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Behavioral and physiological adaptations of mammals and birds to anthropogenic disturbances

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Behavioral and physiological adaptations of mammals and birds to anthropogenic disturbances

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Table of contents

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Editorial: Behavioral and physiological adaptations of mammals and birds to anthropogenic disturbances

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Anthropogenic activities are expanding exponentially and are transforming large areas of natural habitat dramatically. Habitat loss is not the only anthropogenic disturbance that directly affects wildlife, other disturbances that accompany urbanisation and industrialization can also disrupt natural activities of wildlife. Some species are able to adapt their behaviour to thrive in these transformed environments, whereas others disappear. Anthropogenic activities are regarded as a threat to biodiversity, and there is a dire need for mitigation actions which would allow humans and wildlife to co-exist in the same space.

Construction of roads is an integral part of urbanization, and it can impact wildlife negatively not only by fragmenting their habitat, but also by creating traffic noise. Previous studies frequently investigated the effect of traffic noise on acoustic communication, reproductive success and stress hormones in animals (Crino et al., 2013; Caorsi et al., 2017). Chronic exposure to traffic noise can also affect other factors such as behaviour and metabolic rate. Qu et al. showed that chronic noise exposure increased exploratory behaviour and stress hormones while it decreased the resting metabolic rates of pikas, and it altered the correlations between these factors. Increased stress levels can negatively affect the health and survival of animals.

Anthropogenic disturbances are not exclusively associated with the expansion of cities, but also include other disruptions to natural ecological systems such as those caused by farming practices. Grasslands are frequently degraded by grazing livestock. The timing, duration, and intensity of grazing of large herbivores can change the composition and height of vegetation (Wei et al., 2022a; Wei et al., 2022b), that in turn can affect population densities of small mammals, in particular since there are predation risk implications (Wei et al., 2023). The effect of grazing appears to be species specific, and dependent on whether small mammals are able to take advantage of changes in vegetation and exploit this niche. Changes in the vegetation structure could proportionally increase preferred food sources of small rodents. They spend less energy to on feeding, thereby reducing foraging costs which can make the habitat more appealing for them (Zhang et al.). In addition, the perceived predation risk can further be lowered by living in groups and is evident in the behaviour and physiology of small mammals. Zhou R. et al. showed that at higher population densities, pikas increase their foraging time, and reduce their vigilance, they have lower levels of stress hormones, and higher reproductive hormone levels. Together, these factors increase the reproductive potential of the animals, and can lead to a population explosion, resulting in increased human-wildlife conflict.

Li et al. demonstrated that grazing activities of large herbivores in the Alxa desert (China), changed interspecific interactions between small herbivorous mammals. Grazing reduced plant heights, density, and cover, inducing a significant reduction in the activity of small desert rodents. Increasing competition between the different rodent species effected changes their timing of their activity to reduce the temporal overlap. Desert rodents displayed an increased sensitivity to environmental changes. This is of concern since climate change research suggests drier and warmer climates in future (Idris et al., 2022), which could have serious implications for animals that already live in harsh habitats and appears to be vulnerable to environmental changes.

Grazing by large herbivores can also change the community structure of small mammals. Grazing, and overgrazing typically change the structure of the plant community and can have knock-on effects on small mammals that relies on it. Yuan et al. demonstrated a reduction in the rodent community resilience, evident from the reduced number of r- selected strategists, and an increase in resilience variability as is apparent from larger variability in abundance and species richness of r-selected strategists.

Agricultural land use as intensified in the past few decades and has transformed large areas of natural habitat to arable land (Foley et al., 2005). Monocultures such as maize reduce biodiversity, soil fertility and ecosystem stability (Wang et al., 2019). Although rodents perform useful ecosystem services such as biological pest control and pollination in crop fields, the abundance of food can lead to population explosions of rodent species that can exploit these food sources (Fischer et al., 2018). Monocultures can have how levels of certain organic compounds that are essential that for survival. Niacin is one such a compound and deficiencies thereof affect animals that are already restricted to a suboptimal habitat, adversely. Selimovic A. et al. showed that niacin deficiencies affect the reproduction of European brown hares negatively and contributes to the decline in their local populations. Selimovic A. et al. also undertook a more focused study to investigate the effect of dietary niacin deficiency in European brown hares. They revealed that although there was no difference in the reproductive output and survival rate of leverets in European brown hares, a niacin deficit caused females to have a lower body mass and their leverets did not grow as fast.

The effect of agricultural crop farming on the small mammal community is further illustrated by the reduction in community stability when the temporal variation of the community increases. Yuan et al. showed that when land was reclaimed for agriculture in the Alxa desert, a decrease in rodent community stability was evident from the decrease in the number of K-selected strategists and the increase in their abundance and species richness variability.

We conclude that responses of wildlife to anthropogenic disturbances are species dependent. Many species are well adapted to their natural habitats, such that when the climate and/or vegetation changes, they retain the characteristics of their historical habitats. These species do not respond well to changing environmental conditions as a result of anthropogenic disturbances, and such changes can result in the destabilization of ecosystems by altering the community structures. Some species are more tolerant and flexible to disturbance and are able to take advantage of changed environmental conditions to a certain extent. It is clear that, depending on the life history of the different species, anthropogenic disturbances can have profound implications for biodiversity.

Author contributions

MO: Writing – original draft. WW: Writing – review & editing. DL: Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Increased availability of preferred food and decreased foraging costs from degraded grasslands lead to rodent pests in the Qinghai-Tibet Plateau

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The increased population density of rodent species during ongoing grassland degradation further deteriorates its conditions. Understanding the effects of grassland degradation on rodent feeding habits is of great value for optimizing grassland management strategies. In this study, lightly degraded (LD), moderately degraded (MD), severely degraded (SD), and reseeded grassland (RG) were selected and their plant resources and soil physical properties were investigated. In addition, the study used *ITS2* barcode combined with the Illumina MiSeq sequencing method to analyze the food composition and proportion of plateau zokors in different grassland conditions. The results showed that, with grassland degradation, plant biomass decreased, but the relative proportion of forbs increased (LD: 32.05 ± 3.89%; MD: 28.97 ± 2.78%; SD: 49.16 ± 4.67% and RG: 10.93 ± 1.53%). Forbs were the main food of the plateau zokor, accounting for more than 90% of their diet, and the animal had a clear preference for *Potentilla* species; the soil compaction of feeding habits showed a decreasing trend in the 10–25 cm soil layer, suggesting a decreased foraging cost. Nutritional analysis showed that the stomach content of crude protein in zokors feeding on MD grassland was significantly higher than that of animals feeding on the other grassland types. Structural equation modeling showed that soil physical properties and the relative biomass of forbs had significant ($P < 0.05$) and extremely significant ($P < 0.001$) impacts on the population density of plateau zokors, with direct impact contribution rates of 0.20 and 0.63. As the severity of grassland degradation increased, although the aboveground and underground biomass of the plants decreased, the proportion of food preferred by the plateau zokor increased, and the corresponding changes in the feeding environment resulted in decreased foraging energy expenditure, thereby increasing the suitability of the degraded grassland for the plateau zokor. Compared with degraded grassland, the food diversity and evenness of zokors increased, the food niche width enlarged, and the proportion of weeds decreased in RG, which increased the difficulty of obtaining food. Reseeding in grassland management is therefore an effective way to control plateau zokors.

KEYWORDS

plateau zokor, feeding habits, degraded and reseeded grassland, *ITS2* barcode, illumina MiSeq sequencing

Introduction

Grassland degradation from climate change and human activity is a global environmental problem (Wang and Wesche, 2016). Grassland degradation is a process of retrograde succession of grassland ecosystems, during which the structures, processes and functions within the system have undergone significant changes (Dong et al., 2020; Yu et al., 2022). Owing to the high altitude and unique alpine climate, the alpine ecosystem of the Qinghai-Tibet Plateau (QTP) is very sensitive and fragile, and the impact of grassland degradation is more prominent (Klein et al., 2004; Schleuss et al., 2015). This not only hinders the development of animal husbandry in the country, but also seriously affects the stability of the regional ecosystem and the functioning of the plateau ecological barrier (Harris, 2010; Liu et al., 2021). Recently, the Chinese government has implemented numerous ecological protection projects (Xu et al., 2006; Quan et al., 2011; Zhao et al., 2022). Dealing with the environmental problems of grassland degradation, restoring and improving functions, and connecting ecological protection projects, such as the construction of ecological security barriers, is of great significance to the sustainable development of the region and the construction of ecological security barriers in the future.

Grassland degradation is often accompanied by an increase in the number of rodents, and a large number of rodents aggravate the process of grassland degradation (Miehe et al., 2008; Li et al., 2014). Studying the mechanisms of rodent populations during grassland degradation is of great significance for rodent control and the optimal management of grasslands (Wei et al., 2020). Changes in environmental conditions play an important role in population fluctuations and can have important effects on population numbers either directly or through their interactions (Harris et al., 2015). After grassland degradation, the original environment underwent significant change. Grassland height and coverage have decreased, vegetation community structure and dominant species have changed, aboveground and belowground biomass has decreased, soil bulk density and compactness have increased, and soil water content has decreased (Harris, 2010; Wang et al., 2014). Relevant studies have shown that degraded grassland provides a good open habitat for rodents who prefer to live in these conditions, which is conducive to observing natural enemies from a distance and reducing the risk of predation (Liu et al., 2013). As the main body of rodents builds burrows, soil properties are important factors affecting rodent habitat selection. Soil compaction determines the firmness of the rodent tunnel system and affects the energy consumption in the process of building the tunnel system (Zhang, 2007). Changes in vegetation community structure and dominant species directly affect animal food resources (Tang et al., 2015).

The abundance and quality of food directly affect the population density and distribution areas of animals in the environment (Chua et al., 2021). Optimal foraging theory indicates that animals usually tend to choose food resources of the highest nutritional quality and concurrently modify their foraging strategies in terms of energy and time. They tend to minimize food handling time, reduce exposure to predation threats while decreasing their energy expenditure, and maximize their own nutritional and energy gain (Forbey et al., 2018; Abrahms et al., 2021; Demi et al., 2021). Therefore, when food resources are abundant, animals only select the best dietary items. Under food shortages, generalist herbivores can respond by expanding their dietary selection to low-ranked food types, regardless of whether forage is preferred (Li et al., 2019; Goldberg et al., 2020). However, food types of different qualities are spatially distributed in a mosaic pattern. High-quality food resources often exist on a small scale and are often surrounded by large-scale, low-quality food resources (Hope et al., 2021). The primary vegetation of alpine meadows on the QTP is dominated by grasses and sedges; however, as grassland degradation advances, the proportion of forbs significantly increases (Zhang et al., 2018). In the case of severely degraded (SD) grasslands, a plant community with forbs as the dominant species is often formed, and the soil surface becomes bare and loosely compacted (Wang et al., 2020). Optimal foraging theory also assumes that animals need to spend energy in the search for food, and the difference between the energy gained from food consumption and the energy expended during foraging is the net income of energy gain. The greater the income, the more beneficial the food will be to foragers; therefore, animals prefer to forage in patches of high-quality food (Schai-Braun et al., 2020). The underground niche may impose some constraints on the food selection process of subterranean species, and their foraging mainly relies on digging tunnels, which necessitates extra energy expenditure compared with aboveground animals (Su et al., 2018). For example, the amount of energy an animal uses to move a unit distance underground by digging is 360–3400 times greater than that of moving aboveground (Xie et al., 2013).

The plateau zokor (*Eospalax baileyi*), a small mammal in the alpine meadow, is a typical subterranean rodent endemic to the QTP (Su et al., 2015). As a key species in the alpine meadow ecosystem of the QTP, it performs several vital ecological functions. In natural habitats, digging activities can promote soil renewal, which is helpful for the circulation of soil nutrients and germination of seeds and underground buds. In addition, the underground activities of plateau zokors may be beneficial for the colonization of exotic plants in alpine meadow ecosystems. Therefore, it deserves the reputation of "*Ecosystem engineer*" (Zhang et al., 2010; Eldridge et al., 2016; Miranda and Rothen, 2019). However, when the population density of zokors is high, a series of activities, such as digging and eating plant roots, seriously damage plants and thus affect grassland ecosystems. Burrowing activity causes grassland vegetation to be covered by mounds of soil for a long time, changes the structure of the plant community, reduces the productivity of the grassland, and further aggravates its degradation (Li et al., 2021). In practice, it has been found that the population density of the plateau zokor increases with the aggravation of grassland degradation (Wang et al., 2000a). However, the factors that lead to the increase in the plateau zokor population during grassland degradation remain unclear. The change in the food resources of the plateau zokor under grassland degradation might be a key factor. The adequacy of food resources and environmental safety are priorities for animal habitat selection (Šumbera et al., 2012). For subterranean rodents, security in underground tunnels is guaranteed because of the absence of predation risks. Therefore, food resources may be regarded as crucial factors for subterranean dwellers. High-quality food and loose soil structures are important for the reproduction and development of subterranean rodent populations. Thus, we speculate that degraded grassland provides an optimum foraging environment for plateau zokors (the cost of foraging is reduced after grassland degradation) and a reliable food source.

Our working hypothesis was that the total biomass of plants decreases after grassland degradation, but the proportion of food that the plateau zokor prefers increases, and the cost of obtaining food decreases, making it more suitable for foraging. This might be the main reason for the increase in the population density of plateau zokors in degraded grasslands. In this study, soil bulk density, compactness, and water content were used to indicate the difficulty of food acquisition. Food composition and proportion were used as indicators to analyze whether food changes and how diversity changes. Finally, the stomach nutrients contents were analyzed. The aim of this study was to determine how grassland degradation affects the dietary selection of plateau zokors and to provide a new perspective on the mechanism of rodent damage to the grassland ecosystem.

Materials and methods

Study areas

The experimental area was located in the Luqu County, Gansu Province, QTP (34°14′07′′–34°48′48′′N, 102°10′80′′–102°58′15′′E), with an average altitude of 3550 m. The annual average temperature was 2.6 °C, and the annual frost-free period was 56 days. The annual precipitation ranges from 632 to 781 mm, mainly from May to September. The soil was mainly subalpine and alpine meadow soils. Alpine shrub meadows and alpine meadows accounted for 88.68% and 11.32% of the county's grassland area, respectively. The alpine meadow is one of the main grazing grasslands in the region, and is also the area with the most severe prairie rat infestation. The area of rodent infestation in the county is 14.18×10^4 hm^2, accounting for 33.84% of the county's grasslands. The plateau zokor is one of the dominant groups of rodents in the Luqu County, accounting for ∼13.58% of rodent species (Wu et al., 2020).

Methods

Experimental design

According to the Grading Standard of Natural Grassland Degradation, Desertification and Salinization (GB19377-2003) (Su et al., 2003), vegetation coverage was selected as a classification index of degraded grassland, and the vegetation coverage of each type of degraded grassland was as follows: lightly degraded (LD), 80–100%; moderately degraded (MD), 60–80%; severely degraded (SD), $<$ 60%. The degree of grassland degradation in the study area, LD, MD, and SD grasslands were scored and selected. Another reseeded grassland (RG) was selected (**Figure 1** and **Table 1**). The reseeding was *Elymus nutans* + *Poa pratensis*, and the reseeded period was 4 years.

Plant community survey

Five (0.5 m × 0.5 m) quadrats were randomly selected from different plots to investigate the density, coverage, and height of each plant. All plants in the square were collected and classified, brought back to the laboratory for drying at 95°C, and weighed separately for the aboveground and underground parts to obtain the corresponding biomasses of each species. The coverage, density, height, and biomass of each species in the plant community were standardized using the maximum-standardization method.

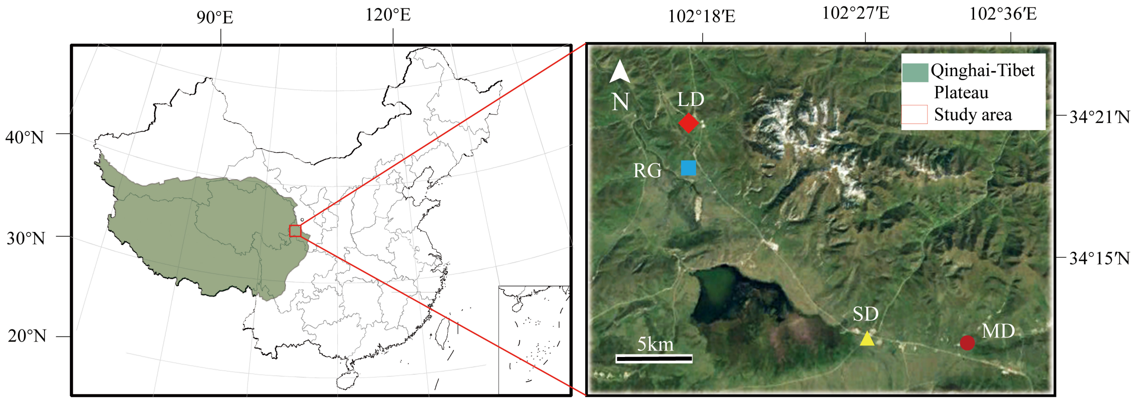

FIGURE 1
Location of the sampling site. LD, lightly degraded grassland; MD, moderately degraded grassland; SD, severely degraded grassland; RG, reseeded grassland.

TABLE 1 Study area.

Plot	Coverage %	Richness	Shannon-Wiener index	Zokor density No./hm^2	Area hm^2
RG	98.00 ± 0.98a	4.92 ± 0.38a	2.56 ± 0.13a	4.69	6.21
LD	89.00 ± 1.87b	4.35 ± 0.43a	2.41 ± 0.21a	5.44	3.66
MD	69.00 ± 1.87c	3.81 ± 0.45a	2.35 ± 0.13a	7.73	5.33
SD	45.00 ± 2.24d	1.55 ± 0.23b	1.64 ± 0.11b	11.2	4.41

LD, lightly degraded grassland; MD, moderately degraded grassland; SD, severely degraded grassland; RG, reseeded grassland. Different letters indicate significant differences between groups ($P < 0.05$).

Species importance value = (relative coverage + relative density + relative height of species + relative biomass)/4. The importance value was used to calculate the diversity (Shannon-Wiener index) of the plant community in the quadrat (Niu et al., 2019).

Soil physical properties

The moisture content and bulk density were determined by the aluminum box drying method, which was repeated three times. Soil compaction at a depth of 0–30 cm was measured using a soil compaction meter (SC-900), recorded every 2.5 cm, and repeated 15 times.

Plateau zokor population density survey and gastric content collection

In the study area, 122 plateau zokors were captured using bow traps, including 25 individuals in RG, 24 individuals in LD, 38 individuals in MD, and 35 individuals in SD. The population density of the plateau zokor per unit area was calculated based on the specific plot area of the captured plateau zokor (Table 1). Ten adult individuals were selected from each grassland type (LD: 4 males and 6 females; MD: 5 males and 5 females; SD: 5 males and 5 females; RG: 3 males and 7 females), euthanized by decapitation, and their gastric contents were collected. Forty samples were used for food habit analysis. Institutional guidelines for the use of animals were followed, the Animal Ethics Committee of Gansu Agricultural University approved the experimental procedure (approval no. GAU-LC-2020-014), and local authorities' approval was also obtained.

Analysis of nutrients in the stomach contents of plateau zokors

Referring to GB/T14924.9-2001 (Zhou et al., 2001), the semi-micro Kjeldahl method was used to determine the crude protein (CP) content, the crude fat was determined by the Soxhlet extraction method, and Van's fiber method was used to determine the acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents.

DNA extraction and illumina MiSeq sequencing of the *ITS2* amplicons

Genetic analysis of 40 samples was performed using Genepioneer Biotechnologies (Nanjing, China), including DNA extraction, *ITS2* gene amplification, and sequencing of the amplified products on an Illumina MiSeq platform.

Data analysis

The QIIME2 software was used for species annotation with default parameters, and the RDP3 Classifier v. 2.2 algorithm

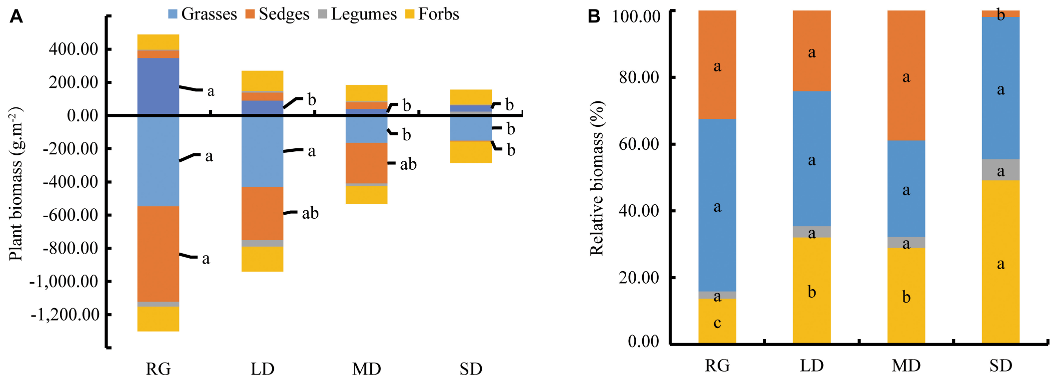

FIGURE 2
Biomass **(A)** and relative biomass **(B)** of different functional groups. LD, lightly degraded grassland; MD, moderately degraded grassland; SD, severely degraded grassland; RG, reseeded grassland. Different letters indicate significant differences between groups ($P < 0.05$).

were used to identify the taxonomic composition (Edgar, 2004). The diversity analyses of the diet were conducted according to output-normalized sequence data. The significance analysis in this study was carried out using one-way ANOVA in SPSS 23.0 (IBM®, New York, United States). The R version 2.6-2 (The R Foundation for Statistical Computing, Vienna, Austria) "vegan" packages was used for non-metric multidimensional scaling (NMDS) analysis (Oksanen et al., 2018), and the Amos Graphics 24.0 (IBM®, New York, United States) software was used to draw structural equations (Grace, 2006). The level of significance at which the null hypothesis was rejected was set at $\alpha = 0.05$.

Results

Plant resources

The survey found that there were 18 families, 37 genera, and 41 plant species in the RG; 16 families, 33 genera, and 38 plant species in LD; 15 families, 30 genera, and 31 species in MD; and 11 families, 16 genera, and 16 species in SD. The aboveground and underground biomasses of RG were the highest, and the aboveground and underground biomasses decreased with increasing degree of grassland degradation (**Figure 2A**). The aboveground biomass of different functional groups and underground biomass of grasses and sedges decreased with an increase in the degree of degradation. The relative biomass of forbs under RG was significantly lower than that of other grassland varieties ($P < 0.05$), the relative biomass of forbs under SD was the highest, and the relative biomass of grasses under SD was significantly lower than that of other grassland types (**Figure 2B**). The biomass proportions of the top 15 genera were 96.46% in RG, 86.59% in LD, 88.35% in MD, and 93.77% in SD. The top four genera of relative biomass in RG were *Elymus* (29.63%), *Poa* (17.82%), *Scirpus* (16.15%), and *Kobresia* (10.62%); *Elymus* (27.86%), *Kobresia* (23.08%), *Poa* (10.92%), and *Potentilla* (7.51%) in LD; *Elymus* (24.07%), *Kobresia* (20.20%), *Scirpus* (12.79%), and *Potentilla* (9.13%) in MD; and *Potentilla* (36.52%), *Elymus* (30.54%), *Poa* (12.12%), and *Astragalus* (6.32%) in SD (**Figure 3**).

Physical properties of soil

The soil water content at LD within 0–10 cm depth was significantly higher than that of other grasslands ($P < 0.05$), whereas the soil moisture at SD at 10–20 cm and 20–30 cm depths was the lowest (**Figure 4A**). There were no significant differences in the soil bulk density at different depths among the different grassland conditions. Under SD, soil bulk densities at depths of 0–10 cm and 20–30 cm were the lowest, and soil bulk density at depths of 10–20 cm increased with the deterioration of grassland conditions (**Figure 4B**). The soil compaction degree of the grassland under different conditions was significantly different at 0–10 cm depth ($P < 0.05$), and the soil compaction degree at 0–10 cm of RG was significantly smaller than those of MD and SD. Soil compaction was the smallest in RG at 10–20 cm depth and the largest in MD at 20–30 cm depth, LD had the smallest soil compaction, and the largest was recorded in SD (**Figure 4C**). The soil compaction degree under different grassland conditions increased gradually at 2.5–7.5 cm, decreased at 7.5–12.5 cm, and showed an increased gradual trend at 12.5–25 cm (**Figure 5**).

Dietary differences under different degrees of degradation and reseeded grasslands

The plateau zokor fed on 21 families and 30 genera of plants in RG, 18 families and 25 genera in LD, 22 families and

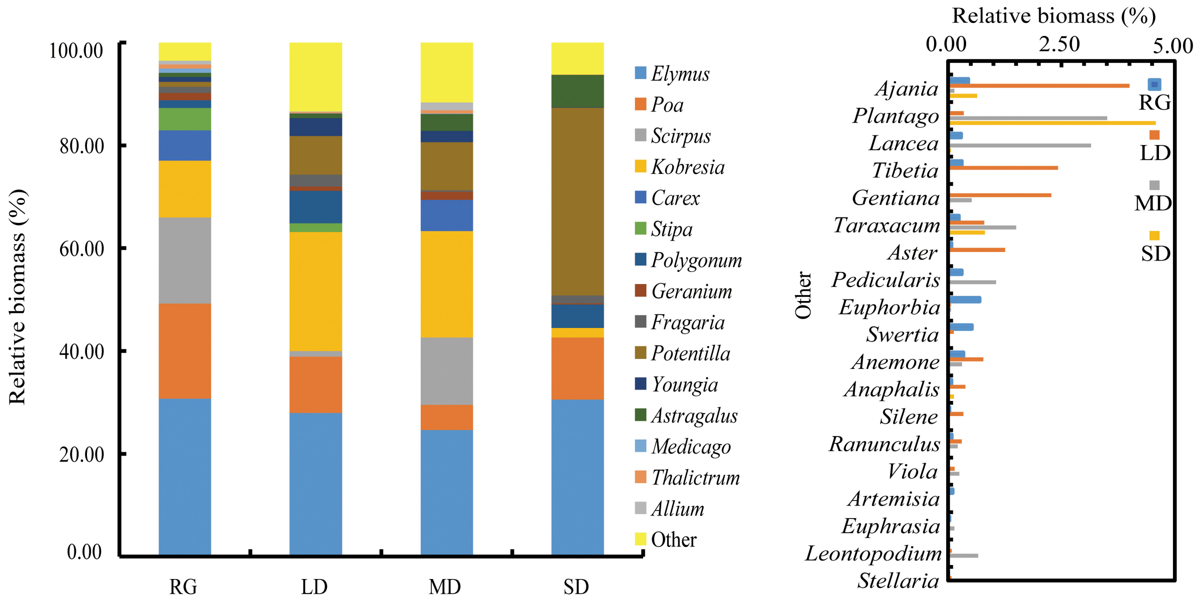

FIGURE 3
Relative biomass of plants at the genus level. LD, lightly degraded grassland; MD, moderately degraded grassland; SD, severely degraded grassland; RG, reseeded grassland.

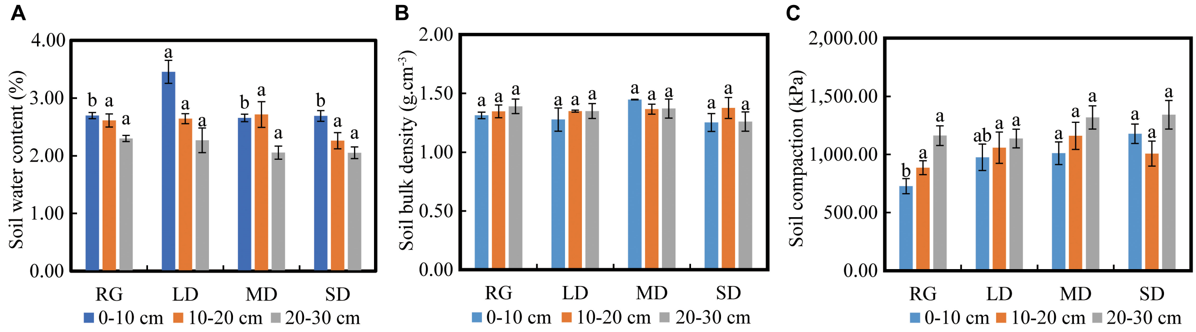

FIGURE 4
Changes in soil water content **(A)**, soil bulk density **(B)**, and soil compaction **(C)** of different grasslands. LD, lightly degraded grassland; MD, moderately degraded grassland; SD, severely degraded grassland; RG, reseeded grassland. Different letters indicate significant differences between groups ($P < 0.05$).

35 genera in MD, and 19 families and 30 genera in SD. The plant species (genus level) eaten by the plateau zokor in various grasslands were roughly the same. The proportions of the top 15 genera in their diets were 99.53% in RG, 99.61% in LD, 97.92% in MD, and 97.51% in SD. *Potentilla* had a large proportion, accounting for 40.57 % in RG, 72.96 in LD, 77.17% in MD, and 86.13% in SD. In addition, other genera with a higher proportion in the diet of plateau zokors in RG were *Geranium* (22.07%), *Taraxacum* (8.68%), and *Tibetia* (7.12%); in LD were *Geranium* (7.56%), *Lancea* (4.94%), and *Taraxacum* (4.49%); in MD were *Youngia* (10.29%), *Tibetia* (4.36%), and *Taraxacum* (3.27%); and in SD were *Stellaria* (6.85%), *Youngia* (1.65%), and *Lepidium* (1.53%) (Figure 6B). According to the statistics of plant functional groups, forbs under the four different grassland types were the main diet of plateau zokors, and their proportions were more than 90% (Figure 6A). The food diversity of the plateau zokor in different types of grasslands was significantly different ($P < 0.05$). The richness and Shannon-Wiener index of RG were significantly greater than those of LD and SD, and the Shannon-Wiener index of SD was significantly lower than that of LD ($P < 0.05$) (Figures 7B,C). The evenness index of RG was significantly larger than those of MD and SD ($P < 0.05$) (Figure 7D). In addition, the food niche breadth of RG was significantly larger than that of other grasslands (Figure 7E). The NMDS results show that the food composition of plateau zokors under different grassland conditions was

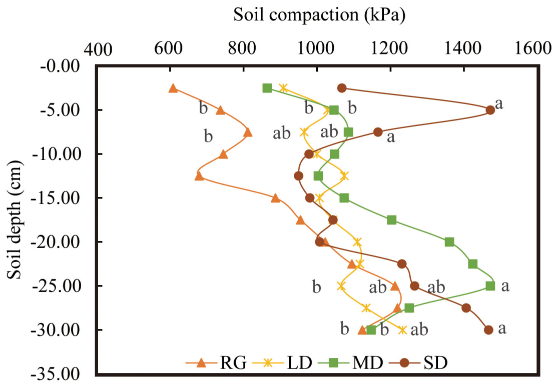

FIGURE 5
Change in soil compactness between different soil layers. LD, lightly degraded grassland; MD, moderately degraded grassland; SD, severely degraded grassland; RG, reseeded grassland. Different letters indicate significant differences between groups ($P < 0.05$).

roughly divided into two groups: the food compositions of RG and LD were similar, and those for MD and SD grasslands were similar (**Figure 7A**).

Nutritional composition of the stomach contents of plateau zokors

The ADF content was the highest in RG and decreased with an increase in grassland degradation degree, which was significantly the lowest in SD ($P < 0.05$). NDF did not show any significant differences among the plots. Crude fat was the highest in RG ($P < 0.05$) and the lowest in MD ($P < 0.05$). The CP contents of MD and LD were significantly higher than those of RG and SD ($P < 0.05$) (**Figure 8**).

Effects of grassland condition on the diet of plateau zokors

During grassland degradation, soil physical properties had a significant impact on the population density of the plateau zokor and the relative biomass of forbs ($P < 0.05$), and the direct impact contribution rates were 0.20 and 0.30, respectively. Plant diversity had an extremely significant negative impact on the nutrient composition of gastric contents ($P < 0.001$), and the contribution rate of the direct impact was 0.48. The relative biomass of forbs had an extremely significant negative correlation with feeding habits ($P < 0.001$) with a direct impact contribution rate of 0.56, and an extremely significant impact on the population density of plateau zokors ($P < 0.001$) with a direct impact contribution rate of 0.63 (**Figure 9**).

Discussion

Changes in the diet of plateau zokors after grassland degradation

In this study, 24 families and 48 genera were identified in all stomachs, whereas only 21 families and 45 genera

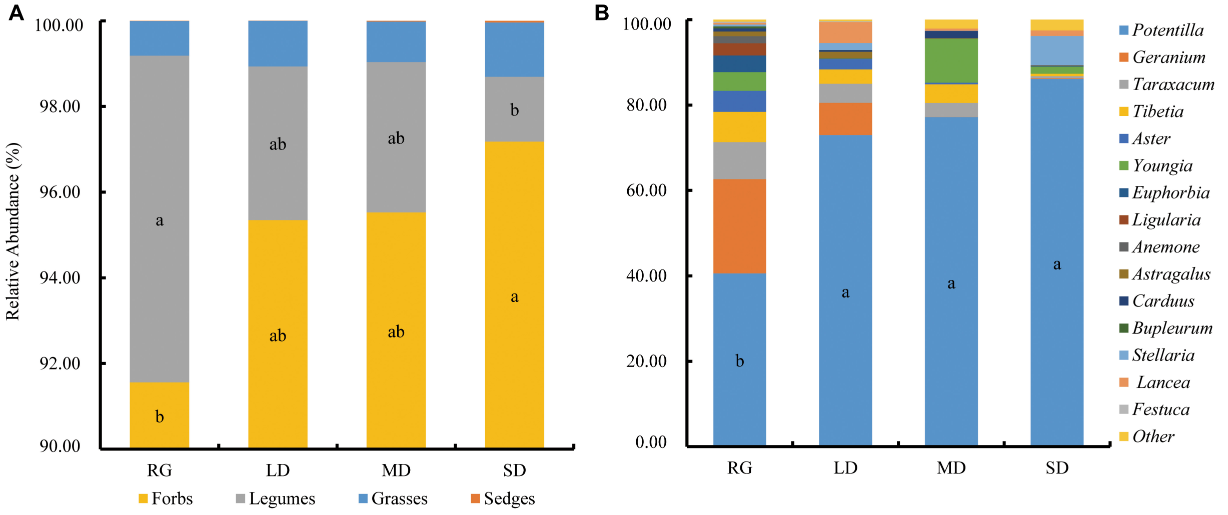

FIGURE 6
Food composition and proportion of plateau zokors with different functional groups **(A)** and plants at the genus level **(B)**. LD, lightly degraded grassland; MD, moderately degraded grassland; SD, severely degraded grassland; RG, reseeded grassland. Different letters indicate significant differences between groups ($P < 0.05$).

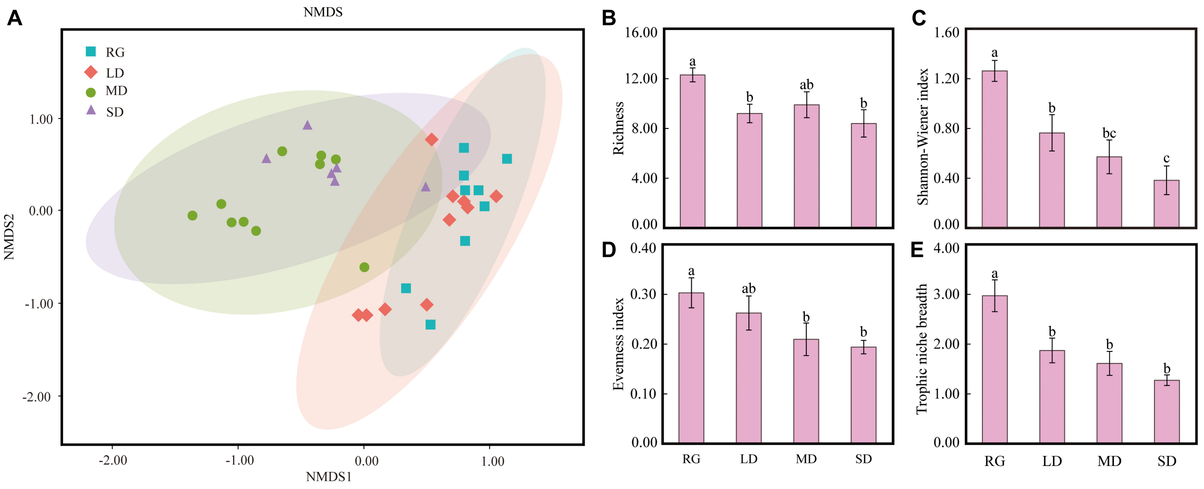

FIGURE 7
NMDS analysis of food structure of plateau zokors **(A)**, food richness **(B)**, Shannon-Wiener index **(C)**, evenness index **(D)**, and food niche width **(E)**. LD, lightly degraded grassland; MD, moderately degraded grassland; SD, severely degraded grassland; RG, reseeded grassland. Different letters indicate significant differences between groups ($P < 0.05$).

were found in the research area. Our results show that the *ITS2* barcode combined with Illumina MiSeq sequencing can effectively identify animal feeding habits. Among the four types of grasslands, forbs were the main food source for plateau zokors. Plateau zokors have a clear preference for *Potentilla* plants, which occupy a considerable proportion of their diet. This result is in agreement with that of Wang et al. (2000a). Plateau zokors dwell in special underground circumstances that require higher energy expenditures for excavation and mound building during foraging (Hu et al., 2017; Lopes et al., 2020). Therefore, forbs with a relatively large biomass were selected as food. Generally, the lower parts of the forbs are enlarged and their nutrient content is more abundant. The distribution of the biomass of forbs determines the population density of plateau zokors in different regions to a certain extent (Zhang et al., 2010, 2022). Plateau zokors at different degradation levels and RG feed on the same species of plants but with different proportions. Animals can adjust their food intake ratios according to changes in food resources and nutritional needs (Egert-Berg et al., 2021; Guo et al., 2021). As the degree of grassland degradation increased, the feed intake of the plateau zokors of forbs increased. The proportion of forbs of plateau zokors under RG was the smallest, which increased the amount of leguminous plant intake to meet their dietary needs.

Effects of feeding cost on plateau zokors

In the present study, plateau zokors preferred to inhabit degraded grasslands, and the population density of plateau zokors increased with the severity of degradation. Habitat selection is the most important step for animals to survive and reproduce successfully, and the adequacy of food resources is a crucial factor in habitat selection (Šumbera et al., 2012). The foraging activities of the plateau zokor were not random but highly selective. Although the diet of plateau zokors contains numerous plant species, *Potentilla* accounted for a large proportion of their dietary choices. Owing to the high energetic cost of burrowing, non-directional underground search patterns, and low food availability, plateau zokors need to consider the benefits and costs of foraging (Xie et al., 2014), but diets of different qualities are distributed in a mosaic model (Hope et al., 2021). The dominant species of RG and LD grassland were mainly grasses and sedges, whereas the forbs favored by plateau zokors were relatively small and embedded within. Among them, forb rhizomes are surrounded by tightly connected rhizomes of grasses and sedges. With the aggravation of grassland degradation, the proportion of grasses and sedges decreased significantly, whereas the proportion of forbs increased. Especially in the SD grassland, the vegetation cover was mainly forbs, and the forbs are the primary dietary selection of plateau zokors, which qualifies the degraded grassland as a suitable habitat for these animals. The nutrient composition of the stomach contents of plateau zokors in different grasslands was analyzed, and it was found that the contents of acidic and neutral detergent fibers in the degraded grassland were lower, and the content of CP was higher, in light and moderately degraded grasslands. Proteins play a key role in growth and metabolism. Too low a protein content in the diet seriously deteriorates the fitness of an individual, leading to weight loss and immune impairment (Kohl et al., 2016). Nutrients in the food of the plateau zokor under the degraded grassland can better ensure and maintain

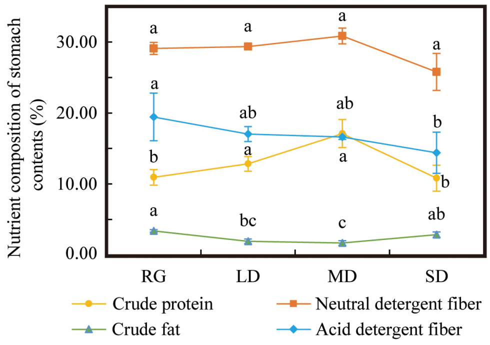

FIGURE 8

Nutrient composition of stomach contents. LD, lightly degraded grassland; MD, moderately degraded grassland; SD, severely degraded grassland; RG, reseeded grassland. Different letters indicate significant differences between groups ($P < 0.05$).

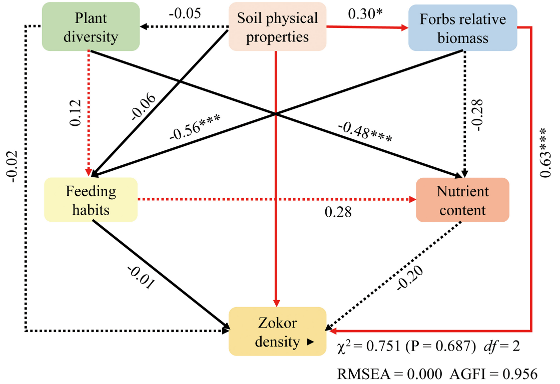

FIGURE 9

Structural equation model between plateau zokor density and soil physical properties, plant resources, and feeding habits. Dotted line indicates that the effect is insignificant and the black line indicates a negative correlation. $^{*}P < 0.05$, $^{***}P < 0.001$.

the normal physiological processes of the animal. Plateau zokors live underground and rely on digging tunnels for daily foraging. Soil hardness not only affects the firmness of rodent tunnels but is also directly linked to the level of energy expenditure during digging, both for foraging and tunnel building. In this study, we found that the compactness of 10–25 cm in SD grassland was less than that in moderately degraded grassland, and the tunnels of the plateau zokor were concentrated at 10–20 cm underground. Therefore, zokors may consume less energy during digging in SD grasslands than in moderately degraded ones. Although the compactness of the RG and LD grassland is low, which is favorable for excavation, the population density of plateau zokor is low. Therefore, we may assume that the relative proportion of high-quality food is the main factor that adjusts the population density of plateau zokors, and that low soil compactness may be an auxiliary factor for the regulation of the plateau zokor population when food is plentiful. After grassland degradation, the relative proportion of forbs preferred by the plateau zokor increased with the low digging costs required for foraging. Therefore, foraging efficiency is improved, and more protein is consumed as the protein content in the stomach is higher. This might help explain, from a dietary point of view, why the population density of the zokors increases after grassland degradation.

A control plan of plateau zokors in light of grassland degradation

In this study, compared with degraded grasslands, the population density of plateau zokors in reseeding grasslands was the lowest. Habitat selection in rodents is imperative for their optimal survival and fitness, and changes in vegetation communities often have an impact on rodent populations in grasslands (Fox and Monamy, 2007).

Additional explanations and mechanisms, such as food resource theory (Ostoja and Schupp, 2009) and movement obstruction theory (Gitzen et al., 2001; Rieder et al., 2010), might help unravel increasing zokor population density with advanced grassland degradation. Plateau zokors live underground, and soil hardness determines the energy expenditure of subterranean rodents when they forage and build burrows. Wang et al. (2000b) found that there is a significant negative correlation between the population density of plateau zokors and soil hardness, and the zokor prefers to live in areas with less soil hardness. In the present study, soil compaction under the RG tended to develop in the direction of the habitat preferred by the plateau zokor. A reasonable explanation is that when the soil compactness is too low, the tunnels constructed by the plateau zokor may collapse readily, and it is difficult to maintain the original state. Therefore, plateau zokors need to re-pair or reconstruct the tunnel, which requires extra time and energy (Zhang, 2007).

