, 2015), after which a calendar year was assigned to each growth ring. Across both watersheds, the average age of the *Quru* trees included in this study was 92 ± 16 years ($N = 41$, mean $\pm$ SD), while the average age of *Litu* trees was 100 ± 21 years ($N = 43$, mean $\pm$ SD). Across both watersheds, *Quru* trees ranged from 68 to 154 years in age, while *Litu* trees ranged from 56 to 154. The ~40 years of juvenile growth preceding 1960 were excluded from our analyses of the influence of environmental factors on the growth and physiology of *Quru* and *Litu*. The expressed population signal (EPS), a measure of how well the sampled trees reflect a chronology developed from an infinite population (≥ 0.85 is often considered a lower threshold) (Wigley *et al.*, 1984), was 0.91 for *Quru* and 0.83 for *Litu* across the two watersheds for the period 1960 – 2015, respectively, with site and species specific EPS values found in Figure 4.1. Chronologies of measured ring widths were converted to basal area increment (BAI), a metric of tree growth that allows for the detection of trends in growth (Peters *et al.* 2015), using

$$BAI = \pi * (R_n^2 - R_{n-1}^2) \quad (1)$$

where R is the radius of the tree at year, n . If a missing ring was identified, its value was estimated using the average ring width for its respective year, so as not to influence subsequent calculations of BAI. Individual tree chronologies of BAI were then averaged across all trees ($N \geq 20$) for both species within each site.

Carbon isotope analysis of tree rings

Preparation of samples for C isotope analysis followed methods from Mathias and Thomas (2018). Within each site, a subset of 5 increment cores were randomly selected from both species for analysis of tree ring C isotope signatures across the mature phase of the chronology (1960 – 2015). Increment cores were dissected under a stereo zoom microscope into individual growth rings at the boundary of earlywood and latewood, ground to a fine powder, and 1.0 ± 0.2 mg of ground whole wood from each tree ring was packed into tin capsules (CE Elantech, Lakewood, NJ) for isotope analysis.

Carbon isotope composition of each tree ring was analyzed using a ThermoFisher Delta V+ isotope ratio mass spectrometer (ThermoFisher, Waltham, MA) interfaced with a Carlo Erba NC 2500 Elemental Analyzer (Carlo Erba, Milan, Italy) at the University of Maryland Center for Environmental Science (UMCES) Appalachian Lab. Tree ring stable C isotope composition was reported in standard delta notation $\delta^{13}\text{C}$ (‰) using

$$\delta^{13}\text{C} (\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) * 1000, \quad (2)$$

where R_{sample} is the ratio of $^{13}\text{C}:^{12}\text{C}$ in the wood sample and R_{standard} is the ratio of $^{13}\text{C}:^{12}\text{C}$ in the standard PeeDee belemnite (PDB) from the PeeDee River Formation in Hemingway, South Carolina. To account for the fractionation associated with the conversion of leaf carbohydrate to wood (Leavitt & Long, 1982; Mathias & Thomas, 2018; Thomas et al., 2013), wood isotope signatures were corrected using fractionation factors specific to each species (Table S1; *Quru*: -0.21 ‰, *Litu*: -0.62 ‰). The resulting leaf-corrected C isotope signatures ($\delta^{13}\text{C}_{\text{leaf}}$) were then converted to leaf C isotope discrimination ($\Delta^{13}\text{C}$) to account for the continuous depletion of atmospheric $\delta^{13}\text{CO}_2$ across the chronology due to the burning of isotopically-light fossil fuels using

$$\Delta^{13}\text{C} = \left(\frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{leaf}}}{1 + \frac{\delta^{13}\text{C}_{\text{leaf}}}{1000}} \right), \quad (3)$$

where $\delta^{13}\text{C}_{\text{air}}$ is the $\delta^{13}\text{C}$ signature of atmospheric CO_2 during the period of tissue formation (Farquhar et al., 1982). Records of $\delta^{13}\text{C}_{\text{air}}$ for the period 1960 – 2004 used in the calculation of $\Delta^{13}\text{C}$ were obtained from Francey *et. al* (1999) and (McCarroll & Loader, 2004), with $\delta^{13}\text{C}_{\text{air}}$ for years 2005 – 2015 calculated from the average change each year (-0.0199 ‰) during 1960 – 2004 (Keeling et al., 2010). We assumed no changes in leaf structure or mesophyll conductance over the study period (Seibt *et al.*, 2008).

Chronologies of leaf internal CO_2 concentration (c_i) were calculated from $\Delta^{13}\text{C}$ using

$$c_i = \frac{\Delta^{13}\text{C} - a}{b - a} * c_a, \quad (4)$$

where c_a is the atmospheric CO_2 concentration (ppm), a is the fractionation factor (4.4‰) associated with the diffusion of CO_2 through the stomata, and b is the fractionation factor (27‰) associated with the preferential utilization of $^{12}\text{CO}_2$ over $^{13}\text{CO}_2$ by Rubisco during carbon fixation (Farquhar et al., 1982). Values for c_a used in the calculation of c_i were obtained from the Scripps CO_2 Program (Keeling et al., 2015). Intrinsic water use efficiency, the ratio of net

photosynthesis (A) to stomatal conductance to water (g_s), was calculated according to Farquhar et al. (1989) using

$$iWUE = \frac{A}{g_s} = (c_a - c_i) * 0.625. \quad (7)$$

where 0.625 is ratio of the diffusivity of CO₂ to H₂O (Farquhar et al., 1989).

Statistical analyses

To explore the relationship between climate variables and tree growth from 1960 - 2015, we first performed bootstrapped Pearson's correlations using the package "treeclim" in R (Bunn *et al.*, 2015). Climate variables examined in this analysis include precipitation, as well as monthly mean, maximum, and minimum temperature spanning May of the year prior to ring formation through August of the year of ring formation (PRISM Climate Group, 2004). In addition to the relationship between single monthly climate variables and tree growth, we also examined climate variables integrated over a range of time, using sums for precipitation and means for temperature (i.e. sum of May – September precipitation, or mean of May – September temperature). We accounted for temporal autocorrelation in these analyses.

Kendall's rank correlation, a nonparametric correlation analysis, was used to examine the nature, and strength of the relationships between single environmental factors, including climate, and each response variable, BAI and iWUE during the 1960 – 2015 period for each species as it does not make *a priori* assumptions of the distribution of each factor. Non-climatic environmental parameters examined in these analyses include atmospheric CO₂ concentration (Keeling et al., 2016), U.S. national emissions of SO₂ (Lefohn *et al.*, 1999; EPA, 2015), and U.S. national emissions of NO_x (EPA, 2015). We also considered the effects of sulfate (SO_4^{2-}) and nitrate (NO_3^-) deposition (NADP, 2015) on the growth and physiology of *Quru* and *Litu*; however, given the short history of deposition monitoring in this region (unavailable for >30% of the mature chronology), the high correlation between emissions and deposition at our study locations (Figure S4.1), and the nature of the relationship between emissions and deposition (deposition largely being a product of emissions), we chose to examine the relationship between SO₂ and NO_x emissions and each response variable (Mathias & Thomas, 2018). Climate variables examined in these analyses include monthly, growing season, and annual precipitation and temperature (PRISM Climate Group, 2004), as well as other meaningful combinations (i.e. sum of June and July precipitation) informed by the bootstrapped Pearson's correlations.

To identify temporal trends in BAI and iWUE across both study locations, we

constructed linear mixed effects (LME) models using the package “nlme” in R with ‘Year’ as the sole fixed effect, and ‘Site’ as the random effect for each response variable, accounting for temporal autocorrelation with an AR(1,0) model structure (Pinheiro *et al.*, 2018). To identify temporal trends of each response variable specific to each site and species (i.e. WS10 *Quru* BAI), we used generalized least squares (GLS) regression using the package “nlme”, again with ‘Year’ as the sole fixed effect, and an AR(1,0) autocorrelation structure (Pinheiro *et al.*, 2018). We used ANCOVA to determine if the change in each response variable over time was dependent on study location or species. Further, we used ANOVA to test whether mean BAI or iWUE was different between WS10 and WS13 within a given species, or between species within a given study location.

Model averaging of linear mixed effects (LME) models was used to quantitatively assess the temporal contributions of multiple environmental factors and their potential interactions on BAI and iWUE for both *Quru* and *Litu* using the packages “nlme” (Pinheiro *et al.*, 2018) and “MuMIn” (Bartoń, 2017) in R (Fernández-Martínez *et al.*, 2017; Mathias & Thomas, 2018). We first developed a global model of the environmental factors of interest based on the *a priori* assumption of their physiological importance to BAI and iWUE, in addition to the results of bootstrapped Pearson’s correlations and Kendall’s rank correlation, including all potential interactions up to order two. For these analyses, the final environmental factors examined include atmospheric CO₂, U.S. national emissions of SO₂, U.S. national emissions of NO_x, average minimum May – September temperature, and the total combined June-July precipitation of the current year (except for *Litu* BAI, in which case the previous year’s total combined June-July precipitation was used; see Supplemental Information). We also examined the contribution of additional environmental factors; however, those factors ultimately included in the model consistently captured the greatest variation in either BAI or iWUE, while minimizing unexplained variance from biotic or abiotic factors, thereby reducing the “Unknown” component in the model. From the global model, we explored all possible model combinations (1,450 models for each response variable) fit via maximum likelihood to allow comparison among LME models with different fixed factors, with site as a random effect, and an AR(1,0) temporal autocorrelation structure. All examined models were ranked by AIC_c, and those with a $\Delta AIC_c < 1$ from the best model (*Quru* BAI: 2 models, *Litu* BAI: 2 models, *Quru* iWUE: 4 models, *Litu* iWUE: 2 models) were averaged resulting in a final average model (hereafter “final model”)

which was then used to assess the temporal contribution of environmental factors to BAI and iWUE (Fernández-Martínez *et al.*, 2017; Mathias & Thomas, 2018).

To determine the temporal contribution of each environmental factor to BAI and iWUE, we first predicted each response variable separately for *Quru* and *Litu* over the 1960 – 2015 time period using the final model. We then iteratively forced the final model to run, holding each respective environmental factor at its median value over the study period, while allowing all other factors change as observed. The temporal contribution of a given factor to BAI and iWUE for each species over the study period was then calculated as the difference between slope of the predicted values over time from the final model letting all environmental factors change as observed and the slope of the predicted values over time from the final model when holding a given environmental parameter constant (Fernández-Martínez *et al.*, 2017; Mathias & Thomas, 2018).

We used the R package ‘visreg’ to visualize the averaged LME model interactions among environmental factors and the response variables BAI and iWUE during the study period using the component model with parameter estimates nearest the average model (Breheny & Burchett, 2017). Significance was assigned at the $\alpha = 0.05$ level, and marginal significance at the $\alpha = 0.1$ level. Throughout all analyses, propagation of uncertainty was carried out for addition and subtraction using

$$\varepsilon C = \sqrt{(\varepsilon A)^2 + (\varepsilon B)^2}, \quad (8)$$

while propagation of uncertainty for multiplication and division was calculated using

$$\varepsilon C = C \sqrt{\left(\frac{\varepsilon A}{A}\right)^2 + \left(\frac{\varepsilon B}{B}\right)^2}, \quad (9)$$

where the ε is the propagated uncertainty from the quantities A and B , to the calculated value, C (Fernández-Martínez *et al.*, 2017, 2019; Mathias & Thomas, 2018). All analyses were performed using R version 3.5.0. (R Core Team, 2018).

4.4. Results.

Changes in tree BAI and iWUE throughout the 55-year chronology

From 1960-2015, BAI of *Quru* trees in their mature growth phase in WS10 and WS13 in the Fernow Experimental Forest increased by *ca* 0.32 cm² yr⁻¹, while BAI of *Litu* trees in their mature growth phase increased by *ca* 0.33 cm² yr⁻¹, resulting in a 65% increase in BAI for *Quru* and a 71% increase in BAI for *Litu* across the 55-year chronology (Figure 4.1). Growth rates were not different between species ($F = 1.70$, $p = 0.19$), nor were growth rates different between the two watersheds for *Quru* ($F = 1.23$, $p = 0.26$) or *Litu* ($F = 2.45$, $p = 0.12$).

From 1960-2015, leaf-corrected $\Delta^{13}\text{C}$ of *Quru* trees averaged across both watersheds increased by *ca* 0.020‰ yr⁻² ($p = 0.0001$; Figure 4.2a), while leaf-corrected $\Delta^{13}\text{C}$ of *Litu* trees increased by *ca* 0.011‰ yr⁻² ($p = 0.008$; Figure 4.2b), resulting in a 5.9% increase in leaf $\Delta^{13}\text{C}$ for *Quru* and a 3.2% increase in leaf $\Delta^{13}\text{C}$ for *Litu*. This translated into 10.0% increase in iWUE for *Quru* trees ($p = 0.005$; Figure 4.2c) and a 19.6% increase in iWUE for *Litu* trees ($p = 0.0001$; Figure 4.2d) across the 55-year chronology. Changes across the iWUE chronologies were not different between the two watersheds for *Quru* ($F = 0.125$, $p = 0.72$) or *Litu* ($F = 0.00$, $p = 0.99$), but iWUE was 1.9% greater for *Quru* than in *Litu* averaged across watersheds ($F = 4.33$, $p = 0.04$).

Contribution of environmental factors to changes in BAI across the 55-year tree ring chronologies

Bootstrapped Pearson's correlations highlight the positive relationship between BAI of *Quru* and *Litu* with precipitation, with BAI of *Quru* being positively correlated to total combined June-July precipitation of the current year ($r = 0.18$, $p < 0.05$; Figure S4.2a,c) and BAI of *Litu* being most positively related to total combined June-July precipitation of the previous year ($r = 0.15$, $p < 0.05$; Figure S4.2b,d). Additionally, BAI of *Quru* in WS10 was positively related to previous November precipitation ($r = 0.19$, $p < 0.05$), current year June precipitation ($r = 0.17$, $p < 0.05$), and current year July precipitation ($r = 0.24$, $p < 0.05$), while BAI of *Quru* in WS13 was positively related to current year July precipitation ($r = 0.24$, $p < 0.05$) (Figure S4.2a,c). BAI of *Litu* in WS10 was positively related to previous year October minimum temperature ($r = 0.26$, $p < 0.05$) and current year February temperature ($r = 0.25$, $p < 0.05$), while BAI of *Litu* in WS13 was positively related to previous year July precipitation ($r = 0.21$, $p < 0.05$) (Figure S4.2b,d).

