J. Mt. Sci. (2022) 19(4): 1156-1167 e-mail: jms@imde.ac.cn http://jms.imde.ac.cn
https://doi.org/10.1007/s11629-021-7026-6

Original Article

Short-term effects of yak and Tibetan sheep urine deposition on soil carbon and nitrogen concentrations in an alpine steppe of the northern Tibetan Plateau, China

DU Zi-yin[1,2*] https://orcid.org/0000-0003-3404-8902; e-mail: duzy@cwnu.edu.cn

WANG Xiao-dan[2] https://orcid.org/0000-0002-0545-1041; e-mail: wxd@imde.ac.cn

CAI Yan-jiang[3] https://orcid.org/0000-0002-5376-7884; e-mail: yjcai@zafu.edu.cn

* *Corresponding author*

1 School of Geographical Sciences, China West Normal University, Nanchong 637009, China

2 Xainza Alpine Steppe and Wetland Ecosystem Observation Station, Institute of Mountain Hazards and Environment, Chinese Academy of Sciences & Ministry of Water Resources, Chengdu 610041, China

3 College of Environmental and Resource Sciences, Zhejiang A&F University, Hangzhou 311300, China

Citation: Du ZY, Wang XD, Cai YJ (2022) Short-term effects of yak and Tibetan sheep urine deposition on soil carbon and nitrogen concentrations in an alpine steppe of the northern Tibetan Plateau, China. Journal of Mountain Science 19(4). https://doi.org/10.1007/s11629-021-7026-6

Abstract: Yak (*Bos grunniens*) and Tibetan sheep urine deposited onto the alpine grasslands of the Qinghai-Tibetan Plateau is an important pathway for nutrient return, and it is closely related to soil fertility and alpine grassland productivity. However, hitherto, few studies have reported the effects of yak and Tibetan sheep urine deposition on soil carbon (C) and nitrogen (N) concentrations and the possible functional mechanisms under field conditions in alpine grasslands. To explore the status of soil C and N responding to the immediate N addition from livestock urine, we conducted a 28-d field experiment with three treatments, which include the application of yak urine (YU) and Tibetan sheep urine (TSU) application, and the control (CK, no application of urine). The results showed that YU treatment increased the soil moisture content and pH at 0-10 cm across the 28-day experimental period. Urine application resulted in the fluctuation of soil organic C (SOC) and increased topsoil SOC concentration during the middle and later periods of the experiment. Application of YU evidently increased the soil total N (TN) concentration in the 0-10 cm layer, while it did not affect the SOC concentrations in the 10-20 and 20-30 cm layers. Compared with the control, YU treatment significantly (P<0.05) increased the 0-10 and 10-20 cm soil ammonium-N (NH_4^+-N) concentration throughout the 28 days, while the TSU treatment significantly (P<0.05) increased the 0-10 and 10-20 cm soil nitrate-N (NO_3^--N) concentration. Urine N input changed soil physicochemical properties, nitrification, denitrification, and N leaching processes, and therefore affected the availability of N accumulation and consumption in soil. Under these conditions, the trade-off between soil NH_4^+-N and NO_3^--N under the influence of yak and Tibetan sheep urine application would change the form and concentration of available N, thereby altering the plant N uptake and utilization strategy of alpine grassland. The conclusions of this study could provide theoretical references for exploring the change characteristics of soil nutrient under the deposition of urine and optimizing the management

Received: 28-Jul-2021
Revised: 31-Dec-2021
Accepted: 27-Jan-2022

strategies of livestock urine in the alpine grassland ecosystem.

Keywords: Livestock urine; Mineral nitrogen; Nitrification; Denitrification; Qinghai-Tibetan Plateau

1 Introduction

Livestock urine deposition represents an important pathway for carbon (C) and nitrogen (N) entering grazed grassland ecosystems (Aarons et al. 2009; Haynes and Williams 1993; Lovell and Jarvis 1996), and is of great ecological significance for maintaining grassland soil nutrient balance and vegetation productivity. It has been reported that livestock excreta deposition can influence soil C and N cycling through direct transfer of nutrient and indirect change of the physicochemical and biological properties of soil following its deposition (Yoshitake et al. 2014). Previous studies have suggested that over 60% of bovine excreta N is urine, and the urine N concentration can reach 1000 kg N/ha for a single urine patch (Allen et al. 1996; Haynes and Williams 1993), thereby presenting an important N source to the grassland soil and vegetation. However, the return of a large amount of N generally far exceeds the short-term demand for plant growth, resulting in the loss of excess N through nitrous oxide (N_2O) emission, nitrate-N (NO_3^--N) leaching, and ammonia (NH_3) volatilization (Byrnes et al. 2017; Haynes and Williams 1993). With the rapid development of the economy and society, the increase in livestock carrying capacity will inevitably lead to more urine N being excreted into the grassland ecosystem. It is estimated that, compared with 1900, the global N input from livestock excreta will reach 52 million tons by 2050, and the corresponding NH_3 volatilization, N_2O emission, and N leaching and runoff loss would increase by approximately 9, 3, and 10 times, respectively (Bouwman et al. 2013), which may generate important and potential impacts on global warming, soil nutrient imbalance, and other ecological and environmental problems. Therefore, livestock urine patch nutrient content and migration characteristics should be heeded to when considering nutrient retention efficiency in sustaining soil nutrient balance and plant growth, especially for naturally grazed grassland ecosystems with low soil fertility.

Generally, the N transformation process within livestock urine patches largely affects the form and availability of mineral N, and therefore deeply alters the N demand for plant growth and soil fertility. It has been reported that most of the N in urine exists in the form of urea, which can be readily converted to ammonium-N (NH_4^+-N) through hydrolysis and thereafter be oxidized to NO_3^--N by nitrification to supply the N demand for plant growth (Allen et al. 1996; Dixon et al. 2010). Petersen et al. (1998) indicated that urea hydrolysis can usually be completed within 24 h, and the formation of a large amount of NH_4^+-N can easily generate a burning effect on roots, thus affecting the normal growth of plants. Moreover, the average size of the area affected by cattle urine was 0.68 m^2, and the growth status of the plant around the urine patch was significantly affected by the actual urinary N input, NH_3 volatilization, and nutrient redistribution (Lantinga et al. 1987).

