Evidence of recovery of *Juniperus virginiana* trees from sulfur pollution after the Clean Air Act

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Using dendroisotopic techniques, we show the recovery of *Juniperus virginiana* L. (eastern red cedar) trees in the Central Appalachian Mountains from decades of acidic pollution. Acid deposition over much of the 20th century reduced stomatal conductance of leaves, thereby increasing intrinsic water-use efficiency of the *Juniperus* trees. These data indicate that the stomata of *Juniperus* may be more sensitive to acid deposition than to increasing atmospheric CO₂. A breakpoint in the 100-y δ¹³C tree ring chronology occurred around 1980, as the legacy of sulfur dioxide emissions declined following the enactment of the Clean Air Act in 1970, indicating a gradual increase in stomatal conductance (despite rising levels of atmospheric CO₂) and a concurrent increase in photosynthesis related to decreasing acid deposition and increasing atmospheric CO₂. Tree ring δ³⁴S shows a synchronous change in the sources of sulfur used at the whole-tree level that indicates a reduced anthropogenic influence. The increase in growth and the δ¹³C and δ³⁴S trends in the tree ring chronology of these *Juniperus* trees provide evidence for a distinct physiological response to changes in atmospheric SO₂ emissions since ~1980 and signify the positive impacts of landmark environmental legislation to facilitate recovery of forest ecosystems from acid deposition.

A key uncertainty in coupled biosphere–atmosphere models of climate change is the long-term effects of increasing atmospheric CO₂ on carbon uptake and storage in terrestrial ecosystems (1, 2). Forest ecosystems play a fundamental role in the global C cycle; they contribute ~50% of terrestrial net primary production (3, 4), account for ~45% of terrestrial C (5), and are a major part of the terrestrial C sink that removes nearly 30% of anthropogenic C emissions each year (6). Consequently, state-of-the-art climate models require a mechanistic understanding of how simultaneous changes in key environmental variables affect C cycling in trees and forest ecosystems. Although experiments have examined how numerous environmental factors affect tree growth and forest productivity, accurately forecasting future tree growth and C cycling requires a better understanding of complex environmental interactions across spatial and temporal gradients in natural systems.

For over a century, the combustion of fossil fuels resulted in the widespread alteration of the composition of the atmosphere. For example, though atmospheric CO₂ concentrations increased by almost 40% since the mid-19th century and are continuing to increase at an unprecedented rate (7, 8), SO₂ emissions in the United States also increased rapidly initially but then declined following US environmental legislation (the Clean Air Act) in 1970 and its subsequent amendment in 1990 (Fig. 1) (9, 10). Although the direct effects of elevated CO₂ are positive for plants, generally increasing photosynthesis and decreasing water loss by reducing stomatal conductance (11), SO₂ emissions are transformed into acidic deposition (Fig. S1), which has numerous negative impacts on ecosystem productivity (12).

The Central Appalachian Mountains have historically received some of the highest rates of acid deposition in the United States due to their downwind proximity to the abundance of coal-fired power plants in the Ohio River Valley (12). Despite these historical rates of acid deposition and the potentially long-lasting effects on ecosystems, there are many examples of tree species in the temperate deciduous forest in the eastern United States that have shown recent unexplained increases in growth (13–17). In this study, we used dendroisotopic techniques to examine simultaneous environmental influences on *Juniperus virginiana* trees growing in the eastern panhandle of West Virginia. Using this approach, we were able to estimate how key physiological processes have changed across the past century, and the extent to which temporal changes in atmospheric CO₂ concentrations and acid deposition are responsible.

Results and Discussion

Here, we found that the basal area increment (BAI) of *Juniperus* in an old-growth stand in West Virginia has increased significantly since the enactment of the Clean Air Act of 1970 (Fig. 1), despite being older (118–480 y old) than the age of trees that normally exhibit rapid growth (18, 19). A recent tree ring chronology of *J. virginiana*, incorporating our site and others in this region, indicates an increasing growth trend for these trees since the late 20th century that is greater than any other time over the past 450 y (20). We performed a multivariate correlation analysis using historical climate variables (21), atmospheric CO₂ concentrations (7, 8), and US SO₂ and NOₓ emissions (9, 10), and found that the growth of these *Juniperus* trees over the last century is explained best by increases in atmospheric CO₂ and NOₓ emissions, and decreases in SO₂ emissions (Table S1).