Complete Kendall's rank correlation for BAI of *Quru* and *Litu* using data combined from WS10 and WS13 is listed in Table S4.2. This analysis revealed that BAI of *Quru* was most positively related to atmospheric CO₂ ($\tau = 0.47, p < 0.01$), followed by minimum growing season temperature ($\tau = 0.37, p < 0.01$), growing season precipitation ($\tau = 0.26, p < 0.01$), and July precipitation ($\tau = 0.24, p < 0.05$) (Figure S4.3, Table S4.2). Kendall's rank correlation revealed that BAI of *Litu* was most positively related to atmospheric CO₂ ($\tau = 0.49, p < 0.01$), followed by minimum growing season temperature ($\tau = 0.33, p < 0.01$) (Figure S4.3, Table S4.2). Kendall's rank correlation indicated a strong negative relationship between BAI and U.S. emissions of SO₂ for both *Quru* ($\tau = -0.26, p < 0.01$) and *Litu* ($\tau = -0.32, p < 0.01$) (Figure S4.3, Table S4.2).

Linear mixed effects model (LME) summaries for the 55-year BAI chronologies of *Quru* and *Litu* are shown in Table S4.3. The final model for BAI of *Quru* indicated main effects of climate change on tree growth, whereby growth was positively affected by both minimum growing season temperature ($\beta = 1.20, p = 0.03$) and total combined June-July precipitation ($\beta = 0.08, p < 0.01$). Further, U.S. emissions of NO_x ($\beta = 1.71, p < 0.001$) was found to have a positive effect on BAI of *Quru*. There was a negative effect of U.S. emissions of SO₂ on BAI of *Quru* ($\beta = -0.83, p < 0.01$) (Table S4.3). However, there were interactive effects between total combined June-July precipitation with U.S. emissions of SO₂ ($\beta = -0.002, p = 0.04$) and with NO_x ($\beta = -0.003, p = 0.04$), where the effects of precipitation on BAI were modulated by acidic air pollution (Figure 4.3a,b). We found no effects of increased atmospheric CO₂ on the growth of *Quru* (Table S4.3).

Over the 1960-2015 period, the average model predicts an increase in *Quru* BAI of $0.316 \pm 0.027 \text{ cm}^2 \text{ yr}^{-1}$, in close agreement with the observed BAI ($r = 0.87$). Of the observed $0.318 \text{ cm}^2 \text{ yr}^{-1}$ increase in *Quru* BAI, our analysis of temporal contributions of environmental factors indicated that reductions in U.S. emissions of SO₂ had the greatest impact on BAI, resulting in an increase of $0.326 \pm 0.145 \text{ cm}^2 \text{ yr}^{-1}$ during the study period. This was followed by reductions in U.S. emissions of NO_x, which negatively impacted BAI of *Quru* by $0.108 \pm 0.03 \text{ cm}^2 \text{ yr}^{-1}$ (Figure 4.4a). Increases in minimum growing season temperature contributed positively to BAI of *Quru* by $0.033 \pm 0.039 \text{ cm}^2 \text{ yr}^{-1}$, similar to increased total combined June-July precipitation, which contributed positively by $0.047 \pm 0.04 \text{ cm}^2 \text{ yr}^{-1}$. Unknown contributions to BAI of *Quru* accounted for $0.018 \pm 0.175 \text{ cm}^2 \text{ yr}^{-1}$ (Figure 4.4a).

For the 55-year BAI chronologies of *Litu*, LME model results indicated main effects of atmospheric CO₂ ($\beta = -0.21, p < 0.05$), U.S. emissions of SO₂ ($\beta = -0.87, p = 0.04$), U.S. emissions of NO_x ($\beta = -5.72, p = 0.03$), as well as the total combined June-July precipitation of the previous year ($\beta = 0.018, p < 0.001$) (Table S4.3). Further, there was an interactive effect between atmospheric CO₂ and U.S. NO_x emissions on BAI of *Litu* ($\beta = 0.020, p < 0.01$), where the positive effects of increasing CO₂ were diminished under low NO_x emissions (Figure 4.5a, Table S4.3).

Over the 1960-2015 period, the average model predicts an increase in BAI of *Litu* of $0.287 \pm 0.073 \text{ cm}^2 \text{ yr}^{-1}$, in close agreement with the observed BAI ($r = 0.88$). Of the observed $0.329 \text{ cm}^2 \text{ yr}^{-1}$ increase in BAI of *Litu*, our analysis of the influence of these environmental factors indicated that reductions in U.S. emissions of SO₂ had a large positive contribution to increases in BAI of $0.357 \pm 0.186 \text{ cm}^2 \text{ yr}^{-1}$, while reductions in U.S. emissions of NO_x contributed negatively by $-0.339 \pm 0.158 \text{ cm}^2 \text{ yr}^{-1}$ (Figure 4.4a). The total combined June-July precipitation of the previous year positively impacted BAI of *Litu* by $0.127 \pm 0.131 \text{ cm}^2 \text{ yr}^{-1}$ and atmospheric CO₂ had a small, but positive, contribution of $0.068 \pm 0.077 \text{ cm}^2 \text{ yr}^{-1}$. Unknown contributions to BAI of *Litu* accounted for $0.074 \pm 0.285 \text{ cm}^2 \text{ yr}^{-1}$ (Figure 4.4a).

Contribution of environmental factors to changes in iWUE across the 55-year tree ring chronologies

Kendall's rank correlation revealed that iWUE of *Quru* was most positively related to atmospheric CO₂ ($\tau = 0.24, p < 0.05$) and mean July temperature ($\tau = 0.24, p < 0.05$), followed by minimum July temperature ($\tau = 0.21, p < 0.05$) and maximum July temperature ($\tau = 0.20, p < 0.05$) (Figure S4.3, Table S4.2). Kendall's rank correlation revealed that iWUE of *Litu* was most positively related to atmospheric CO₂ ($\tau = 0.47, p < 0.01$) and there was a strong negative correlation with U.S. emissions of SO₂ ($\tau = -0.39, p < 0.01$) (Figure S4.3, Table S4.2). Further, iWUE of *Litu* was positively related to minimum annual temperatures ($\tau = 0.21, p < 0.05$), minimum growing season temperatures ($\tau = 0.23, p < 0.05$), minimum temperatures in June ($\tau = 0.19, p < 0.05$) and July ($\tau = 0.24, p < 0.05$), as well as mean July temperature ($\tau = 0.22, p < 0.05$) (Figure S4.3, Table S4.2).

Linear mixed effects model summaries for the 55-year iWUE chronologies of *Quru* and *Litu* are shown in Table S4.3. For iWUE of *Quru*, the final model results showed no significant

main effects across the chronology (Table S4.3), although there were marginally significant interactions among four of the environmental factors examined (Figure 4.3c,d,e). The average linear mixed effects model indicated a positive interaction between atmospheric CO₂ and total combined June-July precipitation ($\beta = 0.0003$, $p = 0.07$) (Figure 4.3c) on iWUE of *Quru*, as well as a negative interaction between atmospheric CO₂ and growing season minimum temperature ($\beta = -0.037$, $p = 0.07$) (Figure 4.3d). There was also a negative interaction between minimum growing season temperature and U.S. emissions of NO_x ($\beta = -0.153$, $p = 0.08$) on iWUE of *Quru* (Figure 4.3e).

The average model predicted an increase of 0.098 ± 0.035 mmol CO₂ mol⁻¹ H₂O yr⁻¹ in iWUE of *Quru* during the 1960 – 2015 study period, in close agreement with the observed 0.106 ± 0.037 mmol CO₂ mol⁻¹ H₂O yr⁻¹ increase in iWUE of *Quru* ($r = 0.83$, $p < 0.001$). Increases in atmospheric CO₂ resulted in a positive contribution to iWUE of *Quru* of 0.193 ± 0.053 mmol CO₂ mol⁻¹ H₂O yr⁻¹ (Figure 4.4b). Reduced U.S. emissions of NO_x contributed -0.041 ± 0.036 mmol CO₂ mol⁻¹ H₂O yr⁻¹ to iWUE of *Quru* and minimum growing season temperature contributed -0.039 ± 0.049 mmol CO₂ mol⁻¹ H₂O yr⁻¹. Total combined June-July precipitation had a positive, but highly variable effect on iWUE of *Quru* of 0.013 ± 0.096 mmol CO₂ mol⁻¹ H₂O yr⁻¹. The unknown contribution of biotic or abiotic factors to the increase in iWUE of *Quru* was -0.028 ± 0.110 mmol CO₂ mol⁻¹ H₂O yr⁻¹ (Figure 4.4b).

For the 55-year iWUE chronology of *Litu*, LME model results identified main effects of minimum growing season temperature ($\beta = -3.72$, $p < 0.01$) and total combined June-July precipitation of the current year ($\beta = -0.16$, $p < 0.01$) (Table S4.3). There was a positive interaction between atmospheric CO₂ and U.S. emissions of NO_x on iWUE of *Litu* ($\beta = 0.011$, $p = 0.01$) (Figure 4.5b, Table S4.3), as well as a negative interaction between U.S. emissions of SO₂ and U.S. emissions of NO_x ($\beta = -0.038$, $p = 0.02$) (Figure 4.5c, Table S4.3). The effect of minimum growing season temperature on iWUE of *Litu* depended on the total combined June-July precipitation of the current year ($\beta = 0.014$, $p < 0.01$) (Figure 4.5d, Table S4.3).

The average model predicted an increase of 0.193 ± 0.018 mmol CO₂ mol⁻¹ yr⁻¹ in iWUE of *Litu* during the 1960 – 2015 study period, similar to the observed 0.195 ± 0.038 mmol CO₂ mol⁻¹ yr⁻¹ ($r = 0.80$, $p < 0.001$). Increases in atmospheric CO₂ made the greatest contribution to increases in iWUE of *Litu*, resulting in an increase of 0.209 ± 0.023 mmol CO₂ mol⁻¹ H₂O yr⁻¹

(Figure 4.4b). U.S. emissions of SO₂ had a small, but highly variable, positive contribution iWUE of *Litu* of 0.004 ± 0.020 mmol CO₂ mol⁻¹ H₂O yr⁻¹, while reductions in U.S. emissions of NO_x had a reduced iWUE of *Litu* by -0.113 ± 0.028 mmol CO₂ mol⁻¹ H₂O yr⁻¹. Minimum growing season temperatures had a small, but highly variable, effect on iWUE of *Litu* of 0.010 ± 0.027 mmol CO₂ mol⁻¹ H₂O yr⁻¹, while total combined June-July precipitation of the current year contributed positively by 0.068 ± 0.040 mmol CO₂ mol⁻¹ H₂O yr⁻¹ over the study period. Unknown contributions to iWUE of *Litu* accounted for 0.015 ± 0.053 mmol CO₂ mol⁻¹ H₂O each year (Figure 4.4b).

4.5. Discussion.

Since *ca.* 1960, canopy *Quru* and *Litu* trees in Watershed 10 and Watershed 13 of the Fernow Experimental Forest have experienced sustained increases in BAI, and moderate increases in iWUE, despite having been in their mature growth phase for *ca.* 55 years (Figure 4.1, Figure 4.2c,d). This persistent increase in tree growth is consistent with observations from *Quru* and *Litu* in the eastern U.S. (Johnson & Abrams, 2009; McMahon *et al.*, 2010) and is similar to recent reports of enhanced tree growth that include conifer species (Engel *et al.*, 2016; Fang *et al.*, 2014; Johnson & Abrams, 2009; Kosiba *et al.*, 2018; Mathias & Thomas, 2018; Thomas *et al.*, 2013) and other broadleaf deciduous tree species (Johnson & Abrams, 2009; McMahon *et al.*, 2010). Further, our results reflect recent studies using tree ring carbon isotopes that show increased iWUE in the eastern U.S. (Levesque *et al.*, 2017; Mathias & Thomas, 2018; Thomas *et al.*, 2013), as well in other tree species globally (Peñuelas *et al.*, 2011; Leonelli *et al.*, 2012; van der Sleen *et al.*, 2015; Frank *et al.*, 2015; Guerrieri *et al.*, 2019), and are similar in magnitude to the growing body of literature documenting increasing ecosystem WUE from eddy covariance flux observations (Keenan *et al.*, 2013; Knauer *et al.*, 2016). This study on *Quru* and *Litu* in the central Appalachian Mountains provides multiple lines of evidence that point to the adverse effect of acidic sulfur pollution on BAI and the positive impact of atmospheric CO₂ on iWUE (Figure S4.3, Figure 4.4), but also reveal the importance of considering the simultaneous effects of multiple environmental factors, including interactions with climate, for understanding long-term tree growth and physiology (Figure 4.3, Figure 4.5).

Acidic air pollution from SO₂ emissions may reduce radial stem growth (Savva & Berninger, 2010) and crown volume of trees (Wang *et al.*, 2007) and may also have negative effects on stomatal and photosynthetic physiology (Savard *et al.*, 2004; Choi *et al.*, 2014). Recent work has reinforced findings of the negative impact of SO₄²⁻ deposition on tree growth and survival (Horn *et al.*, 2018), but has also highlighted the ability of forest ecosystems to recover from declining SO₂ emissions and SO₄²⁻ deposition (Boettger *et al.*, 2014; Engel *et al.*, 2016; Marcos Fernández-Martínez *et al.*, 2017; Mathias & Thomas, 2018; Savard *et al.*, 2004; Thomas *et al.*, 2013). Of all of the environmental factors that we examined, national SO₂ emissions, which have decreased by about 86% since 1960, had the largest effect on BAI of *Quru* and *Litu* over the past 55 years. Yet, despite these results, it is clear that the growth of these two broadleaf deciduous tree species is not as sensitive to acidic air pollution as growth of red

spruce trees in the same region (Mathias & Thomas, 2018). Levesque et al. (2017) and Maxwell et al. (2019) concluded that precipitation and climatic water balance were more important than reductions in S acidic pollution as a driver of tree growth of *Quru* and *Litu*. Indeed, our study found that summer precipitation exerted important contributions to increasing BAI of *Quru* and *Litu* in support of research documenting the importance of water availability for the radial growth of *Quru* and *Litu* in the eastern U.S. (Brzostek et al., 2014; Helcoski et al., 2019), especially during the early growing season (Tardif et al., 2006; Keyser & Brown, 2014; Helcoski et al., 2019). For *Quru*, summer precipitation interacted with acidic sulfur pollution, such that the highest values of BAI were observed when SO₂ emissions were low and June and July precipitation was high, and acidic SO₂ pollution was most damaging when precipitation was low (Figure 3b). Li et al. (2010) found that red oak trees growing in highly polluted locations in NE Pennsylvania were highly sensitive to acidic deposition, but are now recovering after pollution levels declined after the Clean Air Act. Our study sites in the Fernow Experimental Forest have a history of reductions from pervasively high levels of acidic pollution; a local NADP monitoring station (WV18, Tucker County) has recorded that wet SO₄²⁻-S deposition has declined 78% from a high of 44.12 kg ha⁻¹ in 1979 (Figure S4.1b), reflected by a 17% drop in S in stream water chemistry in our study site watersheds since monitoring began in 1984 (Figure S4.1d) (Edwards & Wood, 2011), and the history of acidic S deposition in these locations may help explain why our results differ from those of Levesque et al. (2017) and Maxwell et al. (2019). A first approximation using predicted values from our statistical model of tree growth illustrates that without the observed drastic reductions in SO₂ emissions due to the 1990 Clean Air Act Amendments, letting all other environmental factors change as observed, *ca.* 11-15% less carbon would have been stored in woody biomass of *Quru* and *Litu* trees growing in the central Appalachian Mountains over the past 25 years (Figure 4.6a,b).

