

We are grateful to the editor Dr. Edzo Veldkamp and reviewers Drs. Hietz and Pena for their time, expertise, and thoughtful input on our manuscript. We have worked diligently to carefully consider each of the many individual suggestions, and in nearly all cases we incorporated revisions accordingly toward what we believe is an improved manuscript. In the few cases where we disagreed with the reviewer critiques, we modified the manuscript to articulate our rationale and interpretation with greater clarity and expanded justification, and specific rebuttals are detailed in the responses to reviewers text below. Based on these responses to reviewers, and the manuscript changes we propose, as articulated below, we hope the editor will find the revised manuscript to be suitable for publication.

Below, the reviewer comments are copied verbatim in black. We have responded to each comment below in blue, and summarized specific changes made to the manuscript in green.

Author Responses to Peter Hietz (Referee)
(Responses in Blue) (Summary of revisions in green)

The biogeochemical cycle of nitrogen is complex and influenced by human activities in various ways. Understanding and monitoring the slow impact of human interference on N accumulation and losses is important, but limited by the lack of long-term records. A number of studies have therefore measured N15 signals in tree rings to investigate changes that may be interpreted as N availability or losses. The isotopic effects of various processes in the N cycle are variable and not perfectly understood, but reasonably well to draw some general conclusion from differences in N15 signals, or changes thereof, in soils or plants. That said, interpreting any N15 trends in wood poses additional questions and is not as straightforward as with C13. First N concentrations in wood are very low so precise isotopic analysis is more challenging than for C isotopes which means a higher signal-to-noise ratio. Second, at least in sapwood N is somewhat mobile so N isotope in a growth ring do not necessarily reflect the year the wood was formed. From the little that is known it is reasonably assumed that only a small proportion is translocated or not across many years. Third, while N in heartwood is likely limited to cell walls, in sapwood some is in living cells (and presumably more mobile) so concentrations in more recently produced wood tends to be higher (as seen in Fig. 5). Very little is known about related changes in N15 signals of wood. Fourth, changes in N15 might also carry a source signal (N15 trend in deposition). Finally, the discrimination during uptake and transfer in different species can be variable. Trends in N15 in wood are thus open to many interpretations unless some or all of the confounding factors can be controlled and studies such as the one by Tumpeter et al. that do so at least help to clarify how useful trends in tree ring N15 are.

We are grateful for the careful and encouraging review provided by Dr. Hietz.

While the study by Tumber et al. found a clear effect in sapwood on N concentrations, this appears to be unrelated to changes in wood N15, which are all seen in heartwood, so the inter-annual mobility and sapwood/heartwood differences are unlikely to be an issue. Given that the trees are from a small area, changes in N deposition and the N15 signal thereof at least should not affect differences in N15 trends. Also, they used only one species, so at least we should not expect differences in N15 found to be a species effects. That said, the previous documentation of species-specific trajectories (McLauchlan & Craine, 2012 Biogeosciences, 9, 867-874) where N15 can go up in one species while going down in another pours cold water on the use of tree ring N15 as an indicator of changes in the N cycle or availability and questions many interpretation of N15 trends one might suggest. Tumber et al. found significant differences in N15 trends between treatments. Frustratingly, the patterns seen do not offer an easy interpretation and a relationship with fire appears elusive. In the low-fire regime, wood N15 changes approx. with the onset of burns. However, N15 changed at the same time in the no-burn regime, in the medium-fire regime the shift in N15 was much earlier than the burning regime, and in the high-frequency fire there was no change in N15 (Fig. 6). Having to reject their initial hypothesis, they discuss alternative causes of the observed N15 trends, specifically changes in vegetation. Still, if vegetation change has something to do with the fire regime, we would expect an effect to be somehow related to the time or intensity of the burns. Data on the change in vegetation cover might help to support or refute this idea, but are not presented. I am not sure if the aerial photographs available or other records could be analyzed for this.

Historical aerial photos are available for our study area for the years 1938, 1954, 1973, 1991, 2015, and 2016. While we did not conduct a quantitative analysis of landcover as evidenced in these photos, we did conduct a thorough qualitative interpretation of them. For simplicity, we elected to only include the end member years, 1938 and 2016, in Figure 3. Images from the intervening years, not shown, illustrate the intermediate steps of the patterns we discuss in the text, whereby canopy density increased through time in the no-fire, low-fire, and medium-fire stands but remained relatively constant in the high-fire stand. Our results are in general agreement with the results from Peterson and Reich (2001) at our site and Faber-Langendoen and Davis (1995) from an adjacent experimental site. We have added this reference and expanded our interpretation in the manuscript accordingly:

We revised the text starting on p4, l1 to read: “Although systematic vegetation inventories were not conducted prior to the onset of prescribed burning, a series of historical aerial photographs are available for our study area between the years 1938 and 2016 (McAuliffe et al., 2017; MHAPO, 2019). Our qualitative interpretation of these photographs indicates that the savanna vegetation prior to fire treatment at CCESR consisted of mixed woodland and grass communities (Fig. 2). Over time, oak tree vegetation – predominantly bur oak, *Quercus macrocarpa*, and northern pin oak, *Quercus ellipsoidalis* – increasingly dominated fire-excluded stands, while C₄ grasses and sedges increased in abundance in frequently burned stands (Peterson and Reich, 2001; Dijkstra et al., 2006).”

We revised the text starting on p15, l16 to read: “Our qualitative assessment of aerial photographs between 1938 and 2016 indicates that canopy density increased through time in the no-, low-, and medium-fire stands but remained relatively constant in the high-fire stand. Our interpretation is in general agreement with results from other studies at CCSR (Peterson and Reich, 2001) and a directly adjacent experimental site with the same soil, topography, and plant community characteristics (Faber-Langendoen and Davis, 1995).”

A rather serious limitation of the study is that there are no replicates of fire treatment. The four trees per treatment were not from different plots, spatially quite close and at a substantial distance from trees sampled for other treatments. Unfortunately, there is a spatial variability in N15 as trees from different location differ substantially in wood N15 before any treatment. If this also affects N15 trends we do not know, but in the end the data do not convincingly show that N15 trends are related with fire regimes at all.

We agree that the lack of fire treatment replicates imposes a limitation on the interpretation. With the limited resources available for this research project, we made a conscious decision in designing the study to maximize the temporal extent of sampling and evaluate $\delta^{15}\text{N}$ across a range of burn frequency treatments. We did not know beforehand how much variation in $\delta^{15}\text{N}$ values would exist among trees with treatment units. We still believe our sampling design represents a valuable contribution to the literature because it is among the most spatially and temporally dense sampling of tree-ring $\delta^{15}\text{N}$ at a single site.

The rationale of our study design was not adequately articulated in the manuscript. Knowing what we do now, we would advocate that a future study should (1) include replicates of fire treatment and (2) sample trees that were more spread out within treatments. We have expanded the manuscript text with discussion of this limitation:

We added the following text starting on p16, l2: “The relative close proximity of sampled trees within treatment stands may also contribute to the ambiguous relationship between fire and wood $\delta^{15}\text{N}$ at this site. The four trees sampled per burn stand were spatially quite close to each other (on average 34 meters between trees within each burn unit) and we were unable to replicate sampling in other units with the same fire treatment. Given the spatial variability in wood $\delta^{15}\text{N}$ that existed before the fire treatment began, additional replicates of fire treatment may help to clarify the relationship between fire and wood $\delta^{15}\text{N}$.”

The results are presented in a useful way for readers to understand and make up their minds. For me, the clearest message of the manuscript is pointing to the challenges and perhaps limitations of using tree ring N15 and this as well as technical limitations should be acknowledge in the discussion.

We agree that the challenges and limitations of using wood $\delta^{15}\text{N}$ were not made explicit in our original submission. To clarify for the reader and future related studies, we now acknowledge these issues in the discussion section:

We added the paragraph starting on p17, l3: “Recent studies have shown mixed results regarding the reliability of wood $\delta^{15}\text{N}$ as a proxy for terrestrial N availability. In some settings wood $\delta^{15}\text{N}$ appears to reliably integrate spatiotemporal variation in soil N supply relative to plant demand (Elmore et al., 2016; Kranabetter and Meeds, 2017; Sabo et al., 2020), whereas other results suggest an inconsistent relationship between wood $\delta^{15}\text{N}$ and N cycling (Tomlinson et al., 2016; Burnham et al., 2019). Our study sought to clarify the relationships between N availability, wood $\delta^{15}\text{N}$, and long-term disturbance by sampling across four treatment stands to control for the presence and frequency of prescribed burning. Contrasting trends in heartwood $\delta^{15}\text{N}$ across our treatment stands suggest minor roles for the heartwood-sapwood transition and exogenous drivers of wood $\delta^{15}\text{N}$ at this study site. Nonetheless, the apparent lack of wood $\delta^{15}\text{N}$ response to repeated burning at our study site, particularly in the high fire frequency treatment, raises uncertainty about the ability for individual tree-level $\delta^{15}\text{N}$ to record the effects of low intensity fires on N cycling. We recommend that future studies of N cycling using wood $\delta^{15}\text{N}$ utilize diligent site selection criteria to control for as many confounding factors as possible to help clarify the processes controlling variation in wood $\delta^{15}\text{N}$ values.”

Other than that, I suggest a number of relatively small changes or additions that might help interpret the data.

Table 2: please add the treatment (burn regimes) to the individual trees (this is in Table 1) or mark the groups of 4 in some way so that it is easy to compare groups.

Table 2 has been revised to include this information.

Why is $p > 0.05$ considered significant for the MK test for trends but $p < 0.01$ for the Student t-test for shifts in mean?

Thank you for highlighting this unintended inconsistency. We have revised the significance level for the MK test to $p < 0.01$. Testing for significant increases in wood %N at $p < 0.01$ yields similar results: wood %N increased significantly in 11 out of 16 trees at $p < 0.01$ compared to 12 out of 16 trees at $p < 0.05$.

Page 9/ line 12 “Standardizing wood ^{15}N for each tree by subtracting the tree mean ^{15}N from each data point produced similar results: standardized ^{15}N values were different among stands in 1902 ($p = 0.039$).” If you standardize by subtracting the mean, the standardized mean will be the same for all groups. If the values from 1902 are not the same (as the mean of the time series), this simply says that there were different trends, but this is better shown in the trends rather than some standardized values, so simply drop this.

This sentence was a carryover from an early version of the manuscript, attempting to clarify methods relative to a few other key studies. We feel this point is superfluous and we have dropped this sentence from the manuscript.

"Tree age did not have an effect on the mean or trend of $\delta^{15}\text{N}$." This is reassuring, but how was this tested and where is this shown? If there is a trend in individual trees, how do you distinguish an age from an environment effect? Looking at the data, age effect is a very implausible explanation for the trends found, which might be stated.

Thank you for raising this point, which was not sufficiently outlined in the manuscript. As the reviewer implies and the data indicate, the age effect is an implausible explanation for the wood $\delta^{15}\text{N}$ trajectories. The temporal wood $\delta^{15}\text{N}$ patterns in Fig. 6a-c do not appear as a gradual age-related effect, but rather as relatively invariant values, a shift to lower values, followed again by relatively invariant values. Additionally, Fig. 6d does not show any trends that could be related to tree age.

