**Summary of Revisions**
**Biogeosciences Discussions**
**22 May 2015**
**Title: Spatio-temporal analysis of nitrogen cycling in a mixed coniferous forest of the northern United States**
**Author(s): I. Howard and K.K. McLauchlan**
**MS No.: bg-2015-35**
**MS Type: Research Article**
**Iteration: First submission**

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Each of the three reviewers expressed high interest in the wood d15N dataset reported in the manuscript, and found that the study design and analyses had been conducted well. Among all reviewers, a main revision needing to be addressed is a refinement of the interpretations of our findings about the ultimate cause of the declining patterns of δ15N in wood. Because of the temporal synchrony of the declines among individual trees, the timing of the onset of the declines, and the known effects of fire suppression on nitrogen cycling from other forested systems, we posit that the decline is more than likely related to fire suppression in the state park beginning in 1920. However, as the reviews suggest, alternative and more complicated hypotheses for the decline are possible, and it is difficult to test these hypotheses fully without additional theoretical background and incorporation of additional data (such as the precise fire history of the watershed or additional datasets about temporal δ15N patterns in pine species). We have followed the reviewer's suggestions and we now have more deeply examined the possibility that the decline is related to an anthropogenic change in disturbance regime.

Summary of major changes:

1. Incorporated more detailed description of the statistical tests employed and the reasoning for choosing the tests.
2. Incorporated more discussion related to the role of fire within the Deming Lake watershed, using results found from the Clark (1990) study.
3. Changed Figure 2 from a map demonstrating the slope within the watershed to a contoured map to better demonstrate the sampling sites along with their age classes.
4. Added more discussion related to alternative explanations and the ultimate implications of the results.

**Anonymous Referee #1**
Received and published: 31 March 2015
General comments

This MS by Howard and McLauchlan explored temporal and spatial variability of N cycling in a mixed coniferous forest of the northern US, by examining long-term changes in N15 in both wood tissues and lake sedimentary. The authors demonstrated that N15 in wood tissues exhibited a synchronous decline since the 1920s, and N15 data from the lake sedimentary confirmed the declining trend. The authors ascribed the decline of N15 to fire suppression across the study area. These results are pretty interesting, and definitely deserve to be published. I carefully read through the manuscript, and did not find any significant flaw. The field sampling is reasonable, and the statistical analyses are robust. One minor point is that, the explanations for the observed N15 patterns are too much speculative. I am wondering whether the authors could provide some fire dataset, and then explore the quantitative relationship between fire activity and N15 trajectory.

Reviewer one addresses the need for better fire data in the watershed. We have now included the previous work of James S. Clark who reconstructed the fire history for Deming Lake through sediment records and fire scars from *Pinus resinosa* trees. Similar to the pattern seen in wood δ15N and sediment δ15N, the decline in fire frequency began in the early 20th century. We have also contacted Dr. Clark with the goal of obtaining the fire data to potentially assess the spectral properties related to fire frequency and N availability.

**Anonymous Referee #2**

Received and published: 6 April 2015

General Comments

Howard and McLauchlan set out to assess the century-scale trajectory of N availability in the forests around Demming Lake, and to determine influential drivers of d15N trends in tree wood across space and time. This paper makes several exceptional contributions which make this dataset well worth publishing: the ability to compare wood to sediment data, rigorous temporal and spatial analysis, and a wood d15N chronology that is longer than any previously published. Greater organization is primarily what is called for. Particularly in the description of statistical methods, the discussion of the biogeochemical and ecological effects of fire suppression, and in speculation of the ultimate drivers of the observed trends. Their argument for fire suppression as the most likely driver of the temporal shift in d15N, despite an acknowledged lack of expected time lags, needs strengthening. In addition, the wood 15N proxy method has several interpretation challenges, which I believe the authors well understand, but should clarify in the text.

SPECIFIC COMMENTS

**3618:24 – ". . .a variety of negative environmental consequences. . . have been attributed to increases in Nr." It seems appropriate to site more seminal papers of previous decades here, in addition to these recent papers.**

Yes, we agree. We have added:

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and G. D. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications 7:737-750.

Aber, J., Mcdowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., and Fernandez, I.: Nitrogen saturation in temperate forest ecosystems - Hypotheses revisited. Bioscience 48, 921-934, 1998.

3619:5-18 The case for retrospective studies as a means to understanding modern processes, and the particular time scale of interest, could be laid out more clearly here.

We have included a discussion of why retrospective analyses are important for understanding the processes behind elemental cycling and how humans may have altered these processes.

3620:13 – A leap is made from inferring "fractionating pathways" to inferring "N availability;" the link ought to be explained.

A more thorough explanation of how fractionation affects N availability as well as additional references has now been included.

3622:1-10 – It would be useful to know which of the mentioned species are N-fixers. Either here or later in the paper, It would be useful to note how fire return, its reduction, and the growth of hardwood species, is significant for the balance of N fixation at this site.

None of the dominant or subcanopy tree species are known to be associated with N-fixers. The main N inputs to the site are likely from free-living N2-fixing bacteria. While to our knowledge no measurements have been made of this flux in this system, we refer to Smithwick et al. 2005 that reviewed estimated N budgets from coniferous forests.

3623:4-5 – "The wood samples were not subjected to any chemical pretreatments based on results from Doucet et al. 2011." I would like to see the authors elaborate about their choice for foregoing pretreatment of samples, as on reviewing Doucet et al. 2011 and Gerhart McLauchlan 2014, it appears that different authors have concluded that different methods are appropriate at different sites.

Pretreatment has been the focus of a number of studies and there does not appear to be a single methodology that could be deemed "correct." In both Caceres et al. (2011) and Doucet et al. (2011), the authors tested the use of pretreatment on δ15N for one species and found that while there may be effects of pretreatment on labile N, it is not enough to offset the overall trajectory. Further, Caceres et al. (2011) concluded that pretreatment is not necessary when trees are exposed to their natural N concentrations and only need to be pretreated when there are unusually high inputs of N into the soils, such as fertilizer applications. Since fertilizer has not been applied to this watershed or nearby, atmospheric wet and dry N deposition is relatively low (~4-7 kg/ha on average between 2011-2013), and the trajectory of N availability was the most important aspect of the research, there was no need to pretreat the samples prior to examination. This is now addressed in the manuscript.

3623 – Statistical Analyses – This section would benefit from greater organization. Specifics about parameters for the tests are jumped into before an outline of the tests is provided, for example. It would be useful to lay out questions, statistical methods employed and reasons for choosing them, and then specifics on how the methods were employed.
3627 – These paragraphs are a mix of method description and results, which buries the results. I would prefer to see the description of the tests moved to the statistics part methods section.

The methods included in some of the discussion have now been moved into the appropriate section. A more detailed explanation of our choice of certain statistical tests has now been included as well.

3630:8-26 – These two paragraphs could be condensed and combined, as ideas are repeated and scattered between the two of them. The second paragraph has an opening thesis about nutrient status, but the examples given are not nutrient-mediated, but pertain to regeneration from the seed bed, which is mentioned but not explained in the preceding paragraph.

This paragraph has been condensed to address the important aspects related to altered nutrient cycling caused by an altered fire regime.

3630:27 – I am confused by the authors' opening statement, "Altered biogeochemistry would explain the sharp declines in wood d15N in many of the trees following the implementation of a no-burn policy in the 1920s." The authors subsequently say that the effect of such policy on wood d15N would probably be lagged. This is an important point. The authors' concluding statements point to fire suppression as their preferred explanation for the d15N trend, but this paragraph does not make a case for how d15N could respond so quickly (though it does explain why a lag would be expected).

We have given this further discussion in the paragraph related to the varying response times between change in fire regime and N availability.

**3631:24 – What is meant by "a time course" of ecosystem processes in soils? I don't think this is a recognizable term without explanation.**

3632:3-5 – ". . .the fact that both old-growth and younger trees are exhibiting a similar timeline of rapidly declining d15N, an external rather than internal force must be driving this macro-level change." A diameter-independent effect of outer rings on pine wood d15N could be an "internal" cause of this pattern; in the absence of more evidence about tree ring d15N patterns in general an external force would not seem to be required. Are there other trees outside this treatment area ideally of a similar ˇ species that don't show this pattern? Something like a control dataset would be useful.

A diameter-related effect is more than likely the most realistic alternative explanation as to why all the trees within watershed are declining in wood d15N. However, this would only be a viable explanation if the declines began within a certain age of the trees. This is not the case. There is substantial evidence that trees in multiple age classes (250, 200, 150, and 130) all begin to decline in wood d15N at around the same timeframe. Unfortunately, there are no "control" datasets or wood d15N measurements on trees outside the fire suppressed area. However, we have changed the wording to be clearer about the potential internal v. external causes of the synchronous decline.