Nitrogen deposition may act as a source of soil acidity and negatively impact tree growth or it may act as a fertilizer source of N and positively impact tree growth (Thomas et al., 2010; Jennings et al., 2016). Our results support work that documents enhanced productivity across a range of tree species in U.S. forests experiencing elevated rates of nitrogen deposition, including *Litu* and *Quru* (Thomas et al., 2010; Horn et al., 2018). The question remains, however, as to what happens to tree growth when ecosystem N inputs decline as N deposition declines (Gilliam et al., 2019), as it has in the Fernow Experimental Forest where wet NO₃-N deposition has

decreased by 62% from a high of 23.03 kg ha⁻¹ in 1979 to 7.36 kg ha⁻¹ in 2015 (Figure S4.1a). Stream water N has decreased by 53% in the two watersheds across the same time period (Figure S4.1c). Our data indicate that reductions in U.S. emissions of NO_x, and consequently **NO₃⁻** deposition (Figure S4.1a), had a negative influence on BAI of both tree species over the past 55 years, although the relative contribution was greater for *Litu* than for *Quru* (Figure 4.4a). This finding is in line with work showing a shifts in tree species composition to those functionally more similar to *Litu* than *Quru* under high N deposition (Averill *et al.*, 2018). As with SO₂ emissions, our study found an interactive effect of summer precipitation and NO_x emissions on BAI of *Quru*, whereby the highest values of BAI were observed when NO_x emissions and June and July precipitation were both high (Figure 4.3a), again underscoring the importance of water availability for the growth of deciduous hardwood tree species in eastern deciduous forests (Levesque *et al.*, 2017; Maxwell *et al.*, 2019).

Increasing atmospheric CO₂ stimulates tree growth in experimental settings (Ainsworth & Rogers, 2007) and long-term productivity in deciduous broadleaf and coniferous ecosystems (Cernusak *et al.*, 2019; Walker *et al.*, 2019). Yet, isolating the effects of increasing atmospheric CO₂ from the myriad environmental factors known to affect tree growth and ecosystem productivity in unmanaged ecosystems has been difficult (Hyvönen *et al.*, 2007b; Gedalof & Berg, 2010; Fernández-Martínez *et al.*, 2019), and only recently has a positive CO₂ effect been documented in conifer tree species growing in the central Appalachian Mountains (Mathias & Thomas, 2018). This study found differing results for an effect of increasing atmospheric CO₂ on BAI of *Quru* and *Litu*. While we found no effect of CO₂ on BAI of *Quru*, increasing atmospheric CO₂ made a positive contribution to BAI of *Litu*, although a significant interaction between CO₂ and NO_x deposition indicates that the CO₂ fertilization effect for *Litu* was diminished as nitrogen deposition declined (Figure 4.5a). This interactive effect adds support for the hypothesis that N availability constrains the CO₂ stimulation of plant growth in many terrestrial ecosystems (Luo *et al.*, 2004; Reich *et al.*, 2006; Schulte-Uebbing & de Vries, 2018; Terrer *et al.*, 2019), such that continued increases in atmospheric CO₂ may only stimulate productivity when N is sufficiently available.

In both species, increasing atmospheric CO₂ exerted the greatest influence on iWUE. Although the rate of increase in iWUE of *Litu* over the 55-year chronology was approximately twice that of *Quru*, the magnitude of the positive contribution of atmospheric CO₂ was similar

between the two species (Figure 4.4b), highlighting a consistent leaf-level response of iWUE to increasing atmospheric CO₂ that supports previous studies using tree ring chronologies (Frank et al., 2015; Keller et al., 2017; Mathias & Thomas, 2018; Peñuelas et al., 2011; Saurer, Siegwolf, & Schweingruber, 2004; Thomas et al., 2013) and is consistent with leaf physiology theory (Frank et al., 2015; Lavergne et al., 2019a). However, we found several environmental factors interacted with CO₂ to moderate the integrated response of iWUE in these two species (Figure 4.3, Figure 4.5). Interactions between increasing CO₂ and summer precipitation and increasing CO₂ and minimum growing season temperature on iWUE of *Quru* indicated that atmospheric CO₂ had its largest effect when summer precipitation was low and minimum growing season temperatures were low (Figure 4.3c,d). For *Litu*, an interaction between atmospheric CO₂ and NO_x emissions on iWUE indicated that the greatest effect of CO₂ occurred when NO_x emissions were at their peak (Figure 4.5b). Nitrogen deposition has been shown to correlate positively with iWUE in several recent dendroisotopic studies (Guerrieri et al., 2011, 2016; Leonardi et al., 2012; Jennings et al., 2016), possibly due to a N fertilization effect on photosynthesis. We found similar results, with reductions in N deposition in the Fernow Experimental Forest negatively impacting iWUE of both species. For *Quru*, iWUE increases with NO_x emissions, but it increases more when minimum growing season temperatures are lower (Figure 4.3e). NO_x emissions and SO₂ emissions interact such that the positive effect of NO_x emissions on iWUE of *Litu* is greatest when SO₂ is lowest (Figure 4.5c), and minimum growing season temperature and summer precipitation interact such that iWUE increased with precipitation as temperature increases, but the greatest iWUE occurred at low precipitation and low temperatures (Figure 4.5d). Thus, while our study shows that increasing CO₂ is a strong driver of increased water use efficiency in trees, it is clear that one must examine the interactive effects of environmental factors to fully understand the complexity of how iWUE changes over time. To illustrate this, we performed a simple modeling exercise using our final models to simulate iWUE of *Quru* and *Litu* through the year 2050, with atmospheric CO₂ concentrations increasing according to the RCP 4.5 scenario (IPCC, 2014) and climate factors changing $\pm 25\%$ from their previous 30-year averages by the year 2050 (Figure 4.7a,b). While we use our statistical model to force future predictions of iWUE, rather than a mechanistic model, it becomes apparent that future climate change may differentially affect iWUE of the two tree species, with *Litu* showing less sensitivity

to changes in climate than *Quru*, which may be related to *Litu* being more isohydric through greater stomatal regulation (Roman *et al.*, 2015)

In conclusion, our study presents clear evidence of increases in growth of two broadleaf deciduous tree species in the Central Appalachian Mountains that have been sustained since 1960, and builds upon work documenting recent increases in tree growth and forest ecosystem productivity in the eastern U.S. (Engel *et al.*, 2016; Johnson & Abrams, 2009; Kosiba *et al.*, 2018; Levesque *et al.*, 2017; Mathias & Thomas, 2018; McMahon *et al.*, 2010; Thomas *et al.*, 2013) and globally (Fernández-Martínez *et al.*, 2017, 2019) in response to environmental change. Our data from red oak and tulip poplar trees in the Central Appalachian Mountains show the importance of considering the responses of tree growth and physiology to individual environmental factors, but also point to the complexity and consequences of interactions between environmental factors that are driving the responses of trees in forest ecosystems. This study highlights the independent effects of reductions of S and N deposition on tree growth in the Fernow Experimental Forest, whereby reductions in S deposition positively affect tree growth through reductions in acidity, but reductions in N pollution may diminish a source of plant available N and negatively affect tree growth. Yet, these contrasting effects of acid pollution on vegetation dynamics have largely been overlooked when developing models of plant responses to environmental change. To more accurately predict future forest responses to environmental change, it is crucial that we begin to consider not only the independent, but also the interactive effects of multiple environmental factors on long term tree growth and physiology.

4.6. Figures.

Figure 4.1: Chronologies of basal area increment from 1920 – 2015 for *Quercus rubra* (a, c) and *Liriodendron tulipifera* (b, d) from Watershed 10 (a, b) and Watershed 13 (c, d) in the Fernow Experimental Forest, Parsons, West Virginia, USA. Individual tree growth in each panel is denoted by light gray lines, while the average chronology is denoted by the bold purple and orange lines for *Quercus rubra* and *Liriodendron tulipifera*, respectively. The vertical dashed lines at 1960 in each panel represent the beginning of the mature growth phase of each chronology. The slope of the average growth trend from 1960 – 2015 (solid black trendline) is denoted for each respective panel below the expressed population signal (eps), a measure of how well the sampled trees reflect a chronology developed from an infinite population (Wigley *et al.*, 1984).

Figure 4.2. Chronologies of leaf-corrected carbon isotope discrimination (a,b) and intrinsic water use efficiency (c,d) for the period 1960 – 2015 for *Quercus rubra* (a,c; purple) and *Liriodendron tulipifera* (b,d; orange) in Watershed 10 (circles) and Watershed 13 (triangles) at the Fernow Experimental Forest, Parsons, WV, USA. For each year, iWUE was derived from leaf-corrected $\Delta^{13}\text{C}$ from *Quru* and *Litu* trees in each of the two study locations. Data points in gray represent measurements from each tree, while purple and orange represent mean values within a given site for a given year for each chronology. The solid black trendline in each panel is the average trend from 1960 – 2015.

Figure 4.3. Contour plots depicting the interactive effects among environmental factors on BAI (a, b) and iWUE (c, d, e) of *Quercus rubra* in the Fernow Experimental Forest, Parsons, WV, U.S during the 1960 – 2015 study period. Interactions were determined from the best (lowest AIC_c) linear mixed effects model for each response variable and include the sum of June and July precipitation of the current year (JJ_{ppt}), minimum growing season temperature (Growing_{Tmin}), and U.S. emissions of SO₂ (SO₂ Emissions) and NO_x (NO_x Emissions). Units for NO_x and SO₂ emissions are 10⁶ metric tons, while JJ_{ppt} are in mm, Growing_{Tmin} are in °C, and atmospheric CO₂ are in ppm. Each interaction is plotted across the range experienced by each respective environmental parameter, with the value of the response variable (BAI or iWUE) corresponding to the respective color hue (moving from light green toward dark blue indicates increasing BAI, while moving from dark blue toward dark red indicates increasing iWUE).

Figure 4.4. Contributions of multiple environmental factors to the observed changes in BAI (A) and iWUE (B) for the period 1960 – 2015 for *Quercus rubra* (purple) and *Liriodendron tulipifera* (orange) in Watershed 10 and Watershed 13 in the Fernow Experimental Forest, Parsons, WV, U.S. Numbers in parentheses beside each environmental factor denote the direction and magnitude of the respective average yearly change (i.e. slope) for the period 1960 – 2015. Units for Atmospheric CO₂, Growing_{Tmin}, and JJ_{ppt} are ppm, °C, and mm, respectively, while units for both SO₂ Emissions and NO_x Emissions are 10⁶ metric tons. Unknown contributions were determined by subtracting all known contributions from the observed change during the 1960 – 2015 study period. Model summaries for linear mixed effects models for BAI and iWUE of *Quru* and *Litu* used in the calculation of contributions are listed in Table S3.

Figure 4.5. Contour plots depicting the nature of the interactions among environmental factors on BAI (A) and iWUE (B, C, D) of *Liriodendron tulipifera* in the Fernow Experimental Forest, Parsons, WV, U.S during the 1960 – 2015 study period. Interactions were determined from the best (lowest AIC_c) linear mixed effects model for each response variable and include the sum of June and July precipitation of the current year (JJ_{ppt}), minimum growing season temperature (Growing_{Tmin}), and U.S. emissions of SO₂ (SO₂ Emissions) and NO_x (NO_x Emissions). Units for NO_x and SO₂ emissions are 10⁶ metric tons, while JJ_{ppt} are in mm, Growing_{Tmin} are in °C, and atmospheric CO₂ are in ppm. Each interaction is plotted across the range experienced by each respective environmental parameter, with the value of the response variable (BAI or iWUE) corresponding to the respective color hue (moving from light green toward dark blue indicates increasing BAI, while moving from dark blue toward dark red indicates increasing iWUE).

Figure 4.6. Modelled BAI using the final models for *Quercus rubra* (a) and *Liriodendron tulipifera* (b) under observed (*Quru*, purple line; *Litu*, orange line) and high SO₂ emissions (red lines) scenarios. BAI under high SO₂ (red lines in a and b) was predicted using the average SO₂ emissions level from 1970 – 1990 (24.4 metric tons) forced during the entire period from 1991 to 2015. Cumulative basal area increment for each scenario was then converted to diameter at breast height (DBH), and species specific allometric equations between DBH and aboveground biomass (AGBM) (Chojnacky *et al.*, 2014) were used to determine total aboveground biomass carbon at 2015 for each scenario after accounting for %C in wood (AGBM*0.5). The gray shaded area in a and b represents the calculated integrated difference in basal area between the two scenarios, with the percent difference in carbon stored in AGBM listed in each panel, and the corresponding basal area on the bottom right corner of each panel.

Figure 4.7. Modeled changes of *Quercus rubra* (a) and *Liriodendron tulipifera* (b) intrinsic water use efficiency under hypothetical future environmental change predicted using the final model. Data points represent iWUE for each year in the period 2030 – 2050 as climatic factors linearly approach a $\pm 25\%$ change from their previous 30-year averages by the year 2050 and atmospheric CO₂ concentrations increase according to the RCP 4.5 scenario (IPCC, 2014). Horizontal dashed lines represent observed iWUE in 2015 for each species, respectively.

