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Mortality predispositions of conifers across Western USA

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Summary:

- Conifer mortality rates are increasing in western North America, but the physiological mechanisms underlying this trend are not well understood.

- Accepted Article
- We examined tree-ring based radial growth along with stable carbon and oxygen isotope composition ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, respectively) of dying and surviving conifers at eight old-growth forest sites across a strong moisture gradient in the western USA to retrospectively investigate mortality predispositions.
 - Compared to surviving trees, lower growth of dying trees was detected at least one decade prior to mortality at seven of the eight sites. Intrinsic water-use efficiency (iWUE) increased over time in both dying and surviving trees, with a weaker increase in dying trees at five of the eight sites.
 - Carbon starvation was a strong correlate of conifer mortality based on a conceptual model incorporating growth, $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$. However, this approach does not capture processes that occur in the final months of survival. Ultimately carbon starvation may lead to increased mortality vulnerability, but hydraulic failure or biotic attack may dominate the process during the end-stages of mortality in these conifers.

Keywords: Tree mortality, tree growth, stable carbon and oxygen isotope, water-use efficiency, carbon starvation, western USA conifers.

Introduction

Climate-driven tree mortality from rising temperature, vapor pressure deficit (VPD), drought and insect outbreaks is occurring globally (Allen *et al.*, 2010; Hartmann *et al.*, 2018; McDowell *et al.*, 2018). This phenomenon is particularly severe for conifers across western USA, where the tree mortality rates increased significantly in the past four decades (van Mantgem *et al.*, 2009). The physiological mechanisms of climate-induced tree mortality are not well understood, but can include physiological changes promoting hydraulic failure, carbon starvation, and biotic attack (McDowell *et al.*, 2011; Das *et al.*, 2016; Choat *et al.*, 2018). Rising temperature and VPD coupled with soil drought forces lower growth and higher mortality in conifers (Williams *et al.*, 2013) due to increased water loss that can promote hydraulic failure (Choat *et al.*, 2018), and/or via greater stomatal closure to avoid water loss, which can promote carbon starvation (Adams *et al.*, 2017). Despite growing research interest, the physiological mechanisms of conifer mortality, particularly over multi-year periods, is still not clearly documented, which limits our ability to mechanistically predict tree mortality globally (McDowell *et al.*, 2016).

Trees are long-lived species and mortality mechanisms involve complex legacy effects (Manion, 1981; Anderegg *et al.*, 2013; Anderegg *et al.*, 2015). Tree radial growth, inferred from tree-ring width data, provides retrospective information about carbon allocation to stemwood through trees' entire lifespans (Camarero *et al.*, 2015; Cailleret *et al.*, 2017). Tree rings have been used extensively to investigate growth patterns during the years prior to mortality (Christiansen *et al.*, 1987; Waring, 1987; McDowell *et al.*, 2010; Cailleret *et al.*, 2017). Compiling from 2970 dying and 4224 surviving trees across 190 sites globally revealed 84% of the trees experienced a decrease in radial growth before death, with the duration of growth reductions varying from 1 year to 100 years (Cailleret *et al.*, 2017). Moreover, a general increase in inter-annual growth variability was detected during the last 20 years before tree mortality in global gymnosperms, suggesting reduced resilience to environmental extremes (Cailleret *et al.*, 2018).

As tree growth data alone are not adequate to inform on physiological mechanisms of tree mortality, stable isotope composition in tree rings can potentially provide additional retrospective insights into ecophysiological processes related to a tree's death (Gessler *et al.*, 2014; Gessler *et al.*,

2018). Annually-dateable tree-ring stable carbon isotope composition ($\delta^{13}\text{C}$) are directly related to CO_2 uptake and water balance, which generally reflects the trade-off of assimilation-weighted, crown-scale photosynthesis rate (A) and stomatal conductance (g_s). $\delta^{13}\text{C}$ yields estimates of carbon isotope discrimination (Δ), intercellular CO_2 concentration (C_i), and intrinsic water-use efficiency (iWUE) (Farquhar *et al.*, 1989; McCarroll & Loader, 2004; Seibt *et al.*, 2008). Thus, $\delta^{13}\text{C}$ of tree rings allows us to examine if trees that die experienced greater water stress (e.g. lower Δ) or shifts in iWUE prior to mortality. Tree-ring stable oxygen isotope composition ($\delta^{18}\text{O}$) is influenced by the $\delta^{18}\text{O}$ of precipitation, atmospheric humidity, and transpiration, as well as oxygen atom exchange processes at the leaf and xylem levels (Barbour, 2007; Gessler *et al.*, 2014). $\delta^{18}\text{O}$ of stem xylem is not related to A (Roden *et al.*, 2000) but shows a close relationship with g_s either via a joint influence of VPD or via transpirational dilution of evaporatively enriched leaf water (Gessler *et al.*, 2009) and, theoretically, can be applied to distinguish the effects of A and g_s on changes in Δ (Scheidegger *et al.*, 2000; Roden & Siegwolf, 2012). Multi-proxy tree ring records (growth, $\delta^{13}\text{C}$, $\delta^{18}\text{O}$) have been recently used to understand long-term trends in iWUE (Guerrieri *et al.*, 2019) and survival (Giguère-Croteau *et al.*, 2019), and several studies have used multi-proxy tree-ring records to assess tree mortality mechanisms of individual species or site (Voltas *et al.*, 2013; Hentschel *et al.*, 2014; Gaylord *et al.*, 2015; Colangelo *et al.*, 2017). A conceptual framework combining long-term growth and isotope data was developed to identify the predisposition to hydraulic failure (fast or even no growth decline with high A or high g_s) or to carbon starvation (slow or continuous growth decline with low A or low g_s , Gessler *et al.*, 2018). However, no studies, to our knowledge, have combined tree growth, iWUE and $\delta^{18}\text{O}$ to investigate conifer physiological predispositions to mortality across a wide range of species and climates.