TECHNICAL CORRECTIONS
3618:4 – Nr has not yet been defined in the MS

Changed to reactive nitrogen (Nr)

3620:16 – Period missing after "north-central US"
3626:17 – should read "shift beginning in the 1920s."
3628:6 – should read "varies anywhere"
3630:9 – Remove the word "Fire" and start the sentence "Suppression of the type of low intensity ground fires. . ."
3630:18 – Period missing after "US"
3632:3-5 – ". . .the fact that both old-growth and younger trees are exhibiting a similar timeline of rapidly declining d15N, an external. . ." there is a word or phrase missing before this comma.

These all have been addressed and corrected.

**Anonymous Referee #3**
Received and published: 10 April 2015
General comments
This manuscript presents an analysis of tree ring stable N isotopes from an unmanaged pine forest in Minnesota, adjacent to a lake were sediment isotope analyses have also been carried out. The major change in disturbance regime and biogeochemistry in this stand is the suppression of the natural fire regime beginning in the early 20th Century. The data presented in this paper are certainly a valuable contribution to a literature that is still somewhat inconclusive about how to process or interpret N isotopes in tree rings. The length of the record presented is a major strength of this manuscript, as is the ability

to compare with a sediment record. The exploratory work attempting to explain variation in N isotope patterns among individual trees is interesting, but did not add much in the end.
Overall this study was quite well conducted, but the manuscript could be more carefully written to avoid overstating the conclusions. The largest problem with this manuscript is that in several places, it frames its conclusions as relating directly to N availability, indicating that the accumulation of biomass in the absence of fire disturbance has more than compensated for the global and regional increases in DIN deposition. This is a reasonable explanation for the observed pattern, but is not conclusively proven. These sections (e.g. the second paragraph of the results and first sentence of the discussion) could be rewritten to acknowledge that testing for changes in d15N is not a very direct test of the hypothesis presented, though it is helpful evidence. The linkage between d15N and N availability at the ecosystem level has a firm theoretical grounding but mixed empirical support in the literature, as there are many other factors affecting d15N (which are described elsewhere in the manuscript).

I am curious what the authors think about the rather striking similarity in long-term trend and breakpoint between this site and Mirror Lake NH site, (McLaughlin et al. 2007). This is especially interesting given the very different natural disturbance and land-use histories of the two sites. Are there other sites in the central and eastern parts of North America that show similar patterns? If so, is there a more parsimonious explanation than the various changes in disturbance regime at each site?

Extremely interesting point! This is an ongoing active area of research for us and several other groups. At this point, there are not enough samples to say how ubiquitous and synchronous these N isotopic declines might be. If there were a large number of heterogeneous sites exhibiting this signal, it seems that regional or global-scale drivers would be the most parsimonious explanation. We have highlighted this on line 630 to 634.

Abstract: In the concluding sentence, “consequences of global-scale alterations : : :” is a bit ambiguous, and could be worded more precisely.

“Global-scale alterations” has been removed from the abstract and replaced with more precise wording related to human changes to global N cycling.

Introduction: The description of how fire and human disturbances affect the N cycling and N availability could be more thorough; the conclusions hinge on the reader understanding
these links.

Another good point. We have added some text to lines 151-153 mentioning that severe stand-replacing fires can affect N cycling and reference a good review paper. Unfortunately, fire and N cycling is not a simple relationship. There is less work on how fire suppression affects N cycling. We believe there is a good discussion of how fire affects N cycling in the discussion section.

Study Site: Unpacking the fire history some more might help the reader better understand the site. Also, how are stand-initiating fires occurring every 10 years while the return interval is 22 years? Are these two metrics at different scales (stand vs. landscape?) This should be clarified.
Section 2.1. As this is a global journal, a bit more information about the soils and climate at the study site might be helpful to readers who are not familiar with the region.

Very good point and this has now been included in the description of Deming Lake and Itasca State Park.

Section 2.2. – the writing is awkward and difficult to follow in several places
I would also like to see the authors briefly discuss their choice to analyze total N rather than conducting some sort of extraction or separation procedure, rather than simply citing one reference. There is no single standardized approach that is universally accepted, so this choice is reasonable, but should be discussed.

Per the request of reviewer two as well, this has been addressed in the manuscript.

Section 2.3, line 22: Is this really a Z-score treatment? Were values expressed as # of standard deviations?

Yes, these are considered z-scores ($x- \mu / \sigma$) for each of the values and thus represents the number of standard deviations below the mean for any given data point.

P3627, L20: what else might be different near the shoreline? Soil moisture, soil texture, soil organic matter? How would these relate to N cycling? What about fire frequency? Also, is the lake level steady over time or not?

This is a very interesting question about what else might be different near the shoreline. Because the catchment is not steeply-sloped (i.e. X% slope near shoreline), and the parent material is glacial till, soil properties do not seem to vary with distance from shoreline. Although it is possible that local topographic differences would alter fire frequency near the lake, anecdotal evidence from park managers indicates that the low-intensity prescribed burns in the late 1990s burned to the edge of the water. Lake level has been relatively stable during the Holocene, even during dramatic climate shifts, because the lake is groundwater-fed. (This was established by Colin Plank and Bryan Shuman, we are not sure if the data are published.)

P3631, L19: can the authors be more specific about the “low levels” of N deposition?

Though there is little agriculture in the region and no direct influence from industry, there is some inorganic N deposition in this region compared with other regions throughout the United States. The National Atmospheric Deposition Program data indicates that annual N deposition rates from wet and dry deposition averaged ~4-7 kg/ha in this region during the period 2011-2013. We have added these data.

Figure 2: What is meant by "% change in slope"? Is this the second derivative of elevation? Or should it just say "slope (%)"? Also, the scale bar is a bit ambiguous – is the entire bar 100m, or each marked interval?

As mentioned in the summary, this figured has been changed to better depict the changes in elevation in the watershed.

Figure 4: this does not appear to be the correct caption for this figure.

The caption was incorrect and now correctly reflects the figure.

Cited references:

Cacerces, M.K., Mizota, C., Yamanaka, T., Nobori, Y.: Effects of pre-treatment on the nitrogen isotope composition of Japanese black pine (Pinus thunbergii) tree-rings as affected by high N input. Rapid Commun. Mass Spectrom, 21, doi: 10.1002/rcm.5227, 2011.

Clark, J.S.: Fire and climate change during the last 750 yr in northwestern Minnesota, Ecol. Monogr., 60, 135-159, http://dx.doi.org/10.2307/1943042, 1990a.

Doucet A., Savard, M.M., Bégin, C., and Smirnoff, A.: Is pre-treatment essential for tree-ring nitrogen concentration and isotope analysis?, Rapid Commun. Mass Spec., 25, 469-475, doi: 10.1002/rcm.4876, 2011.

McLauchlan, K.K., and Craine, J.M.: Species-specific trajectories of nitrogen isotopes in Indiana hardwood forests, USA, Biogeosciences, 9, 867-874, doi:10.5194/bg-9-867-2012, 2012.

National Atmospheric Deposition Program. 2013 Annual Summary. http://nadp.sws.uiuc.edu/lib/data/2013as.pdf, 2013.

Smithwick, E.A.H., Turner, M.G., Mack, M.C., and Chapin, F.S.: Postfire Soil N Cycling in Northern Conifer Forests Affected by Severe, Stand-Replacing Wildfires. Ecosystems 8, 163-181, 2005.

# Spatio-temporal analysis of nitrogen cycling in a mixed coniferous forest of the northern United States

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# Abstract

Nitrogen (N) is the limiting nutrient to primary productivity in a variety of temperate forests, and N cycling is undergoing a variety of anthropogenic changes, notably a doubling of reactive N (Nr) on a global scale. Yet, the local scale impacts of 20th century changes to N cycling have been difficult to document in terrestrial ecosystems, especially old-growth forests. To determine the spatial and temporal variability of anthropogenic effects on old-growth forest N dynamics, we measured the composition of stable nitrogen isotopes ($\delta^{15}$N) in wood from living red pine trees (*Pinus resinosa*) at a single site in northern Minnesota, USA. A synchronous decline in wood $\delta^{15}$N values began approximately in the 1920s C.E. in 18 individual trees at different topographic positions, indicating a common driver. The decline in wood $\delta^{15}$N values corresponded with declines in sedimentary $\delta^{15}$N recorded in lacustrine sediments of the same catchment. Disturbance regime and species composition began to change at the turn of the 20th century with park establishment, providing a likely mechanism of decline in $\delta^{15}$N values toward present. While other mechanisms are possible, we conclude that the consequences of while increased influxes of various forms of anthropogenic nitrogenNr into terrestrial ecosystems through anthropogenic sources global-scale alterations to N cyclinghave potentially altered global-scale N cycling, these changes are not being expressed at a local level in this temperate forest ecosystem.

