Biogeochemistry **68:** 169–178, 2004.

The role of ammonia volatilization in controlling the natural ^{15}N abundance of a grazed grassland

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Received 6 August 2002; accepted in revised form 19 March 2003

Key words: ^{15}N, Ammonia, Grassland, Herbivory, Ungulate, Volatilization, Yellowstone National Park

Abstract. Although the variation in natural ^{15}N abundance in plants and soils is well characterized, mechanisms controlling N isotopic composition of organic matter are still poorly understood. The primary goal of this study was to examine the role of NH_3 volatilization from ungulate urine patches in determining ^{15}N abundance in grassland plants and soil in Yellowstone National Park. We additionally used isotopic measurements to explore the pathways that plants in urine patches take up N. Plant, soil, and volatilized NH_3 δ^{15}N were measured on grassland plots for 10 days following the addition of simulated urine. Simulated urine increased ^{15}N of roots and soil and reduced ^{15}N of shoots. Soil enrichment was due to the volatilization of isotopically light NH_3. Acid-trapped NH_3 δ^{15}N increased from −28‰ (day 1) to −0.3‰ (day 10), and was lighter than the original urea-N added (1.2‰). A mass balance analysis of urea-derived N assimilated by plants indicated that most of the N taken up by plants was in the form of ammonium through roots. However, isotope data also showed that shoots directly absorbed ^{15}N – depleted NH_3-N that was volatilized from simulated urine patches. These results indicate that NH_3 volatilization from urine patches enriches grassland soil with ^{15}N and shoots are a sink for volatilized NH_3, which likely leads to accelerated cycling of excreted N back to herbivores.

Introduction

A large proportion of the annual N budget of grasslands can cycle through herbivores. For example, migratory herds of ungulates in temperate grassland and tropical savanna ecosystems graze as much as 45% and 65% of the aboveground plant production, respectively (Frank et al. 1998), and in rangeland with livestock, consumption ranges 20–75% (Oesterheld et al. 1992). Thus grazers can function as a conduit for a substantial amount of the biologically active N that is annually cycled in grassland ecosystems. Because 65% or more of the N ingested by grazers is excreted in urine (Mould and Robbins 1981; Ruess 1987), urine deposition by ungulates can be a major flux of N to the soil in grasslands that support abundant herbivores (McNaughton et al. 1988; Hobbs 1996).

The δ^{15}N of vegetation and soil is a function of the rate and isotopic composition of inputs and outputs, and the internal N transformations that occur in a plant-soil system (Mariotti et al. 1982; Nadelhoffer and Fry 1994). Different ^{15}N abundance of coexisting plants may provide information about differences in N_2 fixation (Shearer and Kohl 1993), rooting depth (Schulze et al. 1994), or levels of mycorrhizal colonization (Michelsen et al. 1998; Hobbie et al. 2000) among species. At the ecosystem scale, measurements of natural ^{15}N abundance have been useful in exploring the influence of anthropogenic N deposition on the porosity of forest N cycling (Durka et al. 1994), the integrity of a desert N cycle in response to disturbance (Evans and Ehleringer 1993), N loss in relation to climatic variables (Austin and Vitousek 1998; Handley and Raven 1992), and rates of grassland N cycling and retention (Frank et al. 2000). However, direct study of the controls of ^{15}N abundance in organic material currently is hampered by difficulties in determining the δ^{15}N of potential N sources of transformations (Högberg 1997). Thus, an understanding of the mechanisms that control ^{15}N abundance in plants and soils is still rudimentary.

The principal goal of this study was to examine a possible mechanism for the previously reported (Frank and Evans 1997) grazer-^{15}N-enrichment of Yellowstone grassland soil. Because ammonia volatilization from grassland soils amended with urea-N can be high (Ruess and McNaughton 1988; Frank and Zhang 1997), and ^{15}N is discriminated against during volatilization in open, laboratory systems (Handley and Raven 1992), ammonia loss from urine patches in the field has been proposed as a mechanism that enriches grazed grassland with ^{15}N (Frank and Evans 1997). Here we directly measured the rate and isotopic composition of NH_3 volatilized from simulated urine patches and the resulting effects on plant and soil ^{15}N abundance in Yellowstone grassland. The experiment was designed to determine isotopic effects during the short period that NH_3 volatilization was enhanced after simulated urine was applied. In addition, we used isotope measurements to investigate plant uptake of N and report an important pathway for N uptake by plants in urine patches, absorption of NH_3 by shoots.

