

Feces nitrogen release induced by different large herbivores in a dry grassland

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Abstract. Large herbivores have pronounced effects on nutrient cycling in grasslands. These organisms are known to alter the quality and quantity of plant production as well as the amounts and quality of plant litter and animal wastes. The generalization that the relative quality of detritus inputs is enhanced by herbivores is well known, but how this process is affected by diet selection processing and feces production of different large herbivores remains largely unstudied. Here, we measured how these differences for cattle and sheep on a dry grassland might influence nitrogen (N) mineralization from feces. We found that cattle of larger body size tended to select the low quality grass *Stipa grandis* as their major food source. In contrast, the subdominant grass *Leymus chinensis*, with relatively high N content, was a majority in the diet of smaller sheep, when palatable forbs were insufficient in the field. This diverse diet quality resulted in a C:N ratio of cattle feces that was higher than that of sheep feces. Relatively higher labile C availability in the cattle feces, namely relatively higher cellulose/hemicellulose contents, promoted microbial growth and in turn accelerated cattle feces decomposition. A surprise finding was that the feces from cattle mineralized about twice as much N as feces from sheep, despite the latter having slightly higher N content. From a grassland productivity perspective, increasing the proportion of large body-sized species in grazing herbivore assemblages perhaps is beneficial to forage productivity and nutrient recycling by the rapid degradation of feces.

Key words: cattle; diet selection; feces decomposition; N cycling; N mineralization; sheep.

INTRODUCTION

Large herbivores play a major role in regulating the nutrient cycling and primary production in grassland ecosystems. These animals have a variety of effects on nutrient fluxes, including herbivore-mediated changes in plant community composition, production and the deposition of feces (Frank and McNaughton 1992, Ritchie et al. 1998, Bagchi and Ritchie 2010). Previous studies indicate that the variation of nitrogen (N) losses and gains in typical steppe systems are closely related to grazing, which has been both diverse in terms of grazers and highly variable over time (Mack and Thompson 1982, Gong et al. 2008). The often low substrate quality of plant litter limits decomposition rates of this material while feces and urine provides a fast decomposition pathway in grasslands by returning highly decomposable resources that are rich in plant-

available nutrients (Bakker et al. 2004). The direct effect of herbivores on nutrient dynamics through feces production is potentially overshadowed by the indirect effect that herbivores have on litter quality and quantity (e.g., the higher quality litter produced in the presence of herbivores) (Pastor et al. 1993, Sirotinak and Huntly 2000), and most terrestrial studies focus on litter decomposition and often ignore feces decomposition. However, and especially in grasslands, where 30–50% of aboveground plant biomass is consumed annually by herbivores (Towne et al. 2005, Giese et al. 2013), feces decomposition is a significant, codominant pathway for nutrient cycling within those intensive grazing systems. Moreover, the return of nutrient-rich feces can provide rapid nutrient pulses that significantly increase N availability (Frost and Hunter 2004), which, if timed appropriately, could stimulate plant production and soil microbial activity (Tracy and Frank 1998, Van der Wal et al. 2004, 2011). Heavy grazing, hay-making, and sheep excrement are important pathways of N losses from dry grasslands in China (Giese et al. 2013). Obviously, the application of effective grazing management strategies is very important for maintaining ecosystem

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functioning in China and in similar grasslands found elsewhere, and more attention needs to be paid on the impact of large herbivores on nutrient cycling through feces returning.

Feces decomposition can be a complex process, because many factors influence rates, such as the presence or absence of dung beetles, changeable environments where the feces are deposited, and fecal chemical composition. For example, dung beetles can prevent the loss of N through ammonia (NH₃) volatilization, accelerate N mineralization and alter the microorganism fauna in feces by brood balls during feeding and nesting (Gillard 1967, Kazuhira et al. 1991). Likewise, the rates of decomposition are suppressed by sun baking of the feces (Horgan 2005). The chemical composition of feces and its C:N ratio are critical for predicting rates of feces decomposition and nutrient release. Usually, a low C:N ratio of relatively undecomposed organic matter may accelerate metabolic efficiency of decomposer microflora that in turn increases decomposition rates (Garret 1963, Swift and Anderson 1989) and increase N release rates (Sitters et al. 2014).

Chemical composition of feces can immediately be altered by subtle variations in diet (Coates et al. 1991, Codron et al. 2007), and this means that fecal chemical composition is affected by the diet selection of herbivores. Small herbivores have selective consumption of palatable plants by reason of comparatively high metabolic demands and small gut capacities, whereas larger herbivores have higher tolerance of low plant nutrient content, but demand a greater quantity of food to maximize performance (Olofsson et al. 2004, Wang et al. 2010). Previous work has reported that the feces of browsers has higher N content than that of grazers, and the N content in feces decrease with increasing body size (Codron et al. 2007). Focusing only on ruminant grazers, herbivores still differ significantly in fecal nutrient contents. For instance, the N content in feces of reedbuck is twice as high as that of hartebeest (Sitters et al. 2014). Likewise, there is the same trend in N content between cattle of large body size and sheep of small body size (Esse et al. 2001). Consequently, the behavioral and physiological traits of herbivores are most likely to determine the chemical composition of their feces.

