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HIGHLIGHTED STUDENT RESEARCH

Soluble soil aluminum alters the relative uptake of mineral nitrogen forms by six mature temperate broadleaf tree species: possible implications for watershed nitrate retention

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Abstract Increased availability of monomeric aluminum (Al^{3+}) in forest soils is an important adverse effect of acidic deposition that reduces root growth and inhibits nutrient uptake. There is evidence that Al^{3+} exposure interferes with NO_3^- uptake. If true for overstory trees, the reduction in stand demand for NO_3^- could increase NO_3^- discharge in stream water. These effects may also differ between species that tolerate different levels of soil acidity. To examine these ideas, we measured changes in relative uptake of NO_3^- and NH_4^+ by six tree species in situ under increased soil Al^{3+} using a ^{15}N-labeling technique, and measured soluble soil Al levels in a separate whole-watershed acidification experiment in the Fernow Experimental Forest (WV). When exposed to added Al^{3+}, the proportion of inorganic N acquired as NO_3^- dropped 14% across species, but we did not detect a reduction in overall N uptake, nor did tree species differ in this response. In the long-term acidification experiment, we found that soluble soil Al was mostly in the free Al^{3+} form, and the concentration of Al^{3+} was ~65 µM higher (~250%) in the mineral soil of the acidified watershed vs. an untreated watershed. Thus, increased levels of soil Al^{3+} under acidic deposition cause a reduction in uptake of NO_3^- by mature trees. When our ^{15}N uptake results were applied to the watershed acidification experiment, they suggest that increased Al^{3+} exposure could reduce tree uptake of NO_3^- by 7.73 kg N ha^{-1} $year^{-1}$, and thus increase watershed NO_3^- discharge.

Keywords Acid deposition · Nitrogen cycle · Nitrogen export · Tree nutrition · ^{15}N tracer

Introduction

The eastern US has a history of elevated acid deposition. Emissions of SO_2 and NO_x from the combustion of fossil fuels in power plants in the Ohio River Basin and automobiles throughout the region have caused acidic deposition and elevated inputs of nitrogen (N) and sulfur (S) during the late-20th and early 21st centuries (Driscoll et al. 2001; Galloway et al. 2004). The increased deposition of these materials onto downwind ecosystems can increase soil acidity, especially in poorly buffered soils, and lead to a variety of adverse effects (Lovett et al. 2009). These effects include loss of base cations (i.e., Ca, Mg, etc.), altered plant mineral nutrition, reduced root growth, and reduced forest productivity. Through time, elevated supply of N could also exceed forest N demand and cause N saturation (Aber et al. 1998). Thus, acid deposition has the potential to significantly impact the biogeochemistry of temperate forest ecosystems through soil acidification and N saturation.

An increase in soil acidity typically causes higher solubility of monomeric aluminum (Al^{3+}) (de Vries et al. 2003). We define monomeric aluminum as Al^{3+}, but other studies sometimes include different inorganic complexes in surface soils, such as various oxides of Al. Some discrepancy in

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We found that soil solution Al reduces the uptake of NO_3 by mature trees. This effect and the impact on watershed NO_3 export are novel findings important in areas impacted by acid deposition.

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plant responses to Al between studies could be caused, in part, by measurements of different forms of aluminum. We focused on Al^{3+} because of its increase in concentration at low pH and severe impact on plant roots. Root growth is severely reduced when exposed to Al^{3+} in solution (Delhaize and Ryan 1995; Poschenrieder et al. 2009), and while this alone can inhibit plant development, Al^{3+} also has a number of secondary effects on plant roots, including reduced water and nutrient uptake (Kochian 1995). The effects of Al^{3+} on plants have been studied extensively in the lab, and particularly on herbaceous plants and tree seedlings. However, its impact on plant growth in field conditions can be much more variable than in the lab. Al has relatively complex dissolution reactions in the soil that are dependent on the soil composition. Buffering by base cation release (i.e., calcium) (Monterroso et al. 1999; de Vries et al. 2003) and the formation of Al complexes with organic acids (Mulder and Stein 1994; Brumme et al. 2009) may lead to varying levels of free Al^{3+} species, and diverse effects, across a landscape (de Vries et al. 2003; Li and Johnson 2016). For example, Rosenberg and Butcher (2010) found no correlation between foliar and $BaCl_2$-extractable soil Al concentration for red spruce in acid forest soils. In addition, de Wit et al. (2010) found that 7 years of $AlCl_3$ addition to a Norway spruce forest did not impede root growth as seen in lab studies with seedlings of other tree species (e.g., Lux and Cumming 1999), but the additions did reduce foliar magnesium (Mg) concentration. Therefore, while soluble Al^{3+} in soil may not affect the growth of mature trees in the field to the degree suggested by laboratory studies, other aspects of their function may be altered, such as mineral nutrition.

Because of these potential negative effects, many plants reduce their exposure to Al^{3+} by altering the Al species present in the rhizosphere. When soil Al^{3+} increases, plant roots exude organic acids, such as citrate and malate, which chelate free Al^{3+} and reduce negative growth and nutritional effects (Delhaize and Ryan 1995; Kochian 1995). Thus, while bulk soil Al^{3+} may increase under acid deposition, its effect would be lower in the rhizosphere of Al-tolerant plants that exude chelating organic acids. This further complicates the potential biogeochemical effect of acid deposition-induced Al^{3+} solubility, and therefore, it is necessary to measure the chelation of Al^{3+} in rhizosphere and bulk soil to adequately assess its impact on stand-scale growth and nutrient cycling.

