Title: North American temperate conifer (*Tsuga canadensis*) reveals complex physiological response to climatic and anthropogenic stressors

Running Title: Climatic and anthropogenic stressors on conifer physiology

Rayback, Shelly A.[1], Belmecheri, Soumaya[2], Gagen, Mary H.[3], Lini, Andrea[4], Gregory, Rachel[5] and Jenkins, Catherine[5]

[1]Department of Geography, 207 Old Mill Building, 94 University Place, University of Vermont, Burlington, Vermont 05405 USA, P: 802-656-3019, E: srayback@uvm.edu (corresponding author).

[2]Laboratory of Tree-Ring Research, University of Arizona, Tucson, Arizona 85721, USA.

[3]Department of Geography, Swansea University, Singleton Park, Swansea SA2 8PP Wales, UK.

[4]Department of Geology, 319 Delehanty Hall, 180 Colchester Avenue, University of Vermont, Burlington, Vermont 05405 USA.

[5]School of Earth and Ocean Sciences, Cardiff University, Main Building, Park Place, Cardiff CF10 3AT Wales, UK.

Social media:

Instagram: @uvmgeography; @uvmgeology; @swansea_geography; @TreeRingLabUA

Facebook: UVM Geography Dept.; UVM Geology

Twitter: @DesertRym; @TreeRingLabUA; @swanseageog

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Summary

1) Rising atmospheric CO_2 (c_a) is expected to promote tree growth and lower water loss via changes in leaf-gas exchange. However, uncertainties remain if gas-exchange regulation strategies are homeostatic or dynamical in response to increasing c_a, as well as evolving climate and pollution inputs.

2) Using a suite of tree-ring-based $\delta^{13}C$-derived physiological parameters ($\Delta^{13}C$, c_i, iWUE) and tree growth from a mesic, low elevation stand of canopy-dominant *Tsuga canadensis* in northeastern USA, we investigated the influence of rising c_a, climate and pollution on, and characterized the dynamical regulation strategy of, leaf gas exchange at multi-decadal scales.

3) Isotopic and growth time series revealed an evolving physiological response where the species dynamically shifted its leaf gas-exchange strategy (constant c_i; constant c_i/c_a; constant $c_a - c_i$) in response to rising c_a, moisture availability and site conditions over 111 years. Tree iWUE plateaued after 1975 driven by greater moisture availability, and changing soil biogeochemistry that may have impaired stomatal response.

4) Results suggest trees may exhibit more complex physiological responses to changing environmental conditions over multi-decadal periods, complicating parameterization of earth-system models and the estimation of future carbon sink capacity and water balance in mid-latitude forests and elsewhere.

Keywords: acid deposition, carbon dioxide, climate, isotopic discrimination ($\Delta^{13}C$), intrinsic water use efficiency (iWUE), stable carbon isotopes ($\delta^{13}C$), tree-rings, conifer

1. Introduction

Rising atmospheric carbon dioxide (c_a) is expected to promote tree growth through adjustments in leaf-gas exchange resulting in enhanced photosynthetic assimilation rates (A) and lower water loss via reduced stomatal conductance (g_s). As c_a rises causing an increase in internal leaf CO_2 concentration (c_i), stomata may adjust their conductance and move toward a proportional ratio of c_i to c_a with the associated benefit of reduced water loss to the atmosphere (i.e., improved water-use efficiency: WUE) and enhanced photosynthesis. Controlled CO_2-enhancement experiments (Ceulemans & Mousseau, 1994; Ainsworth & Rogers, 2007) and tree-ring studies (Bert *et al*., 1997; Duquesnay *et al*., 1998; Saurer *et al*., 2004; 2014) have reported such findings.

Yet uncertainties remain around C3 plant physiological response to increasing c_a concentrations, alone and in combination with other drivers in ecosystems (Marshall & Monserud 1996). Metabolic set points were first proposed and explored by Ehleringer (1993) and Ehleringer & Cerling (1995) to understand compensatory changes in leaf gas exchange as c_a increased or decreased over time. Others (Saurer *et al.*, 2004; Gagen *et al*., 2010; Frank *et al.*, 2015) broadened this concept to examine isotopic discrimination $\Delta^{13}C$ time series (i.e., isotopic difference of $\delta^{13}C$ of air to that of the plant) derived from whole-tree, tree-ring $\delta^{13}C$ values under rising c_a, assigning one of three homeostatic gas-exchange regulation strategies to investigated tree species (Voelker *et al*., 2016). The strategies, representing the degree to which c_i follows increases in c_a, include: maintenance of constant leaf internal CO_2 (c_i), a constant c_i/c_a or a constant drawdown of CO_2 (c_a - c_i). Voelker *et al.* (2016) demonstrated leaf gas exchange responses may be evolutionarily prescribed, with C3 plants maximizing carbon gain or moisture stress avoidance. They suggested that no single strategy prevails within or between species, but that shifts may be dynamical over time, occurring along a continuum in response to longer-term changes in c_a. These responses, however, were only evaluated in the context of rising c_a and did not explicitly take into account other environmental controls like climate and pollution.

Physiological processes like stomatal conductance (g_s) and photosynthetic assimilation (A) regulate the amount of c_i in the leaf (Farquhar *et al*. 1982; 1989), and in turn, g_s and A are influenced by environmental drivers, both natural and anthropogenic including rising c_a (e.g., Cernusak *et al*., 2013; Loader *et al*., 2011; Saurer *et al*., 2014), changing moisture availability (e.g., Dupouey *et al*., 1993; Saurer *et al*., 1995; Warren *et al*., 2001) and pollutant deposition (e.g., Guerrieri *et al*., 2006; Rinne *et al*., 2010; Savard, 2010; Thomas *et al*., 2013; Boettger *et al*., 2014). On-going climate change and/or legacy effects of pollution in midlatitude forests have been shown to influence tree carbon and water dynamics (Thomas *et al.,* 2013; Saurer *et al*., 2014; Mathias *et al*., 2018; Maxwell *et al*., 2019). Moisture stress can cause stomatal closure and increased WUE with (Peñuelas *et al*., 2008; Andreu-Hayles *et al*., 2011; Nock *et al*., 2011; Silva & Anand, 2013) or without (Farquhar *et al.*, 1980; Yi *et al.*, 2019) a decline in A and growth. Disrupted nutrient cycles due to acid deposition of anthropogenically-generated acids and acid-forming substances (e.g. SO_X, NO_X) cause base cation leaching and depletion from soils (DeHayes *et al*., 1999; Driscoll *et al.*, 2001) and negatively affect stomatal control. Calcium ($Ca+$), a crucial cation, helps to regulate stomatal functioning via its movement into and out of guard cells adjacent to the stomatal opening (Mcainish *et al*., 1997; Hetherington & Woodward, 2003; Wang & Song, 2008; Wang *et al.*, 2012). The removal of $Ca+$ from leaves and needles via direct acid deposition and its longer term loss from soils have been shown to influence stomatal function (Borer *et al.,* 2005), reduce tree physiological responsiveness to environmental change, (DeHayes *et al.,* 1999; Schaberg *et al.,* 2001) and may have important implications for plant carbon-water fluxes across a range of scales (Lanning *et al.,* 2019).

