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The response of tree ring $\delta^{15}N$ to whole-watershed urea fertilization at the Fernow Experimental Forest, WV

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Abstract Plant tissue $\delta^{15}N$ is frequently used as a proxy for N availability and N cycle dynamics, and the $\delta^{15}N$ signature of tree rings could potentially be used to reconstruct past changes in the N cycle due to forest disturbance or anthropogenic N deposition. However, there are substantial uncertainties regarding how effectively tree ring $\delta^{15}N$ records N cycle dynamics. We used increment tree cores from a forested watershed that received a one-time application of urea, along with the long-term stream water chemistry record from that watershed and a nearby reference watershed, to determine the effectiveness of tree ring $\delta^{15}N$ in recording a change in N availability, and whether its effectiveness differed by species or mycorrhizal type. Tree ring $\delta^{15}N$ of three species increased rapidly (within ~1 to 3 years) following fertilization (*Quercus rubra*, *Fagus grandifolia*, and *Prunus serotina*), while that of *Liriodendron tulipifera* did not respond to fertilization but increased ~10 years later. Tree ring $\delta^{15}N$ tended to remain elevated throughout the measured time period (1967–2000), well past the pulsed fertilization response in stream water. This extended $\delta^{15}N$ response may be partially caused by chronic atmospheric N deposition in the region, which also contributed to greater losses of nitrate in stream water by ~1980. Additionally, local recycling of N compounds, and retranslocation of N within the trees, may account for the persistence of elevated tree ring $\delta^{15}N$ levels beyond the direct fertilization effects. Collectively, these results confirm that tree ring $\delta^{15}N$ from some species can document the onset of historical changes in the N cycle. We suggest that studies utilizing tree ring $\delta^{15}N$ as a proxy for long-term N cycle dynamics should look for a consistent pattern of change among several species rather than relying on the record from a single species.

Keywords $\delta^{15}N$ · Nitrogen deposition · Nitrogen cycle · Tree rings

Introduction

Anthropogenic reactive N input into terrestrial ecosystems has more than doubled over the past century (Galloway et al. 2004), stimulating extensive research on the short- and long-term effects of N deposition, and the recovery of natural ecosystems as deposition has declined in some regions (Gundersen et al. 1998;

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Adams et al. 2007; Likens and Buso 2012). However, investigating long-term changes requires long-term records of N cycling in order to identify trends and characterize baseline conditions. Unfortunately, continuous measurements of stream water N are spatially and temporally limited, with the longest record, that we are aware, beginning in 1964 (Knapp et al. 2012; Argerich et al. 2013). In the absence of numerous, long-term records of N cycling, tree ring $\delta^{15}N$ could serve as an indicator of the N status of an area over time and yield valuable information about the timing and extent of the impacts resulting from N deposition.

Stable isotopes are used to study numerous biogeochemical and physiological processes, and ^{15}N has emerged as a tool in N cycling research (Pardo et al. 2006; Pardo and Nadelhoffer 2012). In particular, plant tissue $\delta^{15}N$ can act as an integrator of complex N cycle processes occurring in the soil (Robinson 2001), and the use of tree ring $\delta^{15}N$ to study past N cycle dynamics has increased over the past two decades (Gerhart and McLauchlan 2014). When N availability increases, elevated rates of nitrification can lead to the loss of ^{15}N-depleted NO_3 in stream water, resulting in an increase in the $\delta^{15}N$ of the remaining plant available N pool (Hogberg 1997; Pardo et al. 2002). Elevated N availability can also increase the otherwise low levels of gaseous N losses in deciduous broadleaf forests (Peterjohn et al. 1998; Venterea et al. 2004; Wallenstein et al. 2006), which favors the removal of ^{15}N-depleted N compounds (Yoshida 1988; Barford et al. 1999; Sebilo et al. 2003) and can have a substantial impact on soil $\delta^{15}N$ (Houlton et al. 2006; Wexler et al. 2014). The potential usefulness of plant tissue $\delta^{15}N$ as a record of shifts in the N cycle is supported by evidence from disturbance events such as clear-cutting or selective tree removal (Pardo et al. 2002; Bukata and Kyser 2005; Beghin et al. 2011; Falxa-Raymond et al. 2012), from studies of N deposition gradients (Saurer et al. 2004), and from long-term N deposition data (McLauchlan et al. 2007; Hietz et al. 2010; Sun et al. 2010). However, there is still a high degree of unexplained variation in wood stable N isotope records.

Some variability among species in tree ring $\delta^{15}N$ response could be due to their type of mycorrhizal association, especially in mixed forests where anthropogenic N deposition is prevalent. While arbuscular mycorrhizae (AM) are thought to have a minor role in organic N mobilization, ectomycorrhizal (ECM) fungi can cleave organic polymers to access bound N (Read and Perez-Moreno 2003) and transfer strongly ^{15}N-depleted compounds from ECM fungi to the host plant (Hobbie and Hobbie 2006; Hobbie and Högberg 2012). It is also thought that ECM plants may be less dependent on organic N in temperate ecosystems where mineral N availability is higher than in more northern latitudes (Lilleskov et al. 2002; Mayor et al. 2015). However, when N availability changes, it is unclear how rapidly the ECM community composition might shift, and how rapidly the N acquisition role of ECM fungi might change (Treseder 2004; Hawkins et al. 2015). If a reduction in the reliance on organic N is slow (or doesn't occur), then the transfer of ^{15}N-depleted compounds to the host plant by ECM fungi may delay the appearance of a plant $\delta^{15}N$ response to changes in inorganic N availability. Thus, we expect that the record of tree ring $\delta^{15}N$ in AM species should be more responsive to changes in the availability of inorganic N than the record of tree ring $\delta^{15}N$ in ECM tree species, but changes in the reliance by ECM trees on organic N sources could make the interpretation of tree ring $\delta^{15}N$ signals in these species more challenging.

