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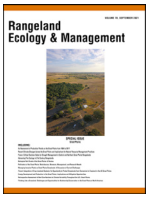

Seed Bank of Livestock Dung in the Qilian Mountain Grassland: A Potential Resource for Vegetation Recovery[☆]

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ABSTRACT

The Qilian Mountain Grassland is an important animal husbandry production base in northwestern China. Horses, cattle, and sheep are the main livestock, which are widely distributed in the desert grasslands and alpine meadows around the Qilian Mountains. Grazing livestock produce large amounts of feces, and the germinable seeds in feces constitute the seed banks. Research on the size, plant species composition, and distribution of livestock dung seed banks in the Qilian Mountain grasslands may help understand the interactions between grass species and livestock and inform the comprehensive management practices for grazing livestock. In mid-October 2018, we collected the dung of horses, cattle, and sheep in the alpine meadows and desert grasslands of the Qilian Mountains and estimated the composition and size of the dung seed bank by the greenhouse germination method. Seeds of aboveground vegetation in the same location were also collected to determine the relationships between the size and composition of dung seed banks and the seed traits (i.e., mass and shape). A total of 30 plant species germinated from the dung seed banks of the three livestock species, of which 22 species (73%) were perennial. The seedling densities for horse, cattle, and sheep dung were 11.91, 10.80, and 7.60 seedlings per gram dung, respectively. The species richness, species diversity, and Jaccard coefficients of similarity between dung seedling and aboveground vegetation of horse dung were significantly greater than that of cattle and sheep dung. Regression analyses indicated that medium-sized (10–30 mg) and spherical (0.04–0.10 shape index) seeds had the greatest germination potential. Our study suggests that, of the three livestock species tested, the horse dung seed bank contributes most to grassland recovery and restoration of the Qilian Mountains.

Introduction

Viable seeds in herbivore feces constitute the dung seed bank (Iravani et al. 2011), which is an important source of vegetation renewal and an important supplement to the soil seed bank. The structure of dung seed bank depends on rangeland composition and the selective feeding of livestock. The dung seed bank is a special form of soil seed bank, as once dung decomposes, seeds in the feces are eventually incorporated into the soil, contributing to the soil seed bank. Therefore, the dung seed bank is a key factor determining pasture seed dispersal, soil seed bank composition, and seedling density, inducing changes in grassland vegetation composition (Elisabeth and Han 2003). A variety of germinable plant seeds accumulate in the feces, so the dung seed bank is also an important driving force for promoting the formation of grassland patches (Myers et al. 2004). Fecal sedimentation, dung-borne seed germination, and establishment of seedlings in feces increases the similarity of plant communities between different types of grazed grasslands and fosters diversity among grassland plants within the local community type (Malo and Suárez 1995). Therefore, research on the composition, size, and ecological characteristics of the dung seed bank is essential for studies of grazing ecology (D'Hondt and Hoffmann 2015).

A number of studies have indicated that seedling emergence and growth are promoted by the organic matter and nutrients in livestock dung (Woldu and Saleem 2000; Traveset et al. 2001;

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Nchanji and Plumptre 2003). It is assumed that seed ingestion by livestock can increase plant species richness and influence large-scale spatial community composition in grazed ecosystems by intensifying intercommunity seed flow. A substantial quantity of seeds and range of seed species dispersed by herbivores in this way can substantively affect the dynamics and species richness of these grazed ecosystems (Pakeman et al. 2002).

Factors affecting the properties of dung seed bank primarily include the species of both livestock and indigenous animals, seed traits (mass and shape), grazing management (e.g., use of antibiotics of veterinary origin may affect dung physical and microbiotic properties of the dung and, in turn, affect somehow seed germination [Minden et al. 2017; Kavanaugh and Manning 2020]), and external environmental conditions. First, the size of dung seed banks varies with livestock species. For example, the percentage of germinable seeds from yak dung (28.1%) is significantly higher than that from Tibetan sheep dung (9.4%) (Yu et al. 2012), and the mean dung seedling densities from red deer, wild boar, and roe deer are 27, 5, and 4 seedling/100 g dung, respectively (Picard et al. 2015). Seed traits also have a significant effect on the ability of seeds to germinate successfully after passing through the digestive tract of animals (Wang et al. 2017). Some studies have shown that medium-sized and spherical seeds have higher germination potential after passing through the sheep gut (Manzano et al. 2005; Wang et al. 2018). External environmental conditions mainly affect the survival and establishment of fecal seedlings. In North America, the effectiveness of seed dispersal through seed consumption by white-tailed deer is greater in a southern Connecticut reservoir (Williams and Ward 2006) than in a New York state forest (Myers et al. 2004), due to the different local environmental conditions (Wang et al. 2017). In addition, external environmental conditions (time of grazing and plant community composition) will also affect the seed plant availability and degree of seed maturity in the dung, especially if there are C3 and C4 species in the grassland ecosystem, as C3 and C4 species will produce seeds in different times of the year (Hammouda and Afify 1999).

The size of a dung seed bank has been found to be affected by livestock chewing, amount of seed intake, and physical and chemical properties of the feces (Milotić and Hoffmann 2016), as well as seed traits (Pakeman et al. 2002). Compared with horse and cattle, the chewing method of sheep causes the most serious damage to seeds (Manzano et al. 2005; Wang et al. 2017). For example, fragments of chewed Mediterranean shrub seeds are often found in sheep dung (Manzano et al. 2005). In addition to chewing, rumen digestion of animals such as the sheep and cattle may also destruct plant seeds via a ruminant process (Wang et al. 2017). By contrast, in monogastric animals, such as the horse, food is chewed roughly (Zang 2015). Therefore, the number of germinable seeds in horse dung is greater than that in cattle and sheep dung. However, Mouissie et al. (2005) reported that the mean seedling density of cattle dung is greater than that of horse dung. Subtle variation in grazing behavior and diet selection could partially explain the observed difference in germinating seed content between cattle and horse (Malo 2000).

The Qilian Mountains are important for the grassland livestock production in northwest China (Liu et al. 2008). The most common management method of grasslands in those areas is nomadic, where the transhumant flocks move seasonally between the warm and cold pastures (Wang et al. 2012). Horses *(Equus caballus),* cattle (*Bos mutus* and *Bos taurus*), and sheep *(Ovis aries)* are the main species of grazing livestock and are widely distributed in different types of pastures around the Qilian Mountains. Research on the size, plant species composition, and distribution of livestock dung seed banks in the Qilian Mountain grasslands may help understand the interactions between grass species and livestock and inform the comprehensive management practices for grazing livestock.

