Variation with slope aspect in effects of temperature on nitrogen mineralization and nitrification in mineral soil of mixed hardwood forests

Frank S. Gilliam, Julia E. Galloway, and Jacob S. Sarmiento

Abstract: This study examined the effects of temperature on soil nitrogen (N) dynamics and variation with slope aspect (northeast (NE) versus southwest (SW)) at two forested sites in West Virginia — Beech Fork Lake (BFL) and Fernow Experimental Forest (FEF) — with similar soil and overstory characteristics but with different latitudes and elevations. Previous work on mineral soil from both sites had shown sharp differences in microbial communities between SW slopes and NE slopes. Mineral soil was sampled from three and eight plots per aspect at FEF and BFL, respectively. Inorganic N was extracted from samples, which were then divided into polyethylene bags for 7-day incubations at 4 °C, 15 °C, 25 °C, and 35 °C. Following incubation, soils were extracted and analyzed for inorganic N. Net N mineralization varied significantly between aspects and temperatures but did not vary between sites; net nitrification varied significantly between aspects, temperatures, and sites. Net N mineralization increased with incubation temperature at all aspects and sites. Net nitrification rates increased with incubation temperature for BFL soils; however, maximum net nitrification rates occurred at 20–25 °C for FEF soils. Net nitrification was essentially undetectable for SW soils at either site. Results underline the complexities of the N cycle in temperate forest ecosystems, representing challenges in predicting alterations in soil N dynamics under conditions of global climate change.

Key words: nitrogen mineralization, nitrification, forest ecology, global warming, temperate forests.

Introduction

Predicted increases in global temperatures have important implications for future changes in the dynamics of soil nitrogen (N) in many regions, especially those that are dominated by temperate forests (Rustad et al. 2001; Booth et al. 2005). Cycling of soil N is facilitated in large part by microbial communities, including prokaryotes, protists, and fungi, with one prominent process — nitrification — being carried out by bacteria (McArthur 2006). Soil microbes display a high level of sensitivity to variation in temperature, with microbial activity generally increasing with increasing temperatures throughout typical ambient ranges (Schütt et al. 2014). At high temperatures, microbial activity can be greatly inhibited, especially for nitrifying bacteria, which generally display temperature optima (Stark 1996).

Forests of montane regions exhibit topographic complexity relevant to the response of soil N dynamics to temperature because of the widely contrasting microclimates created by complex terrain, including elevation and slope aspect. It is widely recognized that north- versus south-facing slopes experience notable differences in net solar radiation ($R_n$), which drives a number of other factors important in forest ecosystems such as soil weathering and ambient air and soil temperatures (Bennie et al. 2008; Beaudette and O’Geen 2009).

Forests throughout most of the Appalachia, especially those comprising the mixed mesophytic forest region (MMF) (Braun 1950; Dyer 2006), are well recognized for their high plant species richness in both the woody overstory and the herbaceous layer...
communities. Indeed, West Virginia is notable for having the highest relative cover of MMF in the United State (US), with ~75% of the state forested (ranked third in US forest cover) and virtually all of it being MMF (Dyer 2006). Previous work at two MMF sites varying in elevation and latitude in West Virginia found sharp, aspect-related contrasts in several mineral soil parameters, including soil microbial groups and in situ rates of net N mineralization and net nitrification. Working at the Fernow Experimental Forest (FEF; higher elevation and latitude), Gilliam et al. (2011) found the predominance of fungal microbial markers and indicators of higher microbial stress in soils of the southwestern (SW) aspect versus soils of the northeastern (NE) aspect, along with lower extractable NH4+ and NO3− and a virtual absence of extractable NO3− in soils of the SW aspect (Table 1). Working at the Beech Fork Lake State Wildlife Area (BFL; lower elevation and latitude), Gilliam et al. (2014) found similar contrasts when comparing forest stands on SW aspects versus NE aspects (Table 1). Further evidence (e.g., soil pH) indicated that for both sites, SW soils were more highly weathered than NE soils and that observed aspect-related differences in soils arose largely from differential weathering of soils, i.e., higher weathering in SW soils that receive higher net Rn, which drives weathering processes.