When soluble Al^{3+} increases in the soil, several negative effects on plants could translate to changes in N demand and thus an impact on the N biogeochemistry of forest catchments. Should soil Al^{3+} rise to a level that reduces plant growth, overall N uptake by vegetation would be reduced, leading to elevated stream water N export. Even in the absence of a reduction in growth, the presence of soluble Al^{3+} can impede other aspects of tree nutrition that may alter N demand (de Wit et al. 2010). In particular, Al^{3+} exposure can reduce NO_3^- uptake by plants (Jarvis and Hatch 1986; Durieux et al. 1993; Calba and Jaillard 1997; Watanabe et al. 1998; Jerzykiewicz 2001; Pal'ove-Balang and Mistrík 2007; Zhou et al. 2016). While the exact mechanism is not established, soluble soil Al^{3+} can interfere with cell membrane H^+-ATPase activity, reducing the cell's capacity to pump out H^+ (Zhou et al. 2016). This would strongly reduce the cell's ability to transport NO_3^- across the cell membrane, since NO_3^- cotransporters require $2H^+$ per NO_3^- moved (Britto and Kronzucker 2005). Thus, the result could be a shift in relative uptake of mineral N forms, towards greater uptake of NH_4^+ and reduced uptake of NO_3^- (Cumming 1990). Since NO_3^- is highly mobile in soils, any reduction in the uptake of NO_3^- induced by higher levels of Al^{3+} has the potential to increase stream water NO_3^- export.

Increased N supply by acid deposition could cause elevated NO_3^- in stream water due to N saturation, and an Al^{3+}-mediated decrease in stand NO_3^- demand would compound this effect. In a long-term, whole-watershed fertilization/acidification experiment at the Fernow Experimental Forest, N added as $(NH_4)_2SO_4$ has caused a persistent reduction in the pH, and increase in the stream water NO_3^- concentration and discharge (Fig. 1; Adams et al. 1997; Edwards et al. 2006). While an initial increase in net nitrification was measured in the fertilized watershed relative to the reference watershed, more recent in situ and lab estimates of net nitrification rates in the upper 10 cm of mineral soil, collected at 100 points within each watershed, were unable to detect any difference in the net rate of NO_3^- production (Gilliam and Peterjohn, unpublished data), despite the persistence of elevated NO_3^- concentration in stream water leaving the fertilized watershed (Fig. 1). This suggests that elevated NO_3^- loss from the fertilized/acidified watershed may be influenced by a decrease in NO_3^- demand, potentially due to elevated Al^{3+} in the soil under acidified conditions. Therefore, the main objectives of this study were to determine if tree roots are exposed to higher levels of free, unchelated Al^{3+} under experimental soil acidification, if this exposure could change the relative uptake of different forms of mineral N by important tree species in situ, and to provide an initial assessment of the potential impact that any such change might have on stream water NO_3^- export from a forested watershed. We hypothesized that (1) an increase in tree root exposure to soluble Al^{3+} would shift the relative uptake of mineral N away from NO_3^- and towards NH_4^+ due to the hindrance of NO_3^- uptake pathways, (2) that species would vary in their sensitivity, with species that are more tolerant of acidic soils, such as *Acer rubrum* and *Quercus rubra*, being less affected by increased levels of soluble Al^{3+}, and (3) that soil acidification causes levels of

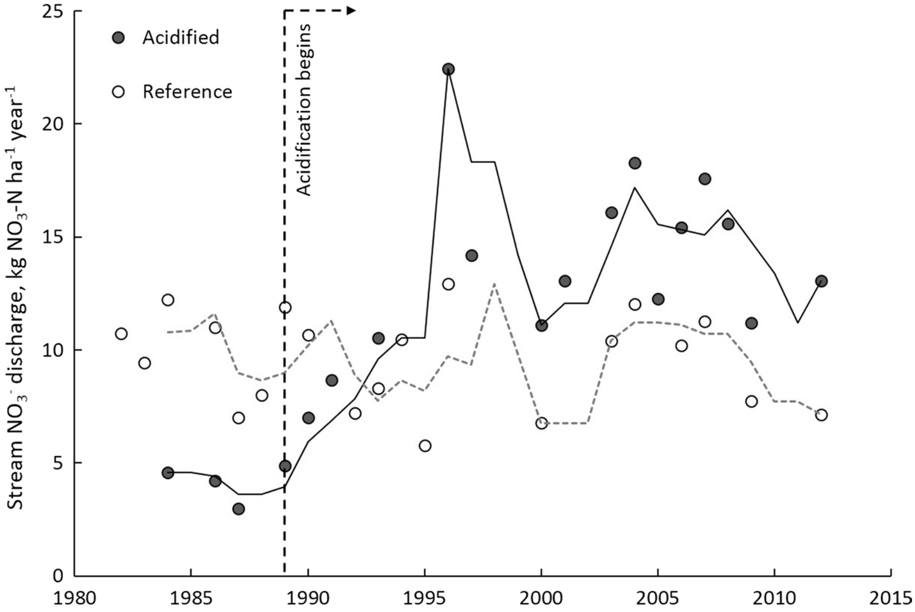

Fig. 1 Annual stream water NO_3^- discharge from the acidified (WS3) and reference (WS7) watersheds. Vertical dashed line indicates the start of the annual addition of 35 kg N ha^{-1} $year^{-1}$ as $(NH_4)_2SO_4$ to the acidified watershed. Three-year running averages are displayed to better visualize temporal trends in the data. Only years with values for all months were included for a given watershed

soluble Al^{3+} that have the potential to elevate stream water NO_3^- discharge from a watershed if N uptake by most of the species present were Al sensitive.