Previous research on the grazing ecosystem of Qilian Mountains mainly focuses on the impacts of livestock on soil physical and chemical properties (Liu et al. 2019) and vegetation diversity (Wu et al. 2019). However, the ecological significance of livestock feces, especially the important role of dung seed bank in vegetation recovery has been largely neglected. In this study, we collected horses, cattle, and sheep, as well as plant seeds (i.e., the dominant species) from the desert grasslands and alpine meadows around the Qilian Mountains. The objectives of this research were to 1) compare the composition and size of dung seed banks from different livestock and grassland types, 2) study the relationships between the dung seed bank and aboveground vegetation, and 3) investigate the relationships between seed traits (i.e., mass and shape) and seedling density of different livestock-egested dung. The results of this research will provide insight into the understandings of the mechanisms of grass-animal interactions and the significance of the dung seed bank in vegetation renewal throughout the Qilian Mountain grassland.

Material and Methods

Study area

The study sites comprised six districts around the Qilian Mountains of northwestern China (Fig. 1): Aksay County, Pingshan Lake, Minqin County, Qilian County, Dahe township, and Gangcha County. Among these sites, Aksay County, Pingshan Lake, and Minqin County are located on the northern side of the mountains, with a mean daily air temperature of 6.9°C and a mean annual precipitation of 177.5 mm during 1988–2018. The rangeland is typical desert grassland, and the dominant grass species are *Salsola passerina, Nitraria tangutorum,* and *Kalidium foliatum*. Qilian County, Dahe Township, and Gangcha County are located on the southern side of the mountains, with a mean daily air temperature of 5°C and a mean annual precipitation of 364 mm during 1988–2018 (Yu et al. 2019). The grassland type is alpine meadow, and the dominant grass species are *Elymus nutans, Kobresia pygmaea,* and *Kobresia graminifolia*. All species are native, representing both current and historical species of the local grassland on the Qilian Mountains (Feng and Pan 2016). The main three grazing livestock are horses, cattle, and sheep, and these animals are not treated with antibiotics. Wild ungulates are rarely seen in our sampling sites, and livestock were evenly distributed across the landscape.

Aboveground vegetation

The aboveground vegetation survey was carried out in mid-August 2018. Due to special climatic conditions and the phenology of the vegetation, the species richness and biomass of aboveground vegetation peak (ca. 35 species for alpine meadow and ca. 15 species for desert grassland) during this period, which reflects the productivity of the grassland around the Qilian Mountains (Liu et al. 2008). Since the vegetation distribution characteristics of alpine meadow and desert grassland are different, different distances and quadrat sizes were used for sampling the two kinds of grasslands (Golodets et al. 2013; Wang et al. 2018). At each dung collection site, three planting lines were selected (40-m intervals for desert grassland and 20-m intervals for alpine meadow), and three quadrats (40 m intervals) were established for each line; the size of each quadrat was 4 × 4 m for desert grassland and 0.5 × 0.5 m for the alpine meadow. Therefore, each sampling site comprised nine quadrats. Species richness (number of plant species) and density were recorded for each quadrat at each sampling site.

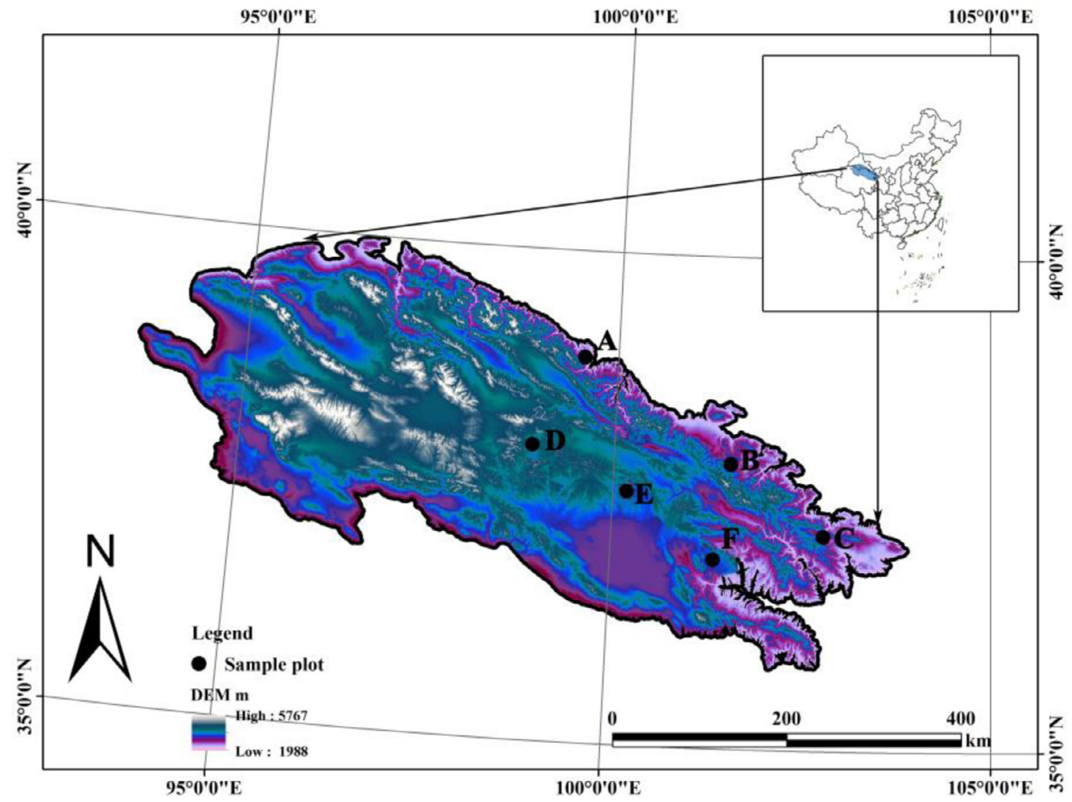

Figure 1. Desert grassland: (**A**) Aksay County (99°47′E, 38°23′N, 2 840 m a.s.l.), (**B**) Pingshan Lake (101°42′E, 37°11′N, 1 875 m a.s.l.), and (**C**) Minqin County (102°47′E, 36°12′N, 1 376 m a.s.l.). Alpine meadow: (**D**) Qilian County (99°11′E, 37°50′N, 3 682 m a.s.l.), (**E**) Dahe township (100°31′E, 36°54′N, 2 996 m a.s.l.), and (**F**) Gangcha County (101°35′E, 36°17′N, 3 351 m a.s.l.).

