Spatial and seasonal variation of gross nitrogen transformations and microbial biomass in a Northeastern US grassland

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Abstract

Understanding how N-cycling processes in unmanaged grassland vary spatially and seasonally would aid the development of management strategies which capitalize on the behavior of its N cycle, in order to manage N losses when such systems are altered. Our objectives were: (1) to quantify gross rates of internal N-cycling processes (i.e. mineralization, nitrification, and immobilization) from an unmanaged grassland, and (2) to investigate the role of topography and climatic factors on the spatial and seasonal variation of these processes. We delineated our study site into three topographic units based on soil and drainage types: upper, lower, and drained lower slopes. The dynamics of NH$_4^+$ in the internal N cycle was influenced by the spatial and seasonal variation of microbial biomass, in which the spatial variation resulted from long-term topographic influence and the seasonal variation from seasonal flushes of available organic matter. The drained lower slope had the highest microbial biomass (647 mg C kg$^{-1}$ and 90 mg N kg$^{-1}$), gross N mineralization (8 mg N kg$^{-1}$ d$^{-1}$), NH$_4^+$ immobilization (6 mg N kg$^{-1}$ d$^{-1}$), and fastest NH$_4^+$ turnover (0.5 d), indicating that the drainage favored microbial growth and activity. The seasonal pattern of NH$_4^+$ transformations and microbial biomass showed that the increases in microbial N (in fall and late spring towards summer) were paralleled by high NH$_4^+$ immobilization and a decrease in the microbial C–N ratio. Spatial variation of NO$_3^-$ transformations showed the effect of topography through water redistribution; higher gross nitrification was observed in the upper (1.7 mg N kg$^{-1}$ d$^{-1}$) than lower slopes (1.1 mg N kg$^{-1}$ d$^{-1}$), with drainage also favoring gross nitrification (1.4 mg N kg$^{-1}$ d$^{-1}$) through improved soil aeration. The seasonal pattern of gross nitrification was related to soil moisture ($r = -0.79$, $P = 0.01$) and temperature ($r = 0.55$, $P = 0.05$). Gross nitrification accounted 32% of the NH$_4^+$ produced. NO$_3^-$ immobilization was about 50% of NH$_4^+$ immobilization. NO$_3^-$ immobilization was highest when microbial immobilization was most favorable and available NH$_4^+$ was insufficient to meet microbial demand. NO$_3^-$ was rapidly produced and consumed (0.7 d average turnover time), and hence its usually small pool size in unmanaged grassland cannot be used as basis to dismiss its importance in the internal N cycle. Our study supported the concept of considering both the spatial and seasonal variation of soil biochemical processes in refining nutrient management strategies in an ecosystem. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Gross nitrogen mineralization; Gross nitrification; Microbial immobilization; Microbial biomass; Spatio-seasonal variation

1. Introduction

In unfertilized grassland, external sources of N come mainly from atmospheric deposition and, depending on grass species composition, possibly also from N$_2$ fixation. Hence, a considerable portion of available N for plant and microbial use must be provided by microbially-mediated N processes in the soil. Mineral N pool sizes in such systems are typically low (Stienstra et al., 1994), with tight coupling between consumptive and productive N-cycling processes (Davidson et al., 1990). An uncoupling of such processes, as a result of management changes, is likely to alter the soil N status and undesirable N losses from the system could occur (Ihori et al., 1995).

The grassland studied here is located in Pennsylvania, USA. It had native perennial vegetation and no fertilization, grazing, or cultivation for at least 30 yr before 1998 summer, when thereafter (after our study year) cows were allowed to graze. The total atmospheric N deposition in the area is only about 11 kg N ha$^{-1}$ yr$^{-1}$ (Williard et al., 1997). In this humid, temperate continental climate, grass biomass yield is not limited by soil moisture availability but rather by N availability (Stout and Schnabel, 1997). With the recent introduction of intensive rotational grazing systems to dairy farming in northeastern USA, farmers commonly convert such grasslands to managed pastures. This usually involves changes in N management, but in the case of poorly drained soils can also include drainage. Understanding on how soil
Table 1
Soil characteristics (0–10 cm depth) measured at the beginning of the study (August 1997) prior to the installation of perforated drains to the lower slope (October 1997). Means (standard errors) followed by the same letter were not significantly different (one-way ANOVA, Bonferroni test at $P \leq 0.05$)

