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Philip M. Crim 1,2,*; Louis M. McDonald 3; and Jonathan R. Cumming 2

1 Department of Physical and Natural Sciences, The College of Saint Rose, Albany, NY 12203, USA
2 Department of Biology, West Virginia University, Morgantown, WV 26506, USA; jcumming@wvu.edu
3 Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506, USA; lmmcdonald@mail.wvu.edu

* Correspondence: pcrim@strose.edu; Tel.: +1-518-454-2910

Received: 5 September 2019; Accepted: 9 December 2019; Published: 11 December 2019

Abstract: Anthropogenic and industrial emissions 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 impact 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 Brit., and Picea rubens Sarg., were sampled. Bulk soil beneath the canopies of individual trees were collected from the top 15-cm and separated into organic and mineral fractions for analysis. Mehlich-III soil extracts of soil fractions and foliar digests from these trees were subjected to elemental analysis. Soil N concentrations supported the presence of a N deposition gradient: in organic horizon soil fractions, N concentrations were driven by precipitation volume and elevation; whereas in mineral soil fractions, N concentration was explained by modeled N deposition rate and elevation. In organic fractions, significant reductions in Ca, K, and P were evident as N deposition increased, whereas the Ca:Sr ratio increased. Foliar Ca, K, and Sr declined in foliage with increasing N deposition, with concomitant increases in foliar Ca:Sr ratios. 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 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 that 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 indicates 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 and 7.4 kg ha\(^{-1}\) yr\(^{-1}\) from 1985 to 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; and (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.

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% to 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 to 2012. Data for dry deposition of N (HNO$_3$, NO$_3^-$, NH$_4^+$) were available for the years 2000–2012, and were 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].

| Site          | Code  | Location         | Total N Deposition (kg N/ha) | Elevation (m) |
|---------------|-------|-------------------|-----------------------------|---------------|
| Flat Ridge    | FLR   | Slaty Fork, WV    | 399.6                       | 1320          |
| McGowan Mtn.  | MCG   | Dry Fork, WV      | 380.2                       | 1270          |
| Cranberry Glades | CGL | Hillsboro, WV     | 366.4                       | 1160          |
| Little Spruce Bog | LSB | Pembroko, VA       | 326.1                       | 1179          |

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. Despite differences in parent material and soil development, pH exhibits little variation across sites or horizons (Table 1).
Table 1. Soil characteristics of the four high-elevation sites. Soil classifications and descriptions were obtained from the NRCS Soil Survey Geographic (SSURGO) Database [42]. Mean soil pH values for soil fractions at each site (organic O; mineral M) were measured in H₂O using 5-g subsamples of homogenized soil from each sample location and each soil fraction.

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

Soil and foliar samples were collected in July 2014. Five individual trees of *A. rubrum*, *B. alleghaniensis*, and *P. rubens* were selected at each site (Table 2). Only 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) fractions and then composited by soil fraction from each individual tree for each species by site. 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   | 14.0   | 21.4                       | 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 65 °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 (Al, B, Ca, Cd, Co, Fe, K, Mg, Mn, Ni, P, Pb, S, Sr, Zn) following the technique outlined by Zhang et al. [43] and adapted from Mehlich [44]. Extracts were analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES) to determine the concentration of macronutrients, micronutrients, and metals. The soil metals Zn and Pb are often-used proxies for atmospheric deposition [45]. 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 mid-canopy sun leaves with a southeastern to southwestern exposure \([46]\). 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 \([22,47,48]\).

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 \(H_2O\) to remove foliar surface contaminants potentially present on leaves \([49]\). 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 CyclotecTM 1093 sample mill. Ground tissue was digested in 75 mL Teflon digestion tubes containing 4 mL of trace-metal-grade concentrated \(HNO_3\) and 1 mL of 30% \(H_2O_2\). Microwave-assisted digestion was performed using a MARSXpress™ 5 with settings optimized for foliar tissue \([50]\). 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.