## 1 Introduction

Global biogeochemical cycles have been altered by human activities, such as elevated levels of atmospheric CO$_2$, global increases in temperatures, and increased deposition of reactive nitrogen (Nr) through anthropogenic sources (fossil fuel burning and fertilizer production) (Vitousek et al. 1997). In forested ecosystems, increases in atmospheric nitrogen (N) deposition have been of particular concern, because a variety of negative environmental consequences, such as reduced forest growth, eutrophication, acid rain, and biodiversity loss, have been attributed to increases in Nr (Hietz et al. 2011; Houlton et al. 2013). Despite global-scale changes to N cycling, it has been difficult to pinpoint effects of Nr on a local scale in terrestrial ecosystems, due to an additional suite of activities that affect local N cycling such as management practices, intensive logging, disturbance regimes, alteration of species composition, and regional-scale variability in climate (Kareiva et al. 2007; McLauchlan and Craine 2012).

Although understanding how these factors affect the N cycle is of paramount importance to understanding recent changes in forest dynamics, long-term records of N cycling in forests are rare and generally restricted to modern measurements that might span a few decades at most (Gerhart and McLauchlan 2014). Long-term monitoring of N cycling metrics such as stream nitrate export indicates an unexpected ability of many terrestrial ecosystems to retain added N, with stable or declining trends in N availability since monitoring began in the late 20th century (Bernal et al. 2012). A more temporally complete understanding of the modern state and trajectory of N cycling in forests requires extending records back in time prior to settlement by Euro-Americans (Galloway et al. 2004). This retrospective approach, which uses N cycling proxies preserved in wood or sedimentary archives, has supported and extended the long-term monitoring studies. Some retrospective studies have indicated that regional and local-scale processes may be altering N availability to varying degrees during the past 500 years (McLauchlan et al. 2013a).

While our understanding of N availability in terrestrial ecosystems has improved, the lack of long-term records and relatively poor spatial coverage limits the ability to fully assess the consequences of anthropogenic disturbance to the N cycle. Retrospective analyses of biogeochemical cycling provides a unique opportunity to assess natural variability of many elemental cycles. Given that humans have altered, and in many cases increased, the fluxes between major pools of elements, it is important to develop records that document the behavior of these cycles on various spatial scales prior to and following human disturbance. Fortunately, analysis of the standardized natural abundance ratios of $^{15}$N to $^{14}$N ($\delta^{15}$N) stored in tree rings is a relatively new technique that serves as a proxy record for the history of N availability in past terrestrial ecosystems (Gerhart and McLauchlan 2014). Of the approximately 50 published wood $\delta^{15}$N studies so far, the majority have been in secondary forests, with trees less than a century old (*e.g*. Poulson et al. 1995; Bukata and Kyser 2007; Hietz et al. 2010; Beghin et al. 2011; Stock et al. 2012; McLauchlan and Craine 2012). The oldest published wood $\delta^{15}$N value is from a sample dated to 1835 C.E. (McLauchlan et al. 2007). Because secondary forests are accruing biomass, with high demand for N, these types of sites potentially alter conclusions about the biogeochemical consequences of anthropogenic N deposition.

Interpretation of wood $\delta^{15}$N trajectories depends on the relationship between soil N availability, foliar $\delta^{15}$N, and wood $\delta^{15}$N, providing another proxy for measuring N availability in soils through tree ring analysis (Craine et al. 2009). Briefly, stable N isotopes preserved in wood reflect multiple fractionating pathways within the N cycle. The main fractionating pathways are gaseous N losses to the atmosphere via microbially-mediated processes. Conversion of ammonium to nitrate (nitrification), leaching of nitrate, mycorrhizal fungi providing depleted $\delta^{15}$N to plants, and preferential uptake of ammonium by plants can ultimately alter the signal of plant $\delta^{15}$N. In most cases though, higher gaseous losses through denitrification and increased nitrification equates to higher N availability in the system and a more enriched $\delta^{15}$N signal in the plant (Houlton et al. 2006). In forests, high $\delta^{15}$N values are found in soils and leaves of ecosystems with high rates of nitrification (Pardo et al. 2006), high rates of denitrification (Houlton et al. 2006), partial nitrification followed by nitrate leaching (Pardo 2002), and low reliance on mycorrhizal fungi for plant N uptake (Hobbie and Colpaert 2003; Cuoto-Vazquez and Gonzalez-Prieto 2010). Stable N isotopes of wood from dated tree rings can produce high-resolution terrestrial records of past N cycling at specific locations that extend back in time several centuries. Thus, wood $\delta^{15}$N provides a useful proxy for analysing spatio-temporal patterns of terrestrial N availability.

Old-growth forests dominated by red pine (*Pinus resinosa*) and white pine (*Pinus strobus*) once covered large areas of the north-central U.S. Today, protected remnants of these forests provide valuable information regarding the role of natural disturbances like fire and windstorms in determining vegetation dynamics (Peet 1984, Webb 1989). Pre-settlement *P. resinosa* stands exhibit a variety of age structures (Fraver and Palik 2012), with maximum tree ages observed to be approximately 200 to 300 years old (D'Amato et al. 2010, Silver et al. 2013). The disturbance regimes have been typically dominated by low-intensity surface fires that maintain an open understory, with occasional high-intensity stand-replacing fires (Clark 1988, 1989). Severe stand-replacing wildfires can have a variety of effects on soil N cycling in northern conifer forests (Smithwick et al. 2005). During the past 100 years, *P. resinosa* forests have experienced substantial regeneration declines, expansion of hardwood species, and the development of dense undergrowth composed of pyrogenic species like fir (*Abies balsamea*) (Frissell 1973). Increases in deer populations, along with severe drought episodes have also hindered regeneration of pine species. As a result, neither *P. resinosa* nor *P. strobus* have been reproducing successfully since fire suppression began in the early 1920s (Ahlgren 1976). One of the best-protected areas of old-growth *P. resinosa* occurs at Itasca State Park in northern Minnesota (Minnesota DNR). Due to significant logging and intensification of agriculture in the region, Itasca is considered a relict of the vast northern coniferous forests. Additionally, given the well documented timeframe of park establishment (1891) and implementation of fire suppression (~1920s), N availability can be analyzed prior to and following human involvement, providing insight into how anthropogenic drivers can influence biogeochemical cycles in local terrestrial ecosystems.

Here, we analyze the spatio-temporal characteristics of N availability of *P. resinosa* within a mixed coniferous forest in Itasca State Park located in northwest Minnesota through use of nitrogen isotopes in tree rings. Our main objectives were to assess the long-term (century-scale) trajectory of N availability through isotopic analysis and additionally determine the influential drivers across space and time. We hypothesized: 1) Nitrogen availability to the dominant tree species, *P. resinosa,* would be declining toward present at most sites within the watershed due to management-driven changes in fire regime in the early 20th century, and 2) There would be spatial heterogeneity within the temporal trend in the watershed, with individual *P. resinosa* trees exhibiting variable $\delta^{15}N$ trajectories based on location and geographic features.

## 2 Methods

### 2.1 Study site:

Itasca State Park (ISP) in northern Minnesota is a large park (13,229 hectares) and lies on the Itasca moraine, deposited by the Laurentide Ice Sheet 13K YBP (47°14'N 95°12'W). The upland soils of ISP are a mixture of sandy outwash and glacial till. Climate is typical of a northern temperate forest, with approximately 65 cm of annual precipitation and a mean annual temperature of 3°C. The vegetation prior to Euro-American settlement in the region was a mixture of red pine (*P. resinosa*), white pine (*P. strobus*), and jack pine (*P. banksiana*) forests and savannas, prairie openings, deciduous northern hardwood forests, and boreal bogs. Our study site, the Deming Lake watershed, lies within a mixed coniferous forest of Itasca State Park (Fig. 1). It is a relatively small lake (5.4 ha surface area) with soils derived largely from glacial till. The climate of the region is dominated by cold arctic air masses in the winter and warm humid air masses from the gulf during the summer, with average precipitation ranging from 22-26 inches per year. Fire was common in the region prior to human involvement, with varying fire return intervals anywhere from ~8 to 50 years over the last 750 years (Clark 1990). In recent years, several deciduous hardwood species have been increasing in abundance, including *Acer saccharum* (sugar maple), *Betula papyrifera* (paper birch), *Corylus cornuta* (beaked hazel), *Populus tremuloides* (aspen), *P. grandidentata* (big-toothed aspen), *Quercus borealis* (northern red oak), and *Tilia americana* (basswood). *Corylus cornuta* now dominates the understory (Kurmis and Sucoff 1989). In the last 20 years, prescribed burns have been implemented in an attempt to stimulate regeneration of *P. resinosa* and reduce fuel loads (Santoro et al. 2001).

