Response of foliar nutrients of *Rubus allegheniensis* to nutrient amendments in a central Appalachian hardwood forest

Frank S. Gilliam⁎, Jeffrey D. May, Mary Beth Adams

**Departments of Biological Sciences, Marshall University, Huntington, WV 25705, United States**

**U.S.D.A. Forest Service, 180 Canfield Street, Morgantown, WV 26506, United States**

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**A B S T R A C T**

Foliar nutrient concentrations are often useful in inferring soil fertility and plant-soil interactions. This study examined pre- and post-senescent foliar macronutrients and Mn of *Rubus allegheniensis* to assess potential effects of two nutrient amendments on mineral soil nutrient availability in aggrading hardwood stands: additions of (NH₄)₂SO₄ (simulating increases in acid deposition) and additions of (NH₄)₂SO₄ with dolomitic limestone (simulating mitigation of effects of acidification). Foliar Mn analysis further addressed a prediction of the nutrient redistribution hypothesis, i.e., *Rubus* accumulates Mn in pre-senescent leaves and further concentrates Mn in post-senescent leaves to produce high-Mn litter. The study used a four plot × four block randomized design with three experimental treatments (Control, NS, NS + Lime). Rubus leaves were harvested randomly in plots of all treatment before and after senescence. Experimental treatments appeared to increase availability of N and P, with little effect on K. Simulated acidification decreased availability of Ca and Mg, an effect mitigated for Mg by addition of dolomitic limestone. By contrast, Mn in *Rubus* foliage was up to 100 times more concentrated than in most plant species, and was further concentrated by ~60% during senescence. These latter observations support predictions of the nutrient redistribution hypothesis that *Rubus* redistributes Mn from the rooting zone to surface soil.

**1. Introduction**

Foliar nutrient concentrations typically integrate the balance between nutrient supply in the soil and immediate demand by plants (Barber, 1995; Marschner, 2002; Hobbie, 2015). Consequently, variation in foliar nutrient content of wild plants has long been used by plant ecologists and biogeochemists as an indicator of soil fertility and soil nutrient availability (Garten, 1978; Chapin, 1980). Analyses of foliar nutrients also can enhance our understanding of how factors, such as natural and anthropogenic disturbances, affect nutrient availability. Miller and Watmough (2009) examined foliar chemistry of sugar maple in south-central Ontario, finding that 20-yr declines in foliar sulfur (S) reflected decreases in atmospheric deposition of S. Schreep et al. (2014) found that foliar nitrogen:phosphorus (N:P) ratios in tropical woody species were indicative of the balance of N and P availability in soils. In fact, foliar N:P ratios have been used in a variety of studies of terrestrial ecosystems as indicators of nutrient limitation and other factors, such as nitrogen saturation (Koerselman and Meuleman, 1996; Tessier and Raynal, 2003). Such utility in inferring soil fertility from foliar chemistry, however, may be limited in some cases by species-specific variation in nutrient use among plants. Some plant species are known for their ability to accumulate and hyperaccumulate nutrients, for example, flowering dogwood (*Cornus florida*) and calcium (Ca) (Thomas, 1969), although this is more typically associated with micronutrients (Pollard et al., 2002). Hyperaccumulation is a phenomenon that occurs in a narrow group of angiosperms with an extreme affinity for particular metal and metallloid elements (Baker and Brooks, 1989; van der Ent et al., 2013). Manganese (Mn) is distinctive among hyperaccumulated elements in that it is relatively abundant in many soils and is a plant nutrient essential for photosynthesis (Loneragan, 1988; Fernando et al., 2006, 2010).

