

## RESEARCH ARTICLE

# Higher dung seedling density increases livestock dung greenhouse gas emissions in an alpine meadow

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**Handling Editor:** Emanuela Weidlich**Abstract**

1. The dung seed bank (i.e. vegetation renewal) and greenhouse gas (GHG, e.g. carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O), which contributing to global warming) emissions (i.e. materials circulation) are two of the most important ecological functions of livestock dung in grassland ecosystems. With decomposition of faeces, dung seedling density (e.g. increase) and GHG emission (e.g. decrease) changes proceeded simultaneously. Although these two processes have so far been studied in isolation, almost no studies have explored the effects of dung seedlings on GHG emissions.
2. We collected the dung of three livestock species—yak, sheep and horse—during late April (i.e. early warm season) in an alpine meadow in the northeastern Qinghai-Tibetan Plateau (QTP). The livestock dung seedling composition and GHG fluxes were determined on Days 1, 6, 11, 16, 21, 26, 31, 37, 53 and 60 of germination, and we used frozen and ground faeces as the control group.
3. The average seedling density of horse dung was significantly greater than that of yak and sheep. The GHG fluxes in the collected native dung samples were significantly greater than those of the dung controls, and the fluctuations in CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O all showed a pattern of yak dung > horse dung > sheep dung. Over the course of the experiment, the GHG fluxes of both the experimental and control groups gradually decreased; however, the GHG emissions of the experimental group declined faster than those of the control group.
4. There was a significant negative correlation between dung seedling density and GHG fluxes. Dung seedlings provide a reference for estimation of GHG emissions from livestock faeces.
5. *Synthesis and applications.* Our research has revealed the features of and interactions between two important ecological functions of livestock dung in the grazing system. Furthermore, dung seedlings could substantially affect GHG fluxes and emission rates of livestock dung patches. The contributions of dung seeds to GHG emissions should be considered when estimating global warming potential from grazing livestock on the QTP for the purposes of determining national and regional land use policies and compiling global GHG inventories.

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## KEYWORDS

endozoochory, grazing ecosystem, livestock dung, QTP, relationship

## 1 | INTRODUCTION

Livestock dung plays a significant role in grassland ecosystems (Li et al., 2021; Yang et al., 2019). On the one hand, livestock faeces constitute the dung seed bank of the grassland; that is, mature plant seeds are sometimes consumed by livestock while foraging, and some seeds survive passage through the digestive tract and are ultimately deposited in dung (referred to as endozoochorous seed dispersal). These viable seeds within large-herbivore faeces form the dung seed bank (Wang et al., 2019, 2021; Wang & Hou, 2021a). Dung seed banks are ubiquitous in grazing ecosystems, especially during the season in which seeds ripen and are important drivers of vegetation renewal and a vital supplement to the soil seed bank (Wang et al., 2021). The germination of dung seeds and the growth of dung seedlings occur at the same time as the process of faeces decomposition. Moreover, seedling emergence and growth are promoted by animal dung (Nchanji & Plumptre, 2003; Traveset et al., 2001), as dung can enhance microsite fertility, increases the water-holding capacity of the soil, increases the local organic matter and nutrients, offers a short-term reduction in competition with extant vegetation and offers short-term protection from grazing near the dung (Ocumpaugh et al., 1996).

On the other hand, livestock dung is also an important source of greenhouse gas (GHG) emissions, especially carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O), which have been identified as the main contributors to global warming, in grassland ecosystems (Lin et al., 2009; Liu et al., 2017). GHG emitted directly or indirectly by livestock production systems accounts for ~18% of the global GHG emissions due to human activities (Liu, 2018), which could potentially increase the atmospheric GHG concentration and impact global climate change (Cai, Wang, et al., 2014). Livestock dung contains substantial amounts of organic matter and mineral salt ions (e.g. NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>), as well as microorganisms (Maljanen et al., 2007). The pool of available nitrogen and the carbon added to the grassland via livestock excreta provide substrates for the production of N<sub>2</sub>O, CO<sub>2</sub> and CH<sub>4</sub> by dung microorganisms (Boon et al., 2014).

Most of the CO<sub>2</sub> and CH<sub>4</sub> associated with animals is released directly from the animals, whereas a smaller amount of both gases results from the degradation of organic matter in dung patches (Saggar et al., 2004). Meanwhile, N<sub>2</sub>O fluxes result from biological processes of nitrification and denitrification. In general, nitrification involves the aerobic microbial oxidation of ammonia into nitrate (NO<sub>3</sub><sup>-</sup>), whereas denitrification involves the anaerobic microbial reduction of NO<sub>3</sub><sup>-</sup> to NO, N<sub>2</sub>O and N<sub>2</sub>. In nitrification, N<sub>2</sub>O is by-product while in denitrification it is an intermediate product (Liu et al., 2017). Defecation by grazing livestock can give rise to 'hot spots' that represent the high local addition of nitrogen and readily

available carbon, which can stimulate surface emissions of GHG (Cardenas et al., 2007; Saggar et al., 2004). GHG emissions from livestock grazing systems cannot be ignored for regional or global GHG emission inventories (Cai, Wang, et al., 2014; Liu et al., 2017). Therefore, researching the mechanisms of GHG emission associated with livestock dung in grazing ecosystems is essential for maintaining the sustainable development of animal husbandry and assessing regional as well as global warming.

