Assessing the Linkages between Tree Species Composition and Stream Water Nitrate in a Reference Watershed in Central Appalachia

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Abstract: Many factors govern the flow of deposited nitrogen (N) through forest ecosystems and into stream water. At the Fernow Experimental Forest in WV, stream water nitrate (NO_3^-) export from a long-term reference watershed (WS 4) increased in approximately 1980 and has remained elevated despite more recent reductions in chronic N deposition. Long-term changes in species composition may have altered forest N demand and the retention of deposited N. In particular, the abundance and importance value of *Acer saccharum* have increased since the 1950s, and this species is thought to have a low affinity for NO_3^-. We measured the relative uptake of NO_3^- and ammonium (NH_4^+) by six important temperate broadleaf tree species and estimated stand uptake of total N, NO_3^-, and NH_4^+. We then used records of stream water NO_3^- and stand composition to evaluate the potential impact of changes in species composition on NO_3^- export. Surprisingly, the tree species we examined all used both mineral N forms approximately equally. Overall, the total N taken up by the stand into aboveground tissues increased from 1959 through 2001 (30.9 to 35.2 kg N ha^{-1} yr^{-1}). However, changes in species composition may have altered the net supply of NO_3^- in the soil since *A. saccharum* is associated with high nitrification rates. Increases in *A. saccharum* importance value could result in an increase of 3.9 kg NO_3^--N ha^{-1} yr^{-1} produced via nitrification. Thus, shifting forest species composition resulted in partially offsetting changes in NO_3^- supply and demand, with a small net increase of 1.2 kg N ha^{-1} yr^{-1} in NO_3^- available for leaching. Given the persistence of high stream water NO_3^- export and relatively abrupt (~9 year) change in stream water NO_3^- concentration circa 1980, patterns of NO_3^- export appear to be driven by long-term deposition with a lag in the recovery of stream water NO_3^- after more recent declines in atmospheric N input.

Keywords: watershed biogeochemistry; nitrogen cycle; nitrification; nitrogen uptake; nitrate export; *Acer saccharum*

1. Introduction

The northeastern United States experienced relatively high atmospheric N deposition during the latter half of the 20th century [1,2], increasing N supply into some forested ecosystems enough that the availability of N exceeded stand N demand—a situation that can cause significant nitrate (NO_3^-) leaching [3]. Substantial loss of NO_3^- contributes to an associated leaching of base cations, such as calcium and magnesium, which are important to plant growth [4–6], and may also have negative effects downstream [1]. Since the passage and subsequent amendment of the Clean Air Act, national emissions of NO_x and atmospheric N deposition have steadily declined; however, the response of forested catchments is variable. Some have lower N export following national emission

and deposition trends, while the levels of N export in others remain high and result in declining inorganic N retention [7–9]. Given the ecological implications of N export into stream water, it is important to understand what controls watershed responses to changes in N deposition through time.

Many factors (both belowground and aboveground) can affect the retention and export of N deposited into forests [10]. Below ground, soil organic matter is the largest pool of N in temperate forests and is a major sink for added N [11]. Microbial immobilization, plant uptake, mineralization, and nitrification control mineral N availability in the soil [12], and net nitrification has a large impact on N export due to the mobility of NO_3^- in soils. Above ground, stand age has a large impact on N retention, as young, aggrading stands usually retain more N due to greater N demand [10]. Even between stands of similar age, differences in species composition can lead to differences in N retention and loss [3,13–16]. As a result, gradual changes in species composition through time could also impact watershed N retention but are more challenging to study due to the need for long-term records.

Fortunately, there are long-term records of changes in both stream-water NO_3^- (since 1970) and the composition of tree species (since 1959) in a reference watershed (WS 4) at the Fernow Experimental Forest (FEF) in the central Appalachian Mountains of West Virginia. From 1975 to 1984, there was a 435% increase (1.3 to 6.9 kg N ha^{-1} yr^{-1}) in stream water NO_3^- export, and one assessment of 24 watersheds in the eastern United States found that WS 4 at the FEF had the lowest retention of inorganic N among those examined [17]. This relatively abrupt increase in stream water NO_3^- export along with other changes in stream water chemistry were likely symptoms of nitrogen saturation caused by long-term N deposition [18]. In addition, nearby measurements show a significant increase in the importance of *A. saccharum* through time [19], which is a species associated with high rates of NO_3^- production. The maintenance of high NO_3^- export from WS 4 despite a reduction in N deposition suggests that long-term changes within the watershed may be responsible, and that these changes may not be quickly reversed. Thus, long-term data sets for WS 4 afford the unique opportunity to assess the potential impact of changes in stand species composition on stream water NO_3^- loss and its potentially long-lasting effect on inorganic N retention.

Tree species composition could impact N retention due to interspecific differences in rate of total N uptake, and interspecific differences in their reliance on different forms of mineral N. Relatively slow-growing *Fagus* species, as well as coniferous species, tend to have lower rates of total N uptake, while other species, including *A. saccharum* and European *Fraxinus* and *Tilia* species, have higher rates of N uptake [20–23]. Therefore, should species with different N uptake requirements change in relative abundance, the overall stand demand for N could shift and alter watershed N retention.