Many species of *Rubus* (blackberry), including *R. allegheniensis*, have been shown to accumulate foliar Mn at high concentrations (Kula et al., 2012). Although these species rarely fit the criterion for true hyper-accumulation of Mn (≥10,000 ppm—see van der Ent et al., 2013 for further discussion), foliar Mn concentrations for several *Rubus* species have been reported to be 6000–8000 ppm (Kula et al., 2012; Gilliam et al., 2016a), well above typical ranges for most plant species (50–800 ppm—Fernando et al., 2010). Gilliam et al. (2016a) found

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⁎ Corresponding author.

E-mail address: gilliam@marshall.edu (F.S. Gilliam).

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that > 20 yr of whole-watershed additions of N increased both concentrations and spatial heterogeneity in extractable soil Mn at Fernow Experimental Forest (FEF), WV. By superimposing mean cover of *Rubus* (hereafter, *Rubus*) in permanent sample plots to kriged maps of soil Mn, they found that cover of *Rubus* and the patchiness in soil Mn were highly spatially correlated (Fig. 1). As high *Rubus* cover was closely coincident with discreet areas of high soil Mn, they proposed a hypothesis—the nutrient redistribution hypothesis—to explain observed patterns.

This hypothesis is based on the observation that soil Mn is more soluble under acid conditions (Barber, 1995; Blake and Goulding, 2002), and that nitrification in excess of uptake of NO₃⁻ by plants is acidifying (Barber, 1995; Marschner, 2002). Accordingly, the N treatment at FEF not only increased soil acidity and extractable NO₃⁻ pools (Gilliam, 2014), but also enhanced mobility and availability of Mn. Although Gilliam et al. (2016b) found that *Rubus* cover increased sensitively to N added to a whole watershed at FEF, Walter et al. (2016) demonstrated that *Rubus* at FEF responds interactively to both N and light. Despite that the experimental watershed has a closed canopy, there persists notable heterogeneity in light availability formed by canopy gaps, with the gap fraction within high-*Rubus* plots being seven times greater than on low-*Rubus* plots (Fig. 1). Thus, the hypothesis predicts that uptake and foliar accumulation of Mn by *Rubus*, followed by subsequent release of Mn during decomposition (Keiluweit et al., 2015), will redistribute extractable Mn to surface soil from throughout the rooting zone, which can be as deep as 0.5 m for *Rubus* (Böhm, 1979). Although nutrient redistribution has been reported for dominant tree species both in native forests and plantations for macronutrients and Mn (Thomas, 1969; Jobbágy and Jackson, 2004), this is, to our knowledge, the first suggestion that nutrient redistribution can be facilitated by a forest understory species.

The effects of acid deposition on forest soil fertility comprise a well understood phenomenon (Johnson and Lindberg, 1992; Driscoll et al., 2003; Gilliam, 2016). Because increased acidity generally caused...
leaching losses of base cations, which historically led to soil acidification and decreased fertility, lime additions were initiated as early as the late 1980s in some forests as a mitigation strategy for impacted stands (Driscoll et al., 1996; Cho et al., 2010) to both neutralize excess soil acidity and restore base cation fertility.

The purpose of this study was to examine pre- and post-senescence foliar macronutrients and Mn of Rubus to gain further insight into the effects of two nutrient amendments on soil nutrient availability in an aggrading central Appalachian hardwood forest. The first amendment (additions of N and S) was to simulate increases in acid deposition, whereas the second amendment (Mg and Ca) was to simulate a treatment (dolomitic limestone) to mitigate effects of acid deposition (Driscoll et al., 1996). In addition, we use foliar Mn analysis to address a prediction of the nutrient redistribution hypothesis, namely that Rubus accumulates Mn in pre-senescent leaves and further concentrates Mn in post-senescent leaves to produce high-Mn litter.

For the latter purpose, we took advantage of archived, but previously unanalyzed, samples of Rubus foliar material that were initially taken as part of the National Long-term Soil Productivity (LTSP) Study at our research site (see Methods for details). These samples were dried, ground, and stored following standard archiving protocol (Miller and Watmough, 2009).

