Ecosystem Processes

Herbaceous Layer and Soil Response to Experimental Acidification in a Central Appalachian Hardwood Forest

Frank S. Gilliam,* Nicole L. Turrill, Staci D. Aulick, Dan K. Evans, and Mary Beth Adams

ABSTRACT

The herbaceous layer (vascular plants ≤1 m in height) is an important component of forest ecosystems and a potentially sensitive vegetation stratum in response to acid deposition. This study tested several hypotheses concerning soil and herbaceous layer response to experimental acidification at the Fernow Experimental Forest in north-central West Virginia. Fifteen circular sample plots (0.04 ha) were established in each of three watersheds: WS3 (an ≈20-yr-old watershed receiving acidification treatment with $(NH_4)_2SO_4$), WS4 (>80-yr-old control), and WS7 (≈20-yr-old control). The herb layer was sampled intensively in 10 1-m^2 subplots within each sample plot, including determination of species composition, cover, and random biomass harvests. Harvested plant material was separated by species and analyzed for macronutrients, micronutrients, and Al. Soil was sampled from harvest subplots and analyzed for texture, pH, organic matter, and macro- and micronutrients. Few differences among watersheds for virtually all measured soil variables indicated minimal response of soil fertility to the acidification treatment. The herbaceous layer was also quite similar among watersheds with respect to cover–biomass and species diversity; WS7, however, had ≈70% higher herb layer cover that both Ws3 and WS4, a result of the predominance of a few high-cover fern species and attributable to the north-facing aspect of WS7 vs. south-facing aspects of WS3/WS4. There was a high degree of species similarity among watersheds, suggesting no shift in species composition in response to acidification. There was also minimal response of element concentrations to acidification, although Fe and Al exhibited evidence of increased uptake in WS3. We conclude that, contrary to our expectations, there has been little substantive response of the soil and herb layer to acidification, but hypothesize that herbaceous layer species may experience toxicity problems with increased mobility of Al and micronutrients in the future.

RECENT STUDIES have supported the conclusion that current levels of acid deposition have contributed to a decline in productivity of forest ecosystems of the eastern United States (Johnson and Taylor, 1989; Adams and Eagar, 1992; Eagar and Adams, 1992). There has been lack of general agreement, however, among these studies as to the extent of forest damage directly attributable to increased atmospheric acidity. Such lack of agreement results from several factors, including the problems associated with smaller-scale experimental plot (e.g., Bergkvist and Folkeson, 1992) and greenhouse (e.g., Haines et al., 1980) designs that must extrapolate results to the large-scale level of the ecosystem. These are important problems to address, however, because acid deposition effects ultimately must be assessed at the ecosystem level.

In 1988, the USDA Forest Service funded a project at the Timber and Watershed Laboratory, Parsons, WV (Northeastern Forest Experiment Station), to experimentally acidify an entire watershed at the Fernow Experimental Forest (FEF) (Adams et al., 1993). This experimental treatment offers unique opportunities to study directly the potential effects of acid deposition at the level of the ecosystem. While cooperative studies within this project have looked at several components of the ecosystem, the purpose of this study was to examine the response of herbaceous layer vegetation and soil nutrients to acidification. The whole ecosystem approach of the present study is unique from other studies on effects of acidification on herb layer species in which the treatments are based on experimental plots (e.g., Nygaard and Abrahamsen, 1991).

Although considerable research has been directed at the potential effects of acid deposition on forest tree species, far fewer studies have focussed on responses of the herbaceous layer to ecosystem acidification (Thimonier et al., 1992). The herb layer, usually defined as all vascular plants ≤0.5 to ≤1.5 m in height, is an important stratum of forest vegetation in terms of its relationship to soil fertility (Siccama et al., 1970; Peterson and Rolfe, 1982; Gilliam and Christensen, 1986; Gilliam, 1988). Indeed, because of its sensitivity to site conditions, the herb layer (also referred to as ground layer, ground vegetation, or herbaceous understory) has been used as an indicator of edaphic factors, landform types, and forest site quality (Pregitzer and Barnes, 1982; Cserep et al., 1991; Strong et al., 1991; Meilleur et al., 1992; Host and Pregitzer, 1992). The herb layer is also sensitive to natural disturbance (Moore and Vankat, 1986) and forest management practices (Gilliam and Christensen, 1986; Duffy and Meier, 1992; Gilliam and Turrill, 1993).

The herbaceous layer plays an important role in initial competition among seedling and sprouting individuals of potential forest overstory canopy dominants. We view the herb layer as a dynamic assemblage of resident and transient species. Resident species are those vascular plants (e.g., annuals, herbaceous perennials, and low-growing shrubs) whose life history characteristics confine them to

F.S. Gilliam, N.L. Turrill, S.D. Aulick, and D.K. Evans, Dep. of Biological Sciences, Marshall Univ., Huntington, WV 25755, and M.B. Adams, USDA Forest Service, Timber and Watershed Laboratory, Parsons, WV 26287. This research was funded by USDA-FS Cooperative Grant no. 23-590 through NE-4301, Fernow Experimental Forest, Parsons, WV. The use of trade, firm, or corporation names in this paper is for the information and convenience of the user. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture or the Forest Service of any product or service to the exclusion of others that may be suitable. We are indebted to Steve Horsley for helpful discussion of fern data. Received 7 June 1993. *Corresponding author (bsc022@marshall.wvnet.edu).

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Abbreviations: FEF, Fernow Experimental Forest; WS4, Watershed 4; WS7, Watershed 7; WS3, Watershed 3; MUHW, Marshall University Herbarium; CEC, cation exchange capacity; IV, importance value.

this lowest vascular stratum. Transient species are those plants, such as larger shrubs and trees, which exist in the herb layer for a short period and then either die or develop and emerge into higher strata (e.g., shrub, understory, and overstory layers). Since seedlings and sprouts of regenerating overstory species must pass through this layer and compete as transient species with resident species (Morris et al., 1993; Wilson and Shure, 1993), herb layer species changes from acidification have consequences potentially significant to the response of the forest to acid deposition.