Similarly, differences among species with respect to the mineral forms of N they prefer could also affect watershed N retention if the composition of tree species is altered. The relative uptake of different forms of N varies from species that rely mostly on NO_3^- [24], to species that prefer NH_4^+ [25–28], to species that change their preference to match the form that is most available [29,30]. More specifically, *A. saccharum* trees, which are often abundant in northeastern and Appalachian deciduous forests, may have a strong preference for NH_4^+ [21,31–34]. While many other trees also preferentially take up NH_4^+, some acquire most of their N as NO_3^- [22]. Indeed, seedlings of several species found in central Appalachian forests (*Fagus grandifolia*, *Tsuga canadensis*, *Quercus rubra*, and *Betula lenta*) either take up more NO_3^- than NH_4^+ [21], or grow better under NO_3^- additions [35]. Thus, both the total uptake of N and the variability in relative uptake of different mineral N forms by overstory trees could impact NO_3^- losses following shifts in stand species composition.

Given the variation between species in both total N uptake and relative utilization of different mineral forms, it is interesting that the importance of *A. saccharum* in the FEF has increased substantially over the past century [19]. Since this species appears to strongly prefer NH_4^+, a shift towards a greater influence of *A. saccharum* on the overall

community could partially explain the maintenance of stream water NO_3^- exhibited in FEF WS 4 despite recent reductions in N deposition, particularly if the species it replaces preferentially utilizes NO_3^-. In addition, *A. saccharum* in the FEF is associated with soils having higher NO_3^- production rates and higher soil water NO_3^- concentrations at the scale of individual trees, plots, and entire watersheds [15]. Thus, an increase in the relative importance of this species may not only diminish the demand for NO_3^- but also increase its supply. These combined effects indicate that shifts in species composition and stand NO_3^- utilization may contribute to the temporal trends observed in stream NO_3^- export from WS 4.

To assess whether changing tree species composition in WS 4 could reduce long-term watershed N retention, we took advantage of the relatively unique stand inventory and stream water chemistry data at the FEF by coupling these data with in situ measurements of NO_3^- versus NH_4^+ preference for the dominant, overstory tree species found at this location. This combination of data was then used to estimate total N uptake and temporal changes in stand composition in order to evaluate the hypothesis that changes in species composition at this site have contributed to elevated NO_3^- export in stream water.

2. Materials and Methods

2.1. Study Site

The focus of this study was a long-term reference watershed and a nearby untreated stand at the FEF. The reference watershed (WS 4) is 39 ha at an average elevation of 792 m and has a southeastern aspect. The predominant soil type is a Calvin channery silt loam (loamy-skeletal, mixed, mesic Typic Dystrochrept), and the average annual precipitation is ~145 cm [36]. The forest in WS 4—and the entire FEF—was heavily cut in approximately 1905–1910, and since that time the forest in WS 4 has been left uncut and untreated. WS 4 is dominated by temperate broadleaf trees, with *Quercus* spp., *Acer* spp., *Liriodendron tulipifera*, and *Prunus serotina* making up >75% of the tree stems. In this watershed, the forest canopy is closed along the drainage and there is no clear delineation separating the riparian zone from surrounding areas and no discernable difference in riparian vegetation compared to that of the surrounding slopes.

Continuous stream flow measurements for WS 4 began in 1951 [37], and weekly or bi-weekly stream water samples have been analyzed for their NO_3^- concentration since 1970 [36]. All precipitation and stream water chemistry variables were measured using EPA-approved protocols by the USDA Forest Service's Timber and Watershed Laboratory in Parsons, WV. The analyses and quality control measures are detailed by Edwards and Wood, 1993 [38]. From 1975 through 1984, NO_3^- export in stream water increased by 5.6 kg N ha^{-1} yr^{-1} (~435%); since that time, NO_3^- levels have remained elevated, with fairly regular ~ 5–10 year oscillations (Figure 1). Stream water NH_4^+ concentrations average ~0.05% of NO_3^- concentrations, and although dissolved organic N is not regularly measured in stream water at this site, one year of measurements in the 1995 show that ~87% of N export is as NO_3^-; thus, we focused on stream water NO_3^- export. Historically, the area has received high rates of N deposition (Figure 1), with total (wet + dry) deposition estimated to be ~10 kg N ha^{-1} year^{-1} from 1986 to 2002 [15].

