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ECOSYSTEM ECOLOGY – ORIGINAL RESEARCH

Assessing tree ring δ^{15}N of four temperate deciduous species as an indicator of N availability using independent long-term records at the Fernow Experimental Forest, WV

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Abstract
Nitrogen deposition in the northeastern US changed N availability in the latter part of the twentieth century, with potential legacy effects. However, long-term N cycle measurements are scarce. N isotopes in tree rings have been used as an indicator of N availability through time, but there is little verification of whether species differ in the strength of this signal. Using long-term records at the Fernow Experimental Forest in West Virginia, we examined the relationship between soil conditions, including net nitrification rates, and wood δ^{15}N in 2014, and tested the strength of correlation between tree ring δ^{15}N of four species and stream water NO_3^- loss from 1971 to 2000. Higher soil NO_3^- was weakly associated with higher wood δ^{15}N across species, and higher soil net nitrification rates were associated with higher δ^{15}N for *Quercus rubra* only. The δ^{15}N of *Liriodendron tulipifera* and *Q. rubra*, but neither *Fagus grandifolia* nor *Prunus serotina*, was correlated with stream water NO_3^-. *L. tulipifera* tree ring δ^{15}N had a stronger association with stream water NO_3^- than *Q. rubra*. Overall, we found only limited evidence of a relationship between soil N cycling and tree ring δ^{15}N, with a strong correlation between the wood δ^{15}N and NO_3^- leaching loss through time for one of four species. Tree species differ in their ability to preserve legacies of N cycling in tree ring δ^{15}N, and given the weak relationships between contemporary wood δ^{15}N and soil N cycle measurements, caution is warranted when using wood δ^{15}N to infer changes in the N cycle.

Keywords Dendroisotope · Wood δ^{15}N · Nitrogen deposition · Watershed · Fernow Experimental Forest

Introduction

Forests in the northeastern United States experienced high deposition of reactive nitrogen (N) over the last half-century (Galloway et al. 2004). Since primary production in these ecosystems is often N-limited, the retention of N in forests is typically high and supply is low relative to demand (a “closed” N cycle). However, high inputs of N from atmospheric deposition can alter N transformations within the system and reduce forest watershed N retention. For example, elevated N deposition can cause high rates of N mineralization and nitrification, and elevated losses of mobile nitrate (NO_3^-) in stream water (Peterjohn et al. 1996; Aber et al. 1998; Lovett and Goodale 2011), with possible long-term impacts including the loss of important base cations and changes in understory and overstory species composition (Edwards and Helvey 1991; Gilliam et al. 1996, 2016; May et al. 2005). Changes in species composition, caused by climate change or forest pests, can also alter N availability and promote N leaching losses (Lovett et al. 2002; Peterjohn

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et al. 2015; Crowley and Lovett 2017), and watershed N losses shift throughout the course of succession (Vitousek and Reiners 1975; Lovett et al. 2018). However, few long-term records of changes in N cycling exist, complicating rigorous assessment of the legacy effects of N deposition and other long-term changes in N retention. Sediment cores are an effective proxy of catchment N cycling over extended time scales (McLauchlan et al. 2007), but lack the potential for fine temporal or spatial resolution in forests affected by N deposition. However, the δ^{15}N signature of plant tissue could potentially serve as such an indicator of soil N cycling.

Plant tissue δ^{15}N should reflect changes in the local soil N cycle (Robinson 2001; Pardo et al. 2006; Pardo and Nadelhoffer 2012; Falxa-Raymond et al. 2012). In the absence of fractionation during N uptake, N assimilated by plants and incorporated into tissue should have a similar isotopic signature as the soil N pool from which it was acquired. In the N cycle of a temperate forest, the primary fractionating step in well-drained soils with little denitrification is microbial nitrification, a process that preferentially uses the lighter ^{14}N isotope, and that results in a ^{15}N-enriched NH_4^+ substrate pool and a ^{15}N-depleted NO_3^- product pool (Handley and Raven 1992). As an anion, NO_3^- is readily leached into stream water, while NH_4^+ remains associated with cation exchange sites in the soil. So, when N deposition causes an increase in nitrification, more $^{14}NO_3^-$ is leached from the plant available N pool, resulting in a shift towards higher ^{15}N abundance under more "open" N cycling (Martinelli et al. 1999; Pardo and Nadelhoffer 2012). Although plants acquire N as both NH_4^+ and NO_3^-, over time, the loss of ^{15}N-depleted NO_3^- increases the overall δ^{15}N of N retained in the system. One complication is the potential for fractionation upon the uptake of N, particularly in species associated with ectomycorrhizal fungi (ECM) (Hobbie and Högberg 2012), which would decouple the δ^{15}N signatures of plant tissue and available soil N. However, ECM colonization and abundance typically declines in areas of high N deposition (Kjøller et al. 2012; Bahr et al. 2013), reducing the effect of ECM on plant δ^{15}N Central Appalachian forests. So, if the δ^{15}N signature of plant tissue reflects the plant available N pool, it would shift accordingly when the supply of N into the system exceeds N demand and could be used as a proxy for reduced N retention in the absence of direct measurements.

Since central Appalachian temperate broadleaf forests historically received high anthropogenic N deposition, which is now declining, the δ^{15}N signature of tree rings may be particularly useful as a long-term indicator of changes in N cycling in these ecosystems. Annual preservation of tissue in tree rings potentially make them a powerful tool for studying temporal changes in the N cycle (Gerhart and McLauchlan 2014). Past research using tree ring δ^{15}N to detect distinct isotope signatures from N pollution from fossil fuel burning (Saurer et al. 2004; Bukata and Kyser 2007; Savard et al. 2009) and track N cycle perturbations after known disturbances and fertilizer additions provide support for the signal preservation in wood N isotopes (Elhani et al. 2003, 2005; Hart and Classen 2003; Burnham et al. 2016). Generally, the direct influence of point source pollution tends to drive tree ring δ^{15}N signatures towards the signature of the source, which is typically high δ^{15}N for fossil-fuel pollution sources (Elliott et al. 2007). In addition, elevated long-term deposition may cause a change in soil nitrification, N isotopic fractionation, and mycorrhizal function, increasing the δ^{15}N of plant available N over time (Högberg and Johannisson 1993; Bukata and Kyser 2007; Högberg et al. 2014). As a result, the record of δ^{15}N in tree rings has been used to infer changes in ambient N availability. For example, declines in tree ring δ^{15}N have been detected in North America (Poulson et al. 1995; Härdtle et al. 2013; Kranabetter et al. 2013), and are often attributed to lower N availability and reduced ecosystem N losses (McLauchlan et al. 2007, 2017; McLauchlan and Craine 2012; Elmore et al. 2016). Thus, tree ring δ^{15}N could be used as an indicator of past N cycle perturbation leading to low N retention in Central Appalachian watersheds and a measure of changes in the cycling of N as anthropogenic deposition declines.

