



Different effects of sheep excrement type and supply level on plant and soil C:N:P stoichiometry in a typical steppe on the loess plateau

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Abstract

Aims Deposition of urine and dung is a key mechanism by which herbivores influence the nutrient cycling of terrestrial ecosystems. Yet, little is known about the roles of urine, dung and their mixtures in regulating the plant and soil carbon:nitrogen:phosphorus (C:N:P) stoichiometry and their interactions.

Methods We explored how different excrement types (urine, dung and their mixtures) and supply levels [urine: 0.5, 1.0 and 1.5 L m⁻²; dung: 4.7, 9.4 and 14.2 g m⁻²; mixtures (urine+dung): 0.5 + 4.7, 1.0 + 9.4 and 1.5 + 14.2] altered the plant and soil C:N:P stoichiometry in a semi-arid grassland on the Loess Plateau.

Results We found that the aboveground biomass of the whole community was highest at the highest mixtures application rate, while the highest aboveground biomass of the three dominant species was detected at applications of 0.5 L m⁻² urine for *Stipa bungeana*, 9.4 g m⁻² dung for *Lespedeza davurica* and 1.0 L m⁻² urine + 9.4 g m⁻² dung for *Artemisia capillaris*. Urine and dung alone increased plant N concentration, which

peaked at 1.0 L m⁻² urine. Plant P concentration at 1.5 + 14.2 mixtures was significantly higher than that at control without significant difference between excrement types. Compared to the control, excrement deposition did not significantly change plant C concentration and C:N, C:P and N:P ratios. However, plant C:P and N:P ratios under the mixtures were significantly lower than those under urine or dung. Excrement applications led to more soil (total, microbial and organic) C, N and P accumulations. Compared to urine and dung, the mixtures induced higher total P concentration but lower total N concentration in soil.

Conclusions Our study demonstrated that the impacts of sheep urine and dung on plant and soil C and nutrient concentrations and stoichiometry differed from that of mixtures. The plant N:P ratio indicated balanced N:P supply for urine and dung applications but N limitation for mixtures application. This study provides experimental evidence that the excrement of herbivores plays an important role in altering ecological stoichiometry in plants and soil.

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Introduction

Herbivores play a key role in nutrient cycling through altering the quantity and quality of litter, depositing urine and dung, and trampling topsoil and vegetation, thereby changing ecosystems fertility, and ultimately

affecting the structure and functions of terrestrial ecosystems (Barthelemy et al. 2018; Sitters and Olde Venterink 2015; Wang et al. 2017). Herbivores act as spatial conveyors of carbon (C) and nutrients [e.g. nitrogen (N) and phosphorus (P)] within ecosystems, due to the fact they regularly consume these elements in the form of plant material in one location and transport them as urine and dung to another location (Augustine 2003; Le Roux et al. 2018; Veldhuis et al. 2018). Additionally, herbivores promote nutrient cycling through excrement deposition, thereby developing hotspots of plant and soil nutrients within a nutrient-poor system (Barthelemy et al. 2015; Dennis et al. 2011; Lezama and Paruelo, 2016). White et al. (2001) reported that 70–90% of N, P and other elements were recirculated in an intensive pasture with dairy cattle. However, the livestock-driven spatial-scale hotspots of plant and soil nutrients contribute to heterogeneity and patchiness of grasslands. On one hand, the plant and soil nutrient concentrations within excrement accumulation are higher than outside. On the other hand, the nutrients between the hotspots are different depending on many factors, such as different N:P supply ratios caused by urine and dung deposition (Schnyder et al. 2010), and overlapping and mixing density of urine and dung patches under different stocking rates, livestock types and gender ratios of the herd.

It is commonly accepted that within the urine and dung patches, livestock excrement enhances the nutrient cycling between the atmosphere, plant, soil and animal (Barthelemy et al. 2018; Buckthought et al. 2015; Leiber-Sauheittl et al. 2015). Despite a well-established theoretical framework on these cycling processes, experimental evidence remains scarce. By increasing mineralization of C, N and P through activities of soil microbes, urine and dung from livestock as highly decomposable nutrient resources increase soil nutrient availability and plant nutrient uptake (Barthelemy et al. 2018; Valdés-Correcher et al. 2019). The type of excrement can affect nutrient conditions in different ways. N from urine is usually rapidly dissolved into soluble N via hydrolyzation and easily taken up by plants in 2 weeks (Barthelemy et al. 2018; Witte 2011). N in dung, however, is less readily available for plants than most N compounds in urine (Barthelemy et al. 2018), because most of N and P in dung exist in organic forms, and the rates of release are regulated by environmental conditions and C:N:P stoichiometry of dung (Sitters et al. 2014). However, when urine and dung are mixed, more

N in the mixtures may be lost through NH_3 volatilization and leaching than that in urine or dung (Augustine et al. 2003; Frank and Zhang 1997; Ruess and McNaughton 1988). The different ratios of N:P supply by urine, dung and their mixtures may finally result in unbalanced plant and soil C:N:P stoichiometry within the patches, thereby influencing the productivity and species composition of plant communities (Cech et al. 2008). The ecological stoichiometry (relative element concentration) provides useful criteria for tracking the C, N and P cycles between the excrement, soil and plants in ecosystems (Zechmeister-Boltenstern et al. 2015).