4.7. Literature Cited.

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Chapter 5. General conclusions about the trends and drivers of tree growth and intrinsic water use efficiency during the 20th century

5.1. General Conclusions.

My dissertation examines the trends and drivers of tree growth and intrinsic water use efficiency (iWUE) during the 20th century. Since the onset of the industrial revolution, there have been drastic increases in atmospheric CO₂ concentrations (C_a) (Keeling et al., 2016), significant changes in climate (Dai, 2012; Trenberth *et al.*, 2014), and pronounced changes in acidic air pollution (WHO, 2016) across the globe. While increasing atmospheric C_a is spatially consistent, climate and acidic air pollution are highly variable across both space and time. Importantly, as trees dynamically respond to environmental change, there will be cascading effects throughout the Earth-climate system at local to continental scales (Swann *et al.*, 2016; Kooperman *et al.*, 2018). Given the central role of trees in linking the carbon and water cycles, the overall aim of this research project was to 1) identify those factors most important in driving global scale changes in tree iWUE, 2) determine at a regional scale how environmental change and reductions in acid pollution affect the growth and physiology of an environmentally sensitive tree species and 3) examine the drivers of long term growth and physiology of two broadleaf tree species in the Central Appalachian Mountains where moderate changes in climate and large reductions in acid pollution have occurred.

In *Chapter 2*, I synthesized annually-resolved tree ring carbon and oxygen isotope data from 49 published articles to 1) examine the global trends and environmental drivers of tree ring $\delta^{13}\text{C}$ derived iWUE and 2) investigate whether trends in tree iWUE are overwhelmingly driven by changes in leaf photosynthesis or stomatal conductance through a combined analysis of tree ring $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$. For this chapter, I compiled 113 unique tree ring $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ chronologies that represent 34 tree species across 84 sites in 10 biomes that span a spectrum of leaf and wood types. Based on experimental (Battipaglia *et al.*, 2013) and long-term observational studies (Frank et al., 2015; Giguère-Croteau et al., 2019; Guerrieri et al., 2019), I hypothesized iWUE would increase across all tree species, regardless of plant functional type and would be driven primarily by increasing atmospheric CO₂. Further, I expected reductions in stomatal conductance (g_s) to overwhelm potential increases in photosynthesis (A_{net}) in cases where iWUE increased. I used a model selection approach to determine the importance and contribution of the environmental factors atmospheric CO₂, growing season precipitation, growing season temperature, and growing season vapor pressure deficit to changes in iWUE for the period 1901

– 2015. To do this, I compared 1,450 unique linear mixed effect model structures, examining every fixed effect combination, including second order interactions, after which models were ranked by the corrected Akaike’s Information Criterion (AIC_c). The best model, as characterized by having the lowest AIC_c, revealed tree-level iWUE increased, on average, by *ca.* 40% over the twentieth century. I identified a breakpoint in the combined iWUE chronology at 1963, after which iWUE increased linearly at a rate of 1.67% per year, approximately 3.9 times faster than the previous 63 years. Across all species and study locations since 1963, model results indicated increasing iWUE was strongly, positively related to atmospheric CO₂, supporting my hypothesis. However, I also found iWUE was also positively related to growing season vapor pressure deficit and was negatively related to growing season precipitation. Of all chronologies examined since the 1963 breakpoint, 18.6% showed decreasing $\Delta^{18}\text{O}_{\text{lw}}$, 71.7% showed constant $\Delta^{18}\text{O}_{\text{lw}}$, and 9.7% showed increasing $\Delta^{18}\text{O}_{\text{lw}}$, despite consistent increases in iWUE. This points to enhanced A_{net} overwhelming increases in g_s where $\Delta^{18}\text{O}_{\text{lw}}$ declined, as well as driving increasing iWUE under constant g_s where $\Delta^{18}\text{O}_{\text{lw}}$ remained constant, such that increasing iWUE was driven by stimulated A_{net} in *ca.* 90% of chronologies examined. These data highlight the complexity of tree responses to environmental change and reinforce the importance of increased photosynthesis, and not reductions in leaf g_s , as the primary driver in widespread iWUE increases.

In *Chapter 3*, I focused my analysis of the drivers of tree-level iWUE to red spruce in the Central Appalachian Mountains, which experienced some of the highest levels of acidic pollution in the eastern United States prior to the Clean Air Act Amendments of 1990 (EPA 2015). Reductions in acidic pollution since *ca.* 1990, coupled with increasing atmospheric CO₂ and moderate changes in climate, provide a unique opportunity to disentangle how multiple environmental factors simultaneously influence the growth and physiology of red spruce. Given the previously documented negative effects of acid deposition on red spruce function (Borer *et al.*, 2005; Engel *et al.*, 2016), I hypothesized growth would be negatively influenced by acidic deposition, and would modulate any CO₂ effect on iWUE. To test this hypothesis, I collected increment cores for growth and carbon and nitrogen isotope analysis from three study locations spanning 100 kilometers in the Monongahela National Forest, West Virginia where red spruce are co-dominant canopy trees. I developed site-level growth chronologies using at least 20 individual trees at each site, after which five randomly sampled cores were analyzed for annual

tree ring $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. While tree ring $\delta^{13}\text{C}$ can be used to calculate tree-level iWUE, tree ring $\delta^{15}\text{N}$ serves as an index of ecosystem nitrogen (N) status, whereby higher $\delta^{15}\text{N}$ signatures correspond to increased nitrogen supply relative to demand. Last, I collected physiological measurements on the red spruce trees (A-C_i curves) in order to reconstruct historical seasonally-integrated photosynthesis and stomatal conductance. I found growth declined at each of the red spruce stands from 1940 to 1989, after which growth increased through 2014. The rates of decline between 1940 and *ca.* 1989 were not different between sites; nor was the rate of recovery after *ca.* 1989. I found the enhanced growth of red spruce after 1989 was driven by increasing atmospheric CO₂, warmer April temperatures, and reductions in acidic sulfur pollution, highlighting the positive effects of increasing atmospheric CO₂ on tree growth in a period of rapidly declining acidic pollution. Using a robust statistical model, I calculated, as a first approximation, the potential consequences of acid pollution on carbon sequestration in forests of the northeastern United States by coupling land cover, biomass, and acid pollution data products and showed since 1940, *ca.* 0.75 Pg C may not have been sequestered in aboveground woody biomass due to high levels of acidic pollution.

Red spruce tree ring carbon isotope analysis showed iWUE increased from 1940 to *ca.* 2005, after which iWUE partially declined, resulting in an overall *ca.* 51% increase in iWUE over the 75-year period. A simple modeling exercise showed CO₂ effects alone correctly predicted iWUE at 2014, following the downturn in iWUE in 2005. Importantly, I found acidic pollution had a larger effect on iWUE of the red spruce trees than did atmospheric CO₂ over the same time period, likely through reductions in stomatal conductance (Borer *et al.*, 2005). Nitrogen isotope analysis showed $\delta^{15}\text{N}$ signatures did not change from 1940 to 1989, after which they sharply declined at all three study locations, suggesting a decrease in ecosystem nitrogen supply relative to demand. Together, this multiproxy analysis shows high acid pollution led to reduced tree growth and N demand from 1940 to 1989, after which tree growth increased as inputs declined, leading to lower N supply relative to demand. In summary, this chapter documents the complex environmental interactions that have contributed to the recovery of red spruce forest ecosystems from pervasive acidic air pollution beginning in 1989, about 15 years after acidic pollutants started to decline in the United States.

In *Chapter 4* of this research project, I examined the historical environmental drivers of northern red oak (*Quercus rubra*) and tulip poplar (*Liriodendron tulipifera*) in two reference watersheds in the Fernow Experimental Forest (FEF), West Virginia. Furthermore, I extended my analysis by performing a simple modelling exercise to examine the potential effects of the Clean Air Act on carbon sequestration in eastern U.S. forests, as well as how iWUE may be expected to change under future climate scenarios. While *Chapter 2* provided insight over the broad-scale controls over tree iWUE and *Chapter 3* underscored the influence of acidic pollution on a highly sensitive tree species, *Chapter 4* provides a highly localized investigation of the controls over growth and iWUE of two tree species largely representative of the ecology of forests of the eastern U.S. in the context of historical and future environmental change. I hypothesized the broadleaf deciduous trees may be sensitive to acidic pollution but will also be highly sensitive to climate. Furthermore, I hypothesized red oak and tulip poplar trees will show increasing iWUE over since 1960, driven primarily by increasing atmospheric CO₂. I conducted this experiment in two adjacent watersheds, WS10 and WS13, in the FEF where I cored approximately twenty trees of each species, northern red oak and tulip poplar. I randomly selected a subset of five trees of each species within each watershed for physiological measurements (A-C_i curves), in addition to $\delta^{13}\text{C}$ analysis to calculate iWUE. I found sustained increases in basal area increment (BAI) of both mature tree species since 1960 that were mainly driven by reduced acidic sulfur pollution and increasing summer precipitation, while declining acidic nitrogen pollution negatively impacted tree BAI. Increasing atmospheric CO₂ largely contributed to increasing iWUE but increases in iWUE were attenuated by declining acidic nitrogen pollution. Interactive effects between environmental factors reveal that responses to environmental drivers of growth and physiology are complex and do not operate in isolation—for example, I found no evidence of CO₂ stimulating growth of red oak trees, but an interaction between N emissions and CO₂ showed tulip poplar growth was stimulated under elevated CO₂, but only when N was sufficient to sustain growth. As such, an important finding from this chapter is how environmental factors interact to influence the growth and physiology of broadleaf deciduous trees over multi-decadal time scales. Given the negative impact of acidic sulfur pollution on tree growth, I used a statistical model to examine the potential impact of having not implemented the Clean Air Act and subsequent amendments on both species. My results show since 1990, both tree species would have stored *ca.* 13% less carbon in

aboveground biomass without this landmark environmental legislation in the absence of NO_x effects that showed species-specific positive associations with growth. I further examined how future climate change may be expected to influence iWUE of both tree species and showed iWUE will continue to increase as atmospheric CO₂ rises, but climate may modulate iWUE much more for red oak trees than for tulip poplar trees. Overall, results from this chapter showcase the importance of considering the interactive effects of air pollution and climate change and on tree growth and physiology over multi-decadal time scales and highlights the need to include acidic air pollution and acid deposition in Earth System Models.

During this research project I found evidence for consistent increases in tree iWUE (*ca.* +40% since 1901) through a global meta-analysis of 34 unique tree species, as well as two observational studies conducted in the Central Appalachian Mountains in the eastern United States. In each study, increasing iWUE was largely driven by increasing atmospheric CO₂, though climate factors such as precipitation and vapor pressure deficit modulate this response, which may be particularly important under future climate scenarios (Figure 4.7). Furthermore, a global meta-analysis using a dual isotope approach showed in approximately 90% of cases examined, increasing iWUE resulted from stimulated leaf photosynthesis overwhelming changes in stomatal conductance (Figure 2.4). These findings are in line with observations of enhanced leaf photosynthesis (Haverd *et al.*, 2020) and vegetation productivity (Cernusak *et al.*, 2019) at the global scale and underscore the dynamic response of leaf physiology over a range of atmospheric CO₂.

A focused analysis of temporal iWUE changes in a region with a history of acidic deposition also showed iWUE may also be modulated by non-climatic factors. For example, iWUE of tulip poplar increased with atmospheric CO₂, but this effect was exacerbated under high N levels (Figure 4.5b), likely through enhanced photosynthesis under increasing N availability (Jennings *et al.*, 2016). Furthermore, I found red spruce trees that had experienced high acidic pollution loads generally showed increasing iWUE over the late twentieth century, although acidic pollution effects on the underlying component parts, photosynthesis and stomatal conductance, altered this response (Figure 3.3). Together, these results underscore the general influence of atmospheric CO₂ and climate on tree iWUE across large spatial scales, but also

highlight the complexity of this response when additional factors such as acidic pollution are considered.

A regional analysis of historical growth of three tree species in the Central Appalachian Mountains reinforces the importance of considering the controls of multiple environmental factors over tree growth and productivity. While we did find evidence for a stimulatory CO₂ effect on growth of two of the three tree species examined, climate also played an important role. Importantly, reductions in sulfur pollution had large, persistent effects on the growth of all three species, and negatively impacted total carbon sequestration in these forest stands as shown through simple modeling exercises (Figure 4.6, Figure S3.7). Further, this research highlights a spectrum of sensitivity to acidic pollution among different tree species.

In conclusion, the results of this research project showcase the dynamic regulation of tree growth and physiology under environmental change. Importantly, I have shown acidic pollution negatively impacts tree growth, and modulates tree iWUE responses to increasing CO₂ and climate change, yet cutting-edge process-based models do not consider these processes. This will introduce uncertainty in projections of forest responses to environmental change, potentially overestimating carbon sequestration especially in areas where acidic pollution is high. However, this research also underscores the ability of forests to recover from the negative impacts of acidic pollution and may provide a promising outlook under future scenarios with stringent environmental legislation.

5.2. Literature Cited.

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Appendix. Supplementary Tables and Figures

Table S2.1. List of sites and species and their associated attributes included in the examination of changes in iWUE and $\Delta^{18}\text{O}_{\text{lw}}$ for the periods 1901 – 2015 and 1963 – 2015. For a given published study, information regarding the location all sites (latitude/longitude) and the number of trees of each species used in this analysis are listed, along with wood type, leaf type, biome, and the trend in iWUE and $\Delta^{18}\text{O}_{\text{lw}}$ for the time periods 1901 – 2015 and 1963 – 2015, respectively. Significance of trends in are denoted by ** for $p < 0.05$, * for $p < 0.01$, and ^{ns} where no significant trend was detected.

