

POTENTIAL FOR ASSESSING LONG-TERM DYNAMICS IN SOIL NITROGEN AVAILABILITY FROM VARIATIONS IN $\delta^{15}\text{N}$ OF TREE RINGS

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Numerous researchers have used the isotopic signatures of C, H, and O in tree rings to provide a long-term record of changes in the physiological status, climate, or water-source use of trees. The frequently limiting element N is also found in tree rings, and variation in its isotopic signature may provide insight into long-term changes in soil N availability of a site. However, research has suggested that N is readily translocated among tree rings of different years; such infidelity between the isotopic compositions of the N taken up from the soil and the N contained in the ring of that growth year would obscure the long-term N isotopic record. We used a 15-year ^{15}N -tracer study to assess the degree of N translocation among tree rings in ponderosa pine (*Pinus ponderosa*) trees growing in a young, mixed-conifer plantation. We also measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in unlabeled trees to assess the degree of their covariance in wood tissue, and to explore the potential for a biological linkage between them. We found that the maximum $\delta^{15}\text{N}$ values in rings from the labeled trees occurred in the ring formed one-year after the ^{15}N was applied to the roots. The $\delta^{15}\text{N}$ value of rings from labeled trees declined exponentially and bidirectionally from this maximum peak, toward younger and older rings. The unlabeled trees showed considerable interannual variation in the $\delta^{15}\text{N}$ values of their rings (up to 3 and 5‰), but these values correlated poorly between trees over time and differed by as much as 6‰. Removal of extractives from the wood reduced their $\delta^{15}\text{N}$ value, but the change was fairly small and consistent among unlabeled trees. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of tree rings were correlated over time in only one of the unlabeled trees. Across all trees, both $\delta^{13}\text{C}$ values of tree rings and annual stem wood production were well correlated with annual precipitation, suggesting that soil water balance is an important environmental factor controlling both net C gain and transpirational water loss at this site. Our results suggest that interannual translocation of N among tree rings is substantial, but may be predictable enough to remove this source of variation from the tree-ring record, potentially allowing the assessment of long-term changes in soil N availability of a site.

Keywords: Carbon 13; Nitrogen 15; Nitrogen cycling; Ponderosa pine; Soil water availability; Tree rings

INTRODUCTION

The availability of the element nitrogen (N) often limits net primary production in temperate forest ecosystems [1]. Despite the importance of this element, few long-term studies have assessed changes in N cycling over multiple years. Over the past decade, interest in N has

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changed from its role as a limiting nutrient to how the saturation of forest ecosystems with N alters ecosystem structure and function [2, 3]. Nitrogen saturation of forests occurs through increased rates of N deposition from the atmosphere driven by fossil fuel combustion, and leads to a number of potential deleterious effects, such as decreased water quality, decreased soil fertility, and increased emissions of heat-trapping trace gases [2, 3]. Given the important roles that N plays in ecosystems, it would be advantageous if a method could be developed to assess retrospectively long-term changes in N dynamics within a forest.

The isotopic signatures of “light” elements in tree rings have been used in a variety of ecological studies to assess the long-term record of tree water use [4, 5], tree physiological status [6–8], and climate [9, 10]. Nitrogen is also found in tree rings, albeit in relative low concentrations [11], and the isotopic signatures of N isotopes might also provide a historical record of N cycling processes. Several investigators have proposed that the natural abundance of N isotopes in tree leaves might provide useful information on soil N cycling processes [12–17]. As N is cycled through an ecosystem, slight fractionation, or discrimination against the heavier isotope ^{15}N , is usually observed [18]. On this basis, some ecologists have predicted that the natural ^{15}N abundance would increase over time in plants from ecosystems that experience high rates of N deposition from the atmosphere, or in ecosystems that have high ambient N availability [19]. This is because both high rates of N deposition and high ambient N availability lead to increases in net nitrification, which leaves behind ^{15}N -enriched NH_4^+ and produces ^{15}N -depleted NO_3^- . Given that most trees prefer NH_4^+ to NO_3^- as an N source, trees should become ^{15}N -enriched under these conditions. If these annual patterns in leaves are retained with high fidelity in tree xylem, then a long-term record of changes in N cycling processes may be preserved.

One of the requisites for the use of isotopic signatures in tree rings as an ecological or climatic index of the past is that the isotopic ratio of the element taken up by the plant in a given year is reflected in the isotopic ratio of the element in the xylem produced in that year. Any fractionation in the isotopic composition of the element during transport to the tree bole, or any translocation of the element among tree rings of different ages, would make it difficult to utilize the isotopic record preserved in these rings. There have been several reports of translocation of N among tree rings of different ages in the literature using both mass balance [20] and ^{15}N fertilizations studies [21–23] to denote movement of N. This radial movement of N occurs apparently along the living ray tissue within the xylem [22, 24]. However, in the few studies that have assessed N movement among annual rings, the pattern of N translocation appears relatively predictable [21–23]. Hence, it may be possible to uncover the natural abundance ^{15}N signal in tree rings by removing this inter-ring translocation “noise.” Alternatively, it might be possible to chemically remove the mobile N in wood that contributes to the inter-ring transfer of N, and thus uncover a natural abundance ^{15}N pattern with high fidelity to the ^{15}N abundance of the N assimilated from the soil [25].