As far as natural grasslands are concerned, livestock urine N input is an important source of soil nutrients (Cai et al. 2017), and the associated N transformation process (*e.g.,* nitrification and denitrification) could change the trade-off between soil NH_4^+-N and NO_3^--N concentrations. Somda et al. (1997) reported that cattle and sheep urine deposition in the semi-arid region of West Africa affected soil pH and mineral N gradients, such that the values tended to decline toward the periphery and deeper soil layers. Moreover, soil NH_4^+-N and NO_3^--N concentrations increased markedly after urine application and then decreased over time, probably owing to N losses by volatilization and leaching (Somda et al. 1997). In addition, it has been shown that bovine urine deposition in tropical and temperate pastures largely increases soil moisture, inorganic N content, and dissolved C content to accelerate soil nitrification and denitrification, thereby promoting the N transformation process and changing the available N content (de Klein and Vanlogtestijn 1994; Di et al. 2014; van Groenigen et al. 2005). This was partly consistent with the finding that cattle urine application increased the solubility of soil C and contributed to the increase in the decomposition of soil C (Clough and Kelliher 2005; Lambie et al. 2013; Singh et al. 2008). Furthermore, Cai et al. (2017) indicated that compared with no N addition, urea led to greater increases in soil NH_4^+-N concentration, while yak urine resulted in a greater increase in soil dissolved organic C (DOC) concentrations. Similarly, Ambus et al. (2007) assessed the short-term cycling processes of C and N in urine patches by using carbon-13 and nitrogen-15 labeling, and the results suggested that application of urine changed soil available C content and mineral N transformation, which could alter soil respiration and the balance of soil NH_4^+-N and NO_3^--N pools. Moreover, the denitrification of soil NO_3^--N was thought to be the sole source of N_2O production in the urine-affected soil after 12 days of incubation. In contrast, some organic compounds in urine (*e.g.*, hippuric acid and benzoic acid) can act to reduce N_2O emissions (Bertram et al. 2009; van Groenigen et al. 2006). Bertram et al. (2009) reported that NH_3 volatilization did not increase significantly with increased hippuric acid or benzoic acid concentrations in the applied urine, elevating hippuric acid in the urine had a marked negative effect on the rates of both nitrification and denitrification. Generally, factors such as temperature, precipitation, and available C content are considered to affect soil physicochemical properties and microbial activity, which subsequently alter the associated N transformation processes and mineral N concentrations. It has been reported that once the bulk of mineral N has been nitrified, leaching of NO_3^--N occurs when excess precipitation occurs, whereby water moves down the profile (Haynes and Williams 1993). In contrast, NH_4^+-N does not leach readily owing to its positive charge, which binds electrostatically to negatively charged material. Most cases of NH_4^+-N leaching are owing to the movement of the solute following the runoff or leachates (Monaghan et al. 1989; Sharpley 1991). Furthermore, Di and Cameron (2002) indicated that most soils in the temperate region are negatively charged, and that NO_3^--N is not retained by the soils and thus acts as the dominant form of N leached. The trade-off between NH_4^+-N and NO_3^--N leaching may be closely associated with nitrification, which can convert NH_4^+-N into NO_3^--N. Thus, more NH_4^+-N leaching may lead to less NO_3^--N leaching (Cai and Akiyama 2016).

As the largest grassland unit on the Eurasian continent, the mean altitude of the Qinghai-Tibetan Plateau is over 4,000 m above sea level (m a.s.l.) and covers an area of approximately 2.5 million km^2 (Du et al. 2016; Lu et al. 2012; Yue et al. 2010). Yak and Tibetan sheep are the two main species of livestock in this region, with an estimated 13 million yaks and 30 million Tibetan sheep (Lin et al. 2009; Miller 1990); therefore, large amounts of yak and Tibetan sheep urine can be directly deposited onto alpine grasslands. Urine deposition is an important source of soil nutrients in natural alpine grasslands (Cai et al. 2017), and sufficient attention should be paid to the potential complicated effects of external urine N input on internal soil N migration and transformation processes, so as to scientifically evaluate urine N returning efficiency and its possible environmental effects. Previous studies have primarily focused on simulated N addition under laboratory conditions or investigated the effect of synthetic urea rather than livestock urine on alpine plants and soils (Cai et al. 2017; Fu and Shen 2016; Fu and Shen 2017), similar studies highly emphasized the reporting of the complete effects of grazing on the alpine grassland ecosystem (Fu et al. 2012; Sun et al. 2021; Zhang and Fu 2021), nevertheless the individual and short-term effects of livestock urine on alpine grassland soils on the Qinghai-Tibetan Plateau remain unclear. As such, yak and Tibetan sheep urine deposition may change soil C and N concentrations, which may affect soil fertility and grassland productivity. We hypothesized that yak and Tibetan sheep urine deposition would largely alter soil available C and N concentrations and the N transformation process of the alpine grassland ecosystem. The objectives of this study were to: (1) investigate the variation in soil physicochemical properties following yak and Tibetan sheep urine application, and (2) evaluate the change in the trade-off between soil NH_4^+-N and NO_3^--N concentrations that are associated with the N transformation process mainly controlled by microbes.

2 Materials and Methods

2.1 Site description

This study was conducted at the Xainza Alpine Steppe and Wetland Ecosystem Observation Station in Northern Tibet, China (30°57' N, 88°42' E, 4,675 m a.s.l.). This region is characterized by a cold and semi-arid plateau monsoon climate, with an average annual air temperature and precipitation of 0°C and 300 mm, respectively (Du et al. 2021). Most rainfall occurs from May to September, which is known as the vegetation-growing season. There is no absolute frost-free season, such that frost occurs over 279 days (Du et al. 2017). The dominant species of alpine steppe are *Stipa purpurea* and *Carex moorcroftii*, and the accompanying species include *Artemisia nanschanica*, *Leontopodium alpinum*, and *Oxytropis glacialis* (Hong et al. 2016). The soil is classified as Cryic Aridisol according to the Chinese soil taxonomy (Gong 1999) and can also be classified as Cryosol according to the World Reference Base (IUSS Working Group WRB 2014).

2.2 Experimental methods

The field experiment was conducted from August 12 to September 8, 2019. Three treatments were used: (i) control, (CK, no urine application), (ii) soil amended with 8.13 g N/L yak urine (YU), (iii) soil amended with 4.07 g N/L Tibetan sheep urine (TSU) (Table 1). Each treatment was replicated three times. As such, nine experimental plots (three treatments× three replications) were arranged for soil sampling at a specific time. Fresh yak and Tibetan sheep urine samples were manually collected from a randomly selected group of eight animals located in a camping area adjacent to the study site. These grazing animals were enclosed within the camp at night, and their fresh urine was collected in plastic buckets the next morning until the amount collected was sufficient for the experiment. The total urine samples collected from the yak and Tibetan sheep were respectively mixed and stored for nearly six hours at ambient temperature prior to use. The fresh yak and Tibetan sheep urine respectively contained 11.7 and 8.06 mg/L of NH_4^+-N; however, NO_3^--N contents were not detected (Table 1).

For the YU treatment, 1 L of fresh yak urine was uniformly sprinkled in each plot (1×1 m^2) by watering to form a typical cattle urine patch (40 cm in diameter). For the TSU treatment, 70 ml of fresh Tibetan sheep urine was also uniformly sprinkled in each plot (1×1 m^2) by watering differently to form a typical sheep urine patch (16×20 cm^2). These simulated urine patches were similar to the volume and size of cattle and sheep urine that were naturally deposited in the field (Ma et al. 2006). To reduce the influence of soil sampling on its physical structure and microbial process, six batches of parallel plots (*n* = 54 in total for soil sampling) were simultaneously arranged to demand independent soil sampling at 1, 3, 7, 14, 21, and 28 d after application of urine. At each sampling time, soil samples covered by each urine patch were manually collected at depths of 0-10, 10-20, and 20-30 cm. For each soil layer, soil samples were carefully mixed; then a partial composite sample was manually collected and transported to the laboratory to determine the moisture content and the concentrations of NH_4^+-N and NO_3^--N using an ice cooler. Another part of the composite sample was collected, air-dried, and ground to measure the pH, TOC, and TN concentrations.

