



# Defoliation, trampling and nutrient return differentially influence grassland productivity by modulating trait-dependent plant community composition: insights from a simulated grazing experiment

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## Abstract

Ungulate grazing involves multiple components, including defoliation, dung and urine return, and trampling, which supply offsetting or synergistic effects on plant community composition and productivity (ANPP), but these effects have not been fully studied. Plant functional traits may reflect the response of plants to disturbance and their impact on ecosystem functions. Species turnover and intraspecific trait variation (ITV) are important drivers of community trait composition. We conducted a simulated grazing experiment in a steppe grassland in northern China to examine the effects of defoliation, dung and urine return, and trampling on community-weighted mean (CWM), functional diversity (FD) and ANPP, and to disentangle the roles of species turnover and ITV in driving these changes. We found that defoliation had a dominant effect on CWMs and FDs of all four traits through species turnover and ITV, respectively, resulting in a convergence of traits towards a more resource-acquisitive strategy. Dung-urine return resulted in more resource-acquisitive community traits mainly through ITV, whereas there were no significant effects on FDs except for leaf C/N. Trampling increased CWM of leaf dry matter content primarily driven by ITV, and had no significant effect on FDs. Furthermore, our simulated grazing positively affected ANPP, primarily due to nutrient additions from dung and urine, and ITV largely explained the variation in ANPP. These findings highlight the multifaceted effects of grazing components on community structure and ANPP, and the significance of ITV in shaping grassland plant communities and productivity.

**Keywords** Community-weighted mean · Functional diversity · Intraspecific variability · Species turnover · Grassland productivity

## Introduction

Grasslands, which constitute approximately 41% of the earth's ice-free land (~52.5 million km<sup>2</sup>), play a crucial role in providing various ecosystem services (White et al. 2000). Ungulate

grazing is a widespread land use practice in grasslands. It is a significant driver of plant community structure, which is fundamental to ecosystem function, including aboveground net primary productivity (ANPP) (Li et al. 2017; Piipponen et al. 2022). Grazing is not just a physical disturbance to plant productivity, but also has indirect effects mediated by diversity and community structure (Zhang et al. 2018). Grazing is a complex biotic disturbance involving multiple components, including

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defoliation, trampling, and dung and urine return, which collectively influence grassland ecosystems, shaping plant community composition and productivity (Liu et al. 2015). However, there remains a knowledge gap in understanding the mechanistic links between community functional composition and productivity under grazing disturbance, particularly in exploring how these multiple disturbances operate, which could advance our understanding of the biodiversity-productivity relationship in managed grasslands.

Plant functional traits can serve as indicators of plant response to disturbance at both species and community levels (Díaz et al. 2007; Jäschke et al. 2019), which help us to understand the mechanisms underlying the effects of grazing on plant communities. Generally, the community functional composition is described by both community-weighted mean (CWM) and functional diversity (FD). CWM reflects the dominant trait value within the community, while FD quantifies the trait dissimilarity among coexisting species. Previous studies examining the response of community functional composition to grazing components have primarily concentrated on interspecific trait variation (caused by species turnover), assuming that such variation captures the majority of the underlying processes (Violle et al. 2012). For instance, defoliation diminishes the dominance of taller species pursuing a resource-conservative strategy and mitigates community light limitation (Borer et al. 2014), creating opportunities for shorter species with larger SLA to thrive and shifting CWM towards resource-acquisitive strategies. Nutrient return favors tall species as they have an advantage in acquiring light, thereby driving the change of CWM through species turnover. As competition for light intensifies, shorter-statured species may be excluded from the community gradually (Wang et al. 2022). Defoliation and nutrient return can potentially reduce functional diversity by selecting species with specific traits (Zhang et al. 2023; Zheng et al. 2021). Additionally, few studies have found that trampling affects community-level functional traits and FD by causing species turnover (Li et al. 2023).

However, plant traits are not fixed and can be shaped by biotic or abiotic factors via intraspecific trait variation (ITV). A global meta-analysis reported that ITV explained ~25% of the trait variation within communities and ~32% among communities (Siefert et al. 2015). Consequently, the effects of ITV on community functional composition, community assembly, and ecosystem functions have gained increasing attention in recent years (Wright et al. 2016). In response to ungulates, ITV reflects different species' adaptive strategies to mammalian herbivory (Amaral et al. 2021). The species with grazing-avoidance strategies tend to reduce their palatability, such as short stature, tough leaves with high leaf dry matter content (LDMC), and small specific leaf area (SLA) (Archibald et al. 2019). Conversely, grazing-tolerant plants are associated with high SLA or leaf N concentration (LNC), enabling fast compensatory growth, which trade-offs exist with grazing avoidance strategies (Li et al. 2017; Wright et al. 2004). These response strategies are

primarily related to defoliation, but the contributions of dung and urine return and trampling to the ITV response in relation to ungulate grazing have not been adequately documented. Similar to nutrient addition, dung and urine return may lead to changes in community functional composition by intraspecific variation (Wang et al. 2022). Specifically, increased soil nutrient availability increased individual plant height and SLA (Zhang et al. 2019). Moreover, trampling with moderate intensity can induce compensatory growth in specific species, such as *Medicago ruthenica* in the Tibet Plateau (Xiao et al. 2018). However, understanding the contribution of these species-level changes to community-level functional composition under trampling is still limited (Li et al. 2021b).

Plant functional traits can also provide insights into the relationship between plant communities and ANPP (Hanisch et al. 2020). According to the classical theoretical framework of biodiversity-ecosystem functioning, changes in the species composition (species turnover) are an essential factor influencing productivity. The relationship of CWMs with ANPP suggests that species with a high (or low) trait value will promote productivity (Zuo et al. 2022), which may emphasize the role of dominant species in driving ecosystem function. Traits, such as SLA and leaf nitrogen content, which are thought to be positively correlated with photosynthetic rate, can ultimately affect ANPP (Ali et al. 2013). On the other hand, communities with higher FD may exhibit higher niche differentiation, resource utilization, and thus higher productivity, an idea derived from diversity theory (Bongers et al. 2021). However, the maintenance of grassland productivity is not only dependent on species turnover, intraspecific trait variation may also play an important role. A study in a semi-arid grassland found that ITV of plant stem/leaf ratios, influenced by changes in grazing density, was a crucial factor mediating the relationship between interspecific trait variation and ANPP (Zheng et al. 2021). Additionally, intraspecific trait variation due to nutrient addition (e.g., increased SLA) is an important pathway through which nutrient inputs increase ANPP. The impact of species turnover and intraspecific trait variation on ANPP under different grazing components is still unknown, limiting our understanding of the relationship between plant communities and ecosystem functioning in disturbed grassland.

In this study, we conducted a long-term field experiment in a semiarid grassland in Inner Mongolia, China, simulating the disturbances from ungulate herbivores through various treatments, including defoliation, dung and urine return, trampling, and their combinations. We measured four traits (plant height, SLA, LDMC, and leaf C/N) linked to resource uptake strategies, the competitive ability for light, and adaptation to grazing (Díaz et al. 2001). We asked the following questions: (a) How do plant diversity and community functional composition (CWM and FD) respond to simulated grazing? (b) To what extent is the response of functional composition to simulated grazing due to species

turnover vs. ITV? (c) How does the simulated grazing affect ANPP through intra- and interspecific trait variations, and FD? Specifically, we tested the following hypothesis: (a) Defoliation is a dominant factor in the effects of grazing on plant diversity and functional structure, leading to community convergence (i.e., a community composed of species with similar functional traits or having a low FD) toward resource-acquisitive strategies (higher SLA, lower leaf C/N, and LDMC). Dung and urine return increase plant height and specific leaf area, while trampling decreases plant height. (b) Variations in CWM and FD under defoliation will be mainly explained by species turnover, but the effects of dung and urine return and trampling are more interpreted through ITV due to the change of soil properties. (c) CWM and FD together drive the productivity response to simulated grazing through species turnover.

## Material and methods

### Study site and experimental design

This experiment was carried out in a temperate steppe at the Duolun Restoration Ecology Station of the Institute of Botany of the Chinese Academy of Sciences (42.02°N, 116.17°E, 1324 m a.s.l.), Inner Mongolia, China. The study area has a semiarid continental monsoon climate with a mean annual temperature of 2.1 °C and annual precipitation of 378 mm (Xu et al. 2022). The soils belong to Haplic Calcisols according to ISSS Working Group RB (Deckers and Nachtergaele 1998). Dominant plant species in this area are perennial grasses, forbs and subshrub, including *Stipa krylovii* Roshev. (Poaceae), *Cleistogenes squarrosa* Keng (Poaceae), *Leymus chinensis* Tzvel. (Poaceae), *Potentilla acaulis* L. (Rosaceae), and *Artemisia frigida* Willd. (Asteraceae). Our study site was fenced since 2003 to exclude large herbivores and for hay-making at the end of the growing season until 2014.