Even within an individual tree, the N content (%N) of tree rings typically increases dramatically in the outermost rings due to the movement of labile N compounds toward actively growing tissue (Elhani et al. 2003; Hart and Classen 2003; Härdtle et al. 2014). This could occur due to direct movement of mobile N compounds across rings, or internal recycling of N compounds (Hagen-Thorn et al. 2006). Thus, the movement of N compounds within the tree has the potential to blur the isotopic signal by spreading it over multiple years (Hart and Classen 2003; Tomlinson et al. 2014). Furthermore, some of the physiological transformations N compounds undergo from uptake to storage in woody tissue can discriminate against $\delta^{15}N$ (Kalcsits et al. 2014). For example, Pardo et al. (2013) found variability in the $\delta^{15}N$ signal between different tree tissues, pointing to fractionation as N is transported throughout the tree. However, if the fractionations that impact the $\delta^{15}N$ composition of transported N are consistent across years, then the signal preserved in tree rings should still reflect temporal changes in the openness of the N cycle.

To determine the effectiveness of different tree species as recorders of past N cycling, a known shift or disturbance in the N cycle can be used as a reference point. Past studies have used events such as forest disturbance to investigate tree ring $\delta^{15}N$ response (Bukata and Kyser 2005; Falxa-Raymond et al. 2012), and numerous studies have attributed a change in plant tissue $\delta^{15}N$ to increases in N deposition (Choi et al. 2005; Bukata and Kyser 2007; Savard et al. 2009; Hietz et al. 2011; Jung et al. 2013). McLauchlan and Craine (2012) found differences in the temporal trends of tree ring $\delta^{15}N$ between species, but no study has directly compared the temporal response of $\delta^{15}N$ in tree rings of multiple co-existing species to a known, and independently-measured past disturbance to the N cycle. Thus, the purpose of this study was to examine the effectiveness of different species in recording a known shift in N cycle dynamics in tree ring $\delta^{15}N$. Similar to a pulse-chase experiment, we used a one-time, whole-watershed, fertilization event from 1971 that caused a distinct, short-term increase in a continuously measured stream water N record. By comparing the tree ring and stream water records from both within this single-dose fertilized watershed, as well as a nearby reference watershed, we examined the following hypotheses:

(1) Tree ring $\delta^{15}N$ would increase in response to fertilization, followed by a decline back to pre-fertilization levels.

(2) The reduction of $\delta^{15}N$ back to pre-fertilization levels would not be as rapid as the return of stream water chemistry because tree-ring N could be retranslocated from senescent tissues and reused.

(3) The tree ring $\delta^{15}N$ record in AM species would be more responsive to changes in N cycling than that of ECM species, and more closely parallel changes in stream water NO_3 concentration.

Methods

Study site

We sampled tree rings from multiple species in a 30-ha experimental watershed (WS 1), as well as from one tree species in a 39-ha reference watershed (WS 4) at the Fernow Experimental Forest (FEF) in Tucker County, WV. The predominant soil is Calvin channery silt loam and is relatively acidic (pH ~ 4.5–5). The FEF receives approximately 145 cm annual precipitation (Kochenderfer 2006). Stream flow in both watersheds is continuously monitored using 120° V-notch weirs (Trimble 1977), and monthly stream water conductivity and flow-weighted NO_3 concentration have been measured since 1958 and 1970, respectively. Peterjohn et al. (1996) estimated that the average wet N deposition rate was ~6.7 kg N ha^{-1} $year^{-1}$ from 1982 to 1993. The experimental watershed was commercially clear-cut in the winter of 1957–1958, with all merchantable trees removed down to approximately 15 cm DBH; prior to this cut, the watershed was a 50-year-old uneven aged stand dominated by *Quercus, Acer, Liriodendron, Prunus,* and *Fagus* species (Reinhart et al. 1963). In 1970, the stand averaged ~10 m in height and was dominated by these same species as well as *Tilia americana* (Patric and Smith 1978). In May, 1971, the experimental watershed received a one-time, 617.75 kg ha^{-1}, aerial application of urea, which added 288 kg N ha^{-1} and caused a rapid, short-lived increase in stream water conductivity and NO_3 (Patric and Smith 1978). Based on recent measurements from a nearby watershed, the N content in the top 5 cm of mineral soil was ~1514 kg ha^{-1}, and so the added N likely was ~14 to 20 % of the N originally present in top 5 cm of soil. Although no $\delta^{15}N$ measurement was made on the applied urea at that time, typical $\delta^{15}N$ values for urea range from −2.3 to −1 ‰ (Nommik et al. 1994; Choi et al. 2002; Zhou et al. 2013), and potentially up to 1.3 ‰ (Li and Wang 2008). While no measurements of net N mineralization or nitrification rates have ever been made in WS 1, evidence for a positive relationship between net nitrification rates and NO_3 level in soil and stream water exists for other areas of the FEF, including the reference watershed (Peterjohn et al. 1996, 1999; Gilliam and Adams 2011). From these results, we think it is likely that the rate of net nitrification in the soils of WS 1 increased rapidly after fertilization, causing the observed increase in stream water NO_3 concentration.

