

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

3  
4 **I. Howard<sup>1</sup>, K.K. McLauchlan<sup>2</sup>**

5  
6 <sup>1</sup>Department of Geosciences, University of Arkansas, 216 Ozark Hall,  
7 Fayetteville, AR, 72701

8  
9 <sup>2</sup>Department of Geography, Kansas State University, 118 Seaton Hall, Manhattan, KS  
10 66506

11  
12 Correspondence to: I. Howard ([ihowardksu@gmail.com](mailto:ihowardksu@gmail.com))

13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38

## 39 Abstract

40

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

58

## 59 1 Introduction

60

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

73

74 Although understanding how these factors affect the N cycle is of paramount  
75 importance to understanding recent changes in forest dynamics, long-term records of N  
76 cycling in forests are rare and generally restricted to modern measurements that might  
77 span a few decades at most (Gerhart and McLauchlan 2014). Long-term monitoring of N  
78 cycling metrics such as stream nitrate export indicates an unexpected ability of many  
79 terrestrial ecosystems to retain added N, with stable or declining trends in N availability  
80 since monitoring began in the late 20<sup>th</sup> century (Bernal et al. 2012). A more temporally  
81 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.

82 2004). This retrospective approach, which uses N cycling proxies preserved in wood or  
83 sedimentary archives, has supported and extended the long-term monitoring studies.  
84 Some retrospective studies have indicated that regional and local-scale processes may be  
85 altering N availability to varying degrees during the past 500 years (McLauchlan et al.  
86 2013a).

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

104 Interpretation of wood  $\delta^{15}\text{N}$  trajectories depends on the relationship between soil  
105 N availability, foliar  $\delta^{15}\text{N}$ , and wood  $\delta^{15}\text{N}$ , providing another proxy for measuring N  
106 availability in soils through tree ring analysis (Craine et al. 2009). Briefly, stable N  
107 isotopes preserved in wood reflect multiple fractionating pathways within the N cycle.  
108 The main fractionating pathways are gaseous N losses to the atmosphere via microbially-  
109 mediated processes. Conversion of ammonium to nitrate (nitrification), leaching of  
110 nitrate, mycorrhizal fungi providing depleted  $\delta^{15}\text{N}$  to plants, and preferential uptake of  
111 ammonium by plants can ultimately alter the signal of plant  $\delta^{15}\text{N}$ . In most cases though,  
112 higher gaseous losses through denitrification and increased nitrification equates to higher  
113 N availability in the system and a more enriched  $\delta^{15}\text{N}$  signal in the plant (Houlton et al.  
114 2006). In forests, high  $\delta^{15}\text{N}$  values are found in soils and leaves of ecosystems with high  
115 rates of nitrification (Pardo et al. 2006), high rates of denitrification (Houlton et al. 2006),  
116 partial nitrification followed by nitrate leaching (Pardo 2002), and low reliance on  
117 mycorrhizal fungi for plant N uptake (Hobbie and Colpaert 2003; Cuoto-Vazquez and  
118 Gonzalez-Prieto 2010). Stable N isotopes of wood from dated tree rings can produce  
119 high-resolution terrestrial records of past N cycling at specific locations that extend back  
120 in time several centuries. Thus, wood  $\delta^{15}\text{N}$  provides a useful proxy for analysing spatio-  
121 temporal patterns of terrestrial N availability.

122 Old-growth forests dominated by red pine (*Pinus resinosa*) and white pine (*Pinus*  
123 *strobus*) once covered large areas of the north-central U.S. Today, protected remnants of  
124 these forests provide valuable information regarding the role of natural disturbances like

125 fire and windstorms in determining vegetation dynamics (Peet 1984, Webb 1989). Pre-  
126 settlement *P. resinosa* stands exhibit a variety of age structures (Fraver and Palik 2012),  
127 with maximum tree ages observed to be approximately 200 to 300 years old (D'Amato et  
128 al. 2010, Silver et al. 2013). The disturbance regimes have been typically dominated by  
129 low-intensity surface fires that maintain an open understory, with occasional high-  
130 intensity stand-replacing fires (Clark 1988, 1989). Severe stand-replacing wildfires can  
131 have a variety of effects on soil N cycling in northern conifer forests (Smithwick et al.  
132 2005). During the past 100 years, *P. resinosa* forests have experienced substantial  
133 regeneration declines, expansion of hardwood species, and the development of dense  
134 undergrowth composed of pyrogenic species like fir (*Abies balsamea*) (Frissell 1973).  
135 Increases in deer populations, along with severe drought episodes have also hindered  
136 regeneration of pine species. As a result, neither *P. resinosa* nor *P. strobus* have been  
137 reproducing successfully since fire suppression began in the early 1920s (Ahlgren 1976).  
138 One of the best-protected areas of old-growth *P. resinosa* occurs at Itasca State Park in  
139 northern Minnesota (Minnesota DNR). Due to significant logging and intensification of  
140 agriculture in the region, Itasca is considered a relict of the vast northern coniferous  
141 forests. Additionally, given the well documented timeframe of park establishment (1891)  
142 and implementation of fire suppression (~1920s), N availability can be analyzed prior to  
143 and following human involvement, providing insight into how anthropogenic drivers can  
144 influence biogeochemical cycles in local terrestrial ecosystems.

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

155

## 156 **2 Methods**

### 157 **2.1 Study site:**

158

159 Itasca State Park (ISP) in northern Minnesota is a large park (13,229 hectares) and  
160 lies on the Itasca moraine, deposited by the Laurentide Ice Sheet 13K YBP (47°14'N  
161 95°12'W). The upland soils of ISP are a mixture of sandy outwash and glacial till.  
162 Climate is typical of a northern temperate forest, with approximately 65cm of annual  
163 precipitation and a mean annual temperature of 3°C. The vegetation prior to Euro-  
164 American settlement in the region was a mixture of red pine (*P. resinosa*), white pine (*P.*  
165 *strobus*), and jack pine (*P. banksiana*) forests and savannas, prairie openings, deciduous  
166 northern hardwood forests, and boreal bogs. Our study site, the Deming Lake watershed,  
167 lies within a mixed coniferous forest of Itasca State Park (Fig. 1). It is a relatively small  
168 lake (5.4 ha surface area) with soils derived largely from glacial till. Fire was common in

169 the region prior to human involvement, with varying fire return intervals anywhere from  
170 ~8 to 50 years over the last 750 years (Clark 1990). In recent years, several deciduous  
171 hardwood species have been increasing in abundance, including *Acer saccharum* (sugar  
172 maple), *Betula papyrifera* (paper birch), *Corylus cornuta* (beaked hazel), *Populus*  
173 *tremuloides* (aspen), *P. grandidentata* (big-toothed aspen), *Quercus borealis* (northern  
174 red oak), and *Tilia americana* (basswood). *Corylus cornuta* now dominates the  
175 understory (Kurmis and Sucoff 1989). In the last 20 years, prescribed burns have been  
176 implemented in an attempt to stimulate regeneration of *P. resinosa* and reduce fuel loads  
177 (Santoro et al. 2001).