Citation	Latitude	Longitude	Latin binomial	N	Wood Type	Leaf Type	Biome	iWUE trend 1901 – 2015	iWUE trend 1963 – 2015	$\Delta^{18}\text{O}_{\text{lw}}$ trend 1901 – 2015	$\Delta^{18}\text{O}_{\text{lw}}$ trend 1963 – 2015
Awada et al. 2019	40.25	-100.27	<i>Populus deltoides</i>	4	Diffuse Porous	Broadleaf Deciduous	Temperate Grasslands, Savannas & Shrublands	1.066**	1.066**	0.020 ^{ns}	0.020 ^{ns}
Colombaroli et al. 2016	0.23	25.25	<i>Pericopsis elata</i>	4	Diffuse Porous	Broadleaf Deciduous	Tropical & Subtropical Moist Broadleaf Forests	0.412**	0.483**	0.037**	0.036**
Hartl-Meier et al. 2014	47.90	14.44	<i>Fagus sylvatica</i>	8	Diffuse Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.429**	0.429**	0.045*	0.045*
Hartl-Meier et al. 2014	47.80	14.44	<i>Fagus sylvatica</i>	8	Diffuse Porous	Broadleaf Deciduous	Temperate Conifer Forests	0.344**	0.344**	0.039 ^{ns}	0.039 ^{ns}
Hartl-Meier et al. 2014	47.70	14.44	<i>Fagus sylvatica</i>	8	Diffuse Porous	Broadleaf Deciduous	Temperate Conifer Forests	0.371**	0.371**	0.031 ^{ns}	0.031 ^{ns}
Lavergne et al. 2017	-41.19	-71.62	<i>Nothofagus pumilio</i>	12	Diffuse Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.366**	0.459**	0.022**	0.024**
Levesque et al. 2019	41.42	-74.01	<i>Liriodendron tulipifera</i>	5	Diffuse Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.043 ^{ns}	0.014 ^{ns}	-0.020**	-0.031**
Levesque et al. 2019	37.07	-89.60	<i>Liriodendron tulipifera</i>	5	Diffuse Porous	Broadleaf Deciduous	Temperate Grasslands, Savannas & Shrublands	0.255**	0.255**	-0.040**	-0.040**
Levesque et al. 2019	34.67	-84.18	<i>Liriodendron tulipifera</i>	5	Diffuse Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.220**	0.269**	-0.012 ^{ns}	-0.006 ^{ns}
Liu et al. 2014	40.95	100.18	<i>Populus euphratica</i>	5	Diffuse Porous	Broadleaf Deciduous	Deserts & Xeric Shrublands	0.119**	0.161**	0.004 ^{ns}	0.044**
Sladden et al. 2018	51.31	-0.84	<i>Fagus sylvatica</i>	1	Diffuse Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.503**	0.490**	0.012*	0.010 ^{ns}
Awada et al. 2019	40.25	-100.27	<i>Elaeagnus angustifolia</i>	3	Ring Porous	Broadleaf Deciduous	Temperate Grasslands, Savannas & Shrublands	0.401 ^{ns}	0.401 ^{ns}	0.029 ^{ns}	0.029 ^{ns}
Danis et al. 2006	45.71	6.15	<i>Quercus R</i>	6	Ring Porous	Broadleaf Evergreen	Temperate Conifer Forests	0.416**	0.416**	0.001 ^{ns}	0.001 ^{ns}
Danis et al. 2006	45.75	6.19	<i>Quercus R</i>	9	Ring Porous	Broadleaf Evergreen	Temperate Conifer Forests	0.400**	0.400**	-0.010 ^{ns}	-0.010 ^{ns}
Etien et al. 2009	48.38	2.67	<i>Quercus petraea</i>	15	Ring Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.202**	0.412*	-0.002 ^{ns}	0.007 ^{ns}
Granda et al. 2018	42.68	-2.49	<i>Quercus pyrenaica</i>	5	Ring Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.598**	0.639**	0.029**	0.029**
Granda et al. 2018	42.68	-2.49	<i>Quercus robur</i>	5	Ring Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.519**	0.550**	0.038**	0.042**
Granda et al. 2018	43.15	-1.60	<i>Quercus pyrenaica</i>	5	Ring Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.338**	0.363**	0.025**	0.024**

Granda et al. 2018	43.15	-1.60	<i>Quercus robur</i>	5	Ring Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.329**	0.341**	0.004 ^{ns}	0.002 ^{ns}
Haupt et al. 2011	48.19	16.21	<i>Quercus petraea</i>	7	Ring Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.239**	0.278*	0.005 ^{ns}	0.047**
Hilasvuori and Berninger 2010	60.00	23.08	<i>Quercus robur</i>	4	Ring Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.222**	0.300**	0.004 ^{ns}	-0.042*
Levesque et al. 2019	41.42	-74.01	<i>Quercus rubra</i>	5	Ring Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.121**	0.135**	-0.031**	-0.040**
Levesque et al. 2019	34.67	-84.18	<i>Quercus rubra</i>	5	Ring Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.128**	0.082*	-0.004 ^{ns}	-0.005 ^{ns}
Levesque et al. 2019	34.68	-94.63	<i>Quercus rubra</i>	5	Ring Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.311**	0.326**	-0.016 ^{ns}	-0.027 ^{ns}
Loader et al. 2008	55.67	-3.95	<i>Quercus robur</i>	4	Ring Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.132**	0.022 ^{ns}	0.000 ^{ns}	0.002 ^{ns}
Raffalli-Delerce et al. 2004	48.10	-1.68	<i>Quercus robur</i>	4	Ring Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.148**	0.307**	0.004 ^{ns}	0.025 ^{ns}
Sladden et al. 2018	51.31	-0.84	<i>Castanea sativa</i>	1	Ring Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.713**	0.817**	0.026**	0.020**
Sladden et al. 2018	51.31	-0.84	<i>Quercus robur</i>	1	Ring Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.465**	0.546**	0.025**	0.024*
Szczepanek et al. 2006	50.04	20.34	<i>Quercus robur</i>	4	Ring Porous	Broadleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.162**	0.297 ^{ns}	0.004 ^{ns}	0.019 ^{ns}
Anderson et al. 1998	47.18	8.26	<i>Picea abies</i>	4	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.661**	0.634**	-0.041**	-0.025 ^{ns}
Awada et al. 2019	40.25	-100.27	<i>Juniperus virginiana</i>	3	Tracheids	Needleleaf Evergreen	Temperate Grasslands, Savannas & Shrublands	1.455**	1.455**	-0.046 ^{ns}	-0.046 ^{ns}
Battipaglia et al. 2009	41.13	14.58	<i>Abies alba</i>	4	Tracheids	Needleleaf Evergreen	Mediterranean Forests, Woodlands & Scrub	0.212**	0.290*	0.041*	0.049 ^{ns}
Battipaglia et al. 2009	41.13	14.58	<i>Picea abies</i>	4	Tracheids	Needleleaf Evergreen	Mediterranean Forests, Woodlands & Scrub	0.391 ^{ns}	0.184 ^{ns}	0.021 ^{ns}	0.016 ^{ns}
Boettger et al. 2014	49.15	9.53	<i>Abies alba</i>	6	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.312**	-0.050 ^{ns}	0.005 ^{ns}	0.004 ^{ns}
Churakova et al. 2016	46.68	10.17	<i>Larix decidua</i>	4	Tracheids	Needleleaf Deciduous	Temperate Conifer Forests	0.354**	0.699**	0.001 ^{ns}	0.007 ^{ns}
Churakova et al. 2016	46.68	10.17	<i>Pinus mugo</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.295**	0.695**	-0.010*	-0.049*
Churakova et al. 2016	46.67	10.17	<i>Larix decidua</i>	4	Tracheids	Needleleaf Deciduous	Temperate Conifer Forests	0.338**	0.657**	-0.011*	-0.017 ^{ns}
Churakova et al. 2016	46.67	10.17	<i>Pinus mugo</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.280**	0.608**	-0.011*	-0.022 ^{ns}
Cullen and Grierson 2007	-22.85	118.63	<i>Callitris columellaris</i>	11	Tracheids	Needleleaf Evergreen	Deserts & Xeric Shrublands	0.033 ^{ns}	-0.089 ^{ns}	-0.060**	-0.112 ^{ns}
Esper et al. 2017	45.71	6.15	<i>Picea abies</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.235**	0.246 ^{ns}	0.03**	0.019 ^{ns}
Esper et al. 2018	67.95	20.03	<i>Pinus sylvestris</i>	4	Tracheids	Needleleaf Evergreen	Tundra	0.074**	0.046 ^{ns}	-0.020**	-0.026 ^{ns}
Esper et al. 2018	68.00	20.03	<i>Pinus sylvestris</i>	4	Tracheids	Needleleaf Evergreen	Tundra	0.071**	0.041 ^{ns}	-0.016**	-0.013 ^{ns}
Esper et al. 2018	59.44	18.00	<i>Pinus sylvestris</i>	4	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.196**	0.184**	-0.017**	-0.046**

Esper et al. 2018	59.55	18.00	<i>Pinus sylvestris</i>	4	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.174**	0.150*	-0.009*	-0.027*
Gennaretti et al. 2017	54.23	-71.39	<i>Picea mariana</i>	5	Tracheids	Needleleaf Evergreen	Boreal Forests/Taiga	0.235**	0.335*	0.002 ^{ns}	0.019 ^{ns}
Giguère-Croteau et al. 2019	48.47	-79.33	<i>Thuja occidentalis</i>	11	Tracheids	Needleleaf Evergreen	Boreal Forests/Taiga	0.262**	0.401**	-0.018**	0.010 ^{ns}
Gori et al. 2013	46.08	11.05	<i>Picea abies</i>	10	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.444**	0.369**	0.018 ^{ns}	-0.016 ^{ns}
Gori et al. 2013	46.25	11.50	<i>Picea abies</i>	10	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.228**	0.301 ^{ns}	-0.011 ^{ns}	-0.059**
Gori et al. 2013	46.29	11.61	<i>Picea abies</i>	10	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.310**	0.362**	0.023**	0.022 ^{ns}
Granda et al. 2017	42.65	0.83	<i>Pinus uncinata</i>	4	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.180**	0.296*	-0.012 ^{ns}	-0.048*
Granda et al. 2017	42.66	0.02	<i>Pinus uncinata</i>	4	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.039 ^{ns}	0.330 ^{ns}	-0.012 ^{ns}	-0.080 ^{ns}
Granda et al. 2017	42.35	1.96	<i>Pinus uncinata</i>	4	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.266 ^{ns}	0.544**	0.004 ^{ns}	0.001 ^{ns}
Grießinger et al. 2019	31.08	96.98	<i>Juniperus tibetica</i>	5	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.176**	0.465**	0.031**	-0.007 ^{ns}
Grießinger et al. 2019	31.08	96.98	<i>Picea balfouriana</i>	5	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.062**	0.241**	0.009 ^{ns}	0.020 ^{ns}
Hafner et al. 2011	46.39	14.22	<i>Larix decidua</i>	9	Tracheids	Needleleaf Deciduous	Temperate Conifer Forests	0.155**	0.291**	-0.028**	-0.003 ^{ns}
Hartl-Meier et al. 2014	47.90	14.44	<i>Larix decidua</i>	8	Tracheids	Needleleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.512**	0.512**	0.010 ^{ns}	0.010 ^{ns}
Hartl-Meier et al. 2014	47.90	14.44	<i>Picea abies</i>	8	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.570**	0.570**	-0.026 ^{ns}	-0.026 ^{ns}
Hartl-Meier et al. 2014	47.80	14.44	<i>Larix decidua</i>	8	Tracheids	Needleleaf Deciduous	Temperate Conifer Forests	0.561**	0.561**	0.031 ^{ns}	0.031 ^{ns}
Hartl-Meier et al. 2014	47.80	14.44	<i>Picea abies</i>	8	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.475**	0.475**	-0.001 ^{ns}	-0.001 ^{ns}
Hartl-Meier et al. 2014	47.70	14.44	<i>Picea abies</i>	8	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.682**	0.682**	-0.013 ^{ns}	-0.013 ^{ns}
Hilasvuori et al. 2009	68.93	28.32	<i>Pinus sylvestris</i>	4	Tracheids	Needleleaf Evergreen	Boreal Forests/Taiga	0.109**	0.240**	-0.017**	-0.025 ^{ns}
Hilasvuori et al. 2009	62.98	31.27	<i>Pinus sylvestris</i>	4	Tracheids	Needleleaf Evergreen	Boreal Forests/Taiga	0.104**	0.212**	-0.009 ^{ns}	-0.079**
Knorre et al. 2010	54.40	89.95	<i>Larix sibirica</i>	5	Tracheids	Needleleaf Deciduous	Temperate Conifer Forests	0.140**	0.149 ^{ns}	-0.026**	-0.081**
Konter et al. 2014	42.58	-0.97	<i>Pinus uncinata</i>	5	Tracheids	Needleleaf Evergreen	Mediterranean Forests, Woodlands & Scrub	0.223**	0.459**	-0.015**	-0.022 ^{ns}
Kress et al. 2010	46.41	7.79	<i>Larix decidua</i>	5	Tracheids	Needleleaf Deciduous	Temperate Conifer Forests	0.168**	0.353**	-0.012*	0.001 ^{ns}
Lavergne et al. 2017	-41.16	-71.78	<i>Fitzroya cupressoides</i>	12	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.432**	0.523**	0.019**	0.022*
Levesque et al. 2014	45.78	7.55	<i>Larix decidua</i>	4	Tracheids	Needleleaf Deciduous	Temperate Conifer Forests	0.348**	0.347**	-0.025 ^{ns}	-0.020 ^{ns}
Levesque et al. 2014	45.78	7.55	<i>Picea abies</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.531**	0.499**	-0.006 ^{ns}	-0.006 ^{ns}