2.3 Urine and soil samples measurement

The urine pH was measured using the glass electrode method. Urine total N was determined using the automatic Kjeldahl apparatus method, and the concentrations of NH_4^+-N and NO_3^--N were measured using a San^{++} Continuous Flow Analyzer (Skalar, Netherlands). Soil moisture content was determined by oven-drying at 105°C to a constant weight. Soil pH was measured using a ratio of 1: 2 solids: water (Diaz et al. 2008). To determine soil inorganic N (NH_4^+-N and NO_3^--N), the supernatant extracted from 0.5-g of soil (on an oven-dried basis), using 50 ml solution of 2 M KCl for 60 min in a rotary shaker, was measured using a segmented flow analyzer (Skalar, Breda, The Netherlands) (Diaz et al. 2008). Soil TOC and TN concentrations were measured using the potassium dichromate external heating and wet micro-Kjeldahl methods, respectively (Cai et al. 2013).

2.4 Data analysis

All data were statistically analyzed using the SPSS software package for Windows (Version 18.0, SPSS Inc., Chicago, IL, USA) and presented using Origin 8.0 (Origin Lab Corp., Northampton, MA, USA). A series of one-way analyses of variance, followed by least significant difference (LSD) tests at $P<0.05$, were performed to determine the significant differences in soil NH_4^+-N or NO_3^--N concentrations among the three treatments at the same sampling time or among the different sampling times for the

Table 1 Background information of yak and Tibetan sheep urine used for field experiment in this study.

Type of urine	pH	TN (g/L)	NH_4^+-N (mg/L)	NO_3^--N (mg/L)
Yak urine	7.94	8.13	11.7	Not detected
Tibetan sheep urine	7.90	4.07	8.06	Not detected

same treatment. Repeated measures of ANOVA and the post hoc multiple comparisons followed by LSD tests at $P<0.05$, were used to test the differences in soil variables (soil moisture, pH, SOC, and TN) among the treatments and the difference at each time at 0-10 cm soil depth. Pearson correlation analysis was used to evaluate the relationships among the soil properties.

3 Results

3.1 Air temperature and precipitation

The daily air temperature and precipitation fluctuated between August and September (Fig. 1). Air temperature first increased several days after urine application and then gradually decreased during the later period of the experiment (Fig. 1). The mean air temperature during the entire 28 days was 11.3°C and the days of mean value > 10°C mostly occurred before September. The cumulative precipitation for the 28 days experimental period was 33.7 mm, and most rainfall events occurred during the former and later periods of the experiment, respectively (Fig. 1). Precipitation from September 1 to 3 accounted for 42.7% of the total amount across the entire experimental period.

3.2 Soil moisture and pH

Compared to the CK, YU treatment markedly increased the soil moisture content in the 0-10 cm layer 1 d after urine application, with the peak value reaching 8.34% (Fig. 2(a)). With an increase in the number of days after urine application, yak urine application increased the soil moisture content in the 0-10 and 10-20 cm layers during the first 21 days of the experimental period (Fig. 2(a) and 2(b)). In contrast, TSU treatment generally showed a decrease in soil moisture content for the three soil layers, except for a minor increase in the 10-20 cm layer one day after urine application (Fig. 2(b)). In addition, yak urine application evidently increased 0-10 cm soil pH compared to the control, and the mean value 8.75 was greater than that of other treatments. This is contrary to the fact that Tibetan sheep urine application largely decreased soil pH, especially during the first 7 days after urine application (Fig. 2(d)). Generally, YU and TSU treatments failed to markedly alter soil pH in the 10-20 cm layer, but caused fluctuations in 20-30 cm soil pH (Fig. 2(e) and 2(f)). At the end of the experiment, TSU treatment significantly decreased soil pH in the 0-10, 10-20, and 20-30 cm layers contrastingly to the control. Repeated measures of ANOVA showed that YU treatment significantly increased ($P<0.05$) the 0-10 cm soil moisture and pH compared to other treatments throughout the experimental period (Table 2). TSU treatment significantly decreased ($P<0.05$) the 0-10 cm soil pH compared to the CK and YU treatments (Table 2).

3.3 SOC and TN

The SOC concentration in the 0-10 cm topsoil for CK treatment (11.0 g/kg) was greater than that in the deeper soil layer 1 d after the beginning of the experiment. It first decreased and then increased throughout the 28 d experimental period (Fig. 2(g)). In contrast, 10-20 and 20-30 cm SOC concentrations showed repeated increasing and decreasing trends, respectively (Fig. 2(h) and 2(i)). YU and TSU

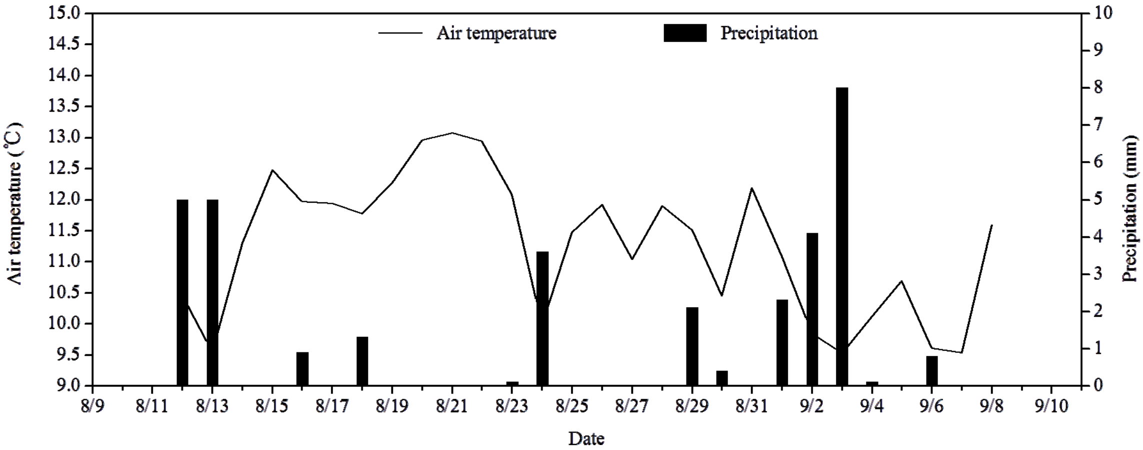

Fig. 1 Temporal variation of air temperature and precipitation across the 28 days experimental period in 2019.

