

IMPACTS OF GRAZING INTENSITY ON NITROGEN POOLS AND NITROGEN CYCLE IN AN ALPINE MEADOW ON THE EASTERN TIBETAN PLATEAU

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Abstract. Grazers may influence nitrogen (N) pools and alter N inputs and outputs (losses) to the ecosystem in a number of ways. In this study, we evaluated N pools within the plant-soil system (0-30 cm) and soil N transformation under three different grazing intensities by yaks (light: 1.2, moderate: 2.0, and heavy: 2.9 yaks ha⁻¹) in an alpine meadow on the eastern Tibetan Plateau. Total plant and soil N at 0-30 cm depth tended to increase as grazing intensity increasing (plant N: 26.6, 31.4 and 36.7 g m⁻²; soil N: 905, 939 and 1125 g m⁻² for light, moderate and heavy grazing, respectively). Soil N transformation rates, such as net N mineralization, gross nitrification, denitrification and N₂O emissions, generally increased under heavy grazing intensity. Results indicate that heavy grazing intensity accelerated the N cycling rates between system components and led to increases in plant-soil system N in this alpine meadow.

Keywords: biomass; grassland; N stocks; soil N transformation; Tibet Plateau

Introduction

Grasslands occupy approximately one-third of the earth's terrestrial surface and are subject to varying degrees of management by humans [1, 2]. Grazing by livestock is a widely used management tool in grasslands, impacts ecosystem structure and plays a role in regulating nutrient cycling and energy flow [3]. N limits primary production in many terrestrial ecosystems [4]. Determining the role that herbivores play in regulating N storage and cycling in grassland ecosystems is important to understanding not only the grasslands themselves, but also the contribution of grasslands to global nitrogen fluxes.

Grazers can return large amounts of N to the soil through urine and feces, increasing levels of available soil N [3, 5, 6, 7, 8]. Herbivores also increase decomposition rates by reducing C/N ratios of plants [3, 9, 10]. Furthermore, plants often respond to defoliation by decreasing root production [10, 11, 12] that can result in reduced soil C and C/N ratios [10]. Lower C/N ratios in grazed plant material and soils increases net N mineralization by reducing microbial demand for N (i.e., microbial immobilization) during decomposition [10, 13]. However, grazing can also reduce N turnover and availability, as grazers feed selectively on plants with high N content and thus increase the dominance of plant species with low N content, and litter from those species decomposes slowly. Moreover, reducing N availability favors N-poor species because

they are often better nitrogen competitors [14, 15, 16]. In this case, grazers reduce aboveground productivity and the rate of N cycling. Thus, grazers may have different or even opposite effects on N cycling in different systems and may consequently shift ecosystem productivity in either a positive or negative direction.

The Tibetan Plateau, the largest geo-morphological unit on the Eurasian continent, is an important part of the global terrestrial ecosystem, and one of the major pasture lands in China. Alpine meadows, covering about 35% of the plateau area, are a representative vegetation type and the major grazing land of the region, especially in eastern areas [17]. Long-term overgrazing in the areas has resulted in considerable deterioration and even desertification [18]. However, few data exist quantifying the magnitude and distribution of N stored, and the effects of grazing management on the biogeochemical processes controlling the exchanges of N between the soil and atmosphere.

This study was conducted to examine impacts of three grazing intensities on N pools and soil N cycling in alpine meadow on the eastern Tibetan Plateau. Objectives were to quantify and compare the effects of grazing intensity on (1) plant species composition; (2) above- and belowground biomass; (3) N storage in plants and soil; and (4) soil N transformations, including net N mineralization, net nitrification, gross nitrification, denitrification and N₂O emissions.

Materials and methods

Study site

The study site is approximately 140 ha and located at Hongyuan County, Sichuan Province, China (33°03'N, 102°36'E) and has been previously used as traditional winter pasture (early November to mid-May) by local Tibetan nomads with light grazing intensities [19]. It is 3462 m above sea level, with a continental harsh climate. Annual precipitation averages 752 mm, with about 86% received from May through September. Mean annual temperature is 1.1°C and there is not an absolute frost-free period. The highest monthly mean temperature is 10.9°C in July and the lowest is -10.3°C in January. The dominant species in the whole area was *Clinelymus nutans* and *Roegneria nutans*, accompanied by *Koeleria litwinowii*, *Agrostis schneideri*, *Kobresia setchwanensis* and *Anemone rivularis*. The vegetation covered over 90% [19]. Soils are Mat Cry-gelic Cambisols [20]. Soil organic matter and total N were 61.20 and 3.42 g kg⁻¹, respectively [21].