Changes in the plant community structure also alter the original food resources (Ostoja and Schupp, 2009). In this study, the vegetation composition of the grassland in the original habitat of the plateau zone changed significantly after the grassland was reseeded. Vegetation coverage and height increased, and the reseeded grass species, *Elymus nutans* and *Poa pratensis*, became the dominant species. In general, the proportion of grasses and sedges increased, whereas the proportion of forbs favored by plateau zokors decreased, resulting in food availability reduction and food shortage for the animals. Therefore, the plateau zokor increased the food intake of legume plants in the RG, its food diversity and evenness increased, and the vegetative niche breadth was significantly widened. Owing to the characteristics of subterranean life, foraging underground adopts different mechanisms compared to aboveground species, and its normal foraging activities need to rely on tunnels. However, the dominant groups of grasses and sedges in the RG have developed underground rhizomes and are closely connected, and the favorite forbs are often embedded in the sedges and grasses, which greatly increases the foraging difficulty and time of plateau zokors. Colloquially, the decrease in population density of plateau zokors in RG is closely linked to the reduction of preferred food availability and increasing foraging difficulty, which qualifies the grassland replantation strategy as an optimum policy to, on the one hand, decrease the population density, and, on the other hand, to combat grassland degradation.

Briefly, grassland degradation aggravates the occurrence of rodent pests, the aggravation of rodent pests in degraded grasslands should be regarded as the result of the comprehensive effect of a series of factors such as food resources, soil characteristics, intraspecies relationship, and aboveground environment. The present study focused on two factors, food resources and soil characteristics. Subterranean mammals live mainly in open habitats, and their evolution is tightly connected with the emergence of seasonal habitats and prolonged droughts (Nevo, 1999). In these habitats, plants storing energetic reserves in bulbs, roots, and rhizomes have evolved, and these parts of plants are staple foods for subterranean mammals (Nevo, 1999; Busch et al., 2000); in addition, plants in arid regions allocate more underground biomass, providing them with more food resources. Nevertheless, soil water content needs to be kept within a reasonable threshold, because the extremely dry or humid habitat cannot form the tunnel for subterranean mammals to inhabit, and the underground biomass will also be greatly reduced, resulting in a shortage of food resources. Therefore, soil properties have always been an essential factor affecting the distribution of subterranean mammals, and the plateau zokor is no exception.

Conclusion

This study showed that plateau zokors prefer to inhabit degraded grasslands, and the population of plateau zokors increased with grassland deterioration. The mechanisms underlying this phenomenon were explored from the perspective of feeding habits. *ITS2* barcoding combined with Illumina MiSeq sequencing was used to analyze the feeding habits of different degrees of degraded and reseeded grasslands. Forbs were found to be the main food source of plateau zokors, and *Potentilla* occupies a considerable proportion of its diet. After grassland degradation, the relative proportion of forbs that plateau zokors preferred increased, the feeding cost reduced, the feeding efficiency improved, and the content of CP in the stomach contents increased. The foraging difficulties for plateau zokors to obtain the plants they preferred significantly increased after the grassland was reseeded. Reduced food availability has led to a decline in the population density of plateau zokors. Affecting rodent eating habits through reseeding is an effective way to prevent and control rodent ecology. In the future, it is necessary to perform monitoring trial to observe the long-term effects of grassland degradation and reseeding on plateau zokor population.

Data availability statement

The data presented in this study are deposited in the NCBI repository, bioProject ID: PRJNA867532, accession numbers: SAMN30207155–SAMN30207194.

Ethics statement

The animal study was reviewed and approved by Institutional guidelines for the use of animals were followed, the Animal Ethics Committee of Gansu Agricultural University approved the experimental procedure (approval no. GAU-LC-2020-014), and local authorities' approval was also obtained.

Author contributions

KA, QP, and ZW were responsible for the majority of data collection. JS guided the primary writing. YK, XS, and BY assisted with revisions of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Maize monoculture causes niacin deficiency in free-living European brown hares and impairs local population development

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Maize (*Zea mays*) is the most produced crop worldwide and the second most important bio-energy plant. Huge maize monoculture is considered a threat to biodiversity in agricultural landscapes and may also contribute to the decline of European brown hares (*Lepus europaeus*, Pallas 1778). Indeed, the intensification of agriculture has been identified as one of the main factors responsible for the decline of brown hare populations. A reason why large maize cultures can be particularly detrimental to animals consuming this plant is its poor nutritional value with respect to niacin. In this study, we investigated the effects of the proportion of area under maize crops on liver concentrations of niacin in free-living hares, on the reproductive output of does (females), and on the development of local populations, at nine study sites in Lower Austria. Hare numbers were estimated from spotlight counts in spring and autumn. Liver samples and uteri were obtained from hares shot in the same areas during regular autumn hunts. Number of offspring born to an individual female during the preceding reproductive period was determined by counting placental scars. Our results show a significant negative effect of the area under maize crops on liver concentrations of niacin of does and on their reproductive output. Further, we found a significant negative effect of the area under maize on the development of a population. Altogether, our findings indicate that high proportions of the area under maize crops contribute to the decline of brown hares by reduced fecundity of does and impaired development of local populations.

KEYWORDS

niacin, placental scars, maize crops, *Lepus europaeus*, monoculture, fecundity, population development

Introduction

Maize (*Zea mays*) is the most produced crop on a global scale (Nuss and Tanumihardjo, 2010), and is, with rapeseed, one of the two main energy crops cultivated in Europe (Klenke et al., 2017). Maize is considered as having the highest yield potential of energy crops grown in central Europe (Amon et al., 2007), and will therefore probably become increasingly important for energy/biogas production (Nielsen and Oleskowicz-Popiel, 2008). However, large-scale cultivation of maize is increasingly threatening biodiversity (Fargione et al., 2009), and affects most farmland animals negatively (Klenke et al., 2017). Low habitat quality, e.g., loss of rotational set-aside or loss of habitat heterogeneity due to maize cultivation, with a landscape structure no longer meeting the ecological requirements of many species, seems to be responsible (Gevers et al., 2011).

Population trends of a typical farmland species, the European brown hare (*Lepus europaeus*, Pallas 1778), show a strong decline in many European countries over the last decades (Mary and Trouvilliez, 1995). As this species also represents an important game species (Pielowski, 1976), long-term trends can be monitored by hunting bag data (Tapper and Parsons, 1984; Langbein et al., 1999). These records show a dramatic population decline throughout Europe [Poland: Pielowski and Raczynski (1976), Denmark: Madsen et al. (1996), Wincentz Jensen (2009), parts of Croatia: Pintur et al. (2006); Popović et al. (2008), Serbia: Ristić et al. (2021), Germany, Austria, Bulgaria, Luxembourg, Netherlands, Slovakia, Switzerland: Mary and Trouvilliez (1995), United Kingdom: Smith et al. (2005)].

A large number of studies tried to identify causes for this decline (reviewed in Smith et al., 2005). Negative effects of predators like a red fox (*Vulpes vulpes*, Linnaeus 1758) on brown hare abundance were reported from Poland, Germany, and the United Kingdom (Reynolds and Tapper, 1995; Panek et al., 2006; Kalchreuter, 2015). However, Weber et al. (2019) showed that populations could grow despite high predator abundance when sufficient habitat structures offering cover to leverets were present. There was no evidence for reduced fertility of brown hare does as a potential cause of the decline (Hackländer et al., 2001; Schai-Braun et al., 2020), but low temperatures and high precipitation during spring increase leveret mortality (Hackländer et al., 2002). Farming practice, particularly the intensification of agriculture, was identified as a significant factor negatively affecting the abundance and population dynamics of European brown hares (Smith et al., 2005), presumably due to poor nutrition caused by low crop diversity and large fields (Tapper, 1987). However, Smith et al. (2005) found no effect of field size *per se* on population densities, but report a negative effect of extensive monocultures on hare abundance. Accordingly, population decline in Bulgaria, for instance, began simultaneously with the increase of monocultures (Petrov, 1976). Increase in maize monoculture was also identified as a potential cause of a population decline in Germany (Sliwinski et al., 2019). A decline in hare densities of up to 90% after a change to maize monoculture was reported by Bertóti (1975).

Altogether, loss of cover and malnutrition are considered important causes of low reproduction, and especially high postnatal mortality of brown hares in Europe (Hansen, 1992; Edwards et al., 2000). The diet of hares is twice as diversified in mixed compared to monocultural farmed landscapes (Frylestam, 1986). There is, however, local variation according to the type of cultivated crops, field size, and alternative field habitats (Reichlin et al., 2006; Petrovan et al., 2012). The brown hare is highly selective in its food consumption and is, to some extent, actively searching for plants rich in fat (Schai-Braun et al., 2015). To meet their nutritional requirements, hares rely strongly on weeds (Reichlin et al., 2006; Schai-Braun et al., 2015), and specific crops such as beets (*Beta vulgaris*), soybean (*Glycine max*), and fodder crops like alfalfa (*Medicago sativa*) or red clover (*Trifolium pratense*). However, most weeds have vanished from farmed landscapes because of the high utilization of herbicides in conventional farming (Wilson et al., 1999; Stoate et al., 2001; Gaba et al., 2016), and fodder crops are nowadays far less cultivated at the expense of energy crops or cereals (Klenke et al., 2017).

An alternative, but not mutually exclusive hypothesis for the negative effects of maize on animals involves its low nutritional value for species consuming this crop. Indeed, although maize is particularly valued for its fatty acid composition (especially adapted for livestock diets), and its high sugar level (for ethanol production), it contains low proportions of many essential micronutrients including calcium, manganese, copper, and iron (Nuss and Tanumihardjo, 2010; INRA et al., 2011). Moreover, maize seeds and leaves are highly deficient in niacin and its precursor tryptophan (Hogan et al., 1955; Henderson et al., 1959; Goss, 1968; Mawson and Jacobs, 1978). Tryptophan (trp) is an essential amino acid for all eukaryotes and even some prokaryotes (de Groot, 1953; Meisinger, 1978; Kantak et al., 1980; Walz et al., 2013). It is the precursor of 5-hydroxytryptophan, an important monoamine neurotransmitter, and of niacin (Kohlmeier, 2003). Niacin can be decomposed into two molecules, nicotinic acid and nicotinamide, essential for the *in vivo* synthesis of nicotinamide adenine dinucleotide (NAD) (Wan et al., 2010). NAD is indispensable for the effective functioning of the Krebs cycle and therefore for cell respiration and ATP synthesis. Animals must obtain trp and most of their niacin from food on a daily basis. Only plants and microorganisms can synthesize trp, and niacin synthesis from trp is very inefficient in many animal species (Baker, 2008). However, in addition to the deficiency of trp in maize, up to 90% of niacin is present as niacytin in mature maize grains, i.e., bound up in a complex with hemicellulose which renders it unavailable to vertebrates (Ammerman et al., 1995; Ball, 2005; Baker, 2008). Deficiencies in trp and its derivatives, especially nicotinamide, lead to dementia, diarrhea, and dermatitis (i.e., skin rashes) in humans and hamsters (Hegyi et al., 2004; Wan et al., 2010; Tissier et al., 2017), the black-tongue syndrome in dogs (Baker, 2008), and aggressiveness and growth retardation in rats (Krehl et al., 1945; Kantak et al., 1980; Walz et al., 2013). Tissier et al. (2017) recently highlighted that niacin deficiency causes high rates of maternal infanticides in European hamsters fed a diet dominated by maize, reducing reproductive success by up to 70%.

Considering the increase in field size and the amount of maize cultivation, as well as the fact that brown hares select the maize plant as a food source in certain periods of the year (Schai-Braun et al., 2015), and the negative effects of maize dominated diets, we expected with increasing proportions of maize crops in the agricultural landscape

1. Negative effects on niacin concentrations in the liver of brown hares where "*de novo* biosynthesis" of nicotinic acid solely takes place (Yang and Sauve, 2016), and reserves of this vitamin are stored (Podlogar and Smollich, 2019).
2. Negative effects on hare densities due to detrimental effects of niacin and trp malnutrition on survival and reproductive output of does.

Materials and methods

We gathered data at nine study sites in Lower Austria during 2017 and at one site also in 2018 (for more detailed information about each study site, see **Supplementary Table 1**). From these sites, we selected 226 does for tissue sampling from a total of 1,054 hares shot during regular autumn hunts. Selected animals were shot during our presence. The selection was made to guarantee high-quality, and fresh samples. Of these 226 carcasses, only 117 had intact uteri. We assumed random shooting and hence considered local hunting bags as representative samples of hare populations. Carcasses were sexed by optical inspection of secondary sexual characteristics by experienced biologists. After the first examinations in the field, carcasses were cooled to 2°C. Liver samples (1 g of tissue) and uteri were stored at −18°C until further analyses.

In the laboratory, we determined in liver samples three forms of niacin, free nicotinic acid, free nicotinamide, and nicotinamide moiety of NAD+/NADP+, by gas chromatography with flame ionization detection (Hämmerle et al., 2020). Total niacin concentrations were expressed as the sum of these three forms in μg per g of liver tissue. Reproductive output of does during the preceding season of reproduction was determined by counting placental scars (Hackländer et al., 2001). *In situ* age classification was verified by the weight of dried eye lenses (Suchentrunk et al., 1991).

Population sizes (individuals per 100 ha) were estimated by spotlight counts (Langbein et al., 1999; Schai-Braun et al., 2013) in spring (late March/early April) and autumn (October) at the nine study sites in Lower Austria during years 2002–2015.

The proportion of arable land cultivated with maize crops was obtained for each study site and year from Statistik Austria (2018).

Statistical analyses

All statistical analyses were performed using R v4.2.1 (R Core Team, 2021). If necessary for obtaining the normal distribution of residuals, we log-transformed variables. We investigated the effects of the area under maize crops, and of liver vitamin B3 concentrations, on the difference between spring and autumn counts of hares with linear mixed effects models [lme, package "nlme"; Pinheiro et al. (2021)]. The study area was included in these models as a random effect to account for repeated measurements at one site. To identify a potential effect of maize monoculture on the reproductive output of does, independent of the effect of niacin concentration in the liver, we calculated a generalized linear mixed effects model with family "poisson" (glmer, package "lme4; Bates et al. (2015). The response variable was in this model the number of uterine scars. The concentration of niacin in the liver and proportion of area under maize crop were entered as fixed effects, study area as random effect. Plots were generated using the "ggplot2" package (Wickham, 2016) with a back transformation of logarithmized values.

Results

The proportion of area under maize crops of total arable land at our study sites had a significant negative effect on the development of the local hare populations (**Figure 1**, $p < 0.005$, t-value = −2.872, $R^2 = 0.063$). For every 10% increase of the area under maize crops, the difference between spring and autumn counts of hares decreased on average by 2.2 hares per 100 ha. To some degree, this negative influence was caused by an increasingly lower fecundity of does. The more maize was cultivated at a study site, the lower the number of uterine scars found in does shot there (**Figure 2**, glmer: $p = 0.002$, z-value = −3.097, $R^2 = 0.083$).

We further found that the proportions of arable land used for growing maize had a significant negative relation with niacin concentrations in liver samples of does living there (**Figure 3**, $p = 0.013$, t-value = −2.497, $R^2 = 0.182$), with a decrease of on average 0.97 μg/g with every 10% increase of the area under maize crops. In line with these results, we also found a negative association between niacin concentrations in livers and the population development of

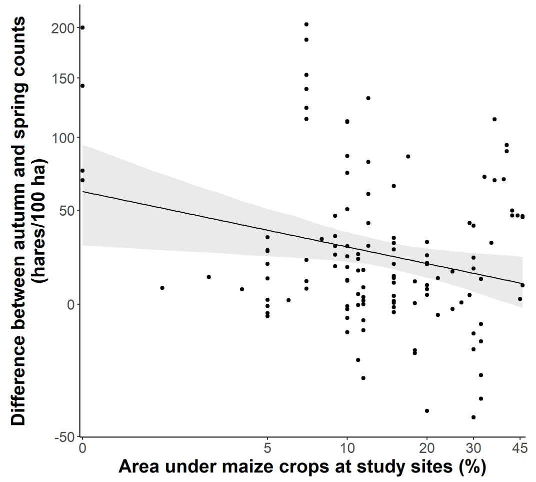

FIGURE 1
Proportion of maize of total arable land and differences between autumn and spring population counts of hares per 100 ha (light shaded: 95% confidence interval). Both variables were transformed with natural logarithm for analysis and back-transformed for the plot.

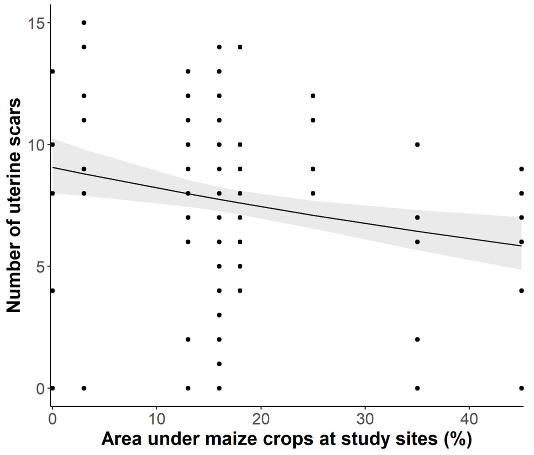

FIGURE 2
Proportion of maize of total arable land and numbers of uterine scars as a measure of fecundity, i.e., total reproductive output of a female during the preceding period of reproduction (light shaded: 95% confidence interval).

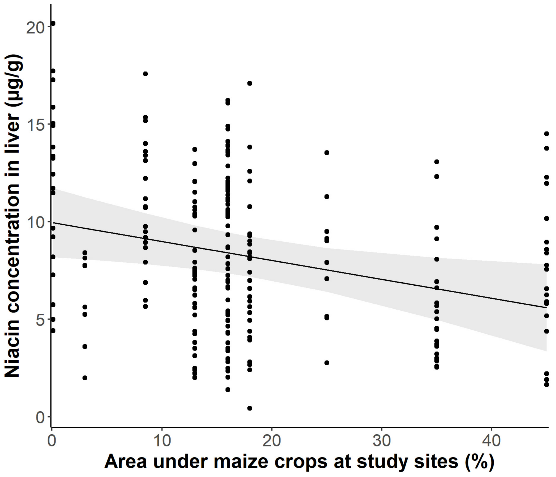

FIGURE 3
Proportion of maize of total arable land and concentrations of niacin in liver samples (light shaded: 95% confidence interval).

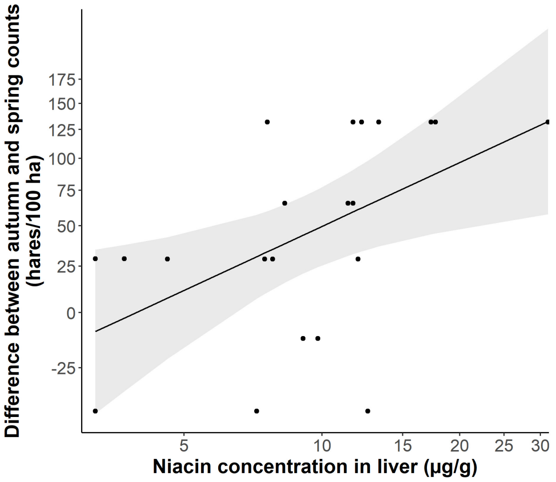

FIGURE 4
Differences between autumn and spring population counts of hares and its relation to the liver concentrations of niacin found at a study site (light shaded: 95% confidence interval). Both variables were transformed with natural logarithms for analysis and back-transformed for the plot.

local hare populations (**Figure 4**, p = 0.018, t-value = 2.627, R^2 = 0.257). However, a direct effect of niacin deficiency on the number of uterine scars, independent of other potential effects of maize abundance, was statistically not detectable (partial regression coefficient −0.008, z-value = −0.934, p = 0.346). Conversely, the negative effect of the proportion of arable land used for maize production on the reproductive output of does remained significant when adjusting for a direct effect of niacin (partial regression coefficient −0.01, z-value = −3.186, p = 0.001), suggesting that other aspects of maize cultivation were also detrimental, on top of an undersupply of niacin.

Discussion

In this study, we discovered several important effects of maize monoculture on local brown hare populations. Our results show that populations rather declined from spring to autumn, or grew less, the higher the proportion of maize crops on total arable land was. A similar negative effect of maize cultures on brown hare abundance has been reported by Mayer and Sunde (2020). Potential causes could be temporal migration of hares and a shift or increase in their home ranges after harvest events, as described by Ullmann et al. (2018) and Schai-Braun and Hackländer (2014). However, a recent study by Ullmann et al. (2020) showed, based on telemetry data, that hares specifically shift their home ranges toward maize fields after the harvest of maize. These areas are selected because of potential cover and forage (Mayer et al., 2018). It therefore seems unlikely that dispersal contributes significantly to the change of hare abundance in a given area from spring to autumn. Instead, our findings suggest that the detrimental effects of maize monoculture on survival and reproduction are responsible.

Effects of maize monoculture on survival of hares

Brown hares select plants rich in fat and protein and strongly rely on weeds (Reichlin et al., 2006). Similar feeding behavior was also observed in the Mountain hare (*Lepus timidus*, Linnaeus 1758) (Dingerkus and Montgomery, 2001), in the Arctic hare (*Lepus arcticus*, Ross 1819) (Klein and Bay, 1994), in the Italian hare (*Lepus corsicanus,* de Winton 1898) (Rizzardini et al., 2019), and the Granada hare (*Lepus granatensis,* Rosenhauer 1856) (Paupério and Alves, 2008). Therefore, brown hares likely suffer malnutrition when living in areas with monocultures poor in plant biodiversity (Schai-Braun et al., 2015). It is well known that malnutrition negatively impacts body condition and hence the survival of brown hares (Hansen, 1992; Edwards et al., 2000). Further, malnutrition impairs reproductive success, because lactating does experience high energetic costs. Particularly for nursing litters in spring, does rely to a certain degree on energy reserves stored in body fat depots (Parkes, 1989; Valencak et al., 2009). Female hares nurse their offspring only once a day (Broekhuizen and Maaskamp, 1980). Further, leverets lack shelters like burrows and are therefore exposed to unfavorable weather conditions, particularly in spring (Andersen, 1952; Broekhuizen and Maaskamp, 1980; Hackländer et al., 2002; Karp and Gehr, 2020). It is therefore pivotal for leverets to obtain milk with a high energy content. To meet this requirement, does produce milk with a high-fat content, which in spring is even fatter than average (30% vs. 20%, Valencak et al., 2009). Sufficient plant biodiversity is absent in areas of maize monoculture even after harvest in autumn and winter (Fuchs et al., 2021), presumably resulting in malnutrition of does during the period when they accumulate body fat for subsequent reproduction. This reduces the survival chances of leverets, particularly in spring litters, which are most important for population development (Olesen and Asferg, 2006).

Effects of maize monoculture on fecundity

We also found a decrease in reproductive output of does with the proportion of maize in arable land. Our measure of reproductive output is the number of scars found in the uteri of does shot during the autumn hunting season. These numbers reflect the total number of juveniles born by a doe during the preceding season of reproduction (Hackländer et al., 2001), and are therefore a measure of female fecundity. Numbers of uterine scars could be affected either by direct negative effects of the area of maize monoculture on brown hare nutrition, and therefore body condition, or by general detrimental effects of monoculture. However, a more likely explanation for the effects on uterine scars is changes in the age structure of a population, because we did not find a direct effect of niacin stored in the liver on the number of uterine scars. Schai-Braun et al. (2021) showed that heavier does have larger litters. The same study reported that primiparous does, which are lighter, had smaller litters than an adult does. Unfortunately, we have no reliable information about the age structure of females at our study sites to confirm that a higher number of juvenile does in the bag was responsible for lower numbers of uterine scars. Alternatively, the direct effects of niacin stores on the reproductive output of does could have remained undetected due to the small sample size. From 223 uteri gathered, only 117 were suitable for analysis, and few were obtained from areas with no maize monoculture or with high proportions of maize fields ($n = 5$ and $n = 10$ respectively).

Maize monoculture and niacin deficiency

For the first time, we identified here that maize monoculture is detrimental to brown hare populations because it leads to niacin deficiency. The concentration of this vitamin in liver tissue of does decline with the area under maize crops. The concentration of niacin is very low in maize. Further, niacin in maize is mainly present in its bound form, rendering it inaccessible to vertebrates (Ammerman et al., 1995; Ball, 2005; Baker, 2008). In addition to the low content of niacin, maize is also poor in its precursor tryptophan (Carpenter, 1983). Consumption of maize therefore also impairs the potential, for an inefficient pathway of biosynthesis of niacin from tryptophan. Fragments of maize plants were found in the stomach contents of brown hares even at low proportions of areas under maize crops, showing that brown hares readily consume maize plants as well as corn (Steineck, 1978; Reichlin et al., 2006).

Diets with maize as a major component can lead to severe health problems. In humans, for instance, such a diet causes the pathology of "pellagra"

(WHO [World Health Organization], 2000). In European hamsters (*Cricetus*, Linnaeus 1758), less than 12% of pups born to mothers fed a niacin deficient diet survived a 30-day period (Tissier et al., 2017). As a reason for infanticide and insufficient maternal care, Tissier et al. (2017) suggested low levels of oxytocin concentrations in the blood of the mothers, caused by niacin deficiency, as this peptide, which acts as a neurotransmitter, plays an important role in maternal and sexual behavior. Stronger effects of maize diets on niacin deficiency on the reproductive output of hamsters could be a result of less pronounced "caecotrophy" compared to hares (National Research Council [NRC], 1995; Weinhold and Kayser, 2006; Miśta et al., 2015). If niacin deficiency has similar effects in hares, does could show abnormal maternal behavior and give up nursing. As a result, offspring survival would be jeopardized because leverets strongly rely on milk supply during the first three weeks of life (Hackländer et al., 2002). Keeping in mind that hare offspring require daily nursing with milk rich in fat, as described above, additional negative effects of niacin deficiency on the fat content of milk, as reported by Havlin et al. (2017), could have further impaired offspring survival.

Conclusion

Our study clearly identified the negative impacts of maize monoculture on population dynamics and reproductive output of European brown hares. Most importantly, we discovered that niacin deficiency, resulting from the consumption of maize, is a likely, so far unknown mediator. However, whether a maize-dominated diet results in hares in similar severe health issues and impairment of reproduction like in other mammals remains an open question. In contrast to humans and mice, brown hares regularly consume soft feces with caecum content, a nutritional pathway making nutrients and vitamins produced by caecal microorganisms available to the host. Therefore, brown hares may be less affected than other species by consuming a maize-dominated diet, a hypothesis that needs to be investigated experimentally.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the animal study because samples analysed in this study were taken from legally shot animals during seasonal hunts according to the hunting laws in Austria.

Author contributions

WA and MT conceived the study. AS performed field work. WA and AS analyzed the data. AS, WA, and MT wrote the manuscript. All authors approved the final version and are accountable for all aspects of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.1017691/full#supplementary-material

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Corrigendum: Maize monoculture causes niacin deficiency in free-living European brown hares and impairs local population development

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WA and MT conceived the study. AS performed field work. WA and AS analyzed the data. AS, WA, and MT wrote the manuscript. All authors approved the final version and are accountable for all aspects of the work.

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Comparison of breeding strategies of two sympatric thrush species in an alpine environment

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Closely related birds that occur sympatrically will inevitably differ in the acquisition of food resources, nest site selection, and breeding times. The successful coexistence of birds that breed in the same area can be determined by investigating how they divide limited resources and reduce interspecific competition. Our study area is located at the eastern foot of the Qinghai-Tibet Plateau, with a high altitude and low annual average temperature. In this study, the life history characteristics and reproductive ecological parameters of two closely related species, the Chestnut Thrush (*Turdus rubrocanus*) and the Kessler's Thrush (*Turdus kessleri*), were compared in detail in April–July of 2020, 2021, and 2022 in the scrub along the Liqiu River in Xinduqiao Town, Tibetan Autonomous Prefecture of Garzê, Sichuan. The results showed no differences in nesting material, nest cup size, clutch size, feeding strategy of nestlings, brood food type, and reproductive effectiveness between the Chestnut Thrush and the Kessler's Thrush. However, the Kessler's Thrush had a higher nesting height, thicker nest wall, earlier breeding time, smaller eggs, fewer brooding frequent and a longer brooding time compared to the Chestnut Thrush. In addition, they invested more time in parental care, resulting in a faster growth and development rate. The results indicate that birds with different ranges and breeding in the same range show convergent and divergent life history characteristics in terms of reproductive biology. Chestnut Thrush with low reproductive altitude shows the characteristics of low-altitude bird reproduction in part of reproductive biology, while Kessler's Thrush with high reproductive altitude shows the characteristics of high-altitude bird reproduction in part of reproductive biology.

KEYWORDS

sympatric breeding, alpine environment, nest site selection, brooding behavior, nestling growth

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Introduction

The characteristics of avian life history are underpinned by ecological and behavioral patterns that formed during a long-term natural selection process and are adapted to their living environment (Martin, 2004). The evolution of avian life history is currently one of the hot topics in ornithological research. Among these, breeding is the most important part of the life history of birds, and research on avian breeding mainly focuses on the response of avian breeding characteristics to the environment (including biotic and abiotic factors; Partridge and Harvey, 1988; Southwood, 1988). Avian breeding traits have both a genetic aspect and an evolutionary aspect under the influence of the environment. In high-altitude alpine and subalpine mountains, avian breeding is subject to a series of environmental stressors, such as short breeding seasons, unpredictable climatic conditions, and limited food resources (Hille and Cooper, 2015). Under the influence of natural selection, these species of birds show large differences in many aspects, for example, breeding season, hatching period, clutch size, nestling stage, nestling growth pattern, reproductive success rate, mating system, and parental care strategy (Walters, 2003). We know very little about how the life history characteristics of birds change on the altitude gradient, especially the lack of in-depth and systematic research on how bird reproduction adapts to the high altitude environment.

According to the study of similar species at different breeding altitudes, birds at high altitudes have different life history characteristics. Compared with those at low altitudes, birds at high altitudes have fewer clutch sizes, fewer annual breeding nests, larger male investment, and longer nesting periods, hatching periods, nestling periods, and care periods for young birds leaving the nest (Badyaev, 1997; Badyaev and Ghalambor, 2001). High-altitude breeding Black Redstart (*Phoenicurus ochruros*) lay small clutches of large eggs, and increased parental input to the chicks did not accelerate chick development but rather slowed it down compared to chicks of lower altitude relatives (Mu et al., 2008). White-bellied Redstarts (*Hodgsonius phaenicuroides*) inhabiting high-elevation environments have a shorter breeding period, smaller clutch size, and larger egg size than their low-elevation counterparts (Lu et al., 2010). Compared to its low-altitude congeners, the Fire-tailed Sunbird elevated parental investments in its offspring by producing fewer eggs and providing more parental care to nestlings (Liang et al., 2020).

Most mountain birds breed at a limited altitude (Johnson et al., 2006). Compared with low-altitude areas, the climate in high-altitude areas is more unpredictable, with lower temperatures, stronger wind, greater frequency and depth of snow, and shorter seasons suitable for bird breeding (Johnston, 1954; Stewart et al., 1977). Under the condition of limited space and resources, birds cannot avoid the competition for breeding resources in order to survive. Previous studies have found that due to competition, two ecologically similar species cannot occupy the same ecological niche but replace each other to a certain extent with regard to food or other lifestyle characteristics (Sun, 2001). The habitats of sympatrically breeding birds can overlap greatly (Minot, 1981). The utilization of similar food sources or overlapping breeding domains will lead to competition, which can directly lead to a decrease in breeding performance (Minot, 1981; Minot and Perrins, 1986). Therefore, in order to maximize their fitness, in the long-term natural evolution process, the species formed a series of breeding and survival strategies (Atramentowicz, 1992; Sinervo et al., 1992).

The Chestnut Thrush (*Turdus rubrocanus*) and the Kessler's Thrush (*Turdus kessleri*) belong to the genus *Turdus* in the Passerine family and are taxonomically closely related. During the breeding period, the Chestnut Thrush primarily inhabits forests (mountain and coniferous broad-leaved, coniferous, bamboo and mixed) at altitudes between 2,000 and 3,500 m, in particular the dense coniferous and coniferous broad-leaved mixed forest. Kessler' Thrush, on the other hand, breeds in the bushes in rocky areas above the forest line at an altitude of 3,600–4,500 m (Mackinnon et al., 2000). Both the Chestnut Thrush and the Kessler's Thrush occur in Xinduqiao Town, Tibetan Autonomous Prefecture of Garzê, Sichuan Province, and breed sympatrically there. The region is characterized by high altitude, a harsh climate, strong solar radiation, large differences in daily temperature, and a short season that is suitable for bird reproduction.

Because the altitude of the breeding distribution of Chestnut Thrush and Kessler's Thrush is different, in this study area, it is the overlap area of the two species of blackbird breeding distribution altitude. It provides good natural study conditions to study what reproductive strategies and life history characteristics are adopted by two similar species breeding in the same domain in the alpine environment. Although Chestnut Thrush is a common species, few people have published about its reproductive ecology before, and there are some reports about it in Lianhua Mountain, Gansu Province. Nest site characteristics and nest success (Zhao and Sun, 2018), egg rejection and recognition mechanisms (Yi et al., 2020b), nestling discrimination and feeding habits during brooding (Yi et al., 2020a), behavioral plasticity in relation to head volume (Zhao and Sun, 2016), and Parental attendance reduce nest predation during the incubation period (Hu et al., 2017), and brief field observations describing the breeding habits of the Chestnut Thrush in Wanglang Nature Reserve, Sichuan (Zhang et al., 1986), while more detailed studies on the breeding biology of the Chestnut Thrush are less available. There is little research on the reproductive habits of Kessler's Thrush, only Yang et al. (2012) and Gao et al. (2022) have reported on the reproductive habits of Kessler's Thrush, lacking detailed reproductive biology information (Yang et al., 2012; Gao et al., 2022). In addition, there is no report on the comparison of reproductive parameters and behaviors of two species of thrush distributed in the same domain.

We aimed to study the breeding biology (nest, egg, brooding behavior, nestling development, and breeding success) of the Chestnut Thrush and the Kessler's Thrush. By comparing the breeding biology of the two species of thrush, we will gain a better understanding of the convergence and divergence of the breeding characteristics of the thrushes inhabiting the same area in an alpine environment. It can provide a reference for future studies regarding the relationship between the breeding behavior and environment of birds, and the coexistence mechanisms of similar species, and further enrich the breeding biology data of the Chestnut Thrush and Kessler's Thrush.

Materials and methods

Study area

The research site is located in the scrub along the Liqiu River in Xinduqiao town, Kangding city, Tibetan Autonomous Prefecture of Garzê, Sichuan Province (101°20′-101°50′E, 29°50′-30°10′N; Figure 1). The plant community is composed of *Salix cupularis, Hippophae rhamnoides, Berberis sibirica, Cerasus serrula, Malus hupehensis, Lonicera rupicola,* and *Caragana sinica*. The area has a mountainous cold temperate climate with an average altitude of 3,460 m, strong ultraviolet radiation all year round, a large daily temperature difference, low night temperatures, an annual average temperature of 5.2°C, and an annual average rainfall of 950 mm. The main natural disasters are hail, floods, low temperatures, lightning, etc. Hail and floods are common in June and July every year.

Methodology

From April to July during 2020, 2021, and 2022, we conducted a systematic search for Chestnut Thrush and Kessler's Thrush nests in the study area. After finding the nest, we used the mobile phone software Two Step Road Outdoor Assistant to record and number the nest site. If there were no eggs in the nest, it was observed every 3 days, and the nests with eggs were observed every other day to determine their breeding status. Clutch sizes, egg weight, egg size, hatching period, brood period, number of nestlings, nestling weight and tarsus length were recorded and measured for each nest. Egg size and nestling tarsus length were measured with a digital vernier caliper (0.01 mm), and egg weight and nestling weight were measured with a portable electronic scale (0.01 g). The egg volume (cm^3) was calculated as $V = K_v \times L \times B^2/1{,}000$, where K_v is a constant equal to 0.51, L is the maximum egg length (mm) and B is the maximum egg width

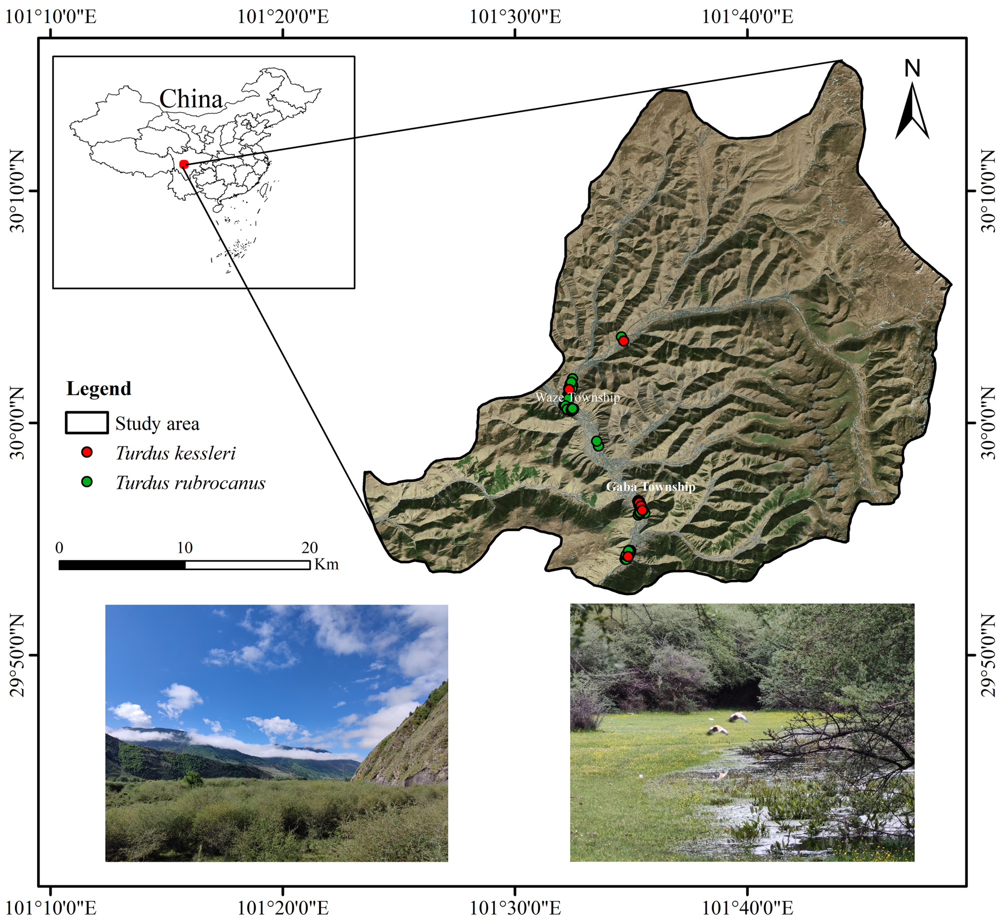

FIGURE 1
Study area and nest sites.

(mm) (Hoyt, 1979). To reduce disturbance to breeding activities, the nest site was measured after the nestling had left the nest. The measured parameters included the species of the tree where the nest was located, the height of the tree, the distance of the nest site from the ground, and the canopy density above the nest site.

During the nestling stage, the brooding behavior and nestlings were recorded. A miniature camera (DS-200S, Wireless Low-Power Battery Camera, Guangzhou, P.R. China) was used to monitor the breeding behavior of the Chestnut Thrush and the Kessler's Thrush, and 3 nests of each species were selected for monitoring. Cameras are installed daily from 9 am to 11 am and retrieved from 5 pm to 7 pm. By analyzing the videos at a later stage, we summarized the daily brooding (i.e., behavior that keeps nestlings warm) frequency and time, feeding frequency per hour from the beginning to the end of the monitoring period, daily feeding frequency, and the frequency of cleaning feces. Daily measurement of nestling growth parameters (body weight and tarsus length) was done at the same time each day. Breeding success was evaluated by hatching rate, fledgling rate, breeding success rate, and nest success rate. The hatching rate is the percentage of the number of hatched eggs in the total number of successful nests, the breeding success rate is the proportion of the number of successful nests in the total number of nests, and breeding success means that at least one nestling survived until it left the nest. The fledgling rate is the ratio of a fledgling number to hatched nestling, and the success rate of nesting is the percentage of nests that hatched at least one nestling to the observed nest.

Data treatment and statistical analyses

Fitting nestling growth curve by Logistic regression (Ricklefs, 1967, 1968),

$$W = \frac{a}{\left(1 + e^{-k(t-b)}\right)}$$

where W is the measure of body weight, a is the asymptotic value, k is the instantaneous growth rate, and b is the inflection point time value (d). t 10–90 is the time it takes for the nestling to reach 10–90% of the asymptotic value. Data analyses were performed using IBM SPSS Statistics for Windows version 24.0 (IBM Corp., Armonk, NY, United States). Kolmogorov–Smirnov was used to test the normal distribution of the data. The Mann–Whitney U-test is used to compare the continuous variables of non-normal distribution, and the Independent Samples t-test is used to compare the continuous variables of normal distribution. The Chi-square test is used to compare probability (such as hatching rate, fledgling rate, etc.). All of the tests were two-tailed and data were presented as mean ± SD, and $p < 0.05$ was considered statistically significant.

Results

Nests and nest site selection

We found a total of 100 Chestnut Thrush nests (56 nests in 2020, 33 nests in 2021, 11 nests in 2022) and a total of 19 Kessler's Thrush nests (9 nests in 2020, 7 nests in 2021, 3 nests in 2022). The main nests were on shrubs such as *Salix cupularis, Malus hupehensis, Hippophae rhamnoides, and Cerasus serrula*, and the nesting time varied from 5 to 7 days. There were no differences in nest type and nesting material between the two species of thrushes. The nests were bowl-shaped with the opening pointing upward (Figure 2). It was mainly composed of bryophytes, mud, twigs, fine grass stems, fine root whiskers, and household garbage (soft plastic film, nylon, etc.).

Overall, the nests of the two species of thrushes were very similar. There was no difference in the size of the cup of the nest. There were also no differences in nest diameter (Chestnut Thrush: 9.645 ± 0.816 cm, Kessler's Thrush: 9.778 ± 0.647 cm, $Z = -0.553$,

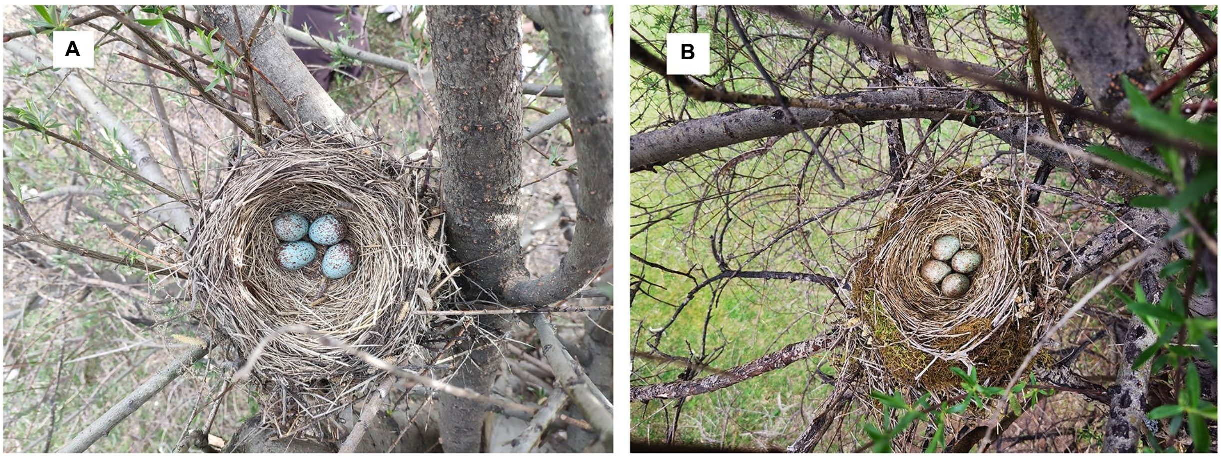

FIGURE 2
(A) Chestnut Thrush eggs and nests. **(B)** Kessler's Thrush eggs and nests.

$p = 0.580$, Mann–Whitney U test) and cup depth (Chestnut Thrush: 6.256 ± 0.765 cm, Kessler's Thrush: 6.914 ± 1.417 cm, $Z = -1.680$, $p = 0.093$, Mann–Whitney U-test) between the Chestnut Thrush and Kessler's Thrush. However, the outer diameter of the Kessler's Thrush nest (15.821 ± 1.55 cm) was larger than that of the Chestnut Thrush nest (15.003 ± 1.161 cm), and the difference was significant (independent t-test: $t = -2.221$, $p < 0.05$). The nested wall of Kessler's Thrush was thicker than that of Chestnut Thrush.