In this study, we assessed a whole-tree 111 year tree-ring $\delta^{13}C$ chronology developed from a mesic eastern hemlock (*Tsuga canadensis* (L.) Carr.) old growth stand in the northeastern North American (NENA) forest. This research is a step toward understanding the longer-term physiological response of a temperate conifer species in the NENA forest to rising c_a, changing climate and pollution inputs, as well as exploring dynamical change in leaf gas exchange to rapidly evolving environmental conditions. Across New England, rising c_a has been accompanied by rising annual (1.7 ºC since 1901 or 0.09ºC decade^{-1}; 1901-2011), as well as winter, spring and fall temperatures (Janowiak *et al.,* 2018). The freeze-free growing season lengthened by 10 days (1960-1990 vs. 1991-2010) (Kunkel *et al.,* 2013), with end of the growing season occurring later in the fall (Dragoni & Rahman, 2012). Annual $PRCP_{mean}$, while variable across space and time, increased by 175 mm (1901-2011) (Janowiak *et al.,* 2018) in New England, with an increase in the occurrence of heavy precipitation events (Kunkel *et al*., 2013). Following the mid-1960s drought, the region experienced a strong increase in precipitation and is currently in an extended pluvial (Pederson *et al.,* 2013; Melillo *et al.*, 2014). Drought incidence, duration and severity, particularly during the growing season, did not change or decreased slightly (1885-2011; Kunkel *et al.,* 2013; NOAA National Climatic Data Center, 2014). However, while the NENA forest is typically characterized as mesic, soil moisture availability can limit tree growth (Martin-Benito & Pederson, 2015; D'Orangeville *et al.*, 2018), an additional potential stress factor as climatic regimes shift and c_a rises. Vapor pressure deficit (VPD) is also predicted to rise in the 21st century, but the influence of atmospheric water demand vs. soil water deficit on stomatal response is not completely understood (Ficklin & Novick, 2017). As well, soil nutrient depletion and recovery and an accelerated nitrogen cycle are linked to 20th century acid deposition and its legacy (Likens *et al*., 1996; 1998; Groffman *et al*., 2018).

Research examining NENA forest conifer and deciduous tree species' responses to environmental change have provided insight into the multiple drivers of gas exchange and growth response (Thomas *et al*., 2013; Belmecheri *et al*., 2014; Levesque *et al*., 2017; Mathias & Thomas, 2018; Maxwell *et al.,* 2019). These studies have identified and articulated the various importance that drivers such as moisture availability, pollution inputs and rising c_a, alone and in combination, have had on tree physiological response and growth. However, previous work focused on the mid to late-20th and early 21st century and did not address longer-term isotopic trends and/or explore dynamical changes in leaf-gas response as driven by concomitant changes in c_a, climate and pollution.

Based on our understanding of changing moisture availability in the NENA forest and atmospheric VPD dynamics, we hypothesize climate and pollution are as important as rising c_a in modulating stomatal leaf-gas exchange and ultimately A at local to regional scales. Thus, the goals of this study were to, 1) evaluate the influence of rising c_a, climate and pollution on leaf gas exchange, 2) characterize the dynamical leaf gas exchange regulation strategy at multi-decadal scale by examining a suite of $\delta^{13}C$-derived physiological parameters ($\Delta^{13}C$, c_i, iWUE) and tree growth (basal area increment: BAI), and 3) examine if regional acid deposition has influenced tree physiology over time. Such information is needed to better parameterize Earth system models which link future biosphere-atmosphere-hydrosphere interactions with biochemical cycling under changing climatic and atmospheric conditions.

2. Materials and Methods

2.1 Study site and species

Abbey Pond (ABP) (Table **S1a**), located in the Green Mountains National Forest, Vermont (USA) is an example of the Eastern Hemlock-White Pine-Northern Hardwood Forest (Thompson & Sorenson, 2005). The stand contains a mixture of canopy-dominant eastern hemlock and white pine (*Pinus strobus* L.), interspersed with sub-dominant sugar maple (*Acer saccharum* Marshall var. *saccharum*), American beech (*Fagus grandifolia* Ehrh.) and yellow birch (*Betula alleghaniensis* Britton). Eastern hemlock is a long-lived (~400-500 years), shallow-rooted, late-successional conifer, capable of existing in the shade of a hardwood canopy for decades before becoming dominant (Marshall, 1927; Fowells, 1965; Kelty, 1986). It is considered moisture sensitive (Cook, 1991; Cook & Cole, 1991) and its tree-ring chronologies are widely used in climate and stream flow reconstructions in eastern North America (Cook & Jacoby, 1977; Pederson *et al*., 2013; Maxwell *et al*., 2017). The stand is old growth and shows no evidence of logging or other anthropogenic disturbances (Cogbill, C.V., *pers. communication*). Twentieth century natural disturbances events (e.g., tropical storms) affected <15% of the eastern hemlock in the stand (Belmecheri, S. *et al.*, unpublished) and hemlock wooly adelgid was not present.

ABP is a humid-temperate, mid-latitude, continental site (Zielinski & Keim, 2003; Leathers & Luff, 2007). The 30-year July average temperature is 20°C with a daily maximum of 27°C and a range of 11-16°C (https://www.usclimatedata.com/climate/salisbury/vermont/united-states/usvt0489). In winter, January average temperature is -7°C with a daily range of >11°C. Precipitation is well distributed throughout the year with average annual totals reaching 1100 mm.

2.2 Ring-width and BAI chronologies

Nineteen canopy-dominant eastern hemlock trees were sampled in late August 2010 using a five mm increment borer (2 cores/tree; opposite sides of the tree; perpendicular to the slope at breast height, ~1.07 m above ground level) (Table **S1b**). Samples were prepared using standard dendrochronological techniques (Stokes & Smiley, 1996) and crossdated using COFECHA (Homes, 1983). Tree-ring width chronologies were converted into a basal area increment (BAI, cm^2 $year^{-1}$) time series to detect growth changes in stem woody biomass over time. This technique standardizes annual increments relative to basal area (assuming a circular stem cross section), addressing the issue of declining tree-ring width with increasing tree diameter as a tree matures (West, 1980; Biondi & Qeadan, 2008). BAI was averaged over all sampled trees for the period 1849-2010.

