Ecosystem nutrient responses to chronic nitrogen inputs at Fernow Experimental Forest, West Virginia

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Abstract: Among the current environmental concerns for forests of the eastern United States is nitrogen (N) saturation, a result of excessive inputs of N associated with acidic deposition. We studied nutrient responses on N-treated and untreated watersheds of the Fernow Experimental Forest, West Virginia, to test for evidence of N saturation on the treated watershed. The watersheds were WS7 (23-year-old even-aged control), WS4 (mature mixed-aged control), and WS3 (23-year-old even-aged treatment). WS3 has received aerial applications of $(NH_4)_2SO_4$ from 1989 to the present (a total of 4 years for the study period) at 3× ambient inputs of N and S (54 and 61 $kg \cdot ha^{-1} \cdot year^{-1}$, respectively). Base-flow stream samples were collected weekly from each watershed and analyzed for NO_3^- and Ca^{2+}. Mineral soil was incubated in situ, placed in bags, and buried about every 30 days during the growing season in each of seven sample plots within each watershed. Moist samples of soil from the bags were analyzed for extractable NH_4^+ and NO_3^-. In addition, forest floor material and leaves of an herbaceous species (*Viola rotundifolia* Michx.) from each plot were analyzed for N and other nutrients. *Viola rotundifolia* was present on all 21 plots and used as an additional indicator of N availability and soil fertility. Foliage tissue was sampled from overstory tree species (*Liriodendron tulipifera* L., *Prunus serotina* Ehrh., *Betula lenta* L., and *Acer rubrum* L.) from WS3 and WS7 and analyzed for nutrient content. Results from the 1993 growing season showed few, if any, differences among watersheds for (1) N content and C/N ratio of the mineral soil and forest floor and (2) relative proportion of NH_4^+ and NO_3^- produced in the buried bags. Nitrification rates were equally high in soils of all watersheds; N concentrations were significantly higher in foliage tissue of overstory tree species and of *V. rotundifolia* in the treatment versus control watersheds; plant tissue Ca was significantly lower for the treatment watershed than for the control watersheds. Our results support the conclusions of earlier studies that high amounts of ambient N deposition have brought about N saturation on untreated watersheds at the Fernow Experimental Forest. This is suggested by minimal differences among watersheds in N mineralization and nitrification and soil and forest floor N. However, aggravated N saturation on our treated watershed can be seen in differences in plant tissue nutrients among watersheds and streamflow data, indicating increased losses of NO_3^- with accompanying losses of Ca^{2+} in response to further N additions to a N-saturated system.

Résumé : La saturation en azote (N) résultant des apports excessifs en N associés aux dépositions acides dans les forêts de l'Est des États-Unis est l'une des préoccupations environnementales de l'heure. Nous avons étudié les réponses en nutriments dans des bassins traités avec N ou non traités de la forêt expérimentale de Fernow, en Virginie de l'Ouest, pour confirmer la saturation en N dans le bassin traité. Ces bassins étaient WS7 (peuplement équienne de 23 ans comme témoin), WS4 (peuplement mature d'âge mixte comme témoin) et WS3 (peuplement équienne de 23 ans comme traitement). WS3 a reçu des applications aériennes de $(NH_4)_2SO_4$ pendant 4 ans à partir de 1989 à 3× les apports ambiants de N (54 $kg \cdot ha^{-1} \cdot an^{-1}$) et de S (61 $kg \cdot ha^{-1} \cdot an^{-1}$). Les eaux des ruisseaux de drainage de chacun des bassins ont été récoltées hebdomadairement et analysées pour NO_3^- et Ca^{2+}. Du sol minéral a été placé dans des sachets et enfoui environ 30 jours in situ durant la saison de croissance dans chacune des sept parcelles d'échantillonnage de chacun des bassins. Les échantillons humides de sol dans les sachets ont été analysés pour le NH_4^+ et le NO_3^- extractibles. En plus, le matériel de la couverture morte et les feuilles d'une espèce herbacée (*Viola rotundifolia* Michx.) de chacune des parcelles ont été analysés pour N et d'autres nutriments. *Viola rotundifolia* était présente dans chacune des 21 parcelles et a été utilisée comme indicateur additionnel de la disponibilité de N et de la fertilité du sol. Le feuillage des arbres dominants (*Liriodendron tulipifera* L., *Prunus serotina* Ehrh., *Betula lenta* L. et *Acer rubrum* L.) a été échantillonné dans les bassins WS3 et WS7 et analysé pour sa teneur en nutriments. Les résultats de la saison de croissance de 1993 n'ont pas montré ou ont montré peu de différences entre les bassins pour (1) le contenu en N et le rapport C/N

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du sol minéral et de la couverture morte et (2) la proportion relative de NH_4^+ et NO_3^- produits dans les sachets enfouis. Les taux de nitrification étaient élevés et semblables dans tous les écosystèmes; les concentrations de N étaient significativement plus élevées dans le feuillage des arbres et de *V. rotundifolia* dans le bassin traité que dans les bassins témoins alors que l'inverse était observé pour Ca. Nos résultats supportent les conclusions d'études antérieures à l'effet que les forts apports de N par les dépositions acides ont conduit à une saturation en N à la forêt expérimentale de Fernow. Ceci est suggéré par des différences minimales dans la minéralisation de N, la nitrification et les pools de N du sol minéral et de la couverture morte entre les bassins. Toutefois, la plus grande saturation de N dans notre bassin traité se manifeste par des différences dans les concentrations de nutriments des plantes entre les bassins. De plus, les données de l'eau d'écoulement indiquent une augmentation des pertes de NO_3^- avec une augmentation concomitante des pertes de Ca^{2+} en réponse à des ajouts additionnels dans un système déjà saturé en N.

[Traduit par la Rédaction]

Introduction

A current environmental concern is nitrogen (N) saturation, which may result from excessive inputs of N associated with acidic deposition. Although forest ecosystems typically are assumed to be N limited, the general N saturation hypothesis posits that N supply (via acidic deposition) exceeds demand by the biota. There are several direct and indirect consequences of N saturation. Some of these consequences are (1) increases in NO_3^- loss via leaching, (2) decreases in foliar lignin concentration, (3) increases in foliar N concentration, and (4) decreases in soil C/N ratios (Aber 1992). It is suggested that such changes could lead to forest decline (Aber et al. 1989; Skeffington 1990; Johnson and Ball 1990).