To examine changes in leaf physiology that influence tree growth over time, whole wood from tree rings from the years 1909–2008 was analyzed for δ¹³C (Fig. 2A), corrected to account for the differences between leaf and wood isotopic signatures, and δ³⁴S (Fig. 2B), corrected to account for within-plant isotopic fractionation.

Significance

This study shows that red cedar trees growing in the Central Appalachian Mountains of West Virginia are recovering from decades of acidic pollution. Our study shows the efficacy of the Clean Air Act in a region where acidic pollution levels were some of the highest in the United States before the Clean Air Act. We demonstrate that a large portion of the increase in water use efficiency of trees that is often attributed to increasing atmospheric CO₂ over the last century may be caused by acid deposition. This study has important implications for carbon cycling in forests, showing an interaction between decreasing SO₂ emissions and increasing CO₂ that is not currently accounted for in biosphere–atmosphere models of climate change.

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converted to $\Delta^{13}C$ (isotopic discrimination) to account for the anthropogenic increase in isotopically light atmospheric $\delta^{13}CO_2$ and used in standard equations to calculate leaf physiological characteristics (22). We found a striking nonlinear trend in $\Delta^{13}C$ (Fig. 2B) that indicates a fundamental shift in foliar gas exchange of these Juniperus trees, resulting in a 66% increase in intrinsic water use efficiency (WUE), or the ratio of carbon gained through photosynthesis ($A$) to the stomatal conductance to water ($g_w$), over the 100-y time period (Fig. 2C). This isotopic signal indicates a progressive reduction in the discrimination against the heavy C isotope across the tree-ring chronology until later in the 20th century, when increased isotopic discrimination resulted in a lighter C isotopic signature being incorporated into the wood (Fig. 2B). We fit a third-order polynomial function to the sequence of $\Delta^{13}C$ to estimate the year when the isotopic trend changed from enrichment to depletion of the heavy C isotope ($y = 0.0001227x^3 - 0.07179x^2 + 139.9x - 90869; r^2 = 0.86, P < 0.0001$). The breakpoint was identified as 1982, which corresponds to the approximate time when $SO_2$ emissions began to decline in the United States. This result was surprising because it is counter to the widely observed stomatal response of plants to increasing $CO_2$ in the 20th century (11, 23). Here, decreasing $\Delta^{13}C$ before ~1980 suggest stomatal closure as if the trees were responding to increasing atmospheric $CO_2$, yet the reversal and increases in $\Delta^{13}C$ after 1980 suggest reduced stomatal regulation to $CO_2$ (Fig. 2F).

Similar isotopic trends in trees growing in the northern hemisphere have been observed by other investigators who have suggested that these recent shifts in $\delta^{13}C$ are indicative of a physiological change in guard cells where stomata become uncoupled from $CO_2$ concentration as atmospheric $CO_2$ began to increase rapidly in the 1980s (24). After converting $\Delta^{13}C$ to $C_i/C_a$ (Fig. S2A), the ratio of internal leaf $CO_2$ to atmospheric $CO_2$, a metric of leaf physiology that provides a relative assessment of the stomatal limitation of photosynthesis, it is clear that stomatal limitations for these Juniperus trees increased until ~1980, at which point the trend reversed and stomatal limitations to $CO_2$ uptake are reduced. Several lines of evidence point to the declining levels of $SO_2$ emissions around the 1980s that drive these stomatal responses in Juniperus rather than a decoupling of stomatal sensitivity to atmospheric $CO_2$.