Johnson and Taylor (1989) argued strongly that environmental stresses on plants from pollution (e.g., anthropogenic acidification) are less influential from direct effects (such as foliar lesions) than from indirect effects (such as alteration of a plant's ability to obtain resources), including alteration of plant–soil nutrient relationships (Runge and Rode, 1991). The functional processes of herb layer species' responses to increasing acidity are quite complex. Following ≈20 yr of artificial acidification Nygaard and Abrahamsen (1991) found substantial changes in what they termed *ground vegetation*. Although most of these changes were for nonvascular species (predominantly mosses), they discussed possible direct (foliar) and indirect (soil) effects and concluded that acid-mediated changes in competitive interactions and decreases in soil fertility best explained the response of vascular plants to the acidification treatment. Regardless of the specific mechanisms of response, given the sensitivity of the herbaceous layer to soil conditions, any effects of increased acid deposition on soil nutrients might first be seen in the response of plants within the herb layer.

We address several interrelated hypotheses concerning the potential response of soils and herb layer plants to ≈3 yr of experimental acidification treatment (3 times annual ambient N and S deposition). These will be tested as null hypotheses (no differences between experimental watersheds, implying lack of treatment effects); however, our specific predictions are that the alternate hypotheses will be accepted (i.e., there will be differences between watersheds resulting from acidification treatment effects).

Null Hypotheses of Herbaceous Layer Response to Acidification Treatment

Null Hypothesis 1. *Soil fertility will not vary significantly between treatment and control watersheds.* We expect, however, that the treatment will cause fertility changes in WS3. Natural factors influencing rates of ecosystem acidification include interactions between precipitation amounts and soil age, which lead to weathering and base cation leaching, and substantial nutrient uptake by trees (Binkley and Richter, 1987). Whether acidification is from natural or anthropogenic factors, higher acidity in forest soils should result in (and be related to) lower fertility from lower nutrient cation availability and from lower nitrification. Thus, we predict that extractable base cations and NO_3^- will be lower on the treatment watershed.

Null Hypothesis 2. *Herb layer cover and species diversity will not vary between treatment and control watersheds.* Sparse and species-poor herb layers often are associated with highly acidic forest soils (Gilliam and Christensen, 1986). This is usually the result of a negative response of herb layer species to acidic soil conditions that are accompanied by low nutrient availability and high Al mobility and toxicity (Gilliam and Christensen, 1986; Gilliam, 1991; Runge and Rode, 1991). We therefore anticipate that our data will reject the null hypothesis and we predict substantially lower herb layer cover and species diversity on the treatment (acidification) watershed.

Null Hypothesis 3. *Acidification treatment will result in no change in herb layer species composition and dominance.* Plant species generally are well-separated with respect to their soil pH tolerance ranges, with only a relative few species surviving well under broad ranges of soil acidity (Runge and Rode, 1991). Accordingly, plants often are categorized as either acidophobic or acidophilic (calcicoles or calcifuges, respectively, *sensu* Hope Simpson, 1938). We predict that the treatment watershed will have a higher number and relative cover of acid tolerant (acidophilic) species, again rejecting the null hypothesis of no response.

Null Hypothesis 4. *Herb layer tissue nutrients will not be different between treatment and control watersheds.* Due to the potential complexity of functional responses of the herb layer to acidification treatment, it may be anticipated that growth responses to the treatment might occur without changes in tissue concentrations. It may also be anticipated, however, that treatment responses of herb layer tissue nutrient concentrations might precede growth responses. Regardless, we predict that herb layer concentrations of elements made less available–mobile by acidification will be lower on the treatment watershed. Those made more available–mobile by acidification should have higher tissue concentrations in the treatment watershed.

MATERIALS AND METHODS

Study Site

The study was conducted at the Fernow Experimental Forest (FEF), a 1900 ha area of the Allegheny Mountain section of the unglaciated Allegheny Plateau in Tucker County, West Virginia (39° 03′15″N, 79° 49′15″W). Precipitation for FEF averages ≈1430 mm yr^{-1}, with precipitation generally increasing through the growing season and with higher elevations. It is notable that concentrations of acidic species in precipitation (H^+, NO_3^-, and $SO_4^=$) at FEF are among some of the highest in North America (F.S. Gilliam and M.B. Adams, 1993, unpublished data). Based on input-output budgets, incoming H^+ and $SO_4^=$ is accumulating within the watersheds (Helvey and Kunkle, 1986). Soils of the study watersheds are largely Inceptisols of the Berks (loamy-skeletal, mixed, mesic Typic Dystrochrept) and Calvin series (loamy-skeletal, mixed, mesic Typic Dystrochrept), derived from sandstone, and are generally coarse-textured sandy loams, well-drained, and ≈1 m in depth (Forest Service, 1987).

As part of the FEF Watershed Acidification Project, three watersheds were used for the location of sample plots: Watershed 4 (WS4), Watershed 7 (WS7), and Watershed 3 (WS3), with WS3 serving as the *treatment* watershed, receiving additions of $(NH_4)_2SO_4$, and WS4 and WS7 serving as the controls. Ammonium sulfate has been demonstrated to be an effective acidifying agent in experimental acidification studies (Fernandez and Kosian, 1986). Watershed 3 received three aerial applications of $(NH_4)_2SO_4$ per year from 1989 to the initiation of our study. March and November applications are 33.6 kg/ha of fertilizer,

or ≈ 7.1 and 8.1 kg/ha of N and S, respectively. July applications are 100.8 kg/ha fertilizer (21.2 and 24.4 kg/ha N and S, respectively). These rates were chosen as approximately twice the ambient rates of N and S deposited on the watersheds via throughfall. The total amount of N and S deposited on WS3 (application plus atmospheric deposition) is ≈ 54 and 61 kg/ha per yr, or about three times pretreatment levels (Adams et al., 1993).

Watershed 3 is a ≈ 20-yr-old even-aged stand, whereas WS4 is a >80-yr-old mixed-aged stand. Thus, to better account for differences in forest stand age, WS7 (also ≈ 20 yr old) was included in the study as an additional control. WS7 received herbicide treatment for ≈ 6 yr prior to release in 1969 (Kochenderfer and Wendel, 1983).