Previous research suggests that higher foliar δ^{15}N is associated with elevated nitrification and soil N availability in tropical (Martinelli et al. 1999) and temperate forests (Garten 1993; Falxa-Raymond et al. 2012). However, species may differ in the degree to which tree ring δ^{15}N would reflect soil processes. The form of N taken up can differ between species (Templer and Dawson 2004), and fractionation by soil N transformation processes changes the δ^{15}N signature of different N pools. Also, there is evidence of fractionation against ^{15}N during the transfer of N compounds to plants by ectomycorrhizal (EM) fungi (Hobbie and Högberg 2012; Kranabetter et al. 2013), decoupling the δ^{15}N of EM species' tree rings from that of the soil when trees are well colonized with their EM symbionts. Arbuscular mycorrhizal (AM) species are often associated with greater rates of nitrification relative to EM trees (Phillips et al. 2013; Lin et al. 2017), further distinguishing their potential tree ring δ^{15}N trends. Furthermore, differences in wood anatomy and function could lead to varying N retranslocation (Del Arco et al. 1991), lateral mobility of N within bole wood (Elhani et al. 2003; Tomlinson et al. 2014; Goodale 2017), and internal cycling of N within active woody tissue (El Zein et al. 2011) between species. So, it is important to determine how well soil N processes and availability relate to wood δ^{15}N of different species and mycorrhizal types, and to use existing records of N retention and loss to validate tree ring δ^{15}N as a proxy of changes in N cycling.

Final considerations that could impact the tree ring δ^{15}N record are any temporal changes in the δ^{15}N signature of N deposition due to different emission sources (Elliott et al. 2007). For example, the δ^{15}N of deposition tends to be higher during the dormant season as a result of the greater influence of coal-fired power generation (Elliott et al. 2007; Rose et al. 2015). If the δ^{15}N signature from power generation is typically high (Heaton 1990), then vegetation downwind from power plants might have tree rings enriched in δ^{15}N regardless of the ecosystem's inherent rates of nitrification and N retention. A reduction in the amount of power plant-derived deposition would reduce its influence on the δ^{15}N of the system and the temporal trend in tree ring δ^{15}N. Thus, it is currently far from established that tree ring δ^{15}N effectively records and preserves the N status of ecosystems through time.

Given the uncertainty in how well tree ring δ^{15}N of different species reflects soil N processes and availability and how closely tree ring δ^{15}N relates to watershed N retention and loss through time, we investigated how nitrification and other soil factors influenced wood δ^{15}N of two arbuscular mycorrhizal (AM) and two ectomycorrhizal (EM) tree species within a reference watershed (WS 4) at the Fernow Experimental Forest (FEF) that exhibits very low N retention. We also determined if tree ring δ^{15}N reflected temporal changes in stream water NO_3^- discharge. Differences in the δ^{15}N of tree rings should reflect the microbially mediated processes of nitrification and mycorrhizal N contribution. As such, we expected to find a positive relationship between wood δ^{15}N and both nitrification and soil NO_3^-, with AM trees having stronger relationships due to the lower N contribution, and thus reduced fractionation, by their mycorrhizal symbionts. This watershed has one of the longest continuous stream water NO_3^- records in the eastern United States, and exhibited a 145% increase in stream water NO_3^-, which has been attributed to long-term N deposition and N saturation (Peterjohn et al. 1996). There is a positive correlation between nitrification, soil NO_3^-, and stream water NO_3^- concentration through time at the FEF (Gilliam and Adams 2011), and so the increase in WS 4 stream water NO_3^- was likely caused by temporal trends towards greater soil nitrification and reduced N retention. Thus, we expected tree ring δ^{15}N to be positively related to stream water NO_3^- concentration through time. Finally, we expected a stronger relationship between tree ring δ^{15}N and stream water NO_3^- in portions of the watershed with higher nitrification and extractable NO_3^- pools.

Methods

The FEF is a US Forest Service research site located in Tucker County, WV, USA (39.054839°N, 79.688580°W). Watershed 4 (WS 4, 38.7 ha) (Fig. 1) is a long-term reference watershed, last commercially logged circa 1910 and allowed to naturally regrow since that time. The predominant soil is a Calvin channery silt loam (loamy-skeletal, mixed, mesic Typic Dystrochrept), elevation ranges from approximately 760–840 m, and the slope averages 16% at a southeasterly aspect (Kochenderfer 2006). Nearly continuous hydrologic