The nest site selection of the two thrushes showed both similarities and differences. There was no difference in the tree height for nesting between the two species of thrush (Chestnut Thrush: 4.095 ± 1.367 m, Kessler's Thrush: 5.083 ± 2.17 m, $Z = -1.789$, $p = 0.074$, Mann–Whitney U-test). There was, however, a significant difference in height of the nest in the tree between the two types of thrush ($Z = -2.264$, $p < 0.05$, Mann–Whitney U-test), the nests of Kessler's Thrushes (2.72 ± 1.37 m ($n = 12$)) were higher than that of Chestnut Thrushes (2.14 ± 0.80 ($n = 59$)). The results showed that there was a differentiation in nesting between the two species of thrushes in vertical distribution, and the nesting of Kessler's Thrush was higher.

Breeding time and eggs

The earliest egg of Chestnut Thrush was laid on May 3 and the latest on July 2, while the earliest egg of Kessler's Thrush was laid on April 15 and the latest on June 13. The laying period of both species was nearly 2 months, the earliest laying date of Kessler's Thrush was 18 days earlier than that of Chestnut Thrush, and the latest laying date of Kessler's Thrush was 19 days later than that of Chestnut Thrush. The two species of thrushes usually lay one egg a day, occasionally one egg every other day, and lay eggs in 3–5 days.

The average clutch size of Chestnut Thrush was 3.25 ± 0.63 (range: 2–4, $n = 60$), the eggs were oval, the average egg weight was 7.91 ± 0.79 g, the long diameter of the eggs was 31.83 ± 2.25 mm, and the short diameter of the eggs was 22.15 ± 1.13 mm, the eggshell was light blue, with irregular brown and purple patches and spots, denser at the blunt end of the egg (Figure 2A). The average clutch size of Kessler's Thrush was 3.29 ± 0.62 (range 2–4, $n = 14$), the eggs were obtuse oval, the average egg weight was 6.90 ± 1.36 g, the long diameter of the eggs was 29.60 ± 2.23 mm, and the short diameter of the eggs was 21.70 ± 1.22 mm, the eggshell was light green, with rose-tan, light gray-brown, coffee-colored fine spots and stains, and the blunt end was dense (Figure 2B).

There was no significant difference in clutch size between Chestnut Thrush and Kessler's Thrush ($Z = -0.163$, $p = 0.870$, Mann–Whitney U-test). However, the egg size of the two thrushes was significantly different. The mean egg weight of the Chestnut Thrush was significantly higher than that of Kessler's Thrush ($Z = -3.888$, $p < 0.01$, Mann–Whitney U-test). The egg length diameter of the Chestnut Thrush was significantly larger than that of Kessler's Thrush ($Z = -5.616$, $p < 0.01$, Mann–Whitney U-test). The egg short diameter of Chestnut Thrush was significantly larger than that of Kessler's Thrush ($Z = -2.082$, $p < 0.05$, Mann–Whitney U-test). The egg volume of Chestnut Thrush (8.01 ± 1.06 cm^3) was significantly larger than that of Kessler's Thrush (7.18 ± 1.25 cm^3) ($Z = -3.789$, $p < 0.01$, Mann–Whitney U-test). Three eggs are the most common clutch size for both species of thrush, accounting for 52.33 and 57.14%, respectively. The hatching of both the Chestnut Thrush and Kessler's Thrush was done by the females, while the males were on guard. Both species of thrushes are asynchronous hatching birds. The hatchlings do not hatch on the same day but usually happen over 2–3 days, with most hatching within 2 days.

Parental care

The brooding period of both species of thrushes is 15–17 days. A total of 12,216 min of effective video were recorded during the brooding stage of the Chestnut Thrush and 15,221 min of effective video were recorded during the breeding stage of the Kessler's Thrush. The brood was undertaken by the male and the female in both thrush species, and once the eggs hatched, the eggshells were immediately removed by the female bird. Nestlings will immediately accept insects. The brooding behavior included feeding and nestling brooding and removing the fecal sac. Both species of thrushes are fed in three ways: female-to-nestling, male-to-nestling, and male-to-female-to nestling (this pathway existed only in the early stage of the nestling feeding period).

The types and quantities of food fed by parents of two types of thrushes to their nestlings during their brooding period were statistically analyzed. Both species of thrushes fed their nestlings with animal food, which mainly included worms, earthworms, arthropods, and some insects that could not be recognized because of their small size or blurred video. According to the video recordings, Chestnut Thrushes fed 2,500 insects, of which 39.64% were worms, 40.96% were earthworms, 5.8% were arthropods, and 13.6% were unidentified worms. A total of 3,040 insects were fed to Kessler's Thrush chicks, among which worms accounted for 56.95%, earthworms accounted for 15.49%, arthropods accounted for 17.37%, and unidentified worms accounted for 10.20%.

Statistical analysis of the feeding frequency of the two thrushes during the brooding period showed that the feeding frequency of Kessler's Thrushes was significantly higher than that of the Chestnut Thrush (Kessler's Thrush was 12.511 ± 4.148 times/h, Chestnut Thrush was 6.8093 ± 1.26 times/h, independent t-test: $t = -4.555$, $p < 0.01$). The feeding frequency of Chestnut Thrush increased first and remained stable after the chicks were 5 days old. The feeding frequency of Kessler's Thrush increased at first, then increased slowly after the chicks were 5 days old, and decreased after they were 8 days old, showing a unimodal pattern (Figure 3A). The study on the brooding time and frequency of the two thrush species during the monitoring period showed that the brooding time of both decreased as the nestlings aged (Figure 3B).

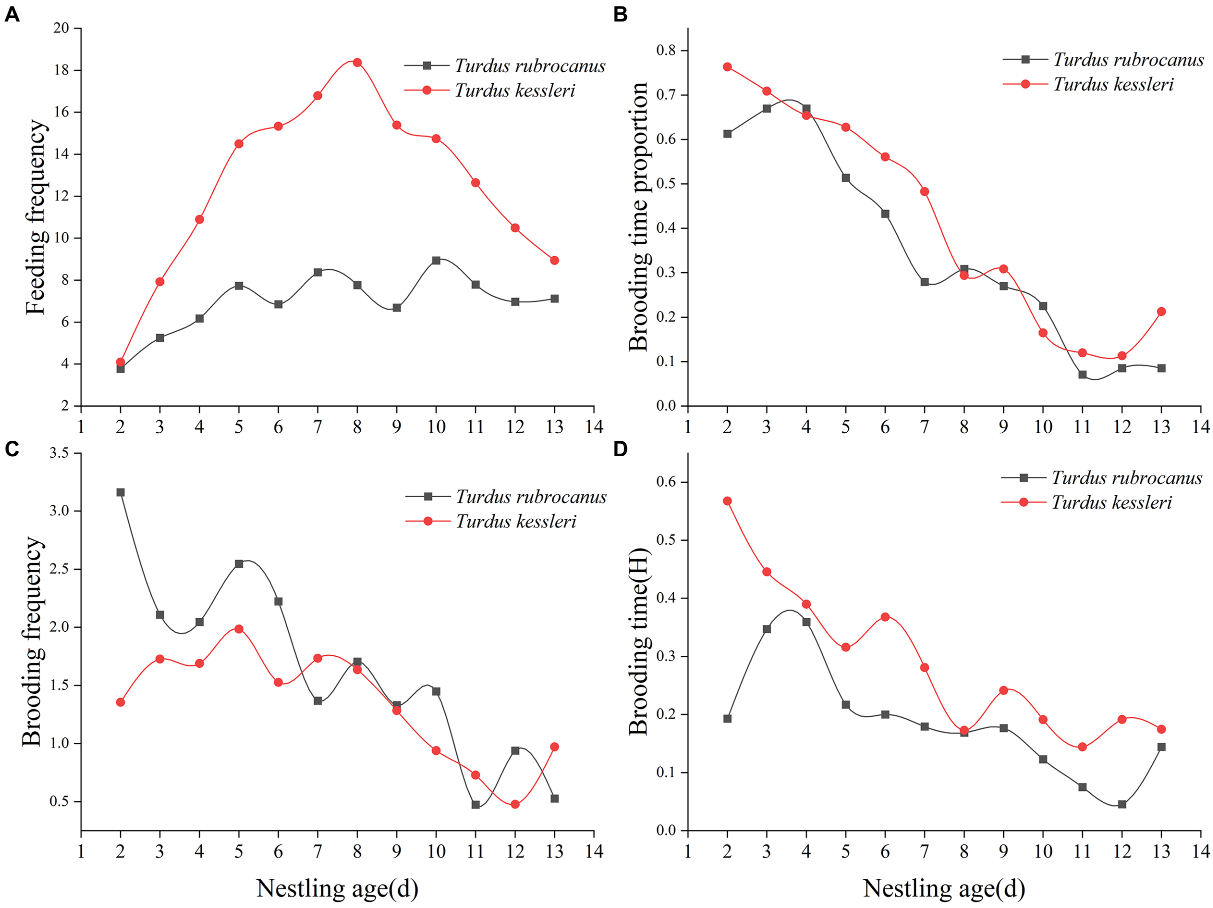

FIGURE 3

(A) Average feeding times per hour of two kinds of thrush in daily monitoring time. **(B)** The proportion of nesting time of female birds of the two thrush species were monitored daily. **(C)** The average number of times that two thrushes warmed their nestling per hour during daily monitoring. **(D)** The average warming time per hour of two thrush species was monitored daily.

There is no significant difference between the two female thrush birds in their daily brooding time (Chestnut Thrush: 0.3519 ± 0.224; Kessler's Thrush: 0.4158 ± 0.242; Independent *t*-test: $t = -0.670$, $p = 0.510$). However, the two thrush species adopted different ways of brooding their chicks. Chestnut Thrush females brooded nestlings more frequently but for a shorter time, while Kessler's Thrush brooded the nestlings less frequently but for a longer time period (Figures 3C,D).

Growth and development of nestlings

Nestlings from both Chestnut Thrushes and Kessler's Thrushes are naked with red skin when they emerge from the eggs, light brown fetal feathers occur on both sides of the top of the head, middorsal line (upper back to tail base), and on both wings, and eyes are not open. At days 7 to 8, the eyes open in an oval shape, most of the body has feathers, the tail feathers and primary flight feathers are out of the feather sheath, the baby bird can crawl, and there is avoidance behavior. At days 12–13, the chicks are fully feathered, when frightened, they immediately leave the nest, however, their flying ability is poor. Once on the ground, they can fly jump, and take swift action. After diving into the dense forest, they do not return to the nest.

We fitted the measurements of body weight and tarsus length of 11 nestlings from 3 nests of Chestnut Thrushes and 9 nestlings from 3 nests of Kessler's Thrushes using Logistic curve equations. The growth curves of body mass and tarsus length were both well-fitted by logistic regression (Figure 4), and the observed value is significantly correlated with the fitting value.

Nestlings grew rapidly, and Chestnut Thrush nestlings reached 90% of their asymptote weight at 9.96 days of age. The average weight of the hatchling is 8.22 ± 0.90 g, and the average weight on the 10th day is 74.74 ± 2.08 g, with an average increase of 7.39 ± 0.31 g/day. At the age of 8.29 days, the weight of Kessler's Thrush nestlings reached 90% of the body weight of the asymptote. The average weight of the hatchling was 6.66 ± 0.59 g, and the average weight of the nestling on the 9th day was 67.00 ± 6.22 g, with an average daily increase of 8.24 ± 0.63 g. The hatchlings of Chestnut Thrushes were heavier than those of

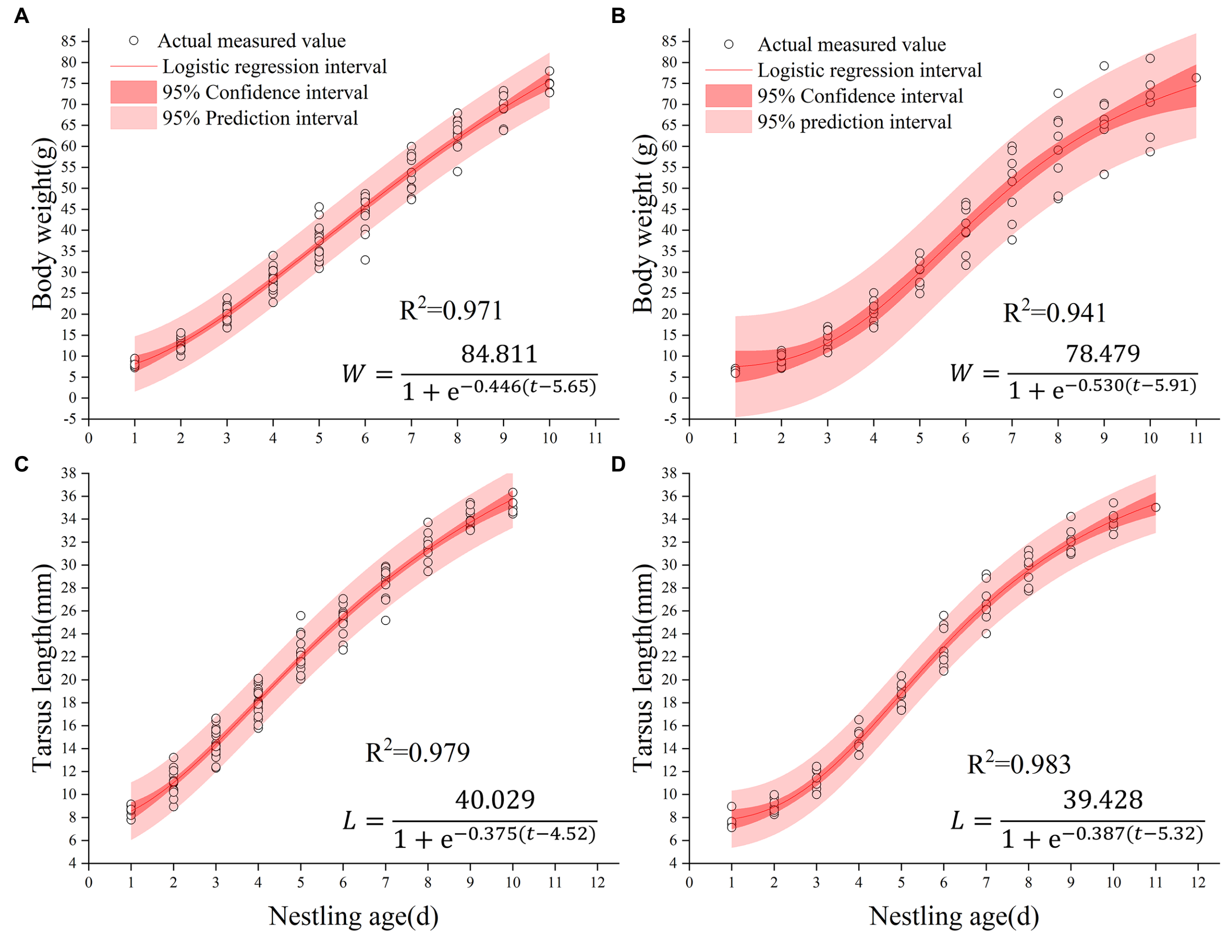

FIGURE 4
(A) Body weight fitting curve of Chestnut Thrush. **(B)** Body weight curve of Kessler's Thrush. **(C)** Tarsus fitting curve of Chestnut Thrush. **(D)** Tarsus fitting curve of Kessler's Thrush.

Kessler's Thrushes, and the growth rate and relative growth rate of Kessler's Thrush nestlings were higher than those of Chestnut Thrushes (Figure 5A). It took fewer days to complete the development of 10–90% of the body weight than that of Chestnut Thrush. The relative growth rate of the tarsus of Kessler's Thrushes was higher than that of Chestnut Thrushes (Figure 5B). Kessler's Thrush nestlings adopted a rapid development growth strategy.

Breeding success

Of the 63 Chestnut Thrush nests with known breeding conditions, 36 nests successfully hatched birds (3 nests failed, 3 nests were predated, and 1 nest failed with an unknown cause). Of the remaining 27 nests, 5 nests were destroyed by humans, 15 nests were predated (28.57%), and 7 nests were abandoned (11.11%). The breeding success rate was 46%, the hatching rate was 95%, the fledging rate was 78% and the nesting success rate was 46%.

Of the 14 Kessler's Thrush nests, 9 nests hatched successfully (2 nests were preyed on), 3 of the remaining 5 nests were preyed on (35.71%), and 2 nests were abandoned (14.29%). The breeding success rate was 50%, the hatching rate was 96.77%, the fledging rate was 80%, and the nesting success rate was 50%. There is no significant difference in the breeding success rate, hatching rate, fledging rate, and nesting success rate between the two thrushes (Pearson Chi-square test $p > 0.05$).

Discussion

Nest and nest site selection

Nest site selection is a critical component of habitat selection, and its effectiveness directly affects breeding performance (Macdonald et al., 2016; Maisey et al., 2016). Nest site selection is influenced by different combinations of biotic and abiotic factors in the environment. Birds that coexist in the same environment will inevitably have different needs for food resources, nesting

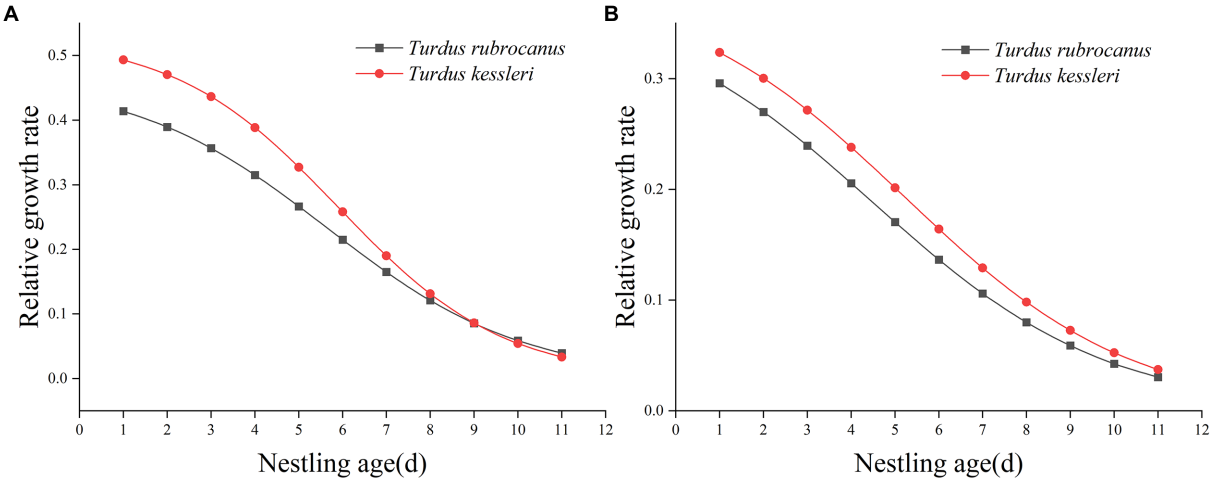

FIGURE 5
(A) The relative growth rate of body weight of two kinds of thrush. **(B)** The relative growth rate of tarsus of two kinds of thrush.

sites, and breeding phenology. The results of our study showed that the nest sites of the Chestnut Thrush and Kessler's Thrush overlapped, but the nesting height was stratified. Kessler's Thrushes nested significantly higher than Chestnut Thrushes. Sympatrically breeding birds may coexist successfully by dividing limited resources and reducing interspecific competition within the same area (Sommer and Worm, 2002; Atiénzar et al., 2013). For example, two sympatric Alpine Rose finches that breed in the Tibetan mountains divide their ecological niches by nesting on shrubs at different heights (Lu et al., 2011).

There is no difference in the size of the inner nest cup between Chestnut Thrush and Kessler's Thrush, but the nest wall of Kessler's Thrush is thicker than that of Chestnut Thrush. Kessler's Thrush can breed at higher altitudes than Chestnut Thrush (Mackinnon et al., 2000), and with increasing altitude, temperatures decrease, and Kessler's Thrush has thicker nest walls to adapt to colder conditions. It has been found that as the temperature decreases with the increase of latitude, the thickness of the wall of the open nest also increases, whereas the diameter of the inner nest cup does not change (Mainwaring et al., 2014a). It is also possible that Kessler's Thrush breeds earlier than Chestnut Thrush start breeding earlier, starts breeding when the temperature is low and increases the thickness of the nest wall in order to maintain the temperature.

Breeding time and eggs

The specialized natural environment at high altitudes, such as the short season suitable for bird breeding and the colder climate, has certain restrictions on the breeding of birds (Kovshar, 1981; Badyaev, 1987). Breeding timing is considered a life history strategy, and different timing of breeding can also reduce the stress of reproductive competition (Minot and Perrins, 1986; Dhondt, 2010). Our research shows that the breeding time of Kessler's Thrush is about half a month earlier than that of Chestnut Thrush. In previous studies of birds breeding in the same domain, similar trends have been confirmed. Birds breeding in the same domain can reduce the overlapping degree of the breeding period with other birds by adjusting their breeding time, to reduce the resource competition among different species during the breeding period (Macarthur, 1958; Prokesova and Kocian, 2004; Atiénzar et al., 2013).

The food restriction hypothesis suggests that the clutch size of birds is determined by the maximum food resources available (Lack, 1947, 1948; Lack, 1968; Martin, 1987). Under certain conditions, birds can ensure the maximum number of viable offspring by selecting the optimal clutch size and achieving the maximum reproductive effect (Charnov and Krebs, 1974). In addition to being influenced by food resources, avian clutch size is related to a variety of factors including latitude, altitude, nest structure, and nest predation rate (Skutch, 1985; Kulesza, 1990). Due to the nature of the environmental factors and food resources, the clutch sizes of sympatric species should tend to be similar. This is also supported by our results, which show no difference in clutch size between sympatrically breeding Chestnut Thrushes and Kessler's Thrushes. In terms of egg size, the eggs of Chestnut Thrush are larger and heavier than those of Kessler's Thrush. Compared to egg volumes of conspecifics at other elevations, egg volumes were larger with increasing elevation. The egg volume of Chestnut Thrush at different elevations is 2,100 m $V = 7.05 \pm 0.38\,\text{cm}^3$ (Zhang et al., 1986), 2,700 m $V = 7.5 \pm 0.04\,\text{cm}^3$ (Zhao and Sun, 2018), 3,400 m $V = 8.01 \pm 1.06\,\text{cm}^3$ (this article), and the egg volume of Kessler's Thrush at different elevations is 3,400 m $V = 6.9 \pm 1.36\,\text{cm}^3$ (this article), 4,100 m $V = 7.99 \pm 0.84\,\text{cm}^3$ (Yang et al., 2012). Toward higher elevations, organisms are increasingly exposed to cold, seasonal climates, strong solar radiation, low atmospheric pressures, and poor oxygen availability (Körner, 2007). Large eggs seem to have an advantage in harsh environments (Smith et al., 1995; Fox and Czesak, 2000). By lowering the surface area-to-volume ratios (Mcnab, 2002), larger offspring may buffer heat or water loss caused by the temperature and atmospheric conditions of high elevations. This will improve the adaptability of nestlings in harsh environments to make up for the cost of the environment (Smith et al., 1995; Fox and Czesak, 2000).

Parental care

Parental care is important for offspring survival and fitness (Buitron, 1988; Ghalambor et al., 2013), species living at higher elevations increase their parental care to compensate for low fecundity, thus allowing their nestling to survive in extreme environments (Badyaev and Ghalambor, 2001; Hille and Cooper, 2015; Boyle et al., 2016; Liang et al., 2020). The results showed that both species of thrushes were raised jointly by their parents, and the frequency of feeding nestling by Kessler's Thrush was higher than that by Chestnut Thrush because Kessler's Thrush nestling developed more rapidly and had more food requirements. The two thrush brood diets overlapped substantially, but the two thrush showed different preferences for the types of food. Chestnut Thrush preferred worms and earthworms, while Kessler's Thrush primarily fed worms but incorporated a larger proportion of arthropods. The two species of thrush reduce competition among themselves through their preference for different foods. In terms of brooding nestlings, Chestnut Thrush adopted a strategy of brooding nestling more frequently and for a short time, while Kessler's Thrush brooded nestlings less frequently but for a longer time. Species dwelling in the same environment may use different life history strategies to cope with the local environment (Auer et al., 2007).

Nestling growth

Nestlings are affected by a variety of internal and external factors during the process of growth and development. We found that the nestling growth and development of Kessler's Thrush is faster than that of Chestnut Thrush. Because Kessler's Thrush is distributed at higher altitudes, high-altitude birds increase the feeding of their parents and adapt to low temperatures, and develop faster than nestlings at low altitudes (Badyaev and Ghalambor, 2001). In addition, rapid growth is also an adaptation to the short breeding seasons in alpine environments.

Breeding success

The breeding success of birds is restricted by many factors, including the nutritional conditions during laying eggs, the weather conditions during the hatching or brooding period, predation, egg quality, and human disturbance (Angelstam et al., 1984; Moss and Watson, 1984; Brittas, 1988). As a result of low temperatures, strong seasonality, short breeding seasons and the strong fluctuation of food availability at high altitudes (Kovshar, 1981), the production of small clutches and large eggs is beneficial to increase the investment in each offspring to improve survival (Berven, 1982a, b; Blanckenhorn, 1997). Our results show that the breeding success rate, hatching rate, fledgling rate, nesting success rate, and predation rate of the Chestnut Thrush and Kessler's Thrush are similar. Since the breeding distribution of the Chestnut Thrush and Kessler's Thrush is sympatric in time and space, and they have the same environment and nest predation risk, they have the same breeding success. In the study area, the main cause of breeding failure was nest predation. The nest predation rate of Kessler's Thrush was slightly higher than that of Chestnut Thrush. There are two possible reasons for this: (1) it is related to the feeding frequency of the parent birds, which is higher for the Kessler's Thrush than for the Chestnut Thrush. High nest attendance and feeding frequencies of high-altitude species may in turn result in high exposures to potential predators (Conway and Martin, 2000) and therefore lower their breeding successes. (2) It is related to the nesting location because Kessler's Thrush nests higher than Chestnut Thrush. Nest height may directly affect the breeding success of birds, as taller nests are more vulnerable to aerial predators, while lower nests are more vulnerable to ground predators (Piper and Catterall, 2004; Mainwaring et al., 2014b; Tabib et al., 2016).

In our study area, the primary threat comes from aerial predators, therefore higher nesting sites are more at risk. Although we have not been able to identify the predators of the two species of thrush in the study area, we have observed *Eudynamys scolopaceus* preying on *Trochalopteron elliotii* nestlings, *Lanius tephronotus* attacking *T. elliotii* nestlings, and studies have also found *L. tephronotus* predation on the eggs of *Turdus merula maximus* (Purbu et al., 2018). The second was nest abandonment and human destruction. The nest abandonment rate of Chestnut Thrush was higher than that of Kessler's Thrush. A part of the study area is in a wetland Park which is a tourist attraction and is greatly disturbed by humans. A total of 5 Chestnut Thrush nests in this area were destroyed by human beings. The impact of extreme weather is also an important reason for forcing birds to abandon their nests. The study area usually experiences extreme weather such as snow, hail, strong winds, and heavy rain during the breeding season.

Conclusion

The results show that the breeding biology of the sympatrically breeding Chestnut Thrush and Kessler's Thrush in the alpine environment shows convergent and divergent characteristics. There were no differences between the two thrushes in terms of nest material, clutch size, type of food fed to the nestling, and breeding performance. In terms of breeding time, Kessler's Thrushes bred half a month earlier than Chestnut Thrushes and Chestnut Thrushes lay heavier and larger eggs. Kessler's Thrushes invest more in their nestlings compared to Chestnut Thrushes and brooded their chicks for longer periods of time but less frequently than Chestnut Thrushes. The nestling growth rate of Kessler's

Thrushes is higher and they develop faster. These results suggest that the Chestnut Thrush with a lower breeding distribution, exhibits characteristics similar to that of low-altitude birds, and the Kessler's Thrush with a higher breeding distribution resembles high-altitude birds with its breeding characteristics. This indicates that the breeding strategies of birds in the alpine environment are not only affected by environmental factors but also may be affected by genetics or phylogeny.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Research Ethics Review Committee of China west Normal University (CWNU2020D08).

Author contributions

HZ and YG: conceived and designed the study. HX, HZ, and YG: collected data and samples in the field. YG: analyzed the data. HZ and YG: wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effect of chronic traffic noise on behavior and physiology of plateau pikas (*Ochotona curzoniae*)

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During the last two decades, numerous studies have shown the effects of traffic noise on animal vocal communication. However, studies on the influences of traffic noise on wildlife behavior and physiology are scarce. In the present study, we experimentally manipulated the traffic noise exposure of plateau pika, a native small mammal widely distributed in the alpine meadow of Qinghai-Tibet Plateau, to explore the effects of traffic noise exposure on its behavior and physiology. We showed that noise exposure increased the pika's exploration and cortisol concentration (CORT) but decreased the resting metabolic rate (RMR). In addition, the relationships between RMR and exploration or CORT appeared under traffic noise treatment. This study suggests that traffic noise plays a large role in the behavior and physiology of plateau pikas and may have a long-term negative effect on the fitness of rodent populations. Generalizing these non-lethal effects to different taxa is crucial for the conservation and management of biodiversity in this increasingly noisy world.

KEYWORDS

behavior, cortisol, metabolism, road ecology, Qinghai-Tibet Plateau, rodent, small mammal, non-lethal effect

Introduction

From the last century, worldwide human population growth dramatically influenced the Earth through urbanization and industrialization (Sih et al., 2011). Transportation construction developed in an unprecedented scale, which helped us live a more convenient life. Transportation has become a fundamental element of human well-being. Although about 80% of Earth's terrestrial surface remains roadless, more than half is located in <1 km^2 buffer to roads (Ibisch et al., 2016). Simultaneously, traffic brings significant impacts, which are usually negative, on native species and ecosystems;

such impacts include natural landscape change (Fahrig and Rytwinski, 2009; Kati et al., 2020), biological invasion (Iacarella et al., 2020), habitat fragmentation (Ware et al., 2015), biodiversity loss (Secco et al., 2022). Following a huge anthropogenic road-building program, road ecology is now increasingly being studied globally (Ibisch et al., 2016). Road and traffic can affect terrestrial animal populations *via* fragmenting them into smaller sub-populations, which are more vulnerable to local extinction, increasing mortality directly or decreasing habitat amount and quality (Rytwinski and Fahrig, 2015). Around 194 million birds and 29 million mammals may be killed each year on European roads (Grilo et al., 2020); with the increase in traffic density, roadkills consisting of mammals and birds increased in Southern Spain (Canal et al., 2019); road reduced the natural area and space use of jaguars (*Panthera onca*) and wolf (*Canis lupus*), which prefer to cross low-speed and low-traffic-volume roads (Cerqueira et al., 2021; Dennehy et al., 2021); forest road can serve as barriers that impede movements and space use of red squirrel (*Tamiasciurus hadsonicus grahamensis*; Chen and Koprowski, 2016). Traffic also decreases animals' accessibility to food, mate, and resources, thus reducing the reproductive fitness and threatening population persistence (Fahrig and Rytwinski, 2009; Rytwinski and Fahrig, 2015; Secco et al., 2022). In 2050, a 60% increase of global road length or 25 million kilometers of new-building roadway is predicted compared with that in 2010 (Dulac, 2013). Therefore, road ecology should pay more attention to the effects of traffic on the biodiversity and conservation in the world.

In the last two decades, an increasing number of studies demonstrated that traffic noise has become one of the most important noise pollutions and has significant effects on wildlife (Halfwerk et al., 2011). The impact of traffic noise has been expended to variety of taxa, such as frogs (Troïanowski et al., 2017), bats (Berthinussen and Altringham, 2012), and songbird (Potvin and MacDougall-Shackleton, 2015). Traffic noise has negative effects on animals, i.e., disturbing their acoustic communication, reducing their reproductive success, embryo survival rate, and nestling growth, increasing stress hormone level, and influencing the population maintenance (Crino et al., 2013; Caorsi et al., 2017). Birds show behavioral flexibility under noise and adjust their song features to adapt to the variable-noise environment (Slabbekoorn, 2013). Bischoff's treefrog (*Boana bischoffi*) moves away from the noise source and decreases its advertisement call rate and dominant frequency (Caorsi et al., 2017), whereas the European treefrog (*Hyla arborea*) increases corticosterone levels and decreases immune function under traffic noise (Troïanowski et al., 2017). Shipping noise drives right whale (*Eubalaena* spp.) to shift their call frequencies, whereas male fin whales (*Balaenoptera physalus*) modify their song characteristics (Pirotta et al., 2019). However, most studies on noise effects on animals focus on the acoustic communicating species, with approximately two-thirds of such research mentioning songbirds and marine mammals (Shannon et al., 2016); a limited number of studies centered on non-lethal effects of traffic noise on terrestrial mammals (Naidenko et al., 2021), especially on small mammals such as rodents. Rodents represent around 2,277 (42%) of the world's mammalian species with different life forms (Wilson and Reeder, 2005), and they are widely distributed in variable environments of the Earth. Studying the effects of traffic noise on rodents is crucial for their conservation and management.

The transportation construction has developed rapidly in China. By 2018, more than 5,280,700 km of highways, including 117,000 km of national expressways and 40,000 km of high-speed railways, have been built (Ministry of Transport of China, 2022). In 2035, the lengths of railway and expressway are predicted to reach 70,000 and 160,000 km, respectively (Xinhua News Agency, 2021). Sanjiangyuan National Park is one of the first batch of National Parks and also the largest National Park in China, occupying an area of 190,700 km^2. This park is a biodiversity hotspot with more than 270 wild animals; thus, balancing the ecological conservation and livelihood development is a crucial issue in Sanjiangyuan National Park (Cheng et al., 2020). Qinghai-Tibet highway and railway, which cross the Sanjiangyuan National Park, are major human and transportation routes and are thus also extremely busy traffic channels. The streams of traffic produce noise and light pollution along traffic channels. It has demonstrated that both richness and abundances of ground-dwelling birds was decreased near the Qinghai-Tibet highway and railway (Li et al., 2010), and the behaviors of Tibetan antelope were obviously affected by the highway (Ru et al., 2022). Plateau pikas are native small Lagomorphs that are widely distributed in the alpine meadow of Qinghai-Tibet Plateau (Smith and Xie, 2008; Wei et al., 2022). They are regarded as keystone species in the ecosystem of the plateau (Wei et al., 2019), and commonly found near the highway slope (Zhang et al., 2021). Previous studies have demonstrated that the Qinghai-Tibet highway and railway have changed the local rodent community structure (Yang et al., 2006) and restricted plateau pika's genetic exchange, leading to several genetic differentiations (Zhou et al., 2006). However, the knowledge of how traffic noise affects the plateau pikas is limited. This study aimed to test whether traffic noise affects the plateau pika's behavioral and physiological traits and their correlations. We predicted that (i) chronic exposure to traffic noise induces stress in plateau pika; (ii) traffic noise increases pika's exploration and metabolism; (iii) the correlations between behavior and physiology may change under traffic noise.

Materials and methods

Experiment animals

Plateau pikas were live-trapped using the using nooses anchored at burrow entrances with chopsticks (see details in the

Qu et al., 2018) at the alpine meadow of Geermu, Qinghai in 10th-12th August 2019, brought back and raised in the animal laboratory at the Northwest Institute of Plateau Biology, Chinese Academy of Sciences. The trap site was about 2.0 km away from the road to ensure that the plateau pikas were not exposed to traffic noise in the natural environment.

Plateau pikas were maintained in separate polypropylene cages (47 cm × 35 cm × 20 cm) under natural light cycle condition, and the ambient temperature was kept 23 ± 1°C. Their gender was tested, and ID information were marked on the cages. The pikas were fed standard rabbit pellet (Beijing Ke Ao Food, Co., China) and pure water *ad libitum*. They were kept in the laboratory and adapted for 2 weeks. Then, 16 adult males and 12 adult females were used for the following experiment.

Traffic noise preparation and broadcasting

We recorded the traffic noise for playback experiments at Qinghai-Tibet highway (G109) at about 3,002 milestone. Recordings were collected 20 m away from the edge of the paved road at a height of 1 m, where plateau pikas are frequently found, on August 15th, 2019 from 09:00 am to 12:00 am, and lasted for 3 h. A total of 1,430 vehicles were recorded during this period. We selected such date and time because they represented the normal vehicle flux during the season, according to the records of the local department of transportation. A portable sound level meter (DELIXI 2201, 0.1 dB precision) was used to measure the mean amplitude (dB) of the traffic noise, and SONY PCM–D100 recorder was utilized to obtain sounds. From the 2 h recording, a 30 min segment was selected and normalized to a mean amplitude level using Praat v 6.2 software.

Experimental procedure

The behavior and physiology of all pikas were measured at the start of the experiment in early September. Then, the pikas were randomly divided into two treatment groups ("treat" for short), i.e., "*Noise*" and "*Control*" groups, and housed in separate but adjoining rooms (18 m^2) with the same conditions. In the "*Noise*" group room, traffic noise was broadcasted. In the "*Control*" group room, a silent broadcast was conducted during the whole experiment. The treatment lasted for 30 days. At the end of the experiment, the behavior and physiology of all pikas were measured once again using the same procedure.

Traffic noise was broadcasted using an amplified loudspeaker connected to a radio at the center of the "*Noise*" group room, with all the cages spread around the room to ensure that all the pikas heard the same noise. The noise was loop broadcasted during daytime from 07:00 am to 19:00 pm at 80 ± 2 dB sound pressure level to simulate the natural acoustic environment. Although a certain traffic noise was observed at night in G109, we did not broadcast it in the experiment because pikas are inactive and live underground overnight (Zong and Xia, 1987).

Behavioral and physiological measurement

We conducted behavioral and physiological assays in early September and early October 2019, i.e., "*Pro*" and "*Post*" ("trial" for short) the traffic noise treatments, respectively. The behavioral and physiological parameters were tested in the following sequence:

Heart-rate test

A digital voice recorder (Sony PCM-D100) was placed on the left thorax of a pika to record the heart rate. Individuals were held gently by an experimenter, and the heart rate was measured for 30 s by another experimenter. The intervals between two adjacent heartbeats at 12 randomly selected points were detected from each audio file using Praat v 6.2 software. Previous studies demonstrated that this approach is feasible for the heart-rate measurement of plateau pikas (Qu et al., 2018).

Open-field test

After the heart-rate test, pikas were gently transferred to an open-field arena. The arena was a black acrylic box (50 cm × 50 cm × 50 cm) with a white floor. We recorded the behavior exhibited by each pika for 2 min using a digital camera that was fixed above the arena using a tripod. Then, the pika was removed from the arena, and its ID and gender were recorded. After each trial, the arena was cleaned using 75% alcohol and air-dried for the subsequent trial. Using EthoVision XT 9.0 (Noldus Inc., Beijing, China) software, we measured the distances covered by the pika for 2 min as a proxy of exploration (Réale et al., 2007).

Resting metabolic rate (RMR)

We used an 8-channel FMS (Sable Systems International, Henderson, NV, United States) portable respiratory metabolism system to measure the RMR at 27°C using a biochemical incubator. Oxygen consumption per hour (mL O_2/[g h]) was expressed as the index of RMR. After the pikas had acclimatized in the chamber for 30 min, the RMR of plateau pikas was measured. Four rounds of metabolism were measured in 120 min, with each round lasting for 30 min. We obtained the average of the lowest consecutive records as the RMR (Zhu et al., 2021).

Fecal cortisol concentration (CORT)

After each test, fresh feces were immediately collected and transferred to a −20°C refrigerator. Fecal CORT was measured using rabbit cortisol enzyme-linked immunosorbent assay kit following the operation manual (Shanghai DUMA Biotechnology Co., Ltd., China). The assay sensitivity was 1.0 ng/ml with the degree of confidence above 99.8%. The intra-and inter-assay coefficients of variance were 10.3 and 6.8%, respectively.

TABLE 1 Unadjusted and adjusted repeatability (*R*) estimates with 95% *CI*.

Variables	Unadjusted *R*	Adjusted *R*
Heart rate	0.333 (0.115, 0.702)	0.333 (0.099, 0.707)
Exploration	0.324 (0.087, 0.735)	0.303 (0.068, 0.673)
RMR	0.333 (0.112, 0.702)	0.333 (0.120, 0.698)
CORT	0.322 (0.018, 0.674)	0.321 (0.025, 0.667)

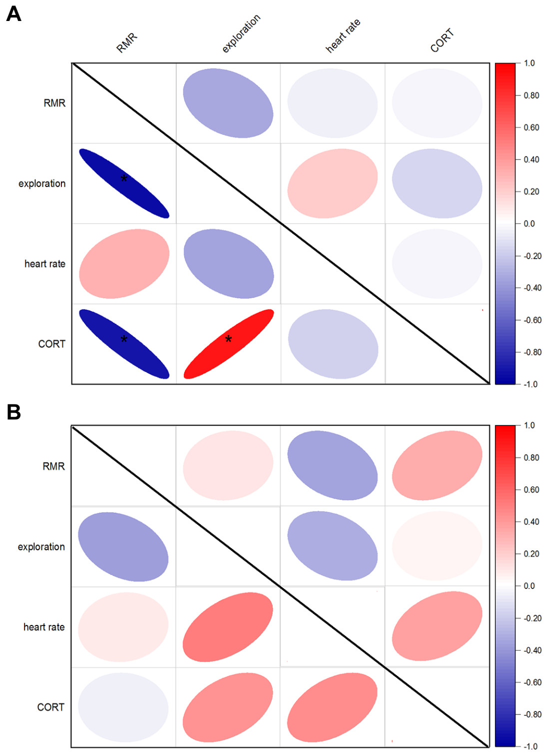

FIGURE 1
Correlations between behavioral and physiological traits *pre* and *post* experiment [**(A)**: *Noise*; **(B)**: *Control*]. Upper and under the diagonal indicated *pre* and *post* experiments, respectively. *Suggests significant correlations between two traits.

Statistical analysis

We calculated the repeatability of the heart rate, exploration, RMR, and CORT as an intra-class correlation coefficient from one-way ANOVA with treat as a fixed factor using the "*rptR*" package (Stoffel et al., 2017). Linear mixed-effects models (LMMs) were used to test the potential effect of traffic noise on these behavioral and physiological traits. We assessed the existence of correlations between the heart rate, exploration, RMR, and CORT of each individual from different treatment and trial groups by Spearman rank correlations using the "*Hmisc*" package. The LMM allows the estimation of the effects of explanatory variables and their interactions as fixed effects, with ID as random effects. LMMs were fitted using the "*lmer*" package, and "*lmerTest*" package was used to obtain the summary table and *p*-values for LMMs *via* Satterthwaite's degrees of freedom method (Kuznetsova et al., 2017).

R 4.2.1 software (R Development Core Team, 2022) was used for all statistical analyses.

Ethical statement

The animal study was reviewed and approved by The Ethics Committee of Northwest Institute of Plateau Biology, Chinese Academy of Sciences (NWIPB-20190727). The procedure for capturing, feeding, and operating on the plateau pikas followed the guidelines of the ethical approval standard of the committee. No effect of transport and experiment was observed on pika mortality.

Results

Repeatability and correlations

According to the variance proportions explained by between-individual variations, the behavioral and physiological traits of plateau pikas were repeatable (Table 1). The repeatability of heart rate and RMR showed similar patterns ($R_{adj} = 0.333$ [0.099, 0.707], $R_{adj} = 0.333$ [0.120, 0.698], respectively), followed by CORT ($R_{adj} = 0.321$ [0.025, 0.667]) and exploration ($R_{adj} = 0.303$ [0.068, 0.673]), which suggest that all behavioral and physiological traits measured were medium repeatable over time. At the start of the experiment, all traits were not significantly correlated with each other in the two groups. However, at the end of the experiment, exploration and CORT were significantly positively correlated, and RMR was significantly negatively correlated with exploration or CORT in the *Noise* group ($p < 0.001$; Figure 1A). However, all traits were not significantly correlated with each other in the *Control* group (Figure 1B).

Main and interaction effects

Before the experiments, the heart rates of plateau pikas were in the range of 140.70–413.82 beats per min, with an average of 342.22 ± 2.13 beats per min. At the end of the experiment, the average exploration of the *Noise* and *Control* groups changed to 383.60 ± 4.24 and 372.93 ± 2.67 beats per min, respectively. Treatment, trial, and their interaction and gender had no significant effects on heart rate (Figure 2A; Table 2).

Before the start of experiments, the exploration ranged from 0.50 to 6.09, with an average of 4.44 ± 0.08. By contrast, at the end of the experiment, the average exploration of *Noise* and *Control* groups changed to 42.93 ± 0.76 and 4.13 ± 0.06, respectively. The treatment and its interaction with the trial had significant effects on exploration (Figure 2B; Table 2).