2. Methods

2.1. Study site and experimental design

The study was carried out at FEF, located in Tucker County, West Virginia (39° 03' 15"N, 79° 49' 15"W), a ~1900 ha area of the Allegheny Mountain section the unglaciated Allegheny Plateau. This is part of long-term, on-going research, established in 1996, that is affiliated with the National Long-term Soil Productivity (LTSP) Study (Adams et al., 2004; Powers et al., 2005). The LTSP Study is a network of sites to evaluate impacts of timber management on soil productivity and sustainability of forest stands.

Common tree species at this site are mixed hardwoods, including sweet birch, yellow poplar, black cherry, and red maple. Mineral soils are generally acidic and moderately fertile and of the Calvin, Berks, or Hazleton series (loamy skeletal, mixed mesic Typic Dystrochrepts). Mean soil pH was 4.24, while C and N were 6.58 and 0.42%, respectively. Extractable Ca, Mg, K, and Al were 0.54, 0.18, 0.33, 3.52 cmol c/kg, respectively; CEC was 28.70 cmol c/kg (Adams et al., 2004). Depth of rooting zone at this site generally varies from 45 to 100 cm.

The LTSP at FEF was initiated as a four plot × four block randomized design including three experimental treatments and one uncut area per block (Fig. 2). Because this work used foliar material from Rubus and because Rubus cover is light-limited and minimal under forest canopies (Walter et al., 2016), uncut areas were not included in this study (Walter et al., 2017). All plots were initially whole-tree harvested in the winter of 1996 (Adams et al., 2004). Since then, four plots have been hand-fertilized with (NH4)2SO4 at a rate of 35 kg N ha−1 yr−1 (NS), four other plots have been fertilized with (NH4)2SO4 at the same rate and treated with dolomitic limestone at a rate of 22.5 kg Ca++ ha−1 yr−1 (NS + lime); four additional plots have been allowed to regrow naturally with no chemical additions (Control). Each growth (measurement) plot is 0.2 ha, with another 0.2 ha in the treated buffer. Total plot size is 0.405 ha (Fig. 2).  

2.2. Field sampling and laboratory analysis

Foliar material of Rubus was sampled at two times periods—prior to leaf senescence (hereafter, pre-senescence) and during the later period of leaf senescence before abscission (hereafter, post-senescence). Pre-senescence sampling took place in late June of 2000, whereas post-senescence sampling took place in mid to late October 2000. This sampling period (i.e., four years post-harvest) coincides with maximum cover of Rubus (Kochenderfer and Wendel, 1983), and prior to the rapid decline in Rubus cover as the forest canopy develops, creating light limitations (Walter et al., 2016). During all sampling, Rubus leaves were hand-harvested from random plants using latex gloves in each of the 12 experimental plots until enough foliar material was obtained for drying, grinding, and analysis.

Upon return to Marshall University, all foliar material was oven-dried at 50°C overnight and ground in a Wiley mill to pass a 40-mesh screen. Dried and ground samples were archived under controlled conditions until later analysis (Miller and Watmough, 2009). Samples were analyzed in 2015 at the University of Maine Soil Testing Service and Analytical Laboratory for analysis for N, P, Ca, Mg, K, and Mn. Total Kjeldahl N was determined with autoanalysis following block digestion with H2SO4 and K2SO4/CuSO4; NBS1 572 Citrus Leaf was used as standard. All other elements were analyzed with plasma emission spectrophotometry following dry ashing and extraction with HCl and HNO3.

2.3. Data analysis

Means were compared for significant differences among treatments and sample times (pre- versus post-senescence) using ANOVA and post hoc least significant difference tests (Zar, 2010). A priori significant differences were accepted for all statistical tests at P < .10.

3. Results

Foliar N concentrations in Rubus varied significantly with both treatment and sample time, being higher in both NS and NS + Lime plots than the Control in pre-senescent leaves, and higher in NS plots in post-senescent leaves. Foliar N was sharply lower in post-versus pre-senescent leaves across all three plot types (Fig. 3). Across all treatments, post-senescent leaves averaged 38% lower N than pre-senescent leaves.

