

Research Article

Effects of Nitrogen on Temporal and Spatial Patterns of Nitrate in Streams and Soil Solution of a Central Hardwood Forest

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This study examined changes in stream and soil water NO_3^- and their relationship to temporal and spatial patterns of NO_3^- in soil solution of watersheds at the Fernow Experimental Forest, West Virginia. Following tenfold increases in stream NO_3^- concentrations over a 13-year period (1969–1981) on untreated WS4, concentrations have declined through 2006. Following fourfold increases in stream NO_3^- on treatment WS3 from pretreatment levels to a 1998 maximum, concentrations have declined through 2006, despite additions of N. Concentrations of soil water NO_3^- were consistently lower for WS4 compared to WS3. Data for soil water NO_3^- on WS3 versus WS4 followed patterns of net mineralization and nitrification for these watersheds. Nitrogen additions to WS3 decreased spatial heterogeneity of N processing, which was largest in the pretreatment year and decreased significantly to a minimum by 2000–2001. Concurrently, soil water NO_3^- increased on WS3 from 1.3 mg $\text{NO}_3^- \cdot \text{N L}^{-1}$ in pretreatment 1989 to a maximum of 6.4 mg $\text{NO}_3^- \cdot \text{N L}^{-1}$ in 2001. Spatial heterogeneity in soil water NO_3^- on WS4 remained high during this period. Data suggest that temporal patterns of stream NO_3^- may be influenced by spatial heterogeneity of watershed processes which vary over time in response to N availability.

1. Introduction

The structure and function of terrestrial ecosystems are maintained by processes that vary temporally and spatially. Furthermore, these scales of variation—through time and over space—can be highly codependent. Thus, the degree to which an ecosystem process, for example, nitrification, varies spatially is not a static property; rather, that degree of spatial variability changes over time. To borrow from Cowles' [1] description of the nature of plant succession, a given ecosystem process can often be thought of as “variable approaching a variable rather than a constant.” Recently, there has been an interest in temporal phenomena as *pulses* [2] and spatial phenomena as *subsidies* [3]. Anderson et al. [3] concluded that the synergistic effects of temporally and spatially variable resources help explain the complexities of food web structure. Rietkerk and van de Koppel [4] reviewed several studies demonstrating that spatial pattern

of ecosystems innately varies over time, which they called “spatial self-reorganization.”

Although temporal patterns of ecosystem processes (e.g., ecological succession, responses to disturbance) have long served as a cornerstone of ecological research, considerable current interest in characterizing spatial heterogeneity in terrestrial ecosystems has arisen from an awareness of the intimate relationship between spatial heterogeneity of soil resources and maintenance of plant species diversity [5, 6]. For example, working on limestone pavements of southern Ontario, Canada, Lundholm and Larson [7] found that, among 144 0.18 m² plots, peaks in species richness were most closely correlated with maximally heterogeneous plots. Hutchings et al. [8] suggested that the mechanism for such relationships arises from the alteration of interspecific competition brought about by spatial heterogeneity in soil resources. Significant responses of plant diversity to soil resource heterogeneity have been observed in studies that

span virtually all spatial scales, from the plot (e.g., 1-m²) [9, 10] to the landscape [11], and even on a global scale [12].

Indeed, although it has long been the bane of designs for field experiments [13], spatial heterogeneity is increasingly being considered an important, integral facet of terrestrial ecosystems [14, 15]. The cycling of nutrients, particularly nitrogen (N), in soils of terrestrial ecosystems is controlled by processes, such as microbial activity and plant nutrient dynamics, that often are quite spatially heterogeneous [16–19]. Relatively undisturbed terrestrial ecosystems typically display a high degree of spatial heterogeneity, with spatially discrete areas of high N mineralization rates in soils. These have been referred to as “islands of fertility” by Schlesinger et al. [17] and as “hot spots” by McClain et al. [14]. Other sources of heterogeneity of N in watersheds include the dispersion pattern within populations of different tree species [20], spatial variability in hydrology [21], interspecific variation in fine root structure and N content of trees, and the patchiness associated with snow and soil freezing events [22]. Many of these factors not only can vary over successional time but can also exhibit distinct seasonal patterns.