All three study watersheds are composed primarily of mixed hardwood stands. Overstory dominant species include 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.) (Gilliam and Turrill, 1993). The herbaceous layer is spatially quite heterogeneous, but is dominated by stinging nettle [*Laportea canadensis* (L.) Wedd.] and violet (*Viola* spp.) on WS3 and WS4 (Gilliam and Turrill, 1993) and by several fern species [including shield fern (*Dryopteris marginalis* L. Gray) and Christmas fern (*Polystichum acrostichoides* Michx. Schott] on WS7 (Aulck, 1993; Turrill, 1993). The seedbank, including buried seed, rootstocks, and rhizomes, for both woody overstory and herbaceous layer species is substantial (Wendel, 1987).

Field Sampling

The herbaceous layer was sampled using methods described in Gilliam and Turrill (1993). Fifteen circular 0.04-ha plots (11.3 m radius) were established in each watershed, for a total of 45 plots. Plots were located adjacent to lysimeters already established on WS3 and WS4. Plots were located in WS7 on sites similar to those in WS3 and WS4 in terms of elevation, slope, and aspect. Ten circular 1-m^2 subplots were established within each sample plot (for a total of 450 subplots for all watersheds). These subplots were located with the polar coordinate method of Gaiser (1951), permitting the use of stratified-random subplot location within a circular plot, while avoiding the tendency to over sample the inner one-half of the plot (Gaiser, 1951).

All vascular plants ≤ 1 m in height within each of the 10 1-m^2 circular subplots per sample plot were identified to species and estimated for cover (%) following the visual estimation method of Gilliam and Christensen (1986). The two subplots with the highest total herbaceous layer cover in each plot were designated as harvest subplots, within which all herb layer vegetation was clipped at the soil surface. We chose these subplots to provide enough tissue material for nutrient analysis. Also, as a result of the wide range of herb cover values for all plots of the study watersheds, the degree of cover (%) of these maximum cover subplots varied greatly from plot to plot, allowing for an adequate comparison of estimated cover to harvested biomass (see Gilliam and Turrill, 1993). There were a total of 90 harvest subplots for all watersheds. All harvested plant material was separated by species, oven dried, and weighed. Following removal of organic forest floor material, a sample of mineral soil was taken to a 0- to 10-cm depth from each of the two harvest subplots per sample plot. All sampling was carried out during a 1-wk period in mid to late July 1991, following 3 yr of artificial acidification on the treated watershed.

To better describe the species composition of the study watersheds, a floristic survey was made by traversing each watershed four times during two growing seasons, spring and summer of 1991 and 1992 (Aulick, 1993). All vascular plants were noted and identified during each traverse and a voucher specimen of each species was collected and deposited in the Marshall University Herbarium (MUHW), Dr. D.K. Evans, Curator (Aulick, 1993). No quantitative measurements (e.g., cover) were made on these plants, but floristic affinity among watersheds was assessed with the following modification of Sorensen's Coefficient of Similarity (Barbour et al., 1987):

$$C_s = 3D/(A + B + C) \quad [1]$$

where C_s is the coefficient of similarity, D is the number of species common to all three study watersheds, and A, B, and C are the numbers of species for WS3, WS4, and WS7, respectively. Nomenclature follows Gleason and Cronquist (1991).

Laboratory Analyses

Following separation into species, oven drying, and weighing, plant samples (one sample being each species in each harvest subplot) were ground in a Wiley mill to pass a 1-mm screen. Plant tissue samples were analyzed at the University of Maine Soil Testing Service and Analytical Laboratory for macronutrient (N, P, Ca, Mg, K), micronutrient (B, Cu, Fe, Mn, Zn), and Al concentrations. Total Kjeldahl N was determined with autoanalysis following block digestion with H_2SO_4 and $K_2SO_4/CuSO_4$; NBS 1572 Citrus Leaf was used as standard. All other elements were determined with plasma emission spectrophotometry following dry ashing and extraction with HCl and HNO_3.

Soils were analyzed as described in Gilliam and Turrill (1993). Analyses included (i) particle-size (texture) analysis, (ii) water-extractable pH (1:1, w/v), (iii) 1 *M* KCl-extractable Ca, K, Mg, and P (plasma emission), (iv) 1 *M* KCl-extractable NO_3 and NH_4 (flow-injection colorimetry), (v) soil organic matter (loss-on-ignition), and (vi) cation-exchange capacity (CEC) (estimated from sum of exchangeable acidity and extractable base cations).

All subplot values of soil variables and herb layer species cover were averaged to give mean values per plot. Herb layer tissue element concentrations were weighted by harvested biomass (per species per subplot) to yield biomass-weighted concentration means per plot. Mean plot values were averaged to give mean values per watershed. Significant differences among watersheds were assessed using analysis of variance and Duncan's multiple range testing (SAS Inst., 1982; Zar, 1984). All stated differences are statistically significant at the $P < 0.05$ level unless otherwise indicated.

It should be mentioned that the design of this project is an example of simple pseudoreplication, since each watershed represents an experimental condition with a sample size of one (Hurlbert, 1984). Therefore, our data will be interpreted with caution. Although pseudoreplication can create some statistical problems, it is common among watershed ecosystem studies (Likens et al., 1977) and is related to the logistical difficulties of (i) finding watersheds across a landscape that are true replicates of one another (i.e., finding two or more watersheds that are identical in most respects is either rare or perhaps impossible) and (ii) accommodating the high cost of watershed-level treatments.

RESULTS

There were few substantial differences between watersheds for soil physical and chemical characteristics (Table 1). Based on particle-size analysis, soils for all watersheds would be classified as sandy loams. Soil organic matter was ≈ 14% for all watersheds and CEC ranged between 40 and 50 meq/kg (Table 1). WS4 had a significantly lower mean soil pH than did WS7, but WS3 was not significantly

Table 1. Mean physical and chemical characteristics of soils from the study watersheds. Values given are mean ± 1 SE.