We have added the following text starting on p10, l3: "Two approaches revealed that tree age likely did not have an effect on the mean or trend of $\delta^{15}\text{N}$. First, we compared wood $\delta^{15}\text{N}$ with tree biological age rather than Gregorian calendar year using inner ring dates to estimate tree age. Tree age and $\delta^{15}\text{N}$ were negatively correlated ($r = -0.35$; $p < 0.001$), however this negative relationship may be expected given the negative temporal trends in $\delta^{15}\text{N}$ in the majority of trees. Additionally, we tested for an age effect on wood $\delta^{15}\text{N}$ by dividing trees into four age classes based on ring counts (Table 1, Table 2). A Kruskal-Wallis test of wood $\delta^{15}\text{N}$ across age classes revealed that tree age did not have a significant effect on the mean ranks of $\delta^{15}\text{N}$ ($p = 0.242$)."

Table 1 and Table 2 were revised to include tree age class information with the following description: "Trees in age class 1 contain <129 rings, trees in age class 2 contain 130-159 rings, trees in age class 3 contain 160-189 rings, and trees in age class 4 contain > 190 rings".

9/22 "Declines in wood $\delta^{15}\text{N}$ were roughly synchronous in these stands, beginning primarily between 1940 and 1965" I would not call events that differ by 20 years or more to be synchronous, not even roughly.

We agree. We followed the suggestion of the reviewer by removing mention of synchrony here and throughout the manuscript.

Wood % N was negatively correlated with wood $\delta^{15}\text{N}$ across all trees. This is potentially problematic: if there is a trend in %N that is caused by (recent) sapwood having higher N, and N and $\delta^{15}\text{N}$ are correlated, you might get trends in $\delta^{15}\text{N}$ that are related to changes in %N from heartwood to sapwood and not driven by external changes. Ideally, heartwood (the last 20 yrs or so) should thus be excluded. Given that changes in $\delta^{15}\text{N}$ occurred much earlier than 20 yrs ago, this appears not to be a problem, but should perhaps be mentioned in the discussion. In any case I suggest to test if the $\text{N} \sim \delta^{15}\text{N}$ relationship also holds true if you exclude sapwood.

We further examined the relationship between wood $\delta^{15}\text{N}$ and %N, as suggested.

We added the sentence starting on p13, l14: “Excluding all samples from the sapwood, wood % N and wood $\delta^{15}\text{N}$ remained negatively correlated, statistically significant although the relationship weakened ($r = -0.30$; $p < 0.001$).”

We added the sentences starting on p13, l32 : “Wood % N and $\delta^{15}\text{N}$ were negatively correlated across all samples and this negative relationship diminished when excluding samples from the sapwood. Although we do not have further evidence to suggest that this is a meaningful relationship, future studies should examine relationships between wood % N and $\delta^{15}\text{N}$ because their covariance could be problematic if trends in $\delta^{15}\text{N}$ are strongly related to % N in addition to external factors.”

“Higher resolution sampling using annual or sub-annual growth rings may provide greater insights into short-term effects of fire on wood $\delta^{15}\text{N}$ and N availability and is recommended for future study” – I do not see how this would improve the outcome and would rather invest more analyses in sampling more trees and sites. Perhaps even pool wood across several years to dampen noise caused by short-term inter-annual variation, unless this is of specific interest (mostly not).

We agree with this critique. Higher resolution sampling may provide greater insights into the short-term effects of fire, although there is substantial uncertainty surrounding this question of N translocation. Translocation of N likely limits the extent to which annual wood $\delta^{15}\text{N}$ patterns reflect inter-annual disturbances to the N cycle. We clarify in the manuscript:

We added the following sentences starting on p16, l24: “It is uncertain whether higher resolution sampling using annual growth rings would provide greater insights into short-term effects of fire on wood $\delta^{15}\text{N}$, as N translocation may smooth out inter-annual disturbances to the N cycle.”

In the discussion studies that looked at N mineralization and total N at specific (and different) time points in the experiment are mentioned. These are published data, but it would be useful to present them together with (perhaps only recent) wood N15 in a table or figure to see if there is any relationship.

Thank you for raising this point, we agree that this is a data rich site and it would be beneficial to add more data for comparison. We included a new table with data from Reich et al. (2001) who examined net N mineralization rates in three of four stands that we sampled in 1995, as well as data from Pellegrini et al. (2020) who gathered soil % N and soil $\delta^{15}\text{N}$ data at multiple soil depths in two of four stands that we sampled in 2016. Although these additional data add context to our results, they ultimately do not clarify interpretation of our observed wood $\delta^{15}\text{N}$ patterns.

Year	Data	Soil Depth (cm)	Low-fire	Medium-fire	High-fire
1992	Wood $\delta^{15}\text{N}$	NA	-2.46	-1.06	0.13
1995	Net N mineralization	0–15	7.21	10.42	2.31
1997	Wood $\delta^{15}\text{N}$	NA	-1.84	-1.94	-0.76
2016	% N	0–5	0.12	NA	0.1
2016	% N	5–10	0.06	NA	0.06
2016	% N	10–20	0.05	NA	0.06
2016	% N	20–60	0.02	NA	0.04
2016	% N	60–100	0.01	NA	0.03
2016	$\delta^{15}\text{N}$	0–5	1.13	NA	2.25
2016	$\delta^{15}\text{N}$	5–10	4.15	NA	3.57
2016	$\delta^{15}\text{N}$	10–20	4.76	NA	3.85
2016	$\delta^{15}\text{N}$	20–60	NA	NA	5.14
2016	$\delta^{15}\text{N}$	60–100	NA	NA	2.93
2017	Wood $\delta^{15}\text{N}$	NA	-2.54	-1.58	-0.32

Table 4. Comparison of previously collected soil N data and our wood $\delta^{15}\text{N}$ data from CCESR. Values represent the mean of all samples within each burn unit. Net N mineralization data from Reich et al. (2001), and soil % N and $\delta^{15}\text{N}$ data from Pellegrini et al. (2020).

We revised the paragraph starting on p15, l1 to reflect these additional data. This paragraph now reads: “Prior studies at CCESR have examined N cycling in the same stands we sampled for wood $\delta^{15}\text{N}$ across the burn experiment. Reich et al. (2001) measured net N mineralization in 1995 in three of the four stands we sampled (low-, medium-, and high-fire). Net N mineralization decreased nonlinearly with increasing fire frequency among these stands. However, our wood $\delta^{15}\text{N}$ samples in 1992 and 1997 increased with increasing fire frequency among these stands. Wood $\delta^{15}\text{N}$ values from 1992 and 1997 were lower in the low- and medium-fire stands compared to the high-fire stand, despite relatively higher net N mineralization in the former stands in 1995 (Table 4; Reich et al. 2001). This could point to large inter-annual variation in wood $\delta^{15}\text{N}$

or that soil net N mineralization may not be strongly related to wood $\delta^{15}\text{N}$ at our study site. More recently, Pellegrini et al. (2020) measured soil $\delta^{15}\text{N}$ and cumulative N stocks at multiple depths in two of the four stands we sampled (low- and high-fire) in 2016. They found $\delta^{15}\text{N}$ decreased with more frequent burning and $\delta^{15}\text{N}$ increased with depth across the entire fire frequency gradient, while fire did not have a clear effect on total soil N between the low- and high-fire frequency stands we sampled (Pellegrini et al., 2020). In contrast, our wood $\delta^{15}\text{N}$ in 2017 was significantly lower in the low-fire stand compared to the high-fire stand and we found limited evidence for variation in wood $\delta^{15}\text{N}$ related to long-term fire frequency. Altogether, these nuanced results from previous studies add context but do not clarify interpretation of our observed wood $\delta^{15}\text{N}$ patterns.”

Author Responses to Rodica Pena (Referee)
(Responses in Blue) (Summary of revisions in green)

The manuscript by Trumper et al. reports on a fascinating study examining the ^{15}N enrichment in the savanna oak wood rings under the influence of fire events of different frequency. The basis of this study is the assumption that ^{15}N levels in the wood are determined by the ^{15}N enrichments of the taken-up N. Thus, interrogating the wood ^{15}N enrichments, the authors may catch a glimpse of the effect of fire frequency on the soil N cycling or directly N availability in the soil. They demonstrated once more that the complex contributions of various processes in the nitrogen cycle make impossible assigning the same pattern of ^{15}N enrichments to different trees/plots. This has been the case, even if the trees were from plots that look similar in overstory vegetation, topography, and soil properties (1st part of the hypothesis). Maybe it depends a bit more on the soil properties. The authors may want to show the soil characteristics in the four plots.

We are grateful for the careful and encouraging review provided by Dr. Pena.

We have added some clarifying description of the soil characteristics in the four plots:

In the revisions, we added the following text starting on p5, l10: “Trees in the low-fire stand grew on Sartell fine sand mapped in soil surveys with 0–6% slopes and trees in no-, medium-, and high-fire stands grew on Sartell fine sand mapped in soil surveys with 6–15% slopes. In the localized vicinity of the trees sampled across these sites, slopes rarely exceeded 8%. The Sartell series consists of excessively drained soils that are rapidly permeable and have low available N, low organic matter content, and low available water capacity (Grigal et al., 1974). The upper 15 cm of Sartell fine sand has a pH of 5.3, 0.025% total nitrogen (18 $\mu\text{mol/g}$ dry soil), and ~0.3% organic matter (Grigal et al., 1974; Tilman, 1984).”

The 2nd part of the hypothesis, based on the assumption that soil N availability decreases (on long timescale) after burning, predicts a decrease in the wood ^{15}N proportionally to the fire frequency. My major concern is that the experimental design does not enable precise statistical testing of this hypothesis. This is because there are

no replicates for the treatments (only one plot with four trees) and the plots, localised at different distances, bring a high heterogeneity in the analysis anyhow. I do not think that the way the authors try to present the testing of this hypothesis is the most adequate. I am surprised by the regression analysis between a continuous ($\delta^{15}\text{N}$) and a categorical (Year) variable. I suggest evaluating the effect of time but no differences among treatments, using the trees as replicates in a repeated measured ANOVA. We all know the high variability among measurements that make the data difficult to meet the normality assumptions of ANOVA, but you can give a try. There is also a possibility to use GLMM and consider the tree as a random factor. I see in the text different p-values or expressions referring to the comparison among treatments (i.e., stands). I do not understand how were those comparisons done.

We agree that the lack of fire treatment replicates is a limitation. We addressed this concern, as described above in response to a similar comment from the first reviewer. In terms of regression with a categorical variable, this is not uncommon in the literature (Cottingham et al., 2005; McLauchlan and Craine, 2012; Howard and McLauchlan, 2015). Furthermore, we believe that our nonparametric analytical approach is both conservative and pragmatic in terms of expectations for evaluation of results. We describe our approach to post-hoc testing in greater detail in the revised version:

We added the following sentences starting on p9, l4: “A Kruskal-Wallis test of wood $\delta^{15}\text{N}$ across the time series for the four stands revealed that $\delta^{15}\text{N}$ was significantly different among stands ($p < 0.001$). We used the nonparametric Games-Howell test for post-hoc analysis due to unequal variances and group sizes between burn treatment stands. The Games-Howell test revealed that $\delta^{15}\text{N}$ in the no-fire stand was lower than all other stands ($p < 0.001$).”

I miss a table/figure presenting the mean value or min, median, max of data in each plot at each time point.

While we strived to ensure that the time series plots represented clearly legible values for individual and population data (including minimum and maximum values), we have created a new table of mean wood $\delta^{15}\text{N}$ in each plot at each time point.