Levesque et al. 2014	45.78	7.55	<i>Pinus nigra</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.387**	0.372**	-0.033 ^{ns}	-0.037 ^{ns}
Levesque et al. 2014	45.78	7.55	<i>Pinus sylvestris</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.130**	0.086 ^{ns}	-0.055 ^{ns}	-0.061 ^{ns}
Levesque et al. 2014	45.78	7.55	<i>Pseudotsuga menziesii</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.432**	0.396**	-0.020 ^{ns}	-0.016 ^{ns}
Levesque et al. 2014	47.17	7.27	<i>Larix decidua</i>	4	Tracheids	Needleleaf Deciduous	Temperate Broadleaf & Mixed Forests	0.353**	0.370**	-0.011 ^{ns}	-0.015 ^{ns}
Levesque et al. 2014	47.17	7.27	<i>Picea abies</i>	4	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.471**	0.468**	0.001 ^{ns}	-0.002 ^{ns}
Levesque et al. 2014	47.17	7.27	<i>Pinus nigra</i>	4	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.282**	0.341**	-0.010 ^{ns}	-0.009 ^{ns}
Levesque et al. 2014	47.17	7.27	<i>Pinus sylvestris</i>	4	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.282**	0.279**	-0.014 ^{ns}	-0.018 ^{ns}
Levesque et al. 2014	47.17	7.27	<i>Pseudotsuga menziesii</i>	4	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.246*	0.198 ^{ns}	-0.024 ^{ns}	-0.026 ^{ns}
Liu et al. 2014	30.28	99.47	<i>Abies georgei</i>	5	Tracheids	Needleleaf Evergreen	Montane Grasslands & Shrublands	0.210**	0.255**	0.013 ^{ns}	0.021 ^{ns}
Liu et al. 2018	51.39	123.34	<i>Larix gmelinii</i>	5	Tracheids	Needleleaf Deciduous	Temperate Conifer Forests	0.181**	0.432**	-0.014**	-0.010 ^{ns}
Liu et al. 2018	51.45	122.76	<i>Pinus sylvestris</i>	5	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.127**	0.439**	-0.029**	-0.054**
Loader et al. 2010	50.40	87.55	<i>Pinus sibirica</i>	4	Tracheids	Needleleaf Evergreen	Montane Grasslands & Shrublands	0.154**	0.238 ^{ns}	-0.016**	-0.077**
Porter et al. 2009	64.40	-133.81	<i>Picea glauca</i>	3	Tracheids	Needleleaf Evergreen	Tundra	0.192**	0.402**	0.003 ^{ns}	-0.010 ^{ns}
Roden and Ehleringer 2007	44.40	-121.67	<i>Pinus ponderosa</i>	3	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.361**	0.361**	-0.042 ^{ns}	-0.042 ^{ns}
Roden and Ehleringer 2007	38.43	-119.43	<i>Pinus ponderosa</i>	3	Tracheids	Needleleaf Evergreen	Deserts & Xeric Shrublands	0.569**	0.569**	-0.002 ^{ns}	-0.002 ^{ns}
Roden and Ehleringer 2007	36.27	-110.00	<i>Pinus ponderosa</i>	3	Tracheids	Needleleaf Evergreen	Deserts & Xeric Shrublands	0.412**	0.412**	-0.032 ^{ns}	-0.032 ^{ns}
Roden et al. 2011	40.87	-124.05	<i>Sequoia sempervirens</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.387**	0.43**	-0.017 ^{ns}	-0.017 ^{ns}
Roden et al. 2011	41.80	-124.10	<i>Sequoia sempervirens</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.383**	0.304**	0.004 ^{ns}	0.003 ^{ns}
Roden et al. 2011	41.40	-124.03	<i>Sequoia sempervirens</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.221**	0.268**	-0.003 ^{ns}	-0.006 ^{ns}
Roden et al. 2011	38.40	-122.99	<i>Sequoia sempervirens</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.276**	0.249**	-0.010 ^{ns}	-0.017 ^{ns}
Seftigen et al. 2011	63.17	13.50	<i>Pinus sylvestris</i>	7	Tracheids	Needleleaf Evergreen	Boreal Forests/Taiga	0.062**	0.117**	-0.008 ^{ns}	-0.051**
Sensula and Wilczynski. 2018	50.24	19.25	<i>Pinus sylvestris</i>	10	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.564**	0.564**	0.059 ^{ns}	0.059 ^{ns}
Sensula and Wilczynski. 2018	50.20	18.19	<i>Pinus sylvestris</i>	10	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.525**	0.525**	0.006 ^{ns}	0.006 ^{ns}
Sensula and Wilczynski. 2018	50.90	18.56	<i>Pinus sylvestris</i>	10	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.647**	0.647**	0.054 ^{ns}	0.054 ^{ns}
Shestakova et al. 2017	54.26	89.62	<i>Pinus sylvestris</i>	5	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.293**	0.339**	-0.021*	-0.031*

Shestakova et al. 2017	53.72	91.84	<i>Pinus sylvestris</i>	5	Tracheids	Needleleaf Evergreen	Temperate Grasslands, Savannas & Shrublands	0.222**	0.266**	-0.035**	-0.059**
Sidorova et al. 2009	64.53	100.23	<i>Larix gmelinii</i>	8	Tracheids	Needleleaf Deciduous	Boreal Forests/Taiga	0.299**	0.416**	-0.018**	-0.056**
Sidorova et al. 2013	72.00	102.00	<i>Larix gmelinii</i>	4	Tracheids	Needleleaf Deciduous	Tundra	0.212**	0.404*	0.008 ^{ns}	-0.006 ^{ns}
Sladden et al. 2018	51.31	-0.84	<i>Pinus sylvestris</i>	1	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.420**	0.371**	-0.002 ^{ns}	-0.013 ^{ns}
Sohn et al. 2014	35.27	-111.74	<i>Pinus ponderosa</i>	3	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.254 ^{ns}	0.034 ^{ns}	0.106 ^{ns}	0.200 ^{ns}
Szczepanek et al. 2006	50.04	20.34	<i>Pinus sylvestris</i>	4	Tracheids	Needleleaf Evergreen	Temperate Broadleaf & Mixed Forests	0.097 ^{ns}	0.253 ^{ns}	0.001 ^{ns}	-0.004 ^{ns}
Szejner et al. 2016	35.27	-107.62	<i>Pinus ponderosa</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.449**	0.524**	-0.037 ^{ns}	-0.023 ^{ns}
Szejner et al. 2016	32.72	-109.97	<i>Pinus ponderosa</i>	4	Tracheids	Needleleaf Evergreen	Tropical & Subtropical Coniferous Forests	0.446**	0.482**	-0.005 ^{ns}	-0.006 ^{ns}
Szejner et al. 2016	34.24	-105.79	<i>Pinus ponderosa</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.68**	0.694**	0.003 ^{ns}	0.013 ^{ns}
Szejner et al. 2016	36.76	-112.26	<i>Pinus ponderosa</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.484**	0.574**	-0.041*	-0.046*
Szejner et al. 2016	35.36	-111.62	<i>Pinus ponderosa</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.270**	0.316**	0.002 ^{ns}	0.009 ^{ns}
Szejner et al. 2016	39.36	-111.26	<i>Pinus ponderosa</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.431**	0.485**	-0.008 ^{ns}	-0.003 ^{ns}
Szejner et al. 2016	40.54	-110.64	<i>Pinus ponderosa</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.381**	0.462**	-0.014 ^{ns}	-0.008 ^{ns}
Szejner et al. 2016	33.81	-110.91	<i>Pinus ponderosa</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.501**	0.570**	-0.005 ^{ns}	-0.005 ^{ns}
Szejner et al. 2016	32.75	-105.77	<i>Pinus ponderosa</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.526**	0.564**	-0.025 ^{ns}	-0.021 ^{ns}
Szejner et al. 2016	35.21	-106.42	<i>Pinus ponderosa</i>	4	Tracheids	Needleleaf Evergreen	Temperate Conifer Forests	0.669**	0.802**	-0.132**	-0.131**
Szejner et al. 2016	32.41	-110.70	<i>Pinus ponderosa</i>	4	Tracheids	Needleleaf Evergreen	Tropical & Subtropical Coniferous Forests	0.327**	0.368**	-0.077 ^{ns}	-0.085 ^{ns}
Szymczak et al. 2012	42.00	9.00	<i>Pinus nigra</i>	5	Tracheids	Needleleaf Evergreen	Mediterranean Forests, Woodlands & Scrub	0.307**	0.344**	-0.029*	-0.040**
Xu et al. 2018	44.00	87.00	<i>Picea schrenkiana</i>	10	Tracheids	Needleleaf Evergreen	Deserts & Xeric Shrublands	0.206**	0.219**	-0.050**	-0.052**
Zeng et al. 2017	29.50	94.00	<i>Abies georgei</i>	6	Tracheids	Needleleaf Evergreen	Montane Grasslands & Shrublands	0.411**	0.472**	0.010 ^{ns}	-0.023 ^{ns}

Table S2.2. Trends in iWUE and the associated standard error across all sites and species within a given biome for the period 1963 – 2015. Those biomes with the same letter in the third column, group, have temporal trends that are not significantly different from one another.

Biome	Δ iWUE ($\mu\text{mol CO}_2$ $\text{mol}^{-1} \text{H}_2\text{O yr}^{-1}$)	S.E.	Group
Mediterranean Forests, Woodlands & Scrub	0.611	0.074	a
Temperate Conifer Forests	0.482	0.021	a
Tropical & Subtropical Moist Broadleaf Forests	0.472	0.136	abc
Tropical & Subtropical Coniferous Forests	0.426	0.085	abc
Montane Grasslands & Shrublands	0.422	0.083	abc
Boreal Forests/Taiga	0.412	0.058	ab
Temperate Broadleaf & Mixed Forests	0.409	0.022	ab
Temperate Grasslands, Savannas & Shrublands	0.182	0.082	bc
Tundra	0.111	0.069	c
Deserts & Xeric Shrublands	0.101	0.070	c

Table S2.3. Linear mixed effect model results and attributes for the best model (lowest AICc) examining the drivers of tree ring derived iWUE for the period 1963 – 2015 when considering factors climate, CO₂, and wood (2nd column), or leaf type (3rd column) in the model, including interactions with wood and leaf type. Intercept values for each model represent the value of iWUE when each numerical environmental factor included in the model is at its mean value during the study period. Leaf and wood type parameter estimates indicate the difference from the original intercept value for each respective variable, with comparisons made with “tracheids” at the base level for wood type and “needleleaf evergreen” as the base level for leaf type. Parameter estimates for interactions between wood and leaf type and environmental factors indicate the change from the slope of the original estimate for that parameter and when considering the respective leaf or wood type. The marginal R² describes the goodness of model fit given fixed effects only, while the conditional R² describes the goodness of model fit including fixed and random effects (tree species nested within site). Model parameter significance is denoted by an asterisk where ~ denotes p < 0.06, *denotes p < 0.05, **denotes p < 0.01, and ***denotes p < 0.001, while ^{ns} denotes not significant.

Attribute	1963 – 2015 _{wood}	1963 – 2015 _{leaf}
Intercept (μmol mol ⁻¹)	87.338 ± 1.293 ^{***}	87.584 ± 1.327 ^{***}
CO ₂ (ppm)	0.248 ± 0.008 ^{***}	0.244 ± 0.008 ^{***}
PPT _{grw} (mm)	-0.010 ± 0.001 ^{***}	-0.009 ± 0.001 ^{***}
TMP _{grw} (°C)	0.288 ± 0.157 ^{ns}	0.148 ± 0.155 ^{ns}
VPD _{grw} (kPa)	13.523 ± 1.761 ^{***}	14.292 ± 1.506 ^{***}
Diffuse Porous	-17.656 ± 2.854 ^{***}	-
Ring Porous	-18.340 ± 2.729 ^{***}	-
Needleleaf Deciduous	-	-3.068 ± 2.669 ^{ns}
Broadleaf Evergreen	-	-10.760 ± 7.960 ^{ns}
Broadleaf Deciduous	-	-18.946 ± 2.286 ^{***}
CO ₂ :VPD _{grw}	-0.094 ± 0.054 ^{ns}	-0.161 ± 0.073 [*]
CO ₂ :TMP _{grw}	-	0.011 ± 0.007 ^{ns}
CO ₂ :Diffuse Porous	-0.056 ± 0.021 ^{**}	-
CO ₂ :Ring Porous	-0.017 ± 0.018 ^{ns}	-
CO ₂ :Needleleaf Deciduous	-	0.013 ± 0.021 ^{ns}
CO ₂ :Broadleaf Evergreen	-	-0.100 ± 0.084 ^{ns}
CO ₂ :Broadleaf Deciduous	-	-0.030 ± 0.015 [*]
PPT _{grw} :TMP _{grw}	-	0.001 ± 0.001 ^{ns}
TMP _{grw} :VPD _{grw}	2.123 ± 0.901 [*]	2.485 ± 1.004 [*]
TMP _{grw} :Diffuse Porous	-0.996 ± 0.452 [*]	-
TMP _{grw} :Ring Porous	0.058 ± 0.365 ^{ns}	-
TMP _{grw} :Needleleaf Deciduous	-	0.822 ± 0.289 ^{**}
TMP _{grw} :Broadleaf Evergreen	-	1.618 ± 0.790 [*]
TMP _{grw} :Broadleaf Deciduous	-	-0.340 ± 0.207 ^{ns}
VPD _{grw} :Diffuse Porous	7.019 ± 3.832 ^{ns}	-
VPD _{grw} :Ring Porous	-5.151 ± 3.541 ^{ns}	-
Marginal R ²	0.38	0.39
Conditional R ²	0.89	0.89

Table S2.4. Linear mixed effect model results and attributes for the best model (lowest AICc) examining the drivers of tree ring derived iWUE for the period 1901 – 2015 when only considering environmental factors climate and CO₂ (2nd column), including wood type as a fixed effect in the model (3rd column), and when including leaf type as a fixed effect in the model (4th column). Intercept values for each model represent the value of iWUE when each numerical environmental factor included in the model is at its mean value during the study period. Leaf and wood type parameter estimates indicate the difference from the original intercept value for each respective variable, with comparisons made with “100% tracheids” at the base level for wood type and “needleleaf evergreen” as the base level for leaf type. The marginal R² describes the goodness of model fit given fixed effects only, while the conditional R² describes the goodness of model fit including fixed and random effects (tree species nested within site). Model parameter significance is denoted by an asterisk where ~ denotes $p < 0.06$, *denotes $p < 0.05$, **denotes $p < 0.01$, and ***denotes $p < 0.001$, while ^{ns} denotes not significant.