Sites were characterized by cumulative total N deposition from 1985 to 2012 rather than mean annual total N deposition due to wide annual variation (Supplementary Materials Figure S1). We used stepwise regression using site variables to evaluate the impacts of N deposition or different site variables on soil elemental profiles using SAS JMP Pro 13 \([51]\). Mixed model analyses of variance (ANOVA) using species nested within sites and N deposition were used to evaluate depositional and species effects on soils and N deposition effects on species foliar elemental profiles. Data were log-transformed to meet assumptions of normality. Further, regressions were undertaken using log-transformed deposition values. The resulting coefficients (slopes) are then interpretable as % change in response for each % change in the independent (deposition) variable \([52]\). Pearson product–moment correlations for the pairwise comparisons of foliar element values by soil fraction were also calculated, and heat maps representing the correlations between foliar element concentrations and their soil counterparts by each fraction were generated in Microsoft Excel.

3. Results

3.1. Indicators of Anthropogenic Deposition Across the Gradient

Soil pH varied significantly by fraction \((O = 3.38, M = 3.50)\), and increased slightly in each fraction across the N deposition gradient (Supplementary Materials Figure S2). Stepwise regression analysis indicated that soil N was primarily influenced by precipitation and elevation in organic fractions and by N deposition and elevation in mineral fractions (Table 3). Other indicators of historic atmospheric deposition, S, Zn, Pb, and Al, were variably influenced by N deposition, precipitation, and elevation (Table 3). Given that N deposition is partially correlated with precipitation volume and elevation, it is not surprising that these three variables interact in various ways to explain observed soil chemical indicators of historic air pollution deposition.

N in soils increased with increasing N deposition in both organic and mineral fractions (Figure 2). In contrast, S in organic fractions declined with N deposition, but increased in mineral fractions with increasing N deposition (Figure 2). Zn concentration declined in organic fractions with modeled N deposition, precipitation, and elevation but increased in mineral fractions across the N deposition gradient (Figure 2). Pb concentrations exhibited a similar pattern, declining with N deposition, precipitation and elevation in the organic fraction; Pb in mineral fractions was positively correlated with total N deposition across the gradient (Table 3, Figure 2). Al in organic fractions declined with deposition, whereas there was no change in mineral fraction Al with N deposition (Figure 2).
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.

| Model Parameter                  | Organic Fraction | Mineral Fraction |
|----------------------------------|------------------|------------------|
|                                  | 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,     | 51.6 | 121.5| 127.3*| 135.8 | 145.6| 91.2 | 82.9 | 120.2| 146.4 | 155.2|
| elevation                        |      |      |      |       |      |      |      |      |       |      |
| R² (best model)                  | 0.30 | 0.46 | 0.32 | 0.36  | 0.38 | 0.29 | 0.41 | 0.17 | 0.25  |      |

Figure 2. Indicators of acidic deposition vs. estimated total N deposition (kg N ha\(^{-1}\)) within the organic and mineral fractions of montane soils.
Percent broadleaf tree in the stepwise regression, following Smith et al. (2016), did not explain 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).

3.2. Deposition and Species Effects on Soil Elemental Pools

Sites exhibited differences in soil element concentrations due to N deposition and these varied by soil fraction, but typically not by species (Table 4). C and N in both organic and mineral soil fractions increased beneath the three species with increasing N deposition, but did not vary between species. C:N ratios did not vary in either soil fraction.