<table>
<thead>
<tr>
<th>Topographic units</th>
<th>Soil type</th>
<th>Drainage type</th>
<th>Soil texture (%)</th>
<th>Soil pH (1:1 H₂O)</th>
<th>Total soil organic C (mg C kg⁻¹)</th>
<th>Total soil N (mg N kg⁻¹)</th>
<th>Total soil C–N ratio</th>
<th>Biodegradable organic C (mg C kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper</td>
<td>Aeric</td>
<td>Well</td>
<td>27-sand</td>
<td>5.9 (0.1)</td>
<td>18070 (636)</td>
<td>1612 (55)</td>
<td>11.5 (0.1a)</td>
<td>8.5 (0.7b)</td>
</tr>
<tr>
<td></td>
<td>Fragiqualt</td>
<td>Drained</td>
<td>44-silt</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>29-clay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower</td>
<td>Typic</td>
<td>Poorly</td>
<td>26-sand</td>
<td>5.9 (0.1)</td>
<td>18472 (825)</td>
<td>1681 (82)</td>
<td>10.6 (0.3b)</td>
<td>19.1 (3.5a)</td>
</tr>
<tr>
<td></td>
<td>Fluvaquent</td>
<td>Drained</td>
<td>39-silt</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>35-clay</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drained Lower</td>
<td>Typic</td>
<td>Poorly</td>
<td>26-sand</td>
<td>5.9 (0.2)</td>
<td>18351 (725)</td>
<td>1650 (76)</td>
<td>10.5 (0.3b)</td>
<td>18.5 (4.0a)</td>
</tr>
<tr>
<td></td>
<td>Fluvaquent</td>
<td>Drained</td>
<td>39-silt</td>
<td></td>
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<td></td>
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<td>35-clay</td>
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<td></td>
</tr>
<tr>
<td>$P$ values</td>
<td></td>
<td></td>
<td>0.1</td>
<td>0.78</td>
<td>0.81</td>
<td>0.59</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>
and climatic factors control the N-cycling dynamics in unmanaged grassland is essential to managing N losses when such system is altered.

Topography influences hillslope hydrology or water redistribution (Haggott, 1975), microclimate (Rowe, 1984), soil type (Pennock et al., 1987), and differences in vegetation (Burke et al., 1989). The influence of topography on N-cycling processes in agricultural and forest ecosystems is well documented; compared to upper slope well-drained soils, lower slope poorly-drained soils had higher microbial respiration, N mineralization (Merrill and Zak, 1992; Goovaerts and Chiang, 1993), net nitrification, microbial biomass N (Groffman et al., 1993), and denitrification (Davidson and Swank, 1986; Groffman and Tiedje, 1989; Pennock et al., 1992; Van Kessel et al., 1993), and lower N immobilization (Groffman et al., 1993). These results suggest that the topographic units are functionally distinct units, requiring different management strategies. Nevertheless, little is known on the role of topography on N-cycling processes in temperate grassland. To our knowledge, no study has been conducted on spatial and seasonal dynamics of internal N cycling in Northeastern US grassland. Microbial response to topographic controls on water redistribution, and on the consequent redistribution of materials carried by water, could alter N-cycling processes. Understanding how N-cycling processes vary spatially and seasonally would aid in devising management strategies, which capitalize on the behavior of the grassland N cycle. Our objectives were: (1) to quantify gross rates of internal N-cycling processes (i.e. mineralization, nitrification, and immobilization) from an unmanaged grassland, and (2) to investigate the role of topography (signifying differences in soil and drainage types) and climatic factors (i.e. precipitation and temperature) on the spatial and seasonal variation of these processes.

2. Materials and methods

2.1. Site description and sampling design

The study site was located in the Cove Mountain Farm near Hancock, Pennsylvania (78°1'45"W, 39°44'30"N). During the year of study (August 1997–July 1998), the total rainfall was 132 cm and the mean January and July temperatures were 3 and 22 °C, respectively. We delineated our study site into three topographic units based on soil and drainage types (Table 1): upper slope, lower slope, and drained lower slope. The upper and lower slopes had each 100 m transect, and in each transect measurements were taken from ten sampling points spaced at 10 m apart. These transects were parallel to each other and separated by a distance of 100 m, with an average elevation difference of 2 m and average slope of 2.1%. The drained lower slope was about 100 m away from the transect of the lower slope and was established in first October 1997 with perforated drains installed between 0.5 and 1.0 m depth. In this topographic unit, measurements were taken from two transects (perpendicular to the lower slope transect), each with five sampling points (45 m apart); these transects ran along the direction of the perforated drains. The transects at each topographic unit were on the same location for all sampling months, and samples were taken within 1 m² area around the sampling points. The study site had spatially-continuous vegetation cover and there was no difference in vegetation species among the topographic units, with the following five most dominant species: tall fescue (Festuca arundinacea Schreb.; comprised ~50%), white clover (Trifolium repens L.), kentucky bluegrass (Poa pratensis L.), reed canary (Phalaris arundinacea L.), and slender rush (Juncus tenuis Willd.).

Gross mineralization, gross nitrification, N immobilization, microbial biomass N and C, mineral N pool, and soil moisture were all measured at each sampling point at each sampling time: once in summer 1997 (August 6), once in fall 1997 (November 1), and once a month from spring to summer 1998 (April 1, May 19, June 30, and July 27). Soil moisture content was expressed as water-filled pore space (ratio of volumetric soil water content to total soil porosity). Other soil characteristics (Table 1) were also measured at each sampling point in August 1997, before the drainage system was installed in the drained lower slope. Total organic C and N were measured from air-dried, ground samples using CHN elemental analyzer (1100 CE Instruments, Elantech, NJ, USA). For biodegradable organic C, 150 g fresh soil was extracted with 300 ml distilled water and filtered through 2 μm filter paper. A measure (25 ml) of the filtrate was again filtered (0.2 μm filter) for dissolved organic C (DOC) analysis. Another 50 ml of the 2 μm filtrate was placed in an Erlenmeyer flask, covered with perforated parafilm to allow exchange of air, and incubated at 22 °C for 30 days. At the end of incubation the solution was filtered through 0.2 μm filter for DOC analysis. DOC was analyzed by dry combustion at 680 °C using TOC5000A Shimadzu carbon analyzer (Shimadzu, MD, USA). Biodegradable organic C was calculated from the difference in DOC before and after 30 d incubation (Corre et al., 1999). Rainfall and air temperature (at 1.5 m above the ground) were recorded continuously by an on-site meteorological station.

