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The effects of α-cellulose extraction and blue-stain fungus on retrospective studies of carbon and oxygen isotope variation in live and dead trees†

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Tree-ring carbon and oxygen isotope ratios from live and recently dead trees may reveal important mechanisms of tree mortality. However, wood decay in dead trees may alter the $\delta^{13}C$ and $\delta^{18}O$ values of whole wood obscuring the isotopic signal associated with factors leading up to and including physiological death. We examined whole sapwood and α-cellulose from live and dead specimens of ponderosa pine (*Pinus ponderosa*), one-seed juniper (*Juniperous monosperma*), piñon pine (*Pinus edulis*) and white fir (*Abies concolor*), including those with fungal growth and beetle frass in the wood, to determine if α-cellulose extraction is necessary for the accurate interpretation of isotopic compositions in the dead trees. We found that the offset between the $\delta^{13}C$ or $\delta^{18}O$ values of α-cellulose and whole wood was the same for both live and dead trees across a large range of inter-annual and regional climate differences. The method of α-cellulose extraction, whether Leavitt-Danzer or Standard Brendel modified for small samples, imparts significant differences in the $\delta^{13}C$ (up to 0.4‰) and $\delta^{18}O$ (up to 1.2‰) of α-cellulose, as reported by other studies. There was no effect of beetle frass or blue-stain fungus (*Ophiostoma*) on the $\delta^{13}C$ and $\delta^{18}O$ of whole wood or α-cellulose. The relationships between whole wood and α-cellulose $\delta^{13}C$ for ponderosa, piñon and juniper yielded slopes of ~1, while the relationship between $\delta^{18}O$ of whole wood and α-cellulose was less clear. We conclude that there are few analytical or sampling obstacles to retrospective studies of isotopic patterns of tree mortality in forests of the western United States. Published in 2011 by John Wiley & Sons, Ltd.

Tree mortality associated with climate warming and drying threatens to upset ecosystems and economies that rely on forests.[1,2] Carbon starvation, runaway embolism, overwhelming beetle infestation and their complex interactions have been ascribed to particular mortality events, but the mechanisms driving widespread increases in vegetation mortality remain unclear.[3] The isotopic compositions of carbon and oxygen ($\delta^{13}C$ and $\delta^{18}O$, respectively) in tree-rings are used frequently to reconstruct past climates,[4] to examine the fall of civilizations,[5] and recently to examine the relationship between climate and forest mortality.[6,7] While tree-ring isotope studies of mortality have proven valuable, some methodological issues associated with using trees that died many years prior to sampling remain unsolved. In this study, we examine the $\delta^{13}C$ and $\delta^{18}O$ of tree-rings collected from the sapwood of both living and dead trees, the effects of α-cellulose extraction on each, and the impact of frass (the powdered waste product deposited by wood-boring beetles) and fungal colonization on isotopic redistribution in rings of dead trees.

α-Cellulose is the main component in the cell walls of tree-ring wood. It is robust, non-labile and resists post-formational oxygen exchange with environmental water.[8] For this reason, α-cellulose is considered a reliable recorder of sub-annual and annual variations in tree-ring $\delta^{13}C$ and $\delta^{18}O$ studies.[4,9] Common methods of α-cellulose extraction from tree-rings are based on techniques described or modified from Brendel *et al.*[10] modified for small samples[11,12] or Leavitt and Danzer[13] (with an additional NaOH step, Steven Leavitt, personal communication). There is now general agreement that these methods yield similar results, although they require vastly different materials and allocations of laboratory resources.[14] Some studies have shown that the extraction of α-cellulose from the whole wood component of live sapwood is unnecessary to obtain accurate annual records of carbon and oxygen isotopic variation.[15–17]

After tree death, the isotopically distinct components of whole wood undergo decay at different rates.[18,19] For example, Schleser *et al.*[19] found that wood kept in conditions that accelerated decay underwent rapid degradation of cellulose compared with lignin with a consequent 1 ‰ decrease in the $\delta^{13}C$ ratio of the remaining bulk carbon in just 600 days. Biotic mortality agents, particularly insects and fungal pathogens, further complicate efforts to recreate isotopic compositions laid down during growth.[3] The dominant mortality agent in the northern hemisphere are bark beetles, many of which inoculate the tree stems they infest with fungi carried from other infected trees.[20,21] Tree-killing beetles, beetles that

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infest wood after death, and colonizing fungi may obscure inter-annual variations in $\delta^{13}C$ and $\delta^{18}O$ by liberating carbon and oxygen from cell walls and transporting these elements, and their associated isotope ratios, across rings.