## 2.2 Field and laboratory methods:

Increment cores of 5.15 mm diameter were obtained at 1m above the forest floor from twenty-four individual *P. resinosa* trees surrounding the Deming Lake watershed in March 2014 with a Haglöf borer. *P. resinosa* was the only species sampled because of its dominance, importance and legacy to the area, and the need to use a single species to test our hypotheses. Samples were gathered from five stands comprising four to five trees with aspects of west, north, east, and south (Fig. 2). Trees were sampled at different elevations, slopes, and proximity to the shoreline to capture the spatial variation in $\delta^{15}N$ that may arise from small-scale topographic features or geographical position.

The cores were stored in plastic bags and dried at 65°C. Each core was sanded then scanned at 1200dpi. Ring widths of the cores were analyzed using both Cybis and the CDendro software (Saltsjöbaden, Sweden). The pith was reached on 11 out of the 24 samples, therefore the absolute age could not be determined for most trees but could nevertheless be dated based on cross dating techniques and the last ring formation of 2013. The longest chronology contained 237 rings, while the shortest had 79 rings. Cores on average contained 145 rings (Table 1). Cores were weighed and divided into 10 mg segments along ring boundaries that maximized temporal resolution while maintaining enough N in the sample for mass spectrometry. We did not apply any chemical pretreatments to the samples, as no universally accepted standardization method of sampling total N within wood has been developed. Pretreatment has been the focus of a number of studies and has been deemed unnecessary when trees are exposed to natural N inputs (i.e. unfertilized) (Caceres et al. 2011). In both Caceres et al. (2011) and Doucet et al. (2011), the authors tested pretreatment methods on $\delta^{15}N$ for one species and found that while there may be effects of pretreatment on labile N, pretreatment does not offset the overall trajectory. Since fertilizer has not been applied to this watershed or nearby, atmospheric N deposition is relatively low (average of ~4-7 kg/ha per year between 2011-2013), and the trajectory of N availability was the most important aspect of the research, pretreatment did not seem necessary (NAPD 2014). On average, wood samples comprised 2 or 3 annual rings, with a total of 1224 samples measured. Two cores were lost during measurement, and two were not measured for $\delta^{15}$N, therefore a total of 20 trees were used for subsequent analysis (Table 1).

The standardized ratio of $^{15}$N:$^{14}$N relative to air ($\delta^{15}$N) was determined for segments of wood at the University of Maryland Center for Environmental Science, Central Appalachian Stable Isotope Facility (CASIF) Laboratory. The instrument is a ThermoFisher Delta V+ isotope ratio mass spectrometer fitted with sequential traps of $MgClO_4$, NaOH on solid support (Carbosorb), and a cold trap in liquid $N_2$.

**2.3 Statistical analyses:**

In order to develop a spatio-temporal understanding of N cycling within the watershed, a number of statistical methods were employed. We set out to understand whether the effects of geography, geomorphology, and proximity to the shoreline of Deming Lake affected the overall mean values of $\delta^{15}$N for each tree and the stands sampled. For instance, do trees closer to the lake exhibit a more depleted signal of $\delta^{15}$N compared to those areas further away from the water body? To test for differences in mean, we utilized the Kruskal-Wallis and applied it to the averaged $\delta^{15}$N values based on the stand level mean, topography (based on slope percentage), elevation, and the trees location relative to the shoreline. For the latter test, distances from the shoreline were divided into three groups: Those within 13.7m of the shoreline (7 trees), between 13.7m to 16.8m (7 trees) and greater than 16.8m (6 trees). Three groups were selected in an attempt to provide an adequate number and equal distribution of samples. For temporal analyses, we calculated standardized values by subtracting the mean $\delta^{15}$N from each sample for each core, giving each a core a mean value of 0‰. This is essentially a Z-score treatment, used in other isotopic analysis (McLauchlan et al. 2007, Craine and McLauchlan 2010). To test hypothesis #1 (evidence of trend), simple linear regression along with the rank-based, non-parametric Mann Kendall Trend test (MK-Test) were applied to each sample. Given its robustness for non-normally distributed data, the MK-Test is frequently applied to hydroclimatic time series data. The MK-Test determines the presence of a monotonic increasing or decreasing trend (given by the MK-Tau value), along with the magnitude of the slope calculated through the Sen's Slope estimator. Trends determined by the test were deemed significantly different from zero if p-values were <.05 (Mann 1945; Sen 1968).

After determining the trends and slopes of each core, a supplemental analysis sought to determine the date range of when the potential shift in mean occurred. We hypothesized that changes in N availability (particularly decreases) would be the result of a reduction in fire frequency through park management practices. Testing for a break-point allows us to assess whether a definitive change in N availability can be detected. Time-series related studies, particularly in the climate and atmospheric sciences, often employ change point analysis using a suite of tests. We applied four separate tests of change in the mean in order to improve bolster confidence that a change in mean does exist. The Pettit's test (Pettitt 1979) Buishand's Bayesian Test (Buishand 1984), the standard normal homogeneity test (Alexandersson 1986) as well the Cumulative deviation test (Rebstock 2002) were applied to all samples. Relevant to our study, we used these tests to aid in our determination of whether a detectable human disturbance signature exists in the record. A great deal of research has been dedicated towards the study of detection procedures for identifying a potential change point in a data series. Many have argued that trends must be removed prior to detection of inhomogeneities, as trends (or autocorrelation) can create synthetic change points in a dataset (Beaulieu et al. 2012). However, other studies have claimed that removing the trend component has the same adverse affect (Guerreiro et al. 2013). In our study we manipulated the data as little as possible (the exception being standardization) and applied no detrending methods prior to tests for shifts in mean. Regardless, using a diverse suite of change-in-mean tests allows for comparison of different tests on the same datasets. The use of multiple analyses allows a rigorous test of whether a detectable shift in $\delta^{15}$N values exists.

## 3 Results

### 3.1 Trends in $\delta^{15}$N:

Within the Deming Lake watershed, wood $\delta^{15}$N has been significantly declining over the period of record as evident by the trajectories of individual trees. Of the twenty trees sampled, 18 demonstrated significantly declining trends toward present ($p<0.05$) based on both simple linear regression and the MK-Test. Trends were significantly positive in the other two trees. The steepest declines tended to occur in trees located in stands two and five (northeast and south areas of the lake), although no clear cohesive spatial pattern could be detected (Table 2). Similarly, the length of the chronology had no apparent effect on the steepness of slope of the samples, as trees with similar ring counts had different rates of change.

To test our first hypothesis— that management practices have influenced N availability in *P. resionsa* in this watershed— we employed four statistical analyses to detect temporal breakpoints in the wood $\delta^{15}$N time series. Of the 18 trees with negative trajectories, a significant breakpoint ($p < 0.05$) could be detected based on each test in 15 cases. In 13 of the 15 trees, a similar breakpoint for each individual tree (within 1 data point) was evident for the four tests (Table three). In the case of S2TA where significant break points were evident but the timing differed, extreme values occurring within a 20-year period more than likely distorted the signal creating the differences, as removal of the outliers in the period resulted the temporal agreement of a breakpoint.

Breakpoint tests were then applied to larger-scale watershed-level wood $\delta^{15}$N based on averaging the time series for all trees. Using this chronology, a significant breakpoint of 1927 C.E. was detected (Fig. 3). A piecewise regression was also applied to the time series to show the varying trajectories prior to and post-1927 C.E. A slight positive trend exists (not significant) prior to 1927 C.E., and the major decline in wood $\delta^{15}$N did not begin until the 1930s.