178

## 179 **2.2 Field and laboratory methods:**

180

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

189 The cores were stored in plastic bags and dried at 65°C. Each core was sanded  
190 then scanned at 1200dpi. Ring widths of the cores were analyzed using both Cybis and  
191 the CDendro software (Saltsjöbaden, Sweden). The pith was reached on 11 out of the 24  
192 samples, therefore the absolute age could not be determined for most trees but could  
193 nevertheless be dated based on cross dating techniques and the last ring formation of  
194 2013. The longest chronology contained 237 rings, while the shortest had 79 rings. Cores  
195 on average contained 145 rings (Table 1). Cores were weighed and divided into 10 mg  
196 segments along ring boundaries that maximized temporal resolution while maintaining  
197 enough N in the sample for mass spectrometry. We did not apply any chemical  
198 pretreatments to the samples, as no universally accepted standardization method of  
199 sampling total N within wood has been developed. Pretreatment has been the focus of a  
200 number of studies and has been deemed unnecessary when trees are exposed to natural N  
201 inputs (i.e. unfertilized) (Caceres et al. 2011). In both Caceres et al. (2011) and Doucet et  
202 al. (2011), the authors tested pretreatment methods on  $\delta^{15}\text{N}$  for one species and found that  
203 while there may be effects of pretreatment on labile N, pretreatment does not offset the  
204 overall trajectory. Since fertilizer has not been applied to this watershed or nearby,  
205 atmospheric N deposition is relatively low (average of ~4-7 kg/ha per year between  
206 2011-2013), and the trajectory of N availability was the most important aspect of the  
207 research, pretreatment did not seem necessary (NAPD 2014). On average, wood samples  
208 comprised 2 or 3 annual rings, with a total of 1224 samples measured. Two cores were  
209 lost during measurement, and two were not measured for  $\delta^{15}\text{N}$ , therefore a total of 20  
210 trees were used for subsequent analysis (Table 1).

211 The standardized ratio of  $^{15}\text{N}:^{14}\text{N}$  relative to air ( $\delta^{15}\text{N}$ ) was determined for  
212 segments of wood at the University of Maryland Center for Environmental Science,

213 Central Appalachian Stable Isotope Facility (CASIF) Laboratory. The instrument is a  
214 ThermoFisher Delta V+ isotope ratio mass spectrometer fitted with sequential traps of  
215  $\text{MgClO}_4$ , NaOH on solid support (Carbosorb), and a cold trap in liquid  $\text{N}_2$ .

216

### 217 **2.3 Statistical analyses:**

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

241 After determining the trends and slopes of each core, a supplemental analysis  
242 sought to determine the date range of when the potential shift in mean occurred. We  
243 hypothesized that changes in N availability (particularly decreases) would be the result of  
244 a reduction in fire frequency through park management practices. Testing for a break-  
245 point allows us to assess whether a definitive change in N availability can be detected.  
246 Time-series related studies, particularly in the climate and atmospheric sciences, often  
247 employ change point analysis using a suite of tests. We applied four separate tests of  
248 change in the mean in order to improve bolster confidence that a change in mean does  
249 exist. The Pettit's test (Pettitt 1979) Buishand's Bayesian Test (Buishand 1984), the  
250 standard normal homogeneity test (Alexandersson 1986) as well the Cumulative  
251 deviation test (Rebstock 2002) were applied to all samples. Relevant to our study, we  
252 used these tests to aid in our determination of whether a detectable human disturbance  
253 signature exists in the record. A great deal of research has been dedicated towards the  
254 study of detection procedures for identifying a potential change point in a data series.  
255 Many have argued that trends must be removed prior to detection of inhomogeneities, as

256 trends (or autocorrelation) can create synthetic change points in a dataset (Beaulieu et al.  
 257 2012). However, other studies have claimed that removing the trend component has the  
 258 same adverse affect (Guerreiro et al. 2013). In our study we manipulated the data as little  
 259 as possible (the exception being standardization) and applied no detrending methods prior  
 260 to tests for shifts in mean. Regardless, using a diverse suite of change-in-mean tests  
 261 allows for comparison of different tests on the same datasets. The use of multiple  
 262 analyses allows a rigorous test of whether a detectable shift in  $\delta^{15}\text{N}$  values exists.

263  
 264

### 265 **3 Results**

#### 266 **3.1 Trends in $\delta^{15}\text{N}$ :**

267

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

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

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

292 Both parts of our first hypothesis are supported by these results, if tests for  
 293 breakpoints are good estimates of when shifts in mean occurred. Fire suppression began  
 294 in the 1920s, and in 13 cases a change in mean could be detected within the period of  
 295 1920 to 1947. It is important to note that it would take time for the potential mechanisms  
 296 affecting levels of N availability to change after a change in disturbance regime. As a  
 297 result, there would be a lagged effect from the time of altered disturbance regime to when  
 298 significant declining N availability would be evident in the wood  $\delta^{15}\text{N}$  record of already  
 299 established species such as *P. resinosa*. Without using homogeneity tests to assess

300 whether a change-point exists, trees with sufficient data records prior to 1925 C.E. (>15  
 301 samples before 1925) were divided into two periods:  $\delta^{15}\text{N}$  prior to 1925, and  $\delta^{15}\text{N}$  after  
 302 1925. Tests for trend were applied to the two time series to see if slopes significantly  
 303 differed. Indeed, in the analysis of the 15 trees used, 10 exhibited no significant trend ( $p$   
 304  $> 0.5$ ), three trees had significantly increasing trends, while two had significantly  
 305 downward trends. Tests of the data following 1925, however, reveal similar trends to  
 306 those discussed initially, with 16 trees having significantly negative trends, one tree with  
 307 a negative trend, two trees with significantly upward trends, and one with no significant  
 308 trend. Therefore, even without removing any trends, it is clear that these trees exhibit  
 309 breakpoints in wood  $\delta^{15}\text{N}$  trajectories, with a shift beginning in the 1920s.

310  
 311

### 312 **3.2 Spatial Characteristics of $\delta^{15}\text{N}$**

313

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

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

336 One unexpected result from the proximal tests was a change in average wood  
 337  $\delta^{15}\text{N}$  value with distance from the shoreline. Values of wood  $\delta^{15}\text{N}$  for trees closest to  
 338 Deming Lake averaged -1.54‰, followed by -0.77‰ for the second closest group, and  
 339 finally -0.64‰ for the group furthest from the lake. Inversely, higher variation in  $\delta^{15}\text{N}$   
 340 was present in those trees furthest away from Deming Lake (1.47 standard deviations),  
 341 followed by 1.26 for the intermediate distance group and 1.10 for the trees closest to the  
 342 lake. The decline in  $\delta^{15}\text{N}$  from trees sampled further out to trees close to the shoreline  
 343 could be the result of trees incorporating a more enriched signal of  $\delta^{15}\text{N}$  in higher