Tree core collection and analysis

We collected tree cores from four *Fagus grandifolia* and *Quercus rubra* trees (ECM) and five *Prunus serotina* and *Liriodendron tulipifera* trees (AM) in the fertilized watershed (WS 1), and from three large *Liriodendron tulipifera* trees located near the weir used for stream water measurements in the reference watershed (WS 4). Using a 5-mm increment borer (Mora of Sweden, Mora, Sweden), we extracted two cores parallel to the topographical contour from each tree, rinsing the increment borers with deionized water between trees. Trees were selected at 5 points along a mid-elevation band to be evenly spaced through the fertilized watershed to control for potential elevational effects on the δ^{15}N signal in plant available N pools (Garten 1993). At each point, we cored the largest canopy tree within ~30 m, with a minimum DBH of 30 cm. *F. grandifolia* trees tended to be smaller in girth, and so a minimum DBH of 25 cm was used for this species. We sampled the wood tissue from each individual tree ring between 1967 and 1980—a range surrounding the year of urea application (1971). In addition, we pooled 5-year tree-ring segments for 1981–1985, 1986–1990, 1991–1995, and 1996–2000. Since the temporal dynamics of fertilizer application and stream water chemistry response were known, this made it possible to detect any inward translocation of the δ^{15}N signal to earlier tree rings, and also whether changes in the tree ring δ^{15}N signal lasted longer than those in stream water chemistry (Elhani et al. 2003).

We mounted, sanded, measured, and cross-dated one core from each tree (Stokes and Smiley 1996), calculated basal area increment (BAI) using ring widths and tree diameter measurements at breast height, and assessed cross-dating accuracy using the dplR package in R (Bunn 2010). The second core from each tree was sanded only lightly to minimize cross-contamination between rings. We separated years selected for isotope analysis from the core using a razor blade and ground the tissue to a fine powder using a dental amalgamator (Henry Schein, Inc., Melville, NY), wrapping approximately 5 mg of ground tissue in tin capsules for isotope ratio gas chromatography–mass spectrometry analysis. Isotope analysis was completed by the University of Maryland Central Appalachians Stable Isotope Facility (Frostburg, MD). Due to variable results of wood N extraction techniques (reviewed by Gerhart and McLauchlan 2014), we analyzed raw wood tissue rather than performing any N extraction.

Statistical analysis

To reduce tree-to-tree differences in absolute δ^{15}N level while preserving the temporal trend, we standardized the tree ring δ^{15}N values for each tree by subtracting the within-tree average from each ring's value (Gerhart and McLauchlan 2014). While Gerhart and McLauchlan (2014) suggest that some studies standardize to the same mean within site to focus on temporal trends, we standardized within each tree due to species differences in δ^{15}N at our single site and tree differences within species at different locations within the watershed. Data were analyzed using a nested two-way factorial design with tree ring δ^{15}N as the response variable. For this analysis, we used the 4 years prior to fertilization (1967–1970) as a pretreatment reference time period, while considering the 4 years following fertilization (1972–1975) to be the treatment time period. A two-way model was constructed with species nested within mycorrhizal type and year nested within pre- versus post-fertilization time period. To test our hypotheses we focused on detecting a significant effect ($\alpha = 0.05$) due to the time period (pre- vs. post-fertilization), and due to the mycorrhizal type by time period interaction. A significant time period effect would indicate a change in tree ring δ^{15}N from the 4 years prior to fertilization to the 4 years after, and a significant interaction effect between time period and mycorrhizal type would indicate that the change in tree ring δ^{15}N from years prior to fertilization to years post-fertilization differs by mycorrhizal association (ECM or AM).

Results

Stream NO_3 and tree growth

Stream water conductivity (not shown) and NO_3 were strongly correlated ($r = 0.765, P < 0.001$) and peaked shortly after urea fertilization (Fig. 1) (Patric and Smith 1978). The peak in stream water NO_3 was short-lived (lasting ~3 years), but NO_3 concentrations never completely returned to pre-fertilization levels – with levels in 2006 (~100 µM) still 4× greater than pre-fertilization levels (~25 µM in 1970). In addition, there was a 57 % increase in NO_3 concentration from 1978–1979 (75 µM) to 1980–1981 (117 µM), an increase that coincided with a 145 % increase (17–42 µM) in stream NO_3 concentration in the nearby reference watershed (WS 4).

Since not all trees were harvested from WS 1 in 1957–1958, ~50 % of the trees we cored were

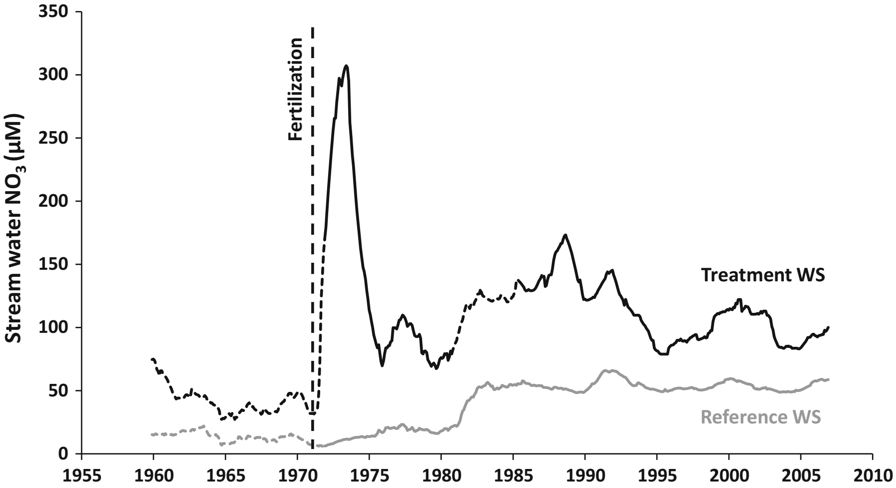


Fig. 1 24-month running mean of flow weighted monthly stream water NO_3 in Fernow Experimental Forest watershed 1 (clear-cut in 1957, fertilized in May, 1971) and watershed 4 (reference, cut circa 1900). *Dashed line* segments include estimated values based on the relationship between NO_3 and stream water conductivity