2.2. Species Composition and Stand N Uptake

Complete inventories of all trees in WS 4, including the total number of live trees of all species in 2 inch diameter at breast height (DBH) categories, were completed by the US Forest Service in 1959, 1964, 1972, 1984, and 2001 [39]. To investigate changes in species composition, we calculated relative importance value (RIV) for each species in each inventory year as the average of its relative abundance (RA, the number of stems of that species divided by the total number of tree stems) and its relative basal area (RBA, the basal area of that species divided by the total tree basal area).
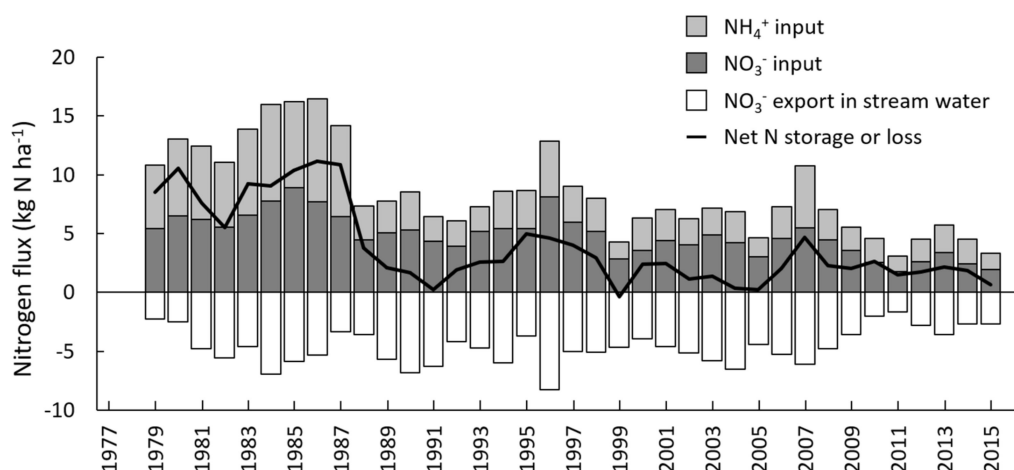
Figure 1. Annual NH_4^+ and NO_3^- inputs into and stream NO_3^- export from FEF WS 4, and the net N storage or loss from the catchment. Export of NH_4^+ in stream water is negligible (~0.05% of NO_3^- export).

We estimated the total N uptake by the trees in WS 4 as the sum of annual N storage in aboveground woody biomass and annual N return to the soil via litterfall. Complete forest inventory data (1959–2001) were used to estimate annual woody N storage, and since these were 100% live-tree inventories, tree death is accounted for in these measurements, and in our estimates. To determine the N concentration in aboveground woody tissue, trees greater than 8 cm in DBH were cored in 16 plots (10 m radius) spread evenly throughout WS 4 in the summer of 1998 (Christ and others 2002). Using these cores, the width of the last 5 growth rings was measured, and the wood within 1 cm of the bark was ground and analyzed for N concentration by Dumas combustion [40] using a Carlo Erba 1500 CNS elemental analyzer. The total aboveground woody biomass of each tree was estimated with FEF-specific allometric equations [41], and annual N storage was then calculated as the product of annual biomass increment and woody tissue N concentration. Using the DBH and annual N storage, a regression equation was built to estimate the annual woody N storage based on the DBH of any tree in the watershed ($R^2 = 0.790$):

$$\log(\text{annual woody N storage}) = -2.256 + 2.182 \log(\text{DBH}) + a$$

where a is a species-specific constant (Table A1) based on the average residual for each species (Christ and Peterjohn, unpublished data).

Total autumnal litter fall mass (~September through December) was collected annually beginning in 1988 by the US Forest Service using 25 litter traps throughout the watershed (0.7679 m^2 wooden frames with bottoms of ~0.625 × 0.625 cm-opening metal mesh). A relationship between autumnal litter fall and total stand basal area was created using the total basal area measured at 13 long-term growth plots in WS 4, and the total litter fall measured in 1989, 1994, 1999, and 2009 ($R^2 = 0.887$). Using this relationship, we estimated total litter fall for the years of stand inventories prior to the start of the collection of litterfall data (1959, 1964, 1972, and 1984). We then estimated each species' litter N returns for all inventory years using the relationships between a tree species' RBA and the species-specific litterfall N contents at 16 plots in 1998 [13].

2.3. ^{15}N Labeling

To avoid affecting the δ^{15}N of materials in the long-term reference watershed, we used a "test area" located in a nearby untreated area of the FEF (<1 km from WS 4) to measure the relative uptake of NO_3^- versus NH_4^+. This area has a similar elevation, slope, and tree composition to WS 4, and an east-northeasterly aspect. Unlike WS 4, small (0.2-ha) plots in this portion of the FEF were harvested to selected basal areas in the 1980s. However,

for this study we selected trees within an area showing no signs of harvest, and the trees selected were of similar size to those in WS 4.