In 1997, the study site was segregated into several pastures and contracted out to different farmers who established fences to enclose their own pastures. This caused a shift and redistribution of livestock across the study site with grazing intensities varying by farmer, but consistent among years for a given pasture. Three adjacent experimental sites, each with a different grazing intensity, were chosen for study. All the sites had been used as winter pasture with a continuous grazing period from early November to the end of May. Light grazing intensity (LG) was 1.2 yaks ha⁻¹ which resulted in 20 to 35% utilization of annual forage production for the 16 ha pasture area, and vegetation was dominated by *Roegneria nutans*, *Deschampsia caespitosa*, and *Elymus nutans*. Moderate grazing intensity was 2.0 yaks ha⁻¹, resulting in 40-50% utilization over the 28 ha pasture, with vegetation dominated by *Kobresia setchwanensis*, *Kobresia pygmaea*, and *Roegneria nutans*. Heavy grazing intensity was 2.9 yaks ha⁻¹, resulting in 60-75% utilization over the 20 ha pasture, with vegetation dominated by *Kobresia pygmaea*, *Kobresia setchwanensis*, and *Potentilla anserina*.

Field sampling and investigation

In August 2005, five 10 m×10 m plots were randomly selected in each experimental site. Each plot was located at least 12 m from the next nearest replicate. In each plot five 50 × 50 cm quadrates were randomly selected for biomass sampling. Aboveground biomass was clipped to ground level as living biomass and dead biomass (standing dead and litter). Root biomass was measured by collecting 5 soil cores (20 cm in diameter) from depths of 0-30 cm in each plot, which were co-located with the aboveground biomass measurement quadrates. The soil cores (20 cm diameter) were cut into segments corresponding to sampling depths of 0-10, 10-20, and 20-30 cm. These cores were immediately washed over a 1-mm mesh screen to remove soil.

Plant community characteristics were determined from two systematically located transects (50cm×500cm) of ten continuous quadrates (50cm×50cm) in each plot. Plant species were identified and recorded, the total ground cover, species canopy cover, and height determined from 0.25 m² quadrates. The frequency of each plant species was calculated for each plot. Importance data for individual species were calculated as averages of their relative abundance in terms of canopy cover, height, and frequency.

Within each plot, composite soil samples consisting of 5 soil cores 7.5 cm in diameter of 30-cm depth were taken from the same five quadrates in which biomass was harvested and root cores were taken. All plant litter was removed from the soil surface before the sampling. Soil samples were segregated into 0-10, 10-20, 20-30 cm increments. Duplicate soil cores were also taken at each sampling quadrate for soil bulk density determination, which were used to convert soil N concentrations (in grams per kilogram) to N mass (in grams per square meter) in the soil.

Rates of in situ net N mineralization and nitrification were evaluated in the field using the buried-bag technique [22]. Five paired soil cores (5 cm diameter, 5 cm depth) were taken from random locations in each plot. In each pair, one soil core was sealed in a gas permeable polyethylene bag and buried at a depth of 5 cm. The other core (initial) was taken adjacent to each buried bag and kept in cooler bags during the transportation to the laboratory prior to freezing for analyses. The buried bags were retrieved after 30 days of incubation and analyzed for NH₄⁺-N and NO₃⁻-N. Net N mineralization was calculated by subtracting initial NH₄⁺-N plus NO₃⁻-N concentrations from final concentrations. Net N nitrification was calculated as the difference in corresponding NO₃⁻-N concentrations.

We measured gross rates of nitrification, denitrification and N₂O emissions on intact cores using the Barometric Process Separation (BaPS) technique adapted for laboratory incubations [23]. Five intact soil cores (5.6 cm diameter, 4 cm depth) from each plot were collected, which co-located with the other soil cores. The cores were cooled with freezer blocks and returned to the laboratory in insulated boxes for immediate analysis.

Laboratory and statistical analyses

Soil samples intended for N analyses were passed through a 2-mm screen to remove plant crowns, visible roots and root fragments. Samples were air-dried and analyzed for total N by the semi-micro Kjeldahl procedure [24]. Soil extracts were analyzed for NH₄⁺-N with the potassium chloride-indophenol blue colorimetric method, and NO₃⁻-N with calcium sulfate-phenol disulfonic acid method [24].

When soil N transformations were measured, five intact soil samples were directly filled into the BaPS instrument and the system was closed gas tight and incubated at a

temperature of 11.7°C (average air temperature in August). Determination of soil N processes via the BaPS technique lasted approximately 12 hours [23].

All plant samples were oven-dried for 48 h at 65°C and weighed. Dry samples were then milled and analyzed for N content with the same method as soil sample.