Patterns for foliar P concentrations were similar to those for foliar N. Although mean concentrations were higher for both treatments than the Control in pre-senescent leaves, neither was significantly different, as was the case for post-senescent leaves. As with N, foliar P was lower in post-versus pre-senescent leaves for all treatments (Fig. 4), averaging 40% lower across treatments.

Foliar K concentrations did not vary significantly across treatment in either sampling period. Although generally lower in post- versus pre-senescent leaves, this difference was statistically significant only for the NS + Lime treatment (Fig. 5).

The pattern of response in foliar Ca concentrations contrasted with N, P, and K, being significantly lower in treatment versus Control plots in pre- and post-senescent leaves. Also, foliar Ca was generally higher in post- versus pre-senescent leaves, but this was only significant for the Control (Fig. 6).

The response of foliar Mg concentrations was inconsistent, relative to the previous analytes, with respect to experimental treatments and variation with sample time. Means were significantly lower in NS plots than the Control, whereas NS + Lime concentrations did not differ significantly from the Control in pre-senescent leaves. In post-senescent leaves, foliar Mg for NS + Lime was significantly higher than both Control and NS plots. Means were significantly lower in post-versus pre-senescent leaves for Control and NS + Lime plots, but not for the NS treatment (Fig. 7).

Manganese was distinctive among all foliar nutrients in that it was consistently significantly higher in post-versus pre-senescent leaves, regardless of treatment. Although foliar Mn was significantly lower for pre-senescent leaves in NS + Lime compared to NS treatments, neither treatment differed significantly from the Control (Fig. 8). There were no statistically significant differences in post-senescent leaves.
4. Discussion

The results of this field experiment are interpreted in two ways. First, it will be assumed that nutrient concentrations of pre-senescent Rubus leaves reflect relative levels of available nutrients in the upper mineral soil (Wang and Klinka, 1997; Schreeg et al., 2014; Hobbie, 2015). Thus, variation in foliar nutrients among treatments should be generally indicative of treatment effects on soil nutrient availability of (1) simulated acid deposition (NS) and (2) mitigation of soil nutrient response to acidity (NS + Lime). Second, changes in nutrient levels between sample times (pre- versus post-senescence) is assumed to be indicative of the dynamics of nutrient resorption/accumulation by leaves (Killingbeck, 1996; May et al., 2005; Reed et al., 2012).

4.1. Effects of experimental treatments on foliar nutrients

Data suggest that both amendments increased N availability, an unsurprising result considering that both involved the direct additions of an available form of N as (NH4)2SO4. Although the differences were not significant (P > .10), a similar pattern was found for foliar P as for foliar N. Consistently lower concentrations of N and P in post- versus pre-senescent leaves, regardless of treatment, is evidence of resorption of both nutrients, a common phenomenon for mobile nutrients as a mechanism for nutrient conservation of potentially growth-limiting nutrients (Killingbeck, 1996). Gress et al. (2007) found compelling evidence for a P limitation induced from the whole-watershed fertilization treatment at FEF, which may be related to alleviation of N limitation. Nutrient resorption has been shown to enhance plant fitness (May and Killingbeck, 1992; May et al., 2005), responding both to internal nutrient status and several environmental factors (May and Killingbeck, 1995). Although sampling time differences were not significant for Control and NS treatments, the same general pattern of lower concentrations post- versus pre-senescence suggests resorption for K (Fig. 5). Alternatively, given its highly mobile nature in leaves,
much of this difference may have arisen from leaching from foliar tissue. For example, K+ in throughfall collected beneath plant canopies can be up to 20 times greater than in wetfall encountering the top of the canopy (Sigmon et al., 1989).