The dung seed bank (i.e. vegetation renewal) and GHG emissions (i.e. materials circulation) are two of the most important ecological functions of livestock dung in grassland ecosystems (Cai et al., 2013; Liu et al., 2017; Wang & Hou, 2021b). With decomposition of faeces, dung seedling density (e.g. increase; Wang & Hou, 2021a) and GHG emission (e.g. decrease; Lin et al., 2009) changes proceeded simultaneously. Therefore, we inferred that there is a negative correlation between dung seedling density and GHG fluxes. However, these two factors have not been studied in combination, and the effects of dung seedlings on GHG emissions are largely unknown.

The Qinghai-Tibetan Plateau (QTP) or 'Third Pole' of the Earth, so named because of its extensive ice fields, extends for ~2.5 million km<sup>2</sup> (Cai, Wang, et al., 2014). About 65% of the QTP is covered by alpine meadows (Ma et al., 2022), where ~3.3 million yaks (*Bos grunniens*; Yu et al., 2013), ~30 million Tibetan sheep (*Ovis aries*; Lin et al., 2009) and ~1 million horses (*Equus caballus*) graze freely. Annually, a large amount of livestock dung is deposited directly onto the QTP grassland (including >40 million tons of yak dung alone; Liu, 2018). During the warm season (July–September) of an alpine meadow on the QTP, average emissions of CH<sub>4</sub>, CO<sub>2</sub> and N<sub>2</sub>O from a yak dung patch were 586, 2816 and 745 mg m<sup>-2</sup> h<sup>-1</sup>, respectively (Lin et al., 2009). Meanwhile, the dung seedling density of yak, sheep and horse was 9.1, 7.4 and 10 seedlings/g dung, respectively (Wang & Hou, 2021b). One hypothesis is that dung seedling growth and establishment accelerates GHG emissions by absorbing dung nutrients and promoting dung decomposition (Nchanji & Plumptre, 2003).

For this study, we collected the dung of yak, Tibetan sheep and horse during the warm season in an alpine meadow in the north-eastern QTP, and the livestock dung seedling composition and GHG fluxes were determined. The objectives of this research were to study (i) the size and species composition of the livestock dung seed bank during the warm grazing season, (ii) the GHG fluxes of the dung of different livestock species and (iii) the relationship between dung seedling density and GHG fluxes. We hypothesized that dung seed could contribute to dung GHG emissions as dung seedling growth promotes the uptake and transfer of dung nutrients (i.e. dung decomposition). This study was the first to assess the integration of two important ecological functions of livestock dung in a grazing ecosystem. The results should enhance our understanding of the ecological significance of livestock dung in grasslands worldwide.

## 2 | MATERIALS AND METHODS

Our study did not require ethics approval and permission for fieldwork.

### 2.1 | Study site

This study was performed at the Maqu Grassland Agricultural Research Station of Lanzhou University in Maqu County (i.e. Maqu Research Station), Gansu Province, China (33°36' to 33°43' N, 101°44' to 101°48' E; 3520 m a.s.l.), located in the northeastern QTP (Figure 1). The climate is cold and humid, with only a warm season (late April–October) and a cold season (November–April; Sun et al., 2015), and there is no absolute frost-free period throughout the year. Based on local weather data for the past 30 years, the annual average temperature is  $-1.2^{\circ}\text{C}$ ; the highest temperatures occur from June to August with an average of  $-12^{\circ}\text{C}$ , and the lowest temperatures (average,  $-10^{\circ}\text{C}$ ) occur from December to February. The average annual precipitation is  $\sim 620\text{ mm}$ , occurring mainly during the herbage-growing season (May–September).

