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Soil Variability along a Nitrogen Mineralization and Nitrification Gradient in a Nitrogen-Saturated Hardwood Forest

Frank S. Gilliam,* Nikki L. Lyttle, Ashley Thomas, and Mary Beth Adams

ABSTRACT

Some N-saturated watersheds of the Fernow Experimental Forest (FEF), West Virginia, exhibit a high degree of spatial heterogeneity in soil N processing. We used soils from four sites at FEF representing a gradient in net N mineralization and nitrification to consider the causes and consequences of such spatial heterogeneity. We collected soils with extremely high vs. low rates of N processing within each of two watersheds: WS3, treated for 15 yr with $(NH_4)_2SO_4$, and WS4, untreated reference (control). Mineral soil was analyzed for extractable NH_4, NO_3, Ca, and Al before and during 28-d incubations at 10, 20, and 30°C after 7, 14, 21, and 28 d. To address the fourth question, we decreased C:N ratios in the soil exhibiting lowest field rates of net N mineralization and no net nitrification (control–low N) by adding glycine and increased C:N ratios in the soil exhibiting highest field rates of net N mineralization and nitrification by adding sucrose. Incubations under controlled conditions supported the N-processing gradient found in the field under in situ conditions in the following order from highest to lowest rates of N mineralization and nitrification: control–high N > N-treated–high N > N-treated–low N > control–low N, the latter exhibiting no net nitrification in the field. Net Ca mineralization increased with net nitrification along the gradient from zero to highest rates. Soil Al appeared to inhibit net nitrification, being lowest in the soil with highest net nitrification and highest in the soil exhibiting no net nitrification. Glycine additions to this latter soil greatly stimulated net N mineralization but failed to initiate net nitrification. Sucrose additions resulted in net immobilization of NH_4 and NO_3 in soil with highest net N mineralization and nitrification. Results demonstrate that increased nitrification may enhance Ca mobility in N-saturated soils and further demonstrate that substrate quality alone is not necessarily a good predictor of soil N processing.

The dynamics of N in soils of terrestrial ecosystems are controlled by processes that often exhibit a high degree of spatial heterogeneity. Although such heterogeneity can represent a challenge in the design of appropriate sampling in the field, ecosystem ecologists are becoming increasingly aware of the ecological significance of spatial heterogeneity in ecosystem function and even as a component of biodiversity (McClain et al., 2003; Roberts and Gilliam, 1995).

Several factors potentially interact to influence spatial heterogeneity of N in forest soils. Morris and Boerner (1998) found considerable variability at the landscape scale in patterns of nitrification and microbial biomass in eastern hardwood forest soils, relating these patterns to spatial patterns of soil moisture. Some investigations have found spatial variability of N processing in forest soils to be related to leaf litter chemistry of contrasting tree species (Ferrari, 1999). Other studies suggest that spatial heterogeneity is largely determined by the direct effects of plants on soil processes (e.g., Chen and Stark, 2000), effects that are often mitigated by other spatially variable factors, such as fire (Hirobe et al., 2003b). Venterea et al. (2003) demonstrated that forest landscape-scale variation in net nitrification is best explained by a suite of vegetation, physiographic, and soil factors.

The Watershed Acidification Study (WAS) was initiated by the USDA Forest Service in 1988 at the FEF, Parsons, WV, to examine ecosystem responses to aerial applications of $(NH_4)_2SO_4$ to an entire watershed (WS3). Results from this project suggest that untreated watersheds of FEF already have become N saturated from high ambient levels of atmospheric deposition of N (Gilliam et al., 1996; Peterjohn et al., 1996; Adams et al., 1997). Despite this, several studies (e.g., Peterjohn et al., 1999; Gilliam et al., 2001a, 2001b; Christ et al., 2002) found that untreated watersheds exhibited a relatively high degree of spatial heterogeneity in soil water NO_3 and net N mineralization and nitrification. Gilliam et al. (2001b) further concluded that one of the more pronounced effects of experimental additions of N to the treatment watersheds was to decrease spatial heterogeneity of soil N processing.

Spatial patterns of net nitrification and NO_3 in soil water of one untreated watershed in the WAS at FEF (WS4) suggest that, although WS4 shows general signs of N saturation, microenvironmental variability (e.g., soil factors) may greatly limit rates of N processing in some areas (Gilliam et al., 2001a). Some data suggest that these rates may be maintained at low levels by presence of ericaceous species, such as *Vaccinium* spp., in the herbaceous layer that may inhibit activity of microbial populations responsible for the conversion of organic forms of N into inorganic forms of N that are potentially available for uptake by plants (Bradley et al., 2000; Gilliam et al., 2001b; Read and Perez-Moreno, 2003). Although a valid alternative hypothesis for the lack of net nitrification in soils within this otherwise N-saturated watershed would be the absence of nitrifier populations in such soils, this was rejected by Gilliam et al. (2001a) using microbial gene amplification techniques; that is, they demonstrated evidence for the presence of nitrifying bacteria in nonnitrifying soils.

A serious consequence of N saturation is enhanced leaching of Ca from the mineral soil, usually seen as increases in stream concentrations of NO_3 and Ca over time. Data gathered by the USDA Forest Service demonstrate that increases in leaching of NO_3 over time have

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Published in Soil Sci. Soc. Am. J. 69:247–256 (2005).
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Abbreviations: FEF, Fernow Experimental Forest; WAS, Watershed Acidification Study.