This study examined the effects of temperature on net N mineralization and net nitrification and how these effects may vary with slope aspect. Further emphasis was placed on comparing these relationships between two forested sites with similar soil and overstory characteristics but different latitudes and elevations. We hypothesized that (i) soils from NE aspects will exhibit a more sensitive response of net N mineralization and nitrification rates than soils from SW aspects, and (ii) soils from lower elevations and latitudes have higher temperature optima (T opt) for both net N mineralization and net nitrification than soils from higher elevations and latitudes.

Materials and methods
Study sites

Soil samples for this experimental study were taken from two sites in West Virginia, namely BFL and FEF. BFL is in Wayne County, West Virginia (38°18’N, 82°25’W), located on the far western edge of the Appalachian Plateau. FEF is adjacent to the Monongahela National Forest in Tucker County, West Virginia (39°03’N, 79°49’W), and occupies ~1900 ha of the Allegheny Mountain section of the state. Mean precipitation at these sites is approximately 1123 mm·year−1 and 1430 mm·year−1 at BFL and FEF, respectively. Mean monthly temperatures for BFL and FEF vary from a minimum in January of 0.0 °C and ~2.7 °C, respectively, to a maximum in July of 23.7 °C and 20.4 °C, respectively (Fig. 1). Differences between sites in long-term temperatures were most pronounced for the minimum monthly temperatures and least pronounced for the maximum monthly temperatures (Fig. 2). The long-term reference watershed at FEF (i.e., watershed 4 (WS4)) was used in this study.

### Table 1. Study site characteristics (mean ± 1 standard error) on contrasting slope aspects of Beech Fork Lake (BFL) and Fernow Experimental Forest (FEF).

<table>
<thead>
<tr>
<th>Site and aspect</th>
<th>Elev. (m)</th>
<th>BA (m²·ha⁻¹)</th>
<th>OM (%)</th>
<th>pH</th>
<th>FB</th>
<th>SI</th>
<th>NH₄⁺ (µg N·(g soil)⁻¹)</th>
<th>NO₃⁻ (µg N·(g soil)⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BFL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northeast</td>
<td>213</td>
<td>28.3±2.5</td>
<td>12.5±1.0</td>
<td>5.35±0.22</td>
<td>0.20±0.01</td>
<td>0.51±0.04</td>
<td>2.32±0.2</td>
<td>2.1±0.2</td>
</tr>
<tr>
<td>Southwest</td>
<td>221</td>
<td>21.6±2.4</td>
<td>8.3±0.9</td>
<td>4.44±0.22</td>
<td>0.27±0.01</td>
<td>0.88±0.15</td>
<td>2.62±0.2</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td><strong>FEF</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Northeast</td>
<td>833</td>
<td>39.3±2.6</td>
<td>13.7±0.6</td>
<td>5.20±0.08</td>
<td>0.12±0.06</td>
<td>0.88±0.04</td>
<td>6.6±1.5</td>
<td>9.1±1.1</td>
</tr>
<tr>
<td>Southwest</td>
<td>808</td>
<td>33.3±1.4</td>
<td>14.1±1.1</td>
<td>4.85±0.14</td>
<td>0.24±0.12</td>
<td>1.97±0.44</td>
<td>5.4±1.2</td>
<td>0.7±0.1</td>
</tr>
</tbody>
</table>

Note: Data were summarized from Gilliam et al. (2014) and Gilliam et al. (2011) for BFL and FEF, respectively. Variables included are elevation (Elev.), overstory basal area (BA), soil organic matter (OM), microbial fungi to bacteria ratio (FB), and microbial stress index (SI; the ratio of fatty acid methyl esters cy19 to 18:1n7c; see Kaur et al. (2005)); NH₄⁺ and NO₃⁻ represent soil-extractable NH₄ and NO₃, respectively. For BFL, N = 8 for each aspect; for FEF, N = 3 for each aspect.

**Fig. 1.** Long-term (1981–2010) mean monthly temperatures (°C) for Beech Fork Lake, West Virginia (open squares), and Fernow Experimental Forest, West Virginia (solid squares) (data source, http://www.ncdc.noaa.gov/).

**Fig. 2.** Differences in mean monthly temperatures (1981–2010) between Beech Fork Lake (BFL) and Fernow Experimental Forest (FEF) sites, West Virginia, calculated as BFL – FEF. Minimum temperatures (open diamonds), mean temperatures (solid squares), and maximum temperatures (open squares) (data source, http://www.ncdc.noaa.gov/).