In comparison with whole wood, α-cellulose is expected to be less affected by post-death contamination.[13] Here, we examine if it is necessary to extract cellulose from tree-rings of live and dead individuals to accurately determine inter-annual $\delta^{13}C$ and $\delta^{18}O$, and, if so, which extraction methods yield stable and consistent results. First, we evaluated whether or not using the Standard Brendel or Leavitt-Danzer method results in significant differences in the $\delta^{13}C$ and $\delta^{18}O$ of α-cellulose extracted from whole wood of living and dead ponderosa (*Pinus ponderosa*), piñon pine (*Pinus edulis*) and one-seed juniper (*Juniperous monosperma*). Second, we examined if the $\delta^{13}C$ and $\delta^{18}O$ offset between whole wood and α-cellulose in living and dead trees is consistent. Third, we determined if there is a consistent effect of α-cellulose extraction on $\delta^{13}C$ and $\delta^{18}O$ from tree-rings grown in wet, average or dry years sampled from living and dead piñon, juniper and white fir (*Abies concolor*). Last, we determined if beetle frass or blue-stain fungus (*Ophiostomas*) in juniper and piñon wood, respectively, alters the $\delta^{13}C$ and $\delta^{18}O$ in tree-rings. If the offset between whole wood and α-cellulose is consistent across all of these tests, it would then negate the need for costly and laborious α-cellulose extraction from tree-rings of these species when determining inter-annual $\delta^{13}C$ and $\delta^{18}O$.

EXPERIMENTAL

Comparisons of whole wood versus α-cellulose

We sampled whole sapwood, including multiple years, from one living and one dead ponderosa pine, one live and one dead piñon pine, and one live and one dead juniper to examine the effects of cellulose extraction, tree status (live or dead) and extraction method on $\delta^{13}C$ and $\delta^{18}O$ (Table 1). Cross-sections of tree trunks (i.e. cookies) were cut from living and dead piñon and juniper individuals in Technical Area 49 (TA-49) at the Los Alamos National Laboratory (N35.818° N, 106.305° W, 2175 m). Samples of ponderosa were acquired using an increment borer from a live tree in Los Alamos (35.888°N, 106.270° W, 2158 m) and a cookie taken from a dead tree in Bandelier National Monument (35.779° N, 106.270° W, 1859 m). Dead trees in this study died during a prolonged drought between 2000 through 2004 and were collected between 2007 and 2010. Except for the live ponderosa pine, we used a 2.5 cm wood borer to obtain samples of wood from cross-sections of the live and dead trees. All tree cores and cookies were dated and cross-dated at the University of Arizona Tree-Ring Laboratory in Tucson, Arizona. The bored wood-debris was ground and homogenized to a fine powder by passing it twice through a cyclone mill (18 mesh, 1 mm; Udy Corporation, Fort Collins, CO, USA). Wood powder from each tree was subsampled 10 times each to be run as whole wood, the α-cellulose extracted using the Leavitt-Danzer method (LD), and the α-cellulose extracted using the Standard Brendel (SB) method (see below for further detail).

We also sampled frass left behind by wood-boring beetles in juniper to see if contamination of rings by this material would affect the isotopic results. While we do not believe that the beetles killed this particular juniper, we were curious to determine if there are differences in material type (whole wood vs. frass) that could result in altered isotopic ratios when processed to α-cellulose. No attempt was made to sample from consistent years between samples because it is impossible to associate the frass with specific years; therefore, only comparisons between differences in whole wood/frass and α-cellulose of each sample can be made. Throughout this study, only sapwood was sampled, and these results cannot be extrapolated to heartwood.

Cellulose extraction using Leavitt-Danzer (LD)

To extract α-cellulose using LD, we followed the method detailed in Leavitt and Danzer.[13] We placed ~3 mg of material in sealed mesh pouches (ANKOM, Macedon, NY, USA) and inserted these into a Soxhlet extraction device plumbed

Table 1. Carbon and oxygen isotope values (‰) of standards, whole wood and α-cellulose extracted using Standard Brendel and Leavitt-Danzer methods

	Whole wood		α-cellulose (Standard Brendel)*		α-cellulose (Leavitt-Danzer)[13]	
	$\delta^{13}C_{WW}$	$\delta^{18}C_{WW}$	$\delta^{13}C_{SB}$	$\delta^{18}C_{SB}$	$\delta^{13}C_{LD}$	$\delta^{18}C_{LD}$
IAEA-C3 cellulose	−24.91±*0.05*	32.1±*0.10*	- -	32.1±*0.11*	- -	- -
Pinus ponderosa						
Live	−22.83±*0.11*	28.6±*0.35*	−21.48±*0.09*	34.1±*0.31*	−21.34±*0.22*	33.23±*0.12*
Dead	−24.51±*0.03*	28.65±*0.18*	−23.54±*0.25*	34.01±*0.59*	−23.28±*0.18*	33.08±*0.92*
Pinus edulis						
Live	−22.54±*0.03*	27.87±*0.15*	−21.16±*0.04*	33.87±*0.43*	−21.09±*0.06*	32.69±*0.21*
Dead	−22.86±*0.06*	27.88±*0.22*	−21.61±*0.03*	33.11±*0.51*	−21.43±*0.12*	32.58±*0.13*
Juniperus monosperma						
Live	−22.48±*0.03*	25.32±*0.10*	−20.53±*0.05*	32.4±*0.26*	−20.35±*0.12*	31.04±*0.13*
Dead	−21.02±*0.02*	26.54±*0.06*	−19.07±*0.03*	34.24±*0.27*	−18.67±*0.22*	33.04±*0.28*
Beetle frass	−21.68 ±*0.17*	24.32±*0.23*	−19.91±*0.15*	32.10 ±*0.53*	-	-

