



Article

Soil and tree nutrient status of high elevation red spruce (*Picea rubens* Sarg.) forests

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Abstract: Anthropogenic emissions stemming from industry have resulted in historically high levels of acidic deposition into central Appalachian forests. Despite the reduction in acidic inputs due to legislation curbing industrial emissions in the United States, continued N deposition may contribute to legacy effects in these forest ecosystems. Soil and foliar samples were collected from four high elevation red spruce sites along a modeled gradient of historic N deposition. The three most abundant tree species at all sites, *Acer rubrum* L., *Betula alleghaniensis* Britt., and *Picea rubens* Sarg., were sampled. Mehlich-III soil extracts of both organic and mineral horizons and foliar digests from these trees were subjected to elemental analysis. Soil N concentrations supported the presence of a N deposition gradient: in the O horizon, N concentrations were driven by precipitation volume and elevation; in the B horizon, N concentration was explained by modeled deposition rate and elevation. Cation depletion was evident in O horizons, with significant declines in Ca, Mg, and K as N deposition increased. Foliar Ca, K, and Sr declined in foliage with increasing N deposition. Although the three species were sympatric in mixed stands at all four sites, the foliar-soil nutrient associations differed among them across the gradient, indicating differential uptake and cycling of nutrients/metals by these forest tree species.

Keywords: *Acer*, acid deposition, *Betula*, biogeochemistry, forest recovery, *Picea*

1. Introduction

Nutrient relations of forest trees play a central role in forest ecosystem function and productivity. Nutrient acquisition is influenced by physiological adaptations of tree species, interactions with microbial symbionts in the rhizosphere, and the nature of soils supporting forests [1–4]. The production of enzymes and other compounds by roots and root-associated microbes, differential uptake of nutrients and other elements from the soil, and their return in litter result in tree species acting as “ecosystem engineers” by accumulating and redistributing these elements throughout the soil profiles. The complex interplay between different tree species, mycorrhizal fungi, and the bacterial communities in the rhizosphere determines how tree species respond to nutrient limitation, soil pH, and metal availability and further determines patterns of nutrient cycling that vary across the landscape [5–7].

Since the advent of heavy industry and power generation in the late 19th century, forested ecosystems worldwide have been exposed to high levels of anthropogenically-derived nitrogen (N) and sulfur (S) in acidic deposition. The deposition of acidic compounds varies spatially across the landscape, with the magnitude of inputs strongly influenced by topographic factors such as elevation, slope, and aspect [8]. High elevation ecosystems, such as the red spruce (*Picea rubens* Sarg.) forests in

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the Appalachian Mountains in the United States, are spatially predisposed to high inputs of acidic deposition [9–12]. With implementation of the Clean Air Act and supporting legislation in the early 1970s in the United States, however, anthropogenic inputs of N and S into the atmosphere have declined, although areas downwind of the Ohio River Valley, including central Appalachia, still receive some of the highest levels of N and S deposition in the United States [13].

Forest ecosystems are affected by acidic deposition through a variety of pathways, including reductions in plant-available nutrient cations in soils, elevated metals in the soil solution, leaching of cations from foliage, and N fertilization effects [14–16]. With continued N inputs into these ecosystems, changes in soils and tree nutrient acquisition continue, including increases in soil and foliar N, decreases in nutrient cations, and decreases in Ca/Al ratio [17–20]. Diminished soil Ca^{2+} has been directly correlated with decreases in winter hardiness of red spruce foliage, leading to region-scale winter dieback events occurring as late as 2003 [21–23] and potentially significant reductions in C storage at the landscape scale [24]. Disruptions in plant-soil nutrient relations and C assimilation due to acid deposition also increase the vulnerability of red spruce to secondary pests and pathogens [25]. These direct and indirect effects of acidic deposition can have wide-reaching implications for ecosystem function and productivity [16,26,27].

There have been numerous studies on biogeochemistry and nutrient relations in Appalachian red spruce stands. Robarge et al. (1989) conducted a survey of red spruce foliar elemental status in southern Appalachia and consolidated the reported literature values for red spruce foliar chemistry available at that time. Foliar Ca in spruce was at the extreme low and Al at the high ranges of values reported for this species in the eastern USA, suggesting these systems were at risk for Ca deficiency and Al toxicity [28]. Johnson et al. (1991) linked the presence of plant-available Al to soil solution pulses of NO_3^- and SO_4^{2-} from acid deposition in southern Appalachia, but failed to detect signs of soil base cation depletion or nutrient deficiency in spruce foliage [29]. More recent studies in the region indicate that soil Ca/Al ratios still represent a threat to red spruce in the region, although comparison to earlier reported foliar concentrations indicate possible recovery in nutrition since the previous decade [30].

A transect containing high elevation red spruce forests in central Appalachia corresponding to a gradient of historic acidic deposition was established by Smith et al. (2016) using National Atmospheric Deposition Program (NADP) data [31]. These sites experienced cumulative wet N inputs ranging between 117.5 and 206.9 kg ha⁻¹ and average annual wet N additions between 4.2 to 7.4 kg ha⁻¹ yr⁻¹ from 1985–2013 [32]. These values are comparable to N deposition ranges observed in studies in other regions in the eastern United States experiencing elevated rates of N deposition, such as the Adirondack Mountains of New York [33] and at sites throughout New England [34,35]. Smith et al. (2016) found that broadleaf deciduous tree relative importance value rather than N deposition rate had the biggest effect on site N status in these red spruce forests [31]. Species-specific differences in nutrient retention have long been considered important drivers of N availability and cycling [36–38].

Building on the Smith et al. (2016) study, we evaluated soils beneath and foliage of three dominant tree species, *Acer rubrum* L. (red maple), *Betula alleghaniensis* Britt. (yellow birch), and *P. rubens* (red spruce), in four central Appalachian forest stands across this historic N deposition gradient to assess soil and plant nutritional status of these mixed spruce-hardwood stands. Although anthropogenic acidic inputs have declined over the last three decades, continued N deposition and legacy effects may still have a significant influence over soil chemistry. While soils provide insight into the status of plant-available nutrients and metals, foliage allows us to gauge element acquisition by the principle tree species at these sites. We hypothesized that: (1) legacy soil elemental profiles would exist across this historic N deposition gradient and that these profiles, characterized by low cations and high metals in high deposition sites, would be manifest in foliar elemental concentrations; (2) despite all sites being mixed stands, there will be local, species-specific effects on soil nutrient concentrations resulting from differential nutrient cycling among the trees species in these ecosystems.