We developed a tree-ring multiproxy dataset of growth, $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$ from eight undisturbed old-growth conifer forests in western USA. The eight sites spanned a broad range of climate conditions from the Pacific Northwest to Southwestern USA and all demonstrated increasing mortality trends over recent decades (van Mantgem *et al.*, 2009). At each site, we compared surviving and dying trees to investigate the trajectories of whole-crown, assimilation-weighted gas exchange (from tree-ring $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) and growth prior to mortality, with the goal of detecting the physiological predispositions associated with tree mortality. We compared the difference of dying and

surviving tree growth, $\delta^{18}\text{O}$ to identify the growth pattern and gas-exchange predispositions prior to tree mortality. We also assessed the resilience difference of dying and surviving tree growth, Δ and their response to growing season VPD. Using these data within the dual-isotope approach conceptual framework (Gessler *et al.*, 2018), we additionally investigated the evidence for contributions of carbon starvation and hydraulic failure to tree mortality. We tested two hypotheses regarding the physiological predispositions of tree mortality: (1) dying trees experienced consistent multi-year reductions in radial growth and increases in inter-annual variability compared to neighboring living congeners; and (2) $\delta^{18}\text{O}$ of dying trees would be greater than that of surviving trees, reflecting more extreme water stress in the dying trees.

Materials and Methods

Study region

All sampling was performed in western USA (Fig. S1). The study area includes two predominant climate systems, with the Pacific Northwest and California influenced by a Mediterranean climate, and the Southwest dominated by the North American monsoon. Annual precipitation exhibits a substantial gradient, ranging from 3988 mm in the Pacific Northwest to 461 mm in the Southwest during the period 1975 to 2009, while annually averaged VPD ranges from 0.38 kPa in the Pacific Northwest to 0.97 kPa in the Southwest. Though the climate types are different, temperature and drought have risen significantly in all of the western USA (Karl *et al.*, 2009). All selected sites have old-growth conifer forests that have experienced increasing rates of tree mortality thought to be driven by drought and insect attack in recent decades (van Mantgem *et al.*, 2009; Stephenson *et al.*, 2019).

Field sampling and growth.

The field sites were distributed in or near networks of long-term forest monitoring plots located in intact, old-growth forests, including seven sites from van Mantgem *et al.* (2009) plus an eight site near Los Alamos, New Mexico (Breshears *et al.*, 2009) (Table 1). Field sites were selected to cover a wide moisture gradient, from presumably temperature-limited sites in the temperate rainforests of the Pacific Northwest to presumably water-limited sites in the arid Southwest. We

sampled the dominant tree species at each site, including: Hoh rainforest, Olympic National Park, WA (*Picea sitchensis*), Wind River Canopy Crane Site, WA (*Pseudotsuga menziesii*), HJ Andrews Experimental Forest, OR (*Pseudotsuga menziesii*), Rocky Mountain National Park, CO (*Picea engelmannii*), Sequoia National Park, CA (*Pinus ponderosa*), Sequoia National Park, CA (*Abies concolor*), Fraser Experimental Forest, CO (*Pinus contorta*), Los Alamos, NM (*Pinus edulis*). To facilitate comparison, we used three or four letters abbreviation to represent sampling site and four letters from tree species as site code: HOH PISI, WIND PSME, HJA PSME, ROMO PIEN, SEKI PIPO, SEKI ABCO, FRAS PICO, and LANL PIED (Table 1). At each site, we selected between five and eight dead trees with the following characteristics: standing and having died recently, as indicated by the presence of foliage and fine twigs and branches; being canopy dominant or co-dominant; receiving abundant light on their southern aspect; and having no evidence that the mortality was due to mechanical causes (as indicated by broken stems and tops). For each sampled dead tree, we then chose the closest nearby live tree that met the same criteria as the dead individual. In total, we sampled 98 trees across all sites (Table S1). For each tree, two tree cores were collected with a 12 mm diameter increment borer, generally at 1.37 m in height, but occasionally above or below this height if necessary to avoid wounds or defects in the trunk.

Cores were air dried and sanded with successively finer sandpaper prior to further analysis. Cores were cross dated visually using narrow marker years from tree-ring chronologies. For the surviving trees, the outer ring date was known, being the collection year (2009). Each ring was precisely cross-dated to its year of formation, using standard dendrochronological methods (Stokes & Smiley, 1968). For the dead trees, we cross-dated it by comparing to the nearest living trees and assuming the year following the last tree-ring dated year was the year of tree death (Table S1). Surviving and dying trees had similar dated age but relatively low expressed population signal (EPS) at the site level (Table S1).