Materials and methods

Site description

This research was conducted in the Fernow Experimental Forest (FEF) in Tucker County, WV, USA. This site is a mixed hardwood forest, and the soil is primarily a Calvin channery silt loam (loamy-skeletal, mixed, mesic Typic Dystrochrept). Elevation ranges from 762 to 854 m, and average annual precipitation totals ~145 cm (Kochenderfer 2006). To test if Al^{3+} affects the relative uptake of NO_3^- vs. NH_4^+, we used an area of the FEF with no assigned long-term treatment, to avoid affecting the $\delta^{15}N$ of the experimental areas. The area was last used in the 1980s when 0.2 ha plots were harvested to varying levels of basal area. However, we selected mature canopy trees that were similarly sized to those in the nearby acidified watershed (<1 km away), and we avoided areas with signs of harvest. To assess the potential effects of acidification on plant available Al in the soil, we used the long-term watershed acidification experiment at the FEF. This is a paired watershed experiment consisting of two adjacent watersheds—an acidified 34-ha watershed (WS 3, 1883 tree stems ha^{-1}) and a similarly aged, 24-ha reference watershed (WS 7, 1473 tree stems ha^{-1}) (Kochenderfer 2006). The forest on the acidified watershed is currently dominated by *Prunus serotina* (52% of the total basal area), *A. rubrum* (10.9%), *Betula lenta* (7.2%), and *Liriodendron tulipifera* (6.4%). In 1969–1970, the watershed was clearcut, and then allowed to naturally regrow thereafter. To experimentally acidify the soils in WS 3, 35 kg N ha^{-1} $year^{-1}$ of $(NH_4)_2SO_4$ have been aerially applied in three doses per year since 1989. The reference watershed (WS 7) is currently dominated by *P. serotina* (29.4% of the total basal area), *B. lenta* (19.1%), *L. tulipifera* (17.9%), *Acer saccharum* (11.3%), *A. rubrum* (6%), and *Q. rubra* (4%). This watershed was clearcut between 1963–1964 and 1966–1967 (lower half, then upper half) (Patric and Reinhart 1971). The reference watershed has never received additions of $(NH_4)_2SO_4$. In 2011, after 21 years of treatment, the pH of the top 10 cm of mineral soil was significantly lower in the acidified watershed than in the reference watershed (pH 4.2 vs. 4.6), and the extractable soil Al (extracted with 1 N ammonium acetate) was significantly higher in the acidified watershed than in the reference watershed (0.45 ± 0.03 vs. 0.32 ± 0.01 meq 100 g^{-1}; Peterjohn, unpublished data).

Relative uptake of NO_3^- and NH_4^+

In the early July of 2014, we used an in situ ^{15}N-labeling method to determine the relative uptake of NO_3^- and NH_4^+ by mature overstory trees (McKane et al. 2002; Andresen and Michelsen 2005). NO_3^- and NH_4^+ pools under canopy trees were labeled with sub-fertilization amounts of either $^{15}NH_4Cl$ or $K^{15}NO_3$. Five canopy trees of six important species found in WS 3 and WS 7 (*A. saccharum, A. rubrum, B. lenta, L. tulipifera, P. serotina,* and *Q. rubra*) were selected from an area adjacent to the experimental watersheds in the FEF to avoid labeling the natural ^{15}N pool in the soils of the long-term experimental areas. Under each tree's canopy, and within 4 m of the stem, four 625-cm^2 plots were used for the injection of labeled N solutions. One of the four solutions was applied to each plot: (1) $^{15}NH_4Cl$; (2) $^{15}NH_4Cl + Al^{3+}$; (3) $K^{15}NO_3$; and (4) $K^{15}NO_3 + Al^{3+}$. The N concentrations in each treatment solution were 3.5 mM. The past measurements of lysimeter soil water Al^{3+} from the acidified watershed yielded concentrations from zero to nearly 600 μM (Lux 1999). We used 600 μM Al^{3+} in our treatment solutions assess the potential of Al^{3+} to impact tree N form uptake. Since some added Al^{3+} would rapidly associate with exchange sites on soil particles, the resulting Al^{3+} concentration in solution was in the range of measured lysimeter values, up to 600 μM. Al^{3+} was added as $Al_2(SO_4)_3$, and all solutions were acidified to pH 4.0–4.5 using HCl, to best match the soil pH. Each plot consisted of a 100-hole grid frame (10 × 10 hole commercial pegboard and 2.54 cm between holes) laid on the ground to guide the injection of labeled N solutions. At each hole, 1 mL of N solution was injected at a depth of 3 cm (approximately the top of the A horizon) using a side-port syringe needle for a total of 52.5 mg ^{15}N added to each plot.

After 3 h, a sample of fine roots (<2 mm diameter) of the nearby canopy tree was removed from a depth of ~3 cm. In addition to the ^{15}N-labeled plots, we collected roots from an unlabeled area around each tree for measurement of root ^{15}N natural abundance. To maximize our confidence that the roots were from the intended tree, the roots were traced as far as possible towards the canopy tree. In addition, we compared the morphology of the collected roots to the fine roots of nearby seedlings of the same species. Four of the species had distinct root characteristics; however, the roots of the two *Acer* spp. were very similar. Thus, we selected *A. saccharum* trees that had no nearby *A. rubrum* trees within ~15 m, and vice versa. We placed all collected roots on ice and transported them to the lab, where they were immediately placed in 1 M $CaSO_4$ for 1 min to remove unassimilated nutrients from the Donnan free space (Thornton et al. 2007). This was done to isolate the signal to N that had been transported across a cell membrane, and remove N that was passively present in the root apoplast. This may be a low amount of N, but even a small amount could greatly influence the results when working with highly $\delta^{15}N$-enriched solutions. Root samples were then dried at 65 °C for 48 h, and then ground in a dental amalgamator (Henry Schein, Inc., Melville, NY, USA). From each plot, powdered root samples (~5 mg each) were wrapped in tin capsules and analyzed for tissue $\delta^{15}N$ and N content (% N) by the Central Appalachian Stable Isotope Facility at the Appalachian Laboratory of the University of Maryland (Frostburg, MD, USA).