We conducted two field experiments to investigate the processes of feces decomposition and nutrient release from cattle and sheep, two dominant grazers of different body size in a dry grassland ecosystem in China. This study provided a direct experimental approach to measure how herbivores affect C and N cycling via specific plant selection and their respective digestive abilities. We tested the following hypotheses: (1) fecal chemical composition is potentially connected with the diet selection of herbivores, (2) fecal decomposition and N release is closely related to their chemical composition, and (3) herbivores of different body sizes can regulate N flux by excreting qualitatively different feces.

MATERIALS AND METHODS

Study area and experimental design

Our study was carried out at the field observation station of National Climate Observatory in Xilinhot (44°08' N, 116°20' E; 1,100 m elevation), Inner Mongolia Autonomous Region, China. It is semiarid continental climate characterized by dry, cold winters and wet, mild summers. Based on meteorological data provided by the National Meteorological Administration of China, mean monthly temperatures range from −19.5°C in January to 21.2°C in July, with a mean annual temperature of 2.4°C (data available online).⁶ Annual precipitation averaged around 281 mm from 1953 to 2013, and nearly 85% occurring in the growing season from May to September, coinciding with the peak temperatures (see Appendix S1: Fig. S1). Annual potential evapotranspiration is approximately 70% more than mean annual precipitation (Zhang et al. 2011), and mean annual relative humidity is 57.13%.

The common soil type in this area is a kastanozems soil with poor fertility, low organic matter content (less than 4%), and a marked calcic horizon (Wu and Loucks 1992), while sandy soil and meadow soil are two major non-zonal soil types. The vegetation is typical steppe dominated by perennial grasses *Stipa grandis* P. Smirn., often accompanied by subdominant species *Leymus chinensis* (Trin.) Tzvel, which represents the most widely distributed grassland communities in the eastern Eurasian steppe region (Zhu 1993). In addition, more than 85 vascular plant species have been found in the study area, including *Cleistogenes squarrosa* (Trin.) Keng, *Agropyron cristatum* (L.) Gaertn., and *Allium tenuissimum* L. (Wu et al. 2015).

The study area has a long history of free-range grazing by domestic livestock, such as native cattle, horses, sheep, and goats. Cattle and sheep are the dominant, large herbivores in recent decades. Previous studies have found that there is normally a different dietary preference between cattle and sheep. Sheep favor the plants with high content of nutrients while cattle demand a greater quantity of food to maximize performance (Liu et al. 2015).

Differences in diet selection between different body-sized herbivores

A grazing experiment was set out in the field to reveal the relationship between the foraging behavior of herbivores and the chemical composition of their feces. Nine plots were established nearby the decomposition experiment region. All the plots were arranged in a randomized complete block with three replicates. Three plots in each block were randomly assigned to the following grazing treatments: (1) no grazing (control), (2) grazing by cattle, (3) grazing by sheep. Stocking rate was designed for a moderate intensity level of grazing

⁶ <http://data.cma.cn>

(amounted to removal of approximately 50% of above-ground plant biomass) that was achieved by using 10 adult Simmental cattle or 85 adult Inner Mongolian Fine-wool sheep. Three grazing intervals averaging two weeks in duration occurred from June to August.

In early June, we commenced herbivore manipulations within the blocks. The cattle and sheep were trained to accompany experimenters in the first two grazing trials so that they were habituated to grazing under monitoring. After these adjustment periods, in the third grazing trial (in August), the foraging behavior of individual sheep and cattle was carefully monitored once a day from 08:00 to 08:30. We randomly selected three individuals from each species and observed them at a short distance to record plant species eaten and the number of bites taken. These data were used to calculate the diet selection of cattle and sheep as percentage of total vegetation.

Aboveground biomass in the plots was sampled during late August by clipping all plants inside 12 quadrats of 0.25 m² that were located on two diagonal lines within each plot and by drying samples to a constant mass. The dry mass of each plant species per quadrat averaged over the three blocks was used to calculate its aboveground biomass. Five subsamples of *S. grandis*, *L. chinensis*, and *Anemarrhena asphodeloides* were obtained from plant samples inside the quadrats in the no-grazing treatment and were ground for nutrient analyses. Both of total organic carbon (TOC) and total nitrogen (TN) contents in the plants were measured with an element analyzer (vario EL cube; ELEMENTAR, Langensfeld, Hesse, Germany).

Feces nutrient release associated with the chemical composition

A decomposition experiment using the litterbag technique was carried out in the field between June and October in 2013. Feces bags used in this experiment were constructed as 30 × 20 cm rectangles of 1-mm polyester mesh, sewn together on three sides. The mesh size permitted entry of bacteria, fungi, and small invertebrates, but prevented dung beetles from entering. When discussing the results, we will refer to the effect of microbes and microinvertebrates only, although we recognize that dung beetles can have large effects on dung decomposition and nutrient release rate (Jay-Robert et al. 2008, Nichols et al. 2008). Fresh feces used in bags were collected from the soil surface in livestock pens. Sheep and cattle, which lived in the pens at night, were also used to record their diet selection. Preexisting feces on the ground had been removed about one week before and we only collected the fresh feces. According to our observation, there were few coprophagous macroinvertebrates (e.g., dung beetles) in the pens, and they did not have enough time to colonize the fresh feces. It had not rained in the past one week before our feces collection and daily mean temperature was around 15°C. The collected cattle and sheep feces were put in plastic containers separately and mixed evenly. We did

not destroy the physical structure of the feces during mixing. Afterward, the fresh feces were placed on ice in a cooler while fieldwork was in progress and taken to the field laboratory at the end of each day for storage at 4°C.