Material and methods

Site description

We examined the rate of ammonia volatilization and the resulting isotopic changes to soil and plant material in simulated urine patches in a *Stipa occidentalis*, *Poa pratensis*, and *Juncus balticus*-dominated grassland at Mammoth Hot Springs, Yellowstone National Park. Mammoth Hot Springs is located on Yellowstone National Park's northern winter range. Large herds of elk (*Cervus elaphus*) and bison (*Bison bison*) graze senesced plant tissue during the winter and young green tissue in the spring on the northern winter range, before migrating to high elevation summer range. Soil in the major rooting zone (0–20 cm) at the site was a sandy loam, with a pH (in water slurry) and organic matter content (loss on ignition) of 5.81 and 8.2%, respectively.

The experiment

An artificial urine solution, approximating the chemical composition of bovine urine (Stillwell 1983) and containing 50 g urea-N, was applied to each of three, 1 m^2 quadrats. Urine was applied on May 15, 1997, when grazing and urinating elk and bison were common in the Mammoth vicinity. Quadrats were randomly located within a 6 × 6 m^2 plot that was fenced to keep large mammals off during the experiment. Ammonia volatilized from artificial urine patches was trapped in 4% boric acid for 10 days after application; volatilization declined to background levels by day 11. We used a modified version of the dynamic chamber of Ruess and McNaughton (1988) to capture ammonia. Briefly, air was drawn under vacuum through a 25 cm long, 10 cm diam PVC pipe driven 5 cm into the soil. A Plexiglas top was glued to form an air- and water-tight seal. Tops were recessed 5 cm so that water could be added to the tops of chambers to absorb radiant heat and reduce chambers from overheating. Air for each of the three chambers and a blank control was drawn from a common point off the plot and delivered to each of the chambers or directly to an acid trap (blank) through tygon tubing. Chambers were moved to a new location within each quadrat every 24 hrs. Traps were changed every 5–7 hrs throughout the day and night. Ammonia-N absorbed in traps was determined by back-titration with 0.01 N HCl and bromocresol green (Ruess and McNaughton 1988; Frank and Zhang 1997). ^{15}N abundance of the urea-N added to plots and the NH_3-N in dried-down boric acid traps was determined by mass spectrometry, see below. The amount and isotopic composition of trapped ammonia-N were determined for pooled daytime and nighttime collections separately through day 6 of the experiment. Measurements were made on pooled 24 hr samples for days 7–10.

Soil cores (3.1 cm diam, 0–20 cm deep) were collected on and off artificial urine-treated quadrats the day before and 11 days following application. Roots were handpicked from soil samples and cleaned by soaking (2 hr) and then thoroughly rinsing in distilled water. Aboveground biomass was estimated by clipping three 20 × 50 cm quadrats located randomly throughout the 36 m^2 fenced plot at the beginning of the experiment, and paired quadrats located on and off each treated quadrat ($n = 3$) 11 days following urine addition. Vegetation was not clipped within designated urine-treated quadrats at the start of the experiment to reduce disturbance.

Plant and soil N content (%) were determined on a CE Instruments 2100 CN analyzer. ^{15}N abundance of plant and soil samples and dried-down boric acid from NH_3 traps was determined with a Finnigan MAT model delta S mass spectrometer at the Boston University Stable Isotope Laboratory.

Data analysis

Isotopic composition is reported as δ^{15}N (‰), following Mariotti (1984). Whole-plant δ^{15}N was calculated using the equation

$$\delta^{15}N_E = \frac{\delta^{15}N_R{*}X_R + \delta^{15}N_S{*}X_S}{X_R + X_S}$$

where X denotes the amount of N in each organ, and the subscripts E, R, and S refer to entire plant, root, and shoot, respectively (Shearer and Kohl 1993). The amount of N assimilated was calculated as the difference between the patch plots and controls. The δ^{15}N of the N assimilated during the experiment was calculated as

$$\delta^{15}N_A = \frac{\delta^{15}N_U{*}X_U - \delta^{15}N_C{*}X_C}{X_A}$$

Where the subscript A represents new N assimilated during the experiment, U is the urine patch, and C is the control (Shearer and Kohl 1993). One-way analysis of variance was used to examine effects of urine addition on plant biomass, plant and soil N content, and plant and soil δ^{15}N.

Results

Plant and soil N

Plant biomass, and plant and soil N content and δ^{15}N did not differ between samples collected from the plot at the start of the experiment before urine addition and in unamended areas at the end of the experiment. Consequently, these samples were pooled and used as controls in analyses to examine the effect of urine amendment on plant and soil properties. Amendments enhanced N content and reduced δ^{15}N of shoots (Table 1). In contrast, ^{15}N abundance was increased in root, whole plant, soil, and the plant – soil system. The calculated N assimilated by plants on urine plots was 1.1 g/m^2 and the composition of that N was + 14.0‰. Contrary to findings in other grasslands that urine stimulates aboveground biomass (Day and Detling 1990; Jarmillo and Detling 1992), urine amendment had no effect on plant biomass 11 days after addition at our study site in Yellowstone (Table 1), probably due to the short duration of the experiment.