Most of the research on N saturation in forest ecosystems has been carried out in Europe, particularly at the ecosystem level (see Wright and van Breemen 1995 and references cited therein). Several studies have concluded that the forests of Europe are becoming N saturated (Skeffington 1990; Sullivan 1993; Stein and van Breemen 1993; Tietema et al. 1993; Jacks et al. 1994; Wright and van Breemen 1995). Much of the research using experimental manipulations on N saturation in the United States has been done in hardwood and conifer forests of the Northeast (Aber et al. 1989, 1993; McNulty and Aber 1993). Inputs of N have been manipulated by applications of varying amounts of N to individual plots. Such experimental designs allow for more appropriate statistical tests of N treatment effects, but they do not provide an adequate treatment scale to examine effects at the ecosystem level.

Less research has been conducted in other forest ecosystem types of the United States. Because many of the hardwood forests of the southern and central Appalachian region receive extremely high rates of N deposition, the potential for N saturation may be particularly great in this region (see Stoddard 1994 and references therein). Wetfall inputs of N can be greater than 10 kg $N \cdot ha^{-1} \cdot year^{-1}$, with deposition rates increasing with increasing elevation (F.S. Gilliam and M.B. Adams, submitted[2]). Total N deposition may be as much as 4–5 kg $N \cdot ha^{-1} \cdot year^{-1}$ higher than wetfall amounts when dry deposition of N is included (Lovett and Lindberg 1993).

[2] F.S. Gilliam and M.B. Adams. Wetfall deposition and precipitation chemistry for a central Appalachian forest. J. Air Waste Manage. Assoc. Submitted.

Previous studies on effects of chronic N additions on stream chemistry have shown that these treatments can result in elevated losses of N (as NO_3^-) and base cations, especially calcium (Ca^{2+}) (Adams et al. 1993; Kahl et al. 1993; Rustad et al. 1993; Norton et al. 1994). This has important implications for forest health because it has been suggested that increased losses of base cations can lead to growth limitations in forests previously limited by N or phosphorus (Gilliam and Richter 1991; Eagar and Adams 1992).

In 1988, the USDA Forest Service began a project at the Fernow Experimental Forest (FEF), Parsons, West Virginia, to provide additions of $(NH_4)_2SO_4$ to an entire watershed (Adams et al. 1993). Although this should have allowed us to test the effects of initiating N saturation on a hardwood forest, Stoddard (1994) reviewed compelling data to indicate that one of our untreated watersheds (WS4) was already N saturated. Thus, the objective of this study was to examine the effects of additional inputs of N to an already N-saturated system. We accomplished this by measuring (1) concentrations of NO_3^- and Ca^{2+} in streamflow, (2) soil N dynamics, and (3) forest floor and plant tissue chemistry.

Methods

Study site

Research was conducted at FEF, about 1900 ha of the Allegheny Mountain section of the unglaciated Allegheny Plateau, Tucker County, West Virginia (39°03′N, 79°49′W). Precipitation averages around 1430 $mm \cdot year^{-1}$, with precipitation generally highest in June and lowest in January and increasing with higher elevations (F.S. Gilliam and M.B. Adams, submitted (see footnote 2)). Soils of the study watersheds are Inceptisols of the Berks and Calvin series, both loamy-skeletal, mixed, mesic Typic Dystrochrepts. These are derived from sandstone and are generally coarse-textured sandy loams, well drained, and approximately 1 m deep.

Three watersheds were used for sampling: WS4, WS7, and WS3, with WS3 serving as the treatment watershed, receiving additions of $(NH_4)_2SO_4$, and WS4 and WS7 serving as controls for different stand ages. WS3 received three aerial applications of granular $(NH_4)_2SO_4$ per year beginning in 1989, for a total of 4 years of treatment prior to this study. March and November applications were 33.6 $kg \cdot ha^{-1}$ of fertilizer, or approximately 7.1 kg $N \cdot ha^{-1}$; July applications were 100.8 $kg \cdot ha^{-1}$ fertilizer, or 21.2 kg $N \cdot ha^{-1}$. These rates were chosen as approximately 2× the ambient rates of N deposited on the watersheds via throughfall. The total amount of N deposited on WS3 (application plus

Table 1. Important overstory species of three watersheds of Fernow Experimental Forest, West Virginia.

	WS7	WS3	WS4
Acer pensylvanicum L.	4.4	4.5	9.7
Acer saccharum	44.3	33.7	74.7
Betula lenta	37.4	—	—
Fagus grandifolia	—	9.6	18.7
Fraxinus americana L.	12.3	7.9	—
Liriodendron tulipifera	20.7	10.4	2.2
Prunus serotina	51.0	100.9	13.1
Quercus prinus L.	—	—	8.9
Quercus rubra	2.7	11.4	43.6
Robinia pseudoacacia L.	11.5	—	—
Sassafras albidum (Nutt.) Nees	5.3	6.8	—

Note: Data are importance values based on sum of relative basal area and relative density. (From Gilliam et al. 1995, reproduced with permission of Ecol. Appl., Vol. 5, ©1995 The Ecological Society of America.)

atmospheric deposition) is approximately 54 kg $N \cdot ha^{-1} \cdot year^{-1}$, or about 3× pretreatment levels (Adams et al. 1993). The total amount of S deposited on WS3 is approximately 61 kg $S \cdot ha^{-1} \cdot year^{-1}$, also 3× pretreatment levels (Adams et al. 1993). Ambient deposition of N and S averages about 15 and 20 $kg \cdot ha^{-1} \cdot year^{-1}$, respectively.

The WS3 stand is about 23 years old and even aged; WS4 is a >80-year-old mixed-aged stand. To better account for differences in stand age, WS7 (also ~23 years old) was included in the study as an additional control. WS7 received herbicide treatment for about 6 years prior to release in 1969 (Kochenderfer and Wendel 1983). Both WS3 and WS7 were typical of second-growth hardwood forests of this region (e.g., WS4) before treatments.