Dung collection

The peak period of plant seed maturation in Qilian Mountains is from October to December. During this period, a large number of matured seeds are retained on the plants, which constitute the canopy seed bank (Oudtshoorn and Rooyen 1998). Livestock are evenly distributed across the landscape, allowing them access to seed-bearing vegetation.

We sampled ca. 3 kg fresh dung of horse, cattle, or sheep near the quadrats at each study site during the fruit peaking period in mid-October 2018. Dung samples were placed separately in canvas bags and brought to the laboratory. Samples were dried at 35°C in a drying oven for 72 h to prevent both decay and premature seeds in the dung for analyses. All dried samples for each livestock type at each sampling site were then divided into three equal subsamples (ca. 500 g per subsample, $n = 18$) and stored in dark at room temperature.

Seed mass and shape index

From mid-October to mid-November 2018, we collected seeds from the aboveground vegetation by sampling at least 20 individual plants for each species at each sampling site. We weighed 100 seeds without appendages per species and randomly selected 30 seeds per species to measure the seed length, width, and height using an electronic Vernier Caliper under a stereomicroscope (Nikon SMZ 1500, Shanghai, China). The seed shape index *(I),* or divergence from sphericity, was calculated according to Wang et al. (2017) as the variance of the three main dimensions:

$$I = \frac{[3(X_L^2 + X_W^2 + X_H^2) - (X_L + X_W + X_H)^2]}{3^2} \quad (1)$$

where I is the seed shape index and X_L, X_W, and X_H are seed length, width, and height divided by seed length, respectively. The I value may range from 0 to 1, with $I = 0$ representing a perfect sphere and $I = 1$ representing completely flat or elongated seeds.

Germination

We gently broke the dry manure into pieces but did not damage the seeds. A 100-g portion was taken from each subsample, mixed with ca. 50 g sterile sand, and spread in a pot (35 cm length, 18 cm width, 10 cm height) at 2-cm thickness on a 5-cm-thick bed of vermiculite. Pots were placed in a greenhouse (Yuzhong campus, Lanzhou University, 35°56′N, 104°9′E, elevation ca. 1 755 m a.s.l.) with 70–80% humidity, 15–20°C, and 16 h light/d. The pots were watered twice a day from January until June 2019. A temperature regime of 20°C during the 16 h light and 15°C during the 8 h darkness was chosen to mimic the early-spring germination temperature range experienced by most of the grass species typically found in the Qilian Mountains. The experiment was terminated after 6 mo because no substantive additional germination could be detected after this time (Malo 2000). The number of emerging seedlings was recorded, and seedlings were removed soon after identified or transplanted into separate pots for later identification. Whenever seedlings were removed, the dung/sand mix was stirred to facilitate germination of the remaining buried seeds.

Diversity and similarity index

The number of emerged seedlings (seedlings g^{-1} dung) and species richness (species g^{-1} dung) were determined on the basis of the data collected from the three replicates of each dung sample. For each seedling pot, a Shannon-Wiener diversity index (H')

was calculated as:

$$H' = -\sum_{i=1}^{S} p_{\mathrm{i}} \ln p_{\mathrm{i}} \tag{2}$$

where p_{i} is the relative proportion of species of the total community (in this study, community refers to the dung seed bank) and s is the total number of species for each dung sample.

The Jaccard coefficient of similarity (S_{J}) was used to test similarities in species composition between the dung seed bank and aboveground vegetation for each livestock in the desert grassland or alpine meadow:

$$S_{\mathrm{J}} = \frac{a}{b} \tag{3}$$

where a is the number of common species found in both the dung seed bank and aboveground vegetation at the same site, and b is the total number of species identified in the dung seed bank and aboveground.

Data analysis

Data were analyzed using the Statistical Package for the Social Sciences (ver. 25.0 for Windows; SPSS, Inc., Chicago, IL). Data were checked for normal distribution using the Shapiro-Wilk test. Data on dung seedling species richness, species diversity, and seedling density were $\log_{10}$-transformed to pursue the assumption of normality and homogeneity of variances. One-way analysis of variance was used to assess differences in dung seedling density, functional groups, dung seed bank species richness, species diversity, and similarity coefficient affected by livestock at different sampling sites. The level of significance used was $P < 0.05$. Dung seedling density in relation to single-seed mass and seed shape index were determined by regression analyses. All figures were constructed using Origin 9.1.

Results

Germinated species from dung

A total of 30 species germinated from the dung samples. In samples from Dahe Township, *Oxytropis kansuensis,* and *K. pygmaea* germinated from horse, cattle, and sheep dung (Table 1). *E. nutans* and *Agropyron cristatum* germinated only from horse dung samples, whereas *Potentilla bifurca* and *Kobresia humilis* germinated only from sheep dung samples. In samples from Qilian County, *Potentilla ancistrifolia* germinated from horse (5.26 ± 1.41), cattle (5.88 ± 1.52), and sheep (4.23 ± 1.44) dung. *Stipa purpurea* and *Artemisia scoparia* germinated only from horse (7.23 ± 2.14) and sheep (3.12 ± 0.14) dung samples, respectively. In samples from Gangcha County, *K. graminifolia* and *Thermopsis lanceolata* germinated from horse, cattle, and sheep dung, whereas *Lancea tibetica* germinated only from sheep (3.13 ± 0.27) dung samples.

In samples from Aksay County, *S. passerina* germinated from horse (12.77 ± 6.27), cattle (22.14 ± 3.25), and sheep (8.23 ± 4.12) dung (see Table 1). *Zygophyllum fabago* germinated only from cattle (6.11 ± 3.42) dung samples. In samples from Pingshan Lake, *Sympegma regelii* germinated only from horse (12.11 ± 2.21) dung samples. In samples from Minqin County, *Chloris virgata* and *Stipa glareosa* germinated only from cattle (15.32 ± 3.78) and horse (8.28 ± 3.27) dung samples.