At our "test area" in early July 2014, we conducted a ^{15}N-labeling experiment similar to one by performed by McKane et al. [42] to determine the relative uptake of NH_4^+ vs. NO_3^- for 6 major tree species at the FEF: *A. rubrum, A. saccharum, B. lenta, L. tulipifera, Q. rubra,* and *P. serotina*. We used the holes in pieces of commercial peg board (625 cm^2 each, with 10 rows $\times$ 10 columns of holes spaced 2.54 cm apart) to evenly space injections of 3.5 mM ^{15}N as $K^{15}NO_3$ in one area (1 mL per hole), and 3.5 mM ^{15}N as $^{15}NH_4Cl$ in another area under the canopy (within ~3 m of the trunk) of five mature trees of each species. The solutions were injected midday at approximately the boundary between organic and mineral soil horizons—a depth of ~3 cm—using a syringe needle with four side ports. Based on the soil NH_4^+ and NO_3^- concentrations, we estimate that this injection increased background N concentrations by 10% and 5%, respectively. After three hours, we harvested fine roots (<2 mm diameter) from a depth of ~3 cm at each injection site, and roots from one unlabeled area under each tree to measure the natural ^{15}N abundance of root tissue. The sampled roots were traced as far as possible towards the target canopy tree, and we compared the morphology of the collected roots to the fine roots of nearby seedlings of the same species. All species had distinct root characteristics except the two *Acer* species. Thus, we selected *A. saccharum* and *A. rubrum* trees that had no nearby *Acer* spp. within ~15 m.

All harvested roots were placed on ice and transported to the lab, where they were soaked in 1 M CaSO$_4$ for 1 min to remove unassimilated N from the Donnan free space [43]. They were then dried at 65 °C for 48 h and ground to a fine powder in a dental amalgamator (Henry Schein, Inc., Melville, NY, USA). Approximately 5 mg of each sample was wrapped in tin capsules and analyzed for δ^{15}N via isotope ratio gas chromatography–mass spectrometry at the Central Appalachian Stable Isotope Facility that is part of the University of Maryland Center for Environmental Science Appalachian Laboratory (Frostburg, MD, USA).

We calculated root uptake of ^{15}N from the labeled N pool as described in Burnham and others [44]. We first converted δ^{15}N values to the fraction of the heavy isotope in the sample (F) using the ^{15}N/^{14}N ratio in each sample (R_{sample}) [45]:

$$R_{sample} = \left(\left(\frac{\delta^{15}N}{1000} \right) * R_{std} \right) + R_{std}$$

$$F = \frac{R_{sample}}{1 + R_{sample}}$$

where $R_{std} = {}^{15}$N/^{14}N ratio in atmospheric N$_2$ (0.0036764). Using the root tissue N content and F, we calculated the µmol ^{15}N g^{-1} root, and then estimated the rate of ^{15}N uptake from the ^{15}N-labeled pools by dividing the ^{15}N excess (^{15}N content of labeled—unlabeled roots from the same tree) by the exposure time (3 h). Finally, we calculated total uptake of ^{15}N label ($^{15}NH_4^+ + {}^{15}NO_3^-$) and the percent that was taken up as NH_4^+ and NO_3^-.

2.4. Data Analysis

Our overall ^{15}N label study design included six species, and five trees per species, with a measurement of NO_3^- vs. NH_4^+ uptake associated with each tree. We used a nested ANOVA with Tukey's HSD post hoc test ($\alpha = 0.05$) to determine if the percent of total N taken up as NO_3^- varied by species. The model included the effect of tree nested within species. We then performed one-tailed *t*-tests to determine if the contribution of NO_3^- to total uptake of N from the labeled pool was greater than 50%, which would indicate a significant preference of NO_3^- over NH_4^+.

We used the error terms in our plot-level RBA vs. leaf litter N return and BA vs. woody N storage models to run a Monte Carlo simulation to estimate the uncertainty in our total stand N uptake calculations. For this simulation, we assumed errors were normally distributed and randomly sampled 100 times from the error distribution, and we

report uncertainty estimates in woody N storage, litter N return, and total N uptake are reported as 95% confidence intervals.

3. Results

From 1959 to 2001, total stand density in WS 4 decreased 18% (from 372 to 305 trees ha^{-1}) and total stand basal area increased 45% (from 24.3 to 35.2 m^2 ha^{-1}). In 2001, eight species accounted for ~85% of the stand composition (84.6% of stems and 85.8% of basal area): *Quercus rubra*, *Q. prinus*, *Acer saccharum*, *A. rubrum*, *Liridendron tulipifera*, *Prunus serotina*, *Betula lenta*, and *Fagus grandifolia*. Over the study period, five of these species increased in RIV, and three decreased (Figure 2). The RIVs of *A. saccharum* and *A. rubrum* increased 5.8 and 8.5%, respectively, the most of any species. While the RIV of *A. saccharum* increased, its relative basal area decreased slightly (1.4%) and the number of stems increased substantially (from 8.9% to 21.9%) throughout the period examined. The RIV of *Q. rubra* increased to a more modest degree (2.9%), with its relative basal area increasing from 22.6% to 32.3% and its relative abundance decreasing from 20.4% to 16.7% throughout the study period. The RIV of *Q. prinus*, *B. lenta*, and *F. grandifolia* all declined through the study period (Figure 2). The RIV of *Q. prinus* fell from 6.8% to 5.6%, and the RIV of *B. lenta* fell from 6.9% to 3.8%. While there was only a slight decline in the RIV of *F. grandifolia*, from 4.1% to 3.7%, its relative basal area fell from 5.4% of the stand to 3.4%, but its relative abundance increased from 2.8% to 4.0%.