Calcium and Mg are similar in many respects in mineral soil, e.g., both are divalent base cations with generally similar mineralogy (Burns et al., 2000; Sheldon et al., 2002). In plants, however, they exhibit widely contrasting characteristics. In addition to several functions within plant cells, Ca is used with pectic acid to bind cell walls in plant tissue (Hepler, 2005); Mg is an essential part of the structure of chlorophyll (Fiedor et al., 2008). Another difference, one which is relevant to results of this study, regards mobility in plants. In contrast to Ca, which is relatively immobile once it is in place in plant tissue, Mg is both mobile and transported readily via phloem (Steucek and Koontz, 1970). This explains, in large part, differences between the two base cations in pre- versus post-senesence foliar concentrations, especially for the Control, i.e., Mg was effectively resorbed, whereas Ca was not (Figs. 6 and 7).

Consistently lower concentrations of both foliar Ca and Mg in NS versus Control plots suggests that additions of (NH4)2SO4 did, indeed, simulate acidic deposition by causing leaching of these ions from the rooting zone, a phenomenon wide-spread among impacted forests of the eastern United States (Rosi-Marshall et al., 2016). Wallenstein et al. (2006) demonstrated high rates of nitrification for these plots from approximately the same time frame, and Gilliam et al. (in review) reported similar results in mineral soil of watersheds at FEF adjacent to the LTSP study plots. Because net nitrification in excess of plant uptake of NO3− is an acidifying process in soil (Barber, 1995), additions of (NH4)2SO4 likely mobilized Ca and Mg via increased acidification and facilitated base cation leaching with NO3−. It is not clear why additions of dolomitic limestone in the NS + Lime treatment mitigated this response for Mg, but not for Ca. Based on the formula for dolomite—CaMg(CO3)2—ratios of added Ca:Mg theoretically are 1:1 (Burns et al., 2000), although great compositional variability can exist, depending on the source of dolomite (Drits et al., 2005). Alternatively, it is also possible that Ca is available in sufficient amount in the soil, but Mg is not.

Foliar Mn in pre-senescent leaves ranged from ∼3000 to 5000 ppm, whereas values for post-senescent leaves were ∼6000 to 7500 ppm. This is in sharp contrast to typical foliar Mn concentrations for most other plant species which generally range from 50 to 800 ppm (Fernando et al., 2010). Although the difference was not significant for pre-senescent foliage between Control and NS treatments for Mn, results suggest that soil acidification may have caused some mobilization of Mn in the soil (Fig. 8), a response demonstrated for FEF watersheds (Gilliam et al., 2016a) and well-documented in other studies (Hue et al., 2001; Blake and Goulding, 2002). Certainly, addition of lime appeared to significantly decrease Mn uptake, as indicated by lower foliar Mn in NS + Lime versus NS plots (Fig. 8). In contrast to the macronutrients,
foliar Mn was consistently higher in post-versus pre-senescent leaves, independent of treatment. Manganese further contrasts with macronutrients regarding mobility, being initially highly mobile, yet largely immobile in foliar tissue (Loneragan, 1988). Xu et al. (2006) found that the distribution of foliar Mn in *Phytolacca acinosa*, a Mn hyperaccumulator, was controlled primarily by transpiration rate with Mn found in high concentrations in xylem tissue. Once it reached the leaves, however, it did not easily remobilize, a conclusion supported by our findings (Fig. 8).

4.2. Nutrient redistribution hypothesis

As originally articulated in Gilliam et al. (2016a), the nutrient redistribution hypothesis addresses the levels and spatial patterns of extractable soil Mn in response to chronic experimental additions of N to an entire watershed (WS3) at FEF, i.e., that N additions increased both overall concentrations of extractable Mn, as well as its spatial heterogeneity, in surface mineral soil of WS3 (Fig. 1). Although additions of N likely had notable direct effects on Mn mobility in the soil, the hypothesis states most of the pattern observed in Fig. 1 arose from N-mediated increases in *Rubus* that, in turn, redistributed Mn from the depths of the rooting zone to surface soils. Given that *Rubus* displays a significant light/N interaction with respect to growth response (Walter et al., 2016), spatial patterns of *Rubus* cover likely resulted from heterogeneity in light availability to the forest floor. High-*Rubus* plots (20–37% cover) received 7× more light via canopy gaps than did low-*Rubus* plots (1–6% cover) (Fig. 1). Furthermore, additions of N to WS3 increased foliar Mn in *Rubus* from ∼3500 to ∼5500 ppm Mn (Gilliam et al., 2016a).