Since the δ values of the labeled samples were highly enriched, we converted $\delta^{15}N$ values to R_{sample}, the ratio of ^{15}N to ^{14}N in the root sample, and calculated the value of F, the fraction of the heavy isotope in the sample (Fry 2006):

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

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

where R_{std} = $^{15}N/^{14}N$ ratio in atmospheric N_2 (0.0036764). We then used the tissue N content, and F values to determine the μmol $^{15}N\ g^{-1}$ in root tissue. Finally, we estimated the rate of ^{15}N taken up by roots from the labeled N pools by dividing the ^{15}N excess (^{15}N content of labeled–unlabeled roots from the same tree) by the exposure time (3 h). The total uptake rate of inorganic N from the labeled pools was the sum of our estimate of NO_3^- and NH_4^+ uptake rates.

Soil Al determination

To determine the effect of whole-watershed acidification on both chelated and free monomeric soluble soil Al, we measured aqueous Al in organic and mineral soils from the two watersheds in the whole-watershed acidification study. We collected organic and mineral soil (top 15 cm) from ten plots in each watershed, combining four separate subsamples collected within each ~10-m radius plot into two composite samples—one for the organic and one for the mineral soil. In the lab, the mineral soils were further separated into mineral bulk soil and mineral rhizosphere soil. Any roots in the mineral soil were gently shaken to remove excess soil, and any soil remaining attached to the root was considered mineral rhizosphere soil. Due to the high density of roots in the organic horizon, this fraction was considered all rhizosphere soil. We sieved all soils through a 2-mm mesh and stored them at 4 °C. Soil moisture content was measured on a subsample from each soil by mass loss after drying for 48 h in a 65 °C oven. To measure total (chelated + monomeric) aqueous Al in soil solution, we used undried, fresh, sieved soil samples, combining 10 mL of distilled H_2O with 10 g of mineral soil, and 20 mL of H_2O with 10 g of organic soil. The goal of this procedure was to collect Al that is currently present in soil water close to the soil surface (top 15 cm). This region of soil has high fine root density (~57 g m^{-2} in the O-horizon and ~230 g m^{-2} in the top 15 cm of mineral soil in the acidified watershed; Carrara unpublished data). Our water addition diluted the existing soil water ~3:1 and allowed us to collect now-diluted soil solution after centrifugation. We chose to measure only the Al in soil water rather than using an ionic extractant to best estimate the Al that is delivered to the root surface via the soil solution. Thus, the Al values that we present are concentrations (μM) in aqueous soil solution after accounting for the dilution factor using the initial soil moisture content, which is intended to be similar to what would be measured in lysimeters (Lux 1999; Edwards et al. 2002). All soils were shaken for 30 min and centrifuged for 5 min at 4400×*g*, and then, the supernatant passed through a 0.45 µm filter. To separate free monomeric Al^{3+} from chelated Al in solution, we passed each sample through a Cleanert SCX cation exchange column (Bonna-Agela Technologies, Inc., Wilmington, DE, USA) to remove Al^{3+} from solution. The concentration of Al in the filtered and deionized (after the exchange column) solutions was then analyzed using a Varian SpectrAA 220FS graphite tube atomic absorption spectrometer (Varian, Inc., Palo Alto, CA, USA). The amount of chelated Al was subtracted from the total water-soluble Al to obtain the monomeric Al^{3+} content of each extract. Using soil moisture measurements for each sample, we adjusted the diluted Al values to the concentrations of the original soil water in each sample.

Statistical analyses

We used a complete three-way ANOVA and a Tukey's HSD post hoc analysis ($\alpha = 0.05$) to test for differences in soil Al between watersheds, soil fractions (organic, mineral bulk, and mineral rhizosphere), forms of Al (chelated vs. unchelated), and to test all interactions between the three factors. We focused on the differences in unchelated Al^{3+} between watersheds in the mineral rhizosphere and organic horizon, since these soil fractions should best characterize the exposure of tree roots to potentially damaging Al^{3+}.

To determine if Al^{3+} addition affected total uptake of N from the ^{15}N-labeled pool ($^{15}NH_4$ uptake + $^{15}NO_3$ uptake), we used a two-way ANOVA with ^{15}N uptake as the response variable and species and Al^{3+} addition as factors. The residuals for the rates of N uptake from the labeled pool were non-normal, so we natural log-transformed these data to fulfill the normality assumption of ANOVA. Thus, the reported rates of uptake of N from the ^{15}N labeled pools are back-transformed means (±SE). To test for an effect of Al^{3+} on NO_3^- uptake, we used the NH_4^+ and NO_3^- uptake rates from the labeled pools for each tree to calculate the total ^{15}N uptake from the labeled pools, as well as the percentage taken up as NO_3^-, both in the presence and absence of added Al^{3+}. We then used a two-way ANOVA with a Tukey's HSD post hoc analysis ($\alpha = 0.05$) to determine the effects of Al^{3+} and species on the percentage of ^{15}N uptake that was NO_3^-, and to test if the effect of Al^{3+} depended on species. The model included the effect of tree nested within species. To determine if any species took up significantly more NO_3^- than NH_4^+ without added Al^{3+}, or significantly