treatments immediately decreased the 0-10 and 10-20 cm SOC concentrations to some extent after urine application, and the negative effects weakened during the middle period of the experiment (Fig. 2(g) and 2(h)). Large differential effects of YU and TSU treatments on SOC concentration in the 0-10, 10-20, and 20-30 cm soil layers mainly occurred in the last week of the experiment from 21 to 28 d. Compared with the CK, the YU treatment resulted in the SOC concentration in the 10-20 and 20-30 cm layers to respectively decrease to 7.12 and 3.95 g/kg at the end of the experiment (28 d), while TSU treatment resulted in 0-10 and 10-20 cm SOC concentration to respectively increase to 10.7 and 8.87 g/kg (Fig. 2(g), 2(h), and 2(i)). In addition, soil TN concentration for CK treatment decreased with increasing soil depth, and the values at 0-10, 10-20, and 20-30 cm were 0.99-1.17, 0.71-0.89, and 0.42-0.65 g/kg, respectively, during the entire experimental period. YU treatment largely increased the 0-10 cm soil TN concentration, with a mean value of 1.30 g/kg greater than that of other treatments (Fig. 2(j)). The priming effect decreased and was sustained at a relatively steady state 3 days after urine application (Fig. 2(j)). In contrast, TSU treatment decreased the 10-20 cm and increased the 20-30 cm soil TN concentrations 3 days after urine application, and exhibited an increase soil TN concentration in the 0-10 and 10-20 cm layers from 21 to 28 d (Fig. 2(j), 2(k), and 2(l)). The ratio of SOC to TN for CK decreased with increasing soil depth, with values of 0-10, 10-20, and 20-30 cm respectively ranging from 8.43 to 9.43, 7.24 to 9.31,

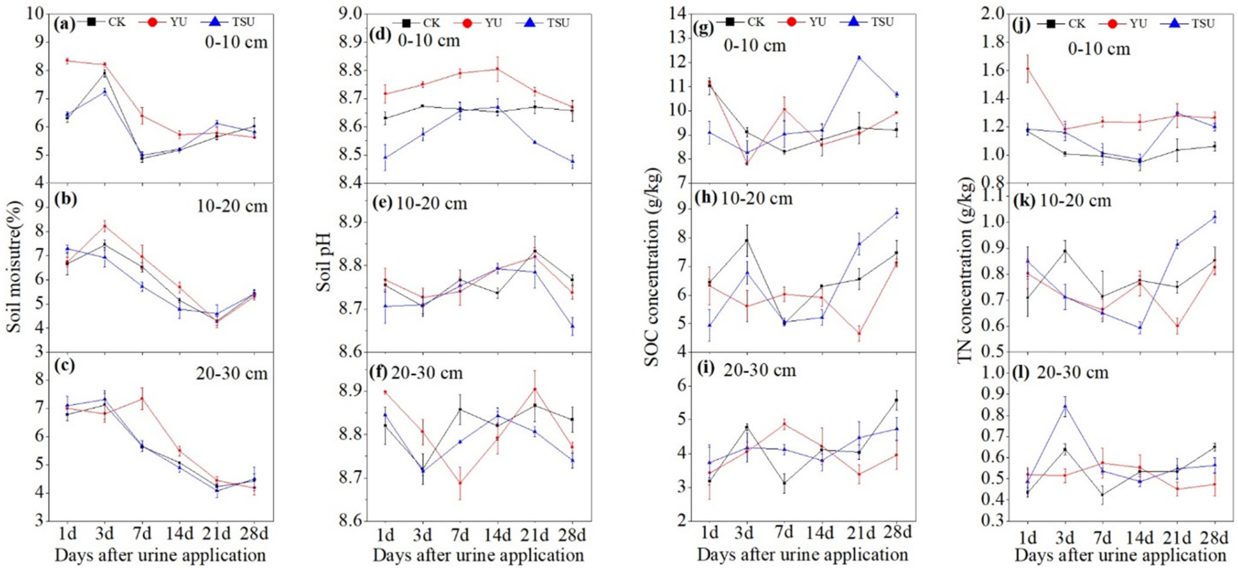

Fig. 2 Temporal variation of soil moisture at the depth of (a) 0-10 cm, (b) 10-20 cm, and (c) 20-30 cm, soil pH at (d) 0-10 cm, (e) 10-20 cm, and (f) 20-30 cm, SOC at (g) 0-10 cm, (h) 10-20 cm, and (i) 20-30 cm, and TN at (j) 0-10 cm, (k) 10-20 cm, and (l) 20-30 cm across the 28 days experimental period for the control (CK), yak urine (YU), and Tibetan sheep urine (TSU) treatments.

Table 2 Repeated measures of ANOVA were conducted to test the difference in soil variables among the treatments and the difference at each time at 0-10 cm soil depth

Type	/	Moisture (%)	pH	SOC (g/kg)	TN (g/kg)
Treatment	CK	5.98±0.07 b	8.66±0.01 b	9.29±0.09 b	1.03±0.01 b
	YU	6.67±0.07 a	8.74±0.01 a	9.44±0.11 b	1.31±0.01 a
	TSU	5.97±0.06 b	8.57±0.01 c	9.74±0.16 a	1.14±0.02 b
Sampling time	T1	7.03±0.09 b	8.61±0.03 b	10.4±0.06 a	1.34±0.03 a
	T3	7.78±0.02 a	8.67±0.01 ab	8.40±0.16 c	1.12±0.03 b
	T7	5.42±0.17 c	8.70±0.02 a	9.14±0.33 ab	1.08±0.05 b
	T14	5.36±0.08 c	8.71±0.02 a	8.86±0.15 b	1.05±0.01 b
	T21	5.85±0.07 c	8.65±0.01 ab	10.2±0.22 a	1.20±0.06 ab
	T28	5.81±0.13 c	8.60±0.01 b	9.93±0.10 ab	1.17±0.02 ab

Notes: Mean±SE. CK, control; YU, yak urine; TSU, Tibetan sheep urine. T1, T3, T7, T14, T21, and T28 represent the soil sampling times at 1, 3, 7, 14, 21, and 28 d after urine application. SOC = soil organic carbon, TN = total nitrogen. Different lowercase letters within the same column indicate the differences among the treatments or the differences among the different soil sampling times at P<0.05.

and 7.30 to 8.56 (data not shown). This was partly comparable to the variation in the ratio that was amended with TSU treatment. In contrast, the ratio of SOC to TN for YU treatment in the 0-10 cm layer (6.63-8.14) was lower than that for the CK and increased to reach the maximum in the 10-20 cm layer (7.38-9.17) throughout the experimental period. Overall, YU and TSU treatments significantly increased (P<0.05) 0-10 cm soil TN and SOC concentrations during the 28 days of the experiment, and the increasing effects were clearly detected at the first sampling time (T1), in contrast to other times (Table 2).