Year	No-fire mean $\delta^{15}\text{N}$	Low-fire mean $\delta^{15}\text{N}$	Medium-fire mean $\delta^{15}\text{N}$	High-fire mean $\delta^{15}\text{N}$
1902	-2.19*	0.38	1.65	-1.16
1907	-2.91*	0.54	2.21	-1.58
1912	-1.14*	0.77	1.93	-0.98
1917	-0.03	1.19	1.94	-0.46

1922	-1.81	0.86	1.32	-1.24
1927	-0.7	0.98	1.37	-0.09
1932	-1.24	1.17	1.37	-0.53
1937	-0.65	0.94	1.25	-0.66
1942	-1.06	0.07	0.9	-0.62
1947	-1.27	1.26	0.26	-0.5
1952	-1.54	-0.18	-0.68	-0.72
1957	-1.63	0.05	-0.35	-0.09
1962	-0.9	-0.4	-0.94	-0.71
1967	-2.34	-2.2	-2.07	-0.6
1972	-3.12	-2.35	-2.06	-0.47
1977	-3.15	-2.54	-1.94	-0.67
1982	-2.81	-2.09	-2.14	-0.91
1987	-2.6	-2.45	-2.25	-1.24
1992	-3.08	-3.38	-1.9	-1.32
1997	-3.28	-2.65	-2.29	-1.33
2002	-3.3	-3.25	-2.44	-1.15
2007	-3.2	-3.04	-2.41	-0.93
2012	-3.27	-3.16	-2.54	-0.83
2017	-3.53	-2.87	-2.16	-1.03

Table 3. Mean wood $\delta^{15}\text{N}$ (‰) by year in each burn unit. Mean values * indicate years with only one $\delta^{15}\text{N}$ sample in that year.

The authors acknowledged that the fire differentially affects N soil cycle on a short vs long timescale. I have understood the reason, but I have missed the algorithm of their selection of the two samples per decade.

In order to clarify our rationale, we have added text, as indicated below. Our decision to select two samples per decade was motivated by two factors. First, we had to optimize the spatial and temporal coverage of sampling against analytical cost constraints. Choosing two wood $\delta^{15}\text{N}$ samples per decade rather than annual sampling allowed us to expand our temporal coverage and sample more trees. Second, N translocation likely inhibits true annual resolution of wood $\delta^{15}\text{N}$ as a proxy of N availability. Two samples per decade therefore seemed like a reasonable trade-off between temporal resolution and the length and spatial coverage of the record.

This section starting on p6, l10 was revised to read: “Although wood samples were partitioned at annual resolution, we did not analyze all wood samples for $\delta^{15}\text{N}$ due to cost and time constraints. Rather, we selected two wood samples per decade for $\delta^{15}\text{N}$ measurement. The decision to forego annual resolution and analyze our data at supra-annual timescales also aimed to mitigate the known phenomenon of inter-ring mobility of N-containing compounds that could smooth out inter-annual variation in $\delta^{15}\text{N}$ (Hart and Classen, 2003; McLauchlan et al., 2017).”

Specific comments Page 2-line 18: The main factor is the reduced losses that result in a lower enrichment of the soil N pools, the mycorrhizal transfer is added to that.

We agree with this point and the primary importance of reduced N losses was not sufficiently expressed in this section. We propose the following revisions:

This section starting on p2, l18 now reads: “When N availability is low, reduced N losses result in lower $\delta^{15}\text{N}$ values of remaining N pools. In addition, plants are more likely to receive N from mycorrhizal fungi than from direct uptake from inorganic N pools; mycorrhizal fungi are known to provide N with relatively low $\delta^{15}\text{N}$ values to plants (Hobbie and Högberg, 2012).”

Fig 1 & Fig 2: Fig 1 is almost identical with the figure from van der Sleen et al. 2017 (you probably need the copyright). The predictions shown in Fig. 2 are based on your box additions to the Fig 1. Maybe it would be sufficient enough to include those boxes as an inset in Fig 2.

We agree that Fig. 1 of the manuscript is quite similar to the figure from van der Sleen et al. (2017). Rather than pursue copyright permission, we removed Fig. 1 from the manuscript. In addition, we moved the boxes from the old Fig. 1 to the new Fig. 1. These steps address both suggestions from the reviewer and we believe that the relevant fire- $\delta^{15}\text{N}$ processes are summarized well in the new Fig. 1.

The revised Fig. 1 is shown below:

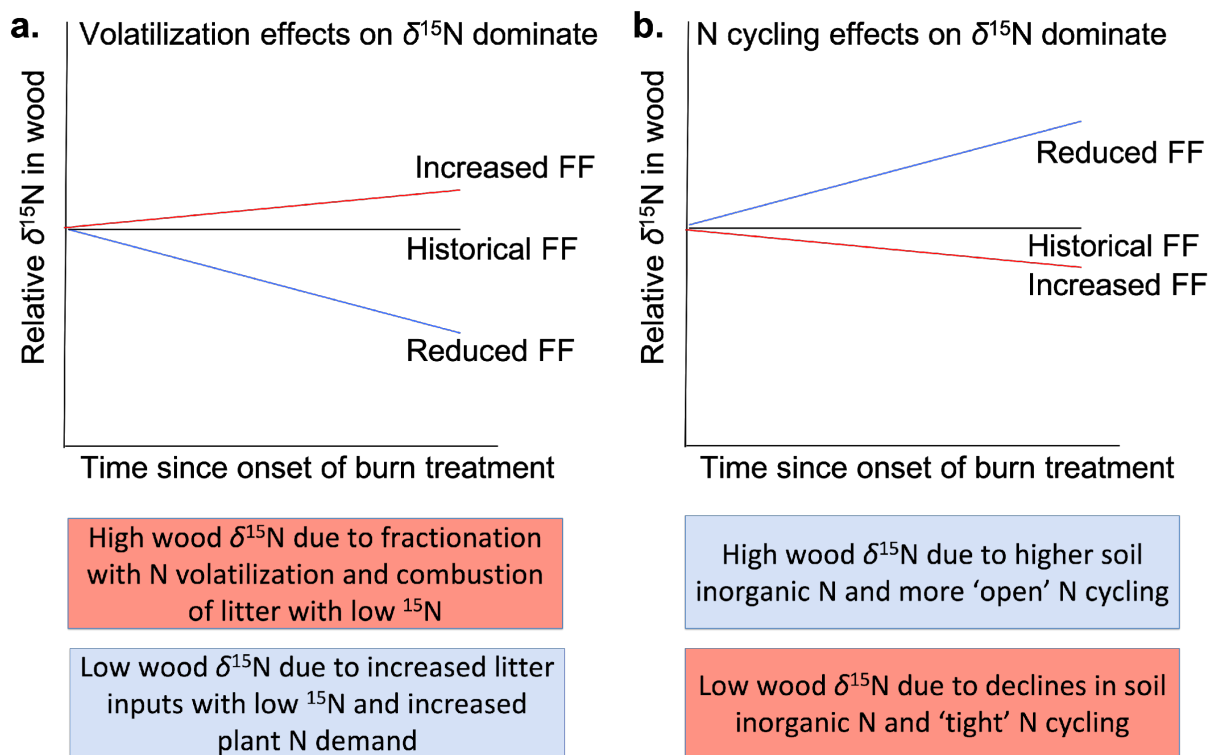


Figure 1: Hypothesized effects of fire experimentation on wood $\delta^{15}\text{N}$ values relative to the historical fire frequency (FF). Increased FF would lead to higher relative wood $\delta^{15}\text{N}$ values if combustion and isotopic fractionation with N volatilization dominate (A). In contrast, increased FF would lead to lower relative wood $\delta^{15}\text{N}$ values if N cycling effects such as reduced N stocks and non-fire N losses dominate the trajectory of wood $\delta^{15}\text{N}$ values (B). Colored boxes indicate potential contrasting effects of fire frequency on wood $\delta^{15}\text{N}$ values. Pink and blue boxes indicate processes resulting from higher and lower FF, respectively.

Page 3-lines 6-7: I think the soil N availability matters in those patterns. Maybe it is worth it to discuss the low N availability that is the case at CCESR.

Additional information regarding the soil N status at CCESR was included in the same revised section (above) that describes soil characteristics in more detail.

The following text was added starting on p6, l1: “The Sartell series consists of excessively drained soils that are rapidly permeable and have low available N, low organic matter content, and low available water capacity (Grigal et al., 1974). The upper 15 cm of Sartell fine sand has a pH of 5.3, 0.025% total nitrogen (18 $\mu\text{mol/g}$ dry soil), and ~0.3% organic matter (Grigal et al., 1974; Tilman, 1984).”

Page 6-line 19: a mean value of ring width would be helpful.

This was added.

The following sentence was added on p6, l8: “Mean ring width from 1902–2017 across all N cores was 1.11 mm.”

Page 6-line 20: Is this an issue here? The inter-ring $\delta^{15}\text{N}$ mobility is relatively restricted to the youngest rings.

Although inter-ring $\delta^{15}\text{N}$ mobility is relatively restricted to the youngest rings, we believe that this remains an issue because all of the rings were once young and therefore influenced by $\delta^{15}\text{N}$ mobility.

Page 8-line 20: the regime shift detection analysis is less known to some readers. Could you please add a short explanatory phrase.

Thank you for bringing this to our attention. We added a short clarification to the manuscript.

We added the text starting on p8, l7: “This method applies sequential *t*-tests to time series data to statistically identify shifts in the mean state (Rodionov, 2004).”

Page 9-line 3: In the case you used a nonparametric test, the median value is more appropriate to be shown.

The regime shift algorithm we used was a parametric test, therefore the mean value is appropriate. Although we chose nonparametric tests for testing significant mean differences and trend, we chose a parametric test for regime shift detection because this method has been shown to be quite robust to violations of the normality assumption of the data (Rodionov, 2004). We also felt that the apparent mean states in our data suggested that a test for mean differences was more appropriate than a piecewise regression approach.

Page 9-lines 10-20: please specify where are all these data displayed.

These data were not displayed in our original submission. Rather than creating a new figure, we more explicitly outline our post-hoc tests for $\delta^{15}\text{N}$ differences between burn treatment stands. We also summarize the corresponding range of p-values.

We added the following sentences starting on p9, l5: “We used the nonparametric Games-Howell test for post-hoc analysis due to unequal variances and group sizes between burn treatment stands. The Games-Howell test revealed that $\delta^{15}\text{N}$ in the no-fire stand was lower than all other stands ($p < 0.001$). In contrast, the Games-Howell test revealed nonsignificant differences in $\delta^{15}\text{N}$ between the low- and medium-fire stands ($p = 0.385$), low- and high-stands ($p = 0.944$), and medium- and high-fire stands ($p = 0.441$).”

Figure 5: please also include the heartwood-sapwood transition in the left panel.

We revised this figure accordingly.

Page 12-line 27: The correlation figure between N and $\delta^{15}\text{N}$ could be of interest as the negative correlation is a bit unexpected.

This was addressed above in response to a similar comment from the other reviewer.

Page 13-line 1: but see Meerts 2002(doi.org/10.1051/forest:2002059) and Martin et al. 2014 (doi.org/10.1111/nph.12943).

We appreciate the reviewer for bringing these papers to our attention. Our manuscript shows that wood % N increased in the sapwood in all individual trees. We suggested that this result matches well documented patterns in the literature. However, these papers shared by the reviewer show insignificant differences between heartwood % N and sapwood % N, indicating more mixed results in the literature. We revised the manuscript to reflect the uncertainty regarding wood % N variation within trees.

We revised the text starting on p13, l24 to read: “In contrast to wood $\delta^{15}\text{N}$, wood % N trajectories were positive and consistent among stands. Wood % N is not as commonly reported in wood N isotope studies because this metric likely incorporates tree physiology and thus may not reflect ecosystem N cycling (Gerhart and McLauchlan, 2014). Positive wood % N trajectories shown here match documented patterns of increasing wood % N after the heartwood-sapwood transition in the wood $\delta^{15}\text{N}$ literature (Gerhart and McLauchlan, 2014), yet this pattern has seen more mixed results in the wood chemistry literature (Meerts, 2002; Martin et al., 2014).”