Attribute	1901 – 2015	1901 – 2015 _{wood}	1901 – 2015 _{leaf}
Intercept ($\mu\text{mol mol}^{-1}$)	80.242 $\pm$ 1.437***	84.373 $\pm$ 1.361***	84.821 $\pm$ 1.379***
CO ₂ (ppm)	0.251 $\pm$ 0.005***	0.251 $\pm$ 0.005***	0.251 $\pm$ 0.005***
PPT _{grw} (mm)	-0.010 $\pm$ 0.001***	-0.010 $\pm$ 0.001***	-0.010 $\pm$ 0.001***
TMP _{grw} (°C)	0.190 $\pm$ 0.099~	0.190 $\pm$ 0.099~	0.190 $\pm$ 0.099~
VPD _{grw} (kPa)	14.509 $\pm$ 1.187***	14.503 $\pm$ 1.187***	14.504 $\pm$ 1.187***
CO ₂ :TMP _{grw}	0.011 $\pm$ 0.005*	0.010 $\pm$ 0.005*	0.010 $\pm$ 0.005*
CO ₂ :VPD _{grw}	-0.121 $\pm$ 0.051*	-0.120 $\pm$ 0.051*	-0.120 $\pm$ 0.051*
Diffuse Porous	-	-17.159 $\pm$ 2.831***	-
Ring Porous	-	-18.543 $\pm$ 2.780***	-
Needleleaf Deciduous	-	-	-4.835 $\pm$ 2.584 ^{ns}
Broadleaf Evergreen	-	-	-7.874 $\pm$ 8.359 ^{ns}
Broadleaf Deciduous	-	-	-19.246 $\pm$ 2.274***
Marginal R ²	0.15	0.37	0.38
Conditional R ²	0.89	0.89	0.89

Table S3.1. Kendall's rank correlation coefficients (τ) between environmental parameters and the regional chronologies from 1940 to 2014 of basal area increment (BAI), $\Delta^{13}\text{C}$, seasonally integrated net photosynthesis (A), and seasonally integrated stomatal conductance (g_c) of red spruce trees from three locations in the central Appalachian Mountains. Environmental parameters included national SO_2 and NO_x emissions (Lefohn *et al.*, 1999; EPA, 2015), atmospheric CO_2 concentrations (Keeling *et al.*, 2015), and monthly, growing season, and annual precipitation and temperatures (WV Climate Division 4, NOAA, 2017). τ is shown for significant correlations only, and * denotes $p < 0.01$, ** denotes $p < 0.001$, while *** denotes $p < 0.0001$.

Environmental Parameter	BAI	$\Delta^{13}\text{C}$	A	g_c
NO_x	-0.341***	-0.498***	-0.265***	-0.659***
SO_2	-0.116		-0.386***	
Atmospheric CO_2	-0.142*	-0.416***	0.202***	-0.426***
MAT			0.223***	
MGT			0.146*	0.119
January temperature			0.103	
March temperature		-0.106		-0.107
April temperature			0.168**	
May temperature		0.094		0.126*
June temperature		0.105	0.142*	0.146*
August temperature			0.095	
October temperature		0.103		0.135*
November temperature		-0.099		-0.104
December temperature				
May precipitation			0.128*	
June precipitation		0.103	0.110	0.140*

Table S3.2. Bootstrapped Pearson's correlation coefficients between tree growth and maximum annual temperature ($T_{\max}$), minimum annual temperature ($T_{\min}$), and the Palmer Drought Severity Index (PDSI) for months of the current (capital letters) and previous year (lowercase letters). There were no significant relationships detected at the $\alpha = 0.05$ level for each of the climate variables listed.

Month	<u>MCG</u>			<u>SOR</u>			<u>CGL</u>		
	$T_{\max}$	$T_{\min}$	PDSI	$T_{\max}$	$T_{\min}$	PDSI	$T_{\max}$	$T_{\min}$	PDSI
Jun	0.062	-0.044	-0.025	-0.039	0.058	0.148	-0.06	0.143	0.043
Jul	-0.071	-0.04	-0.043	-0.054	0.108	0.097	-0.013	0.119	0.042
Aug	0.033	-0.013	-0.048	-0.008	0.045	0.081	0.122	0.056	0.018
Sep	-0.118	0.066	-0.013	0.115	0.158	-0.076	0.084	0.016	-0.005
Oct	0.089	-0.005	-0.029	-0.077	0.054	-0.088	0.037	0.052	-0.041
Nov	0.068	-0.051	-0.003	0.039	0.026	-0.056	0.097	0.019	-0.053
Dec	0.02	-0.096	0.058	-0.05	-0.04	0.014	-0.115	-0.107	-0.104
JAN	0.075	0.056	0.062	-0.141	-0.092	0.07	-0.071	-0.056	-0.103
FEB	-0.04	-0.058	-0.055	-0.085	-0.129	-0.028	0.04	0.023	-0.064
MAR	-0.041	-0.046	-0.034	-0.092	-0.047	-0.111	-0.095	-0.058	-0.013
APR	-0.036	0.145	0.008	0.017	0.001	-0.086	0.023	0.126	0.035
MAY	0.051	0.089	0.018	0.046	-0.069	0.037	0.034	-0.012	0.029
JUN	0.134	0.04	-0.026	-0.01	0.107	0.097	0.008	-0.058	0.036
JUL	0.066	0.138	-0.059	-0.055	-0.01	0.109	0.033	-0.098	-0.002
AUG	0.031	0.04	-0.077	0.125	-0.008	0.075	0.207	0.087	-0.01
SEP	0.029	-0.031	-0.095	0.032	-0.099	-0.102	-0.059	-0.064	0.009

Table S3.3. Summary of the average model (generated from models with $\Delta AICc < 4$ from the best model) used to determine the contribution of each environmental parameter to changes in BAI each year. The saturated model included April temperature (WV Climate Division 4, NOAA, 2017), atmospheric CO₂ (Keeling et al., 2015), SO₂ emissions (Lefohn *et al.*, 1999; EPA, 2015), and NO_x emissions (EPA, 2015), as well as all possible combinations. Significance at the $p = 0.001$ level is denoted by **, while *** denotes significance at the $p < 0.0001$ level.

Model Parameter	Estimate	Std.Error	z-value	p
(Intercept)	-8.683	62.15	0.139	0.889
April temperature	0.509	0.15	3.284	0.001**
SO ₂ emissions	-0.243	1.89	0.129	0.898
NO _x emissions	-1.066	3.62	0.294	0.769
NO _x emissions:SO ₂ emissions	-0.060	0.02	3.853	0.000***
Atmospheric CO ₂	0.078	0.22	0.348	0.728
Atmospheric CO ₂ :NO _x emissions	0.022	0.01	3.511	0.000***
Atmospheric CO ₂ :SO ₂ emissions	0.016	0.00	4.323	0.000***

Table S3.4. Estimates of the reduction in carbon sequestration for each respective zone outlined in the Multi-Resolution Land Characteristics Consortium’s 2011 National Land Cover Database. Values represent the average reduction over the 75-year chronology and are given for each land cover class (evergreen, deciduous, and mixed) for each zone. To determine this, we first calculated the cumulative basal area over the 75-year chronology for an average red spruce tree at our study locations with (1177 cm²), and without (1446 cm²), the influence of acid pollution (assuming BAI at 1940 would approach BAI at 2014 linearly without pollution effects). Each respective cumulative basal area was converted to diameter at breast height, used to predict aboveground biomass (AGBM) (Siccama *et al.*, 1994), and converted to kilograms C tree⁻¹ in AGBM using the %C of our red spruce samples (48 ± 0.05%). The predicted difference in C storage (+25.3% without pollution) was then applied to zones 53, 57, and 61-66 outlined in the MRLC’s Land Cover Data Base for Land cover estimates for (Homer *et al.*, 2015) using biomass estimates from the National Biomass and Carbon Dataset for the Year 2000 (Kellndorfer *et al.*, 2012). Columns 2-4 indicate the C reduction on a mass basis, whereas columns 5-7 indicate reductions on a mass per area basis. Total sink reduction due to pollution (Pg C) for each land cover class was taken as the sum of all zones examined; total sink reduction per land area due to pollution for each land cover class (kg C m⁻²) was taken as the average of all zones examined. Summing the total sink reduction for each land cover class (evergreen, deciduous, and mixed) provided the total sink reduction estimate of 0.79 Pg C.

Zone	1940 – 2014 Carbon Sink Reduction for Land Cover Class (Pg C)			1940 – 2014 Carbon Sink Reduction for Land Cover Class (kg C m ⁻²)		
	Evergreen	Deciduous	Mixed	Evergreen	Deciduous	Mixed
53	0.002	0.093	0.005	1.37	1.73	1.56
57	0.006	0.077	0.003	1.66	1.99	1.64
61	0.008	0.128	0.006	1.62	1.80	1.72
62	0.002	0.084	0.001	1.90	1.76	1.97
63	0.007	0.057	0.017	1.96	1.82	1.99
64	0.017	0.066	0.013	1.83	1.86	1.93
65	0.015	0.049	0.013	2.12	1.88	2.07
66	0.032	0.038	0.050	1.41	1.64	1.54
Total	0.090	0.591	0.108	1.66	1.81	1.71

Table S4.1. Leaf and wood carbon isotope signatures $\delta^{13}\text{C}$ (‰) for *Quercus rubra* and *Liriodendron tulipifera* used to determine the fractionation associated with the conversion of leaf to wood carbohydrate (Leavitt & Long, 1982). Leaf isotope signatures integrate sun and shade leaves. Leaf and wood samples were collected at WS10 and WS13 throughout June, July, and August of 2016.

Species	Tissue	$\delta^{13}\text{C} \pm \text{S.E. (‰)}$	N
<i>Quercus rubra</i>	Wood	-28.08 ± 0.34	10
<i>Quercus rubra</i>	Leaf	-28.29 ± 0.16	38
<i>Liriodendron tulipifera</i>	Wood	-27.39 ± 0.31	10
<i>Liriodendron tulipifera</i>	Leaf	-28.80 ± 0.25	29

Table S4.2. Complete table for Kendall's rank correlation coefficients between all considered environmental factors and BAI and iWUE for *Quru* and *Litu* in Watershed 10 and Watershed 13 in the Fernow Experimental Forest, Parsons, WV, U.S for the period 1960 - 2015. Climate variables include minimum, mean, and maximum monthly, growing season, and annual temperature, as well as monthly, growing season and annual precipitation (PRISM Climate Group, 2004). Non-climatic environmental factors include SO₂ and NO_x emissions (EPA, 2015), as well as atmospheric CO₂ concentrations (Keeling et al., 2015). Significance at the $\alpha = 0.05$ level is denoted by the presence of one asterisk (*), while significance at the $\alpha = 0.01$ level is denoted by the presence of two asterisks (**).

Environmental Factor	<i>Quercus rubra</i>		<i>Liriodendron tulipifera</i>	
	BAI	iWUE	BAI	iWUE
Atmospheric CO ₂	0.47**	0.24*	0.49**	0.47**
SO ₂ Emissions	-0.26**	-0.06	-0.32**	-0.39**
NO _x Emissions	0.00	0.16	-0.04	-0.15
January _{ppt}	0.01	0.01	0.10	0.12
January _{tmin}	0.05	0.04	0.14	0.15
January _{tmean}	0.00	0.05	0.08	0.12
January _{tmax}	-0.04	0.05	0.02	0.08
February _{ppt}	0.04	-0.06	0.00	0.00
February _{tmin}	0.13	0.13	0.20*	0.08
February _{tmean}	0.12	0.16	0.20*	0.08
February _{tmax}	0.08	0.17	0.17	0.08
March _{ppt}	0.03	0.02	-0.03	0.05
March _{tmin}	0.09	-0.01	0.09	0.00
March _{tmean}	0.05	-0.01	0.04	-0.04
March _{tmax}	0.02	0.01	0.00	-0.04
April _{ppt}	-0.07	-0.06	-0.05	-0.04
April _{tmin}	0.07	0.02	0.10	0.07
April _{tmean}	0.06	0.02	0.11	0.10
April _{tmax}	0.03	0.04	0.08	0.10
May _{ppt}	0.15	0.06	0.11	0.10
May _{tmin}	0.21*	-0.06	0.15	0.08
May _{tmean}	0.12	-0.07	0.08	0.04
May _{tmax}	0.02	-0.07	0.01	-0.02
June _{ppt}	0.17	-0.13	0.12	-0.13
June _{tmin}	0.28**	0.03	0.23*	0.19*
June _{tmean}	0.12	0.03	0.08	0.14
June _{tmax}	-0.08	0.03	-0.08	0.03
July _{ppt}	0.24*	-0.03	0.10	-0.01
July _{tmin}	0.22*	0.21 *	0.23*	0.24*

July _{tmean}	0.08	0.24*	0.13	0.22*
July _{tmax}	-0.05	0.20*	0.02	0.14
August _{ppt}	0.08	-0.06	-0.02	-0.06
August _{tmin}	0.18	0.11	0.19*	0.17
August _{tmean}	0.08	0.11	0.16	0.17
August _{tmax}	-0.06	0.08	0.07	0.13
September _{ppt}	-0.06	0.03	-0.12	-0.02
September _{tmin}	0.15	-0.09	0.11	-0.03
September _{tmean}	0.09	-0.10	0.09	-0.02
September _{tmax}	0.01	-0.08	0.05	-0.01
October _{ppt}	-0.01	-0.05	0.03	-0.08
October _{tmin}	0.21*	-0.05	0.14	-0.01
October _{tmean}	0.16	-0.04	0.09	-0.02
October _{tmax}	0.07	0.00	-0.01	-0.02
November _{ppt}	0.01	0.00	0.02	-0.07
November _{tmin}	0.04	-0.03	0.01	-0.10
November _{tmean}	0.04	0.01	0.04	-0.05
November _{tmax}	0.02	0.03	0.04	-0.02
December _{ppt}	0.11	-0.06	0.01	-0.01
December _{tmin}	0.20*	0.09	0.11	0.11
December _{tmean}	0.15	0.10	0.07	0.09
December _{tmax}	0.11	0.09	0.05	0.07
Annual _{ppt}	0.23	-0.09	0.07	-0.07
Annual _{tmin}	0.38**	0.13	0.37**	0.21*
Annual _{tmean}	0.25**	0.14	0.27**	0.18
Annual _{tmax}	0.07	0.13	0.11	0.09
Growing _{ppt}	0.26**	-0.03	0.11	0.00
Growing _{tmin}	0.37**	0.04	0.33**	0.23*
Growing _{tmean}	0.16	0.04	0.17	0.15
Growing _{tmax}	-0.05	0.02	0.01	0.06

Table S4.3. Model summaries for the average models used to determine the temporal contribution of each environmental parameter on the growth (BAI) and physiology (iWUE) of *Quercus rubra* (*Quru*) and *Liriodendron tulipifera* (*Litu*) in Watershed 10 and Watershed 13 at the Fernow Experimental Forest in Parsons, WV, U.S. for the period 1960 – 2015. Environmental factors included in the saturated model included atmospheric CO₂, minimum growing season temperatures (May – September; Growing_{tmin}), the sum of June and July precipitation (JJ_{ppt}), U.S. emissions of SO₂, and U.S. emissions of NO_x. All possible combinations of models were examined using the previously mentioned parameters, including interactions up to order two, resulting in a total of 1,450 models examined for each response variable. Models with $\Delta AICc < 1$ from the best model were averaged resulting in the final model. Significance at the $p < 0.05$ level is denoted by *, while significance at the $p < 0.09$ level is denoted by •.