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increased loss of Ca from soils at FEF (Adams et al., 1997). This relationship between NO_3 and Ca was highly significant during a >30-yr period in an untreated watershed (WS4) and during the >10 yr since time of initial N treatment in an N-treated watershed (WS3), consistent with responses to N additions in soil solution chemistry at FEF (Edwards et al., 2002) and with studies of stream chemistry responses to N additions at other eastern forest sites (Fernandez et al., 2003; Jefts et al., 2004).

The purpose of this study was to consider the causes and consequences of spatial heterogeneity of N processing at FEF by using soils from four sites within FEF that represented a gradient in net N mineralization and nitrification measured in situ during a 3-yr period. We also examined the relationship between extractable cations and rates of net N mineralization and nitrification along this gradient. We addressed the following four questions: (i) How do rates of net N mineralization and nitrification found in the field compare to rates found under controlled conditions at varying temperatures? (ii) What is the effect of soil NO_3 production on extractable Ca and how does this effect vary along the N gradient? (iii) What soil chemical variables best explain the observed N gradient? (iv) What is the effect of varying substrate quality on laboratory rates of net N mineralization and nitrification in soils of the extremes (highest vs. lowest) of this gradient?

MATERIALS AND METHODS

Study Site

The FEF lies adjacent to the Monongahela National Forest and occupies ≈1900 ha of the Allegheny Mountain section of the unglaciated Allegheny Plateau in Tucker County, West Virginia (39°03′ N, 79°49′ W). Averaging approximately 1430 mm yr^{-1}, precipitation at FEF varies seasonally and with elevation, generally greater during the growing season and at higher elevations (Gilliam et al., 1996).

The sample sites were located in two experimental watersheds at FEF: WS4 (≈100-yr-old mixed-aged stand) and WS3 (≈30-yr-old even-age stand); WS3 serves as the treatment watershed for the WAS, receiving three aerial applications of $(NH_4)_2SO_4$ yr^{-1}, totaling 35 kg N ha^{-1} yr^{-1} since 1989. WS4 was the untreated control watershed. Although the two watersheds vary in stand age, their soils are similar, predominantly coarse-textured Inceptisols (loamy-skeletal, mixed mesic Typic Dystrochrept) of the Berks and Calvin series, sandy loams derived from sandstone. Within each of these watersheds, sampling took place on sites designated "low N" and "high N" (see *Field Sampling* below).

The woody overstory is characterized by a high diversity of tree species, including several species common to all sample sites, including sugar maple (*Acer saccharum* Marshall), black cherry (*Prunus serotina* Ehrh.), and northern red oak (*Quercus rubra* L.). Other species, such as white ash (*Fraxinus americana* L.) and yellow poplar (*Liriodendron tulipifera* L.) are found only on N-treated sites. The herbaceous layer of these sites comprises species typical of montane eastern deciduous forests (Gilliam and Roberts, 2003). Herb layer species common to all sites include seedlings of striped maple (*A. pensylvanicum* L.), sugar maple, and black cherry and several species of *Viola*. Species with relatively high biomass are associated with single sites—southern groundcedar [*Lycopodium flabeliforme* (Fern.) Blanch] at the N-treated–high N site and hillside blueberry (*Vaccinium vacillans* Aiton) at the untreated–low N site.

Field Sampling

Sampling was done at four sites, one low-rate site and one high-rate site in each of the two watersheds, that were shown by previous investigations to represent a gradient in rates of net N mineralization and nitrification (Gilliam et al., 2001a). Determination of this gradient was based on 3-yr means of rates of net N mineralization and nitrification measured in situ and reported in Gilliam et al. (2001b). The sites were not defined by specific boundaries, thus precluding accurate estimates of area. Rather, they were essentially subcatchments within each of the two watersheds.

Mineral soil samples were taken to a 5-cm depth from three randomly located, 4- by 4-m plots within each site. Soil was taken at points randomly within each plot and combined to yield a single composite sample per plot. Surgical gloves and trowels were used for soil sampling and were washed with an antibacterial solution between plots within each site and between sites within each watershed to eliminate contamination between samples.

Laboratory Analyses

All soil samples were brought back to the laboratory at Marshall University for determination of preincubation levels of extractable ions and for incubation under controlled conditions. Soil from each plot was divided into three subsamples and placed in sterilized polyethylene bags, with each group of subsamples being incubated at three temperatures: 10, 20, and 30°C. All subsamples were extracted following incubation at 3, 7, 14, 21, and 28 d for NH_4, NO_3, Ca, and Al. Soil moisture was monitored for all subsamples before and during incubation, and neither varied among samples nor through time of incubation. Extraction and analysis for all ions followed methods described in Gilliam et al. (2001a). Briefly, moist soils were extracted with 1 *M* KCl at an extract/soil ratio of 10:1 (v/w). Extracts were analyzed colorimetrically for NH_4 and NO_3 with a Bran+Luebbe TrAAcs 2000 automatic analysis system. Extractable Ca and Al were analyzed with a Varian inductively coupled plasma emission spectrophotometer. Net N mineralization and nitrification rates (in μg N g^{-1} soil d^{-1}) were determined via regression analysis as the slope of the line comparing extractable NH_4 plus NO_3 (for N mineralization) and extractable NO_3 (for nitrification) vs. incubation time (in d). These relationships were linear and significant ($P < 0.05$) for all sites.