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Century-scale wood nitrogen isotope trajectories from an oak savanna with variable fire frequencies

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Abstract. Fire frequency exerts a fundamental control on productivity and nutrient cycling in savanna ecosystems. Individual fires often increase short-term nitrogen (N) availability to plants, but repeated burning causes ecosystem N losses and can ultimately decrease soil organic matter and N availability. However, these effects remain poorly understood due to limited long-term biogeochemical data. Here, we evaluate how fire frequency and changing vegetation composition influenced wood

5 stable N isotopes ($\delta^{15}\text{N}$) across space and time at one of the longest running prescribed burn experiments (established in 1964). We developed multiple $\delta^{15}\text{N}$ records across a burn frequency gradient from precisely dated *Quercus macrocarpa* tree-rings in an oak savanna at Cedar Creek Ecosystem Science Reserve, Minnesota, USA. Sixteen trees were sampled across four treatment stands that varied in temporal onset of burning and burn frequency, but were consistent in overstory species representation, soil characteristics, and topography. Burn frequency ranged from an unburned control stand to a high fire-frequency stand that

10 burned in four of every five years during the past 55 years. Because N stocks and net N mineralization rates are currently lowest in frequently burned stands, we hypothesized that wood $\delta^{15}\text{N}$ trajectories would decline through time in all burned stands, but at a rate proportional to fire frequency. We found that wood $\delta^{15}\text{N}$ records within each stand were remarkably coherent in their mean state and trend through time. A gradual decline in wood $\delta^{15}\text{N}$ occurred in the mid 20th century in the no-, low-, and medium-fire stands, whereas there was no trend in the high-fire stand. The decline in the three stands did not

15 systematically coincide with the onset of prescribed burning. Thus, we found limited evidence for variation in wood $\delta^{15}\text{N}$ that could be attributed directly to long-term fire frequency in this prescribed burn experiment in temperate oak savanna. Our wood $\delta^{15}\text{N}$ results may instead reflect decadal-scale changes in vegetation composition and abundance due to early to mid 20th century fire suppression.

1 Introduction

Fire is a fundamental control of species composition, diversity, and nutrient cycling in savanna ecosystems. Fire affects N pools and cycling in myriad ways that vary spatially and temporally (Pellegrini et al., 2015). On timescales of days to years, fire often enhances N availability to plants – possibly due to direct ash deposition or increases in microbial mineralization (Wilson et al., 2002; Boring et al., 2004). Furthermore, the loss of plant biomass during fire reduces plant N uptake, thereby increasing inorganic N pools (Ficken and Wright, 2017). In the long-term (decades to centuries), fire may reduce ecosystem N stocks due to elevated N volatilization and leaching losses, as well as diminished plant biomass (Raison, 1979; Ojima et al., 1994; Reich et al., 2001). Changes in vegetation composition may also influence the trajectory of soil N biogeochemistry, as the production of plant litter with low N concentration can slow decomposition and net N mineralization, reducing N supply and inorganic N pools (Ojima et al., 1994; Dijkstra et al., 2006). However, there remains uncertainty regarding multi-year to multi-decadal effects of fire on N cycling in savanna ecosystems because there is a paucity of long-term records of N cycling from areas with different fire-return intervals (Reich et al., 2001; Coetsee et al., 2008).

In the absence of long-term baseline biogeochemical data, the ratio of ^{15}N to ^{14}N (i.e., $\delta^{15}\text{N}$) in wood from trees can provide a proxy of N pools and cycling that extends decades or centuries into the past (Gerhart and McLauchlan, 2014). Plant $\delta^{15}\text{N}$ can be a useful metric of plant N availability, defined as soil N supply relative to plant demand for N (Craine et al., 2015). When N availability is high, N is cycled and lost primarily as inorganic N, and relatively high rates of processes such as nitrification, nitrate leaching, ammonia volatilization, and denitrification lead to increased $\delta^{15}\text{N}$ values of remaining N pools (Craine et al., 2015; van der Sleen et al., 2017). When N availability is low, reduced N losses result in lower $\delta^{15}\text{N}$ values of remaining N pools. In addition, plants are more likely to receive N from mycorrhizal fungi than from direct uptake from inorganic N pools; mycorrhizal fungi are known to provide N with relatively low $\delta^{15}\text{N}$ values to plants (Hobbie and Högberg, 2012). Prior studies have used natural-abundance measurements of wood $\delta^{15}\text{N}$ to better understand the consequences of natural and human-induced disturbance to the N cycle, such as changes in N cycling due to fire (Beghin et al., 2011; Kranabetter and Meeds, 2017), tree-clearing (Bukata and Kyser, 2005; Hietz et al., 2010), and changing management practices (Howard and McLauchlan, 2015). These studies demonstrate the ability of tree-ring $\delta^{15}\text{N}$ values to capture the role of disturbance and land-use change in altering N cycling at local scales.

Demonstrated effects of fire on N cycling (Pellegrini et al., 2015) and ability of wood $\delta^{15}\text{N}$ to capture variation in N cycling suggest that long-term wood $\delta^{15}\text{N}$ records can provide insight on past effects of fire on N cycling. Yet few studies have focused on wood $\delta^{15}\text{N}$ response to fire, and thus the relative importance of fire-related processes that affect wood $\delta^{15}\text{N}$ remain poorly understood. On the one hand, increased N volatilization and combustion of surface soils that typically have relatively low $\delta^{15}\text{N}$ values are expected to increase plant $\delta^{15}\text{N}$ values after fire events (Högberg, 1997). Alternatively, by reducing N stocks and rates of cycling, frequent fire might reduce non-fire N losses (e.g., via leaching, denitrification, and/or ammonia volatilization) and thereby decrease plant $\delta^{15}\text{N}$ values. The net effect of these opposing effects of fire on $\delta^{15}\text{N}$ values likely depends on the degree to which fire leads to isotopic fractionation associated with combustion processes versus N loss pathways (Fig. 1). For example, incomplete combustion of plant biomass can cause volatilization of NH_3 , thereby increasing $\delta^{15}\text{N}$ in the remaining

pool (Pellegrini et al., 2014). Strong isotopic fractionations associated with gaseous loss pathways can lead to N pools with relatively high $\delta^{15}\text{N}$ values (Houlton et al., 2006), and nitrification followed by leaching of nitrate can also cause higher $\delta^{15}\text{N}$ values of the remaining N (Gerhart and McLauchlan, 2014). Therefore, fire-induced “tightening” of the N cycle through reductions in non-fire N losses might only alter plant $\delta^{15}\text{N}$ values if gaseous losses are high in the unburned ecosystem. Beghin et al. (2011) observed increases in wood $\delta^{15}\text{N}$ in the five years after a large fire, consistent with isotopic fractionation effects during combustion, but 6–10 years after fire $\delta^{15}\text{N}$ values returned to pre-disturbance levels. Field studies show both increases and decreases in soil $\delta^{15}\text{N}$ in days to decades after fire, underlining the importance of post-disturbance processes that shape ecosystem $\delta^{15}\text{N}$ (Grogan et al., 2000; Aranibar et al., 2003; Perakis et al., 2015).

In this study, we use one of the longest running savanna burn experiments worldwide to evaluate how frequent fire influenced wood $\delta^{15}\text{N}$ as an indicator of effects on N availability across space and time. The experiment is located at Cedar Creek Ecosystem Science Reserve (CCESR), a Long Term Ecological Research (LTER) site in eastern Minnesota, USA where 55 years of prescribed fire have been linked to substantial declines in N stocks, net N mineralization rates, and litter N cycling in frequently burned stands (Reich et al., 2001). We examined wood $\delta^{15}\text{N}$ patterns across four oak woodland/forest and savanna plots that were similar in overstory species representation, soil characteristics, and topography in an ongoing long-term controlled burn experiment at CCESR. We hypothesized that the mean and trajectory of wood $\delta^{15}\text{N}$ values would be coherent (i.e., vary simultaneously) across treatment stands before the onset of prescribed fire, whereupon wood $\delta^{15}\text{N}$ values would decrease thereafter in burned plots at rates proportional to the frequency of prescribed fire.

2 Methods

2.1 Study Site

CCESR in eastern Minnesota is located within the continental-scale ecotone between the northern Great Plains prairie, the eastern deciduous woodlands, and the southwestern extent of the northern mixed conifer forests. Many upland and lowland plant communities exist at CCESR. Of particular interest is the oak savanna, which was once an important ecosystem at the prairie-forest border (Nuzzo, 1986), but now survives as a relatively rare component of the post European-settlement landscape. The oak savanna terrain has low relief with sandy, excessively drained soils that are N-poor (Udipsammments, Grigal et al., 1974; Tilman, 1984). Throughout our wood nitrogen isotope record spanning 1902–2017, mean annual temperature was 4.8°C and mean annual precipitation was 72 cm. Wet plus dry atmospheric N deposition inputs at CCESR are ~ 4–10 kg N·ha⁻¹·yr⁻¹ (NADP, 2018). Annual NH_4^+ inputs have marginally increased and NO_3^- inputs have marginally decreased between 1997–2017. Combined, total N deposition inputs at CCESR have largely remained stable since data collection began in 1984. Fire suppression was the management practice within the reserve from at least 1938–1964; however, this area burned periodically before and after Euro-American settlement in the late 19th century (Leys et al., 2019).

The burn experiment at the oak savannas of CCESR was established in 1964 to preserve vegetation communities and to investigate ecosystem response to fire treatment (Peterson and Reich, 2001; Reich et al., 2001). Nineteen units of 2.4 to 30 ha were each assigned a burn frequency treatment, ranging from fire exclusion to a four out of five year burn frequency (Fig. 2).

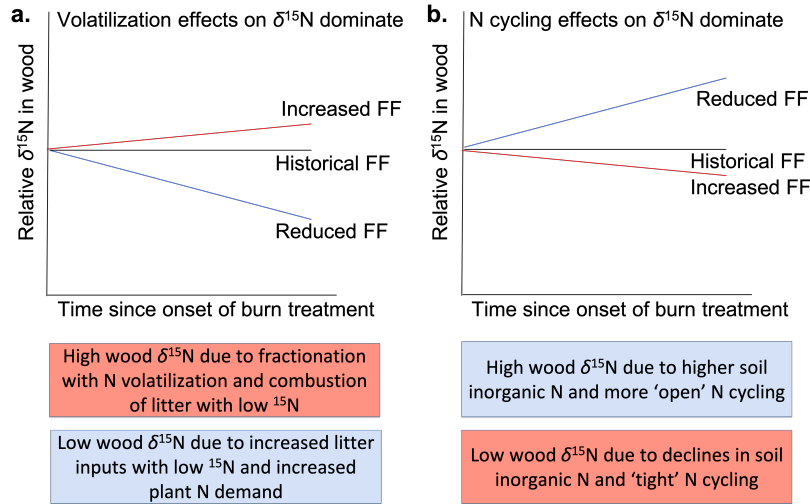


Figure 1. Hypothesized effects of fire experimentation on wood $\delta^{15}\text{N}$ values relative to the historical fire frequency (FF). Increased FF would lead to higher relative wood $\delta^{15}\text{N}$ values if combustion and isotopic fractionation with N volatilization dominate (a). In contrast, increased FF would lead to lower relative wood $\delta^{15}\text{N}$ values if N cycling effects such as reduced N stocks and non-fire N losses dominate the trajectory of wood $\delta^{15}\text{N}$ values (b). Colored boxes indicate potential contrasting effects of fire frequency on wood $\delta^{15}\text{N}$ values. Pink and blue boxes indicate processes resulting from higher and lower FF, respectively.

