

Response of soil fertility to 25 years of experimental acidification in a temperate hardwood forest

Frank S. Gilliam<sup>\*1</sup>

Mary Beth Adams<sup>2</sup>

William T. Peterjohn<sup>3</sup>

<sup>1</sup>Department of Biology  
University of West Florida  
Pensacola, FL 32514

<sup>2</sup>Forest Sciences Laboratory  
U.S.D.A. Forest Service  
Morgantown, WV 26505

<sup>3</sup>Department of Biology  
West Virginia University  
Morgantown, WV 26506

Corresponding author: [fgilliam@uwf.edu](mailto:fgilliam@uwf.edu)

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Abstract

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The effects of enhanced acid deposition from the atmosphere, and the elevated inputs of nitrogen (N) associated with it, are evident even in remote areas of the North America. Critical loads for N in the U.S. are widely exceeded for many ecosystem types. This is especially the case for forests, where excess N has led to a variety of deleterious effects, including declines in biodiversity, a response that will likely require considerable time for recovery. The purpose of this study was to determine the response of plant nutrient availability in the upper portion of the mineral soil to 25 years of experimental acidification/N addition in a central Appalachian hardwood forest ecosystem. We hypothesized that chronic additions of  $(\text{NH}_4)_2\text{SO}_4$  increased available N pools ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ), decreased soil pH, phosphorus, and base cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{K}^+$ ), increased micronutrients ( $\text{Mn}^{2+}$  and  $\text{Fe}^{2+}$ ), and increased levels of phytotoxic  $\text{Al}^{3+}$ . Results generally supported these predictions, except that  $\text{Mn}^{2+}$  did not exhibit a significant response. Earlier work on the same plots found no response of any of the extractable nutrients to 3 years of treatment; yet after 25-years, our results suggest that impacts are apparent in surficial portions (top 5 cm) of the A horizon. We surmise that impacts in the surface soils may have lagged behind the onset of acidification treatments or that several years of treatment were required to overcome pre-existing differences in soil ions. In general, current findings confirm that  $(\text{NH}_4)_2\text{SO}_4$  treatments have lowered the pH, enhanced levels of exchangeable  $\text{Al}^{3+}$ , and increased stream-water exports of  $\text{NO}_3^-$  and base cations—a process which further acidifies soil. The combination of these changes in surface soils, where there is a high proportion of fine roots, may contribute to the reduced growth and competitiveness of some hardwood species in the acidified site.

**Key words:** eastern deciduous forests, nitrogen saturation, soil acidification, base cation leaching, aluminum toxicity

## Core Ideas

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1. Long-term acidification and excess nitrogen (N) can alter surface soil fertility of forest ecosystems.
2. Previous work at our site found no effects after 3 yr of experimental acidification on soil nutrients.
3. This study found notable change in nutrient availability after 25 yr of acidification.
4. Concentrations of available N, iron, and aluminum increased, whereas pH and base cation concentrations decreased.
5. Results have implications for management of impacted forests based on interspecific variation in nutrient demands.

The efficacy of the Clean Air Act in the United States has led to notable declines in acidic deposition, including atmospheric deposition of N, although much of the latter is in the form of oxidized N, as reduced N has increased in some regions (Du et al. 2014, 2019, Gilliam et al. 2019). Deposition of N remains high in parts of the conterminous U.S. and beyond, with the biogeochemical signature of elevated N being evident even in remote areas of the Northern Hemisphere (Holtgrieve et al. 2011, Vet et al. 2014). For areas where chronic atmospheric deposition of N has led to N saturation—wherein the supply of available N exceeds plant and microbial demand for N (Aber et al. 1998)—excess N still represents a chronic threat to ecosystem structure and function. This has been especially the case for

temperate forest ecosystems, with a high spatial coincidence of dense human populations and elevated levels of acid and N deposition (Gilliam 2016). Critical loads for N in the U.S. are still widely exceeded for many ecosystem types, particularly forests (Pardo et al. 2011), leading to a variety of deleterious effects, including loss of biodiversity (Clark et al. 2013, 2019b, Simkin et al. 2016). Also, recovery of acidified and N-impacted sites may require extended periods of time (Stevens 2016, Schmitz et al. 2019, Gilliam et al. 2019), especially in locations where base cations, such as  $\text{Ca}^{++}$ , have been depleted by accelerated  $\text{NO}_3^-$  leaching (Likens et al. 1996, Likens et al. 1998). Thus, understanding the potential biogeochemical responses of forest ecosystems to chronically-elevated acidification and N inputs still remains essential.

In addition to evidence that symptoms of N saturation have developed on a long-term reference watershed at Fernow Experimental Forest, WV (FEF) (Peterjohn et al. 1996), an on-going experiment at this site—the Watershed Acidification Study (WAS; Adams et al. 2006a)—has examined the response of a small (35 ha) forested watershed to aerial applications of  $(\text{NH}_4)_2\text{SO}_4$  (Figure 1). To date, results have demonstrated numerous treatment-mediated changes in plant communities (e.g., Gilliam et al. 2016a, b, 2018) and watershed biogeochemistry (e.g., Edwards et al. 2006), but some of these changes have taken considerable time to emerge.

For example, based on permanent plots in the three watersheds that comprise the WAS, Gilliam et al. (1994) was unable to detect a response in the levels of exchangeable soil ions in 1991, after three years of experimental additions of  $(\text{NH}_4)_2\text{SO}_4$ . This delay was unexpected considering that (1) treatment-mediated increases in stream  $\text{NO}_3^-$  had already commenced, along with notable increases in stream concentrations of base cations, as well as similar results for soil solutions (Edwards et al. 2006, Adams et al. 2006b), and (2) studies in

other hardwood forests have shown relatively rapid responses to experimental manipulations such as ours (Moore and Houle 2009, 2013).