Regardless of sampling site, horse dung contained the greatest number of germinated seeds, with an average seedling density of 11.91 g^{-1} dung, which was significantly greater than cattle (10.80 g^{-1} dung) and sheep (7.60 g^{-1} dung) dung samples ($P < 0.05$) (Fig. 2).

Functional group of dung seeds

The 30 plant species that germinated from the livestock dung belonged to nine families (Chenopodiaceae, Poaceae, Asteraceae, Leguminosae, Rosaceae, Cyperaceae, Zygophyllaceae, Plumbaginaceae, and Scrophulariaceae) (Table 2), with three subshrub species (*S. regelii, K. foliatum*, and *S. passerina*); three annual species (*Halogeton arachnoideus, Eragrostis Pilosa,* and *C. virgata*); and two shrub species (*Caragana jubata* and *N. tangutorum*). The remaining 22 plant species (73% of total) were perennials.

The number of perennial species detected in horse dung (15 ± 2.13) was significantly greater than that in cattle dung (12 ± 1.98) or sheep dung (10 ± 1.22) ($P < 0.05$), and the number of shrub species found in horse dung (2 ± 0.73) and cattle (2 ± 0.57) dung was significantly higher than that in sheep dung (1 ± 0.23) ($P < 0.05$). However, the number of annual species in the dung of the three livestock species was not significantly different ($P > 0.05$).

The number of perennials species found in livestock dung was significantly greater than that of annuals, shrubs, or subshrubs ($P < 0.05$). There was significantly more subshrub species than shrub and annual species from the horse dung ($P < 0.05$), with no significant difference in number among annual, shrub, and subshrub species from cattle dung ($P > 0.05$). The numbers of shrub species detected from sheep dung was significantly smaller than those of subshrub and annual species ($P < 0.05$) (Fig. 3).

Seedling richness of dung sampling sites

The seedling richness of horse dung in Gangcha County was significantly less than that in other sampling sites ($P < 0.05$). The cattle dung seedling richness in Dahe County (7.23 ± 0.05) and Qilian County (4.12 ± 0.16) was significantly greater than that in Gangcha County (3.13 ± 0.14), Minqin County (3.34 ± 0.12), Aksay County (3.02 ± 0.21), and Pingshan Lake (3.11 ± 0.11) ($P < 0.05$). Sheep dung seedling richness was significantly greater in Dahe County than in the other five sampling sites ($P < 0.05$). The mean seedling richness of the three alpine meadows (4.33 ± 0.96) (Dahe County, Qilian County and Gangcha County) was significantly greater than that of the other three desert grasslands (2.37 ± 0.31) (Minqin County, Aksay County, and Pingshan Lake, $P < 0.05$).

Except for Gangcha County, the seedling richness of sheep dung was significantly less than that of horse or cattle dung in the other five sampling sites ($P < 0.05$). In addition, the mean seedling richness of horse dung (4.67 ± 1.07) was significantly greater than that of cattle (3.83 ± 1.04) or sheep (2.67 ± 0.75) dung ($P < 0.05$) (Fig. 4A).

Seedling diversity of dung from three different types of livestock

The dung seedling diversities of livestock from the alpine meadows in Dahe County, Qilian County, and Gangcha County were significantly greater than that from the desert grasslands in Minqin County, Aksay County, and Pingshan Lake ($P < 0.05$). In addition, the mean seedling diversity of horse dung (1.24 ± 0.45) was significantly greater than that of cattle (0.87 ± 0.31) or sheep (0.49 ± 0.17) dung ($P < 0.05$) (see Fig. 4B).

Similarity of dung seedling bank and aboveground vegetation

The Jaccard coefficients of similarity between livestock dung seedlings and aboveground vegetation were < 0.5 for the alpine meadows, significantly smaller than those calculated for the desert grasslands ($P < 0.05$). However, the coefficients of similarity between horse dung seedlings and aboveground vegetation were significantly greater than for cattle or sheep dung for all six sampling sites ($P < 0.05$) (see Fig. 4C).

Table 1
Mean (± standard error) germination density (seedlings g^{-1} dung) of species from livestock dung collected at different sites of the Qilian Mountains, China.

Species	Alpine meadow								
	Dahe			Qilian			Gangcha		
	Horse	Cattle	Sheep	Horse	Cattle	Sheep	Horse	Cattle	Sheep
Oxytropis kansuensis	9.38 ± 3.27	5.17 ± 2.54	3.12 ± 1.27						
Potentilla bifurca			6.67 ± 2.14						
Elymus nutans	8.21 ± 2.13								
Stipa purpurea				7.23 ± 2.14			23.12 ± 4.38	14.25 ± 2.57	
Caragana jubata	7.31 ± 2.12	5.34 ± 1.21							
Kobresia graminifolia							13.15 ± 2.17	9.27 ± 4.36	5.14 ± 1.23
Lancea tibetica									3.13 ± 0.27
Carex kansuensis	13.32 ± 7.34	33.12 ± 3.33							
Leontopodium nanum	6.42 ± 2.22	5.23 ± 2.18		4.12 ± 1.23	3.21 ± 0.89				
Agropyron cristatum	8.11 ± 3.12								
Poa pratensis	8.34 ± 3.37	6.19 ± 3.23		8.34 ± 1.23	5.13 ± 2.22				
Thermopsis lanceolata	13.12 ± 2.22	7.33 ± 1.67					6.24 ± 2.26	3.23 ± 0.47	1.38 ± 0.22
Kobresia pygmaea	18.34 ± 3.33	14.32 ± 2.17	17.32 ± 4.14						
Kobresia humilis			22.31 ± 5.34						
Artemisia scoparia						3.12 ± 0.14			
Potentilla ancistrifolia				5.26 ± 1.41	5.88 ± 1.52	4.23 ± 1.44			

Species	Desert grassland								
	Aksay			Pingshan Lake			Minqin		
	Horse	Cattle	Sheep	Horse	Cattle	Sheep	Horse	Cattle	Sheep
Halogeton arachnoideus							11.23 ± 4.57	6.22 ± 2.17	4.46 ± 2.17
Eragrostis pilosa							9.19 ± 3.11		4.29 ± 3.11
Chloris virgata								15.32 ± 3.78	
Zygophyllum mucronatum							12.11 ± 1.23		
Astragalus scaberrimus								10.22 ± 2.78	8.23 ± 3.14
Stipa glareosa							8.28 ± 3.27		
Nitraria tangutorum	23.47 ± 5.37		12.67 ± 3.14	25.23 ± 5.43	25.32 ± 4.33				
Salsola passerina	12.77 ± 6.27	22.14 ± 3.25	8.23 ± 4.12	35.13 ± 4.57	21.14 ± 3.32	12.12 ± 2.21			
Limonium otolepis	8.32 ± 2.13	4.27 ± 3.54							
Limonium aureum	3.37 ± 4.27								
Zygophyllum fabago		6.11 ± 3.42							
Kalidium foliatum				12.23 ± 3.34	9.22 ± 2.15	5.22 ± 1.23			
Sympegma regelii				12.11 ± 2.21					