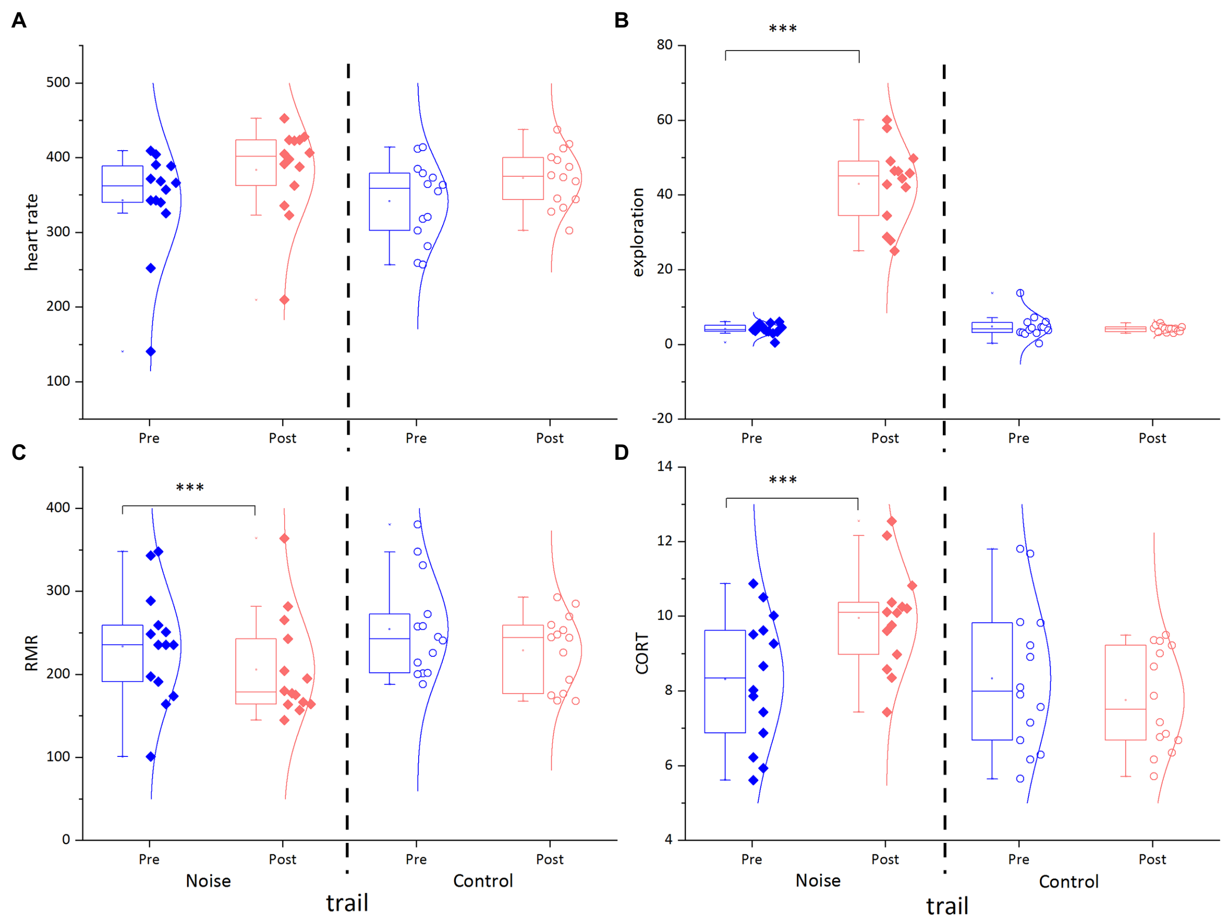

FIGURE 2
Effect of treatment on behavioral and physiological traits *pre* and *post* experiment in the *Noise* and *Control* groups. **(A)** Heart rate under handling, **(B)** exploration, **(C)** RMR, and **(D)** fecal CORT.

Before the experiments, the RMR of plateau pikas ranged from 100.83 ml O_2/[g·h] to 348.01 ml O_2/[g·h], with the mean of 244.07 ± 2.26 ml O_2/[g·h]. At the end of the experiment, the mean RMR of the *Noise* and *Control* groups changed to 205.83 ± 4.40 and 228.74 ± 3.17 ml O_2/[g·h], respectively. Although the trial had no significant effects on RMR, treatment, gender, and the interaction between treatment and trial had significant effects on RMR (Figure 2C; Table 2).

Similarly, before the start of the experiment, the CORT of plateau pikas ranged from 5.612 ng/mg to 11.799 ng/mg, with a mean of 8.32 ± 0.06 ng/mg. At the end of the experiment, the mean CORT of the *Noise* and *Control* groups changed to 9.88 ± 0.06 and 7.76 ± 0.09 ng/mg, respectively. The treatment and its interaction with trial had significant effects on CORT (Figure 2D; Table 2).

Discussion

The present study aimed to examine whether traffic noise has effects on the behavioral and physiological traits of plateau pikas. We observed significant increases in exploration and CORT but a significant decrease in the RMR of plateau pikas. Correlations between several traits were detected after exposure to traffic noise compared with the controls, supporting our prediction that traffic noise constitutes a chronic stressor to rodents. The results of one-month experimental studies focusing on the effects of artificial noise on rodent behavior and physiology (e.g., cortisol) were consistent with those of previous research (Cui et al., 2016, 2018).

Test sequence: Habituation to trial

When wild animals face repeated stimulus, their behavior may be modified, a process called habituation (Rankin et al., 2009). Heart rate and RMR tests involve the capture and handling of subjects, which may elicit significant stress responses (Careau et al., 2008). In this study, although neither behavioral (i.e., exploration) nor physiological traits (heart rate, RMR, and CORT) were singly affected by trial, exploration, RMR, and CORT were interactively affected by trial and traffic noise, suggesting that plateau pikas habituated to the experimental trial, which may

TABLE 2 Results from the LMM analyses with traffic noise and time as fixed effects and ID as the random effect.

Variables	Fixed effects	Estimate	*SE*	*t*-value	*p*-value
Heart rate	Intercept	366.884	17.683	20.747	**<0.001**
	treat	10.670	21.658	0.493	0.624
	trial	−31.298	21.658	−1.445	0.155
	gender	10.574	15.473	0.683	0.497
	treat × trial	−9.488	30.629	−0.310	0.758
Exploration	Intercept	7.470	3.161	2.363	0.022
	treat	26.910	3.871	6.952	**<0.001**
	trial	−0.633	3.871	−0.163	0.871
	gender	−4.737	2.766	−1.713	0.093
	treat × trial	−26.930	5.474	−4.919	**<0.001**
RMR	Intercept	174.9	16.95	10.317	<0.001
	treat	48.64	19.87	2.448	**0.018**
	trial	22.91	15.8	1.45	0.159
	gender	54.12	16.6	3.26	**0.003**
	treat × trial	−43.7	22.35	−1.965	**0.049**
CORT	Intercept	7.875	0.515	15.305	<0.001
	treat	2.306	0.619	3.724	**0.001**
	trial	0.153	0.573	0.267	0.791
	gender	0.541	0.473	1.144	0.263
	treat × trial	−2.330	0.810	−2.875	**0.008**

The last column reports the marginal R^2 with 95% *CI* of the model (row: fixed effects) and the variance explained by treatment and trial (reported as semi-partial R^2, see Methods for details). The bold value means significant effect of variables.

weaken the impacts of traffic noise, as shown by the decreases or homogenizations in the exploration, RMR, and CORT of the *Control* group (Figure 2). In this scenario, we still detected the significant effects of traffic noise on exploration, RMR, and CORT, suggesting the crucial effects of traffic noise on pikas.

Numerous studies have reported the high repeatability estimate for animal behavior over time and/or context environment, which is termed personality (Réale et al., 2010). When animals face external challenges or disturbances, they may reflect behavioral variations, which can be explained through individual consistency and plasticity (Clermont et al., 2019; Couchoux et al., 2021). Using meta-analysis, Bell et al. (2009) observed a mean repeatability of 0.37; our previous study showed that the repeatability of exploration and CORT ranged from 0.34 to 0.63 in the natural population of plateau pikas (Qu et al., 2018). In the present study, the repeatability of exploration, heart rate, RMR, and CORT was around 0.32, indicating that plateau pikas showed lower behavioral and physiological consistency, but higher plasticity under traffic noise stress compared with the natural environment. Plasticity benefits animals to alter their behavior and physiology to better cope with variable environments. For example, urban birds show behavioral plasticity of the acoustic phenotype to increase their survival and reproduction rate during the urbanization process following artificial noise (Slabbekoorn, 2013). A number of insect species show behavioral plasticity in foraging, reproduction, habitat choice, and modified social behavior to cope with artificial rapid environmental changes (van Baaren and Candolin, 2018). This study demonstrated that rodents can change their behavior and physiology to mitigate the potential adverse effects of traffic noise.

Behavioral, physiological variations and correlations

Heart rate under handling indicates the individual physiological index of coping style and reflects the response of the animal autonomic nervous system to predation risk or fearful stimuli rather than disturbance stress (Koolhaas et al., 2010). Our previous studies demonstrated that in the natural population, the heart rate and personality were correlated in plateau pikas, where the predation risk is high (Qu et al., 2018; Wei et al., 2020), as pikas are the primary prey for almost all predators on the Qinghai-Tibetan Plateau (Harris et al., 2014). In this study, the heart rate was consistent at the start and end of the experiment, indicating that plateau pikas maintained a stable response to human handling, regardless of whether they were exposed to traffic noise exposure; thus, artificial traffic noise may have different affecting mechanisms for wildlife compared with predation risk, and animals show variable coping styles to predation and artificial challenges (Francis and Barber, 2013).

As an external stressor, noise can hyperactivate the sympathetic autonomic nervous system and activate the hypothalamic–pituitary–adrenal axis, which increase the organism CORT and finally alter metabolism (Schmidt et al., 2013). Two weeks of noise enhanced the blood glucose and CORT levels and varied the glucose metabolism and gut microbiota composition in rats (Cui et al., 2016). Noise exposure also increased the corticosterone level of male treefrog but decreased their immune function (Troïanowski et al., 2017). Our results showed that at the end of the experiment, the CORT of plateau pikas in the *Noise* group was significantly higher than that at the start, whereas no significant variations in the CORT *pre* and *post* experiment were detected in the *Control* group. Thus, traffic noise caused evident stress to the pikas, and the significant interactions between treatment and trial on CORT suggested that pikas might have accustomed to the experimental environment over 1 month, which relieved the potential stress from an unfamiliar environment. Exploration reflects an animal's response to space, and it is associated with anxiety; anxious animals consistently show heightened exploration (Karl et al., 2006). In this study, the increased CORT and exploration suggest that persistent traffic noise is an interference pressure resource to pikas, and it affects their hormone sensitivity and anxiety, finally influencing their body conditions.

Metabolism is a proximate mechanism underlying behavioral and physiological variations (Careau et al., 2008). According to the Pace-of-life Syndrome (POLS) hypothesis, animal life history, behavior, and physiology are correlated, and their coupling is used to cope with external challenges (Réale et al., 2010; Boulton et al., 2015; Houslay et al., 2017). In this study, at the start of the experiment in both groups and at the end of experiment in the *Control* group, no significant correlations were detected among traits, whereas significant correlations between exploration, RMR, and CORT appeared in the *Noise* group at the end of the experiment, suggesting that traffic noise promoted the correlations among traits, that is, the occurrence of coping style. Although the presence of personality traits has been found in different external stresses, such as predation risk (Dingemanse et al., 2007, 2009) and artificial harvest (Biro and Post, 2008), we emphasized for the first time a significant relationship among behavior, RMR, and CORT induced by traffic noise.

Two distinct conceptual models explain the relationships between animal metabolism and behavior (Careau et al., 2008). The *performance model* refers to the situation in which a high metabolic rate requires more active behavioral traits. Thus, exploration, aggressiveness and boldness should positively correlate with energy metabolism. A positive correlation was detected between boldness and metabolism in wild-caught orb-weaving spider (*Larinioides cornutus*; Shearer and Pruitt, 2014) and fall field crickets (*Gryllus pennsylvanicus*; Careau et al., 2019). By contrast, the *allocation model* argues that with individuals having to allocate a fixed amount of energy to maintain their normal metabolism, limited energy is expended in the behaviors associated with activity, exploration, etc. The active mosquitofish (*Gambusia holbrooki*) has a low RMR, which suggests that high activity rates require individuals to allocate less energy toward maintenance (Biro et al., 2020). In this study, the decrease of RMR, as well as negative relationship between RMR and exploration in the *Noise* group at the end of experiment, provided evidence for the *allocation model* of energy management. Under *ad libitum* food conditions in the laboratory, traffic noise caused trade-offs between RMR and exploration. The relationships between behavior and metabolism are not always stable (Mathot et al., 2019). For instance, the risk-taking behavior (i.e., boldness) and basal metabolic rate of the great tit (*Parus major*) changed from positive to negative in two consecutive years (Mathot et al., 2015). Under artificial light stimulus, the common toad (*Bufo bufo*) allocates less energy on activities but more energy on RMR (Touzot et al., 2019). Similarly, given that hormone production is a high-energy-consuming process, the negative relationships between CORT and RMR occurred under noise exposure conditions, which is consistent with the result of Guenther et al. (2014), who observed negative correlations between CORT and RMR in juvenile and mature cavies (*Cavia aperea*).

Conclusion

This study provided experimental evidence of the effects of chronic noise exposure on the behavior and physiology of wild rodents. Our results indicate that traffic noise exposure altered the exploration, metabolism, and CORT and their correlations in plateau pikas. The behavioral and physiological effects of traffic noise are currently overlooked in rodents, and their ecological consequences on the conservation and management are unclear. Our study showed that they should receive more attention, and traffic noise should be regarded as a potential influencing factor when assessing the effects of transportation on ecosystems. In the future, generalizing the non-lethal effects of traffic noise to different taxa, especially non-flagship or endangered species, is also crucial for the conservation and management of the whole ecosystem in this increasingly noisy world.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by The Ethics Committee of Northwest Institute of Plateau Biology, Chinese Academy of Sciences (NWIPB-20190727). Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

JQ and YW conceived the study. HZ, YY, and LZ completed the majority of the experiments, and collected samples. JQ wrote the manuscript. YW reviewed the manuscript. All authors contributed to the articles and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.1065966/full#supplementary-material

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Dynamic changes in the home range of the subterranean rodent *Myospalax baileyi*

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As ecosystem engineers, subterranean rodents excavate and inhabit burrow systems. However, the changes in their use of underground space are poorly recorded. There is conflicting evidence about whether the burrow systems of subterranean rodents, once established, are relatively stable as a result of the high energy costs of digging. We monitored the size of the home ranges of the plateau zokor (*Myospalax baileyi*) during different stages of its life cycle to show whether mating behavior and the characteristics of its habitat influence the size and location of its home range. We used radio-tracking to quantify the changes in, and overlap of, the home range of *M. baileyi* during a one-year period. The average size of the home ranges of male zokors was 6.5 times larger than that of female zokors during the mating season. The males expanded their burrows to overlap with multiple females to increase their chances of mating. However, there was no overlap between estrus females or males, perhaps to reduce the number of encounters and unnecessary fights. The home ranges of male and female zokors were similar in size after courtship and the home ranges of single zokors overlapped with those of several neighbors. Most individuals remained territorial and excluded intraspecific interactions from their home ranges. The location of female zokors was stable throughout the year, but half of the males changed the location of their nests and established completely new home ranges in the non-breeding season, mainly in October. The use of space by *M. baileyi* is flexible in response to a need for physical contact during the mating season and food resources. The home ranges of subterranean plateau zokors are dynamic and the home ranges of male zokors can change within one breeding cycle.

KEYWORDS

subterranean rodents, home range, dynamics, overlap, radio-telemetry

Introduction

Basic theoretical studies of the use of space by animals are based on the concept of a home range, which was initially defined as the area in which an animal can meet its daily needs (Burt, 1943). The home range has more recently been defined as the area that an animal repeatedly traverses (Kenward et al., 2001; Powell and Mitchell, 2012). Most resident mammals limit their movements to a fairly well-defined area rather than just wandering randomly. The original definition of range has been explored in a variety of ways, including empirical and theoretical methods. The fact that the home range of most animals is dynamic in both size and location in spatiotemporal sequences There are thousands of articles quantifying seasonal dynamics of home ranges going back decades (Wada and Ichiki, 1980; Schoen and Kirchhoff, 1985; Lesage et al., 2000; Powell and Boitani, 2012). Ecologists have been linking animal movement with population dynamics for decades (e.g., Cooper, 1978; Boutin, 1979; Turchin, 1998). The seasonal changes in the home range of subterranean rodents were mainly concentrated in a few species, but the research objects based on different populations, and the radio-tracking based on the changes of home range in same population was rare (Šklíba et al., 2009). Most studies have focused on the factors that influence the size of the home range at a given point in time (Fisher and Owens, 2012).

Subterranean rodents successfully adapted to the underground environmental condition (Lessa, 2000; Shao et al., 2015). Compared with ground-dwelling species, their scope of activity and dispersal are greatly restricted by their underground environment. Most of their activities take place in underground burrow systems occupied by solitary and society individuals. Subterranean rodents mainly rely on the underground storage organs of plants for food and they must continuously extend their burrow systems to ensure adequate food resources. Begall et al. (2007) suggested that the burrow systems of subterranean rodents are continuously transformed by excavating new tunnels while abandoning part of the older tunnel network. Some subterranean rodents maintain an optimum size of their burrow systems through backfilling the tunnels with soil (Fisher and Owens, 2012). If the size of the home range of an animal is too large, it will be detrimental to the domain's occupant because the cost of defending their territory increases dramatically as the home range expands (Hinsch and Komdeur, 2017). The food resource and soil characteristics directly influence the size of the home range and the dynamics of the burrow systems of subterranean rodents in different types of habitat (Fisher and Owens, 2012; Lovari et al., 2013; Lövy et al., 2015; Kubiak et al., 2017). Larger animals don't 'require' more mates. They may have access to more mates through a competitive advantage, but it is not a requirement. Furthermore, home range size has been linked to a wide range of other biological factors beyond body size including habitat productivity, population density, etc. The range of activities of subterranean rodents is restricted by the underground physical environment. Analysis of home-range size could be useful also for revealing mating strategies. Lager male home ranges are expected to be used to encounter potential mates or recruiting. Cutrera et al. (2010) observed the intraspecific variation in home range size between two different sites in the South American Talas tuco-tuco (*Ctenomys talarum*). The size of the home range was influenced by both body size and the differences in soil characteristics (especially soil hardness) between the two study sites (Cutrera et al., 2006).

Live trapping and radio-tracking are valid tools with which to systematically collect data on the characteristics of movement and the use of space by small mammals inhabiting the underground environment. Studies of the size of home ranges have been carried out by radio-tracking a few species of *Bathyergus*, *Ctenomys* and *Spalax* (Rado and Terkel, 1989; Narins et al., 1992; Šumbera et al., 2008; Šklíba et al., 2016; Kubiak et al., 2017). Surprisingly, there have been few studies on the long-term use of space by subterranean rodents. Although there have been studies of the seasonal changes in the dynamics of the home range (Zhou and Dou, 1990; Rado et al., 1993), most previous studies have been conducted in different populations of the same species in different seasons. Nevo (1999) reported that the home ranges of underground mammals, once established and used for one breeding season, become essentially permanent for life. However, constantly shifting home ranges have been described (but not quantified) in solitary subterranean rodents, such as the blind mole rat (*Spalax ehrenbergi*) (Zuri and Terkel, 1996). Šklíba et al. (2009) tracked silvery mole rats

(*Heliophobius argenteocinereus*) for three months in the non-breeding season and found that their home ranges were dynamic and continuously changing in space. We therefore assume that home ranges are dynamic patterns of the use of space by animals and result from life-history strategies or interactions between individuals and the external environment.

We analyzed the use of space and the stability of the home range in the plateau zokor *Myospalax baileyi* (Rodentia: Myospalacinae), a widely distributed solitary subterranean rodent from the Qinghai–Tibetan Plateau (Zhang, 2007). Adult females of plateau zokors give birth once a year between April and July. The periods of gestation and lactation each last about 50 days (Zheng, 1980). *M. baileyi* occurs in various habitats with different food supplies and soil properties in an environment with alternating cold and warm seasons and it exemplifies a successful adaptation to an extreme subterranean environment (Shao et al., 2015).

We investigated individual home ranges using a radio-telemetry system. We tracked individuals of *M. baileyi* from June 2016 to May 2017 to cover the physiological changes in the plateau zokor during the year and changes in the food supply and soil characteristics. *M. baileyi* occupies different habitats with varying characteristics of soil and food supply in a seasonal environment with regular changes between dry and rainy seasons. Our main objectives were (1) to quantify the home range dynamics in different months: the home range overlap between neighbors, the number of new mounds, changes in the location and size of the home range of single individuals and Whether the nest has moved; and (2) to assess how these animals explore their underground environment over a full year.

Materials and methods

The study was conducted in Gansu Province in the eastern part of the Qinghai–Tibetan Plateau (**Figure 1**) at the Tianzhu alpine grassland system station of Gansu agricultural university (37° 18′ 34′′ N, 102° 36′ 22′′ E, elevation 2,892 m). The alpine meadow vegetation at this site consists of a mosaic of grasses (mainly *Elymus nutans*), sedges (mainly *Kobresia humilis*) and forbs. The climate in Tianzhu is characterized by a warm season (May–September) and a cold season (October–December/January–April) with precipitation mainly concentrated in June–September. The rainfall from June to September 2016 was 372.2 mm, accounting for 73.4% of the total rainfall in the research year (**Figure 1**). The average temperature and the soil temperature 15 cm underground were both < 0°C from November 2016 to March 2017 (**Figure 1**). The meteorological data were obtained from the China Meteorological Administration[1].

Radio-tracking

Radio-tracking was carried out from June 2016 to May 2017. We captured 14 *M. baileyi* adults (seven females and seven males) in 2016 and 12 adults (five females and seven males) in 2017 (**Table 1**). We also captured a sub-adult (ID M981) in 2017, which was not included in the statistical analysis. The animals were captured using a live trap for subterranean rodents (Hua et al., 2015). To ensure the integrity of the tunnel, we did not recapture individual animals, so we did not obtain weight data for the other months of 2016. After capture the animals were anesthetized (1% pentobarbital, 0.5 mg/100 g body mass), weighed, sexed and fitted with a radio-collar (Ag 357, Biotrack Ltd., Wareham, Dorset, UK; battery life about nine months). The animals were released at the trap site when they were fully recovered from the effects of the anesthetic. The radio-collars weighed 4.75 g (<4% of the body weight of the smallest zokor in this study) (Zhou et al., 2013). The experimental procedures involving capture, handling and the use of radio-collars were approved by the Institutional Animal Care and Use Committee of the Grassland Science College of Gansu Agricultural University (GSC- IACUC-2018-0011).

We used a Sika radio-tracking receiver (Biotrack Ltd.) and two-element Yagi antennas (Sirtrack Ltd., Havelock North, New Zealand) to locate the zokors. In 2016, radio fixes were taken during 24-h periods for 10 days each month (15th to 25th of each month). To ensure independence of the data collection, there was a 2-h time interval between fixes (Kubiak et al., 2017). In 2017, radio-tracking began 36–48 h after the release of the animal. All the individual animals were tracked until May 25. Radio fixes were taken over 12-h periods (08:00–20:00) with 2-h intervals between fixes. In total, we successfully radio-tracked 13 zokors (seven females and six males) from June to August, 11 zokors (five females and six males) in September and October 2016 and 12 (five females and seven males) in April and May 2017.

The radio-collars of two zokors (ID: F533, F663) fell off in September 2016 and we were not able to recapture the animals, so we did not obtain any further data from them. Another radio-collared male (ID M425) was not radio-tracked because it left its burrow system two days after release and moved above ground to outside the study area, where it created new caves in June 2016. We radio-tracked more than ten zokors continuously for more than ten months without damaging their burrow systems. The radio-collars did not affect their normal activities, including mating, excavating and building new surface mounds. The radio-collars could precisely locate the zokors, as shown by the fact that we were able to use the receiver to accurately find the two radio-collars that dropped off zokors F521 and F663. The data from individual animals with missing or incomplete transmission were not used in our analyses (see **Supplementary Data 1**). The battery power of the radio-collars

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[1] http://data.cma.cn/data/detail/dataCode/A.0012.0001.html

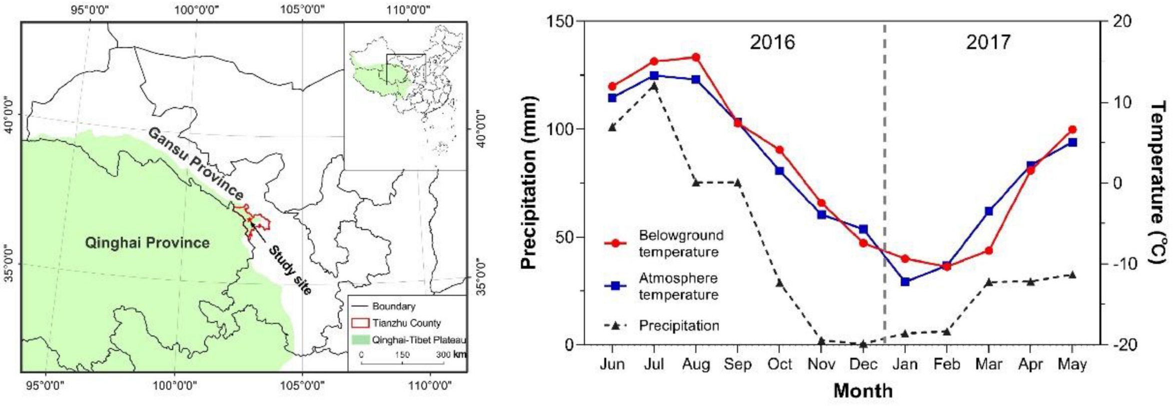

FIGURE 1

(Left-hand panel) Location of the study site. **(Right-hand panel)** Changes in temperature (air and 15 cm underground) and precipitation from June 2016 to May 2017.

TABLE 1 Overlap area (OA) of a single individual's home range with other individuals and the percentage of overlap (OP) in its home range in different months.

ID	2016										ID	2017	
	Jun		Jul		Aug		Sep		Oct			Apr + May	
	OA[a]	OP	OA	OP	OA	OP	OA	OP	OA	OP		OA	OP
F304	0	0	0	0	0	0	0	0	0	0	F147	49.16	39.59
F233	52.45	43.83	0	0	0	0	0	0	0	0	F533	11.34	100
F663	13.87	6.43	0	0	0	0	-	-	-	-	F638	27.69	100
F638	40.57	29.48	0	0	0	0	0	0	0	0	F521	8.9	25.31
F533	0	0	0	0	0	0	-	-	-	-	F616	23.41	45.27
F169	0	0	0	0	0	0	0	0	0	0	M370	8.61	6.94
F521	0	0	0	0	0	0	0	0	2.78	3.64	M266	43.66	17.79
M039	2.4	1.81	0	0	0	0	0	0	0	0	M697	36.85	21.98
M288	0	0	0	0	0	0	0	0	0	0	M198	53.99	6.87
M008	11.41	4.8	0	0	0	0	0	0	0	0	M455	11.53	3.24
M104	0	0	0	0	0	0	0	0	2.78	7.21	M856	0	0
M442	2.4	2.44	0	0	0	0	0	0	0	0	M929	72.87	23.65
M357	27.85	16.51	0	0	0	0	0	0	0	0			
Mean	16.56	9.97	0	0	0	0	0	0	0.51	0.97	Mean	29	32.55
SD	23.33	15.13	0	0	0	0	0	0	1.13	2.34	SD	22.45	34.38

[a] Unit of overlap area is m^2.

of most individual animals was exhausted by March 2017, so we recaptured the individuals wearing the radio-collars and replaced them with a new one in April. The recaptured zokors and recycled radio-collars were used for studies in other areas (or other studies in this area).

The size of individual home ranges size was calculated using 95% kernel-density estimation (KDE) methods (Fleming et al., 2015). We also calculated 50% KDE. This method is commonly used to evaluate the size of the home range of subterranean rodents. To accurately record the current position of the zokors, we set up a georeferenced grid (5 m × 5 m cell size) above all the burrow systems before radio-tracking. By measuring the vertical distance between the fixes and the nearest tunnel, we estimated the accuracy of radio-tracking points to be <1 m. We found that the home ranges of some individuals overlapped and we calculated the degree of overlap by dividing the area of overlap of one individual with other intraspecific individuals by the total area of the zokor population. We calculated the KDE using all the tracking points from each individual for comparison with other studies. KDE and overlap calculations were performed using Ranges 8 version 2.1.6 software (Anatrack Ltd., Wareham, UK).

Measurement of environmental factors

To evaluate the characteristics of soil and vegetation, we considered each individual nest as a central point. The plant biomass and soil compaction were sampled at each of the central points in the three sampling units located at a distance of 3 m in the three cardinal directions. We used the method of Galiano et al. (2014) to estimate the plant biomass and soil hardness. In brief, the vegetation present in a 0.25 m^2 × 0.3 m sample was collected and separated into above-ground and subterranean portions, dried for 24 h at 80°C and then weighed to the nearest 0.1 g. Soil samples were taken from near the capture points. Soil compaction was measured at 12.5, 15, and 17.5 cm soil depth using an SC-900 soil compaction meter (Spectrum, USA). We calculated the mean of three depths as the compaction (units: $kg \cdot cm^{-2}$) of one sampling point because the tunnel depth at the capture point of the zokors was between 10 and 20 cm (Li et al., 2004). We calculated the mean of the three sampling units and then the mean in the analysis of the soil compaction and vegetation variables.

Statistical analysis

We applied Student's *t*-tests or Mann–Whitney *U*-tests (where the assumptions of the *t*-test were not fulfilled) to compare the differences in the size of the home range and the overlap between sexes in the same months. We used the same analysis methods to compare the differences between male and female body mass over two years. The comparisons of the overlap area of the same individual's home range between June and October and the percentage of the overlap ratio in the two months were examined using Student's *t*-test or a Mann–Whitney *U*-test. A comparison of all variables (home range size, home range overlap and body mass between the sex) was implemented in GraphPad Prism version 8.0.1 (GraphPad Software, San Diego, CA, USA).

We use Kruskal-Wallis one-way ANOVA to compare home range sizes, number of new mounds, soil compaction and plant biomass (aboveground and underground) in different months. The ANOVA analyses were performed with STATISTICA StatSoft (version 17.0) for Windows. The influence of each row representing the matched data (soil compaction and aboveground or underground biomass) on the mean home range of females, males and individuals were tested using a linear regression analysis, as implemented in GraphPad Prism. The map of study area and home range was drawn with ArcGIS 9.2 (ESRI, Inc., CA, US). In all cases the critical significance level was set at $P < 0.05$. All results are given as mean ± SD values.

Results

Use of space in the breeding season

The sizes of the home ranges of females and males changed dynamically throughout the breeding season. Based on 95% KDE methods, the size of the home ranges of males ($n = 7$, average 327.10 ± 218.10 m^2) was significantly larger than the home ranges of females ($n = 5$, average 50.01 ± 43.92 m^2) during the courtship and mating stages (April and May, 2017) (Mann–Whitney *U*-test = 1, $P = 0.005$; **Figure 2**). During the period of pregnancy and lactation (June and July, 2016), the home ranges of males ($n = 7$) averaged 139.10 ± 56.01 m^2 versus 87.66 ± 74.09 m^2 for the home ranges of females ($n = 6$) in June and 34.05 ± 38.62 m^2 versus 51.10 ± 53.74 m^2 in July; these differences in size were not significant (unpaired *t*-test: June, $t = 1.390$, *d.f.* = 11, $P = 0.192$; July, $t = 0.646$, *d.f.* = 11, $P = 0.532$; **Figure 2**). During the mating period, female-male overlapping area accounted for 7.46 ± 5.20% of the male nest area ($n = 5$, male and female pairs), while the overlapping area accounted for 61.92 ± 35.59% of the female nest area ($n = 5$, male–female pairs; **Figure 3F**). No overlap was found between the home ranges of females. In addition, we found that the nests of females were within the overlap of the home ranges of both males and females and we detected the radio-collar signals of the males that overlapped with the female's nest at the same time.

We observed the individual M697 travel above-ground from the position (a) of capture to position (b) after 72 h of radio-tracking and then, after staying at position (c) for two days, it traveled above-ground to position (d) and built a new home range. There was no overlap between the home ranges of other adult males (**Figure 3F**).

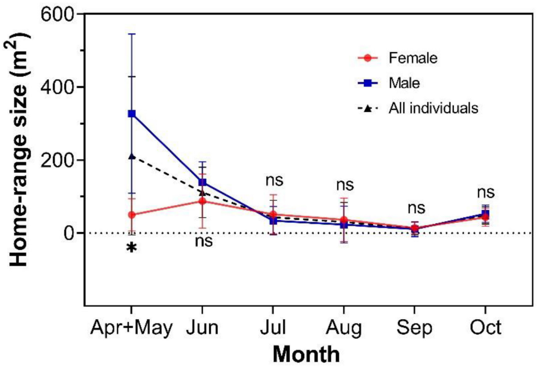

FIGURE 2
Comparison of the sizes of the home range of males and females in different months. The average sizes of the home range of all individuals are represented by the black dotted line. The asterisk indicates a significant difference, and ns means no significant difference in the home ranges of males and females. The results are presented as mean ± SD values.

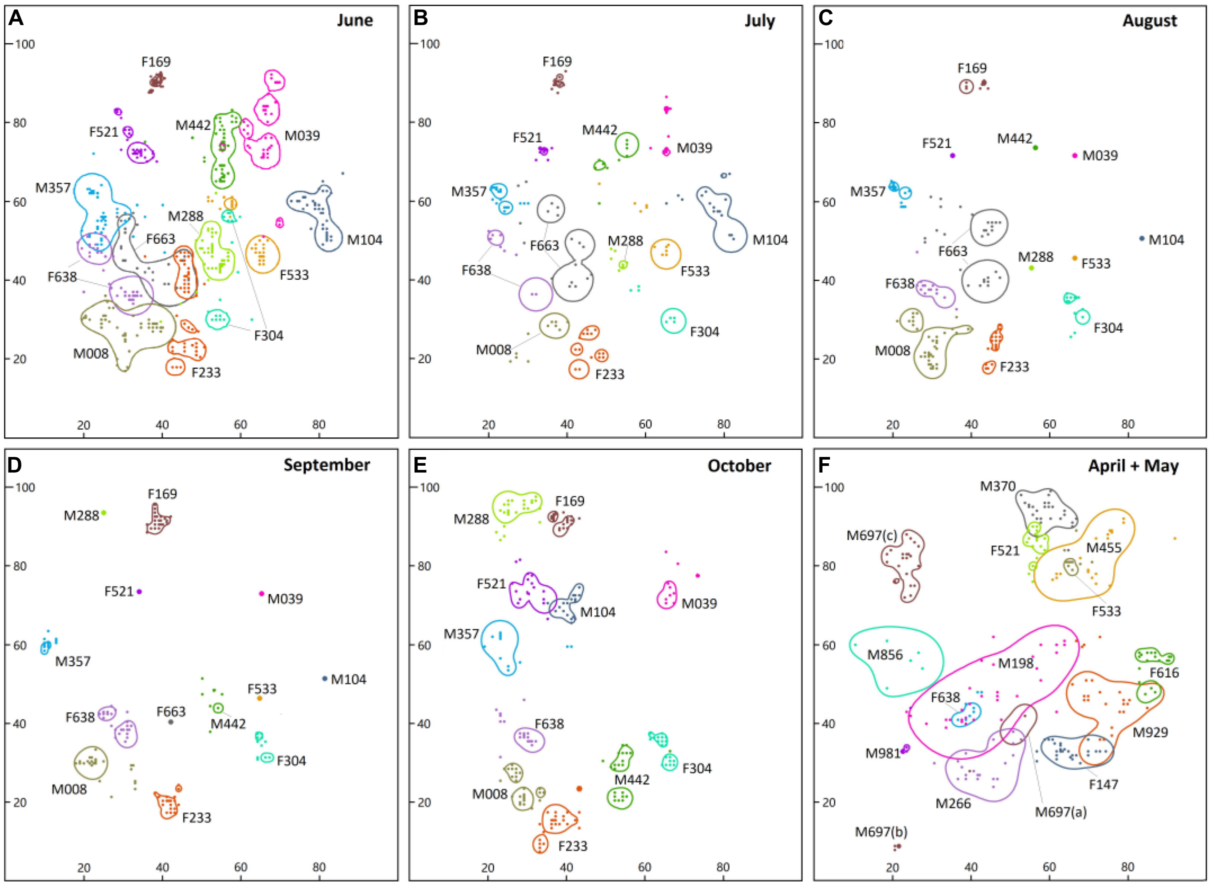

FIGURE 3
Representation of the size of the home range of radio-tracked *Myospalax baileyi* in an alpine meadow in eastern Qilian Mountain estimated by 95% KDE methods. Maps **(A–E)** show the continuous home ranges in 2016 (n = 13), whereas map **(F)** represents the home ranges of recaptured individuals in 2017 (n = 13). The area of the same color in a single month represents the home-range of one zokor. Coordinate axis units are meters.

In June, the home ranges of females overlapped those of males by 3.04 ± 4.75% (n = 7, female-male pairs), and the home ranges of males overlapped those of females by 4.48 ± 6.36% (n = 6, male-female pairs; Figure 3F). The home ranges of two pairs (F663-F233 and F638-F663) of the seven radio-tracked females overlapped by mean of 52.45 m^2 and 7.95 m^2 (Figure 3A), whereas only one pair in the six radio-tracked males overlapped by a mean of 2.4 m^2. There was no overlap between the home ranges of males, females, or female and male from July to September. In October, only one pair (M104–F521) had small overlaps (overlapped area: 2.78 m^2). The mean percentage of overlap of the home range of a single zokor with other individuals was 9.97 ± 15.13% in April and May and 32.55 ± 34.88% in June (Table 1); this difference in the proportion of overlap was significant (Mann–Whitney U-test = 30, P = 0.007). The average area of overlap of the home range of single individuals (16.56 ± 23.33 m^2) in June was smaller than the area of overlap in April and May (29.00 ± 22.45 m^2). Difference was found in these two months (t = 55, $d.f.$ = 23, P = 0.041). In June, no individual nest was occupied by other individuals, and no two individuals were wirelessly traced to the same coordinate point at the same time in the overlap region. No overlap was found between the home ranges of all individuals in July (Figure 3B and Table 1).

Use of space in the non-breeding season

The average sizes of the home ranges of males and females were small during the non-breeding season (Unpaired t-test: August, t_{11} = 0.421, P = 0.682; September, t_9 = 0.321, P = 0.754; October, t_9 = 0.663, P = 0.524; Figure 2). No overlap was found between the home ranges of males and females in August or September (Figures 3C,D). In October, only one pair (M104–F521) of the 11 individuals showed overlap (Figure 3E). The overlap area of the home ranges of M104 and F521 was only 2.78 m^2 (Table 1), accounting for 7.21 and 3.64% of their respective home ranges. However, the overlap area of the home ranges of M442 and F304 reached 14.2 m^2, accounting for 8 and 31.2% of their home ranges, respectively (Table 1). All the radio-tracked individuals stayed in their own nests from November 2016 to March 2017 and the size of the home range of all the individuals was <1 m^2.

New surface mounds in different months

The building of new mounds shows that the plateau zokors are expanding their existing tunnels or establishing a new tunnel system. The plateau zokors mainly built new mounds during the breeding season and in October during the non-breeding season (Table 2). The males built significantly more new surface mounds (average 10.1 ± 3.3) than the females (average 4.0 ± 1.9; $t = 3.736$, *d.f.* = 10, $P = 0.004$) during the courtship and mating stages in 2017. However, we found no significant difference between the number of new mounds built by males (9.5 ± 3.5) and females (8.6 ± 6.1) during the period of pregnancy and lactation in June ($t = 0.328$, *d.f.* = 11, $P = 0.749$). The number of new mounds built by females (average 7.0 ± 4.0) was significantly higher than the number of new mounds built by males in July (average 3.0 ± 1.1; $t = 2.385$, *d.f.* = 11, $P = 0.036$).

No new mound was visible above ground during the non-breeding season in August and only a few new mounds were seen in September (average: females ($n = 5$) 2.6 ± 1.7, males ($n = 6$) 2.5 ± 2.5; $t = 0.075$, *d.f.* = 9, $P = 0.941$). Some individuals did not even leave their own nests in August and September (Figures 3C,D). In October, the number of new surface mounds built by males averaged 9.3 ± 3.0 compared with 5.0 ± 1.2 new mounds built by females; this difference was significant ($t = 2.996$, *d.f.* = 9, $P = 0.015$). No new surface mound was built from November 2016 to March 2017 because the ground was frozen to 15 cm depth (Figure 1). The females captured in our study had a lower body mass than the males (average: females 146.6 ± 20.7g, males 257.7 ± 38.1g; $t = 6.831$, *d.f.* = 10, $P < 0.0001$) and no significant difference (average: females 179.0 ± 43.6g, males 225.8 ± 45.8g; $t = 1.892$, *d.f.* = 11, $P = 0.085$) was observed in 2016 (Table 2).

Movements within home ranges

Except for F304, the home ranges of other individuals in June had overlapped with their home ranges in July. The average overlaps accounted for 31.61 ± 25.72% (females 23.73 ± 29.40% and males 39.50 ± 21.03%) of the home ranges in June, but 87.86 ± 17.00% (females 93.72 ± 9.86% and males 82.01 ± 21.37%) of the home ranges in July (Figure 3). The home ranges of four males (M008, M357, M288 and M039) and two females (F533 and F521) in July were completely contained within their home ranges in June (Figure 4). There was no

TABLE 2 Individual ID (sex + transmitter), capture time (CT), body mass (BM) and number of new mounds (NNM) in different months.

2016								2017			
ID	CT	BM (g)	NNM					ID	CT	BM (g)	NNM
			Jun	Jul	Aug	Sep	Oct				Apr + May
F304	May 31	245	4	14	0	3	5	F147	April 6	128	7
F233	May 31	116	22	9	0	4	6	F533	April 17	140	4
F663	June 2	182	8	9	0	-	-	F638	April 17	182	3
F638	June 2	193	7	6	0	4	5	F521	April 18	138	2
F533	June 2	189	8	5	0	-	-	F616	April 21	145	4
F169	June 6	130	5	3	0	2	3	M370	April 6	208	13
F521	June 12	198	6	3	0	0	6	M266	April 6	320	12
M039	May 30	248	7	2	0	0	9	M697	April 17	319	11
M288	May 31	175	8	2	0	2	13	M198	April 18	270	9
M008	June 2	179	11	5	0	5	10	M455	April 18	261	7
M104	June 7	205	16	3	0	0	12	M929	April 25	272	14
M442	June 7	265	8	3	0	6	7	M856	April 27	280	5
M357	June 11	283	7	3	0	2	5				
Mean		200.6	9.0	5.2	0.0	2.5	7.4	Mean		221.9	7.6
SD		49.1	4.9	3.6	0	2.1	1	SD		73.3	4.1
M425[a]	June 11	170						M981[b]	April 6	150	2

[a] Male's radio-collar fell off 2 days of after release.

[b] M981 is a sub-adult.