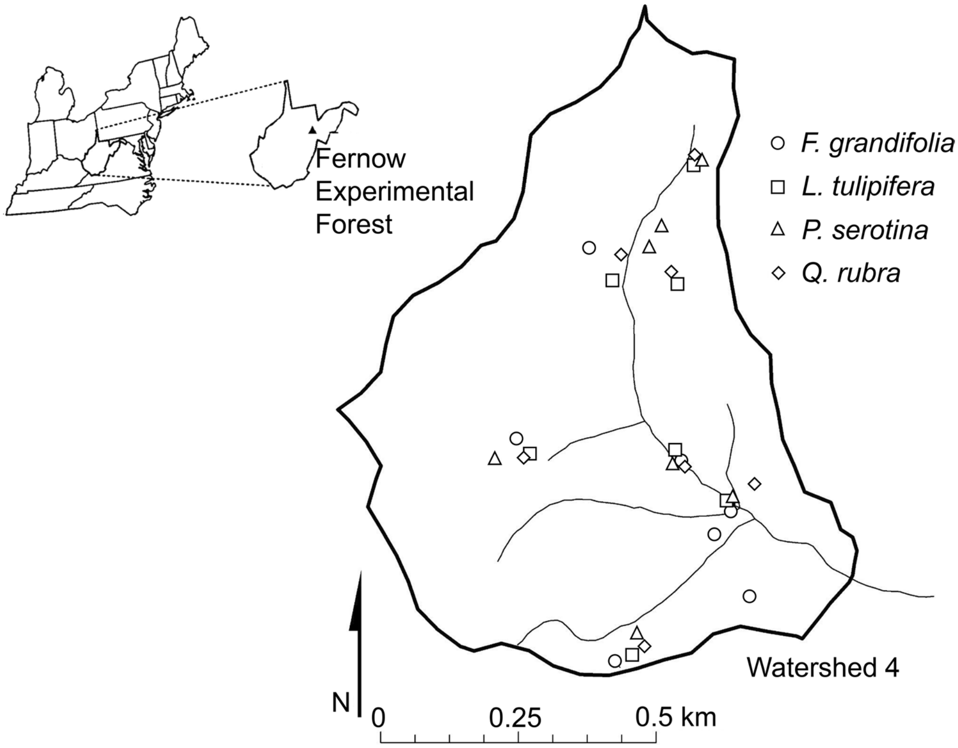
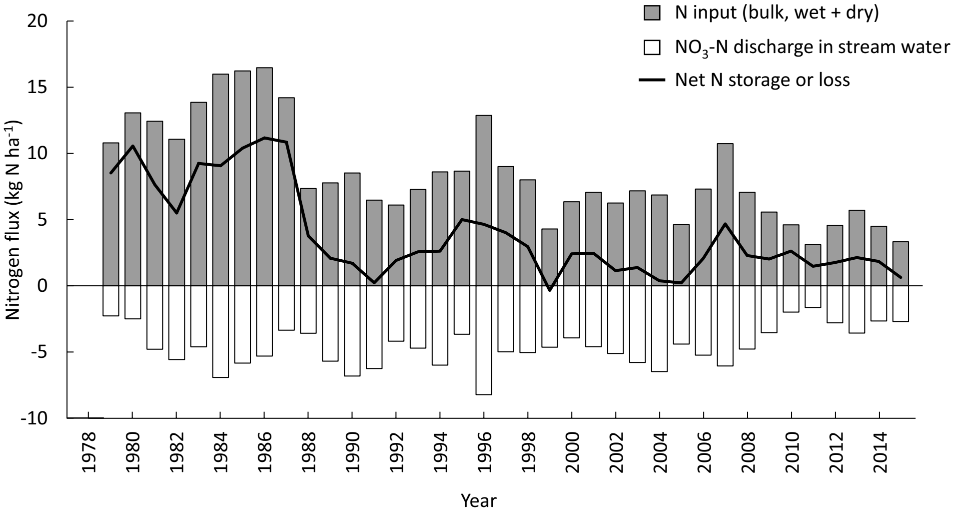


Fig. 1 Location of the Fernow Experimental Forest (FEF) and of the cored trees in watershed 4 (WS 4)

and biogeochemical measurements in the watershed include stream flow and precipitation since 1951 and 1952, stream chemistry including N export since 1970, and bulk N deposition (wet + particulate) since 1983 (Fig. 2). Annually, FEF WS 4 receives an average of ~ 145 cm of rainfall. The forest is a stand of mixed hardwood species consisting of *Quercus rubra* (32.3% of basal area), *Acer saccharum* (13.1%), *Acer rubrum* (11.2%), *Prunus serotina* (9.4%), *Liriodendron tulipifera* (7.9%), *Quercus prinus* (5.9%), and *Fagus grandifolia* (3.4%). Around 1980, stream water NO_3^- concentration increased ~ 145% (Fig. 3a), contributing to a 435% increase in watershed NO_3^-–N discharge from 1975 to 1984. Bulk N deposition data (wet + particulate N), which extend back to 1983 and can be estimated via relationship to wet deposition data back to 1979 (NADP 2016), reveal a long-term decline in deposition (Fig. 2). In contrast, after the increase in N output circa 1980, discharge remained elevated through ~ 2010. The elevated stream water NO_3^- and decrease in N deposition resulted in a shift in N retention in WS 4 from ~ 80% of annual N inputs in 1979–1980 to less than 30% since the early 1990s, and it exhibited the lowest total N retention of measured watersheds in the Northeastern United States over this time frame (Campbell et al. 2004). Although no records of the $\delta^{15}N$ signature of N deposition exist here, if a reduction in isotopically enriched power plant-derived N (Heaton 1990) caused the long-term decline in N deposition, then it is possible that the $\delta^{15}N$ of deposited N declined as well.