Both parts of our first hypothesis are supported by these results, if tests for breakpoints are good estimates of when shifts in mean occurred. Fire suppression began in the 1920s, and in 13 cases a change in mean could be detected within the period of 1920 to 1947. It is important to note that it would take time for the potential mechanisms affecting levels of N availability to change after a change in disturbance regime. As a result, there would be a lagged effect from the time of altered disturbance regime to when significant declining N availability would be evident in the wood $\delta^{15}N$ record of already established species such as *P. resinosa*. Without using homogeneity tests to assess whether a change-point exists, trees with sufficient data records prior to 1925 C.E. (>15 samples before 1925) were divided into two periods: $\delta^{15}N$ prior to 1925, and $\delta^{15}N$ after 1925. Tests for trend were applied to the two time series to see if slopes significantly differed. Indeed, in the analysis of the 15 trees used, 10 exhibited no significant trend ($p > 0.5$), three trees had significantly increasing trends, while two had significantly downward trends. Tests of the data following 1925, however, reveal similar trends to those discussed initially, with 16 trees having significantly negative trends, one tree with a negative trend, two trees with significantly upward trends, and one with no significant trend. Therefore, even without removing any trends, it is clear that these trees exhibit breakpoints in wood $\delta^{15}N$ trajectories, with a shift beginning in the 1920s.

### 3.2 Spatial Characteristics of $\delta^{15}N$

To test our second hypothesis, we examined the spatial patterns of wood $d^{15}N$ values at several levels of spatial organization. First, for individual trees, raw $\delta^{15}N$ values exhibited a high degree of heterogeneity and spatial variation, with maximum $\delta^{15}N$ values for an individual tree ranging from 3.46‰ (S1TC) to -0.06 ‰ (S5TD) and minimum values ranging from -1.76‰ (S4TE) to -4.38‰ (S2TC)(Fig. 4a). The average standard deviation for all trees sampled was 1.22‰. Tests among all trees revealed significantly different mean $\delta^{15}N$ ($p < 0.00001$), not surprising given the multitude of environmental factors that alter levels of N availability. With significantly different variances in the dataset detected, the Games-Howell test was used in the post-hoc tests. Means of $\delta^{15}N$ for each tree significantly differed on average from 9 (8.7) other trees. Outliers in the post-hoc tests included tree S1TD, located in Stand One on the east end of the lake, and S5TC located in Stand Five on the south end of the lake. The former significantly differed from only two trees, while the latter significantly differed from 18 trees.

Second, spatial patterns were also analysed at the level of the stand, a group of neighboring trees. An initial ANOVA test based on group means of the five stands sampled once again revealed significant ($p < 0.00001$) differences among stands, although post-hoc analysis indicated that only Stand Three, consisting of those trees on the north end of the lake, significantly differed from the other four groups. The trees within this stand by far had the lowest $\delta^{15}N$ average (by over 1‰) and the least amount of variation (Fig. 4b). Tests of the group means showed significant differences between the three groups ($p < 0.00001$), but only those trees closest to the lake significantly differed from the other two groups.

One unexpected result from the proximal tests was a change in average wood $\delta^{15}N$ value with distance from the shoreline. Values of wood $\delta^{15}N$ for trees closest to Deming Lake averaged -1.54‰, followed by -0.77‰ for the second closest group, and finally -0.64‰ for the group furthest from the lake. Inversely, higher variation in $\delta^{15}N$

was present in those trees furthest away from Deming Lake (1.47 standard deviations), followed by 1.26 for the intermediate distance group and 1.10 for the trees closest to the lake. The decline in $\delta^{15}N$ from trees sampled further out to trees close to the shoreline could be the result of trees incorporating a more enriched signal of $\delta^{15}N$ in higher elevations and through leaching processes, a more depleted signal is left for trees closest to the shoreline. Since the catchment is not steeply sloped and the parent material is derived from glacial till, soil properties would not appear to be a viable explanation for the watershed-scale differences. Although topographical differences could alter the fire frequency, recent prescribed burns of low-intensity similar to those that occurred naturally burned to the edge of the water. Given the lake is fed by groundwater and has remained relatively stable during the Holocene (McLauchlan et al. 2013), changes in lake levels also would not be a significant influence on N availability. A sampling technique (i.e. cross-section or point-quarter) geared towards assessing the spatial changes in $\delta^{15}N$ would better address the explanations described here

In terms of the variability in trajectories based on location and geographic features, the tests for breakpoints struggle to agree on breakpoint timing for the two groups closest to Deming Lake. Albeit significant, the timing of the breakpoints vary anywhere from 1927 to 1973. Conversely, for the trees furthest from the lake all tests agree on a significant 1924 breakpoint ($p<.0001$). The inability to agree on a similar breakpoint value more than likely results from averaging the $\delta^{15}N$ values for trees in a similar group, creating incredible variability making it difficult to assess whether a change in mean exists. Indeed, using a 5-year smoothing filter results in much better agreement among breakpoint tests for the two groups, with values in the 13.7m-16.7m group varying from 1926-1941, while the closest group of trees having a breakpoint value of 1926 based on all tests ($p<.0001$). Relative to the group trajectories, based on simple linear regression only the slope of the closest group of trees significantly differed from the others ($p<.0001$). The steepest decline was also present in this group. Conversely, when assessing the slopes after 1925, the most negative slopes occurred in the group of trees within 13.7m-16.8m, followed by the group <13.7m to the lake. Only the group furthest away significantly differed from the other two in terms of their trajectories following 1925. The two trees with increasing trajectories toward present appear to have no obvious landscape characteristics that explain those patterns. With respect to the trajectory based on the incline of the surface, once again three groups were created based on the criteria locations with >19% slope, between 14-19% slope, and finally <14%. Trees with the highest % slope had significantly different slope values than the other two groups. While a similar breakpoint could be agreed upon with the two latter groups listed above (1926 and 1927 at $p<.0001$), a breakpoint could not be determined for trees with the highest slopes. Once again this may result from a decreased sample size within the group. The largest decline also occurred within this group, followed by trees residing on terrain with 14-19% slope. In addition to there being significant spatial differences in wood $\delta^{15}N$ demonstrated in this study within a spatial extent of 676 $m^2$, our second hypothesis, about spatial differences in wood $\delta^{15}N$ trajectories, appears to be supported as well despite the overall agreement of declining $\delta^{15}N$.

### 3.3 Terrestrial and Lacustrine records:

Given the strong signal of declines in terrestrial δ$^{15}$N, we compared the wood δ$^{15}$N record to a previously-published sediment record from Deming Lake for the portion of temporal overlap—approximately the last 250 years (McLauchlan et al. 2013b). There is remarkable coherence between these two records. The most noticeable characteristic of both time series is the noticeably similar steep decline of δ$^{15}$N beginning in the 20th century. The same breakpoint tests used on the wood δ$^{15}$N were applied to sedimentary δ$^{15}$N using the period of data from 1750 to 2004 C.E. In three of the four tests, a change point of 1930 C.E. was detected (all significant at $p < .01$). The values of terrestrial δ$^{15}$N, on average, are smaller (more negative) than values of sedimentary δ$^{15}$N. While it would be ideal to test a larger portion of the sedimentary time series to accurately identify the change in mean, the two datasets share a remarkable degree of similarity during the past ~250 years (Fig. 5).

## 4 Discussion

Available nitrogen has clearly been declining over time in *P. resinosa* within the Deming Lake watershed. A robust set of breakpoint analyses identify the early 1920s as the time when a synchronous decline in wood δ$^{15}$N values began. Declines in wood δ$^{15}$N values toward present have been described in a number of studies and attributed to a variety of causes, described below (Gerhart and McLauchlan 2014). Because of the synchrony of the breakpoint, the timing, and the direction of change (decline toward present), we believe the primary reason for the substantial decline in δ$^{15}$N over the past century has been management practices within the state park, namely fire suppression that began in the late 1910s. Disturbance regimes, both human and natural, can have profound impacts on nutrient cycling resulting from complex spatial and temporal patterns that affect primary productivity and biomass accumulation (Clark 1988). In the case of fire, disturbance can influence N availability both on short and long-term scales. In the short term, fire impacts organic-matter pools (Wang et al. 2012), while on longer time scales fire can alter species composition subsequently influencing total carbon and N pools (Clark 1990a; Nave et al. 2011). Fire suppression of the type of low intensity ground fires common to *P. resinosa* forests allows for competing understory species to increase in abundance, both increasing plant demand for N and increasing annual litter input to the forest floor, thereby lowering the availability of nitrogen for tree species dependent on a semi-regular fire regime (Brisson et al. 1988; Tappeiner and Alm 1975). Indeed, fire suppression of ponderosa pine forests in the western U.S. has been demonstrated to reduce N availability up to 130 years after the onset of fire suppression through reduced net N mineralization and nitrification potential (MacKenzie et al. 2006). We suggest that these changes in nutrient status may be contributing to the regeneration problems previously documented for *P. resinosa* in the upper U.S. After a burn, *P. resinosa* invades burned regions by seeding from other trees nearby which were unaffected by the fire. With a reduction in generation, competing species are allowed to propagate. It has been suggested that the old-growth pine forests of not only Itasca but much of the northern United States may eventually succeed to northern hardwoods, as regeneration attempts of pine species in the region have failed with fire suppression (Zenner and Peck 2009).