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2. Materials and Methods

Four high-elevation red spruce stands were selected based upon site characteristics including elevation, tree species composition, and position along a gradient of historic acid deposition in central Appalachia (Figure 1). All sites are at high elevation (>1100 m) and have a southwest aspect with slopes from 0–10% within the plot area. Site selection for historic acid deposition relied upon data from the National Atmospheric Deposition Program (NADP) following the procedure of Smith et al. (2016). Sites were chosen from estimated cumulative total inorganic N wet deposition (NO_3^- and NH_4^+) from 1985–2012. Data for dry deposition of N (HNO_3 , NO_3^- , NH_4^+) was available for the years 2000–2012, and was incorporated into estimates of total inorganic N deposition by calculating the ratio of wet N deposition to total N deposition for those years and applying it to each pixel in the NADP spatial model for the mid-Atlantic region [39]. These data were then used to estimate total N deposition for the years 1985–1999 that lack available dry deposition data for the highly resolved and well-validated spatial model used by NADP. Although S deposition likely represented a significant component of historic acid deposition, historical data for S deposition were neither robust nor adequately validated for modeling, rendering reliable site estimates unavailable. Elevated levels of N and S deposition in this region are known to spatially coincide [13].

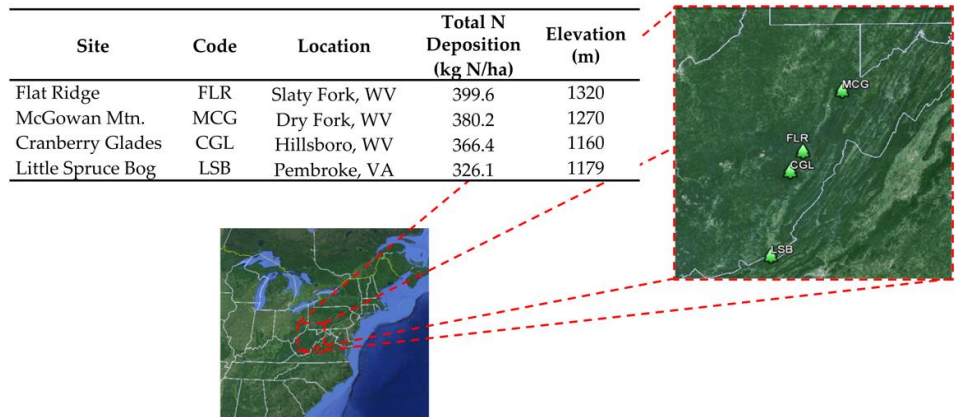


Figure 1. Locations and site characteristics of four high elevation sample sites.

Each site consisted of a single 100-m diameter plot about a center point established in an area with red spruce as the predominant tree species. The Point-Centered Quarter Method [40] was used to analyze forest tree (>10 cm DBH) community composition in these plots using five points randomly located on three parallel 50 m transects each separated by 25-m. Tree species community composition was similar across all sites, with each site belonging to the red spruce-yellow birch cover type defined by the Society of American Foresters [41]. All four sites have a history of disturbance, including fire and, most recently, timber harvest. Smith et al. (2016) determined year of stand establishment for these sites using tree core cross-dating techniques, with stand age at the time of sampling 75 yr for FLR, 149 yr for LSB, and 156 yr for both CGL and MCG, corresponding to timber harvesting dates. Soils at each site are characterized by generally thick organic horizons, high organic matter content, and relatively low pH. Geologically, soils at these sites are derived principally from sandstone, with shale and limestone influences present at FLR and LSB, respectively. Despite differences in parent material and soil development, pH exhibits little variation across sites or horizons (Table 1).

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Cite the source of soil data in Table 1.

Table 1. Soil characteristics of the four high-elevation sites. Soil classifications and descriptions were obtained from NRCS Soil Survey Geographic (SSURGO) Database. Mean soil pH values for each site (organic O; mineral M) were measured using 5-g subsamples of homogenized soil from each sample location and each soil horizon.

Study Site	Soil Series	Taxonomic Class	Drainage Class	Description	Soil pH
FLR	Gauley	Frigid Typic Haplorthods	Moderately deep, well drained	Loamy-skeletal, siliceous, superactive	O: 3.61 M: 3.63
MCG	Ernest	Mesic Aquic Fragiudults	Very deep, moderately well to poorly drained	Fine-loamy, mixed, superactive	O: 3.42 M: 3.36
CGL	Snowdog	Frigid Typic Fragiudepts	Very deep, moderately well drained	Fine-loamy, siliceous, active	O: 3.49 M: 3.47
LSB	Lily (LB Complex)	Mesic Type Hapludults	Moderately deep, well drained	Fine-loamy, siliceous, semi-active	O: 3.18 M: 3.32
	Bailegap (LB Complex)	Mesic Type Hapludults	Deep, well drained		

Soil and foliar samples were collected in July 2014. Five individual trees of *A. rubrum*, *B. alleghaniensis*, and *P. rubens* were selected (Table 2). Canopy dominant or co-dominant individuals greater than 45-cm DBH were selected. Soil samples were collected using a 2-cm diameter soil auger to 15 cm. Prior to sampling, coarse litter was brushed away from the sample area. Sampling points were selected on opposite sides of each trunk, midway between bole and edge of canopy. Samples were carefully separated into organic (O) and mineral (B) horizons and then composited by soil horizon for each individual tree. Poorly-developed spodic horizons were occasionally encountered and, being unrepresentative of the bulk soil, were discarded. No C-horizons were apparent at a depth of 15-cm at any of the sample locations, although some samples, most frequently at FLR, consisted of a thick organic horizon and very little mineral soil (approximately 15-cm total depth) before encountering rock. Conversely, there were some red maple individuals at each site that exhibited virtually non-existent organic horizons.

Table 2. Relative importance values for *A. rubrum* (ACRU), *B. alleghaniensis* (BEAL), and *P. rubens* (PIRU) at each high elevation red spruce site; data from Smith et al. (2016).

Site	% ACRU	% BEAL	% PIRU	% Total Broadleaf Deciduous	% Total Needleleaf Evergreen
FLR	15.1	5.0	75.0	26.2	73.8
MCG	6.4	36.8	21.4	49.8	50.2
CGL	18.2	14.0	50.8	43.1	56.9
LSB	13.0	11.0	23.1	32.6	67.4

Soil samples (n = 5) were sieved using a 2-mm screen and subsamples dried at 105°C for 48 h for extraction and air-dried for C and N analysis. Dried soil samples were stored long-term at 4°C. Oven-dried samples were extracted with Mehlich-III solution to determine elemental content following the technique outlined by Zhang et al. [42] and adapted from Mehlich [43]. Extracts were analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES) to determine the concentration of macronutrients, micronutrients, and metals. Air-dried samples were analyzed for C and N concentration using a Thermoquest Elemental Analyzer.