Variable	WS3	WS7	WS4
Texture classes, %			
Sand	65.7 ± 1.9a†	68.8 ± 1.4a	66.0 ± 1.5a
Clay	12.0 ± 0.9a	9.5 ± 0.6b	10.7 ± 0.7ab
Silt	22.2 ± 1.2a	21.7 ± 1.2a	23.3 ± 1.2a
Organic matter, %	14.2 ± 1.2a	13.4 ± 0.7a	13.8 ± 0.5a
Cation-exchange capacity, meq/100 g	5.1 ± 0.9a	4.0 ± 0.3a	4.1 ± 0.1a
pH	4.3 ± 0.1ab	4.5 ± 0.3a	4.2 ± 0.1b
Macronutrients, μeq/g			
Ca	15.6 ± 9.4a	4.1 ± 0.3a	4.7 ± 0.4a
K	2.3 ± 0.3a	2.2 ± 0.2a	2.1 ± 0.1a
Mg	2.5 ± 0.8a	1.7 ± 0.1a	1.6 ± 0.1a
P	1.2 ± 0.1a	1.3 ± 0.9a	1.4 ± 0.1a
NO_3-N	2.4 ± 0.4a	1.0 ± 0.2b	1.9 ± 0.3ab
NH_4-N	0.9 ± 0.1a	2.0 ± 0.2a	0.7 ± 0.1a
Micronutrients, μeq/g			
Cu	0.01 ± 0.0b	0.01 ± 0.0ab	0.02 ± 0.0a
Fe	2.14 ± 0.4a	1.18 ± 0.3a	2.36 ± 0.6a
Mn	1.75 ± 0.3a	1.47 ± 0.2a	2.00 ± 0.3a
Zn	0.05 ± 0.0a	0.04 ± 0.0a	0.15 ± 0.1a

† Means for a given variable with different letters are significantly different at $P < 0.05$.

different from either WS4 or WS7. Of the macronutrients, only NO_3 showed any significant differences among watersheds, with WS3 being significantly higher than WS7, but not different from WS4. Of the micronutrients, only Cu was significantly different among watersheds; significantly lower on WS3 than on WS4, but no differences between WS7 and either WS3 or WS4 (Table 1).

There were also few differences among watersheds in general characteristics of herbaceous layer vegetation (Table 2). WS3 was not significantly different from WS4 for herb layer cover or biomass, species richness, and species diversity. WS7 had nearly twice the herb layer cover than that which was found on WS3/WS4 (38 vs. ≈23% for WS7 vs. WS3/WS4, respectively) and proportionally more biomass (19 vs. 11 g/m^2). WS7 also had a significantly higher mean per subplot species richness (5 vs. ≈4 species/m^2). Species diversity (H', based on $\log_e$) for all watersheds ranged from 1.6 to 1.9 (Table 2).

The watersheds were similar with respect to herbaceous layer species composition. Subplot sampling of the herb layer encountered 85 vascular plant species for all watersheds combined, whereas the floristic survey, designed to establish a more complete flora, tallied 205 species. Table 3 provides species composition data for the study watersheds based on both subplot and floristic survey information. The listing of the important species (mean cover more than ≈5%), based on overall frequency and cover in the sample plots, of all watersheds combined shows a high number of species common to all three watersheds. Furthermore, of the top 15 species shown in Table 3, 12 species (80%) were found by the floristic survey to be in all three watersheds. The most pronounced difference in herb layer species composition among watersheds was in the predominance of ferns on WS7, particularly shield fern, toothed wood fern [*D. carthusiana* (Villars) H.P. Fuchs], and Christmas fern (Table 3).

Potential species-specific cover responses to acidification were assessed by plotting mean cover values for individual herbaceous layer species occurring in WS3 versus WS4 or WS7 or both (Fig. 1). Thus, each data point in Fig. 1 represents mean cover for a particular species found

Table 2. Characteristics of herb layer vegetation of three watersheds of the Fernow Experimental Forest, Parsons, WV. Values given are means ± 1 SE. Species richness calculated as mean number of species per 1-m^2 subplot. Species diversity calculated with the Shannon–Wiener Index following natural log transformations of cover values. Herb layer biomass values are based on regression equation given in Gilliam and Turrill (1993).

Variable	WS3	WS7	WS4
Herb cover (%)	19.3 ± 3.7a†	37.5 ± 2.7b	26.4 ± 4.3a
Herb biomass (g/m^2)	9.7 ± 1.8a	18.5 ± 1.3b	13.3 ± 2.1a
Species richness (#/m^2)	3.7 ± 0.3a	5.0 ± 0.3b	3.6 ± 0.2a
Species diversity (H')	1.9 ± 0.1a	1.6 ± 0.1a	1.9 ± 0.1a

† Means for a given variable with different letters are significantly different at $P < 0.05$.

Table 3. Important herb layer species of three watersheds of the Fernow Experimental Forest. Importance value (IV) calculated as relative frequency plus relative cover. Nomenclature follows Gleason and Cronquist (1991).

Species	WS3	WS7	WS4
	Importance value		
Acer pensylvanicum L.†	14.7	–	15.5
Sugar maple†	–	–	10.9
Dioscorea quaternata (Walt.) Gmel.	6.0	–	–
Toothed wood fern†	–	4.7	–
Shield fern†	6.2	52.8	7.9
Laportea canadensis (L.) Wedd.†	26.8	7.2	33.9
Lycopodium digitatum Dillen	6.7	–	–
Osmorhiza claytonii (Michx.) Clarke	–	6.4	–
Christmas fern†	–	17.2	11.0
Polygonatum biflorum (Walter) Elliott†	–	–	9.4
Black cherry†	8.4	4.9	9.3
Rubus spp.†	13.2	10.2	7.8
Sassafras albidum (Nutt.) Nees†	10.0	5.7	–
Smilax rotundifolia L.†	21.3	7.5	9.3
Viola spp.†	24.4	14.8	14.5

† Indicates species found in all three watersheds by the floristic survey of Aulick (1993).