Several studies about the effects of excrement on soil and plant C:N:P stoichiometry have been conducted in savanna or humid grassland ecosystems (Cech et al. 2008, 2010; Sitters et al. 2017; Sitters and Olde Venterink 2018). The nutrient enrichment in the form of urine and dung may have special importance on the low-nutrient and semi-arid grasslands, and nutrient availability is one of the main factors besides water, temperature and light regulating plant growth. Previous studies have found that a readily available nutrient source like urine could be expected to be rapidly taken up by plants and thus favour plant growth (Barthelemy et al. 2018; Buckthought et al. 2015). However, the return of nutrients from dung to soil is slow, and it may take a longer time before plant growth is stimulated (Barthelemy et al. 2015; van der Wal et al. 2004), especially in arid grasslands. Moreover, a large amount of nutrients from the urine and dung could be also immobilized by soil microbes rather than directly available for plant growth (Barthelemy et al. 2015, 2018). When urine and dung are mixed, the release rate of nutrients from their mixtures is speeding up due to urine may provide a humid environment for the decomposition of dung (Dickinson et al. 1981). Also, urine-induced increase in soil pH (Rooney et al. 2006) may accelerate the decomposition of organic matter in dung (Cheng et al. 2016). Nutrients returned in form of urine or dung have attracted more attention in the agricultural fields as they are important N and/or P sources for plants and soil in many systems (Barthelemy et al. 2018; Sitters and Olde Venterink 2018). However, only a few studies have investigated the effects of urine and dung as well as their mixtures on rangeland ecosystems.

The patterns of grazing and excretion affect the quantity and spatial distribution of nutrients in grassland ecosystems. First, the grazing system or stocking rate may cause different supply levels of excrement,

resulting in local nutrient depletion or nutrients enrichment in varying degrees. Second, whether urine and dung are excreted in the same areas can affect the densities of urine, dung and their mixtures patches. Hence, livestock may induce different ratios of N:P supplies and potentially shift the nutrient balance. We conducted this research to investigate the effects of excrement type and supply level on plant and soil C:N:P stoichiometry in a typical steppe on the Loess Plateau. We hypothesize that (1) inputs of excrement will increase the plant and soil N and P concentrations, but decrease the plant and soil C:N and C:P ratios, because of nutrient enrichment, (2) due to the various N:P ratios of different excrement types, urine will increase but dung will decrease the plant and soil N:P ratios, and the mixtures will have neutral impacts, and (3) the mixtures will alleviate the P-limitation effects of urine and the N-limitation effects of dung. The results can contribute to a theoretical basis for the sustainable grazing management of grassland in terms of element uptake and allocation.

Materials and methods

Research site

This research was carried out in a typical steppe on the Huanxian Grassland Agriculture Trial Station of Lanzhou University in Huan County, Eastern Gansu Province, China (37.12°N, 106.84°E; 1700 m above sea level). The climate in this region is categorised as a continental monsoon climate (Hu et al. 2019). The annual average air temperature is 8.4 °C and the annual average precipitation is 266.2 mm with over 70% of precipitation occurring from late June to the end of September (Fig. S1). The soil is classified as sandy, free-draining loess (Han et al. 2011). Based on the comprehensive and sequential grassland classification method, the grassland is categorized as a typical steppe (Ren et al. 2008). *Artemisia capillaris*, *Lespedeza davurica* and *Stipa bungeana* are the dominant species (Wang et al. 2019).

Experimental set-up and excrement collection and application

The study was carried out in an ungrazed control site (100 m × 50 m) set up for a long-term rotational grazing

study (Chen et al. 2010; Chen et al. 2015; Hu et al. 2019; Wang et al. 2019). This ongoing long-term experiment consists of four grazing treatments, 0, 4, 8, and 13 Tan sheep, with rotationally grazing in 12 paddocks (0.5 ha for each paddock, 100 m × 50 m) in a typical steppe grassland with visually similar botanical composition, cover and slope (~5°), representing stocking rates of 0, 2.7, 5.3 and 8.7 sheep ha⁻¹, respectively. There were three replicates for each grazing treatment. From early June to mid-September, sheep were rotationally grazed in three of 12 randomly allocated paddocks, with 10 days in each paddock and a rotation cycle of 30 days during the growing season. Tan sheep of about 20 kg in body weight were purchased each spring with a similar condition at the start of each experimental period.

To investigate the effects of excrement on the typical grassland, we set up 10 treatments, i.e. three excrement types (urine, dung and their mixtures) × three excrement supply levels (corresponding to three stocking rates of 2.7, 5.3 and 8.7 sheep ha⁻¹, respectively) and one control treatment with no excrement input (Fig. S2), with three replicates for each treatment and 30 replicates in total. In early June in 2016, an experimental area (20 m × 12 m, slope < 3°) was fenced in one of the 0-Tan sheep-grazing paddocks, and the treatments were randomly applied to 30 plots (2 m × 2 m each) established randomly in the experimental area (Fig. S2). Before the start of the experiment, we measured the soil and plant nutrient status. The soil total C, and total N and total P concentrations in the topsoil (≤ 10 cm) in 2016 were 8.63, 0.78, and 1.39 g kg⁻¹, respectively, soil available N and available P concentrations on the topsoil were 15.9 and 1.34 mg kg⁻¹, and the plant C, N, and P concentration were 426.74, 17.00, and 0.73 g kg⁻¹, respectively. There was no significant difference in soil and plant nutrients between the plots (unpublished data).

The sheep dung corresponding to stocking rates was collected from the grazing paddocks, and the urine was collected while grazing sheep individually stayed overnight in the metabolic cages specially designed for urine collection. After a day of grazing, dung was randomly collected from 10 quadrats (1 m × 1 m) in each grazing paddock, which was repeatedly sampled in three consecutive days. The dung supply levels by grazing sheep were then weighed and we calculated the means based on fresh mass. The amount of urine excreted depended on the supply level of each sheep every day and we then calculated the means. Before they were applied to the

experimental plots, dung and urine samples were stored at 4 °C. Their C, N and P concentrations and stoichiometry were measured (Table 1). The excrements of different supply levels corresponding to stocking rates were randomly applied to the plots in late July in 2016 and 2017. We set the application rates of urine and dung in order to simulate long-term stocking rate and split the function of excrement from the whole grazing process. Therefore, the amount and levels of urine and dung were based on the actual amount of sheep excrement deposited per paddock, instead of considering the proportion of nutrients added.

Measurement of plant aboveground biomass and chemical characteristics

The aboveground biomass was collected from a quadrat (1 m × 1 m) in each plot in late August 2016 and 2017. After sampling, all plants were classified to species, weighed after oven-dried at 60 °C for 48 h. The dominant species of *A. capillaris*, *L. davurica* and *S. bungeana* were used for nutrient analysis at the species level. The relative aboveground biomass was the ratio of aboveground biomass of each species to that of the community based on mass.