2.3 Stable isotope measurement and chronologies

Seven cores (1 core per tree) were selected from the master ring-width chronology to develop the $\delta^{13}C$ chronology (Table **S2**). All cores covered the 1849-2010 period and correlated with the master chronology (Range: $r = 0.55 – 0.71$, $P<0.05$). Individual whole rings were separated from the core with a single-edged razor. Individual rings across the seven cores were pooled for years ending in 1 to 9 (e.g., 1901, 1902…) (Leavitt & Long, 1992; Leavitt, 2008). Before pooling, individual samples were weighed and adjustments made to ensure equal mass contribution from each sample for each year. For years ending in 0 (e.g., 1900, 1910…), individual rings were processed without pooling to examine between-tree variability and establish confidence limits around the chronology mean (McCarroll & Loader, 2004). Samples were milled to a homogeneous fine powder, reweighed and extracted to α-cellulose. The chemical procedure for larger (10-30 mg) and smaller (400-1500 µg) samples followed Brendel & Iannetta (2000) and Evans & Schrag (2004), respectively. Extraction to α-cellulose was based on the simultaneous delignification and removal of non-cellulosic polysaccharides (NCPs) using an acetic acid: nitric acid mixture, followed by sequential washings with ethanol, deionized water, and acetone (Brendel & Iannetta, 2000).

Isotope ratios were measured at the Environmental Stable Isotope Facility, Geology Department, University of Vermont, USA. Samples (~ 0.2 to 2.7 mg of α-cellulose) were prepared using an off-line combustion and cryogenic distillation system followed by analysis on a dual inlet V.G.

SIRA II Stable Isotope Ratio Mass Spectrometer. The results are reported in delta (δ) notation in permil units (‰) relative to the carbonate Vienna Pee Dee Belemnite (V-PDB) standard:

$$\delta^{13}\mathrm{Csample} = \left\lfloor \frac{(^{13}\mathrm{C}/^{12}\mathrm{C})\mathrm{sample}}{(^{13}\mathrm{C}/^{12}\mathrm{C})\mathrm{VPDB}} - 1 \right\rfloor 1000(‰) \qquad \text{(Eqn 1)}$$

Analytical sample precision was ±0.05‰ offline (based on replicate standards).

2.4 Calculations for Δ^{13}C, ci/ca, ci and iWUE

While the original δ^{13}C chronology covered the 1850-2010 period, we truncated the time series to 1900-2010 to account for size effects (i.e., tree diameter, height, canopy position) linked to a tree's position within the canopy. A tree's position can influence trends in Δ^{13}C and iWUE related to increasing height (McDowell *et al*., 2011) via assimilation of δ^{13}C-depleted air at the forest floor (Schleser and Jayasekera, 1985; Buchmann *et al*., 2002), increases in hydraulic resistance as trees become taller (Monserud & Marshall, 2001; McDowell *et al.* 2011) and changes in irradiance and photosynthetic capacity (Francey & Farquhar 1982; Brienen *et al*., 2017). Light attenuation (Brienen *et al.*, 2017) leads to a decrease in assimilation while an increase in hydraulic resistance results in decreased stomatal conductance. Evidence has shown that these effects will manifest, when unaccounted for, in declining trends in Δ^{13}C and an overestimation of iWUE (Francey & Farquhar, 1982; Monserud & Marshall, 2001; Vandeboncoeur *et al.,* 2020). By limiting the period of analysis to 1900-2010, when the trees were in a dominant canopy position, these size effects were largely avoided (Carmean *et al*., 1998; McDowell *et al.*, 2011; Klesse *et al*., 2018). Previous studies provided evidence that prior to the rise in atmospheric CO_2 concentration, trees in their juvenile phase (~50 years) were not characterized by age-related trends in δ^{13}C (Loader *et al*., 2007; Gagen *et al.* 2007; Leavitt 2010; Levesque *et al*., 2017; Vadeboncoeur *et al*., 2020).

Stable carbon isotope discrimination (Δ^{13}C) was calculated from the δ^{13}C time series and is defined as:

$$\Delta = \frac{\delta^{13}\mathrm{C}_{\mathrm{atm}} - \delta^{13}\mathrm{C}_{\mathrm{tree}}}{1 + \delta^{13}\mathrm{C}_{\mathrm{tree}}/1000} \qquad \text{(Eqn 2)}$$

where $\delta^{13}C_{atm}$ is the isotopic value of atmospheric CO_2 and $\delta^{13}C_{tree}$ is the isotopic value of the tree ring, and results from the preferential use of ^{12}C over ^{13}C during photosynthesis. Farquhar *et al.* (1982) described the relationship between carbon isotope discrimination and leaf gas exchange as:

$$\Delta \approx \delta^{13}C_{atm} - \delta^{13}C_{tree} \approx a + (b - a)\, {}^{c_i}/_{c_a}\ , \qquad \text{(Eqn 3)}$$

where *a* is the fractionation between $^{13}CO_2$ and $^{12}CO_2$ during diffusion of CO_2 through the stomata (4.4‰) (O'Leary, 1981), *b* is the discrimination by RuBisCO against $^{13}CO_2$ during carboxylation (27‰) (Farquhar & Richards, 1984), and c_i and c_a are leaf intercellular and ambient (μmol mol^{-1}) CO_2 concentrations, respectively. Corrections for internal leaf (mesophyll) CO_2 conductance (g_m) were not included in this analysis (Seibt *et al.*, 2008; Flexas *et al.*, 2012; Voelker *et al.*, 2014).

To calculate Δ, c_i and iWUE, estimated values of atmospheric CO_2 concentrations and $\delta^{13}CO_2$ from McCarroll & Loader (2004) for the period 1850-2004 were used. Atmospheric CO_2 concentration values were derived from Robertson *et al.* (2001) and $\delta^{13}CO_2$ from long-term Antarctica ice core data from Francey *et al.* (1999). The atmospheric CO_2 data were updated to 2010 using *in situ* measurements from Mauna Loa (HI) and the South Pole (1958-2010) (https://scrippsco2.ucsd.edu/data/atmospheric_co2/sampling_stations.html) (Keeling *et al.*, 2001), and the $\delta^{13}CO_2$ data using direct observations (2004-2010; https://www.esrl.noaa.gov/gmd/dv/data/index.php?category=Greenhouse%2BGases&parameter_name=C13%252FC12%2Bin%2BCarbon%2BDioxide; White *et al.*, 2015).