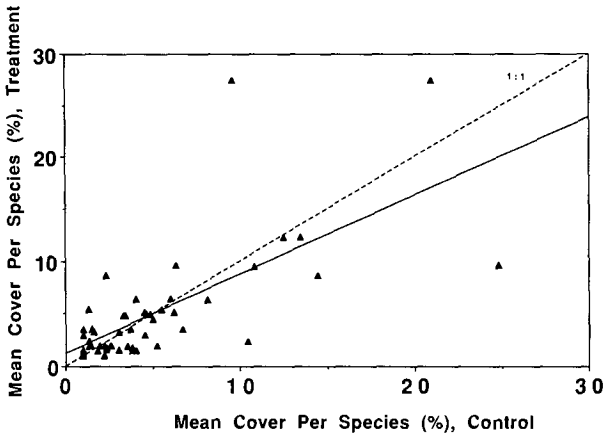

Fig. 1. Species-specific relationships for mean cover on acidification treatment watershed (WS3) vs. two control watersheds (WS4 and WS7). Each data point in the figure represents mean cover for an individual species found in the treatment watershed and in either or both of the control watersheds. Equation for the regression line is: $y = 1.25 + 0.75x$, $r^2 = 0.47$, $P < 0.0001$. Dashed line is 1:1 reference line.

in WS3 (Treatment) and its corresponding mean cover found in either WS4, WS7, or both watersheds (Control). For reference, a 1:1 line is also given to represent a hypothetical situation wherein no change occurred for any species. The regression of mean cover values for individual species of treatment onto control watersheds yielded the equation $y = 1.25 + 0.75x$, $r^2 = 0.47$, which was significant at the $P < 0.0001$ level. Although no specific statistical test was used to compare reference and regression lines, the 1:1 reference line was within the 95% confidence intervals (not shown on figure) throughout the range of cover data in Fig. 1.

There were few differences between watersheds for macronutrient and micronutrient concentrations in herbaceous layer vegetation (Table 4). For the macronutrients, K and N concentrations on WS7 were significantly higher than those on WS4. Ca was significantly lower on WS7 than on WS3 and WS4. Mg and P were not significantly different between any of the watersheds (Table 4). Micronutrient tissue concentrations were also quite similar between watersheds. The only significant difference was for B, which was significantly lower on WS7 than on WS3 and WS4 (Table 4).

Table 4. Mean macronutrient, micronutrient, and Al concentrations of herb layer tissue. Values given are means ± 1 SE. Means for a given element with different letters are significantly different at $P < 0.05$.

Nutrient	WS3	WS7	WS4
Macronutrients (% dry wt)			
Ca	0.8 ± 0.2a	0.4 ± 0.1b	0.7 ± 0.1a
K	2.3 ± 0.3ab	3.1 ± 0.4a	1.9 ± 0.2b
Mg	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a
P	0.2 ± 0.0a	0.1 ± 0.0a	0.2 ± 0.0a
N	2.3 ± 0.1ab	2.4 ± 0.1a	2.0 ± 0.1b
Micronutrients and Al (mg/kg)			
B	24.2 ± 1.6a	14.5 ± 2.2b	28.0 ± 1.9a
Cu	9.8 ± 0.6a	9.0 ± 0.4a	8.3 ± 0.6a
Fe	318.7 ± 80.2a	148.2 ± 12.1a	192.7 ± 65.5a
Mn	839.1 ± 67.5a	974.8 ± 82.0a	1544.3 ± 400.5a
Zn	53.6 ± 5.7a	60.4 ± 4.6a	46.9 ± 2.9a
Al	528.0 ± 141.8a	281.7 ± 19.3a	354.9 ± 96.5a

Species-specific responses of herb layer element concentrations to acidification were assessed by a method similar to that used for cover responses in Fig. 1. Concentrations of each element (macronutrients, micronutrients, and Al) for harvested species were compared between watersheds (Fig. 2a–e; Fig. 3a–f). Treatment versus control watershed relationships were significant at $P < 0.001$ for all macronutrients except K, which was significant at $P < 0.01$ (Fig. 2a–e). The 1:1 reference lines closely approximated the regression lines for all macronutrients, lying within the 95% confidence intervals (not shown) throughout the range of nutrient concentrations in Fig. 2a–e.

Treatment versus control relationships were significant at $P < 0.05$ for all micronutrients except Fe (Fig. 3a–e); this relationship was not significant for Al (Fig. 3f). As with cover (Fig. 1) and the macronutrients (Fig. 2a–e), the 1:1 reference lines generally approximated regression lines for the micronutrients (Fig. 3a–d).

DISCUSSION

Data from this study allow us to test adequately several hypotheses on the effects of 3 yr of acidification treatment on forest soils and the herbaceous layer of FEF watersheds. Four of these, tested as null hypotheses as stated in the Introduction, will be addressed here.

Response of Soils to Acidification

Null Hypothesis 1 predicted that there would be no substantial differences in soil nutrients between watersheds. Indeed, forest soils were remarkably similar for most physical and chemical characteristics (Table 1). This is even more remarkable considering the differences in stand age, >80 yr for WS4 and ≈20 yr for WS3 and WS7. We expected that soil pH would be significantly lower in WS3 compared with WS4 and WS7. Soil pH for the treatment watershed was intermediate between, and not significantly different from, the two untreated watersheds.

A possible contributing factor to the lack of appreciable soil pH differences between watersheds would be the depth of sampling. Soils in this study were taken to a 10-cm depth, whereas other analyses of soils of these same watersheds taken to a 5-cm depth show that WS3 soils are significantly more acidic (F.S. Gilliam and T.K. Pauley, 1993, unpublished data). In a similar study, however, in a Swedish hardwood forest, Bergkvist and Folkeson (1992) also found little change in soil pH in response to simulated deposition (NH_4NO_3), but instead found a marked change in base cation/Al balance in soil solution after ≈5 yr. Based on our results to date and contrary to what we expected, we must accept the null hypothesis that the acidification treatment to data has resulted in no change in fertility within the composite upper 10-cm of soil in WS3. Future research will examine more closely N dynamics (e.g., mineralization and nitrification) in the mineral soil and forest floor to test further this hypothesis.