For each species at each site, foliar samples (n = 3) were collected from a subset of the five individual trees sampled for soils at each site. Foliar samples were obtained using a shotgun to collect

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All mineral soil? So the O horizon collected did not in

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list constituents in parentheses.

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mid-canopy sun leaves with a southeastern to southwestern exposure [44]. Foliage damaged by shot was discarded. Since different cohorts and stages of development for red spruce needles can vary in foliar chemistry, needles were only utilized from the previous year to ensure uniformity of foliar developmental status for that species [22,45,46].

In the field, foliar samples were placed on ice in sealable plastic bags with a damp paper towel to prevent wilting and desiccation. In the lab, foliar samples were washed in a solution of 0.1% Tween-80 and rinsed repeatedly with Nanopure H₂O to remove foliar surface contaminants potentially present on leaves [47]. Approximately 5-g fresh weight samples of foliar tissue were placed into individual paper bags and dried at 70°C for 48 h. Dried foliar tissue was ground using a Cyclotec™ 1093 sample mill. Ground tissue was digested in 75-ml Teflon digestion tubes containing 4 ml of trace-metal-grade concentrated HNO₃ and 1 ml of 30% H₂O₂. Microwave-assisted digestion was performed using a MARSXpress™ 5 with settings optimized for foliar tissue [48]. Digests were then subjected to analysis by ICP-OES. National Institute of Standards and Technology (NIST) standards (Standard Reference Material 1515, apple leaves) were included in this analysis and used to validate elemental concentrations from the ICP-OES data. As with the soils, the C and N content of foliage was determined using a Thermoquest Elemental Analyzer.

We used two-way analysis of variance (ANOVA, species × N deposition), multiple regression, and stepwise regression using site variables to evaluate the impacts of N deposition and species or different site variables, respectively, on soil and foliar elemental profiles using SAS JMP Pro 13 [49]. Data were log- or square root-transformed to meet assumptions of normality. Pearson product-moment correlations for the pairwise comparisons of foliar element values by soil horizon were also calculated, and heat maps representing the correlations between foliar element concentrations and their soil counterparts by horizon were generated in Microsoft Excel.

3. Results

3.1 Evidence of anthropogenic deposition across the gradient

The sites investigated were selected based on modeled N deposition (Figure 1). Sites having received elevated anthropogenic inputs were expected to exhibit characteristic elemental fingerprints of historical deposition, including elevated N and S from atmospherically-borne acids, elevated industrially-released heavy metals, and acid-induced increases in soil Al. Soil pH varied significantly by horizon (O horizon = 3.38, B horizon = 3.50), and increased slightly across the deposition gradient (Figure S1). Stepwise regression was used to evaluate the importance of modeled N deposition, precipitation volume, and elevation on current soil chemical profiles (Table 3, Figure 2). Soil N was primarily influenced by precipitation and elevation in the O horizon and by N deposition and elevation in the B horizon (Table 3). Parameters were all positive indicating increasing N in soils with increasing deposition, precipitation, and elevation. In contrast, S in the O horizons declined with N deposition, but increased in soil B horizons with increasing N deposition (Figure 2). The soil metals Zn and Pb are often used proxies for atmospheric deposition [50]. Zn concentrations in the O horizon declined with modeled N deposition, precipitation, and elevation, but increased in B horizons across the N deposition gradient (Figure 2). Pb concentrations were influenced by precipitation and elevation in the O horizon, decreasing with N deposition; Pb was only a function of N deposition in the B horizon and increased with the N gradient (Table 3, Figure 2). O horizon Al declined with deposition, whereas the change in B horizon with N deposition was not significant (Figure 2). Taken together, these patterns suggest that the sites selected have indeed received a gradient in anthropogenically-generated pollutants. While S, Zn, Pb, and Al have moved from the O to the B horizon where reactions with the CEC and amorphous hydroxides may retain them, N is still accumulating throughout the soil profile.

The percent broadleaf tree representation was also initially used in the stepwise regression following Smith et al. (2016), but this variable did not explain any additional variation in the soil properties measured (data not presented). This discrepancy may reflect the species-specific sampling approach used here compared to stand-level sampling employed by Smith et al. (2016). We

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Table 3. Comparison of Corrected Akaike Information Criterion (AICc) values for linear regression models evaluating drivers of N, S, Zn, Pb, and Al concentrations in montane soils. *Indicates most parsimonious model.

	O Horizon					B Horizon				
Model Parameter	N	S	Zn	Pb	Al	N	S	Zn	Pb	Al
N deposition	55.0	127.8	132.6	141.8	149.3	92.3	79.2*	129.4	142.9*	157.1
Precipitation	64.5	152.9	146.1	157.3	167.9	127.9	95.4	140.7	152.3	156.9
Elevation	58.2	135.1	143.7	138.9	159.1	120.5	94.3	128.6	149.8	163.9
N deposition, precipitation	57.3	123.9	127.4	139.5	143.2*	93.0	88.2	131.7	144.1	156.6
N deposition, elevation	51.8	120.3*	134.9	133.4*	148.8	88.9*	88.7	121.9	144.6	158.8
Precipitation, elevation	49.9*	132.0	145.7	138.4	160.2	106.3	90.8	118.2*	149.7	152.8*
N deposition, precipitation, elevation	51.6	121.5	127.3*	135.8	145.6	91.2	82.9	120.2	146.4	155.2
R ² (*best model)	0.30	0.46	0.32	0.36	0.38	0.57	0.29	0.41	0.17	0.25

Sites exhibited differences in soil element concentrations due to N deposition and elevation and these varied by horizon, but typically not by species (Table 4). C and N in the O horizons increased beneath the three species with increasing N deposition and elevation, but did not vary between species except for the higher concentrations in the B horizon beneath *A. rubrum* (6272 mg kg⁻¹) versus *B. alleghaniensis* (4732 mg kg⁻¹) and *P. rubens* (4350 mg kg⁻¹) (Table 4). C:N ratios did not vary in the O, but declined with N deposition in the B horizon (Table 4). Evidence for cation depletion was evident in O horizons of these montane soils, with significant declines in Ca, Mg, and K in organic soils as N deposition increased (Table 4); P also declined. In contrast to the O horizon, the concentrations of C and N and all macronutrients in the B horizons increased with N deposition, although elevation had no impact (Table 3). Declining Ca/Al, a signal of soil acidification, was not a general characteristic of the sampled soils, but was evident in the O and B horizons only beneath *A. rubrum*; indeed this was the only soil factor for which there was a significant species-by-deposition interaction (Figure S2). Al was greatest beneath *B. alleghaniensis*, intermediate beneath *A. rubrum*, and least below *P. rubens*, reflecting potential differences in uptake and cycling of Al by these species.