For the substrate quality experiment, we utilized soils from two sites only—the extremes of low-N vs. high-N processing sites among the 21 reported in Gilliam et al. (2001b). These were found on the untreated WS4. Four replicate samples were used for each treatment per site. In addition to preincubation extractions for NH_4 and NO_3 as described previously, 70-g subsamples of soil from plots of each site were amended with 10 mL of sucrose, glycine, or deionized H_2O. Solutions of 0.01 and 0.1 *M* sucrose were added to the high-N soil (representing 230 and 2300 mg C added g^{-1} soil, respectively), whereas 0.01 and 0.1 *M* glycine solutions were added to the low-N soil (representing 20 and 200 mg N and 34 and 340 mg C added g^{-1} soil, respectively). Soil samples were incubated at 23°C for 21 d, after which soils were extracted for NH_4 and NO_3. Net N mineralization rates were calculated as postincubation NH_4 and NO_3 minus preincubation NH_4 and NO_3. Nitrification rates were calculated as postincubation NO_3 minus preincubation NO_3.

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Data Analyses

Field vs. laboratory rates (at each of the three temperatures) of net N mineralization and nitrification were compared with Pearson product moment correlation analysis. The effects of temperature on net N mineralization and nitrification were assessed by determining linear and curvilinear relationships between mineralization and nitrification as separate dependent variables vs. incubation temperature as the independent variable. The goal was to find the model that produced the best fit, defined as highest r^2. Potential effects of nitrification on soil Ca extractability were similarly assessed by determining the best fit of extractable Ca vs. extractable NO_3 for all extraction times (0, 7, 14, 21, and 28 d). Finally, the effects of altering substrate quality on net N mineralization and nitrification were assessed by comparing means of control (H_2O added) to those of 0.01 and 0.1 *M* sucrose and glycine additions to the respective soils with one-way ANOVA and Bonferroni multiple range tests (Zar, 1996).

RESULTS AND DISCUSSION

Laboratory Assessment of the Field Gradient in Net Nitrogen Mineralization and Nitrification

Previous work at FEF using in situ field incubations has shown that rates of net N mineralization and nitrification among the four sites were in the following order, from highest to lowest: control–high N > N-treated–high N > N-treated–low N > control–low N, with the control–low N site exhibiting no detectable net nitrification (Fig. 1). Laboratory incubations in the present study exhibited this same gradient in net N mineralization and nitrification rates under temperature-controlled conditions. Rates of net N mineralization in the field were significantly correlated with rates for the incubation at 10°C ($P < 0.05$), whereas net nitrification in the field was significantly correlated with rates for incubations at 10 and 20°C ($P < 0.05$, $P < 0.10$, respectively) (Table 1). The lack of significant correlations at higher temperatures was the result of curvilinear responses to temperature for the two high-N sites (Fig. 2a, 2b). In other words, Pearson product moment correlation analysis tests for linear, rather than curvilinear, relationships. Furthermore, the 10 and 20°C treatments more closely resemble ambient conditions of soil temperature (see below).

Such similarities between in situ and laboratory incubations indicate that spatial heterogeneity of soil N processes observed in the field is the result of innate soil characteristics rather than an artifact of physical differences among sites, such as slope aspect and elevation. For example, some of these differences could arise from spatial heterogeneity in composition and functioning of soil microbial communities and from variation in soil chemical characteristics that can influence soil microbes.

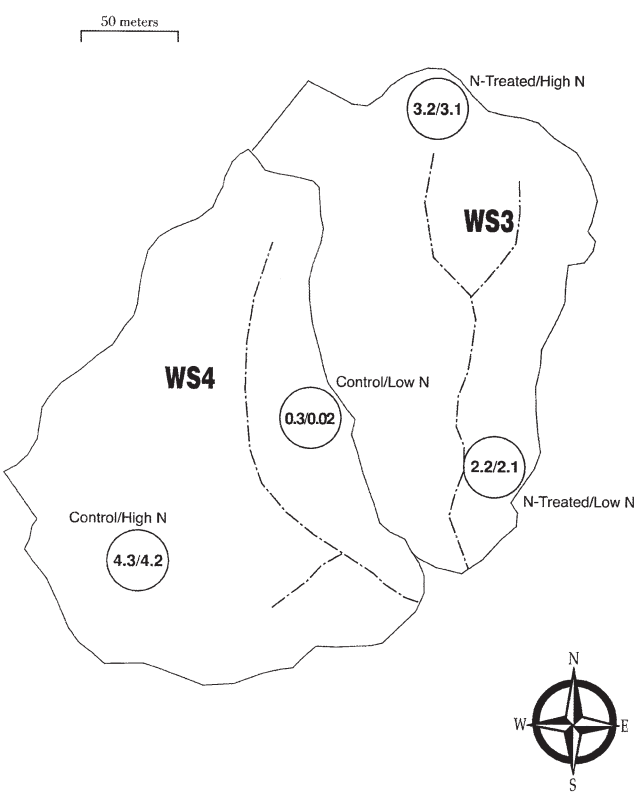

Fig. 1. Map of Watersheds 3 and 4 of Fernow Experimental Forest, West Virginia, showing sample sites for the present study. Values shown are monthly means from a 3-yr period (1993–1995), wherein the first value is net N mineralization and the second value is net N nitrification (g N m^{-2} mo^{-1}). Data taken from Gilliam et al. (2001b).

Effects of Temperature on Net Nitrogen Mineralization and Nitrification

Net N mineralization and nitrification exhibited exponential increases with incubation temperature up to 30°C for the two high-N sites, whereas increases with temperature were linear for the low-N sites (with the exception of no net nitrification at the control–low N site) (Fig. 2a, 2b). Working with N-amended soils from a California oak woodland–annual grassland site, Stark and Firestone (1996) found curvilinear increases of nitrification in response to incubation temperature up to 30°C, finding a significant decline in nitrification at temperatures > 30°C. It was determined that temperature optima for these grassland and oak woodland soils were 35.9 and 31.8°C, respectively, a relationship that was best described by a generalized Poisson density function (Stark, 1996). Comparison of responses of nitrification to temperature between soils of the California site to those of FEF soils suggests that nitrifier populations at FEF may also have temperature optima at >30°C. This is well above the highest mean daily temperature (20°C) during a single growing season reported by Gilliam and Adams (1999) for undisturbed surface soils at another

Table 1. Pearson product moment correlation coefficients (r) and $P > F$ values for field rates of net N mineralization and net nitrification vs. corresponding laboratory rates at varying incubation temperatures. N = 4 for all correlations.