It has been known for some time that dominant tree species are capable of redistributing macronutrients from tree rooting depths to surface soils in both native and plantation forests (Thomas, 1969; Jobbágy and Jackson, 2004). Furthermore, using afforestation of native temperate humid grassland in the Pampas of Argentina with *Eucalyptus* plantations as an experimental system, Jobbágy and Jackson (2003) described this phenomenon for Mn. By comparing grasslands and adjacent plantations of up to 100 yr old with identical soil types (yet contrasting distributions of macro- and micronutrients), they demonstrated that Mn availability in surface soils was enhanced threefold via redistribution by *Eucalyptus* roots.

Implicit in our *Rubus*-based hypothesis is the assumption that *Rubus* both accumulates Mn in foliar tissue during the growing season and then further concentrates it during autumn senescence. Accordingly, we sought to employ a part of our study to address this experimentally. Our data clearly support the first stipulation, i.e., that *Rubus* accumulates Mn in leaves. Whereas pre-senescent leaves ranged from ∼3000 to 5000 ppm Mn, typical foliar Mn concentrations in most plant species range from 50 to 800 ppm (Fernando et al., 2010). Working in experimental watersheds adjacent to the LTSP plots at FEF, Gilliam et al. (1994) found values of 800–1500 ppm Mn for combined herb layer species.

Foliar Mn in post-senescent leaves ranged from ∼6000 to 7500 ppm, approaching the minimum criterion for a plant species to be considered a hyperaccumulator (10,000 ppm Mn; van der Ent et al., 2013). Thus, across all treatments, foliar Mn increased by 55% during leaf senescence, the only nutrient in our study to exhibit such a pattern. This provides firm support for the second stipulation of the nutrient redistribution hypothesis, i.e., that *Rubus* concentrates Mn in post-senescent foliar tissue.

Other work with Mn in plant tissue is further compatible with this scenario. For example, Berg et al. (2007) examined decomposition of foliar material from a wide variety of plant species and climatic regimes (boreal, temperate, and Mediterranean) and found that the best predictor of loss of litter mass was Mn concentration. They further concluded that this relationship is stronger among species with high foliar Mn. This suggests that a positive feedback may exist for *Rubus* at our site. That is, not only do N-mediated increases of *Rubus* cover increase mobility and uptake of Mn, but that further concentration of Mn in senesced leaves enhances decomposition and release of Mn to surface soils. In addition, examining distribution of Mn in parts of the leaf (e.g., midrib, mesophyll), Xu et al. (2006) found highest foliar Mn in the epidermis, further enhancing rapid release during decomposition.

In conclusion, analysis of foliar nutrients of *Rubus* provided insights into the effects of simulated acid deposition and acid mitigation on nutrient availability/uptake. These treatments appeared to increase uptake of N and P, with little effect on K. Simulated acidification decreased uptake of Ca and Mg, likely from acid-induced leaching, an effect apparently mitigated for Mg by addition of dolomitic limestone. Furthermore, pre-versus post-senescence data suggest effective sorption for N and P. By contrast, foliar Mn, which was up to 100 times higher than in most plant species, was concentrated by ∼55% during senescence. These latter observations support predictions of the nutrient redistribution hypothesis that *Rubus* has the ability to redistribute Mn from the rooting zone to surface soil.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2018.01.022.

References