All three study watersheds are composed of mixed hardwood stands. Overstory dominant species include sugar maple (*Acer saccharum* Marsh.), black 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.) (Table 1). The herbaceous layer is spatially heterogeneous, dominated by stinging nettle (*Laportea canadensis* (L.) Wedd.) and species of violet (*Viola* spp.) on WS3 and WS4 (Gilliam and Turrill 1993) and by several fern species on WS7, including shield fern (*Dryopteris marginalis* L. Gray) and Christmas fern (*Polystichum acrostichoides* Michx. Schott.) (Gilliam et al. 1994).

Field sampling

As part of long-term research on factors influencing stream chemistry on watersheds at FEF, weekly grab samples have been taken on WS3, WS4, and WS7 since 1960 (Adams et al. 1994). Flow responding to precipitation events ("storm flow") also is sampled, but these data are not presented here. Data reported in this paper reflect base-flow (non-storm-flow) sampling for January–December 1988 (1 year pre-treatment) and 1993 (4 years post-treatment).

Seven circular 0.04-ha plots (11.3-m radius) were sampled in each watershed, for a total of 21 study plots. Plots were located on all watersheds on similar sites in terms of elevation, slope, and aspect. Five sample points were located by a stratified-random method to avoid oversampling the inner half of the plot (Gaiser 1951). The O horizon was removed, and mineral soil was sampled at each point with a hand trowel to a depth of 5 cm. These five samples were combined and mixed thoroughly to yield a single composite sample per plot. To minimize further disturbance to soils, they were not sieved in the field, but pebbles, rocks, and larger roots were excluded from subsamples. Subsamples of composited mineral soil were placed in polyethylene bags and incubated in situ by burying them 5 cm beneath the mineral soil surface (the "buried bag" technique; Eno 1960) about every 30 days from June to September 1993. The October sample was incubated until May 1994. All samples were refrigerated immediately in the field (using ice packs within backpacks) and then stored prior to extraction at 4°C.

Nutrient analysis of a prominent herbaceous layer species, *Viola rotundifolia* Michx., was used as a further indicator of N availability and soil fertility, as this species was found on all 21 plots. Foliar material of *V. rotundifolia* was harvested during the July sampling. At this same time, a 10 × 10 cm section of forest floor (O1 + O2 horizons, 5–10 cm deep) was taken from three sample points within each plot and composited.

As part of a separate study, five trees were harvested for each of four species (black cherry, red maple, black birch, and yellow-poplar) in both WS3 and WS7 in July 1991. These harvests included only dominant (12–23 cm diameter at breast height) and codominant (5–10 cm) trees. Trees were not harvested from WS4 because that watershed is being maintained in as undisturbed a state as possible. All leaves were removed from harvested trees, mixed, and subsampled randomly for nutrient analysis.

Laboratory analyses

Samples of stream water are routinely analyzed for a variety of dissolved ions. Because of their relevance to the N saturation phenomenon and implications for forest health, only NO_3^- and Ca^{2+} data are reported in this paper. All samples were analyzed at the Northeastern Forest Experiment Station's Timber and Watershed Laboratory at Parsons, West Virginia, using protocols, holding times, and quality assurance – quality control procedures approved by the U.S. Environmental Protection Agency (Edwards and Wood 1993).

Subsamples of soil from paired sample bags (one incubated and one brought to the laboratory immediately after sampling) were extracted for analysis to determine net mineralization and nitrification. Subsamples were extracted with 1 M KCl (10:1 v/w) and analyzed for NH_4^+ with an Orion 720A pH/ISE meter[3] and NH_4^+ electrode; all samples had concentrations well above the detection limit of 0.01 ppm N, and random repeat samples indicated minimal signal drift. Separate subsamples were extracted with deionized H_2O (10:1 v/w) and analyzed for NO_3^- with an Orion 720A pH/ISE meter and NO_3^- and reference electrodes; again, all concentrations were well above the detection limit (0.1 ppm N) and random repeat samples indicated minimal signal drift. Net mineralization was calculated as incubated (buried) soil NH_4^+ plus NO_3^- minus initial soil NH_4^+ plus NO_3^-. Net nitrification was calculated as incubated soil NO_3^- minus initial soil NO_3^-.

Following oven-drying and weighing, *V. rotundifolia*, tree foliage, and forest floor samples were ground in a Wiley mill to pass a 40-mesh screen. The samples were analyzed at the University of Maine Soil Testing Service and Analytical Laboratory for macronutrient (N, P, Ca, Mg, K) concentrations. Total Kjeldahl N was determined with autoanalysis following

[3] Mention of a proprietary product constitutes neither endorsement nor recommendation by the USDA Forest Service or Marshall University.

block digestion with H_2SO_4 and K_2SO_4–$CuSO_3$; National Bureau of Standards NBS 1572 citrus leaf was used as a standard. All other elements were determined with plasma emission spectrophotometry following dry ashing and extraction with HCl and HNO_3.

Since each watershed represents an experimental condition with a sample size of one, the design of this project is an example of simple pseudoreplication (Hurlbert 1984). Accordingly, our data should be interpreted with caution. Although pseudoreplication creates statistical problems, it is common among watershed ecosystem studies (cf. Likens et al. 1977) and is related to the logistical difficulties of carrying out studies at the ecosystem level. For this paper we are assuming that differences among watersheds for any measured variable are due to the $(NH_4)_2SO_4$ treatment applied to WS3 and not to previous watershed treatments. Indeed, earlier studies on these same watersheds revealed minimal differences for virtually all inherent soil characteristics, e.g., texture, cation exchange capacity, organic matter, and base saturation (Gilliam et al. 1994; Gilliam and Adams 1995). Also, to test effects of chronic N additions, we are assuming that the untreated watersheds are N saturated. We feel this is valid considering findings of Stoddard (1994), who concluded that WS4 at FEF might be the best example, among numerous watersheds of the eastern United States, of a watershed in the later stages of N saturation.

Statistical analyses

Means of all measured variables were compared for significant differences among watersheds using analysis of variance (ANOVA) and Duncan's multiple range test (SAS Institute Inc. 1990). Mean rates of N mineralization and nitrification, when found not to vary significantly among watersheds, were pooled for each incubation period (all watersheds combined). Pooled means of rates were then compared for significant differences among incubation periods using ANOVA and Duncan's multiple range test. Finally, concentrations of N and Ca in tree foliage sampled from WS3 were compared with concentrations in foliage sampled from WS7 using linear regression (SAS Institute Inc. 1990).