Uncertainties are 95% (α = 0.05) confidence intervals. For most treatments, $n = 10$, all treatments $n \geq 7$.
*Modified for small samples.[10–12]

with cool water circulated from a constant temperature bath and filled with toluene and 100% ethanol. We heated and circulated the mixture through the samples overnight. We then rinsed the samples with boiling, circulating 100% ethanol overnight, and subsequently removed and rinsed them three times with deionized water (>17.5 MΩ) and, after drying, boiled them for 6 h in deionized water. We then bleached the samples in a dilute solution of sodium chlorite and glacial acetic acid at 70 °C overnight and rinsed them with deionized water until the rinse-water conductivity was less than 5 mΩ cm^{-1} as measured by a handheld conductivity meter. We then soaked the samples in a solution of 17% sodium hydroxide for 1 h. Finally, we rinsed and dried the samples in an oven at 70 °C overnight and then stored them in a vacuum desiccator until they were analyzed.

Cellulose extraction using Standard-Brendel (SB)

To extract α-cellulose from wood using SB, we followed the methods detailed in Brendel *et al*.[10] modified for small samples.[11,22] We placed 800 μg of ground wood tissue in a 1.5 mL centrifuge tube with a screw-on cap and a silicone gasket. We added 120 μL of 80% acetic acid and 12 μL of 69% nitric acid to each tube. We tightly sealed the tubes and placed them in an aluminum heating block at 120 °C (±3 °C) for 30 min. After removing the tubes and allowing them to cool, we rinsed, centrifuged and decanted them using ethanol, deionized water (>17.5 MΩ), ethanol again and finally acetone. We opened the tubes and let them dry in an oven at 70 °C for 30 min and then placed them in a vacuum desiccator until weighed for isotopic analysis.

Whole wood vs. α-cellulose comparisons in wet, average and dry years

We sampled individual rings in crossdated (white fir, piñon) and undated (juniper) cores and cookies of live and dead trees to examine if wet and dry years have differential effects on isotope values when $\delta^{13}C$ and $\delta^{18}O$ are measured in whole wood and α-cellulose. We crossdated the piñon and fir using standard dendrochronological procedures,[22] while juniper was not dated because of its frequent missing and false rings (Chris Baisan, personal communication). We selected and cut from each fir and piñon core a whole ring associated with wet, average and dry years (Table 2) as measured by long-term meteorological stations located at Los Alamos National Laboratory (TA-54, 35.847° N, 106.268° W, 2121 m; piñon/juniper forest) and on nearby Pajarito Mountain (35.886° N, 106.389° W, 3076 m; alpine spruce/fir forest). In the juniper, we selected wide rings to represent wet periods, narrow rings to represent dry periods and intermediate-width rings to represent periods with average precipitation. We ground selected rings in a ball mill (shredded fragments no longer than 1 mm). We used SB to extract α-cellulose from a subsample of each ring.

Blue-stain fungus

We measured $\delta^{13}C$ and $\delta^{18}O$ in both whole wood and α-cellulose (using SB) from circumferential transects within a ring and radial transects across rings in a cross-section taken from a dead piñon at Los Alamos National Laboratory (TA-49) that had been killed in 2000–2001 by beetles carrying the blue-stain fungus (Fig. 4). We used a 3.175 mm drill bit to remove samples along and between rings in two parts of the cookie where blue-stain fungus was present. We drilled slowly so that no combustion occurred. Each circumferential

Table 2. Carbon and oxygen isotope values (‰) of whole wood (WW) and α-cellulose extracted via Standard Brendel (SB) in annual tree-rings