Such a plant-driven scenario in the temporal and spatial heterogeneity of N dynamics is consistent with the challenge made by Schimel and Bennett [23] to the classic paradigm of controls on N cycling in terrestrial ecosystems, which generally considers plants to be consistently poor competitors with microbes for available N. Accordingly, this long-held paradigm essentially relegates plants to a passive role in N cycling of terrestrial ecosystems and taking up largely only the available N not utilized to fulfill microbial demand. By contrast, Schimel and Bennett [23] consider plants as active drivers in soil N processing, including creating and maintaining microsites for N dynamics, thus directly contributing to a degree of spatial heterogeneity that does indeed vary temporally.

Among the body of work published from the Fernow Watershed Acidification Study (WAS) at Fernow Experimental Forest, West Virginia, are several studies that have reported a high degree of spatial heterogeneity in N dynamics in the long-term reference watershed—WS4 [24]. Peterjohn et al. [25] and Christ et al. [26] suggested that spatial patterns of N processing within WS4 may be controlled in large part by variation in species composition of the overstory, with areas of high rates associated with sugar maple and low rates associated with oaks. Gilliam et al. [27] found that watershed-scale patterns of N processing were even more highly correlated with species composition of the herb layer than with overstory canopy composition.

Also as part of the WAS, Gilliam et al. [27] reported the effects of experimental additions of N to an entire watershed (WS3) on several indices of N availability, including *in situ* net N mineralization/nitrification, from 1993 to 1995. Whereas most of these indices (e.g., extractable NO₃⁻ pools) were significantly higher on the treatment WS3 than on the control WS4, not all (e.g., net N mineralization) varied significantly between experimental watersheds. By contrast, virtually all indices of N availability displayed lower spatial heterogeneity on WS3, suggesting that additions of

N decreased spatial heterogeneity in N availability at the watershed scale.

The primary objective of this paper is to examine long-term patterns of stream NO₃⁻ in control and treatment watersheds at FEF in the context of temporal changes in spatial heterogeneity of soil N processing. As an indicator of soil N processing we use concentrations of NO₃⁻ in soil water, a parameter that generally correlates well with other measures of soil N processing, such as net nitrification and extractable N pools [28]. We tested the following hypotheses: (1) changes in stream NO₃⁻ for both untreated and N-treated watersheds will follow recent observations of decline in stream NO₃⁻ at various sites in the eastern United States, and (2) experimental additions of N will decrease spatial heterogeneity in soil N processing at the watershed scale in a central Appalachian hardwood forest.

2. Methods

2.1. Study Site. This study was conducted at the Fernow Experimental Forest, occupying ~1900 ha of the Allegheny Mountain section of the unglaciated Allegheny Plateau in Tucker County, West Virginia (39°03'N, 79°49'W). Annual precipitation for this region is approximately 1430 mm, with amounts being generally higher during the growing season and with increasing elevation [29]. Soils of the study watersheds are coarse-textured Inceptisols (loamy-skeletal, mixed mesic Typic Dystrochrept) of the Berks and Calvin series, sandy loams derived from sandstone [30].

Samples were collected from two watersheds. WS4 currently supports a mixed-aged hardwood stand last cut between 1904 and 1911; this watershed served as the untreated control. WS3 supports an even-age hardwood stand last cut in 1969, wherein ~90% of tree basal area was removed, except for a 3 ha shade strip left along the stream channel; this served as the treatment watershed, receiving additions of (NH₄)₂SO₄. Beginning in 1989, WS3 has received three aerial applications of (NH₄)₂SO₄ yr⁻¹: 9 kg N ha⁻¹ in the spring, 17 kg N ha⁻¹ in the summer, and 9 kg N ha⁻¹ in late fall, for a total of 35 kg N ha⁻¹ yr⁻¹. Mean ambient N deposition (wetfall only) at FEF is ~10 kg N ha⁻¹ yr⁻¹ [29]; dry deposition of N is ~2 kg N ha⁻¹ yr⁻¹ [31].

Tree species were generally similar on these watersheds, with the primary difference being one of dominance. Early successional species, such as black birch (*Betula lenta* L.), black cherry (*Prunus serotina* Ehrh.), and yellow poplar (*Liriodendron tulipifera* L.) were dominant on WS3, whereas late-successional species, such as sugar maple (*Acer saccharum* Marshall) and northern red oak (*Q. rubra* L.) were dominant on WS4. Dominant herbaceous layer species included violets (*Viola* spp.), blackberry (*Rubus* spp.), stinging nettle (*Laportea canadensis* (L.) Wedd.), and several ferns [32].