Soils were sampled for nutrients in some of these watersheds were in 1994 (Adams et al. 2006b), 1995 (Tepp 1995), 2000 (Odenwald 2002), and 2002 (Adams et al. 2006b). There are also a limited number of pre-treatment data from soils collected in 1988. By the time the samples were collected for this study in 2015, WS 3 had undergone treatment for 25 years, or more than eight times longer than when the initial comparison was done in 1991. Although these studies differed in methodology and sampling locations, they suggest that patterns of change in the surface soils have been emerging as the experimental treatment continued, especially a decrease in soil pH and base cation availability (Figure 2).

To more conclusively assess whether detectable changes in soil fertility have emerged after 25 years of whole-watershed acidification, in this study we measured extractable ions in surface mineral soils collected from 7 of the 15 plots where soils were collected in 1991 (Gilliam et al. 1994). We also expanded on the earlier study by measuring extractable Al because of its potential influence on plant root dynamics, nutrient uptake, and forest health.

Changes in soil fertility, especially under deposition of excess acidity, often occur via leaching of inorganic nutrients through the soil column and into streams. Thus, a continuous record of stream chemistry may help us assess, and deepen our understanding of, apparent temporal patterns inferred from periodic measurements of soil nutrients as they respond to chronic acid deposition. Accordingly, along with soil data from these sites, we also present a unique combination of nearly 30 years of pH, electrical conductivity, and ionic concentrations in stream flow for the same treatment period.

We then used these measurements to test the following four hypotheses: Twenty-five years of acidification treatment will (1) increase pools of inorganic N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ), (2) decrease soil pH, P, and base cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{K}^+$ ), (3) increase extractable

micronutrients ( $\text{Mn}^{2+}$  and  $\text{Fe}^{2+}$ ), and (4) elevate levels of phytotoxic  $\text{Al}^{3+}$ . In addition, we were interested in determining how quickly changes in surface soils might be reflected in altered stream-water chemistry.

## Methods

### Study site

This study comprises over a quarter century of on-going research (the WAS—Adams et al. 2006b) that has focused on the response of a temperate hardwood forest ecosystem to experimental soil acidification at FEF, a ~1900 ha research area in the Allegheny Mountain section on the unglaciated Allegheny Plateau, and located in Tucker County, West Virginia ( $39^{\circ} 03' 15''\text{N}$ ,  $79^{\circ} 49' 15''\text{W}$ ). Mean precipitation at this site is  $\sim 1430 \text{ mm yr}^{-1}$ , relatively evenly distributed seasonally (Kochenderfer 2006), and increasing with higher elevation (Gilliam and Adams 1996).

Consistent with the earlier study (Gilliam et al. 1994), three watersheds were used in this study: WS3, WS4, and WS7 (Figure 1). As the treatment watershed, WS3 has received aerial additions of  $(\text{NH}_4)_2\text{SO}_4$  since 1989. WS4 and WS7 serve as unfertilized reference watersheds. Watershed soils are primarily Inceptisols of the Berks and Calvin series, both of which are loamy-skeletal, mixed, mesic Typic Dystrochrepts. These soils are derived from sandstone and shale, and are mostly coarse-textured sandy loams that are well-drained, ~1 m in depth, and are generally acidic (Table 1) and high in organic matter (Adams et al. 2006a).

Experimental additions of  $(\text{NH}_4)_2\text{SO}_4$  to WS3 were made three times per year, administered by either rotary- or fixed-wing aircraft. March and November applications are  $\sim 7 \text{ kg/ha}$  of N, whereas July applications are  $\sim 21 \text{ kg/ha}$  N. The total annual additions reflect a rate approximately twice the ambient rates of N and S deposited as throughfall on the watersheds in the early 1980's (Adams et al. 2006a). WS3 and WS7 support even-aged, 45 yr-old hardwood stands, with stand initiation on WS3 arising from clearcutting the watershed

in 1969, and stand initiation on WS7 arising from release in 1969 from herbicide treatment following clearcutting between 1963 and 1967. WS4 supports an even-aged hardwood stand ~100 yr old (Kochenderfer 2006).

Stands on all watersheds are dominated by mixed hardwood species, including sugar maple (*Acer saccharum* Marsh.), sweet birch (*Betula lenta* L.), American beech (*Fagus grandifolia* Ehrh.), yellow poplar (*Liriodendron tulipifera* L.), black cherry (*Prunus serotina* Ehrh.), and northern red oak (*Quercus rubra* L.) (Kochenderfer 2006). Despite stand age and management differences, the composition of the herb layer community was initially similar between watersheds (Gilliam et al. 1994). Prominent species were *Viola* spp., *R.*

*alleggheniensis*, mixed ferns, and seedlings of *Acer pensylvanicum* L. and *A. rubrum* L.

During the course of the experiment, *R. alleggheniensis* increased significantly on WS3 and WS4, but especially on treatment WS3 where it now represents ~50% of total herb-layer cover (Gilliam et al. 2016b).

### Field sampling and laboratory analyses

In July 2015, mineral soil was sampled to a depth of 5 cm by hand trowel at five points within each of seven circular 0.04-ha plots in each watershed. As this paper focuses on response of plant-available nutrients to experimental treatments, we confined our sampling to surface mineral soils where fine roots (Jackson et al. 1996) and the availability of most nutrients (Jobbágy and Jackson 2001) is typically most abundant. Also, these are the same depth and plot locations used in on-going, long-term investigations into effects of acidification on soil N dynamics via *in situ* incubations (i.e., buried bags) (Gilliam et al. 2018). The five individual samples from each plot were combined and thoroughly mixed into a single composite sample, placed in a sterile polyethylene bag, and refrigerated immediately upon return to the laboratory.

