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Original Research

Effect of Grazing Intensity on Soil and Plant $\delta^{15}N$ of an Alpine Meadow

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Abstract

The mechanisms underlying the retention of inorganic N were still not well understood in an alpine meadow on the Tibetan Plateau as well as in other high-altitude meadow sites greatly grazed and disturbed. We conducted field soil and dominant species foliar nitrogen natural abundance of stable isotope ratios ($\delta^{15}N$) under four grazing intensities. It was demonstrated that soil $\delta^{15}N$ decreased significantly from 5.83±0.20‰ to 2.17±0.48‰ at 0-10 cm with the elevation of grazing intensity. Grazing reduced the degree of ecosystem N openness. The $\delta^{15}N$ value of surface soil was mainly affected by soil total nitrogen. Furthermore, the degree of nitrogen limitation increased with grazing elevation for sedge family and Gramineae family plants.

Keywords: alpine meadow, soil $\delta^{15}N$, foliar $\delta^{15}N$, grazing intensity

Introduction

Nitrogen is essential for the synthesis of nucleic acids and proteins, and is known as the important limiting nutrient for plant growth in mid-latitude and high-latitude regions [1-2]. N cycling is a fundamental ecological process, and it is rapidly changing due to anthropogenic perturbation such as nitrogen fertilization, grazing, and reclamation because of many concerns about the impact of excess N and N_2O emissions [3]. N resource supply is presumed to be the key mechanism explaining terrestrial ecosystem primary production.

Grassland was one of the most important vegetation types covering approximately 40.0% of the world land surface [4]. Furthermore, grasslands covered 41.7% of the area of China, and these were mainly distributed in the north and northwest, and on the Tibetan Plateau at very high elevations (over 4,000 m). Because of the extensive ecological and economic importance of an alpine meadow, many attempts have been made to conserve its surrounding [5].

However, the degradation of alpine meadow was one of the main environmental problems on the Tibetan plateau [6]. Overgrazing was the most significant factor causing grassland degradation [5, 7]. Previous studies showed that grazing altered plant-competitive interactions, decreasing the cover of Gramineae and increasing the cover of forbs for alpine meadow [8]. Intensive livestock grazing could strongly change soil fertility dynamics and nitrogen losses in grassland [9-10].

The N isotopic ratio ($\delta^{15}N$) provides an integrated indicator of relative rates of N inputs and losses [9]. The fractionations were 0‰, -3-3‰, and 28-60‰ from the inputs of biological N fixation and atmospheric

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deposition, and the losses of gas flux [11]. A considerable variation in grassland farm soil $\delta^{15}N$ values ranged from 1.47‰ to 7.91‰ [12]. Soil $\delta^{15}N$ in the Tibetan alpine grasslands (4.1‰) was significantly larger than the global average (2.4‰) [13]. The higher $\delta^{15}N$ values indicated the accelerated N cycling and rich supply [11]. Grazing intensity significantly decreased soil available N and $\delta^{15}N$ in alpine meadow [5, 14-15].

Grazing reduced soil $\delta^{15}N$ from 5.8-6.8‰ for *Elymus* pasture to 4.3-5.7‰ for *Kobresia* pasture on the Tibetan alpine grasslands [5]. Soil $\delta^{15}N$ values decreased significantly from 5.25‰ to 4.67‰ with grazing in the steppe [16]. Soil $\delta^{15}N$ increased with deepening soil depth because there was much litter of depleted ^{15}N input into the surface layer [9]. In addition, soil N availability could regulate leaf $\delta^{15}N$ at a regional level [13].

Foliar $\delta^{15}N$ varied from -4.04‰ to 4.34‰ in the steppe pasture, and the value was the lowest in the leguminosae plant; grazing could increase its quantity [16-17]. There was an ecological niche differentiation in N uptake and a trade-off between N absorption capacity and resource allocation strategy among species.