2.2. 15N pool dilution for measurement of gross N mineralization and gross nitrification

At each sampling point, four aluminum cores (5 cm diameter × 10 cm deep) were driven into the soil in a square format with a distance of 1 cm between cores. The soil surrounding these cores were taken for initial mineral N determination; the soil was mixed in a plastic bag and immediately subsampled for extraction with 2 M KCl (approx. 5:1 ratio of solution to dry mass soil). The remaining soil was kept on ice during transport to the laboratory
Table 2
Comparison of NH$_4^+$ transformation processes and microbial biomass among topographic units for spatial pattern determination and among sampling months for seasonal pattern determination. Means (standard errors) among slopes and among months followed by the same letter(s) were not significantly different (Repeated Measures ANOVA, Tukey’s HSD at $P \leq 0.05$)

<table>
<thead>
<tr>
<th>Slope/month</th>
<th>Gross N mineralization (GNM) (mg N kg$^{-1}$ d$^{-1}$)</th>
<th>NH$_4^+$ immobilization (AI) (mg N kg$^{-1}$ d$^{-1}$)</th>
<th>NH$_4^+$ pool (mg N kg$^{-1}$)</th>
<th>NH$_4^+$ turnover (d)</th>
<th>Microbial C (mg C kg$^{-1}$)</th>
<th>Microbial N (N$_{fib}$) (mg N kg$^{-1}$)</th>
<th>Microbial C–N ratio</th>
<th>GNM–N$<em>{fib}$ ratio (mg N d$^{-1}$ mg$^{-1}$ N$</em>{fib}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper</td>
<td>6.0 (0.6)b</td>
<td>3.1 (0.5)b</td>
<td>2.9 (0.2)b</td>
<td>1.4 (0.4)a</td>
<td>455.1 (38.1)b</td>
<td>65.3 (6.0)b</td>
<td>9.4 (1.3)</td>
<td>0.09 (0.17)</td>
</tr>
<tr>
<td>Lower</td>
<td>5.4 (0.6)b</td>
<td>3.2 (0.5)b</td>
<td>2.8 (0.2)b</td>
<td>0.8 (0.2)ab</td>
<td>614.2 (38.1)a</td>
<td>75.3 (5.9)ab</td>
<td>9.3 (0.7)</td>
<td>0.09 (0.01)</td>
</tr>
<tr>
<td>Drained lower</td>
<td>8.2 (0.6)a</td>
<td>6.2 (0.5)a</td>
<td>3.7 (0.2)a</td>
<td>0.5 (0.1)b</td>
<td>646.7 (38.1)a</td>
<td>90.4 (6.2)a</td>
<td>8.6 (0.9)</td>
<td>0.14 (0.04)</td>
</tr>
<tr>
<td>Month</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov</td>
<td>6.7 (0.8)</td>
<td>5.5 (0.9)ab</td>
<td>2.2 (0.2)b</td>
<td>1.2 (0.3)ab</td>
<td>625.8 (51.8)a</td>
<td>65.0 (7.6)a</td>
<td>11.5 (1.3)b</td>
<td>0.14 (0.04)ab</td>
</tr>
<tr>
<td>April</td>
<td>4.9 (0.8)</td>
<td>0.6 (0.2)c</td>
<td>3.8 (0.2)a</td>
<td>1.8 (0.7)a</td>
<td>404.9 (50.2)b</td>
<td>35.3 (8.5)b</td>
<td>16.6 (2.8)a</td>
<td>0.64 (0.40)a</td>
</tr>
<tr>
<td>May</td>
<td>7.5 (0.6)</td>
<td>3.0 (0.5)b</td>
<td>3.6 (0.3)a</td>
<td>0.7 (0.1)b</td>
<td>703.3 (50.2)a</td>
<td>81.6 (6.0)a</td>
<td>9.4 (0.7)</td>
<td>0.10 (0.01)b</td>
</tr>
<tr>
<td>June</td>
<td>6.4 (0.8)</td>
<td>4.9 (0.8)ab</td>
<td>3.4 (0.3)a</td>
<td>0.7 (0.1)b</td>
<td>579.3 (45.6)a</td>
<td>101.0 (7.6)a</td>
<td>6.0 (0.4bc)</td>
<td>0.06 (0.01b)</td>
</tr>
<tr>
<td>July</td>
<td>7.1 (0.6)</td>
<td>6.7 (0.7)a</td>
<td>2.5 (0.1)b</td>
<td>0.4 (0.1)b</td>
<td>546.7 (24.4)a</td>
<td>102.2 (6.0a)</td>
<td>5.7 (0.3c)</td>
<td>0.08 (0.01b)</td>
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$P$ values

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<tr>
<th>Slope</th>
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<th>0.01</th>
<th>0</th>
<th>0</th>
<th>0.02</th>
<th>0.42</th>
<th>0.06</th>
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<tbody>
<tr>
<td>Month</td>
<td>0.14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>Slope – month</td>
<td>0.17</td>
<td>0.05</td>
<td>0.02</td>
<td>0</td>
<td>0</td>
<td>0.91</td>
<td>0.5</td>
<td>0.27</td>
</tr>
</tbody>
</table>
### Table 3
Comparison of NO$_3^-$ transformation processes among topographic units for spatial pattern determination and among sampling months for seasonal pattern determination. Means (standard errors) among slopes and among months followed by the same letter(s) were not significantly different (Repeated Measures ANOVA, Tukey’s HSD at $P \leq 0.05$)