Foliar elemental concentrations varied strongly by species across the modeled N input gradient (Figure S3), and Ca, K, and Sr were sensitive to deposition (Table 5). Ca, K, and Sr exhibited strong evidence for N deposition-induced limitation, declining consistently in *B. alleghaniensis* with N inputs; Ca and Sr declined with N deposition in *A. rubrum* and *P. rubens* as well (Figure 3). Foliar Mg and Al were relatively unaffected by N deposition across the modeled N input gradient (Figure 3, Figure S3).

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I'm not sure what you mean by this. If species was not significant in the stepwise regression--that's one thing. Was species significant in the ANOVA?

This type of discussion should be included in the statistics section of the methods--not in the results section.

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New paragraph? It may be more clear if C and N are presented together--where there is no spp effect. Then present the elemental results--where there is spp effect.

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If spp differ significantly, how can the change related to N dep be presented as a single value in Table 5. Perhaps it would be more clear to present the spp change in elemental concentration by just using Figure 3.

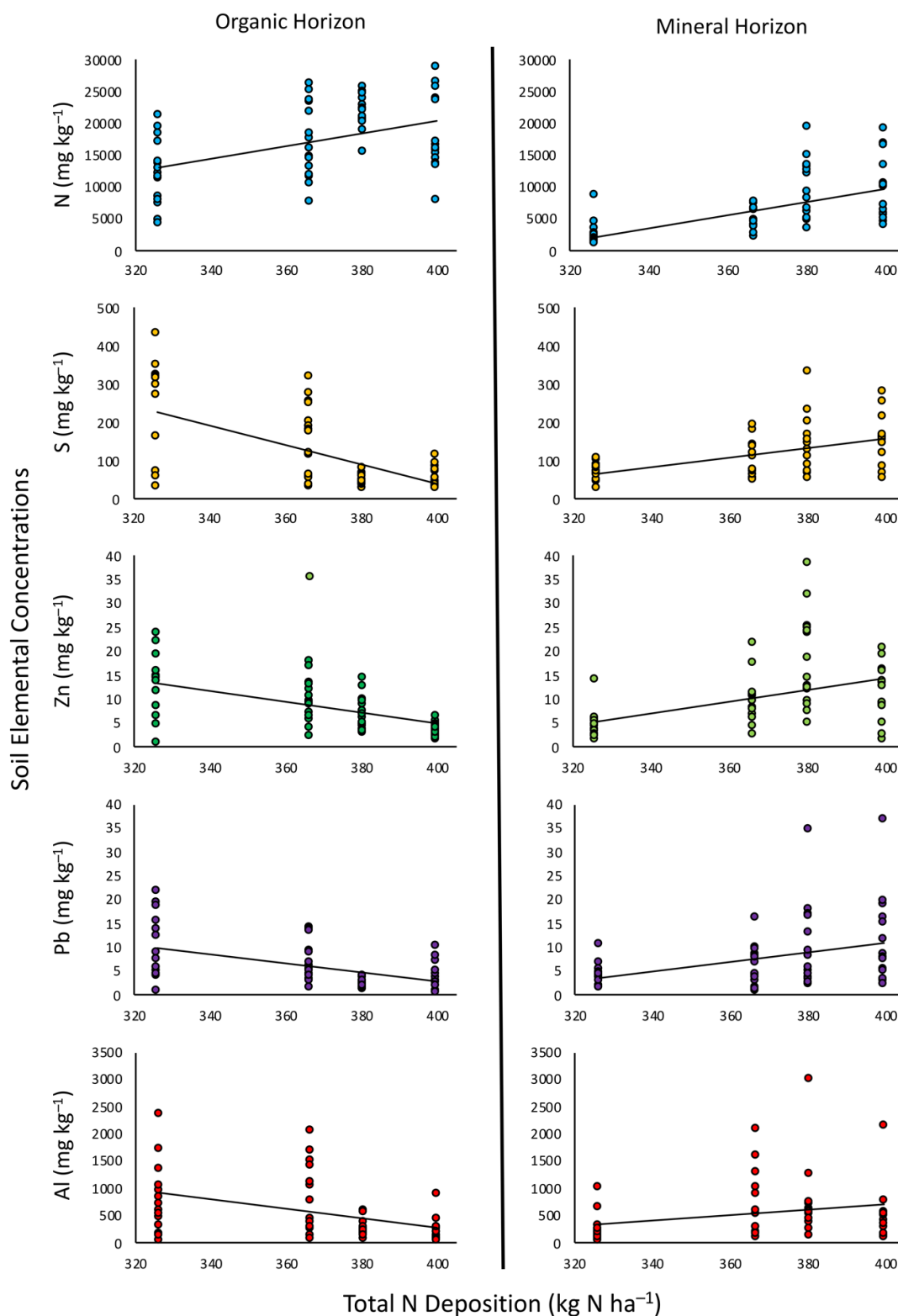


Figure 2. Soil elemental concentrations vs. total estimated total N deposition (kg N ha⁻¹) of selected indicators of deposition within the organic and mineral horizons of montane soils.

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Table 4. Slope coefficients and probabilities for mixed multiple regression for selected elements in montane soils across a modeled N deposition gradient.

Horizon	Nutrient	Deposition Slope (% (% kg N ha ⁻¹) ⁻¹)	Elevation Slope (% (% m ⁻¹) ⁻¹)	P _{Species}
O Horizon	C	1.81*	2.01	0.296
	N	2.00*	2.48*	0.941
	C:N	0.06	−0.42	0.008
	Ca	−3.09**	−6.93***	0.383
	Mg	−3.86**	−4.67*	0.039
	K	−4.59***	−4.98**	0.175
	P	−4.54***	−5.60**	0.182
	Al	−5.73***	−4.03	0.033
	Ca/Al	1.81	−2.93	0.118
B Horizon	C	3.84***	5.19**	0.222
	N	5.98***	3.46*	0.043
	C:N	−1.42***	1.16*	0.173
	Ca	5.01***	−1.57	0.765
	Mg	5.68***	0.785	0.078
	K	4.69***	1.06	0.838
	P	5.14***	−0.41	0.725
	Al	4.66**	1.49	0.155
	Ca/Al	0.45	−3.41	0.181

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.**Table 5.** Slope coefficients and probabilities for mixed model regression for elements in foliage of *A. rubrum*, *B. alleghaniensis*, and *P. rubens* across a modeled N deposition gradient.