3.4 Soil NH_4^+-N and NO_3^--N

Soil NH_4^+-N and NO_3^--N concentrations in the CK treatment decreased with increasing soil depth (Fig. 3), and 0-10 cm soil NH_4^+-N concentration significantly (P<0.05) decreased to 1.70 mg/kg at the end of the experiment (Fig. 3(a)). However, there were no statistical differences (P > 0.05) in soil NH_4^+-N concentration in the 10-20 and 20-30 cm layers between 1 and 28 d (Fig. 3(b) and 3(c)). In contrast, soil NO_3^--N concentration in the 0-10, 10-20, and 20-30 cm layers significantly (P<0.05) increased at the end of the experiment, and the peak values reached 8.45, 3.74, and 3.06 mg/kg. YU treatment significantly (P<0.05) increased the soil NH_4^+-N concentration in the 0-10 and 10-20 cm layers immediately after urine application, with the maximum reaching 140 and 18.3 mg/kg in 1 d, which were statistically (P<0.05) greater than those of the other two treatments (Fig. 3(a)). During the whole experiment, the 0-10 cm soil NH_4^+-N concentration for YU treatment significantly decreased from 1 to 28d, and ultimately declined to 52.3 mg/kg at the end of experiment; whereas the 20-30 cm soil NH_4^+-N concentration significantly increased to 2.00 mg/kg (Fig. 3(a) and 3(c)). In contrast, compared with the CK treatment, soil NH_4^+-N concentration in the 0-10 cm during the first 14 days increased for the TSU treatment (P < 0.05), and the variation of soil NH_4^+-N concentration in 0-10 and 20-30 cm layers across the whole experimental period were similar to that of soil NH_4^+-N amended with yak urine application (Fig. 3(a) and 3(c)). Meanwhile, TSU treatment significantly (P<0.05) increased the 0-10 and 10-20 cm soil NO_3^--N concentrations from 1 to 28 d, which differed significantly from the YU treatment (P<0.05) where the NO_3^--N concentration decreased in the 0-10 cm layer on 1 d after urine application (Fig. 3(d) and 3(e)). Soil NO_3^--N concentration for YU treatment in the 0-10, 10-20, and 20-30 cm layers gradually increased from 1 to 28 d, and the value respectively reached 11.9, 6.50, and 3.21 mg/kg by the end of the experiment

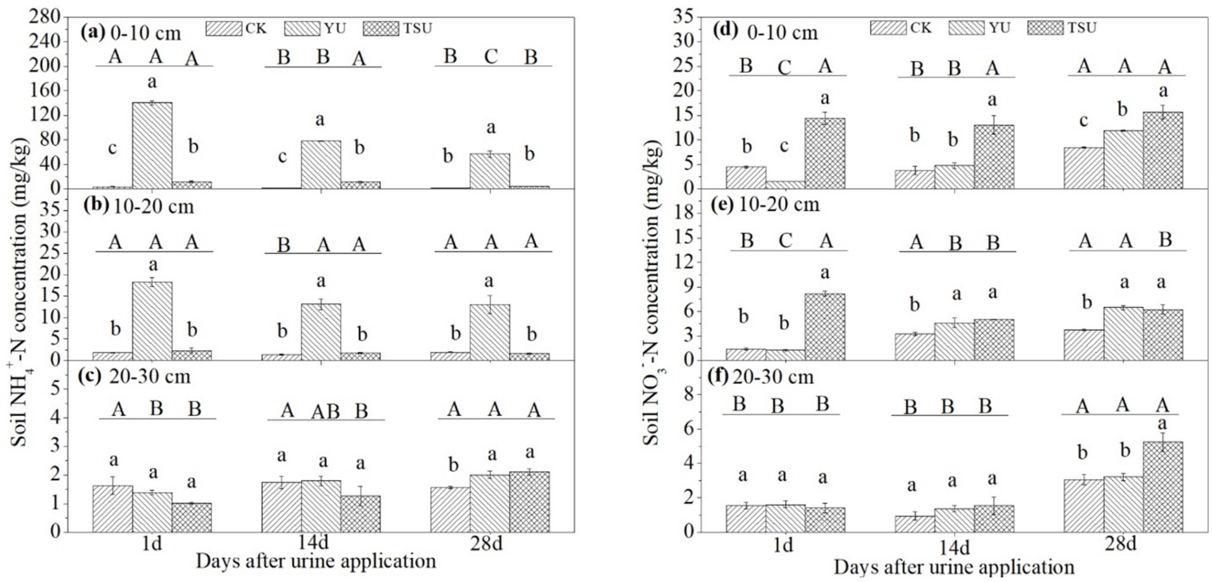

Fig. 3 Soil NH_4^+-N concentrations in the (a) 0-10 cm, (b) 10-20 cm, and (c) 20-30 cm layers, and NO_3^--N concentrations in the (d) 0-10 cm, (e) 10-20 cm, and (f) 20-30 cm layers at the beginning (1d), middle (14d), and end of the experimental period (28d) for the control (CK), yak urine (YU), and Tibetan sheep urine (TSU) treatments. Different lowercase letter at the same sampling time indicate the significant differences among the three treatments at P<0.05, while different capital letter for the same treatment indicate the significant differences among the different sampling time at P<0.05.

(Fig. 3(d), 3(e), and 3(f)).

The ratio of NH_4^+-N to NO_3^--N for YU treatment was significantly greater than that of the other treatments (P<0.05), with a value of 90.1 on 1 d after urine application, gradually decreased to 4.82 by the end of the experiment. Moreover, the ratio of NH_4^+-N to NO_3^--N for YU treatment decreased with increasing soil depth from 0 to 30 cm, while the value for TSU treatment decreased and then increased trends for 0-10, 10-20, and 20-30 cm. It was contrary to the increasing of the ratio for CK treatment from 0-10 cm (0.14-0.80) to 10-20 cm (0.32-1.57). Pearson correlation analysis showed that soil pH at depths of 0-10, 10-20, and 20-30 cm was negatively correlated with soil NO_3^--N concentration (r=−0.674, P<0.01; r=−0.346, P<0.05; r=−0.285, P<0.05, respectively) (Tables 3, 4, and 5). Soil moisture content was positively correlated with soil NH_4^+-N concentration in the 0-10 cm layer (r=0.476, P<0.01) (Table 3) and negatively correlated with soil NO_3^--N concentration in the 0-10 and 20-30 cm layers (r=−0.301, P<0.05; r=−0.315, P<0.05, respectively) (Tables 3 and 5). Soil NH_4^+-N was negatively and positively correlated with NO_3^--N at 0-10 cm (r=−0.428, P<0.01) and 20-30 cm (r=0.499, P<0.01) soil layers (Tables 3 and 5).

4 Discussion

4.1 Urine application promote the migration of topsoil C and N to deep soil layer

A previous study reported that livestock urine had high N concentration and moisture content, and urine deposition would alter soil C and N concentrations by directly inputting the nutrients from itself and indirectly changing soil physicochemical and biological properties (Haynes and Williams 1993; Yoshitake et al. 2014). In this study, YU treatment immediately increased the topsoil moisture content of the 0-10 cm layer 1 day after urine application, but no evident differences were found in the deeper soil layer between urine and CK treatments (Fig. 2), indicating that yak urine application quickly increased soil water supply and therefore resulted in a short-term increase in soil moisture content. This was similar to the findings by de Klein and Vanlogtestijn (1994), who determined

Table 3 Correlation analysis among the related soil properties at 0-10 cm soil depth (n = 54)

Soil properties	pH	Moisture	SOC	TN	NH_4^+-N	NO_3^--N
pH	1	0.108	−0.26	0.107	0.586**	−0.674**
Moisture		1	0.073	0.476**	0.476**	−0.301*
SOC			1	0.515**	0.086	0.337*
TN				1	0.706**	−0.051
NH_4^+-N					1	−0.428**
NO_3^--N						1

Table 4 Correlation analysis among the related soil properties at 10-20 cm soil depth (n = 54)

Soil properties	pH	Moisture	SOC	TN	NH_4^+-N	NO_3^--N
pH	1	−0.494**	−0.383**	−0.412**	0.199	−0.346*
Moisture		1	−0.062	0.064	−0.097	−0.012
SOC			1	0.687**	−0.147	0.121
TN				1	−0.010	0.332*
NH_4^+-N					1	−0.038
NO_3^--N						1