Plant C was analysed by using the Walkley-Black modified acid-dichromate FeSO₄ titration method (Sparks et al. 1996). Total N was determined by a Nitrogen Analyzer System (KJELTEC 2300 AUTO SYSTEM II, Foss Tecator AB, Hoganas, Sweden) after Kjeldahl digestion. Total P was measured by the H₂SO₄-HClO₄ fusion method (Sparks et al. 1996). The

C, N and P stoichiometry for each species were calculated based on mass.

For each quadrat, we determined the total means of C, N and P concentrations, and C to N, C to P, and N to P ratios of the three dominant species according to Garnier et al. (2007):

$$Nutrient = \sum_{i=1}^n p_i \times nutrient_i$$

where *Nutrient* is the total nutrient value of the three dominant species collected in a specific quadrat, *p_i* is the proportion of the aboveground biomass of species *i* to aboveground biomass of the community collected in the quadrat, and *nutrient_i* is the value of nutrient for species *i*.

Measurement of soil physical and chemical characteristics

Using a bucket auger (10 cm in diameter), 5 cores of soil were sampled in each quadrat at 0–10 cm depth after aboveground vegetation was clipped in 2017. Soil samples from the same quadrat were mixed, air-dried, and then sieved by a 1-mm and then a 0.25-mm sieve for nutrient measurements.

An elemental analyzer (Vario EI, Elementar, Germany) was used to measure soil total C and soil total N. The soil organic C concentration of the HCl-treated samples was then measured using an elemental analyzer. The soil inorganic C was the difference between soil total C and soil organic C. The soil inorganic N was determined by the continuous alkali-hydrolyzed reduction diffusing method (Stanford 1982). Soil organic N was equal to

Table 1 Nutrients of excrement and their stoichiometry (based on fresh mass) before experiments

Excrement		C:N:P stoichiometry					
type	level	C (g m ⁻²)	N (g m ⁻²)	P (g m ⁻²)	C:N	C:P	N:P
Urine	0.5	12	6	0.007	2	1724	876
	1.0	24	12	0.014	2	1724	876
	1.5	36	18	0.021	2	1724	876
Dung	4.7	0.663	0.041	0.026	16	26	2
	9.4	1	0.081	0.051	16	26	2
	14.2	2	0.123	0.077	16	26	2
Mixtures	0.5+4.7	13	6	0.033	2	391	190
	1.0+9.4	26	12	0.065	2	391	190
	1.5+14.2	38	19	0.098	2	391	190

soil total N minus soil inorganic N. Soil total P and inorganic P were determined by melt molybdenum, antimony, and scandium colorimetry (Olsen and Sommers 1982). The soil organic P was the difference between soil total P and soil inorganic P.

Microbial biomass for C, N and P concentrations were analyzed by the chloroform fumigation extraction method (Brookes et al. 1985; Vance et al. 1987). In brief, for each soil sample, pre-incubated soil (10 g, fresh weight) was used for chloroform fumigation for 24 h at 25 °C, and the same weight of soil without chloroform fumigation was used for the control. Then, 100 ml 0.5 M K₂SO₄ was used to extract microbial biomass C and N, and 0.5 M NaHCO₃ was used for the extraction of microbial biomass P. The nonfumigated soil was also extracted with 100 ml 0.5 M NaHCO₃. Concentrations of extracted C and N were determined with a TOC analyzer (Liqui TOC II, Elementar, Germany), while concentrations of extracted P were determined calorimetrically with the molybdenum blue method. The experimentally derived conversion factors for microbial biomass for C, N and P concentrations were 0.45, 0.54 and 0.40, respectively (Joergensen and Mueller 1996).

Statistical analysis

All data were analysed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The rejection level of H₀ was set at P<0.05. Before the statistical analysis, the normality of the data was confirmed by Shapiro-Wilk test (UNIVARI-ATE procedure). A generalized multivariate nested linear model (Proc GLMMIXM) was applied to test the effects of year, excrement type (urine, dung and mixtures) and supply level, and their interactions on the C, N and P concentrations and stoichiometry of the three dominant plants and

aboveground biomass of the community (Table 2). A Proc GLMMIXM was applied to test the effects of year, plant species, excrement type (urine, dung and mixtures) and supply level, and their interactions on plant C, N and P concentration and stoichiometry, aboveground biomass and relative aboveground biomass (Table S1). The supply level was nested into the excrement type. Two-way ANOVA was applied to test the effects of excrement type and supply level on soil (total, organic, inorganic and microbial) C, N and P and their stoichiometry (Table 3). An analysis of variance (ANOVA) with an adjust Tukey’s HSD post hoc test was used to compare the difference in plant C, N and P concentration and their stoichiometry, soil (total, organic, inorganic and microbial) C, N and P concentration and their stoichiometry, aboveground biomass, relative aboveground biomass among excrement supply levels (Figs. 1, 2, 3 and 4; S3-4).

Results

Plant aboveground biomass and relative aboveground biomass

Excrement deposition had weak and often no significant effects on plant aboveground biomass (Fig. 1). Compared to the control, aboveground biomass of the community was the highest at applications of 1.5 + 14.2 mixtures (Fig. 1a; Tables 2 and S1). However, for the dominant species, the highest aboveground biomass was detected at applications of 0.5 L m⁻² urine for *Stipa bungeana*, and 9.4 g m⁻² dung for *L. davurica* (Fig. 1a; Tables 2 and S1), while the relative aboveground biomass was significantly higher at applications of 0.5 L m⁻² urine and 14.2 g m⁻² dung for *S. bungeana* and 9.4 g m⁻² dung for *L. davurica* (Fig. 1b). The

biomass of community (CAGB) affected by year, excrement type (urine, dung and their mixtures) and excrement supply level

Table 2 The F and P values from the generalized multivariate nested linear model for plant C, N and P concentration and stoichiometry of the three dominant species, and aboveground