Nutrient	Deposition Slope (% (% kg N ha ⁻¹) ⁻¹)	P _{Species}
C	0.086	0.009
N	0.083	<0.001
Ca	−2.198***	<0.001
Mg	−0.308	<0.001
K	−1.248*	<0.001
P	0.076	<0.001
S	0.338	<0.001
Fe	0.964	<0.001
Mn	−0.484	<0.001
Cu	1.142*	<0.001
Zn	0.794	<0.001
B	−1.111	<0.001
Ni	−0.279	<0.001
Sr	−4.285***	<0.001
Al	−0.551	<0.001

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

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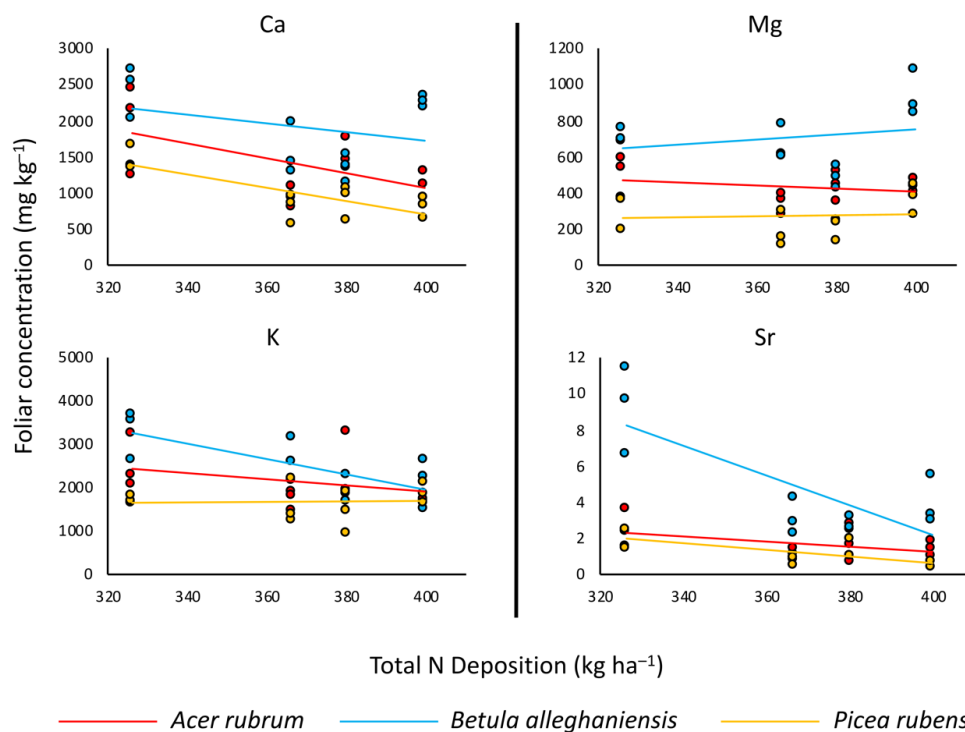


Figure 3. Depletion of selected cations in foliage across a gradient of modeled N deposition.

3.4 Correlations between foliar and soil elemental profiles

Although the tree species were sympatric at all four sites, the foliar-soil nutrient associations differed among them. Pearson product-moment correlations indicated broad differences in foliar element concentrations and their counterparts in each soil horizon (Figure 4). There were no obvious diagonal associations in the matrices in Figure 4, indicating that foliar elemental profiles were broadly independent of organic or mineral elemental availability. For example, while Mn concentrations in *A. rubrum* were positively correlated with both O and B horizon Mn concentrations, Ca, Mg, and Fe were not (Figure 4). For some elements such as K, Al, and Ni in yellow birch, and Ca, Zn, and Sr in red spruce, the correlations are significantly positive in one horizon and significantly negative in the other. This latter pattern indicates that the tree species at these sites preferentially uptake selected elements from different depths of the soil profile.

4. Discussion

The negative effects of soil acidification, especially the depletion of major nutrient cations and the liberation of Al³⁺, are major challenges to plant growth and survival [51,52]. The negative impacts and ecological degradation associated with these effects have been the subject of numerous ecological studies in temperate regions, but nutritional data demonstrating evidence of legacy effects or recovery from N deposition are rarer [53]. In the current study, soil N in both the O and B horizons increased with N deposition, precipitation volume, and elevation, reflecting the confounded nature of these variables in affecting the deposition of N into high-elevation ecosystems, but suggesting that N inputs into high elevation forests continues. In the mid-Atlantic region of the United States, Pb, Cu, and Zn from industrial emissions have been shown to occur in elevated concentrations in the organic horizon, although these concentrations have been declining as total deposition from anthropogenic emissions have declined [54]. In the current study, the concentrations of Zn and Pb (Figure 2), as well as Cu and Ni, increased with N deposition in B horizons, but, as with other cations, declined in the O. The mobility of metals in soils is pH dependent [55], and this migration from the O to the B may reflect

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the broad reductions in current rates of deposition and the movement/retention of historically-deposited heavy metals from the O to subsurface horizons.