2.2. Field Sampling and Analyses. One year prior to the initiation of N treatments on WS3 in 1989, 15 sample areas were established on each of WS3 and WS4 (Figure 1), following criteria detailed in Adams et al. [24]. Briefly, these locations were selected to represent the full range of

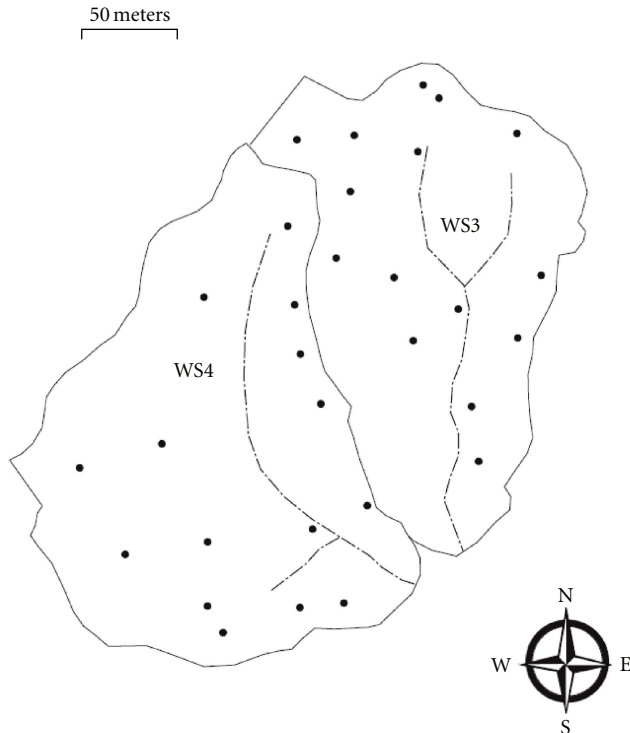


FIGURE 1: Map of study watersheds at Fernow Experimental Forest, West Virginia; each point on map represents a sample area including lysimeters used in this study. WS4 is the control watershed; WS3 is the treatment watershed, receiving $35 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ as $(\text{NH}_4)_2\text{SO}_4$.

elevations and slope aspects of these watersheds. A zero-tension lysimeter was installed at each sample area at each of two soil horizons: A (mean depth 13 cm) and B (mean depth 79 cm). Beginning January 1989 and currently on-going, soil water samples are taken on an approximately monthly basis when soil moisture is sufficient to produce samples. Stream water is similarly sampled on an on-going basis and is done in a variety of ways [33]. For this paper, stream water was grab-sampled weekly from 1984 through 2004 by field personnel at the Timber and Watershed Laboratory, Parsons, West Virginia. Further details for sampling and analysis can be found in Adams et al. [34] and Edwards et al. [33].

Stream samples were analyzed for NO_3^- with ion chromatography using a Dionex Model 10 IC until 2 November 1987. Since this date, all water samples, including stream and soil solution, have been analyzed for NO_3^- with a Dionex 400i HPLC [35].

2.3. Data Analysis. Annual concentrations of NO_3^- in stream water and soil solution were calculated as volume-weighted means [36]. The reporting period for stream data was from 1983 to 2004, whereas the period for soil water was 1989 to 2006.

Relationships between measured variables for both watersheds were assessed with linear regression, including stream NO_3^- concentrations versus time, stream versus soil water NO_3^- concentrations, soil water NO_3^- concentrations versus time, and coefficient of variation (CV) versus mean