344 elevations and through leaching processes, a more depleted signal is left for trees closest  
345 to the shoreline. Since the catchment is not steeply sloped and the parent material is  
346 derived from glacial till, soil properties would not appear to be a viable explanation for  
347 the watershed-scale differences. Although topographical differences could alter the fire  
348 frequency, recent prescribed burns of low-intensity similar to those that occurred  
349 naturally burned to the edge of the water. Given the lake is fed by groundwater and has  
350 remained relatively stable during the Holocene (McLauchlan et al. 2013), changes in lake  
351 levels also would not be a significant influence on N availability. A sampling technique  
352 (i.e. cross-section or point-quarter) geared towards assessing the spatial changes in  $\delta^{15}\text{N}$   
353 would better address the explanations described here

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

383

### 384 **3.3 Terrestrial and Lacustrine records:**

385

386 Given the strong signal of declines in terrestrial  $\delta^{15}\text{N}$ , we compared the wood  $\delta^{15}\text{N}$   
387 record to a previously-published sediment record from Deming Lake for the portion of

388 temporal overlap—approximately the last 250 years (McLauchlan et al. 2013b). There is  
389 remarkable coherence between these two records. The most noticeable characteristic of  
390 both time series is the noticeably similar steep decline of  $\delta^{15}\text{N}$  beginning in the 20<sup>th</sup>  
391 century. The same breakpoint tests used on the wood  $\delta^{15}\text{N}$  were applied to sedimentary  
392  $\delta^{15}\text{N}$  using the period of data from 1750 to 2004 C.E. In three of the four tests, a change  
393 point of 1930 C.E. was detected (all significant at  $p < .01$ ). The values of terrestrial  $\delta^{15}\text{N}$ ,  
394 on average, are smaller (more negative) than values of sedimentary  $\delta^{15}\text{N}$ . While it would  
395 be ideal to test a larger portion of the sedimentary time series to accurately identify the  
396 change in mean, the two datasets share a remarkable degree of similarity during the past  
397 ~250 years (Fig. 5).

398

#### 399 **4 Discussion**

400

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

431 Altered biogeochemistry would explain the sharp declines in wood  $\delta^{15}\text{N}$  in many of  
432 the trees following the implementation of a no-burn policy in the 1920s. Significant  
433 declines in wood  $\delta^{15}\text{N}$  of many of the trees sampled here did not begin until the early-mid  
434 20<sup>th</sup> century. It is logical to believe that if this practice did in fact alter nutrient cycling at  
435 Deming Lake, it would have been a lagged effect. However, it is noteworthy to mention  
436 that some trees decline immediately following fire suppression while with others the  
437 sharp decline does not begin for up to 15 to 20 years later. While we do believe the  
438 evidence highly suggests a large-scale disturbance altered the biogeochemistry of the  
439 watershed, there are various stand-level and micro-level spatial effects that may alter the  
440 timing of disturbance-response. Further, the last recorded fire at Deming Lake occurred  
441 in 1917 and was not a watershed-scale fire, therefore certain areas affected by the burn  
442 may have been more resistant to understory growth compared to unaffected areas in the  
443 watershed (Clark 1990). Studies have documented the effects on N availability following  
444 human-disturbance. Bukata and Kyser (2007) analyzed the effects of tree-clearing and  
445 land-use change on *Quercus alba* (white oak) and *Quercus rubra* (red oak) in forest  
446 stands in Ontario, Canada. Their results showed that trees on the outer edges of the stand  
447 showed an almost immediate marked increase in  $\delta^{15}\text{N}$  compared to those in the center,  
448 coinciding with the tree-clearing event and land-use change. This study emphasizes the  
449 role of shifting ecosystem composition on  $\delta^{15}\text{N}$  values resulting from human activity.

450 There are several alternative explanations for declining wood  $\delta^{15}\text{N}$  values that have  
451 been published in the literature (see Gerhart and McLauchlan 2014 for a summary).  
452 Similar patterns of decline in wood  $\delta^{15}\text{N}$  observed elsewhere have been attributed to  
453 increases in atmospheric N deposition. This might be plausible if  $\delta^{15}\text{N}$  in  $\text{NO}_x$  is much  
454 lower compared to soil  $\delta^{15}\text{N}$ , and if the quantity of deposited N was sufficient to alter  
455 either the isotopic signature or cycling of soil N. Poulson et al. (1995) first hypothesized  
456 that a decreasing trend over time of  $\delta^{15}\text{N}$  in rings of *T. canadensis* (eastern hemlock)  
457 stemmed from increased deposition of depleted  $^{15}\text{N}$  compounds due to an increase of  $\text{NO}_x$   
458 and  $\text{NH}_3$  emissions in the late 20<sup>th</sup> century. Although the area lies on the boundary  
459 between low N deposition levels and higher levels in the southern Minnesota and Iowa,  
460 the lack of agriculture large-scale industry in northwest Minnesota as well as the declines  
461 predating widespread human manipulation of the global N cycle, it is difficult to posit  
462 this as a viable explanation for the patterns evident at this site.

463 Declining  $\delta^{15}\text{N}$  may also be attributed to a physiological process in plants or a  
464 time course of ecosystem processes in soils. As a tree ages, uptake of N switches from an  
465 open cycle to a closed cycle resulting from decreasing nutrient losses and increasing  
466 stand age (Jussy et al. 2000). Perhaps the sources supplying N to the tree change with age  
467 as well, with sources in the most recent growth years contain N depleted in  $^{15}\text{N}$  than those  
468 which supplied the tree in the early stages of its life (Hobbie and Hobbie 2006). More  
469 than likely some physiological factors influence N cycling within *P. resinosa* and could  
470 perhaps be genus or species dependent, but physiological-related explanations fail to  
471 account for the rapid step-change in  $\delta^{15}\text{N}$  seen in a majority of the trees beginning in the  
472 1920s. With age classes exhibiting declines ranging anywhere from ~100 to ~240 years,  
473 coupled with the high degree of spatial heterogeneity in terms of  $\delta^{15}\text{N}$ , an external  
474 process is the most likely hypothesis for driving declines in N availability.

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

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

503 Overall, the results of our study analysing the spatial-temporal characteristics of  
504  $\delta^{15}\text{N}$  in a pine-dominated forest of northwest Minnesota demonstrate declining N  
505 availability for the past 80 years, which we hypothesize the decline coincides with the  
506 onset of fire suppression in the state park. Three different proxy datasets derived from  
507 the watershed exhibit a decline (Clark (1990) charcoal record,  $\delta^{15}\text{N}$  record in wood,  $\delta^{15}\text{N}$   
508 in the sediment) that coincides with a major ecosystem-scale disturbance. This study is  
509 the longest wood  $\delta^{15}\text{N}$  chronology published, with  $\delta^{15}\text{N}$  values from wood dated to 1775  
510 C.E. (~238 years ago). However, it cannot be said with full certainty that the declines are  
511 the result of a single mechanism. More complicated and alternative hypotheses cannot be  
512 ruled out without incorporation of additional data (i.e. precise fire history from the  
513 watershed calibrated with  $\delta^{15}\text{N}$  data, or additional datasets about the temporal trajectory  
514 of  $\delta^{15}\text{N}$  in pine species). Therefore, until additional work is conducted on the effects of  
515 altered fire regimes on nitrogen availability, the dataset presented here should be treated  
516 as strong evidence but not a direct test of our main hypothesis. Although selection of a  
517 single tree species (*P. resinosa*) provided an intriguing spatial and temporal picture of  
518  $\delta^{15}\text{N}$ , it would be useful to sample additional old-growth forests, and wood  $\delta^{15}\text{N}$  of some

519 of the understory species that became abundant with fire suppression to obtain a more  
 520 complete answer about the mechanism of these declines in  $\delta^{15}\text{N}$ . Other elemental cycles  
 521 (i.e. carbon and phosphorus) and their association with changes in N availability would  
 522 also provide a more coherent explanation of why these drastic changes have occurred and  
 523 the role of changing environmental conditions in altering N cycling on local spatial  
 524 scales.