	Laboratory rates		
Field rates	10°C	20°C	30°C
Net N mineralization			
r	0.953	0.885	0.806
$P > F$	0.047	0.115	0.194
Net nitrification			
r	0.957	0.921	0.835
$P > F$	0.044	0.079	0.165

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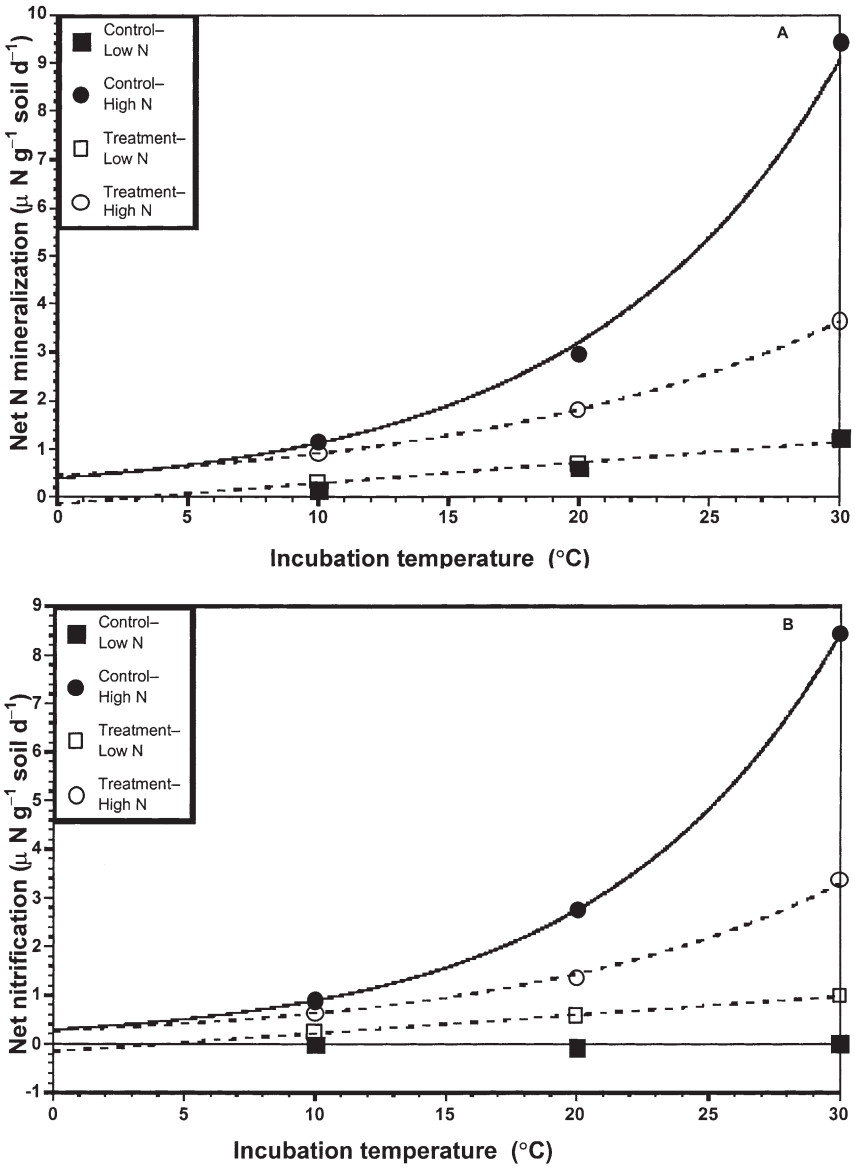

Fig. 2. Effects of incubation temperature on N dynamics of soils from four sites within the Fernow Experimental Forest, West Virginia. (A) Net N mineralization, (B) net nitrification.

FEF site close to these study watersheds. Mean soil temperature throughout that growing season was ≈16°C (Gilliam and Adams, 1999).

Positive relationships between temperature and microbially mediated processes, such as N mineralization and nitrification, are certainly to be expected and indeed have been described for lab-based (e.g., Emmer and Tietema, 1990; Stark and Firestone, 1996; Niklinska et al., 1999) and field-based incubation studies (e.g., Tietema and Verstraten, 1991; Gilliam et al., 2001b), and have been demonstrated across extremely broad spatial scales (Yin, 1992). What is notable about results presented here, however, is that they confirm for N-saturated soils what was concluded by Stark (1996) for soils in woodland and grassland soils—temperature optima for nitrifier populations in the soil are far greater than temperatures typically experienced by those populations. This has important implications for the response of N-saturated forests to increases in temperature in the future that might be brought about by global warming. That is, any negative effects experienced by N-saturated forests (e.g., declines in growth rates from nutrient cation deficiencies) may be exacerbated by increases in ambient temperatures due to global warming.

Potential Effects of Nitrification on Soil Calcium

Nitrate production appeared to have a significant influence on extractable Ca, with positive curvilinear relationships across time found for all sites that exhibited net nitrification (Fig. 3). The range and magnitude of change in extractable Ca varied consistently in the order of net nitrification along the gradient, that is, lowest for the N-treated–low N site, intermediate for the N-treated–high N site, and highest for the control–high N site (Fig. 3).