Altered biogeochemistry would explain the sharp declines in wood $\delta^{15}N$ in many of the trees following the implementation of a no-burn policy in the 1920s. Significant declines in wood $\delta^{15}N$ of many of the trees sampled here did not begin until the early-mid 20th century. It is logical to believe that if this practice did in fact alter nutrient cycling at Deming Lake, it would have been a lagged effect. However, it is noteworthy to mention that some trees decline immediately following fire suppression while with others the sharp decline does not begin for up to 15 to 20 years later. While we do believe the evidence highly suggests a large-scale disturbance altered the biogeochemistry of the watershed, there are various stand-level and micro-level spatial effects that may alter the timing of disturbance-response. Further, the last recorded fire at Deming Lake occurred in 1917 and was not a watershed-scale fire, therefore certain areas affected by the burn may have been more resistant to understory growth compared to unaffected areas in the watershed (Clark 1990). Studies have documented the effects on N availability following human-disturbance. Bukata and Kyser (2007) analyzed the effects of tree-clearing and land-use change on *Quercus alba* (white oak) and *Quercus rubra (*red oak) in forest stands in Ontario, Canada. Their results showed that trees on the outer edges of the stand showed an almost immediate marked increase in $\delta^{15}N$ compared to those in the center, coinciding with the tree-clearing event and land-use change. This study emphasizes the role of shifting ecosystem composition on $\delta^{15}N$ values resulting from human activity.

There are several alternative explanations for declining wood $\delta^{15}N$ values that have been published in the literature (see Gerhart and McLauchlan 2014 for a summary). Similar patterns of decline in wood $\delta^{15}N$ observed elsewhere have been attributed to increases in atmospheric N deposition. This might be plausible if $\delta^{15}N$ in $NO_x$ is much lower compared to soil $\delta^{15}N$, and if the quantity of deposited N was sufficient to alter either the isotopic signature or cycling of soil N. Poulson et al. (1995) first hypothesized that a decreasing trend over time of $\delta^{15}N$ in rings of *T. canadensis* (eastern hemlock) stemmed from increased deposition of depleted $^{15}N$ compounds due to an increase of $NO_x$ and $NH_3$ emissions in the late 20th century. Although the area lies on the boundary between low N deposition levels and higher levels in the southern Minnesota and Iowa, the lack of agriculture large-scale industry in northwest Minnesota as well as the declines predating widespread human manipulation of the global N cycle, it is difficult to posit this as a viable explanation for the patterns evident at this site.

Declining $\delta^{15}N$ may also be attributed to a physiological process in plants or a time course of ecosystem processes in soils. As a tree ages, uptake of N switches from an open cycle to a closed cycle resulting from decreasing nutrient losses and increasing stand age (Jussy et al. 2000). Perhaps the sources supplying N to the tree change with age as well, with sources in the most recent growth years contain N depleted in $^{15}N$ than those which supplied the tree in the early stages of its life (Hobbie and Hobbie 2006). More than likely some physiological factors influence N cycling within *P. resinosa* and could perhaps be genus or species dependent, but physiological-related explanations fail to account for the rapid step-change in $\delta^{15}N$ seen in a majority of the trees beginning in the

1920s. With age classes exhibiting declines ranging anywhere from ~100 to ~240 years, coupled with the high degree of spatial heterogeneity in terms of δ$^{15}$N , an external process is the most likely hypothesis for driving declines in N availability.

Despite the overall synchronous trajectory among trees, certain geographical differences resulted in different spatial signatures of δ$^{15}$N. In old-growth *P. resinosa* forests, Clark (1990) had previously identified topographic position as an important driver of N mineralization rates, with spatial variation in soil moisture correlated with microbial activity. In our study, trees on the north side of the lake in a more open stand structure had the lowest average levels of wood δ$^{15}$N. Scattered and large *P. resinosa* dominated this area (highest average DBH among stands) with relatively few tree/shrub species in the understory present. Average levels of δ$^{15}$N also differed based on proximity to the lake, with samples furthest away containing the highest averages and variances compared to those closer to water body. It would be pertinent in future studies to further investigate the role landscape position plays in δ$^{15}$N signatures of both soil and plants.

Additional questions arise as the result of the striking similarities between the δ$^{15}$N values of the sediment record analysed in McLauchlan et al. (2013b) and our results. The marked decline in δ$^{15}$N in the latter part of the sediment record had been attributed to internally driven processes occurring within the lake, particularly the development of anaerobic conditions in the deeper areas of the lake. Over the past 500 years, δ$^{15}$N in the sediment record has been declining markedly, although a somewhat stable period is present from 1600 to 1900 followed by a drastic decline in the 20th century. The identical temporal pattern of nearby wood δ$^{15}$N suggests either different mechanisms driving the same pattern in the two records, or a need to reassess the contribution of terrestrial inputs to the sedimentary record. This result, of dendrochronological and sedimentary δ$^{15}$N records from the same small catchment showing strong agreement in their respective trajectories, has also been seen at an oligotrophic lake in New Hampshire (McLauchlan et al. 2007) and a high-elevation lake in the western U.S. (Wolfe et al. 2013). Each of these sites has a different land-use history and a different N cycle, but each has demonstrated a decline in δ$^{15}$N in the past century. The ubiquity of 20th century declines in δ$^{15}$N values in various ecosystem pools, and possible regional or global-scale drivers, is an area deserving much further investigation.

Overall, the results of our study analysing the spatial-temporal characteristics of δ$^{15}$N in a pine-dominated forest of northwest Minnesota demonstrate declining N availability for the past 80 years, which we hypothesize the decline coincides with the onset of fire suppression in the state park. Three different proxy datasets derived from the watershed exhibit a decline (Clark (1990) charcoal record, δ$^{15}$N record in wood, δ$^{15}$N in the sediment) that coincides with a major ecosystem-scale disturbance. This study is the longest wood δ$^{15}$N chronology published, with δ$^{15}$N values from wood dated to 1775 C.E. (~238 years ago). However, it cannot be said with full certainty that the declines are the result of a single mechanism. More complicated and alternative hypotheses cannot be ruled out without incorporation of additional data (i.e. precise fire history from the watershed calibrated with δ$^{15}$N data, or additional datasets about the temporal trajectory of δ$^{15}$N in pine species). Therefore, until additional work is conducted on the effects of altered fire regimes on nitrogen availability, the dataset presented here should be treated as strong evidence but not a direct test of our main hypothesis. Although selection of a single tree species (*P. resinosa)* provided an intriguing spatial and temporal picture of $\delta^{15}$N, it would be useful to sample additional old-growth forests, and wood $\delta^{15}$N of some of the understory species that became abundant with fire suppression to obtain a more complete answer about the mechanism of these declines in $\delta^{15}$N. Other elemental cycles (i.e. carbon and phosphorus) and their association with changes in N availability would also provide a more coherent explanation of why these drastic changes have occurred and the role of changing environmental conditions in altering N cycling on local spatial scales.