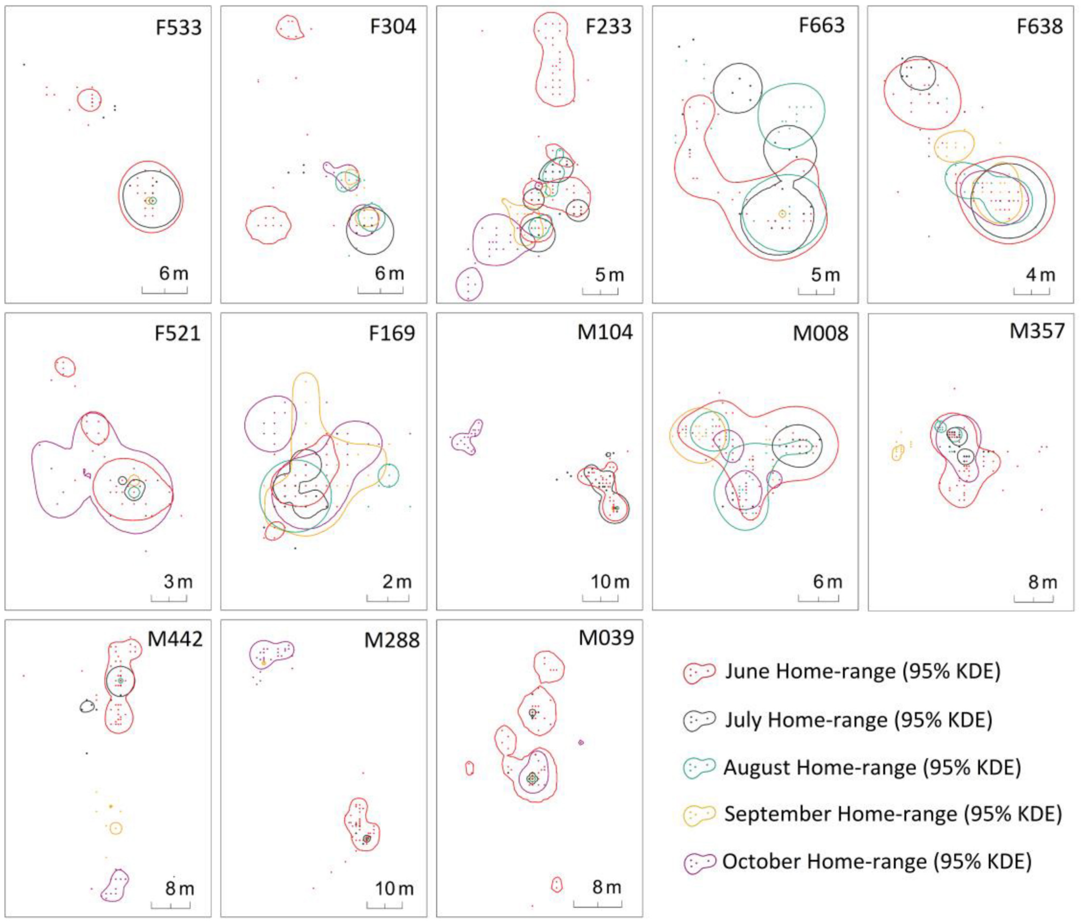

FIGURE 4
Monthly dynamics of each individual home range of *M. baileyi* from June to October 2016.

change in the position of the home range of all individuals at this stage.

Four of the seven females, except for the two individuals (F533 and F663) who shed their radio-collars, had overlapped ranges in June and October (**Figure 4**). The average overlap area of their home ranges was 18.83 ± 16.16 m^2 (range 2.86–34.93 m^2). The proportion of overlap area in the size of the home range in June was 50.80 ± 21.93% and 43.97 ± 20.17% in October (**Table 3**). The movements in the home range of four females (F233, F638, F521 and F169) from June to October was around their respectively original nest. F304 appeared to be building a new nest from July and almost gave up its June home range after October.

The home ranges of only three individual males (M008, M357, and M039) in October overlapped with those of June and the position of their respective nest did not change (**Figure 4**). The average area of overlap was 43.93 ± 32.18 m^2.

The average proportion of overlapping areas in a home range was 28.69 ± 19.39% and 93.76 ± 5.16% in June and October, respectively (**Table 3**). Three male zokors (M104, M442, and M288) completely opened up new home ranges, and established new nests in October and the closest distance from the old nest of their home ranges from October to June were 47.9 m, 53.0 m and 58.2 m, respectively 0.53

Relationships of home range dynamics with plant biomass and soil compaction

There was a significant difference between months on mean soil compaction ($\chi^2 = 18.65$, *d.f.* = 6, $P = 0.002$; **Figure 5B**) and there were statistically significant differences of the above- and below-ground plant biomass (Kruskal-Wallis statistic: above-ground plant biomass, $\chi^2 = 46.41$, *d.f.* = 6, $P < 0.001$; underground plant biomass, $\chi^2 = 43.00$, *d.f.* = 6, $P < 0.001$; **Figure 5A**).

The size of the home range in June for females, males and all individuals did not show a significant correlation with the above-ground plant biomass ($R^2 = 0.0001–0.2398$,

TABLE 3 Overlap area of the same individual's home range between June and October and the percentage of the overlap ratio in each month of 2016.

ID	Overlap area (m^2)	Proportion of overlap area to the size of the home range in June (%)	Proportion of overlap area to the size of the home range in October (%)
F233	2.86	2.39	4.65
F638	34.93	23.58	100
F169	6.26	82.37	30.3
F521	31.28	93.07	40.93
F304[a]	0	0	0
F663[b]	-	-	-
F533[b]	-	-	-
M008	39.12	16.45	88.27
M039	24.55	18.56	98.52
M357	86.12	51.05	94.5
M104[a]	0	0	0
M442[a]	0	0	0
M288[a]	0	0	0

[a]These zokors abandoned their original home ranges and established new ones in October.

[b]This zokor's radio-collar fell off in September and no radio-tracking data was obtained in October.

P = 0.265–0.983, **Figures 5C,D,E**), respectively. And there was no significant relationship between the size of the serial home ranges and soil compaction ($R^2 = 0.057–0.607$, $P = 0.004–0.567$). By contrast, the size of the home range of females, males and all individuals did show a significantly negative correlation with the underground plant biomass regardless of the mating period ($R^2 = 0.589–0.784$, $P = 0.002–0.023$; **Figures 5F,G,H**).

Discussion

Changes in home-range size

Our analysis of the variation in the size and overlap of the home range of plateaus zokors throughout the year suggests that physical contact and environmental characteristics influence the utilization of space by *M. baileyi*. Their home ranges showed dynamic changes in each month and constantly shifted in space. During the mating period, the home ranges of males were 6.5 times larger than the home ranges of females, although both females and males occupied similar sized home ranges at other times. The home ranges of females increased significantly in size in June after mating as the females extended their ranges to 1.8 times larger than these during the mating period to overlap with the home ranges of neighboring individuals. During the non-breeding season, the home ranges of most of the zokors remained within an optimum size and there was little overlap between individuals.

Although we did not excavate the burrow systems of the radio-tracked zokors, the size of their home range in the breeding season was 139.10 ± 56.01 m^2, not significantly different from the average size of the home range (122.7 ± 53.75 m^2) found by Zhou and Dou (1990) using radio-tracking. Studying the secretive habits of subterranean rodents in their natural state is often challenging and has previously been achieved by excavating entire burrow systems (Reichman et al., 1982; Davies and Jarvis, 1986). However, this method only provides snapshots of the tunnel system geometries of individuals or colonies that are then used to estimate the size of their home range (Cutrera et al., 2006). This method cannot perform long-term tracking of the nest domain. Longer studies of the utilization of space by subterranean rodents relies on registering new surface mounds and capture sites, but this method may be not appropriate for *M. baileyi* because two females (F663 and F169) expanded their home range in August (**Figure 3C**), although none of the individuals produced new mounds in this month.

Home range movement and overlap

Plateau zokors show dynamic changes in the size and overlap of their home ranges between intraspecific individuals in their natural habitat. A number of factors may explain the larger home ranges of male *M. baileyi* during the mating period. Many solitary subterranean rodents have a polygynous mating system (Cooper and Randall, 2007). Males try to gain access to multiple potential estrus females by expanding their burrow systems (Schoen and Kirchhoff, 1985), suggesting that females are a finite resource that probably affects the home ranges of males. Although the mating system of plateau zokor is still controversial (Zhang, 2007), we found that a male zokor (M929) entered the nest of different two females (F147 and F616) (**Figure 3F**), suggesting that *M. baileyi* may be a polygynous mating system. Intersexual differences in sizes of the home ranges of *M. baileyi* during the mating season also have been reported by Zhang et al. (1993). Our findings suggest that the home ranges of males remain relatively independent, which avoids competition during the mating period. Similar findings have been reported by Zhou and Dou (1990).

Solitary subterranean males may also search for a mate by traveling above ground; an earlier study of silvery mole rats reported that the long distances between the home ranges of mating partners ruled out an underground search for mates (Patzenhauerová et al., 2010) and showed that males probably look for estrus females by traveling above ground. In our study, a male zokor (M697) traveled above ground to two new locations and eventually established a new home range (with an area of 104 m^2) within 32 days (**Figure 3F**). The capture–mark–recapture rates of male plateau zokors recapture lower than males over three years during the mating seasons

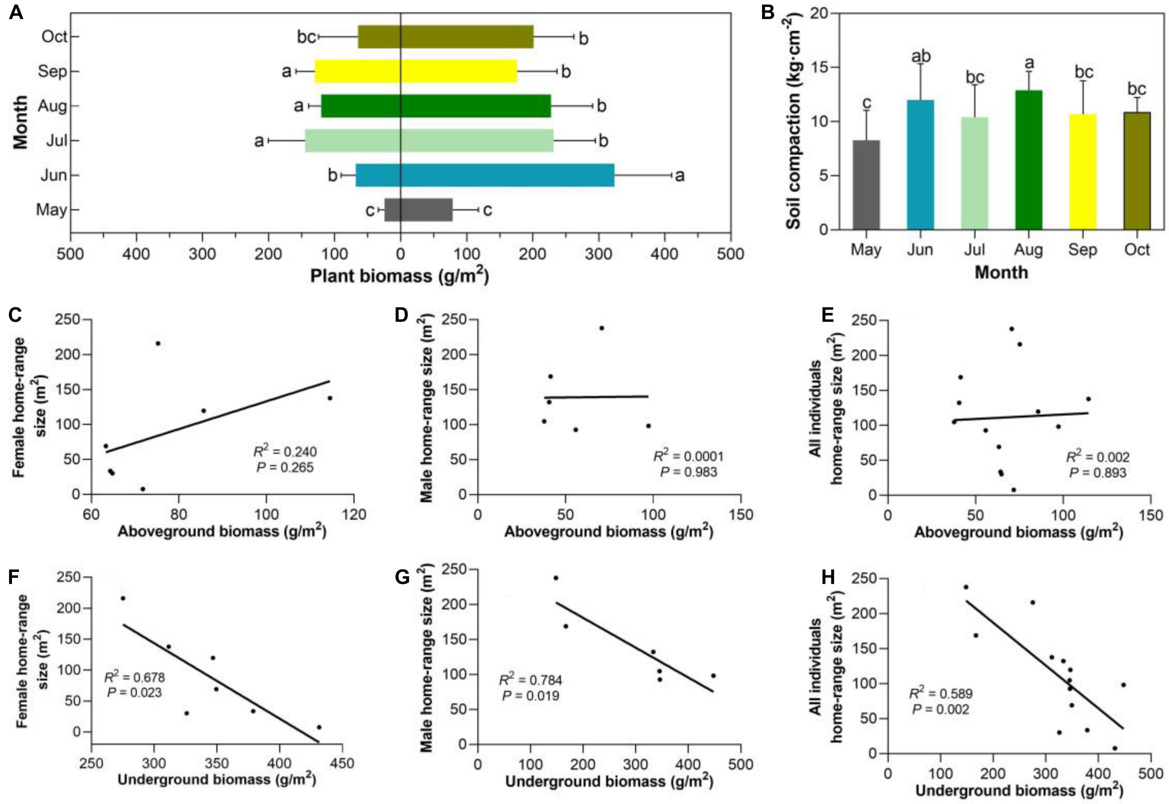

FIGURE 5
(A) Aboveground and underground plant biomass. The lowercase letters represent the differences between months. **(B)** Changes in soil compaction. Relationships between the aboveground (or underground) biomass with the average size of the home range of female **(C,F)**, male **(D,G)**, and all individuals **(E,H)** in June, 2016.

(Zhang et al., 1993) and zokor skulls were found in vomit from many raptors (Cui et al., 2003), which shows that male zokors may be traveling above ground in search of females even though they are at high risk of predation. This may be another factor in the changes in their home ranges.

The home ranges of females often overlapped multiple home ranges of neighbors one month after the mating period, which is unusual among solitary and aggressive subterranean rodents (Tassino et al., 2011). *M. baileyi* seems to show a high tolerance for close neighbors. At this stage, the average size of a female's home range was 1.8 times larger than during mating, which may be related to the large amount of food required during pregnancy and lactation; the size of the home range of males did not significantly change compared with the mating period. However, we estimated the core area of plateau zokors using the 50% KDE method and found that most individuals had no overlap, with only F233 and F663 overlapping by 3.66 m^2; their core areas remained relatively independent and exclusive. Because the core areas are the location of cache and food storage (Zhou and Dou, 1990; Zhang, 2007), the defense of the cache and food resource may be a major factor in the preference of the subterranean rodents for solitude.

The home ranges of the plateau zokor were significantly reduced at the end of the period of reproduction and in the non-breeding season and there was no overlap between the home ranges of most individuals. The home range at this time can even be synonymized to the territory (Nevo, 1999; Winner et al., 2018). This may be because maintaining and defending a large territory could have large costs (e.g., predation risk and energy expenditure). In addition, about half of the individuals did not expand into new spaces in August and September during the non-breeding season, when their activities were mainly concentrated on their own nests, similar to the observations of Zhou and Dou (1990). A decreased intensity of activity of plateau zokor during this period was also reported (Ji et al., 2018). The space utilization patterns of subterranean rodents may reflect an adaptation to the ambient temperature of the tunnels[11] to avoid overheating during the warm season (Rado et al., 1993).

Further evidence of the dynamic home ranges of the plateau zokor is a relatively low overlap between a single individual's successive home ranges within a five-month period in 2016 (**Figure 4**). Females usually have a fixed nest, with new tunnels expanding from the nest. In addition to rearing their young, the changes in the home range may also reflect a balance between foraging strategy and the costs of traveling (Fisher and Owens, 2012). As proposed by Bandoli (1981), Zuri and Terkel (1996) and Smallwood and Morrison (1999), the burrow systems of solitary subterranean rodents are constantly shifted by extending new tunnels while backfilling or abandoning older tunnels. Refilling tunnels and abandoning older burrows makes a significant contribution to the dynamics of the home range of subterranean rodents. For example, Thorne and Andersen (1990) tracked the development of a single burrow system of a pocket gopher (*Geomys bursarius*) for five months. The pocket gopher excavated at least 110 m of tunnel on top of the initial burrow system during that time, while 77 m of the burrow was backfilled and 32 m of an air tunnel were abandoned. Silvery mole rats, strictly solitary subterranean rodents from East Africa, have been reported to continuously rebuild their burrow systems and backfill 64% of older tunnels on average during an 80-day monitoring period (2000). Zhou and Dou (1990) described *M. baileyi* refilling their burrows with excavated soil, although this was not quantified.

Our radio-telemetry data and observations of new surface mounds suggest that male plateau zokors have a more efficient rate of digging. Unlike the home ranges of females, the home ranges of males are unstable in the long term and shift in space, especially during the non-breeding season. Three males in our study built completely new burrows in October (non-breeding season) (**Figure 4**). The mean distance between the new home ranges of these three males and their old nests was 53.03 ± 5.15 m and there was no sign of surface movement and no opening left in the older home ranges. This changes the location of the home range from that during the mating period. For example, M697 left an open cave at two abandoned sites during the mating season, traveling $>$ 88 m above ground. Our results contradict the hypothesis of Nevo (1999) that the home ranges of all subterranean mammals, once established and used for a breeding season, are essentially permanent for life. Shifting home ranges have been reported for some solitary subterranean rodents, such as blind (Zuri and Terkel, 1996) and silvery mole rats (Lesage et al., 2000). Shifts in the home ranges of males may occur to avoid inbreeding or to reduce the competition for food resources and the space caused by future increases in the population (Zhang, 2007). We did not simultaneously capture two zokors in the same burrow systems of females, suggesting that individuals born in the previous breeding season had built their own nests. The migration of the home ranges of solitary subterranean rodents may be a self-regulation strategy for controlling the population density (Galiano et al., 2014), although Šklíba et al. (2009) thought that changes in the home ranges are more likely to reflect a defensive strategy.

Factors influence home-range size

Most subterranean rodents, including plateau zokors, inhabit seasonal environments (Begall et al., 2007). The seasonal availability of food probably influences the use of space by subterranean rodents (Busch et al., 2000). Although the underground storage organs of plants are the main source of food for subterranean rodents, plateau zokor are known to consume the aerial parts of vegetation in addition to subterranean plant organs and even feed on the stems and leaves of grass and sedge which may affect their utilization of space (Wang et al., 2003). The burrowing and foraging activities of *M. baileyi* have a great influence on the soil properties and plant community (Zhang et al., 2014) and there is evidence of overgrazing in the alpine meadows of the Qinghai–Tibetan Plateau, leading to increased degradation of the grasslands (Zhang and Liu, 2003). We found that there was a significant positive correlation between the size of the home range of plateau zokors in different months and the underground biomass.

Soil compaction is also considered to be an ecological constraint in extending the burrows of subterranean rodents (Galiano et al., 2016; Kubiak et al., 2018). Although we found no significant difference, there was such a constraint between the two peaks (June and October) in the activity on the plateau and the average size of the home ranges was significantly larger in June than in October (Mann–Whitney U-test = 30, $P = 0.015$). This may be due to the abundance of underground biomass in June, whereas in October the zokors need to gather a lot of food from underground for the long winter because the above-ground parts of plants are almost inedible. *M. baileyi* did not expand its home range to obtain more food when there was less underground biomass. It is suggested that they may dig more branching tunnels for foraging, but this was not been proved in our study because we did not open the tunnels for long-term monitoring. Exploration of the surroundings of permanent primary tunnels by excavating short, branched foraging tunnels is a typical behavior in many subterranean rodents (Davies and Jarvis, 1986; Šklíba et al., 2009, Tassino et al., 2011). Additional studies, such as the impact of intraspecific seismic communication on the size of the home range, are required to further understand the use of space by *M. baileyi* and other solitary subterranean rodents.

Our monitoring data provide information on the size of the home range of *M. baileyi* in relation to mating behavior and food resources. We have shown that the home ranges of *M. baileyi* are dynamic and that males can change the location of their home range within one breeding cycle. Understanding the use of space by subterranean rodents and their dispersal are crucial to their conservation and management. More comprehensive information on burrows should be combined with subsequent excavation and mapping of burrow systems.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Ethics statement

Institutional guidelines for the use of animals were followed and the Animal Ethics Committee of Gansu Agricultural University approved the experimental procedure (approval no. GSC-IACUC-2018-0011), and local authorities' approval was also obtained.

Author contributions

LH and JZ designed the research project and conceived the manuscript. JZ and CJ wrote the first draft. JZ, KD, and BC collected the data. JZ and LW analyzed the data. JZ, CJ, KD, BC, LW, and LH reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.1041322/full#supplementary-material

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Influence of grazing on the activity pattern and temporal niche of two dominant rodent species in Alxa desert

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Grazing by large herbivores can potentially affect interspecific interactions between small herbivores by reducing the ecological fitness of animals. Desert rodents are important components in desert ecosystems and indicators of environmental change. Grazing reduces food resources, but rodents can decrease interspecific niche overlap by adaptive behavior. However, the key factors driving rodent behavioral activities and coexistence in the Alxa desert remains unstudied. We monitored population density and behavioral activities of Midday gerbil (*Meriones meridianus*) and northern three-toed jerboa (*Dipus sagitta*) in a grazing exclusion experiment in Alxa desert, Inner Mongolia, China, in 2017. We assessed the relationship between environmental factors (such as plant height, density, coverage, rainfall and temperature) and the behavioral activities of two coexisting rodent species. The results showed that: (1) In summer, grazing significantly reduced the activity time of gerbil and jerboa compared to that in grazing exclusion areas (gerbil: $F=5.98$, $p<0.05$, $\eta^2=0.22$; jerboa: $F=8.57$, $p<0.01$, $\eta^2=0.28$). Grazing reduced the temporal niche overlap with an obvious shifting of activity peaks between two species. (2) Grazing exclusion enhanced the temporal niche overlap between the two rodent species due to greater food availability which relieved inter-specific competition in each season. (3) Grazing strengthened the sensitivity of rodents to environmental changes in all seasons. These results indicated that grazing affected competition between the rodent species by altering vegetation conditions, which in turn affected the temporal niche and activity patterns of rodents.

KEYWORDS

desert, rodent, grazing exclusion, activity pattern, temporal niche, coexistence mechanisms

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1. Introduction

Animal behaviour underlies many critical ecological functions, including nutrient cycling, primary productivity, pathogen transmission and habitat provision (Gribben et al., 2009; Barber and Dingemanse, 2010; Palkovacs and Dalton, 2015; Fernandes et al., 2020; Rahelinirina et al., 2021). While every species is inherently linked to ecosystem function, keystone species or ecosystem engineers are far more critical for overall ecosystem function than other species (Wilson et al., 2020). Desert rodents are important components in desert ecosystems and indicators of environmental change (Wu et al., 2016). Human-induced habitat fragmentation has a clear impact on rodents' behavioral fitness (Goulson and Nicholls, 2022). As one of the most extensive land uses worldwide (Wang et al., 2019), grazing causes habitat loss and reduces wildlife (Loggins et al., 2019), which directly contributes to the reduction of biodiversity. In addition, the behavior of species and their interspecific relationships are altered through the influence of indirect and cascading effects (Azevedo et al., 2018). Grazing may change plant quality and food availability for small mammals, and subsequently may affect their population size, survival, body mass and reproduction (Li et al., 2016). The predation risk hypothesis states that trampling by livestock alters the height and coverage of vegetation, making it easier for predators to hunt, and that prey has greater ability to perceive predation risks in sparse, low vegetation (Pereira et al., 2012). Previous studies have shown that animal behavior changed only when human interference reached a certain threshold level. When animals believe that human activities are neither threatening nor beneficial, chronic or repeated interference can promote animal adaptability and tolerance (Smith et al., 2019).

Human activities, such as overgrazing, can reduce the ecological fitness of animals, and potentially affect interspecific interactions (Li et al., 2021). In this context, how to achieve regional coexistence through ecological niche allocation has become a hot issue in conservation biology and animal ecology in recent years (Bu et al., 2016). Temporal niche allocation for sympatric species is one of the main mechanisms supporting stable species coexistence (Sunarto et al., 2015). Animals can adjust their niche width and range through adaptation or behavioral changes to maximize benefits (Kronfeld-Schor and Dayan, 2003). Recently, a growing number of studies have suggested trade-offs in foraging ecology is a mechanism supporting coexistence (Ziv et al., 1993; Brown, 1996). Different activity patterns mean different evolutionary adaptations. Taxonomically close species possess similar activity patterns (Daan, 1981). Temporal partitioning may occur between distantly related species or even within various functional groups (Shuai et al., 2014). Both theoretical and empirical evidence support the hypothesis that interference competition stimulates temporal niche partitioning between sympatric species (Carothers and Jaksić, 1984; Ziv et al., 1993; Navarro-Castilla et al., 2017; Cepeda–Duque et al., 2021).

Direct human presence and indirect impacts on the surroundings of animals could alter their behaviour *via* changing population densities, top-down effects, bottom-up effects and effects on the physical environment (Wilson et al., 2020). Inter-specific competition of sympatric species affects their spatiotemporal activities (Odden et al., 2010; Ayala et al., 2021). In addition, many other biotic and abiotic factors may shift individual activity patterns. Such factors include predation risk (Yuan et al., 2017a; Loggins et al., 2019), intraspecific competition (Alanärä et al., 2001), food availability (Tang et al., 2020) and population size (Cui et al., 2015). However, the relative importance of multiple factors acting on rodent species coexistence and the drivers underlying the activity patterns of sympatric species remain unstudied.

Midday gerbil (*Meriones meridianus*) and northern three-toed jerboa (NTJ, *Dipus sagitta*) are typical nocturnal dominant rodent species, and coexist in the Alxa desert in China (Wu et al., 2016). They are omnivorous species with similar food habits (Yuan et al., 2018; Yang et al., 2020), and compete for food in the same habitats (Li et al., 2020). Midday gerbil is a quadrupedal forager with food-hoarding habits, while NTJ is a bipedal wanderer with larger home range, and does not store food (Zhao, 1964; Song and Liu, 1984; Shuai et al., 2016). The two species seem to compete, because of similar diets and nocturnal activity. However, they are sympatrically distributed in Alxa desert. These facts seem to violate the niche hypothesis. The climate in the desert is arid and food resources are extremely limited, so there is limited room for compromise in the nutritional niche between Midday gerbils and NTJ. However, the reasons for coexistence of the two species remain unknown. Is the coexistence of the two rodent species caused by rational nighttime allocation? Reasonable time allocation is of great significance to the survival and reproduction of animals (Ebensperger and Hurtado, 2005). Therefore, we hypothesized that: (1) There is strong competition between Midday gerbil and NTJ, and they coexist through temporal niche partitioning. (2) In grazing areas, lower food availability would prolong the activity time of rodents, and decreased vegetation coverage and the expansion of bare land would be beneficial to NTJ, while the vigilance behavior of Midday gerbils in grazing areas would increase. Meanwhile, the NTJ would reduce feeding behavior and increase vigilance in grazing exclusion areas because of dense vegetation. (3) Grazing exclusion would change the key pathways affecting temporal niche and behavioral patterns between sympatric Midday gerbil and NTJ.

2. Materials and methods

2.1. Study area

This study was conducted in southern Alxa Desert, Inner Mongolia, China, (E104°10′–105°30′, N37°24′–38°25′). Our study area has a continental climate with cold and dry winters and warm summers. Annual precipitation ranges from 75 mm to 215 mm, about 70% of which falls from June to September. The soil is grey desert soil and grey brown soil. The vegetation is sparse, and the plants are mainly xerophytic, super xerophytic and halophytic shrubs. Midday gerbil, NTJ, desert hamsters (*Phodopus roborovskii*) and Mongolian five-toed jerboa (*Orientallactaga sibirica*) are dominant small-mammal species (Yang et al., 2020). Other natural enemies include Eurasian eagle owl (Bubo bubo), Marbled polecat (*Vormela peregusna*), and Corsac fox (*Vulpes corsac*; Yuan et al., 2018).

In the study area, we established an experimental area using a randomized block design with 3 blocks and 2 treatments (Yuan et al., 2018), grazing exclusion (3 repeated plots) and grazing (3 repeated plots). Each block covered 240 ha and each treatment unit was 60 ha. The distance between each replicate plot is 300–1,000 m. The grazing exclusion area began to be fenced in 1997, and in the grazing plots grazing began in 1995 with a grazing intensity of 1.03 sheep/ha (Yuan et al., 2017a). Before the exclusion areas were set up, the treatment blocks experienced the same grazing events. Grazed and grazing exclusion plots were enclosed by standard sheep fencing (110 cm high) that prevented livestock movement in or out of plots. Other small mammals and natural enemies were allowed to enter and leave the study area freely. We conducted rodent surveys, plant community surveys, and infrared camera monitoring in May, July, September, and October in 2017. According to the local climatic conditions, we defined May as spring, July as summer, September as autumn, and October as early winter.

2.2. Rodent population density survey

A 7 × 8 trapping grid (1-ha) at a 15-m inter-trap distance was established at the center of each plot (60 ha). We placed one wire-mesh live trap (42 cm × 17 cm × 13 cm, Guixi Rodent Equipment Co. Ltd.) at each trap station. Traps were baited with fresh peanuts and checked twice (morning and afternoon) each day. We recorded the species name, sex, reproductive status, capture location, and weight (to the nearest 0.10 g) of the captured individuals. Each captured individual was marked with a passive integrated transponder (PIT; 2.12 mm × 8 mm, Guangzhou Ruimai Intelligent Technology Co. Ltd.) tag with a unique identification number (ID) injected under the pelage. In order to prevent rainfall and other sudden events, a wooden box (15 cm × 10 cm × 10 cm) was placed in the live trap to protect the rodents entering the cage (Yuan et al., 2018). The species and quantity of rodents captured every day in each plot were counted. We live-trapped rodents for four consecutive days in each season. The relative population of captured rodents was calculated according to the hundred cage capture rate (Wu et al., 2016).

2.3. Vegetation sampling

We randomly selected three 100 m^2 plots within each treatment unit to sample shrubs in each season. Within each 100 m^2 plot, we randomly placed three 1 m^2 quadrats to sample grasses and forbs. We measured the height, cover, density, abundance and biomass of shrubs and herbs (Yuan et al., 2018).

2.4. Camera trapping

After the rodent survey, the live trap was retrieved and an infrared camera trap experiment was conducted in the same grid. Before camera trapping, five points were randomly selected in each grid. We placed fresh peanut bait at 18:00 daily, checked and weighed the remaining peanuts at 06:00 in the next morning. We then chose the point with the highest number of harvested peanuts, and set up one infrared camera (ERE-E1B, Shenzhen Ereagle Technology Co. Ltd) at the selected point in each grid. In total, there were three camera points in both the grazing exclusion and grazing areas. The camera was fixed at a distance of 30 ~ 40 cm from the ground, so that it could monitor the feeding, handling and vigilant behavior of rodents as much as possible. Camera monitoring continued from 18:00 to 06:00. Camera trapping was conducted for four consecutive days each month. To effectively observe the foraging behavior of rodents, peanuts (about 10 g) were selected as attractants. Other studies on mammals have shown that the use of attractants does not affect abundance and density estimates, distance moved or activity patterns (Preez et al., 2014; Braczkowski et al., 2016). We recorded the shooting time of each video, species, appearance time, departure time, rodent behavior, and duration time of each behavior.

2.5. Statistical analyses

We used the overlap package in R 4.1.2, which was developed specifically for the visualization and activity patterns analysis for camera trapping data. Activity patterns were performed using kernel density estimates which describe the probability of a camera-trap event occurring at any given time (Linkie and Ridout, 2011). We then ran the procedure developed by Ridout and Linkie (2009) to determine the overlap coefficient of activity patterns between different rodent species. Overlap was defined as the area under the curve formed by taking the minimum of the two kernel density estimates at each point in time (Ridout and Linkie, 2009). We used $\Delta 4$ for large samples (>75 camera records; Azevedo et al., 2018), and the $\Delta 1$ estimator was used when the number of events was less than 50, Confidence intervals were generated using the basic0 estimation for 10,000 bootstrapped samples (Meredith and Ridout, 2017).

A rodent ethogram during foraging was obtained from previous observations (Shang, 2005; Wang et al., 2015; Table 1). We calculated the ratio of a given behavior duration to the total activity time in 1 day, and the duration of the active period (Azlan and Sharma, 2006; Bach et al., 2017). All the data involved in this paper have been Shapiro - Wilk tested, in which activity pattern ($p > 0.05$, $n = 12$) and population density ($p > 0.05$, $n = 12$) all

TABLE 1 Rodent ethogram.

Activity	Definition
Foraging	During the movement process, a series of actions such as crawling to search for food through smell, smelling the ground and vegetation, etc.
Feeding	In standing, sitting, squatting or lying down positions, grabbing food with both front claws to assist in gnawing and chewing food.
Storage	Taking food away from the feeding point and bringing it back to the nest or finding temporary shelter.
Vigilance	Observing the surrounding environment in a standing, sitting or squatting position, often accompanied by head twisting, for longer than 3 s.; or interrupting ongoing behaviors (such as running, feeding, foraging, etc.) and quickly leaving the feeding point.
Fighting	Behavior of two individuals biting, threatening, chasing, fleeing, trembling, avoiding, etc.

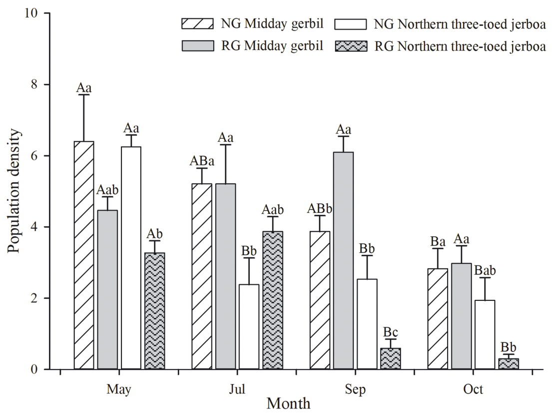

FIGURE 1
Seasonal changes in population densities of Midday gerbil, northern three-toed jerboa. NG represent the grazing exclusion area, RG represent the grazing area. Different capital letters (A and B) indicate that the population density of rodents varied significantly between seasons; different lowercase letters (a and b) indicate significant differences in population density of rodents in the same season.

conform to the normal distribution. In addition, the activity duration have been normalized (log $(n+1)$) prior to the analysis. Activity duration and patterns under different grazing treatments and seasonal difference was analyzed with the two-way repeated measures ANOVA using SPSS Statistics. SigmaPlot 12.0 was used for the plot.

We used random forest (RF) to evaluate the relationship of environmental factors with the duration of each behavior. RF analysis was performed to identify the major driving factors and to estimate the importance of the variables. We used percentage increases in mean squared error (MSE) of variables, where a higher MSE% value implied a more important variable. Analysis was conducted using the "rfPermute" package in R (Jiao et al., 2021). Calculation of the correlation coefficient and the visualization of the heat map are all realized through "link ET" (Huang, 2021).

3. Results

A total of 6,629 videos were shot over the study. The total shooting time was 4,917 min, and the total duration of rodent activity was 1,448 min. Five rodent species, including Midday gerbil, NTJ, desert hamsters, Mongolian five-toed jerboa, and Alashan ground squirrel (*Spermophilus alaschanicus*) were captured by the cameras.

3.1. Population density

There were significant seasonal differences in the population($F_{(3,44)}=9.42$, $p<0.01$, $\eta^2=0.36$). In the grazing exclusion plots, the population density of Midday gerbil decreased from spring to early winter ($F_{(3,44)}=12.34$, $p<0.001$), and NTJ population in spring was significantly higher than in other seasons ($F_{(3,44)}=20.64$, $p<0.001$). The population density of Midday gerbil was significantly higher than that of NTJ in summer ($F_{(3,44)}=3.391$, $p<0.05$, Figure 1).

In the grazing area, there was no significant difference in the population density of Midday gerbil in different seasons ($F_{(3,44)}=0.44$, $p>0.05$), and the NTJ populations in spring and summer were significantly higher than in autumn and early winter ($F_{(3,44)}=52.36$, $p<0.001$). The population density of Midday gerbil was significantly higher than that of NTJ in autumn ($F_{(3,44)}=23.874$, $p<0.001$, Figure 1).

Grazing exclusion promoted higher NTJ population density than in the grazing treatment, both in spring ($F_{(3,44)}=4.314$, $p<0.05$) and autumn ($F_{(3,44)}=23.874$, $p<0.001$). Grazing exclusion induced higher population density of Midday gerbil than that in the grazing area in early winter ($F_{(3,44)}=6.086$, $p<0.01$, Figure 1).

3.2. Daily activity pattern

The activity time of the two species had a large overlap, and there were seasonal differences in the daily activity pattern. In spring, the niche overlap index of Midday gerbil with NTJ was higher than other seasons, both in the grazing exclusion and the grazing areas (Δgrazing exclusion = 0.8994, Δgrazing = 0.9549). The activity peak of the two species was concentrated at 20:00. Grazing promoted higher activity intensity of both species compared to the grazing exclusion area (Figures 2A,E). In summer, the niche overlap index of the two species was 0.8890 in the grazing exclusion area (Figure 2B). Grazing induced a clear staggered peak pattern in the daily activity rhythm pattern of the two species, and the overlap index of two species was 0.6329 (Figure 2F). In autumn, the niche overlap index of the two species was 0.8496 in the grazing exclusion area. There was an obvious staggered activity peak pattern in the grazing area ($\Delta = 0.7957$, Figures 2C,G). In early winter, the overlap index of the two species was 0.7195 in the grazing exclusion area (Figure 2D). There was a staggered peak pattern in the grazing area and the niche overlap index of the two species was 0.8329 (Figure 2H).

3.3. Activity duration of rodent species

There were significant seasonal differences in the activity duration of Midday gerbil and NTJ (Midday gerbil: $F_{(3,44)} = 11.87$, $p < 0.001$, $\eta^2 = 0.35$; NTJ: $F_{(3,44)} = 12.26$, $p < 0.001$, $\eta^2 = 0.358$). In the grazing exclusion area, Midday gerbil had the longest activity duration in spring and autumn ($F_{(3,44)} = 2.94$, $p > 0.05$, $\eta^2 = 0.31$), while the NTJ activity duration reduced in early winter ($F_{(3,44)} = 7.39$, $p < 0.01$, $\eta^2 = 0.53$). In the grazing area, Midday gerbil and NTJ were active longer in spring and autumn than that in other seasons (Midday gerbil: $F_{(3,44)} = 6.55$, $p < 0.01$, $\eta^2 = 0.49$; NTJ: $F_{(3,44)} = 4.52$, $p < 0.05$, $\eta^2 = 0.40$). In summer, the Midday gerbil and NTJ activity duration was significantly longer in the grazing exclusion area than in the grazing area (Midday gerbil: $F_{(1,22)} = 5.98$, $p < 0.05$, $\eta^2 = 0.22$; NTJ: $F_{(1,22)} = 8.57$, $p < 0.01$, $\eta^2 = 0.28$; Figure 3).

3.4. Time allocation pattern

In the grazing exclusion area, Midday gerbil showed significant foraging behavior in spring and summer ($F_{(3,44)} = 6.82$, $p < 0.01$), storing behavior in autumn and early winter ($F_{(3,44)} = 10.48$, $p < 0.01$), and most fighting behaviour in autumn ($F_{(3,44)} = 7.89$, $p < 0.01$; Table 2). NTJ allocated significantly more time for foraging behavior in spring than that in early winter ($F_{(3,44)} = 5.60$, $p < 0.01$), and showed notable vigilance in spring ($F_{(3,44)} = 5.20$, $p < 0.01$). There were no significant seasonal differences in storing, feeding and fighting behavior of NTJ among seasons (Storing: $F_{(3,44)} = 0.86$, $p > 0.05$; Feeding: $F_{(3,44)} = 0.51$, $p > 0.05$; Fighting: $F_{(3,44)} = 2.14$, $p > 0.05$; Table 3). In the grazing area, Midday gerbils showed significant foraging behavior and vigilance behaviour in spring and summer (Foraging: $F_{(3,44)} = 13.47$, $p < 0.01$; Vigilance: $F_{(3,44)} = 3.89$, $p < 0.05$), storing behavior in autumn ($F_{(3,44)} = 4.69$, $p < 0.01$), and feeding behavior peaked in spring ($F_{(3,44)} = 9.64$, $p < 0.01$; Table 2). In the grazing area, NTJ showed more vigilance behavior in spring ($F_{(3,44)} = 4.66$, $p < 0.01$), and no seasonal differences in their other behaviors (Foraging: $F_{(3,44)} = 2.42$, $p > 0.05$; Storing: $F_{(3,44)} = 1.15$, $p > 0.05$; Feeding: $F_{(3,44)} = 0.76$, $p > 0.05$; Vigilance: $F_{(3,44)} = 2.16$, $p > 0.05$; Table 3).

In spring, the vigilance behavior of NTJ in the grazing exclusion area was significantly longer than that in the grazing area ($F_{(1,22)} = 4.95$, $p < 0.05$). In summer, the storing behavior of NTJ in the grazing exclusion area was significantly longer than that in the grazing area($F_{(1,22)} = 4.63$, $p < 0.05$; Table 3). In autumn, the fighting behavior of Midday gerbil in the grazing exclusion area was significantly longer than that in the grazing area ($F_{(1,22)} = 5.99$, $p < 0.05$; Table 2). In early winter, Midday gerbils spent more time on foraging, storing, vigilance and fighting behaviors in the grazing exclusion area than in the grazing area (Foraging: $F_{(3,44)} = 5.58$, $p < 0.05$; Storing: $F_{(3,44)} = 9.42$, $p < 0.01$; Vigilance: $F_{(3,44)} = 4.83$, $p < 0.05$; Fighting: $F_{(3,44)} = 11.63$, $p < 0.01$; Table 2).

3.5. Influencing factors on rodent behavior

Environmental humidity (Hum) and precipitation (Pre) reduced the activity overlap of the two dominant rodent species in the desert area (Grazing: $R_{Hum} = -0.38$, $p < 0.05$; $R_{Pre} = -0.58$, $p < 0.001$; Grazing exclusion: $R_{Hum} = -0.67$, $p < 0.001$; $R_{Pre} = -0.55$, $p < 0.001$; Figure 4). In the grazing exclusion area, environmental factors had non-significant influences on the daily activity rhythm overlap index (Overlap), vigilance behavior of Midday gerbil (M.Vig), and the foraging behavior (D.For), vigilance behavior (D.Vig) and activity duration (D.Dur) of NTJ. In the grazing exclusion area, the key factor affecting animal behaviors was NTJ population density ($R_{Overlap} = 0.95$, $p < 0.001$; $R_{M.Vig} = 0.51$, $p < 0.001$; $R_{D.For} = 0.40$, $p < 0.01$; $R_{D.Vig} = 0.46$, $p < 0.001$; $R_{D.Dur} = 0.44$, $p < 0.01$; Figure 4A). In the grazing area, environmental factors had non-significant influences on the daily activity rhythm overlap index (Overlap), the behaviors of Midday gerbils (M.For, M.Sto, M.Fee, M.Vig, M.Fig, M.Dur), and the foraging and storing behaviors of NTJ (D.For, D.Sto). Key factors affecting the activity overlap of species were shrub density and coverage. The key factor affecting the behaviors of Midday gerbils was their population size ($R_{M.For} = 0.48$, $p < 0.001$; $R_{M.Sto} = 0.54$, $p < 0.001$; $R_{M.Fee} = 0.52$, $p < 0.01$; $R_{M.Vig} = 0.43$, $p < 0.01$; $R_{M.Fig} = 0.43$, $p < 0.01$; $R_{M.Dur} = 0.48$, $p < 0.01$). The population density of Mongolian five-toed jerboa was the key factor affecting vigilance behavior of NTJ ($R_{D.Vig} = 0.31$, $p < 0.05$). Grass density was a key factor affecting the storing behavior of NTJ ($R_{D.Vig} = 0.48$, $p < 0.001$). Grass height was a key factor affecting the vigilance behavior and activity duration of NTJ ($R_{D.Dur} = 0.34$, $p < 0.05$). Rodent activities in grazing areas are affected by more biotic and abiotic factors than activities in grazing exclusion areas (Figure 4B).

4. Discussion

4.1. Coexistence strategies of two rodent species

The activity rhythm overlap index reflects the degree of competition and temporal niche separation between two

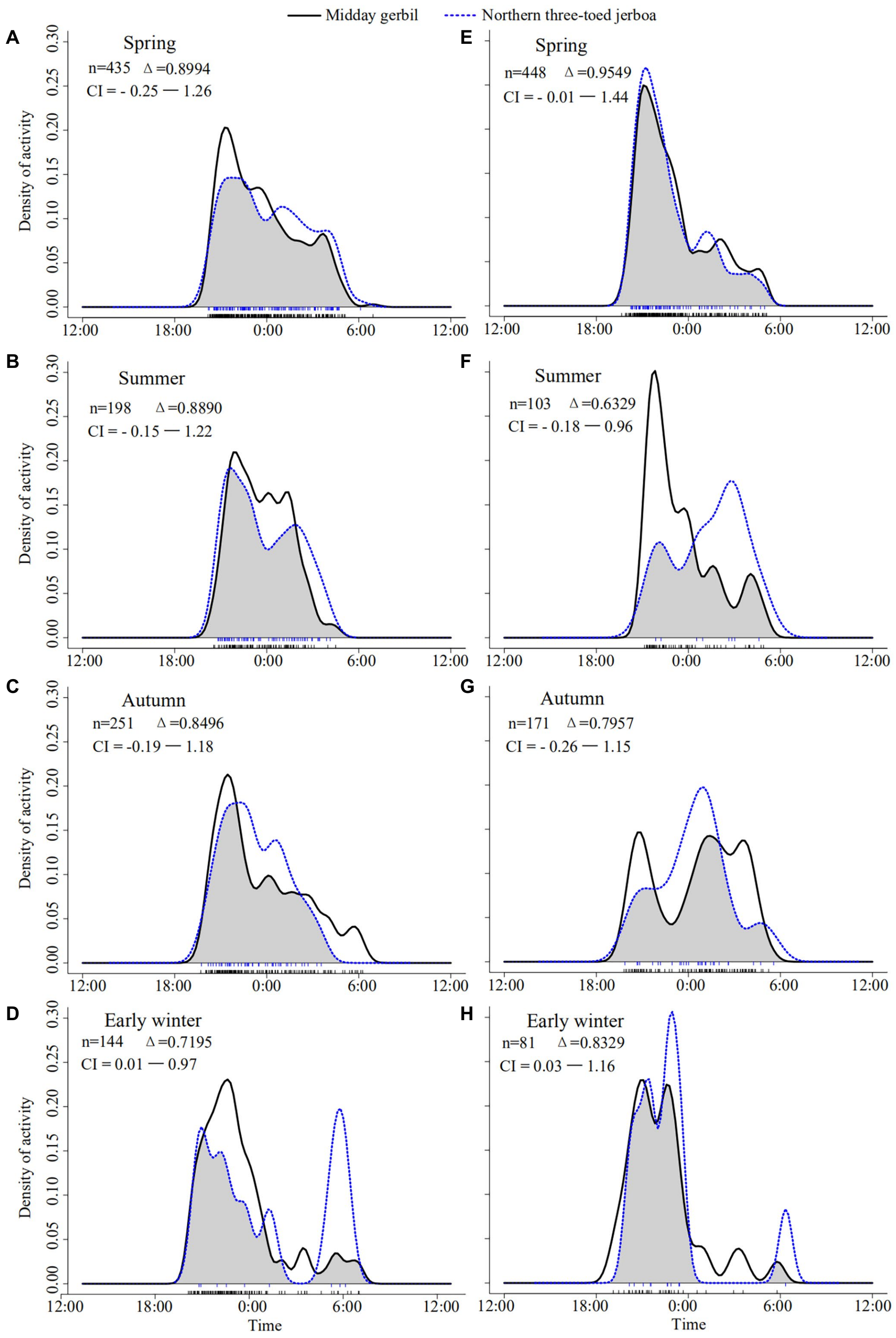

FIGURE 2
Daily activity patterns of Midday gerbil and northern three-toed jerboa. The gray area shows the overlap between activities of the two species and the notches at the bottom of each image illustrate the density of camera-trap events. **(A–D)** represent the daily activity patterns of Midday gerbil and northern three-toed jerboa in the grazing exclusion area in May, July, September and October, respectively. **(E–H)** represent the daily activity patterns of Midday gerbil and northern three-toed jerboa in the grazing area in May, July, September and October, respectively. The number of independent camera-trap records (*n*), classification of daily activity patterns, estimates of coefficients of overlapping (Δ) of Midday gerbil with northern three-toed jerboa and 95% confidence intervals (CI) are given in each plate.