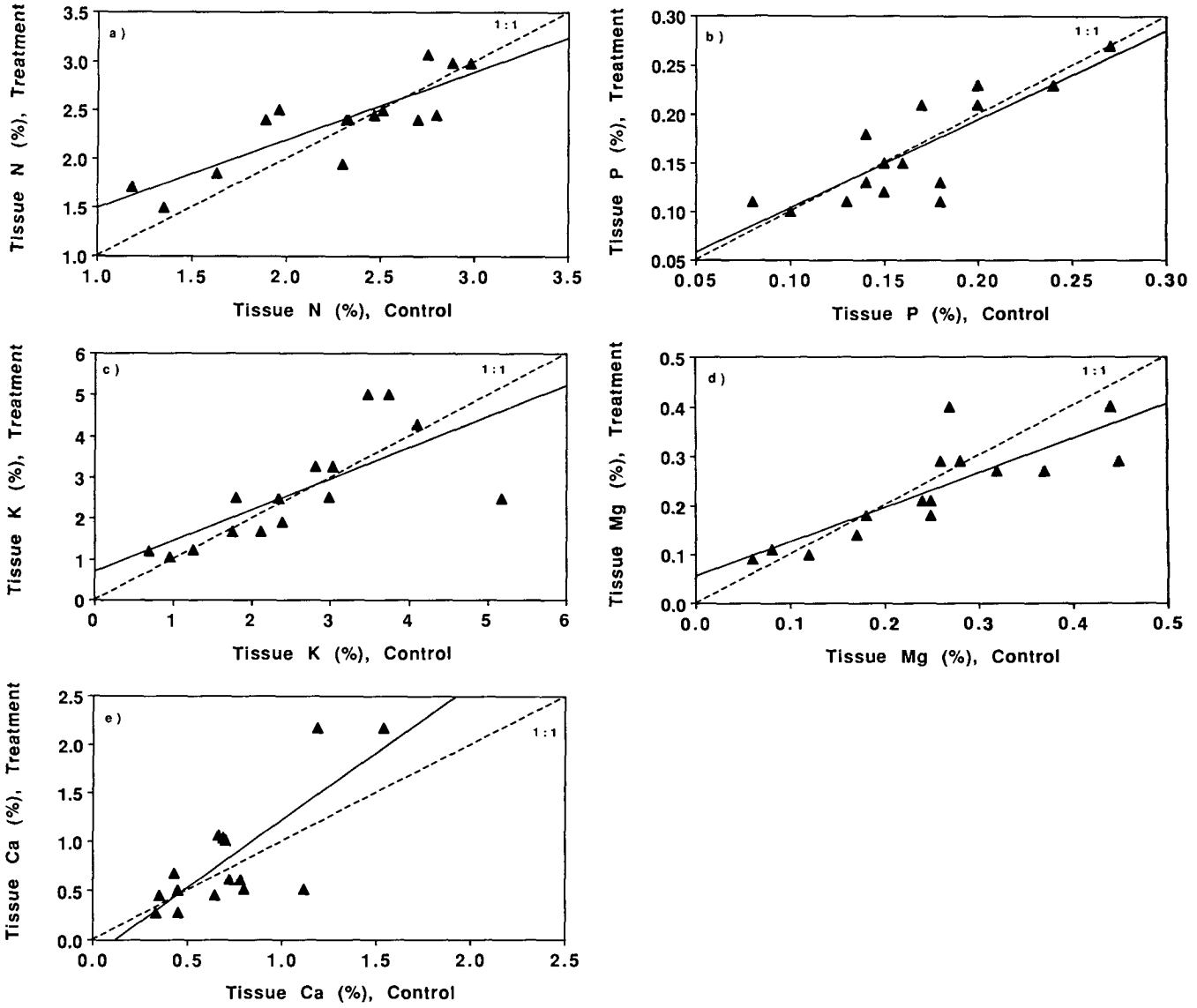

Fig. 2. Species-specific relationships for macronutrient concentrations of herbaceous layer tissue on acidification treatment watershed (WS3) vs. two control watersheds (WS4 and WS7). Each data point in the figure represents mean concentration for a species harvested from the treatment watershed and from either or both of the control watersheds. Dashed line is 1:1 reference line. (a) N: equation for regression line is $y = 0.78 + 0.70x$, $r^2 = 0.72$, $P < 0.0001$; (b) P: equation for regression line is $y = 0.01 + 0.91x$, $r^2 = 0.67$, $P < 0.001$; (c) K: equation for regression line is $y = 0.70 + 0.76x$, $r^2 = 0.51$, $P < 0.01$; (d) Mg: equation for regression line is $y = 0.05 + 0.70x$, $r^2 = 0.68$, $P < 0.001$; (e) Ca: equation for regression line is $y = -0.21 + 1.42x$, $r^2 = 0.61$, $P < 0.001$.

Response of Herbaceous Layer Cover and Species Diversity to Acidification

Null Hypothesis 2 predicted that herbaceous layer cover would not be significantly different between the experimental watersheds. As with soil characteristics, the watersheds were somewhat similar with respect to many general characteristics of the herb layer (Table 2). The most pronounced difference between watersheds was the ≈70% higher herb layer cover–biomass on WS7 relative to WS3 and WS4; there were no significant differences between WS3 and WS4 (Table 2). Since there are no pretreatment period data for these watersheds, it is difficult to determine whether differences between WS3 and WS7 may be related to the acidification treatment of WS3. Aulick (1993) attributed this broad discrepancy, however, to the predominance of fern species on WS7 and related fern dominance to the north- to northeast-facing aspect of the watershed, compared with the south and south-southeast aspects of WS3 and WS4, respectively. The microclimatic conditions of northern aspects generally include lower soil and air temperatures and higher soil moisture (Barbour et al., 1987), all conditions that would benefit fern species. Other studies have found ferns to be quite prevalent in the herbaceous layers of north-facing watersheds in the Appalachian region (Phillippi and Boebinger, 1986).

Microclimatic conditions of predominantly north-facing aspects of WS7 might also explain the slightly (but significantly) higher mean species richness per plot (Ta-