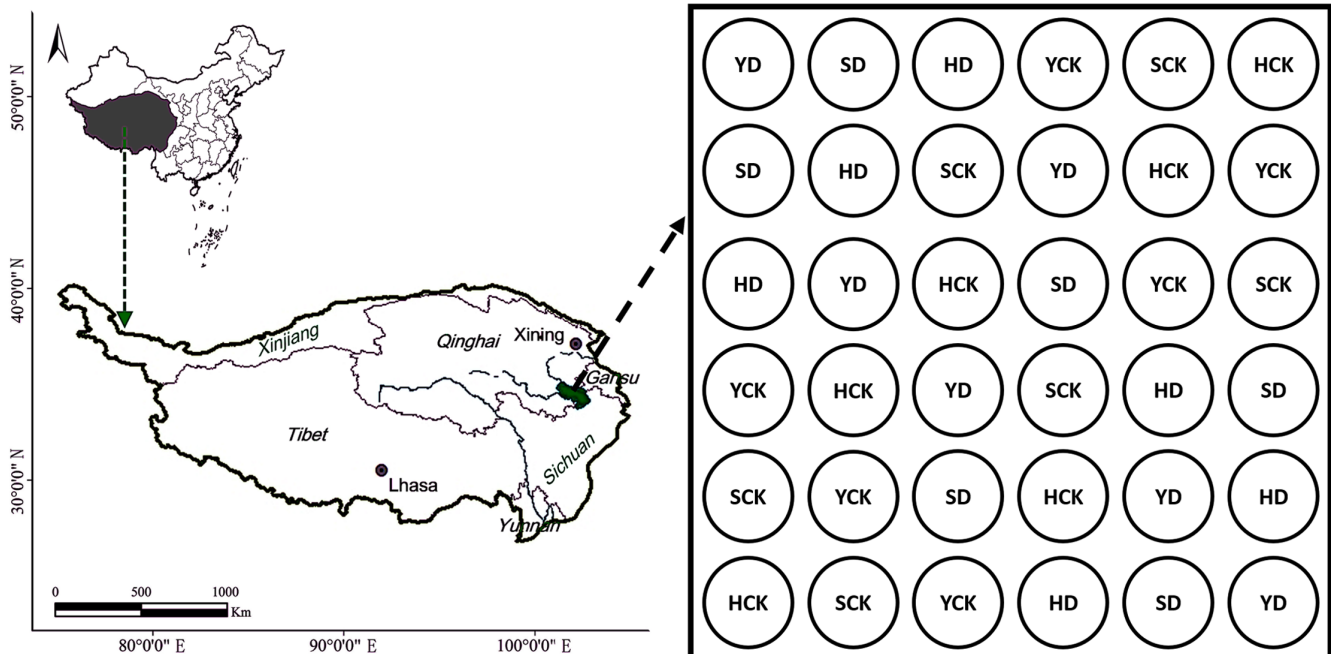
The soils are classified as Mat-Cryic Cambisols (Sun et al., 2015; Wang & Hou, 2021c), and the vegetation is characteristic of a typical alpine meadow (Yang et al., 2019). Dominant species include *Carex hughii* S. R. Zhang, *Elymus nutans* Griseb., *Gentiana lawrencei* var. *farreri* (I. B. Balfour) T. N. Ho, *Anemone rivularis* var. *floreminore* Maxim. and *Aster diplostephioides* (DC.) C. B. Clarke (Wang & Hou, 2021a). Yak ( $\sim 300$  individuals), Tibetan sheep ( $\sim 200$  individuals) and horses ( $\sim 100$  individuals) are the only grazing livestock species in this region.

### 2.2 | Livestock dung collection

Mid-August is the peak period of seed maturity in the study area; however, a substantial number of mature seeds still remain on the reproductive culms of herbage (i.e. the canopy seed bank; Ripa et al., 2020) until the following April (i.e. the climax period of seed germination). During this period, those remaining seeds have the potential to be eaten by livestock. To be consistent with the seed germination time under natural conditions, we collected livestock dung in late April for seed germination and GHG flux detection.

On 20 April 2019, dung from freely grazing yaks (YD, yak dung; fresh weight,  $\sim 40\text{ kg}$ ; moisture content, 84.29%), Tibetan sheep (SD, sheep dung; fresh weight,  $\sim 3.5\text{ kg}$ ; moisture content, 61.62%) and horses (HD, horse dung; fresh weight,  $\sim 45\text{ kg}$ ; moisture content, 82.40%) were collected from a natural pasture. To avoid collecting seeds that were deposited by wind or other agents onto/into the dung, only fresh dung (i.e. recently defecated) was collected. To prevent above-ground seeds from sticking to the dung, we carefully removed the soil and/or litter from the underside of each dung piece (Yu et al., 2013).

After dung from each of the livestock species was thoroughly mixed, we took about one-fifth of each respective mixture, placed it in a sealed plastic bag and stored it in a refrigerator at  $4^{\circ}\text{C}$  (i.e. experimental group), and another one-fifth of each sample was frozen and ground (Freeze Grinder, SDL-500) to destroy any dung-borne seeds and then stored at  $4^{\circ}\text{C}$  (i.e. control group). Freezing and grinding does not substantially change the initial livestock dung physical properties (e.g. aeration) as well as chemical properties (Figure S1). Both groups were subjected to the germination and GHG assays. For each dung mixture, the remaining approximately three-fifths of the



**FIGURE 1** Location of the Maqu Research Station on the Qinghai-Tibetan Plateau in China and the arrangement of the germination pots. HCK, horse dung control; HD, horse dung; SCK, Tibetan sheep dung control; SD, Tibetan sheep dung; YCK, yak dung control; YD, yak dung.

sample was used to detect the decomposition dynamics (i.e. decomposition group).

## 2.3 | Germination

The germination experiment started on 25 April 2020. For both the experimental group and control group, a sample of fresh dung was measured according to the weight of a single defecation of each livestock species: yak dung, 1200g; horse dung, 1400g; and Tibetan sheep dung, 60g. Each sample was mixed with ~50g sterile sand and placed in round plastic pots (50cm in diameter×30cm in depth) with drainage holes on a 5-cm-thick bed of vermiculite. The dung/sand mixture was placed on the vermiculite in accordance with the shape of the native dung patches on the natural grassland as follows: YD was cylindrical (19 cm in diameter×4 cm in height), HD was cylindrical (25 cm in diameter×13cm in height), and SD was rectangular (25 cm in length×13 cm in width×1.4 cm in height). A total of six treatments (three experimental group samples [i.e. YD, HD and SD]+three control group samples [i.e. YCK, HCK and SCK]) were set up, and each treatment had six replicates. Therefore, 36 pots (6 treatments×6 replicates) were obtained. Pots were arranged in a randomized complete block design on an enclosed flat grassland (10m×10m, from which all above-ground vegetation was removed during the study period) under natural conditions (Figure 1). The pot bottom was buried ~3cm underground to allow the exchange of material between the dung and the soil below through the drainage holes. In addition, six pots containing only sterilized sand and vermiculite were placed alongside the dung pots to check for contamination by seeds blown in by the wind (or other potential means; no germination was detected in these control samples during the experimental period).