525

### 526 **Acknowledgements**

527

528 This work was supported by NSF BCS-0955225 to K.M. We thank Jon Ross at Itasca  
 529 State Park for logistical assistance, and the Minnesota Department of Natural Resources  
 530 for a scientific research permit. Courtney Howard provided field assistance, and Robin  
 531 Paulman conducted the isotopic analyses at CASIF. P. R. Leavitt produced sediment data  
 532 about Deming Lake, and we thank him, Laci Gerhart Barley, and Kyleen Kelly for  
 533 helpful discussion.

534

### 535 **References**

536

- 537 Aber, J. D., Nadelhoffer, K.T. Steudler, Melillo, J.M.: Nitrogen saturation in northern  
 538 forest ecosystems, *BioScience*, 39, 378-386, [dx.doi.org/10.2307/1311067](https://doi.org/10.2307/1311067), 1989.
- 539 Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M.,  
 540 McNulty, S., Currie, W., Rustad, L., and Fernandez, I.: Nitrogen saturation in  
 541 temperate forest ecosystems - Hypotheses revisited. *Bioscience* 48, 921-934, 1998.
- 542 Ahlgren, C.E.: Regeneration of red pine and white pine following wildfire and logging in  
 543 northeastern Minnesota, *J. Forest.*, 74, 135-140,  
 544 <http://dx.doi.org/10.3375/043.029.0208>, 1975.
- 545 Alexandersson, H.: A homogeneity test applied to precipitation data, *J. Climatol.*, 6, 661-  
 546 675, doi: 10.1002/joc.3370060607, 1986.
- 547 Beaulieu, C., Chen, J., and Sarmiento, J.L.: Change-point analysis as a tool to detect  
 548 abrupt climate variations, *Philos. T. R. Soc. A.*, 370, 1228-1249,  
 549 [dx.doi.org/10.1175/JCLI4291.1](https://doi.org/10.1175/JCLI4291.1), 2012.
- 550 Beghin, R., Cherubini, P., Battipaglia, G., Siegwolf, R., Saurer, Matthias, and Bovio, G.:  
 551 Tree-ring growth and stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) detect effects of wildfires on  
 552 tree physiological processes in *Pinus sylvestris* L, *Trees*, 25, 627-636, doi:  
 553 10.1007/s00468-011-0539-9, 2011.
- 554 Bernal, S., Hedin, L.O., Likens, G.E., Gerber, S., and Buso, D.C.: Complex response of  
 555 the forest nitrogen cycle to climate change, *P. Natl. Acad. Sci. Usa.*, 109, 3406-  
 556 3411, doi: 10.1073/pnas.1121448109, 2012.
- 557 Brisson, J., Bergeron, Y., and Bouchard, A.: Secondary succession on mesic sites in the  
 558 Upper St. Lawrence Region, Quebec, Canada, *Can. J. Botany*, 6, 1192-1203,  
 559 doi:10.1139/b88-170, 1988.
- 560 Buishand, T.A.: Tests for detecting a shift in the mean of a hydrological time series, *J.*  
 561 *Hydrol.*, 73, 51-69, doi:10.1016/0022-1694(84)90032-5, 1984.

- 562 Bukata, A.R., and Kyser, T.K.: Carbon and nitrogen isotope variations in tree-rings as  
563 records of perturbations in regional carbon and nitrogen cycles, *Env. Sci. Tech.*, 41,  
564 1331-1338, doi: 10.1021/es061414g, 2007.
- 565 Cacerces, M.K., Mizota, C., Yamanaka, T., Nobori, Y.: Effects of pre-treatment on the  
566 nitrogen isotope composition of Japanese black pine (*Pinus thunbergii*) tree-rings as  
567 affected by high N input. *Rapid Commun. Mass Spectrom*, 21, doi:  
568 10.1002/rcm.5227, 2011.
- 569 Clark, J.S.: Effect of climate change on fire regimes in northwestern Minnesota, *Nature*  
570 334, 233-235, doi: 10.1038/334233a0, 1988.
- 571 Clark, J.S.: Effects of long-term water balances on fire regime, north-western Minnesota,  
572 *J. Ecol*, 77, 989-1004, 1989.
- 573 Clark, J.S.: Landscape interactions among nitrogen mineralization, species composition,  
574 and long-term fire frequency, *Biogeochemistry*, 11, 1-22, doi: 10.1007/BF00000849,  
575 1990.
- 576 Clark, J.S.: Fire and climate change during the last 750 yr in northwestern Minnesota,  
577 *Ecol. Monogr.*, 60, 135-159, <http://dx.doi.org/10.2307/1943042>, 1990a.
- 578 Cuoto-Vázquez, A., and González-Prieto, S.J.: Effects of climate, tree age, dominance  
579 and growth on  $\delta^{15}\text{N}$  in young pinewoods, *Trees*, 24, 507-514,  
580 <http://dx.doi.org/10.1007/s00468-010-0420-2>, 2010.
- 581 Craine, J.M., Elmore, A.J., Aidar, P. and co-authors: Global patterns of foliar nitrogen  
582 isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient  
583 concentrations, and nitrogen availability, *New Phytol.*, 183, 980-992, doi:  
584 10.1007/s00468-010-0420-2, 2009.
- 585 D'Amato, A.W., Palik, B.J. and Kern, C.C.: Growth, yield, and structure of extended  
586 rotation *Pinus resinosa* stands in Minnesota, USA, *Can. J. For. Res.*, 40, 1000-1010,  
587 doi: 10.1139/X10-041, 2010.
- 588 Doucet A., Savard, M.M., Bégin, C., and Smirnov, A.: Is pre-treatment essential for tree-  
589 ring nitrogen concentration and isotope analysis?, *Rapid Commun. Mass Spec.*, 25,  
590 469-475, doi: 10.1002/rcm.4876, 2011.
- 591 Flannigan, M.D., and Bergeron, Y.: Possible role of disturbance in shaping the northern  
592 distribution of *Pinus resinosa*, *J. Veg. Sci.*, 9, 477-482, doi: 10.2307/3237262, 1998.
- 593 Fraver, S., Palik, and Brian J.: Stand cohort structures of old-growth *Pinus resinosa*-  
594 dominated forests of northern Minnesota, USA, *J. Veg. Sci.*, 23, 249-259, doi:  
595 10.1111/j.1654-1103.2011.01348.x, 2012.
- 596 Frissell, J.: The importance of fire as a natural ecological factor in Itasca State Park,  
597 Minnesota, *Quaternary Res.*, 3, 397-407, doi:10.1016/0033-5894(73)90005-7, 1973.
- 598 Galloway, J.N., Dentener, F.J., Capone, D.G. and co-authors: 2004. Nitrogen cycles:  
599 past, present, and future, *Biogeochemistry*, 70, doi: 10.1100/tsw.2001.326, 153-226.
- 600 Gerhart, L., and McLauchlan, K.K.: Reconstructing terrestrial nutrient cycling using  
601 stable nitrogen isotopes in wood, *Biogeochemistry*, 120, 1-21, doi: 10.1007/s10533-  
602 014-9988-8, 2014.
- 603 Guerreiro, S., Kilsby, C.G., and Serinaldi, F.: Analysis of time variation of rainfall in  
604 transnational basins in Iberia: abrupt changes or trends?, *Int. J. Climatol.*, 34, 114-  
605 133, doi: 10.1002/joc.3669, 2013.