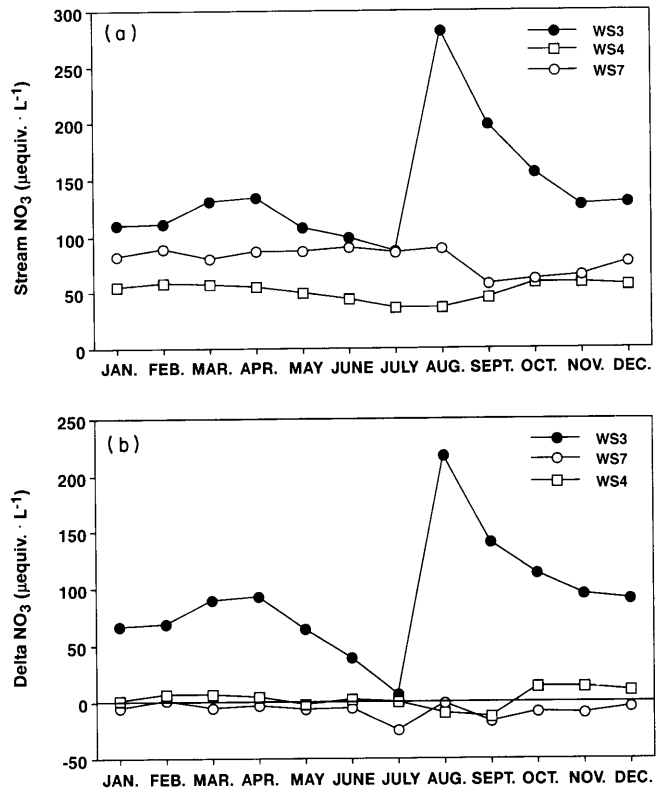
Results and discussion

Stream chemistry

Nitrification long has been known to be an acidifying process that includes the bacterial oxidation of NH_4^+ and subsequent release of H^+ into the soil (Tietema and Verstraten 1991). One of the consequences of increased acidification (from increases in both acidic deposition and nitrification) is elevated mobility and stream concentrations of base cations, especially Ca^{2+}. Theoretical studies and modeling have shown that Ca^{2+} and other base cations decrease on cation-exchange sites (and subsequently increase in soil solution and streamflow) with increasing acidity (Reuss and Johnson 1986; Gilliam 1991; Gilliam and Richter 1991), particularly when base saturation is >20% (Turner et al. 1986). Base saturation of soils of our watersheds was approximately 25% and did not vary significantly among watersheds (F.S. Gilliam, unpublished data). As an environmental concern, Ca deficiencies have been linked to declines in productivity of some high-elevation forest ecosystems (Eagar and Adams 1992).

At FEF, Adams et al. (1993) found that increased outputs of Ca^{2+} were associated with increased outputs of NO_3^- and related to the experimental treatment of WS3. They concluded that increased outputs of Ca^{2+} were not associated with SO_4^{2-} mobility because of the high capacity of these soils for SO_4^{2-} adsorption (Helvey and Kunkle 1986) and because of low SO_4^{2-} losses in streamflow (Adams et al. 1993) or soil solution (M.B. Adams et al., to be published). Thus, we were interested in examining base-flow concentrations of NO_3^- and Ca^{2+} as evidence of aggravated N saturation on WS3.

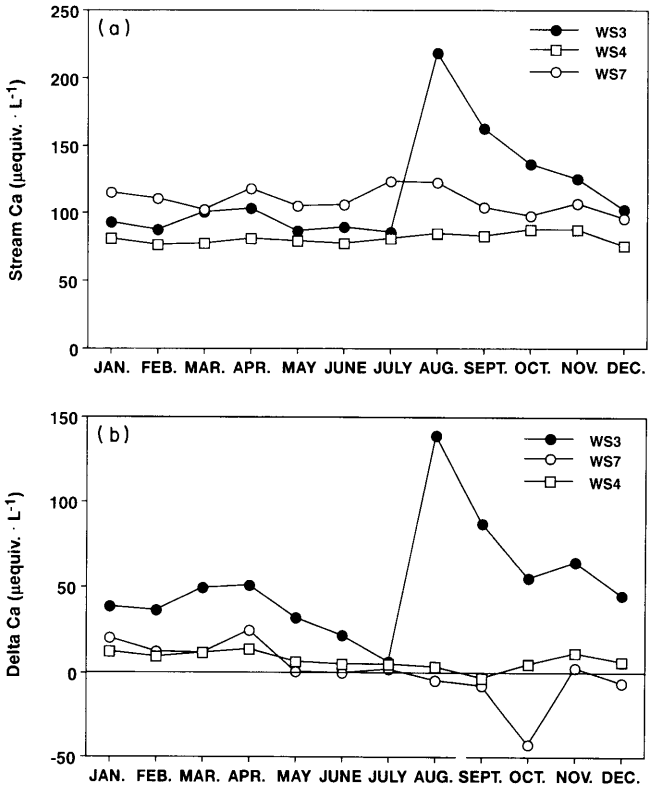
Fig. 1. Volume-weighted concentrations of NO_3^- in base flow of WS3, WS4, and WS7 for (*a*) 1993 data only and (*b*) pre- to post-treatment change in NO_3^- concentrations, calculated as monthly means for 1993 (post-treatment) minus those for 1988 (pre-treatment). Note that delta NO_3^- calculations for August are based on 1988 means for WS3 and WS4, which were determined by interpolation because of lack of flow for that month.



For the study period (calendar year 1993), except for the months of May–July, stream NO_3^- was substantially higher on WS3 than on the control watersheds (Fig. 1*a*). Stream water draining WS7 was slightly higher in NO_3^- than that draining WS4 for most of 1993. Stream Ca^{2+} showed little seasonal pattern on the control watersheds and was slightly higher on WS7 than on WS4 (Fig. 2*a*). The sharp seasonal increase of stream Ca^{2+} on WS3 was coincidental with that of stream NO_3^- (Figs. 1*a* and 2*a*). Pre- to post-treatment differences in stream NO_3^- and Ca^{2+} concentrations were evident on WS3, but not on either WS7 or WS4 (Figs. 1*b* and 2*b*).