Sample	$\delta^{13}C_{WW}$	$\delta^{13}C_{SB}$	$\delta^{18}C_{WW}$	$\delta^{18}C_{SB}$
Wet Year				
Abies Concolor (2000†, 592 mm††)				
Live 1	−25.76	−25.81	24.81	28.88
Live 4	-	−24.80	20.28	23.99
Live 8	−25.54	−24.30	23.71	27.40
Dead 1	−25.87	−24.62	22.35	27.97
Dead 4	−25.47	−24.48	21.04	24.80
Dead 8	−26.13	−25.53	20.77	27.34
Pinus edulis (1998†, 501 mm†)				
TA51 Live 3	−21.72	−21.03	27.08	32.11
TA51 Live 4	−23.87	−22.01	27.24	-
TA51 Live 6	−23.45	−22.28	27.31	-
TA49 Dead 11	−22.12	−22.73	26.51	27.07
*Juniperus monosperma**				
TA71 Live 4	−24.70	−24.01	25.65	28.04
TA71 Dead 6	−23.89	−22.95	23.72	28.38
Average Year				
Abies Concolor (2003†, 414 mm†)				
Live 1	−26.12	−24.67	24.76	28.96
Live 4	−26.54	−25.32	21.1	23.47
Live 8	−26.01	−25.08	24.95	28.65
Dead 1	−25.96	−24.86	23.83	28.68
Dead 4	−25.75	−24.77	22.21	23.98
Dead 8	−26.38	−24.83	24.02	29.35
Pinus edulis (2003†, 336 mm†)				
TA51 Live 3	−21.23	−20.26	28.93	34.32
TA51 Live 4	−22.74	−21.50	27.60	
TA51 Live 6	−22.90	−21.50	26.63	30.81
TA49 Dead 11	−22.56	−22.14	29.02	32.02
*Juniperus monosperma**				
TA71 Live 4	−24.06	−24.00	23.53	25.25
TA71 Dead 6	−23.21	−22.71	24.10	27.00
Dry Year				
Abies Concolor (2001†, 215 mm†)				
Live 1	-	−26.46	23.75	26.54
Live 4	−26.12	−24.31	21.97	26.46
Live 8	−25.56	−24.51	23.10	26.81
Dead 1	−25.74	−24.17	22.29	27.40
Dead 4	−25.07	−24.01	21.68	26.20
Dead 8	−25.71	−24.31	21.70	26.48
Pinus edulis (2001†, 171 mm†)				
TA51 Live 3	−21.86	−20.73	26.89	31.97
TA51 Live 4	−25.65	−22.41	28.20	-
TA51 Live 6	−23.00	−21.65	27.28	-
TA49 Dead 11	−23.94	−23.36	26.36	31.46
*Juniperus monosperma**				
TA71 L4	−23.49	−23.19	26.93	27.61
TA71 D6	−23.42	−22.75	-	27.36

*Samples of *J. monosperma* are poorly dated, so we used ring width as a proxy for annual rainfall (e.g. wide ring = wet year).
†Year of the ring sampled.
††Precipitation and snowfall (water equivalent) amount in respective water year.

transect was drilled at ~3 mm intervals, one along the 1987 ring and one along the 1985 ring, and crossed from non-stained wood into wood visibly colonized by blue-stain fungus and then back into non-stained wood. We note here that wood may still be colonized by blue-stain fungi even without visible signs of colonization (i.e. blue-stain). These rings, 1987 and 1985, presented the best balance between sampling ease and a position to be affected by the blue-stain fungus. The annual time-series across rings (radial transects, starting at 1976 and 1979) take place wholly within areas affected by blue-stain fungus. We then measured the whole wood and α-cellulose $\delta^{13}C$ and $\delta^{18}O$ of each sample from the circumferential and radial transects.

Isotopic and statistical analyses

We measured the $\delta^{13}C$ and $\delta^{18}O$ of whole wood, α-cellulose and frass at the Los Alamos National Laboratory Earth and Environmental Sciences Stable Nuclide-Isotope Facility for Forensics and Ecology Research (EES SNIFFER). We measured $\delta^{13}C$ and $\delta^{18}O$ using a CHN elemental analyzer (Costech Analytical Technologies, Valencia, CA, USA) and a thermal combustion elemental analyzer (Thermo Electron Corp., Waltham, MA, USA), each attached to a continuous flow isotope ratio mass spectrometer (MAT 253, Thermo Electron Corp.). The reported values are in per mil (‰) relative to VPDB for $\delta^{13}C$ and V-SMOW for $\delta^{18}O$ analyses. The precision (σ) of our method was 0.07‰ for $\delta^{13}C$ and 0.16‰ for $\delta^{18}O$, based on a mean of means of laboratory standards (σ-cellulose from Sigma-Aldrich, St. Louis, MO, USA) for each analysis run. An international standard (IAEA C3) run periodically yielded a mean value of -24.91 ± 0.05‰ for $\delta^{13}C$ and 32.1 ± 0.1‰ for $\delta^{18}O$ (± values are 95% confidence intervals calculated with $\alpha = 0.05$). To compare the effects of α-cellulose extraction methods on $\delta^{13}C$ and $\delta^{18}O$ we used JMP IN 5.1.2 (SAS Institute Inc., Cary, NC, USA) to perform simple linear regressions and one-way analysis of variance (ANOVA) ($\alpha = 0.05$) for each species and live/dead treatment with equal variances. If a two-sided F-test determined that the variances of treatments were unequal, we then used a Welch ANOVA to test for differences in means.

RESULTS

Comparisons of whole wood, α-cellulose and extraction method

Extracted α-cellulose had greater $\delta^{13}C$ (1.0 to 2.4‰) and $\delta^{18}O$ (4.4 to 7.7‰) values than whole wood for all treatments (Table 1, Fig. 1). Regardless of the extraction method, the $\delta^{13}C$ values of whole wood and α-cellulose plot were within error of the 1:1 line (simple linear regressions for whole wood vs. SB and LD, $P < 0.001$, 95% confidence intervals for both slopes between 1.0 and 1.6; Fig. 1). The $\delta^{13}C$ and $\delta^{18}O$ means of the α-cellulose extracted by SB were greater and less, respectively, than those of the α-cellulose extracted by LD in all ponderosa, piñon and juniper (Table 3, Fig. 2). Except in four cases ($\delta^{13}C$ in dead ponderosa and live piñon, and $\delta^{18}O$ in dead ponderosa and piñon), the difference in the means of the α-cellulose extracted by SB and LD was significant ($P < 0.05$; Table 3). The mean differences between SB- and