Source	Plant nutrient and stoichiometry								Plant production						
	C	N		P		C:N	C:P	N:P		CAGB					
	df	F	P	F	P	F	P	F	P	F	P	F	P		
Year	1,59	13.21	0.0006	5.89	0.0183	77.58	<0.0001	42.77	<0.0001	44.99	<0.0001	50.90	<0.0001	0.39	0.5337
Type	2,59	4.42	0.0163	3.98	0.0238	0.81	0.4494	0.70	0.4990	12.98	<0.0001	9.68	<0.0001	0.59	0.5580
Level	9,59	3.58	0.0013	2.90	0.0066	2.00	0.0550	2.62	0.0128	2.75	0.0094	1.90	0.0690	2.95	0.0026

Table 3 The F and P values from the two-way ANOVA for soil (total, organic, inorganic, microbial) C, N and P concentration and stoichiometry affected by excrement type (urine, dung, and their mixtures) and excrement supply level

Source	df	C		N		P		C:N		C:P		N:P	
		F	P	F	P	F	P	F	P	F	P	F	P
<i>Total</i>													
Type	2,24	18.34	<0.0001	64.49	<0.0001	135.52	<0.0001	65.49	<0.0001	53.21	<0.0001	148.62	<0.0001
Level	9,24	95.15	<0.0001	31.16	<0.0001	65.23	<0.0001	19.22	<0.0001	49.35	<0.0001	21.4	<0.0001
<i>Organic</i>													
Type	2,24	6.24	0.0066	45.97	<0.0001	135.26	<0.0001	31.72	<0.0001	30.18	<0.0001	104.38	<0.0001
Level	9,24	26.53	<0.0001	23.25	<0.0001	65.13	<0.0001	11.62	<0.0001	26.37	<0.0001	15.53	<0.0001
<i>Inorganic</i>													
Type	2,24	0.10	0.9064	0.62	0.5467	47.31	<0.0001	0.30	0.7438	2.26	0.1262	3.84	0.0357
Level	9,24	2.11	0.0699	0.69	0.7151	23.78	<0.0001	0.89	0.5481	1.85	0.1104	0.83	0.5922
<i>Microbial</i>													
Type	2,24	21.16	<0.0001	121.86	<0.0001	69.74	<0.0001	168.95	<0.0001	60.43	<0.0001	347.68	<0.0001
Level	9,24	321.45	<0.0001	217.59	<0.0001	15.04	<0.0001	347.30	<0.0001	56.81	<0.0001	161.18	<0.0001

aboveground biomass and relative aboveground biomass of *A. capillaris* were not significantly affected by excrement deposition (Fig. 1).

Plant C, N and P concentration and stoichiometry

Excrement deposition had limited effects on plant C, N and P concentration and stoichiometry (Fig. 2). The effects of excrement on plant C, N and P concentration and stoichiometry significantly differed between 2 years, where plant C and P concentrations and C:N ratio were significantly higher but plant N concentration and C:P and N:P ratios were significantly lower in 2016 (Table 2). Compared to the control, excrement deposition did not significantly change the plant C concentration and C:N, C:P and N:P ratios (Fig. 2a, d-f). However, plant C:P and N:P ratios under the mixtures were significantly lower than those under urine or dung. Plant N and P concentrations were significantly higher at 1.0 L m⁻² urine and 1.5 + 14.2 mixtures compared to the control, respectively (Fig. 2b-c).

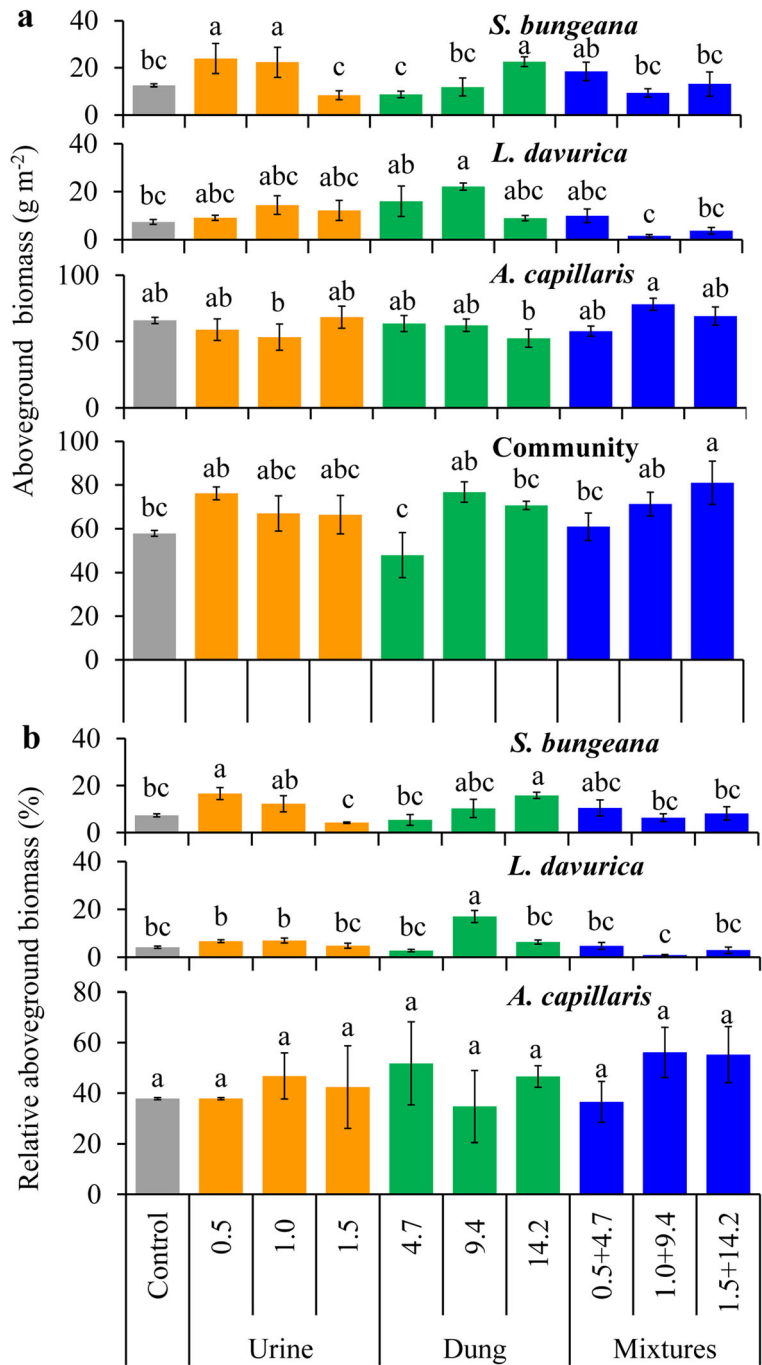
Excrement deposition did not significantly affect C concentration in *L. davurica* and *S. bungeana*, while in *A. capillaris* C concentration was significantly higher in the control (Fig. S3a-c; Table S1). N concentration was significantly higher at 1.0 L m⁻² urine for *A. capillaris*, 4.7 g m⁻² dung for *L. davurica*, and 1.0 L m⁻² urine for *S. bungeana* (Fig. S3d-f; Table S1). Excrement deposition did not alter P concentration in *A. capillaris*, while