Prior to sieving/air drying, subsamples of soil were extracted with 1 M KCl and analyzed for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  colorimetrically with an AutoAnalyzer III system in the Marshall University Weeds and Dirt Laboratory. All remaining soil material was sieved to pass a 1-mm mesh and air-dried for shipment to the University of Maine Soil Testing Service and Analytical Laboratory for determination of pH (in distilled  $\text{H}_2\text{O}$ ), and  $\text{NH}_4\text{Cl}$ -extractable macronutrients (P, Ca, Mg, K), micronutrients (Fe and Mn), and Al (inductively coupled plasma optical emission spectrometry). Organic matter was measured by loss on ignition at  $550^\circ\text{C}$ . Exchangeable acidity was extracted in KCl and measured by titration.

Each of the experimental watersheds used in this study is gauged with a V-notch weir and a water level recorder. Water samples collected at a fixed location upstream from the weir have been analyzed weekly since 1970 for a variety of analytes, including dissolved nitrate concentrations (Edwards and Wood 2011). These and other data are currently available at: [www.nrs.fs.fed.us/ef/locations/wv/fernow/data/](http://www.nrs.fs.fed.us/ef/locations/wv/fernow/data/) and [www.as.wvu.edu/fernow/data.html](http://www.as.wvu.edu/fernow/data.html).

## Data analysis

As this study is an example of simple pseudoreplication (Hurlbert 1984), with replications represented as plots within single watersheds, interpretation of data should be made with that in mind. It is our contention, however, that effects reported are most appropriately interpreted as resulting from experimental treatment, rather than pre-existing differences among watersheds, especially considering that the apparent changes are consistent with those commonly expected in acidified soils.

Means of all measured variables were compared for significant differences among watersheds using analysis of variance (ANOVA) and least significant difference (LSD) tests. *A priori* significant differences were accepted for all statistical tests at  $P < 0.10$  to accommodate naturally high spatial variability at the watershed scale (Zar 2009).

## Results and Discussion

Although Gilliam et al. (1994) were unable to detect a response in exchangeable soil nutrients after three years of experimental acidification, continued treatment over an additional 22 years has altered extractable ions in the surface mineral soils in ways that are largely consistent both with theoretical expectations and with patterns suggested from findings from other research (Table 1; Figure 2). After 25 years of  $(\text{NH}_4)_2\text{SO}_4$  additions, extractable concentrations of both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were higher, whereas soil pH and extractable  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ , and P concentrations generally were lower on the treated watershed (WS3), relative to the reference watersheds (WS4 & WS7) (Table 1). Although concentrations of extractable  $\text{Fe}^{2+}$  and  $\text{Al}^{3+}$  were significantly greater on WS3, extractable  $\text{Mn}^{2+}$  did not vary significantly between treatment and reference watersheds, contrary to expectations. There were no detectable differences in soil organic matter among watersheds which averaged ~12%. This was consistent with Gilliam et al. (1994) who also reported no differences in organic matter for soils collected in 1991 from the same watersheds, and with values reported for surface soils in WS3 and WS4 that were collected in 1988, 1994, and 2002 (Adams et al. 2006).

The higher  $\text{NH}_4^+$  in soils of treatment WS3 is likely the direct result of the experimental additions of  $(\text{NH}_4)_2\text{SO}_4$ . Extractable  $\text{NH}_4^+$  on WS3 was >2 times the levels measured in either WS4 or WS7 (Table 1). Surprisingly, although the levels of extractable  $\text{NO}_3^-$  were ~ 3 times higher on WS3 than on the reference watersheds, no detectable treatment effect on net  $\text{NO}_3^-$  production rates in these soils has been found since 1995 (Gilliam et al. 2018b). If net  $\text{NO}_3^-$  production rates are indeed the same across all watersheds, then the differences in the extractable pools, and higher stream  $\text{NO}_3^-$  concentrations, may reflect treatment-induced differences in vegetative or microbial demand. For example, Burnham et al. (2017) provided support for the idea that elevated  $\text{Al}^{3+}$  levels in

the soils of the acidified watershed might lower the demand for  $\text{NO}_3^-$  by several of the dominant tree species. Regardless, it is clear that the acidification treatment has not only increased soil acidity in WS3, but has also greatly enhanced the leaching of mobile  $\text{NO}_3^-$  (Figure 3A), a result which is also important in understanding the response of soil cations.

The previously documented lack of a detectable difference in net  $\text{NO}_3^-$  production rates in the well-drained soils at these sites (Gilliam et al. 2018b) is not likely to be attributable to substantially greater rates of gaseous N loss by either nitrification or denitrification in the fertilized watershed. Indeed, a limited number of measurements in these watersheds found that, although losses of N gases in WS3 were greater, the magnitude of the combined loss of N as  $\text{N}_2\text{O}$  and  $\text{NO}$  was small, and likely to be less than  $0.1 \text{ g N/m}^2/\text{yr}$  (Peterjohn et al. 1998; Venterea et al. 2004). However, although rates of net  $\text{NO}_3^-$  production in the surface mineral soils of the fertilized watershed were not elevated, the significant reduction of soil pH in WS3 (Table 1) suggests the interesting possibility that the microbial community may have shifted towards a more prominent role for acid-stimulated  $\text{NH}_4^+$  oxidation via archaea (Li et al. 2018). Although low soil pH has traditionally been thought to inhibit nitrification (Barbour et al. 1999), recent work has shown that nitrification can not only occur at a pH as low as 3.0, but also that obligate acidophilic  $\text{NH}_4^+$ -oxidizing archaea (e.g. *Nitrosotalea* spp.) are stimulated at low soil pH (Norton and Stark 2011).