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**Table 1**: List of the trees sampled, ring counts, the number of $\delta^{15}$N samples obtained from that tree, elevation in meters, and distance from shore in meters.

| ***Tree*** | ***Ring Counts*** | ***# δ15N Samples*** | ***Elevation (m)*** | ***Distance from Shore (m)*** |
|---|---|---|---|---|
| ***S1TC*** | 238 | 99 | 468 | 23.69 |
| ***S1TD*** | 122 | 44 | 469.5 | 20.63 |
| ***S1TE*** | 215 | 84 | 472.2 | 16.58 |
| ***S2TA*** | 175 | 98 | 474.5 | 14.28 |
| ***S2TB*** | 158 | 74 | 467.1 | 14.31 |
| ***S2TC*** | 162 | 55 | 465.2 | 12.57 |
| ***S2TD*** | 158 | 63 | 464.8 | 17.00 |
| ***S2TE*** | 79 | 50 | 467.7 | 13.20 |
| ***S3TA*** | 99 | 60 | 467 | 13.65 |
| ***S3TC*** | 118 | 49 | 469.5 | 10.29 |
| ***S3TD*** | 128 | 45 | 471.2 | 11.97 |
| ***S4TA*** | 95 | 35 | 470.4 | 9.35 |
| ***S4TB*** | 86 | 78 | 471 | 17.77 |
| ***S4TC*** | 171 | 64 | 468 | 16.82 |
| ***S4TD*** | 178 | 56 | 458 | 14.88 |
| ***S4TE*** | 159 | 51 | 466.1 | 9.30 |
| ***S5TA*** | 132 | 69 | 462.2 | 20.50 |
| ***S5TB*** | 110 | 68 | 462.2 | 20.50 |
| ***S5TC*** | 153 | 45 | 465.8 | 22.12 |

**Table 2**: Slope based on simple linear regression (m), Sen's Slope and Kendall's tau value based on Mann-Kendall Trend Test for each tree. The Hamed and Rao method takes into account autocorrelation and adjusts the Sen's Slope estimate. Tau values with * indicate p < .05, ** p <.01, and *** p <.0001, NS = not significant.

| ***Tree*** | ***m*** | ***p-value*** | ***Sen's Slope (Hamed and Rao method)*** | ***Kendall's Tau*** |
|---|---|---|---|---|
| ***S1TC*** | -0.0196 | <0.0001 | -0.029 | -0.600*** |
| ***S1TD*** | 0.0101 | <0.0001 | 0.064 | 0.638*** |
| ***S1TE*** | -0.0067 | <0.0001 | -0.024 | -0.355*** |
| ***S2TA*** | -0.0085 | <0.0001 | -0.022 | -0.418*** |
| ***S2TB*** | -0.0163 | <0.0001 | -0.041 | -0.657*** |
| ***S2TC*** | -0.0105 | <0.0001 | -0.045 | -0.532*** |
| ***S2TD*** | -0.0150 | <0.0001 | -0.043 | -0.518*** |
| ***S2TE*** | -0.0101 | <0.0001 | -0.080 | -0.532*** |
| ***S3TA*** | 0.0061 | <0.05 | 0.017 | 0.214* |
| ***S3TC*** | -0.0078 | <0.0001 | -0.037 | -0.461*** |
| ***S3TD*** | -0.0077 | <0.0001 | -0.051 | -0.554*** |
| ***S4TA*** | -0.0003 | NS | -0.003 | -0.025NS |
| ***S4TB*** | -0.0170 | <0.0001 | -0.042 | -0.576*** |
| ***S4TC*** | -0.0145 | <0.0001 | -0.038 | -0.587*** |
| ***S4TD*** | -0.0014 | <0.05 | -0.013 | -0.176* |
| ***S4TE*** | -0.0075 | <0.0001 | -0.039 | -0.442*** |
| ***S5TA*** | -0.0095 | <0.0001 | -0.050 | -0.546*** |
| ***S5TB*** | -0.0156 | <0.0001 | -0.040 | -0.606*** |
| ***S5TC*** | -0.0128 | <0.0001 | -0.044 | -0.674*** |
| ***S5TD*** | -0.0094 | <0.001 | -0.058 | -0.371** |

**Table 3**: Estimated breakpoint for each tree based on the four homoegeneity tests. Asterisk indicates a significant break point ($p < .05$) detected by that test, regardless of whether the tests agree.

| ***Tree*** | ***Buishand's Test*** | ***CUSUM*** | ***Pettit's Test*** | ***SNTH Test*** |
|---|---|---|---|---|
| ***S1TC*** | 1941* | 1942* | 1941* | 1942* |
| ***S1TE*** | 1925* | 1925* | 1925* | 1925* |
| ***S2TA*** | 1928* | 1942* | 1927* | 1949* |
| ***S2TB*** | 1930* | 1930* | 1930* | 1930* |
| ***S2TC*** | 1935* | 1935* | 1935* | 1935* |
| ***S2TD*** | 1958* | 1956* | 1956* | 1958* |
| ***S2TE*** | 1973* | 1975* | 1973* | 1983* |
| ***S3TC*** | 1942* | 1942* | 1942* | 1942* |
| ***S3TD*** | 1917* | 1917* | 1920* | 1917* |
| ***S4TA*** | 1980 | 1996 | 1980 | 2008 |
| ***S4TB*** | 1947* | 1947* | 1926* | 1947* |
| ***S4TC*** | 1943* | 1943* | 1933* | 1943* |
| ***S4TD*** | 1941* | 1941* | 1941* | 1941* |
| ***S4TE*** | 1968* | 1968* | 1968* | 1968* |
| ***S5TA*** | 1941* | 1941* | 1943* | 1939* |
| ***S5TB*** | 1924* | 1923* | 1923* | 1923* |
| ***S5TC*** | 1923* | 1923* | 1929* | 1923* |
| ***S5TD*** | 1927* | 1927* | 1927* | 1927* |

**Figure 1**: The Deming Lake watershed, looking west-southwest. *P. resinosa* and *P. strobus* are the most common species in this area, though understory vegetation includes smaller hardwoods, shrubs and various grasses. Photo credit: Amy Myrbo.

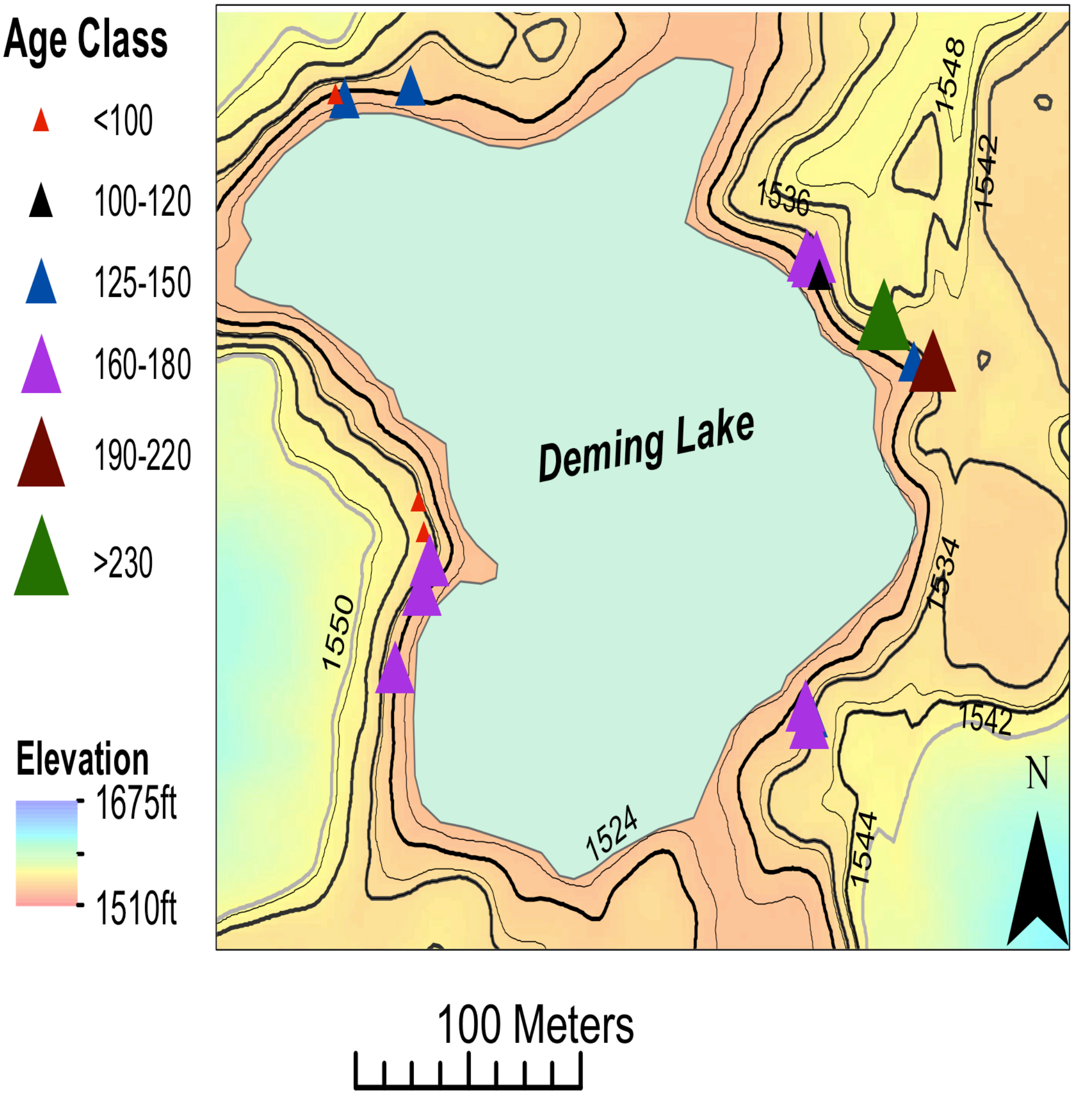

**Figure two:** Contoured elevation map of Deming Lake (in feet) along with labeled contoured lines. The locations of the trees sampled are included, along with their respective age classes based on the size of the triangle. Note some trees were sampled within close proximity to one another so not all samples may be represented on the map.