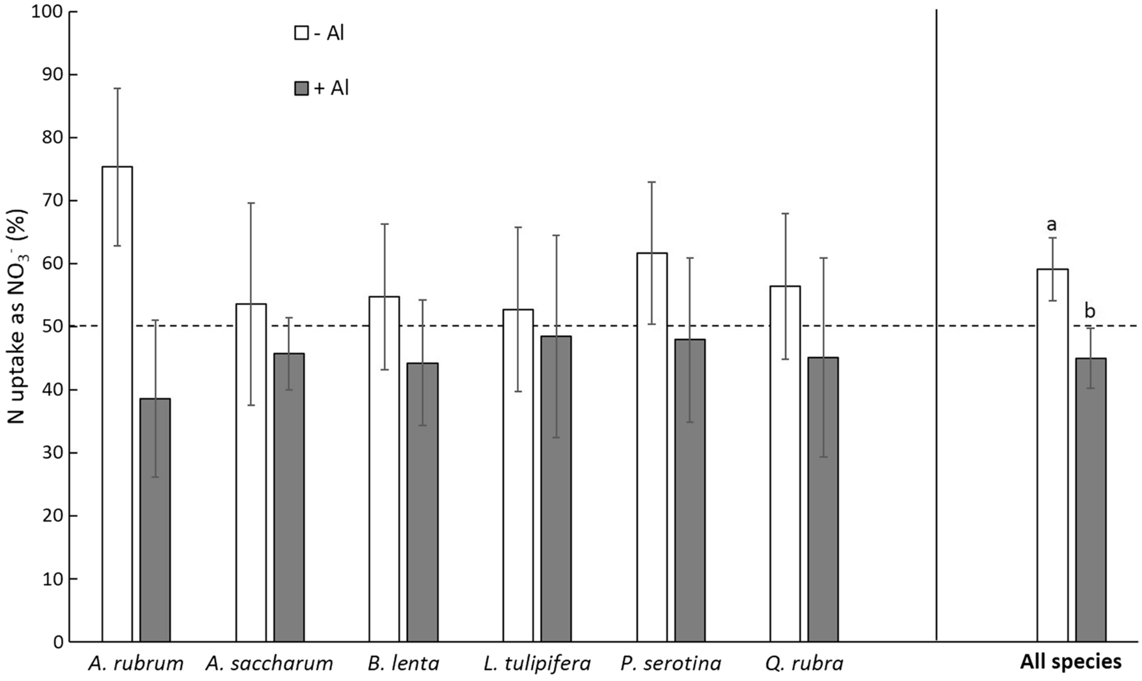

Fig. 2 Percent of ^{15}N taken up from the labeled pools as NO_3^- in the presence or absence of added Al^{3+} for the six temperate broadleaf tree species, and averaged across all species (far right). Bars that do not share a like letter are significantly different (Tukey's HSD post hoc analysis, $\alpha = 0.05$). No individual species percent ^{15}N uptake as NO_3^- was significantly affected by Al^{3+} addition. Dotted line shows 50% threshold of ^{15}N uptake as NO_3^- for visual comparison

less NO_3^- than NH_4^+ with added Al^{3+}, we performed one-tailed *t* tests to determine if the contribution of NO_3^- to total uptake of N from the labeled pool was greater (no added Al^{3+}) or less (added Al^{3+}) than 50%.

Results

Relative uptake of NO_3^- and NH_4^+

Across tree species, the total N uptake rate from the labeled pool ($^{15}NH_4^+ + {}^{15}NO_3^-$) was 0.120 μmol $^{15}N\ g^{-1}\ h^{-1}$, which is similar to rates measured in prior studies from the ^{15}N pool (McKane et al. 1990). There was no significant effect of species or Al treatment on total uptake of N from the labeled pool, and the effect of Al did not depend on species. Among all species, 59% (±5.2%) of N from the labeled pool was taken up as NO_3^- (0.074 ± 0.02 μmol $^{15}N\ g^{-1}\ h^{-1}$), and 41% as NH_4^+ (0.046 ± 0.05 μmol $^{15}N\ g^{-1}\ h^{-1}$), in the absence of added Al^{3+}, and these proportions were not significantly different between species. However, under added Al^{3+}, NO_3^- uptake from the labeled pool decreased to 44.6% (±5.0%) of total N uptake (0.065 ± 0.03 μmol $^{15}N\ g^{-1}\ h^{-1}$) ($F = 4.38$, $P = 0.047$) (Fig. 2), and NH_4 accounted for 55.4% of total N uptake from the labeled pool (0.094 ± 0.03 μmol $^{15}N\ g^{-1}\ h^{-1}$). While the mean percent of N uptake as NO_3^- declined from >50% for all species without added Al^{3+} to <50% under added Al^{3+}, no individual species decline was significant. For *A. rubrum*, there was a trend towards NO_3^- uptake contributing >50% to total uptake of N from the labeled pool ($t = 2.03$, $P = 0.056$), but no other species' NO_3^- uptake significantly differed from 50% of total uptake of N, regardless of Al treatment.

Table 1 Total soil solution Al (μM) (monomeric Al^{3+} + chelated Al) in three soil fractions within the acidified and reference watersheds, and the percent of total Al that was chelated

	Acidified Mean (SE)	Reference Mean (SE)
Organic soil		
Total Al	46.0 (7.3)^{bc}	76.9 (12.2)^{ab}
Percent chelated	38% (5.1)	32% (3.1)
Mineral bulk soil		
Total Al	103.3 (11.1)^{a}	29.9 (6.3)^{c}
Percent chelated	16% (2.8)	23% (9.5)
Mineral rhizosphere soil		
Total Al	113.2 (9.4)^{a}	41.8 (5.5)^{bc}
Percent chelated	11% (2.2)	20% (4.0)

Total Al values that do not share a like letter are significantly different (Tukey's HSD post hoc analysis, $\alpha = 0.05$)

Soil Al determination

The total soil solution Al (across all soil forms and fractions) was 77% higher in the acidified watershed than the reference, an increase of 37.9 μM Al (SE 7.3, $F = 5.19$, $P < 0.001$). Total Al was higher in the fertilized watershed in both the mineral bulk (245%) and mineral rhizosphere (171%) soil fractions, whereas there was no significant difference in total Al in the organic horizon (Table 1). Within each soil fraction (organic and mineral), <50% of the total soil solution Al was chelated in both watersheds