The germinated seedlings were identified and recorded 1, 6, 11, 16, 21, 26, 31, 37, 53 and 60 days after the start of the germination test. It was difficult to identify the dung seedlings during the early stages of germination. We took photographs of each dung pot and marked the position of the seedlings in the picture (i.e. Digital Repeat Photography; Songsom et al., 2021; Figure S2). When the seedlings were identifiable, the number of individual species (i.e. richness) and species density were recorded for each pot.

## 2.4 | Determination of decomposition

The same arrangement of pots was used for the decomposition group. There were three replicates of the experimental and control groups for each livestock species. Therefore, 54 pots ([3 experimental group samples + 3 control group samples]×3 livestock species×3 replicates) were obtained for the decomposition group. We used 18 pots ([3 experimental group samples + 3 control group samples]×3 livestock species) for materials determination on Day 0 (i.e. the initial sample) and at 31 and 60 days, respectively. When the experimental

group samples were analysed, any seedlings were first removed. See Table S1 for the determination methods.

## 2.5 | GHG emissions

We used an online observation system instrument (LI-COR 8150; Figure S3) to determine GHG emissions on Days 1, 6, 11, 16, 21, 26, 31, 37, 53 and 60 of dung seedling germination (i.e. simultaneously with the identification of dung seedlings) to measure the CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub> emission dynamics of both the experimental and control pots. The GHG observation instrument is composed of an analysis system (CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub> analysers), a control system (multiplex system), a main control box and six GHG efflux chambers. Each component is connected by wires and rubber tubes. Before measuring the GHG, we prepared six trays (110 cm<sup>2</sup>), and we moved six pots from the same row to the six trays and covered them with the efflux chambers before filling each tray with the proper amount of water to seal the chamber bottom (Zhao et al., 2021). GHG measurement started at 09:00, and the time to analyse each efflux chamber (i.e. one pot) was 15 min. The measurement of one row (six pots) thus required 1.5 h, and six rows (36 pots) could be finished in 9 h. The formula for the GHG emission fluxes is as follows:

$$F = \frac{\alpha \times (C_f - C_i) \times V}{\Delta t \times A} \times \frac{P}{P_0} \times \frac{T_0}{T}, \quad (1)$$

where  $F$  is the gas flux (mg m<sup>-2</sup> h<sup>-1</sup>);  $\alpha$  is the conversion coefficient (1.94);  $C_i$  and  $C_f$  are the initial and final gas concentrations, respectively;  $V$  is the system volume (including the total volume of the tubes and chambers, cm<sup>3</sup>);  $A$  is the area at the bottom covered by the chamber (1 m<sup>2</sup>);  $\Delta t$  is the measurement time (15 min);  $P_0$  is the standard atmospheric pressure (101.325 kPa);  $T_0$  is the absolute temperature under standard conditions ( $T_0=273.15$  K); and  $P$  and  $T$  are the atmospheric pressure of the sampling site (kPa) and the temperature inside the chamber (K), respectively.

## 2.6 | Data analysis

For the experimental group, dung seedling density (number of seedlings germinated per unit of dung weight, i.e. seedlings/g dung) and dung seedling richness (number of seedling species, i.e. species/g dung) were determined based on data collected from six replicates of each dung sample in each pot after 60 days of the germination test. For each seedling pot, a Shannon diversity index ( $H'$ ) was calculated as follows:

$$H' = - \sum_{i=1}^s P_i \ln P_i, \quad (2)$$

where  $P_i$  is the relative proportion of seedling density of the total community (in this study, community refers to the dung seedling bank), and

S is the total number of dung seedlings per dung sample (i.e. the richness of dung seedlings per pot).

We used the least significant difference method to compare differences in the data for dung seedling density, species richness and species diversity, as well as materials decomposition dynamics (Figure S1) among different livestock species. GHG fluxes between the experimental and control group over time for each livestock species were compared using an independent t-test method with a linear mixed-effects model. Livestock type (i.e. horse, cattle and sheep) or treatment (i.e. experimental and control group) was treated as the fixed effect. A Shapiro–Wilk test was used to test the normality of the residuals before comparing mean values. The error bars and numbers following averages are the standard error of the mean. The level of significance was  $p < 0.05$ .

GHG fluxes ( $Z$ ,  $\text{mg m}^{-2} \text{h}^{-1}$ ) in relation to germination time ( $X$ , days) were determined by the general linear model:  $Z = aX + c$ . GHG fluxes in relation to germination time as well as dung seedling density ( $Y$ , seedlings/g dung) were determined by multiple linear regression models:  $Z = aX + bY + c$ , where  $a$  and  $b$  are estimated regression coefficients and  $c$  is the intercept. Only significant coefficients were included in the final model. All statistical analyses were conducted with Statistical Package for the Social Sciences (ver. 26.0 for Windows; SPSS, Inc.), and all figures were constructed using Origin 9.1.