Data were statistically analyzed by one-way analysis of variance (ANOVA) and significant differences were tested by the least significant difference (LSD) at $p < 0.05$.

Results

Plant species composition

Difference in plant species composition was observed between the treatments (Table 1).

Table 1. Composition of species and their importance value at the three study sites

Species name	Light grazing	Moderate grazing	Heavy grazing
<i>Roegneria nutans</i>	18.48	8.72	6.06
<i>Elymus nutans</i>	8.54	4.20	3.75
<i>Deschampsia caespitosa</i>	11.43	4.20	
<i>Agrostis schneider</i>	4.18		
<i>Koeleria litwinowii</i>	4.67	5.68	
<i>Kobresia setchwanensis</i>	7.68	17.43	23.32
<i>Kobresia pygmaea</i>	1.70	5.25	12.54
<i>Gueldenstaedtia diversifolia</i>	3.76	3.79	3.34
<i>Oxytropis ochrocephala</i>	2.09	2.78	4.25
<i>Astragalus polycladus</i>		2.09	
<i>Aster alpinus</i>	4.81	6.99	2.78
<i>Saussurea hieracioides</i>		4.60	2.28
<i>Taraxacum maurocarpum</i>	2.20	1.60	1.36
<i>Ligularia virgaurea</i>		1.85	
<i>Leontopodium longifolium</i>	2.60	2.82	6.09
<i>Anemone rivularis</i>	7.33	4.90	3.41
<i>Consolida ajacis</i>	2.32	1.86	
<i>Anemone trullifolia</i>		2.48	2.51
<i>Thalictrum alpinum</i>	1.80	2.26	1.80
<i>Ranunculus brotherusii</i>	1.53	1.74	3.24
<i>Potentilla anserina</i>	1.41	2.13	8.35
<i>Potentilla discolor</i>	1.66		
<i>Geranium phlżowianum</i>	4.19	2.04	1.86
<i>Polygonum viviparum</i>	2.55	5.42	4.11
<i>Stellera chamaejasme</i>	1.32	1.81	3.78
<i>Plantago depressa</i>	1.45	1.15	2.27
<i>Gentiana algida</i>	2.30	1.88	2.88
Total species number	23	25	20
Total cover (%)	89.7	92.6	73.6

Dominant plant species in the LG site were *Roegneria nutans* (18.48% as importance value), *Deschampsia caespitosa* (11.43%), and *Elymus nutans* (8.54%), and *Kobresia setchwanensis* (7.68%). Major species in the MG site were *Kobresia setchwanensis* (17.43%), *Roegneria nutans* (8.72%), *Aster alpinus* (6.99%), and *Koeleria litwinowii* (5.68%). In the HG site, *Kobresia pygmaea* (12.54%), *Kobresia setchwanensis* (23.32%), *Potentilla anserine* (8.35%), and *Leontopodium franchetii* (6.09%) were most dominant species. Vegetation coverage was highest in the MG site, intermediate in the LG site, and lowest in the HG site.

Above- and belowground biomass

Live, dead and total aboveground biomass was lower in the HG site compared to the other two sites, which did not differ (Table 2).

Across all sites, more than 85% of the total belowground biomass (0-30cm) was in the surface 0-10cm soil depth (Table 2). Total root biomass was lower in the LG site compared to the MG and HG sites, which did not differ. Root/shoot ratio was higher in the HG site, with MG and LG sites similar.

Table 2. Plant biomass (g m^{-2}) as affected by grazing intensity

System components	Light grazing	Moderate grazing	Heavy grazing
Above ground			
Live biomass	359.2±53.4a	412.3±65.7a	281.0±39.0b
Dead biomass	162.2±16.7a	177.2±30.1a	111.1±20.1b
Total above ground biomass	521.4±60.4a	589.4±91.4a	392.1±53.6b
Roots			
0-10cm	1523.1±184.1c	2147.0±335.8b	2686.9±449.6a
10-20cm	196.7±52.4	228.5±82.7	152.6±41.2
20-30cm	78.7±14.8	107.0±24.3	84.1±16.0
Total roots	1798.5±179.5b	2482.6±356.2a	2923.6±481.3a
Roots/shoot ratio	3.46±0.23b	4.27±0.77b	7.51±1.33a
Total plant biomass	2319.9±232.6b	3072.0±397.1a	3315.6±504.7a

Within rows, means ± S.D. Different letters represent statistically significant at $p < 0.05$. $n = 5$.