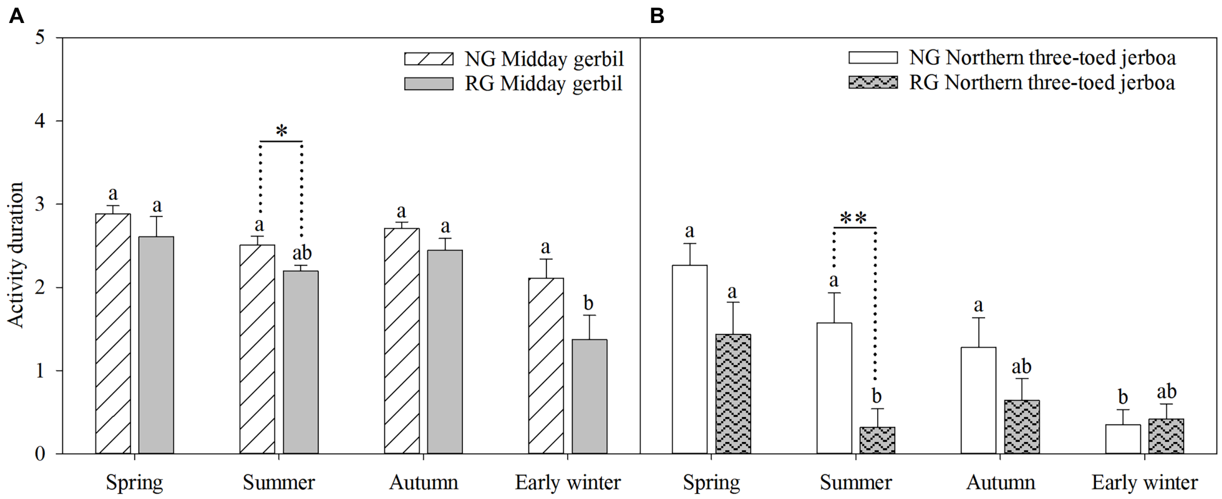

FIGURE 3
Activity duration of Midday gerbil **(A)**, northern three-toed jerboa **(B)**. NG represent the grazing exclusion area, RG represent the grazing area. Different lowercase letters (a and b) indicate that the activity duration of rodents varied significantly between seasons. Asterisk denotes statistically significant differences, $*p<0.05$, $**p<0.01$.

species (Sunarto et al., 2015). The activity patterns of Midday gerbil and NTJ in the grazing exclusion area showed high similarity in different seasons, and the separation degree in the temporal niche between two species was low. Studies in the same study area showed that food availability in the grazing exclusion area was significantly higher than that in the grazing area (Yang et al., 2020). Midday gerbils and NTJ have a similar diet, but higher food availability alleviated the pressure of food competition. Thus, despite high temporal niche overlap of Midday gerbil with NTJ in the grazing exclusion area, they can coexist in the grazing exclusion area due to sufficient food and the lack of large competitors such as livestock. In the grazing areas, the activity patterns of Midday gerbil and NTJ begin to stagger significantly in summer, with a separation of the temporal ecological niches of the two species. This is because summer is a critical period for the growth and development of individual herbivores and determines breeding success (Wang et al., 2021). Interspecific competition among animals is particularly intense in summer, and the presence of competitors can influence the behavioral patterns of species (Ritchie and Johnson, 2010). In contrast, temporal niche separation of species usually occurs in situations where alternative mechanisms of coexistence cannot operate (spatial and trophic niche; Harrington et al., 2009; Anna et al., 2015). To reduced competition, Midday gerbil and NTJ have adopted a strategy of temporal niche separation to achieve coexistence. Therefore, hypothesis (1) was supported, as desert rodents with similar a diet used a strategy of temporal (grazing areas) niche separation to reduce niche overlap and achieve coexistence.

4.2. Time allocation for the two dominant rodent species

There were seasonal differences in the activity duration of Midday gerbil and NTJ. Their activity durations were significantly higher in spring. In spring, rodents have a higher energy requirement caused by limited food availability (Yang et al., 2020), lower ambient temperatures (Sun, 2006), and frequent reproduction behavior (Wei et al., 1996; Topilko et al., 2022). In summer, the activity duration of Midday gerbil and NTJ was significantly curtailed, and the high temperatures reduced the animals' foraging efficiency (Plessis et al., 2012). Moreover, food was sufficiently abundant to meet rodents' energy needs and there was no need to let itself under the risk of predation. Desert rodents maximize their foraging efficiency by reducing the time spent outside their burrows to reduce water loss (Hut et al., 2012) and predation risk, which facilitates their adaptation to arid desert environments (Ji et al., 2021). Due to the higher predation risk from low vegetation coverage for quadrupedal gerbils in the grazing area (Pereira et al., 2012), the activity duration of Midday gerbil in the grazing area in summer was significantly lower than that in the grazing exclusion area. In autumn, there were differences in the activity allocation of the two dominant rodents, because they had different overwintering patterns (Shuai et al., 2016; Yuan et al., 2018; Yang et al., 2020). Midday gerbils are non-hibernators, so they increased food storing behavior and showed confrontation behavior in autumn. NTJ are hibernators (Zhao, 1964). Because they need to store body fat reserves prior to hibernation, and fighting behaviour can be energy draining, so fighting behaviour

TABLE 2 Difference analysis of time distribution ratio of Midday gerbil.

Behavior	Month		Grazing exclusion (mean±SE)	Grazing (mean±SE)	Grazing disturbance
Foraging	Simple Effects	May	57.705 ± 3.462 Aa	58.438 ± 2.889 Aa	$F_{(1,22)} = 0.026, p > 0.05$
		July	59.530 ± 9.099 Aa	65.166 ± 7.165 Aa	$F_{(1,22)} = 0.237, p > 0.05$
		September	24.150 ± 3.484 Ab	33.058 ± 7.215 Ab	$F_{(1,22)} = 1.236, p > 0.05$
		October	38.846 ± 7.640 Aab	13.689 ± 7.431 Bb	**$F_{(1,22)} = 5.572, p < 0.05$**
		Season	**$F_{(3,44)} = 6.818, p < 0.01$**	**$F_{(3,44)} = 13.472, p < 0.01$**	
	Repeated Measures	Season	**$F = 17.562, p < 0.001, \eta^2 = 0.444$**		
		Grazing	$F = 0.288, p > 0.05, \eta^2 = 0.013$		
		Season × Grazing	$F = 2.912, p > 0.05, \eta^2 = 0.117$		
Storage	Simple Effects	May	25.356 ± 3.949 Ab	21.899 ± 3.261 Aab	$F_{(1,22)} = 0.456, p > 0.05$
		July	13.347 ± 3.486 Ab	27.289 ± 8.095 Aab	$F_{(1,22)} = 2.502, p > 0.05$
		September	57.235 ± 7.371 Aa	46.962 ± 8.961 Aa	$F_{(1,22)} = 0.784, p > 0.05$
		October	40.561 ± 7.476 Aa	11.029 ± 6.059 Bb	**$F_{(1,22)} = 9.418, p < 0.01$**
		Season	**$F_{(3,44)} = 10.477, p < 0.01$**	**$F_{(3,44)} = 4.690, p < 0.01$**	
	Repeated Measures	Season	**$F = 10.474, p < 0.001, \eta^2 = 0.323$**		
		Grazing	$F = 2.473, p > 0.05, \eta^2 = 0.101$		
		Season × Grazing	**$F = 3.968\ p < 0.05, \eta^2 = 0.153$**		
Feeding	Simple Effects	May	6.879 ± 1.539 Aa	11.580 ± 3.549 Aa	$F_{(1,22)} = 1.476, p > 0.05$
		July	7.386 ± 3.965 Aa	0.822 ± 0.729 Ab	$F_{(1,22)} = 2.651, p > 0.05$
		September	9.176 ± 6.185 Aa	0.108 ± 0.108 Ab	$F_{(1,22)} = 2.149, p > 0.05$
		October	1.223 ± 0.965 Aa	0.094 ± 0.094 Ab	$F_{(1,22)} = 1.356, p > 0.05$
		Season	$F_{(3,44)} = 0.826, p > 0.05$	**$F_{(3,44)} = 9.642, p < 0.01$**	
	Repeated Measures	Season	$F = 2.845, p > 0.05, \eta^2 = 0.115$		
		Grazing	$F = 2.007, p > 0.05, \eta^2 = 0.084$		
		Season × Grazing	$F = 2.147, p > 0.05, \eta^2 = 0.089$		
Viligance	Simple Effects	May	9.715 ± 1.248 Aa	7.668 ± 1.098 Aa	$F_{(1,22)} = 1.517, p > 0.05$
		July	3.032 ± 1.170 Ab	6.569 ± 3.033 Aa	$F_{(1,22)} = 1.184, p > 0.05$
		September	6.540 ± 1.519 Aab	3.004 ± 1.254 Aab	$F_{(1,22)} = 3.223, p > 0.05$
		October	2.206 ± 0.899 Ab	0.188 ± 0.188 Bb	**$F_{(1,22)} = 4.825, p < 0.05$**
		Season	**$F_{(3,44)} = 7.887, p < 0.01$**	**$F_{(3,44)} = 3.893, p < 0.05$**	
	Repeated Measures	Season	**$F = 8.792, p < 0.05, \eta^2 = 0.286$**		
		Grazing	$F = 0.784, p > 0.05, \eta^2 = 0.034$		
		Season × Grazing	$F = 2.279, p > 0.05, \eta^2 = 0.094$		
Fighting	Simple Effects	May	0.344 ± 0.106 Ab	0.415 ± 0.162 Aa	$F_{(1,22)} = 0.134, p > 0.05$
		July	0.039 ± 0.026 Ab	0.155 ± 0.134 Aa	$F_{(1,22)} = 0.712, p > 0.05$
		September	2.899 ± 1.095 Aa	0.201 ± 0.132 Ba	**$F_{(1,22)} = 5.985, p < 0.05$**
		October	0.497 ± 0.146 Ab	0.000 ± 0.000 Ba	**$F_{(1,22)} = 11.628, p < 0.01$**
		Season	**$F_{(3,44)} = 5.626, p < 0.01$**	$F_{(3,44)} = 1.900, p > 0.05$	
	Repeated Measures	Season	**$F = 5.441, p < 0.05, \eta^2 = 0.198$**		
		Grazing	**$F = 7.049, p < 0.05, \eta^2 = 0.243$**		
		Season × Grazing	**$F = 5.430, p < 0.05, \eta^2 = 0.198$**		

Different uppercase letters (A and B) indicate differences in grazing practices, and different lowercase letters (a and b) indicate significant seasonal differences. Bold values indicate the significant difference.

TABLE 3 Difference analysis of time distribution ratio of northern three-toed jerboa.

Behavior	Month		Grazing exclusion (mean±SE)	Grazing (mean±SE)	Grazing disturbance
Foraging	Simple Effects	May	57.602 ± 8.447 Aa	47.388 ± 10.708 Aa	$F_{(1,22)} = 0.561, p > 0.05$
		July	32.119 ± 11.180 Aab	13.086 ± 9.210 Aa	$F_{(1,22)} = 1.727, p > 0.05$
		September	24.050 ± 10.301 Ab	39.270 ± 12.309 Aa	$F_{(1,22)} = 0.899, p > 0.05$
		October	6.674 ± 4.250 Ab	18.657 ± 9.561 Aa	$F_{(1,22)} = 1.312, p > 0.05$
		Season	**$F_{(3,44)} = 5.598, p < 0.01$**	$F_{(3,44)} = 2.418, p > 0.05$	
	Repeated Measures	Season	**$F = 6.818, p < 0.01, \eta^2 = 0.225$**		
		Grazing	$F = 0.005, p > 0.05, \eta^2 = 0.000$		
		Season × Grazing	$F = 1.554, p > 0.05, \eta^2 = 0.066$		
Storage	Simple Effects	May	3.665 ± 1.193 Aa	2.353 ± 1.984 Aa	$F_{(1,22)} = 0.321, p > 0.05$
		July	0.419 ± 0.195 Aa	0.000 ± 0.000 Ba	**$F_{(1,22)} = 4.626, p < 0.05$**
		September	1.011 ± 0.536 Aa	0.798 ± 0.581 Aa	$F_{(1,22)} = 0.072, p > 0.05$
		October	6.702 ± 6.036 Aa	0.000 ± 0.000 Aa	$F_{(1,22)} = 1.233, p > 0.05$
		Season	$F_{(3,44)} = 0.864, p > 0.05$	$F_{(3,44)} = 1.152, p > 0.05$	
	Repeated Measures	Season	$F = 0.969, p > 0.05, \eta^2 = 0.042$		
		Grazing	$F = 1.456, p > 0.05, \eta^2 = 0.062$		
		Season × Grazing	$F = 0.950, p > 0.05, \eta^2 = 0.041$		
Feeding	Simple Effects	May	20.730 ± 6.616 Aa	13.565 ± 4.758 Aa	$F_{(1,22)=}0.773, p > 0.05$
		July	13.074 ± 6.017 Aa	3.481 ± 3.481 Aa	$F_{(1,22)} = 1.905, p > 0.05$
		September	23.061 ± 10.035 Aa	8.961 ± 4.263 Aa	$F_{(1,22)} = 1.673, p > 0.05$
		October	11.624 ± 8.358 Aa	13.512 ± 8.203 Aa	$F_{(1,22)} = 0.026, p > 0.05$
		Season	$F_{(3,44)} = 0.505, p > 0.05$	$F_{(3,44)} = 0.760, p > 0.05$	
	Repeated Measures	Season	$F = 0.742, p > 0.05, \eta^2 = 0.225$		
		Grazing	$F = 1.854, p > 0.05, \eta^2 = 0.078$		
		Season × Grazing	$F = 0.528, p > 0.05, \eta^2 = 0.023$		
Viligance	Simple Effects	May	8.823 ± 2.381 Aa	3.013 ± 1.072 Ba	**$F_{(1,22)} = 4.948, p < 0.05$**
		July	4.380 ± 2.313 Aab	0.034 ± 0.034 Aa	$F_{(1,22)} = 3.532, p > 0.05$
		September	1.296 ± 0.898 Ab	0.972 ± 0.923 Aa	$F_{(1,22)} = 0.063, p > 0.05$
		October	0.000 ± 0.000 Ab	1.164 ± 0.935 Aa	$F_{(1,22)} = 1.550, p > 0.05$
		Season	**$F_{(3,44)} = 5.204, p < 0.01$**	$F_{(3,44)} = 2.163, p > 0.05$	
	Repeated Measures	Season	**$F = 7.479, p < 0.01, \eta^2 = 0.254$**		
		Grazing	$F = 3.958, p > 0.05, \eta^2 = 0.153$		
		Season × Grazing	**$F = 3.501, p < 0.05, \eta^2 = 0.137$**		
Fighting	Simple Effects	May	0.847 ± 0.353 Aa	0.347 ± 0.138 Aa	$F_{(1,22)} = 1.739, p > 0.05$
		July	0.008 ± 0.008 Aa	0.067 ± 0.067 Ab	$F_{(1,22)} = 0.771, p > 0.05$
		September	0.584 ± 0.460 Aa	0.000 ± 0.000 Ab	$F_{(1,22)} = 1.607, p > 0.05$
		October	0.000 ± 0.000 Aa	0.000 ± 0.000 Ab	——
		Season	$F_{(3,44)} = 2.142, p > 0.05$	**$F_{(3,44)} = 4.662, p < 0.01$**	
	Repeated Measures	Season	$F = 3.156, p > 0.05, \eta^2 = 0.125$		
		Grazing	$F = 3.722, p > 0.05, \eta^2 = 0.145$		
		Season × Grazing	$F = 1.145, p > 0.05, \eta^2 = 0.049$		

Different uppercase letters (A and B) indicate differences in grazing practices, and different lowercase letters (a and b) indicate significant seasonal differences.

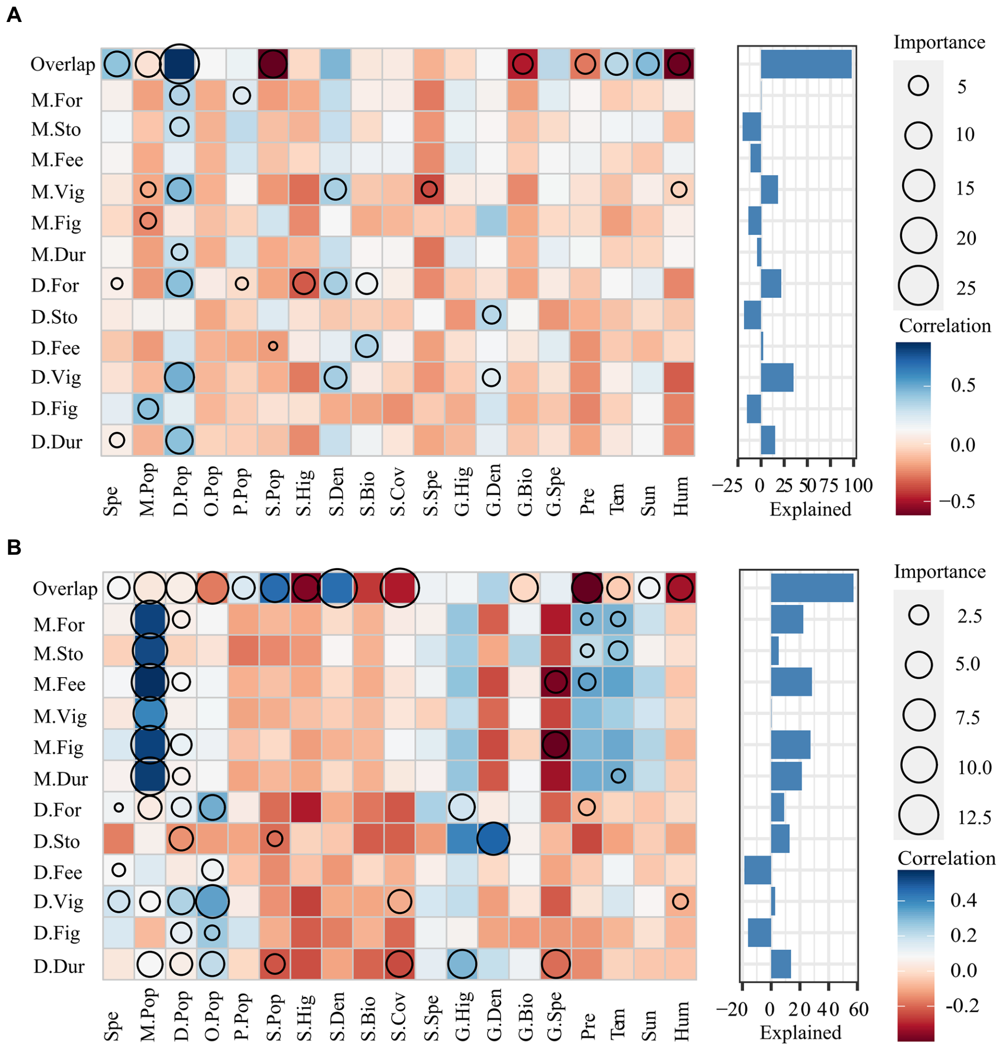

FIGURE 4
Relationship of Midday gerbil and NTJ activities with environmental factors in grazing exclusion areas **(A)**and Grazing areas **(B)**. The horizontal axis in the figure represents the predictor variable and the vertical axis represents the response variable. The size of the circle represents the importance of the variable. The larger the circle, the stronger the predictive effect of the predictor on the response variable. Different colors represent Spearman correlations. Red represents a negative correlation, blue represents a positive correlation, and the depth of the color represents the size of the correlation. The deeper the color, the stronger the correlation. The bar chart describes the total explanatory value of the predictor variable to the response variable. The variables are: degree of activity overlap of Midday gerbil and north three-toed jerboa (Overlap); Feeding behavior duration of Midday gerbil (M.Fee); Foraging behavior duration of Midday gerbil (M.For); Storage behavior duration of Midday gerbil (M.Sto); Vigilance behavior duration of Midday gerbil (M.Vig); Fighting behavior duration of Midday gerbil (M.Fig); Feeding behavior duration of northern three-toad jerboa (D.Fee); Foraging behavior duration of northern three-tord jerboa (D.For); Feeding behavior duration of northern three-tord jerboa (D.Sto); Vigilance behavior duration of northern three-tord jerboa (D.Vig); Fighting behavior duration of northern three-toed jerboa (D.Fig); Spe, Number of captured rodent species; Population density of Midday gerbil (M.Pop); Population density of northern three-toed jerboa (D.Pop); Population density of Mongolian five-toed jerboa (O.Pop); Population density of desert hamsters (P.Pop); Population density of Alashan ground squirrel (S.Pop); Average height of shrubs (S.Hig); Average shrub density (S.Den); Shrub biomass (S.Bio); Shrub coverage (S.Cov); Number of shrub species (S.Spe); Average height of grass (G.Hig); Average density of grass (G.Den); Grass biomass (G.Bio); Number of grass species (G.Spe); Precipitation (Pre); Temperature (Tem); Hours of sunshine (Sun); Humidity (Hum).

of NTJ was significantly decreased in autumn. In early winter, the population density of NTJ in the grazing area decreased significantly, while in the grazing exclusion areas it remained at a high level. An increase in population size augments the movement distance and activity duration of mammals (Grove, 2012), The proportion of activity duration spent on each behavior was significantly higher in the grazing exclusion area than in the grazing area. The time allocation strategies of the Midday gerbils and NTJ in the desert area varied with seasons, which not only facilitated their sympatric coexistence, but also enabled them be in a favorable position with regard to natural selection.

4.3. Drivers of behavioral activity and coexistence

Synergies between land use and climate change have more profound negative impacts on biodiversity than single drivers (Brodie, 2016). Our results also demonstrate that rodents respond differently to grazing. Rodent activities in grazing areas had more influencing factors than in grazing exclusion areas. Livestock grazing made desert rodents more sensitive to environmental changes. Both environmental humidity and rainfall had a significant negative effect on the activity overlap of the two rodent species. Midday gerbil and NTJ had different responses to changes in ambient temperature and humidity. Midday gerbil are highly tolerant to rainfall, while NTJ are highly tolerant to high temperature (Wu et al., 2016). The different environmental tolerances of the Midday gerbil and NTJ reduced the overlap between their activities, and facilitated coexistence in the same domain. Our results did not support hypothesis 3, as the key pathways of the effects of grazing on the temporal niche changes and behavioral patterns of sympatric Midday gerbil and NTJ were inconsistent. Based on rodent natural histories, cattle grazing may favor bipedal heteromyids more than quadrupedal species (Jones and Longland, 1999; Wu et al., 2016) because of grazing-induced changes in vegetative structure (Pereira et al., 2012). NTJ and Mongolian five-toed jerboa require similar habitat conditions (Jones and Longland, 1999; Shuai et al., 2016), Therefore, the key factor affecting the activity duration of NTJ in grazing areas was interspecific competition with the Mongolian five-toed jerboa. Grazing expanded the NTJ home range (Yuan et al., 2017b), which then resulted in limited space available for the Midday gerbil in the grazing area. Therefore, the key factor affecting the Midday gerbil behaviors in the grazing area was intraspecific competition. In the grazing exclusion area, the available space for NTJ was limited, so it was most affected by intraspecific competition (Zhang et al., 2022). Therefore, the greatest impact of grazing exclusion on Midday gerbil activity was interspecific competition with NTJ. Previous studies showed that ungulate trampling altered habitat traits and indirectly affected intraspecific competition among rodents (Navarro-Castilla et al., 2017), and intensified intraspecific competition can lead to behavioral adaptation (Murray et al., 2006; Karanth et al., 2017). This is consistent with our results.

Rodent communities in nature are intrinsically dynamic, and some of the dynamics noted during the relatively short duration of our study differed systematically between grazing and grazing exclusion areas, indicating that these animals are sensitive to livestock grazing. There were seasonal differences in the activity pattern and temporal niche of the dominant rodent species in the desert area, and they adjusted their activity strategies according to the habitat characteristics. Grazing increased the competitive pressure on desert rodents, and long-term overgrazing is inevitably unfavorable to species coexistence. Ranchers should formulate suitable grazing regimes based on grass yield to maintain healthy pastures, so that cattle have adequate forage.

Data availability statement

The original contributions presented in the study are included in the article further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by Research Ethics Review Committee of Inner Mongolian Agricultural University (NND2017012).

Author contributions

XL and SY analyzed the data and wrote the manuscript. LLi, HZ, YJ, LLiu, RZ, SS, and FB collected data and samples in the field. XW and HF mapped out the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The effect of grazing and reclamation on rodent community stability in the Alxa desert

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Ecosystem stability has been of increasing interest in the past several decades as it helps predict the consequences of anthropogenic disturbances on ecosystems. A wild rodent community under reclamation and different grazing disturbances in the Alxa Desert was investigated using live trapping from 2006 to 2011. We studied the rodent community composition, community diversity, and variability of different life history strategies. These results showed that reclamation reduced rodent community stability by increasing temporal variability of community, reducing rodent community resistance as shown by decreasing dominance of *K*SS strategists, and increased the resistance variability of the rodent community by increasing the variability of abundance and richness for *K*SS strategists. Grazing reduced rodent community resilience by reducing the dominance of *r*RF strategists, and increased the resilience variability of the rodent community by increasing the variability of abundance and richness for *r*RF strategists. Those results may answer the three ecological questions about how ecosystems respond to disturbances from a diversity perspective. The ecosystems with intermediate disturbance are more stable, in other words, with higher resistance and resilience. The increase of *K*SS strategists means the increase of resistance of the community. The increase of *r*RF strategists means the increase of community resilience.

KEYWORDS
life history strategies, community stability, diversity, resistance, resilience, rodent

1. Introduction

Disturbances are an essential component of desert ecosystems. While they play an important role in desert renewal and diversification, disturbances can generate profound changes in desert composition and structure. With the global environmental change, the occurrence and severity of biotic and abiotic disturbances have increased, affecting desert ecosystems around the world (Grimm and Fisher, 1989; Frank et al., 2013). Such alterations in disturbance regimes may change desert responses to disturbances, resulting in altered ecosystems and affecting their function and provision of services (Eldridge et al., 2009; Quoreshi et al., 2019). The concept of ecological disturbance originated from ecological studies at the community level, and the influence of interference on this scale was obvious. Many species groups are closely related to the disturbance, and one prominent function of the disturbance is to lead to the change of various resources in the ecosystem and the reorganization of the ecosystem structure leading to the formation of heterogeneous environment (Chen and Fu, 2000). Two major foci of ecological research involve reciprocal views of the relationship between biodiversity and disturbance: Disturbance determines community diversity or diversity determines realized disturbance severity. A largely separate body of ecological research has examined how biodiversity affects community or ecosystem properties. Experimental explorations of the biodiversity–stability relationship often focus on the effect of diversity on community response to specific disturbance events, revealing several sound theoretical and biological mechanisms by which diversity might influence the magnitude of loss to (resistance) or rate of recovery from (resilience) disturbance (Sánchez-Pinillos et al., 2019).

Understanding desert responses to disturbances requires considering the mechanisms that enhance ecosystems to persist through time around an equilibrium state subjected to disturbances (Scheffer et al., 2012; Willis et al., 2018). Several studies recognize the important insights gained by considering resistance and resilience as related but distinct measurable components of ecosystem responses to disturbances (Hodgson et al., 2015; Nimmo et al., 2015). Resistance can be defined as the ability of ecological systems to persist through the disturbance event (Tilman and Downing, 1994; Connell and Ghedini, 2015), while resilience is the ecosystem's capacity to recover or "bounce back" after the disturbance has been alleviated (Gunderson, 2000). Given the variety of meanings around these broad concepts, it is important to establish procedures to specifically measure these two properties when assessing responses of natural communities to disturbances (Hodgson et al., 2015; Nimmo et al., 2015). The resistance of an ecosystem to a given disturbance can be measured by the change in ecosystem structure and composition caused by the disturbance agent and can be estimated by comparing ecosystem characteristics before and immediately after the disturbance. Measuring resilience requires considering changes over a longer post-disturbance period to evaluate the degree to which an ecosystem returns to pre-disturbance conditions (Díaz-Delgado et al., 2002; Bagchi et al., 2017).

Desert resistance and resilience to disturbances depend on multiple variables acting at different levels of organization. Species responses to disturbances mediate changes in their abundance, affecting the composition and structure of the entire community, and therefore, forest functioning as well (Oliver et al., 2015). Different quantitative indices have been successfully applied to identify such variables and to compare forests based on their species responses (Bruelheide and Luginbuhl, 2009; Sánchez-Pinillos et al., 2019). While useful, the simplification inherent in quantitative, univariate indices does not capture the complexity of the entire system (Quinlan et al., 2016). Further insights into desert resistance and resilience can be gained by combining quantitative indicators based on cross-scale temporal dynamics with descriptive analyses of post-disturbance ecosystem-level variants. This approach allows us to comprehensively compare the responses of desert while assessing potential factors affecting resistance and resilience.

Stability may be caused in two ways: through the pattern of interaction between species in the community, or through the inherent stability of the species (MacArthur, 1955). Much subsequent research focused on the first pathway, namely the relationship between diversity and stability as well as the interaction between species (Hairston et al., 1968; Pimm, 1984), while less research has focused on the inherent stability of species. McCann (2000) suggested that the ability of a community to accommodate species or functional groups with different responsiveness limits stability. Functional and compositional stabilities were two aspects of stability in ecological communities (Huelsmann and Ackermann, 2022). In previous studies, life history characteristics are often used as the main indicators of community functional (Kirwan et al., 2009; Rodríguez and Ojeda, 2014; Lin et al., 2022). Life history theory (Stearns, 1992) predicts that certain environments favor specific suites of traits. Species with different life history strategies take different behavioral responses to environmental changes, which can adapt quickly to environmental changes to survive. While other species have taken non-adaptive responses to human disturbances which not encountered in their evolutionary history (Ghalambor et al., 2007). Reducing population density or lead to extinction in non-suitable habitats by emigration or death (Badyaev, 2005), and non-adaptive responses leading to changes in species distribution, diversity and community patterns (Tuomainen and Candolin, 2011). MacArthur and Wilson (1967) introduced *r*-*K* theory to account for linkages between life history strategies and specific environmental conditions. The *r*-*K* model suggests that life history strategies represent a continuum bounded by two end points: The *r*-strategy, characteristic of small, high-fecundity animals, and the *K* strategy, characteristic of large, low-fecundity animals. The model implies moving from disturbed environments. The increase in *K* strategists in a community imply an increase in community resistance, whereas an increase in *r* strategists implies an increase in community resilience (Lepš et al., 1982). Studying how different systems are dominated by animals with different life history strategies can help us solve the problem of resistance and resilience (Pimm, 1984).

Alxa desert is located in the Gobi and Karakum regions of Asia. It is an interior continental desert far away from the ocean and receiving air that has been depleted of moisture. This desert and the Great Basin of North America exemplify interior continental deserts (Kelt, 2011). In the 1990s, a large agricultural population migrated to the Alxa desert. With the increase in the agricultural population, land reclamation and heavy grazing became the main human disturbances affecting this desert ecosystem. Rodents are an important part of the environment, and an important indicator of environmental change in desert ecosystems (Jones and Longland, 1999; Flowerdew et al., 2004). Studies of rodents have contributed significantly to the development of desert ecology theory and to understanding animal adaption to arid environments (Ojeda and Tabeni, 2009). Understanding the mechanisms involved in rodent community stability has great significance for further evaluation of the stability of desert ecosystems subjected to human disturbance. However, studies on Chinese desert rodent community stability are not common.

In this study, based on Pianka's classification principle for r and *K* strategists (Pianka, 1970) and Harvey' classification principle (Read and Harvey, 2009) for fast-slow life history strategies, we further divided rodents into different life history strategies. Then we adapted a method of measuring and assessing ecosystem resistance and resilience to disturbances by comparing species composition and structure among different strategies, combining dissimilarity indices from ecosystem composition and structure with variability analyses of these descriptors in different disturbances. This method can help to answer three questions considered essential in the understanding of ecosystem responses to disturbances (see for example Willis et al., 2018): (i) Which types of ecosystems are the most resistance and resilience (ii), what factors contribute to resistance and resilience, or

(iii) how prone is an ecosystem to losing resilience? In this article, we evaluate rodent community stability subjected to different grazing patterns and to land reclamation in the Alxa desert, investigating species diversity, resilience, resistance, and variability of the rodent community. Specifically, we assume that: (1) Land reclamation and heavy grazing would decrease rodent community stability by reducing rodent community diversity; and (2) Land reclamation and grazing change resilience, resistance or variability of the desert rodent community by regulating the composition of different bionomic strategists in the community.

2. Materials and methods

2.1. Study site

This study was conducted in the southern part of Alxa Zuo Qi at the eastern edge of the Tengger Desert, Inner Mongolia, China (E104°10′–105°30′, N37°24′–38°25′), from April to October each year between 2006 and 2011. Our study area has a continental climate with cold and dry winters and warm summers. Annual temperatures range from −36 to 42°C with a mean of 8.3°C. Annual precipitation ranges from 45 to 215 mm, but about 70 percent falls from June to September. Potential evaporation ranges from 3,000 to 4,700 mm, and the annual frost-free period is 156 days. Approximately 5–15% of the ground is covered with shrubs, forbs, and some gramineous plants. Shrubs mainly consist of *Zygophyllum xanthoxylon*, *Nitraria tangutorum*, *Caragana brachypoda*, *Ceratoides latens*, *Oxytropis aciphylla*, *Artemisia sphaerocephala,* and *Artemisia xerophytica* with *Reaumuria soongorica* as the dominant species. The major grasses/forbs species are *Cleistogenes squarosa*, *Peganum nigellastrum*, *Cynanchum komarovii*, *Salsola pestifer*, *Suaeda glauca*, *Bassia dasyphylla*, *Corispermum mongolicum*, *Artemisia dubia*, and *Plantago lessingii* (Yuan et al., 2018).

2.2. Methods

The experiment, established in 2006, adopted a randomized block design with three blocks and four treatments, including light sheep grazing, heavy sheep grazing, grazing exclusion and land reclamation, to assess the effect livestock grazing and agricultural reclamation on small mammal communities. Each block encompassed 240 ha and each treatment unit was 60 ha. The two grazing treatments and grazing exclusion were established with standard sheep fencing (110 cm high). In the light grazing sites, sheep grazing intensity was controlled within the range of 0.83–1.00 sheep per ha. In the heavy grazing sites, sheep grazing intensity was controlled within the range of 3.75–4.23 sheep per ha. This was close to the common grazing intensity in the study area, but significantly higher than the Inner Mongolia government standards, which ranged from 0.603 to 1.120 sheep per ha in the study area. The land reclamation plots previously had plant species similar to the grazing exclusion plots, but were reclaimed in 1994 by planting saxaul (*Haloxylon ammodendron*), sunflowers (*Helianthus annuns*), and maize (*Zea mays*). A 7 × 8 trapping grid (0.96 ha) at a 15 m inter-trap distance was established at the center of each treatment unit. Twelve trapping grids were established in the study area. One wire-mesh live trap (42 cm × 17 cm × 13 cm) was placed at each trap station.

2.3. Trapping of rodents

Rodents were live trapped for four consecutive days at 4-week intervals from April 2006 to October 2011. Trapping was not run during winter (from November to March). Traps were baited with fresh peanuts, and checked twice (morning and afternoon) each day. Considering that the average survival time of jerboa was greater than that of other non-jerboa species in our study areas. The life span of jerboa is longer than 2 years, and the average life span of non-jerboa species is shorter than 2 years. The electronic chips be used have a life span of 2 years. Each captured jerboa individual was sexed, marked with a 1.5 g aluminum leg ring (0.4 cm diameter) with a unique identification number (ID) attached to the left hind foot, and weighed to the nearest 1 g. Each captured non-jerboa individual was sexed, marked with a electronic chip with a unique identification number (ID). The sex, capture station, body mass, and reproductive condition of each capture were recorded. Males were considered in reproductive condition if they had scrotal testes. Females were considered reproductive if they possessed enlarged nipples surrounded with white mammary tissue, or a bulging abdomen. In order to avoid accidental death, traps were closed on extremely warm or rainy days, and trapping time was extended after extremely warm or rainy days to ensure 4 days of trapping in each month (Wu et al., 2016).

To assess the effectiveness of the aluminum leg rings, whether the leg rings are lost, we conducted another experiment in 2018 and 2019. In April and May 2018, both the leg rings and electronic chips were used to mark jerboas at the same time, and the loss of the leg rings and electronic chip was recorded in September of the same year. At the beginning of this pre-experiment, we captured and marked 21 Northern three-toed Jerboa (NTJ, *Dipus sagitta*) individuals and 15 Mongolian five-toed Jerboa (MFJ, *Orientallactaga sibirica*) individuals in 2018. In September of 2019, six NTJ marked individuals and seven MFJ marked individuals were recaptured. And there was no loss of leg rings or chips.

In this study we calculated population abundance of rodent with a minimum number known to be alive (MNA) (Krebs, 1966; Hilborn et al., 1976).

$$\text{Proportion of the i}'\text{th rodent (\%)} = \frac{\text{The population density of the i}'\text{th species}}{\text{The total density of rodents in a community}} \times 100\%$$

2.4. Vegetation sampling

We conducted vegetation sampling monthly from April 2006 to October 2011. During each month's sampling, we randomly selected three 100-m^2 plots within each treatment unit to sample shrubs. Within each 100-m^2 plot, we randomly selected three individuals of small, medium, and large size of each species of shrub present. We removed a tenth of aboveground material (decided by cover) from the three shrubs and airdried samples. We then calculated the biomass of a shrub species using the formula $TB = 10 \times DW \times D/100$, where DW is mean dry weight of 1–10th of aboveground material averaged over the three individuals, D is the density of a shrub species (individuals/100 m^2), and TB is total aboveground standing biomass (g/m^2) (Yuan et al., 2018).

Within each 100-m^2 plot, we randomly placed three 1-m^2 quadrats to sample grasses and forbs. We clipped aboveground plant

TABLE 1 Biological features of captured rodent species in different treatments in Alxa desert, Inner Mongolia, China, from April 2006 to October 2011.

Species	Body length c (mm)	Body mass (g)	Births number (birth/year)	Litter size	Pregnancy (day)	Reproductive period (month)	Longevity (month)	Hibernation (yes or no)
Spermophilus alaschanicus	163–230	173.7–220.4	1	4–5	28–30	2	About 72	Yes
Orientallactaga sibirica	112–160	72.2–108.9	1	4–5	>20	3	>24	Yes
Dipus sagitta	114–135	86.4–95.9	1	4–6	28	3	>24	Yes
Stylodipus andrewsi	110–136	83.1–88.4	2	2–4	—	—	>24	Yes
Meriones meridianus	68–136	46.5–64.1	2–3	6	22–28	7	<12	No
Meriones unguiculatus	90–130	46.8–57.5	2–3	6–7	20–25	12	18–24	No
Cricetulus barabensis	70–110	17.7–25.9	4–5	4–8	22	9–10	About 10	No
Cricetulus eversmanni	76–180	21.0–27.4	2–3	4–6	—	6	—	No
Phodopus roborovskii	73–75	10.37–20.14	≥2	5–6	20–22	7	<24	No

"—": Missing data.

material of grasses and forbs within each 1-m^2 quadrat. We air-dried all aboveground plant material for >30 days until there were no changes in dry weight. We weighed dry plant material to the nearest 0.01 g to estimate aboveground standing biomass of grasses and forbs with an electronic balance (Model TD5002, Jingnuo, Yuyao, China) (Yuan et al., 2018).

2.5. Community diversity

There is no one indicator that can be used to evaluate stability (Sutherland, 1981; Pimm, 1984). In order to understand the effect of different disturbances on desert rodent community stability, our study analyzed the disturbed rodent community from the aspects of community diversity, variability of species richness and population abundance, community resistance and community resilience.

Species richness, Shannon–Wiener index, Pielou evenness index, and Simpson's diversity index were calculated by years and treatments. These indices were analyzed using a mixed effects model (Proc mixed, SAS9.2) with a significance level of α = 0.05, using Tukey's test for means comparison. Blocks and years were set as random effects. All values are given as the mean ± 1 standard error of mean (sem), unless otherwise noted.

2.6. Community resistance and resilience: Segmentation by bionomic strategies

Based on Pianka's classification principle for *r* and *K* strategists (Pianka, 1970) and Harvey' classification principle (Read and Harvey, 2009) for fast-slow life history strategies, statistical analysis was applied to six biological indicators, i.e., body length, litters, litter size, pregnancy, reproductive period, and longevity (Zhang and Wang, 1998; Wu et al., 2009; **Table 1**).

Cluster analysis was performed using the unweighted pair-group method with arithmetic means (due to incomplete data on individual species, pregnancy was not included, **Table 1**). In high latitude areas, hibernation contributes significantly to the survival rate of species under adverse environmental conditions (Bakran-Lebl et al., 2011; Bieber et al., 2012; Armitage, 2017). Hence, whether a species hibernates or not was added into the classification index (Armitage, 2017). The result of cluster analysis suggested that rodents could be divided into two categories. The Alashan ground squirrel (AGS, *Spermophilus alaschanicus*), MFJ, NTJ, and Mongolian thick-tailed three-toed jerboa (MTTJ, *Stylodipus andrewsi*), with larger body size, long longevity, short reproductive period and relatively weak fecundity, were clustered into one category. We defined this category as *K*-stress tolerant slow strategists (*K*SS). Other rodent species with smaller body size, short longevity, long reproductive period, and high fertility were clustered into the other category, and, were categorized as *r*-ruderal-fast strategists (*r*RF) (**Figure 1**). According to the classification by bionomic strategy, we calculated the species richness and community structure of rodents with different bionomic strategies in different disturbed habitats. We defined a community with more *K*SS strategists dominance shows greater resistance, while a community with more *r*RF strategists dominance shows greater resilience.

2.7. *W*-statistic calculation

We also used the *W*-statistic to analyze the dominance of different life strategists in different treatments, using the formula as follows (Clarke, 1990):

$$W = \sum_{j=1}^{S} [(\sum_{j=1}^{i} b_j) - (\sum_{j=1}^{i} a_j)]/50(S-1)$$

Where S is the number of species in the community, b_j is the cumulative biomass ratio of the j species before order by biomass from the highest to the lowest; a_j is the cumulative abundance ratio of the j species before order by abundance from the highest to the lowest. When W is close to 1, the biomass of the community is gradually dominated by a single species, while the abundance of each species tends to be the same. When W approaches −1, the opposite situation is indicated.