Aboveground woody N storage increased from 6.4 (6.1–6.7 95% CI) to 9.8 (9.2–10.4) kg N ha^{-1} yr^{-1} (+53.5%) and litter N return increased from 24.5 (19.3–29.7) to 25.4 (21.8–29.0) kg N ha^{-1} yr^{-1} (+3.5%) over this period. In total, stand N uptake increased from 30.9 (25.7–36.1) kg N ha^{-1} yr^{-1} in 1959 to 35.2 (31.7–38.7) kg N ha^{-1} yr^{-1} in 2001 (+13.8%). The percent of mineral N uptake as NO$_3^-$ ranged from 52.7% (*L. tulipifera*) to 75.3% (*A. rubrum*) but was not significantly different between species (Table 1). When these rates of NO$_3^-$ vs. NH$_4^+$ uptake were applied to the estimates of total N uptake within the watershed, NO$_3^-$ uptake increased from 18.7 to 21.4 kg N ha^{-1} yr^{-1} (14.5%), and NH$_4^+$ uptake increased from 12.2 to 13.8 kg N ha^{-1} yr^{-1} (12.8%) from 1959 to 2001. The percent of total stand uptake of N taken up as NO$_3^-$ thus increased only 0.4%.

Table 1. The percent of total uptake of mineral N as NO$_3^-$ for six major overstory trees at the FEF, measured in situ using ^{15}N-labeled NO$_3^-$ and NH$_4^+$.

Species	Percent of N Uptake as NO$_3^-$ ($\pm$SE)
A. rubrum	75.3 ($\pm$12.5)
A. saccharum	53.6 ($\pm$16.0)
B. lenta	54.7 ($\pm$11.5)
L. tulipifera	52.7 ($\pm$13.0)
P. serotina	61.6 ($\pm$11.3)
Q. rubra	56.4 ($\pm$11.5)

Prior studies, using other methods and some using more sampling dates, found much lower rates of N uptake as NO$_3^-$ by *A. saccharum* (average of 15.8%, vs. 53.6% in this study) (Table 2). Given the range of values reported for the affinity of *A. saccharum* for NO$_3^-$, we assessed the potential impact that changes in this particular species might have on stand uptake of NO$_3^-$ by considering two scenarios. First, we used the average relative contribution of NO$_3^-$ to tree uptake of N (15.8%) reported in previous studies. Second, we used the average of all available estimates of NO$_3^-$ uptake by *A. saccharum*, which raised the average to 23.4%. In both scenarios, to estimate stand uptake of NO$_3^-$ we used the average of our measured values of NO$_3^-$ uptake for all unmeasured species. For the first scenario, when values from previous studies were applied to the estimates of total N uptake within WS 4 at the FEF, NO$_3^-$ uptake increased from 17.5 to 20.3 kg N ha^{-1} yr^{-1} (2.8%) and NH$_4^+$ uptake increased from 13.4 to 14.9 kg N ha^{-1} yr^{-1} (1.5%) from 1959 to 2001. Under this scenario, the percent of total stand uptake of N as NO$_3^-$ increased slightly,