Raw basal area increment (BAI, $\text{cm}^2 \text{yr}^{-1}$) was calculated from tree ring width measurements starting from the earliest ring in each core (the first full year of growth data) through the last year, along with diameter at breast height (corrected for phloem and bark thickness). Ring widths were converted to BAI using tree-specific circumferential growth (inside bark) and assuming concentric circularity as follows (McDowell *et al.*, 2010):

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

where R is trunk radius, t is the year of tree growth.

Isotopic composition.

After the increment cores had been cross-dated, we used one tree core from each tree to split annual rings using a scalpel under a dissecting scope. Because the last year of dying trees was different at each site, the timespan at different sites was not equal, but all tree cores were split from 1975 to include at least 25 years from the earliest year of death in the dying trees at each site for $\delta^{13}\text{C}$. After reviewing the literature, we found that it was unnecessary to extract cellulose prior to measurement of $\delta^{13}\text{C}$ because there was a consistent offset (on average 1.24‰) between whole-wood and cellulose $\delta^{13}\text{C}$ (Figure 1a). Additionally, there was no detectable effect of decomposition upon $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ within our samples (Figure 1c, d; English *et al.*, 2011). We did not separate early from late-wood (Livingston & Spittlehouse, 1996; Leavitt *et al.*, 2002) because our objective was to examine the isotopes as a proxy for assimilation-weighted gas exchange characteristics at the crown-scale along a complete growing season that the isotopes represent. Most cross-ring contamination is associated with carbon movement from the sapwood into the heartwood (S. Leavitt, *personal communication*) and all of our isotopic sample rings were located within the sapwood as estimated using the clear color difference between sapwood and heartwood. On rare occasions when the boundary was indistinguishable, the cores were not utilized. Each ring was individually diced and ground to a fine powder in a ball mill.

To extract α -cellulose from wood for $\delta^{18}\text{O}$ analysis we used the standard Brendel method modified for small samples (Brendel *et al.*, 2000; Anchukaitis *et al.*, 2008). Briefly, 800 μg of ground wood tissue was placed in a 1.5 ml centrifuge tube with screw on cap and silica gasket. We added 120 μL 80% acetic acid and 12 μL 69% nitric to each tube. The tubes were tightly sealed and placed in an aluminum heating block at $120^\circ (\pm 3^\circ)$ for 30 minutes. The tubes were then removed, allowed to cool and rinsed, centrifuged and decanted using ethanol, deionized water ($>17.5 \text{ M}\Omega$), ethanol again and finally acetone. The tubes were opened and left to dry in an oven at 70° overnight and then placed in a vacuum desiccator until weighed out for isotopic analysis. The length of the tree-ring $\delta^{18}\text{O}$ time series is shorter than that for $\delta^{13}\text{C}$ because there was insufficient wood for α -cellulose extraction at some

sites, but we were able to ensure a consistent $\delta^{18}\text{O}$ data record from at least six years prior to mortality per site.

The powder collected from each ring was analyzed on a CHN elemental analyzer (Costech Analytical Technologies, CA) and a Thermal Combustion Elemental Analyzer (Thermo Electron Corp, Waltham, MA), each attached to a continuous flow isotope ratio mass spectrometer (MAT 253, Thermo Electron Corp, Waltham, MA). Reported values are in per mil (‰) relative to V-PDB for $\delta^{13}\text{C}$ analyses and V-SMOW for $\delta^{18}\text{O}$ analyses. The precision of our method based on means of laboratory standards (Aldrich σ -cellulose) for each analysis run was 0.07‰ for $\delta^{13}\text{C}$ and 0.16‰ for $\delta^{18}\text{O}$. An international standard (IAEA C3) run periodically yielded a mean value of -24.91 ± 0.05 ‰ for $\delta^{13}\text{C}$ and 32.1 ± 0.1 ‰ for $\delta^{18}\text{O}$ ($\pm$ values are 95% confidence intervals). In this effort, we analyzed 1627 $\delta^{13}\text{C}$ samples and 508 $\delta^{18}\text{O}$ samples respectively, though the EPS of tree-ring width and $\delta^{13}\text{C}$ series were mostly below 0.85.

Tree-ring $\delta^{13}\text{C}$ was converted to carbon isotope discrimination Δ (Farquhar & Sharkey, 1982):

$$\Delta = \frac{\delta^{13}\text{C}_a - \delta^{13}\text{C}_p}{1 + \delta^{13}\text{C}_p} \quad (2)$$

where $\delta^{13}\text{C}_a$ and $\delta^{13}\text{C}_p$ (in ‰) are the carbon isotope compositions of the ambient air and tree rings, respectively. The $\delta^{13}\text{C}_a$ values since 1975 were derived from the observed values at Mauna Loa observatory. We assumed temporal variation of $\delta^{13}\text{C}_a$ among the eight sites were the same, and we used the same $\delta^{13}\text{C}_a$ data for the eight different sites.