For several years prior to the start of acidification treatments, stream concentrations of cations and electrical conductivity (EC) exhibited generally similar temporal patterns among the three watersheds (Figure 3). Following the initiation of experimental additions of  $(\text{NH}_4)_2\text{SO}_4$ , reference WS4 and WS7 revealed minimal changes in stream chemistry over time, whereas WS3 showed notable increases in  $\text{NO}_3^-$ ,  $\text{H}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{K}^+$ , and EC (Figure 3). Furthermore, all analytes with notable responses to treatment also displayed a time lag in such response—from 1 to 2.5 years (Edwards et al. 2006). This time lag is also consistent

with the mean transit time of ~1.5 years that has been estimated for baseflow in these watersheds using measurements of seasonal patterns in  $^{18}\text{O}$  (DeWalle et al. 1997).

Despite differences in methods, our results, when coupled with those from previous measurements of exchangeable ions in the surface soils of these watersheds (Figure 2), suggest a pattern of change that is generally consistent with theoretical predictions for ion leaching following soil acidification and enhanced  $\text{NO}_3^-$  mobility (e.g., Galloway et al. 1983, Fernandez et al. 2003). In addition to the measurements reported here for 1991 and 2015, comparisons of extractable nutrients have also been made between WS3 and WS4 for samples collected in 1994 and 2002, and between WS3 and WS7 for soils collected in 1995 and 2000 (Tepp 1995, Odenwald 2002, Adams et al. 2006). Pre-treatment values for a limited number of samples from the upper mineral soils (0-10 cm depth) have been reported for WS3 and WS4 (Adams et al. 2006), allowing some insight into the existence of any pre-treatment differences between these two watersheds. For the Ca, Mg, and K, the relative differences in average extractable concentrations between surface soils in the acidified (WS3) and mature reference (WS4) watershed have diminished over time (Figure 2).

Twenty-five years of the soil acidification treatment decreased extractable Ca in the surface mineral soil of WS3 relative to the two reference watersheds (Table 1). These results are consistent with previous observations indicating that enhanced leaching of  $\text{NO}_3^-$  is coupled to greater losses of  $\text{Ca}^{2+}$  in stream water leaving WS3 (Peterjohn et al. 1996, Edwards et al. 2006) (Figure 3C). This is also in agreement with findings from the Hubbard Brook Experimental Forest based on a 30-year data set that show a tight coupling of  $\text{NO}_3^-$  and  $\text{Ca}^{2+}$  losses in stream water (Likens et al. 1998) that arises from the electrostatic anion/cation binding of  $\text{NO}_3^-$  and  $\text{Ca}^{2+}$  (Schlesinger 1997).

The Ca/Mg ratios of ancient silicate sediments that gave rise to soils such as those of the study watersheds at FEF are close to 1 (van Moort 1973). So, because  $\text{Mg}^{2+}$  is

biogeochemically similar to  $\text{Ca}^{2+}$ , it was not surprising that the response of  $\text{Mg}^{2+}$  to experimental N treatments closely resembled that of  $\text{Ca}^{2+}$  (Table 1). As found in earlier studies focusing on plant nutrient balances (Garten 1976, Gilliam 1988), Ca and Mg comprise the same foliar nutrient functional group, i.e., photosynthetic/structural (Garten 1978). Previous results from the FEF found similar responses between  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  regarding N-mediated leaching and tree and herb foliar concentrations (Peterjohn et al. 1996, Adams et al. 2006b, Gilliam et al. 2016a). Fernandez et al. (2003) also reported similarities between soil  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in response to 9 years of  $(\text{NH}_4)_2\text{SO}_4$  additions at the Bear Brooks Watersheds in Maine.

Although extractable  $\text{K}^+$  displayed a response to the acidification treatment that was similar to other base cations (Table 1), we suggest that this may have been influenced more by enhanced plant uptake than from N-mediated leaching (as appeared to have occurred for  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) for two reasons: (1) treatment-mediated increases in stream  $\text{K}^+$  have been small, relative to  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  (Edwards et al. 2006, Figure 3), and (2) foliar K concentrations of dominant herb layer species were significantly greater on treated WS3 relative to the reference watersheds (Gilliam et al. 2016a), and overall herb layer biomass on WS3 is significantly higher than on WS4 (Gilliam et al. 2016b). Combining foliar K concentrations and biomass data from the two studies suggests that uptake of  $\text{K}^+$  by herb-layer plants on WS3 may be ~2.5 times that on WS4 (note: similar current herb biomass data are unavailable for WS7).

Although soil P levels exhibited a similar response to acidification as the base cations (Table 1), the mechanisms for this are likely quite different. The biogeochemistry of soil P is complex (Marschner 1995), as P can bind with other nutrients (e.g., Ca) and oxides of Al and Fe (Borggaard et al. 1990). At the pH values of our soils (~4.0 to 4.4—Table 1), however, adsorption by oxides of Al and Fe are more likely (Borggaard et al. 1990) and capable of

influencing P availability (Tahovská et al. 2018). Furthermore, our results support earlier findings at FEF that excess N can initiate a greater biotic demand for P (Gress et al. 2007), and are consistent with other studies showing that alleviation of N limitation can give rise to P limitation (Elser et al. 2007, Vitousek et al. 2010). At FEF, Gress et al. (2007) used several approaches to demonstrate the onset of P limitation by N additions to WS3. One facet of their study analyzed activity of phosphomonoesterase (PME), a plant enzyme produced under extreme P limitation, in roots of *Viola rotundifolia*, and found significantly higher root-associated PME in *V. rotundifolia* on WS3. Additionally, they employed a root in-growth study in which they varied macronutrient additions (N, Ca, and P) to mesh bags containing vermiculite, and measured the degree to which fine roots grew into the bags. They reported that the greatest fine root in-growth biomass occurred in bags treated with P, further demonstrating that additions of  $(\text{NH}_4)_2\text{SO}_4$  may have led to P limitation (Gress et al. 2007).