Stable carbon isotope analysis of tree rings have been used as long-term and sensitive indicators of carbon uptake limitations [26] and the efficiency of water use [27] in trees. At the leaf level, $\delta^{13}\text{C}$ values are also regulated by the photosynthetic capacity of the leaf, which is strongly correlated with leaf N concentration [28]. If the relative availability of soil N controls both the N concentration of leaves [1] as well as the $\delta^{15}\text{N}$ values assimilated by the roots [19], then this provides a direct mechanistic link between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in tree tissues, including wood. Alternatively, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in tree tissues could be correlated indirectly due to the dual effect of soil water availability on the $\delta^{15}\text{N}$ values of available soil pools [29] and on leaf stomatal conductance [26, 30].

We took advantage of a ^{15}N tracer experiment where individual ponderosa pine (*Pinus ponderosa* Laws.) trees were labeled with small amounts of highly enriched ^{15}N 14 years previously. Our primary objective was to assess the degree of translocation of assimilated

N among rings in this species in an attempt to develop a method for the long term, retrospective assessment of changes in N availability to trees. Our secondary objective was to evaluate the potential for a biological linkage between ^{13}C and ^{15}N values in tree wood by comparing the degree of correspondence between them over time.

MATERIALS AND METHODS

Our study was conducted in a young mixed-conifer plantation within the Blodgett Forest Research Station (BFRS), located on the western slope of the Sierra Nevada Mountains of California ($38^{\circ}52'$, $120^{\circ}40'$ W). The elevation of the plantation is about 1300 m, and it was established in 1981 following clearcutting the previous stand in 1980. Mean annual air temperature in this region is 11°C and mean annual precipitation is 162 cm, with about 85% of the precipitation falling as snow between October–March (Mediterranean-type climate). The soil is classified as a member of the Ultic Haploxeralf Soil Taxonomic subgroup and has a sandy-loam surface texture.

During 1985, three, five-year-old ponderosa pine trees were selected at random within a 100-m^2 area of a mixed-conifer plantation. These trees were labeled with ^{15}N by locating the lateral roots within the soil, carefully removing the surface soil to expose the roots, and then applying a ^{15}N solution (67 atom % ^{15}N -enriched urea, 3.8 L per tree) directly to these roots. This method was used to increase the proportion of the label assimilated by the trees rather than by the soil microbial heterotrophs, which have been shown to assimilate the majority of ^{15}N label when applied directly to the bulk soil [31]. Furthermore, this approach should also increase the likelihood that most of the label would be taken up by the tree and assimilated during the 1985 growing season. The roots were reburied immediately after labeling. Trees were labeled in this manner twice during the 1985 growing season; two grams of urea were applied to each tree on May 22, and one additional gram of urea was applied on June 23. Additionally, 17.3 L of tap water were applied to the bulk soil under each tree on July 14 to facilitate the continued uptake of label during this period of low soil water content. Approximately 2 g of ^{15}N were applied to each tree.

In September 1999, the three, 19-year-old labeled trees were located, and two unlabeled trees of similar height and diameter growing within 10 to 20 m of the labeled trees were also selected (Table I). The unlabeled trees were used to assess the annual variation of natural ^{15}N abundance in tree rings at this site. The canopy of the mixed conifer plantation was still partially open in 1999, but adjacent trees crowns were beginning to overlap with each other.

At approximately 40 cm above ground, a 12-mm diameter increment core was taken to the tree pith from both the south and the west sides of each tree. The tree cores were soaked in deionized water for two days (changing the water frequently) to help remove any readily

TABLE I Selected Tree Characteristics of Unlabeled (UL) and ^{15}N Labeled (L) Trees in 1999 Prior to Tree-ring Sampling. Stem Wood Biomass was Estimated Using Allometric Equations (See Materials and Methods).

<i>Tree</i>	<i>Diameter at 1.4 m (cm)</i>	<i>Diameter at 0.4 m (cm)</i>	<i>Height (m)</i>	<i>Stem wood biomass (kg)</i>
UL 1	31.3	38.1	13.6	148
UL 2	29.0	33.1	14.2	119
L 1	32.0	38.4	15.5	157
L 2	31.0	36.5	15.0	144
L 3	30.2	39.0	15.4	133

soluble N compounds present in the wood. The cores were then air-dried and temporarily mounted in a vice-like holder [32]. Using stainless steel razor blades, a sliver of wood was cut from a transverse surface in order to see the rings clearly using a dissecting microscope. Ring-widths were measured to the nearest 0.001 mm under 20X magnification with a banister connected to a micrometer [32]. Cross dating was unnecessary because each ring could be accounted for with the innermost ring outside the pith having a 1984 date. After the tree-ring analysis, annual rings from the cores taken from the south side of the trees were dissected from each core using a fine-toothed coping saw. The increment cores from the west side were not analyzed for elemental concentrations and isotopic compositions because the annual rings in one increment core provided enough mass for all analyses. All of the wood on these trees was sapwood.