compared to the control, P concentration was significantly higher at applications of 0.5 + 4.7 mixtures for *L. davurica*, and 1.0 + 9.4 mixtures for *S. bungeana* (Fig. S3g-i; Table S1). The inputs of excrement did not cause significant changes in C:N ratio for *A. capillaris* and *L. davurica*, while C:N ratio for *S. bungeana* was the highest in the control (Fig S4a-c). Compared to the control, excrement deposition did not significantly alter C:P and N:P ratios for *A. capillaris*, but significant differences in C:P and N:P ratios were found between excrement types (Fig. S4d, g; Table S1). C:P and N:P ratios were significantly lower at applications of mixtures regardless of the supply levels for *L. davurica* than in the control (Fig. S4e, h). Dung tended to increase C:P and N:P ratios for *S. bungeana*, while mixtures decreased them (Fig. S4f, i).

Soil C:N:P stoichiometry

Excrement deposition significantly increased soil total, organic and microbial C concentrations, with the highest values at applications of 9.4 g m⁻² dung and 1.0 + 9.4 mixtures (Fig. 3a-b, d; Table 3). Urine and dung promoted soil total and organic N concentrations, while the mixtures had no significant impact on these two parameters (Fig. 3e-f). Excrement supply did not significantly affect soil inorganic N concentration but increased soil microbial N concentration (Fig. 3g-h). Dung increased soil total, organic, and inorganic P and mixtures increased soil total, organic,

Fig. 1 Mean (\pm SE) of plant aboveground biomass and relative aboveground biomass among excrement supply levels for each dominant plant species (*A. capillaris*, *L. davurica* and *S. bungeana*) and aboveground biomass of the community from 2016 to 2017. For each category, columns with the same letters are not significantly different (Tukey’s HSD post hoc tests: $P > 0.05$). Relative aboveground biomass was the ratio of aboveground biomass of each species to that of the community



inorganic and microbial P, while urine had no significant influence on soil P (Fig. 3i-l).

Urine did not significantly change soil total, organic and inorganic C:N ratios, while dung and mixtures increased soil total and soil organic C:N ratios except at high supply levels (Fig. 4a-c). The excrement supply level had no significant impact on soil inorganic C:N ratio, but decreased

soil microbial C:N ratio except at moderate mixtures supply level (Fig. 4c-d). Urine significantly increased soil total, organic and microbial C:P ratios regardless of supply levels, while dung significantly increased soil total C:P ratio at any supply levels, soil organic C:P ratio at moderate level, and soil microbial C:P ratio at moderate and high levels (Fig. 4e-h). The mixtures significantly increased soil total C:P