Prescribed burns typically occurred in April or May and fires were of low intensity. Although systematic vegetation inventories were not conducted prior to the onset of prescribed burning, a series of historical aerial photographs are available for our study area between the years 1938 and 2016 (McAuliffe et al., 2017; MHAPO, 2019). Our qualitative interpretation of these photographs indicates that the savanna vegetation prior to fire treatment at CCESR consisted of mixed woodland and grass

5 communities (Fig. 2). Over time, oak tree vegetation – predominantly bur oak, *Quercus macrocarpa*, and northern pin oak, *Quercus ellipsoidalis* – increasingly dominated fire-excluded stands, while C_4 grasses and sedges increased in abundance in frequently burned stands (Peterson and Reich, 2001; Dijkstra et al., 2006). The cumulative effects of direct fire-induced N losses and indirect fire-induced shifts in vegetation composition since 1964 have caused the vegetation and soil characteristics of these burn stands to diverge dramatically (Fig. 3). Unburned stands are characterized by high oak dominance, increased

10 woody plant biomass (including mesic taxa such as *Acer*), and high rates of soil N cycling and productivity, whereas frequently burned stands show high grass dominance, scattered bur and northern pin oak, and low rates of N cycling and productivity (Reich et al., 2001). We selected four stands for this study to span the gradient of fire frequency (from unburned to burned every 4 in 5 years) and the temporal onset of the fire experiment.

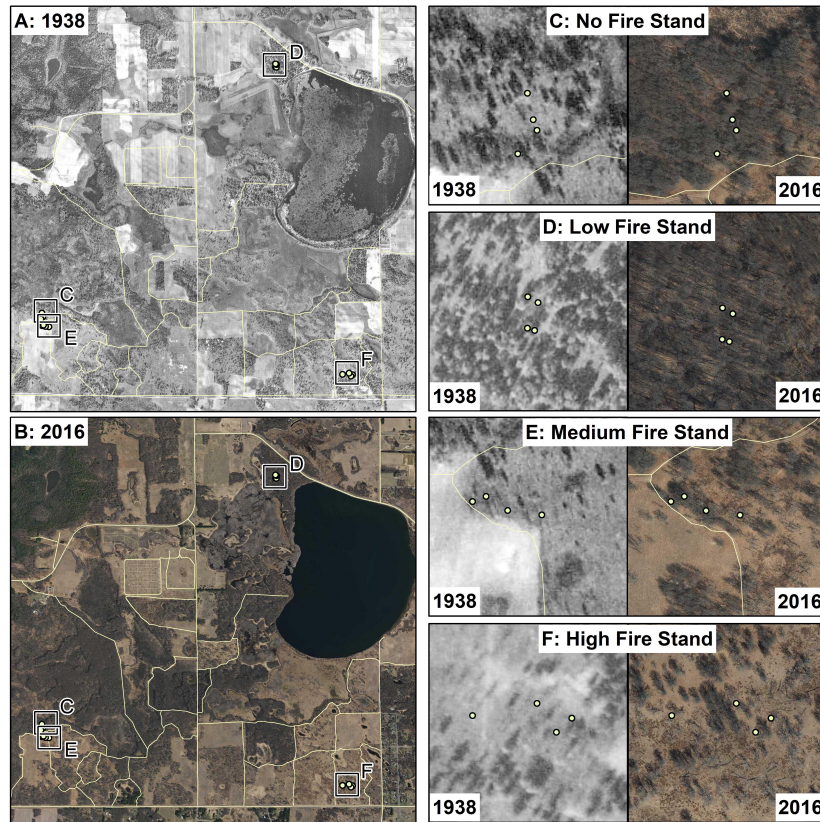


Figure 2. Historical aerial imagery for 1938 (A) and 2016 (B) for the savanna burn experiment and locations for 16 trees analyzed for wood $\delta^{15}\text{N}$ at 1:50,000. Polygons illustrate roads at CCEsr. Panels C - F illustrate stand level differences in canopy openness from 1938 to 2016 across the four fire treatments at a scale of 1:5,000. Imagery from Minnesota historical aerial photographs online (MHAPO, 2019).

2.2 Sample Collection

Increment cores were collected within ~1 m of the root collar from 16 *Q. macrocarpa* trees at CCEsr in March 2018 with a 5.15 mm diameter Hagl f borer. *Q. macrocarpa* was the only tree sampled for wood N isotope analysis because this is the dominant overstory species (in age and frequency) of the savanna where the burn experiment is located. Sampling a single

5 species also allowed us to control for potential species effects on wood $\delta^{15}\text{N}$. Trees spanned four stands in the fire frequency experiment: a no-fire stand (control), a low fire-intensity stand burned every 1 in 10 years (BU113), a medium fire-intensity stand burned every 1 in 3 years (BU115), and a high fire-intensity stand burned every 4 in 5 years (BU104). The onset of burning in the fire treatment stands was 1968, 1992, and 1965, respectively. Prior to that, fire episodes in a nearby stand had a median return interval of 15 years between 1822–1924, followed by a period of localized suppression (Leys et al., 2019). Broad

10 soil characteristics were similar across sampled stands. Trees in the low-fire stand grew on Sartell fine sand mapped in soil surveys with 0–6% slopes and trees in no-, medium-, and high-fire stands grew on Sartell fine sand mapped in soil surveys with



2 in 3 years \longrightarrow **no burn**

Figure 3. The Cedar Creek Ecosystem Science Reserve burn experiment. The difference in vegetation between unburned and frequently burn treatments stands is clearly shown. On left, a frequently burned stand is characterized by increased canopy openness and higher grass dominance. On right, an unburned control stand is characterized by dense undergrowth and high oak dominance. Photo credit: Peter Reich.

6–15% slopes. In the localized vicinity of the trees sampled across these sites, slopes rarely exceeded 8%. The Sartell series consists of excessively drained soils that are rapidly permeable and have low available N, low organic matter content, and low available water capacity (Grigal et al., 1974). The upper 15 cm of Sartell fine sand has a pH of 5.3, 0.025% total nitrogen (18 $\mu\text{mol/g}$ dry soil), and ~0.3% organic matter (Grigal et al., 1974; Tilman, 1984).

- 5 Following sampling, the cores were stored in paper straws and dried at 65° C. After drying, each core was surfaced using a core microtome, visually cross-dated (Stokes and Smiley, 1996), and measured with micrometry. The pith was within 25 rings in 13 out of 16 samples. The mean series length was 157 years, with the oldest tree containing 252 rings and the youngest containing 109 rings (Table 1). Mean ring width from 1902–2017 across all N cores was 1.11 mm. Cores for $\delta^{15}\text{N}$ analysis were cut into 10-mg samples using a razor blade along annual ring boundaries. Samples on average contained 1.35 years of
- 10 wood; 70% of samples contained only one year and 95% of samples contained one or two years. Although wood samples were partitioned at annual resolution, we did not analyze all wood samples for $\delta^{15}\text{N}$ due to cost and time constraints. Rather, we selected two wood samples per decade for $\delta^{15}\text{N}$ measurement. The decision to forego annual resolution and analyze our data at supra-annual timescales also aimed to mitigate the known phenomenon of inter-ring mobility of N-containing compounds that could smooth out inter-annual variation in $\delta^{15}\text{N}$ (Hart and Classen, 2003; McLauchlan et al., 2017). N translocation has
- 15 long been recognized as an issue in interpreting wood $\delta^{15}\text{N}$. We did not perform chemical pretreatment on wood samples, as no pretreatment protocols have been widely shown to remove labile N in wood (Sheppard and Thompson, 2000; Bunn et al., 2017).

Wood samples were analyzed for $\delta^{15}\text{N}$ and wood % N at the Central Appalachians Stable Isotope Facility (CASIF) of the University of Maryland Center for Environmental Science. Samples were combusted in a Carlo Erba NC2500 elemental

20 analyzer (CE Instruments). Carbosorb and MgClO_4 were used to absorb CO_2 and H_2O , respectively, prior to the introduction

of N_2 to a Thermo Fisher Delta V+ isotope ratio mass spectrometer for $\delta^{15}N$ analysis. The $\delta^{15}N$ data were normalized to the AIR scale using a two-point normalization curve with internal standards calibrated against USGS40 and USGS41. Analytical precision for $\delta^{15}N$ of an internal wood standard analyzed along with samples was 0.3 ‰. A mass series of an atropine standard was used to calculate % N. In total, 367 wood samples were analyzed for $\delta^{15}N$ and % N across 16 trees. Samples correspond to the second and seventh calendar year in each decade and temporal coverage spans 1902-2017.

Burn Unit	Burn Stand	Tree	Specimen ID	Ring Counts	Tree Age Class	First $\delta^{15}N$ Sample Year	# $\delta^{15}N$ Samples	Fire Median Return (Years)	Burning Onset (Year)
Control	No-fire	1	CCS033	109	1	1927	19	0	NA
Control	No-fire	2	CCS034	132	2	1937	17	0	NA
Control	No-fire	3	CCS038	107	1	1917	21	0	NA
Control	No-fire	4	CCS071	131	2	1927	19	0	NA
BU113	Low-fire	1	CCS135	190	4	1902	24	10	1968
BU113	Low-fire	2	CCS136	197	4	1902	24	10	1968
BU113	Low-fire	3	CCS139	167	3	1902	24	10	1968
BU113	Low-fire	4	CCS141	206	4	1902	24	10	1968
BU115	Medium-fire	1	CCS236	129	1	1902	23	3	1992
BU115	Medium-fire	2	CCS271	150	2	1902	24	3	1992
BU115	Medium-fire	3	CCS273	152	2	1902	24	3	1992
BU115	Medium-fire	4	CCS274	161	3	1902	24	3	1992
BU104	High-fire	1	CCS545	116	1	1922	20	1	1965
BU104	High-fire	2	CCS556	144	2	1902	23	1	1965
BU104	High-fire	3	CCS558	161	3	1967	11	1	1965
BU104	High-fire	4	CCS559	252	4	1902	24	1	1965

Table 1. List of the trees sampled, ring counts, first sample year for $\delta^{15}N$, number of $\delta^{15}N$ samples, fire median return, and the first year of prescribed burning. Ring count is the total number of years for each core and does not represent specific tree age, as pith was not present for all cores. Trees in age class 1 contain <129 rings, trees in age class 2 contain 130-159 rings, trees in age class 3 contain 160-189 rings, and trees in age class 4 contain >190 rings.

2.3 Statistical Analysis

Beyond understanding essential differences in mean $\delta^{15}N$ values across burn stands, a primary goal was to understand whether varying fire treatment frequency and temporal onset of fire affected the mean and long-term trajectory of $\delta^{15}N$ values. We used several statistical methods to assess time series patterns of wood $\delta^{15}N$ and N cycling across the CCESR burn experiment. Although many studies standardize wood $\delta^{15}N$ values for each core with respect to that core mean (McLauchlan et al., 2007, 2017), we chose not to do so in order to maintain patterns that might otherwise be obscured with detrending or standardization

techniques. Differences in wood $\delta^{15}\text{N}$ across burn treatment stands and individual trees were tested using the Kruskal-Wallis test, a nonparametric test to identify significant differences on a continuous dependent variable by a categorical independent variable. To test for evidence of long-term trends in $\delta^{15}\text{N}$, we used simple linear regression and the nonparametric Mann-Kendall trend test (MK test). The MK test looks for evidence of monotonic trend in a time series and is robust to non-normally distributed data. For linear regression and the MK test, trends were deemed significant for p values < 0.05 . In addition, we conducted regime shift detection analysis on $\delta^{15}\text{N}$ values by burn stand to statistically identify changes in the mean state of $\delta^{15}\text{N}$ following the method of Rodionov (2004). This method applies sequential t -tests to time series data to statistically identify shifts in the mean state (Rodionov, 2004). Significant shifts in mean $\delta^{15}\text{N}$ were identified using the two-tailed Student t -test and the $\alpha = 0.01$ threshold was used. A 6-year (1/4 series) cut-off length was used to allow flexibility in detecting mean shifts. The Huber's weight parameter was set to 1 and red noise was not estimated.