Model	Model Parameter	Estimate	Adjusted SE	z value	P
<i>Quru</i> BAI	(Intercept)	-4.879	10.406	0.469	0.639
	Growing _{tmin}	1.200	0.556	2.158	0.031*
	JJ _{ppt}	0.077	0.028	2.775	0.006*
	NO _x Emissions	1.706	0.477	3.579	0.000*
	SO ₂ Emissions	-0.829	0.317	2.615	0.009*
	JJ _{ppt} *NO _x Emissions	-0.003	0.001	2.049	0.040*
	JJ _{ppt} *SO ₂ Emissions	-0.002	0.001	2.011	0.044*
<i>Quru</i> iWUE	(Intercept)	-61.200	111.000	0.553	0.580
	CO ₂	0.364	0.273	1.337	0.181
	Growing _{tmin}	9.810	9.980	0.983	0.326
	JJ _{ppt}	-0.113	0.078	1.443	0.149
	NO _x Emissions	1.410	1.050	1.342	0.180
	CO ₂ *Growing _{tmin}	-0.037	0.021	1.803	0.071•
	CO ₂ *JJ _{ppt}	0.0003	0.000	1.826	0.068•
	Growing _{tmin} *NO _x Emissions	-0.153	0.089	1.724	0.085•
<i>Litu</i> BAI	(Intercept)	95.838	38.919	2.463	0.014*
	CO ₂	-0.214	0.108	1.977	0.048*
	JJ _{ppt}	0.018	0.005	3.711	0.000*
	NO _x Emissions	-5.725	2.600	2.202	0.028*
	SO ₂ Emissions	-0.867	0.431	2.011	0.044*
	CO ₂ *NO _x .Emissions	0.020	0.007	2.855	0.004*
	Growing _{tmin}	0.756	0.594	1.273	0.203
<i>Litu</i> iWUE	(Intercept)	78.186	44.216	1.768	0.077•
	CO ₂	0.078	0.102	0.764	0.445
	Growing _{tmin}	-3.723	1.340	2.778	0.005*
	JJ _{ppt}	-0.163	0.054	3.018	0.003*
	NO _x Emissions	-0.741	2.386	0.311	0.756

SO ₂ Emissions	0.541	0.533	1.015	0.310
Growing _{tmin} *JJ _{ppt}	0.014	0.005	2.818	0.004*
NO _x Emissions*SO ₂ Emissions	-0.038	0.016	2.366	0.018*
CO ₂ :NO _x .Emissions	0.011	0.004	2.439	0.015*

Figure S2.1. Chronologies of iWUE for each biome over time (a) and mean iWUE for each biome (b) for the period 1963 – 2015, representing the part of the chronology following the breakpoint where iWUE began rapidly, and linearly increasing. Biomes were determined according to Dinerstein et al. (Dinerstein *et al.*, 2017) with biome numbers in panel (b) classified as follows: 1) temperate conifer forests, 2) montane grasslands and shrublands, 3) tropical and subtropical coniferous forests, 4) deserts and xeric shrublands, 5) temperate grasslands savannas, and shrublands, 6) boreal forests/taiga, 7) temperate broadleaf and mixed forests, 8) tundra, 9) Mediterranean forests, woodlands, and scrub, and 10) tropical and subtropical moist broadleaf forests. Solid lines in (a) represent the average trend for each biome over the chronology. Center lines in boxplots in (b) represent the median value of iWUE for each group, while upper and lower box borders represent the 75th and 25th percentile values, respectively. Within each panel in (b) different letters indicate groups with means that are significantly different.

Figure S2.2. Chronologies of iWUE for each wood type (a), mean iWUE for each wood type (b), chronologies of iWUE for each leaf type (c), and mean iWUE for each leaf type (d) for the period 1963 – 2015, representing the part of the chronology following the breakpoint where iWUE began rapidly, and linearly increasing. Solid lines in (a) and (c) represent the average trend for each wood or leaf type over the chronology, respectively. Center lines in boxplots in (b) and (d) represent the median value of iWUE for each group, while upper and lower box borders represent the 75th and 25th percentile values, respectively. Within each panel in (b) and (d) different letters indicate groups with means that are significantly different.

Figure S2.3. Visualization of the nature of the interaction between growing season vapor pressure deficit and atmospheric CO₂ (a) and growing season temperature and CO₂ (b) on iWUE during the period 1901 – 2015. The interactions shown represent a given predicted value of iWUE throughout the range experienced by each group mean-centered environmental factor over the 1901 – 2015 period. Values listed for each environmental factor represent its value with respect to the mean during the study period. Parameter estimates for each interaction are listed in Table 2.1.

Figure S2.4. Visualization of the nature of the interaction between growing season temperature (TMP_{growing}) and growing season precipitation (PPT_{growing}) on those tree species with 100 % tracheids (a) and diffuse porous trees (b), and between growing season vapor pressure deficit (VPD_{growing}) and PPT_{growing} for ring porous trees (c). The interactions shown represent a given predicted value of iWUE throughout the range experienced by each group mean-centered environmental factor over the 1963 – 2015 period. Values listed for each environmental factor represent its value with respect to the mean during the study period.

Figure S2.5. Trends in $\Delta^{18}\text{O}_{\text{lw}}$ for each leaf ring type (a) and leaf type (b) across all tree species and sites for the period 1963 – 2015. The bold lines each panel represent, in turn, the average trend in $\Delta^{18}\text{O}_{\text{lw}}$ for each ring type or leaf type. The F-statistic and p-value associated with ANCOVA testing for differences in the rate of change among each trait within each panel are listed.

Figure S2.6. Trends in $\Delta^{18}\text{O}_{\text{lw}}$ for each of the 113 unique chronologies examined in this study as a function of mean TMP_{grw} (a), PPT_{grw} (b), and VPD_{grw} (c), and as a function of the average trend in TMP_{grw} (d), PPT_{grw} (e), and VPD_{grw} (f) for the period 1963 – 2015. The slope ($\pm$ s.e.) and p-value for the linear relationship between the trend in $\Delta^{18}\text{O}_{\text{lw}}$ and each respective environmental factor is listed in each panel. Trends in $\Delta^{18}\text{O}_{\text{lw}}$, as well as each environmental factor, were determined using generalized least squares regression, including an AR(1,0) autocorrelation structure.

Figure S2.7. Global concentrations of annual atmospheric CO₂ from 1901 – 2015. The vertical blue dashed line at 1963 represents the breakpoint where iWUE averaged across all 113 chronologies began to rapidly increase, while the vertical red dashed line at 1969 represents the breakpoint where concentrations of atmospheric CO₂ began increasing more rapidly than the 69 years prior.

Figure S3.1. National emissions of sulfur dioxide (SO_2) estimated by the EPA (2015) and regional sulfate (SO_4^{2-}) deposition measured at the NADP's WV18 site in Parsons, WV (a) (NADP 2015), national emissions of nitrogen oxides (NO_x) estimated by the EPA (2015) and regional deposition of nitrate (NO_3^-) measured at the NADP's WV18 site in Parsons, WV (b) (NADP 2015), atmospheric CO_2 concentrations measured at the Mauna Loa Observatory (c) (Keeling *et al.* 2015), and mean April temperatures for WV Climate Division 4 (d) (NOAA, 2017). The unshaded region indicates the time period after the critical year when a directional shift in BAI, $\Delta^{13}\text{C}$, and $\delta^{15}\text{N}$ occurs. Coefficients of determination for the relationship between emissions and deposition of oxidized forms of S and N are shown for the unshaded region in panels (a) and (b), respectively. For mean April temperatures (d), trend lines are for each period (1940-1989 and 1989-2014).

Figure S3.2. Temporal trends in mean annual temperature (a), mean annual precipitation (b), mean growing temperature (c), and mean growing precipitation (d) for WV Climate Division 4. Vertical dashed lines are at 1989, regional critical year, and trend lines for each period (1940-1989 and 1989-2014) are displayed on each panel. ANCOVA indicated that rates of change before and after the 1989 critical year were not different for these climate parameters. Climate data are from West Virginia Division 4, which is the region that contains the three red spruce stands in this study (NOAA 2017). Over the entire 75 year chronology, (a) yearly temperature ($T = -3.81 + 0.007 \cdot \text{year}$, $R^2 = 0.04$, $p = 0.04$), (b) yearly precipitation ($y = -686.15 + 0.97 \cdot \text{year}$; $R^2 = 0.02$, $p = 0.22$), (c) growing season temperature ($y = 14.69 + 0.002 \cdot \text{year}$; $R^2 = 0.003$, $p = 0.65$), and (d) growing season precipitation ($y = 8.37 + 0.29 \cdot \text{year}$; $R^2 = 0.004$, $p = 0.58$). The only significant change has been a 0.50°C increase in mean annual temperature over 1940-2014. ANOVA comparing the means before and after 1989 indicates that annual temperature was 0.54°C greater after 1989 than for the annual temperature during 1940-1989 ($F = 14.99$, $p < 0.01$).

Figure S3.3. Wood $\delta^{15}\text{N}$ as a function of U.S. NO_x emissions during 1989-2013 (a), and wood $\delta^{15}\text{N}$ as a function of NO_3^- wet deposition during 1989-2013 (b). The three red spruce forest sites are McGowan Mountain (MCG), Span Oak Run (SOR), and Cranberry Glades (CGL). NO_x emissions are from the EPA (2015) and NO_3^- wet deposition are from (NADP 2015).

Figure S3.4. Bootstrapped Pearson's correlations calculated between tree ring indices and current year's (months in all capital letters) and previous year's (months in lowercase) climate predictor variables for MCG (a), SOR (b), and CGL (c) for the period 1940-2014. Correlations between tree ring indices and mean monthly temperatures are in blue, while correlations between tree ring indices and monthly precipitation sums are in red. A solid vertical line indicates a significant relationship at the $\alpha = 0.05$ level, while dashed lines indicate a non-significant relationship.

Figure S3.5. Predicted BAI from the GLMM average model as a function of observed BAI across all red spruce forest sites (MCG, SOR, and CGL) for the 1989-2014 period. Models with $\Delta\text{AICc} < 4$ were included in the average model. Average model structure can be found in Table S3.

Figure S3.6. Chronology of stomatal conductance to CO_2 (a), and observed and modeled intrinsic water use efficiency (b) from 1940 to 2014 for each red spruce site ($N = 5$ per site). In panel (a), if we assume a complete recovery of g_c after 1989, the 15% decline in g_c that occurred between 1940 and 2014 can be attributed to the effect of increasing CO_2 (dashed line). The 47% decline in g_c can be partitioned with 10% attributed to increasing CO_2 and 37% attributed to acid deposition ($47\% - 10\% = 37\%$). In panel (b), modeled $i\text{WUE}$ was calculated assuming a constant C_i/C_a (0.72, 1940 mean) using the Ball-Berry model (solid line) (Knauer *et al.*, 2016) and changes in leaf physiology predicted from CO_2 enrichment studies (Ainsworth & Rogers, 2007), combined with changes in g_c attributed to increasing CO_2 (dashed line). The three red spruce forest sites are McGowan Mountain (MCG), Span Oak Run (SOR), and Cranberry Glades (CGL).

Figure S3.7. Map of the U.S. regions (53, 57, and 61 – 66) used in estimation of the reduction in carbon sequestration due to pollution (Table S4), from the National Biomass and Carbon Dataset (Kelldorfer *et al.*, 2012). Forest land cover estimates from the Multi-Resolution Land Characteristics Consortium's 2011 Land Cover Database (Homer *et al.*, 2015) were coupled with biomass estimates from the National Biomass and Carbon Dataset for the year 2000. Map was made using ArcMap 10.3.1 (<http://desktop.arcgis.com/en/arcmap/>).

Figure S4.1. U.S. national emissions of NO_x and local deposition of NO_3^- (a) national emissions of SO_2 and local deposition of SO_4^{2-} (b), national emissions of NO_x and stream water NO_3^- in WS10 and WS13 (c), and national emissions of SO_2 and stream water SO_4^{2-} in WS10 and WS13 in the Fernow Experimental Forest, Parsons, WV (d). Emissions of NO_x (black line in a and c) and SO_2 (black solid line in b and d) are available for the entire 1960 – 2015 study period (EPA, 2015), while deposition of NO_3^- (green solid line in a) and SO_4^{2-} (blue solid line in b) are shown beginning in 1979 NADP, 2015), and stream water chemistry available beginning in 1984 (Edwards & Wood, 2011). The Pearson's correlation coefficient between emissions and deposition of N and S for the period 1979 – 2015 is denoted in the lower left corner a and b. Concentrations of stream water NO_3^- (c, red lines) and SO_4^{2-} (d, brown lines) for WS10 and WS13 are shown in dashed and solid lines, respectively.

Figure S4.2. Bootstrapped Pearson's correlations between climate variables and basal area increment for the period 1960 – 2015 for *Quercus rubra* (a,c) and *Liriodendron tulipifera* (b,d) in Watershed 10 (a,b) and Watershed 13 (c,d) in the Fernow Experimental Forest, Parsons, WV, U.S. Green bars represent correlations between temperature and BAI, while blue bars represent correlations between precipitation and BAI. Solid bars indicate a significant correlation between either previous year's (lowercase months) or current year's (uppercase months) climate variable and BAI at the $\alpha = 0.05$ level, while dashed bars indicate a non-significant correlation at the $\alpha = 0.05$ level. For precipitation, JJ_{ppt} is the sum of the current year's June and July precipitation, while jj_{ppt} is the sum of the previous June and July's precipitation. G_{tmin} represents the mean of May – September minimum temperatures.

Figure S4.3. Heatmap of Kendall's rank correlation coefficients between environmental factors and BAI and iWUE for *Quru* and *Litu* for the period 1960 – 2015 across WS10 and WS13 in the Fernow Experimental Forest, Parsons, WV, U.S. Climate variables included in the analysis are temperature and precipitation (PRISM Climate Group, 2004), while non-climatic environmental factors are atmospheric CO₂ (Keeling et al., 2015), SO₂ emissions, and NO_x emissions (EPA, 2015). Minimum, mean, and maximum temperatures are denoted by T_{min}, T_{mean}, and T_{max}, respectively. Significant correlations ($p \leq 0.05$) between a given environmental factor and response variable are denoted by the presence of an asterisk, with the cell hue corresponding to the correlation coefficient, with blue being positive and red being negative. Environmental parameters with at least one significant relationship with a given response variable are shown. A full correlation summary can be found in Table S2.