Dijkstra (2003) used an approach similar to ours in

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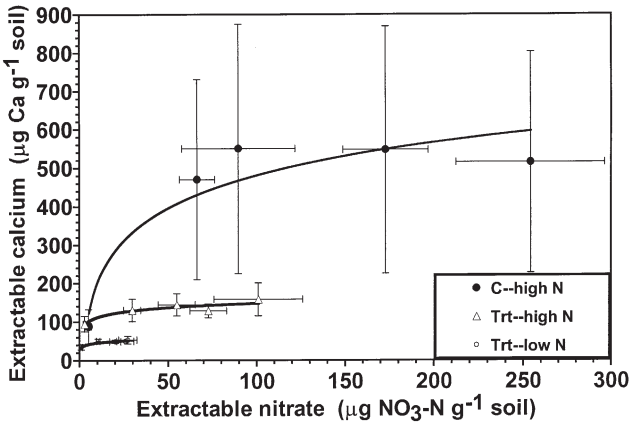

Fig. 3. Relationship between extractable Ca and extractable NO_3 in soils from the Fernow Experimental Forest, West Virginia. Points shown are means of three replicates for each of five incubations periods (30°C only)—0 (preincubation), 7, 14, 21, and 28 d. Error bars (±1 SE of the mean) are shown for mean extractable Ca and NO_3 for all significant relationships with extractable NO_3: control–high N, $y = -85.1 + 112.9\ln(x)$, $r^2 = 0.90$, $P < 0.01$; N-treated–high N, $y = 74.9 + 15.6\ln(x)$, $r^2 = 0.84$, $P < 0.01$; N-treated–low N, $y = 32.9 + 5.3\ln(x)$, $r^2 = 0.92$, $P < 0.01$.

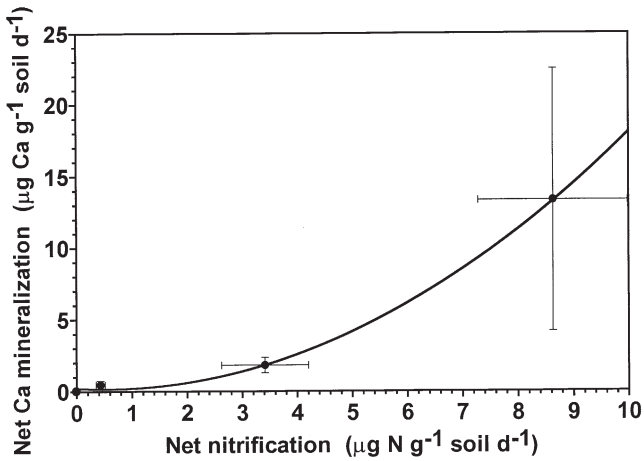

Fig. 4. Net Ca mineralization vs. net nitrification at 30°C across all four study sites. Equation for curve is $y = 0.21 - 0.20x + 0.20x^2$, $r^2 = 0.998$, $P < 0.01$. Points shown are means of three replicates; error bars shown are ±1 SE of the mean.

investigating changes in extractable Ca over time (referring to such changes as "Ca mineralization") at a second-growth mixed hardwood–hemlock stand in Connecticut. Differences between our study and Dijkstra (2003) include his (i) inclusion of forest floor material and focus on individual trees of six species, (ii) use of longer incubation periods performed in situ, and (iii) lack of attempt to relate Ca mineralization to nitrification. We calculated net Ca mineralization for the 30°C incubation using an approach similar to that used for net nitrification (i.e., slopes of linear regressions of extractable Ca across time). Other than the control–low N plot, which had neither detectable net nitrification (Fig. 2b) nor detectable net Ca mineralization, our rates of net Ca mineralization (0.4, 1.8, and 13.3 μg Ca g^{-1} soil d^{-1} for N-treated–low N, N-treated–high N, and control–high N sites, respectively) were generally much higher than those found by Dijkstra (2003), who found highest rates (≈0.3 μg Ca g^{-1} soil d^{-1}) in mineral soil beneath sugar maple. In fact, net Ca mineralization for mineral soil at our control–high N was within the range of the highest rates for forest floor (13–14 μg Ca g^{-1} soil d^{-1} for sugar maple and white ash) reported by Dijkstra (2003). Net Ca mineralization at the control–high N site is 0.31 kg Ca ha^{-1} yr^{-1}, which is low relative to stream flow outputs of Ca at the study watersheds (11–12 kg Ca ha^{-1} yr^{-1}) (Adams et al., 1997).

Such large differences between studies are quite notable and likely arise for several reasons. One possibility is related to the conditions under which the samples were incubated (variable and lower in situ temperatures vs. constant 30°C). We suggest, however, that most of the difference is related to length of incubation period. Because we found measurable increases in extractable Ca even at the 7-d incubation period, it is probable that the relatively low values reported by Dijkstra (2003) were in part an artifact of the lengthy incubation periods (4 and 7 mo for summer and winter incubations, respectively) he used for both forest floor and mineral soil materials (i.e., although his soils may have had high short-term rates of Ca mineralization, the rates—expressed on a d^{-1} basis—would have been calculated by dividing by the very large number of days in the incubation period).

We found a highly significant curvilinear pattern for net Ca mineralization along the gradient of net nitrification across all four study sites at FEF (Fig. 4), suggesting a direct connection between the two processes. Furthermore, these results are consistent with the observation that increased nitrification can increase mobility of Ca in soils at FEF, and are consistent with the findings of Edwards et al. (2002) at FEF. That study demonstrated that the $(NH_4)_2SO_4$ treatments on WS3 have increased movement of NO_3 in soil solution from A and B horizons to accumulate in the C horizon, and have concurrently increased Ca concentrations in soil water of the C horizon beneath the rooting zone for most plants (Edwards et al., 2002).