Burn Unit	Burn Stand	Specimen ID	Tree Age Class	m	p value	Kendall's Tau
Control	No-fire	CCS033	1	-0.19	< 0.0001	-0.70***
Control	No-fire	CCS034	2	-0.05	NS	-0.23
Control	No-fire	CCS038	1	-0.25	< 0.0001	-0.85***
Control	No-fire	CCS071	2	-0.16	< 0.0001	-0.49**
BU113	Low-fire	CCS135	4	-0.20	< 0.0001	-0.74***
BU113	Low-fire	CCS136	4	-0.21	< 0.0001	-0.54**
BU113	Low-fire	CCS139	3	-0.25	< 0.0001	-0.75***
BU113	Low-fire	CCS141	4	-0.25	< 0.0001	-0.72***
BU115	Medium-fire	CCS236	1	-0.20	< 0.0001	-0.82***
BU115	Medium-fire	CCS271	2	-0.26	< 0.0001	-0.59***
BU115	Medium-fire	CCS273	2	-0.22	< 0.0001	-0.78***
BU115	Medium-fire	CCS274	3	-0.28	< 0.0001	-0.66***
BU104	High-fire	CCS545	1	0.02	NS	0.12NS
BU104	High-fire	CCS556	2	-0.03	NS	-0.27NS
BU104	High-fire	CCS558	3	-0.02	NS	-0.07NS
BU104	High-fire	CCS559	4	-0.03	< 0.01	-0.35*

Table 2. Estimated slope coefficient based on linear regression (m), p -value based on linear regression, and Kendall's Tau based on the Mann Kendall test (MK test). The MK test looks for evidence of monotonic trend in a time series and is robust to non-normally distributed data. Tau values * indicate $p < 0.05$, ** $p < 0.01$, and *** $p < 0.0001$; NS is not significant. Trees in age class 1 contain <129 rings, trees in age class 2 contain 130-159 rings, trees in age class 3 contain 160-189 rings, and trees in age class 4 contain >190 rings.

Year	No-fire mean $\delta^{15}\text{N}$	Low-fire mean $\delta^{15}\text{N}$	Medium-fire mean $\delta^{15}\text{N}$	High-fire mean $\delta^{15}\text{N}$
1902	-2.19*	0.38	1.65	-1.16
1907	-2.91*	0.54	2.21	-1.58
1912	-1.14*	0.77	1.93	-0.98
1917	-0.03	1.19	1.94	-0.46
1922	-1.81	0.86	1.32	-1.24
1927	-0.7	0.98	1.37	-0.09
1932	-1.24	1.17	1.37	-0.53
1937	-0.65	0.94	1.25	-0.66
1942	-1.06	0.07	0.9	-0.62
1947	-1.27	1.26	0.26	-0.5
1952	-1.54	-0.18	-0.68	-0.72
1957	-1.63	0.05	-0.35	-0.09
1962	-0.9	-0.4	-0.94	-0.71
1967	-2.34	-2.2	-2.07	-0.6
1972	-3.12	-2.35	-2.06	-0.47
1977	-3.15	-2.54	-1.94	-0.67
1982	-2.81	-2.09	-2.14	-0.91
1987	-2.6	-2.45	-2.25	-1.24
1992	-3.08	-3.38	-1.9	-1.32
1997	-3.28	-2.65	-2.29	-1.33
2002	-3.3	-3.25	-2.44	-1.15
2007	-3.2	-3.04	-2.41	-0.93
2012	-3.27	-3.16	-2.54	-0.83
2017	-3.53	-2.87	-2.16	-1.03

Table 3. Mean wood $\delta^{15}\text{N}$ (‰) by year in each burn unit. Mean values * indicate years with only one $\delta^{15}\text{N}$ sample in that year and burn unit.

3 Results

To test whether wood $\delta^{15}\text{N}$ values were broadly similar before fire and diverged after fire, we first examined spatial differences in $\delta^{15}\text{N}$. In the first decade of the 20th century, wood $\delta^{15}\text{N}$ values varied among stands ($p = 0.023$). Average wood $\delta^{15}\text{N}$ values in 1902 ranged from -2.2 to 1.7‰ among stands (Table 3). A Kruskal-Wallis test of wood $\delta^{15}\text{N}$ across the time series for the
5 four stands revealed that $\delta^{15}\text{N}$ was significantly different among stands ($p < 0.001$). We used the nonparametric Games-Howell
test for post-hoc analysis due to unequal variances and group sizes between burn treatment stands. The Games-Howell test
revealed that $\delta^{15}\text{N}$ in the no-fire stand was lower than all other stands ($p < 0.001$). In contrast, the Games-Howell test revealed
nonsignificant differences in $\delta^{15}\text{N}$ between the low- and medium-fire stands ($p = 0.385$), low- and high-stands ($p = 0.944$), and

medium- and high-fire stands ($p = 0.441$). Wood $\delta^{15}\text{N}$ was also significantly different between stands prior to the onset of the prescribed burn experiment in 1964 ($p < 0.001$). Wood $\delta^{15}\text{N}$ differences among individual trees were small within each stand; only tree CCS545 in the high-fire stand was significantly different from the other three trees in that stand ($p < 0.001$). Two approaches revealed that tree age likely did not have an effect on the mean or trend of $\delta^{15}\text{N}$. First, we compared wood $\delta^{15}\text{N}$ with tree biological age rather than Gregorian calendar year using inner ring dates to estimate tree age. Tree age and $\delta^{15}\text{N}$ were negatively correlated ($r = -0.35$; $p < 0.001$), however this negative relationship may be expected given the negative temporal trends in $\delta^{15}\text{N}$ in the majority of trees. Additionally, we tested for an age effect on wood $\delta^{15}\text{N}$ by dividing trees into four age classes based on ring counts (Table 1, Table 2). A Kruskal-Wallis test of wood $\delta^{15}\text{N}$ across age classes revealed that tree age did not have a significant effect on the mean ranks of $\delta^{15}\text{N}$ ($p = 0.242$).

10 Over time and in aggregate, wood $\delta^{15}\text{N}$ values have declined at CCESR since approximately 1967, although specific trajectories varied by fire frequency (Fig. 4, Table 2). Negative trends in wood $\delta^{15}\text{N}$ were evident in 12 out of 16 trees sampled, 11 of which were in the no-fire, low-fire, and medium-fire stands. Declines in wood $\delta^{15}\text{N}$ occurred in the mid 20th century in these stands, beginning primarily between 1940 and 1965. In contrast, there was little evidence of declining wood $\delta^{15}\text{N}$ in the high-fire stand, where $\delta^{15}\text{N}$ values were relatively stable over time. Results from the regime-shift analysis were consistent with
15 the trend tests; shifts in the mean state of $\delta^{15}\text{N}$ were detected in the no-fire, low-fire, and medium-fire frequency stands ($p < 0.01$), whereas there was not a $\delta^{15}\text{N}$ shift in the high-fire stand at the $\alpha = 0.01$ significance level (Fig. 5).

In the no-fire stand, three out of four sampled trees had significantly declining trends in wood $\delta^{15}\text{N}$ to present based on simple linear regression ($p < 0.0001$) and the MK test ($p < 0.01$; Fig. 4a). Although the wood $\delta^{15}\text{N}$ trend for tree CCS034 was not significant, this tree had a negative $\delta^{15}\text{N}$ slope that matched the overall negative trend in this stand. Regime shift analysis
20 detected one mean shift in the 1960s, reflecting a stable mean of higher wood $\delta^{15}\text{N}$ between 1902 to 1962 followed by a stable mean of lower wood $\delta^{15}\text{N}$ from 1967 to 2017. Wood $\delta^{15}\text{N}$ in this stand had a maximum of 1.8 ‰, a minimum of -4.7 ‰, and standard deviation 1.3 ‰.

In the low-fire stand, all four trees demonstrated significant declines in wood $\delta^{15}\text{N}$ towards present based on the two trend tests ($p < 0.0001$; Fig. 4b). Regime shift analysis identified one mean wood $\delta^{15}\text{N}$ shift in the 1960s, separating a stable mean
25 of higher wood $\delta^{15}\text{N}$ between 1902 and 1962 and lower mean $\delta^{15}\text{N}$ from 1967 to 2017. Fire treatment in this stand began in 1968 and with a 1 in 10-year fire median return, and this stand has burned five times throughout the experiment. The wood $\delta^{15}\text{N}$ values ranged from 2.8 to -4.1 ‰ with standard deviation 1.9 ‰. There was tight coherence between individual cores during the period 1942–1962, including a steep decline in all four cores between samples 1962 and 1967. While this steep single increment decline bracketed the beginning of the burn experiment in 1964, prescribed fire had not yet occurred in this
30 stand.

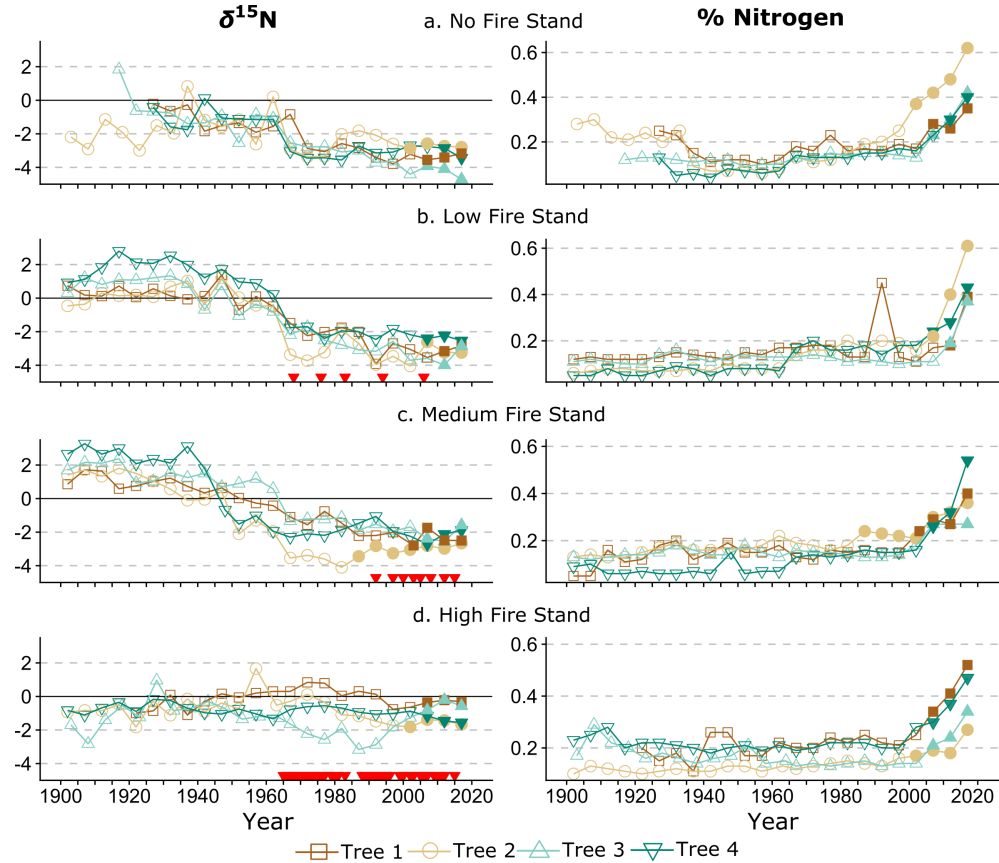


Figure 4. Wood $\delta^{15}\text{N}$ (left) and % N (right) data over time. Lines connect data for individual trees within each stand. Shapes and colors correspond to trees 1–4 in each stand (Table 1). On left, red triangles indicate individual years of prescribed fire in each treatment stand. Filled shapes indicate wood sampled after the heartwood-sapwood transition. Wood % N increased in all trees over the last 115 years, with the sharpest increases after the heartwood-sapwood transition (average transition year 2004).

