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

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- μ / σ) 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.

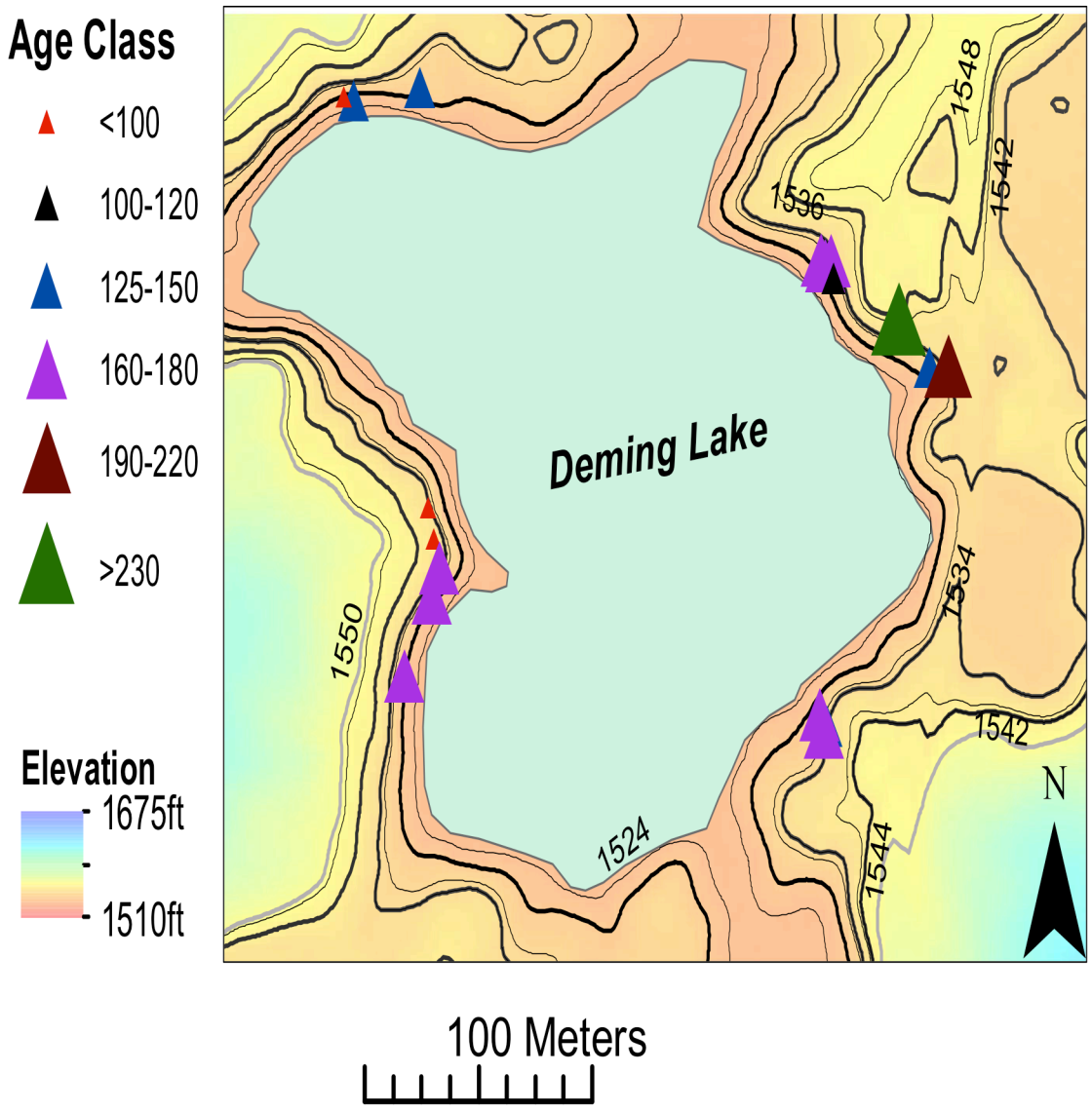
Clark, J.S.: Fire and climate change during the last 750 yr in northwestern Minnesota, Ecol. Monogr., 60, 135-159, http://dx.doi.org/10.2307/1943042, 1990a.

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.



Revised figure two.