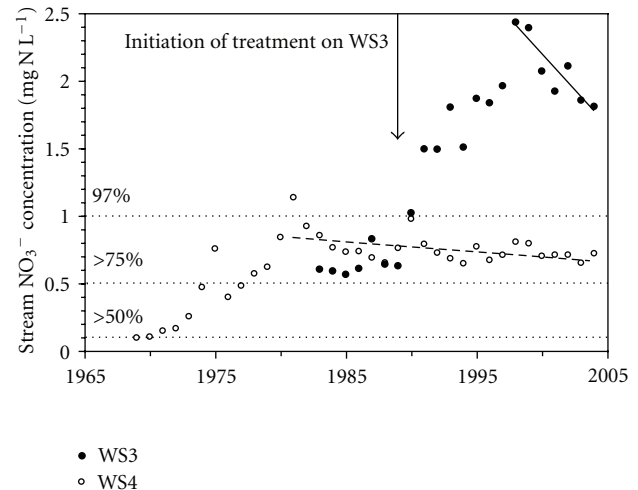


FIGURE 2: Mean annual stream water NO_3^- for WS3 (treatment: solid symbols) and WS4 (control: open symbols) at Fernow Experimental Forest, West Virginia. Arrow indicates initiation of N treatments on WS3. Horizontal dashed lines represent thresholds based on *The State of the Nation's Ecosystems* (see text). Heavy dashed line represents correlation between mean annual stream water NO_3^- and time from 1981 to 2004 for WS4 ($r = -0.51$, $P < .05$); solid line represents relationship from 1998 to 2004 for WS3 ($r = -0.90$, $P < .01$).

annual soil water NO_3^- concentration. Temporal patterns of soil water NO_3^- concentrations and associated CV were assessed with second-order polynomial functions.

We quantified spatial heterogeneity on the watershed scale by calculating the coefficient of variation (CV) for each watershed and year. Use of CV has considerable precedence in the ecological literature for studies that assess spatial heterogeneity, including those such as ours that use permanent plots. Guo et al. [37] used CV to examine temporal changes in spatial variability among permanent plots of soil moisture in a slash pine (*Pinus elliotii* Engelm.) forest over a 2.5-year period. In their review that defined and quantified spatial heterogeneity, Li and Reynolds [38] referred to CV as a measure of the magnitude of variance in spatial data.

3. Results and Discussion

For watersheds with contrasting stand age and history, WS3 and WS4 have displayed surprising similarity in general long-term patterns of stream NO_3^- concentrations (Figure 2). The pattern for stream NO_3^- on N-treated WS3 was as follows over the nearly 20 years of the WAS: (1) lack of rapid, initial response to the N treatment begun in 1989, (2) increase in stream NO_3^- to a maximum in 1998 following this lag period, and (3) significant decline since 1998 (Figure 2). Although receiving no experimental manipulations of any kind, WS4 had a similar trend for stream NO_3^- as did WS3, but over a longer time period (Figure 2). A highly significant linear increase ($r = 0.79$, $P < .01$) in streamwater NO_3^- was reported by Peterjohn et al. [39] for WS4 from 1969 to 1990.

Since the maximum annual concentration in 1981, however, NO_3^- has declined significantly ($r = -0.51$, $P < .05$) to the present time.

The similarity in temporal patterns of stream NO_3^- between these two watersheds is notable for two reasons. First, they support forests of sharply contrasting stand ages, with WS4 being typical of mature second-growth stands of this region [40] and WS3 being in an aggrading phase for most deciduous forest types [41]. Second, throughout the period since 1981 WS4 has received a cumulative total of just under 300 kg N via ambient atmospheric deposition (based on long-term means of annual N deposition), yet basal area of trees on WS4 has increased only minimally during this period; similarly, WS3 received a cumulative total of 245 kg N ha^{-1} during the period of 1998 to the present as part of the WAS (see Methods) and yet there were actually decreases in growth rates for some tree species [42].

Certainly, temporal patterns for WS3 and WS4 at FEF are consistent with studies of hardwood forests of the northeastern USA that report recent declines in stream NO_3^- concentrations [43–45]. Martin et al. [43] reported this pattern after resampling streams within the Bowl Research Natural Area in New Hampshire, finding decreases in stream NO_3^- over a 20-year period beginning in the mid 1970s. Goodale et al. [44] found similar declines when they resampled most of the streams throughout the New England region that were initially sampled and reported by Vitousek and Reiners [46], suggesting that interannual variation in climate and its effects on biotic retention of N was the most likely mechanism, a conclusion challenged by Huntington [47].

Goodale et al. [48] further suggested that increases in dissolved organic carbon (DOC), which can facilitate net immobilization of mineral N, might provide the most plausible mechanism for regional declines in stream NO_3^- . Dittman et al. [45] examined this phenomenon for Hubbard Brook Experimental Forest using a 12-year sampling period. They concluded that hydrologic flow paths exert an influence on stream N concentrations that can override both biotic and abiotic mechanisms of N retention.