Individual annual tree-rings were initially ground using a Thomas Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) through a 40-mesh screen and then finely ground to a powder using a homemade bar mill. Total N and carbon (C) concentrations and $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ ratios in the wood samples were analysed on a Finnigan Delta Plus XL isotope ratio mass spectrometer coupled to a CE 2100 Elemental Analyzer at the Colorado Plateau Stable Isotope Laboratory, Northern Arizona University. Nitrogen and carbon were analysed in separate runs; approximately 25 mg samples were used for N analyses, while approximately three mg samples were used for C analyses. Values are reported in the standard notation relative to atmospheric N_2 for N and Pee-Dee Belemnite for C, using NIST Peach Leaves no. 1547 as a standard, where $\delta X(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ and R is the molar ratio of $^{\text{heavy}}X/^{\text{light}}X$ [33]. The standard deviations ($n = 12$) of repeated analysis of a bulk wood sample (generated from one western increment core from an unlabeled tree) measured throughout the sample runs were 0.0010%, 0.39%, 0.25‰, and 0.10‰ for total N, total C, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$, respectively.

We correlated annual rates of N deposition with $\delta^{15}\text{N}$ values observed in rings in the unlabeled trees to assess whether the variation in the natural ^{15}N abundance of tree rings was due to changes in atmospheric N inputs experienced by these trees. Nitrogen deposition measurements were unavailable at BFRS, so we estimated these rates based on measured precipitation at BFRS and precipitation-weighted N concentrations from the closest National Atmospheric Deposition Program site (<http://nadp.sws.uiuc.edu/>), located in Yosemite National Park (37°47'46" N, 119°51'29" W) at a similar elevation (1408 m) and about 170 km to the southeast of BFRS.

We determined stem wood biomass, stem wood production, and the total amount of the added ^{15}N label contained within stem wood produced in different years using allometric equations developed for ponderosa pine that relate stem diameter to wood biomass (W.W. Covington, Northern Arizona University, unpublished data, [34]). Stem diameter was determined retrospectively using measured diameter in 1999 and tree-ring annual growth data. Stem wood production was calculated as the difference in stem wood biomass between successive years. The ^{15}N excess for each growth year was calculated by subtracting the mean natural ^{15}N abundance of the unlabeled trees from the ^{15}N enrichment values of the labeled tree, and then multiplying this value by the N concentration and stem wood production in the labeled tree for that year.

We assessed how elemental concentrations and isotopic compositions of N and C change following removal of extractives (*i.e.*, waxes, oils, resins, some inorganic salts, and low molecular weight polysaccharides [35]). Wood produced in each tree during 1987 (selected randomly) was used for this test, with an additional year (1984) selected from one of the unlabeled trees in order to have a sample size of three for both labeled and unlabeled wood. Extractives were removed following the procedures of Leavitt and Danzer [35], and the extracted wood samples were then analyzed for their N and C concentrations and isotopic

compositions as described previously. All N and C concentrations are expressed on an oven-dry basis (70 °C).

We used repeated measures analysis of variance (RM ANOVA) to compare tree characteristics between labeled and unlabeled trees over time. Friedman repeated measures ANOVA on ranks was used when the data did not meet the assumptions of parametric statistical analyses. We used correlation analyses (Pearson product-moment) to explore covariance in the tree ring $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopic values, as well as between these values and environmental variables. We used a p -value ≤ 0.05 to denote statistical significance. All statistical analyses were conducted using SigmaStat (version 2.0, Jandel Scientific, San Rafael, CA, USA).

RESULTS

There was no significant difference in tree ring widths or calculated stem wood biomass between labeled and unlabeled trees (RM ANOVA, $p=0.335$ and 0.540 , respectively; Fig. 1). Tree ring widths increased over time until about 1993, when they began declining. Despite an initial lag early on, stem wood biomass increased more or less linearly over the study.

There was no significant difference (RM ANOVA, $p=0.890$) in N concentration of tree rings between labeled and unlabeled trees (Fig. 2). Nitrogen concentration in tree rings declined slightly over time until about 1990, when N concentration increased. Tree ring N concentration increased in the most recent (1999) year, where N concentration was about 2-fold higher than in the other years.

The temporal patterns of $\delta^{15}\text{N}$ values in all three labeled trees were highly correlated with each other ($r > 0.964$, $p < 0.001$; Fig. 3). Maximum $\delta^{15}\text{N}$ values in the tree rings occurred in 1986, one year after the label was applied to the fine roots, and $\delta^{15}\text{N}$ values decreased exponentially in both older and younger rings from the maximum value. However, labeled