Aber (1992) reviewed research on changes in concentrations of stream NO_3^- as a forest ecosystem progresses toward a condition of N saturation. At stage 0, the system is severely N limited and there are rarely higher than detectable levels of NO_3^- in stream water throughout the

Fig. 2. Volume-weighted concentrations of Ca^{2+} in base flow of WS3, WS4, and WS7 for (*a*) 1993 data only and (*b*) pre- to post-treatment change in Ca^{2+} concentrations, calculated as monthly means for 1993 (post-treatment) minus those for 1988 (pre-treatment). Note that delta Ca^{2+} calculations for August are based on 1988 means for WS3 and WS4, which were determined by interpolation because of lack of flow for that month.



year. At stage 1, a transitional stage during which N demand by the biota is beginning to be met by increased N deposition, there are elevated concentrations of NO_3^- during non growing season periods of streamflow, but decreases toward nondetectable levels during the growing season. At stage 2, when the ecosystem is N saturated, concentrations of stream NO_3^- are elevated at all times, though they may decrease slightly during the growing season.

Based on concentrations of stream NO_3^- alone (Fig. 1*a*), it appears that all watersheds had reached stage 2 by 1993, exhibiting the pattern described for a N-saturated system and confirming earlier conclusions concerning WS4 (Stoddard 1994). The effects of further N additions on a saturated system are particularly apparent when examining change in NO_3^- concentrations from 1993 (post-treatment) to 1988 (pre-treatment) monthly means. Whereas stream NO_3^- showed little change from 1988 to 1993 on WS7 and WS4 (especially from May to December), increases in stream NO_3^- were quite pronounced on WS3 (Fig. 1*b*).

The response of Ca^{2+} closely resembled that of NO_3^-, with substantial increases in concentrations from 1988 to 1993 on WS3, but not on either control watershed (Fig. 2*b*). Further, in 1993, seasonal patterns of Ca^{2+} in stream water followed those of NO_3^- on WS3 but not on WS7 or WS4. For example, although NO_3^- was highest on WS3 for virtually all of 1993, Ca^{2+} concentrations were highest on WS7 for most of the year (Figs. 1*a* and 2*a*). The most notable pattern of similarity in seasonal concentrations of NO_3^- and Ca^{2+} is for August–December 1993 on WS3. The sharp increase in concentrations of both ions in August streamflow was the result of extremely low flow typical of summer drought conditions, i.e., the data points for both ions represent only a single week of streamflow sampling, since there was no flow during the other weeks. However, as flow increased following the growing season, concentrations of both NO_3^- and Ca^{2+} remained elevated on WS3 but showed little change on WS7 and WS4. Finally, NO_3^--mediated leaching of Ca^{2+} on WS3 is indicated further by a significant correlation ($r = 0.98$, $\alpha = 0.001$) between the two ions for monthly mean concentration differences (1993 minus 1988). Neither control watershed showed such correlations, suggesting that base cation leaching may represent a later stage of N saturation for these forests.

Our results are consistent with the seasonal patterns in soil-solution chemistry found by Rustad et al. (1993), who reported increased leaching of Ca^{2+} (and Mg^{2+}) associated with increased leaching of NO_3^- from a high-N treatment applied to a northern hardwood forest in Maine. In that study, the soil solution of the N-treated area consistently showed elevated concentrations of NO_3^- during the non growing season months (Rustad et al. 1993); these were similar to our seasonal streamflow concentrations. Feger (1992) found that Mg^{2+} responded similarly in $(NH_4)_2SO_4$-treated spruce stands in Germany.

Soil

If our untreated watersheds are N saturated, we would expect minimal differences in soil and forest floor chemistry between WS3 and the control watersheds, especially with respect to N. In fact, we found few differences in soil and forest floor chemistry among watersheds attributable to the chronic N additions on WS3. There were no differences in soil organic matter, total N, and C/N ratios among watersheds (Table 2). The most pronounced difference among watersheds for soil variables was for pH, which was significantly lower on WS3 than on the control watersheds (Table 2). This may be related to the direct effects of $(NH_4)_2SO_4$, an acidic salt (Nebergall et al. 1972) that is commonly used in watershed studies as an effective acidifying agent (Fernandez and Kosian 1986; Feger 1992). Differences in pH between WS4 and WS7 likely were related to stand age, considering that increased leaching through time generally decreases soil pH in more mature stands (Jenny 1980). The only difference with respect to soil N among watersheds attributable to the N additions on WS3 was for preincubation extractable N, wherein WS3 had significantly higher levels than the control watersheds (Table 2).

For the incubation periods in this study, there were no significant differences in net N mineralization among watersheds. When data for all watersheds were pooled by incubation period, there were significant differences in monthly N mineralization rates among periods in the order June > August > September = July > October (Fig. 3). Comparisons of net nitrification were similar to those for net

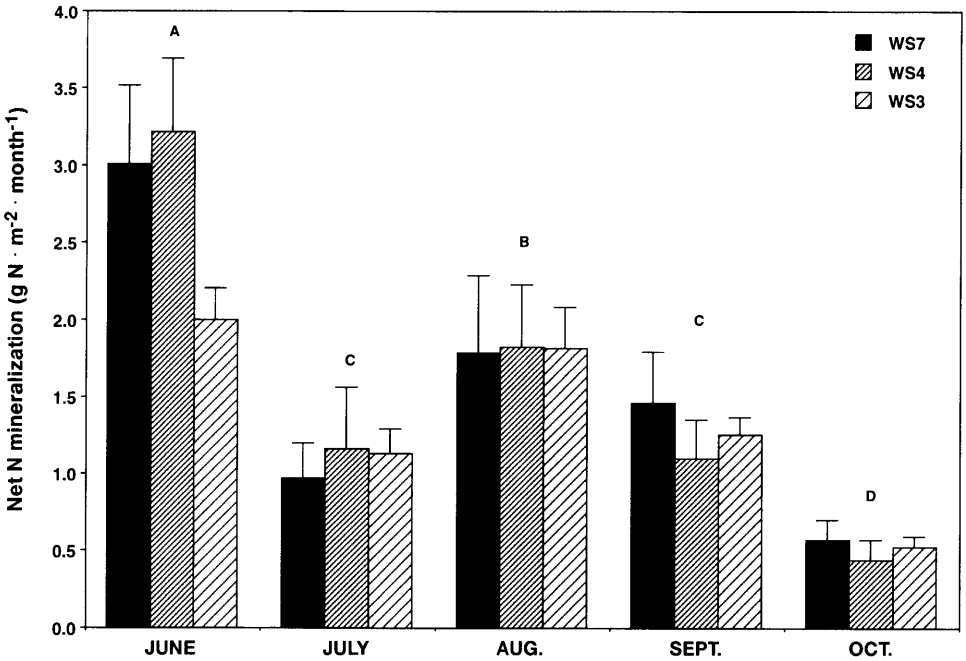
Table 2. Soil characteristics for three watersheds of Fernow Experimental Forest, West Virginia.