Such a wide-spread occurrence of temporal declines in stream NO_3^- of relatively mature and undisturbed forests challenges predictions of traditional successional theory of nutrient cycling, as originally articulated by Odum [49] and further developed by Vitousek and Reiners [46]. Briefly, this theory recognizes that young, aggrading forest ecosystems should conserve NO_3^- because demand for available N by rapidly growing vegetation would exceed supply by atmospheric inputs and N mineralization. As these forests mature, net primary productivity (NPP) would decline and relative demand for N would attenuate proportionally, resulting in increases in loss of N via NO_3^- in streams. However, the synoptic scale of the observed decline of stream NO_3^- suggests that processes other than those associated with stand age-related change in NPP may be more important than once thought.

At FEF, the <40-year-old stand on WS3 would be considered to be in an aggrading phase by most forest models (e.g., [41]) and, indeed, there was an initial positive growth

response to the initiation of the N treatment [50]. However, current growth rates of some of the dominant tree species (e.g., *P. serotina*, *L. tulipifera*, and *A. rubrum*) on WS3 have decreased by as much as 50% [42] and have done so at a time when stream NO_3^- is also declining, indicating that patterns of decline in stream NO_3^- are not the result of increased uptake by rapidly growing trees of an aggrading ecosystem.

It is further notable that the reference watershed at FEF (WS4) has been cited as one of the better examples of an N-saturated watershed in North America [39, 51, 52]. To put our results in the context of stream NO_3^- throughout the United States, we compare data for WS4 to those from the Heinz Center for Science, Economics and the Environment that, as part of *The State of the Nation's Ecosystems*, summarized stream NO_3^- data from the United States Geological Survey National Water Quality Assessment. They found that as of 1998, 97% of forest streams in the United States had NO_3^- concentration less than 1 mg N L^{-1} , >75% had concentrations less than 0.5 mg N L^{-1} , and >50% had concentrations less than 0.1 mg N L^{-1} [53]. Thus, over the period 1969 to 1981, the stream draining WS4 increased from a level typical of over half the forested streams in the USA to one found in only ~3% of forested streams (Figure 4).

4. Alternative Hypotheses for Temporal Declines in Stream NO_3^-

In addition to mechanisms proposed by earlier studies (e.g., [44, 45, 47, 48, 54]) to explain temporal declines in stream NO_3^- that appear to be occurring on a synoptic scale in eastern USA and even in parts of Europe [55], we suggest that these temporal patterns can also be a function of spatial variability in soil N processing and that the degree of this spatial heterogeneity can change over time. In this section, we discuss long-term patterns of stream NO_3^- in the context of temporal variability in spatial heterogeneity of soil water NO_3^- . Finally, we discuss two processes—microbial population dynamics and decomposition—as potential mechanisms to relate temporal and spatial pattern. These processes share two characteristics: (1) they are sensitive to increases in N availability and (2) they potentially vary both temporally and spatially in watersheds of eastern deciduous forests.

4.1. Spatial Heterogeneity in Soil Water NO_3^- . For FEF during the period 1989 to 2004, annual mean concentrations of stream NO_3^- were significantly correlated with those of soil water NO_3^- on both WS3 ($r = 0.70$, $P < .05$) and WS4 ($r = 0.60$, $P < .05$) (Figure 3). This correlation is also apparent in similarities in long-term temporal patterns between stream and soil water NO_3^- for both watersheds. For WS4, this was a significant decline in stream and soil water NO_3^- from the period of 1988 to the present (Figure 4(a)). For WS3, there was an initial lack of response to the N treatment, followed by an increase of stream NO_3^- to a maximum concentration in 2000 and subsequent decline (Figure 4(b)).