Therefore, understanding how grazing intensity changes affect N absorbing dynamics is meaningful for restoring degraded grasslands. However, we are lacking in soil nitrogen utilization under diverse grazing intensity. Our objective was (1) to determine the ^{15}N signature of soils and foliar in different grazing intensity grassland to investigate how $\delta^{15}N$ values in an ecosystem are affected by grazing, and (2) to compare the different reactions among six dominant species under grazing. We hypothesized that grazing increased soil and plant $\delta^{15}N$ values probably due to more loser N with grazing intensity.

Materials and Methods

Research Site

This study was conducted at the Haibei alpine meadow ecosystem station in Qinghai Province (N37º36′, E101º19′, and 3,200 m). Annual temperature and annual precipitation averaged -1.7ºC and 560 mm, of which 85% (precipitation) was concentrated in growing seasons from May to September.

Alpine meadows have been fenced and used as winter grazing pasture from late September to the end of April since 1982. Grazing intensity was investigated by inquiring about the local herdsman. These grasslands could be divided into undegraded grassland (four Sheep ha^{-1}), light-degraded grassland (eight Sheep ha^{-1}), medium-degraded grassland (12 Sheep ha^{-1}), and heavily degraded grassland (16 Sheep ha^{-1}). Every year, grazing started on 5 October and ended on 20 May. The Kobresia meadow is dominated by *K. humilis, K. pygmaea, Saussurea superba, Potentilla saundersiana, Elymus nutants, Oxytropis ochrocephala, Gentiana farreri, Gentiana straminea, Leontopodium nanum, Lancea tibetica, Festuca ovina, Festuca rubra, Stipa aliena, Helictotrichon tibetica,* and *Poa crymophila*.

The soils were classified as Mat-Cryic Cambisol, corresponding to Gelic Cambisol. These were rich in organic carbon content with an Udic soil moisture regime.

Sampling and Analyses

Three subplots (5 × 5 m) were randomly selected and mixed together in each replicate plot, at 0-10 cm and 10-20 cm depths. After field collection, these samples were immediately transferred to the laboratory. Then, living roots were carefully removed from soil and were sieved to 2 mm. About 20 g fresh soil was dried at 75°C and ground to fine power using a ball mill. The remaining soil was stocked at 20°C for bacteria biomass measurements.

Six species were selected from four types of functional vegetation, i.e., sedge family taxon (*K. humilis, K. pygmaea*), gramineae family taxon (*E.nutants*), leguminosae family taxon (*O.ochrocephala*), and forb family taxon (*G. farreri, G. straminea*). During the peak growing season (middle of August), all mature leaves were collected with six replicates during random sampling from every species. Samples were dried at 70ºC for 48 hours and were mulled to fine power using a ball mill.

Total N and $\delta^{15}N$ from soil and foliar were analyzed by continuous-flow isotope ratio mass spectrometry coupled with an elemental analyzer (EA 1110, Milan, Italy), and IRMS (MAT 253, Bremen, Germany). The $\delta^{15}N$ abundance in soil is expressed in δ units as related to V_{PDB} standard:

$$\delta^{15}N = R_{sample}/R_{standard} - 1$$

…where R is the ratio of the abundance of $^{15}N/^{14}N$ of sample and standard. The analytical precision was 0.2‰.

Calculations and Statistics

One-way ANOVA was performed to estimate the effect of grazing on the soil and foliar $\delta^{15}N$ values. Simple line regression analyses were conducted to assess the relationship between soil $\delta^{15}N$ values and soil characteristics. All statistical analyses were conducted by SPSS 16.0 software with significance at $p<0.05$ (SPSS Inc., Chicago, USA).

Results

Soil $\delta^{15}N$ Values Decreased with Grazing Intensity Elevation

With the elevation of grazing intensity, soil $\delta^{15}N$ was reduced significantly from 5.83±0.20‰ to 2.17±0.48‰ at 0-10 cm (Fig. 1). The decreased ranges were 6.2%, 32.6%, and 62.8% in comparison with undegraded grassland,