from 56.7% to 57.7% (Figure 3). For the second scenario, using all available estimates of NO_3^- uptake, the stand uptake of NO_3^- increased from 17.6 to 20.5 kg N ha^{-1} yr^{-1} (2.6%) and uptake of NH_4^+ increased from 13.1 to 14.7 kg N ha^{-1} yr^{-1} (1.5%) from 1959 to 2001. In addition, the percent of total stand uptake of N as NO_3^- increased slightly 57.4% to 58.3% (Figure 3). Thus, in neither of the two scenarios did the observed change in the importance of *A. saccharum* reduce the absolute amount NO_3^- uptake, and in only one scenario was the relative amount of NO_3^- uptake reduced—but this apparent reduction was extremely small.
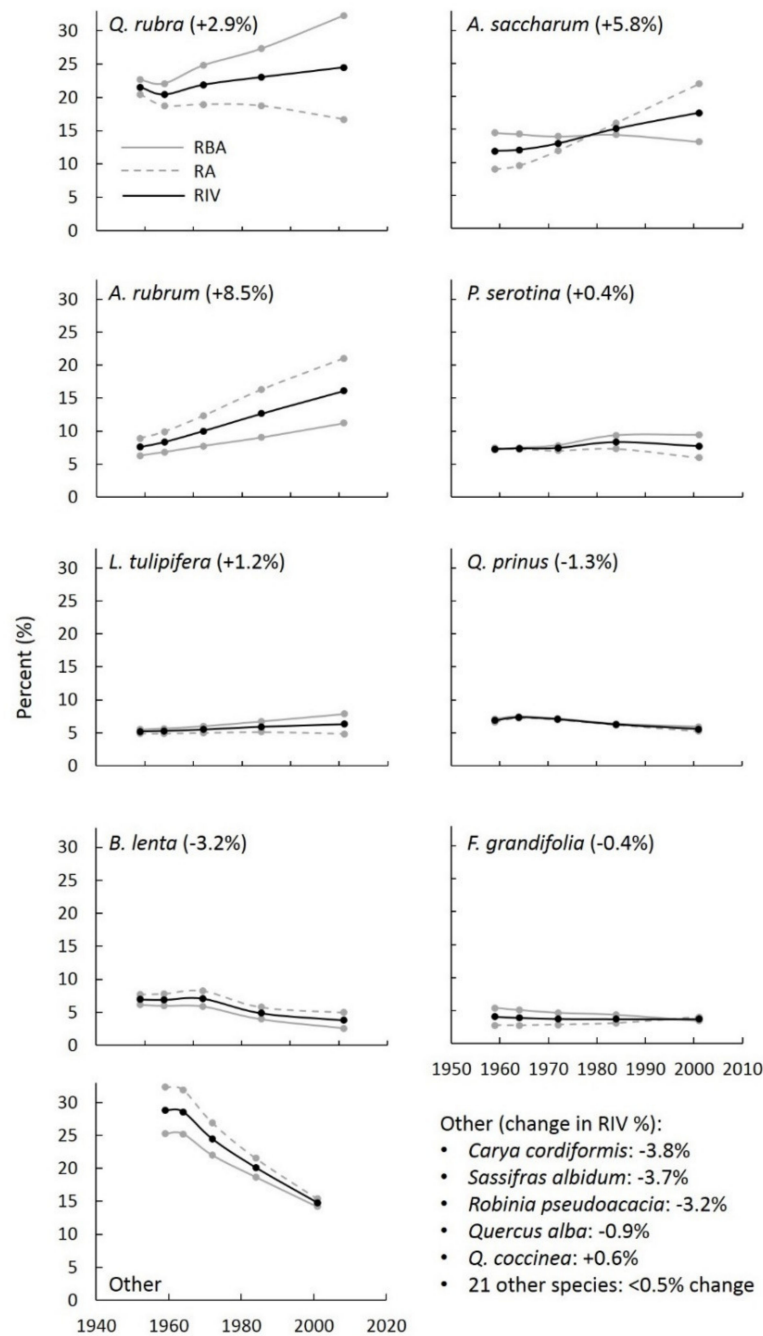

Figure 2. Tree species' relative importance, abundance, and basal area (%) in FEF WS 4 from 1959 to 2001. The percent changes for species listed under "other" are changes in RIV. Data from the USDA Forest Service Northern Research Station [39].

Table 2. All available estimates of the percent of total uptake of mineral N as NO_3^- and estimated N uptake rates (μmol NO_3^--N g dry root^{-1} hr^{-1}) for *A. saccharum*. Measurement methods and parameters varied by study.

Study	Method	*A. saccharum* N Uptake as NO_3^- (%)	Estimated Uptake Rate (μmol N g^{-1} h^{-1})
BassiriRad et al. (1999)	In situ N depletion, excavated intact roots, V_{max}	31	9
Eddy et al. (2008)	Excised root ^{15}N uptake, V_{max}	11.2	0.63
Rothstein et al. (1996)	Excised root ^{15}N uptake, V_{max}	3	1.0
Templer and Dawson (2004)	^{15}N addition to seedlings, greenhouse, roots in native soil	18	1.0 [1]
This study	In situ ^{15}N addition to mature trees, roots left in native soil	53.6	11.6 [2]

[1] Estimated using the reported values of root biomass, total plant biomass, and N uptake per total plant biomass. [2] Estimated assuming that the soil ^{15}N atom percent after labeling was similar to that of the root after 3 h of uptake.
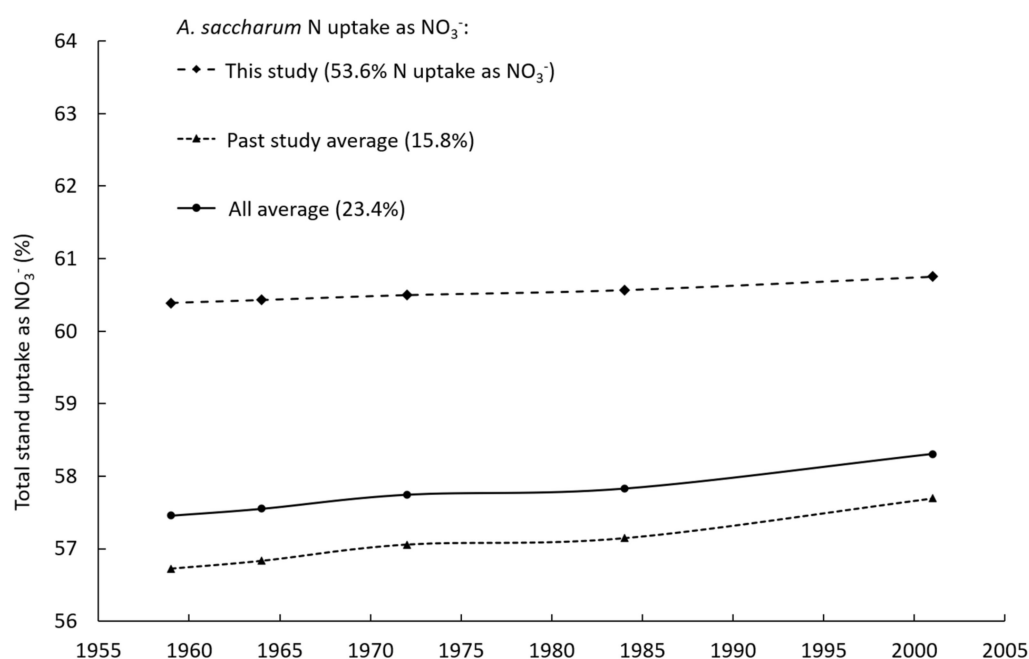