Ammonia volatilization

Ammonia volatilization peaked on day 3 after urine addition (Figure 1). Rates returned to background levels by day 11. During this period an estimated 14.8 gN/m^2

Table 1. Plant biomass, and plant and soil N content and $\delta^{15}N$ off (control) and on plots treated with simulated urine. Mean (1 SE).

	Biomass (g/m^2)			N content (%)			$\delta^{15}N$ (‰)		
	Control	Urine Treated	*P*	Control	Urine Treated	*P*	Control	Urine treated	*P*
Plant									
Shoot	25.1 (1.1)	25.6 (2.0)	NS	2.3 (0.23)	4.4 (0.29)	0.002	1.1 (0.2)	−10.8 (0.6)	0.00001
Root	1082 (193)	1012 (30)	NS	1.07 (0.19)	1.2 (0.3)	NS	2.0 (0.5)	4.5 (0.9)	0.024
Whole	1107 (192)	1038 (29)	NS	1.10 (0.56)	1.28 (0.28)	NS	2.0 (0.5)	3.0 (0.6)	0.0118
Soil				0.39 (0.05)	0.43 (0.02)	NS	6.1 (0.2)	6.9 (0.1)	0.012
Plant & Soil							6.1 (0.2)	6.9 (0.1)	0.0118

(derived from the amount of ammonia trapped during the experiment) or 30% of the urea-N originally added, was volatilized. The $\delta^{15}N$ of the volatilized NH_3-N increased through the experiment from −28‰ the first day to −0.3‰ 10 days after urine was added. By comparison, the composition of the original urea-N added was 1.2‰.

We explored how effective our NH_3 trapping method was by comparing two independent calculations of the amount of the ^{15}N originally added in urine remained at the end of the experiment: (1) the ^{15}N volatilized during the experiment (calculated from rates and isotopic composition of NH_3 lost, determined from the trapping method) was subtracted from the ^{15}N added in urine, and (2) the amount of plant-soil ^{15}N before urine application was subtracted from the plant-soil ^{15}N at day 10. We found close agreement between the two methods: 0.132 g $^{15}N/m^2$ (SE = 0.0006) vs 0.135 $^{15}N/m^2$ (SE = 0.0003) using the former and latter methods, respectively. However, we also found that the estimate derived from rates and isotopic composition of the NH_3-N lost (method 1) was significantly lower ($P = 0.01$, $t_5 = 4.6$) than the amount of ^{15}N required to have enriched the plant-soil system by the measured amount (method 2), suggesting that NH_3 trapping was incomplete, or the chamber method reduced losses, or N was lost by other pathways.

Discussion

Ammonia volatilization can be an important pathway of N loss from grassland urine patches. Estimated losses usually range from 10% to 40% of the urea-N added in simulated patches (e.g., Musa 1968; Stewart (1970); Vallis et al. 1985; Ruess and McNaughton 1988; Frank and Zhang 1997), in agreement with volatilization from

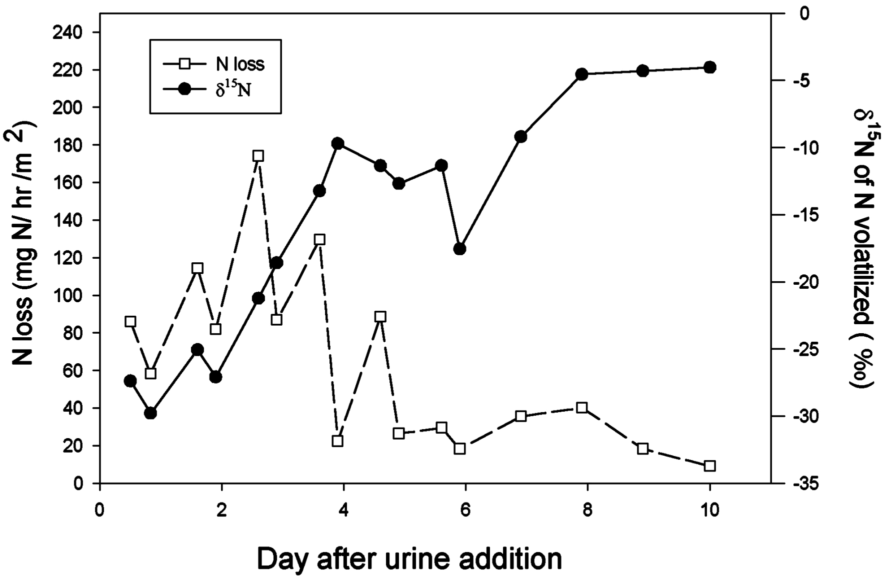

Figure 1. The relationships between rate of N volatilization and the isotopic composition of N lost with days after artificial urine was added to grassland plots near Mammoth Hot Springs in Yellowstone National Park.