established prior to 1957. The ring width and BAI of all four species increased markedly (51.4 % for *L. tulipifera* to 178 % for *F. grandifolia*) after the watershed was commercially clear-cut in 1957 (Fig. 2). This BAI increase was most apparent for *F. grandifolia* trees whose growth had been suppressed in the understory prior to 1957. A second increase in BAI ($P < 0.001$) occurred during the 5 years after urea fertilization compared to the 5 years prior for three of the species we examined; *L. tulipifera* (189 %), *P. serotina* (118 %), and Q. *rubra* (45 %). There was no significant change ($P = 0.101$) in *F. grandifolia* BAI following urea fertilization (Fig. 2).

General species differences in $\delta^{15}N$

The non-standardized average wood $\delta^{15}N$ signature across all years differed between species. Specifically, we found that *F. grandifolia* and *Q. rubra* had the highest mean $\delta^{15}N$ values (−0.322 and −0.556 ‰, respectively), while the mean $\delta^{15}N$ value for *P. serotina* was significantly lower (−1.480 ‰), and the value for *L. tulipifera* was significantly lower than all other species (−2.603 ‰). There was a positive correlation between ring width and tree ring $\delta^{15}N$ for *P. serotina* ($r = 0.623$, $P < 0.001$) and *Q. rubra* ($r = 0.378$, $P = 0.006$), and a negative correlation for *F. grandifolia* ($r = -0.473$, $P = 0.002$), while the correlation for *L. tulipifera* was not statistically significant. Non-standardized wood $\delta^{15}N$ also differed between species ($P < 0.001$) for pre-fertilization rings and followed the same pattern as $\delta^{15}N$ averaged over all years. *F. grandifolia* and *Q. rubra* had the highest pre-fertilization $\delta^{15}N$ values (−1.039 and −1.201 ‰, respectively), while *P. serotina* $\delta^{15}N$ was lower (−2.340 ‰) and *L. tulipifera* was lowest of all species (−2.943 ‰).

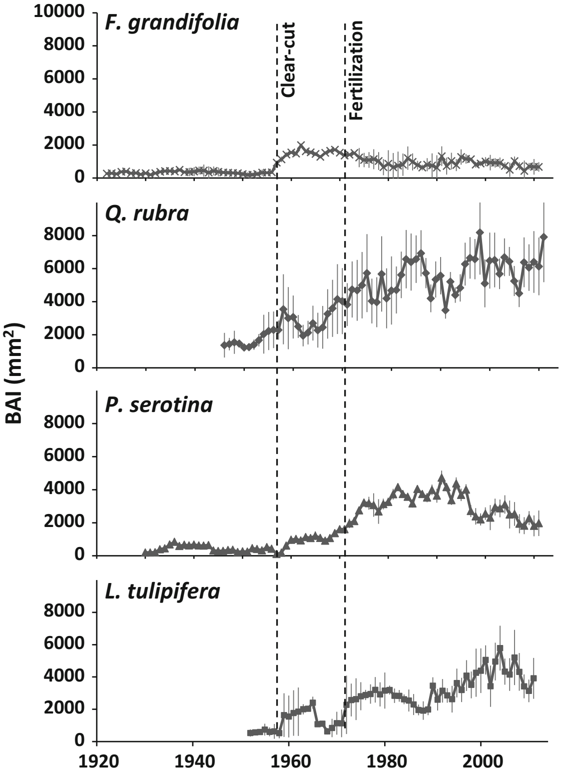


Fig. 2 Mean basal area increment (BAI) of each species through time (±SE)

Species differences in fertilization effects on $\delta^{15}N$

When averaged across all species, standardized tree ring $\delta^{15}N$ increased 0.84 ‰ from the 4 years before urea fertilization to the 4 years after ($P < 0.001$). However, the magnitude of the increase differed by species, with *Q. rubra*, *F. grandifolia*, and *P. serotina* all showing a >1 ‰ increase in tree ring $\delta^{15}N$, while *L. tulipifera* did not respond noticeably to the fertilization event (Fig. 3). In *Q. rubra*, tree ring $\delta^{15}N$ increased 1.56 ‰ from 1968 through 1973, while *F. grandifolia* tree ring $\delta^{15}N$ increased 1.16 ‰ between 1970 and 1972. *P serotina* tree ring $\delta^{15}N$ increased 1.41 ‰ from 1971 through 1974.

Grouping tree species by mycorrhizal type indicated that the tree ring $\delta^{15}N$ of ECM species increased more strongly due to fertilization than that of AM species ($P = 0.0099$). However, this difference was driven by the tree ring $\delta^{15}N$ signal for one of the two AM species examined (*L. tulipifera*), and when *L. tulipifera* was not considered, the three other species showed similar increases in tree ring $\delta^{15}N$ after fertilization with respect to their timing and overall magnitude.