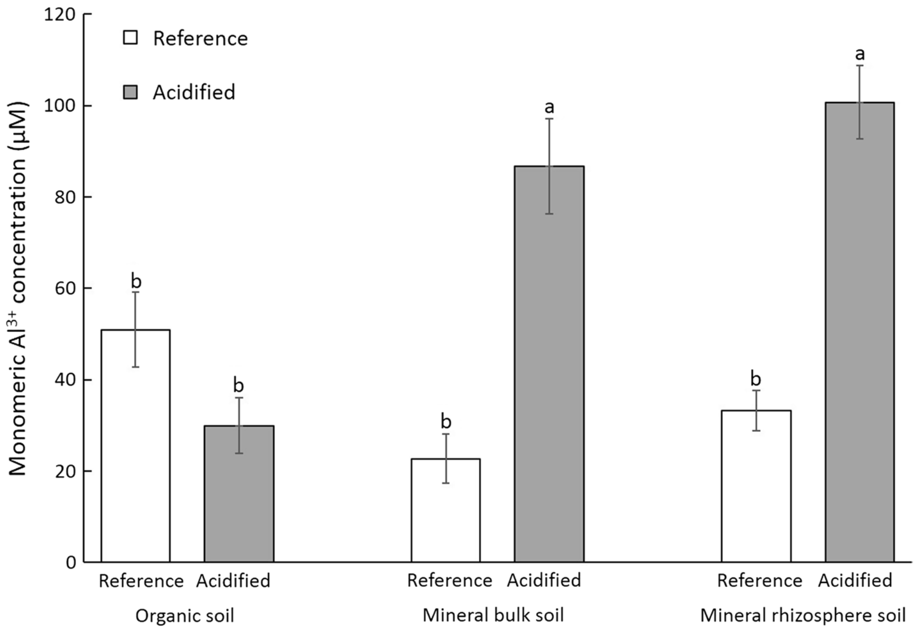

Fig. 3 Monomeric soil solution Al^{3+} (μM) in three soil fractions of the acidified and reference watersheds. Bars that do not share a like letter are significantly different (Tukey's HSD post hoc analysis, $\alpha = 0.05$)

(Table 1), and the percent chelated did not significantly differ between watersheds.

Monomeric soil solution Al^{3+} was 103% higher (36.9 μM) in the acidified watershed than the reference (Tukey's $t = 6.12$, $P < 0.001$), and within the different soil fractions, it was 64.1 μM higher (283%) (Tukey's $t = 6.14$, $P < 0.001$) in mineral bulk soil and 67.5 μM higher (203%) (Tukey's $t = 6.47$, $P < 0.001$) in mineral rhizosphere soil in the acidified watershed compared to the reference watershed (Fig. 3). In the organic soil, there was no significant difference in monomeric soil solution Al^{3+} between the watersheds, despite a high statistical power (>0.98) to detect a similar difference in this soil horizon as the bulk and rhizosphere mineral soils.

Discussion

In the absence of added Al^{3+} from the ^{15}N label addition, we found little difference in relative uptake of NO_3^- vs. NH_4^+ for six temperate tree species under field conditions, whereas many prior studies found that NH_4 is the dominant mineral N form utilized by tree species (Buchmann et al. 1995; Gessler et al. 1998; Kronzucker et al. 1997; Lovett and Mitchell 2004; Malagoli et al. 2000; McFarlane and Yanai 2006; Min et al. 2000; Rothstein et al. 1996; Socci and Templer 2011; Templer and Dawson 2004). Our study differs from most of these in two important ways. First, the studies that tend to show the highest relative uptake of NH_4^+ over NO_3^- used coniferous species, whereas we studied temperate deciduous species that have been exposed to decades of elevated atmospheric N deposition. For example, Buchmann et al. (1995) labeled the soil of a *Picea abies* plantation and estimated that uptake of $^{15}NH_4^+$ was between two and four times higher than $^{15}NO_3^-$. Second, many previous studies placed live or excised roots directly into nutrient solutions containing one or both mineral N forms. While this is valuable when studying the physiology of N uptake at the root surface, the higher diffusional resistance of NH_4^+ vs. NO_3^- in soil results in a greater delivery of NO_3^- to the root surface under natural conditions (Chapman et al. 2012). As a result, nutrient solution studies may underestimate the relative contribution of NO_3^- to tree N nutrition under field conditions. Similarly, the use of excised roots severs the transpiration stream, which drives mass flow to the root surface and is an important factor in plant NO_3^- uptake (Oyewole et al. 2014). Under more natural conditions, NO_3^- is more mobile than NH_4^+, and the movement of NO_3^- via diffusion to the root surface may lead to greater relative uptake of NO_3^- than can be measured using nutrient solutions (Fahey and Yavitt 2005). Indeed, the keystone species *A. saccharum* (sugar maple) may be a good example of how N uptake assessments under artificial conditions may be misleading. Sugar maples are typically thought to utilize NH_4^+ as the primary mineral N source (Lovett and Mitchell 2004), a conclusion supported by excised root (Rothstein et al. 1996; Eddy et al. 2008; Socci and Templer 2011), nutrient solution depletion (McFarlane and Yanai 2006; Socci and Templer 2011), and greenhouse seedling studies (Templer and Dawson 2004). However, when we measured the relative importance of NO_3^- uptake in situ, we found a much higher relative contribution of NO_3^- to total uptake of N for mature trees than was indicated by many previous studies. Furthermore, the only other study that measured uptake of NO_3^- by mature *A. saccharum* trees under nearly in situ conditions found significant uptake of NO_3^-, and also found that *A. saccharum* took up NO_3^- at a higher rate than three other temperate broadleaf species (Fahey and Yavitt 2005). Therefore, we suggest that in situ ^{15}N-labeling techniques may provide meaningful insight into the mineral N uptake dynamics of mature trees under natural conditions.