During July 2014, we collected increment cores from four major tree species within FEF WS 4 (seven individual trees per species): *Q. rubra*, *P. serotina*, *L. tulipifera*, and *F. grandifolia* (Fig. 1). These represent both arbuscular mycorrhizal (*P. serotina* and *L. tulipifera*) and ectomycorrhizal (*Q. rubra* and *F. grandifolia*) species, and Burnham et al. (2017) found that they take up both NO_3^- and NH_4^+ in approximately equal proportion. To ensure that the tree ring record extended back to the commencement of stream water NO_3^- measurements (1970), we chose mature, canopy trees of each species > 40 cm in diameter at breast height (DBH), except for one somewhat smaller *F. grandifolia* tree (32 cm DBH). Since each species is not evenly distributed across the watershed, we opportunistically selected trees that were near (within ~ 25 m) trees from the other species being examined, when possible, to minimize species differences in $\delta^{15}N$ due to their spatial location and elevation (Garten 1993). Due to the relative scarcity of canopy beech trees, two cored trees were spatially separated from the other species (Fig. 1). We collected two increment cores from each tree, one from either side of the tree parallel to the contour of the land, using 5-mm diameter increment borers (Mora of Sweden, Mora, Sweden). Increment corers were rinsed with deionized water between trees, and the cores were air dried prior to processing for ring width and wood $\delta^{15}N$. One core from each tree was mounted and sanded to better visualize the annual growth rings. The ring widths were measured and cores were cross-dated, using the dplR package for R to assess cross-dating accuracy (Bunn 2010). The second core from each tree was used for $\delta^{15}N$ analysis. Due to the lack of an established, consistent labile-N extraction method (Gerhart and McLauchlan 2014), we analyzed raw, non-extracted wood. The most recently formed wood, from the 2014 tree ring, was separated from each core to compare its $\delta^{15}N$ signal with contemporary N cycle measurements in nearby soil. To analyze the temporal relationship between tree ring $\delta^{15}N$ and stream water NO_3^- concentration, each core was cut into 3-year segments from 1971 to 2000, which included the ~ 1980 increase in stream NO_3^- and the subsequent discharge dynamics through the year 2000. Each ring from 2014, and the 1971–2000 ring segments, were ground into a fine powder using a dental amalgamator (Henry Schein, Inc., Melville, NY), and 8–10 mg of tissue from each were wrapped in tin capsules. The $\delta^{15}N$ of each segment was measured via isotope ratio gas chromatography–mass

Fig. 2 Annual bulk (wet + dry) N depositional inputs into and stream discharge from FEF WS 4, and the net N storage or loss from the catchment



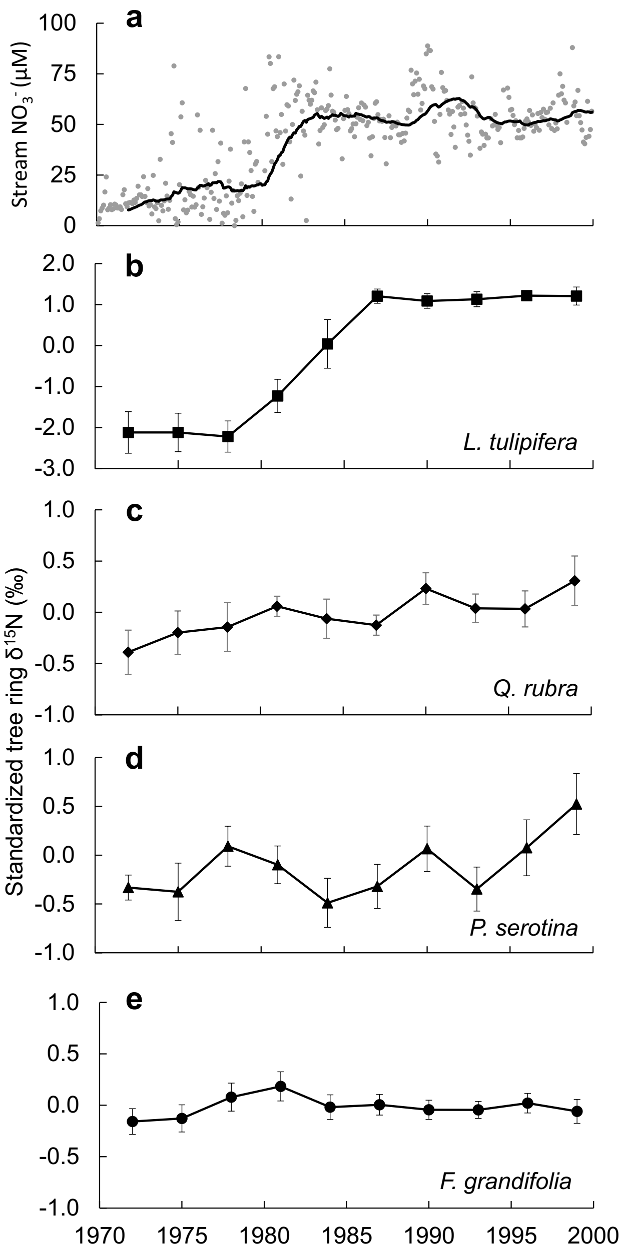


Fig. 3 Stream water NO_3^- concentration (**a**) and tree ring $\delta^{15}N$ signature (**b**–**e**) since 1971. The temporal trend in stream water NO_3^- is visualized using a 3-year running average of the monthly, flow-weighted stream water NO_3^- concentration. The average tree ring $\delta^{15}N$ ($n = 7$ for each species) is shown for each 3-year time segment, 1971–2000. Note the difference in *y*-axis scale for *L. tulipifera*

spectrometry by the Central Appalachians Stable Isotope Facility at the University of Maryland Center for Environmental Science Appalachian Laboratory (Frostburg, MD). The mobility of non-structural N compounds within the tree spurred efforts to extract labile N prior to isotopic analysis (Gerhart and McLauchlan 2014), but these have met mixed results, with some reduction of $\delta^{15}N$ variability (Elhani et al. 2003), but little change in temporal trends (Elhani et al. 2003; Caceres et al. 2011; Bunn et al. 2017). As such, we did not perform any labile N extraction.

To determine if measures of soil N status are related to tree ring $\delta^{15}N$, we collected mineral soils from under each cored tree in July of 2014. The area of the vertically projected canopy under each tree was divided into four quadrants, and two soil cores were extracted from each quadrant, within ~ 3 m of the trunk, using a 2.2-cm inner diameter soil-recovery probe (AMS, Inc., American Falls, ID, USA). The top 5 cm of mineral soil from the soil cores were pooled into one sample for each tree. Prior to sieving, the soils were weighed to calculate their total dry mass (using the gravimetric water content) and bulk density. The soils were then sieved to pass through a 5.6-mm (#3.5) mesh testing sieve, and a subsample used for gravimetric determination of moisture content, in which 5–6 g of soil were weighed before and after drying for 48 h at 65 °C.