Physiological or intrinsic water-use efficiency (iWUE) is defined as the ratio of the fluxes of carbon assimilation (*A*) and stomatal conductance (g_s) (Feng, 1999; Ehleringer *et al.*, 1993) and is estimated from $\Delta^{13}C$ and c_a values as (Farquhar & Richards, 1984):

$$\text{iWUE} = \frac{A}{g_s} = (c_a - c_i)\frac{1}{1.6} \qquad \text{(Eqn 5)}$$

where 1.6 is the ratio of diffusivities for water vapor relative to CO_2. Unlike iWUE, actual WUE is calculated at the whole plant level and is dependent upon evaporative demand, influenced by vapor pressure differences with the atmosphere and the leaf and stomatal conductance. As iWUE takes into account neither this constraint nor respiratory losses, it is treated as potential WUE (Seibt *et al*., 2008).

2.5 Data Standardization

The $\Delta^{13}C$ chronology was standardized using a cubic smoothing spline with a frequency response cut-off at 0.50 and a wavelength of 50 years (ARSTAN; Cook, 1985; Cook & Holmes, 1986) to reduce the influence of spurious longer-term trends and to retain multi-decadal and interannual variability. Time series were transformed into dimensionless indices by dividing the raw values with the spline function estimates (Fritts, 2001) and then averaged using the bi-weight robust mean (Cook, 1985; Cook & Briffa, 1990). Chronology quality ($\delta^{13}C$, BAI) was evaluated using the RBAR (Fritts, 1976; Wigley *et al*., 1984), Expressed Population Signal (EPS) and Subsample Signal Strength (SSS) (Briffa, 1984; Wigley *et al*., 1984, but see Buras, 2017). The residual chronology was used in subsequent correlation-based analyses.

2.6 Climate, pollution and atmospheric carbon dioxide data

Monthly climate data (1900-2010) at 4 km resolution were obtained from the PRISM Climate Group (http://prism.oregonstate.edu; 2004) including: mean, minimum and maximum temperature (T_{mean}, T_{min}, T_{max}, °C), total precipitation (PRCP, mm) and maximum vapor pressure deficit (VPD_{max}, mb). The average of multi-month (e.g., May-September) periods were calculated to account for integrated seasonal effects. Monthly Palmer drought severity index (PDSI) for the western division (2) of Vermont was also explored in analysis (https://www.esrl.noaa.gov/psd/data/timeseries/). The three moisture-related variables (VPD, PRCP, PDSI) were included in the analysis to explore the influences of atmospheric water demand and/or soil moisture on $\delta^{13}C$-derived physiological parameters during periods of greater or reduced moisture availability in the environment. Increasing temperatures under climate change will influence atmospheric water demand and soil moisture differently (Novick et al., 2016; Ficklin & Novick, 2017) and thus, greater understanding of the influence on these variables on g_s is needed (e.g., Yi *et al*., 2019; Zhang *et al*., 2019). Trends over time in dominant climate variables were characterized through linear regression analysis and differences among periods were examined through analysis of variance. Homogeneity of variance was examined with Levene's test and post-hoc analysis using Dunnett T3 test.

Pollutant deposition data (NO_3^-, NH_4^+, SO^{2-}_4, mg/L) were derived from volume-weighted, average monthly concentration of bulk precipitation from watershed 6 (W6) at the Hubbard Brook Experimental Forest LTER (HBEF; Woodstock, NH, USA; 1966-2010; https://hubbardbrook.org/d/hubbard-brook-data-catalog; Likens, 2010). Pollutant data were also averaged across months to create seasonal (e.g., June-August) and water year (previous October-September) variables (Fig. **S1**). The HBEF W6 dataset was selected over the Underhill, Mount Mansfield, VT (USA) site (National Atmospheric Deposition Program, (http://nadp.slh.wisc.edu/data/ntn/; 1984-2010) due to its longer time span and the high correlation between the two time series ($r = 0.94$, $p<0.0001$). It was also selected over longer pollutant time series (e.g., Thomas *et al.*, 2014; Mathias *et al.*, 2018) due to the high quality of field measurements and their proximity to the study site. Trends in pollutant data over time were evaluated using linear regression analysis.

The target climate and pollutant time series were also detrended using a cubic smoothing spline with a frequency cutoff at 0.50 and a wavelength of 50 years (ARSTAN; Cook, 1985; Cook & Holmes, 1986) to remove anthropogenically-driven trends in climate and reductions in pollutants associated with the Clean Air Act and its Amendments (Driscoll *et al*., 2001). As the time series explored in this study were 111 years ($\Delta^{13}C$, climate) and 45 years (pollutants), we were limited to the identifiable and interpretable higher to medium frequencies (e.g., interannual to multi-decadal). By filtering the time series used in the correlation analyses ($\Delta^{13}C$, climate, pollutants) (see below), we sought to reduce the influence of lower frequency climatic and pollution variance that might be indistinguishable from non-climatic/non-pollutant variance and, to avoid the influence of artificial, lower frequency trends.

2.7 Data analysis

Correlation analyses (DendroCLIM2002; Biondi & Waikul, 2004) were used to evaluate relationships between the standardized $\Delta^{13}C$, climate (1900-2010) and pollutant (1966-2010) time series. DENDROCLIM2002 employs bootstrapped confidence intervals to compute the significance of correlation coefficients at the $P<0.05$ level. Correlation coefficients were calculated for a 17-month period (previous May-current September), as well as for multi-month periods (e.g., May-September). DendroCLIM was also used to explore the persistence and changing significance of $\Delta^{13}C$, climate and pollutant relationships using a forward evolving interval of 30-years (30-year window length is incremented by one, starting from the least recent year with each iteration) for 1900-2010. The length of the HBEF pollutant record limited time series comparison to the 1966-2010 period. A rank-based non-parametric Pettitt test (1979) was used to detect shifts in the central tendency of the c_i time series (Killick & Eckley, 2014). The Pettitt test is considered distribution free and insensitive to outliers. Based on identified time periods with statistically significant differences in the mean c_i, temporal trends in the $\Delta^{13}C$, c_i, c_i/c_a, iWUE and BAI time series were assessed using linear regression analysis. Analysis was carried out in IBM SPSS 24 (2018) and DendroCLIM2002 (Biondi & Waikul, 2004).