Although it is not within the scope of this study to determine the source of increased extractability of Ca associated with nitrification in FEF soils, there are three possible sources, the first two of which are related to net production of H^+ resulting from NO_3 produced in excess of uptake, which is characteristic of N-saturated systems: (i) weathering of Ca-bearing minerals, (ii) mobilization of Ca from exchange sites, and (iii) conversion of organically bound Ca to inorganic Ca.

We suggest that the first potential source of Ca is not important at our N gradient sites. Because soils of the study watersheds were derived from sandstone parent materials, they are very low in Ca-bearing minerals. Indeed, an extensive mineralogical characterization of several soil types at FEF (Lusk 1998) concluded that there is little, if any, ecologically significant geochemical source of Ca in FEF soils. Lusk (1998) found a virtual absence of apatite, the Ca-bearing mineral found in some northern hardwood forests (Blum et al., 2002, Hamburg et al., 2003). Thus, most of the Ca that increases with nitrification is either being released from the cation exchange sites associated with both clay and organic colloids (Gil-

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liam et al., 1994) or being directly mineralized from organic matter. Ultimately, the mechanism of nitrification-enhanced extractability of Ca suggested in Fig. 3 and 4 is likely one that diminishes the readily available pool for soil Ca required by plants for uptake. This conclusion is consistent with findings of Gilliam et al. (1996), who found that increased NO_3 production decreased uptake of Ca by roundleaf yellow violet (*Viola rotundifolia* Michx.) on FEF watersheds.

Effects of Altering Substrate Quality on Net Nitrogen Mineralization and Nitrification

Because of its importance in influencing microbial processing of N, the quality of litter and soil organic matter has long been of interest in the study of N cycling of forest ecosystems, whether quality is defined by lignin/N ratio (Melillo et al., 1982; Stump and Binkley, 1993) or C:N ratio (Hirobe et al., 2003a; Springob and Kirchmann, 2003). The potential effects of variation in litter quality on N mineralization and/or nitrification are often assessed on the landscape scale by correlation among sites with contrasting litter and soil organic matter quality (Scott and Binkley, 1997; Venterea et al., 2003). Experimentally, however, this is usually assessed with manipulation of C:N ratios by adding C-rich sources, such as sugars (e.g., Davidsson and Ståhl, 2000; Magill and Aber, 2000) and sawdust (e.g., Gulledge and Schimel, 2000), or N-rich sources, such as amino acids (Jones and Shannon, 1999; Garten, 2004).

In this part of our investigation of the gradient in soil N processing, we used a C-rich source (sucrose) to determine if adding C without N (increasing soil C:N) would stimulate net immobilization in the soil with highest rates of net N mineralization and nitrification. Concurrently, we used an N-rich source (glycine) to determine if decreasing C:N would stimulate net N mineralization and initiate net nitrification in the soil exhibiting lowest rates of N mineralization and no net nitrification.

Sucrose Additions

Adding sucrose solutions to soil from the site with the highest rates of net N mineralization and nitrification significantly decreased rates of both processes, indicating that both NH_4 and NO_3 were immobilized by soil microbes (Fig. 5), although immobilization of NO_2, which was not assessable in this study, is also possible (Fitzhugh et al., 2003). Net immobilization of inorganic N increased significantly as a logarithmic function of sucrose concentration.

Working in wetland soils of southern Sweden, Davidsson and Ståhl (2000) used added glucose as a source of increased energy availability for immobilizing microbes, and found inconsistent responses with no substantial increases in immobilization from glucose additions. In addition to potentially stimulating net N immobilization, Jordan et al. (1998) found that sucrose additions significantly increased rates of denitrification in surface soils of a riparian forest.

Magill and Aber (2000) used two concentrations of glucose, along with leaf leachate, cinnamic acid, and humic

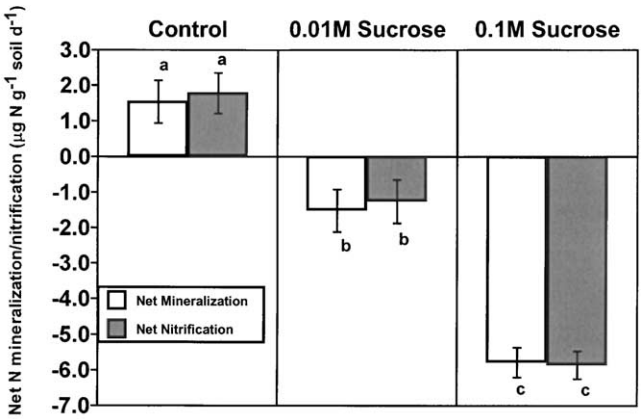

Fig. 5. Effects on net N mineralization and nitrification of additions of sucrose to soils with high N processing, Fernow Experimental Forest, West Virginia. Error bars (n = 4) shown are ±1 SE of the mean. Means with the same superscript are not significantly different at $P < 0.05$.

acid, as sources of added C to soils of mixed hardwood forests and found that N immobilization responded more sensitively to glucose than to the other C sources. Overall, they found that both low and high levels of glucose additions (high glucose = 2 × low glucose) immobilized 6 and 15 mg N kg^{-1} mineral soil in 5 wk, respectively. Using field applications of sucrose and extracts of roots and litter to soils of a Tennessee hardwood forest, Johnson and Edwards (1979) found that adding sucrose rapidly increased net N immobilization, and did so more than less-labile sources of C, such as root and litter leachates. They found that sucrose additions decreased net N mineralization by nearly 70% in a 19-d period. Although our experiment did not include less-labile sources of C, our results are consistent with the findings of Johnson and Edwards (1979) and Magill and Aber (2000) for the effects of experimental additions of labile C on microbial immobilization of available N.