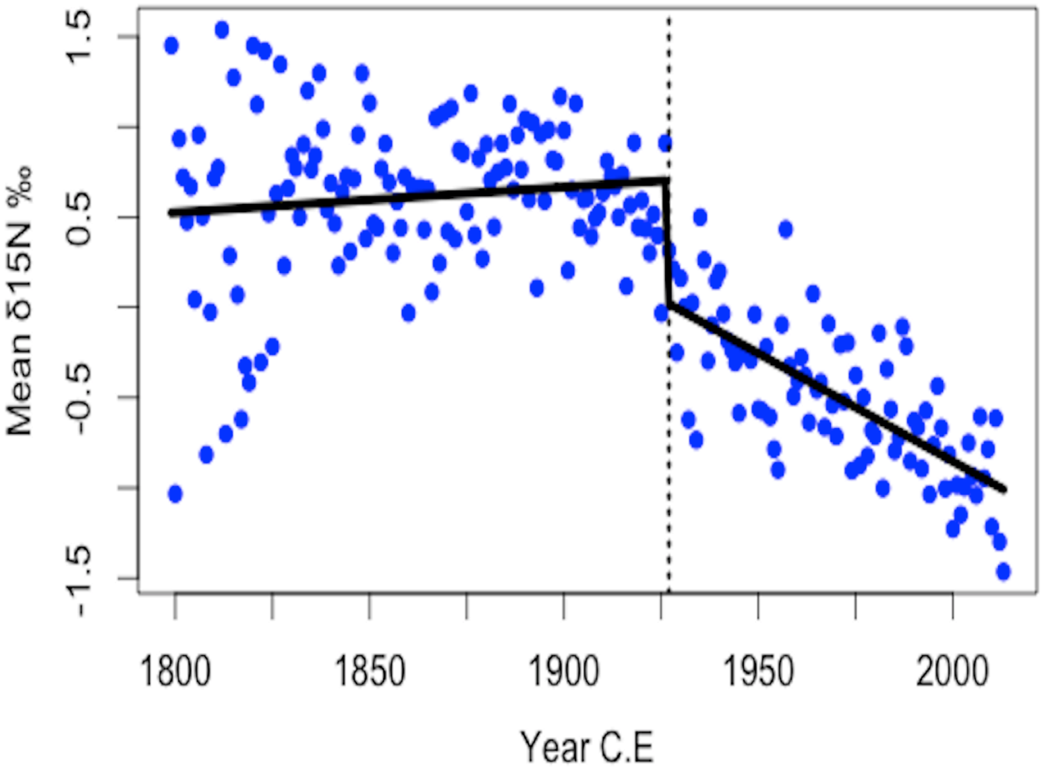

**Figure 3**: Piecewise regression line fit to the standardized $\delta^{15}$N for the entire study site. For purposes of larger sample size, data for the period 1800-2013 were selected. Dashed line represents the significant change point of 1927 determined by change point tests. Sen's Slope prior to the determined 1927 breakpoint based on Mann-Kendall Trend Test is slightly positive albeit not significant (p = .972). The period of 1927-2013 has a significant (p<.00001) Sen's Slope of -0.012.

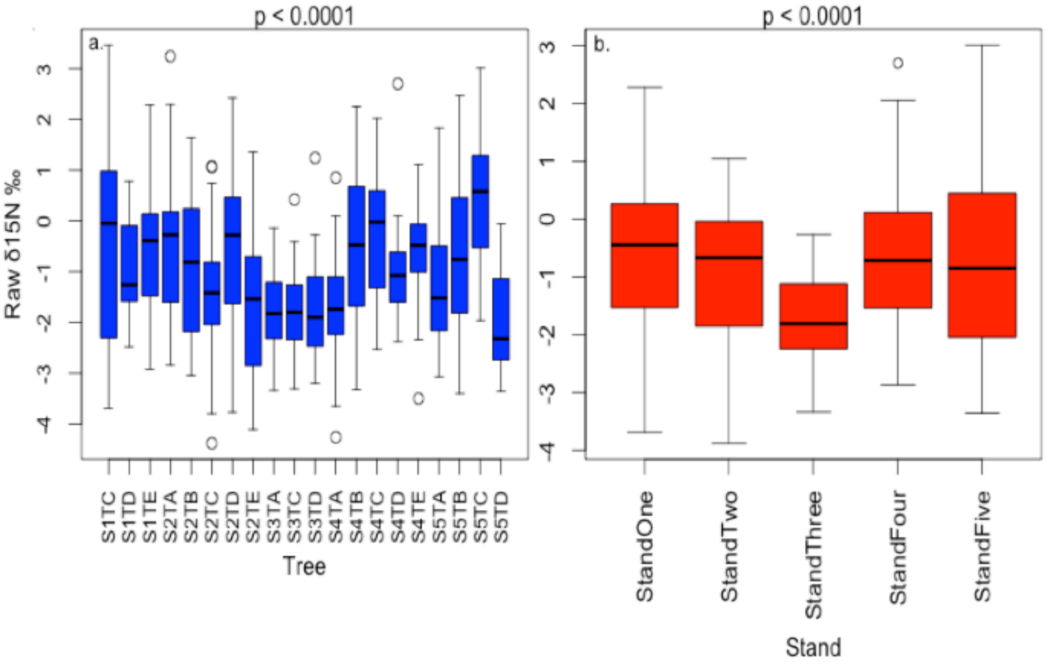

Figure 4: Box plots showing differences in means between trees (a) and stands (b). a.) Box plot of $\delta^{15}N$ for each tree. S refers to the Stand (i.e S1= Stand One), T refers to tree, (T1= Tree One). b.) Same as Fig. 4a but for the five stands analysed. The box plots are based on the averages from each stand. Data averaged over the time series.

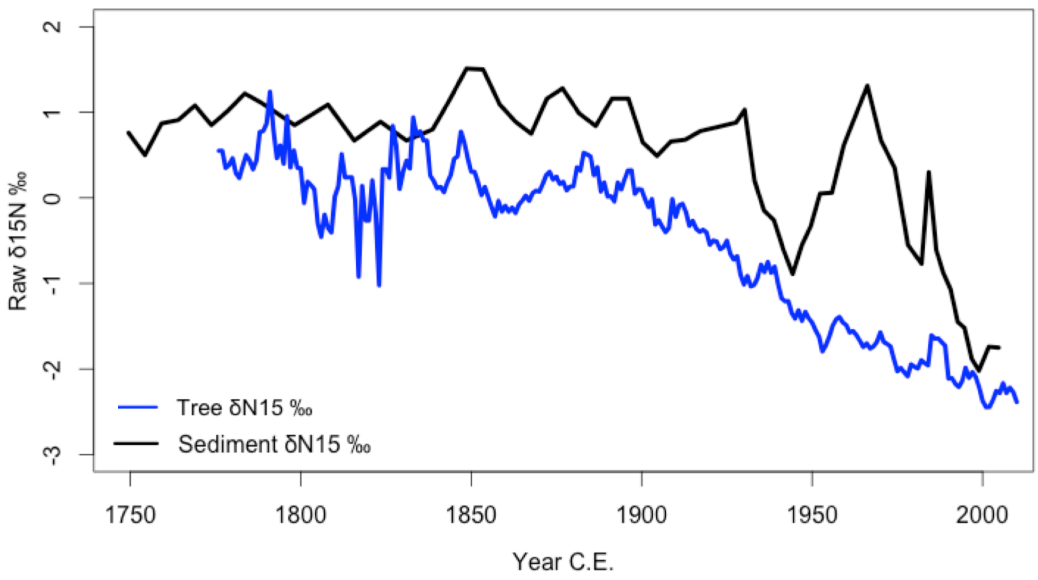

Figure 5: Comparison of sediment $\delta^{15}N$ record with a 5-year smoothed raw wood $\delta^{15}N$ record. Blue line indicates the sediment $\delta^{15}N$ record (1749.5-2004.7), black line indicates smoothed wood $\delta^{15}N$ record (1776-2013).