Δ can also be expressed as the ratio of intercellular (C_i) and atmosphere (C_a) CO_2 concentration when post-photosynthetic fractionation processes are assumed to be negligible (but see Gessler *et al.*, 2014):

$$\Delta = a + (b - a) \times \frac{C_i}{C_a} \quad (3)$$

where a is the fractionation associated with diffusion in air (4.4‰), b is the net fractionation associated with carboxylation by Rubisco (27‰). The atmospheric CO_2 concentration since 1975 was

derived from direct measurements at the Mauna Loa observatory
(<https://www.esrl.noaa.gov/gmd/ccgg/trends/data.html>)

The rates of carbon fixation (A) and stomatal conductance (g_s) are the primary factors determining carbon isotopic discrimination, and thus the iWUE is related to Δ according to (Farquhar *et al.*, 1982; Seibt *et al.*, 2008),

$$iWUE = \frac{A}{g_s} = \frac{C_a - C_i}{1.6} = \frac{C_a(1 - C_i / C_a)}{1.6} \quad (4)$$

Δ provides an estimation of crown-scale gas-exchange, because tree-rings integrate assimilation weighted iWUE of the entire canopy.

Climate data

Not all sites had available climate data so gridded data (4 km resolution) were used for each site over the period 1975 to 2009. For each location we used monthly gridded mean temperature and monthly total precipitation, monthly VPD (averaged from monthly maximum and minimum VPD) data from the PRISM Climate Group (Di Luzio *et al.*, 2008). For the analyses, we used annual average VPD through averaging the twelve months data.

Analyses.

To test for the difference between dying and living trees' growth across western USA, we calculated the annual growth ratio between dying and surviving trees ($BAI_{dying}/BAI_{surviving}$) at each site, then, we averaged all sites into a temporal series prior to tree mortality (Cailleret *et al.*, 2017). At each site, we analyzed the difference of dying and surviving tree growth, iWUE since 1975 and $\delta^{18}O$ over last six years through paired t -test. The interannual variability in growth and isotopes were assessed using the coefficient of variation (CV) before tree mortality. For each tree, we calculated the CV during 1-5th years, 6-10th years, 11-15th years, 16-20th years and 21-25th years before tree mortality, then averaged the CV from every individual dying and surviving trees. The CV is an index reflecting resilience in response to external stresses, *e.g.* drought (Zang *et al.*, 2014; Cailleret *et al.*, 2018). The difference of CV between dying and surviving trees for each five-year block was assessed using two-factor analysis of variance (ANOVA). Relationships of tree growth, Δ , iWUE, $\delta^{18}O$ and VPD were calculated from linear regression. Covariance analysis was applied to identify the heterogeneity of

slopes of iWUE vs $\delta^{18}\text{O}$, VPD vs $\delta^{18}\text{O}$, VPD vs iWUE between dying and surviving trees. We also used one-sample t test to detect the difference of zero of dying and surviving tree growth, $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$ in the conceptual framework. Statistical analyses were performed in SPSS 22.0 (IBM Corporation).

Results

We observed significantly lower BAI of dying trees compared to surviving trees across the western USA. BAI of dying trees in the year before mortality was only 56% of the BAI of surviving trees, and the duration of persistently lower radial growth was approximately three decades before tree mortality (Figure 2a). The dying trees sampled at seven of the eight sites had significantly ($p < 0.05$) lower radial growth than surviving trees since 1975 (Figure 3, Table S2). The single exception was *Pinus contorta* at Fraser experimental forest, Colorado, where BAI of dying trees exhibited significantly ($p < 0.05$) higher growth than surviving trees (Figure 3). At the remaining seven sites, the duration of lower radial growth prior to mortality exhibited large variability, ranging from 10 years to >100 years (Figure 3), however our results show no geographical pattern associated with the duration of lower radial growth across western USA.

There was a significant increasing trend of iWUE for both dying and surviving trees at all sites since 1975 (Figure 4). The increasing rate of iWUE at five of the eight sites was significantly smaller ($p < 0.05$) for dying trees compared to surviving trees. Averaged iWUE across all sites, the rate of iWUE increase in dying trees was significantly ($p < 0.001$) smaller than those of surviving trees, with $3.57 \mu\text{mol mol}^{-1}$ per decade in dying trees compared to $5.86 \mu\text{mol mol}^{-1}$ per decade in surviving trees (Fig. S2). Mean iWUE of dying trees was significantly greater ($p < 0.05$) than that of surviving trees at WIND PSME, HJA PSME, ROMO PIEN, while dying trees had lower iWUE values ($p < 0.05$) than surviving trees at HOH PISI, SEKI PIPO, LANL PIED (Table S2). There were no significant ($p > 0.05$) differences between dying and surviving iWUE for the two remaining sites (Table S2). However, iWUE of dying trees during the last two years prior to mortality was significantly lower than that of surviving trees on average (Figure 2b).

For tree-ring $\delta^{18}\text{O}$, there were no overall significant ($p > 0.05$) differences between dying and surviving trees observed prior to mortality (Figure 2c). Rings of dying trees had more positive $\delta^{18}\text{O}$ at SEKI ABCO than of surviving trees but dying trees had more negative $\delta^{18}\text{O}$ at WIND PSME and HJA

PSME (Figure 5, Table S2). However, there was no difference between dying and surviving $\delta^{18}\text{O}$ at the other five sites (HOH PISI, SEKI PIPO, ROMO PIEN, FRAS PICO, LANL PIED. Figure 5, Table S2).