3. Results

3.1 BAI and $\delta^{13}C$ chronologies

The ABP BAI and $\delta^{13}C$ chronology and its derivatives provide a 111-year perspective (1900-2010) of an eastern North American, mid-latitude, conifer species' growth trajectory and gas exchange response to environmental change in the 20th and 21st centuries. The mean length of the xylem increment cores used in this study was 144.9±25.37 years (range: 87-183 years) with a mean DBH of 58.3±10.28 cm (range: 49-92 cm) (Table **S1b**). All trees used for stable isotope analysis began growing before 1850 and thus, were at least 50 years old and ~18-22 m in height at the start of the 20th century (Carmean *et al.*, 1998). We assume that increases in height would likely have had minimal effects on the suite of tree-ring-based $\delta^{13}C$-derived physiological parameters values (Carmean *et al.*, 1998; McDowell *et al*., 2011; Levesque *et al*., 2017; Klesse *et al.*, 2018). Based on $\delta^{13}C$ measurements for every tenth year, mean $\delta^{13}C$ values fell within the 95% confidence interval (Fig. **S2b**). Based on shifts in the central tendency of the c_i time series (Pettitt, 1979) and the predominant trend in the data, three periods were delineated including, an initial stable period (1900-1956), a shift downward (1957-1975), and a third period (1976-2010) characterized by a continuous upward trend (Fig. **S3**).

3.2 Climatic influences on eastern hemlock $\Delta^{13}C$

Analysis of $\Delta^{13}C$ and climate variables indicate the importance of growing season VPD_{max}, followed by T_{max} and moisture. Correlations between $\Delta^{13}C$ and PRISM climate data (1900-2010) were significant ($P<0.05$-0.01) for individual months and growing season multi-month periods (May-September) (Fig. **1**). The strongest correlations with individual months included, May VPD_{max} ($r = -0.42$, $P<0.01$), May T_{max} ($r = -0.40$, $P<0.01$), July PRCP ($r = 0.30$, $P<0.05$) and July PDSI ($r = 0.47$, $P<0.01$). The $\Delta^{13}C$ chronology was most highly correlated with multi-month periods: May-September VPD_{max} ($r = -0.57$, $P<0.01$), May-July T_{max} ($r = -0.47$, $P<0.01$), May-August PRCP ($r = 0.41$, $P<0.01$), and July-August PDSI ($r = 0.47$, $P<0.01$). Correlation analyses of these multi-month periods for each of the three time periods (Table **S3**) showed all but May-July T_{max} (1976-2010) were significant ($P<0.05$-0.01). Further, 30-year forward evolving intervals revealed persistent and significant ($P<0.05$) relationships with the same four climate variables (1900-2010) (Fig. **2)**. Moving correlations of $\Delta^{13}C$ with May-July T_{max} and May-September VPD_{max} were consistent across the 111-year period, with VPD_{max} of slightly greater importance after 1950 as the influence of T_{max} declined. Both May-August PRCP and July-August PDSI correlation values increased from ~1930 to the early-1950s, declined and remained low through the mid-1960s, increased again into the mid-1970s, and then declined slightly to 2010.

3.3 Shifts in local climate conditions

Rising growing season temperature and greater moisture availability, as well as strong atmospheric water demand characterized the ABP site (1900-2010). Based on the previously identified multi-month climate variables, there was an upward trend in May-July T_{max} ($P<0.01$), May-August PRCP ($P<0.0001$) and July-August PDSI ($P<0.0001$) (Fig. **S4**). May-September VPD_{max} also increased, but was not significant ($P=0.138$). Across the three time periods, these climate variables showed positive trends for May-July T_{max} and May-September VPD_{max} in the early period (1900-1956; $P<0.01$-0.001), May-August PRCP and July-August PDSI in the middle period (1957-1975; $P<0.05$-0.01), and May-September VPD_{max} and July-August PDSI in the late period (1976-2010; $P<0.05$) (not shown). The four climate variables showed intercorrelations (1900-2010; $P<0.05$), including strong relationships between May-July T_{max} and May-September VPD_{max} ($r=0.69$, $P<0.01$) and May-August PRCP and July-August PDSI ($r=0.72$, $P<0.01$) (Table **S4**) – indicative of the local hydroclimate. Analysis of variance and post-hoc tests comparing the three periods revealed May-August PRCP was significantly different ($P<0.05$) between 1900-1956 and 1976-2010, indicating an increase in moisture availability between the start and end of the 111-year period. No other climate variables were significantly different among periods.

3.4 Trends in Δ^{13}C, c_i/c_a, c_i, iWUE and BAI

Based on the three time periods, the Δ^{13}C series showed a declining trend from 1900-1956 ($R^2 = 0.21$, $P<0.0001$), no trend from 1957-1975 ($R^2 = 0.09$, $P>0.05$), and an increasing one from 1976-2010 ($R^2 = 0.40$, $P<0.0001$) (Fig. **3a**, Fig. S**5a**). This pattern was mirrored in the c_i/c_a time series (Fig. **3b**, Fig. **S5b**). Intercellular CO_2 concentration (c_i) remained relatively unchanged from 1900-1956 ($R^2 = 0.00$, $P>0.05$), showed a positive but not significant slope from 1957-1975 ($R^2=0.002$, $P>0.05$), and an increase after 1976 ($R^2 = 0.78$, $P<0.0001$) (Fig. **3c**, Fig. **S5c**).

The iWUE increased from 1900 to 1956 ($R^2 = 0.53$, $P<0.0001$) and again from 1957 to 1975 ($R^2 = 0.35$, $P<0.01$) (Fig. **3d**, Fig. **S5d**). From 1976 to 2010, iWUE continued to rise ($R^2 = 0.19$, $P<0.01$) and reached its highest measured value during this period (2007: 130.69 µmol/mol). Overall, the percentage increase in iWUE was 28.01%, relative to the 1900-1910 period. However, iWUE began to plateau after 1975 with the rate of increase decelerating from 0.51 ± 0.17 ppm year^{-1} (1957-1975) to 0.19 ± 0.07 ppm year^{-1} (1976-2010). Further, when iWUE was compared against c_a (1900-2010), the relationship was more variable after the mid-1960s and the rate of increase in iWUE plateaued and then declined at recent c_a concentration (Fig. **4a**).

For the combined period of 1900-1975, a flat BAI trend prevailed (1900-1956, $R^2 = 0.04$, $P>0.05$; 1957-1975, $R^2 = 0.07$, $P>0.05$) and then it increased (1976-2010; $R^2 = 0.45$, $P<0.0001$) (Fig. **3e**; Fig. **S5e**). BAI remained consistently near the chronology mean (17.15 cm^2 year^{-1}) until 1975. A regression of BAI over iWUE (1900-2010) showed a positive relationship ($R^2 = 0.30$, $P<0.0001$) (Fig. **4b**), but when examined over the three periods no trends were significant (1900-1956, $R^2 = 0.00$, $P>0.05$; 1957-1975, $R^2 = 0.00$, $P>0.05$; 1976-2010, $R^2 = 0.03$, $P>0.05$) (Fig. **S6**).