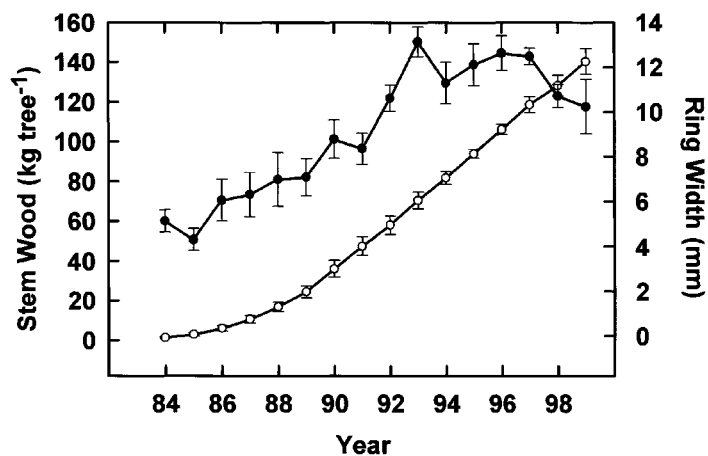


FIGURE 1 Cumulative increase in stem wood (open symbols) and annual tree ring width (closed symbols) of ponderosa pine trees from 1984 to 1999. Trees were four-years old in 1984. There was no statistically significant effect of ^{15}N addition on tree ring width or stem wood biomass (RM ANOVA, $p=0.335$ and 0.540 , respectively), so mean values of all five trees are shown. Vertical bars denote ± 1 standard error.

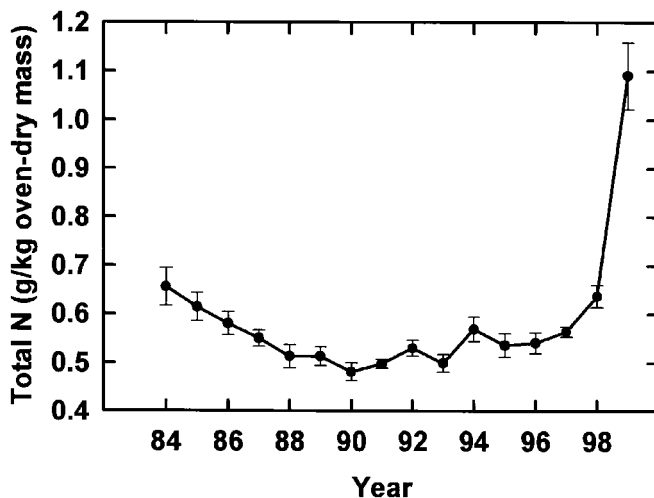


FIGURE 2 Annual variation in tree ring N concentration in ponderosa pine trees. There was no statistically significant effect of ^{15}N addition on tree ring N concentration (RM ANOVA, $p = 0.890$), so mean values of all five trees are shown. Vertical bars denote ± 1 standard error.

tree L3 had significantly lower ($p < 0.05$) $\delta^{15}\text{N}$ values than trees L1 and L2 (RM ANOVA on ranks, $P < 0.001$, Tukey multiple comparison test).

In general, annual variation in ^{15}N excess contained in the stem wood in the labeled trees followed a similar pattern as the $\delta^{15}\text{N}$ values (Fig. 4). However, because of increased stem wood production over time, the ^{15}N excess in stem wood tended to be more evenly distributed between 1986 and 1998. In 1999, increases in ^{15}N excess in stem wood were driven by increased N concentration of this current year wood increment (Fig. 2). Prior to 1986, little ^{15}N excess was found due to low stem wood biomass and

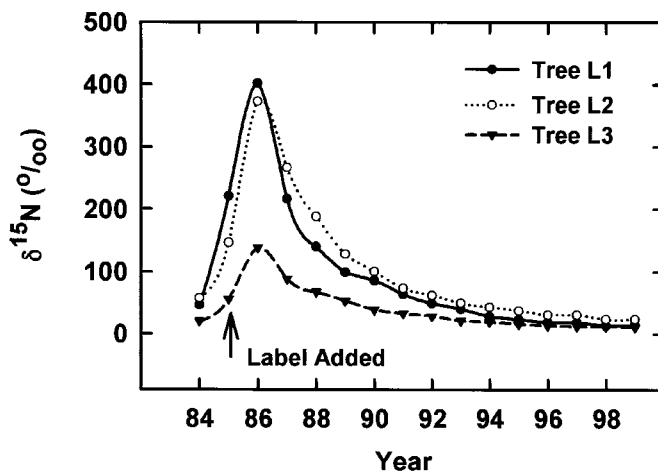


FIGURE 3 Annual variation in tree ring $\delta^{15}\text{N}$ values of three ponderosa pine trees labeled with 2 g of ^{15}N during the 1985 growing season.

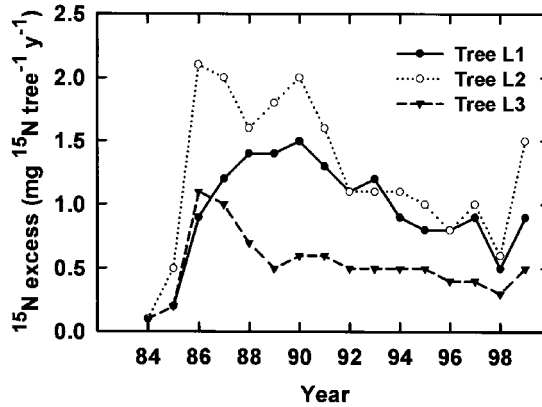


FIGURE 4 Estimated ^{15}N excess (above the mean background ^{15}N value of two unlabeled trees) recovered in annual stem wood production in the three ponderosa pine trees labeled with 2 g ^{15}N during the 1985 growing season.

relatively low $\delta^{15}\text{N}$ values for those years. We estimate that a total of 0.8, 1.0, and 0.4% of the added ^{15}N label was recovered in the stem wood from 1984 to 1999 in labeled trees L1, L2, and L3, respectively.