- 606 Heitz, P., Dünish, O, and Wanek, W.: Long-term trends in nitrogen isotope composition  
607 and nitrogen concentration in Brazilian rainforest trees suggest changes in nitrogen  
608 cycle, *Env. Sci. Tech.*, 44, 1191-1196, doi: 10.1021/es901383g, 2010.
- 609 Hietz, P., Turner, B.L., Wanek, W., and Richter, A., Nock, C.A., Wright, S.J.: Long-term  
610 change in nitrogen cycle of tropical forests, *Science*, 334, 664-666, doi:  
611 10.1126/science.1211979, 2011.
- 612 Hobbie, J.E., and Colpaert, J.V.: Nitrogen availability and colonization by mycorrhizal  
613 fungi correlate with nitrogen isotope patterns in plants, *New Phytol.*, 157, 115-126,  
614 doi: 0.1046/j.1469-8137.2003.00657.x, 2003.
- 615 Hobbie, J.E., and Hobbie, E.A.: <sup>15</sup>N in symbiotic fungi and plants estimates nitrogen and  
616 carbon flux rates in arctic tundra, *Ecology*, 87, 816–822, doi: 10.1890/0012-  
617 9658(2006)87(816:NISFAP)2.0.CO;2, 2006.
- 618 Houlton, B.Z., and Sigman, D.M., Hedin, L.O.: Isotopic evidence for large gaseous  
619 nitrogen losses from tropical rainforests, *P. Natl. Acad. Sci. Usa.*, 103, 8745-8750,  
620 doi: 10.1073/pnas.0510185103, 2006.
- 621 Houlton, B.Z., Boyer, E., Finzi, Adrien, Galloway, J., Leach, A., Liptzin, D., Melillo, J.,  
622 Rosenstock, T.S., Sobota, and D., Townsend, A.R.: Intentional versus unintentional  
623 nitrogen use in the United States: trends, efficiency and implications,  
624 *Biogeochemistry*, 114, 11-23, doi: 10.1007/s10533-012-9801-5, 2013.
- 625 Jussy, J.H., Colin-Belgrand, and M., Ranger, J.: Production and root uptake of mineral  
626 nitrogen in a chronosequence of Douglas-fir (*Pseudotsuga menziesii*) in the  
627 Beaujoalis Mountains, *Forest Ecol Manag.*, 128, 197-209, doi: 10.1007/s10533-012-  
628 9801-5, 2000.
- 629 Kurmis, V., and Sucoff, E.: Population density and height distribution of *Corylus cornuta*  
630 in undisturbed forests of Minnesota, *Can. J. Botany*, 67, 2409-2413, doi:  
631 10.1139/b89-308, 1989.
- 632 MacKenzie, M. D., Luca, T.H., and Sala, A.: Fire exclusion and nitrogen mineralization  
633 in low elevation forests of western Montana, *Soil Biol. Biochem.*, 38, 952-961, doi:  
634 10.1016/j.soilbio.2005.08.008, 2006.
- 635 Mann, H.B.: Non-parametric test against trend, *Econometrica*, 13, 245-249, doi:  
636 10.1080/10629360600564924, 1945.
- 637 McLauchlan, K.K., Craine, J.M., Oswald, W.W., Leavitt, P.R., and Likens, G.E.:  
638 Changes in nitrogen cycling during the past century in a northern hardwood forest, *P.*  
639 *Natl. Acad. Sci. Usa.*, 104, 7466-7470, doi: 10.1073/pnas.0701779104, 2007.
- 640 McLauchlan, K.K., and Craine, J.M.: Species-specific trajectories of nitrogen isotopes in  
641 Indiana hardwood forests, USA, *Biogeosciences*, 9, 867-874, doi:10.5194/bg-9-867-  
642 2012, 2012.
- 643 McLauchlan, K.K., Williams, J.J., Craine, J.M., and Jeffers, E.S.: Changes in global  
644 nitrogen cycling during the Holocene epoch, *Nature*, 495, 352-355,  
645 doi:10.1038/nature11916, 2013a.
- 646 McLauchlan, K.K., Lascu, I., Myrbo, A., and Leavitt, P.R.: Variable ecosystem response  
647 to climate change during the Holocene in northern Minnesota, USA, *Geol. Soc. Am.*  
648 *Bull.*, 125, 445-452, doi: 10.1130/B30737.1, 2013b.