Species richness, capture rate, proportion of captured rodents with different bionomic strategies and *W*-values were calculated by years and treatments. These indices were analyzed using a mixed effects model (Proc mixed, SAS9.2) with a significance level of α = 0.05, and using Tukey's test for means comparison. Blocks and years were set as random effects.

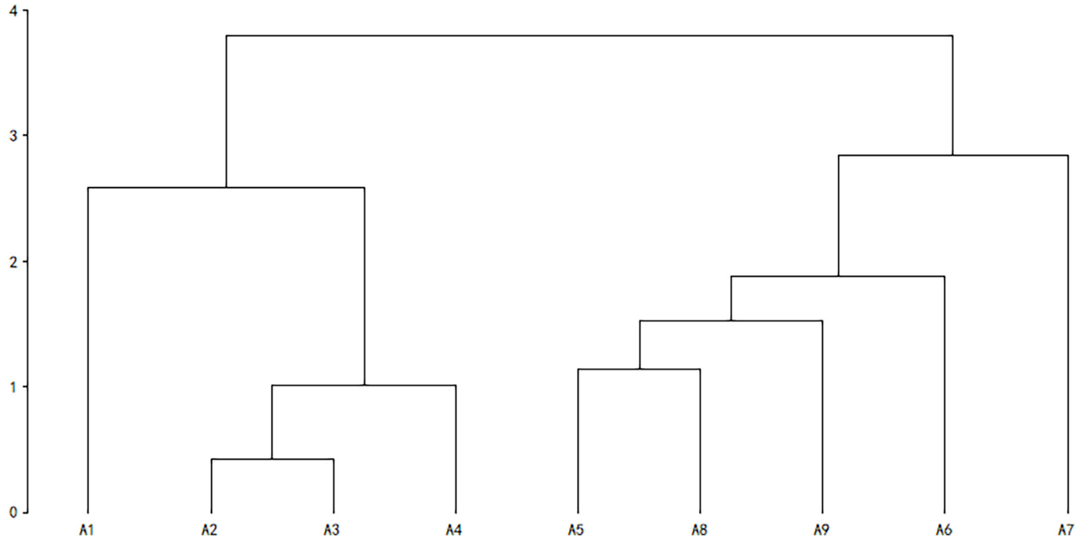

FIGURE 1
Cluster analysis of rodents with different bionomic strategies. Cluster analysis was performed using UPGMA, A1–A9 indicate, respectively: Alashan ground Squirrel (*Spermophilus alaschanicus*), Mongolian five-toed jerboa (*Orientallactaga sibirica*), Northern three-toed jerboa (*Dipus sagitta*), Mongolian thick-tailed three-toed jerboa (*Stylodipus andrewsi*), Midday gerbil (*Meriones meridianus*), Mongolian gerbil (*Meriones unguiculatus*), Chinese hamster (*Cricetulus barabensis*), Eversmann's hamster (*Cricetulus eversmanni*), and Desert hamster (*Phodopus roborovskii*).

2.8. Community variation

The community stability is expressed by temporal variability of community (Thebault and Loreau, 2005). The coefficient of variation (C.V.) is usually taken as an indicator of temporal variability of community (Bai and Chen, 2000; Thebault and Loreau, 2005). Lower temporal variability of community indicates higher community stability (Yang et al., 2022).

$$\text{C.V. (\%)} = \frac{\text{Standard deviation of population abundance or species richness}}{\text{Mean of population abundance or species richness of 6 years}} \times 100\%$$

2.9. Habitat suitability

Habitat suitability was investigated in different treatments. Habitat suitability refers to the possibility of an animal taking advantage of a particular habitat (Wang et al., 2008). Vegetation characteristics are often used as indicators of the suitability or quality of small mammal habitats (Jorgensen, 2002). Habitat suitability in different treatments were evaluated from the aspects of productivity, disturbance intensity and habitat shelter. Habitat productivity was defined as the total biomass of plants (i.e., the sum of above ground shrub biomass and herbaceous biomass). Habitat disturbance intensity was measured using stocking capacity, grazing frequency, human activity, and plant diversity in different treatments. Habitat shelter was measured using plant height, coverage and density.

Total biomass and Shannon–Wiener index of plant species, shrub height, and coverage, density and biomass of shrubs and herbaceous plants in different treatments were investigated. These indices were also analyzed by the mixed effects model (Proc mixed, SAS9.2) with a significance level of $\alpha = 0.05$, using Tukey's test for means comparison, with blocks and years set as random effects.

3. Results and analysis

3.1. Habitat suitability

Total plant biomass of the reclamation area was significantly higher than that of the other three disturbed areas [$F_{(3,687)} = 48.91$, $P < 0.001$, **Table 2**]. Total biomass in the grazing areas were lower than in the grazing exclusion area, although there were no statistically significant differences (**Table 2**). The nutrition balanced value of *Reaumuria soongorica* was lower than that of other plants (Wu et al., 2010), and *R. soongorica* was not main food for desert rodents in our study area (Wu et al., 2009). There were significant differences in total biomass removed *R. soongorica*, which followed in the order: Grazing exclusion area > light grazing area > heavy grazing area [$F_{(2,513)} = 61.77$, $P < 0.001$, **Table 2**].

We measured the degree of habitat disturbance using stocking capacity and plant diversity. Stocking capacity in each habitat were described above, and analysis focused on the plant diversity of disturbed habitats. The Shannon–Wiener index of shrubs showed a significant difference among different treatments [$F_{(3,706)} = 280.38$, $P < 0.001$, **Table 2**].

There were significant differences in height, coverage and density of plants in different treatments. Shrub height followed a significant order: Reclamation area > grazing exclusion area > light grazing area > heavy grazing area [$F_{(3,687)} = 1044.47$, $P < 0.001$, **Table 2**). Shrub coverage was highest in the light grazing area, and was lowest was in the heavy grazing area [$F_{(3,687)} = 3.08$, $P < 0.05$, **Table 2**). Shrub density in grazing exclusion area was significantly higher than that in light grazing area, heavy grazing area, and reclamation area. Reclamation decreased shrub density significantly than other treatments [$F_{(3,691)} = 68.95$, $P < 0.001$, **Table 2**). Herbaceous height was higher significantly in reclamation than that in other treatment areas, while the lowest was in the grazing exclusion area [$F_{(3,691)} = 110.72$, $P < 0.001$, **Table 2**). Herbaceous coverage in the

TABLE 2 Vegetation characteristics in different treatments in the Alxa desert, Inner Mongolia, China, from April 2006 to October 2011.

Indices	Reclamation	Grazing exclusion	Light grazing	Heavy grazing	sem	F-value	P-value
Total biomass (g/m^2)	97.23a	50.93b	39.53b	42.86b	10.36	48.91	<0.001
Total biomass (remove *Reaumuria soongorice* biomass) (g/m^2)	–	50.65a	24.25b	8.61c	5.71	61.77	<0.001
Shannon–Wiener index of plant	0.22d	0.91b	1.07a	0.38c	0.03	280.38	<0.001
Shrub height (cm)	133.22a	43.72b	26.67c	18.80d	2.08	1044.4	<0.001
Shrub coverage (%)	15.65ab	16.64ab	22.39a	12.55b	2.95	3.08	0.03
Shrub density (plants/m^2)	0.20c	0.78a	0.56b	0.49b	0.07	68.95	<0.001
Shrub biomass (g/m^2)	55.01a	40.29b	28.52c	35.96bc	7.02	12.75	<0.001
Herbaceous height (cm)	7.08a	3.27b	2.59b	1.58c	0.49	110.72	<0.001
Herbaceous coverage (%)	8.29a	1.80b	1.80b	0.57b	0.73	93.50	<0.001
Herbaceous density (plants/m^2)	179.45a	67.19c	103.63bc	123.60b	23.71	17.96	<0.001
Herbaceous biomass (g/m^2)	42.22a	10.64b	11.02b	6.90b	5.76	68.90	<0.001

Different letters in columns indicate significant differences ($P < 0.05$) according to Tukey's test.
"—": No presence of this species, sem: Standard error of means between treatments.

reclamation area was significantly higher than that in the other three disturbed habitats [$F_{(3,460)} = 93.50$, $P < 0.001$, Table 2]. Herbaceous coverage in the grazing exclusion area and light grazing area were higher than that in the heavy grazing area, although there was no significant difference in herbaceous coverage among different grazing habitats.

3.2. Rodent community structure and diversity

We captured 5,129 rodent individuals belonging to nine species, seven genus, and three families in 6 years. There were three species of Dipodidae, including NTJ, MFJ, and MTTJ; five species of Cricetidae, including midday gerbil (MIG, *Meriones meridianus*), Mongolian gerbil (MOG, *Meriones unguiculatus*), desert hamster (DH, *Phodopus roborovskii*), Chinese hamster (CH, *Cricetulus barabensis*), and Eversmann's hamster (EH, *Cricetulus eversmanni*); one species of Sciuridae: Alashan ground squirrel (*S. alaschanicus*). The total capture rates both in grazing exclusion area and light grazing area were significantly higher than that in the reclamation area [$F_{(3,15)} = 13.40$, $P < 0.001$, Figure 2]. The reclamation area had a rodent community dominated by MIG [$F_{(4,20)} = 9.48$, $P < 0.001$, Table 3]. NTJ and MIG were the dominant species in the grazing exclusion area [$F_{(6,30)} = 10.77$, $P < 0.001$, Table 3]. Rodent communities in the light grazing [$F_{(6,30)} = 11.43$, $P < 0.001$] and heavy grazing areas were dominated by NTJ and MFJ [$F_{(8,40)} = 18.78$, $P < 0.001$, Table 3].

Species richness in the reclamation area was significantly lower than in the other three treatments [$F_{(3,15)} = 20.76$, $P < 0.001$), but there was no significant difference between the grazing exclusion area, light grazing area and heavy grazing area (Table 4). Shannon–Wiener index [$F_{(3,15)} = 10.19$, $P < 0.01$) and Simpson's diversity index [$F_{(3,15)} = 9.09$, $P < 0.01$, Table 4] of the rodent community in the reclamation area were significantly lower than in the other treatments. Both indices showed the following order: Grazing exclusion < light grazing < heavy grazing treatment, although the differences were not statistically significant. The evenness index was not significantly different between the four disturbances (Table 4).

3.3. The composition of life history strategists in different disturbed communities

The species richness of *r*RF strategists was significantly greater than that of *K*SS strategists in the reclamation area [$F_{(1,10)} = 19.29$, $P < 0.01$, Figure 3A]. Reclamation decreased *K*SS strategists' species richness significantly ($F_{(3,15)} = 58.85$, $P < 0.001$), but did not decrease *r*RF strategists' species richness (Figure 4A). *K*SS strategists had higher species richness than *r*RF strategists in the other three treatments, although there was no significant difference between the species richness of *r*RF strategists and *K*SS strategists in the other three treatments (Figure 3A).

The proportion of *r*RF strategists was significantly higher than that of *K*SS strategists in the reclamation area [$F_{(1,10)} = 334.86$, $P < 0.001$, Figure 3B], while the opposite was the case in the other three areas [grazing exclusion: $F_{(1,10)} = 5.99$, $P < 0.05$, light grazing: $F_{(1,10)} = 14.81$, $P < 0.01$, heavy grazing: $F_{(1,10)} = 54.59$, $P < 0.001$, Figure 3B], and the proportion of *K*SS-strategists significantly increased with grazing intensity [$P = 0.034, 0.003, 0.0001$, Figure 3B]. The capture rate of *r*RF strategists in the grazing exclusion areas was the highest among all treatments, while the lowest was in the heavy grazing treatment [$F_{(3,15)} = 67.36$, $P < 0.001$, Figure 4B]. The capture rate of *K*SS strategists in the reclamation area was the lowest among all treatments [$F_{(3,15)} = 67.35$, $P < 0.001$, Figure 4B]. There was no significant effect of grazing on the *K*SS strategist capture rate (Figure 4B).

3.4. Rodent community stability

The W-statistic values in the reclamation area and heavy grazing area fluctuated greatly, but the fluctuation in the light grazing area and grazing exclusion area were smaller than in the other areas

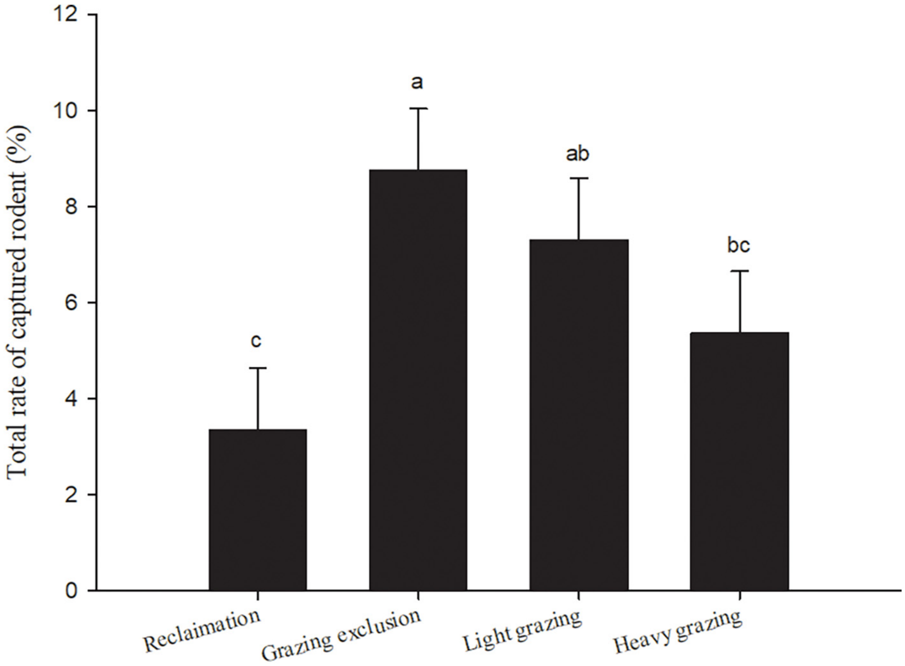

FIGURE 2
Total rate of captured rodents (means + sem) in different treatments in Alxa desert, Inner Mongolia, China, from April 2006 to October 2011. Different lowercase letters indicate significant differences ($p < 0.05$) among treatments, according to Tukey's test.

TABLE 3 Total proportion (%, means + sem) of each rodent species in the rodent community in different treatments in the Alxa desert, Inner Mongolia, China, from April 2006 to October 2011.

Species	Reclamation	Grazing exclusion	Light grazing	Heavy grazing
Spermophilus alaschanicus	7.09b	4.72cd	1.97bc	8.88b
Orientallactaga sibirica	1.14b	3.17cd	16.21b	42.75a
Dipus sagitta	–	53.40a	52.23a	30.02a
Stylodipus andrewsi	–	–	–	0.48b
Meriones meridianus	74.07a	21.47b	16.48b	6.16b
Meriones unguiculatus	7.79b	0.07d	1.48bc	4.45b
Cricetulus barabensis	9.92b	0.18d	0.16c	0.05b
Cricetulu eversmanni	–	–	–	0.27b
Phodopus roborovskii	–	16.99bc	11.49bc	6.94b
Sem	3.21	3.56	3.5	3.14
F-value	89.72	28.99	26.89	22.82
P-value	<0.001	<0.001	<0.001	<0.001

Different letters in rows indicate significant differences ($P < 0.05$) according to Tukey's test.
"—": No individual of this species was captured, sem: Standard error of means between rodent species.

TABLE 4 Rodent community diversity (means + sem) in different treatments in the Alxa desert, Inner Mongolia, China, from April 2006 to October 2011.

Diversity indices	Types of disturbance						
	Reclamation	Grazing exclusion	Light grazing	Heavy grazing	sem	*F*-value	*P*-value
Species richness	3.50b	5.50a	5.33a	5.50a	0.34	20.76	<0.01
Shannon–Wiener index	0.76b	1.14a	1.18a	1.31a	0.09	10.19	<0.01
Pielou evenness index	0.56a	0.65a	0.68a	0.73a	0.05	2.68	0.08
Simpson's diversity index	0.45b	0.62a	0.65a	0.68a	0.05	9.09	<0.01

Different letters in each row indicate significant differences ($P < 0.05$) according to Tukey's test. sem: Standard error of means between treatments.

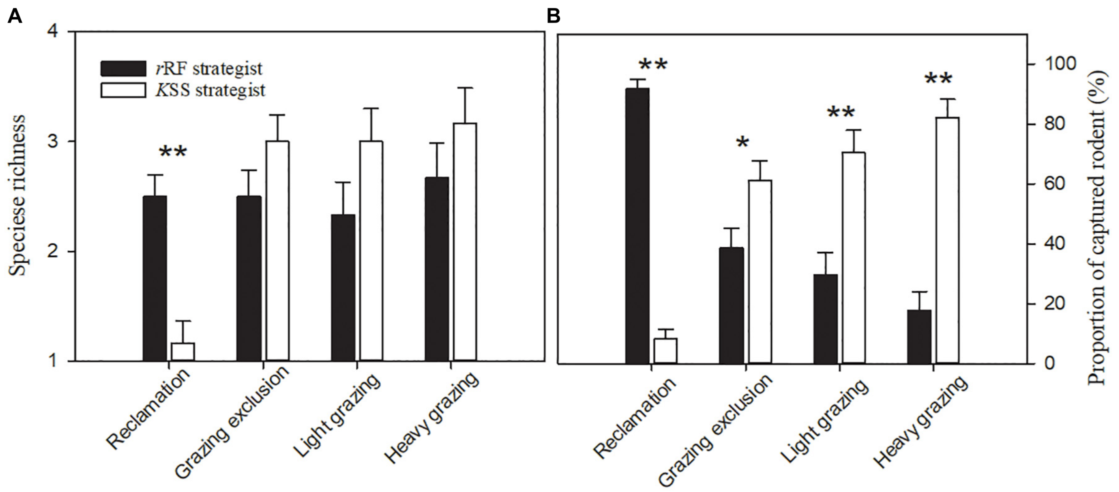

FIGURE 3
Species richness **(A)** and proportion of captured rodents **(B)** (means + sem) with different bionomic strategies in the same treatment in Alxa desert, Inner Mongolia, China, from April to October, 2006 to 2011. Asterisks indicate significant differences (*$P < 0.05$; **$P < 0.01$) according to Tukey's test.

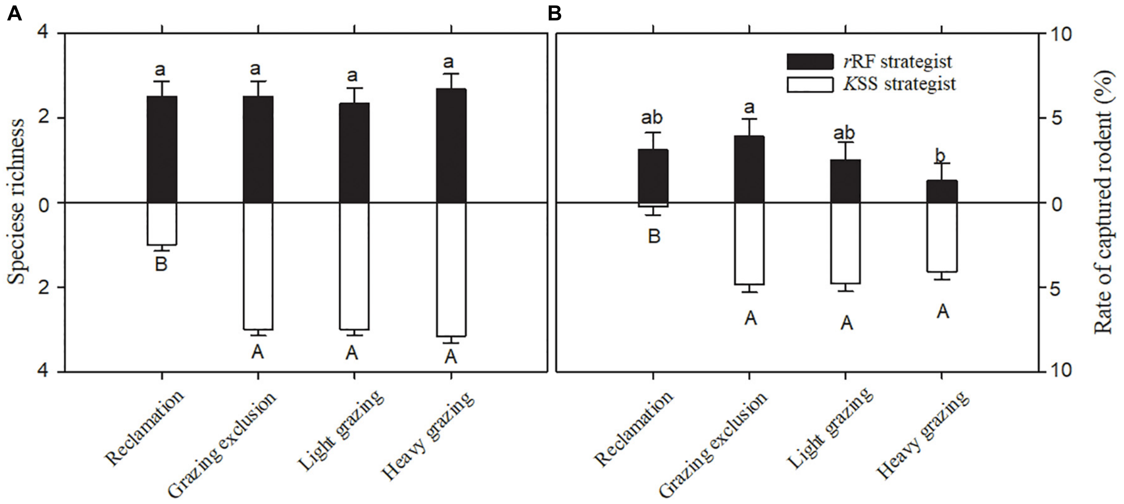

FIGURE 4
Speciese richness **(A)** and rate of captured rodents **(B)** (means + sem) with the same bionomic strategy in different treatments in Alxa desert, Inner Mongolia, China, from April 2006 to October 2011. Different letters indicate significant differences ($P < 0.05$) of *r*RF (lowercase letters) and KSS (capital letters) according to Tukey's test.

(Figure 5). The analysis of variance showed that the *W*-statistic values in the heavy grazing treatment were significantly higher than that in the reclamation area [$F_{(3,20)} = 4.58$, $P < 0.05$, Figure 5]. The *W*-statistic value in the reclamation area was less than 0 in most years, but was greater than 0 in most years in the other treatments. The cumulative biomass dominance of the rodent community in the grazing exclusion, light grazing, and heavy grazing treatments were dominated by individual species, while the cumulative abundance dominance of the rodent community was influenced by multiple species. However, the opposite result was observed in the reclamation area (Figure 5).

Regardless of treatment, variability of dominant species richness in all rodent communities was the lowest, while the richness of the non-dominant species showed stronger variability (Table 5). In the grazing exclusion, light grazing and heavy grazing treatments, rodent species with larger body size had a low variation coefficient, and large variation mainly occurred in rodent species with smaller body size (Table 5). In the reclamation treatment, variability of all rodents was high (>70%, Table 5). *S. andreusi* and *C. eversmanni* were rare species in the study sites, with a low capture rate, reflected in greater variability (Table 5). Variability of total abundance in reclamation treatment was the highest (Table 5).

Regarding the community structure of different strategists, variation in the population abundance, and species richness of *K*SS strategists in the reclamation area was greater than that of *r*RF strategists, but in other treatments the opposite was found (Table 6). The variability of population abundance and species richness of *K*SS strategists in the grazing exclusion area were the lowest (Table 6). The abundance variability of *r*RF strategists in the heavy grazing area was the highest (Table 6). The species richness variability of *r*RF strategists in the grazing exclusion and light grazing areas were the highest (Table 6). The maximum variation of rodent total abundance

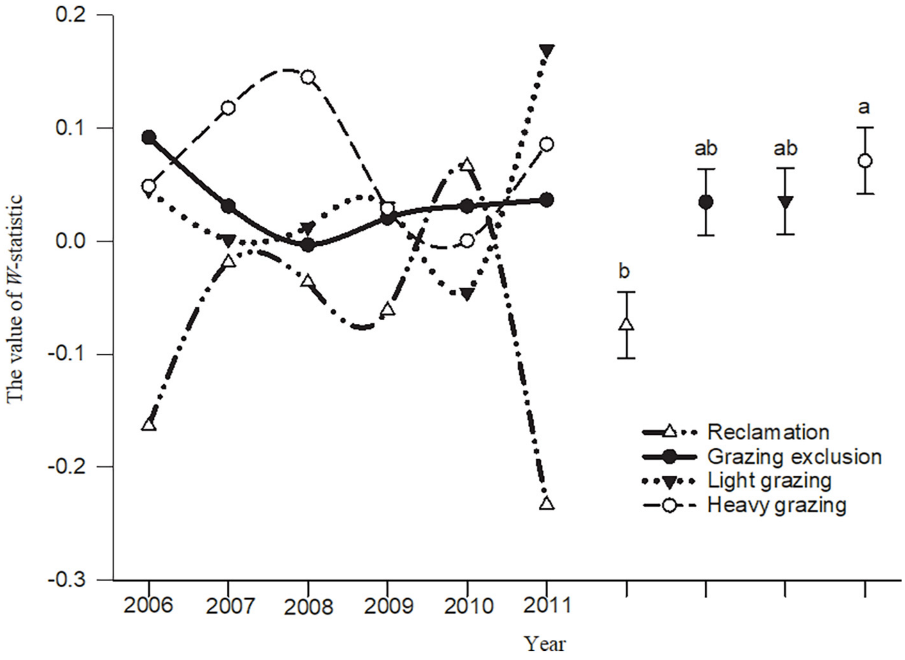

FIGURE 5

W-statistic dynamic and mean comparison (means ± sem) in different treatments in Alxa desert, Inner Mongolia, China, from April 2006 to October 2011. Different letters indicated significant differences ($P < 0.05$) according to Tukey's test.

was in the reclamation area, while the minimum was in the light grazing sites (**Table 6**). The maximum variation of total species richness was in the light grazing treatment, while the minimum was in the grazing exclusion treatment (**Table 6**).

4. Discussion and conclusion

4.1. Habitat suitability

The relationship between stability and disturbance may depend on productivity (Fränzle, 1979). The plant productivity of the reclamation area was the highest compared to the other areas. There was no significant difference in productivity among the grazing exclusion areas and grazing areas, but heavy grazing significantly reduced the biomass of plants with higher nutrient value. This indicates that grazing did not affect plant total biomass but did affect plants with high nutritional value. Studies have shown that herbivorous feeders are selective to the forage due to the characteristics of growth and development and nutritional of the plants (Nagaishi and Takemoto, 2018). Some studies have found that livestock in grassland tend to choose high-quality plants rather than plants with low palatability (Peco et al., 2005). Fleischner (1994) also argued that heavy grazing results in a decline in pasture quality, so plant species that are resistant to grazing become dominant. Therefore, our results support the view that livestock grazing affected plant quality rather than plant total biomass. Heavy grazing reduced the shrub height and coverage of plant. Plant diversity indicates that the intensity of interference was the largest in the reclamation area, followed by the heavy grazing area, light grazing area and grazing exclusion area (**Table 2** and **Figure 6**). According to habitat productivity, plant quality and animal shelter, rodents in the heavy grazing area faced the greatest environmental pressure, followed by the light grazing area, grazing exclusion area, and reclamation area (**Figure 6**). The intermediate disturbance hypothesis suggests that moderate interference suppresses competitive exclusion of dominant species and thus has a higher diversity than under severe and mild interference (Connell, 1978). There are some evidences from previous studies that moderate disturbance increases rodent diversity in forest ecosystems (Sullivan et al., 2000; Yang et al., 2018, 2022). However, we did not observed higher diversity of rodents in light grazing area, and dominant species was not suppressed under light grazing. These results may be caused by the differences from disturbance type, species in desert ecosystem. Disturbances cause habitat fragmentation, which will reduce the number of species and species diversity (Krishna et al., 2008; Hagen et al., 2012). According to human activities, rodent in reclamation area experience more disturbance from various farming activities than that in grazing area, and the habitat is completely changed, therefore the rodent number and diversity rate at the minimum level in the reclamation area.

4.2. Stability and diversity

In our study, the reclamation area had a significantly lower rodent community diversity. Reclamation reduced community stability by increasing temporal variability of community. The relationship between diversity and stability has been debated for a long time (Hu et al., 2022). The diversity-stability hypothesis suggested that the higher the species diversity in a community, the more stable the community (MacArthur, 1955). May has also argued that simple communities are more stable (May, 1973). However, some scholars believe that these two views are not in conflict. Tilman (1995) suggested that when external interference damages some species, the number of other non-injured species who are competing with the

TABLE 5 Variability of rodent populations subjected to different disturbances (%) in Alxa desert, Inner Mongolia, China, from April 2006 to October 2011.

Species	Reclamation	Grazing exclusion	Light grazing	Heavy grazing
Spermophilus alaschanicus	79.59	27.70	79.63	39.19
Orientallactaga sibirica	244.95	52.87	37.01	21.98
Dipus sagitta	–	25.08	38.19	58.24
Stylodipus andrewsi	–	–	–	244.95
Meriones meridianus	74.52	123.99	114.59	131.92
Meriones unguiculatus	137.83	244.95	244.95	178.71
Cricetulus barabensis	118.98	195.10	167.33	244.95
Cricetulus eversmanni	–	–	–	244.95
Phodopus roborovskii	–	70.69	102.89	122.59

"—": Not captured.

TABLE 6 Variability of rodent communities subjected to different disturbances (%) in Alxa desert, Inner Mongolia, China, from April 2006 to October 2011.

		Reclamation	Grazing exclusion	Light grazing	Heavy grazing
Abundance	*K*SS strategist	83.25	19.97	32.63	30.67
	*r*RF strategist	72.60	89.18	96.58	124.28
	Σ	70.80	46.38	44.33	50.51
Species richness	*K*SS strategist	63.25	0.00	14.41	12.89
	*r*RF strategist	21.91	55.78	55.78	38.73
	Σ	15.65	15.21	19.36	16.85

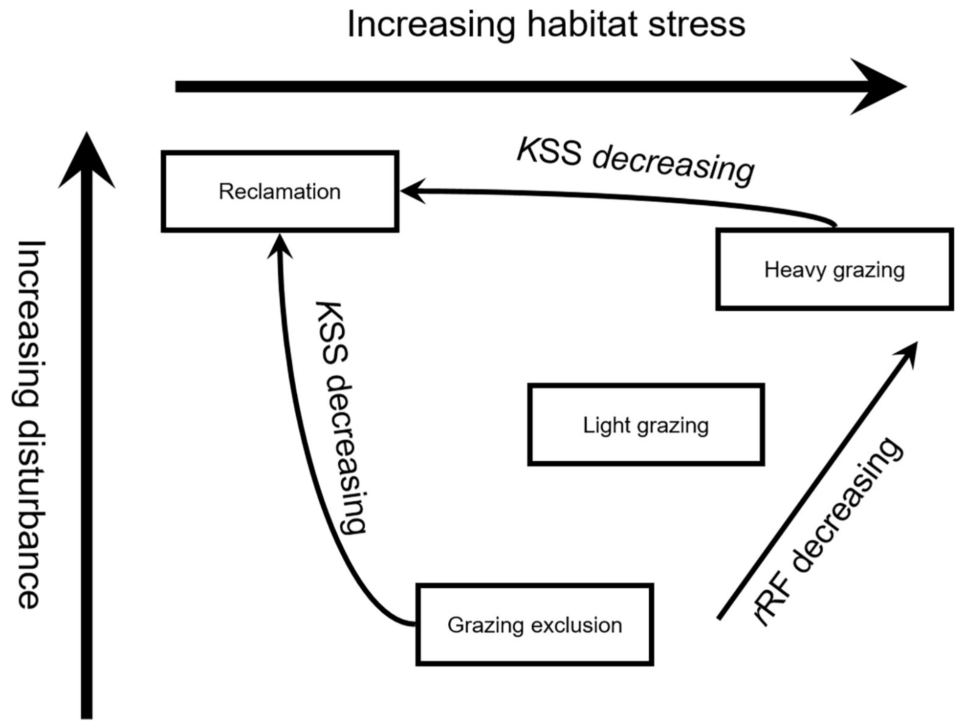

FIGURE 6
Effect of disturbances on rodents with different bionomic strategies.

injured species in the community will increase. This compensation can increase community total biomass stability, but also cause variation in species abundance. He argued that this conclusion not only supported May's consideration of the impact of diversity on the population, but also applied the diversity-stability hypothesis to community and ecology processes. McCann (2000) showed that diversity usually contributes to stability, but that diversity is not the driving force of stability. Grazing did not make significant difference in the diversity of rodent community, but, changed the dominant species among different grazing disturbances. The effect of grazing on the rodent community stability may come from the response of species or functional groups with different responsiveness to disturbances (de Valpine, 2000). Life history characteristics are often used as the main indicators of community functional (Kirwan et al., 2009; Rodríguez and Ojeda, 2014; Lin et al., 2022). Functional stability was one of the important aspects of stability in ecological communities (Huelsmann and Ackermann, 2022).

From the habitat suitability and the characteristics of different strategists, we can understand the different changes in the reclamation and grazing areas among different strategists. For animals, habitat shelter has an important impact on habitat selection, and predation risk may result in the specialization of smaller mammals in habitats with excellent shelter. The reclamation areas with high shrub height, herbaceous height, herbaceous coverage, and herbaceous density, these conditions provided excellent shelter for *r*RF strategists with small body size (Cui et al., 2005). On the other hand, *r*RF strategists with a higher reproductive rate and a longer reproductive period, which is beneficial to increase the population in a short time. Studies have shown that when habitat heterogeneity occurs and the original ecosystem becomes less stable, a higher population size is better able to cope with the impact of heterogeneity (Shadrina and Vol'pert, 2016). Above, we can understand the changes in the reclaimed areas among different strategists. Grazing areas have higher biodiversity than reclaimed areas, which means they have higher stability. How do these communities acquire the stability in response to disturbances? It is universally acknowledged that increase in animal body size reduces environmental resistance in many ways. It maybe that there is an increase in the number of *K*SS strategists in the community, the increased resistance of the community. In our study, our proposed hypothesis was verified. At the same time, our research may answer these three questions from the respect of life history strategy. From a diversity perspective, the ecosystems with intermediate disturbance are more stable, in other words, with higher resilient and resilient.

4.3. Stability and resistance, resilience, and variability

McNaughton (1977) argued that species with different adaptation patterns, whose abundance is volatile, may be a mechanism for the community to remain stable in a volatile environment. The adaptation patterns here were likely to be related to life history strategies of the species (McNaughton, 1977). Studying how different systems are dominated by animals with different life history strategies can help us solve the problem of resistance and resilience (Pimm, 1984).

Pianka (1970) argued that increase in animal body size reduces environmental resistance in many ways. Animals with larger body size have fewer potential predators, and more adaptable to the environment (Meador and Brown, 2015). The creatures living longer than 1 year have to deal with physical or biological conditions of the whole range in order to survive, while those living less than 1 year will only experience part of that range during their short- life time. So animals with longer longevity and larger size can better buffer changes in the environment, and their population is not subject to such dramatic change as relatively smaller and shorter-lived animals. A stable population also provides the necessary conditions for a stable community. Studies in the same study area have shown that the survival rate of the large jerboas is less affected by grazing than MIG which is smaller in size (Yuan, 2013). Mammals with a fast strategy have relatively higher reproductive elasticity and lower adult survival elasticity, while mammals with a slow strategy have lower reproductive elasticity and higher adult or juvenile survival elasticity. Since the decline in adult survival is not conducive to the slow strategy mammalian population, the resilience of the slow strategist will be slower once the outside interference exceeds its ability to resist environmental resistance (Heppell et al., 2000). MacArthur and Wilson (1967) argued that high-fecundity strategists can recover from frequent density independent deaths through higher population growth rates. Therefore, *K*SS strategists have higher resistance and lower resilience to external environmental changes. The *r*RF strategists have lower resistance and higher resilience to external environment changes (MacArthur and Wilson, 1967; Pianka, 1970; Heppell et al., 2000). Lepš et al. (1982) argued that two different aspects of stability may be determined by life strategy and external variables of the dominant species. The increase in *K*SS strategists in a community imply an increase in community resistance, whereas an increase in *r*RF strategists implies an increase in community resilience. Variability in community stability reflects the effect of external variables on the community (Lepš et al., 1982). In our study, the reclamation disturbance significantly reduced species richness, the capture ratio, and the capture rate of *K*SS strategists. Therefore, reclamation had a significant negative effect on *K*SS strategists, so the reclamation area had lower community resistance. The resistance variability of rodent community in reclamation was also greater than in the other three disturbed areas. So reclamation reduced rodent community resistance. With an increase in grazing intensity, the capture rate of *K*SS strategists and *r*RF strategists showed a downward trend, and the declining trend of *r*RF strategists was more significant, which caused the dominant advantage of *K*SS strategists in the community to gradually increase. The results from *W*-values also indicate that *K*SS strategists in the grazing exclusion area and grazing areas were dominant, particularly in the heavy grazing treatment. So grazing has a greater impact on *r*RF strategists than on *K*SS strategists (**Figure 6**). Because the heavy grazing area had lower *r*RF strategy abundance than the other treatments, the heavy grazing area had lower community resilience, and resilience variability was higher than in the other three disturbed regions. There was no significant difference in species richness and population abundance for *K*SS strategists among the heavy grazing area and other areas, and the variability of this resistance was not markedly different among the other three disturbance treatments, so the effect of grazing on community resistance was not significant. Because the heavy grazing area had a lower *r*RF strategist abundance than the grazing exclusion area, so the heavy grazing area had lower community resilience, and resilience variability was larger than in the other two disturbed areas. Therefore, grazing reduced community resilience.

In summary, the increase of *K*SS strategists means the increase of resistance of the community. The increase of *r*RF strategists means the increase of community resilience. Reclamation reduced rodent community stability by increasing temporal variability of community, reducing rodent community resistance as shown by decreasing dominance of *K*SS strategists, and increased the resistance variability of the rodent community by increasing the variability of abundance and richness for *K*SS strategists. Grazing reduced rodent community resilience by reducing the dominance of *r*RF strategists, and increased the resilience variability of the rodent community by increasing the variability of abundance and richness for *r*RF strategists. Despite the limitations of the theory and data analysis, these findings enhance the understanding of the structure and resilience of ecosystems. Sensitivity to disturbances varies between species, so it is difficult to further explain the hidden mechanisms behind this response (Ma et al., 2004). However, some insights can be gained if sensitive species are classified in terms of bionomic strategy, as this helps elucidate which kind of bionomic strategy sensitive species take when subject to varying disturbances.

Data availability statement

The datasets analyzed for this study can be found in the Science DB: https://doi.org/10.57760/sciencedb.07040.

Ethics statement

This animal study was reviewed and approved by the Research Ethics Review Committee of Inner Mongolia Agricultural University (NND2017012).

Author contributions

SY, H-PF, and X-DW designed the experiments and mapped out the manuscript. SY analyzed the data and wrote the initial draft of the manuscript. H-TZ, XL, and X-XY were responsible for the majority of data collection and assisted with revisions of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Publisher's note

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The effect of dietary niacin deficiency on reproduction of European brown hares: An experimental study

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Large plots of maize in agricultural landscapes are associated with reduced reproductive output of females and impaired population development of free-living populations of European brown hares (*Lepus europaeus*, Pallas 1778). We investigated in captive brown hares experimentally whether these effects could result from an undersupply of niacin due to a suspected maize-biased diet in such areas. We repeatedly mated adult female hares, fed *ad libitum* either with a niacin deficient pellet mainly consisting of maize plant parts, or with the same pellet enriched with niacin to meet physiological requirements. We measured effects of the experimental feeding on body mass of females, reproductive output, growth and survival of leverets. We found significantly higher body mass of females fed the control pellet, and faster body mass gain of their leverets until standardized weaning. We found no significant difference between niacin deficient and control diet groups in reproductive output of females and survival rates of leverets. Our results show that even a diet severely depauperate of niacin affects reproductive success of female brown hares only slightly, presumably because of sufficient conversion of tryptophan to niacin, or of additional supply of niacin by caecal microorganism.

KEYWORDS

maize biased diet, growth, survival, pellagra, nicotinamide, tryptophan, *Lepus europaeus*, niacin

1. Introduction

Maize (*Zea mays*) is not only the most produced crop in the world (Nuss and Tanumihardjo, 2010), but also one of the main energy crops cultivated in Europe (Klenke et al., 2017). High energy yield per area unit and high economical profit will make maize the most cultivated plant in Europe in the future (Nielsen and Oleskowicz-Popiel, 2008). Economic advantages come, however, with a high price regarding ecological aspects of maize cultivation. Intensive maize cultivation is blamed for increasing threats to biodiversity, with particularly negative effects on farmland animals (Fargione et al., 2009; Klenke et al., 2017). One of these species is the European brown hare (*Lepus europaeus*, Pallas 1778). Brown hare populations show drastic declines in many European countries since the 1960s (Edwards et al., 2000). Hunting bag records, reliably reflecting population trends (Langbein et al., 1999), demonstrate this decline in several European countries (Poland: Pielowski and Raczynski (1976), Denmark: Madsen et al. (1996); Wincentz Jensen (2009), parts of Croatia: Pintur et al. (2006); Popović et al. (2008), Serbia: Ristić et al. (2021), Germany, Austria, Bulgaria, Luxembourg, Netherlands, Slovakia, Switzerland: Mary and Trouvilliez (1995), United Kingdom: Smith et al. (2005)).

Several studies searched for causes of the hare decline. Reported main driving factors are changes in climate (Hackländer et al., 2002a; Smith et al., 2005; van Wieren et al., 2006), and intensification of agricultural practices resulting in a loss of set asides, grass stripes and habitat heterogeneity (Vaughan et al., 2003; Jennings et al., 2006; Schai-Braun et al., 2020; Johann and Arnold, 2021). Particularly maize affects hare populations negatively when cultivated in large monoculture (Meriggi and Alieri, 1989; Smith et al., 2005; Sliwinski et al., 2019). Due to the loss of habitat heterogeneity and plant biodiversity, hares are unable to meet their nutritional requirements there, because these picky herbivores search for plants rich in fat and protein (Schai-Braun et al., 2015). Left with no better choice, hares select green parts of maize plants as well as grains throughout the year in lower Austria (Steineck, 1978; Reichlin et al., 2006). Consequently, large areas under maize have negative effects on population development, i.e., the difference between autumn and spring population densities, and reproductive output of females, likely mediated by impaired supply of niacin and tryptophan (trp) (Selimovic et al., 2022). Both maize seeds and leaves are poor in niacin and trp, an essential amino acid (Hogan et al., 1955; Henderson et al., 1959; Goss, 1968; Mawson and Jacobs, 1978). The maize plant is not only poor in niacin and trp. In addition to such deficiencies, up to 90% of niacin is present as niacytin in mature maize grains, i.e., bound up in a complex with hemicellulose which renders it unavailable to vertebrates (Ammerman et al., 1995; Ball, 2005; Baker, 2008).

Nicotinic acid and nicotinamide are products of niacin decomposition, and are essential for *in vivo* synthesis of nicotinamide adenine dinucleotide (NAD) (Wan et al., 2010). Too low supply of trp and niacin with the diet can lead to severe health issues like dementia, skin rashes, diarrhea, or aggressiveness and growth retardation (Krehl et al., 1945; Kantak et al., 1980; Hegyi et al., 2004; Wan et al., 2010; Walz et al., 2013; Tissier et al., 2017). Tissier et al. (2017) further demonstrated in European hamsters (*Cricetus cricetus*, Linnaeus 1758), that niacin deficiency, resulting from a maize dominated diet, severely reduces reproductive output mainly due to increased infanticide by mothers.

We investigated in this study experimentally in captive brown hares the effects of solely feeding pellets mainly consisting of maize plant parts, but containing sufficient amounts of trp. Control diet groups received the same pellet further enriched with niacin to meet physiological requirements. We tested for effects of the diet poor in niacin on survival, body mass, reproductive success, and on growth of leverets. We expected severe health issues and high mortality in both mothers and leverets receiving the experimental pellet, or milk from mothers on the niacin depauperate diet.

2. Materials and methods

All procedures were carried out in accordance with the Austrian legislation as assessed and approved by the institutional ethics commission of the University of Veterinary Medicine Vienna (GZ: BMBWF-68.205/0080-V/3b/2019).

2.1. Animal keeping

The study was conducted during 2021. Experimental hares were kept in a large barn with open sidewalls at natural ambient temperatures. Twenty adult females, 2.5–4.5 years old, were used for experimental feeding and reproduction twice, once at begin of the annual reproductive season (Mai-April), and once at the end (August–September). Four of the experimental does were primiparous during the first experimental period. Daily mean air temperature in the barn was during the first experimental period 12.3°C (min = 6.2°C and max = 21.4°C), during the second 18.0°C (min = 11.6°C and max = 26.7°C). Each doe was kept in a fenced 6 m*2 m enclosure with two wooden boxes as cover (size 60 cm*60 cm*100 cm). The ground was filled and regularly re-filled with wood chips. Additionally, each enclosure contained as enrichment two 50 cm*40 cm wooden frames, height 4 cm, regularly filled with fresh soil. Further, fresh branches were provided once a week. In order to exclude possible effects of consummation of wood chips and branches on our results, these were tested for niacin in our lab and tested samples lacked any detectable niacin concentrations. Food and water was available *ad libitum* in two bowls, cleaned and refilled daily. Does were mated by releasing an adult male into an enclosure for two consecutive days.