Watershed	OM* (%)	TN* (%)	C/N	pH_{H_2O}	pH_{KCl}	Preincubation (mg N·kg^{-1}) NH_4^+	Preincubation (mg N·kg^{-1}) NO_3^-	Postincubation (mg N·kg^{-1}) NH_4^+	Postincubation (mg N·kg^{-1}) NO_3^-
WS3	15.1*a* (0.6)	0.40*a* (0.01)	18.9*a* (0.5)	4.26*a* (0.08)	3.75*a* (0.03)	7.3*a* (1.1)	9.5*a* (0.9)	5.7*a* (1.4)	36.5*a* (1.4)
WS4	16.6*a* (1.0)	0.42*a* (0.03)	19.9*a* (0.9)	4.44*b* (0.07)	3.73*a* (0.07)	4.5*b* (1.0)	6.0*b* (1.2)	7.6*a* (1.7)	33.0*a* (4.4)
WS7	15.6*a* (1.5)	0.47*a* (0.05)	16.8*a* (1.1)	4.68*c* (0.05)	3.93*b* (0.01)	6.4*ab* (1.4)	6.4*b* (1.0)	9.2*a* (3.0)	32.7*a* (4.0)

Note: Values are means with SE given in parentheses (n = 7). Means followed by the same letter are not significantly different among watersheds for a given soil variable at α = 0.10 using Duncan's multiple range test (SAS Institute Inc. 1990).

*OM, organic matter; TN, total nitrogen.

Fig. 3. Net N mineralization for mineral soil of three watersheds of the Fernow Experimental Forest, West Virginia. Values are mean monthly mineralization rates for each monthly period. Error bars represent 1 SE. There were no significant differences among watersheds for any month. Months with the same letter above are not significantly different at α = 0.05.



N mineralization for both watersheds and incubation periods. There were no differences in monthly nitrification rates among watersheds for any period, but, with data pooled by incubation period, there were significant differences among periods in the order June ≥ August ≥ July ≥ September > October (Fig. 4).

Estimates of annual net N mineralization and nitrification are 6.7 and 7.1, 7.8 and 7.0, and 7.7 and 7.0 g·m^{-2}·year^{-1} for WS3, WS7, and WS4, respectively. Thus, N mineralization and nitrification rates in the mineral horizons of FEF soils are quite high. For example, these rates are higher than those found by Aber et al. (1993) for mineralization and nitrification in mineral soil of a fertile, high-N-treated (receiving 113 kg N·ha^{-1}·year^{-1}) red pine (*Pinus resinosa* Ait.) stand at Harvard Forest, Massachusetts (5.8 and 3.8 g·m^{-2}·year^{-1} for N mineralization and nitrification, respectively). Also, net nitrification represents an extremely high proportion of net mineralization on an annual basis; it exceeded 100% for the treated WS3 and was about 90% for the untreated watersheds. Net nitrification at the high-N red pine stand at Harvard Forest was 66% of net N mineralization (Aber et al. 1993). The high proportion of

Fig. 4. Net nitrification for mineral soil of three watersheds of the Fernow Experimental Forest, West Virginia. Values are mean monthly nitrification rates for each monthly period. Error bars represent 1 SE. There were no significant differences among watersheds for any month. Months with the same letter above are not significantly different at α = 0.05.

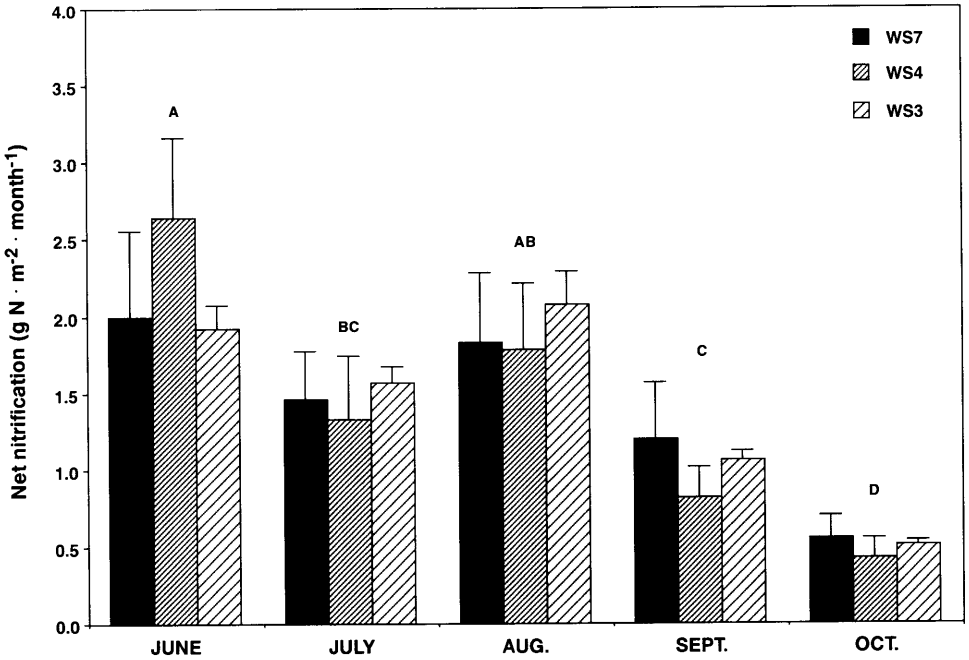


Table 3. Mass, nutrient concentrations, and C/N ratios of forest floor (O1 + O2) for watersheds of Fernow Experimental Forest, West Virginia.