In unlabeled trees, substantial annual and between-tree variation in $\delta^{15}\text{N}$ values of tree rings occurred (RM ANOVA, $p < 0.001$; Fig. 5). This variation in $\delta^{15}\text{N}$ was correlated positively with changes in total N concentration in unlabeled tree UL1 ($r = 0.648$, $p < 0.007$, $n = 16$), but not in unlabeled tree UL2 ($p = 0.140$, $n = 16$). The absolute values of $\delta^{15}\text{N}$ in tree rings from the two unlabeled trees differed by as much as 6‰, and their annual patterns were uncorrelated across all 16 years studied ($r = 0.058$, $p = 0.831$, $n = 16$). Even when only the years from 1984 to 1995 were included in the analysis, a period where the values appeared to covary between the two trees (Fig. 5), the correlation was weak and non-significant ($r = 0.55$, $p = 0.066$, $n = 12$).

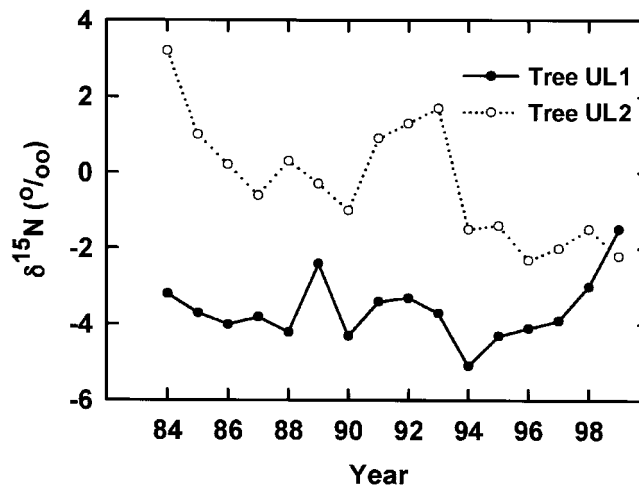


FIGURE 5 Annual variation in tree ring $\delta^{15}\text{N}$ values of unlabeled ponderosa pine trees.

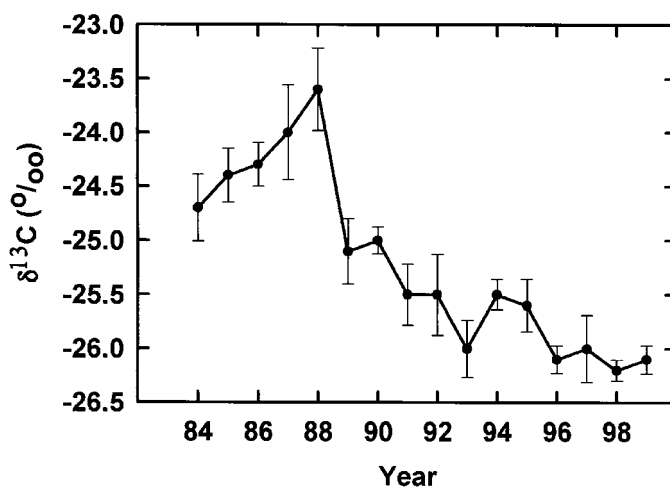


FIGURE 6 Annual variation in tree ring $\delta^{13}\text{C}$ values in ponderosa pine trees. There was no statistically significant effect of ^{15}N addition on tree ring $\delta^{13}\text{C}$ values (RM ANOVA, $p = 0.715$), so mean values of all five trees are shown. Vertical bars denote ± 1 standard error.

Estimated rates of annual N deposition were low at BFRS (between about 0.2 and $0.7 \text{ g N m}^{-2} \text{ y}^{-1}$), but did vary considerably between 1984 and 1999 (data not shown). However, no significant temporal trend in N deposition occurred over that time period ($r = 0.036$, $p = 0.895$, $n = 16$). Nitrogen deposition rates were uncorrelated with $\delta^{15}\text{N}$ values of the two unlabeled trees ($p = 0.335$ and 0.970 for trees UL1 and UL2, respectively). Estimated N deposition rates were also uncorrelated with mean N concentrations of tree rings across the five trees ($p = 0.970$, $n = 16$).

Carbon isotopes values of tree rings were similar among labeled and unlabeled trees (RM ANOVA, $p = 0.715$), but there was considerable variation among years (almost 3‰; Fig. 6). Carbon isotope values from individual trees were highly correlated with each other among years (range of r values from 0.621 to 0.824, $p < 0.010$, $n = 16$). Mean tree $\delta^{13}\text{C}$ values of tree rings increased steadily from 1984 to 1988, declined dramatically in 1989, and then continued to decline more slowly until sampled in 1999. Mean $\delta^{13}\text{C}$ values across all five trees correlated with mean tree stem wood production ($r = -0.780$, $P < 0.001$) and annual precipitation ($r = -0.555$, $p = 0.026$), but not with mean daily maximum air temperature

TABLE II Changes in Total N and $\delta^{15}\text{N}$ Values of Selected Tree Rings Following Removal of Extractives.