Our in situ findings of a reduction in the relative amount of NO_3^- uptake under Al exposure in our ^{15}N labeling experiment support our first hypothesis, and these results generally agree with prior greenhouse- and laboratory-based studies on herbaceous and woody plants. NO_3^- uptake reductions in plants exposed to Al have been found in maize (Durieux et al. 1993; Calba and Jaillard 1997), cucumber (Jerzykiewicz 2001), barley (Watanabe et al. 1998), and *Lotus* (Pal'ove-Balang and Mistrík 2007), as well as in the tropical tree *Melaleuca cajuputi* (Watanabe et al. 1998) and coniferous tree *Pinus rigida* (Cumming 1990). In addition, Al had a greater impact on growth when *P. rigida* seedlings were grown with primarily NO_3^- vs. NH_4^+ or mixed N sources (Cumming and Weinstein 1990). Thus, our in situ measurement of this pulse effect on six important tree species suggests that acidic deposition has the potential to reduce stand NO_3^- demand in a temperate deciduous forest, at least short term, as Al^{3+} becomes soluble in the soil under field conditions. Should the Al^{3+} effect on NO_3^- uptake persist, reduced stand NO_3^- demand would be sustained and impact longer term discharge of N.

Our experiment of Al^{3+} addition to ^{15}N-labeled solutions was a pulse addition of Al^{3+}, which contrasts somewhat with the long-term effects of whole-watershed acidification. We altered the Al^{3+} concentration at the interface of the organic and mineral soil (3 cm depth), but we only measured a long-term acidification effect on Al^{3+} concentration in the mineral soil. Therefore, one assumption of our method was that Al^{3+} would similarly impact fine root uptake of NO_3^- in deeper mineral soil (up to 15 cm) as at the interface between mineral and organic soil. Pulses of Al^{3+} exposure could result from rain storms that increase soil moisture and mobilize Al^{3+} in acidic soils, leading to greater movement of Al^{3+} to the root surface via mass flow. The spike of Al^{3+} in soil solution caused by our experimental addition was similar to what we observed in the soil of the long-term acidified watershed, so the physiological responses of the trees may also be similar. However, it is unknown if trees acclimate to long-term Al^{3+} exposure, thus recovering their uptake of NO_3^- under more natural conditions. Some evidence from herbaceous plants suggests that the effect of Al^{3+} persists. Maize plants showed no signs of short-term acclimation to Al^{3+} after 8 h of exposure (Durieux et al. 1993), although they recovered rapidly once they were removed from Al^{3+} solutions. The uptake of NO_3^- by white clover was also affected by Al^{3+} over a period of 5 weeks (Jarvis and Hatch 1986). In trees, there is also evidence of long-term effects on growth and tissue Ca:Al ratios (Vanguelova et al. 2007), although the effects vary between methods and species. Phillips and Yanai (2004) added $AlCl_3$ to *A. saccharum* trees in the field for 2 years, and found that Al content in the rhizosphere was reduced relative to bulk soil, suggesting that Al leached from the rhizosphere due to increased organic acid efflux from tree roots. However, we did not find a decrease in soluble Al^{3+} in the rhizosphere soils of our paired watershed study, so the effects of Al^{3+} would not be relieved in this manner. While a reduction in the uptake of NO_3^- may lessen over time, prior evidence suggests that at least some effect of Al^{3+} persists while it remains in soil solution.

Surprisingly, contrary to our second hypothesis, our ^{15}N labeling results suggest that the tree species we studied did not differ in the impact of Al^{3+} on percent of N uptake as NO_3^-. This contrasts with prior evidence of variable Al^{3+} sensitivity between species (Kochian 1995; Watanabe et al. 1998), including temperate deciduous trees (Halman et al. 2015). Since we collected roots 3 h after treatment application, we measured their initial response to added Al^{3+}. It is possible that some species would increase Al^{3+}-resistance over a longer time period by, for example, increasing root efflux of organic acids to chelate rhizosphere Al^{3+} (Kochian 1995). In addition, our treatment levels of Al^{3+} were relatively low to mimic the measured increase in the soil of the acidified watershed. We estimate that our levels of added Al^{3+} (~50–100 μM exposed to plant roots, or 2.16–4.32 mg Al m^{-2}) were approximately 2% of the treatment level of Halman et al. (2015) (182 mg Al m^{-2} $year^{-1}$), who also studied temperate forest trees. It is possible that the species reacted similarly because these levels were lower than the threshold for Al^{3+} response by sensitive species (Vanguelova et al. 2007). Thus, low levels of Al^{3+} in acidified soils can rapidly affect uptake of NO_3^- across dominant temperate tree species.