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

| Fraction       | Nutrient | Deposition Slope % (% kg N ha\(^{-1}\)) | \(P_{\text{Species}}\) |
|----------------|----------|----------------------------------------|-------------------------|
| Organic Fraction | C        | 2.30 **                                  | 0.353                   |
|                 | N        | 2.50 **                                  | 0.998                   |
|                 | C:N      | 0.19                                    | 0.881                   |
|                 | Ca       | -4.89 *                                 | 0.797                   |
|                 | Mg       | -4.47                                   | 0.485                   |
|                 | K        | -5.55 *                                 | 0.744                   |
|                 | P        | -5.65 *                                 | 0.684                   |
|                 | S        | -6.86 *                                 | 0.628                   |
|                 | Fe       | -3.87                                   | 0.538                   |
|                 | Mn       | -5.84                                   | 0.572                   |
|                 | Cu       | -4.22 *                                 | 0.945                   |
|                 | Zn       | -5.21 **                                | 0.533                   |
|                 | Ni       | -3.76                                   | 0.553                   |
|                 | Al       | -5.73 *                                 | 0.473                   |
|                 | Sr       | -8.14 **                                | 0.628                   |
|                 | Ca:Al    | 1.81                                    | 0.346                   |
|                 | Ca:Sr    | 2.97 *                                  | 0.671                   |
| Mineral Fraction | C        | 5.89 ***                                 | 0.196                   |
|                 | N        | 6.97 ***                                 | 0.212                   |
|                 | C:N      | -1.06 *                                 | 0.437                   |
|                 | Ca       | 4.56 **                                 | 0.715                   |
|                 | Mg       | 5.86 ***                                 | 0.077                   |
|                 | K        | 4.99 ***                                 | 0.758                   |
|                 | P        | 4.86 ***                                 | 0.418                   |
|                 | S        | 3.59 **                                 | 0.750                   |
|                 | Fe       | 5.24 **                                 | 0.217                   |
|                 | Mn       | 4.34 *                                  | 0.559                   |
|                 | Cu       | 5.59 ***                                 | 0.119                   |
|                 | Zn       | 5.36 **                                 | 0.402                   |
|                 | Ni       | 5.62 **                                 | 0.219                   |
|                 | Al       | 4.66 **                                 | 0.155                   |
|                 | Sr       | 2.21 **                                 | 0.192                   |
|                 | Ca:Al    | -0.24                                   | 0.464                   |
|                 | Ca:Sr    | 2.47 **                                 | 0.791                   |

* \( p \leq 0.10 \), ** \( p \leq 0.01 \), *** \( p \leq 0.001 \).
Evidence for nutrient depletion and redistribution was evident in these montane soils. Significant declines in Ca, K, Cu, Zn, P, and S occurred in the organic fraction as N deposition increased (Table 4). Organic fraction Al and Sr concentration similarly declined with increasing N deposition (Table 4). In contrast, Ca, Mg, K, P, S, Fe, Mn, Cu, Zn, and Ni all increased in mineral fractions with increasing N (Table 4). Declining Ca:Al, a signal of soil acidification, was not noted for these sites. However, soil Ca:Sr ratios, considered an indicator of mineral weathering, increased with N deposition in both soil fractions, reflecting potential differences in uptake and cycling of Ca and Sr in soils beneath these species.

3.3. Species Nutrient Profiles and Deposition Effects on Foliar Elements

Foliar elemental concentrations varied strongly by species (Figures 3–5). *B. alleghaniensis* generally had higher concentrations of nutrient elements, and especially micronutrients and metals, than the other species (Figure 3). *A. rubrum* exhibited intermediate macronutrient levels, but the lowest trace elements of the three species (Figure 3). Across the modeled N input gradient, Ca, K, and Sr concentrations declined in *B. alleghaniensis* with N inputs; Ca and Sr declined with N deposition in *A. rubrum* and *P. rubens* as well, leading to increases in the Ca:Sr ratio in all species (Figure 4).

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 fraction (Figure 5). There were no obvious diagonal associations in the matrices in Figure 5, indicating that foliar elemental profiles were broadly independent of organic or mineral soil elemental availability. For example, while Mn concentrations in *A. rubrum* were positively correlated with both organic and mineral fraction Mn concentrations, Ca, Mg, and Fe were not (Figure 5). 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 fraction 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.

![Figure 3. Foliar elemental profiles by tree species.](image-url)
Figure 4. Depletion of selected cations and changes in Ca:Sr molar ratios in foliage across a gradient of modeled N deposition.

Figure 5. Pearson product–moment correlations for the pairwise comparisons of foliar element values by soil fraction. Color ramp indicates direction of correlation and significance.
3.4. Correlations Between Foliar and Soil Elemental Profiles

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4. Discussion

The negative effects of soil acidification, especially the depletion of major nutrient cations and the liberation of Al$^{3+}$, are major challenges to plant growth and survival [53,54]. 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 more scarce [55]. In the current study, soil N in both the organic and mineral fractions 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 fraction, although these concentrations have been declining as total deposition from anthropogenic emissions have declined [56]. In the current study, the concentrations of Zn and Pb (Figure 2), as well as Cu and Ni, increased with N deposition in mineral fractions, but, as with other cations, declined in the organic fractions. The mobility of metals in soils is pH dependent [57], and this migration from the organic to the mineral fraction may reflect the broad reductions in current rates of deposition and the movement/retention of historically deposited heavy metals from the upper organic to subsurface horizons.