Watershed	Mass ($Mg \cdot ha^{-1}$)	C (%)	N (%)	Ca (%)	K (%)	Mg (%)	P (%)	C/N
WS3	89*b*	44.1*a*	1.66*ab*	0.67*b*	0.11*a*	0.06*a*	0.08*a*	26.6*ab*
	(9)	(1.1)	(0.03)	(0.06)	(0.01)	(0.01)	(0.01)	(0.5)
WS4	123*a*	44.8*a*	1.57*b*	0.86*ab*	0.11*a*	0.06*a*	0.08*a*	28.5*a*
	(9)	(0.8)	(0.07)	(0.15)	(0.01)	(0.01)	(0.01)	(1.2)
WS7	63*c*	44.5*a*	1.80*a*	0.97*a*	0.12*a*	0.07*a*	0.09*a*	24.7*b*
	(3)	(1.5)	(0.12)	(0.07)	(0.01)	(0.01)	(0.01)	(1.2)

Note: Values are means with SE given in parentheses (n = 7). Means followed by the same letter are not significantly different among watersheds for a given soil variable at α = 0.10 using Duncan's multiple range test (SAS Institute Inc. 1990).

nitrification to mineralization in soils of all watersheds, coupled with the lack of differences among treatment and control watersheds in rates of soil N mineralization and nitrification, is consistent with the conclusion (based on stream chemistry data) that all watersheds in our study are N saturated.

Forest floor

The forest floor also showed few differences in nutrient concentrations among watersheds (Table 3). Forest floor mass on mature, mixed-aged WS4 was approximately 1.5× to 2× that on 23-year-old, even-aged WS3 and WS7, respectively. The only significant differences for forest floor nutrients were N (significantly higher on WS7 than on WS4), Ca (significantly higher on WS7 than on WS3), and litter C/N ratios (significantly higher on WS4 than on WS7).

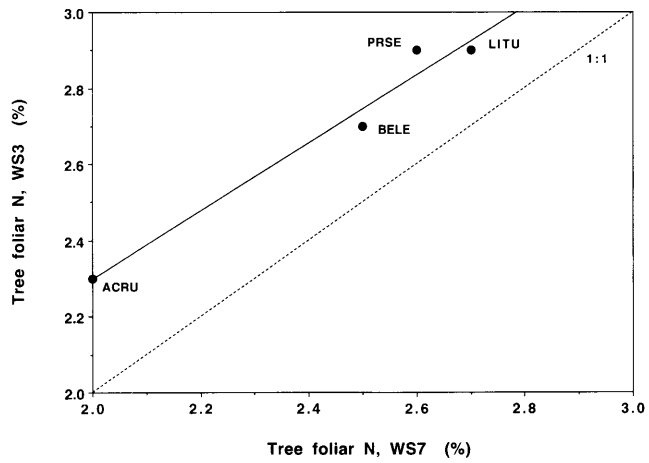
Differences among watersheds for forest floor Ca are consistent with litter chemistry data of individual woody species of WS3 and WS7 (see overstory species below), wherein Ca is generally lower (and N generally higher) for WS7. For example, litter N for WS3 was 1.46, 1.55, 1.16, and 0.88% for black birch, yellow-poplar, black cherry, and red maple, respectively; for WS7 it was 1.36, 1.45, 0.85, and 0.92%, respectively (Adams et al. 1995). Nutrient content of forest floor at FEF (Table 3) was similar to that of northern hardwood stands reported by Snyder and Harter (1987).

Table 4. Macronutrient concentrations of an indicator species, *Viola rotundifolia*, that was present on all sample plots.

Watershed	N (%)	Ca (%)	K (%)	Mg (%)	P (%)
WS3	2.75*a* (0.06)	0.61*b* (0.04)	5.49*a* (0.18)	0.34*b* (0.02)	0.13*a* (0.02)
WS4	2.31*b* (0.12)	0.72*ab* (0.06)	5.04*ab* (0.30)	0.40*a* (0.02)	0.15*a* (0.01)
WS7	2.37*b* (0.20)	0.86*a* (0.08)	4.82*b* (0.29)	0.38*ab* (0.02)	0.13*a* (0.02)

Note: Values are means with SE given in parentheses (n = 7). Means followed by the same letter are not significantly different among watersheds for a given soil variable at α = 0.10 using Duncan's multiple range test (SAS Institute Inc. 1990).

Fig. 5. Concentrations of foliar N of four overstory tree species in treatment versus control watersheds. Broken line represents a 1:1 relationship between watersheds indicating no change in response to N addition treatment. Solid line represents the following equation: $y = 0.51 + 0.89x$, $r^2 = 0.96$, $\alpha = 0.01$. ACRU, red maple; BELE, black birch; PRSE, black cherry; LITU, yellow-poplar.



Overstory and herbaceous layer species

Aber (1992) predicted elevated concentrations of foliar N as characteristic of a N-saturated forest ecosystem. Therefore, if all watersheds were N-saturated ecosystems, we might expect few differences in N concentrations in plants harvested from WS3 compared with WS4 or WS7. Aber (1992) did not indicate types of species in his comparison of N-limited and N-saturated ecosystems. We made these comparisons both with woody species sampled from the overstory on WS3 and WS7 and with *V. rotundifolia* sampled across all three watersheds. This species-specific approach was warranted because the response to elevated N may vary greatly among species. For example, Gilliam et al. (1994) found minimal differences among these same watersheds for concentrations of tissue nutrients for the herbaceous layer as a whole. However, some species were found to be more sensitive than others to the N addition treatment. *Viola* spp. as a group were among the most sensitive herbaceous species (Gilliam et al. 1994).

There were numerous differences among watersheds for plant tissue nutrients. For *V. rotundifolia*, N and K were significantly higher (α = 0.10) on WS3 than on WS4 and WS7, whereas Ca and Mg were significantly lower on WS3 than on WS4 and WS7. Tissue concentrations of P were not different among watersheds (Table 4).