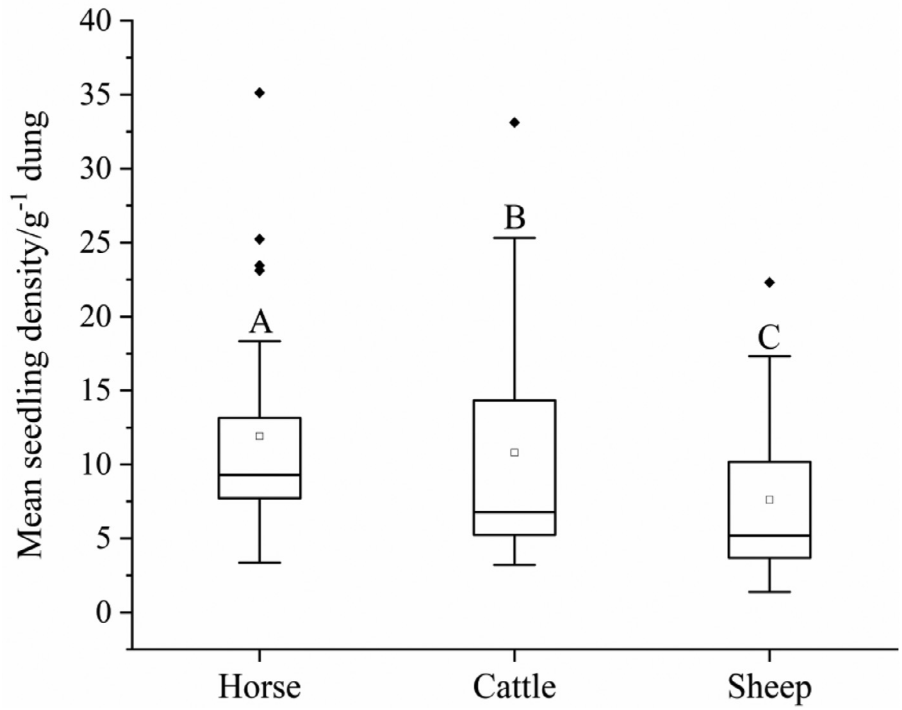

Figure 2. The size of dung seed bank of different livestock types. Different letters indicate significant differences at $P < 0.05$.

Table 2
Plant species that germinated from livestock (cattle, sheep, and horses) dung collected in Qilian Mountain grassland, China, showing functional group, seed mass (mean ± standard error [s.e.]), and shape index (mean ± s.e.) of germinated species in livestock dung.

Family	Species	Functional group	Mass (mg)	Shape index
Poaceae	*Agropyron cristatum*	Perennial	1.30 ± 0.05	0.15 ± 0.01
	Chloris virgata	Annual	0.08 ± 0.02	0.12 ± 0.01
	Elymus nutans	Perennial	4.10 ± 0.02	0.17 ± 0.04
	Eragrostis pilosa	Annual	2.60 ± 0.02	0.07 ± 0.01
	Koeleria cristata	Perennial	0.16 ± 0.01	0.14 ± 0.01
	Poa pratensis	Perennial	0.70 ± 0.23	0.07 ± 0.01
	Stipa glareosa	Perennial	0.50 ± 0.00	0.13 ± 0.01
	Stipa purpurea	Perennial	3.87 ± 0.57	0.15 ± 0.01
Leguminosae	*Astragalus scaberrimus*	Perennial	3.72 ± 0.10	0.12 ± 0.02
	Caragana jubata	Shrub	47.02 ± 2.02	0.07 ± 0.00
	Oxytropis kansuensis	Perennial	2.63 ± 0.03	0.07 ± 0.00
	Thermopsis lanceolata	Perennial	44.84 ± 3.12	0.001 ± 0.00
Cyperaceae	*Carex kansuensis*	Perennial	3.20 ± 0.003	0.16 ± 0.00
	Kobresia graminifolia	Perennial	2.02 ± 0.02	0.10 ± 0.01
	Kobresia humilis	Perennial	0.65 ± 0.01	0.08 ± 0.01
	Kobresia pygmaea	Perennial	1.43 ± 0.27	0.09 ± 0.00
Asteraceae	*Artemisia scoparia*	Perennial	0.57 ± 0.04	0.11 ± 0.01
	Leontopodium nanum	Perennial	0.03 ± 0.002	0.11 ± 0.01
Rosaceae	*Potentilla ancistrifolia*	Perennial	0.46 ± 0.03	0.08 ± 0.01
	Potentilla bifurca	Perennial	0.51 ± 0.02	0.08 ± 0.01
Chenopodiaceae	*Halogeton arachnoideus*	Annual	0.81 ± 0.02	0.07 ± 0.01
	Kalidium foliatum	Subshrub	2.01 ± 0.12	0.09 ± 0.01
	Salsola passerina	Subshrub	6.11 ± 0.19	0.12 ± 0.04
	Sympegma regelii	Subshrub	0.58 ± 0.02	0.12 ± 0.00
Zygophyllaceae	*Nitraria tangutorum*	Shrub	2.63 ± 0.03	0.07 ± 0.00
	Zygophyllum mucronatum	Perennial	42.89 ± 2.06	0.09 ± 0.05
	Zygophyllum fabago	Perennial	0.61 ± 0.12	0.12 ± 0.00
Plumbaginaceae	*Limonium aureum*	Perennial	2.15 ± 0.01	0.08 ± 0.01
	Limonium otolepis	Perennial	2.17 ± 0.02	0.08 ± 0.00
Scrophulariaceae	*Lancea tibetica*	Perennial	2.01 ± 0.03	0.09 ± 0.01

"Perennial" and "annual" refer to perennial grasses or herbs.