3.5 Acid deposition and its influence on Δ^{13}C

Downward trends characterized water year NH_4^+, NO_3^- and SO_4^{2-} (previous October-September; 1966-2010) series, but only SO_4^{2-} was significant ($R^2=0.87$, $P<0.0001$) (Fig. **S1**). The $\Delta^{13}C$ series were negatively correlated with individual months at the end of the previous and current growing season including, previous September NO_3^- ($r= -0.29$, $P<0.05$) and SO_4^{2-} ($r= -0.32$, $P<0.05$) and August NO_3^- ($r= -0.26$, $P<0.05$) (1966-2010; Fig. **5**). No correlations with NH_4^+ were significant ($P>0.05$). Thirty-year forward evolving intervals (1966-2010) indicated a persistent and mostly significant ($P<0.05$) correlation with previous September SO_4^{2-}, with the relationship becoming more negative until the early 21st century and then less negative over the next decade (Fig. **6**). Previous September NO_3^- moving correlations followed a similar trend, but overall were less negative and not statistically significant.

A comparison of static correlations between $\Delta^{13}C$ and climatic and pollutant variables over two periods common to all datasets (1966-1990; 1991-2010) showed $\Delta^{13}C$ was most strongly related ($P<0.05$-0.01) with climate (Fig. **S7**). This relationship held for the period of greatest pollutant input (1966-1990) prior to the Clean Air Act (CAA) and its amendments and the period directly following its implementation (1991-2010). The $\Delta^{13}C$ and VPD_{max} correlations were strongest for both periods examined (1966-1990, $r=0.63$, $P<0.01$; 1991-2010, $r=0.45$, $P<0.05$). Correlations with previous September SO_4^{2-} and NO_3^- were not significant ($P>0.05$) for either period.

4. Discussion

4.1 Dynamic trends: Shifts in gas exchange and growth response to climate and c_a

The water balance of the NENA forest changed over the 20th and 21st centuries, becoming wetter and warmer (Janowiak *et al.*, 2018), with a clear shift to wetter conditions after 1975. Within this context, eastern hemlock is characterized by an evolving physiological response whereby the species rapidly and dynamically shifted along a continuum of leaf gas-exchange strategies (constant c_i; constant c_i/c_a; constant $c_a - c_i$) in response to rising c_a (Saurer *et al.*, 2004; Voelker *et al.*, 2016).

The 1900-1956 period was characterized by constant c_i. This strategy requires a dramatic increase in A, or a decrease in g_c, or both (Voelker *et al.*, 2016) and is described as an active response to rising c_a (McCarroll *et al.*, 2009). ABP iWUE rose rapidly as $\Delta^{13}C$ declined and c_a rose; on the other hand growth remained flat. Our analysis of 20th century regional climate drivers suggest atmospheric demand and moisture availability were likely as influential on stomatal response as rapidly increasing c_a. Stomatal conductance (g_s), in response to these variables, moderates $\Delta^{13}C$ (Comstock & Ehleringer, 1992; Saurer *et al.*, 1997; Roden & Ehleringer 2007). In the northeastern United States, the first half of the 20th century was drier than the second, involving drought area and total annual and summer precipitation (Pederson *et al.*, 2013). Predominantly negative summer PDSI values (1900-mid-1930s; not shown) suggest soil moisture deficit in the region. Thus, greater atmospheric water demand resulted in a decrease in g_s over an increase in A (i.e., reduced transpiration at the expense of CO_2 uptake and tree C gain) during this period. Other studies in arid forests reported similar tree response to moisture deficit despite rising iWUE trends (Andreu-Hayles *et al.*, 2011; Peñuelas *et al.*, 2011; Voltas *et al.*, 2013; Lévesque *et al.*, 2014).

Constant c_i/c_a (Saurer *et al.*, 2004) typified the 1957-1975 period. Termed an active response (McCarroll *et al.*, 2009), the maintenance of a constant c_i/c_a occurs through the simultaneous changes in g_s and A in response to rising c_a (Saurer *et al.*, 2004). While eastern hemlock continued to respond to *rising* c_a, climate drivers including higher atmospheric demand and low soil moisture availability influenced g_s as evidenced by declining $\Delta^{13}C$, steady c_i and rising iWUE, particularly in 1964, 1965 and 1975 (Fig. **4a**). During this period, the northeastern US experienced the most intense drought (mid-1960s) of the last five centuries in the region (Namias, 1966; Cook & Jacoby, 1977; Pederson *et al.*, 2013). The influence of another variable on g_s (i.e., climate) is further suggested by the greater variability of iWUE values around the trend line when regressed on c_a (Fig. **4b**). As well, reduced g_s resulted in limited A, as evidenced by continued level radial growth. Observational and experimental studies from multiple sites showed similar homeostatic c_i/c_a trends (Williams & Ehleringer 1996; Bert *et al.*, 1997; Duquesnay *et al.*, 1998; Saurer *et al.*, 2004; Ward *et al.*, 2005; Linares *et al.*, 2009; Andreu-Hayles *et al.*, 2011; Bonal *et al.*, 2011; Peñuelas *et al.*, 2011; Leonardi *et al.*, 2012; Saurer *et al.*, 2014; Frank *et al.*, 2015; Guerrieri *et al.*, 2019).

The final period (1976-2010) follows the constant $c_a - c_i$ scenario (Saurer *et al.*, 2004). This strategy is characterized by minor increases in A and/or minor decreases in g_s (Voelker *et al.*, 2016), and is described as a passive response (McCarroll *et al.*, 2009). We hypothesized that leaf-gas exchange, as evidenced by rising Δ^{13}C, c_i/c_a and c_i and a leveling-off of iWUE, was driven primarily by climate and site conditions that resulted in eastern hemlock maintaining open stomata. After the mid-1960s drought, northeastern North America experienced rising moisture levels (Pederson *et al*., 2013; Maxwell *et al.*, 2017). Six of the region's 20 wettest growing seasons (May-September PDSI; 1900-2010; not shown) occurred between 1990 and 2010 and 12 of the top 20 since 1975. This suggests that while the overall rise in T_{max} was sufficient to maintain the dominance of VPD_{max} over g_s, after 1975 the rise in soil moisture availability and an upward trend in summer (JJA) relative humidity in the northeastern US (Brown & DeGateano, 2013; Ficklin & Novick, 2017) established a lower gradient of moisture demand and higher leaf water potentials (Ψ_L). Rising evapotranspiration (ET) (Huntington & Billmire, 2014; Kramer *et al.*, 2015) and declining trends in daily temperature ranges in the northeastern US (Lauritsen & Rogers, 2012) may have resulted in reduced or stabilized daytime VPD despite warming temperatures (Ficklin & Novick, 2017).