Sample	Total N (g N/kg oven-dry mass)			$\delta^{15}\text{N}$ (‰)		
	Non-extracted (NE)	Extracted (E)	Difference (NE-E)	Non-extracted (NE)	Extracted (E)	Difference (NE-E)
UL1, 1987	0.50	0.44	0.06	-3.80	-6.55	2.75
UL2, 1987	0.58	0.49	0.09	-0.65	-2.90	2.25
UL2, 1984	0.58	0.52	0.06	3.16	1.20	1.96
L1, 1987	0.57	0.50	0.07	217	206	10.8
L2, 1987	0.52	0.50	0.02	267	233	34.3
L3, 1987	0.58	0.52	0.06	88.3	84.2	4.10

($p = 0.267$) or mean daily minimum air temperature ($p = 0.359$). Additionally, in the unlabeled trees, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were correlated with each other in one tree (UL2; $r = -0.553$, $p = 0.027$, $n = 16$), but not in the other (UL1; $p = 0.501$, $n = 16$).

Removal of extractives from the wood decreased the N concentration and $\delta^{15}\text{N}$ values in both labeled and unlabeled trees (Table II). The change in $\delta^{15}\text{N}$ following extractive removal was relatively small and consistent in unlabeled trees (about 2 to 3‰), but was much larger and variable in the highly ^{15}N -enriched, labeled trees (about 4 to 34‰). In contrast, removal of extractives increased the C concentration (mean increase across all six samples of 71 ± 5.5 g C/kg) and the $\delta^{13}\text{C}$ values of the wood (mean increase across all six samples of 0.31 ± 0.05 ‰).

DISCUSSION

$\delta^{15}\text{N}$ values in tree rings

Only a few studies have assessed annual variation in the natural ^{15}N abundance values of tree wood. Poulson *et al.* [36] reported considerable variation over several decades in two 50-year-old eastern hemlock (*Tsuga canadensis*) trees in New Hampshire, USA. They found absolute differences (range from 0.6 to 5.3‰) in $\delta^{15}\text{N}$ values in two trees separated by only five meters similar to what we found in two ponderosa pine trees located a comparable distance apart. Furthermore, the $\delta^{15}\text{N}$ values of the two trees used in their study were also well correlated with each other across years ($r = 0.726$, $p < 0.001$, $n = 33$; statistics calculated from their published data), and the values generally declined over time. Peñuelas and Estiarte [17] found a decreasing trend in $\delta^{15}\text{N}$ values of tree rings of *Quercus pubescens* in Spain (0.014‰/y, $r = -0.91$, $p < 0.05$, $n = 6$) over the 20th Century. In contrast, we found a poor inter-tree correlation between natural ^{15}N abundance values of annual tree rings, and only one of the two trees (UL2) showed a statistically significant decline in $\delta^{15}\text{N}$ values over time. The weaker correlations that we found may be due, in part, to the shorter tree-ring record used in our analysis (16 y) compared to these previous studies (50–70 y). Taken together, these studies suggest that: (1) there is substantial between-tree variation in $\delta^{15}\text{N}$ values of growth rings produced in the same year even for trees of similar age growing in close proximity to each other; and (2) $\delta^{15}\text{N}$ values of tree rings generally decrease over the past several decades in several temperate forests.

Paramount to using either the isotopic composition of elements or elemental concentrations in tree rings for historical reconstructions of past environments (*i.e.*, in dendrochemistry [37]) is the need for fidelity between the values preserved in tree rings and those experienced by the tree in the past [36, 38]. Past dendrochemical research has concluded that the N concentrations in tree rings cannot provide information on past conditions of N availability because of the high radial mobility of xylem N within trees [25, 36, 37]. Such inter-ring mobility of N should also confound ^{15}N isotopic signatures among rings.

In isotopic analyses of carbon, oxygen, and hydrogen in tree rings, cellulose is typically analyzed because it is resistant to isotopic changes after original synthesis [4, 5, 39–41]. Extraction pretreatments have also been suggested for elemental analyses of tree rings in dendrochemical studies where the mobility of the element is high, such as in the case of N [38]. However, based on our limited number of analyses (Table II), removal of more mobile N fractions of tree wood will not result in large changes in the total N concentration or $\delta^{15}\text{N}$ values in tree rings. This result contrasts with the recent work of Sheppard and Thompson [25] who found that wood extraction substantially changed the inter-ring pattern in stem wood of ponderosa pine and Douglas-fir (*Pseudotsuga menziesii*). In their study,

N concentrations of stem wood decreased on average by 45% in ponderosa pine. We found only about a 10% decrease in N concentration. Smaller reductions in N concentration in our study may be due to the fact that we leached all of our tree increment cores in deionized water prior to removal of wood extractives with toluene and ethanol. If this preleaching treatment effectively removed a majority of the extractable N from wood, then this might account for the small changes in total N concentration that we observed following the removal of extractives [35]. The success of our extraction procedure is evidenced by the similar increase in the $\delta^{13}\text{C}$ values of extracted compared to whole wood, as has been shown in many different studies [10, 42]. The removal of extractives has a relatively small and consistent change in the $\delta^{15}\text{N}$ values of the wood. Hence, our results suggest that simply extracting the wood prior to isotopic analysis will not result in the removal of the “noise” in the isotopic record generated by inter-ring transfer of N.