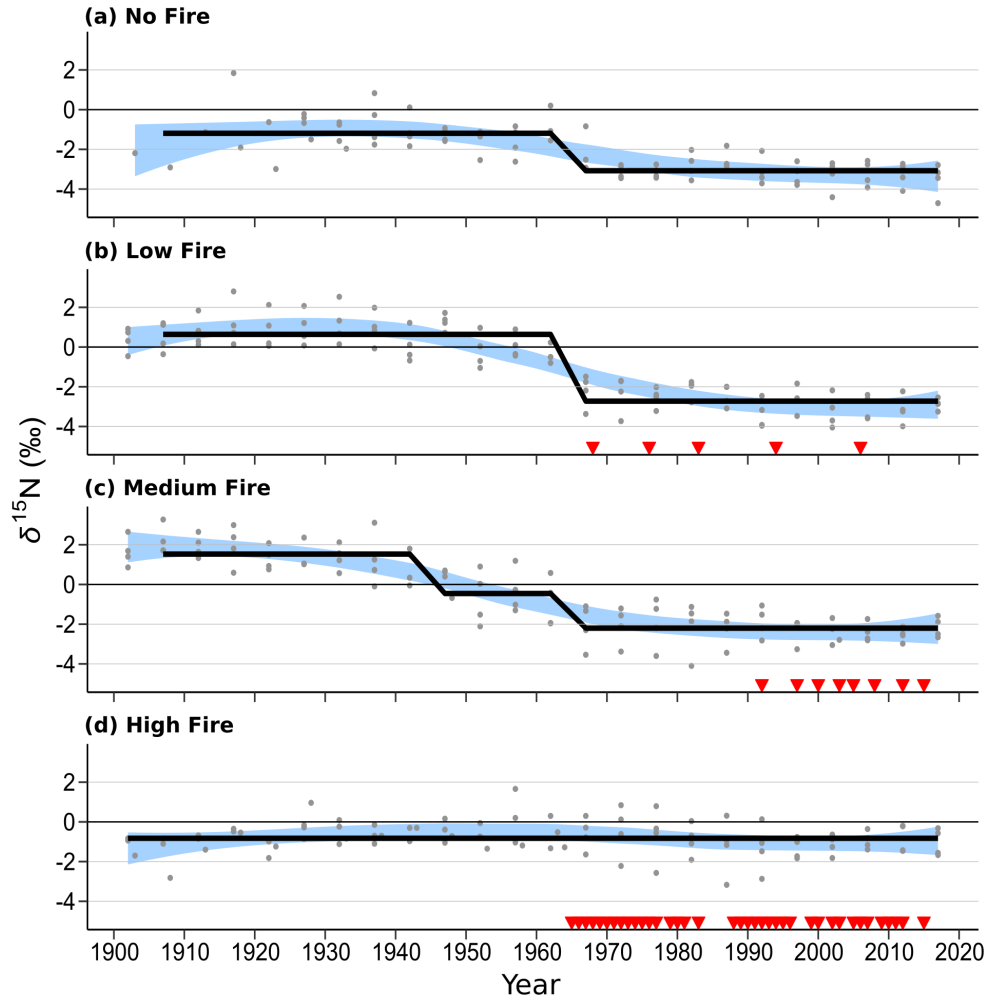


Figure 5. Wood $\delta^{15}\text{N}$ patterns of mature *Quercus* trees in each of four burn stands. Four trees were sampled in each stand. Blue shaded areas represent 99% confidence intervals of a LOWESS curve fit and the thick black line shows mean $\delta^{15}\text{N}$ states determined using regime shift analysis. Red triangles indicate individual years of prescribed fire in each treatment stand. Mid-20th-century declines in wood $\delta^{15}\text{N}$ are readily seen in the no-fire, low-fire, and medium-fire stands.

In the medium-fire frequency stand, all four trees showed significantly declining trends toward present based on the two trend tests ($p < 0.0001$; Fig. 4c). Two mean shifts were detected in the medium-fire stand in the 1940s and 1960s using regime shift analysis. Fire treatment in this stand began in 1992 with a 1 in 3-year fire median return, totaling eight prescribed burns to the date of sampling. Trees in this stand exhibited the steepest declines in wood $\delta^{15}\text{N}$ over time, ranging from a maximum of 3.3 ‰ in 1907 to a minimum of -4.1 ‰ in 1982. The standard deviation of wood $\delta^{15}\text{N}$ values (1.9 ‰) was highest in this burn stand relative to all others.

In contrast to the other stands we sampled, the high fire frequency stand displayed little evidence for declining wood $\delta^{15}\text{N}$. Out of the four sampled trees, only CCS559 demonstrated a significantly declining trend in wood $\delta^{15}\text{N}$ toward present based on the linear trend test (Fig. 4d). Similarly, no shifts in mean wood $\delta^{15}\text{N}$ were detected using regime shift analysis. Fire treatment began in this stand in 1965 and has continued at a four in five-year annual fire return. In total, 38 fires occurred in this stand between 1965 and 2017, likely surpassing the pre-settlement fire frequency in this region (Leys et al., 2019). Wood $\delta^{15}\text{N}$ values ranged from 1.7 to -3.2 ‰ and the standard deviation of $\delta^{15}\text{N}$ (0.8 ‰) was lowest in this burn stand.

Wood % N increased in all trees over the last 115 years, with significant increases in 11 out of 16 trees based on the MK test ($p < 0.01$). A sharp increase in % N occurred after the heartwood-sapwood transition in most trees (Fig. 4). For these trees, the sapwood included the outer 10–20 years of growth, and the heartwood-sapwood transition consistently occurred in one ring and never in more than three. For each tree, % N increased consistently through the last sample, and the last two to three samples exhibited markedly higher % N than in previous years. This result is consistent with the well-documented pattern of increasing wood % N after the heartwood-sapwood transition with highest values in most recent rings (Poulson et al., 1995; McLauchlan and Craine, 2012). Wood % N was negatively correlated with wood $\delta^{15}\text{N}$ across all trees ($r = -0.38$; $p < 0.001$). Excluding all samples from the sapwood, wood % N and wood $\delta^{15}\text{N}$ remained negatively correlated, statistically significant although the relationship weakened ($r = -0.30$; $p < 0.001$). Small differences in wood % N trajectories among treatment stands were evident in our data and these differences were not statistically significant.

4 Discussion

Q. macrocarpa wood $\delta^{15}\text{N}$ values were significantly different among sampled stands at the beginning of our 115-year record and before the onset of prescribed burning in 1964. Spatial differences indicate lack of support for the first part of our hypothesis, specifically that mean $\delta^{15}\text{N}$ values would be similar across stands before the onset of the burn experiment. These differences in wood $\delta^{15}\text{N}$ among stands suggest spatial heterogeneity in N availability before the onset of fire treatment. Over the full record, among-stand wood $\delta^{15}\text{N}$ variability was higher than within-stand variability, potentially reflecting larger differences in vegetation composition and measured N cycling metrics between stands.

In contrast to wood $\delta^{15}\text{N}$, wood % N trajectories were positive and consistent among stands. Wood % N is not as commonly reported in wood N isotope studies because this metric likely incorporates tree physiology and thus may not reflect ecosystem N cycling (Gerhart and McLauchlan, 2014). Positive wood % N trajectories shown here match documented patterns of increasing wood % N after the heartwood-sapwood transition in the wood $\delta^{15}\text{N}$ literature (Gerhart and McLauchlan, 2014), yet this pattern has seen more mixed results in the wood chemistry literature (Meerts, 2002; Martin et al., 2014). Although wood % N may not reflect N availability, coherence in wood % N patterns among individual trees and stands suggests that these data contain a strong signal that is independent from wood $\delta^{15}\text{N}$ at this site. If this result reflects radial nitrogen translocation across ring boundaries, then the greatest mobility would have occurred within the last five years of growth, with a decay in mobility that extends over fifteen years. Annual-scale analyses might further elucidate these patterns. Wood % N and $\delta^{15}\text{N}$ were negatively correlated across all samples and this negative relationship diminished when excluding samples from the sapwood. Although

we do not have further evidence to suggest that this is a meaningful relationship, future studies should examine relationships between wood % N and $\delta^{15}\text{N}$ because their covariance could be problematic if trends in $\delta^{15}\text{N}$ are strongly related to % N in addition to external factors.

Our results do not support the second part of our original hypothesis; wood $\delta^{15}\text{N}$ values did not vary among stands proportional to fire frequency and known dates of fire and differences in fire frequency did not correspond to temporally consistent shifts in mean wood $\delta^{15}\text{N}$. The negative trend in wood $\delta^{15}\text{N}$ for the majority of trees we analyzed matched our a priori expectation of declining wood $\delta^{15}\text{N}$ based on evidence for long-term declines in rates of net N mineralization and tree productivity in frequently burned stands at CCEsr (Reich et al., 2001; Dijkstra et al., 2006). However, wood $\delta^{15}\text{N}$ was not inversely related to fire frequency. Trees in the low-fire frequency stands displayed significant negative trends in wood $\delta^{15}\text{N}$ as expected. But trees in the no-fire stand showed similar declines in $\delta^{15}\text{N}$ to that of trees in the low- and medium-fire stands, despite fire exclusion and higher measured N stocks and net N mineralization rates in this unburned stand toward present (Reich et al., 2001). Further, wood $\delta^{15}\text{N}$ declines in the low- and medium-fire stand predated the onset of experimental prescribed burning, and trees in the high-fire stand demonstrated no significant positive or negative trend in $\delta^{15}\text{N}$ in response to frequent fire beginning in 1965. Although regime shift analysis identified mean shifts that nearly aligned in time in the no-, low-, and medium-fire stands, all shifts preceded the onset of prescribed fire and overall $\delta^{15}\text{N}$ declines were gradual. In combination with wood $\delta^{15}\text{N}$ values in the high-fire stand showing no discernible response to fire, this evidence indicates that the onset of prescribed fire in the mid 20th century did not cause declines in wood $\delta^{15}\text{N}$ at CCEsr.