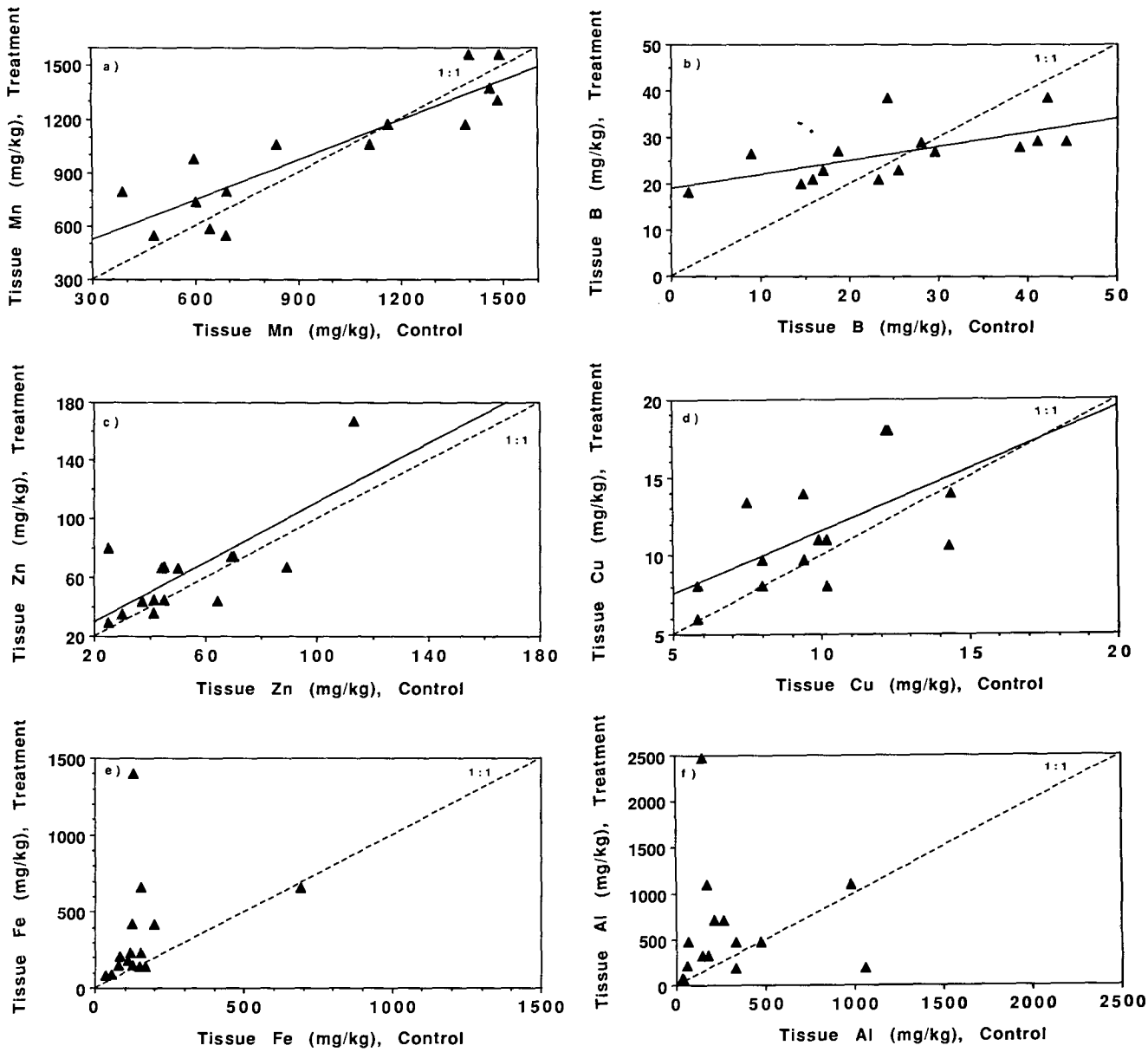

Fig. 3. Species-specific relationships for herbaceous layer tissue micronutrient and Al concentrations on acidification treatment watershed (WS3) vs. two control watersheds (WS4 and WS7). Each data point in the figure represents mean concentration for a species harvested from the treatment watershed and from either or both of the control watersheds. Dashed line is 1:1 reference line. (a) Mn: equation for regression line is $y = 293.15 + 0.75x$, $r^2 = 0.79$, $P < 0.0001$; (b) B: equation for regression line is $y = 19.11 + 0.30x$, $r^2 = 0.39$, $P < 0.05$; (c) Zn: equation for regression line is $y = 9.32 + 1.02x$, $r^2 = 0.56$, $P < 0.01$; (d) Cu: equation for regression line is $y = 3.55 + 0.80x$, $r^2 = 0.37$, $P < 0.05$; (e) Fe: regression not significant at $P < 0.05$; (f) Al: regression not significant at $P < 0.05$.

ble 2) and the higher total species richness per watershed from the floristic survey (127 species vs. 91 and 103 species for WS7 vs. WS3 and WS4, respectively) (Aulick, 1993). However, when species richness from the plot data is combined with species evenness (i.e., Shannon-Weiner diversity), there were no significant differences among watersheds (Table 2). Thus, although the results are not entirely conclusive for these variables, we would tend to accept the null hypothesis, and conclude that the acidification treatment has had minimal effects on herb layer cover and species diversity. This is perhaps not surprising, given the minimal treatment effects found on the soil.

Response of Herbaceous Layer Species Composition to Acidification

The lack of cover and species richness responses to acidification may have been the result of acidophilic species replacing acidophobic species in the herbaceous layer (Nygaard and Abrahamsen, 1991; Runge and Rode, 1991; Thimonier et al., 1992). Accordingly, Null Hypothesis 3 addresses a potential change in herb layer species composition and dominance in response to acidification. The alternate hypothesis would suggest that there would be a shift towards a higher number and relative cover of acid-tolerant

(acidophilic) species on WS3. Comparisons of the dominant herbaceous layer species of each of the study watersheds, however, suggest that, other than the fern predominance in WS7, there is little difference in species composition among watersheds (Table 3). The 15 species listed in Table 3 represent the top 10 species of each watershed according to an importance value (IV) based on the sum of relative frequency and relative cover. We feel that these species are truly representative of the overall dominance of the herb layer, since for a given watershed the 10 species combine to represent $\approx 70\%$ of the total IV (200). Furthermore, of the 15 dominant species listed in Table 3, 80% were found in all three watersheds by the floristic survey. Therefore, we conclude that there is an extremely high degree of species compositional similarity between WS3 and the other watersheds, consistent with predictions of Null Hypothesis 3.

Further evidence of similarities in species composition were seen in analysis of the floristic survey data. The modified Sorensen's index (Eq. [1]), based on the total number of species found in the floristic survey, was ≈ 0.40, from a possible range of 0 (no species in common) to 1 (all species in common). Using the floristic similarity dendrogram method of Sokal and Sneath (1963), Aulick (1993) found a similarity coefficient of 0.58 between WS3 and WS4 and a coefficient of 0.51 between WS7 and a cluster of WS3 and WS4. Based on this high degree of similarity in species composition, we accept Null Hypothesis 3.