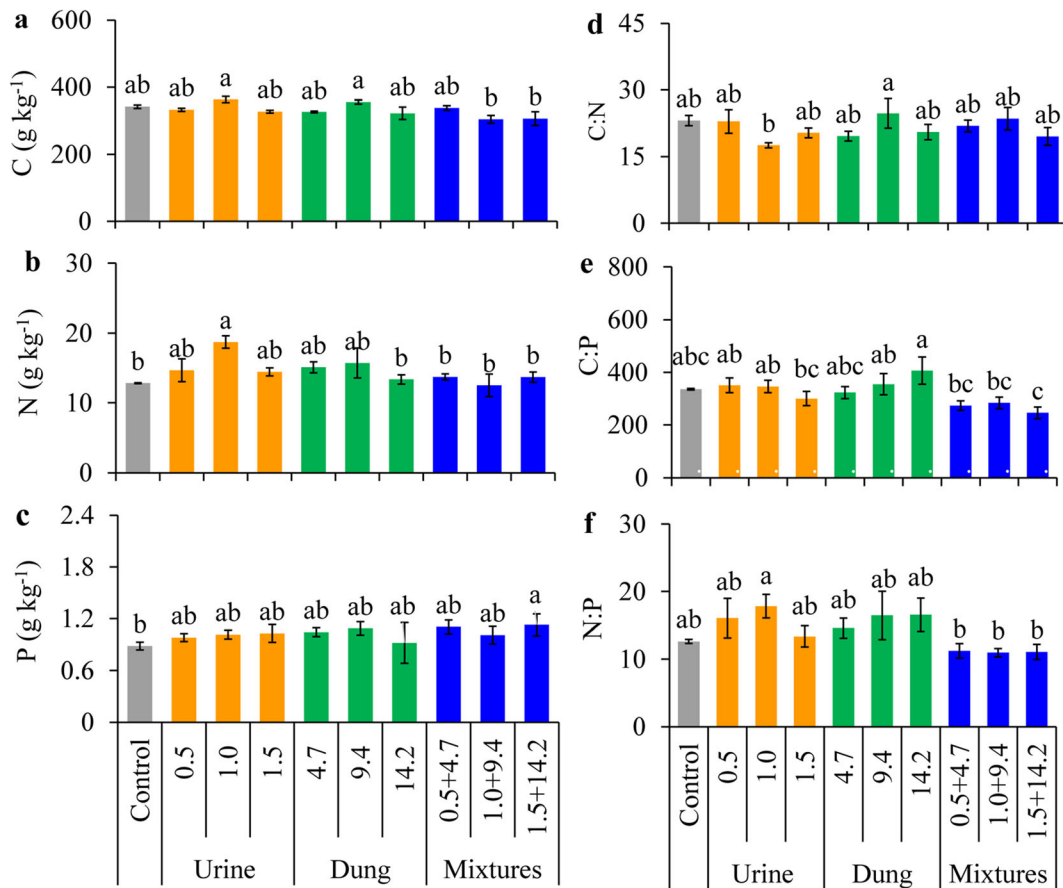


Fig. 2 Total mean (\pm SE) C, N and P concentration (g kg^{-1}) and stoichiometry of the three dominant species affected by excrement types (urine, dung, and the mixtures of urine and dung) and their

supply levels from 2016 to 2017. For each category, means with the same letters are not significantly different (Tukey's HSD post hoc tests: $P > 0.05$)

ratio at low and moderate supply levels and organic and microbial C:P ratios at moderate supply levels, while decreased them at high supply level (Fig. 4e–h). Urine significantly increased soil total, organic and microbial N:P ratios, while dung significantly increased soil total and organic N:P ratios at high supply level and microbial N:P ratio regardless of supply level. Mixtures decreased soil total and organic N:P ratios, but significantly increased soil microbial N:P ratio at high supply level (Fig. 4i–l).

Discussion

Effects of excrement on plant C:N:P stoichiometry

Herbivores regulate nutrient cycling in natural ecosystems by taking in plant biomass and depositing dung and urine (Bardgett and Wardle 2003), which has been

suggested to not only increase nutrient availability for plants directly but also stimulate microbial activities, and N and P mineralization in the soil (Bardgett et al. 1998; Frank and Evans 1997). As reported in other studies (Barthelemy et al. 2015; Barthelemy et al. 2018; Sitters et al. 2017), our results show that urinary N of livestock could be efficiently utilised by plants and increase plant N concentration, supposing the important role of urine in a fast pathway of nutrient return into the system. We further found that N of urine was more incorporated in plants at 1.0 L m^{-2} than at higher levels (Fig. 2b), which is likely that more N at higher levels was immobilized by microorganisms in soil (Fig. 3h). Different from urine, N in dung was more likely to be immobilized in soil and microbial biomass than utilised by plants, because microbes respond to the supply of the nutrients much faster than plants and the short experiment period could not induce significant changes in

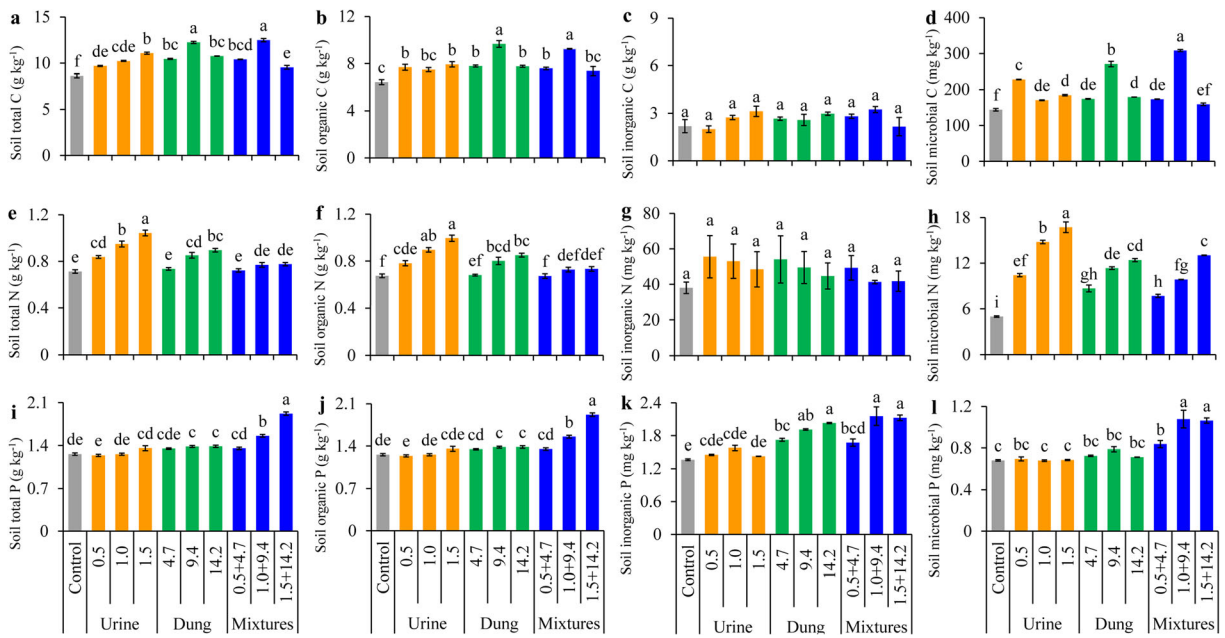


Fig. 3 Mean (\pm SE) of soil (total, organic, inorganic and microbial) C, N and P concentration among excrement supply levels. For each category, means with the same letters are not significantly different (Tukey’s HSD post hoc tests: $P > 0.05$)

plants. Although the amount of N from dung was about 100 times lower than that from urine, the effects of urine and dung on plant N did not show particularly significant differences (Fig. 2b), probably due to the short experiment period as mentioned above. Moreover, among the three excrement types, only urine and dung

increased plant and soil N concentrations (Figs. 2b and Figs. 3e), which partially agreed with our first hypothesis. This suggests that most N from mixtures were not efficiently transferred to plant canopy and soil, and thus the mixtures reduced N retention in the typical steppe. Wu et al. (2020) suggested that mixtures were more