### 3 | RESULTS

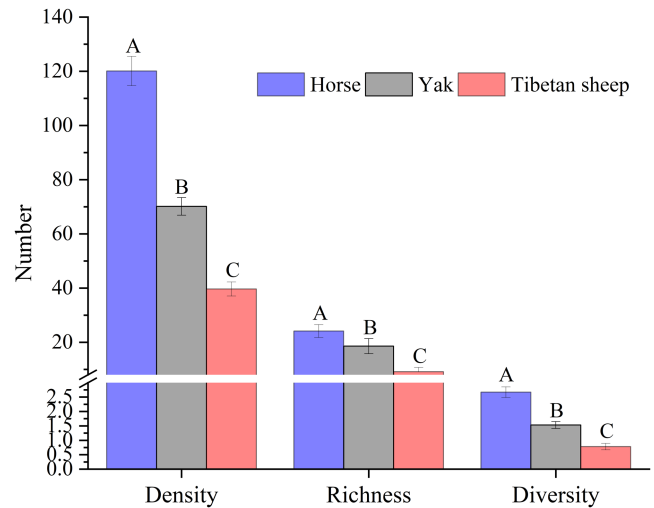
#### 3.1 | Dung seedling density, richness and diversity

The number and species of seedlings that germinated from the dung samples were assessed over 60 days. As the germination time progressed, the number of seedlings that germinated from the dung of the three livestock species gradually increased (Table S2). No new seedlings were detected in horse dung after 26 days of germination. A total of 24 seedling species (14 families) were identified from horse dung, and the density of the horse dung seed bank was 120.10 seedlings/g dung. No new seedlings were detected in yak dung after 21 days of germination. From yak dung, a total of 18 seedling species (10 families) were identified, and the density of the dung seed bank was 70.15 seedlings/g dung. There were no new seedlings detected in Tibetan sheep dung after 31 days of germination. Nine seedling species (five families) were identified from the Tibetan sheep dung, and the density of the Tibetan sheep dung seed bank was 39.66 seedlings/g dung (Table S2).

Seedling density, richness and diversity values were significantly greater for horse dung than for yak and Tibetan sheep dung ( $n = 6$  replicates,  $F_{2,15} = 3.68$ ,  $p = 0.0023$ ; Figure 2).

#### 3.2 | GHG emissions

The  $\text{CO}_2$ ,  $\text{CH}_4$  and  $\text{N}_2\text{O}$  fluxes all showed the pattern  $\text{YD} > \text{HD} > \text{SD}$ . In addition, the GHG fluxes of the experimental group and the



**FIGURE 2** Density (seedlings/g dung), richness (species/g dung) and diversity (Shannon diversity index) of seedlings germinated from horse, yak and Tibetan sheep dung during the warm grazing season. The indices were significantly different for each livestock species, as indicated by different uppercase letters. Data are shown as the mean  $\pm$  standard error of the mean.

control group gradually decreased (Figure 3). The GHG emissions of the experimental group declined faster than those of the control group (Figure 3; Table S3). A t-test showed that the GHG fluxes measured from the experimental group were significantly higher than those of the control group (Table S4).

#### 3.3 | Relationship between livestock dung GHG fluxes and seedling density

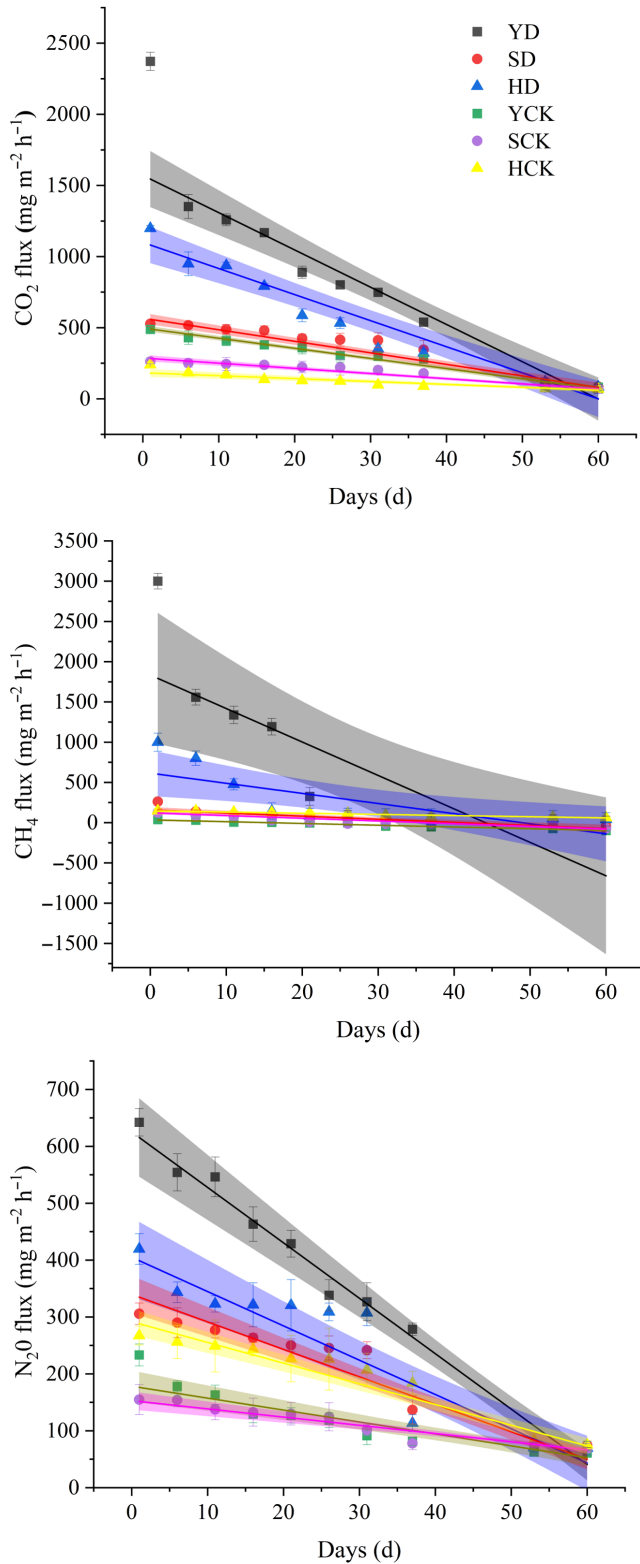
With progressing germination time, the density of the livestock dung seedlings gradually increased, whereas the GHG fluxes gradually decreased (Figure 4). The regression analysis showed that there was a significant negative correlation between the GHG fluxes and the dung seedling density and germination time (Table S5).