<table>
<thead>
<tr>
<th>Slope/Month</th>
<th>Gross nitrification (GN) (mg N kg$^{-1}$ d$^{-1}$)</th>
<th>NO$_3^-$ immobilization (NI) (mg N kg$^{-1}$ d$^{-1}$)</th>
<th>NO$_3^-$ pool (mg N kg$^{-1}$)</th>
<th>NO$_3^-$ turnover (d)</th>
<th>GN–GNM (Table 2) ratio (%)</th>
<th>NI–GN ratio (%)</th>
<th>NI–AI (Table 2) ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper</td>
<td>1.7 (0.2) a</td>
<td>1.6 (0.3)</td>
<td>0.6 (0.1)a</td>
<td>0.5 (0.1)</td>
<td>31 (5)</td>
<td>85 (20)</td>
<td>52 (19)</td>
</tr>
<tr>
<td>Lower</td>
<td>1.1 (0.2)b</td>
<td>1.5 (0.2)</td>
<td>0.4 (0.1)b</td>
<td>0.7 (0.1)</td>
<td>23 (4)</td>
<td>174 (32)</td>
<td>47 (21)</td>
</tr>
<tr>
<td>Drained lower</td>
<td>1.4 (0.2)ab</td>
<td>1.9 (0.4)</td>
<td>0.5 (0.1)ab</td>
<td>0.9 (0.2)</td>
<td>22 (4)</td>
<td>223 (51)</td>
<td>50 (16)</td>
</tr>
<tr>
<td>Month</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov</td>
<td>0.7 (0.2)b</td>
<td>0.2 (0.1)c</td>
<td>0.2 (0.1)b</td>
<td>0.4 (0.1)b</td>
<td>16 (6)b</td>
<td>31 (22)b</td>
<td>2 (1)</td>
</tr>
<tr>
<td>April</td>
<td>0.5 (0.1)b</td>
<td>0.7 (0.1)b</td>
<td>0.3 (0.0)b</td>
<td>0.8 (0.2)b</td>
<td>15 (4)b</td>
<td>30 (15)b</td>
<td>73 (54)</td>
</tr>
<tr>
<td>May</td>
<td>2.8 (0.3)a</td>
<td>2.6 (0.4)a</td>
<td>0.9 (0.2)a</td>
<td>0.4 (0.1)b</td>
<td>40 (5)a</td>
<td>124 (33)a</td>
<td>98 (35)</td>
</tr>
<tr>
<td>June</td>
<td>0.3 (0.1)b</td>
<td>0.8 (0.1)b</td>
<td>0.4 (0.0)b</td>
<td>2.0 (0.4)a</td>
<td>7 (2)b</td>
<td>290 (25)a</td>
<td>20 (8)</td>
</tr>
<tr>
<td>July</td>
<td>2.7 (0.4)a</td>
<td>2.7 (0.3)a</td>
<td>0.7 (0.1)a</td>
<td>0.3 (0.1)b</td>
<td>35 (6)a</td>
<td>212 (51)a</td>
<td>67 (16)</td>
</tr>
</tbody>
</table>

*P* values
- Slope 0.05 0.75 0.14 0.08 0.06 0.51 0.52
- Month 0 0 0 0 0 0.02 0.33
- Slope × Month 0.39 0.03 0.04 0.02 0.22 0.36 0.68
and was used for microbial biomass C and N assays and for gravimetric moisture determination. We followed the procedure described by Davidson et al. (1991) for $^{15}$N injection into the soil core and subsequent extraction. Two soil cores were injected with ($^{15}$NH$_4$)$_2$SO$_4$ solution and the other two with K$^{15}$NO$_3$ solution, using 18 gauge side-port spinal needles. Each soil core received six 1 ml injections of the solutions, containing 30 $\mu$g N ml$^{-1}$ with 98% $^{15}$N enrichments. One core of each labeled pair was immediately broken up, mixed well in a plastic bag, and subsampled for 2 M KCl extraction. The time elapsed between injection and extraction was about 10 min ($T_0$). The $T_0$ cores were used to correct for the reaction(s) that occur immediately after injection, as recommended by Davidson et al. (1991) and Hart et al. (1994). The other core of the labeled pair was capped, reburied, and extracted 24 h later ($T_1$); the remaining soil was kept on ice during transport to the laboratory and was used for N immobilization assay. The moisture content in each soil core was measured gravimetrically.