We measured net mineralization, ammonification, and nitrification potentials in the collected soils using a lab incubation. One ~ 10-g subsample of soil was incubated in the dark at room temperature for 5 days to account for any collection disturbance effect on N transformation rates, and a second subsample was incubated for an additional 28 days to measure the rate of NH_4^+ and NO_3^- production in the soil. After incubation, both subsamples were extracted in 1 M KCl by gently shaking for 30 min, filtering through a polyethersulfone filter with 0.45 µM pore size (Supor membrane, Pall Life Sciences, Ann Arbor, MI, USA), and storing at − 20 °C until NO_3^- and NH_4^+ analyses were performed using a Lachat QuikChem 8500 Series 2 Auto-analyzer (QuikChem Methods 12-107-04-1-B and 12-107-06-2-A).

Statistical analyses

We used multiple linear regressions to test if current inorganic soil N pools or soil N transformation rates could predict non-standardized tree ring $\delta^{15}N$ of the most recent wood (2014). We ran a regression with the $\delta^{15}N$ of the 2014 tree ring of each species as the dependent variable and independent variables of soil potential net mineralization, potential net nitrification, the percent of mineralized N nitrified to NO_3^- (percent nitrification), the pool of extractable NH_4^+, and the pool of extractable NO_3^-. Due to the nature of the predictor variables examined, we assessed multicollinearity using variance inflation factors (VIF values). To adhere to the assumption of independence of predictors, as best as possible, we excluded those variables from the regression model that had VIF values of > 5. In our final models, the VIF values were all < 1.5.

Raw, unstandardized tree ring $\delta^{15}N$ data are supplied as an online resource (ESM 1) and are available in the National Centers for Environmental Information tree ring paleoclimate data repository. Differences in N content and raw δ^{15}N between species and mycorrhizal types were assessed via 2-way ANOVA. To reduce the between-tree variability, and focus on the long-term temporal trend in tree ring δ^{15}N, we standardized the isotope values of the 3-year time segments by subtracting the within-tree mean wood δ^{15}N (Gerhart and McLauchlan 2014; Burnham et al. 2016). The seven replicate trees for each species were then averaged within each 3-year time segment. We used a Pearson correlation analysis to measure the strength and significance ($\alpha = 0.05$) of the relationship between both the basal area-weighted average of δ^{15}N across species and average tree ring δ^{15}N within each species, and the annual average of monthly flow-weighted stream water NO_3^- concentrations through time. To determine if the strength of the relationship between tree ring δ^{15}N and stream water NO_3^- concentration depended on tree species, we tested this interaction via ANCOVA with tree ring δ^{15}N as the response, species as a categorical factor, and stream water NO_3^- concentration as a covariate. In all analyses, we originally included a categorical watershed position variable to account for the loose clustering of sampled trees (Fig. 1), and a covariate of elevation, but these never had significant effects and were not included in the final results. All statistical analyses were completed using Minitab 17 statistical software (Minitab, Inc., State College, PA, USA) and the R statistical environment (R Core Team 2018).

Results

The average net mineralization and nitrification potentials under the tree canopies were 1.02 μg N g^{-1} dry soil day^{-1} and 1.01 μg N g^{-1} dry soil day^{-1}, respectively, and they did not significantly differ between species (Table 1). The net nitrification potential was 96.7% of the net mineralization potential (i.e. "relative nitrification"), and this, as well as extractable soil NO_3^- levels, also did not differ between species. However, the extractable NH_4^+ pool was different between species ($F = 6.45$, $P = 0.002$), with more extractable NH_4^+ present in soils under *P. serotina* and *Q. rubra* trees (6.1 and 5.2 μg N g^{-1} dry soil, respectively) than under *L. tulipifera* (2.0 μg N g^{-1} dry soil). Soils under *F. grandifolia* had intermediate levels of extractable NH_4 (2.9 μg N g^{-1} dry soil) (Table 1).

We did not find any concurrent changes in BAI across species, and within-species mean inter-series ring-width correlations were relatively low (*Q. rubra* = 0.09, *L. tulipifera* = 0.16, *P. serotina* = 0.13, *F. grandifolia* = − 0.05). This is likely because our site is a relatively undisturbed, mesic forest and not a sensitive site typical of dendrochronological studies that investigate strong growth responses to changes in climate or disturbance. The mean wood N content differed between species ($F = 28.9$, $P < 0.01$), with *P. serotina* having lower mean N content from 1971 to 2000 (0.07% ± 0.01%) than the other species (0.10% ± 0.01%). However, in 2014, the species did not differ in N content. The mean raw, non-standardized δ^{15}N of 2014 wood was 1.44‰, and did not differ between species ($F = 0.04$, $P = 0.96$) nor by mycorrhizal type ($F = 0.17$, $P = 0.69$). However, there was an 8.6‰ range of 2014 tree ring δ^{15}N values, from − 3.04 to 5.56‰ (Table ESM1), and the overall mean raw δ^{15}N value, across all species, increased from − 0.13 to 1.44‰ from 1971 to 2014. Across all species examined, higher soil NO_3^- was weakly positively associated with 2014 tree ring δ^{15}N ($R^2 = 18.3\%$, $F = 6.27$, $P = 0.022$), and there was a trend towards this effect depending on species ($F = 2.50$, $P = 0.092$), with 2014 tree ring δ^{15}N of *Q. rubra*, but not other species, increasing with higher soil NO_3^- ($P = 0.029$) (Fig. 4). For *L. tulipifera* trees, extractable soil NH_4^+ was negatively associated with the 2014 tree ring δ^{15}N ($R^2 = 59.9\%$, $F = 7.45$, $P = 0.041$, data not shown). For *Q. rubra*, the 2014 tree ring δ^{15}N was positively associated with net nitrification potential ($R^2 = 79.7\%$, $F = 19.6$, $P = 0.007$) (Fig. 4). There were no soil N pool or transformation rate variables that were significantly associated with the 2014 tree ring δ^{15}N of *P. serotina* or *F. grandifolia*.