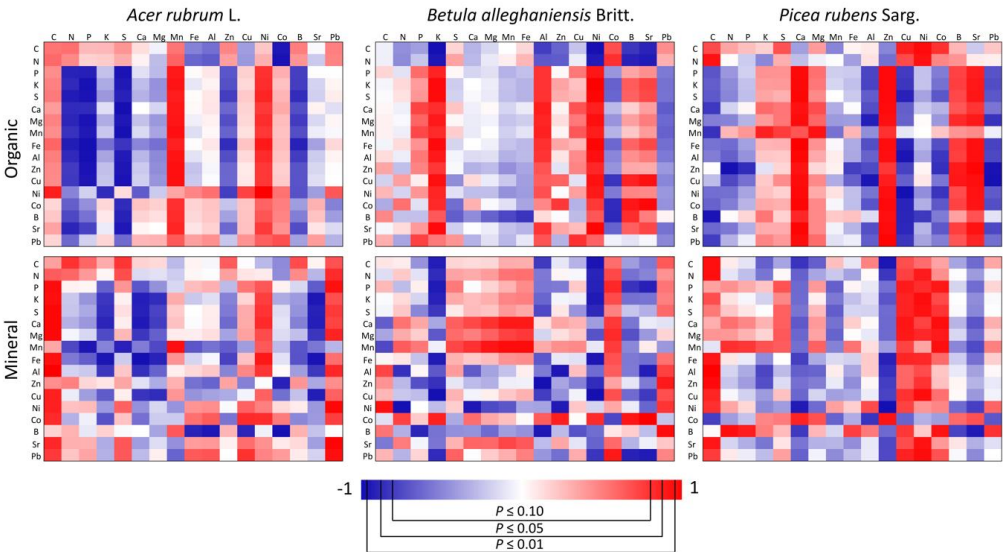


Figure 4. Pearson product-moment correlations for the pairwise comparisons of foliar element values by soil horizon. Color ramp indicates direction of correlation and significance.

The distributions of Mehlich-III extractable soil elements also displayed patterns suggesting that historical acid deposition has depleted cations from O horizons and moved these to B horizons (Table 4), where they may be retained on soil CEC when NO_3^- and SO_4^{2-} mobility is reduced by metal sesquioxides [56]. P also declined in the O horizon with increasing N deposition and increased in the B (Table 4). Depending on the horizons from which trees obtain nutrients for uptake [57–59], these changes may reflect and/or interfere with tree nutrition and productivity. For example, Dijkstra and Smits (2002) noted that uptake of Ca by deep roots of sugar maple (*Acer saccharum*) was responsible for maintaining high Ca cycling beneath maple compared to hemlock (*Tsuga canadensis*) [60]. Elevated Ca in the litter layer beneath maple was associated with high root density, linking Ca cycling and the deployment of roots by maple to retrieve Ca mineralized from litter (Dijkstra and Smits 2002). In the current study, even though the three tree species exhibited characteristic foliar nutrient profiles (Figure 4), we found little evidence that individual tree species were influencing the local distribution of nutrients through the soil profile (Table 4), except for Mg in the O horizon, which was less beneath *A. rubrum*, and N in the B horizon, which was elevated beneath *A. rubrum* (Figure S3a). Increases in Al and reductions in the Ca/Al ratio are often indicators of soil acidification. In the current study, Al concentrations declined in the O with increasing N deposition, but increased in the B horizons (Figure 2, Table 4). Ca/Al ratios remained unchanged overall, but decreased beneath *A. rubrum* (Table 4, Figure S2). The divergent responses beneath the species investigated may reflect differential sequestration of Ca in biomass and/or differential mobilization of Ca and Al by each species (Dijkstra and Smits 2002).

Foliar nutrient profiles indicated that the trees along the currently-studied N deposition gradient exhibited some symptoms of cation depletion, although species varied in this response (Table 5). Ca and Sr declined in the foliage of all species across the N gradient, change in K depended on species, but Mg was unaffected (Figure 3). A comparison of organic horizon soil solution Al, Ca, and Mg and red spruce foliar concentrations in central and southern Appalachia presented similar results as reported here: trees appeared to be nutrient-sufficient and there was no relationship between nutrient status and estimated site differences in acid deposition [61]. In the Great Smoky Mountains of southern Appalachia, soil solution and species foliar Al, Ca, and Mg were not correlated [30]. These studies are consistent with our results, which demonstrate a consistent lack of foliar response to soil

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solution nutrient concentrations (Figure 4). The paucity of field-based red spruce studies in the literature showing nutrient deficiency except in visibly-stressed trees suggests that red spruce may be most vulnerable when foliar cation-leaching from acid deposition is accompanied by very base-poor, poorly buffered soil substrates, as observed for *Picea abies* (L.) Karst. forests of Europe [62].

Although stand-level soil chemistry, resulting from depositional history interacting with local parent material differences, seemed to override tree species effects on soil elemental profiles (Table 4), *A. rubrum*, *B. alleghaniensis*, and *P. rubens* exhibited distinct differences in foliar nutrient concentrations (Figure 4, Figure S3a,b), which may contribute to stand-level nutrient cycling. Pearson product-moment correlations indicate large differences in pair-wise comparisons between foliar element concentrations and their counterparts in each soil horizon (Figure 4). Leaves of *B. alleghaniensis* consistently exhibited the greatest concentrations of nutrients and metals, followed by *A. rubrum* and *P. rubens* (Figure S3a,b). These differences were typically two- to three-fold greater and as high as eight-fold for Zn in *Betula*. Zn hyperaccumulation is a trait known to exist within *Betula* [63]. For some elements, such as K, Al, and Ni in yellow birch and Ca, Zn, and Sr in red spruce, the correlations are significantly positive in one horizon and significantly negative in the other. This could indicate that the tree species at these sites preferentially uptake selected elements from different horizons of the soil profile, which has been observed in other systems [58,64–66]. That these differences did not translate to alterations in local soil concentrations may reflect broad redistribution of litter throughout the stands or efficient resorption by *Betula* before leaf drop. The functional roles of tree species as drivers of biogeochemical differences in high-elevation red spruce stands is supported by the observations of Smith et al. (2016) at these and other sites in central Appalachia [31]. Although there were no effects explained by estimates of historic acid deposition in that study, there were positive effects due to relative importance value of broadleaf deciduous tree species on patterns of N availability: greater N availability from a larger number of broadleaf tree species with lower C:N ratios and higher litter quality diluted any evidence for acid deposition effects on N cycling processes [38]. In the current study, undertaken at a subset of the same sites but sampling beneath specific trees and not at a stand basis [38], soil N levels were sufficiently explained by N deposition, elevation, and/or precipitation volume without evidence of significant species-level effects (Table 3).

Ratios of certain elements can be informative as to source, as well as providing an indication for the presence of processes such as chemical weathering. For example, charge and ionic radius result in Ca and Sr displaying similar behaviors in the plant-soil continuum, with the Ca/Sr ratio effective for integrating soil and tree Ca status [67]. Trees preferentially uptake Ca rather than Sr due to its importance as a macronutrient, while Sr is not known to have any necessary biological function [68]. Plant discrimination of Ca over Sr allows scrutiny of plant Ca availability and source, with lower Ca/Sr ratios a potential indication of low plant-available Ca or interference by Al [69]. In the current study, O and B horizon Ca/Sr ratios increased and foliar Sr declined to a greater degree than Ca as modeled estimates of N deposition increased (Figure 3), also leading to increases in the foliar Ca/Sr ratio in *B. alleghaniensis* and *P. rubens*, but not *A. rubrum*. It thus appears that N deposition is not/no longer limiting Ca or that the trees are extracting Ca from the B horizon where Ca is accumulating along the N gradient.