The first line of evidence is the close correspondence between the timing of the change in direction of $C_i/C_a$ with the decline in $SO_2$ emissions that occurred in the United States following the Clean Air Act. The directional change in the isotopic signal follows reductions in $SO_2$ emissions that began in the 1970s, but precedes reductions in $NO_x$ emissions by over a decade (Fig. S3) (10). Although changes in tropospheric ozone might also impact $C_i/C_a$ over time, a direct assessment of the influence of $O_3$ is not possible because historical records of $O_3$ are not available for the same time period as our tree-ring chronology. Regardless, because $NO_x$ is a precursor to $O_3$ (25), it is reasonable to assume similar historical patterns of $O_3$ and $NO_x$, and because contemporaneous historical $NO_x$ emissions do not show a breakpoint around...
the year 1980, it is unlikely that changes in O₂ explain the pattern of Cᵢ/Cₑ in our tree-ring chronology. In addition, the open canopy and relatively high light levels of this Juniperus/hardwood forest suggests that canopy closure, or increased shading, around 1980 was not likely the cause for this chronology of Cᵢ/Cₑ. Likewise, we found no evidence of fire, timber harvesting, or any other type of disturbance that may have increased the light levels in this forest, thereby influencing the isotopic signatures of the tree rings. There has been an increase in yearly precipitation in this region in West Virginia over 1909–2008 (~1.2 mm y⁻¹; P < 0.012), and there has been a trend toward warmer temperatures (~ºC; P < 0.057), but year-to-year variability of both of these climatic factors were high (Fig. S4). If these changes in climate were responsible for patterns of Cᵢ/Cₑ, we would expect a gradual change in the isotopic signature over the entire century rather than the observed breakpoint at ~1980.

To further examine the consistency between the timing of the increase in Cᵢ/Cₑ in the tree rings of Juniperus with the timing of the decline in US SO₂ emissions, we compared δ¹³S in tree rings formed during preindustrial years to δ¹³S in tree rings before and after 1980 (Fig. 3). Wood δ¹³S is infrequently used in dendroisotopic studies because of the very low concentration of S in wood (26, 27). Sulfur can enter the plant through their roots or leaves, but either route of uptake results in very little fractionation when S is incorporated into biomass, allowing δ¹³S to typically reflect the sources of S incorporated by the tree (28). In this study, the sources of S used by these trees over the tree-ring chronology are unknown, but we assume that the δ⁴S from 1853 to 1870 shows little anthropogenic influence. The large depletion of the heavy S isotope between the preindustrial period and 1960–1975, as US SO₂ emissions were increased by ~260% compared with the mid-1800s (9), reflects a strong anthropogenic S input at the same time that δ¹³C indicates a decline in leaf Cᵢ/Cₑ. The increase in δ¹³S between 1960–1975 and 1995–2010, as US SO₂ emissions declined by ~41% (9, 10) and SO₄ deposition declined by ~66% (29), indicates a reversal back toward preindustrial δ¹³S signatures and a reduced anthropogenic influence. Thus, just as the tree ring δ¹³C after ~1980 indicates Cᵢ/Cₑ increased at the leaf level, tree ring δ¹³S indicates a synchronous change in the sources of S used at the whole-tree level. Sulfur concentrations in wood were constant across the chronology despite large changes in SO₂ emissions, reflecting the fixed physiological processes that regulate S deposition in wood, as has been shown previously (27).

The second line of evidence is that the changes in the C isotopic signature of Juniperus tree rings late in the 20th century were not unique to this time period. Instead, the patterns of change in δ¹³C, Δ¹³C, and Cᵢ/Cₑ after 1980 were not significantly different from the isotopic signatures found during the 1930s, when SO₂ emissions were sharply reduced during the US Great Depression (Fig. 2D and Fig. S5). As with the C isotopic signatures after 1980, no other environmental factors explain the observed changes during the 1930s. In addition, the growth of these Juniperus trees during 1930–1940 was inversely correlated to SO₂ emissions (y = -0.46x + 6.48; r² = 0.72; P < 0.0009), suggesting that the trees experienced a short-term recovery from acid deposition during the Great Depression years (Fig. S6).

The third line of evidence is from the chronology of stomatal conductance to CO₂ (gₛ) of the Juniperus foliage inferred from tree ring C isotopes. Acid misting experiments and field-based isotopic studies have indicated that SO₄ deposition affects stomatal function, including reducing gₛ of leaves (30–32). Based on these studies, we postulated that gₛ would decrease before 1980 when SO₂ emissions were high, but would subsequently increase in response to reductions in SO₂ emissions. The chronology of Cᵢ/Cₑ suggests a strong link between increased SO₂ emissions and the regulation of stomata, but Cᵢ/Cₑ incorporates both A and gₛ. To evaluate drivers of Cᵢ/Cₑ independently, we performed a simulation analysis of changes in A and gₛ using foliar Cᵢ derived from tree ring δ¹³C of the Juniperus trees (Fig. S2B), atmospheric CO₂ concentrations (7, 8), and the physiological relationship between light-saturated A and Cᵢ of Juniperus foliage measured onsite.