The monthly flow-weighted mean stream water NO_3^- concentration in FEF WS 4 increased from ~ 12 μM in the early 1970s to ~ 50 μM in the early 1980s (Fig. 3a). Despite long-term declines in N deposition (Fig. 2), stream water NO_3^- concentration and N discharge remained constant from

Table 1 Mean KCl-extractable soil N pools and transformation rates (*NMP* net mineralization potential, *NNP* net nitrification potential) under the canopies of each tree species

	n	NH_4^+ (μg N g^{-1} soil)	NO_3^- (μg N g^{-1} soil)	NMP (μg N g^{-1} soil day^{-1})	NNP (μg N g^{-1} soil day^{-1})	Relative nitrification
P. serotina	7	6.1^a (1.0)	7.7^a (1.9)	1.00^a (0.19)	1.10^a (0.15)	117.6^a (9.8)
Q. rubra	7	5.2^{ab} (0.8)	6.2^a (1.8)	1.05^a (0.20)	0.99^a (0.24)	83.2^a (12.7)
F. grandifolia	7	2.9^{bc} (0.7)	7.8^a (2.0)	0.95^a (0.14)	0.95^a (0.16)	97.3^a (6.6)
L. tulipifera	7	2.0^c (0.4)	8.9^a (1.7)	1.09^a (0.16)	1.01^a (0.18)	88.5^a (10.2)

Parenthetical values are standard error about the mean. Means that do not share a letter are significantly different ($P < 0.05$)

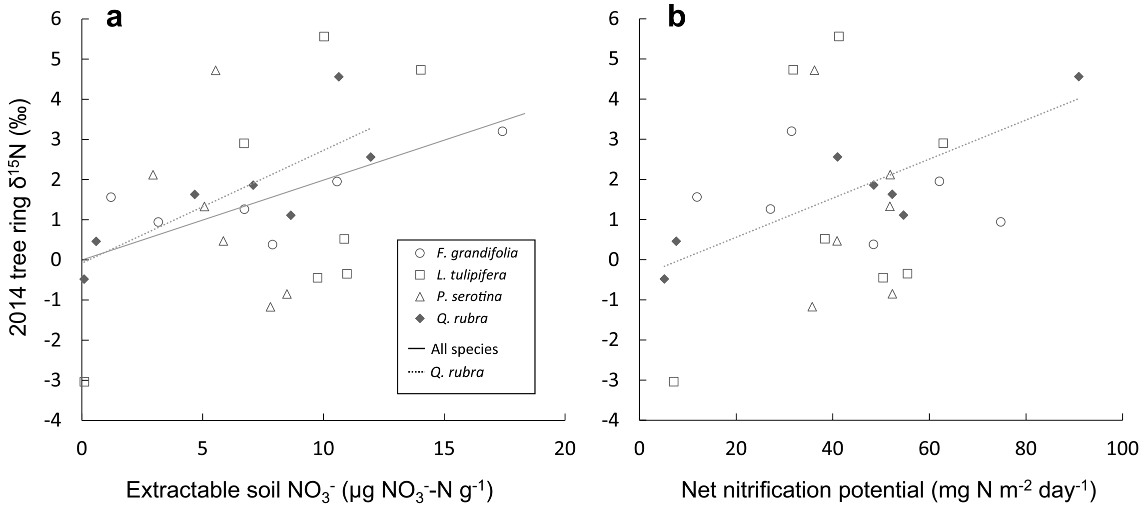


Fig. 4 Relationship between non-standardized 2014 tree ring $\delta^{15}N$, 2014 soil KCl-extractable NO_3^- (**a**), and net nitrification potential (**b**). Only significant ($P < 0.05$) trend lines are shown: all species (solid line) and *Q. rubra* (dashed line) for soil NO_3^-, *Q. rubra* only (dashed line) for net nitrification potential

the mid-1980s through 2000 (Fig. 3a), only starting to decline around 2009 (Fig. 2). Across species, the basal area-weighted tree ring $\delta^{15}N$ was positively correlated with stream water NO_3^- concentration from 1971 to 2000 ($r = 0.88$, $P < 0.001$). However, this was driven by two of the four species. Within tree species, the average *Q. rubra* ($r = 0.81$, $P = 0.004$) and *L. tulipifera* ($r = 0.91$, $P < 0.001$) standardized tree ring $\delta^{15}N$ were positively correlated with stream water NO_3^- concentration from 1971 to 2000, but significant correlations were not found for *P. serotina* ($P = 0.46$) or *F. grandifolia* ($P = 0.44$). As such, the relationship between stream water NO_3^- and tree ring $\delta^{15}N$ differed between species ($F = 27.3$, $P < 0.001$). The temporal dynamics found in stream water NO_3^- concentrations (1971–2000) were well characterized by the temporal changes in the standardized tree ring $\delta^{15}N$ of *L. tulipifera* (Fig. 3b). Over the ~10-year period from 1977 to 1979 until 1986–1988, *L. tulipifera* standardized tree ring $\delta^{15}N$ increased from − 2.2 to 1.2‰ (+ 3.4‰). While the temporal pattern found in the $\delta^{15}N$ record of *Q. rubra* tree rings captured the overall trend in stream water NO_3^- concentrations (Fig. 3c), it was less sensitive to the abruptness of change than the record contained in the rings of *L. tulipifera*. The standardized tree ring $\delta^{15}N$ of *Q. rubra* increased from − 0.39 to 0.06‰ (+ 0.45‰) from 1971–1973 through 1980–1982, and later increased to 0.23‰ in 1989–1991 and 0.31‰ in 1998–2000.