An additional way to test Null Hypothesis 3, focussing on a more species-specific response, is to plot the mean cover for each species found in WS3 and its corresponding mean cover in WS4 or WS7 (i.e., all species found in the treatment watershed and at least one control watershed). If there is no species-specific response to acidification (accepting the null hypothesis), then all data points together should closely approximate a 1:1 reference line (a regression line with a slope of 1 and an intercept of 0). The regression equation for all species is: $y = 1.25 + 0.75x$, $r^2 = 0.47$, $P < 0.0001$. We compared the two lines (reference and regression) and determined if the reference line occurred within the 95% confidence intervals of the regression as generated by Statistix 4.0 (Statistix, 1992). The reference line was indeed within these confidence intervals for the entire range of cover data presented in Fig. 1. Thus, based on this species-specific response analysis, we again accept Null Hypothesis 3 that there is no substantial shift in species dominance on WS3 in response to acidification treatment.

Response of Herb Layer Tissue Element Concentrations to Acidification

Null Hypothesis 4 predicted that there would be little measurable response of herbaceous layer tissue element concentrations to acidification treatment. This hypothesis is accepted for the macronutrients. Although K and N concentrations were significantly different between WS4 and WS7, WS3 was not significantly different from either WS7 or WS4 for any of the macronutrients analyzed in this study, except Ca (Table 4).

The species-specific responses generally support the conclusions based on overall watershed herb layer means. Regression lines relating individual species mean concentrations for control watersheds to corresponding means for WS3 were significant for all macronutrients (Fig. 2a–d); furthermore, 1:1 reference lines occurred within 95% confidence intervals for regression lines of all macronutrients. Again, there appears to be little, if any, response of herbaceous layer macronutrient concentrations to acidification.

The response of tissue micronutrient concentrations to the acidification treatment is less clear than that of the macronutrients. There were no significant differences in micronutrient concentrations between WS3 and either WS7 or WS4, except for B (Table 4). Apparent differences ($P < 0.10$) were observed for tissue concentrations of Mn and Fe among watersheds. Thus, micronutrient data presented in Table 4 alone appear inconclusive with respect to Hypothesis 4.

Unlike that for the macronutrients, the species-specific response analysis for the micronutrients was not always consistent with data for overall watershed herb layer means. This discrepancy was quite pronounced for Mn. Manganese concentrations were lowest on WS3 (Table 4), yet the 1:1 reference line very closely approximated the regression line of treatment vs. control watersheds (Fig. 3a). The discrepancy was the result of three high-Mn species [cucumber tree (*Magnolia accuminata* L.), cinnamon fern (*Osmunda cinnamomea* L.) and black cherry] that occurred in the harvest subplots of WS4, but not in those of either WS3 or WS7. We therefore accept Null Hypothesis 4 for Mn.

We also accept Null Hypothesis 4 for B, Cu, and Zn. Although the mean for B was significantly higher on WS3 than on WS7 (Table 4), the 1:1 reference line closely approximated the regression line in Fig. 3b. Copper and Zn concentrations were extremely similar between watersheds (Table 4) and, except for a few outliers, species' data points were generally closely clustered around the reference line (Fig. 3c and d).

Higher herb layer Fe concentrations in response to acidification were indicated both by watershed means comparisons (Table 4) and by the species-specific analysis (Fig. 3e). The regression line of WS7/WS4 vs. WS3 was not significant for Fe, however, virtually all data points were above the 1:1 reference line (Fig. 3e), indicating a tendency for most species to have higher Fe concentrations in response to the acidification treatment. Thus, we reject Null Hypothesis 4 for Fe, suggesting that the acidification treatment has resulted in greater uptake of Fe by plants of the herbaceous layer. This is understandable considering that Fe is taken up largely as Fe^{2+} (Larcher, 1975) and Fe^{2+} mobility increases substantially with increased acidity (Sumner et al., 1991).

Comparisons among watersheds of herb layer tissue concentrations for Al also are inconsistent with Null Hypothesis 4. Aluminum concentrations were substantially higher on WS3 than on both WS7 and WS4 (Table 4). Although these differences were not significant, even at $P < 0.10$, the lack of significance was likely an artifact of the great variation in Al concentrations between species (ranging from <100 to ≈ 2500 ppm). Similar to Fe, the regression line of WS7/WS4 vs. WS3 was not significant for Al and

all data points but three were above the 1:1 reference line (Fig. 3e), indicating a tendency for most species to have higher Al concentrations in response to the acidification treatment. As with most of the micronutrients, Al mobility increases sharply with increasing acidity (Sumner et al., 1991). Although high Al mobility is toxic to most plant species (Runge and Rode, 1991), we suggest that tissue Al concentrations reported in this study are not at toxic levels, considering the lack of herb layer cover–biomass response to acidification.

CONCLUSIONS

These results suggest minimal changes in the herbaceous layer of this central Appalachian hardwood forest in response to ≈3 yr of experimental acidification treatment, contrary to virtually all of our predictions (i.e., supporting null hypotheses that we did not expect to be supported). This lack of response may in part be related to the minimal changes also found in the mineral soil for pH and extractable macro- and micronutrients. Minimal responses for both soil and herb layer may also be the result of insufficient time to detect such changes. Though herbaceous layer vegetation responds sensitively and rapidly to discrete disturbances, such as treefall gaps (Moore and Vankat, 1986), the acidification treatment on WS3 represents a low-intensity, chronic perturbation on an otherwise intact ecosystem. The existing biotic components (e.g., overstory canopy tree and forest floor and soil microbial populations) may be serving as effective short-term buffers against ecosystem-level effects of acidification. For example, soil solution NO_3^- concentrations exhibited no initial response to acidification treatment, but NO_3^- concentrations are now, after ≈3 yr of treatment, quite elevated in the soil water of the A horizon on WS3 (Edwards et al., 1992). Furthermore, stream water export of NO_3^- showed no response to treatment on WS3 for an entire year following initiation of the project, but is now increasing relative to WS4 (Adams et al., 1993).

Even though most of the herbaceous layer tissue element concentrations exhibited minimal responses to acidification, we feel that the species-specific responses for Fe and Al are real, and we speculate that acid-increased Al and micronutrient mobility, all of which increases substantially with increasing acidity (Sumner et al., 1991; Falkengren-Grerup and Tyler, 1993), may eventually lead to toxicity problems for the more sensitive forest species, especially those of the herb layer. Future work will involve more focus on element concentrations in a single herb layer species across all three watersheds to test this hypothesis.

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