The simulated, moderate-intensity cattle grazing was established in a randomised block design with eight blocks in 2015 (64 plots in total, 4 × 4 m per plot). We choose mowing to a stubble height of 5–6 cm three times per year (in the middle of June, July, and August) to simulate defoliation (Baoyin et al. 2014). In this study, our defoliation has no selectivity. The former results showed that the diet selectivity of cattle was not as strong as that of sheep, and it usually happened under light grazing intensity with higher plant coverage and height (Chen et al. 2015; Fraser et al. 2009). Trampling was performed by a 75-kg man carrying a weight of 25 kg, who wore shoes made from the hind pair of a cow's hooves, and applied three times per year after mowing. It was equivalent to 65% of the pressure of an adult

cow. The trampling induced soil compaction after five years of treatments (Fig. S1a), and the bulk density is equivalent to that under moderate grazing intensity (Ren et al. 2018). The dose of fresh dung and urine mixture applied to the plot was determined by the mean herbage production per hectare in the pasture and the mean digestibility of the grazed herbage (Mikola et al. 2009). A total of 1.5 L urine per m<sup>2</sup> mixed with dung was applied to the soil, split into two applications per year, right after each of the first two mowing treatments and trampling was finished. In total, the two applications of the mixture had 1,152 g of dry matter, 495 g of C, 87 g of N, and 12 g of P per plot per year. Consequently, the experiment included factorial combinations of mowing (M; no mowing vs. mowing), a liquid mixture of dung and urine addition (D; no addition vs. addition), and trampling (T; no trampling vs. trampling), resulting in eight different treatment combinations (CK: control, D: dung and urine return, M: mowing, T: trampling, D + M: dung and urine return + mowing, M + T: mowing + trampling, D + T: dung and urine return + trampling, D + M + T: dung and urine return + mowing + trampling).

### Plant community and trait sampling and measuring

In the middle of August 2019, after five years (2015–2019) of treatment, we surveyed plant communities at their peak standing biomass before the third time treatment implementation. Aboveground vegetation was sampled by clipping all plants using a 0.5 × 0.5 m quadrat randomly placed in the plot. All living vascular plants were sorted into species, oven-dried at 65 °C for 48 h and weighed. All plants were classified into five functional groups (rhizome grass, bunchgrass, legume, forb, and subshrub). The dry weight of all live plants in mid-August was used to estimate aboveground net primary productivity (ANPP) for the unmowed plots. In the defoliated treatment plots, we recorded the biomass removed after each mowing by drying and weighing it. The ANPP for these plots was calculated as the sum of the biomass removed each time and the standing biomass in mid-August.

The effects of our treatments on plant community structure gradually increased from 2016 to 2019 (Fig. S2). To explore the cumulative effects of simulated grazing treatments, we sampled and measured functional traits in 2019 after plant community survey. We measured traits from 3 to 5 individuals for each species in each plot. The functional traits were quantified using methods proposed by Perez-Harguindeguy et al. (2013). Plant height was measured by averaging the height of the three tallest individuals of each species. We randomly sampled at least five healthy, fully expanded leaves from 3 to 5 individuals of each species to measure leaf traits. After sampling, the fresh leaves were immediately scanned (PER-V700P, EPSON, Japan) to measure leaf area and weighed. Then,

the leaves were oven-dried (65 °C) to a constant mass and weighed. LDMC was calculated as dry leaf mass per unit of fresh leaf mass, and SLA was the leaf area per unit of dry leaf mass. These dried leaves were ground in a ball mill (MM400, Retsch, Germany) and used to determine carbon (C) and nitrogen (N) contents (Elementar vario MICRO cube elemental analyzer, Elementar, Germany).

### Soil sampling and physicochemical properties determination

In the middle of August 2019, we sampled soil. A stainless steel cutting ring (5 cm diameter, 100 cm<sup>3</sup>) was used to evaluate soil bulk density and soil water content. Three soil cores (5 cm diameter, 0–10 cm depth) were randomly collected from the quadrats for biomass evaluation in each plot and combined to gain one composite soil sample. After homogenization and removal of roots gently, the soil was sieved through a 2-mm mesh and stored at 4 °C.

Soil pH was measured in soil suspension with a 1:5 ratio of soil to deionized water with a pH/Conductivity Benchtop Meter (ORION STAR A125, Thermo Scientific, USA). Soil ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N) and nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N) were extracted with a 1 M KCl solution and measured with a continuous flow analyzer (AA3, SEAL Analytical, Germany). A subsample of 2.5 g of soil was extracted with 0.5 M NaHCO<sub>3</sub> (pH 8.5) to measure extractable P concentration using the molybdenum blue method to get the content of AP (Olsen 1954).

### Quantification of inter- and intraspecific functional variance

The changes in functional traits at the community level are typically described by community weight means (CWM), reflecting the dominant trait value based on the mass-ratio hypothesis (Grime 1998). Functional diversity (FD) indicates community assembly (Laliberte and Legendre 2010) and describes how dissimilar the coexisting plants are in a community. All traits were weighted by the relative biomass of each species to calculate CWM for each plot using the formula:

$$\text{CWM} = \sum_{i=1}^n p_i \times t_i$$

where  $p_i$  is the relative biomass of species  $i$  in the plot, and  $t_i$  is the mean trait value of species  $i$  in the plot (specific trait values).

We used a method presented by Lepš et al. (2011) based on the sum of squares (SS) decomposition analyses to assess the relative importance of species turnover and ITV

in driving CWM. In this method, we first calculated three CWM parameters for each functional trait: (1) 'Specific average', i.e., CWM as mentioned above, whose response to total change factors can be caused by both species turnover and ITV. (2) 'Fixed average' (CWM\_Fixed), calculated by mean trait values of all plots, and their response variation caused by species turnover. (3) 'Intraspecific variability effect' (CWM\_Intra), i.e., the differences between 'fixed' and 'specific' averages. Next, three-way ANOVA for 'specific', 'intraspecific' and 'fixed' were performed separately with the three grazing components and their interactions as predictors. We extracted the SS for each trait value that was explained by the three grazing components and their interactions, so that SS\_specific, SS\_fixed and SS\_intraspecific represented the treatment-induced total variation, effect of species turnover, and effect of ITV, respectively. Finally, the SS\_cov component, which is the effect of covariation between inter- and intraspecific trait variability, was calculated by subtracting SS\_fixed and SS\_intraspecific from SS\_specific (SS\_total). Positive covariance effects between species turnover and ITV indicate that they change in the same direction, potentially amplifying the response of CWM (or FD) to treatments. In contrast, negative covariance effects between species turnover and ITV suggest that they change in opposite directions. Ultimately, SS can be expressed like this:  $\text{SS}_{\text{total}} = \text{SS}_{\text{fixed}} + \text{SS}_{\text{intraspecific}} + \text{SS}_{\text{cov}}$ .

FD with Rao index for each plot was calculated using relative biomass and standardized specific trait value dissimilarity (Euclidean distance) between species (de Bello et al. 2011).

$$\text{FD} = \sum_i^n \sum_j^n p_i \times p_j \times d_{ij}$$

where  $p_i$  and  $p_j$  are the relative biomass of species  $i$  and  $j$ .  $d_{ij}$  is the specific trait dissimilarity (Euclidean distance) between each pair of species  $i$  and  $j$ .

To quantify the ITV and species turnover in changes of FD, we also calculate the FD parameters based on the methods of Niu et al. (2020) and de Bello et al. (2011). 'Inter\_FD' was calculated using species-level dissimilarity, the distance of mean trait values. We calculated 'Intra\_FD' as the dissimilarity of deviation of trait values from the grand mean value for each species. And then, we calculated rITV as the ratio of Intra\_FD to Inter\_FD. Finally, using a sum of squares decomposition analysis (de Bello et al. 2011), similar to that of Lepš et al. (2011) based on ANOVA, we quantify the contribution of species turnover and ITV to functional diversity. Similarly, SS is expressed by the following formula:  $\text{SS}_{\text{FD}} = \text{SS}_{\text{Inter\_FD}} + \text{SS}_{\text{Intra\_FD}} + \text{SS}_{\text{cov\_FD}}$ .

In order to investigate the impact of treatments on the trait values of various functional groups, we computed the CWMs of different functional groups using the methodology

mentioned above (Fig. S3). Notably, the parameter  $p_i$  in the formula uses the relative biomass of species  $i$  in the functional group to which it belongs.