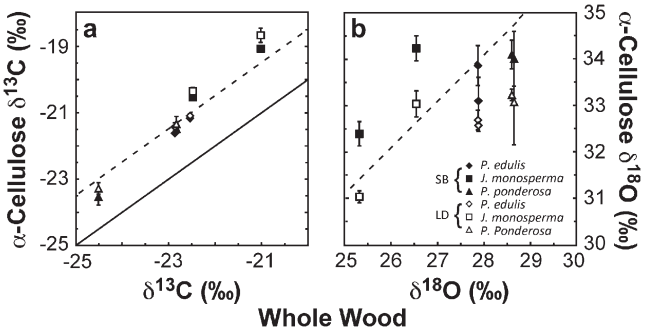

Figure 1. Whole wood and α-cellulose $\delta^{13}C$ (a) and $\delta^{18}O$ (b) for three species of western US conifers (Table 1). Live and dead trees of each species are not differentiated here. Solid line in (a) is the 1:1 line. The dashed line in (a) is the average offset of both SB and LD α-cellulose $\delta^{13}C$ (1.6‰). The dashed line in (b) is the average offset of both SB and LD α-cellulose $\delta^{18}O$ (5.6‰). Closed symbols are Standard Brendel (SB) α-cellulose values, open symbols are Leavitt-Danzer (LD) α-cellulose values. Confidence intervals ($\alpha = 0.05$) for α-cellulose are shown, although whole wood values are smaller than the symbol.

Table 3. Differences in the stable isotope means and variances (σ) of α-cellulose extracted using Leavitt-Danzer (LD) and Standard Brendel (SB) methods

	Difference between means of LD and SB		Difference between σ of LD and SB	
	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$
Pinus ponderosa				
Live	0.3	−0.9	−0.03	−0.29
Dead	0.2	−0.5	−0.19	−0.50
Pinus edulis				
Live	0.1	−1.2	0.04	−0.28
Dead	0.2	−0.5	0.03	−0.40
Juniperus monosperma				
Live	0.2	−1.4	0.15	−0.18
Dead	0.4	−1.2	0.25	0.01

Grey shading indicates significant differences ($P < 0.05$) between the mean and standard deviation (σ) of stable isotope values (‰) from α-cellulose extracted via Standard Brendel (SB; modified for small samples)[11,12] or the Leavitt-Danzer (LD)[13] method.

LD-extracted α-cellulose ranged between 0.1‰ and 0.4‰ for $\delta^{13}C$, and 0.5‰ and 1.4‰ for $\delta^{18}O$ (95% confidence intervals). These differences are large compared with the instrument analytical error (see Experimental section). The variance of the results for the α-cellulose extracted via SB was lower than that of the α-cellulose extracted via LD in some treatments and higher in others; there is no apparent pattern. Because the $\delta^{13}C$ and $\delta^{18}O$ offset between α-cellulose and whole wood was similar for the SB and LD methods, but for small samples SB extraction of α-cellulose was substantially easier, all further results presented here are derived from either whole wood or whole wood treated using SB.

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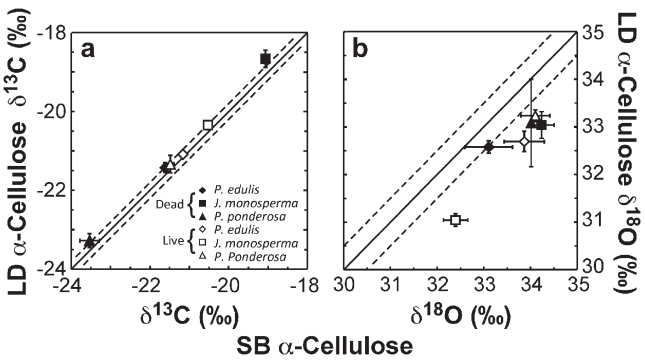

Figure 2. Comparison of α-cellulose $\delta^{13}C$ (a) and $\delta^{18}O$ (b) extracted via Standard Brendel (SB) and Leavitt-Danzer (LD) and between live and dead trees. Solid line is 1:1 line. In (a) and (b) dashed lines represent ±0.2‰ and ±0.5‰ offsets from the 1:1 line, respectively.

Whole wood and α-cellulose comparisons in relation to climate and beetle frass

Whether wood grew in wet, dry or average rainfall years had no demonstrable effect on the difference between $\delta^{13}C$ and $\delta^{18}O$ in whole wood and α-cellulose (SB) of living and dead white fir, piñon and juniper (Table 3, Fig. 3). The α-cellulose plots were near or along the 1:1 slope expected if all the α-cellulose were offset from whole wood by the same amount during SB extraction (dashed line in Fig. 3).

The difference between frass generated by bark beetles in juniper and extracted α-cellulose from the frass (1.8‰, Table 1), while not directly comparable with the whole wood of the live or dead juniper, is almost identical to the difference between whole wood and α-cellulose in dead juniper (1.9‰).