Soils at BFL are moderately deep, well-drained, and primarily of the Gilpin–Upshur complex. Soils on both NE and SW aspects were formed from the same parent material, i.e., largely weathered acidic bedrock from intermixed shale, siltstone, and sandstone. Gilpin soils are fine–loamy, mixed, and mesic Typic Hapludults, and Upshur soils are primarily fine, mixed, and mesic Typic Hapludalfs, both occurring on ridgetops, benches, and side slopes.
Soils are moderately to slightly acidic, with soil pH ranging from 4.4 to 5.4 (Table 1). Soils of WS4 at FEF are predominantly from the Calvin and Berks soil series (loamy-skeletal, mixed, active, and mesic Typic Dystrudepts), originating from uniform parent substrates of the Upper Devonian Hampshire formation (Adams et al. 2006). These soil series are acidic, moderately deep, well-drained, and formed in material that is weathered from interbedded shale, siltstone, and sandstone, with soil pH varying from 4.8 to 5.2 (Table 1).

Common tree species at BFL are sugar maple (Acer saccharum Marsh.), buckeye (Aesculus octandra Marsh.), American beech (Fagus grandifolia Ehrh.), and white oak (Quercus alba L.). The herb layer at this site comprises moist woodland species such as chickweed (Stellaria media (L.) Vill.), harbinger of spring (Erigeria bulbosa (Michx.) Nutt.), and narrow-leaved spring beauty (Claytonia virginica L.), as well as several ericoid and graminoid species (Gilliam et al. 2014).

Common tree species at WS4 of FEF are sugar maple, black cherry (Prunus serotina Ehrh.), and northern red oak (Quercus rubra L.). The herb layer at WS4 also comprises species typical of montane eastern deciduous forests. Common herb-layer species include seedlings of striped maple (Acer pensylvanicum L.), sugar maple, and black cherry, as well as several species of Viola and hillside blueberry (Vaccinium vacillans Kalm ex Torr.) (Gilliam et al. 2011).

Both sites support stands that are typical of the MMF (Braun 1950; Dyer 2006). Although tree species are not identical between sites, they both exhibit similar patterns of aspect-related contrasts in dominant species. For example, sugar maple is prominent on NE slopes of both sites, whereas SW slopes are dominated at both sites, they both exhibit similar patterns of aspect-related contrasts in dominant species. For example, sugar maple is prominent on NE slopes of both sites, whereas SW slopes are dominated at both sites by beech and oak species.

**Field sampling**

Field sampling for this study was carried out as components of two separate studies at the FEF and BFL sites (for summaries, see Gilliam et al. (2011) and Gilliam et al. (2014), respectively). Accordingly, the number of plots varied between sites, with eight and three plots established per aspect (NE and SW) at the BFL and FEF sites, respectively. Plots were circular and 400 m² in area. Mineral soil was sampled from all plots using identical methods. Although, as associated with separate studies, soils were sampled in different years at each site, they were sampled at the same time of year in May. Organic horizons are also important in understanding N dynamics in forest ecosystems; however, we focused solely on the mineral soil to add to previous work that has quantified microbial functional groups (via phospholipid fatty acid analysis) in the mineral soil of these sites (Gilliam et al. 2011, 2014). After removing humus layers, soil was taken at a 5 cm depth, using a hand trowel, at five random locations throughout each plot, mixed into a single composite sample, and placed in 500 mL sterile polyethylene Whirl-Pac bags; these were stored on ice for transport to the Weeds and Dirt Laboratory, Marshall University, Huntington, West Virginia. As stated, soils were sampled in different years as part of the separate studies at each site, so the interpretation of the results should be made with that in mind. It is our contention, however, that site-related differences between sites are more strongly influenced by sharp contrasts in latitude and elevation between sites.

**Experimental treatment and laboratory analyses**

On return to the laboratory, each soil sample was extracted with 1 mol·L⁻¹ KCl at an extract to soil ratio of 10 to 1 (volume to mass) and analyzed for NH₄⁺ and NO₃⁻ with an AutoAnalyzer 3 system. The remaining soil from each sample was separated into four 100 mL sterile polyethylene Whirl-Pac bags and placed into incubators for 7 days at the following temperature treatments: 4 °C, 15 °C, 25 °C, and 35 °C. Polyethylene is permeable to O₂ but not to water vapor; therefore, soil moisture, which was between 20% and 25% for all samples (i.e., did not vary between aspects and sites), did not change during incubation. The length of the incubation period varies greatly among studies such as this, from as short as 1 day (Ross et al. 2006) to up to 4–12 weeks (Rustad et al. 2001). In lieu of other periods of incubation, we chose an incubation period of 7 days for two main reasons. First, previous work with soils from FEF has shown linear relationships between N rates and temperature up to and beyond 7 days. Second, we wanted to maintain methodology consistent with published work from this laboratory (e.g., Gilliam et al. 2011, 2014). Following incubation, soil was extracted and analyzed for extractable N via the same methods as those used prior to treatment.