KCl extracts were shaken for 1 h and filtered through Whatman no.1 filters that had been rinsed with KCl. NH$_4^+$ and NO$_3^-$ contents of the extracts were analyzed colorimetrically using a Lachat QuickChem 8000 Automated Ion Analyzer (Lachat Instruments, WI, USA). For $^{15}$N analysis from KCl extracts, the diffusion procedure described in detail by Stark and Hart (1996) was followed. For the $^{15}$NH$_4^+$-labeled samples, 50 ml of the KCl extract was placed in 150 ml tight-lid, plastic specimen container; MgO was added and the released NH$_3$ was captured in an acid trap (two disks of 7 mm diameter cut from Whatman no. 1 filter paper, acidified with 2.5 M KH$_2$SO$_4$, and encased in polytetrafluoroethylene or teflon tape). The containers were placed in an incubation room set at 22 °C and were diffused for 6 d, during which periodic mixing of the solution was done by swirling the containers. For the $^{15}$NO$_3^-$-labeled samples, the container was left open for 5 d after adding MgO, and the 6 d diffusion proceeded after adding Devarda’s alloy. The $^{15}$N analysis was conducted at the Stable Isotope Laboratory of the University of Saskatchewan, Canada using a RoboPrep sample converter interfaced to a TracerMass mass spectrometer (PDZ Europa Ltd., Crewe, UK). Gross mineralization and nitrification rates were estimated for cores that received $^{15}$NH$_4^+$ and $^{15}$NO$_3^-$, respectively, using the modified calculation procedure of Davidson et al. (1991) from the Kirkham and Bartholomew (1954) model.

2.3. Microbial biomass C and N, and NH$_4^+$ and NO$_3^-$ immobilization assays

We used the fumigation-extraction method (Brookes et al., 1985a; Davidson et al., 1989) for determining microbial biomass C and N. At each sampling point, the soil taken around the cores was mixed well by hand, and two 25 g field-moist subsamples were taken. One pair of the subsamples were immediately extracted with 0.5 M K$_2$SO$_4$ (approx. 5:1 ratio of solution to dry mass soil). The other pair of the subsamples were placed in desiccators containing beakers of distilled CHCl$_3$ with several bioling chips. The desiccator was evacuated and flushed with air several times to distribute CHCl$_3$ vapor, and during the final evacuation it was closed after letting the CHCl$_3$ bubble for 3 min. The samples were removed after 5 d of fumigation and extracted with 0.5 M K$_2$SO$_4$. Extracts were shaken for 1 h and filtered through Whatman no.1 filters that had been rinsed with K$_2$SO$_4$. The organic C in the K$_2$SO$_4$ extracts was analyzed using the same aforementioned DOC analyzer. The total N in the K$_2$SO$_4$ extracts was determined using a modified micro-Kjeldahl digestion procedure in which all NO$_3^-$ present is volatilized as HNO$_3$ in Kjeldahl tubes prior to Kjeldahl digestion (Davidson et al., 1989; Wyland et al., 1994; Stark and Hart 1996). The Kjeldahl N (in NH$_4^+$ form) content was analyzed using the same automated ion analyzer mentioned above. The differences in organic C and Kjeldahl N extracted between the fumigated and nonfumigated soils (C and N fluxes) are assumed to represent the C and N released from lysed soil microbes. The C and N fluxes were converted to microbial biomass C and N, respectively, using $k_{c}$ = 0.45 (Joergensen, 1996) and $k_{N}$ = 0.68 for 5 d fumigated soils (Shen et al., 1984; Brookes et al., 1985a).

The NH$_4^+$ and NO$_3^-$ immobilization was determined from the $T_1$ $^{15}$NH$_4^+$- and $^{15}$NO$_3^-$-labeled cores, respectively. After KCl extraction in the field for the isotopic dilution method, two subsamples were removed for estimation of microbial N flush, as described above. The Kjeldahl digests were prepared for $^{15}$N diffusion following the acid-trap procedure described previously, except that 10 ml of 10 M NaOH rather than MgO was used to buffer the solution and volatilize NH$_3$ (Wyland et al., 1994; Stark and Hart, 1996). The $^{15}$N recovered in nonfumigated soils was subtracted from the $^{15}$N recovered in fumigated soils to estimate the $^{15}$N flush released (presumably from microbial biomass) by fumigation. We used the nonlinear model for calculating immobilization rate as described by Davidson et al. (1991).

2.4. Statistical analyses

Using the N transformation data, the sampling points representing our experimental units (topographic units) were first assessed for statistical independence. This was carried out following the von Neuman’s ratio test (Bartels, 1982). The 10 sampling points in each topographic unit were statistically independent, and hence were considered replicates in the further analysis. Normality was also determined by a goodness of fit test using Kolomogorov–Smirnov D statistic (Sokal and Rohlf, 1981). Differences on variables reported in Table 1 were determined by one-way analysis of variance (ANOVA). For data reported in Tables 2 and 3, repeated-measures ANOVA using PROC MIXED of SAS (SAS, 1992) were used. Crowder and Hand (1990) discussed the applications of repeated-measures analysis in
determining differences among experimental units (topographic units) on variable(s) measured repeatedly over a certain period of time. Sampling points/replications, which were nested within topographic units, were treated as a random effect, while all other effects (topographic units, sampling months, and topographic unit x sampling month interaction) were treated as fixed. Type III sums of squares were computed for the fixed effects. Since F-tests involving mixed model effects are not exact, the Satterthwaite method of adjusting denominator (error term) degrees of freedom was used to obtain accurate approximations. Multiple comparisons of fixed effect least square means was conducted using Tukey’s HSD, corrected for unbalanced replication using the Tukey–Kramer adjustment, at $P \leq 0.05$. Values reported in Tables 2 and 3 for topographic units are an average of all sampling months and for sampling months are an average of all topographic units.