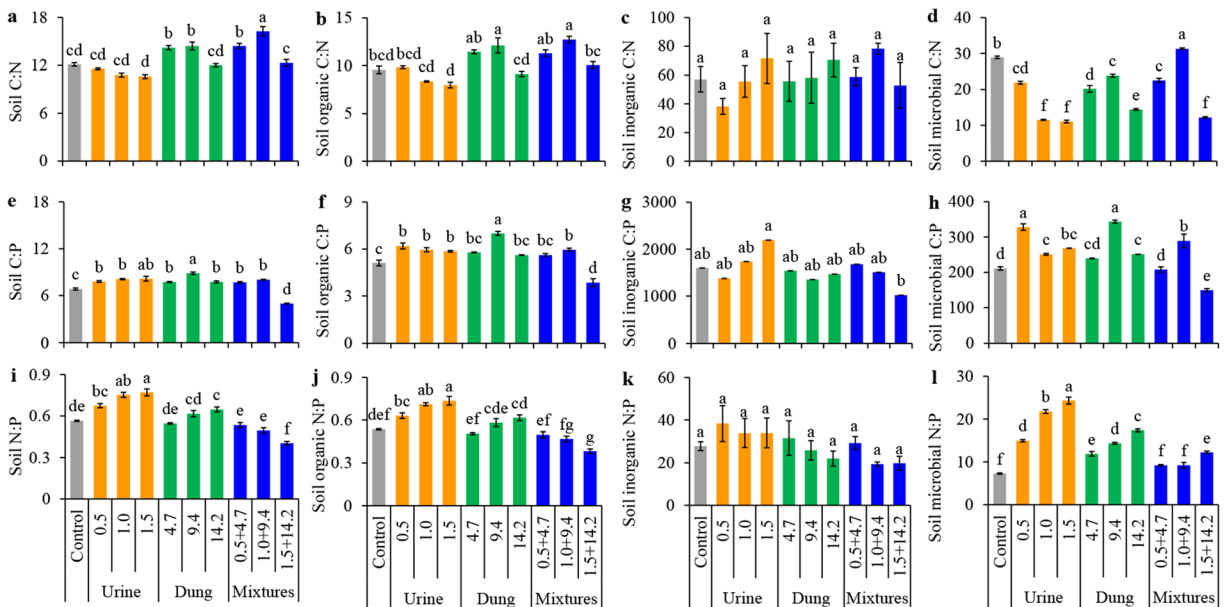


Fig. 4 Mean (\pm SE) of soil (total, organic, inorganic and microbial) C, N and P stoichiometry among excrement supply levels. For each category, means with the same letters are not significantly different (Tukey’s HSD post hoc tests: $P > 0.05$)

likely to regulate soil N transformation and increase nitrous oxide emission compared to the urine or dung alone.

Dung is usually the main source of P for plants and soil compared to urine. Surprisingly, dung had less effect on plant P concentration. This is likely to occur when nutrients are added in a more complex form rather than in the readily available mineral forms, probably due to two reasons. First, the short experiment period did not allow plants to trigger their responses to the low application level of dung. Second, during the sampling period, a large amount of P could be stored in the reproductive organs of the three dominant species, with limited P stored in plant leaves and stems. Compared to the control, the mixtures disproportionately increased plant and soil P concentrations by 22.1% and 27.6%, revealing a phenomenon that the mixtures would accelerate plant P uptake from soil, as evidenced by the increase in soil microbial P and available P (Fig. 2k-l). This is because when urine and dung were mixed, urine induced a more humid condition that could benefit the decomposition of dung. Meanwhile, the mixtures may have stimulated the higher activity of soil microorganisms and soil phosphatase (Olde Venterink 2011) than the urine and dung alone.

Nutrient enrichment can affect grassland C cycling by directly altering soil processes, e.g., nutrient availability, C mineralization and fluxes of greenhouse gases, soil microbes (Chen et al. 2019), and indirectly altering plant-related inputs to soils (Chen et al. 2019; Luo et al. 2019). We found that excrement deposition did not significantly affect plant C concentration, probably because C mainly acts as a relatively stable skeleton of plants (Zhang et al. 2019). Nutrient enrichment may increase soil C, especially soil organic and microbial C (Cusack et al. 2011; He et al. 2018; Lu et al. 2011; Ramirez et al. 2012), as evidenced in our study (Fig. 3a-d). Additionally, as reported by Sitters et al. (2020), herbivore excrement partially compensated for the loss of C in soil. However, dung and mixtures at high supply level did not induce higher soil C than at moderate supply level (Fig. 3a), because the high levels of dung and mixtures may promote soil net export of C (e.g., CO₂ emissions) induced by N deposition (Han et al. 2019), resulting in soil C loss.

The mean plant C:N, C:P and N:P ratios were 21.2, 312 and 15.4, respectively on the Loess Plateau (Zheng and Shangguan 2007). Plant C:N and C:P ratios (24.4 and 395, respectively) in the present study are lower

than those in this region (Zheng and Shangguan 2007). Within extensively managed pastures, plant N and P concentrations are the strong predictors for growth-limiting nutrients, and their transfers are potentially uneven and the patterns of N may be attenuated (relative to P) by other processes (Schnyder et al. 2010). Koerselman and Meuleman (1996) reported that P limitation of plant growth may occur when the N:P ratio in plant tissue is higher than 16, while a lower N:P ratio (< 14) in plant tissue indicates N limitation. The rates of both excrement decomposition and nutrient release were influenced by the C:N:P stoichiometry of excrement (Sitters et al. 2014). We found that the plant N:P ratio indicated balanced N:P supply (N:P = 15) for urine and dung applications or N limitation (N:P = 11) for mixtures application. These results only partially agreed with our third hypothesis that mixtures (N:P = 190) alleviated the P-limitation effect of urine (N:P = 876) on plants. Contrary to our expectation, mixtures did not alleviate the N-limitation effect of dung (N:P = 1.59), probably due to the low application rates of dung, which unlikely caused significant changes in plant nutrients and suggests that the mixtures reduced N retention in the typical steppe. A previous study reported that dung could not alleviate the P limitation in a meadow steppe grazing ecosystem (Schnyder et al. 2010).