## Data analysis

All calculations and statistical analyses were performed in R 4.0.3. We used species richness and Shannon index as indicators of species diversity based on the number and relative biomass of the species present. To visualize the variation of plant composition under simulated grazing, we applied non-metric multidimensional scaling (NMDS) ordination via the metaMDS function in the R package *vegan*. Our ordination was based on the Bray–Curtis dissimilarity matrix of the vegetation datasets with 64 plots. The effects of defoliation, dung and urine return, trampling and their interactions on vegetation composition were tested with a permutational multivariate analysis of variance (PERMANOVA, *adonis* in *vegan*) on the same dissimilarity matrix (999 permutations).

Linear mixed-effects models were employed to evaluate the effects of defoliation, dung and urine return, trampling, and their interactions on species diversity, community functional composition, functional groups' CWM and soil properties. The *lme4* package was utilized to implement the models, with the block included as a random effect to account for potential spatial variability. Prior to analysis, normality of the data was assessed using the Shapiro–Wilk test. For the data that did not meet the assumptions of normality, we used logarithmic or aligned rank transform (a preparation for nonparametric analyses of variance; *ARTool* package).

Finally, a Bayesian linear mixed effect model (*brms* package) was performed to predict the effect of soil physicochemical properties on community functional properties, and to explore the effect of plant richness, intra- and interspecific changes of CWM and FD on ANPP. Before the Bayesian linear mixed effect models of functional properties, all soil factors were standardized, and the *descdist* function (*fidistrplus* package) was used to examine the distribution of response variables. Before the model procedure of ANPP, we conducted principal component analyses (PCAs) to extract the first principal component (PC1) of intra- and interspecific changes of CWM (CWM\_Intra and CWM\_Fixed) and FD (FD\_Intra and FD\_Inter) based on the four functional traits. PC1 explained 37.06% and 69.03% of the total variance in CWM (Fig. S4 a, b) and 44.41% and 65.28% of the total variance in FD (Fig. S4 c, d) for intraspecific and interspecific trait variability. Beta *brms*-model was employed for ANPP divided by its maximum value, and we used block as a random term. Markov Chain Monte Carlo (MCMC) simulations were performed to estimate the fit of the models (median and 95% interval of  $R^2$ ) and range of effect size (95% credibility intervals) of predictors (intra- and interspecific changes of CWM and FD).

## Results

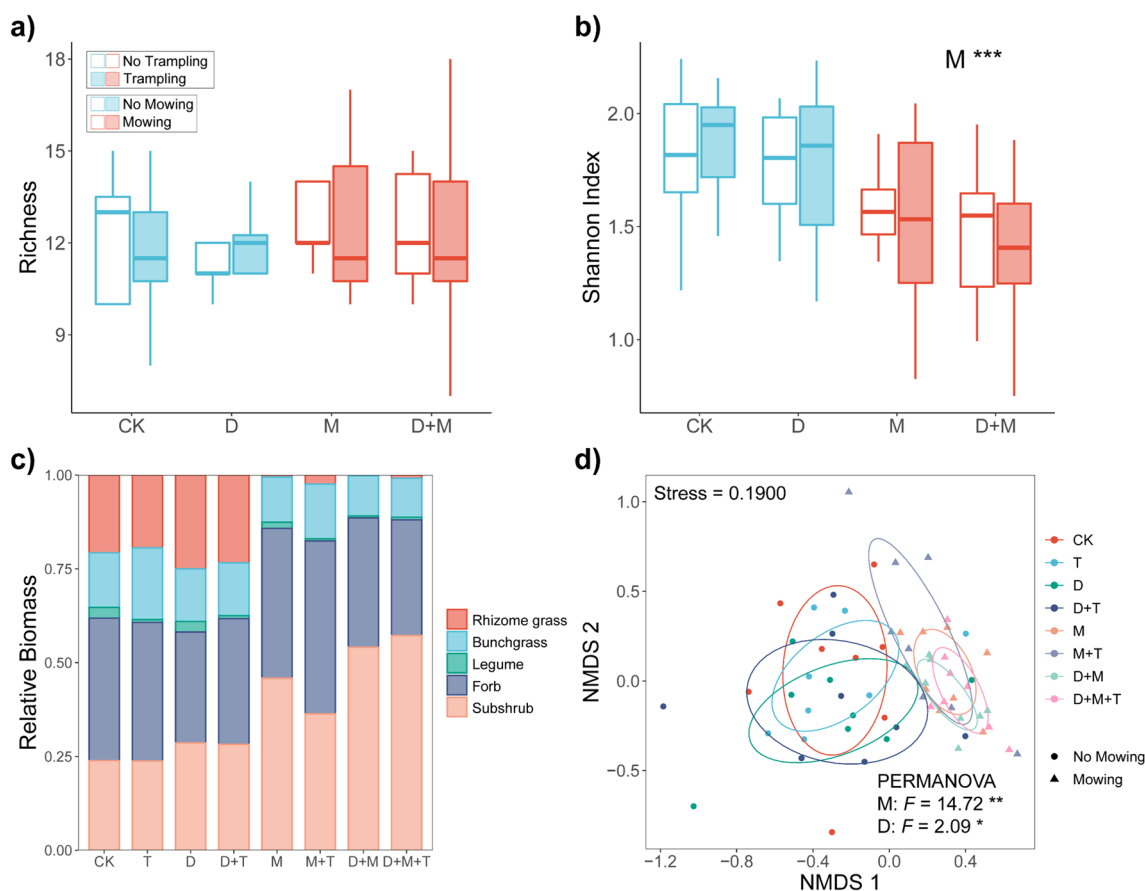
### Plant species diversity and community structure

After five years of simulated grazing treatments, we found that all three grazing components did not significantly affect species richness (Fig. 1a). However, defoliation reduced the Shannon diversity index (Fig. 1b) and significantly changed the community structure (Fig. 1d, Table 1). Specifically, defoliation increased the relative abundance of subshrub *Artemisia frigida* ( $P < 0.001$ ), while sharply decreasing the dominance of perennial rhizome grass (i.e., *Leymus chinensis*, *Agropyron cristatum*) ( $P < 0.001$ ; Fig. 1c, Table S1). PERMANOVA revealed that dung and urine return also caused a significant change in community composition ( $P < 0.05$ ) by reducing the proportion of forbs ( $P < 0.05$ ) and increasing subshrub ( $P < 0.05$ ). Dung and urine addition only promoted the growth of rhizome grasses in un-defoliated communities ( $M \times D$ ,  $P < 0.05$ ; Fig. 1c, Table S1). Trampling had no significant effect on plant community structure.

### Response of community-weighted mean trait values

The defoliation significantly changed CWM values of all plant traits, and overrode other grazing components. Specifically, defoliation decreased plant height, LDMC, and leaf C/N ratio, while increasing the SLA (Fig. 2a–d). Dung and urine addition led to taller stature, higher SLA, and lower leaf C/N ratio (Fig. 2a, b, d,  $P < 0.05$ ). Trampling increased the LDMC at the community level (Fig. 2c,  $P < 0.05$ ). Dung and urine return in combination with defoliation increased LDMC compared to control (CK), but decreased it when mowing was absent ( $D \times M$ ,  $P < 0.01$ ; Fig. 2c).

Overall, species turnover (23.91–68.67%) and ITV (40.61–54.01%) contributed almost equally to the total variation of the four CWM values in this study (Fig. S5). The effect of defoliation on SLA, LDMC, and leaf C/N was mainly achieved by affecting species turnover (Fig. 2). However, the influence of defoliation on plant height was primarily driven by ITV. Furthermore, there was a positive covariation between species turnover and ITV for height and leaf C/N (Fig. 2e, h), but a negative covariation for SLA and LDMC (Fig. 2f, g). Under dung and urine return treatment, ITV explained more variance in CWM values than species turnover, particularly for SLA and leaf C/N. Similarly, the variation in CWM of LDMC under trampling was mainly driven by ITV. Considering the interactive effect between defoliation and nutrient addition, the contribution of species turnover (2.17%) and ITV (2.33%) were almost equal for CWM of LDMC.



**Fig. 1** Effects of defoliation (mowing, M), dung and urine return (D) and trampling (T) on species richness (a), and Shannon diversity index (b). Relative biomass of each functional group in community (rhizome grass, bunchgrass, legume, forb and subshrub) in all eight treatments (c). Ordination of community structure under different treatments by non-metric multidimensional scaling (NMDS) (d).