3. Results

3.1. $\text{NH}_4^+$ transformation processes and microbial biomass

The rates of $\text{NH}_4^+$ transformations in our study site were within the range of those reported from natural grassland in California, USA (Schimel et al., 1989; Davidson et al., 1990). Gross N mineralization, $\text{NH}_4^+$ immobilization and $\text{NH}_4^+$ pool were higher in the drained lower slope than in the lower and upper slopes (Table 2). The microbial biomass showed a slightly different result; microbial C and N contents in the lower and drained lower slopes were comparable and these were higher than in the upper slope (Table 2). The high microbial biomass and chemohetro- trophic activities (i.e. gross N mineralization, $\text{NH}_4^+$ immobilization) in the drained lower slope consequently resulted in a fast turnover time of $\text{NH}_4^+$ ($\text{NH}_4^+$ pool/gross N mineralization); the high microbial biomass but low activities in the lower slope resulted in an intermediate $\text{NH}_4^+$ turnover time; and the low microbial biomass and activities in the upper slope resulted in a slow $\text{NH}_4^+$ turnover time (Table 2).

No significant seasonal pattern was detected for gross N mineralization (Table 2). $\text{NH}_4^+$ immobilization exhibited a clear seasonal pattern that was similar to the pattern of microbial biomass C and N. $\text{NH}_4^+$ immobilization rates (Fig. 2A and Table 2) and microbial biomass (Table 2) were high in the fall (November), reached the lowest in early spring (April), and increased towards summer. As the $\text{NH}_4^+$ immobilization increased, the microbial C–N ratio decreased towards summer (Table 2). The seasonal pattern of $\text{NH}_4^+$ pool was the converse of $\text{NH}_4^+$ immobilization (Fig. 2A and B, and Table 2); when $\text{NH}_4^+$ immobilization was high (i.e. November and July) $\text{NH}_4^+$ pool was low and vice versa. Although $\text{NH}_4^+$ pool is the product of the concurrently occurring $\text{NH}_4^+$-producing process (gross N mineralization) and $\text{NH}_4^+$-consumptive processes (gross nitrification and immobilization), based from the seasonal pattern of $\text{NH}_4^+$ pool this seemed to be the leftover N from $\text{NH}_4^+$ immobilization. The turnover time of $\text{NH}_4^+$ also reflected the seasonal patterns of $\text{NH}_4^+$ immobilization and microbial biomass. The movement of $\text{NH}_4^+$ through the $\text{NH}_4^+$ pool was slowest during early spring when $\text{NH}_4^+$ immobilization and microbial biomass were the lowest (Fig. 2A and C, and Table 2), and the $\text{NH}_4^+$ turnover time was fast during fall and towards summer when microbial biomass and $\text{NH}_4^+$ immobilization were high.
<table>
<thead>
<tr>
<th></th>
<th>NH$_4^+$ immobilization</th>
<th>Microbial N</th>
<th>Microbial C–N ratio</th>
<th>Gross nitrification</th>
<th>NO$_3^-$ immobilization</th>
<th>Water-filled pore space</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
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<td>0.65**</td>
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<td>0.26</td>
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<td>0.15</td>
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<td></td>
<td>0.26</td>
<td>0.43</td>
<td>-0.22</td>
<td>0.47†</td>
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<tr>
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<td></td>
<td></td>
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<td>0.08</td>
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</table>
3.2. NO\textsubscript{3}\ transformation processes

The rates of NO\textsubscript{3}\ transformations were also comparable to those reported by Schimel et al. (1989) and Davidson et al. (1990). NO\textsubscript{3}\ immobilization and turnover time did not differ among slopes, while gross nitrification and NO\textsubscript{3}\ pool were higher in the upper and drained lower slopes than in the undrained lower slope (Table 3). This pattern was parallel with that of the soil moisture contents (Fig. 1). The seasonal patterns of NO\textsubscript{3}\ transformations were related to soil moisture and to a lesser extent, temperature (Table 4). Gross nitrification, gross nitrification–gross N mineralization ratio (Table 3), NO\textsubscript{3}\ immobilization (Fig. 3A), and NO\textsubscript{3}\ pools (Fig. 3B) were higher in months with low moisture contents (May and July) than in months with high moisture contents (November, April and June; Fig. 1). The low temperatures in fall and early spring could have also contributed to the low NO\textsubscript{3}\ transformation activity, but soil moisture effect clearly dominated, as shown by the low activity in June despite high temperatures. The slowest NO\textsubscript{3}\ turnover time (NO\textsubscript{3} pool/gross nitrification) also occurred in June (Fig. 3C and Table 3).

NO\textsubscript{3}\ immobilization to gross nitrification ratios were more than 100% (indicating overestimation of NO\textsubscript{3}\ immobilization) in the lower and drained lower slopes (Table 3), where microbial biomass was high, and from late spring (May) to summer (July), during which microbial biomass was also increasing. Overestimation could result from stimulation of microbial assimilation by the injected N. The immobilization conditions (e.g. soil moisture, temperature, microbial biomass) were generally favorable from late spring towards summer, such that the injected \textsuperscript{15}NO\textsubscript{3} could have been readily consumed by microorganisms to meet their demand for N. The highest overestimation occurred in June (Table 3). During this sampling period NO\textsubscript{3} availability (gross nitrification) was low (Table 3) due to high soil moisture content (Fig. 1), so the microorganisms may have utilized more of the injected \textsuperscript{15}NO\textsubscript{3} instead.