Effects of excrement on soil C:N:P stoichiometry

Previous studies have shown that soil microbes may constitute a considerable ecosystem N pool by sequestering a larger proportion of N added in the form of dung (van der Wal et al. 2004; Barthelemy et al. 2015). Our results demonstrate that a major proportion of N from dung was allocated to soil and microbial biomass (Fig. 3e-f, h), as soil microbes are generally faster in N acquisition from soil than plants. Contrary to our hypothesis, dung did not increase soil total P and microbial P (Fig. 3i-l), probably because of the relatively low levels of dung applied. Also, dung applications may increase the abundance of soil microbial communities with greater N fixation capacity, while limited nutrition and spatial competition may not increase soil microbial biomass P (Xu et al. 2015).

Numerous studies have concluded that the increasing nutrient enrichment will change the C:N:P ratios in plants and soil (Han et al. 2019; Huang et al. 2018). Urine induced decreases of soil C:N ratio (Fig. 4a) but increases of soil C:P and N:P ratios (Fig. 4b-c) due to the

increase of soil total C and N (Fig. 3a, e). The varied absolute soil C, N and P concentrations could be attributed to the increase of soil C:N:P ratios under dung (Fig. 4). In contrast, mixtures-induced decreases in soil C:P and N:P ratios (Fig. 4e-i) may have been affected by mineralization and immobilization of C and P concentrations in soil (Chen et al. 2019; Han et al. 2019). Although application of dung and the mixtures increased soil C:N ratio (Fig. 4a), likely because of addition of C with dung (Fig. 3a), the value of soil C:N ratio in 0-cm soil layer (12.9) was lower than the average value (14.4) in China (Tian et al. 2010). The soil C:P (7.6) and N:P ratios (0.6) in this study were lower than the average values (136.6 and 9.3, respectively) in China (Tian et al. 2010). Compared to urine, the lower N:P ratio (1.5) of dung still caused an increase in soil N:P ratio, indicating that the typical steppe suffers from N limitation. Due to a large amount of N loss, the N:P ratio in the mixtures was lower than that in urine and dung alone, and thus the addition of mixtures decreased soil N:P ratio (Fig. 4i).

Regulation of excrement effects on plant and soil C:N:P stoichiometry by vegetation type and climate

We found that the capacity to take up N from urine at each supply level differed between plant species (Fig. S1d-f; Table S1), which was not consistent with the previous study that different plants, such as graminoids and forbs, effectively sequester N from urine (Barthelemy et al. 2018). *S. bungeana* was the largest sink of N from urine, dung and the mixtures (Fig. S1d-f), because of its larger root surface and thus the greater ability of effective absorption. Unlike *S. bungeana*, *L. davurica*, as a legumes species, responds to dung supply by increasing biomass production (Fig. 2a) as reported by Valdés-Correcher et al. (2019). The dung has a low N:P ratio which may stimulate the growth and competitive ability of N₂-fixing legumes. Approximately 0.04–0.12 N g m⁻² year⁻¹ was deposited as dung, which is much lower than the atmospheric N deposition (1–2 N g m⁻² year⁻¹) in the region (Duan et al. 2002). Thus, dung can not support higher plant production and N uptake. Species with high internal stability generally have an advantageous position in the ecosystem and play an important role in the stability of the ecosystem. Clark and Tilman (Clark et al. 2008) found that species with lower homeostasis under long-term N addition will absorb a large amount of nutrients in the environment

for rapid growth, while nutrients of species with higher homeostasis will not change with environmental nutritional conditions and other factors. Thus, it is possible that other species, especially annual species, are more vulnerable to fertilization to obtain short-term rapid growth opportunities.

The direction and magnitude of excrement effects on C:N:P stoichiometry also depends on the excrement levels, environmental factors, and period of the experiment. In this study, some results reflected the gradual changes in C:N:P stoichiometry in response to the excrement application levels, but others not. These may be that there are several pathways through which the applied nutrients could be lost, including volatilization, leaching, and nutrients uptake by plants outside the treatment plots, and litter and root reallocation inside the plots. We found that plant N was higher while plant P was lower in 2017 (a wet year) (Table 2), suggesting that N limitation is more common in dry biomes whereas P limitation is more common in humid biomes (Bai et al. 2012). Furthermore, higher plant N in 2017 was also attributed to the higher N cycling in grasslands in wet conditions (Bai et al. 2012) and the cumulative effect of two-year excrement deposition. Moreover, soil microbial activity, quantity and community structure can vary within and across sites in different ways that may alter the rate of nutrient supply to plants (Anderson et al. 2018). Therefore, long-term observational studies are required to provide a scientific basis for the selection of the optimal stocking rate on the Loess Plateau.

Conclusions

Many studies reported that different amount of N and P returns from urine and dung may drive the patterns of N and P limitations and affect nutrient allocation between plant and soil, thereby causing patchiness and heterogeneity of grasslands (Haynes and Williams 1993; Schnyder et al. 2010; Scheile et al. 2018). Our study demonstrated that not only excrement level but also the type resulted in spatial C:N:P heterogeneity of the typical steppe on the Loess Plateau. We found that the impacts of sheep urine and dung on soil and plant C and nutrient concentrations and stoichiometry differed from that of mixtures. Surprisingly, the effects of urine and dung on plant N and P did not show a particularly significant difference, probably due to the short experiment period and insufficient dung application levels. In

our study, urine and dung alone indicated balanced N:P ratios, but their mixtures resulted in N limitation. Therefore, our study provides the ranges of N:P availability supply in organic fertilizer and demonstrates that regulating stocking rates can alter the patterns of N and P limitation.

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Author's contributions Fujiang Hou and Lan Li contributed to the study conception and design. Lan Li prepared materials and collected data. Lan Li, Xiong Z. He and Jing Zhang analysed the data. Lan Li wrote the first draft of the manuscript and all authors revised the manuscript. All authors approved the final manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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