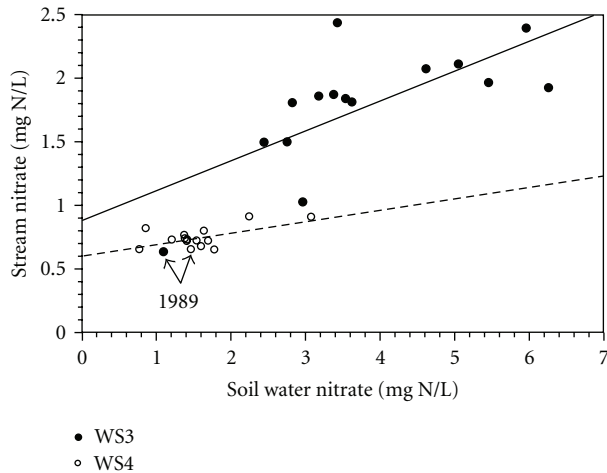


FIGURE 3: Mean annual stream water NO_3^- versus mean annual soil water NO_3^- concentrations for WS3 (treatment: solid symbols) and WS4 (control: open symbols) at Fernow Experimental Forest, West Virginia. Each point represents mean soil water NO_3^- for A and B horizon lysimeters for a given year. Line represents linear correlations: WS3: $r = 0.70$, $P < .003$; WS4: $r = 0.60$, $P < .05$.

Furthermore, data for soil water NO_3^- on the study watersheds over the period 1989 to 2006 at FEF suggest that spatial heterogeneity can decrease with increasing N supply, consistent with a prediction of the N homogeneity hypothesis [56, 57] that increases in N deposition decrease spatial heterogeneity of N processing. In the one-year pretreatment period, mean annual concentrations of soil water NO_3^- were nearly identical, and spatial heterogeneity (CV) was similar between watersheds (Figure 5). Heterogeneity of soil water NO_3^- on the untreated WS4 remained consistently high, with nine years exhibiting CVs $> 100\%$, or essentially half of the years for the sample period; soil water NO_3^- at WS4 remained consistently low (Figure 4(a)). By contrast, WS3 exhibited a maximum heterogeneity (CV) of $\sim 105\%$ for the one year prior to treatment, followed by a significant decrease toward a minimum of $\sim 60\%$ by 2000–2001 (Figure 4(b)). During this time, mean annual concentrations of soil water NO_3^- displayed a notable response to the N treatments on WS3, increasing nearly 5-fold from $1.3 \text{ mg NO}_3^- \cdot \text{NL}^{-1}$ in the pretreatment year (1989) to a maximum of $6.4 \text{ mg NO}_3^- \cdot \text{NL}^{-1}$ in 2001 (Figure 4(b)).

Linear correlation suggests a relationship between concentration of soil water NO_3^- and spatial heterogeneity on the watershed scale at FEF. CV of soil water NO_3^- and mean concentration of soil water NO_3^- were negatively ($P < .05$, $r = -0.60$) correlated for WS3, but not for WS4 for the sample period, 1989–2006 (Figure 5). The pretreatment year (1989) values for CV and, in particular, mean soil water NO_3^- were similar between watersheds: 125.9% and $1.21 \text{ mg NO}_3^- \cdot \text{NL}^{-1}$, respectively, for WS4 and 102.5% and $1.34 \text{ mg NO}_3^- \cdot \text{NL}^{-1}$ for WS3. On average, this varied little over the study period on WS4, with a centroid mean of 105.0% and $1.54 \text{ mg NO}_3^- \cdot \text{NL}^{-1}$; by contrast, the centroid mean for WS3 was 71.6% and $3.85 \text{ mg NO}_3^- \cdot \text{NL}^{-1}$ (Figure 5).

Although the mechanisms whereby added N decreases spatial heterogeneity of processing of soil N are difficult to elucidate at the watershed scale, studies at much finer spatial scales than those of a watershed (e.g., $1\text{--}10 \text{ m}^2$) have demonstrated that maintenance of high spatial heterogeneity in soil nutrients can arise from so-called “islands of fertility” [10, 17, 58, 59]. These are essentially discreet patches of high soil nutrient availability brought about by the clumped distribution of populations of terrestrial plant species which provide inputs of organic matter through litterfall, turnover of fine roots, and species-specific variation in the N content of throughfall and stemflow [60]. We suggest that excess N-mediated decreases in spatial heterogeneity of soil N arise from a “filling in” of the matrix within which these patches naturally occur. That is, inputs of N from atmospheric deposition increase N availability within the low-N matrix to approach that within the patches of high fertility. Indeed, recent studies have demonstrated the active role of plants in influencing soil N dynamics, which represents a departure from a long-held paradigm of N biogeochemistry [23].