Box-plot shows quartile values ( $n = 8$ ) for each treatment.  $\times$  denotes the interaction between grazing components, while the + symbol represents a treatment that combines several grazing components. Significance levels of the factors are as follows: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

## Response of functional trait diversity

Defoliation significantly decreased the functional diversity of the four traits (Fig. 3a–d). Dung and urine return reduced the functional diversity of leaf C/N (Fig. 3d). However, trampling had no significant effect on FD. Compared to the CWM, the variations of FDs were driven more by ITV (Fig. S5). Specifically, for plant height and leaf C/N, defoliation primarily affected FD by reducing ITV (Fig. 3e, h). The relative contributions of species turnover and ITV to LDMC variation were almost equal under defoliation, while the change in SLA was mainly contributed by species turnover (Fig. 3f, g). The variation in leaf C/N under dung and urine return was attributed to species turnover and a positive covariation (Fig. 3g, h).

## Predicting functional properties from soil physicochemical properties

Soil available nutrients (soil inorganic nitrogen and available phosphorus) have a greater impact on functional properties than bulk density and pH (Table 1). The CWM, CWM\_Fixed (calculated by mean trait values of all plots), intraspecific variation, and FD of plant height increased with increasing soil available N. Moreover, both CWM\_Fixed and FD of LDMC exhibited positive correlations with soil available N. Additionally, FD\_intra (FD variation associated with ITV) and rITV (relative importance of ITV for FD) of LDMC were positively influenced by phosphorus availability. The functional properties (CWM, CWM\_Intra, FD, FD\_Intra, and rITV) of leaf C/N ratio showed positive correlations with soil available N and P, while there was a negative and positive effect of bulk

**Table 1** The effect of soil physicochemical properties on plant community properties for four traits

Traits	Functional Properties	BD	pH	IN	AP	$R^2$ (%)
Height	CWM	−0.1387	−0.0385	<b>0.2868*</b>	0.0837	23.69
	CWM_Fixed	−0.0859	−0.0849	<b>0.1565*</b>	−0.0173	22.88
	CWM_Intra	−0.1373	0.0181	<b>0.3321*</b>	0.1704	14.09
	FD	−0.0576	−0.0202	<b>0.1764*</b>	0.0968	10.59
	FD_Inter	0.0109	0.0012	−0.0040	0.0148	18.09
	FD_Intra	0.0412	−0.0487	0.0037	0.0451	17.16
	rITV	0.0127	−0.0131	0.0015	−0.0048	18.78
SLA	CWM	0.0440	0.0259	−0.0074	<b>−0.0775*</b>	29.68
	CWM_Fixed	0.0321	0.0201	−0.0636	−0.0617	15.27
	CWM_Intra	0.0543	0.0225	0.0399	−0.0427	29.60
	FD	−0.0425	−0.0169	−0.0100	0.0983	16.99
	FD_Inter	0.0165	−0.0799	0.0908	0.0662	17.43
	FD_Intra	−0.0778	−0.0041	0.0359	0.0719	22.76
	rITV	−0.0202	0.0257	−0.0191	−0.0001	21.98
LDMC	CWM	0.0203	−0.0367	0.0620	−0.0704	18.88
	CWM_Fixed	0.0417	−0.0732	<b>0.1737*</b>	−0.0417	14.57
	CWM_Intra	0.0474	−0.0198	−0.0640	−0.0833	13.90
	FD	0.0627	−0.0993	<b>0.1605*</b>	0.0576	17.16
	FD_Inter	0.0192	−0.0746	0.0935	0.0507	17.16
	FD_Intra	0.1109	0.0496	0.1332	<b>0.1591*</b>	13.92
	rITV	0.0698	<b>0.1055*</b>	0.0267	<b>0.1051*</b>	14.38
Leaf C/N	CWM	−0.0727	−0.0121	0.0767	<b>0.1445*</b>	17.49
	CWM_Fixed	0.0130	−0.0387	0.1150	0.1362	15.42
	CWM_Intra	<b>−0.0954*</b>	0.0221	0.0649	<b>0.1128*</b>	19.40
	FD	0.0075	−0.0328	0.0915	<b>0.1215*</b>	20.69
	FD_Inter	−0.0415	−0.0675	0.0104	0.0030	20.70
	FD_Intra	0.1310	−0.1065	<b>0.1999*</b>	0.0400	8.69
	rITV	<b>0.1734*</b>	−0.0457	<b>0.2282*</b>	0.0175	13.76

The effects of soil properties on plant community properties are indicated by the slope in Bayesian regression models, and the fit of the models indicated by the median in  $R^2$  (%)

BD bulk density, IN soil inorganic nitrogen, AP soil available phosphorus

\*Indicates the significant effect

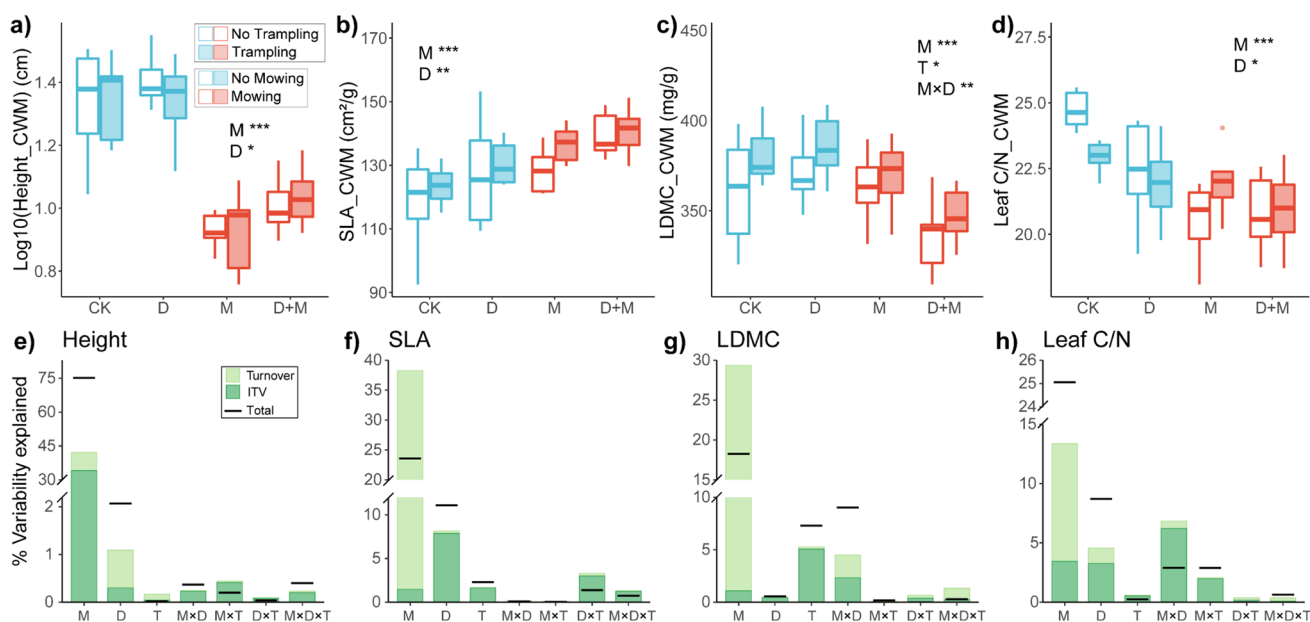
density on CWM\_Intra and rITV, respectively. For SLA, there was only a significant negative effect of phosphorus availability on CWM.

### Responses of aboveground net primary productivity

The ANPP increased significantly under dung and urine return treatment ( $P < 0.05$ , Fig. 4a), while other grazing components did not have significant effects. Bayesian linear mixed effect model demonstrated that functional composition explained 32.05% of the variation in ANPP (Fig. 4b). The intraspecific variation of CWM (CWM\_Intra) had a significant effect on ANPP, and between-population ITV (FD\_Intra) had a large but non-significant effect. However, plant richness and interspecific trait variation did not contribute significantly to ANPP.