Glycine Additions

Adding glycine solutions to soil from the site with the lowest rates of net N mineralization (and no net nitrification) significantly increased net N mineralization, from 0.7 μg N g^{-1} soil d^{-1} for the control to 1.8 and 13.0 μg N g^{-1} soil d^{-1} for 0.01 *M* and 0.1 *M* glycine additions, respectively (Fig. 6). Thus, the 0.01 *M* glycine addition increased mineralization of the low-N soil to a level similar to that of the control–high N soil (Fig. 5). Net N mineralization increased significantly as a linear function of glycine concentration. By contrast, both the 0.01 *M* and the 0.1 *M* glycine concentration failed to stimulate net nitrification (Fig. 6).

These results suggest that net N mineralization may be substrate limited at the control–low N site, considering the dramatic results of adding a low C:N source of N (glycine, C:N = 1.7). Although some models of N mineralization assume that the N in organic substrates is mineralized to NH_4 before being assimilated by soil microbes (i.e., the MIT hypothesis), Barraclough (1997) showed that simple amino acids like glycine are taken up

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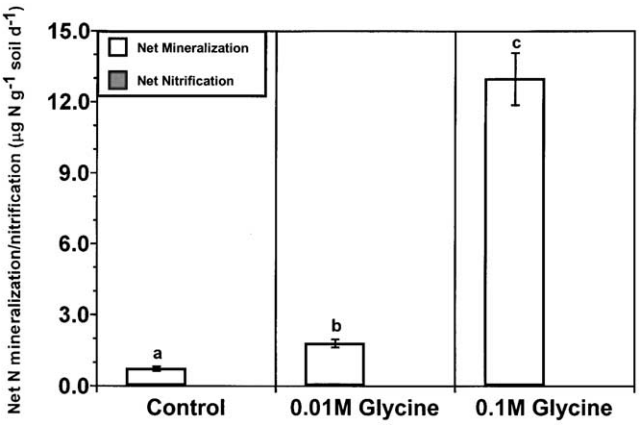

Fig. 6. Effects on net N mineralization and nitrification of additions of glycine to soils with low N processing, Fernow Experimental Forest, West Virginia. Error bars (n = 4) shown are ±1 SE of the mean. Note: lack of error bars for net nitrification due to lack of net nitrification. Means with the same superscript are not significantly different at $P < 0.05$.

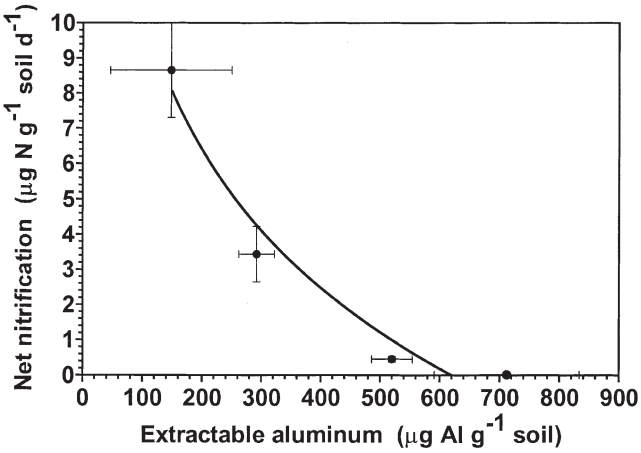

Fig. 7. Net nitrification at 30°C vs. preincubation extractable Al across all four study sites. Equation for curve is $y = 36.37 - 5.66\ln(x)$, $r^2 = 0.96$, $P < 0.01$. Points shown are means of three replicates; error bars shown are ±1 SE of the mean. Note that there are error bars occurring on the line of the x axis that are associated with mean extractable Al for the site that exhibited no net nitrification.

directly by microbes and deaminated, with excess NH_4 released into the soil pool. Indeed, the importance of soil amino acids as sources of dissolved N and as a factor influencing microbial communities has been demonstrated in several studies (e.g., Myers et al., 2001; Vinolas et al., 2001; Yu et al., 2002).

Results for net nitrification suggest that it is limited by neither substrate quality (i.e., C:N ratio) nor availability of NH_4. This is in contrast to results of Hall and Matson (2003) working in severely N-limited soils of Hawaii. Their soils were similar to soils of our untreated low-N site in exhibiting no net nitrification under field conditions. In contrast to our results, however, they were able to initiate net nitrification in their soils by adding high levels of N, concluding that their findings resulted from increases in numbers and activity of nitrifying microbes (Hall and Matson, 2003). Although the most parsimonious explanation for our results would be that FEF soils that fail to produce NO_3 are lacking in nitrifier populations, this explanation was rejected by an earlier investigation (Gilliam et al., 2001a) that demonstrated for these soils the presence of the *amo*A gene, the enzyme that catalyzes the first step in nitrification (NH_4 oxidation to NO_2).