Timing and duration of the $\delta^{15}N$ response

Tree ring $\delta^{15}N$ increased within 2 years of fertilization for three of the four species examined (Fig. 3). Of these three species, the increase did not precede fertilization for *F. grandifolia*. For *P. serotina* the $\delta^{15}N$ signal increased every year from 1967 to 1974, including a trend towards a significant increase from 1967 to 1971 ($P = 0.091$). However, of the total increase found for *P. serotina*, most (76.6 %) of it occurred after fertilization. The increase in tree ring $\delta^{15}N$ for *Q. rubra* appeared to begin ~2 years prior to fertilization, with most (62.8 %) of the maximum increase occurring prior to fertilization. Wood $\delta^{15}N$ for *F. grandifolia* and *P. serotina* increased after fertilization, with *F. grandifolia* reaching a plateau after 1972 (at ~0.1 ‰ non-standardized $\delta^{15}N$) and *P. serotina* peaking in 1974 (at ~0.82 ‰) and stabilizing after 1977 (at ~0.2 ‰). Wood $\delta^{15}N$ for *Q. rubra* began to increase 2 years prior to fertilization and plateaued from 1973 through 1980 (~0.02 ‰ non-standardized). After 1980, the tree ring $\delta^{15}N$ for *Q. rubra* declined and remained ~1 ‰ lower than the years immediately post-fertilization (1973–1980).

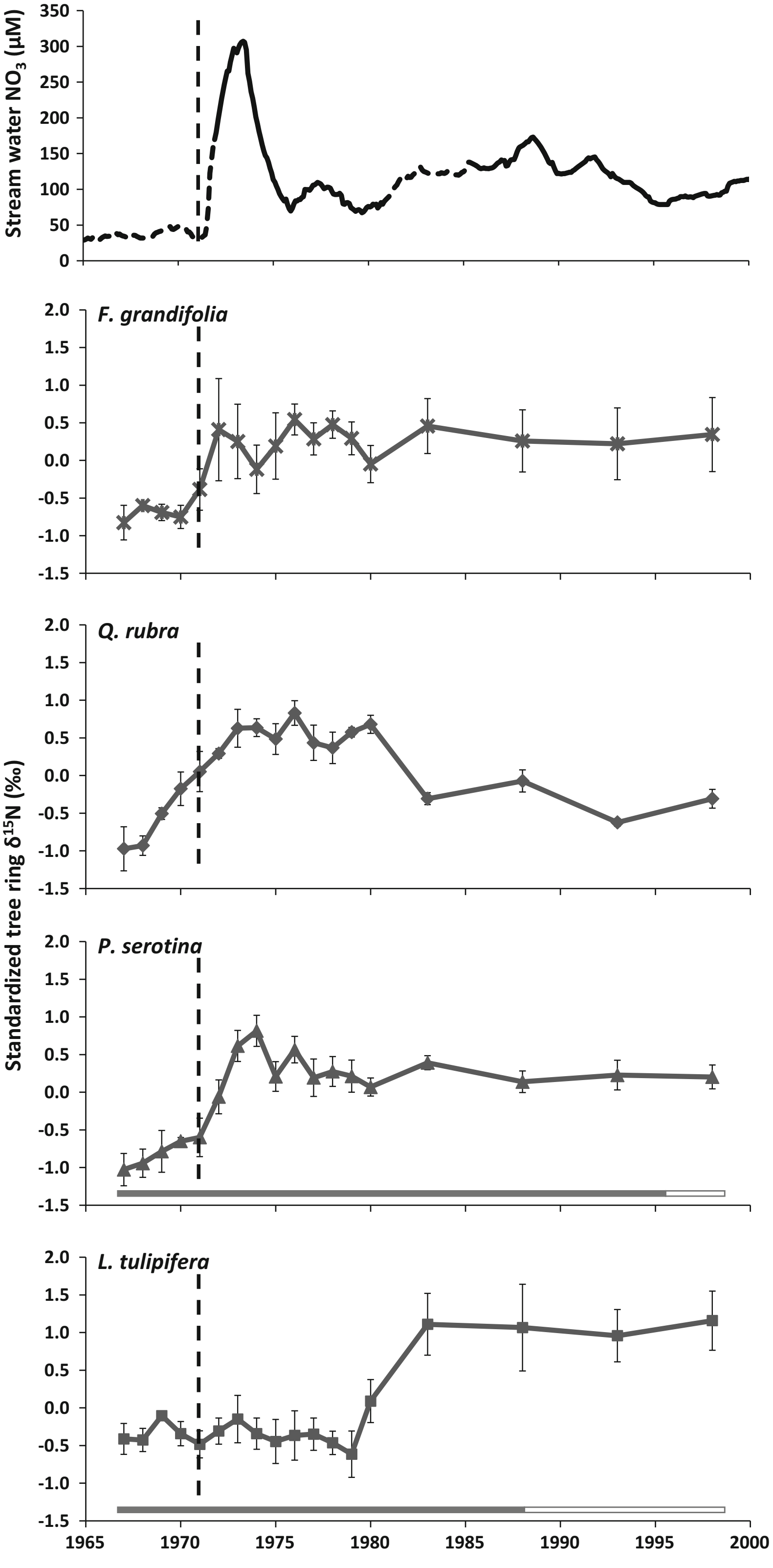
Although there was a distinct, and short-lived, peak in stream water NO_3, this peak was not as evident in the tree ring $\delta^{15}N$ record of any species we examined (Fig. 3). Rather, tree ring $\delta^{15}N$ increased within 2 years of fertilization, but tended to level off near its highest value or only gradually decline. Tukey's HSD post hoc analysis indicated no reduction in tree ring $\delta^{15}N$ during 1976–1980 when compared to 1972–1975, the four years immediately after fertilization. In particular, $\delta^{15}N$ of both *Q. rubra* and *F. grandifolia* remained elevated through 1980. And although the isotopic signature of *P. serotina* trees during 1976–1979 appears to be lower than during the peak years of 1972–1975, this was not statistically significant ($P = 0.713$). Considering the full extent of the post-fertilization tree ring record (through the year 2000), we found that tree ring $\delta^{15}N$ in species responding to fertilization never returned to the pre-fertilization levels (Fig. 2). Even for *Q. rubra* tree ring $\delta^{15}N$, which declined from 1980 to 2000, remained ~0.8 ‰ above the initial pre-fertilization tree ring $\delta^{15}N$. The tree ring $\delta^{15}N$ of both *F. grandifolia* and *P. serotina* remained at levels similar to 1975–1980 throughout the entire tree ring record. However, while *L. tulipifera* tree ring $\delta^{15}N$ did not shift in response to fertilization, a large increase (~1.25 ‰) occurred between 1979 and 1985, and was sustained through 2000.