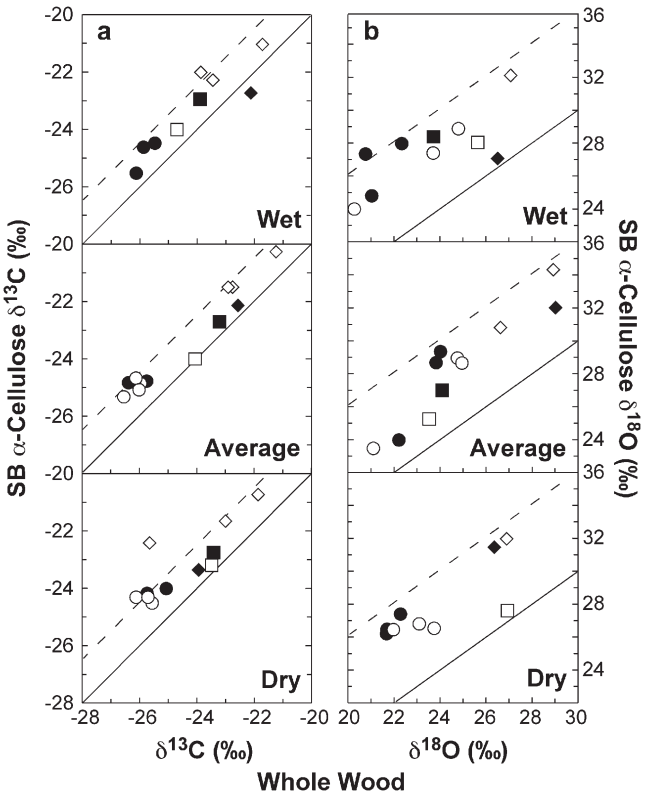

Figure 3. Whole wood and α-cellulose $\delta^{13}C$ (a) and $\delta^{18}O$ (b) for three species of western US conifer (Table 2) in wet, normal and dry years (see text for how these were selected). Circles are white fir, squares are juniper, and diamonds are piñon. Closed symbols are dead trees, open symbols are live trees. α-Cellulose was extracted using SB.

Blue-stain fungus

Blue-stain fungus appears to have no effect on the $\delta^{13}C$ and $\delta^{18}O$ of whole wood or α-cellulose (SB). When samples from all four transects are included, the 95% confidence intervals of the relationship of $\delta^{13}C$ and $\delta^{18}O$ of whole wood and SB cellulose encompass the 1:1 line (simple linear regressions, $P < 0.0001$ and $P < 0.001$, 95% confidence intervals of slopes between 0.7 and 1.1 and 0.3 and 1.0, respectively). Given these data, there are no discernable changes in this relationship across the boundaries of wood colonized by blue-stain fungus (Figs. 4 and 5). Variations that do appear within colonized wood are of the same amplitude and variability as variations outside the colonized wood. Likewise, whole wood and α-cellulose $\delta^{13}C$ and $\delta^{18}O$ time-series across rings colonized by blue-stain fungus (Figs. 5 and 6) show little isotopic variability outside that expected from variations imposed by climate or tree physiology.[23]

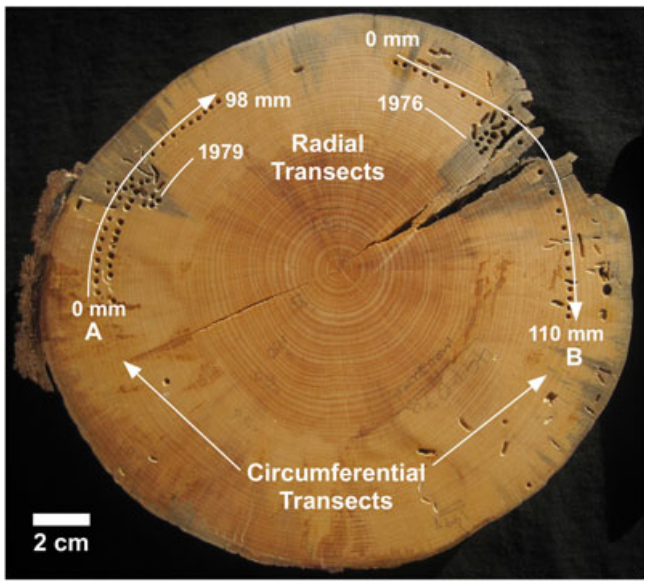

Figure 4. Blue-stain fungi and beetle galleries in a cookie from a dead piñon in northern New Mexico. Areas colonized by blue-stain fungus are visible as darker wedge-shaped discolorations pointing towards the center of the cross-section. Areas colonized by blue-stain fungi may not necessarily be stained blue. Arrows indicate circumferential ring transects (Transects A and B shown in Fig. 5) and a white line and year are the earliest years of radial transects (Fig. 6). Wood-boring beetle galleries containing frass are the ovoid holes aligned parallel to tree-rings.