Soil and foliar elemental profiles for four central Appalachian high elevation forests suggest that historical and ongoing N deposition increases N concentrations of both the O and B horizons, and induces the migration of cations from the O to the B horizon. Although we sampled soils from beneath the canopies of the dominant *A. rubrum*, *B. alleghaniensis*, and *P. rubens*, species effects on soil element concentrations were strongly overshadowed by the differences between the sites across the N deposition gradient. Foliar elemental profiles suggested that a legacy effect of N deposition still influences tree nutrient acquisition, with foliar Ca, K, and Sr declining with increasing N deposition. In addition, the tree species utilized in this study exhibited distinct foliar element profiles, with *B. alleghaniensis* typically accumulating nutrients and metals in excess of two-fold, and up to eight-fold for Zn, compared to *A. rubrum* and *P. rubens*. Despite these differences, species had minimal influence on soil chemical profiles, suggesting that site N deposition, litter mixing, site parent material, and/or litter nutrient dynamics including resorption may dominate soil chemical profiles.

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Supplementary Materials

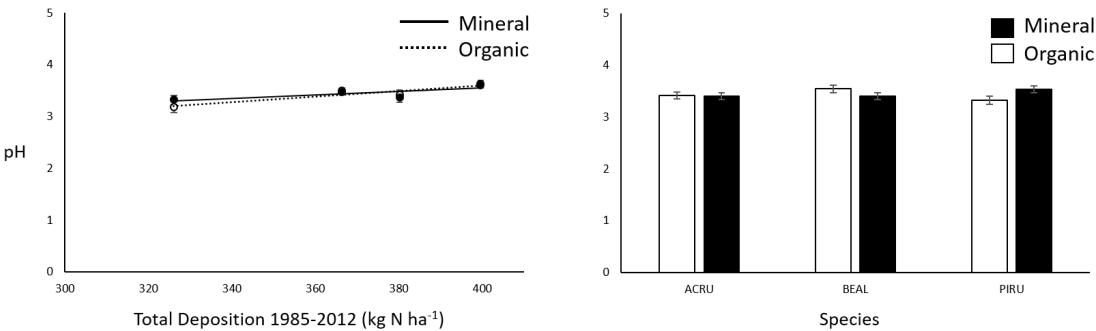


Figure S1. Soil pH as functions of horizon and N deposition (left) and overstory tree species (right).

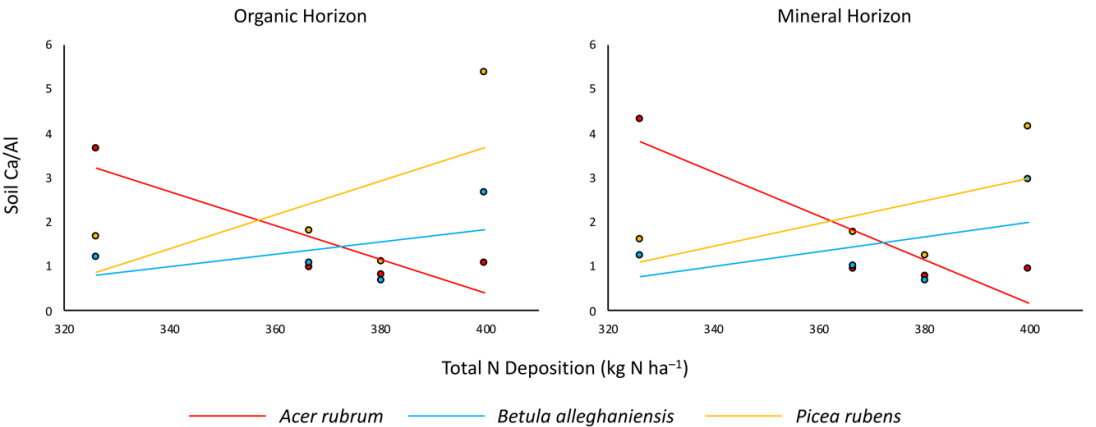


Figure S2. Soil Ca/Al as N deposition and overstory tree species for O (left) and B (right) soil horizons.

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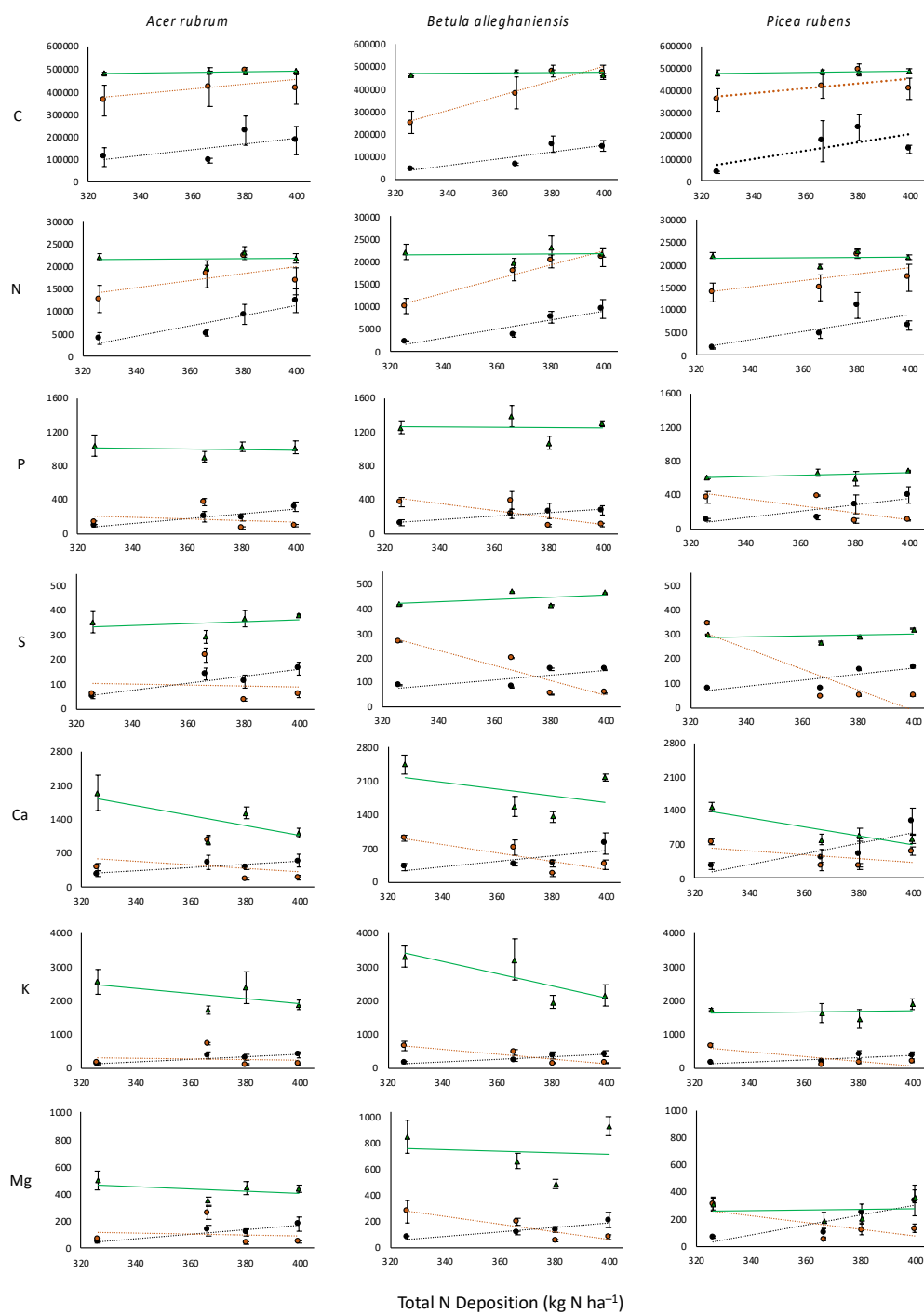