Poulson *et al.* [36] offered three possibilities for their observed patterns in $\delta^{15}\text{N}$ in tree rings: (1) changes in the isotopic composition of the available N pools and the relative proportion of N taken up from each source; (2) isotopic fractionation occurring during N uptake; and (3) isotopic fraction accompanying N translocation. They discounted isotopic fractionation as potential mechanisms because the available data suggests that there is little fractionation during N uptake by roots [43–45] and during other translocation processes within plants (*e.g.*, during reabsorption and retranslocation of N from senescing and older age-classes of foliage [15, 45]). Our ^{15}N -tracer results and those from ^{15}N fertilization [21–23] and N mass balance studies [20] suggest that there is considerable translocation of tree ring N in both radial directions in conifers, and that this redistribution of N among rings could confound the total N and natural ^{15}N abundance signals preserved in growth rings. Data from all these studies suggest that the movement of N from an individual tree ring is relatively systematic. Hence, it may be possible to mathematically remove the translocation “noise” from the isotopic “signal” that represents the N pool taken up during the year that ring was formed.

Despite the retranslocation noise present in the analysis of whole wood or extracted wood samples, there still appears to be a fairly consistent trend of decreasing natural ^{15}N abundance of tree rings over time from different sites. Peñuelas and Estiarte [17] interpreted this decreasing trend in $\delta^{15}\text{N}$ values in tree rings and a decrease in N concentration of plants in herbarium samples over the 20th Century as indicative of decreased ecosystem N loss and increased N fixation and mineralization over this period. Poulson *et al.* [36] hypothesized that the decreasing $\delta^{15}\text{N}$ in tree rings over time was the result of increased input of isotopically light N from the atmosphere over this period. We were unable to correlate estimates of N deposition at our site with these trends, but this failure may be due to inaccuracies in our N deposition estimates. However, until the controls on retranslocation can be understood, we caution the use of ^{15}N signatures in tree rings as a tool for assessing long-term trends in N availability at a site. Furthermore, the large within-site variability in the temporal patterns of $\delta^{15}\text{N}$ among individual trees might also limit the use of these values in comparative studies across sites [46].

Decreasing ^{15}N abundance in growth rings from labeled trees over time (Fig. 5) might also be interpreted as a decrease in residual uptake of the label over time, and not to N assimilated primarily from the soil during the 1985 growing season and then retranslocated among tree rings over time. However, a few lines of evidence suggest that retranslocation is the dominant mechanism responsible for this pattern. Firstly, some of the ^{15}N label was found in the ring formed prior to the addition of the label. Considerable accumulation of ^{15}N in rings formed prior to the addition of a ^{15}N label has been found in other studies as well [21–23]. Secondly, most ^{15}N fertilizer studies suggest that the vast majority of the label is taken up the first year following application. For instance, Preston and Mead [47] found no change in the total ^{15}N assimilated from soil between the end of the first and eighth growing seasons following a

winter application of 10 g N/m^2 to 11-year-old lodgepole pine (*Pinus contorta*) trees. In our study, where the label was applied directly to fine roots, the proportion of the added label assimilated during the first growing season should have been even greater. It is interesting to note, however, that the maximum $\delta^{15}\text{N}$ values in the labeled trees occurred in tree rings formed the year after the label was applied. This lag in translocation to stem wood was not observed by Mead and Preston [22] in their winter application of ^{15}N to lodgepole pine trees. It is possible that we applied the label too late in the growing season for the label to be translocated to stem wood. Measurements of the ^{15}N content of foliage taken after the first growing season in October, 1985 showed that about 2% of the added label was recovered in the foliage that first year, similar to the amount recovered in the entire stem wood in 1999. It is highly unlikely that this pattern could be an artifact of a missing or uncounted ring in the increment cores because all of the rings were wide (Fig. 1), had clear separation between early and late wood, and several expert dendrochronologists at Northern Arizona University verified our growth-year enumeration.

$\delta^{13}\text{C}$ values in tree rings

We measured the $\delta^{13}\text{C}$ values of tree rings along with $\delta^{15}\text{N}$ primarily to determine if the isotopic signals of these two elements would covary over time, indicating a possible coupling between tree physiological condition and soil N availability. We did find a significant correlation between these two isotopes in one of the unlabeled trees but not in the other. Poulson *et al.* [36] also found a significant correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in one of the eastern hemlock trees they studied ($r=0.687$, $p<0.001$, $n=37$) but not in the other ($p=0.960$, $n=33$; statistics calculated from their published data). Hence, it appears that the natural abundances of these two isotopes are correlated sporadically among trees at a given site. Other researchers have also found idiosyncratic covariance between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of leaves (*e.g.*, [29]).