Plant and soil N stocks

Total N storage in the aboveground live and dead biomass was higher in the MG site compared to the LG and HG sites, which did not differ (Table 3). Total root N storage (0-30cm) was higher in the HG site compared to the LG and MG sites, which were similar. Total plant (aboveground + roots) N was greater in the HG site than in the MG and LG sites.

Soil N tended to decrease with increasing of soil depth (Table 3). Soil N storage (0-30cm) was higher in the HG site compared to the LG and MG sites, which did not differ.

Total N was higher in the HG site than the other two grazing intensities, which did not differ (Table 3).

Table 3. Total amounts of N stored in plant and soil pools (g m^{-2}) as affected by grazing intensity

System components	Light grazing	Moderate grazing	Heavy grazing
Above ground			
Live biomass	4.4±0.6b	7.1±1.0a	5.3±0.8b
Dead biomass	1.9±0.3b	2.8±0.6a	1.6±0.2b
Total above ground N	6.3±0.8b	9.9±1.1a	6.9±0.9b
Roots			
0-10cm	18.1±5.2b	19.3±4.6b	28.1±5.6a
10-20cm	1.6±0.4	1.6±0.7	1.1±0.4
20-30cm	0.6±0.1	0.7±0.2	0.6±0.1
Total roots N	20.3±5.1b	21.6±5.0b	29.8±5.8a
Total plant N	26.6±4.7b	31.4±5.0ab	36.7±5.7a
Soil profile			
0-10cm	395.8±43.8b	464.3±93.3ab	541.8±63.2a
10-20cm	289.0±36.1b	289.4±38.9b	359.5±58.6a
20-30cm	219.9±31.0	185.1±30.1	223.7±27.2
Total soil N (0-30cm)	904.7±77.0b	938.8±90.9b	1125.0±81.7a
Total ecosystem N (to 30cm)	931.3±75.4b	970.2±89.2b	1161.7±84.3a

Within rows, means ± S.D. Different letters represent statistically significant at $p < 0.05$. $n=5$.

Soil inorganic N

Soil NO_3^- -N content in 0-5cm depth was lower in the MG site than in the LG site or HG sites, which did not differ (Table 4). NH_4^+ -N increased with increasing grazing intensity ($p = 0.006$). Total inorganic N (NO_3^- -N + NH_4^+ -N) was higher in the HG site than in the LG and MG sites, which did not differ.

Table 4. Mass of soil inorganic N (g m^{-2}) as affected by grazing intensity

	Light grazing	Moderate grazing	Heavy grazing
NO_3^- -N	0.28±0.03ab	0.23±0.02c	0.32±0.05a
NH_4^+ -N	0.37±0.06c	0.50±0.08 b	0.64±0.13 a
Total inorganic N	0.65±0.09 b	0.73±0.09 b	0.96±0.18 a

Within rows, means ± S.D. Different letters represent statistically significant at $p < 0.05$. $n=5$.

Soil N transformations

The HG site had higher net N mineralization, gross nitrification and denitrification rate than the LG site, with the MG site intermediate (Table 5). Net nitrification was higher at the HG compared to the MG site ($p = 0.080$). N_2O flux rate was higher in the HG site compared to the LG and MG sites, with no difference between the LG and MG sites.

Table 5. Soil N transformation rates ($\text{mg N m}^{-2} \text{d}^{-1}$) as effected by grazing intensity

	Light grazing	Moderate grazing	Heavy grazing
Net N mineralization	1.83±0.73b	2.54±0.97ab	4.10±1.80a
Net N nitrification	5.97±1.42ab	3.42±0.99b	7.41±3.00a
Gross N nitrification	226.4±57.9b	272.4±83.2ab	344.3±30.2a
Denitrification	23.79±4.56b	26.79±6.19ab	36.56±10.16a
N ₂ O emission rate	2.98±0.50b	3.15±0.72b	4.62±1.47a

Within rows, means±S.D. Different letters represent statistically significant at $p < 0.05$. $n=5$.

Discussion

The magnitude of impact that livestock grazing may have on a plant community is dependent upon intensity of grazing. In contrast to grazing at a light or moderate grazing intensity, grazing at heavy intensity has tended to decrease the numbers of grasses such as *Roegneria nutans* and *Deschampsia caespitosa* and increased the numbers of sedges such as *Kobresia setchwanensis* and *K. pygmaea*, which is good tolerant to be grazed, specially for yaks [25]. Heavy grazing also markedly reduced vegetation cover compared to light grazing and moderate grazing. This has an important implication for grassland management because vegetation cover is often used to assess spatial extent and degree of desertification [26].