The chronology of A and gₛ in Juniperus shows that A changed very little from 1909–1980 (a 3% decline), whereas gₛ decreased by 35% over the same time period (Fig. 4A and B). After 1980, large increases in both A (27%) and gₛ (33%) occurred. Increases in A after 1980 were correlated with increases in atmospheric CO₂ and decreases in SO₂ emissions (Fig. S7A and B). Likewise, gₛ of Juniperus leaves after 1980 increased as SO₂ emissions declined, despite increasing atmospheric CO₂ (Fig. S7 C and D). Additionally, during 1930–1940, A increased 5.9% and gₛ increased 14.9% (Fig. S8), providing further support to consider that changes in the isotopic signal of Juniperus tree rings late in the 20th century and during the 1930s are both related to the same phenomenon. The chronology of A and gₛ binned by decade reveals that the only two time periods when there were net positive changes in A and gₛ of Juniperus were during the 1930s and after the 1980s (Table 1), and these correspond to time periods when increases in growth of the trees were observed (Fig. 1). During both of these time periods gₛ increased to a greater extent than A, explaining why WUE declined during the 1930s and after ~1990 in the tree ring chronology (Table 1 and Fig. S5D).

Despite decreased WUE of Juniperus during the 1930s and after ~1990, WUE increased 66% over the entire chronology (Fig. 2C) due to a 23% increase in A and an 18.5% decrease in gₛ. Many dendroisotopic studies have concluded that the small but progressive increases in atmospheric CO₂ over the last century have improved WUE (23, 33), and suggest that a fundamental way that increasing atmospheric CO₂ stimulates tree growth and
forest productivity by improving the efficiency that trees are using water. Here, acid deposition over many decades contributed to a large portion of the increase in iWUE of Juniperus, and these data signify that the effects of increasing CO₂ on iWUE can be greatly overestimated if other environmental influences are not considered in these estimates. The mechanisms for increased iWUE over the entire chronology differed between 1909–1980 (small decreases in A, large decreases in gₑ) and 1980–2008 (greater increases in A than gₑ initially, but followed by greater increases in gₑ than A ~1990, whereby iWUE decreases; Table 1). If we assume that the stomatal response between 1909–1980 incorporates stomatal closure due to both a CO₂ response and a response to SO₂ deposition (resulting in a 35% decline in gₑ), and after 1980 there is recovery from SO₂ deposition, then the difference in gₑ between 1909–2008 reflects the effect of increasing CO₂ (a 18.5% decline in gₑ; Fig. S9). Thus, if the recovery of gₑ from the effects of acid deposition was complete by 2008, it appears that the effects of SO₄ deposition on gₑ of Juniperus between 1909–1980 were greater than that of atmospheric CO₂ during the same time period.

SO₂ emissions and acid deposition have been effectively reduced in the United States since the Clean Air Act was enacted, but despite this, acid deposition remains a considerable problem in the United States, as well as other locations globally (34). Though aquatic ecosystems in the United States have shown some improvements as acid deposition has declined (33, 35), recovery of terrestrial ecosystems is expected to be much slower than aquatic systems, and could take decades (12, 36, 37). Our findings indicate that the productivity of this stand of Juniperus growing in the Central Appalachian Mountains is recovering from acid deposition, and confirm the prediction that recovery of trees occurs over long periods of time. It is clear that it will be important to examine the generality of these results from Juniperus to understand how widespread these responses to reduced SO₂ emissions are among co-occurring tree species within eastern temperate forest communities (38). Regardless, the recovery of Juniperus from long-term acid deposition involves gradual stomatal opening over several decades, and thus provides evidence for a physiological response to reductions in SO₂ emissions that is distinct from the response to increased atmospheric CO₂. The slower rate of increase in gₑ, but faster rate of increase in A, that occurred after 1980 compared with the short-term recovery during the 1930s (Fig. S8 A and B) likely reflect the widely observed effects of increased CO₂ concentrations on gₑ and A (11). If we consider A as a diffusive process described by Fick’s law, where 

\[ A = \frac{C_a - C_i}{g_e} \]

then the recovery of Juniperus trees occurs, carbon uptake is enhanced by both increased \( C_a \) as atmospheric CO₂ increases and increased \( g_e \) as SO₂ emissions decline. Taken together, these results highlight the complexities of significant interactions between atmospheric CO₂ and acid deposition on stomatal conductance, photosynthesis, and tree growth that have important consequences for forest C cycling in many northern hemisphere forests.