The decline in $\delta^{13}\text{C}$ values over time observed in our fairly young ponderosa pine trees is similar to that found in some older trees, where there has been a general decline in $\delta^{13}\text{C}$ since about 1940 [17, 36, 48, 49]. These trends have been broadly interpreted as responses to changes in air temperature and relative humidity over that period [49]. Others have attributed the observed tree-ring $\delta^{13}\text{C}$ decline over time as a result of the decline in $\delta^{13}\text{CO}_2$ of the atmosphere caused by continued release of isotopically depleted C from fossil fuel combustion [41, 50–54]. Nevertheless, temporal trends in tree ring $\delta^{13}\text{C}$ values are certainly not universal. For example, Livingston and Spittlehouse [55] found no systematic temporal variation of $\delta^{13}\text{C}$ in 30-year-old Douglas-fir tree rings at two sites located in a rain shadow on Vancouver, Island, Canada. The lack of a temporal trend observed in some studies may be due to the opposing effects of multiple environmental factors on the $\delta^{13}\text{C}$ value of assimilated C in tree leaves [56], as well as the influence of the changing $\delta^{13}\text{CO}_2$ value of the atmosphere over time [41, 50–54].

The initial increases in $\delta^{13}\text{C}$ values in the oldest rings in the trees we studied are consistent with the “juvenile effect” observed by many other researchers [6]. Several explanations for the juvenile effect have been proposed, including intrinsic age-related physiological effects, increasing gradient of CO_2 concentrations near the ground due to soil respiration, and changing proportions of sun and shade leaves in the crown [6, 48]. We speculate that the early increase in $\delta^{13}\text{C}$ values of wood in these widely spaced, young ponderosa pine trees occurs because of increasing drought stress as the saplings accumulated new leaf area faster than they accrued new root area. This would lead to increased water stress in the plant, reducing stomatal conductance and increasing $\delta^{13}\text{C}$ values [49]. Furthermore, we speculate that the sudden drop in the $\delta^{13}\text{C}$ values that occurred in 1989 resulted from the trees reaching a

sufficient size to access deeper stored water pools in lower soil horizons, improving plant water status and leading to higher stomatal conductance and lower $\delta^{13}\text{C}$ values. This pattern is consistent with the fact that trees in this region rely on stored soil water pools for water uptake during the growing season due to low amounts of precipitation during the summer [57]. Alternatively, greater numbers of shaded leaves in more mature crowns might also explain the decline in $\delta^{13}\text{C}$ values in tree rings over time. As crowns expand, a greater proportion of the leaves of a tree are shaded, and this can result in a general increase in stomatal conductance at the tree level and a corresponding decline in $\delta^{13}\text{C}$ values of assimilated C [48]. The significant negative correlation between $\delta^{13}\text{C}$ values and mean annual precipitation but not air temperature parameters is consistent with water availability differences driving the major changes in $\delta^{13}\text{C}$ values in tree rings at our site. Water balance parameters have explained a majority of the variance in tree ring $\delta^{13}\text{C}$ values in several other studies [8, 48, 55, 58]. Furthermore, we found a highly significant negative correlation between $\delta^{13}\text{C}$ values in tree rings and tree growth (*i.e.*, stem wood production), which has also been found by several other investigators [6, 48, 55]. This relationship is likely due to the strong linkage between stomatal conductance and both stem wood production and the $\delta^{13}\text{C}$ values of trees in forests where water availability limits tree growth [48, 55, 57].

CONCLUSION

We found significant interannual variation in the natural ^{15}N abundance values of ponderosa pine tree rings. Although the trees were only meters apart, there was substantial absolute difference in their ring $\delta^{15}\text{N}$ values over time. Additionally, the temporal patterns were poorly correlated between the two trees over the 16 years studied. Changes in the natural ^{15}N abundance found in tree rings did not coincide with estimates of N deposition from the atmosphere over time, but did correlate with changes in $\delta^{13}\text{C}$ values in one, but not the other, unlabeled tree. More individual trees need to be assessed across sites of contrasting fertility to fully evaluate the possible linkages between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in tree rings and their potential ecological significance. Tracer additions of ^{15}N confirmed that inter-ring translocation of N assimilated from the soil is substantial in ponderosa pine sapwood. Removal of wood extractives decreased the $\delta^{15}\text{N}$ values slightly and consistently, and hence will not likely alter the temporal pattern in $\delta^{15}\text{N}$ observed among annual rings. However, given that the translocation pattern we found was systematic across all three labeled trees, it still may be possible to remove the isotopic “noise” due to inter-ring N translocation and generate a useful ^{15}N isotopic “signal” for assessing qualitative changes in N cycling rates in forests over the long term. However, a much stronger mechanistic understanding of retranslocation processes among tree rings needs to be developed before the natural ^{15}N isotopic signal can be interpreted unequivocally.

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