Increases in tree ring $\delta^{15}N$ did not correspond with heartwood-sapwood boundaries in AM species we examined (Fig. 3). The heartwood-sapwood boundaries in *L. tulipifera* trees occurred during 1989–1990, with the exception of one tree in which the transition was in the 1980 ring. In *P. serotina*, all heartwood-sapwood transitions occurred during the late-1990s. In the two ECM species, the heartwood-sapwood transitions were not visible on dried, sanded cores.

Discussion

Following the urea fertilization to WS 1 in 1971, stream water measurements showed a significant increase in NO_3 concentration, very likely due to an increase in the rates of soil net nitrification (Peterjohn et al. 1996, 1999). This increased loss of NO_3 to stream water likely caused a disproportionate amount of the isotopically lighter isotope to leave the forested catchment (Spoelstra et al. 2010), which should increase the $\delta^{15}N$ signal in the residual pool of plant

Fig. 3 Mean annual standardized tree ring $\delta^{15}N$ by species and mean annual stream water conductivity. *Dashed vertical lines* indicate the 1971 urea fertilization. *Dashed line* segment in *top panel* includes estimated values based on the relationship between NO_3 and stream water conductivity. Heartwood-sapwood boundaries are indicated by *shaded* (heartwood) and *open* (sapwood) *horizontal bars* in *P. serotina* and *L. tulipifera* panels



available N. Within ~1 to 3 years of the whole-watershed fertilization event, this increase in $\delta^{15}N$ was preserved in the tree rings of 3 of the 4 species that we examined. We found little evidence for significant movement of the $\delta^{15}N$ signal across more than a few annual rings, with only *Q. rubra* tree ring $\delta^{15}N$ showing a statistically significant increase prior to fertilization, and only by ~2 years. Another species (*P. serotina*) also showed a trend towards an increase prior to fertilization, but the increase was minor relative to the rate of change that occurred after fertilization. Although some N compounds may be mobile within the tree (Elhani et al. 2003), our results show that movement across rings does not substantially impact the tree ring $\delta^{15}N$ signal and its response to local N cycle disturbance—at least for species we examined. Thus, our findings indicate that tree ring $\delta^{15}N$ from some species can effectively document the onset of a known change in the N cycle.

Consistent with our expectations, the reduction of $\delta^{15}N$ back to pre-fertilization levels was not as rapid as the return of stream water chemistry. However, we were surprised to observe that, even 29 years after the fertilization event, the tree ring $\delta^{15}N$ signals showed almost no return to pre-fertilization levels. In fact, the observed short-lived duration of the peak in stream NO_3 levels was not captured by any of the tree ring isotope records. In the species showing an isotopic response to urea fertilization, a decline in tree ring $\delta^{15}N$ either was not detectable (*F. grandifolia* & *P. serotina*) or was significantly delayed (*Q. rubra*) relative to the measured decline in stream NO_3 concentrations.

The mechanisms responsible for the lack of any substantial reduction in the post-fertilization $\delta^{15}N$ signal in tree rings were not determined, but may include both plant and soil processes. The annual retranslocation of approximately 50 % of foliar N during autumn senescence (Hagen-Thorn et al. 2006) causes some N taken up in 1 year to be stored and potentially available for the growth of new tissues in subsequent years. The N lost in litterfall may also be mineralized and taken up by the tree as it cycles through the soils near a given tree (Zeller et al. 2000). In addition, the persistence of elevated stream water NO_3 compared to pre-fertilization estimates (Fig. 1) indicates that the soil N cycle was altered well past the years immediately following fertilization. Thus, it appears that the combination of long-term changes in soil N cycling, internal retranslocation, and local recycling of N may explain the extended duration of elevated tree ring $\delta^{15}N$ beyond the urea fertilization event.

Contrary to our expectations, a clear record of an acute urea fertilization event was present in both ECM and AM tree species. Research in boreal forests and tundra suggests that ECM fungi aid in N mobilization and acquisition by their host plant, and the transfer of N compounds from fungi to the plant host appears to strongly discriminate against ^{15}N, leaving the fungal tissue enriched and the plant tissue depleted in ^{15}N (Hobbie and Hobbie 2006; Craine et al. 2009). However, these findings may apply primarily to low-N cycling ecosystems. Furthermore, there is considerable overlap in $\delta^{15}N$ values between ECM and AM species across the globe (Craine et al. 2009), and the signature is not always lower in ECM species, even in northern alpine climates (Makarov et al. 2014). In temperate forests, ECM tree species can also have higher tissue $\delta^{15}N$ values than AM species (Pardo et al. 2013). This may be especially true in areas of high N availability and regions that have historically received high N inputs from the atmosphere where ECM trees may depend less on their fungal symbionts for meeting their N demand (Read and Perez-Moreno 2003), and the $\delta^{15}N$ of ECM plant tissue should more closely reflect that of the available soil N. Indeed, the $\delta^{15}N$ of ECM species in this study was not consistently lower than that of AM species prior to fertilization, and the observed increase in tree ring $\delta^{15}N$ after fertilization occurred in both AM and ECM species.