### Discussion

Our study first disentangled the effects of individual or combined three grazing components on community functional composition and ANPP. We found that defoliation and dung and urine return significantly altered the community composition and community-level height, SLA, LDMC and leaf C/N. On the other hand, the effect of trampling was comparatively weaker. The variability of CWM induced by defoliation was mainly driven by species turnover, while that induced by dung-urine return and trampling was driven primarily by ITV after 5-year simulated grazing treatments. Consequently, species turnover and ITV were equally important in explaining the simulated grazing effects on CWM trait values. Furthermore, defoliation significantly reduced FD, with ITV explaining most of the variation (Fig. 5). Dung and urine return increased ANPP



**Fig. 2** Community-weighted mean (CWM) of plant height **a**, specific leaf area (SLA), **b**, leaf dry matter content (LDMC), **c** and leaf C/N ratio **d** under control (CK), defoliation (mowing, M), dung and urine return (D), trampling (T), and their combinations. Sum of squares decomposition analyses showing the relative importance of intraspecific trait variability (ITV) vs. species turnover in explaining CWM (**e–h**). In plots **a–d**, box-plot shows quartile values ( $n = 8$ ) for each treatment. The + symbol represents a treatment that combines several grazing components. Significance levels of the factors are as follows:

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . In plots **e–h**, light and dark green bars represent species turnover and ITV effects, respectively, and black lines indicate the total CWM variation (including species turnover, ITV, and their covariation effects) explained by grazing components. Covariation effects are represented by the space between the top of the bar and the black line. The line above the bar denotes positive covariation, and the line across the bar denotes negative covariation. The × symbol denotes the interaction between grazing components

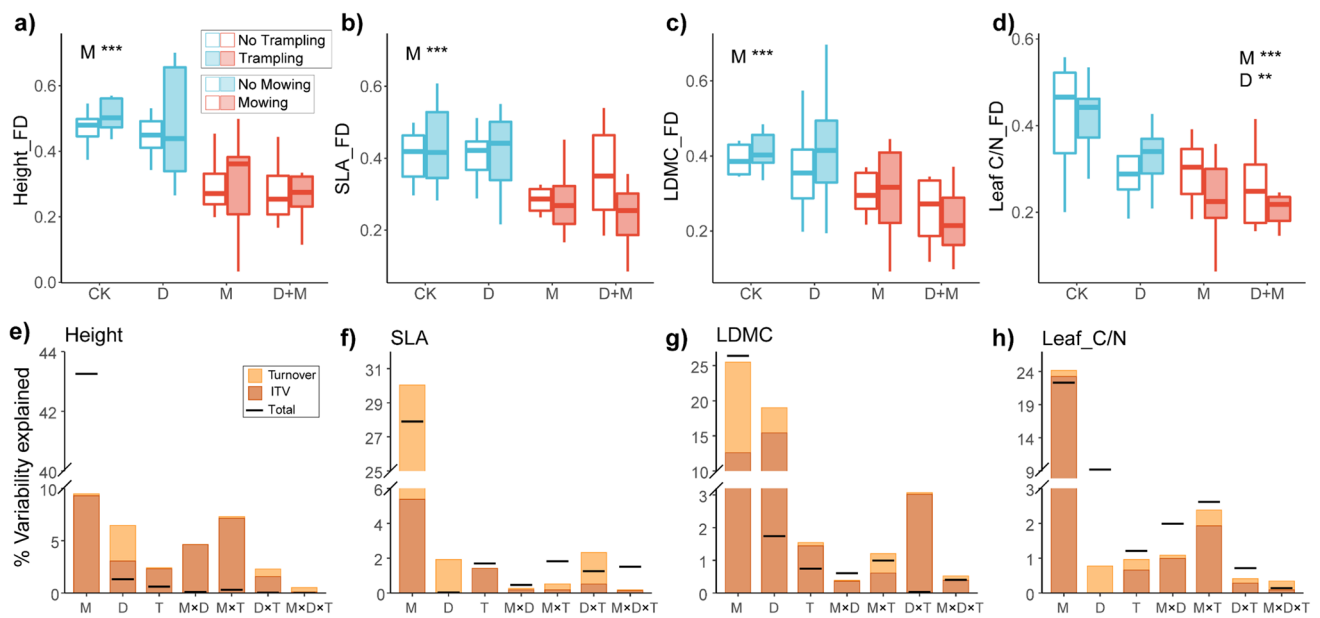
mainly mediated by changes in intraspecific trait values. These findings highlight that grazing may through multiple pathways affect functional composition and their variation to regulate productivity in grassland ecosystems, with ITV playing an important role in the process (Fig. 5).

### Species turnover and ITV together drive the effects of grazing components on CWMs

As hypothesized, defoliation resulted in a shift of traits to the strategies of rapid growth and resource-acquisitive (higher SLA, lower LDMC and leaf C/N) at the community level, and the main driver of changes caused by grazing was species turnover. Defoliation reduced the dominance of species with conservative strategy (*L. chinensis* and *S. krylovii*, Table S2) and decreased litter accumulation (Fig. S6), and consequently altered irradiance and temperature within the community (Jessen et al. 2020; Liu et al. 2019), which optimized the habitat for some forbs and subshrubs colonization. Additionally, high SLA and low LDMC values positively correlate with fast growth rate and grazing tolerance (Laliberté et al. 2012; Zheng et al. 2010), such as the species *A. frigida* in our study.

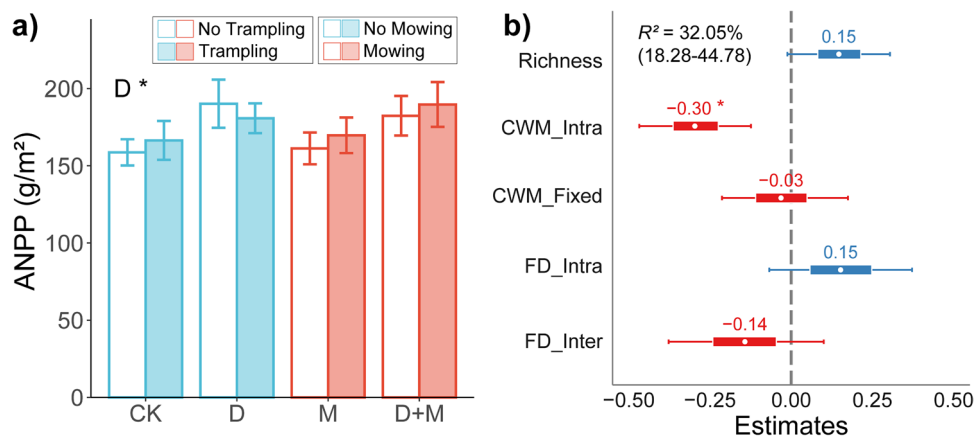
The results of the variance decomposition analysis revealed that species turnover and ITV have different covariance effects on different CWM values under defoliation, suggesting that they can reinforce or counteract each other (Fig. 2e–h). The positive covariations were observed for plant height and leaf C/N, suggesting that defoliation promotes the dominance of plants with short-statured and low leaf C/N at both the community and species levels (Fig. S3). In contrast, although the variation of CWM for SLA and LDMC resulted from species turnover under defoliation, there were negative covariations with ITV. Specifically, defoliation led to higher SLA and lower LDMC at the community level, but some individuals or species tended to decrease SLA (e.g., legumes and forbs) and increase LDMC (e.g., subshrub, Table S3, Fig. S3). These species-level changes may be considered avoidance strategies against grazing, resulting in decreased visibility and palatability to livestock, which differs from the grazing tolerance strategy at the community level (Díaz et al. 2007; Jessen et al. 2020). The differences in grazing response at community and species level may reduce the trade-off between the traits associated with these two strategies, i.e., the community eventually exhibits a simultaneous increase in compensatory growth rate and grazing resistance (Fig. 2a–d). This finding suggests





**Fig. 3** Community functional diversity (FD) of plant height **a**, specific leaf area (SLA), **b**, leaf dry matter content (LDMC), **b** and leaf C/N ratio **d** under control (CK), defoliation (mowing, M), dung and urine return (D), trampling (T), and their combinations. Sum of squares decomposition analyses showing the relative importance of ITV vs. species turnover in explaining the FDs (**e–h**). In plots **a–d**, box-plot shows quartile values ( $n = 8$ ) for each treatment. The + symbol represents a treatment that combines several grazing components. Significance levels of the factors are as follows: \* $P < 0.05$ ,

\*\* $P < 0.01$ , \*\*\* $P < 0.001$ . In plots **e–h**, light and dark orange bars represent species turnover and ITV effects, respectively, and black lines indicate the total FD variation (including species turnover, ITV, and their covariation effects) explained by grazing components. Covariation effects are represented by the space between the top of the bar and the black line. The line above the bar denotes positive covariation, and the line across the bar denotes negative covariation. The  $\times$  symbol denotes the interaction between grazing components