Figure 3. The contribution of NO_3^- to total stand uptake of N from 1959 to 2001. Different lines represent different estimates of uptake of N as NO_3^- for *A. saccharum*, based on prior studies, this study, and the average of all available rates.

4. Discussion

Unexpectedly, the tree species we considered did not differ in their relative uptake of NH_4^+ and NO_3^- and utilized significant amounts of both forms in their mineral N nutrition. This is surprising because prior studies found large differences in the relative uptake of N as NH_4^+ vs. NO_3^- for temperate forest species [21,22]. Notably, in past studies, *A. saccharum* trees took up substantially less NO_3^- than we found using an in situ ^{15}N-labeling technique (Table 2) [21,32–34], and it seems likely that methodological differences could account for the higher relative NO_3^- uptake in this study [23]. Most of the prior research on the form of mineral N uptake utilized seedlings [21], hydroponic techniques [27,46], or N depletion in a simulated soil solution [20,27]—techniques that do not account for some aspects of in situ soil N dynamics. Perhaps most importantly, the differential diffusional resistances of NH_4^+ and NO_3^- in soils [47] are not represented in hydroponic and simulated soil solution techniques. It is possible that tree preferences for NH_4^+ vs. NO_3^- are dynamic through time, particularly as the rate of N deposition changes. However, the relative contributions of NH_4^+ and NO_3^- to total N deposition have not

changed substantially (Figure 1), and we therefore believe that large changes in tree N form preference due to changing relative availability of the two mineral N forms is unlikely. Thus, assuming that our ^{15}N-labeling experiment is representative of the long-term mineral N form preference of these tree species, NO_3^- may contribute more to N nutrition of trees than previously thought due to the greater rates of transfer of NO_3^- to roots in the soil.

Since the species examined did not differ in their relative contribution of NO_3^- to total N uptake, it seems unlikely that changes in stand composition contributed to the relatively rapid increase in NO_3^- export or to the long-term persistence of low N retention via a reduction in the demand by trees for NO_3^-. Furthermore, since the stand N demand may have increased over the second half of the last century, it may have contributed to the gradual and slight decrease in soil and stream water NO_3^- since the early 1980s [48]. Although a forest inventory has not conducted after 2001, there have been no major changes in the stand or significant disturbances in this time. We speculate that the reduction in stream water NO_3^- concentration circa 2010 resulted from decreasing N deposition with a significant lag after this decline in deposition started in the early-1990s. Thus, it appears that the large increase observed in NO_3^- export from WS 4 in approximately 1980 resulted from an enhanced supply of available NO_3^- via deposition, and the long-term trend in stream water NO_3^- is controlled primarily by atmospheric N inputs with a lag in recovery as inputs decline.

Although changes in stand NO_3^- demand do not seem to account for the increase in NO_3^- export in stream water, shifts in stand composition could still affect NO_3^- production in the soil and thus contribute to a lag in the recovery of stream water NO_3^- export after deposition declines. At several locations in the eastern U.S., *A. saccharum* trees are associated with high rates of soil net nitrification and low soil C:N ratios [16,31,32,49,50], including WS 4 and other locations in the FEF [13,15], and nitrification rates are positively associated with stream NO_3^- export [51]. The relationship between *A. saccharum* abundance and nitrification is driven, in part, by relatively labile litter and low N residence time [15,52]. To make an initial assessment of the potential impact of species shifts on soil NO_3^- production and stream water NO_3^- export, we used previous plot-level measurements of net nitrification potential and the relative importance and relative basal area of tree species in WS 4. We estimated that net nitrification potential increases 0.02 kg ha^{-1} day^{-1} for every 1% increase in *A. saccharum* importance value (R^2 = 0.45) and decreases 0.017 kg ha^{-1} day^{-1} for every 1% increase in *A. rubrum* importance value (R^2 = 0.13) [14]. Similarly, net nitrification potential increases 0.017 kg ha^{-1} day^{-1} for every 1% increase in *A. saccharum* relative basal area (R^2 = 0.20) and decreases 0.016 kg ha^{-1} day^{-1} for every 1% increase in *A. rubrum* relative basal area (R^2 = 0.12) When analyzed in the same manner, no other species was associated with significant changes in net nitrification potential. Since *A. saccharum* and *A. rubrum* had large changes in relative importance value and basal area from 1959 through 2001, and have opposite associations with net nitrification potential, we assessed their potential impact on soil NO_3^- supply and NO_3^- loss to stream water. To arrive at an annual estimate, we assumed that: (1) the estimated daily rate of change in net nitrification potential applied during the months of May through August; (2) only 50% of the estimated daily rate occurred during March, April, and September through November, when the rate of nitrification is lower [53]; and (3) the species change had no effect on net nitrification potential during the months of December through February, when very little nitrification takes place.