Among the three responsive tree species, the fertilization event was more apparent in the temporal change in $\delta^{15}N$ than in any change in growth. While tree ring width and BAI trends are commonly used to detect and reconstruct a variety of environmental changes (fire, drought, etc.), our data suggest that tree ring $\delta^{15}N$, rather than growth, is a stronger indicator of a disturbance in the N cycle. This was especially evident in the results obtained from *F. grandifolia* where tree ring $\delta^{15}N$ increased 1.16 ‰ after fertilization with no detectable change in BAI. Since a variety of factors other than N availability (light, water, etc.) can influence growth, we suggest that using tree ring $\delta^{15}N$ is most appropriate when studying changes in the N cycle.

In addition to enhanced nitrification and the loss of ^{15}N-depleted NO_3, other aspects of the N cycle and urea fertilization could have affected the $\delta^{15}N$ of the pool of plant available N. First, the isotopic composition of the fertilizer could have changed the δ^{15}N of the soil N pool regardless of NO_3 leaching. Since samples of the fertilizer used in 1971 were not archived, or their isotopic composition measured, it is impossible to know the exact δ^{15}N of the fertilizer that was applied to WS 1. However, typical δ^{15}N values for urea fertilizer range from −2.3 to −1 ‰ (Nommik et al. 1994; Choi et al. 2002; Zhou et al. 2013) but can be as high as 1.3 ‰ (Li and Wang 2008). Thus, the increase in plant δ^{15}N may be partially a signal from the urea δ^{15}N if it were in the 0–1 ‰ range. Second, an ammonia odor, and moss and leaf damage, were reported in the watershed after fertilization, indicating that there was substantial ammonia volatilization after urea addition (Patric and Smith 1978). Indeed, it is thought that ~50 % of the urea added was volatilized and lost as ammonia compared to an estimated loss of ~20 % in elevated stream-water N losses (Patric and Smith 1978). And any ammonia volatilization should increase the plant tissue δ^{15}N since this process favors the loss of the lighter isotope, leaving the pool of plant-available ammonium more enriched in ^{15}N (Mizutani et al. 1986; Mizutani and Wada 1988). Finally, it is possible that discrimination against ^{15}N by the loss of other N gases—and ^{15}N enrichment of the available N pool—resulted from increased rates of nitrification and denitrification (Wexler et al. 2014; Mnich and Houlton 2015). However, although fertilizer additions can enhance the loss of N gases (Castro et al. 1994; Venterea et al. 2004), the magnitude of these losses in temperate forests is often considered to be low relative to the magnitude of N losses in stream water (Campbell et al. 2004). Thus, the changes in tree ring δ^{15}N we observed may reflect a combination of increased nitrification leading to an enhanced loss of NO_3 in stream water, the δ^{15}N signature of the fertilizer that was added, or increased loss of N gases by ammonia volatilization, nitrification, and/or denitrification. However, the exact manner by which the δ^{15}N signal of plant available N was altered does not change our conclusions regarding the effects of mycorrhizal type on tree ring δ^{15}N response to N cycle disturbance, or the timing and persistence of the signal through time.

A striking and surprising result was the lack of response detected in *L. tulipifera* tree ring δ^{15}N after urea fertilization. The reason behind this result is unclear but may be attributable to an initially strong N limitation on their growth. Indeed, prior to fertilization *L. tulipifera* had the lowest values for tree ring δ^{15}N of any of the species we sampled, and fertilization with urea in 1971 led to substantial increases in BAI (189 % 3 years post-fertilization) and increased bud N concentrations in these trees (Patric and Smith 1978). Collectively, these observations suggest greater N retention, and a reduced loss of ^{15}N-depleted NO_3 in the soils surrounding these young *L. tulipifera* trees. However, a greater N retention associated with this species is not likely to be a sufficient explanation since the large amount of ammonia volatilization should have enriched the residual pool of plant available ammonium with ^{15}N. Furthermore, we estimate the BAI stimulation due to fertilization of *L. tulipifera* would yield ~21.2 kg year^{-1} tree^{-1} of additional growth, or ~14,600 kg year^{-1} ha^{-1} (Brenneman et al. 1978). Assuming a C content of 50 % and a C:N ratio of 165 (Vitousek et al. 1988), then this amount of enhanced growth would sequester ~44 kg N ha^{-1} year^{-1}, or only ~15 % of the added N. However, under a more complex set of circumstances it may be possible that the δ^{15}N of plant tissue could remain relatively unaltered if a given species relied primarily on nitrate, utilized it completely (i.e. little to no nitrate loss from the rhizosphere), and if the enrichment of the ammonium N pool with ^{15}N by volatilization was offset by elevated rates of nitrification which produces NO_3 that is depleted in ^{15}N.

While the reasons for the response of *L. tulipifera* trees compared to the other three species remain unknown, our results highlight how different species' tree ring δ^{15}N can respond differently to changes in local soil N processes. And further research on potential reasons for the surprising *L. tulipifera* result could be valuable, since *Liriodendron* species are common in areas of elevated N deposition and N cycle alteration in the US and China.