Discussion

Across the four species examined, we found only mixed evidence that nitrification and soil NO_3^- affect wood $\delta^{15}N$. Although soil NO_3^- was related to wood $\delta^{15}N$ across species, it explained only a small portion ($R^2 = 18\%$) of the variability in tree ring $\delta^{15}N$ signatures. The strength of this relationship differed between species, with higher nitrification and soil NO_3^- only increasing tree ring $\delta^{15}N$ of *Q. rubra*, and *L. tulipifera* 2014 tree ring $\delta^{15}N$ only responding (negatively) to soil NH_4^+. These effects lend only marginal support for using tree ring $\delta^{15}N$ as an indicator of soil N availability, and only for one species, *Q. rubra*. In the FEF, nitrification is relatively high across the watersheds (Gilliam et al. 2018), particularly mid-season (Gilliam et al. 2001). These site characteristics and our mid-season sampling may have reduced likelihood of detecting a strong relationship between soil N cycling and the $\delta^{15}N$ of contemporary tree rings of different species. In addition, remobilized N contributes substantially to current-year leaf and root growth (Millard and Grelet 2010; Bazot et al. 2016). If internal non-structural N stores contribute to new wood N, then this would decouple wood $\delta^{15}N$ from current-year soil N cycle processes. It is also likely that internal cycling of N within woody tissue, which has been measured in *Quercus* and *Fagus* species (El Zein et al. 2011), is partly responsible for this lack of relationship. The large range and high variability of $\delta^{15}N$ values in newly formed wood (8.6‰) greatly complicated detecting any species differences in effects of soil N cycling on isotope values. Although we found a large range of wood $\delta^{15}N$ values, previous studies report ranges of 4–5‰ (Savard et al. 2009; Reimchen and Arbellay 2018) and often reduce between-tree variability by standardizing $\delta^{15}N$ values within tree core to isolate temporal trends across a site or region (Savard et al. 2009; McLauchlan and Craine 2012; McLauchlan et al. 2017).

Although our results do not support the use of wood $\delta^{15}N$ as an indicator of soil N cycling, other studies have reported positive relationships between soil N and tree ring $\delta^{15}N$. Kranabetter and Meeds (2017) and Kranabetter et al. (2013) found that Douglas fir wood $\delta^{15}N$ was positively associated with N availability and site index. The forest used in these studies was a low N coniferous forest with lower N process rates, whereas we measured high rates of percent nitrification (Table 1), typical across the FEF (Gilliam et al. 2018), and we sampled mid-season when nitrification rates are highest in this forest (Gilliam et al. 2001). Furthermore, Kranabetter et al. (2013) show that the difference between soil and wood $\delta^{15}N$ (termed $\Delta^{15}N$) is negatively associated with soil N supply, suggesting that higher N availability may have caused lower transfer of N from EM fungi to the plant because fractionation during N transfer leads to high $\Delta^{15}N$ values (Kranabetter et al. 2013). If the positive association between soil N availability and Douglas fir wood $\delta^{15}N$ was driven by reductions in mycorrhizal colonization, then such an association would not be detectable in higher N systems already characterized by low transfer of N to plants from mycorrhizae, such as the FEF and Catskill Mountains (Templer et al. 2007).

The inconsistent relationship between wood $\delta^{15}N$ and soil N cycling makes it difficult to support the use of tree ring $\delta^{15}N$ records as a proxy for N retention and loss through time in Central Appalachia. However, our study differs from prior research in that we also used stream water NO_3^- concentration as an independent, long-term index of N retention. In our study, the tree ring $\delta^{15}N$ records for *L. tulipifera* and *Q. rubra* were positively correlated with direct measurements of stream water NO_3^- concentration in FEF WS 4, and the similarity between the tree ring $\delta^{15}N$ record for *L. tulipifera* and the temporal pattern in stream water NO_3^- concentration was particularly striking. The 3‰ increase in $\delta^{15}N$ coincided with a 38 µM increase (317%) in stream water NO_3^- that has been attributed to N saturation under long-term anthropogenic deposition (Peterjohn et al. 1996). The heartwood–sapwood transition for this species did not occur until the rings formed in the late-1980s, and so did not contribute to the prior change in $\delta^{15}N$. Although the tree ring $\delta^{15}N$ of *Q. rubra* reflected the overall trend in stream NO_3^- concentrations, it appears to be less sensitive to shorter term dynamics. Therefore, our results suggest that wood $\delta^{15}N$ is a poor indicator of contemporary nitrification rates, but tree ring $\delta^{15}N$ in some species preserves long-term changes in the $\delta^{15}N$ of the plant available pool under elevated NO_3^- leaching.

It is surprising that tree ring $\delta^{15}N$ of two species appears to relate to N retention and loss through time, but not to current-year N cycle dynamics in a temperate forest with relatively high N availability. As an explanation for this phenomenon, we suggest that changes in tree ring $\delta^{15}N$ through time are controlled most by shifts in mycorrhizal N contribution. Since mycorrhizal transfer of N fractionates against ^{15}N, high rates of colonization and mycorrhizal N contribution would reduce plant tissue $\delta^{15}N$ relative to the soil (Hobbie and Ouimette 2009; Hobbie and Högberg 2012). We then hypothesize that as N availability increased in the FEF under high N deposition, reductions in mycorrhizal colonization and N transfer increased plant tissue $\delta^{15}N$ towards that of the soil, which is typically higher than plant tissue (Templer et al. 2007). However, the extent to which AM fungi contribute to tree N nutrition and the fractionation during their possible N transfer is uncertain (Hobbie and Ouimette 2009). Thus, this may be a particularly important consideration for the use of $\delta^{15}N$ in tree rings of EM species, whereas fractionation by nitrifying soil microbes may be a stronger influence of tree ring $\delta^{15}N$ of AM species. Further measurements of soil and plant $\delta^{15}N$ and mycorrhizal colonization across a gradient of N availability for these EM and AM species are needed to discern the mechanisms behind changes in tree ring $\delta^{15}N$.