The two extractable micronutrients included in this study were Fe and Mn. We hypothesized that extractable levels of both would increase in response to  $(\text{NH}_4)_2\text{SO}_4$  applications to WS3. This was found, however, only for Fe and not for Mn (Table 1). Our expectation was based on (1) the known relationship between soil pH and Fe/Mn mobility (Barbour et al. 1999; Millaleo et al. 2010), and (2) the acidifying nature of net nitrification in excess of plant uptake, which affects changes in several soil nutrients (Lukac and Godbold 2011). The latter appears to have occurred on WS3, considering that soil pH was significantly lower (by nearly 0.5 a pH unit) there than on the reference watersheds (Table 1). Although this explains the significantly higher extractable Fe on WS3, it is less clear why we did not find similar results for Mn.

Our expectation for the response of Mn to acidification is based in large part on an intensive sampling of surface soils at 100 locations within each of WS7 and WS3, which revealed that extractable Mn was ~80% higher on WS3, along with a notable increase in the

spatial heterogeneity in soil Mn levels that correlated closely with high cover of *Rubus allegheniensis*, a species that accumulates Mn at high levels in foliar tissue and litter (Kula et al. 2012, Gilliam et al. 2018a) (Figure 4). It is likely that differences in the response between the two studies is a result of differences in the extractants that were used, wherein the more intensive sampling used ammonium acetate (Gilliam et al. 2018a) and the current study used ammonium chloride.

In contrast to the micronutrients, Al is of no nutritional value to plants, but under conditions of increased mobility can be toxic to plant roots (Lukac and Godbold 2011). Increased N deposition has been shown to increase soil acidity and do so in ways that increases Al mobility in forest soils (Lu et al. 2014). In our study, extractable Al was highest in soils of treatment WS3 (Table 1), which were also the most acidic, suggesting that acidification at FEF has increased mobility of Al. For WS3, extractable Al and soil pH were significantly correlated (Figure 5). These conclusions strongly support a study at FEF by Burnham et al. (2017) that examined soil solution Al in WS3 versus WS7. They not only reported that soil solution Al concentrations were 77% higher in WS3, but also found that elevated Al in the soil solution altered the way in which tree species acquired available soil N—specifically, elevated Al caused a shift from  $\text{NO}_3^-$  uptake (which was inhibited by Al) to uptake of  $\text{NH}_4^+$ . In addition, the response of Al to acidification may have contributed to slowing the growth of some dominant hardwood trees on WS3, as reported by May et al. (2005) and DeWalle et al. (2006).

Gilliam et al. (2016a) assessed the response of foliar Ca:Al ratios of prominent species of the herbaceous layer. Because there had been an N-mediated shift in herb community dominance, these data were for *Viola rotundifolia* at the beginning of the study (1993) and for *R. allegheniensis* later in the study (2014). For *V. rotundifolia*, Ca:Al ratios were significantly lower on treated WS3 than on the reference WS7, but not on reference

WS4. For *R. allegheniensis*, Ca:Al ratios were significantly lower on WS3 than on both WS7 and WS4. Considering that foliar Ca:Al ratios may be useful indicators of soil nutrient conditions (Cronan and Grigal 1995), these contrasts for foliar nutrients support soil nutrient data from this study showing treatment-mediated decreases in available Ca and increases in Al mobility.

## Synthesis and Conclusions

Several studies examining the effects of excess acidity and N deposition on the biogeochemistry of forest ecosystems throughout eastern North America have demonstrated deposition-mediated increases in loss of base cations (Currie et al. 1999, Fernandez et al. 2003, Moore and Houle 2009, 2013). The results for FEF suggest that, despite the apparent lack of an early response of exchangeable soil nutrients to acidification (Gilliam et al. 1994), detectable changes in some nutrient cations emerged after 25 years of simulated acid deposition.

Current patterns in both surface soils and stream water chemistry suggest that chronic excess acidity, coupled with excess N deposition, has generally decreased base cation availability and increased Al mobility in this central Appalachian hardwood forest ecosystem. Not only does this represent a current concern for forest health, as there is evidence that suggests slowing growth rates for some prominent hardwood species at FEF (May et al. 2005, DeWalle et al. 2006), but it also represents an on-going concern for the future management of such forests (Clark et al. 2019a). This is especially relevant considering the high levels of acid and N deposition occurring in global temperate forests (Vet et al. 2014, Gilliam 2016), the wide-spread signature of excess N in the northern hemisphere (Holtgrieve et al. 2014), and the likely long-term nature of recovery from N saturation of impacted forests (Stevens 2016, Gilliam et al. 2019, Schmitz et al. 2019).

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Table 1. Means ( $\pm 1$  SE) of extractable soil analytes for experimental watersheds at Fernow Experimental Forest, WV in 2015. WS3 is the treatment watershed, whereas WS4 and WS7 are untreated reference watersheds. For a given analyte, means with the same superscript are not significantly different at  $P < 0.10$  and significant differences among watersheds are highlighted in bold.