Table 5 Correlation analysis among the related soil properties at 20-30 cm soil depth (n = 54)

Soil properties	pH	Moisture	SOC	TN	NH_4^+-N	NO_3^--N
pH	1	−0.272*	−0.451**	−0.577**	0.043	−0.285*
Moisture		1	−0.076	0.265	−0.380**	−0.315*
SOC			1	0.653**	0.080	0.288*
TN				1	−0.058	0.074
NH_4^+-N					1	0.499**
NO_3^--N						1

Notes: *P<0.05, **P<0.01. SOC = soil organic carbon; TN = total nitrogen; NH_4^+-N = ammonium nitrogen; NO_3^--N = nitrate nitrogen.

that the soil moisture content in urine treated plots was significantly higher than that of the control. Furthermore, the 0-10 cm soil pH treated by yak urine application was markedly higher than that of other treatments simultaneously; thereby proving its priming effect in increasing topsoil pH, which is consistent with the results derived from an experiment conducted in a New Zealand dairy farm pasture (Clough and Kelliher 2005). Compared to TSU treatment, larger yak urine volume and higher initial urine pH may jointly improve topsoil moisture content and pH (Table 1).

The SOC concentration in the 0-10 cm layer decreased from 1 to 3 d, while the corresponding 10-20 and 20-30 cm SOC concentrations gradually increased during the first 3 days after urine application, implying that part of topsoil organic C migrated to the deeper soil layer under the combined leaching effects of urine infiltration and cumulative precipitation of 10 mm during the first 2 days (Fig. 1). Moreover, part of the C derived from urine itself and the increment resulted from the increase in soil C solubility affected by urine (Clough and Kelliher 2005), which may be another reason for the increase in SOC concentration, which could be partly verified by the findings of Cai et al. (2017), who reported that yak urine application resulted in a greater increase in soil dissolved organic C (DOC) concentrations. Nevertheless, the specific contribution of each pathway to increasing SOC has been unclear until now. Conversely, Lambie et al. (2013) reported that cattle urine application would positively promote soil C decomposition, and the leaching of dissolved C and release of gaseous C would jointly lead to a decrease in topsoil organic C concentration. Large differences between YU and TSU treatments on SOC concentration at different soil depths during the later period (14-28 d) might be linked to the discrepancy in the chemical components of urine itself, which therefore altered soil C mineralization and decomposition processes. The relatively higher precipitation (15.3 mm) during the last week would be another important reason explaining in the continuous increase in SOC concentration in the 10-20 and 20-30 cm layers.

In addition, the variation in soil TN concentration was partly similar to that of SOC after urine application, and the amount of N derived from urine itself (Allen et al. 1996; Haynes and Williams 1993) was considered the main factor causing the differences in TN concentration in topsoil. This study determined that YU treatment markedly increased the 0-10 cm topsoil TN concentration during the 28 d experimental period compared with other treatments (Table 2), which was thought to be closely related to the initial high TN concentration (8.13 g/L) which directly increased soil N supply and led to a rapid increase in soil TN. The immediate increase in soil mineral N concentration that was derived from urea hydrolysis (Petersen et al. 1998) and soil organic N mineralization would simultaneously promote the availability of soil N, thus enhancing soil TN concentration to some extent. The decrease in topsoil TN concentration associated with the corresponding increase of TN in deep soil strongly indicated that active N migrated to deeper soil layers, accompanied by precipitation and leaching effects. In contrast, TSU treatment evidently stimulated soil TN concentrations in the 10-20 and 20-30 cm layers during the last week of the field experiment (21-28 d), which might be attributed to the net N accumulation that, based on the processes, includes leaching of mineral N and release of gaseous N; thereby ultimately exceeding other treatments. However, the detailed amounts and functional mechanisms of soil N transformation and consumption after urine application would complicatedly affect soil C and N availability, microbial activity, climatic conditions, and urine properties.

4.2 Trade-off between soil $NH4^+$-N and $NO3^-$-N following urine application

Livestock urine N input is an important pathway for increasing soil N concentration, and the associated N transformation and leaching process could change the trade-off between soil NH_4^+-N and NO_3^--N concentrations (Cai and Akiyama 2016). In this study, soil NH_4^+-N concentration for CK treatment at depth of 0-10 cm significantly decreased; whereas soil NO_3^--N concentrations at depth of 0-10, 10-20, and 20-30 cm significantly increased throughout the 28 d experimental period (Fig. 3), implying that parts of soil mineral N markedly transformed and migrated under natural conditions without disturbing livestock urine N in the alpine grassland. This might be attributed to the combined effects of continuous nitrification promoting part of the oxidation of NH_4^+-N to NO_3^--N; while the precipitation enhanced topsoil NO_3^--N leaching to deeper soil layers (Fig. 1), as Cai and Akiyama (2016) indicated, the trade-off between NH_4^+-N and NO_3^--N leaching was closely associated with nitrification. Furthermore, soil NO_3^--N was easily leached, and the joint effects of heavy rainfall and high NO_3^--N availability generally resulted in greater NO_3^--N leaching (Botter et al. 2006). The significant negative correlation between soil moisture content and NO_3^--N concentration in the 0-10 cm soil layer could prove the statement mentioned above (Table 3).

YU and TSU treatments significantly increased soil NH_4^+-N concentration (Fig. 3). The influential difference between yak and Tibetan sheep urine on soil NH_4^+-N at different depths was thought to be linked to initial N concentrations and chemical component properties of urine *per se*. This was consistent with the findings of Clough and Kelliher (2005), who determined that soil NH_4^+-N concentration increased immediately following urine application. This is similar to the reports of Fu and Shen (2017), who indicated that urea addition significantly increased alpine soil NH_4^+-N and NO_3^--N on the Tibetan Plateau. The significant decrease in the 0-10 cm soil NH_4^+-N concentration that was treated by yak and Tibetan sheep urine at the end of the experiment probably resulted from the combined effects of NH_4^+-N nitrification, leaching, and plant uptake. This could be partly verified by the significant increase in soil NH_4^+-N in the 20-30 cm layer and soil NO_3^--N in the 0-10, 10-20, and 20-30 cm layers (Fig. 3), respectively. Interestingly, YU treatment significantly decreased 0-10 cm soil NO_3^--N concentration in the 0-10 cm layer 1 d after urine application, and its priming effect in improving soil NO_3^--N concentration was reflected at the end of the experiment, implying that the increment of NO_3^--N was largely derived from nitrification rather than the direct supply of urine *per se*. Whitehead (2000) reported that the presence of more water-soluble N in cattle urine could move downward more easily with urine-water infiltration and precipitation. Considering the significant negative correlation between soil moisture content and NO_3^--N in the 0-10 cm layer (Table 3), yak urine application and precipitation (5 mm) on the first day largely increased soil moisture content, thereby resulting in the infiltration of soil NO_3^--N into deeper soil layers, thus decreasing topsoil NO_3^--N accumulation. The corresponding increase of the soil NO_3^--N concentration in the 10-20 cm layer could partly prove the argument mentioned above. This was partly different from the findings of Sun et al. (2021) and Zhang and Fu (2021) who reported that summer grazing in the alpine steppe meadow resulted in a significant decrease in soil moisture and NO_3^--N concentration, and the vigorous water and nutrition uptake by root systems might explain the decrease in soil NO_3^--N concentration. Conversely, de Klein and Vanlogtestijn (1994), Di et al. (2014), and van Groenigen et al. (2005) determined that cattle urine application contributes to altering soil physicochemical properties and enhancing soil denitrification and N_2O emissions in tropical and temperate grasslands. The quick increase in soil moisture, mineral N, and dissolved C following yak urine application in this study may strengthen the soil anaerobic environment and anaerobe activity, thus accelerating soil denitrification and reducing the substrate concentration of NO_3^--N. By contrast, TSU treatment statistically increased the soil NO_3^--N concentrations in the 0-10 and 10-20 cm layers (Fig. 3), probably implying a priming effect in nitrification immediately after the application of Tibetan sheep urine. In natural grassland ecosystems with limited N conditions, the trade-off between NH_4^+-N and NO_3^--N that is affected by urine N input would likely generate non-trivial, spatially heterogeneous impacts on the vegetation growth and community structure of plants with respect to their various N uptake capacities (Hong et al. 2017). More efforts should be made to investigate the detailed microbial functional mechanisms and differences in grassland soil N mineralization, nitrification, and denitrification processes following different types of urine application in the context of climate change, so as to deeply understand the soil C and N processes and formulate scientific urine management strategies to promote the sustainable development of alpine grassland ecosystems on the Qinghai-Tibetan Plateau.