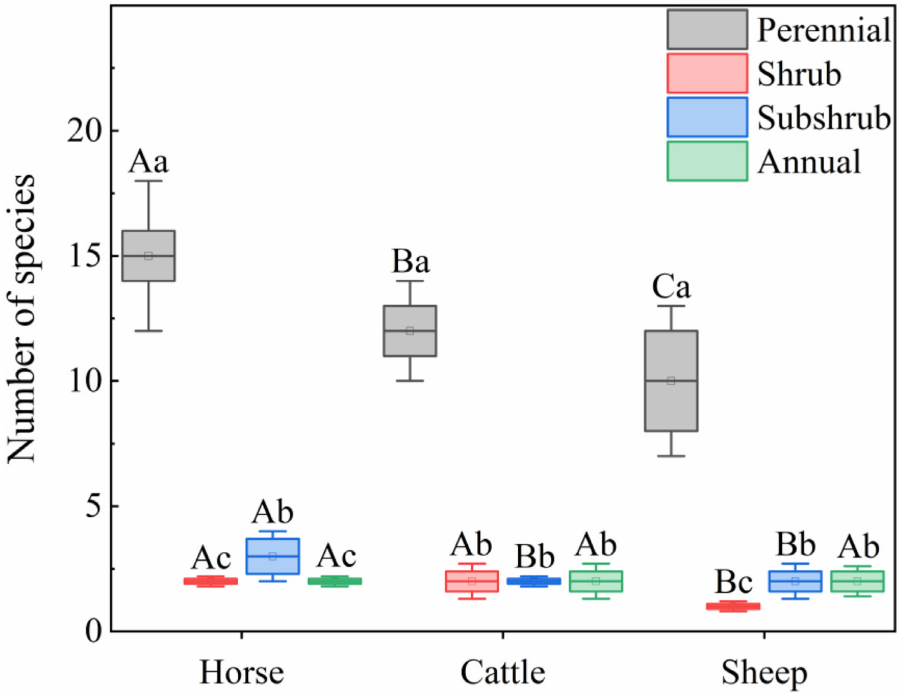

Figure 3. Seedling plant type associated with livestock species. Columns with different lowercase letters are significantly different among shrub, subshrub, and annual species for each livestock ($P < 0.05$), and those with different uppercase letters are significantly different between different livestock for each plant functional group ($P < 0.05$).

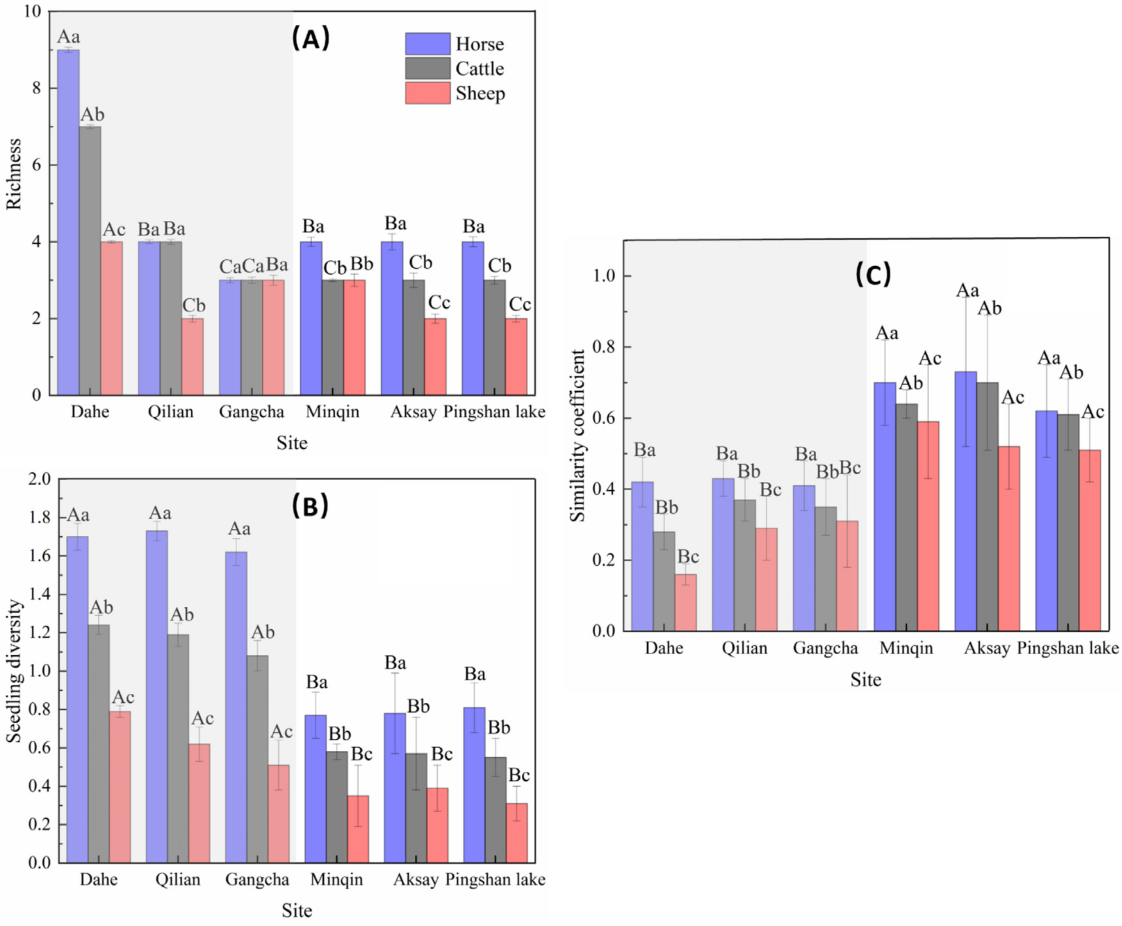

Figure 4. (**A**) Dung seedling richness at different sites on the Qilian Mountain grassland, China. Columns with different lowercase letters are significantly different between livestock types for each sample site ($P < 0.05$), and those with different uppercase letters are significantly different between sample site for each livestock ($P < 0.05$). (**B**) Diversity of dung seedlings at different sampling sites. Columns with different lowercase letters are significantly different between livestock types for each sampling site ($P < 0.05$), and those with different uppercase letters are significantly different between different sampling sites for each type of livestock ($P < 0.05$). (**C**) Jaccard coefficients of similarity for species composition between the dung seed bank and aboveground vegetation for different livestock types at the various sampling sites. Columns with different lowercase letters are significantly different between different livestock types for each sampling site ($P < 0.05$), and those with different uppercase letters are significantly different between sampling sites for each livestock ($P < 0.05$). The shaded section is for the alpine meadows.