The decline in *A. saccharum* and increase in *A. rubrum* relative basal area in WS4 suggest that nitrate production via nitrification was 19.4 kg N ha^{-1} yr^{-1} lower in 2001 than in 1959. However, our plot-level data show a stronger relationship between relative importance value and nitrification potential. Furthermore, past studies have detected a strong relationship between soil NO_3^- concentration and *A. saccharum* abundance [31,54]. Thus, using the relationship between these species' relative importance values and nitrification potential, our initial approximation suggests that the effects of *A. saccharum* and *A. rubrum* on soil NO_3^- production from 1959 to 2001 mostly offset each other, with the

negative effect of *A. rubrum* on nitrification causing a net decrease in the rate of NO_3^- production of 2.6 kg NO_3^--N ha^{-1} yr^{-1} within WS 4. However, the majority of the increase observed in the importance of *A. rubrum* occurred in a silvicultural compartment of the watershed (compartment WS 4c) that produces very little NO_3^- in the soil, and that has very low NO_3^- concentrations in soil water collected by tension-free lysimeters [55]. Thus, it is unlikely that this region of the WS 4 contributed to the observed patterns in stream NO_3^- export. Additionally, this subcompartment contains no *A. saccharum* trees, so the increased importance of this species only occurred in the portions of the watershed where nitrification and soil solution NO_3^- levels are currently much higher [13,55]. Although it is unclear why *A. saccharum* has increased in importance at this site, we believe that this is a long-term successional change due to the decline of other subcanopy species.

Considering these known spatial patterns in NO_3^- availability, we refined our initial assessment to ~86% of WS 4 by excluding compartment WS 4c where NO_3^- availability is very low. Taking this approach, we estimate that the net effect of changes in the importance of *A. saccharum* and *A. rubrum* was to increase soil NO_3^- production by 3.9 kg NO_3^--N ha^{-1} yr^{-1} from 1959 through 2001. The long-term change in species composition resulted in a 2.7 kg N ha^{-1} yr^{-1} increase in NO_3^- demand, which mostly offsets the estimated increase in soil NO_3^- production. Thus, we estimate that a net increase of 1.2 kg NO_3^--N ha^{-1} yr^{-1} was available for leaching into stream water. Consequently, it seems that patterns of NO_3^- export were primarily driven by long-term changes in N deposition, but changes in tree species composition may have contributed an increase in soil NO_3^- production and thus to a lag in the recovery of stream water NO_3^- export, which remained 3.5 kg N ha^{-1} yr^{-1} higher from 1992 to 2001 (~5.0 kg NO_3^--N ha^{-1} yr^{-1}) (Figure 1) than the export that occurred from 1970 to 1979 (~ 1.5 kg NO_3^--N ha^{-1} yr^{-1}).

This first-order estimate illustrates that understanding the effect of N deposition on the temporal dynamics of stream water NO_3^- loss requires a relatively complete understanding of how changes in forest species composition can influence the balance between nutrient supply and demand. Moreover, the spatial patterning of N supply and demand within a watershed and connectivity to stream discharge and N export may also be important. We suggest that the recent reductions in atmospheric inputs of N in the eastern US may result in a delayed return of stream water NO_3^- losses to "baseline" levels in situations where a long-lasting shift in the composition of tree species changes the inherent rates of soil NO_3^- production and biotic NO_3^- demand.

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Data Availability Statement: Relevant data can be accessed in a GitHub repository at https://github.com/markbburnham/Forests-Species-Nitrate-Appalachia (accessed on 3 June 2021).

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Species-specific constants (α) used in woody N storage estimation.

Species	A
Acer rubrum	0.149
Acer saccharum	−0.097
Fagus grandifolia	0.301
Liriodendron tulipifera	0.338
Magnolia acuminata	0.276
Nyssa sylvatica	−0.220
Oxydendrum arboreum	0.187
Prunus serotina	0.222
Quercus alba	−0.041
Quercus coccinea	0.304
Quercus prinus	0.440
Quercus rubra	0.327
Tilia americana	0.000
Other species	0.168

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