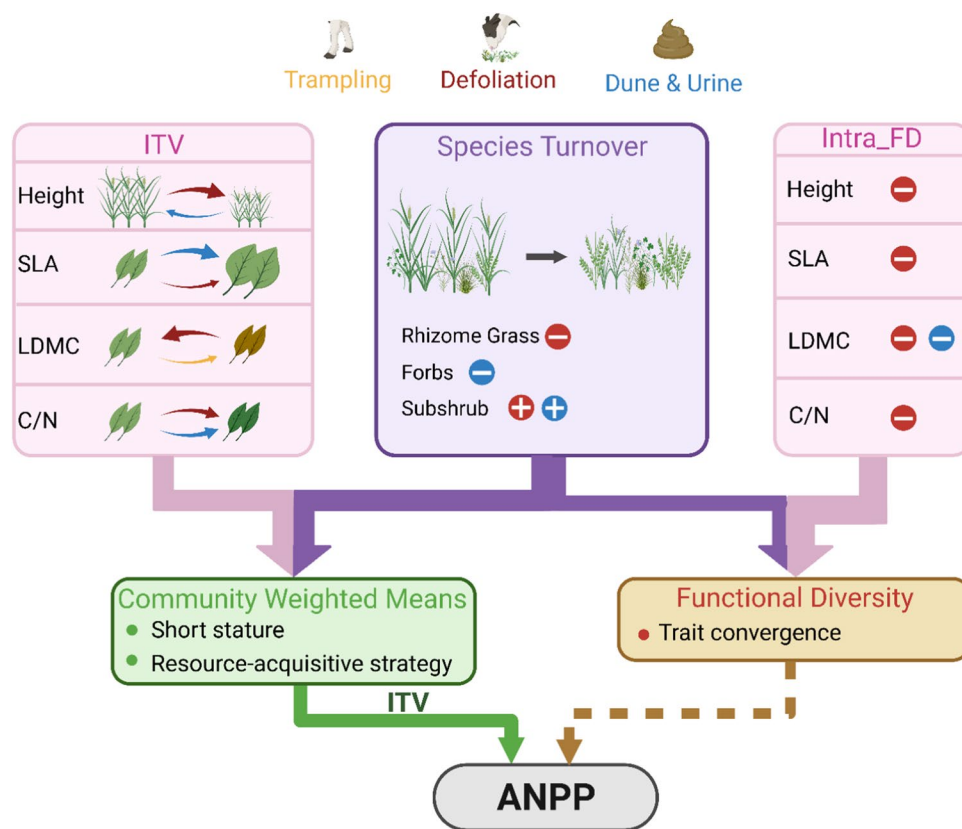
**Fig. 4** ANPP **a** under control (CK), defoliation (mowing, M), dung and urine return (D), trampling (T), and their combinations. \*Indicates the significant effect ( $P < 0.05$ ). Error bars represent  $\pm$  SE. The effect of plant richness, the intraspecific variability of the CWM (CWM\_Intra), the CWM based on fixed trait value (CWM\_Fixed), between-population ITV (Intra\_FD) and interspecific variation

(Inter\_FD) on the ANPP (**b**). The slope in Bayesian regression models indicates the effects with 95% credibility intervals, and the fit of the models is indicated by the median and 95% interval of  $R^2$ . Positive versus negative effects are highlighted in blue and red, respectively, and the significant effects occur when the 95% credibility intervals don't overlap zero

that ITV may cause community-level traits to behave differently from the leaf economic spectrum, complementing the theory of Wright et al. (2004).

In comparison, dung and urine return increased plant community height and SLA, and decreased leaf C/N, mainly explained by ITV (Fig. 2, Fig. S7). These results agreed with observations that dung and urine addition can alleviate

**Fig. 5** A conceptual framework illustrating the mechanisms of grazing mechanisms (defoliation, trampling, and dung and urine return) changes on plant community functional composition and productivity. Red, blue, and yellow arrows represent the effects of defoliation, dung and urine return and trampling respectively, with the thickness of the line indicating the magnitude of the effect. *ITV* intraspecific trait variation, *Intra\_FD* between-population ITV, *SLA* specific leaf area, *LDMC* leaf dry matter content, *C/N* leaf C/N ratio, *ANPP* aboveground net primary productivity



nutrient limitation, stimulate plant growth and increase light demand (Eskelinen et al. 2012; Zhang et al. 2019). Plants tend to develop leaves with higher SLA in nutrient-enriched soil to maximize light capture efficiency (Rozendaal et al. 2006). This plasticity in plant traits can enhance their competitiveness under nutrient enrichment, even when exposed to defoliation (Jessen et al. 2020). Trampling increased the CWM of LDMC, which may be an adaptive response to mechanical stress through trait plasticity. Mechanical stress caused by trampling stimulates plants to produce more mechanical tissue (e.g., collenchyma and fibers) with more structural carbohydrates and lower water content (Leroux 2012). We found significant interactions between defoliation and dung-urine return on the CWM values of LDMC (Fig. 2c), but there were no significant interactions among the three grazing components in the CWM values of the other traits. While previous studies reported interactive or additive effects of the three components (Liu et al. 2015; Zhang et al. 2022), the role of defoliation was dominant in the changes of plant community structure in our simulated experiment. Despite the different pathways of grazing components influencing CWM, the similar contributions of species turnover and ITV to CWM variation under the simulated grazing (Fig. 2, Fig. S5) suggest that defoliation didn't ultimately counteract the effects of dung and urine return and trampling.

### ITV is the main driver of the effects of grazing components on FDs

Grazing is known to reduce FD compared to no grazing in arid or semiarid grassland (Chillo et al. 2017). In our study, defoliation's effects on Rao's FD override those of trampling and dung and urine return (Fig. 3), indicating that defoliation as a habitat filter affects the range of trait values (Fischer et al. 2019). Defoliation leads to a convergence of community traits towards rapid growth and resource-acquisitive strategies. The contribution of deterministic processes to community assembly may increase under the habitat filters, reflecting the mechanism of  $\alpha$ -diversity (Shannon index, Fig. 1b) reduction under grazing (Mammola et al. 2021). In addition, defoliation decreased niche differentiation due to light competition, allowing plants with similar traits to coexist in the community (Keddy 1992).

The changes in FD also indicate that both biotic and abiotic stress tend to alter plant phenotypic plasticity (Niu et al. 2020; Valladares et al. 2007). Our study found that defoliation significantly reduces *Intra\_FD* and *rITV* (Fig. S8), and that *ITV* contributed the most to the variation in FD (Fig. 5, Fig. S5). Defoliation directly damages plants and induces compensatory growth or defense against herbivores (Valladares et al. 2007). The compensatory growth might lead to deficiencies of available soil nutrients (Fig. S1),

indirectly affecting plant functional diversity. Moreover, defoliation removed aboveground biomass and decreased litter inputs (Fig. S6), aggravating these negative effects on soil nutrients and FD (Niu et al. 2016). We found a significant positive effect of soil available nutrients on FD of LDMC and leaf C/N (Table 1), suggesting that nutrient limitation caused by defoliation leads to the convergence of traits related to resource acquisition (Niu et al. 2020). Plants tend to enhance their ability to access resources to increase their inter- and intraspecific competition. Contrary to our initial prediction, dung and urine return did not significantly change soil available nutrient content ( $P > 0.05$ , Fig. S1c, d) in this study, which indicated that nutrient return may not fully compensate for the soil nutrient loss caused by defoliation.

Our study demonstrates that ITV plays a crucial role in shaping changes in plant functional composition (CWM and FD) in response to grazing components (Fig. 5, Fig. S5), suggesting that local plants have likely evolved adaptations to herbivore grazing over a long history of grazing (Adler et al. 2004; Li et al. 2021a). The plasticity of plant traits allows for changes in functional composition under disturbance without relying solely on species turnover, which can serve as a buffer against changes in plant composition and protect species from extinction (Lepik et al. 2005). Hence, this mechanism ensures the relative stability of plant richness in local communities under grazing (Fig. 1a) (Volf et al. 2016).

### ITV-induced changes in CWM mediate the effects of grazing components on ANPP

Our results revealed that the simulated grazing positively affected ANPP, especially under dung and urine return (Fig. 4a). Contrary to our third hypothesis, intraspecific variation-induced changes in CWM played a key role in the changes in productivity, while the effects of both intra- and interspecific FD on ANPP were not significant (Figs. 4b, 5), suggesting that mass-ratio theory mainly explained the increase of ANPP (Mokany et al. 2008). This could be attributed to the fact that nutrient inputs increased SLA and leaf nitrogen content within species (Fig. S7, Table S1), which consequently increased the photosynthetic rate at the community level (Ali et al. 2013). Although the absence of significant effects of defoliation and trampling on ANPP, the positive effect of dung and urine return, along with the compensatory effect of defoliation, explained the mechanism of ANPP enhancement under moderate grazing. We found that *A. frigida* compensated for the biomass loss of perennial rhizome grass caused by defoliation (Fig. 1c, Tabel S3). But this compensation may have a negative impact on animal production performance (Wang et al. 2007). It is worth noting that dung and urine return will not be distributed as evenly for real livestock grazing as in our simulated grazing

(Moir et al. 2011). Therefore, the significant contribution of grazing to productivity may not be observed in some livestock grazing experiments due to the sampling effect.