Fifty bags filled of fresh feces (about 100 g dry matter per bag) were randomly placed in an area (30 × 30 m) with similar soil type and plant species composition, which was fenced by wire mesh (1 m height) to prevent trampling by large herbivores. All the feces bags were put on the soil surface in early June, and five bags of each feces type were retrieved at a 30-d interval from early June to early October. All of the retrieved feces samples were stored at −20°C in an industrial freezer, and taken to the laboratory in the Institute of Grassland Science, Northeast Normal University, Changchun City, Jilin Province, China.

We determined feces microbial biomass carbon (MBC), TOC, TN, and fiber contents (neutral detergent fiber NDF, acid detergent fiber ADF, and acid detergent lignin ADL). MBC were determined using the fumigation–extraction technique. Feces samples were fumigated with CHCl₃ for 24 h at 25°C. After removal of the CHCl₃, soluble C was extracted from fumigated and unfumigated samples with 50 mL of 0.5 mol/L K₂SO₄ for 30 min on an orbital shaker. TOC in filtered extracts was determined using an organic carbon analyzer (vario TOC cube, ELEMENTAR). Microbial C flush was converted to MBC using a *k*_{EC} factor (extraction coefficient which is used for MBC estimations) of 0.45. Afterward, all the feces samples were dried to constant mass and weighed to the nearest 0.01 g. TOC and TN contents were measured on oven-dried milled material with an element analyzer (vario EL cube, ELEMENTAR). Fiber contents were determined by exposure to neutral detergent and acid detergent solutions with a Fibertec M6 Fiber Analyzer (FOSS, Hillerød, Denmark) yielded neutral detergent fiber and acid detergent fiber fractions, respectively. ADL was determined by exposure of the ADF fraction to 72% H₂SO₄. Cellulose and hemicellulose were calculated based on NDF, ADF, and ADL. We defined cellulose/hemicellulose as labile C.

We measured ammonium (NH₄⁺-N) and nitrate (NO₃[−]-N) in soil under the feces bags at the beginning of the experiment (early June) and 30 d later. We took 2 cm diameter, 10 cm deep cores from the soil under five bags of each feces type. Five soil cores under bags without feces were also taken as a control. All soil samples were placed on ice in a cooler and immediately taken to the field laboratory where 10 g subsamples were extracted with 50 mL of 2 mol/L KCl for 30 min on an orbital shaker. NH₄⁺-N and NO₃[−]-N contents per gram of dry soil were determined with a continuous flow analyzer (Futura; AMS France, Frépillon, France). Total soil mineral N was the sum of NH₄⁺-N and NO₃[−]-N contents.

Statistical analysis

All analyses were performed with software R version 3.3.2 (R Core Team 2016). Proportions of each plant species foraged (P) by herbivores were used for representing

dietary preference of cattle and sheep, which were calculated as following formula: $P = \frac{n_i}{N} \times 100\%$, where n_i is the number of bites on plant species (i) and the total number of bites (N). The C:N ratios of diet were calculated from diet records and the C:N ratios of relevant plant species. Relative C loss of feces was calculated by the percentage of the differences between fecal initial and residual C at a given time. C release rate (CRR) of feces was calculated by the differences in the relative C loss between two consecutive retrieved feces samples at a 30-d interval. By using the same method, we calculated feces relative N loss and N release rate (NRR).

Effects of herbivores species and time on relative residual feces mass, nutrient releases, fecal MBC, and fecal C:N ratios were determined with a two-way ANOVA (analysis of variance). The statistical differences between levels were determined by post-hoc Tukey's test with significance for $P < 0.05$. Data of soil mineral N and soil water content were analyzed with a repeated-measures ANOVA. Simple linear regression was used to assess the relationships between the CRR and NRR of feces. A double exponential model was used to fit the dynamics of feces decomposition (Olson 1963, Gong et al. 2016). We modeled the fraction of remaining feces mass (dry matter) to the initial feces mass (dry matter) as a function of time using regression analyses $(M_t/M_0) = \alpha e^{-k_1 t} + (1 - \alpha)e^{-k_2 t}$, where M_t/M_0 is remaining mass ratio and t denotes time; α represents labile fraction, while $(1 - \alpha)$ represents more recalcitrant fraction; k_1 and k_2 are decay (or kinetic) rate constants. Assumptions of normality and heteroscedasticity were tested. If necessary, some data were transformed to meet assumptions of normality and homogeneity of variance. When transformations were unsuccessful, nonparametric tests were used instead of parametric tests.

RESULTS

Vegetation, foraging behavior and the chemical composition of plant and feces

Common plant species in our study area are listed in Table S1 (see Appendix S1), together with plant functional types. Grass species were dominant, and their mean above-ground biomass were higher than that of forbs, which accounted for 95.7% and 4.3% of total aboveground biomass, respectively (Appendix S1: Table S2). In the grass group, *S. grandis* was ranked first (81.1%), followed by *L. chinensis* at 11.4%. For the forb group, the dominant species, *A. asphodeloides*, contributed 2.5% in total aboveground biomass.

A significant difference in foraging behavior was found between cattle and sheep in the grazing experiment (Table 1). The cattle tended to select *S. grandis* as their major food source, but the sheep favored *L. chinensis*. In contrast, while three forbs, *A. asphodeloides*, *A. tenuissimum*, and *Thalictrum petaloideum*, were consumed by the herbivores, only the first species was selected by both

TABLE 1. Proportions of plant species foraged by cattle and sheep (%), and the chemical composition of their fresh feces (mg/g).