Analyte	WS3	WS4	WS7
pH	<b>4.03<math>\pm</math>0.09<sup>b</sup></b>	<b>4.41<math>\pm</math>0.05<sup>a</sup></b>	<b>4.39<math>\pm</math>0.05<sup>a</sup></b>
Acidity (meq/100 gm)	<b>7.9<math>\pm</math>1.0<sup>a</sup></b>	<b>6.5<math>\pm</math>0.5<sup>ab</sup></b>	<b>5.7<math>\pm</math>0.4<sup>b</sup></b>
NH <sub>4</sub> <sup>+</sup> ( $\mu$ g N/g soil)	<b>6.7<math>\pm</math>2.0<sup>a</sup></b>	<b>3.1<math>\pm</math>0.8<sup>b</sup></b>	<b>2.3<math>\pm</math>0.5<sup>b</sup></b>
NO <sub>3</sub> <sup>-</sup> ( $\mu$ g N/g soil)	<b>3.1<math>\pm</math>0.8<sup>a</sup></b>	<b>1.2<math>\pm</math>0.5<sup>b</sup></b>	<b>1.3<math>\pm</math>0.6<sup>b</sup></b>
Ca <sup>++</sup> ( $\mu$ g/g soil)	<b>75.6<math>\pm</math>8.5<sup>b</sup></b>	<b>99.3<math>\pm</math>16.4<sup>b</sup></b>	<b>159.6<math>\pm</math>36.3<sup>a</sup></b>
K <sup>+</sup> ( $\mu$ g/g soil)	<b>80.3<math>\pm</math>4.7<sup>b</sup></b>	<b>99.6<math>\pm</math>8.5<sup>ab</sup></b>	<b>133.9<math>\pm</math>36.0<sup>a</sup></b>
Mg <sup>++</sup> ( $\mu$ g/g soil)	<b>18.0<math>\pm</math>0.8<sup>b</sup></b>	<b>24.6<math>\pm</math>2.3<sup>a</sup></b>	<b>28.4<math>\pm</math>3.1<sup>a</sup></b>
P ( $\mu$ g/g soil)	<b>1.6<math>\pm</math>0.2<sup>b</sup></b>	<b>1.8<math>\pm</math>0.2<sup>ab</sup></b>	<b>2.2<math>\pm</math>0.2<sup>a</sup></b>
Al <sup>+++</sup> ( $\mu$ g/g soil)	<b>513.4<math>\pm</math>81.7<sup>a</sup></b>	<b>398.0<math>\pm</math>42.9<sup>ab</sup></b>	<b>330.4<math>\pm</math>35.9<sup>b</sup></b>
Fe <sup>++</sup> ( $\mu$ g/g soil)	<b>10.5<math>\pm</math>4.3<sup>a</sup></b>	<b>2.0<math>\pm</math>0.6<sup>b</sup></b>	<b>3.01<math>\pm</math>1.1<sup>b</sup></b>
Mn <sup>++</sup> ( $\mu$ g/g soil)	<b>134.1<math>\pm</math>30.1<sup>a</sup></b>	<b>133.0<math>\pm</math>8.7<sup>a</sup></b>	<b>120.6<math>\pm</math>24.3<sup>a</sup></b>

**Figure Legends**

Figure 1. Study watersheds—WS3 (treatment), WS4 (reference), and WS7 (reference) at Fernow Experimental Forest, West Virginia.

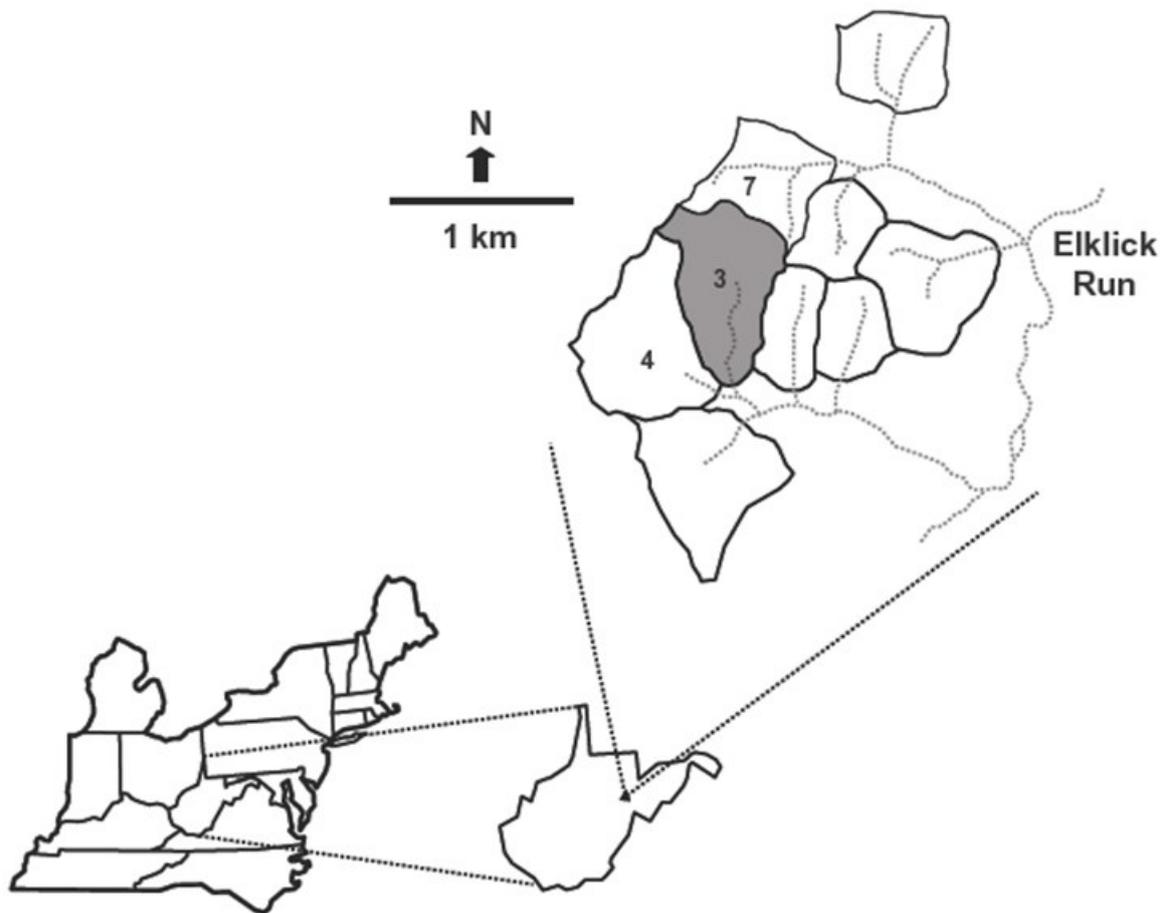


Figure 2. Previously and currently reported mean values for ions in the surface (0-10 cm or A horizon) mineral soils of the acidified (WS3) and reference (WS4) watersheds, Fernow Experimental Forest. (A) pH, (B)  $\text{Ca}^{++}$ , (C)  $\text{Mg}^{++}$ , (D)  $\text{K}^+$ . Change in pH/ion over time shown as linear/curvilinear relationships yielding the best fit per watershed (highest  $r^2$ ). Dashed lines for WS4 in (B) and (C) are not significant at  $P>0.05$ . Data sources: 1988 (pre-treatment)—Adams et al. (2006b)/unpublished (for  $\text{K}^+$ ); 1991—Gilliam et al. (1994); 2002—Adams et al. 2006b); 2015—this study.