Dung seedling density in relation to seed mass and shape index

Thirty plant species were identified from livestock dung (Table 2). The mean seed mass (± standard error [s.e.]) of identified species was 3.64 ± 0.16 mg, ranging from 0.03 mg *(Leontopodium nanum)* to 47.02 mg *(C. jubata),* with 18 species (60% of total) having a seed mass of > 1 mg. The mean seed shape index (± s.e.) was 0.09 ± 0.02, ranging from 0.001 *(T. lanceolata)* to 0.17 *(E. nutans),* with 17 species (56.7% of the total) having a seed shape index of < 0.10 (spherical seeds).

Seed mass had a significant impact on germination success after passage through the livestock digestive tract. Successfully germinated seeds had a range of 10−30 mg (Fig. 5A). Also, as seed shape index increased, dung seedling density decreased (see Fig. 5B).

Discussion

Size and composition of livestock dung seed bank

Of the 30 plant species that germinated from livestock dung, only three were annuals but 22 were perennials (see Table 2), which indicated that the perennial species were the main source of dung seed bank. Annuals rely only on seeds for reproduction and thus tend to produce a larger number of seeds (Smith and Fretwell 1974) with an r-reproductive strategy (i.e., plants produce large numbers of offspring to increase the survival rate; Mac-Arthur and Wilson 1967). However, plants with a greater number of seeds also tend to have poorer seed tolerance to digestion (Lönnberg and Eriksson 2013), which causes low seedling density of annual plants from animal feces. Similar results are also found in Tan sheep dung seed bank on the semiarid Loess Plateau (Wang et al. 2019). Shrubs/subshrubs are the main plant species of desert grassland, and shrubs have greater survival competitiveness than herbs, which is reflected in the hardiness of seedlings (Brown and Mclvor 1998). In this study, we found that a total of five shrubs/subshrubs germinated from livestock dung, suggesting a potential role for herbivore endozoochory for the long-distance dispersal of dry-fruited shrubs and their potential colonization of distant sites (Manzano et al. 2005). In fact, the composition of the dung seed bank is determined by the species of seed ingested by livestock, and selective feeding of livestock has an important impact on this process.

Effect of seed trait on dung seed bank

Seed size and shape are significant plant adaptations for coping with environmental conditions (Wu et al. 2015). Sixty percent of species identified from the aboveground vegetation at the trial sites (see Table 2) had medium or large seeds according to the cri-

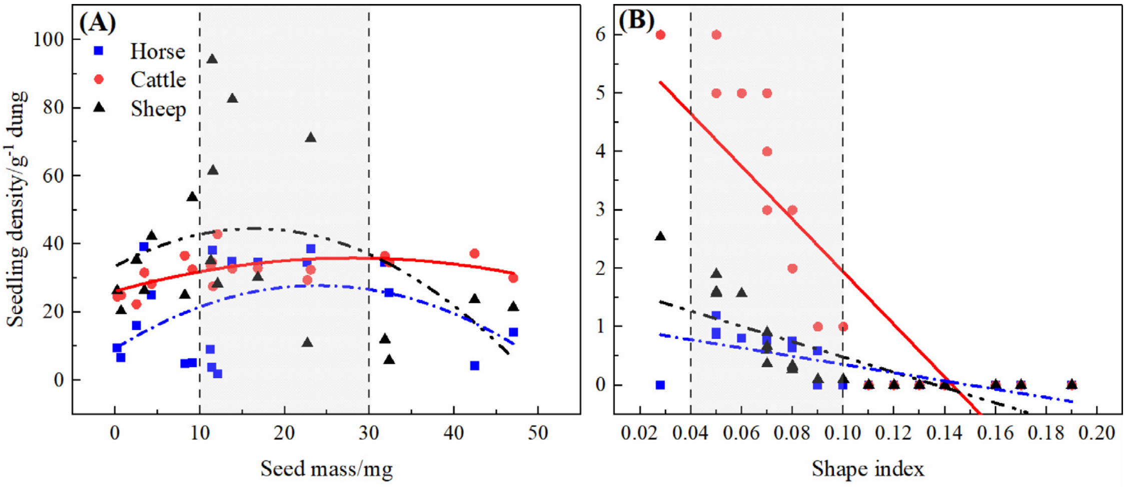

Figure 5. Dung seedling density in relation to seed mass and seed shape index. (**A**) Horse: seedling density = −0.03 mass^2 + 1.54 mass + 9.33, $R^2 = 0.18$, $P < 0.01$. Cattle: seedling density = −0.01 mass^2 + 0.71 mass + 26.14, $R^2 = 0.37$, $P < 0.01$. Sheep: seedling density = −0.04 mass^2 + 1.34 mass + 33.51, $R^2 = 0.16$, $P < 0.01$. (**B**) Horse: seedling density = −7.07index + 1.06, $R^2 = 0.51$, $P < 0.01$. Cattle: seedling density = −45.14 index + 1.06, $R^2 = 0.74$, $P < 0.01$. Sheep: seedling density = −13.12 index + 1.79, $R^2 = 0.59$, $P < 0.01$.

teria of Thompson et al. (1993), and 43% species (see Table 2) had a seed shape index corresponding to flatter or elongated seeds (Peco et al. 2006). Seed mass and shape also have significant effects on seed germination after passing through the digestive tract of animals (Wang et al. 2017). The medium-sized seeds (10–30 mg) with a spherical shape (index, 0.04–0.10) had a higher germinability, indicating that those seeds have superior properties (e.g., dispersal, survival and germination) after passing through the digestive tract. Larger seeds are more easily chewed; for example, in *Retama sphaerocarpa* with large seeds (77 mg), broken seed fragments could be frequently observed in sheep dung (Manzano et al. 2005). Previous studies have indicated that small seeds are capable of escaping from grinding and digesting (Kuiters and Huiskes 2010) or surviving from the gut passage (Pakeman et al. 2002). Small seeds with a spherical shape may shorten the retention duration during digestion, reducing exposure to microbial attack in the rumen and thus eventually promoting the success of survival and dispersal (Traveset 1998). However, other researchers suggest that compared with the large seeds, the high frequency of small seeds presenting in herbivore dung is not due to their better adaptation to environmental conditions of gut passage but rather because of the negative relationship between seed size and seed production (Pakeman et al. 2002; Couvreur et al. 2005) (i.e., small seeds are produced in greater numbers; Eriksson and Jakobsson 1998). Nevertheless, such a relationship is not observed (Bruun and Poschlod 2006; D'Hondt and Hoffmann 2015). The conflicting opinions may be partially resulted from the different plant or animal species used for experiments (Wang et al. 2017).