### 4 | DISCUSSION

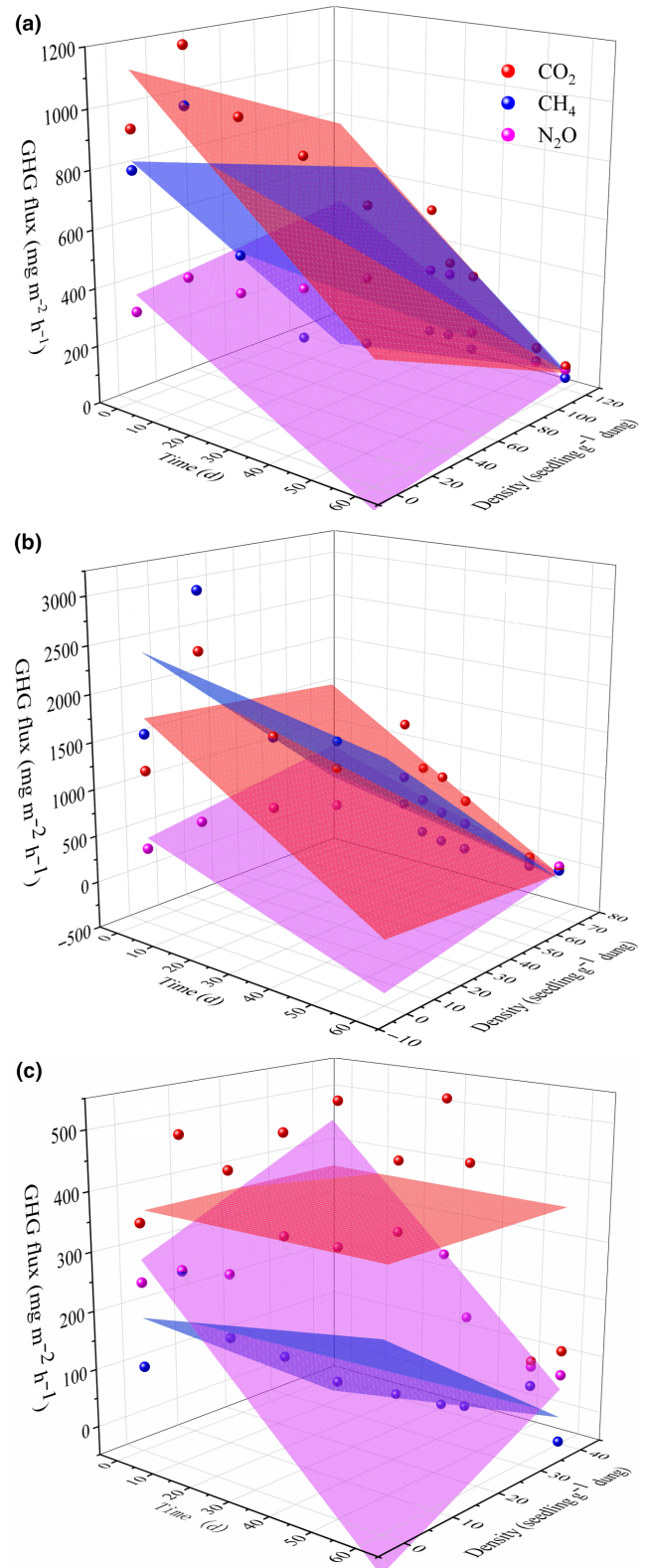
#### 4.1 | Size and composition of livestock dung seed banks

Livestock dung seed bank plays an important role in the regeneration and sustainability of grasslands in grazing ecosystems. A previous study that compared dung samples from cattle, sheep and horse showed that the horse dung seed bank contributes most to grassland recovery and restoration (Egea et al., 2022). The size of the dung seed bank can be affected by the livestock species, the amount of seed intake by the livestock and the physical and chemical properties of the faeces (Milotić & Hoffmann, 2016), in addition to seed traits (Pakeman et al., 2002).