Tree growth and stable isotopes exhibited different interannual variability between dying and surviving trees. We found dying trees had significantly greater CV of BAI than surviving trees over the last five years before tree mortality (Figure 6a). Specifically, the CV of BAI from dying trees increased significantly ($p < 0.05$) over the last five years relative to prior five-year time periods, whereas surviving trees' CV had changed little over time (Figure 6). For tree-ring Δ there was no CV differences at any five-year time periods between surviving and dying trees (Figure 6b). Regressions of normalized BAI vs. VPD at each site revealed that growth of dying trees at the five driest sites had significant negative relationships with evaporative water demand, in contrast to only two sites (one positive, one negative) for surviving trees (Fig. S3a). However, less clear relationships were observed in the dying and surviving Δ response to VPD (Fig. S3b).

In Figure 7a the long-term differences in growth and iWUE are plotted against each other, confirming that although over a 25-year period reduced growth was detected in dying compared to surviving trees at seven out of eight sites, significantly greater ($p < 0.05$) in dying iWUE were detected at three sites (HJA PSME, WIND PSME, ROMO PIEN), while significantly lower ($p < 0.05$) in dying iWUE in the HOH PISI, LANL PIED site. When only the last five years before death are considered, dying trees exhibited a lower iWUE at four sites (Figure 7b). Tree-ring $\delta^{18}\text{O}$, an additional proxy primarily constrained by stomata (Giguère-Croteau *et al.*, 2019; Guerrieri *et al.*, 2019), reveals that more positive $\delta^{18}\text{O}$ in dying trees at three of the four lower iWUE sites (HOH PISI, SEKI PIPO, LANL PIED), which indicates greater fractionation of leaf water by transpiration (Figure 7c; the “Scheidegger plot”).

Discussion

Our study of eight old-growth forests revealed results that were consistent across sites (e.g. growth declines, Figure 3) and divergent across sites (e.g. isotopic signatures related to gas exchange, Figure 4). This suggests that a common process exists, as indicated by the lower growth of dying trees, yet the mechanisms leading to such lower growth may be diverse across our study sites. None of these sites had severe disturbances except FRAS PICO and LANL PIED (both of which experienced an

insect outbreak), thus the increasing “background” mortality (e.g. mortality in the absence of physical disturbances) results potentially from drought and chronic atmospheric drivers such as rising VPD and CO₂ (McDowell *et al.*, 2020). Additionally, at some sites, particularly our wetter sites, declining growth of dying trees may have been associated with increasing competition and (or) ongoing attack by root pathogens or other biotic agents (Cherubini *et al.*, 2002). Using the data within the conceptual model (Gessler *et al.*, 2018) suggests the process of carbon starvation was relatively common across sites (Figure 7). We review the assumptions of these interpretations, and present alternative interpretations, through the discussion section.

The observation that dying trees have lower radial growth than their surviving congeners across western USA (Figure 3) is consistent with prior observations (Voltas *et al.*, 2013; Williams *et al.*, 2013; Camarero *et al.*, 2015; Cailleret *et al.*, 2017). Tree growth decline prior to mortality is expected, as dying trees (particularly under drought and a warming climate) are likely to have low photosynthetic rates due to stomatal closure (McDowell *et al.*, 2016) or leaf loss (Poyatos *et al.*, 2013) and less investment into radial growth (McDowell *et al.*, 2010; Gessler *et al.*, 2014). Low radial growth of dying trees may also be in part caused by relative reduction in allocation to above-ground growth (Mackay *et al.*, 2020). Reduction of radial growth is symptomatic of carbon starvation, though it does not provide direct evidence of this physiological mechanism. Such direct evidence requires measurement of particularly low carbohydrate content in tree tissues prior to death (Dickman *et al.*, 2015). Likewise, long periods of stomatal closure are also associated with prolonged cuticular loss of water and can lead to embolism of the vascular system and complete desiccation of the plant (Martin-StPaul *et al.*, 2017; Cochard, 2019), potentially co-occurring with carbon limitations. While some form of carbon limitation led to lower growth at seven of the eight sites, at FRAS PICO, in contrast, faster growing trees were attacked by mountain pine beetles (*Dendroctonus ponderosae*) that preferentially select larger hosts with thicker phloem (Raffa *et al.*, 2008). Therefore, mountain pine beetles were the proximal or final cause of mortality at this site (Boone *et al.*, 2011). While this occurred only in *Pinus contorta* in our study, this species is particularly widespread in North America (Coops & Waring, 2011), thus such “overwhelming” of apparently healthy trees by mass beetle attack remains an important mechanism at the regional scale. Although dying trees have lower radial growth than their surviving at seven of the eight sites, it is noticeable at two sites (WIND PSME and LANL

PIED) that dying trees experienced a slight growth increase prior to mortality. This phenomenon has been reported in other studies (Rowland *et al.*, 2015; Cailleret *et al.*, 2017), and indicates that hydraulic failure or insect outbreak, rather than carbon starvation, may drive the final mortality of trees. Ultimately, low radial growth of dying trees may be associated with both carbon starvation and hydraulic failure, as both mechanisms may be co-occurring in trees that die (Adams *et al.*, 2017).