4.3 Shortcoming and outlook of the present study

This study theoretically investigated the effects of yak and Tibetan sheep urine application on alpine steppe soil C and N concentrations by conducting a short-term field experiment, and some of the existing shortcomings can be summarized as follow. First, the field experiment in this study was only conducted for one month and focused on the relationship between grazing livestock urine and soil nutrients, while previous studies have found that grazing effects on soil moisture, pH, NH_4^+-N, NO_3^--N, SOC, and TN can vary with measuring years (Fu et al. 2012; Zhang and Fu 2021). Second, previous studies found that summer grazing effects on soil physicochemical properties differ with winter grazing effects (Fu et al. 2012; Zhang and Fu 2021), whereas this study was only conducted in the summer rather than the winter. Therefore, the influence of seasonal differences, temporal range, and nitrogen addition effect (Fu and Shen 2017) on soil properties in the N-limited alpine grassland on the Qinghai-Tibetan Plateau should be considered in future studies.

5 Conclusions

Yak and Tibetan sheep urine deposition affected soil C and N dynamics to varying degrees in the alpine grassland of the northern Tibetan Plateau, China. Compared with the control, urine application led to the fluctuation of SOC and increased topsoil SOC at the middle and later periods of the experiment. Yak urine and Tibetan sheep urine application increased soil NH_4^+-N and NO_3^--N concentrations in the 0-10 and 10-20 cm layers, respectively, and the trade-off between NH_4^+-N and NO_3^--N altered the soil N transformation process and mineral N availability. More attention should be paid to explore the detailed C and N transformation processes that are affected by exogenous N input and microbes following urine deposition by considering the alpine grassland ecosystem nutrient cycling in future studies.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Grant NO. 41807109), the Second Tibetan Plateau Scientific Expedition and Research Program (Grant NO. 2019QZKK0404), the doctoral research launch fund project and National general cultivation project of China West Normal University (Grant NO. 17E042, 18B016).

References

Aarons SR, O'Connor CR, Hosseini HM, et al. (2009) Dung pads increase pasture production, soil nutrients and microbial biomass carbon in grazed dairy systems. Nutr Cycl Agroecosys 84(1): 81-92. https://doi.org/10.1007/s10705-008-9228-5

Allen AG, Jarvis SC, Headon DM (1996) Nitrous oxide emissions from soils due to inputs of nitrogen from excreta return by livestock on grazed grassland in the UK. Soil Biol Biochem 28(4-5): 597-607. https://doi.org/10.1016/0038-0717(95)00186-7

Ambus P, Petersen SO, Soussana JF (2007) Short-term carbon and nitrogen cycling in urine patches assessed by combined carbon-13 and nitrogen-15 labelling. Agr Ecosyst Environ 121(1-2): 84-92. https://doi.org/10.1016/j.agee.2006.12.007

Bertram JE, Clough TJ, Sherlock RR, et al. (2009) Hippuric acid and benzoic acid inhibition of urine derived N_2O emissions from soil. Glob Change Biol 15(8): 2067-2077. https://doi.org/10.1111/j.1365-2486.2008.01779.x

Botter G, Settin T, Marani M, et al. (2006) A stochastic model of nitrate transport and cycling at basin scale. Water Resour Res 42(4): 04415. https: //doi.org/10.1029/2005WR004599

Bouwman L, Goldewijk KK, Van Der Hoek KW, et al. (2013) Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900-2050 period. Proc Nat Acad Sci 110(52): 20882-20887. https://doi.org/10.1073/pnas.1012878108

Byrnes RC, Nunez J, Arenas L, et al. (2017) Biological nitrification inhibition by Brachiaria grasses mitigates soil nitrous oxide emissions from bovine urine patches. Soil Biol Biochem 107: 156-163. https: //doi.org/10.1016/j.soilbio.2016.12.029

Cai YJ, Akiyama H (2016) Nitrogen loss factors of nitrogen trace gas emissions and leaching from excreta patches in grassland ecosystems: A summary of available data. Sci Total Environ 572: 185-195. https://doi.org/10.1016/j.scitotenv.2016.07.222

Cai YJ, Du ZY, Yan Y, et al. (2017) Greater stimulation of greenhouse gas emissions by stored yak urine than urea in an alpine steppe soil from the Qinghai-Tibetan Plateau: A laboratory study. Grassl Sci 63(3): 196-207. https://doi.org/10.1111/grs.12164

Clough TJ, Kelliher FM (2005) Dairy Farm Effluent Effects on Urine Patch Nitrous Oxide and Carbon Dioxide Emissions. J Environ Qual 34(3): 979-986. https://doi.org/10.2134/jeq2004.0360

de Klein CAM, Vanlogtestijn RSP (1994) Denitrification and N_2O emission from urine-affected grassland soil. Plant Soil 163(2): 235-241. https://doi.org/10.1007/BF00007973

Di HJ, Cameron KC (2002) Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. Nutr Cycl Agroecosys 64(3): 237-256. https://doi.org/10.1023/a:1021471531188

Di HJ, Cameron KC, Podolyan A, et al. (2014) Effect of soil moisture status and a nitrification inhibitor, dicyandiamide, on ammonia oxidizer and denitrifier growth and nitrous oxide emissions in a grassland soil. Soil Biol Biochem 73: 59-68. https://doi.org/ 10.1016/j.soilbio.2014.02.011

Dixon ER, Laughlin RJ, Watson CJ, et al. (2010) Evidence for the production of NO and N_2O in two contrasting subsoils following the addition of synthetic cattle urine. Rapid Commun Mass Sp 24(5): 519-528. https://doi.org/10.1002/rcm.4348