Variables	Cattle	Sheep
Grass species (%)		
<i>Stipa grandis</i> P. Smirn.	81.8 ^{Aa} ± 5.3	27.3 ^{Bb} ± 5.7
<i>Leymus chinensis</i> (Trin.) Tzvel.	11.3 ^{Bb} ± 4.6	66.8 ^{Aa} ± 7.3
<i>Cleistogenes squarrosa</i> (Trin.) Keng	–	1.1 ± 0.4
Forb species (%)		
<i>Anemarrhena asphodeloides</i> Bunge	6.9 ± 1.8	4.4 ± 1.9
<i>Allium tenuissimum</i> L.	–	0.3 ± 0.2
<i>Thalictrum petaloideum</i> L.	–	0.1 ± 0.1
C:N ratios of diet	36.5 ^a ± 0.6	32.9 ^b ± 0.2
Feces (mg/g)		
C	396.3 ^a ± 1.0	356.6 ^b ± 0.9
N	22.6 ^b ± 0.2	24.2 ^a ± 0.1
C:N	17.5 ^a ± 0.1	14.7 ^b ± 0.1
ADL	175.4 ^b ± 7.6	214.3 ^a ± 3.4
Hemicellulose	167.3 ^a ± 6.3	144.5 ^b ± 3.2
Cellulose	103.5 ^a ± 8.6	31.8 ^b ± 4.5
ADL/N	7.8 ^b ± 0.3	8.9 ^a ± 0.1

Notes: Values are mean ± SE. Different lowercase letters denote significant differences between herbivores ($P < 0.01$). Different capital letters denote significant differences between plants ($P < 0.05$). A dash denotes that the species were not foraged. ADL, acid detergent lignin.

cattle and sheep, but with no significant difference for their intake proportions.

There were differences in C, N, and C:N ratios of main plant species foraged by cattle and sheep (see Appendix S1: Fig. S2). The C content of grass *S. grandis* and *L. chinensis* were 457.5 ± 1.4 and 445.5 ± 0.8 mg/g (mean ± SE) respectively, which were higher than the forb *A. asphodeloides* (426.6 ± 0.9 mg/g; $F_{2,12} = 198.90$, $P < 0.001$). In contrast, the N content of *A. asphodeloides* was highest (18.1 ± 0.5 mg/g), followed by the other two grasses, *S. grandis* and *L. chinensis* ($F_{2,12} = 54.13$, $P < 0.001$). The variation in the N content was a major factor affecting C:N ratios. Clearly, the larger percentage of *S. grandis* consumed by cattle resulted in significantly higher C:N ratio of their diet ($t_4 = 5.77$, $P < 0.01$).

C, hemicellulose, and cellulose contents in cattle feces were significantly higher than those in sheep feces ($t_8 = 28.79$, $P < 0.001$; $t_8 = 3.20$, $P < 0.05$; $t_8 = 7.36$, $P < 0.001$; Table 1). In contrast, the sheep feces have the relatively higher N and ADL contents ($t_8 = -8.61$, $P < 0.001$; $t_8 = -4.71$, $P < 0.01$). As a result, the ADL/N ratio of sheep feces was significantly higher than that of cattle feces ($t_8 = -3.44$, $P < 0.01$).

Temporal dynamics of feces mass and microbial biomass C

In the feces decomposition experiment, both herbivore species and time significantly influenced feces mass,

TABLE 2. ANOVA results (F values and significance levels) for the effects of herbivores species (S) and time (T) on feces mass (M , %), microbial biomass carbon (MBC), relative C loss, relative N loss, C release rates (CRR), N release rates (NRR), and C:N ratios of feces from feces decomposition experiment.

Factors	df	M	MBC	C loss	N loss	CRR	NRR	C:N ratios
S	1(32)	71.1***	NS	240.9***	233.5***	12.4**	12.0**	224.4***
T	3(32)	62.8***	14.2***	37.4***	16.6***	100.9***	67.1***	128.4***
S \times T	3(32)	NS	5.9*	13.5***	NS	66.0***	16.5***	50.3***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. NS: $P > 0.05$.

microbial biomass C, relative C loss, relative N loss, and relative release rates of C and N (Table 2).

The feces mass (dry matter) consistently declined over 120 d of the experiment, with 30–40% of the feces mass was lost fast within the first 60 d (Fig. 1a). The difference in the feces loss between cattle and sheep was significant from day 30 ($t_8 = -4.80$, $P < 0.01$). At the end of the experiment, relative residual mass of the cattle feces was less than that of the sheep feces ($t_8 = -3.42$, $P < 0.01$). The decomposition dynamics of cattle and sheep feces were fitted well with the double exponential decay model ($R^2 = 0.99$ for cattle feces, $R^2 = 0.99$ for sheep feces, see Appendix S1: Table S3). Cattle feces decomposed faster than sheep feces, and this difference was due to the higher k_1 for cattle feces (0.027 ± 0.006) than that of sheep feces (0.017 ± 0.008).