- 649 Minnesota Department of Natural Resources: Itasca State Park management plan.  
650 files.dnr.state.mn.us/parks\_trails/mgmtplans/Itasca/plan.pdf, 1998.
- 651 National Atmospheric Deposition Program: 2013 Annual Summary.  
652 <http://nadp.sws.uiuc.edu/lib/data/2013as.pdf>, 2013.
- 653 National Climatic Data Center: Climatology of the United States, 1971-2000.  
654 <http://www.ncdc.noaa.gov/DLYNRMS/dnrm?coopid=214106>.
- 655 Nave, L.E., Gough, C.M., Maurer, K.D., Bohrer, G., Hardiman, B.S., Moine, M. Le  
656 Munoz, A.B., Nadelhoffer, K.J., Sparks, J.P., Strahm, B.D., Vogel, C.S., and Curtis,  
657 P.S.: Disturbance and the resilience of coupled carbon and nitrogen cycling in a  
658 northern temperate forest, *J. Geophys. Res-Biogeophys.*, 116,  
659 doi: 10.1029/2011JG001758, 2011.
- 660 Pardo, L.H., Hemond, H.F., Montoya, J.P., Fahey, T.J., and Sicama, T.G.: Response of  
661 the natural abundance of N-15 in forest soils and foliage to high nitrate loss  
662 following clear-cutting, *Canadian Journal of Forestry*, 32, 1126-1136, doi:  
663 10.1139/x02-041, 2002.
- 664 Pardo, L. H., Templer, P. H., Goodale, C. L., Duke and co-authors: Regional assessment  
665 of N saturation using foliar and root delta N-15, *Biogeochemistry*, 80, 143-171, doi:  
666 10.1007/s10533-006-9015-9, 2006.
- 667 Peet, R.K.: Twenty-six years of change in a *Pinus strobus*, *Acer saccharum* forest, Lake  
668 Itasca, Minnesota, *B. Torrey Bot. Club*, 111, 61-68,  
669 <http://dx.doi.org/10.3375/043.029.0208>, 1984.
- 670 Peñuelas, E.: Trends in plant carbon concentration and plant demand for N throughout  
671 this century, *Oecologia*, 109, 69-73, doi: 10.1007/s00442005005, 1997.
- 672 Peterjohn, W.T., Foster, C.J., Christ, M.J., and Adams, M.B.: Patterns of nitrogen  
673 availability within a forested watershed and exhibiting symptoms of nitrogen  
674 saturation, *Forest Ecol. Manag.*, 119, 247-257, doi: 10.1016/S0378-1127(98)00526-  
675 X, 1999.
- 676 Pettitt, A.N.: A nonparametric approach to the changepoint problem, *Applied Statistician*  
677 28, 126-135, doi: 10.2307/2346729, 1979.
- 678 Poulson, S.R., Chamberlian, C.P., and Friedland, A.J.: Nitrogen isotope variation of tree  
679 rings as a potential indicator of environmental change, *Chem. Geol.*, 125, 307-315,  
680 doi: 10.1016/0009-2541(95)00097-6, 1995.
- 681 Rebstock, G.A.: Climatic regime shifts in decadal-scale variability in calanoid copepod  
682 populations off southern California, *Glob. Change Biol.*, 8, 71-89, doi:  
683 10.1046/j.1365-2486.2002, 2002.
- 684 Santoro, A.E., Lombardero, M.J., Ayres, M.P., and Ruel, J.J.: Interactions between fire  
685 and bark beetles in an old growth pine forest, *Forest Ecol. Manag.*, 5148, 1-10, doi:  
686 10.1016/S0378-1127(00)00389-3, 2000.
- 687 Schimel, J.P., and Bennett, J.: Nitrogen mineralization: Challenges of a changing  
688 paradigm, *Ecology*, 85, 591-602, <http://dx.doi.org/10.1890/03-8002>, 2004.
- 689 Sen, P.K.: Estimates of the regression coefficient based on Kendall's tau, *J. Am. Stat.*  
690 *Assoc.*, 63, 1379-1389, doi: 10.1080/01621459.1968.10480934, 2004.



- 691 Smithwick, E.A.H., Turner, M.G., Mack, M.C., and Chapin, F.S.: Postfire Soil N  
692 Cycling in Northern Conifer Forests Affected by Severe, Stand-Replacing Wildfires.  
693 *Ecosystems* 8, 163-181, 2005.
- 694 Spurr, S.H.: The forest of Itasca in the Nineteenth Century as Related to Fire. *Ecology*,  
695 35, <http://dx.doi.org/10.2307/1931399>, 1954.
- 696 Silver, E.J., Fraver, S., D'Amato, A.W., Aakala, T., and Palik, B.J.: Long-term mortality  
697 rates and spatial patterns in an old-growth *Pinus resinosa* forest, *Canadian Journal of*  
698 *Forestry*, 43, 809-816, 10.1139/cjfr-2013-0139, 2013.
- 699 Stock, W.D., Bourke, L., and Froend, R.H.: Dendroecological indicators of historical  
700 responses of pines to water and nutrient availability on a superficial aquifer in south-  
701 western Australia, *Forest Ecol. Manag.*, 264, 108-114, doi:  
702 10.1016/j.foreco.2011.09.033, 2012.
- 703 Tappeiner, J.C., and Alm, A. A.: Undergrowth vegetation effects on the nutrient content  
704 of litterfall and soils in red pine and birch stands in northern Minnesota, *Ecology*, 56,  
705 1193-1200, <http://dx.doi.org/10.2307/1936159>, 1975.
- 706 Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G., Matson, P. A., Schindler, D.  
707 W., Schlesinger, W.H., Tilman, G. D.: Human alteration of the global nitrogen  
708 cycle: sources and consequences. *Ecological Applications* 7, 737-750, 1997.
- 709 Vitousek, P.M., Mooney, H.A., Lubchenco, J., and Melillo, J.M.: Human Domination of  
710 Earth's Ecosystems, *Science*, 277, 494-499, doi: 10.1126/science.277.5325.494,  
711 1997.
- 712 Wang, Q., Zhong, M., and Wang, S.: A meta-analysis on the response of microbial  
713 biomass, dissolved organic matter, respiration, and N mineralization in mineral soil  
714 to fire in forest ecosystems, *Forest Ecol. Manag.*, 271, 91-97, doi:  
715 10.3389/fmicb.2013.00163, 2012.
- 716 Webb, S.L.: Contrasting windstorm consequences in two forests, Itasca State Park,  
717 Minnesota, *Ecology*, 70, 1167-1180, 1989.
- 718 Wolfe, A. P., W. O. Hobbs, H. H. Birks, J. P. Briner, S. U. Holmgren, O. Ingolfsson, S.  
719 Kaushal, G. H. Miller, M. Pagani, J. E. Saros, and R. Vinebrooke.: Stratigraphic  
720 expressions of the Holocene–Anthropocene transition revealed in sediments from  
721 remote lakes, *Earth-Sci. Revs.*, 116, 17-34, <http://dx.doi.org/10.2307/1941384>, 2013.
- 722 Zenner, E.K., and Peck, J.E.: Characterizing structural conditions in mature managed red  
723 pine: Spatial dependency of metrics and adequacy of plot site, *Forest Ecol. Manag.*,  
724 255, 3051-3062, doi: 10.1016/j.foreco.2008.09.006, 2009.
- 725  
726  
727  
728  
729  
730  
731

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

734

<i>Tree</i>	<i>Ring Counts</i>	<i># <math>\delta^{15}\text{N}</math> Samples</i>	<i>Elevation (m)</i>	<i>Distance from Shore (m)</i>	
<i>S1TC</i>	238	99	468	23.69	737
<i>S1TD</i>	122	44	469.5	20.63	738
<i>S1TE</i>	215	84	472.2	16.58	739
<i>S2TA</i>	175	98	474.5	14.28	740
<i>S2TB</i>	158	74	467.1	14.31	741
<i>S2TC</i>	162	55	465.2	12.57	742
<i>S2TD</i>	158	63	464.8	17.00	743
<i>S2TE</i>	79	50	467.7	13.20	744
<i>S3TA</i>	99	60	467	13.65	745
<i>S3TC</i>	118	49	469.5	10.29	746
<i>S3TD</i>	128	45	471.2	11.97	747
<i>S4TA</i>	95	35	470.4	9.35	748
<i>S4TB</i>	86	78	471	17.77	749
<i>S4TC</i>	171	64	468	16.82	750
<i>S4TD</i>	178	56	458	14.88	751
<i>S4TE</i>	159	51	466.1	9.30	752
<i>S5TA</i>	132	69	462.2	20.50	753
<i>S5TB</i>	110	68	462.2	20.50	754
<i>S5TC</i>	153	45	465.8	22.12	