Du ZY, Cai YJ, Yan Y, et al. (2017) Embedded rock fragments affect alpine steppe plant growth, soil carbon and nitrogen in the northern Tibetan Plateau. Plant Soil 420(1): 79-92.

https://doi.org/10.1007/s11104-017-3376-9
Du ZY, Wang XD, Liu XP, et al. (2016) Effects of rock fragments on yak dung greenhouse gas emissions on the Qinghai-Tibetan Plateau. J Mt Sci 13(11): 2006-2014. https://doi.org/10.1007/s11629-015-3798-x
Du ZY, Wang XD, Xiang J, et al. (2021) Yak dung pat fragmentation affects its carbon and nitrogen leaching in Northern Tibet, China. Agr Ecosyst Environ 310: 107301. https://doi.org/10.1016/j.agee.2021.107301
Fu G, Shen ZX, Zhang XZ, et al. (2012) Response of microbial biomass to grazing in an alpine meadow along an elevation gradient on the Tibetan Plateau. Eur J Soil Biol 52:27-29. https://doi.org/10.1016/j.ejsobi.2012.05.004
Fu G, Shen ZX (2016) Response of alpine plants to nitrogen addition on the Tibetan Plateau: a meta-analysis. J Plant Growth Regul 35(4): 974-979. https:// doi.org/10.1007/s00344-016-9595-0
Fu G, Shen ZX (2017) Response of alpine soils to nitrogen addition on the Tibetan Plateau: a meta-analysis. Appl Soil Ecol 114: 99-104. http://dx. doi.org/10.1016/j.apsoil.2017.03.008
Gong ZT (1999) Chinese Soil Taxonomy: Theories, Methods and Applications. Science Press, Beijing (in Chinese)
Haynes RJ, Williams PH (1993) Nutrient cycling and soil fertility in the grazed pasture ecosystem. In: LS Donald (ed) Advances in Agronomy. Academic Press.
Hong JT, Ma XX, Wang XD (2016) Leaf meristems: an easily ignored component of the response to human disturbance in alpine grasslands. Ecol Evol 6: 2325–2332. https://doi.org/10.1002/ece3.2059
Hong JT, Ma XX, Zhang XK, et al. (2017) Nitrogen uptake pattern of herbaceous plants: coping strategies in altered neighbor species. Biol Fert Soils 53: 729-735. https://doi.org/10.1007/s00374-017-1230-0
IUSS Working Group WRB (2014) World Reference Base for Soil Resources 2014. International soil classification system for naming soil and creating legends for soil maps. Food and Agriculture Organization of the United Nations. Rome: pp.154
Lambie SM, Schipper LA, Balks MR, et al. (2013) Priming of soil decomposition leads to losses of carbon in soil treated with cow urine. Soil Res 51: 513-520. https://doi.org/10.1071/SR13148
Lantinga E, Keuning J, Groenwold J, et al. (1987) Distribution of excreted nitrogen by grazing cattle and its effects on sward quality, herbage production and utilization. Animal Manure on Grassland and Fodder Crops Fertilizer or Waste? Springer.
Lin XW, Wang SP, Ma XZ, et al. (2009) Fluxes of CO_2, CH_4, and N_2O in an alpine meadow affected by yak excreta on the Qinghai-Tibetan plateau during summer grazing periods. Soil Biol Biochem 41(4): 718-725. https://doi.org/10.1016/j.soilbio.2009.01.007
Lovell RD, Jarvis SC (1996) Effect of cattle dung on soil microbial biomass C and N in a permanent pasture soil. Soil Biol Biochem 28(3): 291-299. https://doi.org/10.1016/0038-0717(95)00140-9
Lu XY, Yan Y, Fan JH, et al. (2012) Gross nitrification and denitrification in alpine grassland ecosystems on the Tibetan Plateau. Arct Antarct Alp Res 44(2): 188-196. https://doi.org/10.1657/1938-4246-44.2.188
Ma XZ, Wang SP, Wang YF, et al. (2006) Short - term effects of sheep excrement on carbon dioxide, nitrous oxide and methane fluxes in typical grassland of Inner Mongolia. New Zeal J Agr Res 49(3): 285-297. https://doi.org/10.1080/00288233.2006.9513719
Miller DJ (1990) Grasslands of the Tibetan Plateau. Rangelands Archives 12(3): 159-163.
Monaghan RM, Cameron KC, McLay CDA (1989) Leaching losses of nitrogen from sheep urine patches. New Zeal J Agr Res 32(2): 237-244. https://doi.org/10.1080/00288233.1989.10423459
Petersen SO, Sommer SG, Aaes O, et al. (1998) Ammonia losses from urine and dung of grazing cattle: Effect of N intake. Atmos Environ 32(3): 295-300. https://doi.org/10.1016/s1352-2310(97)00043-5
Sharpley AN (1991) Effect of soil pH on cation and anion solubility. Commun Soil Sci Plan 22(9-10): 827-841. https://doi.org/10.1080/00103629109368457
Singh J, Saggar S, Giltrap D, et al. (2008) Decomposition of dicyandiamide (DCD) in three contrasting soils and its effect on nitrous oxide emission, soil respiratory activity, and microbial biomass - An incubation study. Aust J Soil Res 46(7): 517-525. https://doi.org/10.1071/SR07204
Somda ZC, Powell JM, Bationo A (1997) Soil pH and nitrogen changes following cattle and sheep urine deposition. Commun Soil Sci Plan 28(15-16): 1253-1268. https://doi.org/10.1080/00103629709369872
Sun W, Li SW, Wang JH, et al. (2021) Effects of grazing on plant species and phylogenetic diversity in alpine grasslands, Northern Tibet. Ecol Eng 170: 106331. https://doi.org/10.1016/j.ecoleng.2021.106331
van Groenigen JW, Palermo V, Kool DM, et al. (2006) Inhibition of denitrification and N_2O emission by urine-derived benzoic and hippuric acid. Soil Biol Biochem 38(8): 2499-2502. https://doi.org/10.1016/j.soilbio.2006.02.023
van Groenigen JW, Velthof GL, van der Bolt FJE, et al. (2005) Seasonal variation in N_2O emissions from urine patches: Effects of urine concentration, soil compaction and dung. Plant Soil 273(1-2): 15-27. https://doi.org/10.1007/s11104-004-6261-2
Whitehead DC (2000) Nutrient elements in grassland: soil-plant-animal relationships. CABI Publishing.
Yoshitake S, Soutome H, Koizumi H (2014) Deposition and decomposition of cattle dung and its impact on soil properties and plant growth in a cool-temperate pasture. Ecol Res 29(4): 673-684. https://doi.org/10.1007/s11284-014-1153-2
Yue GY, Zhao L, Zhao YH, et al. (2010) Research Advances of Grassland Ecosystem CO_2 Flux on Qinghai-Tibetan Plateau. J Glaciol Geocryol 32: 166-174. https://doi.org/10.3724/SP.J.1077.2010.01263
Zhang HR, Fu G (2021) Responses of plant, soil bacterial and fungal communities to grazing vary with pasture seasons and grassland types, northern Tibet. Land Degrad Dev 32(4): 1821-1832. https://doi.org/10.1002/ldr.3835