Plant biomass is an important measure of ecosystem functioning for alpine meadows. After eight years grazing with different intensity, live, dead and total aboveground biomass was lower with heavy grazing compared to light or moderate grazing intensity. The reason for these results was that the dominator of HG community shifted from grasses-*Roegneria nutans* and *Deschampsia caespitosa* into sedges-*Kobresia pygmaea* and *K. setchwanensis*, which are small and good tolerant to be grazed [24]. Also, as our results demonstrated, a larger proportion of total production was allocated to the belowground biomass with heavy grazing [27]. Aboveground biomass decreased under heavy grazing intensity indicated that the winter forage supply for this region reduced and accordingly the pressure on native grassland productivity increased.

Root biomass responses to grazing are ambiguous. Milchunas and Lauenroth [28], Turner et al. [29], and Frank et al. [30] found mostly no changes, or increases, of root biomass as a function of grazing intensity. Our results suggested belowground biomass was lowest in the LG site and higher in both the MG and HG sites. This can be explained that heavy grazing induced more *Kobresia pygmaea* and *K. setchwanensis*, which have larger root system than that of *Roegneria nutans* and *Deschampsia caespitosa* [31]. This change was reflected in the higher root to shoot biomass ratio under the heavy grazing treatment compared to light grazing treatment. Biomass allocation ratio to root increasing is an adaptive response of plant to grazing. High proportion of root biomass in the total biomass can increase the capacity to tolerate environmental stresses and external disturbances, which is favorable for grassland restoration [32].

N storage of plants may depend on soil nitrogen availability [33, 34]. Our results supported the hypotheses because higher soil N availability occurred when grazing intensity increased. Moreover, greater availability of labile N in the soil on higher grazing intensity sites could increase plant uptake rate of N [30, 35]. In addition, a higher root biomass by increasing grazing intensity was contribution to plant N uptake

[36]. Although high N storage in belowground biomass is common in grasslands [37], the effects of grazing intensity on this pattern were different (i.e., HG amplified this pattern compared to MG and LG). Those results suggested that grazing intensity changed the above- and belowground allocation of N within plant. Belowground N allocation may allow compensatory responses to grazers [29], and might also facilitate the recovery of vegetation after natural disturbance [38].

Soil N storage was higher under the heavy grazing intensity compared to the light grazing intensity, consistent with results for the northern mixed-grass prairie reported by Dormaar et al. [39] and Manley et al. [40]. The higher soil N under grazing intensity could be due to differences in root biomass between the treatments. Schuman et al. [41] and Hibbard et al. [42] reported larger root biomass can contribute more C and N to soil in northern mixed-grass and semi-arid grassland, respectively. In addition, the feces and urine from grazers are also important N source of soil [5, 8].

Heavy grazing had consistently higher soil N transformations than the light grazing in this alpine meadow ecosystem. These findings were more in line with corroborate those of Olofsson et al. [15] and Le Roux et al. [43] who all found that increasing grazing intensity increased soil N cycling rates. There were several mechanisms that could explain the increases of soil N processes under heavy grazing intensity. The N added by grazers in excrement is in forms available to plants and soil microbes [3, 5, 6, 7]. The increased litter decomposition resulted from grazer trampling. Faster decomposition means that less nitrogen was tied up in litter and nitrogen may cycle through the ecosystem more quickly [3, 40]. Another mechanism through which yaks may affect soil N transformations was through altering soil microclimate, such as soil temperature or soil water content [44]. The higher soil N mineralization and nitrification can be responsible for the higher soil inorganic N content under heavy grazing intensity [45, 46]. In comparison, net nitrification did not show a similar pattern as other soil N transformation rates, the lowest value occurred at the MG site. This might be attributed to the excess consumption of NO_3^- -N by the faster accumulating of aboveground biomass at the MG site. Gross nitrification rates were 37-80 times the net nitrification rates, which was similar to the result reported by Sun et al. [47] in a subalpine meadow soil. Higher soil N transformations under heavy grazing intensity also implied that a potential for leaching losses of N as NO_3^- and N_2O losses, via nitrification or denitrification [48].

Conclusion

Although heavy grazing intensity resulted in higher levels of plant and soil N, it decreased vegetation coverage and aboveground biomass, which are undesirable for livestock production and sustainable grassland development. What is more, heavy grazing could also introduce potential N loss via increasing NO_3^- leaching to ground water and N_2O emission to the atmosphere. Grazing at light to moderate intensity resulted in the plant communities dominated by forage grasses with high aboveground biomass productivity and N content. The alpine meadow ecosystems in Tibetan Plateau are very fragile and evolved under grazing by large herbivores; therefore, without an appropriate level of grazing in a long term perspective on an ecological timescale, deterioration of the plant-soil system, and possible declines in soil N, are indicated.

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