755

756

757

758

759

760

761

762

763

764

765

766

767

768

769 **Table 2:** Slope based on simple linear regression ( $m$ ), Sen's Slope and Kendall's tau  
 770 value based on Mann-Kendall Trend Test for each tree. The Hamed and Rao method  
 771 takes into account autocorrelation and adjusts the Sen's Slope estimate. Tau values with  
 772 \* indicate  $p < .05$ , \*\*  $p < .01$ , and \*\*\*  $p < .0001$ , NS = not significant.  
 773

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

774  
 775  
 776  
 777  
 778  
 779  
 780  
 781

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

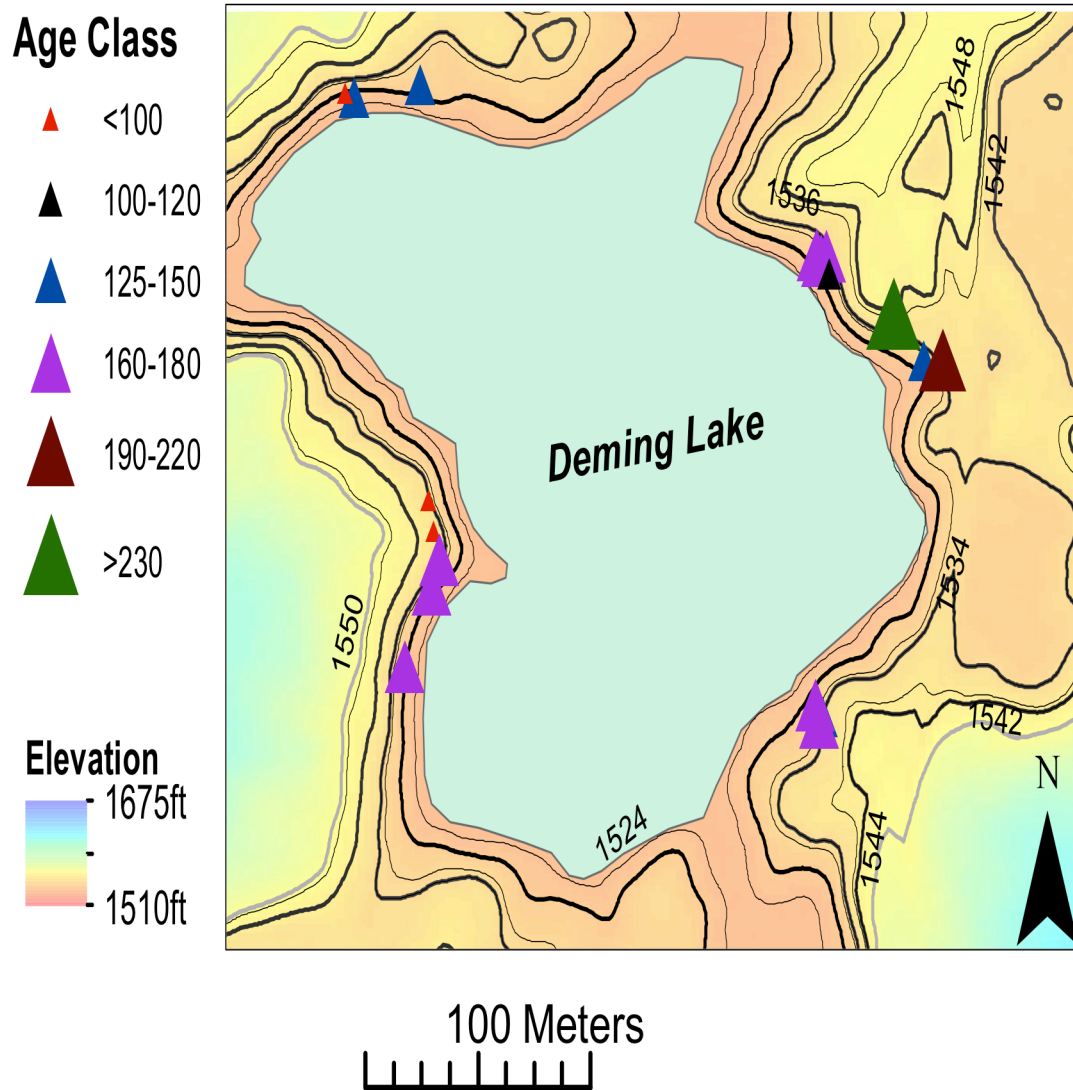
<i>Tree</i>	<i>Buishand's Test</i>	<i>CUSUM</i>	<i>Pettit's Test</i>	<i>SNTH<sup>785</sup> Test</i> 786
<i>S1TC</i>	1941*	1942*	1941*	1942* 787
<i>S1TE</i>	1925*	1925*	1925*	1925* 788
<i>S2TA</i>	1928*	1942*	1927*	1949* 789
<i>S2TB</i>	1930*	1930*	1930*	1930* 790
<i>S2TC</i>	1935*	1935*	1935*	1935* 791
<i>S2TD</i>	1958*	1956*	1956*	1958* 792
<i>S2TE</i>	1973*	1975*	1973*	1983* 793
<i>S3TC</i>	1942*	1942*	1942*	1942* 794
<i>S3TD</i>	1917*	1917*	1920*	1917* 795
<i>S4TA</i>	1980	1996	1980	2008 796
<i>S4TB</i>	1947*	1947*	1926*	1947* 797
<i>S4TC</i>	1943*	1943*	1933*	1943* 798
<i>S4TD</i>	1941*	1941*	1941*	1941* 799
<i>S4TE</i>	1968*	1968*	1968*	1968* 800
<i>S5TA</i>	1941*	1941*	1943*	1939* 801
<i>S5TB</i>	1924*	1923*	1923*	1923* 802
<i>S5TC</i>	1923*	1923*	1929*	1923* 803
<i>S5TD</i>	1927*	1927*	1927*	1927* 804

806  
 807  
 808  
 809  
 810  
 811  
 812  
 813  
 814  
 815  
 816  
 817  
 818  
 819  
 820  
 821  
 822  
 823  
 824