Comparing iWUE of dying and surviving trees potentially gives insight into past dynamics in A and g_s (Farquhar & Sharkey, 1982; Gessler *et al.*, 2014). In the current study, iWUE at six of the eight sites revealed differences between dying and surviving trees predominately in the last decade (Figure 4). One possible explanation is that dying trees have less capacity to adjust stomatal conductance (Martin-StPaul *et al.*, 2017; Zadworny *et al.*, 2019), or that they have larger cuticular conductance (Cochard, 2019). For the wettest site HOH PISI, there is lower iWUE of dying trees than that of surviving trees (Figure 4), which in a first interpretation would imply lower A of dying trees but could also be related to higher g_s (Farquhar *et al.*, 1989; Cernusak *et al.*, 2013; Poyatos *et al.*, 2013). Lower A of the dying trees may be associated with nutrient or light limitations (Craine *et al.*, 2012; Gessler *et al.*, 2017). Concerning g_s , it has been observed that stomatal conductance scales inversely with leaf area i.e., that trees with larger crowns have lower g_s and vice versa (Mencuccini & Comstock, 1999; Levanic *et al.*, 2011) and thus higher g_s might indicate significant foliage loss in the years before mortality as often observed with conifers (Dobbertin & Brang, 2001; Poyatos *et al.*, 2013).

The Scheidegger and Gessler approaches to partitioning carbon starvation and hydraulic failure (Scheidegger *et al.*, 2000; Gessler *et al.*, 2018) provide initial insight into mortality mechanisms (Figure 7). As reduced growth of dying trees might be associated with lower whole-tree carbon assimilation or more investment into the root system, no differences in mean iWUE for three sites either indicates that the ratio of A to g_s may have decreased at a similar rate prior to death or that there was in fact no change in A and g_s but that leaf area of the dying trees decreased (Cernusak *et al.*, 2013; Poyatos *et al.*, 2013). Considering only the last five years (Figure 7b), dying trees at four sites showed lower iWUE and three of those sites experienced $\delta^{18}\text{O}$ enrichment (Figure 7c), which indicating reduced A rather than increased g_s may be responsible for the lower iWUE (Gessler *et al.*, 2018). Constrained carbon uptake of those dying trees in HOH PISI, SEKI PIPO, LANL PIED

suggests these sites most likely suffered from the carbon starvation process in the years before mortality (Gessler *et al.*, 2018). These results confirmed that dying trees at three of the eight sites across western USA could be explained by the synchronic comparison tree growth and dual-isotope conceptual model, and the framework may facilitate to understand long-term tree mortality mechanisms.

Though the Scheidegger/Gessler approach provides initial insights into long-term physiological mechanisms, we acknowledge that there are at least four assumptions associated with Figure 7 that are possibly invalid, and thus lead to alternative interpretations of the dominance of carbon starvation and hydraulic failure upon the death process. Firstly, loss of leaf area such as due to defoliating pathogens or insects can influence the gas exchange (Whitehead, 1998) and hence interpretations from the isotopic results. Additionally, some tree species actively reduce their leaf area by leaf shedding and in the long term by reduced shoot growth (especially for conifers). Defoliation is likely an important factor for SEKI ABCO, where dying trees showed long-term growth reductions but no long-term change in $iWUE$, thus long-term reductions in leaf area with no noticeable effect on leaf level A/g_s would promote carbon starvation if the net, whole-tree photosynthesis is reduced below the carbon demand for survival.

Secondly, the challenge lies in the interpretation of the $\delta^{18}O$ data, which is assumed within the approach to be representative of g_s (Scheidegger *et al.*, 2000; Guerrieri *et al.*, 2019). Differences in source water uptake can confound this interpretation. The $\delta^{18}O$ result from SEKI ABCO was indeed more positive in the dying trees than the surviving trees, but with slight higher $iWUE$, which could thus be due to dying trees using shallower source water (Figure 7c). If dying trees fail to absorb water from deeper layers then a reduction in hydraulic conductance will manifest in reduced g_s and hence higher risk of both hydraulic failure and carbon starvation (Mackay *et al.*, 2020).

Thirdly, by mechanistic resolution we mean the ability of tree-rings to record both carbon starvation and hydraulic failure is not equivalent i.e. the symptoms of carbon starvation (lower growth, shifts in $iWUE$) are recorded within tree-rings while the symptoms of hydraulic failure (xylem embolism, tissue dehydration) are not captured in current research. Thus, interpretation of Figure 7 for hydraulic failure relies in part on the absence of symptoms for carbon starvation (e.g. growth

reduction). But growth reduction can co-occur with hydraulic failure, thus interpretation of the Scheidegger/Gessler approaches as evidence of carbon starvation does not exclude the process of hydraulic failure from occurring simultaneously. The same issue arises when we consider the temporal resolution of tree-ring data. Carbon starvation may be a dominant process over the long-term (inter-annual) record, but hydraulic failure may dominate the death process during the last months of life. In this situation, no evidence of hydraulic failure would emerge from the Scheidegger/Gessler approach even though it could be the dominant process during the final stages of death.