Year	Data	Soil Depth (cm)	Low-fire	Medium-fire	High-fire
1992	Wood $\delta^{15}\text{N}$	NA	-2.46	-1.06	0.13
1995	Net N mineralization	0–15	7.21	10.42	2.31
1997	Wood $\delta^{15}\text{N}$	NA	-1.84	-1.94	-0.76
2016	%N	0–5	0.12	NA	0.1
2016	%N	5–10	0.06	NA	0.06
2016	%N	10–20	0.05	NA	0.06
2016	%N	20–60	0.02	NA	0.04
2016	%N	60–100	0.01	NA	0.03
2016	$\delta^{15}\text{N}$	0–5	1.13	NA	2.25
2016	$\delta^{15}\text{N}$	5–10	4.15	NA	3.57
2016	$\delta^{15}\text{N}$	10–20	4.76	NA	3.85
2016	$\delta^{15}\text{N}$	20–60	NA	NA	5.14
2016	$\delta^{15}\text{N}$	60–100	NA	NA	2.93
2017	Wood $\delta^{15}\text{N}$	NA	-2.54	-1.58	-0.32

Table 4. Comparison of previously collected soil N data and our wood $\delta^{15}\text{N}$ data from CCEsr. Values represent the mean of all samples within each burn unit. Net N mineralization data from Reich et al. (2001), and soil % N and $\delta^{15}\text{N}$ data from Pellegrini et al. (2020).

Prior studies at CCEsr have examined N cycling in the same stands we sampled for wood $\delta^{15}\text{N}$ across the burn experiment. Reich et al. (2001) measured net N mineralization in 1995 in three of the four stands we sampled (low-, medium-, and high-fire). Net N mineralization decreased nonlinearly with increasing fire frequency among these stands. However, our wood $\delta^{15}\text{N}$ samples in 1992 and 1997 increased with increasing fire frequency among these stands. Wood $\delta^{15}\text{N}$ values from 1992 and 1997 were lower in the low- and medium-fire stands compared to the high-fire stand, despite relatively higher net N mineralization in the former stands in 1995 (Table 4; Reich et al., 2001). This could point to large inter-annual variation in wood $\delta^{15}\text{N}$ or that soil net N mineralization may not be strongly related to wood $\delta^{15}\text{N}$ at our study site. More recently, Pellegrini et al. (2020a) measured soil $\delta^{15}\text{N}$ and cumulative N stocks at multiple depths in two of the four stands we sampled (low- and high-fire) in 2016. They found $\delta^{15}\text{N}$ decreased with more frequent burning and $\delta^{15}\text{N}$ increased with depth across the entire fire frequency gradient, while fire did not have a clear effect on total soil N between the low- and high-fire frequency stands we sampled (Pellegrini et al., 2020b). In contrast, our wood $\delta^{15}\text{N}$ in 2017 was significantly lower in the low-fire stand compared to the high-fire stand and we found limited evidence for variation in wood $\delta^{15}\text{N}$ related to long-term fire frequency. Altogether, these nuanced results from previous studies add context but do not clarify interpretation of our observed wood $\delta^{15}\text{N}$ patterns.

Based on the lack of evidence for direct fire effects on short and long-term wood $\delta^{15}\text{N}$ patterns at this site, we believe the primary driver of $\delta^{15}\text{N}$ patterns at this site has been changes in vegetation composition and abundance over time, perhaps driven in part by early to mid 20th century fire suppression. Our qualitative assessment of aerial photographs between 1938 and 2016 indicates that canopy density increased through time in the no-, low-, and medium-fire stands but remained relatively constant in the high-fire stand (Fig. 2). Our interpretation is in general agreement with results from other studies at CCEsr (Peterson and Reich, 2001), and a directly adjacent experimental site (Faber-Langendoen and Davis, 1995) with the same soil, topography, and plant community characteristics. If fire suppression during several decades of this period indeed allowed understory shrubs and young trees to increase in abundance in these plots, total plant N demand likely increased, thereby reducing N availability to mature oaks. In addition, increased litter inputs with low ^{15}N would tend to decrease soil $\delta^{15}\text{N}$ in these stands (Nadelhoffer and Fry, 1988). These processes would explain mid-century wood $\delta^{15}\text{N}$ declines in these plots, particularly in the low- and medium-fire stands where $\delta^{15}\text{N}$ declined most sharply. In contrast, the high-fire plot was more grass-dominated and open relative to surrounding stands in 1938 and has remained so over time. Less ingrowth of understory shrubs and young trees in the high-fire stand could result in low total N demand that was stable over time, perhaps accounting for the lack of negative $\delta^{15}\text{N}$ trend in this stand. Fire suppression was also suggested as a driver of declining N availability based on wood $\delta^{15}\text{N}$ records in an old-growth pine forest in Minnesota (Howard and McLauchlan, 2015). Thus, although we did not find evidence for variation in wood $\delta^{15}\text{N}$ directly related to prescribed fire, the indirect effects of fire suppression on vegetation composition and abundance may have been sufficient to affect nitrogen resource partitioning between mature oaks and competing understory species, ultimately causing declining N availability to mature oaks in three out of four stands.

There are several alternative hypotheses to explain the lack of a direct relationship between prescribed fire and wood $\delta^{15}\text{N}$ trajectories at CCEsr. One hypothesis is that the declines in wood $\delta^{15}\text{N}$ values in trees at CCEsr may reflect a common continental-scale driver such as increased atmospheric CO_2 or N deposition (McLauchlan et al., 2017). However, such drivers changed the most in the past 50 years, not in the 20 years prior when the largest changes in wood $\delta^{15}\text{N}$ values occurred.

Moreover, the large differences in wood $\delta^{15}\text{N}$ mean and trend between burn stands throughout our 115 record also suggest a minor role for continental-scale drivers at the scale of our study. The relative close proximity of sampled trees within treatment stands may also contribute to the ambiguous relationship between fire and wood $\delta^{15}\text{N}$ at this site. The four trees sampled per burn stand were spatially quite close to each other (on average 34 meters between trees within each burn unit), and we were
5 unable to replicate sampling in other units with the same fire treatment. Given the spatial variability in wood $\delta^{15}\text{N}$ that existed before the fire treatment began, additional replicates of fire treatment would help to clarify the relationship between fire and wood $\delta^{15}\text{N}$.

Our results are more likely the result of local-scale influences – apart from the obvious presence of fire – on N cycling and tree N uptake at CCEsr. Despite our limited evidence for variation in $\delta^{15}\text{N}$ of tree-rings due to prescribed fire, it is
10 important to recognize that fire can influence $\delta^{15}\text{N}$ and N availability through a variety of mechanisms. For example, Höglberg (1997) proposed that the combustion of the upper $\delta^{15}\text{N}$ -depleted surface layer would lead to increased $\delta^{15}\text{N}$ values in plants shortly after fire. N losses from volatilization of N during fire events could exceed leaching losses here, given that bursts of plant N-uptake and growth after fire have been shown to limit leaching losses (Boerner, 1982; Dijkstra et al., 2006). However, evidence for this N volatilization mechanism may be limited at CCEsr because fires in the burn experiment were low severity
15 and rarely resulted in complete combustion of the litter layer (Hernández and Hobbie, 2008). Post-fire nitrification is another mechanism that could affect soil and wood $\delta^{15}\text{N}$, causing increased $\delta^{15}\text{N}$ values of NH_4^+ and decreased $\delta^{15}\text{N}$ values of NO_3^- (Höglberg, 1997). This mechanism could affect wood $\delta^{15}\text{N}$ at this site, as there is evidence that *Quercus* seedlings show a greater relative preference for NO_3^- over NH_4^+ (Templer and Dawson, 2004), and preferences for inorganic N were reflected in *Q. alba* wood $\delta^{15}\text{N}$ in an Indiana hardwood forest (McLauchlan and Craine, 2012). Soil net nitrification was negatively related to fire
20 frequency across the CCEsr burn experiment (Reich et al., 2001) but our wood $\delta^{15}\text{N}$ data cannot address the extent to which nitrification is altering the supply of available N. Beghin et al. (2011) invoked both litter combustion and post-fire nitrification in their study of long-term N cycling in a pine forest. They found increased wood $\delta^{15}\text{N}$ in the 5 years after fire disturbance, but a return to pre-disturbance $\delta^{15}\text{N}$ levels 6–10 years after fire. Here, we did not find similar short-term increases in $\delta^{15}\text{N}$ after known prescribed fire events. It is uncertain whether higher resolution sampling using annual growth rings would provide
25 greater insights into short-term effects of fire on wood $\delta^{15}\text{N}$, as N translocation may smooth out inter-annual disturbances to the N cycle.

It is very likely that fire-exclusion since 1964 has increased tree dominance, net N mineralization, and thus overall N availability in the no-fire stand (Reich et al., 2001), yet three out of four trees in this stand demonstrated significant declines in wood $\delta^{15}\text{N}$. In contrast, wood $\delta^{15}\text{N}$ has remained stable throughout the 20th century in trees from the high-fire stand, despite
30 prescribed fire that likely reduced N supply by decreasing rates of soil net nitrification and promoting further establishment of *C₄* grasses in this stand (Reich et al., 2001). Stable wood $\delta^{15}\text{N}$ in the high-fire stand could reflect a decline in N demand that was approximately proportional to declines in N supply, or that decreases in N supply due to fire were too small relative to stable N demand to cause a decline in $\delta^{15}\text{N}$ values. Future studies should consider that wood $\delta^{15}\text{N}$ patterns may result from changes in N supply driven directly by fire, in conjunction with changes in N demand driven indirectly by fire vis-à-vis

altered vegetation composition and abundance. Our results at this temperate oak savanna suggest an important influence of decadal-scale vegetation changes caused by fire on N cycling.

Recent studies have shown mixed results regarding the reliability of wood $\delta^{15}\text{N}$ as a proxy for terrestrial N availability. In some settings wood $\delta^{15}\text{N}$ appears to reliably integrate spatiotemporal variation in soil N supply relative to plant demand (Elmore et al., 2016; Kranabetter and Meeds, 2017; Sabo et al., 2020), whereas other results suggest an inconsistent relationship between wood $\delta^{15}\text{N}$ and N cycling (Tomlinson et al., 2016; Burnham et al., 2019). Our study sought to clarify the relationships between N availability, wood $\delta^{15}\text{N}$, and long-term disturbance by sampling across four treatment stands to control for the presence and frequency of prescribed burning. Contrasting trends in heartwood $\delta^{15}\text{N}$ across our treatment stands suggest minor roles for the heartwood-sapwood transition and exogenous drivers of wood $\delta^{15}\text{N}$ at this study site. Nonetheless, the apparent lack of wood $\delta^{15}\text{N}$ response to repeated burning at our study site, particularly in the high fire frequency treatment, raises uncertainty about the ability for individual tree-level $\delta^{15}\text{N}$ to record the effects of low intensity fires on N cycling. We recommend that future studies of N cycling using wood $\delta^{15}\text{N}$ utilize diligent site selection criteria to control for as many confounding factors as possible to help clarify the processes controlling variation in wood $\delta^{15}\text{N}$ values.

5 Conclusions

In summary, over fifty years of long-term ecological research has provided a uniquely detailed understanding of the N cycle at CCEsr to compare with our results. In contrast to our hypothesis, wood $\delta^{15}\text{N}$ values were significantly different among stands before the onset of prescribed burning and differences in fire frequency did not correspond to long-term $\delta^{15}\text{N}$ trends or temporally consistent shifts in mean $\delta^{15}\text{N}$ across four treatment stands. Combined with findings from previous ecosystem biogeochemistry studies at CCEsr, we interpret declining wood $\delta^{15}\text{N}$ in the no-, low-, and medium-fire stands over the past 115 years to reflect declining N availability to mature oaks at this savanna. No direct effects of fire were consistently supported by our results; instead wood $\delta^{15}\text{N}$ values were perhaps determined by changes in vegetation composition and abundance over time, due in part to early to mid 20th century fire suppression. Specifically, the encroachment of shrubs and juvenile trees in three stands likely increased N demand and competition for N, decreasing N availability for mature oaks, and influencing wood $\delta^{15}\text{N}$; although we note that such an interpretation is more plausible than conclusive.

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