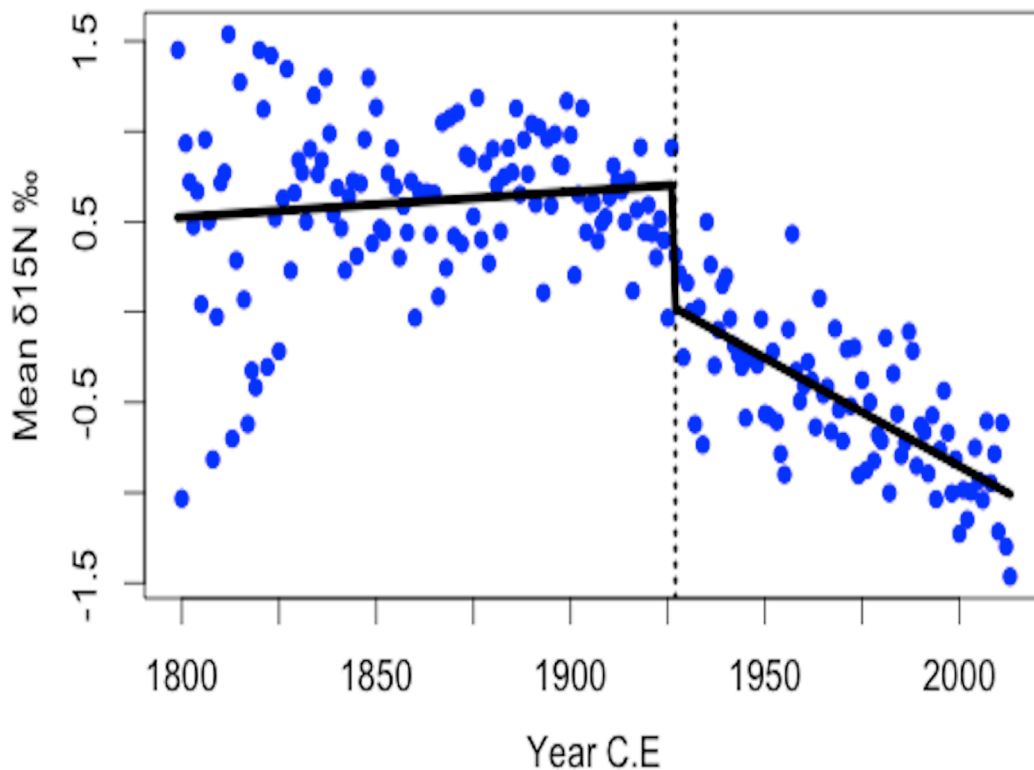
825  
826  
827  
828  
829  
830  
831  
832  
833  
834  
835  
836  
837  
838  
839  
840  
841  
842  
843  
844  
845  
846  
847  
848  
849  
850  
851  
852  
853  
854  
855  
856  
857  
858  
859  
860  
861  
862  
863  
864  
865  
866  
867  
868



**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  $\delta^{15}\text{N}$  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.

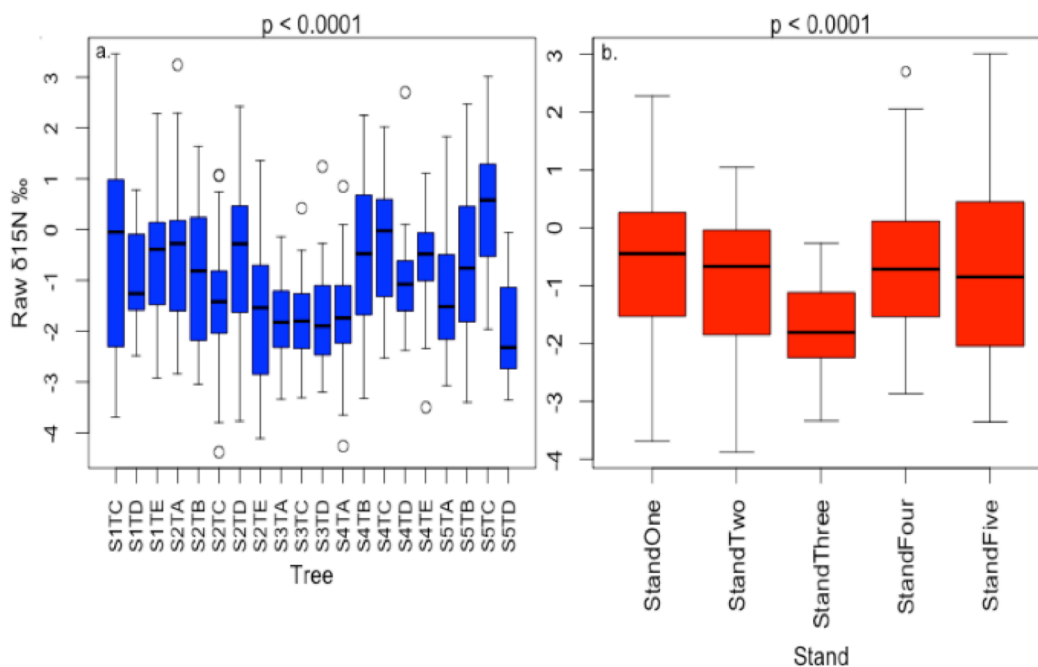


Figure 4: Box plots showing differences in means between trees (a) and stands (b). a.) Box plot of  $\delta^{15}\text{N}$  for each tree. S refers to the Stand (i.e S1= Stand One), T refers to tree, (T1= Tree One). b.) Same as Fig. 4a but for the five stands analysed. The box plots are based on the averages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



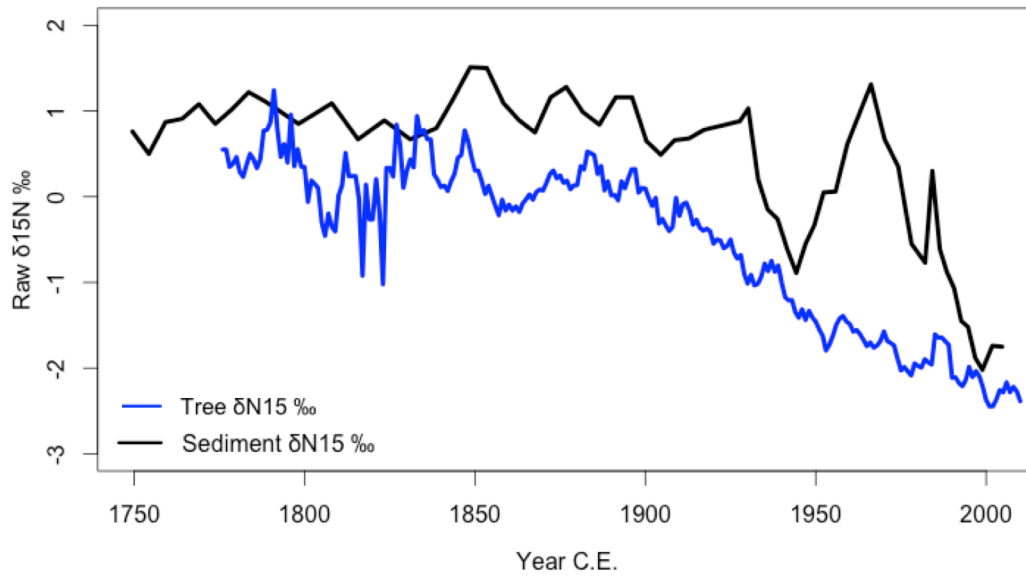


Figure 5: Comparison of sediment  $\delta^{15}\text{N}$  record with a 5-year smoothed raw wood  $\delta^{15}\text{N}$  record. Blue line indicates the sediment  $\delta^{15}\text{N}$  record (1749.5-2004.7), black line indicates smoothed wood  $\delta^{15}\text{N}$  record (1776-2013).