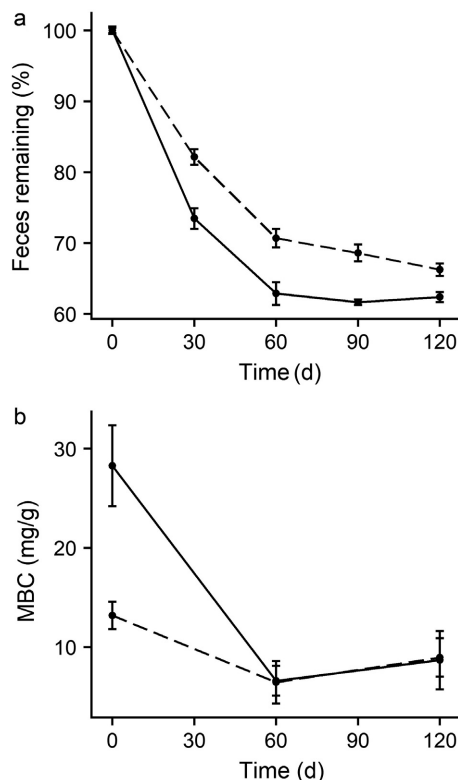


FIG. 1. Dynamics of (a) mass (dry matter) and (b) microbial biomass carbon (MBC, mean \pm SE) of cattle (solid line) and sheep (dashed line) feces.

With loss of the fecal mass, both of the fecal MBC and water content showed a decreasing tendency regardless of cattle or sheep (Fig. 1b; Appendix S1: Fig. S3). The highest MBC and water content were found in the cattle feces at the beginning of the experiment.

Nutrient releases and relationships of variables related to feces decomposition

We found differences in the feces relative C and N losses between cattle and sheep were similar to those of the feces mass losses (Fig. 2a, Table 2). Both of feces C and N released rapidly within the first 60 d and rates subsequently declined, regardless of the source of the feces. There were significant differences in the proportions C and N losses of feces between cattle and sheep at the end of the growing season ($F_{3,16} = 132.80$, $P < 0.001$) with about 43% of the C and 32% of the N in cattle feces released. In contrast, the losses of the C and N in sheep feces were 25% and 18%, respectively. Temporal dynamics of the feces relative C and N losses (Fig. 2a) suggested that feces C and N release rates were different between cattle and sheep during the different periods of time (Fig. 2b, Table 2). The relative C, N release rates of the cattle feces showed a unimodal curve and both peaked at day 30; meanwhile soil mineral N content underneath the cattle feces bags was nearly two times higher than that underneath the sheep feces bags (Fig. 3a), in spite of the overall decrease in mineralization of soil N due to drought (Fig. 3b). In contrast, the relative N release rate of sheep feces reached a peak at day 30, but the peak of the relative C release rate exhibited a lag, and appeared at day 60.

The fecal C:N ratios changed during decomposition and were significantly influenced by the herbivores species and time (Fig. 2c, Table 2). At the start of experiment, the initial C:N ratio of the cattle feces was 17.5 ± 0.1 , significantly higher than the 14.7 ± 0.1 of the sheep feces ($t_8 = 21.58$, $P < 0.001$). Afterward, the C:N ratio of the cattle feces decreased steeply to a low of 14.2 ± 0.2 at mid growing season (day 60; $t_8 = 16.84$, $P < 0.001$). In contrast, the C:N ratio of the sheep feces rose to the peak at 15.3 ± 0.1 at day 30, and then declined to 13.1 ± 0.1 at day 90, which was substantially lower than the initial value ($t_8 = 13.75$, $P < 0.001$). At the end of growing season (in October), the C:N ratio of the cattle feces was again significantly higher than that of the sheep feces ($t_8 = 6.26$, $P < 0.001$).

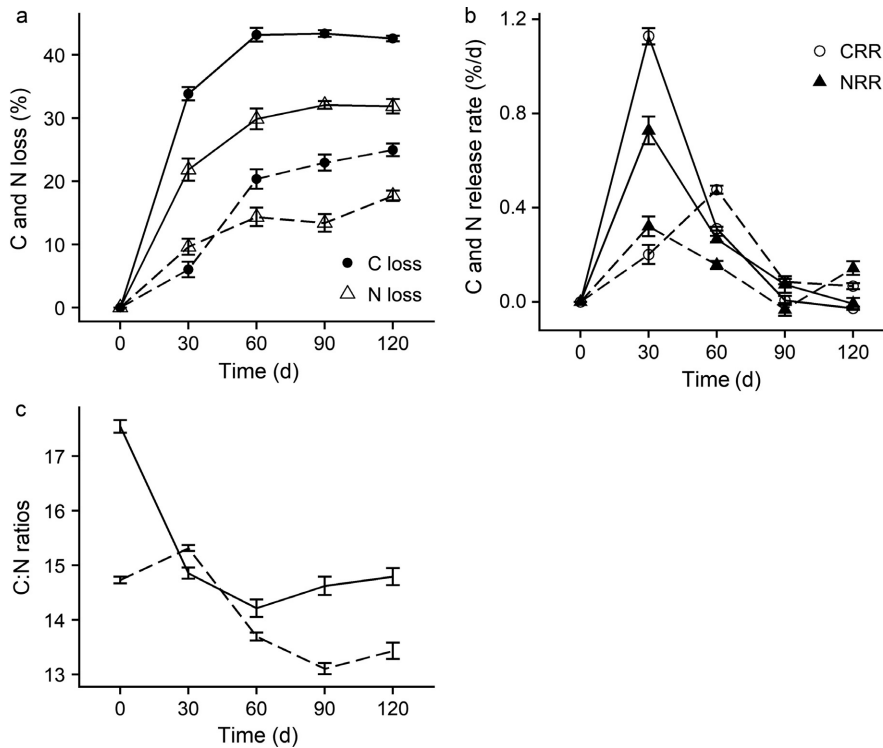


FIG. 2. Dynamics of (a) C and N loss, (b) C and N release rates (CRR, NRR), and (c) C:N ratios (mean \pm SE) of cattle (solid line) and sheep (dashed line) feces.