It is important to acknowledge that our simulated grazing treatments do not fully replicate real grazing by ungulates. Our treatments did not account for the selectivity and persistence of defoliation (Onatibia et al. 2023), and the spatial heterogeneity of defoliation, trampling, and dung and urine return (Liu et al. 2018). Furthermore, it is possible that our anthropogenic disturbance had different effects on other herbivores (such as arthropods) compared with real grazing (Wang et al. 2020), which also has a significant effect on vegetation. Despite these limitations, we believe that our study provides valuable insights into the mechanisms through which management practices, such as grazing and mowing, impact grassland.

## Conclusion

This study provides novel insight into how large herbivores affect plant community functional composition and ANPP, and is crucial to understanding plant–herbivore interactions. Based on a simulated grazing experiment, we demonstrate that defoliation had the greatest influence on CWM and FD. Under defoliation, species turnover accounted for most of the variance of CWM, while intraspecific trait variability played a more critical role in the decrease of FD. Dung and urine return and trampling changed CWM more through ITV than species turnover, and these two components had almost no significant effect on FD. Furthermore, ANPP was increased by dung and urine return and mediated by changes in intraspecific trait values. These results highlighted the key role of ITV in shaping grassland plant community functional composition and productivity under ungulate grazing. Our study also pointed out that even at moderate intensities, continuous removal of aboveground biomass (defoliation) had potential negative effects on individual plants, resulting in soil nutrient deficiencies and a decrease of  $\alpha$  diversity. Therefore, grazing regimes and intensities should be adjusted in grassland management according to key species' phenotypic plasticity and grazing adaptability, and nutrient addition should be implemented for long-term grazing grasslands.

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**Author contribution statement** LN and ZYJ designed the study. WYQ and LN established the field experiment. WYQ, WB, GMQ, GTT performed field and lab work. GTT and LN conducted statistical analysis and wrote the first draft of the manuscript. ZSX and ZYJ assisted with statistical analysis and paper revision.

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**Data availability** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Code availability** Not applicable.

## Declarations

**Conflict of interest** Not applicable.

**Ethical approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

## References

- Adler PB, Milchunas DG, Lauenroth WK et al (2004) Functional traits of graminoids in semi-arid steppes: a test of grazing histories. *J Appl Ecol* 41:653–663. <https://doi.org/10.1111/j.0021-8901.2004.00934.x>
- Ali AA, Medlyn BE, Crous KY et al (2013) A trait-based ecosystem model suggests that long-term responsiveness to rising atmospheric CO<sub>2</sub> concentration is greater in slow-growing than fast-growing plants. *Funct Ecol* 27:1011–1022. <https://doi.org/10.1111/1365-2435.12102>
- Amaral EJ, Franco AC, Rivera VL et al (2021) Environment, phylogeny, and photosynthetic pathway as determinants of leaf traits in savanna and forest graminoid species in central Brazil. *Oecologia* 197:1–11. <https://doi.org/10.1007/s00442-021-04923-w>
- Archibald S, Hempson GP, Lehmann C (2019) A unified framework for plant life-history strategies shaped by fire and herbivory. *New Phytol* 224:1490–1503. <https://doi.org/10.1111/nph.15986>
- Baoyin T, Li FY, Bao Q et al (2014) Effects of mowing regimes and climate variability on hay production of *Leymus chinensis* (Trin.) Tzvelev grassland in northern China. *Rangeland J* 36:593–600. <https://doi.org/10.1071/Rj13088>
- Bongers FJ, Schmid B, Bruelheide H et al (2021) Functional diversity effects on productivity increase with age in a forest biodiversity experiment. *Nat Ecol Evol* 5:1594–1603. <https://doi.org/10.1038/s41559-021-01564-3>
- Borer ET, Seabloom EW, Gruner DS et al (2014) Herbivores and nutrients control grassland plant diversity via light limitation. *Nature* 508:517–520. <https://doi.org/10.1038/nature13144>
- Chen WQ, Wang XY, Zhang YJ et al (2015) Effects of the vertical and horizontal availability of food resources: the diet selection of sheep grazing on natural grassland. *J Agric Sci* 153:322–334. <https://doi.org/10.1017/s0021859614000355>
- Chillo O, Ojeda RA, Capmourteres V et al (2017) Functional diversity loss with increasing livestock grazing intensity in drylands: the mechanisms and their consequences depend on the taxa. *J Appl Ecol* 54:986–996. <https://doi.org/10.1111/1365-2664.12775>
- de Bello F, Lavorel S, Albert CH et al (2011) Quantifying the relevance of intraspecific trait variability for functional diversity. *Methods Ecol Evol* 2:163–174. <https://doi.org/10.1111/j.2041-210X.2010.00071.x>
- Deckers JA, Nachtergaele F (1998) World reference base for soil resources. In: Schulte A, Ruhiyat D (eds) *Soils of tropical forest ecosystems*. Springer, Berlin, pp 21–28
- Díaz S, Lavorel S, McIntyre S et al (2007) Plant trait responses to grazing a global synthesis. *Glob Change Biol* 13:313–341
- Díaz S, Noy-Meir I, Cabido M (2001) Can grazing response of herbaceous plants be predicted from simple vegetative traits? *J Appl Ecol* 38:497–508. <https://doi.org/10.1046/j.1365-2664.2001.00635.x>
- Eskelinen A, Harrison S, Tuomi M (2012) Plant traits mediate consumer and nutrient control on plant community productivity and diversity. *Ecology* 93:2705–2718. <https://doi.org/10.1890/12-0393.1>
- Fischer FM, Bonnet OJF, Cezimbra IM et al (2019) Long-term effects of grazing intensity on strategies and spatial components of functional diversity in subtropical grassland. *Appl Veg Sci* 22:39–47. <https://doi.org/10.1111/avsc.12402>
- Fraser MD, Theobald VJ, Griffiths JB et al (2009) Comparative diet selection by cattle and sheep grazing two contrasting heathland communities. *Agric Ecosyst Environ* 129:182–192. <https://doi.org/10.1016/j.agee.2008.08.013>
- Grime JP (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *J Ecol* 86:902–910. <https://doi.org/10.1046/j.1365-2745.1998.00306.x>
- Hanisch M, Schweiger O, Cord AF et al (2020) Plant functional traits shape multiple ecosystem services, their trade-offs and synergies in grasslands. *J Appl Ecol* 57:1535–1550. <https://doi.org/10.1111/1365-2664.13644>
- Jäschke Y, Heberling G, Wesche K et al (2019) Environmental controls override grazing effects on plant functional traits in Tibetan rangelands. *Funct Ecol* 34:747–760. <https://doi.org/10.1111/1365-2435.13492>
- Jessen MT, Kaarlejarvi E, Olofsson J et al (2020) Mammalian herbivory shapes intraspecific trait responses to warmer climate and nutrient enrichment. *Glob Change Biol* 26:6742–6752. <https://doi.org/10.1111/gcb.15378>
- Keddy PA (1992) Assembly and response rules: two goals for predictive community ecology. *J Veg Sci* 3:157–164. <https://doi.org/10.2307/3235676>
- Laliberte E, Legendre P (2010) A distance-based framework for measuring functional diversity from multiple traits. *Ecology* 91:299–305. <https://doi.org/10.1890/08-2244.1>
- Laliberté E, Shipley B, Norton DA et al (2012) Which plant traits determine abundance under long-term shifts in soil resource availability and grazing intensity? *J Ecol* 100:662–677. <https://doi.org/10.1111/j.1365-2745.2011.01947.x>
- Lepik M, Liira J, Zobel K (2005) High shoot plasticity favours plant coexistence in herbaceous vegetation. *Oecologia* 145:465–474. <https://doi.org/10.1007/s00442-005-0142-0>
- Lepš J, de Bello F, Smilauer P et al (2011) Community trait response to environment: disentangling species turnover vs intraspecific trait variability effects. *Ecography* 34:856–863. <https://doi.org/10.1111/j.1600-0587.2010.06904.x>
- Leroux O (2012) Collenchyma: a versatile mechanical tissue with dynamic cell walls. *Ann Bot* 110:1083–1098. <https://doi.org/10.1093/aob/mcs186>
- Li L, Zhang J, He XZ et al (2021a) Sheep trampling modifies soil and plant C:N: P stoichiometry in a typical steppe of the loess