Similarity of dung seed bank and aboveground vegetation

Many factors, such as the grazing regime, animal species, environmental condition, and the spatial distribution of plant species, may mediate the effects of grazing on the similarity between the dung seed bank and aboveground vegetation (Ungar and Woodell 1996; Peco et al. 1998; Agra and Ne'eman 2012). The similarity index between dung seed banks and the corresponding aboveground vegetation in alpine meadows was significantly lower than that in the desert grasslands (see Fig. 4C). The low similarity index in the alpine meadows is due to the higher species richness of aboveground vegetation but lower dung seed species richness (Yu et al. 2012). However, the high similarity index in the desert grasslands may be attributed to two reasons. First, the species diversity and coverage of desert grasslands are low, resulting in the lack of livestock selective feeding (Chen et al. 2010) and the indiscriminate feeding on all plants. Therefore, in desert grasslands the low seedling richness of livestock dung (see Fig. 4A) may not always induce a low similarity coefficient between dung seedlings and aboveground vegetation (see Fig. 4C) (Wang et al. 2019). Second, in semiarid environments, such as the desert grasslands located on the northern slope of the Qilian Mountains, dung pellets provide substantial protection for the dispersed seeds to survive in the harsh environment until sufficient rainfall occurs, after which seeds successfully germinate and establish.

Role of the dung seed bank in grassland management of the Qilian Mountains

Spatial deposition of herbivore feces in grazing systems is important because it directly affects pasture growth and composition (Malo 2000). The spatial patterns of herbivore defecation result in interesting differences between and within plant communities. These differences are closely linked to the type of herbivore, its grazing behavior, and the distances over which it grazes (Malo 2000). Traditionally, farmland around the Qilian Mountains has been used mainly for grazing and livestock migration between pastures. The majority of seeds passing through the digestive tract of horses, cattle, and sheep are retained respectively for 36–41 h (Illius and Gordon 1992), 74 h (Illius and Gordon 1992), and 24–40 h (Manzano et al. 2005; Mancilla-Leytón et al. 2011; Wang et al. 2017), which is long enough to result in seed dispersal over long distances. Indeed, endozoochorous seed dispersal distances are affected by grazing management. For example, free-grazing Kazakh sheep can disperse over 7–10 km/d (Wang et al. 2016), whereas the transhumant flocks of France and Spain, which move seasonally with their herders between fixed summer and winter pastures (Klein 1981), move approximately 25–30 km/d, allowing seed dispersal over a distance of about 40 km (Manzano et al. 2005). In contrast, horses have greater physical strength to travel greater distances, and the distance of seed dispersal is longer than the distance traveled by sheep. Unfortunately, no studies have reported migration distances of cattle grazing in the Qilian Mountains.

Seed dispersal by livestock is considered as a potential means for introducing desirable plant species into the degraded or over-grazed grasslands (Archer and Pyke 1991), especially in arid and semiarid regions (e.g., the desert grassland on the northern slope of the Qilian Mountains) (Gökbulak 2006). For this approach, the herders feed the animals with appropriate pasture species, which contain seeds that have sufficient dormancy and seed coat durability to survive passage through the gut and retain viability through long periods of extreme dryness with occasional light rain, until there is sufficient precipitation for germinating plants to survive and establish in the silt (loess) soil. Livestock could be used to disperse native seeds with such properties, particularly in remote and inaccessible areas. It should be noted, however, that endozoochorous seed dispersal could potentially threaten functionally rich communities by assisting the spread of invasive weeds (Kuiters and Huiskes 2010). Given these various options, dispersal of desirable seed species can be achieved in the Qilian Mountains through appropriate feed and targeted grazing of livestock (Lerner 2007). Seed germination from animal feces is only the first step toward successful endozoochory, the second vital step being seedling establishment (Calviño-Cancela and Martín-Herrero 2009). Seeds of any plant species able to retain viability after gut passage can be dispersed by animals, germinate, and subsequently establish a seedling (Barrow and Havstad 1992). Our study suggests that the foraging activities of livestock have the potential to contribute to the gathering of plant seeds under traditional rotational grazing in the Qilian Mountains. This is especially true in the cold season (from October to December), when most plants still retain mature seeds. Although the cost of passage through the gut of livestock is undoubtedly high for dry-fruited plant species (present study; Traveset et al. 2002), endozoochory may enhance species colonization through directional dispersal. This mode of dispersal may increase the heterogeneity of plant communities via the rotational grazing of livestock in the Qilian Mountains.

Implications

In the alpine meadows and desert grasslands around the Qilian Mountains, plant seeds are grazed by livestock and then remain in feces after passage through the digestive tract, which results in a large number of seeds, mainly from perennial species, germinating from livestock dung after deposition. The diversity and abundance of horse dung seedlings were significantly greater than that of cattle or sheep dung, indicating that horse dung contributes the most to the soil seed bank. Seed traits (size and shape) influence the spread of seeds via animal dung, with medium-sized (10–30 mg) spherical (0.04–0.10 shape index) seeds being most desirable. The dung seed bank of the Qilian Mountains is widely distributed in the grasslands. Indeed, the dung seed bank of grazing livestock promotes the formation and development of grasslands in the Qilian Mountains and other parts of the world.

This study only examined the dung seed germination ability; however, the survival, establishment, and development of dung seedlings in the desert grasslands and alpine meadows on the Qilian Mountains are largely unknown (Calviño-Cancela and Martín-Herrero 2009). Therefore, further investigations are to 1) explore the contributions of the dung seed bank to grassland vegetation renewal by determining the dung seedling growth and development, and 2) observe the livestock grazing behaviors (e.g., selective feeding, foraging rate and moving distance), aiming to determine the dispersal of specific plant species through dung seeds and to examine coevolution between plants and animals if any (Maron et al. 2019; Valenta and Nevo 2020).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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