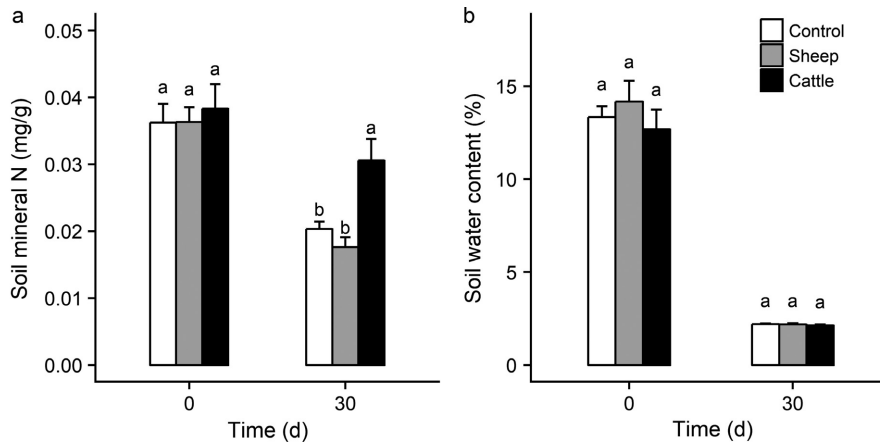


FIG. 3. (a) Soil mineral N content and (b) water content (mean \pm SE) under feces bags at day 0 and day 30. Different letters indicate significant differences among cattle feces, sheep feces, and no feces at the same time ($P < 0.05$).

Regression analyses revealed that, for the cattle feces, the NRR increased linearly with the CRR ($R^2 = 0.96$, $P < 0.001$, Fig. 4a). In contrast, the NRR of sheep feces first increased and then decreased with the CRR ($R^2 = 0.31$, $P < 0.05$, Fig. 4b).

DISCUSSION

Few studies have explored the linkage between the diet selection by more than two large herbivores of grassland

ecosystems and their subsequent impacts on biogeochemical cycling via feces production and decomposition. Our study revealed that feces decomposition is mediated by diet selection and how the specific consumer alters the physical and chemical quality of the substrate via gut passage (Fig. 5). In some respects, we argue that, similar to plant species, consumer species may represent functional groups that contribute differential controls on nutrient feedbacks to plants. Using the lignin : N ratio of substrates to predict decomposition of feces (e.g., Melillo

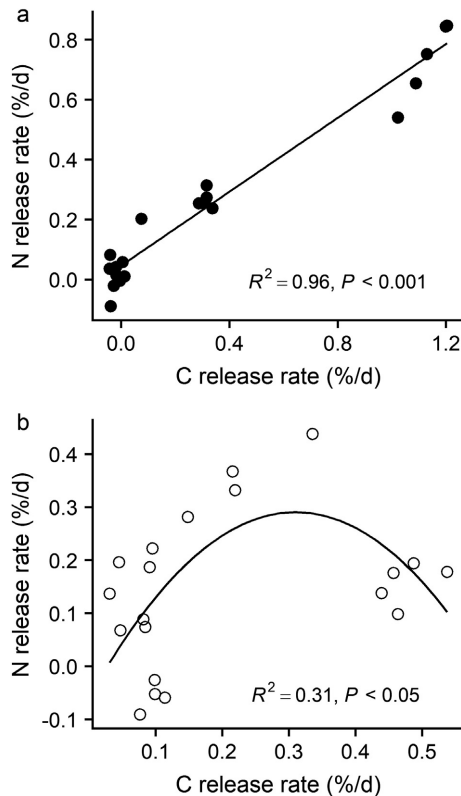


FIG. 4. Measured and fitted data for the relationship between C and N release rates from (a) cattle and (b) sheep feces.

et al. 1982), one finds a 14% difference in lignin : N ratios between two types of feces, and consistent with our study the difference predicts a more rapid decay of feces of cattle. However, the relatively similar, low C:N ratios of the two types of feces translated to a very large difference in N release from feces.

Effects of diet selection on chemical composition of feces

Our results supported the hypothesis that there was a potential connection between feces chemical composition and herbivores diet selection (Fig. 5, part 1). Compared with cattle, sheep of smaller body size ate more of the relatively N-rich *L. chinensis* and their feces also had a higher N content and lower C:N ratio (Table 1; Appendix S1: Fig. S2). Generally, there is a negative correlation between the body size of herbivores and the nutrient content of their feces, which reflects a tendency for declining diet quality with increasing body size (Codron et al. 2007, De Iongh et al. 2011, Sitters et al. 2014). Due to relatively small gut capacity, small herbivores generally require more nutrients and thus have to select higher quality foods, whereas larger herbivores with relatively large gut capacity can digest foods more thoroughly and thus can tolerate a diet of lesser quality (Illius and Gordon 1993). Alternatively, Müller et al. (2013) suggested that the larger species could eat relatively more