**FIGURE 3** CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O fluxes from dung of different livestock species ( $n=10$  replicates). The solid lines are significant regression lines; shaded areas around the regression lines are 95% confidence intervals. HCK, horse dung control; HD, horse dung; SCK, Tibetan sheep dung control; SD, Tibetan sheep dung; YCK, yak dung control; YD, yak dung.



**FIGURE 4** Relationships among greenhouse gas flux of livestock dung ( $Z$ , mg m<sup>-2</sup> h<sup>-1</sup>), seed germination time ( $X$ , days) and dung seedling density ( $Y$ , seedlings/g dung;  $n=10$  replicates). (a) horse dung, (b) yak dung and (c) Tibetan sheep dung.

Compared with horses (cecal digester) and yak (large ruminant), the chewing method of Tibetan sheep (small ruminant) causes the most damage to seeds. For example, in the central region of Spain, fragments of chewed seeds of the Mediterranean shrub *Retama sphaerocarpa* (seed mass, 77 mg seed<sup>-1</sup>) are often found in sheep dung (Manzano et al., 2005). Feeding experiments have shown that the percentage of seeds that germinate from yak dung (28.1% of total seeds collected from dung samples) is significantly higher than that from Tibetan sheep (9.4%; Yu et al., 2012). Additionally, for alpine shrubs of the northeast QTP, the yak dung seedling density is 1.9 times that of Tibetan sheep dung (Wang, 2017). Tibetan sheep and yak are ruminants, and plant seeds are affected not only by chewing and rumen digestion but also by the rumination process, which is quite destructive (Wang et al., 2017). In contrast, horses are monogastric animals, which chew food particularly roughly (Zang, 2015), and there is no rumination-related damage to the seeds (Egea et al., 2022). However, Mouissie et al. (2005) reported that, in the heathlands of the northern Netherlands, the mean seedling density of cattle dung is greater than that of horse dung. Subtle variations in grazing behaviour and diet selection could explain some of the observed differences in the germinable seed content between cattle and horse dung (Malo, 2000). In addition, herbivore species have interspecific differences in functional traits, such as habitat, size, age, mating frequency, cognition and forage preference, which may result in differences in dung seedling density as well as in the seed dispersal service they provide (Cosyns & Hoffmann, 2005; Zwolak, 2017).

## 4.2 | Livestock dung GHG emissions

Cai, Wang, et al. (2014) also reported that, in alpine meadows of northern Tibet, yak dung has a higher N<sub>2</sub>O flux than does Tibetan sheep dung, and the authors posited that most of the CO<sub>2</sub>/CH<sub>4</sub> emitted by the yak dung patch is likely to be released by the yak dung itself, and the lower water content and lower organic carbon content of Tibetan sheep dung probably result in the lower CO<sub>2</sub>/CH<sub>4</sub> emission potential of Tibetan sheep dung as compared to yak dung (Cai, Du, et al., 2014). Furthermore, the C/N ratio varies among different dung patches, which is also an important reason for the difference in GHG emissions from the dung of different livestock species (Jarvis et al., 1995). So far, there have been no reports of research into GHG emissions from horse dung on the QTP.

Livestock dung contains large microbial communities as well as highly degradable organic compounds and, thus, dung is a rich source of CO<sub>2</sub> emissions (Lin et al., 2009; Liu et al., 2017). The CO<sub>2</sub> flux of the experimental group was derived from dung heterotrophic respiration, dung seed germination respiration (Carrillo-Reche et al., 2021) and the autotrophic respiration of dung seedlings from both above and below the dung (Liu et al., 2017). In addition, our experiment was conducted in the early warm season, when the temperatures are relatively high and there is ample rainfall. Previous studies have shown that soil temperature and moisture have a

significant positive correlation with CO<sub>2</sub> flux (Liu et al., 2017; Ma et al., 2006); therefore, the CO<sub>2</sub> flux was very high during this period in both the experimental and control groups. As faeces decompose, the organic matter content of the faeces decreases and, subsequently, CO<sub>2</sub> emissions are gradually reduced (Lin et al., 2009).

The CH<sub>4</sub> flux represents the net flux between consumption and production by methanotrophic and methanogenic microbes, respectively (Liu et al., 2017). Livestock dung shows high levels of CH<sub>4</sub> emissions, which are probably attributable to abundant microbial communities, highly degradable organic compounds and the anaerobic conditions in fresh dung patches (moisture content of 60%–80%; Holter, 1997; Sherlock et al., 2002). Methanogenesis is extremely sensitive to the oxygen concentration, and oxygen diffusion into the manure or slurry inhibits methanogenesis (Chadwick et al., 2000; Jones et al., 2005). Besides, dung seedling root respiration could also reduce oxygen levels in dung, which helps to increase methane production. Therefore, the CH<sub>4</sub> fluxes of the experimental group were significantly higher than the control group (Figure 3; Table S4).