**Data analyses**

Net N mineralization was calculated for each temperature treatment by subtracting the sum of pretreatment NH₄⁺ and NO₃⁻ concentrations from the sum of NH₄⁺ and NO₃⁻ concentrations following incubation, whereas net nitrification was calculated for each treatment by subtracting pretreatment NO₃⁻ concentrations from post-treatment NO₃⁻ concentrations. All difference calculations were divided by seven to yield daily rates (i.e., µg N·(g soil)⁻¹·day⁻¹). Net N mineralization and nitrification rates were compared between aspects, sites, and incubation temperatures with analyses of variance and least significant difference tests, with a priori levels of acceptance of significant differences at P < 0.05 (Zar 2010). Patterns of change in both net N mineralization and net nitrification with temperature were assessed with second-order polynomials. Relationships between net nitrification and net N mineralization were assessed on a plot basis for each site and aspect separately using linear regression (Zar 2010).

To determine the relative contribution of nitrification to the overall N mineralization, relative net nitrification (RNN) was calculated as the following, expressed as %:

$$RNN = \left( \frac{\text{net nitrification rate}}{\text{net N mineralization rate}} \right) \times 100$$

Given the general absence of net nitrification in SW soils for both sites, this calculation was performed only on NE soils. The relationship between RNN and incubation temperature for the NE soils of each site was determined by linear regression.

**Results and discussion**

As predicted, rates of mineral N transformation — both net N mineralization and net nitrification — generally responded sensitively to temperature throughout the experimental range from 4 °C to 35 °C (Tables 2 and 3). However, the nature of this relationship varied substantially with aspect and, to a lesser extent, between sites. Net N mineralization was dominated by net nitrification in NE soils but was predominantly in the form of ammonification in SW soils, consistent with previous work at both sites using in situ (“buried bag”) incubations that demonstrated negligible net nitrification in SW soils (Gilliam et al. 2011, 2014). In general, the results support our first hypothesis regarding the sensitivity of N dynamics to increases in temperature, i.e., greater for NE aspect soils than for SW aspect soils.

Although several studies have shown N mineralization to increase linearly with temperature, at least in the range used in this study (e.g., Myers 1975; Emmer and Tietema 1990; Guntiñas et al. 2012), it increased in a curvilinear fashion for all site-aspect combinations up to 35 °C. Thus, there was no Tₘ₅₀ for N mineralization within this range for soil contrasting in site types and slope aspects. In general, rates of N mineralization differed more between slope aspects than between sites, being higher in soil from NE slopes than in soil from SW slopes. Maximum rates of net N mineralization (at 35 °C) were very similar between sites for soils from NE slopes but were significantly higher for BFL soils from SW slopes than for FEF soils from SW slopes (Fig. 3a).
The most consistent pattern of contrast in this study was the stark contrast between SW and NE soils at both sites. Previous work at BFL and FEF have reported similar aspect-related contrasts in soil N dynamics using in situ incubations, and both provided compelling evidence to suggest that soil weathering, being higher on SW aspects than on NE aspects from higher net radiation, drive such patterns in soil N dynamics. They also concluded that such differences were particularly manifested in soil microbial composition. Using phospholipid fatty acid analysis to characterize microbial functional groups, both studies found the predominance of fungal markers (i.e., higher fungi to bacteria ratios; Table 1) and microbial markers that are indicative of environmental stress in soils from SW aspects (i.e., using the stress index of the ratio of fatty acid methyl esters cy19 to 18:1n7c; see Kaur et al. (2005); Table 1). In contrast, they found a prevalence of Gram-negative bacterial markers in soils from NE aspects, suggesting...
Fig. 1 but is most pronounced during the growing season (Fig. 2) and bacterial groups, further complicates the predictability of climate-driven change in N dynamics.

References


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