- plateau. *Rangeland Ecol Manag* 76:100–108. <https://doi.org/10.1016/j.rama.2021.02.008>
- Li W, He S, Cheng X et al (2021b) Functional diversity outperforms taxonomic diversity in revealing short-term trampling effects. *Sci Rep* 11:18889. <https://doi.org/10.1038/s41598-021-98372-3>
- Li W, Zheng TD, Cheng XP et al (2023) Changes in functional traits and diversity of typical alpine grasslands after a short-term trampling disturbance. *Front Ecol Evol* 11:1154911. <https://doi.org/10.3389/fevo.2023.1154911>
- Li WH, Xu FW, Zheng SX et al (2017) Patterns and thresholds of grazing-induced changes in community structure and ecosystem functioning: species-level responses and the critical role of species traits. *J Appl Ecol* 54:963–975. <https://doi.org/10.1111/1365-2664.12806>
- Liu C, Wang L, Song XX et al (2018) Towards a mechanistic understanding of the effect that different species of large grazers have on grassland soil N availability. *J Ecol* 106:357–366. <https://doi.org/10.1111/1365-2745.12809>
- Liu N, Kan HM, Yang GW et al (2015) Changes in plant, soil, and microbes in a typical steppe from simulated grazing: explaining potential change in soil C. *Ecol Monogr* 85:269–286. <https://doi.org/10.1890/14-1368.1>
- Liu XQ, Zhang X, Zhang LF et al (2019) Using functional trait diversity to infer community assembly mechanisms: an enclosure experiment as an example. *J Plant Ecol* 12:448–459. <https://doi.org/10.1093/jpe/rty030>
- Mammola S, Carmona CP, Guillaume T et al (2021) Concepts and applications in functional diversity. *Funct Ecol* 35:1869–1885. <https://doi.org/10.1111/1365-2435.13882>
- Mikola J, Setälä H, Virkajarvi P et al (2009) Defoliation and patchy nutrient return drive grazing effects on plant and soil properties in a dairy cow pasture. *Ecol Monogr* 79:221–244. <https://doi.org/10.1890/08-1846.1>
- Moir JL, Cameron KC, Di HJ et al (2011) The spatial coverage of dairy cattle urine patches in an intensively grazed pasture system. *J Agric Sci* 149:473–485. <https://doi.org/10.1017/S0021859610001012>
- Mokany K, Ash J, Roxburgh S (2008) Functional identity is more important than diversity in influencing ecosystem processes in a temperate native grassland. *J Ecol* 96:884–893. <https://doi.org/10.1111/j.1365-2745.2008.01395.x>
- Niu KC, He JS, Lechowicz MJ (2016) Grazing-induced shifts in community functional composition and soil nutrient availability in Tibetan alpine meadows. *J Appl Ecol* 53:1554–1564. <https://doi.org/10.1111/1365-2664.12727>
- Niu KC, Zhang S, Lechowicz M (2020) Harsh environmental regimes increase the functional significance of intraspecific variation in plant communities. *Funct Ecol* 34:1666–1677. <https://doi.org/10.1111/1365-2435.13582>
- Olsen SR (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Department of Agriculture, Washington
- Onatibia GR, Aguiar MR, Oesterheld M (2023) Individual-plant selectivity by sheep in drylands scales-up at plant population level and controls the forage supply and its accessibility. *J Appl Ecol* 60:1446–1456. <https://doi.org/10.1111/1365-2664.14427>
- Perez-Harguindeguy N, Diaz S, Garnier E et al (2013) New handbook for standardised measurement of plant functional traits worldwide. *Aust J Bot* 61:167–234. <https://doi.org/10.1071/bt12225>
- Piipponen J, Jalava M, de Leeuw J et al (2022) Global trends in grassland carrying capacity and relative stocking density of livestock. *Glob Change Biol* 28:3902–3919. <https://doi.org/10.1111/gcb.16174>
- Ren H, Gui W, Bai Y et al (2018) Long-term effects of grazing and topography on extra-radical hyphae of arbuscular mycorrhizal fungi in semi-arid grasslands. *Mycorrhiza* 28:117–127. <https://doi.org/10.1007/s00572-017-0812-x>
- Rozendaal DMA, Hurtado VH, Poorter L (2006) Plasticity in leaf traits of 38 tropical tree species in response to light; relationships with light demand and adult stature. *Funct Ecol* 20:207–216. <https://doi.org/10.1111/j.1365-2435.2006.01105.x>
- Siefert A, Violle C, Chalmandrier L et al (2015) A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. *Ecol Lett* 18:1406–1419. <https://doi.org/10.1111/ele.12508>
- Valladares F, Gianoli E, Gómez JM (2007) Ecological limits to plant phenotypic plasticity. *New Phytol* 176:749–763. <https://doi.org/10.1111/j.1469-8137.2007.02275.x>
- Violle C, Enquist BJ, McGill BJ et al (2012) The return of the variance: intraspecific variability in community ecology. *Trends Ecol Evol* 27:244–252. <https://doi.org/10.1016/j.tree.2011.11.014>
- Volf M, Redmond C, Albert AJ et al (2016) Effects of long- and short-term management on the functional structure of meadows through species turnover and intraspecific trait variability. *Oecologia* 180:941–950. <https://doi.org/10.1007/s00442-016-3548-y>
- Wang C, Wang S, Zhou H et al (2007) Effects of forage composition and growing season on methane emission from sheep in the inner Mongolia steppe of China. *Ecol Res* 22:41–48. <https://doi.org/10.1007/s11284-006-0191-9>
- Wang XY, Li FY, Tang KY et al (2020) Land use alters relationships of grassland productivity with plant and arthropod diversity in Inner Mongolian grassland. *Ecol Appl* 30:13. <https://doi.org/10.1002/eap.2052>
- Wang XY, Yan XB, Huang KL et al (2022) Nitrogen enrichment and warming shift community functional composition via distinct mechanisms: The role of intraspecific trait variability and species turnover. *Funct Ecol* 36:1230–1242. <https://doi.org/10.1111/1365-2435.14012>
- White RP, Murray S, Rohweder M et al (2000) Grassland ecosystems. World Resources Institute, Washington
- Wright IJ, Reich PB, Westoby M et al (2004) The worldwide leaf economics spectrum. *Nature* 428:821–827. <https://doi.org/10.1038/nature02403>
- Wright JP, Ames GM, Mitchelll RM (2016) The more things change, the more they stay the same? When is trait variability important for stability of ecosystem function in a changing environment. *Philos Trans R Soc B-Biol Sci* 371:7. <https://doi.org/10.1098/rstb.2015.0272>
- Xiao H, Peng Z, Xu CL et al (2018) Yak and Tibetan sheep trampling inhibit reproductive and photosynthetic traits of *Medicago ruthenica* var. *inschanica*. *Environ Monit Assess* 190:16. <https://doi.org/10.1007/s10661-018-6896-8>
- Xu Q, Yang X, Song J et al (2022) Nitrogen enrichment alters multiple dimensions of grassland functional stability via changing compositional stability. *Ecol Lett* 25:2713–2725. <https://doi.org/10.1111/ele.14119>
- Zhang D, Peng Y, Li F et al (2019) Trait identity and functional diversity co-drive response of ecosystem productivity to nitrogen enrichment. *J Ecol* 107:2402–2414. <https://doi.org/10.1111/1365-2745.13184>
- Zhang L, Bai W, Zhang Y et al (2023) Ecosystem stability is determined by plant defence functional traits and population stability under mowing in a semi-arid temperate steppe. *Funct Ecol* 37:2413–2424. <https://doi.org/10.1111/1365-2435.14401>
- Zhang R, Wang Z, Han G et al (2018) Grazing induced changes in plant diversity is a critical factor controlling grassland productivity in the Desert Steppe, Northern China. *Agric Ecosyst Environ* 265:73–83. <https://doi.org/10.1016/j.agee.2018.05.014>
- Zhang TR, Li FY, Li YL et al (2022) Disentangling the effects of animal defoliation, trampling, and excretion deposition on plant

- nutrient resorption in a semi-arid steppe: the predominant role of defoliation. *Agric Ecosyst Environ* 337:10. <https://doi.org/10.1016/j.agee.2022.108068>
- Zheng SX, Chi YG, Yang XJ et al (2021) Direct and indirect effects of nitrogen enrichment and grazing on grassland productivity through intraspecific trait variability. *J Appl Ecol* 59:598–610. <https://doi.org/10.1111/1365-2664.14078>
- Zheng SX, Ren HY, Lan ZC et al (2010) Effects of grazing on leaf traits and ecosystem functioning in Inner Mongolia grasslands: scaling from species to community. *Biogeosciences* 7:1117–1132. <https://doi.org/10.5194/bg-7-1117-2010>
- Zuo X, Li X, Yue P et al (2022) Drought-driven shifts in relationships between plant biodiversity and productivity in temperate steppes. *Funct Ecol* 36:2917–2928. <https://doi.org/10.1111/1365-2435.14219>

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