**Materials and Methods**

**Tree Selection and Growth Measurements.** The study site is a stand of *J. virginiana* (red cedar) trees located along the south branch of the Potomac River in southern Grant County, WV, on a northwestern facing limestone outcrop located above Smoke Hole Canyon (38° 53′ 02″ N 79° 14′ 12″ W; 670 m above sea level). This site is in the Ridge and Valley Physiographic Province of northeastern West Virginia and has been defined as a cedar glade (39). The soil in this glade woodland has little organic matter and is primarily composed of broken limestone. In addition to Juniperus trees, the overstory vegetation is comprised of Quercus muehlenbergii, Quercus alba, Fraxinus americana, and Juglans nigra, and the open overstory allows most trees to receive high sunlight throughout the day.

*Fig. 4.* Simulated seasonally integrated (A) net photosynthesis and (B) stomatal conductance of *J. virginiana* trees across the tree ring chronology.

*Table 1.* Changes (%) in the photosynthetic variables (PV), net photosynthetic rates (A) and stomatal conductance to CO₂ (gₑ) of *J. virginiana* trees over the 100-yr chronology derived from simulation analysis using Cᵢ from tree-ring isotope data, atmospheric CO₂ (7, 8), and the relationships between A and Cᵢ measured on *J. virginiana* foliage in 2009.

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concentrations between 50 and 1,500 trees from the C was never at saturating ratio (the ratio of the leaf intercellular CO curves, light-saturated photosynthesis was A, respectively) and an (×) (47). Values of 0.65). Our cor-

Carbon Isotopic Analysis of Tree Rings. Tree rings for years 1909–2008 were collected for isotopic analysis by scalpel dissection of cores under a dissecting microscope at the boundary of late wood and early wood. Wood samples were cut to 1 mg and packed into tin capsules for carbon isotopic analysis. Carbon Isotopic Analysis of Tree Rings. 3 was used for 1853 ratios, using

Sulfur Isotopic Analysis of Tree Rings. Five additional Juniperus trees from the same stand were randomly selected and cored in 2013 as described above for BAI and C isotopes. Each core was cross-dated with existing cores left for the 2008 and an n = 3 was used for 1853–1870.

Wood samples were analyzed for δ34S using a PYRO cube elemental analy-

Statistics. To determine if there had been any significant changes in climatic conditions, we examined the linear trends of monthly, seasonal, and yearly means of precipitation and temperature using the 100-y period from 1909 to 2008. Precipitation and temperature data were obtained from the National Climatic Data Center for West Virginia climate region 6 (21), which includes the portion of West Virginia in the Ridge and Valley physiographic province and contains our study site. We used annual average of the atmospheric CO2 concentrations reconstructed from ice cores (7) and recorded at Mauna Loa observatory since 1953 (8); overlapping values were averaged.

Linear regression was used to identify significant temporal trends in BAI of Juniperus trees over the tree-ring chronology (1911–2007). Due to the nonnormal distribution and autocorrelation in BAI, we used Kendall’s tau, a nonparametric correlation coefficient, to identify explanatory variables with the best relationships with BAI. All statistical analyses used single-year BAI data and were not detrended or smoothed, providing interpretable yearly levels of growth rather than a standardized index. Analysis of covariance was used to test for differences in BAI trends for before (1909–1970) and after (1970–2008) the Clean Air Act legislation.
Simple regression analyses were used to examine trends in tree ring δ13C, C/Ca, and NWE, as well as simulated A and g, over the 100-yr tree chronology (1908-2008). Although we only used five individual trees in our isotopic analysis, this number is typically sufficient to characterize stand-level tendencies given the typical uniformity of environmental signals reflected in tree rings for a given species and site (33). Analysis of covariance was used to test for differences in isotopic trends for 1930–1940 and 1980–2008.

One-way analysis of variance was used to examine whether S isotopes differed in tree rings from 1853–1870, 1960–1975, and 1995–2010. Differences between the means of each time category were assessed using a Tukey–Kramer honestly significant difference (HSD) test.

All statistical analyses were performed using JMP software for Macintosh v. 10.0 (SAS Institute).

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