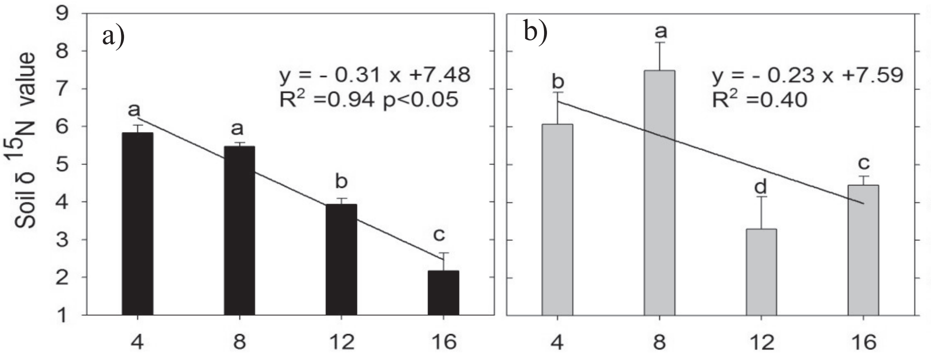

Fig. 1. Values of soil $\delta^{15}N$ under different grazing intensities (sheep ha^{-1}) in alpine meadow (A and B mean values at 0-10 cm and 10-20 cm soil depths).

separately. Furthermore, the regression model was well framed, and the R^2 reached 0.94 ($p<0.05$).

Grazing initially increased soil $\delta^{15}N$ values, yet the value significantly dropped, and then it slightly increased (Fig. 1, $p<0.05$). The highest and lowest $\delta^{15}N$ values were 7.49±0.75‰ and 3.29±0.86‰ while grazing eight and 12 sheep ha^{-1} (Fig. 1).

The $\delta^{15}N$ value of 0-10 cm soil was mainly affected by soil total nitrogen, and the R^2 was 0.97 (Table 2). The latter were higher than the former except for the plot of grazing 12 sheep ha^{-1}. Intensive nitrate leaching (with the residual higher ^{15}N values) enriched 10-20 soil $\delta^{15}N$ values.

Foliage $\delta^{15}N$ Variation under Different Grazing Intensity

The range of foliar $\delta^{15}N$ showed from -3.99‰ in the *K. pygmaea* of 16 sheep ha^{-1} grazing to 5.36‰ in the *G. farreri* of four sheep ha^{-1} grazing on the Tibetan plateau (Fig. 2).

The foliar $\delta^{15}N$ of both *G. farreri* and *G. straminea* significantly decreased, and then increased with grazing intensity elevation from four to 12 sheep ha^{-1}. However, the two kinds of forbs showed a different tendency with the grazing intensity further increased. Heavy grazing sharply decreased the foliar $\delta^{15}N$ in *G. farreri* (0.00‰), but further increased the value in *G. straminea* (1.91‰). Furthermore, most of the foliar $\delta^{15}N$ values were positive except *G. straminea* (-0.71‰), suffering from medium grazing (eight sheep ha^{-1}). However, the foliar $\delta^{15}N$ values of *O.ochrocephala* (Leguminosae plant) increased from 0.36‰ to 1.30‰ with the grazing intensity elevation from 4 to 12, and then it sharply dropped down to -1.02‰ at heavy degraded grassland.

All the foliar $\delta^{15}N$ values of E. *nutants*, *K. humilis,* and K. *pygmaea* were minus ranging from -3.99‰ to -0.41‰ (Fig. 2). These indicated that the growing of three species was limited in alpine meadow. Moreover, with the grazing intensity increasing, all the values were gradually decreased.

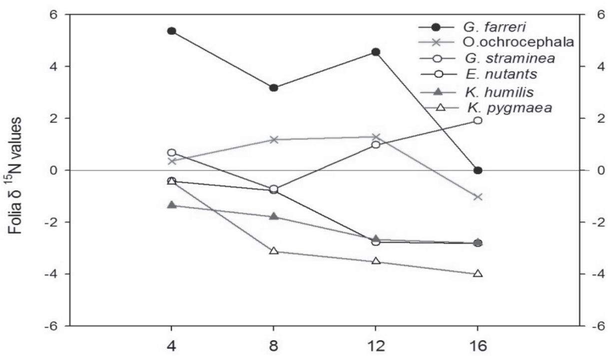

Fig. 2. Foliar $\delta^{15}N$ values from six species under different grazing intensities (sheep ha^{-1}).