The last challenge to the Scheidegger/Gessler approach is that it excludes the potential for neither carbon starvation nor hydraulic failure to be the dominant death process. At FRAS PICO, for example, the overwhelming tree mortality by mass attack of aggressive outbreaking bark beetles was almost certainly much more important than either carbon starvation or hydraulic failure (Raffa *et al.*, 2008), as *Dendroctonus* beetles during outbreaks often attack the most vigorous trees (Boone *et al.*, 2011; de la Mata *et al.*, 2017; Cooper *et al.*, 2018). Hydraulic failure post-attack may occur, but would not be captured by our tree ring measurements because growth is unlikely during the late stages of attack and death. Insect attack is common during the death process in conifers of western USA (Raffa *et al.*, 2008), and it is established that biotic agents, carbon starvation, and hydraulic failure can interact and feedback upon each other (Manion, 1981; McDowell *et al.*, 2008; Gaylord *et al.*, 2013), such that attack is more likely on trees weakened by carbon starvation and hydraulic failure, and likewise, after the pathogens have damaged the carbon and water supply and transport processes in the tree, the trees subsequently dies of rampant carbon starvation or hydraulic failure. This interactive process is not captured by the Gessler/Scheidegger approaches. Overall, multiple assumptions and uncertainties should be considered (Roden & Siegwolf, 2012) when using the conceptual model to understand physiological mechanisms of tree mortality. The low consistency of tree growth and $\delta^{13}\text{C}$ series among trees per site further confound the conceptual model results in our study, therefore large sample size would be suggested to assess physiological processes prior to tree mortality.

While direct or indirect effects of carbon starvation were implicated from the conceptual model to be important processes during death of these old-growth conifers in western USA, direct measurements of the degree of embolism and of non-structural carbohydrates are needed on dying and surviving trees to verify the dominant physiological mechanisms (McDowell & Sevanto, 2010;

Sevanto *et al.*, 2014). The apparent dominance of carbon starvation mechanisms observed from the conceptual approach in our study is consistent with other results from gymnosperms (Adams *et al.*, 2017). As noted above, our study does not demonstrate a lack of hydraulic failure: our tree-ring measurements capture the long-term processes underlying mortality but do not capture short term processes that occur in the last months of life, during which time hydraulic failure may dominate. Thus, while these results suggest carbon starvation matters to the death of most western old-growth conifers, hydraulic failure may still play an important role.

Compared to surviving tree growth, dying trees' BAI were more sensitive to VPD (Fig. S3) suggesting that temperature-induced drought stress may have been a significant contributor to tree mortality (Adams *et al.*, 2009; Allen *et al.*, 2010; Williams *et al.*, 2013). Increased VPD decreases conifer growth (Restaino *et al.*, 2016), and is likely to promote further mortality of conifers in the future (Allen *et al.*, 2015; McDowell *et al.*, 2016). However, no differences in the tree-ring CV of carbon isotope discrimination between surviving and dying trees indicated that all trees may have similar year-to-year variation in Δ and cope with similar environmental stress. Trees that survive may have other strategies (*e.g.* varying depth of source water uptake, higher competitiveness for water, light and nutrient acquisition) to overcome the adverse effects of water deficiency (Barbeta & Penuelas, 2017; McDowell *et al.*, 2019).

Conclusions

Our retrospective dendroecological study revealed that dying conifer trees usually suffered substantially lower radial growth than surviving trees on a subcontinental scale and across diverse climate conditions. These lower radial growths were related to both stomatal and photosynthetic responses, reflected in the iWUE and $\delta^{18}\text{O}$ variation of dying and surviving trees. In addition, we also found dying trees had lower ability to buffer external stress as shown by higher inter-annual CV during the last five years before tree mortality and greater sensitivity to VPD. From the conceptual model based on tree-ring proxies, direct or indirect effects (such as insect or pathogen attack) of carbon starvation were likely important contributors to tree mortality across western USA. However, indirect effects through insects and pathogens attack cannot be discerned from iWUE and $\delta^{18}\text{O}$. At

FRAS, we saw a dramatic example of this, where, in contrast to all other sites, dying trees were growing faster than living trees, almost certainly a consequence of host-tree selection by outbreaks of bark beetles. Our results demonstrate that multi-proxy tree-ring records can yield important insights into the predisposition and physiological mechanisms of conifer mortality; however, the potential for alternative interpretations, such as strong co-occurrence of carbon starvation and hydraulic failure, remains plausible. We strongly recommend that future research should incorporate these methods with explicit identification of biotic mortality factors and direct observations of carbohydrates and hydraulic failure.

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Authors' contributions

NGM, AJD, NLS, and CDA conceived the ideas and designed this project; AJD and NLS conducted the fieldwork; CHB cross-dated and measured all of the increment cores for this study; NBE performed the sample preparation and stable isotope analysis; WW, CG, AG, NGM analysed the data; WW and NGM led the writing of the manuscript; all authors contributed critically to the drafts and gave final approval for publication.

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Supporting information:

Fig. S1. Sampling locations across western USA.

Fig. S2. Dying and surviving tree's intrinsic water-use efficiency (iWUE) variation over time.

Fig. S3. Tree growth and Δ response to growing season VPD.

Table S1. Information about sampling trees and characteristics of the cross-dating information.