An equally striking result was that the δ^{15}N record in tree rings of *L. tulipifera* increased dramatically ~8 years after fertilization. At this time, stream water NO_3 increased in both WS 1 and a nearby mature (last cut ca. 1910), unfertilized watershed (WS 4). Furthermore, the tree ring δ^{15}N of older *L. tulipifera* trees also increased at this time in WS 4 (Fig. 4). The increase in WS 4 stream water NO_3 has been attributed to N saturation caused by chronic additions of N from atmospheric deposition (Peterjohn et al. 1996), and the concurrent increase in WS 1 (Fig. 1) points to a similar

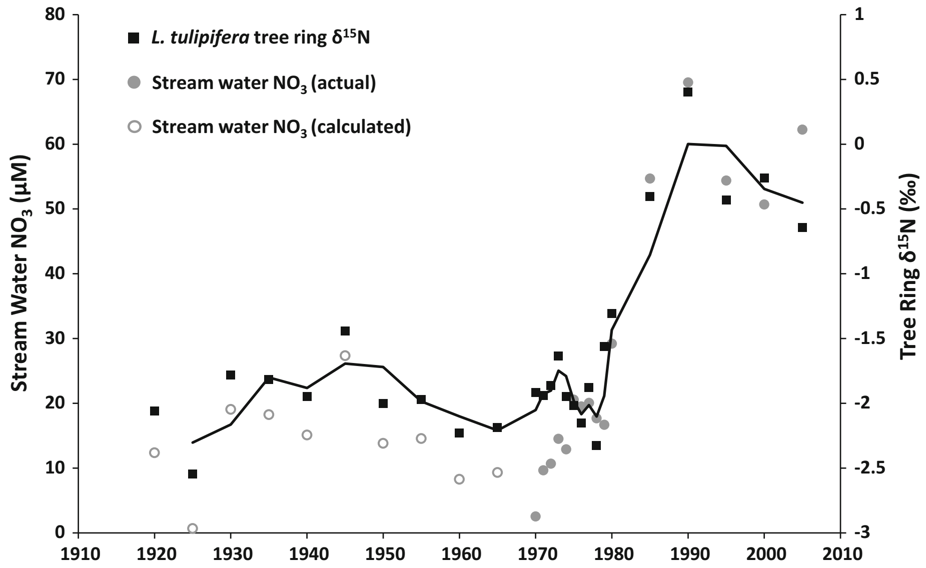


Fig. 4 *L. tulipifera* tree ring $\delta^{15}N$ and annual mean of monthly flow-weighted stream water NO_3 in a long-term reference watershed (WS 4) at the Fernow Experimental Forest. Trend line is a 2-year moving average of *L. tulipifera* tree ring $\delta^{15}N$ to visually depict the long-term trend. Calculated stream water NO_3 values (*open circles*) are based on the linear relationship between tree ring $\delta^{15}N$ and stream water NO_3 measurements 1970–2005 ($P < 0.001$, $r = 0.928$)

effect in this watershed. The soil N pool was likely smaller when signs of N saturation due to long-term deposition appeared than immediately following urea application. The percent of the N pool transformed via nitrification was likely high during the N saturation shift in stream water NO_3 (Peterjohn et al. 1996) compared to urea fertilization, when the soil N pool was much larger. This high percent nitrification, followed by NO_3 loss under N saturation, could have a large impact on the residual plant available N pool. Thus, it is possible that the cumulative effects of N deposition on soil N cycling had a greater effect on *L. tulipifera* tree ring isotope composition than a one-time fertilization, and that the wood $\delta^{15}N$ of this species is a more effective indicator of the effects of long-term N deposition than the effects of a short-term N cycle disturbance.

To demonstrate how tree ring $\delta^{15}N$ might help to extend stream water NO_3 records, we used the strong association between stream water NO_3 concentration and *L. tulipifera* $\delta^{15}N$ from 1970 through 2005 in the WS 4 ($r = 0.928$) to estimate stream NO_3 concentrations between 1920 and 1970 (Fig. 4). These estimates extend the existing long-term record (1970–2010) by an additional 50 years and suggest that prior to ~1980 stream NO_3 concentrations were typically ~15 uM and relatively constant (C.V. ~0.51). While very useful at our study site, the value of using tree ring $\delta^{15}N$ records to reconstruct stream water NO_3 levels at other locations may depend on conditions found at the FEF that may not apply elsewhere. These include high rates of net nitrification (Gilliam et al. 1996), a high percentage of mineralized N that is nitrified (Peterjohn et al. 1996), an apparent relationship between rates of soil nitrification and stream NO_3 level (Gilliam and Adams 2011), and relatively low rates of gaseous N losses (Peterjohn et al. 1998; Venterea et al. 2004). It may also require a stable or relatively slowly changing $\delta^{15}N$ signature in atmospheric N deposition. While this cannot be confirmed at the FEF, Rose et al. (2015) reported precipitation $\delta^{15}N$ values of -0.1 ‰ for the FEF in 2010, which is similar to regional values from 2000 (Elliott et al. 2007) and 1993–1994 (Russell et al. 1998).

In general, the results of this study support the potential utility of tree ring $\delta^{15}N$ in documenting significant changes in soil N cycling dynamics (Pardo and Nadelhoffer 2012; Gerhart and McLauchlan 2014), but show that the temporal record of tree ring $\delta^{15}N$ in different species can vary in response to the same change in the N cycle. As such, we suggest that research using tree ring $\delta^{15}N$ should utilize multiple species to obtain a synthetic view of the N cycle through time. In addition, tree ring $\delta^{15}N$ natural abundance should not be considered a recorder of the local N cycle with annual resolution due to the potential for inter-annual N movement, retranslocation, and recycling. Rather, it would be best used as an indicator of N cycle "openness", i.e. proportion of N lost from the system as NO_3 via nitrification or gaseous N losses, on a decadal time scale. Finally, additional measurements of site-specific soil N cycle processes, current or historic, can aid in the interpretation of the tree ring $\delta^{15}N$ signal and enhance our ability to draw conclusions about long-term N cycling dynamics.

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