Synthesis: Causes and Consequences of Spatial Variability in Nitrogen Processing at Fernow Experimental Forest

Results of experimental investigations in this study support published results based on field studies. The four sites used in this study were chosen because they represented a gradient from extreme low to high rates of N processing, based on in situ incubations. This gradient for net mineralization or nitrification remained under controlled temperature conditions and did so across all three incubation temperatures: 10, 20, and 30°C. The significant increase in Ca extractability in response to increased NO_3 production in these soils is consistent with results of previous studies at sites other than FEF (Lawrence et al., 1995; Currie et al., 1999) and with soil solution and stream chemistry data reported for FEF (Adams et al., 1997; Edwards et al., 2002). Microbial processing of N in the control–high N soil was highly sensitive to labile C, supporting findings of earlier studies (Johnson and Edwards, 1979; Magill and Aber, 2000). The additional source of immediately available energy stimulated soil microbes at this site to take up both NH_4 and NO_3 to meet increased microbial demand for available N. Although ammonifying microbes in soils of the control–low N site were substrate limited (especially with respect to decreasing C:N), nitrifier populations appear to be limited or inhibited by some factor (or suite of factors) other than available NH_4, which increased linearly with glycine addition, but was not accompanied by increases in NO_3.

We hypothesize that factors limiting net N mineralization and/or inhibiting net nitrification at the control–low N site may, in large part, explain the great spatial heterogeneity in N processing within untreated WS4. These include soil chemistry, which is often spatially quite variable (Johnson et al., 2000). This is especially seen in the extremes of high Al and low Ca concentrations, which themselves are often a function of weathering rates of mineral soil. Thus, spatial heterogeneity in N processing at FEF may have arisen, at least in part, from spatially variable rates of weathering in watershed soils.

Net nitrification was negatively related to preincubation levels of Al across the spatial gradient, with the relationship being fit most closely with a logarithmic curve [$y = 36.37 - 5.66\ln(x)$, $r^2 = 0.959$, $P < 0.01$] (Fig. 7). This apparent inhibition of nitrification via Al toxicity is consistent with findings of Illmer et al. (2003), who demonstrated that increases in KCl-extractable Al had a highly significant negative effect on several microbial parameters, including microbial biomass and respiration, in 95 soils from throughout Tyrol, Austria.

In addition to soil chemistry would be influences of plants. For WS4, this appears to be particularly related to the spatial distribution of ericaceous species in the her-

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baceous layer. It is notable that several studies have demonstrated that ericaceous plants can depress availability of N (Van Breemen and Finzi, 1998; Bradley et al., 2000; Van der Krift and Berendse, 2001). Indeed, using multivariate statistics, Gilliam et al. (2001b) demonstrated that barely detectable soil water NO_3 (indicative of severely limited net nitrification) throughout the large area of WS4 that includes our low-N site was closely associated with the dominance of the ericaceous species hillside blueberry in the herbaceous layer.

Roots of ericaceous plants support a special type of mycorrhizae—ericoid mycorrhizae—that secrete organic acids which limit N-mineralizing microbes in general and inhibit nitrifying bacteria in particular (Straker, 1996). Read and Perez-Moreno (2003) showed that, by attacking structural polymers, ericoid mycorrhizae have the potential to render nutrients unavailable to microbes, and that natural selection favors ericoid mycorrhizae with well-developed saprotrophic capabilities to retain N as organic complexes in the soil. Ericaceous plant detritus contains high concentrations of secondary compounds (e.g., polyphenols) that have been shown to shift the pathway of N cycling from inorganic to organic forms (Northup et al., 1998; Schimel et al., 1998). Thus, we hypothesize that the predominance of hillside blueberry on WS4 is part of a positive feedback mechanism between soils with low mineral N and plants that simultaneously (i) are selected for and highly competitive in low-N soils, and (ii) maintain low-N conditions.

An additional positive feedback mechanism at FEF may be related to soil chemistry. Ericoid mycorrhizae have been shown to survive high levels of toxic metals, including Cd, Zn, and Al, in soils (Perotto et al., 2002). Although the specific mechanisms for survival are not completely understood, ericoid fungi have the capacity for rapid adaptation to high metal concentrations. For example, when strains of the ericoid fungus *Oidiodendron maius* were isolated from Cd-, Zn-, and Al-polluted soils, they were found to grow better in vitro in media with those metals than did strains isolated from nonpolluted soils (Lacourt et al., 2000). Furthermore, these mycorrhizae can both (i) increase mobility of toxic metals, which increases toxicity to soil microbes (e.g., Illmer et al., 2003), and (ii) reduce susceptibility of host plants to metal toxicity, which allows ericaceous species to survive in soils with levels of metals that are toxic to other, nonericoid species (e.g., Sharples et al., 2000). Consequently, via its ericoid mycorrhizae, hillside blueberry that dominates the herbaceous layer of the control–low N site at FEF can tolerate the high levels of extractable Al in those soils that exhibited no net nitrification.

Understanding spatial variability in N processing has important practical applications. These include predicting the response of soil N to disturbance and the challenge of sustainable management of N-saturated forest stands (Fenn et al., 1998; Adams 1999; Gilliam et al., 2004). Vitousek et al. (1982) concluded that sites with low rates of N processing would be highly resistant to disturbance, whereas sites with high rates would be much less resistant. Thus, we would expect a disturbance (e.g., wind damage, forest harvesting) involving only the low-N portion of WS4 to bring about few changes in soil N. By contrast, we predict that similar disturbances to other areas of WS4 with high rates of N turnover would further enhance N mobility, which may exacerbate loss of Ca.

ACKNOWLEDGMENTS

We appreciate the helpful comments regarding the manuscript made by Mary Arthur and two anonymous reviewers. Some data in this publication were obtained by scientists of the Timber and Watershed Laboratory and FEF; this publication has not been reviewed by those scientists. The FEF is operated and maintained by the Northeastern Research Station, Forest Service, USDA, Newtown Square, PA. The use of trade, firm, or corporation names in this paper is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the USDA or the Forest Service of any product or service to the exclusion of others that may be suitable.

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