Table S2. Paired t test results of dying and surviving tree growth (BAI), iWUE, and $\delta^{18}\text{O}$ series since 1975 across eight sites in western USA.

Table 1. Tree species, geographical and climate information at the eight sampling sites across western USA.

Site code	Species	Latitude (N)	Longitude (W)	Altitude (m)	Temperature (°C)	Precipitation (mm)
HOH PISI	<i>Picea sitchensis</i>	47°46'	123°58'	710.5	8.3	3988.4
WIND PSME	<i>Pseudotsuga menziesii</i>	45°48'	121°57'	641.9	8.6	2533.7
HJA PSME	<i>Pseudotsuga menziesii</i>	44°14'	122°11'	732.7	9.1	2139.0
ROMO PIEN	<i>Picea engelmannii</i>	40°18'	105°37'	3025.4	0.9	1045.7
SEKI PIPO	<i>Pinus ponderosa</i>	36°33'	118°45'	1966.3	11.3	944.9
SEKI ABCO	<i>Abies concolor</i>	36°33'	118°45'	1966.6	11.3	944.9
FRAS PICO	<i>Pinus contorta</i>	39°53'	105°54'	2944.4	1.5	625.0
LANL PIED	<i>Pinus edulis</i>	35°51'	106°17'	2161.0	9.9	461.2

Annual mean temperature and total precipitation were averaged during the period 1975-2009.

Figures

Figure 1. Sampling and processing impacts on isotopic results. Regressions of (a) *a*-cellulose and whole wood $\delta^{13}\text{C}$ from multiple published studies (Macfarlane *et al.*, 1999; Loader *et al.*, 2003; Verheyden *et al.*, 2005; Harlow *et al.*, 2006; Ares *et al.*, 2008; Eglin *et al.*, 2008; Taylor *et al.*, 2008; English *et al.*, 2011; Szymczak *et al.*, 2011; Schleser *et al.*, 2015; Verlinden *et al.*, 2015; Weigt *et al.*, 2015; Guerrieri *et al.*, 2017), (b) *a*-cellulose and whole wood $\delta^{18}\text{O}$ from four previous studies (English *et al.*, 2011; Szymczak *et al.*, 2011; Weigt *et al.*, 2015; Guerrieri *et al.*, 2017), (c) live and dead whole wood $\delta^{13}\text{C}$ (English *et al.*, 2011), and (d) live and dead *a*-cellulose $\delta^{18}\text{O}$ from our study species and sites (English *et al.*, 2011). All panels include data from English *et al.* (2011), in which the samples originated from the same trees as those in the current manuscript.

Figure 2. Temporals of dying and surviving trees' growth, iWUE and $\delta^{18}\text{O}$ across western USA. (a) Growth ratio (basal area increment (BAI), $\text{BAI}_{\text{dying}}/\text{BAI}_{\text{surviving}}$) of dying and surviving trees from eight conifer sites during the last 100 years before tree mortality; (b) the difference between dying and surviving trees' intrinsic water-use efficiency (iWUE) from eight conifer sites during the last 32 years before tree mortality; (c) difference of dying and surviving tree-ring $\delta^{18}\text{O}$ from the eight conifer sites during the last seven years prior to tree mortality. Error bars in each panel are $\pm 1\text{SE}$ among sites. Dashed lines at each panel represent no difference of dying and surviving trees.

Figure 3. Long-term site-level mean basal area increment (BAI) variation for dying and surviving trees at the eight sites across western USA. The sites are organized from wettest to driest (see supporting information Fig. S1). No error bars are shown to improve clarity.

Figure 4. Intrinsic water-use efficiency (iWUE) of dying and surviving trees at the eight sites across western USA (see supporting information Fig. S1). The slopes $\pm \text{SE}$ (black text is surviving

trees; red text is dying trees) and R^2 of each individual series are shown. Error bars at each panel are ± 1 SE among trees.

Figure 5. Tree-ring $\delta^{18}\text{O}$ variations of dying and surviving trees at the eight sites across western USA. The sites are organized from wettest to driest (see supporting information Fig. S1). Error bars in each panel are ± 1 SE among trees.

Figure 6. Tree growth and Δ variability before mortality across the western USA. Coefficient of variation for all individual (a) basal area increment (BAI), (b) Δ at each 5-yrs block before tree mortality. Error bar represent ± 1 SE. *: $p < 0.05$, **: $p < 0.01$.

Figure 7. Ratios of growth and isotopic composition between dying and surviving trees on different time scales. (a) and (b) shows the growth and intrinsic water-use efficiency (iWUE) ratios in four quadrants indicating a history of reduced growth and increased iWUE (upper left), reduced growth and decreased iWUE (lower left), increased growth and increased iWUE (upper right), and increased growth and decreased iWUE (lower right) of dying trees. (a) comprises long-term information of the last 25 years before mortality (excluding the last five years before mortality) and (b) includes only the last five years before mortality. In (c) ratios in iWUE and $\delta^{18}\text{O}$ for the last five years. Error bars are SE of time period. The relative dominance of carbon starvation (CS) and hydraulic failure (HF) are labeled within the figures according to the concept framework of Gessler et al. (2018). Site locations see supporting information Fig. S1.

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