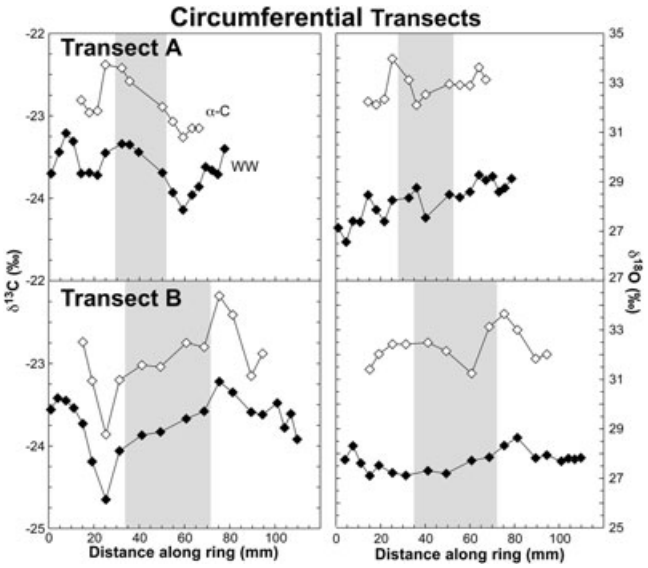

Figure 5. Circumferential ring transect of whole wood and α-cellulose $\delta^{13}C$ (left panels) and $\delta^{18}O$ (right panels). Closed symbols are whole wood values, open symbols are α-cellulose extracted via SB. Shaded area represents wood colonized with blue-stain fungus (circumferential transects are indicated in Fig. 4).

DISCUSSION

In four common species of western US forests (juniper, piñon, ponderosa and white fir) there is no apparent relationship of tree-ring $\delta^{13}C$ and $\delta^{18}O$ to α-cellulose extraction and the tree's status (live or dead). For each tree species and α-cellulose extraction procedure (SB or LD) there are significant differences in the $\delta^{13}C$ and $\delta^{18}O$ means and variances; however, these differences appear to be similar to differences found in other studies[11,14,24] and are not related to tree status when sampled. Our results confirm the finding of Gaudinski *et al.* that α-cellulose extracted using LD yields higher $\delta^{18}O$ values than samples extracted using SB.[14] In cases of incomplete extraction, lower $\delta^{18}O$ values should reflect the presence of hemicelluloses that typically have values lower than those of α-cellulose.[13] Dead trees show fewer instances than live trees (3 vs. 5, respectively) of significant differences between paired means (Table 3). This may suggest that, in some cases, the SB procedure is insufficient to remove the large amounts of sap and resin that exist in living pines. There are three other possibilities (Kevin Anchukaitis, personal communication) that might explain the $\delta^{18}O$ discrepancy between SB and LD: (1) incomplete removal of an as yet unidentified compound by SB; (2) incomplete delignification by SB, although this is not supported by other studies;[11] or (3) an unidentified process in LD that is altering the "true" ratios of α-cellulose. In addition to these three possibilities, acetylation of the hydroxyl groups in the remaining α-cellulose, other non-cellulosic polysaccharides or remaining resins may occur during SB, imparting a partial reagent oxygen signature to the samples (see Anchukaitis *et al.*[11] for a thorough explanation of the evidence for this effect). However, as in other studies,[11] we do not see this effect on the IAEA-C3 samples run through the same procedure (Table 1). The methodological definition of α-cellulose allows for leeway in determining what is "true" α-cellulose and therefore what are the "true" values of $\delta^{13}C$ and $\delta^{18}O$.[11] For our purposes, that of comparing differences in isotopic trends of living and dying trees in the years preceding widespread tree mortality events, the effects of α-cellulose extraction, using either SB or LD, on $\delta^{13}C$ and $\delta^{18}O$ are stable and consistent between live and dead trees (Figs. 2, 3 and 7, Tables 1 and 2).

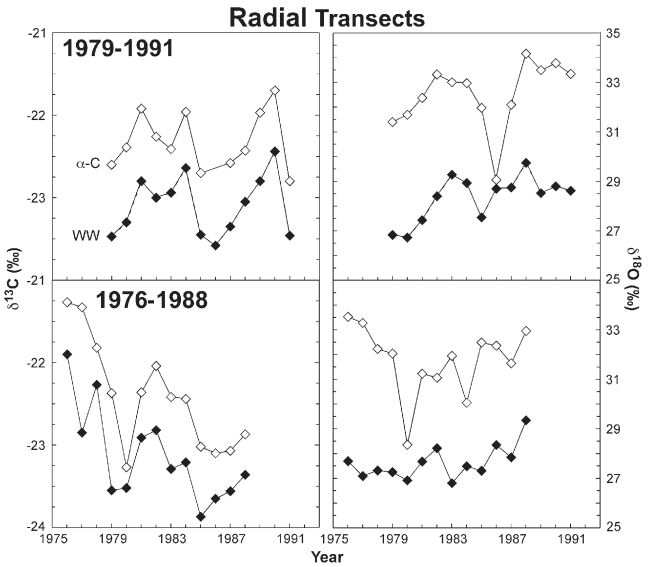

Figure 6. Radial transect of whole wood and α-cellulose $\delta^{13}C$ (left panel) and $\delta^{18}O$ (right panel). Closed symbols are whole wood values, open symbols are α-cellulose extracted via SB. These samples were all taken from within the area colonized by blue-stain fungus (radial transects indicated in Fig. 4).