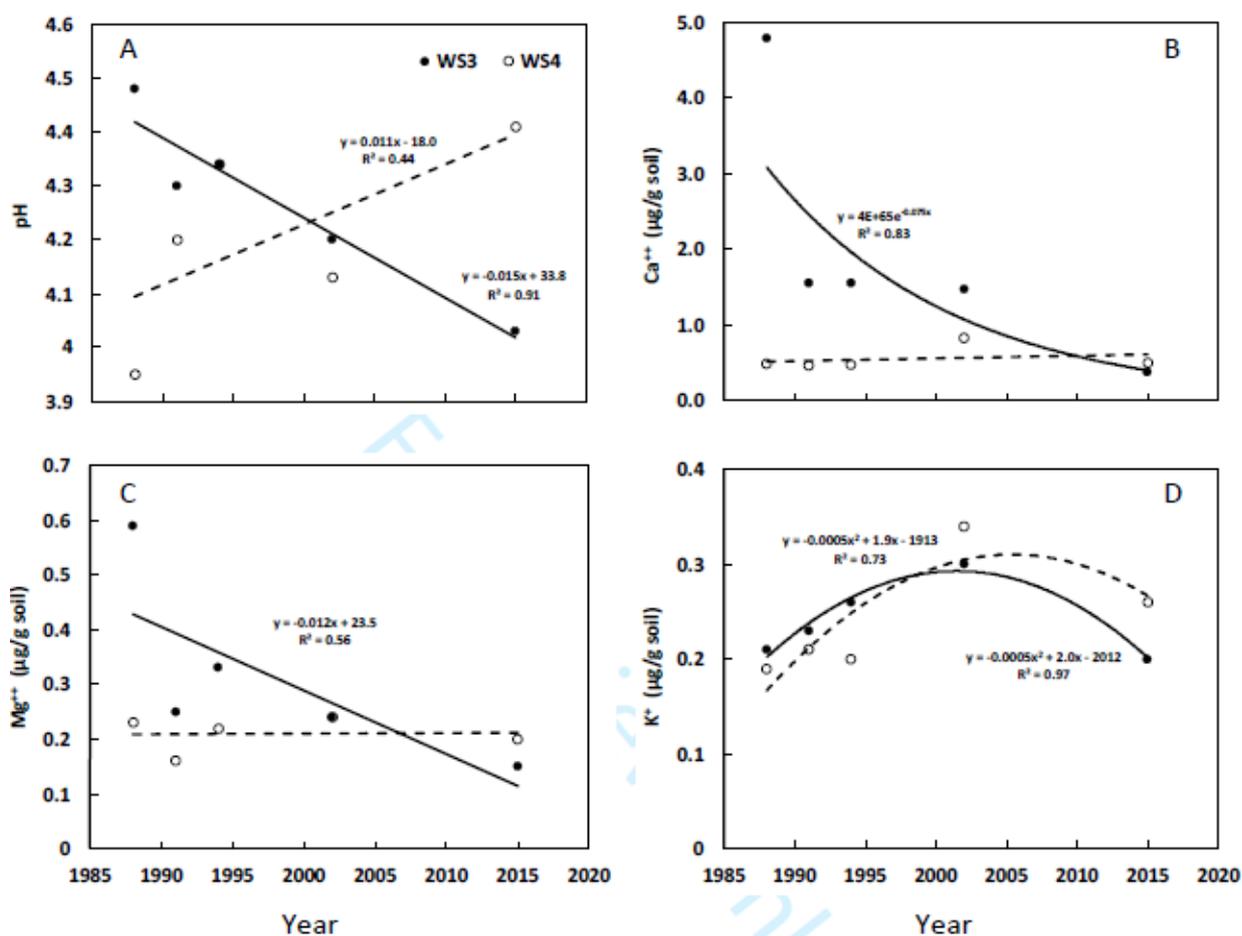


Figure 3. Volume-weighted mean monthly concentrations of major ions in stream water and electrical conductivity from 1989-2014 for three small watersheds at the Fernow Experimental Forest, WV. Trend lines are 24-month running means. Vertical bars indicate when fertilizer applications began.