of a lower quality food without having to increase digestive efficiency, because their intake had a higher allometric scaling than their basal metabolism. Either interpretation results in an outcome that sheep selected the subdominant grass *L. chinensis* that has relatively high N content and low C content as major food sources, especially when palatable forbs were rich in nutrients but insufficient in the field (see Appendix S1: Table S2). In contrast, the low-quality, dominant grass *S. grandis* was a majority of the diet of the larger body-sized cattle. As a result, the C:N ratio of diet consumed by cattle was higher than that consumed by sheep. This diverse diet quality can partly explain the reverse tendency of the C and N contents between sheep and cattle feces. Another factor, digestive efficiency of herbivores, also directly affects the nutrient content in their feces. Owing to a larger gut volume, mean retention time of particles for cattle is nearly 40% more than that for sheep (Steuer et al. 2011) and thus the former have relatively higher digestive efficiency (Poppi et al. 1981). Cattle are better at digesting neutral detergent fiber (Poppi et al. 1981, Reid et al. 1990) because cattle can digest more hemicellulose and cellulose than sheep (Playne 1978). However, a review of published research indicated that N assimilation of cattle was 16% less than that of sheep (Kohn et al. 2005). The different digestive efficiency on the plant fibers and N conflicts with our finding of the relatively high C:N ratio in cattle feces. Many studies have indicated that fecal chemical composition could directly reflect the diet quality of free-ranging herbivores (Wofford et al. 1985, Hodgman et al. 1996, Codron et al. 2005). It is likely that interspecific differences in the digestive efficiency are hidden by variations in diet quality.

Effects of fecal C:N ratios on the decomposition

The outcome of diet selection and gut processing results in four possible outcomes to the C:N composition of fecal material (Table 3). Once below a certain threshold, a low C:N material can be expected to mineralize N (Manzoni et al. 2008, 2010), but the rate can be either rapid or slow depending upon the quality of carbon (Murphy et al. 1998, Hättenschwiler and Jørgensen 2010). In this study, compared with other herbivores (Sitters et al. 2014), both cattle and sheep produce low C:N feces, and our results are clear that the cattle feces contained more labile C components (Table 1). Both the hemicellulose and cellulose contents in cattle feces were higher than those in sheep feces, which can be more easily degraded and utilized by many microorganisms as carbon and energy sources (Sánchez 2009). In contrast, sheep feces had more ADL, which is the most recalcitrant component of plant cell walls. The higher MBC in cattle feces indicated that more labile C and high water content accelerated the feces decomposition by mitigating C and water limitations of coprophilous microorganisms in a low C:N substrate (Part 2 in Fig. 5). The decay rate (k_1) of cattle feces also confirmed that their rapid

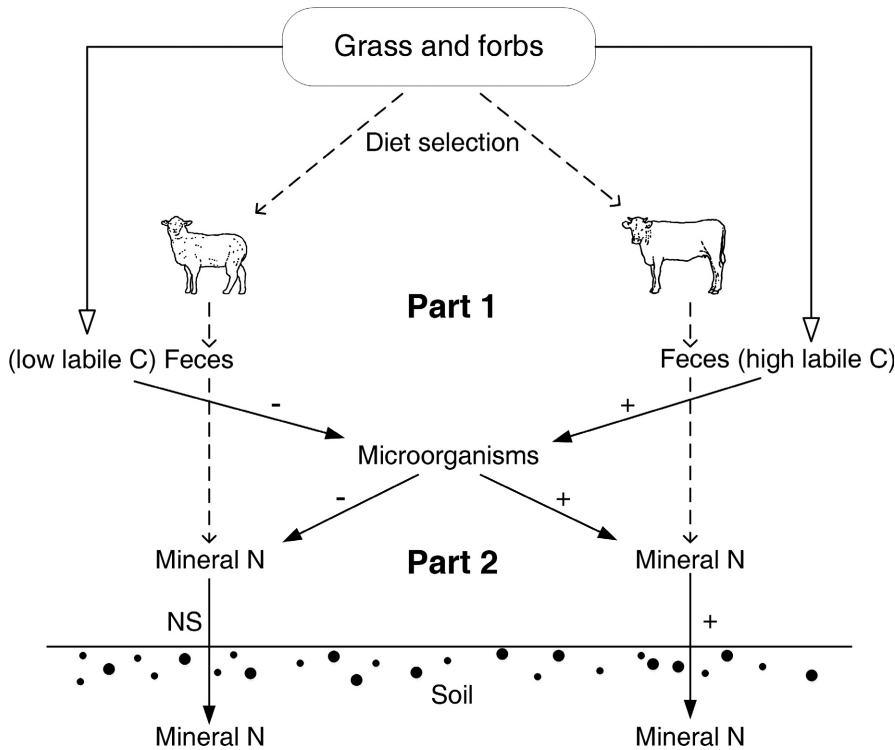


FIG. 5. Proposed mechanisms for how different herbivores alter feces N release in the semiarid steppe. The release is controlled by the N content, C:N ratio, and the quality (carbon composition) of the feces. Effects are noted as NS (not significant), + (positive), and – (negative). Part 1 of the model identifies diet selection and digestive efficiencies, while Part 2 identifies the microbial responses to feces characteristics.

decomposition was closely related to the more labile C in the feces. In addition, the statistically not significant results for k_2 suggested that the recalcitrant components in feces might require long time periods to decompose. Obviously, the rapid decomposition of cattle feces and the slow decomposition of sheep feces respectively confirmed the first two possible outcomes in Table 3. Nonetheless, this outcome was somewhat of a surprise since we assumed that diet quality might be an important factor influencing the outcome of feces decomposition rates. There is only a tiny difference in the gross hemicellulose and cellulose between *S. grandis* and *L. chinensis* (less than 5%), but ADL in the former is

about 16% more than that in the latter (Schiborra 2007). Differences in the fiber contents between diet and feces were probably induced by differential absorption of substrates in the gut of the consumer.