4. Discussion

4.1. Spatial and seasonal pattern of NH\textsuperscript{4+}\ transformation processes and microbial biomass

Our study was conducted in a long-established grassland where microbial biomass should have been in equilibrium with the local conditions. In such conditions, the microbial biomass size is stable, and superimposed on this stable background are short-term fluctuations (Lovell et al., 1995) created by seasonal flushes of labile forms of C (Anderson and Domsch, 1986). This condition was clearly manifested in the upper and lower slopes at our study site, where differences in the size of the microbial biomass reflected the long-term influence of topography on differences in soils, natural drainage, growth performance of the grasses, and water and nutrient redistribution. Considering the biodegradable organic C (Table 1) as a measure of organic C availability to microorganisms, the higher available C in the lower than upper slopes implied that the former could indeed support higher microbial biomass. When we calculated the specific gross N mineralization activity (gross N mineralization–microbial N ratio as an indicator of energy maintenance activity of the microbial biomass, e.g. chemoheterotrophs), the upper and lower slopes were also comparable (Table 2). This signified that the microbial biomass and activity in these topographic units were in concert with the local supply of available organic matter and local environmental conditions (e.g. drainage).

From the soil characteristics shown in Table 1, it is reasonable to assume that the lower and drained lower slopes had similar soil biochemical characteristics before the drainage system in the latter was modified. The evidently high microbial biomass, gross N mineralization, NH\textsubscript{4+}immobilization, and fast NH\textsubscript{4+} turnover time in the drained lower slope indicated that the improved edaphic condition (aeration), through change in soil drainage condition, favored microbial growth and activity. An ancillary survey on above-ground biomass production in 1998 also found that, although there was no apparent change in grass species composition, grass biomass production was higher in the drained than undrained lower slopes (unpublished data; Benjamin Tracy, personal communication). Increased plant production could imply increased return of available organic matter to the soil that, in turn, would enhance microbial growth and activity.

Seasonal patterns of microbial C and N in pasture soils have been reported (Sarathchandra et al., 1988; Bristow and Jarvis, 1991) and are thought to result from increased availability of root exudates from growing grasses (Lovell et al., 1995). Enhanced root growth and increased root exudates are also thought to stimulate bacterial growth (Clarholm, 1985), which have a low C–N ratio (Anderson and Domsch, 1980). We observed a decreasing microbial C–N ratio from spring to summer. As the grasses grew from spring towards summer, root exudates might have also increased and favored the growth of bacteria.

Seasonal changes in soil microbial biomass are proposed to be directly involved in the turnover of organic matter and the rate of cycling of nutrients in the soil, thereby affecting their availability (Jenkinson, 1988; Ross et al., 1995). Our study supported this claim: the high microbial biomass towards summer and in the drained lower slope resulted in a fast turnover time of NH\textsubscript{4+}. Microbial biomass is suggested to not only drive the cycling of nutrients in the soil but also to supply a large proportion of crop nutrient requirements (e.g. phosphorus and sulphur, He et al., 1997; nitrogen, Puri and Ashman, 1998). In the case of N, this function is due to the potential of microbial biomass to release N (when the acted substrate has C–N ratio close to the microbial C–N ratio) or to immobilize N (when the substrate C–N ratio is much higher than the microbial C–N ratio). When a mean
signifies high competition for available N between micro-organism and plants in our study site. Our results wherein NH$_4^+$ pool seemed to be the leftover N from NH$_4^+$ immobilization suggested that the microbial biomass were probably better competitors for available NH$_4^+$ than the grasses. Similar results were reported from a natural grassland, where microbes consumed more NH$_4^+$ than plants in 8 h diurnal $^{15}$N tracer experiment (Schimel et al., 1989), and from a managed long-term pasture soils where the annual flux of N through microbial biomass was greater than the N off-take in harvested grass (Brookes et al., 1985b).

The seasonal patterns of NH$_4^+$ transformations were not directly related to moisture and temperature (Table 4), but possibly indirectly through the effects of these seasonal environmental factors on flushes of available organic matter which, in turn, affect the microbial biomass. The relatively low gross N mineralization–microbial N ratio observed in the fall was accompanied by high microbial biomass and NH$_4^+$ immobilization (Table 2). During the fall sampling (First November 1997), the soil was neither covered with snow nor frozen and the temperature was only starting to decrease (9 °C); this condition combined with the addition of labile C from root and herbage senescence might have still favored microbial growth and activity. Gross N mineralization–microbial N ratio was highest during the early spring (Table 2). In this sampling period (First April 1998), the snow had already melted but the temperature was still low (Fig. 1). This high ratio, which was accompanied by low microbial biomass and NH$_4^+$ immobilization (Table 2), implied that the microbial biomass was engaged more on maintenance metabolism rather than the growth.

The long low temperatures from winter to early spring might have caused a depressive effect on the size of microbial biomass, as indicated by the relationship between microbial N and temperature (Table 4). Brooks et al. (1998) also reported that the microbial biomass decreased during the later stage of snowmelt towards the time the grassland soil became snow free, and this was suggested to be due to exposure of soil to low temperature from loss of snow cover insulation. From early spring towards the summer, the gross N mineralization–microbial N ratios were again low, while the microbial biomass N and NH$_4^+$ immobilization increased (Table 2). This indicated that the microbial biomass, aside from its maintenance metabolism, was also growing. These findings showed that the NH$_4^+$ production and consumption did not vary commensurately across seasons, and hence net N mineralization cannot be used to represent the dynamics of NH$_4^+$ in the internal N cycling in this unmanaged grassland.