Measuring uptake of N in situ by isotopically labeling the available pool presents some significant challenges. First, the use of a labor intensive and higher cost ^{15}N labeling method limited our sample size to five trees of each species. As a result, our ability to detect differences between species was likewise limited. We conducted an iterative post hoc power analysis, following the methods of Sokal and Rohlf (1981), using α of 0.05. This revealed that our sample size led to a relatively low statistical power $(1 - \beta)$ of ~0.1 to detect a similar effect of Al^{3+} on uptake of NO_3^- as a percent of total uptake of N within species as we found across species. The sample size would need to be increased to 66 or greater, depending on species, to reach a statistical power of 0.8. Our results can still be applied to stands given the Al^{3+} effect among species, and further studies on the effects within species could yield interesting results. Second, assimilated N is moved away from the roots into the tree, and the rate at which this happens is difficult to estimate in situ. Given our relatively short time from ^{15}N addition to root excavation (3 h), our estimated uptake rates should be close approximations of the actual uptake of ^{15}N from the labeled pools. The movement of N from the roots into the tree could affect the measured proportion of uptake as NO_3^- vs. NH_4^+ if they have different residence times in the root tissue. The reduction of NO_3^- occurs mostly in leaves in temperate deciduous tree species (Tang et al. 2012), potentially minimizing this effect. However, differential movement of the two N forms out of root tissue could result in an underestimation of the relative contribution of NH_4^+ to overall N uptake if reduced NO_3^- is stored in roots. Finally, it is also difficult to measure total N uptake using an in situ labeling method in undisturbed soil. To do so, an accurate measurement of the ^{15}N atom percent in the soil at the root surface after the label is added would be necessary. As such, we have presented our results as uptake of ^{15}N from the labeled pool, rather than total uptake of N, and focused on the proportions taken up as the two different mineral N forms. With efforts to minimize these methodological concerns, our measurements of root uptake from undisturbed soil provide important advantages that should be considered when conducting research in situ.

Not surprisingly, in the whole-watershed acidification experiment, we found that soluble soil Al^{3+} increased under long-term treatment (since 1989), indicating that soil acidification causes an increase in monomeric Al^{3+} in the upper mineral soil. We found comparable levels of soluble soil Al^{3+} that we measured (87–101 μM in bulk soil, Table 1) to prior measurements in lysimeter-collected soil water in the same watershed (107 μM; Lux 1999), which suggests that our aqueous extraction method yielded accurate measurements of actual soil solution Al^{3+}. Monomeric Al^{3+} was elevated in both the bulk and rhizosphere mineral soils, so Al^{3+} directly impacts tree roots in the mineral soil. There was no significant difference in organic soil Al^{3+} between watersheds, yet the measured levels may still be high enough (30–50 μM) to affect root uptake of NO_3^- in this soil horizon. It is possible that long-term acidic deposition in the region caused these levels of Al^{3+} even in the reference watershed, as was seen by Lux (1999). In addition, additional soluble soil Al^{3+} under experimental acidification of the treated watershed could readily associate with exchange sites on organic material, reducing the treatment's effect on Al^{3+} in soil solution. Since the organic horizon is an area of high root density, the fact that Al^{3+} did not increase in this horizon under experimental acidification could relieve some of the effect of Al^{3+} on root uptake of NO_3^- at the stand level. However, there were actually more roots m^{-2} in the top 15 cm of mineral soil than in the organic horizon in these watersheds (~57 g m^{-2} in the O-horizon vs. ~230 g m^{-2} in the mineral soil in the acidified watershed; Carrara unpublished data). As a result, our results still support the hypothesis that acidification increases Al^{3+} to levels that diminish the relative uptake of NO_3^-, potentially impacting watershed NO_3^- dynamics. Furthermore, we did not detect a decrease in overall N uptake from the labeled pool under Al^{3+} treatment; instead, uptake remained stable, but the proportion of N taken up as NO_3^- decreased. This emphasizes that soluble soil Al^{3+} can impact the pool of mineral N used by overstory trees under long-term acidic deposition, and thus potentially increase NO_3^- discharge from the watershed.

As an initial assessment of the potential impact of Al on the export of NO_3^- in stream water at the scale of a small watershed, we applied the results of our ^{15}N-labeling experiment to estimates of total N uptake by the trees growing in the acidified watershed at the FEF (WS 3). Under whole-watershed fertilization and acidification, the discharge of NO_3-N increased from 4.17 kg N ha^{-1} $year^{-1}$ pre-fertilization (1982–1989) to 13.82 kg N ha^{-1} $year^{-1}$ post-fertilization (1990–2009), an increase of 9.65 kg N ha^{-1} $year^{-1}$ (Fig. 1). However, at the same time, there was no detectable difference in mineral soil net nitrification rate between the two watersheds (Gilliam and Peterjohn unpublished data). Therefore, reduced stand NO_3^- demand due to soil Al may contribute to the higher NO_3^- discharge in stream water in the acidified watershed. We estimated tree uptake of NO_3^- in the acidified watershed by multiplying an estimate of total N uptake (N return in leaf litter + aboveground woody N storage) (50.95 kg ha^{-1} $year^{-1}$) by our ^{15}N-label measurement of percent of uptake as NO_3^- both without and with added Al^{3+} (59 and 44.6% of total N uptake as NO_3^-, respectively). Aboveground woody N storage was calculated by multiplying bole wood N content in the outer 1 cm by the annual stand woody biomass increase reported by DeWalle et al. (2006). The resulting difference between NO_3^- uptake without and with added Al^{3+}, an estimate of unassimilated, excess soil NO_3^- available for leaching due to the impact of Al^{3+}, is 7.73 kg N ha^{-1} $year^{-1}$. If this amount was completely discharged in stream water, the effect of increased Al^{3+} would account for up to 76% of the 9.65 kg N ha^{-1} $year^{-1}$ increase in stream water NO_3^- due to whole-watershed acidification. Perhaps, more realistically, if ~70% of this unassimilated NO_3^- were retained in the watershed, as measured by Adams et al. (2006), then elevated Al^{3+} would still cause 23% of the increase in stream water NO_3^-. While this initial estimate is specific to our study site, the potential magnitude of the effect of elevated soil Al^{3+} on watershed NO_3^- discharge is large enough to warrant more detailed assessments at a variety of locations.

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Author contributions MBB, JRC, and WTP conceived and designed the ^{15}N-labeling experiment, MBA aided with site selection and soil analysis methodology for both the whole-watershed acidification study and the labeling experiment. MBB and WTP analyzed the data and wrote the manuscript; MBA and JRC reviewed the manuscript and provided editorial advice and comments.

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