A second surprise of our study was the difference observed between N releases of cattle and sheep feces (Fig. 2a). Clearly, one might expect N release to mimic rates of mass loss and C release, and this was observed for the cattle (Fig. 4a), in spite of the somewhat higher C:N ratios of the cattle feces. Decomposition from cattle feces released almost twice the amount of N than did the feces of sheep. For cattle, this result indicates that a strong coupling of C and N flux accelerated N release. Since N release is relatively less than C loss, the C:N ratios of cattle feces declined through time during the early stages of decomposition (Fig. 2c). The differences in the fecal N releases between cattle and sheep were in accord with the first two possible outcomes shown in Table 3. We note that sheep feces behaved differently than cattle feces in showing a period where C:N ratios increased, and a pattern where N loss rates during different phases of decomposition were inversely related to C:N ratios. While cattle feces continuously released both C and N, sheep feces immobilized N during a period of this study (Fig. 2b). These results were probably related to relatively low labile C content in sheep feces. Responding to low C availability, microorganisms may enhance C use efficiency and

TABLE 3. A theoretical framework for effects of C:N ratios and carbon quality on decomposition of plant litter and feces.

C:N ratio	Carbon quality†	Effect on decomposition	Effect on N mineralization
Low	High	Rapid	Rapid
Low	Low	Slow	Slow
High	High	Rapid	Immobilization‡
High	Low	Slow	Slow

†High carbon quality is defined as high amounts of hemicellulose and cellulose, low amounts of acid detergent lignin.
‡May also accumulate N from sources other than those contained in the substrate.

utilize more N to produce extracellular enzyme for degrading relatively resistant organic matters (Jeger et al. 2008). Since N release is relatively more than C loss, the C:N ratios of sheep feces rose through time during the first 30 d of decomposition. At this point, the subsequent decomposition of sheep feces was partly accordant with the third possible outcomes for the increasing fecal C:N ratio (Table 3). Increased labile C contents from degraded complex organics accelerated the mass loss and nutrient release of sheep feces through promoting microbial activities. During this period, decreasing N release rates and fecal N immobilization implied that microorganisms may accumulate N from other sources (e.g., obtained by the extracellular enzyme activity on external substrate, from the nearby soil or atmospheric deposition) other than those contained in the substrate until exhaustion of those N sources (Figs. 2a and 4b). Finally, labile C contents in feces were rapidly consumed, and the rates of mass loss and N mineralization both slowed down during the late stages of decomposition. With regard to the last possible outcome in Table 3, it did not occur in our study, and we need to do more work to verify it in the future. Generally, our results partly supported our second hypothesis that the fecal N release and decomposition were related to fecal chemical composition. However, the labile C content in the feces was more important for the decomposition process than fecal C:N ratio.

In contrast with the slow N release of sheep feces, cattle feces significantly increased soil mineral N that is an important plant resource. There was an overall reduction in the soil mineral N, which probably resulted from the suppression of N mineralization due to low soil water content (Fig. 3b; Wang et al. 2006). A large amount of mineral N from cattle feces migrated into the soil and increased the soil mineral N content at the peak phase of fecal N mineralization (Figs. 3a and 4). Cattle feces had a positive effect on soil quality and partly compensated for the suppression of N mineralization by drought. This result is consistent with other studies (Eghball 2000, Edmeades 2003), and supports our last hypothesis that herbivores of different body sizes can regulate N flux by excreting different qualitative feces. Undoubtedly, the enhanced soil mineral N is beneficial to grassland productivity (Elser et al. 2007).

CONCLUSION

Our results show that fecal decomposition process is closely related to the chemical composition of feces, which was potentially regulated by the diet selection of large herbivores. Specially, and somewhat surprisingly, the study demonstrates that cattle feces with relatively higher labile C content compared to that of sheep can enhance and accelerate fecal N release within a short time (e.g., several months or one growing season). Not only the high fecal labile C content but also the higher fecal water content is potentially beneficial to facilitate decomposition activities by coprophilous

microorganisms and to promote N release from these substrates. In consequence, a large amount of mineralized N from cattle feces migrated into the soil and increased soil mineral N contents, which is closely related to grasslands productivity. Such acceleration of N release by the high fecal labile C content is probably more prevalent than previously thought and requires attention at multiple temporal scales. This study only examined the early stages of feces decomposition between two herbivores species, and there is a need for a longer-termed experiment across multiple grasslands. When the two species may have similar diets or sheep may even forage on lower quality items in some habitats/seasons, their feces probably mimic the decomposition of cattle feces in our studies. Additionally, it is an important direction for our future research that a larger analysis across additional herbivore species, along with greater dietary breadth to determine how much diet selectivity affects feces composition and subsequent C and N mineralization for each of the herbivore species. From a grassland productivity perspective, increasing the proportion of large body-sized species in grazing herbivore assemblages is perhaps often beneficial to forage productivity and nutrient recycling by the rapid degradation of feces.

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