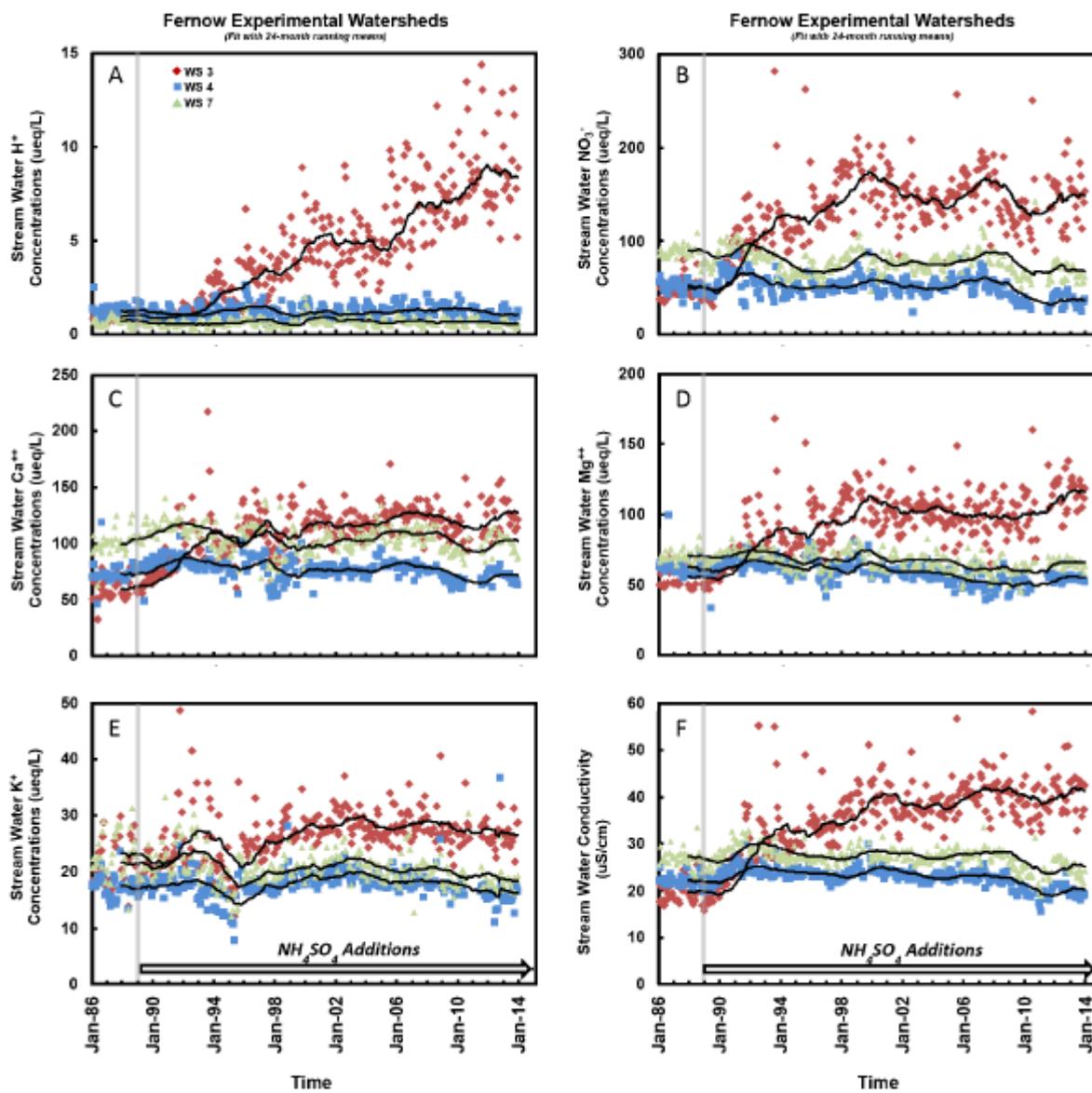


Figure 4. Kriged extractable soil Mn (from 2011 and 2014) for (a) reference WS7 and (b) N-treated WS3. Shown also for WS3 (data not available for WS7) are spatially-explicit mean cover (%) values for *Rubus allegheniensis* in the seven permanent sample plots. Figures taken from Gilliam et al. (2016a).

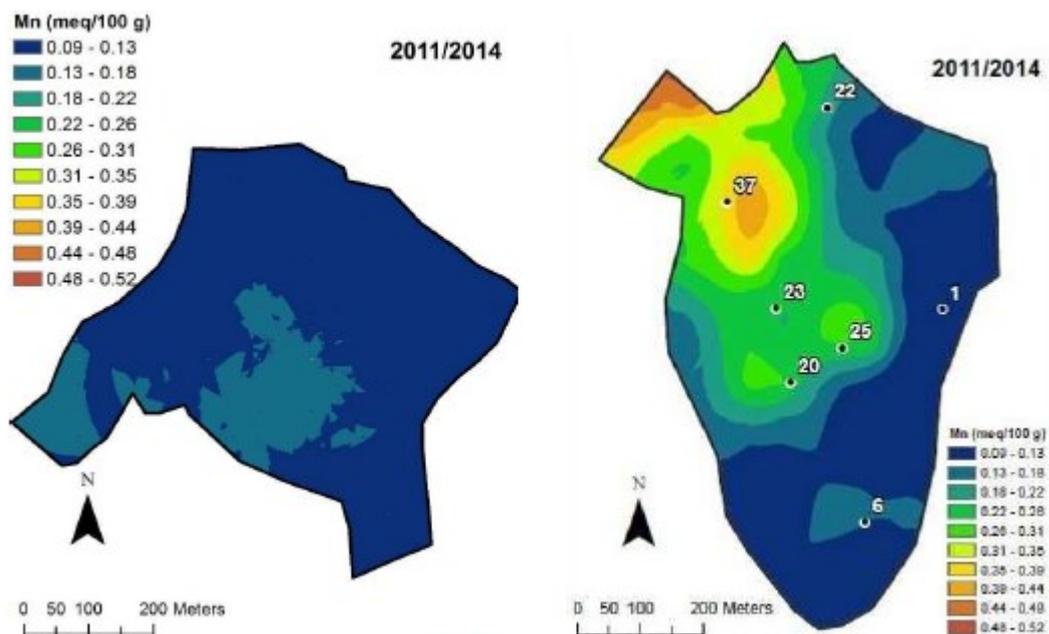


Figure 5. Extractable soil Al versus soil pH for plots on experimental watershed at Fernow

Experimental Forest, WV: WS3 (treatment), black symbols, solid line;  $Y = -964.9X + 4406.0$ ,  $r^2=0.95$ ; WS4 (reference) gray symbols; WS7 (reference), open symbols.