grassland in this study (30%). Our results also showed that adding simulated urine to grassland soil led to ^{15}N enrichment of the plant-soil system. Even though the proportion of Yellowstone grassland that is influenced by urine on an annual basis is small (approx. 2.5%, Augustine and Frank 2001), the cumulative effect over time of urine on ^{15}N enrichment may be significant. Results using long-term ($\geqslant$ 32 yr) exclosures in Yellowstone grasslands indicated that grazers depleted shoots and enriched soil with ^{15}N at the community (approx 10 × 10 m) spatial scale (Frank and Evans 1997), similar to responses measured on urine patches in this short-term study. Thus, NH_3 volatilization may be an important determinant of ^{15}N abundance in Yellowstone grassland, and perhaps other grazed ecosystems.

In a similar study where N_2O was the dominant source of N loss from the soil, Velthof et al. (2000) observed greater soil $\delta^{15}N$ in areas with the highest N_2O flux. Subsequent measurements (Tilsner et al in press a, b) found that N_2O was depleted relative to source and atmospheric values, and gaseous flux was correlated with an increase in the $\delta^{15}N$ of soil inorganic N over time. These results for soil supporting high rates of N_2O loss are qualitatively the same as the isotopic changes associated with simulated urine patches observed in this study.

The mechanism for the increase in soil $\delta^{15}N$ in urine patches in Yellowstone grassland is fractionation during ammonia volatilization. Ammonia volatilized from amended plots became isotopically heavier over time, because fractionation during volatilization resulted in an increase in the $\delta^{15}N$ of the residual ammonium in the soil. Using the equations of Mariotti et al. (1981) that describe a Rayleigh fractionation, the observed fractionation for volatilization in this study was 35‰, which was in close agreement with other observed values of 30–60‰ (Högberg 1997; Robinson 2001).

Additional evidence for enrichment of the soil ammonium pool was provided by the $\delta^{15}N$ of N assimilated by plants during the experiment. According to the mass balance calculation, plants were enriched by 14.0‰, which must have occurred by uptake of isotopically heavy ammonium. For example, if we assume unlimited substrate during the first half of the experiment (Evans 2001), then ammonium in the soil should have increased by + 20‰ (based on a fractionation factor of 35‰). This suggests that plants preferentially absorbed ammonium over the 10 days after urine was added to the plots.

The decline in shoot $\delta^{15}N$ in amended plots suggests that foliage was a sink for N. Shoots of plants grown on ammonium generally reflect whole-plant $\delta^{15}N$ (Yoneyama et al. 1991; Evans et al. 1996; Evans 2001). Thus, the −12‰ shift in shoot $\delta^{15}N$ over the 10 day period after urine was added, while ^{15}N of roots increased, suggests that the primary source of the 91% increase in shoot N content during the experiment was the absorption, through stomata and cuticle (Stutton et al. 1995), of isotopically light NH_3-N. These results are similar to Erskine et al. (1998), who found foliage downwind from a subantarctic penguin colony was isotopically lighter than expected, due to absorption of ^{15}N depleted NH_3-N volatilized from penguin guano. The shoot absorption of NH_3-N lost from urine patches in our study also is corroborated by work showing foliar absorption of NH_3-N by wheat after fertilizer application (Harper et al. 1987; Ping et al. 2000).

There has been substantial progress understanding the influence of ungulate urine deposition on plant N availability, nitrification, and leaching, in addition to NH_3 volatilization rates mentioned above (Schimel et al. 1986; Ruess and McNaughton 1988; Monaghan et al. 1989; Day and Detling 1990; Cuttle and Bourne 1993; Lovell and Jarvis 1996). The data reported here are the first that we are aware of indicating that foliage may be a sink for NH_3 volatilizing from urine patches in a grazed grassland. Because native (Day and Detling 1990; Steinhauer and Collins 2001) and domesticated (Jarmillo and Detling 1992) ungulates graze natural urine patches more intensively than surrounding grassland, shoot absorption of NH_3 may function to increase the flow of N to herbivores.

The similarity in which adding urine affects plant and soil ^{15}N abundance in this short-term study with results from a long-term exclosure study (Frank and Evans 1997) that examined isotopic effects of Yellowstone grazers on grassland at the landscape level suggests that the volatilization of urea-N from urine patches is a determinant of the isotopic composition of Yellowstone grassland. In addition, our results suggest two pathways of short-term plant N uptake in urine patches, root assimilation of mineralized N in the soil, and shoot absorption of volatilized NH_3. The latter pathway could have the effect of accelerating the recycling of excreted N back to herbivores.

Acknowledgements

We thank Center for Resources staff at Yellowstone National Park for logistic support during this study and D. Augustine for critical comments on an early draft. This research was supported by NSF grants DEB-9408771 and DEB-9726569 and a grant from the Inter-American Institute for Global Change Research, CRN-012.

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