4.2. N-Mediated Change in Size, Composition, and Activity of Microbial Communities. Soil microbial communities are extremely complex and diverse, certainly much more so than the plant communities with which they are associated [61]. They are also sensitive and responsive to changes in environmental conditions, such as temperature, and availability of essential resources, such as moisture and nutrients. Although rarely, if ever studied at the scale of a watershed ecosystem, several studies have shown that both microbial biomass and composition change drastically with experimental additions of N [62]. Indeed, Schmidt et al. [63] found that although soil and microbial N pools were higher in N-fertilized plots, both microbial activity and biomass were lower following N fertilization, with negative effects being more profound in the growing season.

Marschner et al. [64] examined the long-term effects of several forms of organic and inorganic N on structure and function of microbial communities of soils in Germany following 31 years of treatment. They found that bacterial communities, but not eukaryotic microbial communities, were particularly sensitive to N treatments. They also found profound changes in microbial composition in response to N treatments. More recently, Treseder [65] performed a meta-analysis of 82 published field studies of the effects of N additions on microbial biomass, finding no significant effects of ecosystem type, form of N fertilizer, level of ambient N deposition, or even microbial biomass methodology. However, she estimated that excess N reduced microbial biomass by 15% across all studies.

As part of the Chronic Nitrogen Amendment Study at Harvard Forest, Frey et al. [66] reported reductions in active fungal, but not bacterial, biomass in response to both low ($50 \text{ kg ha}^{-1} \text{ yr}^{-1}$) and high ($150 \text{ kg ha}^{-1} \text{ yr}^{-1}$) levels of N additions. N treatments also decreased the diversity of the ectomycorrhizal fungal community. Working on the same plots, Compton et al. [67] also found that chronic N additions altered the soil microbial community; results suggested that N treatments inhibited N_2 -fixing populations.

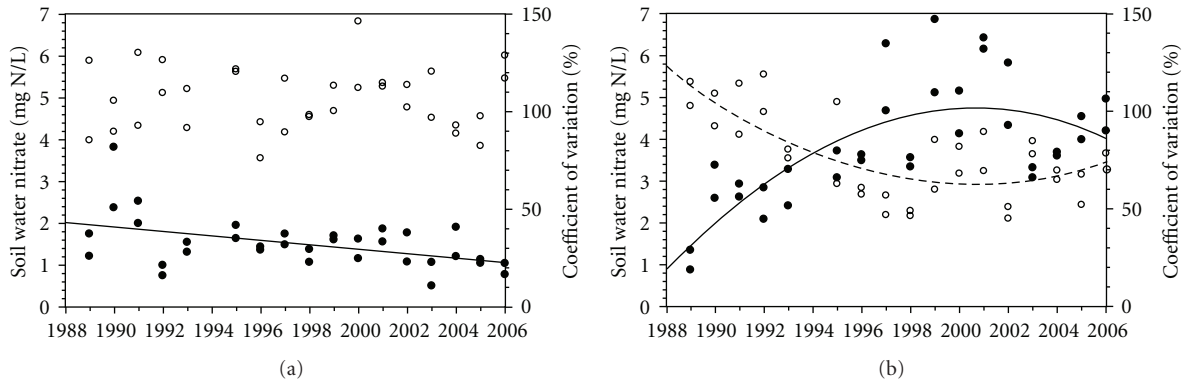


FIGURE 4: Annual mean soil water NO_3^- concentration (solid circles) and corresponding coefficients of variation (CV) (open circles) from 1989 (pretreatment year) to 2006 for (A) WS4 (control): solid line is soil water NO_3^- versus year— $y = 107.8 - 0.053x$, $r^2 = 0.22$, $P < .05$ —and (B) WS3 (treatment): dashed curve is CV versus year and represents the following 2nd-order polynomial: $y = 1504363 - 1503x + 0.36x^2$, $r^2 = 0.53$, $P < .01$; solid curve is soil water NO_3^- versus year and represents the following 2nd-order polynomial: $y = -97016 + 97.0x - 0.024x^2$, $r^2 = 0.53$, $P < .01$.