The distributions of Mehlich-III extractable soil elements also displayed patterns suggesting that historical acid deposition has depleted cations from organic fractions and moved these to the lower mineral fractions (Table 4), where they may be retained on soil CEC when NO$_3^-$ and SO$_4^{2-}$ mobility is reduced by metal sesquioxides [58]. 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 organic to the mineral fractions where reactions with the CEC and amorphous hydroxides may retain them, N is still accumulating throughout the soil profile. P also declined in organic fractions with increasing N deposition while increasing in mineral fractions (Table 4). Depending on the horizons from which trees obtain nutrients for uptake [59–61], 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* Marsh.) was responsible for maintaining high Ca cycling beneath maple compared to hemlock (*Tsuga canadensis* (L.) Carr.) [62]. 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 [62]. In the current study, even though the three tree species exhibited characteristic foliar nutrient profiles (Figures 4 and 5), 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 organic fractions, which was less beneath *A. rubrum*, and N in mineral fractions, which was elevated beneath *A. rubrum* (Supplementary Materials Figure S4a). Increases in Al and reductions in the Ca:Al ratio are often indicators of soil acidification. In the current study, Al concentrations declined in organic fractions with increasing N deposition, but increased in mineral fractions (Figure 2, Table 4). Ca:Al ratios remained unchanged.
overall, but decreased beneath *A. rubrum* (Table 4, Supplementary Materials Figure S3). 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.

Foliar nutrient profiles indicated that the trees along the currently studied N deposition gradient exhibited some symptoms of cation deficiency, although species varied in this response (Figure 3). 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 fraction 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 [63]. 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 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 [64].

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 (Figures 4 and 5, and Figure S4a,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 fraction (Figure 4). Leaves of *B. alleghaniensis* consistently exhibited the greatest concentrations of nutrients and metals, followed by *A. rubrum* and *P. rubens* (Figure 5, Figure S4a,b). These differences were typically two- to three-fold greater and as high as eight-fold for Zn in *B. alleghaniensis*. Zn hyperaccumulation is a trait known to exist within *Betula* [65]. 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 soil fraction 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 [60,66–68]. 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 *B. alleghaniensis* 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 [31,69,70]. 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 [71]. 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 [72]. 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 [73]. In the current study, organic and mineral fraction 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 mineral fraction 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 organic and mineral soil fractions, and induces the migration of cations from the organic to mineral horizons. 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 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.

Supplementary Materials: The following are available online at http://www.mdpi.com/2571-8789/3/4/80/s1, Figure S1: Annual variation in total N deposition from 1985 to 2012 at Flat Ridge (FLR), McGowan Mountain (MCG), Cranberry Glades (CGL), and Little Spruce Bog (LSB), Figure S2: Soil pH as functions of soil fraction and N deposition (left) and overstory tree species (right), Figure S3: Soil Ca:Al molar ratios by N deposition and overstory tree species for organic (left) and mineral (right) fractions, Figure S4a: Soil macro-element concentrations in foliage, and both organic and mineral soil fractions (mg g\(^{-1}\)) by forest tree species and modeled estimates of historic N deposition, Figure S4b: Soil micro-element concentrations in foliage, and both organic and mineral soil fractions (mg g\(^{-1}\)) by forest tree species and modeled estimates of historic N deposition.

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

Funding: This study was supported by the West Virginia University Eberly College of Arts and Sciences Academic Affairs Grant for doctoral students.

Acknowledgments: We would like to thank Richard Thomas, Kenneth Smith, and Justin Mathias for their assistance in both introducing these sites and assisting the first author in gaining familiarity with these locations. Kenneth Smith with Richard Thomas devised the site selection procedure using NADP data for his own work at these and other sites in central Appalachia. We are also grateful to the U.S. Forest Service for granting us access to these sites to complete the fieldwork. Taylor Boone was instrumental in assisting with fieldwork and management of samples in the laboratory. Nicole Herbst, Rachel Michaels, and Jennifer Seabourne assisted with organizing and preparing soil samples. Kyle Videtto assisted with processing foliar samples, and Mathew Aldridge and Tyler Davidson assisted with soil extractions.

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

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