Figure S3a. Soil macro-element concentrations in foliage, and both organic and mineral horizon soils (mg g^{-1}) by forest tree species and modeled estimates of historic N deposition.

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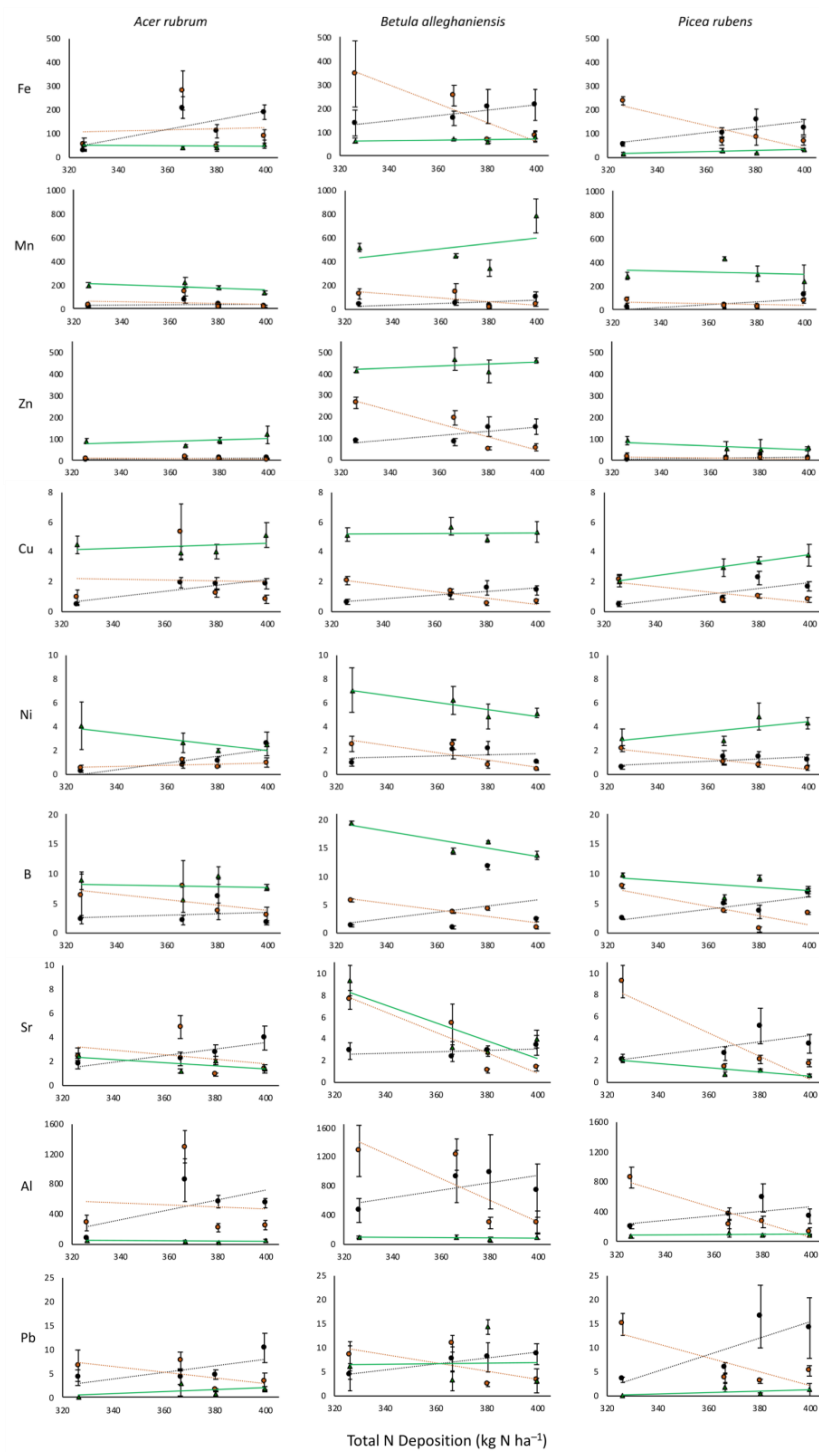


Figure S3b. Soil micro-element concentrations in foliage, and both organic and mineral horizon soils (mg g⁻¹) by forest tree species and modeled estimates of historic N deposition.

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Author Contributions: Conceptualization, P.C.; methodology P.C., J.C., and L.M.; software, P.C.; validation, P.C. and J.C.; formal analysis, P.C. and J.C.; investigation, P.C.; resources, P.C., J.C. and L.M.; data curation, P.C.; writing—original draft preparation, P.C.; writing—review and editing, P.C., J.C. and L.M.; visualization, P.C. and J.C.; supervision, J.C.; project administration P.C. and J.C.; funding acquisition P.C. and J.C.

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

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