N<sub>2</sub>O flux results from the biological processes of nitrification and denitrification. In most grazed grassland ecosystems, ~80% of the consumed N is directly returned in the form of dung (and urine) by grazing animals (Wang & Li, 1997). On a dry matter basis, total N content for YD, Tibetan SD and HD was 2.7% (Cai, Wang, et al., 2014), 1.7% (Cai, Wang, et al., 2014) and 1.6% (data not published), respectively. Hence, livestock dung is considered to be one of the most important sources of N in natural grasslands (Weerden et al., 2011). In our research, the high moisture content of the faeces provided good anaerobic conditions for denitrification by microorganisms and was conducive to the accumulation of N<sub>2</sub>O (Liu et al., 2017). Similar studies have also shown that livestock dung is a strong source of N<sub>2</sub>O emissions (Cai, Wang, et al., 2014; Lin et al., 2009).

## 4.3 | Linkage between livestock dung seedling density and GHG emissions

Nutrient release from livestock dung into soil involves complex processes subject to biological degradation (Wang et al., 2021; Yang et al., 2019). During these processes, the decomposers (i.e. microorganisms) transfer organic C and N into inorganic matter (e.g. CO<sub>3</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>) via respiration, immobilization or mineralization, and those inorganic materials could be absorbed and utilized by dung seedlings (Cai et al., 2013). In this study, the CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O fluxes of the experimental group were significantly higher than those of the control group. However, the GHG emissions of the experimental group declined faster than those of the control group. That is, dung seedlings could substantially increase the fluxes and emission rate of GHG from livestock dung patches. This may be due to an interaction effect between dung seedlings and faecal microbes, such that under the combined action of the seedlings and microbes, the decomposition (e.g. dung pat fragmentation) and absorption of the faecal organic matter and mineral elements are accelerated (Du et al., 2021).

This is also presumably why the GHG emissions of the experimental group declined faster than those of the control group.

With the reduction in organic matter content caused by the decomposition of dung due to microbial community activities and with nitrogenous mineral elements (e.g.  $\text{NO}_3^- - \text{N}$  and  $\text{NH}_4^+ - \text{N}$ ) in the faeces being absorbed by the dung seedlings (Yu, 2010), there was a significant negative correlation between dung seedling density and dung GHG fluxes (Table S5). That is to say, the germination of dung seeds and subsequent growth of the resulting seedlings stimulate the decomposition of faeces (Wang et al., 2021; Figure S1), which further demonstrates that dung is the hot spot for material flow as well as transformation in grassland ecosystems (Yang et al., 2019). As compared to measuring GHG emissions, dung seed germination is easier to detect. Therefore, our research also provides a method to predict GHG emission fluxes according to dung seedling density.

GHG emissions from livestock grazing systems cannot be ignored for regional or global GHG emission inventories (Liu et al., 2017); meanwhile, large herbivores dung seed banks are important drivers of vegetation renewal and a vital supplement to the soil seed bank (Wang & Hou, 2021b). The ecological function of livestock dung for climate warming and the seed dispersal are contrary. Therefore, the rational use of livestock dung for alpine meadow restoration is a great challenge for technicians engaged in grassland restoration on the QTP.

## 5 | CONCLUSIONS

In a grazing ecosystem of an alpine meadow on the QTP during the warm season, livestock dung contains a large number of germinated seeds (horse, yak and Tibetan sheep dung had an average of 120.10, 70.15 and 39.66 seedlings/g dung, respectively). Horses (cecal digester) had the highest efficiency of endozoochorous seed dispersal. Livestock dung is also an important source of GHG emissions, and the fluctuations in  $\text{CO}_2$ ,  $\text{CH}_4$  and  $\text{N}_2\text{O}$  all showed a pattern of yak dung > horse dung > Tibetan sheep dung. Dung seed germination and GHG emission proceed simultaneously, and there is a significant negative correlation between these two processes. Our study shows that the ability of dung seeds to contribute to accelerated GHG emissions should be considered when estimating global warming potential from grazing livestock on the QTP for the purposes of determining national and regional land use policies and compiling global GHG inventories.

### AUTHOR CONTRIBUTIONS

Shulin Wang collected livestock dung, identified the dung seedlings and wrote the manuscript; Fujiang Hou developed the research plan and revised the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

### DATA AVAILABILITY STATEMENT

Data available via the FigShare <https://doi.org/10.6084/m9.figshare.19430432> (Wang & Hou, 2023).

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### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Figure S1.** Changes in the characteristics of experimental and control dung samples over time.

**Figure S2.** Photograph of seedlings germinated from seeds acquired from the dung bank in an alpine meadow.

**Figure S3.** Efflux chamber for greenhouse gas online observation system instrument.

**Table S1.** Determined indicators and methods used with the livestock dung samples.

**Table S2.** Density of plant species that germinated from livestock dung collected from an alpine meadow on the Qinghai-Tibetan Plateau.

**Table S3.** Relationships between livestock dung greenhouse gas fluxes ( $Y$ ,  $\text{mg m}^{-2} \text{h}^{-1}$ ) and germination time ( $X$ , days).

**Table S4.** Greenhouse gas fluxes of experimental (YD, SD and HD) and control (YCK, SCK and HCK) groups.

**Table S5.** Relationship among livestock dung greenhouse gas flux ( $Z$ ,  $\text{mg m}^{-2} \text{h}^{-1}$ ), germination time ( $X$ , days) and dung seedling density ( $Y$ , seedlings/g dung).

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