Likewise, we found no evidence that offsets resulting from SB α-cellulose extraction from whole wood of live and dead trees was influenced by whether or not wood was laid down in wet, dry or average rainfall years. While resins may be mobilized during periods of stress or pathogen attack, at least with respect to annual rainfall, our results suggest that mobile resins, lignin and other components of juniper, piñon and white fir sapwood are not preferentially deposited in rings of any particular year. If this were the case, we might expect to see consistent differences in the offset between whole wood and α-cellulose in years with greater amounts of these compounds.

Our results also suggest that blue-stain fungus in piñon does not appreciably alter the $\delta^{13}C$ and $\delta^{18}O$ of sapwood through the alteration of wood component ratios, or does so in a way that maintains the isotopic ratio of the affected components. Frass deposited by wood-boring beetles in juniper responds identically to whole wood during α-cellulose extraction. This suggests that alteration of the whole wood is limited and small amounts of frass in sampled wood should not affect the offset of whole wood during α-cellulose extraction, assuming that the frass has not been transported from the heartwood. Blue-stain fungi and wood-boring by beetles are commonly found in dead trees. While the evidence presented here is not conclusive, we suggest that studies of both live and dead trees should not be hampered by remobilization of carbon or oxygen due to blue-stain fungi or frass contamination.

Overall, there appear to be few obstacles to comparing $\delta^{13}C$ in whole sapwood of living and dead trees, and some ambiguity in the relationship between $\delta^{18}O$ in whole wood and

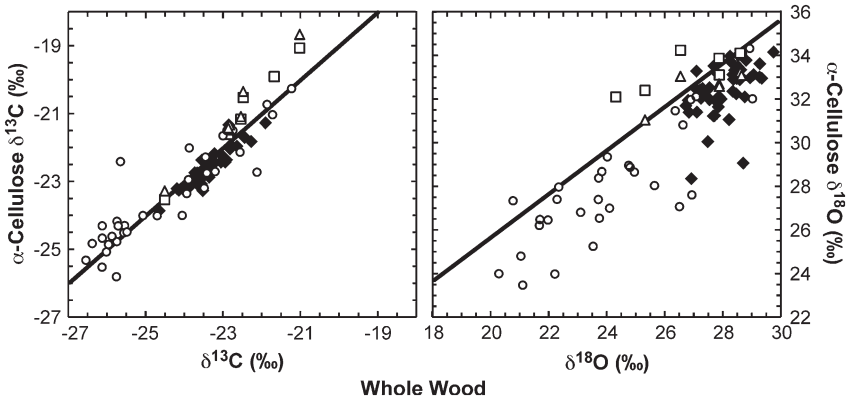

Figure 7. Comparison of whole wood and α-cellulose $\delta^{13}C$ (left panel) and $\delta^{18}O$ (right panel) from all paired samples in this study. Open squares and triangles are the means of live and dead trees from multiple species extracted using SB and LD, respectively (Table 1). Open circles are from individual trees and years in multiple species sampled in wet, average and dry years (Table 2) and extracted using SB. Closed diamonds are samples from circumferential and radial piñon transects (Figs. 5 and 6) extracted using SB. Dark black line is 1:1 line offset by 1.0‰ and 5.8‰ for $\delta^{13}C$ and $\delta^{18}O$, respectively. These data are from sapwood only.

corresponding α-cellulose (Fig. 7). For at least ponderosa, juniper, piñon and white pine, our results suggest that carbon isotopic investigations of mortality in live and dead trees undertaken solely within the sapwood can proceed without costly and labor-intensive α-cellulose extractions. We still suggest that preliminary checks of the relationship between whole wood and α-cellulose $\delta^{13}C$ should be carried out for each system under investigation and, if extractions are undertaken, either the SB or the LD method is appropriate. For $\delta^{18}O$ of sapwood (Fig. 7), the story is less clear and caution should be exercised before proceeding with only $\delta^{18}O$ isotopic analyses of whole wood in studies investigating the causes of mortality in these species.

CONCLUSIONS

The evidence presented here suggests that there are few barriers to comparing the isotopic patterns of $\delta^{13}C$ and $\delta^{18}O$ in the tree-rings of live and dead trees, and that sapwood from recently dead trees can be treated similarly to sapwood from live trees. While we found differences between whole wood and α-cellulose similar to those in other studies, the significance, variance and offset of these differences were not related to the status of the tree (alive or dead) when it was sampled. $\delta^{18}O$ values, and to a lesser extent $\delta^{13}C$ values, from α-cellulose extracted using LD and α-cellulose using SB are probably not directly comparable, and any study that does use α-cellulose should strive to use either LD or SB, but not both procedures. Beetle frass and blue-stain fungi do not appear to detectably alter whole wood or α-cellulose $\delta^{13}C$ and $\delta^{18}O$. The absence of any apparent effect of live and dead tree sampling does not mean that other considerations normally associated with isotope dendrochronology can be ignored,[25] and caution should always be exercised for species or locales where the relationship between climate, whole wood and α-cellulose has not been established.

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