While *Q. rubra* tree ring $\delta^{15}N$ was positively correlated with stream water NO_3^-, it appeared to better capture the long-term trend in stream NO_3^- concentration (Fig. 3), rather than the shorter term dynamics. Though no measurements of mycorrhizal colonization exist at this site, it is possible that N deposition-induced reductions in EM colonization causing lower fungal transfer to *Q. rubra* trees were gradual, since *Quercus* species are typically associated with lower N cycling rates (Templer et al. 2007; Piatek et al. 2009; Peterjohn et al. 2015). Also, there is evidence of the movement of N compounds between rings after ring formation in *Q. rubra* (Burnham et al. 2016) and in other species (Elhani et al. 2003; Hart and Classen 2003). Inter-annual mobility of N would smooth out the preserved signal of any response of tree ring $\delta^{15}N$ to a perturbation in the N cycle and result in the observed response of *Q. rubra* to long-term N cycle changes in WS 4. Interestingly, *Q. rubra* was the only species that had a relationship between soil nitrification and contemporary tree ring $\delta^{15}N$. This suggests that inter-annual mobility of N compounds within this species is partial and/or takes place in subsequent years after wood formation. Finally, a long-term increase in *Acer saccharum* at the FEF (Schuler and Gillespie 2000) might also contribute to gradual changes in N availability and nitrification (Lovett et al. 2002, 2004; Phillips et al. 2013), in turn affecting the $\delta^{15}N$ signature of co-occurring species such as *Q. rubra*. Although *Q. rubra* does not capture short-term dynamics as precisely as *L. tulipifera* in this study, it appears to more consistently respond to both short-term perturbations in the N cycle (Burnham et al. 2016) and the long-term changes in N availability.

It was also notable that two species, *P. serotina* and *F. grandifolia*, showed no relationship between tree ring $\delta^{15}N$

and stream water NO_3^- in WS 4 or between any soil N pools or transformation rates and $\delta^{15}N$ of newly formed woody tissue. It is unclear why *P. serotina* tree ring $\delta^{15}N$ did not respond to changes in watershed N cycling, particularly since urea fertilization increased *P. serotina* tree ring $\delta^{15}N$ in a nearby watershed (Burnham et al. 2016). However, this species had lower mean wood percent N from 1971 to 2000 than the other species, but equal in contemporary 2014 wood. This could indicate higher mobility of N compounds within this species, obscuring any temporal $\delta^{15}N$ trend. For *F. grandifolia*, Elhani et al. (2003) used ^{15}N-enriched label addition to show that N can be mobile within the woody tissue by detecting $\delta^{15}N$ enrichment in wood formed prior to ^{15}N treatment. This species may also be an ineffective recorder of the N saturation signal, because it can be more responsive to surrounding stand dynamics and changes in the light environment than patterns of long-term N deposition. Indeed, we observed that individual *F. grandifolia* trees would often exhibit short-term increases in ring width that did not coincide with other *F. grandifolia* trees in the same watershed, which caused a poor mean ring-width inter-series correlation for this species (−0.05 vs. 0.13 for all other species). This pattern we attribute to the fact that, in WS 4, *F. grandifolia* is common in the sub-canopy, where it grows slowly until the canopy is disturbed. This is consistent with the observation that the annual coefficient of variation in *F. grandifolia* BAI (85% across all years) was much higher than the values for the other species (all ~ 50% or lower). Further evidence was also seen in the concurrent growth response of four *F. grandifolia* trees in a nearby watershed when it was harvested in the late 1950s (Burnham et al. 2016). As a result, we suspect that the short-term increases in *F. grandifolia* N demand during periods of rapid growth generally reduced leaching in the surrounding soil and possibly increased mycorrhizal colonization and N transfer, hindering this species' ability to integrate long-term changes in N supply and demand within WS 4.

Since power plant N pollution is typically enriched in $\delta^{15}N$ in this region (Elliott et al. 2007), the reduction in deposition since the early 1980s (Fig. 2) suggests a reduction in $\delta^{15}N$ of N input into the watershed. Studies that use tree ring isotopes to detect changes in pollution rely on pollution signatures that are distinct from the background $\delta^{15}N$ of the ecosystem and N input rates that are high relative to internal N cycling (Gerhart and McLauchlan 2014). Though long-term data on the $\delta^{15}N$ signal of precipitation do not exist here, Rose et al. (2015) found that within-year precipitation $\delta^{15}N$ values are highly variable at this site, ranging from −6.0 to +5.1‰, with an annual average of −0.1‰. Additionally, inputs of N via deposition, ~ 15 kg N ha^{-1} $year^{-1}$ at their highest, are low compared to internal rates of N cycling, 114 kg N ha^{-1} $year^{-1}$ for nitrification (Gilliam et al. 2001). If the $\delta^{15}N$ of deposition exerted strong control over tree ring $\delta^{15}N$, we would expect a decline in the wood $\delta^{15}N$ through time and similar $\delta^{15}N$ values across trees that reflect the signature of deposition. However, we detected an overall increase in tree ring $\delta^{15}N$ through time, and high variability in raw $\delta^{15}N$ (Table ESM1). Thus, we do not believe that the $\delta^{15}N$ signal of deposited N greatly impacted the $\delta^{15}N$ record in tree rings at this site.

Although tree ring $\delta^{15}N$ has been used as an indicator of changes in N availability and retention through time (Craine et al. 2009; Hietz et al. 2011; Elmore et al. 2016; McLauchlan et al. 2017), there is little verification using independent measurements of N availability and leaching loss. In our study, contemporary measurements of N cycle processes and N availability were not related to $\delta^{15}N$ of newly formed wood. This finding contributes to a small body of evidence that wood $\delta^{15}N$ is, at best, an inconsistent indicator of local N cycling (Templer et al. 2007; Kranabetter et al. 2013; Tomlinson et al. 2016; Kranabetter and Meeds 2017). However, we were able to detect a meaningful temporal signal of N availability, based on stream water NO_3^- losses, in two of the four species we examined. At our site, these mixed results suggest that past changes in mycorrhizal colonization and N transfer caused by increasing N availability under long-term N deposition exerted significant control over tree ring and plant $\delta^{15}N$, at least for EM species, and this mechanism should be further explored across a broader range of N availability. To further our understanding of temporal changes in plant $\delta^{15}N$, studies should also focus on determining the dominant forms of N in wood and how they are formed and cycled within the plant, and any if isotopic fractionation occurs during these processes. Overall, our results warrant caution in interpreting tree ring $\delta^{15}N$ trends until more is known about the mechanisms that govern wood $\delta^{15}N$ variation.

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Author contribution statement MBB and WTP conceived the idea. MBA maintained and processed long-term N data, and MBB and WTP performed field core and soil collection and laboratory analyses. MBA provided editorial advice, and MBB and WTP analyzed the data and wrote the manuscript.

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