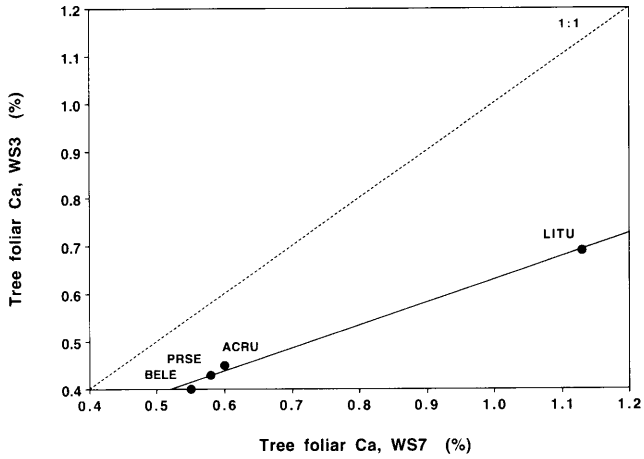
Because biomass of *V. rotundifolia* did not vary significantly among watersheds (Gilliam et al. 1994), we can assume that tissue nutrient differences represent differences in nutrient uptake. Data also suggest that N uptake is predominantly in the form of NO_3^-, since tissue K in *V. rotundifolia* was also higher on WS3 than on the other watersheds. Uptake of N as NO_3^- involves passive uptake of excess cations, especially K^+, whereas N uptake as NH_4^+ often involves competition with K^+ (Waring and Schlesinger 1985).

Significantly lower concentrations of Ca and Mg in *V. rotundifolia* on WS3 are consistent with results of Adams et al. (1993), who found increased stream exports of Ca^{2+} associated with the acidifying conditions brought about by the treatment on WS3; Mg^{2+} was reported to respond similarly. It was concluded that this increased loss of Ca^{2+} was brought about by increased exports of NO_3^- on WS3 (Adams et al. 1993). Thus, a decrease in Ca^{2+} and Mg^{2+} is highly probable, especially considering that soils of these watersheds are derived from sandstone parent materials and are low in exchangeable Ca and Mg. Indeed, sampling of another study of the top 10 cm of soil from these same plots (see Gilliam et al. 1994) showed that exchangeable Ca was only 11–16% and Mg was less than 5% of saturation for all three watersheds.

Concentrations of foliar N and Ca from four canopy species (black cherry, red maple, black birch, and yellow-poplar) were compared between WS3 and WS7. These data are presented in Figs. 5 and 6, respectively, along with a 1:1 reference line indicating no significant difference between the two watersheds. Data points for each species represent means of five trees harvested and sampled. All points were above the 1:1 reference line for N and were fit with the following linear equation: $y = 0.51 + 0.89x$, $r^2 = 0.96$, $\alpha = 0.01$. This line was statistically different from the reference line (determined by comparing the reference line to the 95% confidence limits of the regression line). All points were below the reference line for Ca, and fit the following linear equation: $y = 0.15 + 0.48x$, $r^2 = 0.99$, $\alpha = 0.01$. This line was also statistically different from the reference line at $\alpha = 0.05$.

Data for woody overstory species generally reflected patterns for *V. rotundifolia* in the herbaceous layer (Table 4). Although there was a range of foliar N values associated with the four different species, with red maple the lowest and yellow-poplar the highest, all species had higher concentrations of foliar N on WS3 (Fig. 5). By contrast, concentrations of foliar Ca were consistently lower in trees harvested from WS3 than in trees from WS7 (Fig. 6). As with *V. rotundifolia*, because stand age, stand structure, and species composition did not vary appreciably between WS3

Fig. 6. Concentrations of foliar Ca of four overstory tree species in treatment versus control watersheds. Broken line represents a 1:1 relationship between watersheds indicating no change in response to N addition treatment. Solid line represents the following equation: $y = 0.15 + 0.48x$, $r^2 = 0.99$, $\alpha = 0.01$. ACRU, red maple; BELE, black birch; PRSE, black cherry; LITU, yellow-poplar.



and WS7 (Gilliam et al. 1995), we can assume that differences between watersheds in tree foliar concentrations of nutrients are related to differences in nutrient uptake. This supports conclusions of Adams et al. (1993, 1995) that N addition to WS3 has resulted in increases in NO_3^- production and leaching, accompanied by a loss of Ca^{2+}.

Conclusions

Our results indicate strongly that all study watersheds have become N saturated, regardless of treatment in this field experiment, supporting earlier data (Stoddard 1994). Stoddard (1994) discussed factors that may predispose a watershed to become N saturated, including increased atmospheric input of N, stand age, and high pools of soil N. Striking similarities between WS7 (supporting a 24-year-old, even-aged hardwood stand) and WS4 (supporting a mature second-growth hardwood stand, typical of many central Appalachian forests; Gilliam et al. 1995) for most measured variables (including stream chemistry and rates of soil N transformations) suggest that stand age may be relatively less important in these forests. However, extremely large amounts of N deposition (often as much as 20 kg $N \cdot ha^{-1} \cdot year^{-1}$ as wet- plus dry-fall; F.S. Gilliam and M.B. Adams, submitted (see footnote 2)) have combined with high soil – forest floor N pools (about 1500 kg $N \cdot ha^{-1}$ for both the top 5 cm of soil and forest floor combined) to result in N saturation for these two untreated watersheds.

Of interest in this study, then, is to determine what happens when a saturated system continues to receive excess N inputs. Some processes may change very little. For example, neither N mineralization nor nitrification were higher on the treatment watershed. Thus, rates of N transformation reported here for soils of the control watersheds might represent some maximum rate limit, such that increased N inputs would not increase rates of mineralization and nitrification.

By contrast, our data suggest that other processes may change further with continued excessive inputs of N. For example, comparisons of concentrations of foliar tissue nutrients of *V. rotundifolia*, a prominent herbaceous layer species, and several hardwood canopy species reveal significantly higher uptake of N by plants on the treatment watershed (WS3). Perhaps most significant is that not only were concentrations of stream NO_3^- quite elevated throughout 1993 on WS3, but also WS3 alone exhibited marked increases in base-flow concentrations of both NO_3^- and Ca^{2+} from pre-treatment 1988 to post-treatment 1993. This may indicate that leaching losses of base cations may come after the later stages of N saturation.

Therefore, although admittedly preliminary, our data also support concerns expressed by other studies that N saturation may lead to forest floor base cation (especially Ca^{2+}) loss and therefore deficiency in forest soils. Whereas earlier reports have demonstrated this for conifer forests (e.g., Gilliam and Richter 1991; Eagar and Adams 1992), our study suggests that hardwood forests of the eastern United States also may be susceptible to this effect of N saturation.

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