Further, it is unlikely that increased irradiance modulated Δ^{13}C via photosynthetic rate. Greater irradiance should result in lower Δ^{13}C and greater assimilation or growth (Voelker *et al.*, 2014). However, *both* ABP Δ^{13}C and growth (BAI) increased after 1975. Regional irradiance declined as shown by increases in regional cloud cover (Lauritsen & Rogers, 2012) and local declines in growing season total sunshine hours and percent possible sunshine. These variables may be used as proxies for solar irradiance when photosynthetic active radiation (PAR) is not available (http://www7.ncdc.noaa.gov/IPS/lcd/lcd.html) (Young *et al.*, 2010). Multiple theoretical and observational studies at the leaf and canopy-level have noted an enhancement in canopy photosynthesis under diffuse radiation conditions (Hollinger *et al*., 1994; Gu *et al.* 2002; Gu *et al*., 2003; Niyogi *et al*. 2004; Urban *et al*., 2007; Mercado *et al*., 2009; Zhang *et al*., 2010; Urban *et al*., 2012), and in one modeling study Knohl & Baldocchi (2008) showed an increase in Δ^{13}C. Thus, under higher moisture availability and cloudier conditions, leaf-gas exchange was not limited by g_s, and indeed, g_s likely remained constant or potentially increased as indicated by declining Δ^{18}O values from nearby NENA forests sites (Guerrieri *et al.*, 2019). These conditions allowed the species to maintain open stomata and increase *A*, resulting in increased Δ^{13}C and BAI. Further, the rising trend in the ABP Δ^{13}C time series matches the recent rise in global atmospheric measurements (Keeling *et al.*, 2017).

4.3 Unusual response of iWUE

Contrary to studies showing a secular increase of iWUE in response to rising CO_2 (Ainsworth & Rogers, 2007; Franks *et al*., 2013; Saurer *et al*., 2014), at ABP iWUE plateaued and then declined at more recent c_a levels (~330 ppm). Waterhouse *et al*. (2004) hypothesized trees from European sites characterized by a late 20th-early 21st century plateau in iWUE are no longer physiologically forced by or are insensitive to rising c_a (*i.e.,* saturation effect). *In situ* biochemical processes that respond to c_a, moisture and nutrient availability play a critical role in modulating the leaf-gas exchange strategy in C3 plants (Oren *et al.,* 2001; Becklin *et al*., 2014; Warren *et al.* 2015). Theory posits that as c_a rises, A is less limited by the carboxylation rate of Ribulose-bisphosphate carboxylase/oxygenase (Rubisco) (Farquhar *et al*., 1980; Long & Bernacchi, 2003). Rising c_a increases the efficiency of Rubisco and A can be maintained or rise despite declines in enzyme content, activity or maximum photosynthetic capacity (Warren *et al.,* 2015). However prior to 1975 at ABP, reduced g_s due to lower moisture availability limited any increases in A linked to rising c_a. When c_a exceeds 400 ppm, A will plateau as it is limited by RuBP-regeneration (Long & Bernacchi, 2003). At this point, A is saturated as either soil N availability becomes more limiting or leaf N concentrations are diluted by CO_2-induced growth (Oren *et al.,* 2001; Warren *et al.,* 2015; Voelker *et al.,* 2016). In NENA forests, atmospheric N deposition has declined since the early 2000s (Groffman *et al*., 2018; Gilliam *et al*., 2019). The negative effects of this decline on forest productivity and tree response are thought to be exacerbated by increases in c_a, deacidification of soils, and climate change (Richardson *et al.,* 2010; Groffman *et al*., 2012). Stomatal conductance (g_s) will also begin to decline with rising c_a, but is hypothesized to stabilize at a species-specific minima (Becklin *et al.,* 2014; Voelker *et al.,* 2016). However, a recent study (Haverd *et al*., 2020) using a terrestrial land-based model suggests that as c_a continues to increase, C3 plants may optimize productivity through coordination (Chen *et al*., 1993; Farquhar & von Caemmerer, 1981; Wang *et al*., 2017) whereby, the relative nitrogen investments in carboxylation and electron transport are co-limiting.

It is unknown if this “passive” response in eastern hemlock has only begun and, if it is a short-term acclimation to present c_a or a longer-term physiological response to environmental change. At present, it is unclear how C3 plants in natural environments respond physiologically to the higher c_a over longer periods (but see Becklin *et al*., 2014) and why this plateau occurs in multiple species growing in various ecosystems and under different climatic regimes. Indeed, multiple studies show this non-linear response during the late 20th to 21st centuries from mid-latitude (e.g., Feng, 1998; Waterhouse *et al.*, 2004; Peñuelas *et al.*, 2008; Andreu-Hayles *et al.*, 2011; Belmecheri *et al.*, 2014), high elevation (Marshall & Monserud, 1996; Wu *et al.*, 2015; Wieser *et al.*, 2016) and boreal forests (e.g., Gagen *et al.*, 2011). This anomaly in iWUE requires further investigation as it is clear that the effects are not limited to one region or species.

4.4 Acid deposition, stomatal response and vegetation water use

Acid deposition, first reported in North America in the White Mountains in 1972 (Likens *et al.*, 1972), results in base cation leaching and depletion (e.g., Ca+) from leaves and soils. Calcium helps regulate stomatal response, carbon metabolism, and facilitates plants' ability to sense and respond to stress (Marschner, 2002). The negative response of ABP $\Delta^{13}C$ (1966-2010) to acid deposition indicates net soil leaching of Ca+ was on-going (Talhelm *et al*., 2012; Greaver *et al*., 2012), even post-1990. Thus, in addition to greater moisture availability, changing soil biogeochemistry may be partially responsible for rising $\Delta^{13}C$ (1976-2010), as Ca+ deficit prevented stomatal closure and thus, sustained transpiration. Based on results from a long-term, watershed acidification experiment, Lanning *et al*. (2019) suggested Ca+ leaching altered tree stomatal response and vegetation water use, causing an increase in transpiration that depleted available soil water as measured at the watershed scale. Examining the regional hydrological cycle in the Northeastern US (1960-2012), Vadeboncoeur *et al.* (2018) highlighted higher ET in northern watersheds compared to southern ones (i.e., lower ET). This suggests regional water balance dynamics may be responding to both atmospheric demand and plant physiological effects via stomata response as influenced by soil Ca+ availability. While the effect of CO_2 fertilization on WUE may explain some ET decline in southern watersheds, it did not explain increasing ET trends in the north, which may be driven more by climate (Vadeboncoeur *et al.*, 2018) and legacy pollution effects. Our results, showing iWUE plateaued and stomata responded strongly to increasing moisture availability and net Ca+ leaching from soil since 1975, provide support for observations of increasing ET in northern watersheds. As numerous studies have detected negative effects of acid deposition on NENA tree species (DeHayes *et al*., 1999; Schaberg *et al.*, 2001; Halman *et al.*, 2011; 2013; Thomas *et al*., 2013; Battles *et al*., 2014; Engel *et al*., 2015; Mathias *et al*., 2018; Wason *et al*., 2019), more thorough investigations of leaf-gas exchange response are needed across species and community types before conclusions are drawn. While peak pollution loading has abated since 1990, legacy effects of long-term net soil base cation depletion will delay soil recovery into the 21st century (Lawrence *et al*., 2012).