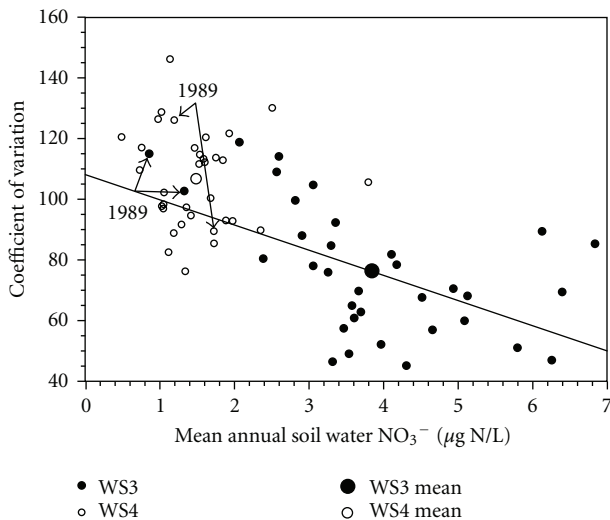


FIGURE 5: Coefficients of variation (CV) versus annual mean soil water NO_3^- concentration from 1989 (pre-treatment year) to 2006 for WS3 (treatment) and WS4 (control). Points in graph are combinations of soil water NO_3^- for A and B horizon lysimeters. Shown also are the combined means of CV and concentration (i.e., centroids) and the location of the pretreatment year (1989) for WS3 and WS4. Line represents linear correlation ($r = -0.60$, $P < .05$) for WS3 only.

Increases in N supply to forests clearly have the potential to alter the microbial communities of forest soils and do so at the watershed scale. Working at another forested site at FEF, Wallenstein et al. [68] found that N amendments did not alter denitrifier communities, indicating that observed decreases in stream NO_3^- are not likely the result of increased denitrification. We suggest that excess N-mediated shifts in microbial communities are toward those that simply process N at much lower rates (i.e., lower net N mineralization and nitrification) rather than those that affect immobilization. Data from on-going *in situ* incubations at

FEF indicate that rates of net nitrification on WS3 declined by 50% from 1995 to 2002 and by a further 80% from 2002 to 2005; declines over the same time intervals for WS4 were 52% and 67%, respectively (Gilliam and Peterjohn, *unpublished data*).

4.3. N-Mediated Decreases in Decomposition. Decomposition rates of organic material in forests are determined largely by litter quality, which is partly defined by N content, with higher rates usually accompanying higher N content [69]. Thus, it may be expected that higher inputs of N to forests might lead to higher rates of decomposition. To the contrary, several recent studies have shown that increased N not only can fail to stimulate rates of decomposition [70] but can often inhibit decomposition [71–74]. A meta-analysis of 24 studies on the effects of N additions on decomposition rates found widely varying results [75]: decomposition was inhibited at low and high rates of N addition (<75 and >125 $\text{kg N ha}^{-1} \text{yr}^{-1}$, resp.), and was stimulated at intermediate rates (75–125 $\text{kg N ha}^{-1} \text{yr}^{-1}$). On our watersheds at FEF, Adams and Angradi [76] found that N additions to WS3 significantly decrease litter decomposition rates of ecologically important species of central hardwood forests, for example, yellow poplar, black cherry, and black birch.

5. Conclusions

Temporal patterns of mean NO_3^- concentrations in soil solution support previous findings for soil N dynamics (i.e., N mineralization and nitrification) at FEF that increases in supply of N decrease spatial heterogeneity in N processing. There was a significant, negative relationship between soil water NO_3^- and spatial heterogeneity on the N-treated WS3. The decline in soil water NO_3^- on WS3 was consistent with the pattern of decline in stream water NO_3^- that has occurred since 1998. A similar decline has been observed for untreated WS4 since 1981 and for several forest streams over the past 20 years in the eastern United States. Although

it has been suggested that successional change in ecosystem demand for N may explain the declines in soil and stream water NO_3^- concentrations, similar temporal trends for two watersheds at different successional stages at FEF indicate that this proposed mechanism is not operating at our site. On the other hand, experimental additions of N have been demonstrated to alter soil microbial communities, including the balance between autotrophic and heterotrophic production of NO_3^- . In addition, N additions have been shown to decrease rates of decomposition of forest litter in several studies. Thus, based on evidence in the literature, we suggest that such effects may explain, at least in part, these observed declines. Such patterns challenge long-held theories of successional change in the biogeochemistry of forest ecosystems and open up new areas for further research.

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