4.2. Spatial and seasonal pattern of NO$_3^-$ transformation processes

The rates of N productive and N consumptive processes (i.e. gross N mineralization to the sum of NH$_4^+$ immobilization (Table 2) and gross nitrification (Table 3), and gross
nitrification to NO$_3^-$ immobilization (Table 3) indicated a tight coupling of microbial N-cycling processes. The fact that the NO$_3^-$ immobilization was overestimated, during favorable immobilization conditions (i.e. late spring to summer), attests to the N-limited condition of our study site such that the microorganisms readily used externally supplied N (injected N). A N limited system is also normally characterized by higher extractable NH$_4^+$ than extractable NO$_3^-$ (Davidson et al., 1990; Stienstra et al., 1994), as was observed in all topographic units at all sampling dates in our study site (Figs. 2B and 3B).

The higher gross nitrification and NO$_3^-$ pool in the upper than lower slopes reflects the effect of topography on water redistribution; nitrification as an aerobic microbial process was more favored in the upper slope with low moisture contents than in the lower slope where water tends to accumulate. Drainage also favored gross nitrification through its effect on improving the aeration status of the soil. Similarly, the seasonal variation of soil moisture and temperature was depicted in the seasonal pattern of NO$_3^-$ transformations.

While in our study, we observed that the low soil moisture contents favored gross nitrification, Davidson et al. (1990) showed that further reduction of soil moisture (during dry summer months of Mediterranean-type grassland in California) reduced nitrification potential due to a decrease in NH$_4^+$ availability brought about by diffusional limitation as the soil dries. In our study site with a humid temperate climate, the lowest water-filled pore space (Fig. 1) measured was in May and July (equivalent to 0.25 g g$^{-1}$) and was well above those reported by Davidson et al. (1990) during the same months. Nitrifiers were able to compete relatively well for NH$_4^+$ in this N-limited grassland (gross nitrification: gross N mineralization ratio, Table 3). Microsite heterogeneity of NH$_4^+$ availability in our undisturbed soil cores may explain the success of nitrifiers in obtaining significant amount of NH$_4^+$ in an N-limited system (Davidson et al., 1990). While the NO$_3^-$ pool was apparently smaller than the NH$_4^+$ pool, the turnover time of NO$_3^-$ indicated that the movement of N through the NO$_3^-$ pool was very rapid. Hence, the size of the NO$_3^-$ pool cannot be used as the basis to dismiss the importance of NO$_3^-$ in the internal N cycle of grassland ecosystem.

Although plant uptake is well recognized as an important sink for NO$_3^-$ (Jackson et al., 1989; Schimel et al., 1989), evidence for the importance of microbial assimilatory sink for NO$_3^-$ is growing (Jackson et al., 1989; Rice and Tiedje, 1989; Schimel et al., 1989; Davidson et al., 1990). In our study, NO$_3^-$ immobilization was on average 50% of the NH$_4^+$ immobilization (Table 3). This significant portion of NO$_3^-$ sink has been attributed to the relative spatial compartmentalization of NH$_4^+$, NO$_3^-$, and C availability (Schimel et al., 1989; Davidson et al., 1990); NH$_4^+$-depleted microsites may occur at sites that have high C availability, and the greater diffusion rate of NO$_3^-$ relative to NH$_4^+$ may then lead to significant NO$_3^-$ assimilation. NO$_3^-$ immobilization was also highly dependent on NO$_3^-$ availability (gross nitrification; Table 4). These results implied that NO$_3^-$ immobilization could increase with increase in NO$_3^-$ availability (for example, as a consequence of N addition in managed pasture) if the size of microbial biomass would be high to impose high demand for N.

5. Conclusions

Our results indicated that the dynamics of NH$_4^+$ in the internal N cycling was influenced by the spatial and seasonal variation of soil microbial biomass; the spatial variation has resulted from long-term topographic influences on differences in soils, natural drainage, growth performance of the grasses, and water and nutrient redistribution, and the seasonal variation from seasonal flushes of available organic matter. The dynamics of NO$_3^-$ in the internal N cycling was closely related to the climatic factors...
(rainfall and temperature), of which the effect of rainfall is further influenced by topography-driven water redistribution in the landscape. These patterns answer to the important requirement of refining N management and reducing N losses to the environment—consideration of the spatial and seasonal variation of microbial biomass and activity. For example, when such unmanaged grassland is converted to managed pasture, our results suggest the need for a variable rate of fertilizer/manure application (e.g. low amount in low-activity area and higher amount in more active area of the landscape) with applications made during the season when microbial biomass is most active and growing (late spring to summer). N retention (through microbial immobilization) was also clearly influenced by microbial biomass. Nutrient management strategies for managed grassland should then be geared towards maintaining the microbial biomass size in its previously unaltered state. Moreover, the normally small NO₃⁻ pool size in unmanaged grassland, which resulted from its rapid turnover, cannot be used as basis to dismiss the importance of NO₃⁻ in the internal N cycling. Models predicting the impact of anthropogenic NO₃⁻ in managed grasslands need to consider the existing pathways of microbial NO₃⁻ consumption in minimally impacted grasslands.

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References