Discussion

The natural abundance of soil $\delta^{15}N$ values reflected the net effect of ecosystem N dynamics [9]. Soil $\delta^{15}N$ was the result of isotopic compositions of N inputs, fractions associated with N transformation, and N losses in terrestrial soils [18]. The $\delta^{15}N$ value was used as an indicator of the grassland ecosystem nitrogen saturation, and higher soil $\delta^{15}N$ indicated the long history of high N availability, more openness, and a more saturated state [19].

Soil mineral nitrogen was the dominant source of plant uptake nitrogen [20], herbivores appeared to have a strong influence on grassland N availability, and some studies showed that grazing could lead to an increase in soil $\delta^{15}N$ [15, 19, 21], while no apparent and negative effects of grazing have also been reported [22]. Ammonia volatilization and gaseous N losses via denitrification resulted in the remaining soil and plant enrichment of ^{15}N in grazed grasslands [12]. However, grazing reduced grassland soil N depletion, and decreased the degree of ecosystem N openness. Grazing reduced soil nitrogen losses in alpine meadow [23], and long-term grazing profoundly decreased surface soil $\delta^{15}N$ in alpine meadow (Fig. 1). Similarly, higher soil $\delta^{15}N$ values in the low-grazing intensity were found in a previous study [21]. Grazing, especially high-intensity grazing, resulted in

Table 1. Grazing intensity and soil basic properties at 0-10 cm.

Grazing intensity Sheep hm^{-2}	pH	Soil organic carbon %	Total nitrogen %	Soil bulk g cm^{-3}	Biomass g m^{-2}
4	7.3±0.4	5.5 b	0.79±0.03 ab	0.75±0.05 b	646.54±23.14 a
8	7.4±0.5	6.2 a	0.72±0.04 a	0.59±0.10 c	530.23±12.14 b
12	7.3±0.5	6.4 a	0.65±0.04 b	0.54±0.04 c	328.16±13.59 c
16	7.5±0.5	3.6 c	0.54±0.02 c	0.99±0.02 a	460.23±15.41 b

Table 2. Stepwise regression models between soil characteristics and 0-10 cm soil $\delta^{15}N$.

Items	R^2	P	Models
pH	0.10	0.75	-
SOC	0.12	0.66	-
Total N (x)	0.97	0.02*	y = 15.41 x - 6.05
Soil bulk	0.11	0.64	-
Biomass	0.05	0.22	-

Note, y was soil $\delta^{15}N$, and * means $P<0.05$

a significant decrease in $\delta^{15}N$ of surface soils in Inner Mongolia grassland [16, 22]. Soil $\delta^{15}N$ values from light grazing intensity grasslands were 2.8‰ units lower than those of heavy grazing grasslands [12]. This was also revealed in drought grassland [24].

The interpretations were indicated for this negative feedback of surface soil $\delta^{15}N$ against grazing in an alpine meadow. Grazed treatments tended to result in relatively lower N_2O emissions, and grazing did not significantly vary grassland ammonia volatilization [25]. Soil aridity retards the revolve rate of microorganisms and denitrification intensity, and consequently it reduced N_2O emissions [11]. Furthermore, alpine ecosystems were extremely depleted in available nitrogen content, and the main resource of nitrogen was from biological nitrogen fixation [13]. Organic N decomposition and inorganic N supply is usually slow in cold regions due to decelerated microbial activity [19]. Livestock consumed 29.22 kg N ha^{-1} one year in alpine meadow ecosystem at medium grazing intensity, but returned only 6.20 kg N ha^{-1} into soils by excreting urine and dung [5]. Grazing significantly decreased soil total nitrogen and N recycling rates [19].

In addition, this paper indicates that reasonably reducing grazing intensity (keeping eight sheep ha^{-1}) would be benefit both soil nitrogen supply, dominant herbages absorbing nitrogen (Figs 1-2), and grassland production (Table 1). However, the effects of other factors and processes were beyond the scope of this analysis. This highlights the need for a further study to ascertain these suggested interpretations. We would attempt to offset these limitations while simultaneously enhancing research capacity.

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