Our study demonstrates that mesic forest ecosystems may exhibit a more physiologically complicated and dynamic response over multi-decadal time scales and driven by climate change, rising c_a, and pollution effects than previously thought. A generalized pattern of leaf-gas exchange dynamics and iWUE over the 20th and 21st centuries and at regional and global scales therefore, is called into question (Silva & Horwath, 2013; Levesque *et al*., 2017). Under the specter of rising temperatures (Crouch *et al.*, 2018), greater ET and VPD (Ficklin & Novick, 2017), and drought in NENA over the next century (Berg *et al*. 2017) and, the fact that the region's forests provide ecosystems services for > 64 million people in urban and rural areas, a clearer understanding of tree physiological response will be an important contribution towards parameterizing earth-system models and estimating future carbon sink capacity and water balance in mid-latitude forests and elsewhere.

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Author Contribution

SAR designed the study. SAR and RG collected and performed tree-ring data analyses. SAR, AL, RG and CJ analyzed isotopic samples. SAR performed isotopic data analyses with input on data interpretation from SB and MHG. SAR wrote the manuscript with contributions from all authors.

ORCID

Shelly A. Rayback: ID 0000-0002-7925-4892
Soumaya Belmecheri: 0000-0003-1258-2741
Mary H. Gagen: 0000-0002-6820-6457
Andrea Lini: 0000-0002-2920-570X

Data Availability

Isotopic and tree-ring data will be available on the NOAA National Centers for Environmental Information Paleoclimatology Data (https://www.ncdc.noaa.gov/data-access/paleoclimatology-data).

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Figure Legend

Fig 1. Pearson's correlation coefficients between Abbey Pond eastern hemlock (*Tsuga canadensis*) stable carbon isotope discrimination ($\Delta^{13}C$) time series and monthly and seasonal climate data (1901-2010). The 17-month period examined (left side) includes the previous May (lower case) to the current September (upper case). Seasonal periods (right side) include two to five month periods during the current growing season. Climate variables include mean maximum temperature (T_{max}, blue), mean maximum vapor pressure deficit (VPD_{max}, green), total precipitation (PRCP, red) and the Palmer drought severity index (PSDI, yellow). Dashed lines indicate significance at $P<0.05$.

Fig 2. Thirty-year running correlation coefficients between Abbey Pond eastern hemlock (*Tsuga canadensis*) stable carbon isotope discrimination ($\Delta^{13}C$) time series and climate variables (1901-2010). Climate variables include mean maximum temperature (T_{max}, blue), mean maximum vapor pressure deficit (VPD_{max}, green), total monthly precipitation (PRCP, red) and the Palmer drought severity index (PSDI, yellow). Correlations with T_{max} and VPD_{max} were inverted to facilitate comparison. Significance level for the 30-year window was $P<0.05$ and is shown by the dashed line.

Fig 3. Linear regression-derived trends for the three periods (1901-1956 (black circles and line); 1957-1975 (orange circles and line); 1976-2000 (blue circles and line)) for Abbey Pond eastern hemlock (*Tsuga canadensis*): (a.) $\delta^{13}C$ discrimination ($\Delta^{13}C$, ‰); (b.) leaf intercellular CO_2 over atmospheric CO_2 concentration (c_i/c_a, ppm); (c.) leaf intercellular CO_2 concentration (c_i, ppm); (d.) intrinsic water-use efficiency (iWUE, µmol/mol); and (e.) basal area increment (BAI, cm^2 $year^{-1}$). Trend lines, slope, confidence interval (high & low CI), coefficient of determination (R^2) and significance (P-value) are provided.

Fig 4. (a.) Abbey Pond eastern hemlock (*Tsuga canadensis*) annual values of intrinsic water use efficiency (iWUE) regressed against annual atmospheric CO_2 concentrations (c_a) for the period (1901-2010). Second-order polynomial trend line is included. (b.) Annual values of Abbey Pond eastern hemlock (*Tsuga canadensis*) basal area increment (BAI) regressed against iWUE for the period 1901-2010. Trend lines, coefficient of determination (R^2) and significance (P-value) are provided.

Fig 5. Pearson's correlation coefficients between Abbey Pond eastern hemlock (*Tsuga canadensis*) stable carbon isotope discrimination ($\Delta^{13}C$) (1966-2010) and pollutants (SO_4^{2-} (brown); NO_3^- (orange)) measured at Hubbard Brook Experimental Forest (New Hampshire, USA). The 17-month period examined (left side) includes the previous May (lower case) to the current September (upper case). Seasonal periods and water year (p October-September) (right side) were also examined. Dashed line indicates significance at $P<0.05$.

Fig 6. Thirty-year running correlations between Abbey Pond eastern hemlock (*Tsuga canadensis*) stable carbon isotope discrimination ($\Delta^{13}C$) time series and pollutants (1966-2010) measured at Hubbard Brook Experimental Forest (New Hampshire, USA). Pollutant variables include mean monthly NO_3^- for the previous September (orange) and August (light orange) and SO_4^{2-} measurements for the previous September (brown). Significance level for the 30-year window was $P<0.05$ and is shown by the dashed line.

Supplemental Figure and Table Legend (Abbreviated)

Fig. S1 Time series and trends of pollutants in bulk precipitation.

Fig. S2 Pettitt test-identified changes in mean of c_i time series.

Fig. S3 Time series of $\delta^{13}C$, confidence interval and BAI.

Fig. S4 Climate variable trends over time.

Fig. S5 Time series of Δ^{13}C, c_i/c_a, c_i, iWUE, and BAI.

Fig. S6 Linear regression of BAI over iWUE for three periods.

Fig. S7 Comparison of Δ^{13}C with climate and pollutant variables.

Table S1 Site information and chronology statistics for eastern hemlock.

Table S2 Sampling and core information.

Table S3 Correlation coefficients between Δ^{13}C and climate time series.

Table S4 Correlation coefficients among climate time series.