For both experimental periods, females were randomly assigned to an experimental and control diet group containing 10 individuals each. Both groups were fed pelleted food, a food source readily accepted by brown hares as known from our long time experience of keeping and breeding brown hares (Hackländer et al., 2002a,b; Valencak and Ruf, 2009; Schai-Braun et al., 2021). During an experimental feeding period, each doe was weighed twice at begin and end to the nearest g. Leverets were weighed to the nearest g every day, beginning with day one after birth. At each weighing, the health status of an individual was checked by a veterinarian. Five leverets reaching our animal welfare endpoint criteria during the experiment (body mass < 20% than the mean body mass of other leverets of the same age, or severe disease symptoms like diarrhea, or neurological disorders), were sacrificed by several blunt blows to the neck. Body mass data from two sacrificed and one other leveret with diagnosed coccidiosis at the age of 18 days were omitted from analyzes because of obviously impaired growth. We kept the body mass data from three sacrificed animals in our dataset, because growth curves of these individuals showed no visible deviation from those of other leverets in the experiment.

2.2. Experimental feeding

For designing an experimental food pellet mirroring a maize biased diet of free-living hares, we used as primary material commercially available pellets made purely from air dried maize plant parts (Mais cobs™, Agrobs GmbH Degerndorf, Germany), and pellets made from air dried alfalfa (Luzerne cobs™, Agrobs GmbH Degerndorf, Germany). Regarding the content of macro-and micronutrients, we aimed at a composition of the experimental food pellet similar to the standard pellet used for many years of successfully keeping and breeding European hares at our institute (Hasenpellet, Garant Pöchlarn, Austria). In order to meet the protein and fat content of the standard pellet, we added pea protein isolate (Erbsenproteinpulver, Erdschwalbe GmbH, Neu-Ulm, Germany), and commercial sunflower oil (Sonnenblumenöl, Delikatessa GesmbH, Wiener Neustadt, Austria). Addition of the pea protein isolate also compensated for the low trp content of maize. As binding material, we used molasses (3% Melasse Königshofer GmbH, Ebergassing, Austria). Vitamin D3 was added in form of cholecalciferol (25 μg/kg pellet). Minerals were added in form of calcium carbonate, calcium dihydrogen phosphate, sodium chloride, magnesium oxide, trace minerals as zinc oxide, copper sulfate, potassium iodide, and sodium selenite. As an additional precaution against unwanted trp deficiency, we supplemented the niacin deficient experimental pellet with 0.109 g L-trp/kg during the first experimental period. Because of the surprisingly low effects of feeding the niacin deficient diet, evident already during the first experimental period, we dispensed L-trp supplementation of the experimental pellet fed during the second experimental period. The control diet group received during both experimental periods the experimental pellet supplemented with niacin to meet a dietary supply as recommended for rabbits (Weisbroth et al., 1974). We chose nicotinamide as source, because this form of niacin is well tolerated, without unwanted side effects known for other forms of niacin. For a full description of the ingredients of the fed pellets, see Table 1.

Food pellets were produced in 15 kg batches. We grounded the maize and alfalfa pellets with an electric grinder (Maximilian Fuchs & Co, Vienna, Austria) with a 3 mm sieve. For each batch, we created a pre-mix by adding all supplements as well as the pea protein isolate, and the sunflower oil to 2 kg of grounded plant components. This pre-mix was then added to the rest of plant components and mixed thoroughly with an electrical mixer (TX-MX1200E™, Einhell Landau/Isar, Germany). This mixture was pelleted with an electric pellet press (Magic PTO™, Ceccato Olindo San Giorgio delle Pertiche, Italy). After cooling and drying overnight, pellets were stored in sun proof plastic containers.

TABLE 1 Ingredients and their quantities in the pellets fed during the experiment.

Ingredients	Quantities per kg of pellet material
Maize pellet (air dried material of whole plants)	520 g
Alfalfa pellet (air dried material of whole plants)	160 g
Pea protein isolate	109 g
Sunflower oil	41 g
Calcium carbonate	11.8 g
Calcium dihydrogen phosphate	21.9 g
Sodium chloride	3.1 g
Magnesium oxide	4.6 g
Zinc oxide	95 mg
Copper sulfate	24 mg
Potassium iodide	5.1 mg
Sodium selenite	0.49 mg
Vitamin D3 (Cholecalciferol)	25 μg
Water	98.5 g
Molasses	30 g
Tryptophan, experimental pellet	1,391 mg
Nicotinamide, experimental pellet	19.7 mg
Tryptophan, control pellet (supplement 109 mg/kg April/May)	1,500 mg
Nicotinamide, control pellet (supplement 130.3 mg/kg)	150 mg

Does were slowly familiarized to consume the experimental pellets during 5 weeks before mating by gradually replacing our standard food pellet with the experimental pellet, either niacin deficient or control. After 5 weeks, does only received experimental pellets and were mated thereafter. Each feeding period with solely experimental pellets lasted for 28 days after removal of the male when a female did not give birth, or for 28 days after giving birth. We selected this time period, because it corresponds to the approximate age of leverets at weaning in free-living hares (Broekhuizen and Maaskamp, 1980; Zörner, 1981).

2.3. Statistics

We calculated linear mixed effects models (lme) using the "nlme" package (Pinheiro et al., 2021) of the statistical package R (R Core Team, 2021) to test for possible effects and interactive effects of diet group and experimental period. We accounted for a possible effect of ambient temperature by including the daily mean air temperature in the stable as a covariate in each model. In the models analyzing body mass of leverets, we further adjusted for possible effects of litter size, mother's age, and mother's body mass at mating, or weaning, respectively. Litter size was analyzed with and without failures to litter. In both models, we adjusted for mother's body mass and age at mating. Further, we analyzed with a binomial test whether diet group influenced failure to litter. When investigating effects on the body mass of mothers, we adjusted for age and initial body mass. In all models, we accounted for repeated measurements of individuals by including a random effect individual ID. For the analysis of growth of juveniles, we nested the random factor "leveret ID" within "mother ID" to further account for potential, but unknown differences in maternal care between mothers. Differences between slopes of regressions were tested with type III Anova. We checked with diagnostic plots the distribution of model residuals, and, if necessary, Box-Cox-transformed response variables to avoid deviation from normality. We used the "visreg" package (Breheny et al., 2020) for plotting results from lme models. In order to test and plot the effect of experimental feeding on survival of leverets, we used the function "survdiff" from package "survival" (Therneau, 2022) which uses tests from the G-rho family and Kaplan–Meier estimates of survival.

3. Results

3.1. Effects on mating success and gestation

The gradual replacement of our standard hare pellet with experimental pellets during the 5 weeks before mating did not result in significant changes of body mass of females at mating, although there was a tendency of lower body mass of females receiving the niacin deficient diet [Table 2, effect of diet type $F_{(1,15)} = 3.0$, $p = 0.106$]. The 40 mating events resulted in 16 litters, with 17 leverets born in April–May and 13 in August–September. Body mass of mothers at mating, leverets at birth, litter size, and failures of giving birth were similar in both the niacin deficient and control diet group (Table 2, all tests for effects of diet group and experimental period, n.s.). However, leveret body mass at birth differed significantly according to diet group and experimental period, with lower body mass at birth during the second experimental period in offspring born to mothers fed the niacin deficient diet [Table 2, interaction $F_{(1,14)} = 6.4$, $p = 0.024$].

3.2. Effects during the nursing period

During both experimental periods, body mass of mothers increased during lactation [$F_{(1,89)} = 8.1$, $p = 0.006$], was higher during the late summer experimental period [$F_{(1,89)} = 19.2$, $p < 0.001$], but lower in both experimental periods of individuals receiving the niacin deficient diet [$F_{(1,89)} = 11.3$, $p = 0.001$, Figure 1]. Independent of these seasonal and diet group difference, mothers' seemed to gain more body mass during the nursing period at higher daily mean ambient temperatures [$F_{(1,89)} = 3.7$, $p = 0.058$].

Leverets increased their body mass after birth rapidly, but slightly slower when nursed by a mother receiving the niacin deficient diet [Figure 2, interaction of leveret's age and diet type fed to mother on leveret body mass, $F_{(1,589)} = 64.3$, $p < 0.001$]. Leverets were during the second lactation period in August – September heavier [$F_{(1,10)} = 6.8$, $p = 0.028$]. Litter size had a borderline significant negative effect on body mass development of leverets [$F_{(1,10)} = 4.2$, $p = 0.068$]. Independent of season, there was a strong negative correlation between body mass of leverets and daily mean ambient temperature in the stable [$F_{(1,589)} = 64.9$, $p < 0.001$].

Of the 30 leverets born during the two experimental periods, two leveret from the niacin deficient diet group and three from the control diet group were sacrificed because they had reached our animal welfare endpoint criteria. Four had severe infectious diseases of the gastrointestinal tract, and one showed severe neurological disorders. For analyzing the effect of diet type on leveret survival, we considered these five individuals as having died during the experiment. However, there was no statistically detectable difference in the survival probabilities of leverets from both diet type groups ($CHI^2 = 0.7$, $p = 0.4$; Figure 3). A similar survival analysis for does was not possible, because all 20 experimental does survived the experiment.

4. Discussion

Survival and reproduction is impaired in hare populations living in areas characterized by maize monoculture. Niacin undersupply due to a maize biased nutrition was suspected to contribute to this phenomenon (Selimovic et al., 2022). We conducted this study to evaluate experimentally effects of dietary niacin deficiency on health and reproductive success of European brown hares. In contrast to our expectation, poor supply of niacin, as a result of feeding exclusively a niacin deficient pellet, did not increase mortality, or cause any severe health issues, neither in the experimental does nor in their offspring. The minimum amount of dietary supply of niacin and trp is unknown for European hares. The closest related species for which such information is available is the rabbit. The amount of niacin provided with our experimental pellet was only 13.1% of the required concentration of niacin per kg food (Weisbroth et al., 1974). Conversely, the pellet contained a sufficient concentration of trp (100% of the recommended amount during the first experimental period, 93% during the second when trp supplementation was suspended; *cf.* Table 1; Adamson and Fisher, 1973).

Reasons for the lack of pathologies in brown hares, well known for other species to result from a dietary undersupply of niacin, could be (i) compensatory synthesis of niacin from trp (Krehl et al., 1945; Wooley, 1947; Hegyi et al., 2004; Terakata et al., 2012; Tissier et al., 2017). The conversion of trp to niacin occurs at maximum rate when trp requirements are covered (Olcese et al., 1949), a condition fulfilled with our experimental pellet. Although this conversion is inefficient (67:1, Olcese et al., 1949; Goldsmith, 1965), it could supply enough niacin when trp rich food is available *ad libitum*, as it was the case in our experiment. However, as maize tissues are not only deficient in niacin but also in trp, this pathway may be limited in the wild. Further, the conversion of trp to niacin becomes more effective during late pregnancy (Fukuwatari et al., 2004), another possible contribution why our experimental females remained healthy despite a severe nutritional undersupply with niacin, (ii) Despite the widely accepted inability of vertebrates to hydrolyze niacytin to make it bioavailable, hares may be able to accomplish this at least to a small degree as reported for rats (Mason and Kodicek, 1970), and (iii) Niacin could be delivered in sufficient amount by caecal microorganism (McBee, 1971; Hunt and Harrington, 1974; Halls, 2010). The caecum is in many small herbivorous mammals the principal site of microbial activity (Hume, 1989). Some of these caecum fermenters excrete and re-ingest fermented caecal material as so-called "soft feces" or

TABLE 2 Descriptive statistics of birth data.

Experimental period	April–May						August–September					
Variable/Diet type	Niacin deficient			Control			Niacin deficient			Control		
	Mean	n	S.E.M.	Mean	n	S.E.M.	Mean	n	S.E.M.	Mean	n	S.E.M.
Body mass of does at mating	3,344	10	61.0	3,612	10	97.6	3,163	9	71.9	3,515	11	85.0
Leverets' body mass at birth	129	10	3.7	120	7	3.6	118	7	6.4	138	6	7.4
Litter size	2.5	4	0.50	1.8	4	0.48	1.8	4	0.25	1.5	4	0.29
No litter		6			6			5			7	

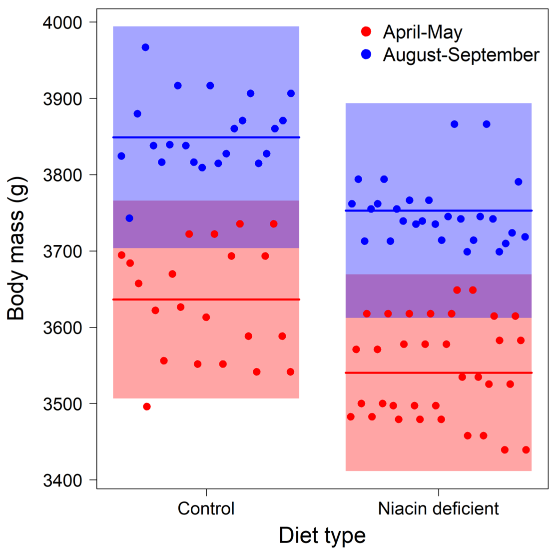

FIGURE 1
Body masses of European brown hare mothers measured during lactation in April–May (red circles) and August–September (blue circles). Animals were fed *ad libitum* exclusively with a niacin deficient pellet, or a niacin supplemented control pellet (horizontal lines indicate group means, shaded areas 95% confidence intervals of the means).

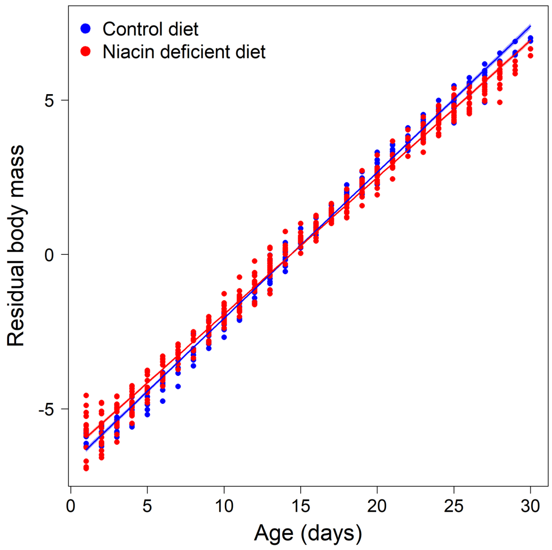

FIGURE 2
Residuals of Box-Cox-transformed body masses of leverets measured during the period of nursing (shaded areas indicate 95% confidence intervals of the estimated regression lines). Leverets had, like their mothers, access *ad libitum* to either a niacin deficient pellet, or a niacin supplemented control pellet.

"caeotrophs." Caecotrophs are rich in nutrients and vitamins, particularly of the B-group, including niacin (Halls, 2010). Caecotrophy is considered most sophisticated in leporids (Hirakawa, 2001). This may explain why a maize-biased diet produced more detrimental effects in European hamsters (Tissier et al., 2017) compared to European brown hares, the subject of our study. Even among leporids, there seem to be differences. Rabbits also use niacin produced by ceacum microorganism (Kulwich et al., 1953; Hunt and Harrington, 1974), but compensation of a dietary undersupply is apparently less efficient than in the European hare, considering the relatively high niacin concentration recommend for rabbit nutrition (Weisbroth et al., 1974). This surprising difference calls for a comparison of the nutrient contents of soft feces and the caecum microbiomes of European hares and rabbits. According to our results, substantial differences may exist.

Despite the lack of increased mortality or health issues when feeding the experimental pellet, we found significant effects on body mass at birth and on growth of leverets. Leveret body mass at birth was significantly lower in the niacin deficient diet group in August–September. An undersupply of trp, and therefore impaired conversion of trp to niacin, could have been responsible for this difference. Although still 93% of the recommended trp supply was provided with the experimental pellet fed during August–September, this difference may have been crucial, because trp plays an important role in fetal development (Badawy, 2015). During the nursing period, offspring of mothers fed the niacin deficient pellet gained mass at a lower rate. A shortfall of niacin availability is known to negatively affect the fat content of milk (Havlin et al., 2017). Apparently, this holds true for European brown hare mothers as well. Although we have no information about the fat content of milk produced by the does in our experiment, a too low fat content of milk most likely explains retarded growth of offspring from does fed the niacin deficient pellet. The effects of dietary niacin deficiency on growth of leverets occurred on top of a strong effect of ambient temperature. The negative effects of higher daily mean ambient temperature on body mass gain could result from impaired dissipation of heat produced as a byproduct of the high metabolic rate of rapidly growing juveniles (Valencak and Ruf, 2009).

Notwithstanding that, an experimentally induced undersupply with niacin had astonishingly little negative effects. However, it has been shown that maize monoculture, probably increasing the consumption of maize by herbivores (MacGowan et al., 2006), impairs the development of free-living European brown hare populations (Selimovic et al., 2022). The maize plant is not only poor in niacin but also in trp. Therefore, detrimental effects of a maize biased diet on European brown hare populations may to a large degree be mediated more by trp deficiency than by niacin undernutrition. Nevertheless, the weak effects of low niacin concentration in the food, found in this study under buffered environmental conditions, could translate into increased mortality under natural conditions. Free ranging leverets are in spring frequently exposed to not only much lower ambient temperatures, but also to wind and precipitation (Hackländer et al., 2001). Such conditions increase the basal metabolic rate of leverets (Hackländer et al., 2002a). In addition, free-living leverets have no shelter and thus have most likely higher energy requirements than leverets in our study, at least in spring. When mothers are unable to deliver enough energy with milk because of malnutrition caused by maize monoculture in their home ranges during lactation, or the fattening period preceding the reproductive season, growth of juveniles may well be much more impaired as found under our experimental conditions. As a result, juvenile mortality may become substantial under natural conditions, considering that weak juveniles are also more likely to fall victim to predators. High juvenile mortality

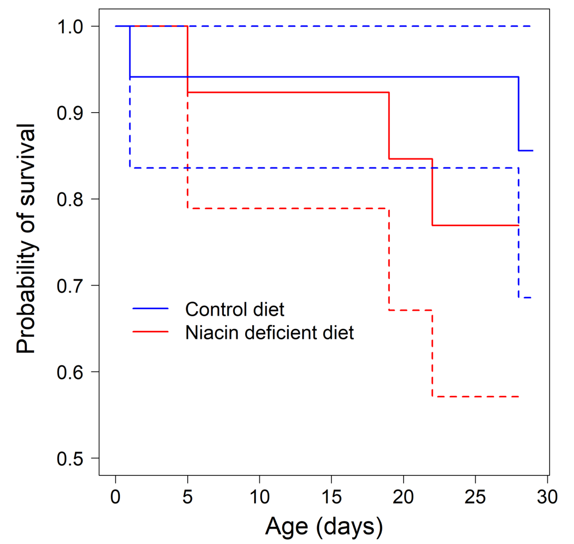

FIGURE 3
Probability of survival of leverets during the nursing period from birth to forced weaning at the age of 28days (solid lines, estimated survival probability, dashed lines 95% confidence intervals of survival estimates). Leverets had, like their mothers, access *ad libitum* to either a niacin deficient pellet, or a niacin supplemented control pellet.

negatively affects population development particularly when spring litters suffer, because early born leverets are most important for population growth (Olesen and Asferg, 2006).

5. Conclusion

Altogether, our study showed that a niacin deficient diet negatively affects growth of brown hare leverets. Combined with low plant biodiversity due to monoculture, and increasingly unfavorable climatic conditions, a maize biased nutrition seem to be an additional, so far neglected factor contributing to the decline of brown hares throughout Europe.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by all procedures were carried out in accordance with the Austrian legislation as assessed and approved by the institutional ethics commission of the University of Veterinary Medicine Vienna (GZ: BMBWF-68.205/0080-V/3b/2019).

Author contributions

AS, WA, and MT conceived the study. AS and WA analyzed data and wrote the manuscript. GS and JP-G contributed to designing the experiment. AS, AH, and HR collected data. AH and HR provided veterinary care. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Group-living decrease predation risk of individual: Evidence from behavior, hormones and reproduction of plateau pika

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Extensive research confirms that abiotic stressors like predation risk can profoundly affect animal condition. However, there is a lack of experimental research assessing the suite of physiological responses to risk. We increased predation risk in free-living plateau pikas (*Ochotona curzoniae*) by simulating natural chases using a model predator (UAV: unmanned aerial vehicle) and monitored behavior, physiology, and reproduction of pikas. We found that: Predation risk affects the body weight of plateau pika under different population density stress, but the effect is not significant. Compared with the non-predation risk treatment (control), the plateau pika under high population density stress mainly responded to the risk of predation by reducing the foraging time and increasing the vigilance time, while plateau pikas under low population density pressure not only reduce foraging time and appropriately increase vigilance time, but also focus on increasing the concealing time in the burrows. The corticosterone (CORT), cortisol and thyroid (T_4) level of plateau pika with low population density under the predation risk was significantly higher than those in the control, and the testosterone (T), progesterone (PROG), leptin (LEP) and testicular weight of plateau pika with low population density in the predation risk was significantly lower than those in the control. There was no difference in the litter size of female pika between predation risk treatments and control. Broadly, our result show that predation risk had significant effects on the behavior, physiology and reproduction of plateau pikas with low population density, but had no significant effect with high population density, and the response of male plateau pika to predation risk was greater than that of female. Therefore, the prevention of plateau pika should focus on the control of population density and the protection of predators, so as to avoid the failure of ecological prevention methods such as natural enemies due to the increase of population density.

KEYWORDS

predation risk, anti-predator behavior, non-consumptive effects, stress, plateau pika (*Ochotona curzoniae*)

1. Introduction

Studying how biotic and abiotic stressors affect animal activity is essential to reveal the complex structure and function of physiological systems (Boudreau et al., 2019). Abiotic stressors include various challenges imposed on wildlife, including predation risks (Turner and Chislock, 2010). Animals respond to predation risks by increasing vigilance, changing group sizes, or finding safe habitat (Childress and Lung, 2003; Creel et al., 2014). Typically, these anti-predation behaviors will reduce the foraging rate of animals, change dietary patterns or cause physiological stress responses, and eventually affect the growth and reproduction of prey (Kotler et al., 2004; Yin et al., 2017). In recent years, the research on the response of animal acute and chronic stress to predation risk has been gradually improved (Clinchy et al., 2013). The predation stress theory states that under the risk of predation, chronic stressors cause the hypothalamus to release corticotropin-releasing factor (CRF) and other hormones, and promote the release of adrenocorticotropic hormone (ACTH) from the hypophysis, which in turn leads to the release of cortisol or corticosterone from the adrenal cortex. The increase of corticosteroid concentration can inhibit the activity of hypothalamus-pituitary-gonad axis through acute stress, If predation stress persists, higher glucocorticoid concentrations in chronic exposure can lead damage to mammals, including immunosuppression and weight loss (Christianson and Creel, 2010; Clinchy et al., 2013). Therefore, physiological changes related to predation risk are usually represented by corticosteroids (cortisol and corticosterone) and sex hormones (testosterone and progesterone) to represent the physiological response of animals (Hawlena and Schmitz, 2010; Zanette et al., 2011).

The level of predation risk is closely related to the population density of small mammals. Under the pressure of population density, the behavior strategy of small mammals is mainly affected by the tradeoff between risk pressure and energy demand. The greater the density pressure, the greater the weight of risk aversion behavior (concealing, vigilance, escape, etc.) in their behavior patterns, while foraging and social behaviors decrease accordingly (Bian and Fan, 1997). At present, domestic and foreign scholars make lots of research to verify the hypothesis of “many eyes effect” in many behavioral studies of small mammals. For example, with the increase of *Octodon degus* population density, the earlier it finds predators (Ebensperger and Wallem, 2002); In addition, group-living *Microtus epiroticus*, *Clethrionomys glareolus* and *Apodemus flavicollis* are less likely to be attacked and hunted by weasels than individuals living alone (Jędrzejewski et al., 1992). *Cynomys ludovicianus* and *Cynomys leucurus* with high population density can find predators earlier and avoid them effectively (Hoogland, 1981). Although numerous studies have demonstrated that increased population density is beneficial for small mammals to cope with predation risks, the physiological mechanism remains unclear.

We conducted a field experiment manipulating predation risk in free-living plateau pika (*Ochotona curzoniae*) to assess their physiological, behavior, reproduction response in a natural setting. Plateau pika (*Ochotona curzoniae*) are small, diurnal lagomorphs that inhabit alpine meadows on the Qinghai-Tibet Plateau (QTP), People’s Republic of China (Wilson and Smith, 2015). They have crucial ecological significance for biodiversity maintenance in alpine rangeland ecosystems and improving soil quality on the QTP (Zhang et al., 2016). Mammalian predators of pikas include Wolves (*Canis lupis*), Tibetan foxes (*Vulpes ferrilata*), Red foxes (*V. vulpes*); avian predators include Upland buzzards (*Buteo hemilasius*), Golden eagles (*Aquila chrysaetos*), Steppe eagles (*A. nipalensis*) and Saker falcons (*Falco cherrug*) (Smith et al., 2018). Therefore, Plateau pikas face high predation risk in alpine meadow ecosystem. Since the 1990s, some scholars have done a lot of research on the behavior and physiological stress response of plateau pikas under population density or predation pressure in laboratory and natural conditions, and the “predator sensitive food hypothesis,” “predator stress hypothesis” and “multi-eye effect hypothesis” were verified (Yang et al., 2007; Yin et al., 2017; Wei, 2019). Among them, Wei et al.’s study on the “multi-eye effect hypothesis” of plateau pikas shows that the higher the population density, the lower the predation risk of plateau pikas in the population, and the vigilance time decreases with the increase of population density. However, the above results are based on the fact that the population density of plateau pikas is within the range of 36–132 effective burrows per hectare, but under the higher population density of plateau pikas, the strategy of pikas to deal with predation risk is also consistent with the theory of “multi-eye hypothesis.” It’s not clear. We selected two plateau pika populations with different densities and used UAVs to simulate natural enemies to interfere with these two populations, and analyze the response of behavior, physiology and reproduction of plateau pika with different population densities to predation risk. We hypothesized that under the predation risk, the anti-predation behavior and physiological response of the high density population were weaker than those of the low density population, and the reproduction of the high density population did not decrease significantly. Based on the hypothesis, we predicted that (1) the plateau pika with low species density reduces the predation risk by increasing the vigilance time, while the plateau pika with high population density reduces the predation risk by increasing the concealing time in the burrows, (2) the predation risk can significantly reduce the contents of testosterone and progesterone in plateau pikas, and increase the contents of cortisol, corticosterone, thyroid hormone and leptin in plateau pikas, (3) regardless of the population density of plateau pika, the predation risk significantly decreased the testicular weight and the litter size of plateau pika.

2. Materials and methods

2.1. Study area

This study is located on the northern Qinghai-Tibet Plateau in Maqu Tibetan Autonomous County in Gansu Province, China (N 33°50′23″, E 102°08′48″). The climate belongs to continental monsoon type dominated by the southeastern monsoon and high pressure from Siberia. Winter is long and severe, and summer is short and cool (Wei and Zhang, 2018). Based on a survey of vegetation and topography in the study area, we selected the pasture of an alpine meadow with an altitude of 3,250 m. Plateau pikas are the main lagomorphs in the study area, with a relative population density of 232 ± 18 effective holes per hm^2. Therefore, this area is an ideal place to study the behavior of plateau pikas.

2.2. Experimental and sampling design

On March 19, 2018 (the breeding start period of plateau pika). Four experimental plots (1 ha per plot) were selected in the study area. The study area was divided into two high population density and two low population density. The vegetation status (coverage, height, dominant plant species, etc.) and topography (slope, height, distance to the road, etc.) between the experimental group and the control group in the same plateau pika population density area are as

TABLE 1 The information of study areas.

Density Treatment	High		Low	
	Predation risk	Control	Predation risk	Control
Coverage of plants (%)	78.49 ± 6.21 a	80.38 ± 11.17 a	86.04 ± 5.83 b	89.71 ± 4.19 b
Height of plants(cm)	4.23 ± 0.91 a	4.76 ± 1.06 ab	5.07 ± 0.55 b	5.10 ± 0.18 b
Number of effective burrows (no/hm^2)	271 ± 23 a	259 ± 17 a	143 ± 21 bc	171 ± 11 b
Grazing intensity (sheeps/hm^2)	0.402	0.402	0.402	0.402

consistent as possible, and the specific information of the experimental sample site is shown in Table 1. The average activity range of plateau pika was 114.2 ± 5.1 m in the study area, in order to avoid the interference between different sample plots, the distance between plots is more than 200 meters. Plots were divided into two temporary sampling plots (fecal sampling and weight measurement) and one behavior detection plot.

2.3. Animal marking

On March 19–23, 2018, plateau pika was caught in the study area trapped using string snares (Dobson et al., 1998). After each capture, the capture position, body weight, sex and reproductive status of each plateau pika were recorded. In order to identify the individual of plateau pika in behavior observation, dyeing agent was used. The head of male and backs of females was also marked with black hair dye, which can determine the sex of the individual at a long distance. Each marked pika was released in the original capture location. Following each capture, the weight, sex, and reproductive condition of each adult pikas were recorded. All procedures involved in animal treatment in this experiment were approved by the animal ethics committee of Gansu Agricultural University (GSC-IACUC-2018-0011).

2.4. Predation risk simulation experiment

In this study, the unmanned aerial vehicle (UAV) was used to simulate the predation risk of raptors to plateau pikas. Before simulating the predation risk experiment, we need to conduct two preliminary experiments to determine the optimal flight parameters of the UAV and the impact of UAV noise on the plateau pika.

2.4.1. Experiment 1

First, we need to determine the optimal flight altitude and velocity of UAV. We used the target animal observation method to record the response of the marked plateau pika to the different flight altitude and velocity of UAV. The ratio of the number of entering burrows and the total number of ground activities of plateau pika was used as the response efficiency to choose the best flight height and velocity of UAV. The result showed that the response efficiency is the most obvious when the height is 10 m and the velocity is 8 m/s (Details refer to the Supplementary Table 1).

2.4.2. Experiment 2

Secondly, we need to verify whether the sound generated by the UAV make an effect on plateau pika. We used PCM-D100 digital recorder (frequency response range: 0.04–1.5khz) and SY-322 external microphone of SONY company to record the sound generated by the UAV, and then imported the sound file into the sound playback device (Bose Sound Link Mini). Put the sound playing equipment into the experimental plot, and observed the behavior changes of plateau pika in the experimental plot and the control plot. Set three observation time points (0900, 1,200, 1,600) in a day, and observe for 20 min each time. The results showed that there was no significant difference in the behavior of plateau pika between the experimental and the control (Details refer to the Supplementary Table 1).

In the previous study on the activity pattern of plateau pika, it was found that the activities of plateau pika in March and April were unimodal, and the activities of the day were mainly concentrated in 9:00–17:00 (Zhou et al., unpublished data, 2022). Therefore, UAV interference is conducted from 9:00 to 17:00 every day. The official interference time starts on March 29 and ends on April 7.

2.5. Behavior variables and measurements

The recorded behaviors of plateau pikas are defined the following: (1) concealing: the animal hid inside the burrow, (2) foraging: gathering or chewing vegetation, and (3) vigilance: sitting in the meadow with neck outstretched, standing with front feet off the ground, or raising head during foraging, (4) others: other behavior except for the above behaviors in the observation period (Yang et al., 2007).

At the active peaks from 0900 to 1700, after most pikas were captured for 3 days, observers measured behaviors according to a focal-animal sampling rule and a continuous recording rule (Martin and Bateson, 1985). Focal individuals were randomly selected from the colony based on color of fur dyeing location. Binoculars were used to identify the focal animal and observe its behavior. During the observation period, in order to avoid the impact of observer, the observer sat at a distance of more than 30 m from the study area (Wei et al., 2019). Observe for 20 min per hour and change observation points every other hour to ensure that each labeled adult pika is observed at least once. Use a stopwatch to record the duration of each behavior of the focus animal every 20 min.

2.6. Feces collection and hormone measurement of plateau pika

We set the fecal collection time to March 29th, April 3rd, April 7th. To facilitate the quantitative collection of all voided feces without handling the animals, we selected 15 active latrines of plateau pikas based on their similar characteristics (Navarro-Castilla et al., 2019). Following the method of Calvete et al.

TABLE 2 The fixed effects and random effects in each liner fixed model.

Index	Sensitivity	Intra-assay coefficients of variation (%)	Inter-assay coefficients of variation (%)
T	1.0 pg./ml	6.1	7.4
PROG	0.1 pg./ml	7.7	6.1
T4	0.1 pg./ml	7.9	8.6
LEP	0.1 pg./ml	6.8	6.9
CORT	1.0 ng/ml	8.9	7.2
Cortisol	1.0 ng/ml	6.3	7.0

(2004), only pellet groups with clear fecal accumulation and with multiple pellets were considered latrines. Individual characteristics (e.g., sex, age and breeding condition) usually influence physiological variation in animals (Cockrem, 2013). Recently, Lafferty et al. (2017) showed that subsamples derived from homogenized whole pellet groups from snowshoe hares (*Lepus americanus*) are the most appropriate and representative of the physiological state when the conditions are not fully controlled (e.g., unknown identity and multiple defecation events). Therefore, feces from the same latrine and same color fresh fecal particles were thawed and thoroughly mixed to minimize potential bias due to both sample and individual variation (Cockrem, 2013). There are many ways to determine the sex of animals by animal feces, one of which is to determine the sex of animals by sex chromosomes. Another is to determine whether male or female is based on the hormones in the feces. At present, it has been found that the ratio of *Tragopan caboti* to testosterone in the feces of female is significantly higher than that of male. This method can be used to successfully distinguish the sex of yellow-bellied pheasant. Therefore, we judged sex by the content of testosterone and progesterone in each sample (each plateau pika individual). Moreover, the sampling time is the early reproductive stage of plateau pikas, and there is a significant difference in the contents of testosterone and progesterone between male and female, which is also helpful for us to judge the sex of fecal samples.

The collected pellets were stored in the freezer at −20°C until analysis. Testosterone (T), progesterone (PROG), thyroid (T_4), leptin (LEP), corticosterone (CORT) and cortisol concentrations in fences were assayed by rat-specific enzyme-linked immunoassay with reagents supplied by Hunan fengrui Biotech Inc. (Hunan, China), and according to the manufacturer's instructions. The sensitivity, intraassay coefficients of variation and inter-assay coefficients of variation for PROG, T_4, LEP, CORT, and cortisol were listed in Table 2. All samples were tested twice. Refer to Wang et al. (2012) for the main steps of fecal hormone extraction.

2.7. Data analysis

We used the growth rate (GR) of each indicator between months (March–May) to determine the changes in body weight and reproduction (litter size and testicular weight) of plateau pikas under predation risk. The formula as follows.

TABLE 3 The fixed effects and random effects in each liner fixed model.

Response variables	Fixed effects	Random effects
Body weight	Control/density/sex	Pika ID
Testicular weight	Control/density	Pika ID
Litter size	Control/density	Pika ID
T4	Control/density/sex	Feces ID
LEP	Control/density/sex	Feces ID
T	Control/density	Feces ID
PROG	Control/density	Feces ID
CORT	Control/density/sex	Feces ID
Cortisol	Control/density/sex	Feces ID
CT	Control/density/sex	Pika ID
FT	Control/density/sex	Pika ID
VT	Control/density/sex	Pika ID

$$GR_i = \frac{\text{value}_{\text{May}} - \text{value}_{\text{March}}}{\text{value}_{\text{March}}} \times 100\%$$

In the formula, GR indicates the growth rate of the indicator, $\text{value}_{\text{May}}$ indicates the value of each indicator in May, and $\text{value}_{\text{March}}$ indicates the value of each indicator in March. i refers to the experimental group in high density area (EXH), the control in high density area (CH), the experimental group in low density area (EXL) and the control in low density area (CL).

The assumption of normality was tested by the Kolmogorov–Smirnov test, and the assumption of homogeneity of variances was tested with Levene's test. If these assumptions were not met, data were logarithmic or square-root transformed. Considering the effects of individual differences on behavior and fecundity, we used a linear mixed effect model to analyze the responses of plateau pikas body weight (BW), testicular weight (TW), litter size (LS), foraging time (FT), vigilance time (VT), and concealing time (CT) to predation risk. BW, TW, LS, FT, VT, and CT were taken as response variables, sex, treatment and different population density as fixed effects, and plateau pika individual ID as random effects. we also used a linear mixed effect model to analyze the responses of plateau pikas thyroid (T_4), leptin (LEP), testosterone (T) and progesterone (PROG), corticosterone (CORT) and cortisol to predation risk, taking T_4, LEP, T, PROG and CORT and cortisol as response variables, and sex, treatment and different population density as fixed effects. The feacal sample ID of plateau pika was taken as a random effect. If the treatment effect is significant, then a simple effect analysis is carried out, and the differences between groups are obtained. The specific settings of fixed effects and random effects in the linear mixed effect model were listed in Table 3.

The linear mixed model is run by lmerTest package in R, and the simple effect analysis is carried out by emmeans package. All statistical analyzes were carried out using R5.5.3 (R Core Team, 2016). Ggplot2 package is used to draw the graph. The data were expressed by mean ± SD. The significant level was $p < 0.05$, and the extremely significant level was $p < 0.01$.

3. Results

3.1. Effect of predation risk on body weight of plateau pika with different population density

The results showed that the body weight of male plateau pika decreased in both the predation risk group and the control under different population density, and the growth rate ($GR_{EXH} = -3.05\%$；$GR_{EXL} = -6.06\%$) of the predation risk was higher than that of the control ($GR_{CKH} = -1.86\%$；$GR_{CKL} = -2.96\%$). The body weight of female plateau pika increased in both the predation risk group and the control under different population density, and the growth rate ($GR_{EXH} = 8.92\%$；$GR_{EXL} = 7.36\%$) of the predation risk was lower than that of the control ($GR_{CKH} = 14.46\%$；$GR_{CKL} = 12.80\%$, Figure 1). The results of liner mixed model (LMM) test showed that the body weight of plateau pikas was significantly different between sex ($F_{1,81} = 4.513, p = 0.0362$), but there was no significant difference between treatment ($F_{1,81} = 0.301$, $p = 0.585$), density ($F_{1,81} = 0.003$, $p = 0.956$) and the interaction of the above three factors (Supplementary Table 2). It can be seen that predation risk can affect the body weight of plateau pikas, but the effect is not significant. It indicates the risk of predation has effect on the body weight of plateau pikas, but the effect is not significant.

3.2. Effect of predation risk on behavior of plateau pika with different population density

A total of 56 individuals were observed in this study (male: 35; female: 21). LMM test showed foraging of plateau pikas varied strongly with density ($F_{1,56} = 18.852$, $p = 0.0001$) and the interaction between treatment and density ($F_{1,56} = 5.4981$, $p = 0.0238$), but had no significant variance with sex, treatment and other interaction of the above three factors. Vigilance time of plateau pikas varied strongly with density ($F_{1,56} = 16.831, p = 0.00013$) and interactive between density and treatment ($F_{1,56} = 6.5565$, $p = 0.0141$). Concealing time of plateau pikas varied strongly with density ($F_{1,56} = 22.727, p < 0.0001$), treatment ($F_{1,56} = 35.249$，$p < 0.0001$) and the interactive between density and treatment ($F_{1,56} = 9.4118$，$p = 0.0038$, Supplementary Table 2). Simple effect analysis result showed foraging time of female plateau pikas in low-density plot decreased in predation risk group compared with no-predation risk group ($p = 0.031$). Foraging ($p = 0.0042$) and vigilance ($p = 0.027$) time of male plateau pikas in low-density plot decreased in predation risk group compared with no-predation risk group, but concealing time ($p = 0.027$) increased significantly (Figure 2). It can be seen that under the risk of predation, plateau pika in the high-density area reduced the time of foraging and increased the time of vigilance; while plateau pika in the low-density area increased the time of concealing time in the burrows in addition to reducing the time of foraging.

3.3. Effect of predation risk on hormone concentration of plateau pika with different population density

A total of 192 fences of individuals were observed in this study. LMM test also showed a significant difference in the treatment ($F_{1,86} = 27.834$, $p < 0.001$), interactive effects between sex and treatment ($F_{1,86} = 6.1552$, $p = 0.0141$) and interactive effects between density and treatment ($F_{1,86} = 37.326$，$p < 0.001$) in the fences T concentrations of male adult plateau pikas. The PROG concentrations in fences of plateau pika had no significant difference among densities, treatments, sex and its interactions. There was significant difference in T_4 concentrations between density ($F_{1,171} = 17.131$，$p < 0.001$) and treatment ($F_{1,171} = 35.809$，$p < 0.001$), but there was no significant difference between sex and other interactions of the density, treatments and sex. The LEP concentrations had significant difference among treatments ($F_{1,171} = 12.7341$，$p = 0.0005$) and the interaction between density and treatment ($F_{1,171} = 10.884$，$p = 0.0012$). CORT concentrations of adult plateau pikas showed a significant difference in the interaction between treatment and density ($F_{1,171} = 9.593$，$p = 0.0023$), and Cortisol concentrations showed a significant effects in the treatment ($F_{1,171} = 16.579$，$p < 0.0001$, Supplementary Table 2). Simple effect analysis result showed T concentrations of male adult plateau pikas in low-density plot significantly lower than that of high-density plot ($p < 0.001$). T ($p < 0.001$), T_4 ($p < 0.001$), LEP ($p = 0.0017$), CORT ($p < 0.01$), and Cortisol ($p = 0.021$) concentrations of adult plateau pikas in low-density plot significantly higher than that of high-density plot. There is no significant difference of these 6 hormones in high-density between predation risk group and control group. The results showed that predation risk had the most significant effect on CORT, cortisol, T_4, T, and LEP of plateau pika in low-density, but had no significant effect on high-density plateau pika (Figure 3).

3.4. Effect of predation risk on reproduction of plateau pika with different population density

The results showed that the testicular weight of male plateau pikas increased at first and then decreased under different population

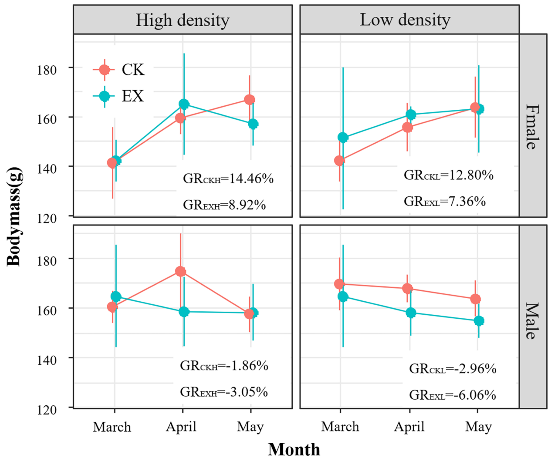

FIGURE 1
Mean (± SE) body mass (g) of adult plateau pikas from March to May between predation risk treatment group (n=69 individuals) and control group (n=58 individuals). The blue solid point represents the predation risk treatment group (EX), red solid point represents the control (CK); dot represents the high population density area (HIGH), triangle represents the low population density area (LOW); GR denotes the change rate.

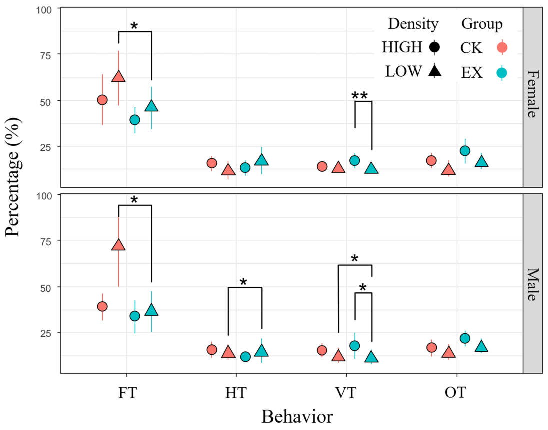

FIGURE 2
Mean time (± SE) allocation pattern of main behavior (FT-foraging time; CT-concealing time; VT-vigilance time; OT-other time) of the plateau pika in predation risk. The blue solid point represents the predation risk treatment group (EX), the red solid point represents the control (CK); dot represents the high population density area (HIGH), triangle represents the low population density area (LOW). *$p<0.05$; **$p<0.01$.

density, and the rate of change ($GR_{EXH} = -23.73\%$; $GR_{EXL} = 79.87\%$) in the treatment group was higher than that in the control ($GR_{CKH} = -6.92\%$; $GR_{CKL} = 79.65\%$), and the female litter size also increased at first and then decreased, but the rate of change in the treatment group ($GR_{EXH} = -42.86\%$; $GR_{EXL} = 31.43\%$) was lower than that in the control ($GR_{CKH} = -41.03\%$; $GR_{CKL} = 9.52\%$, Figure 4). LMM test also showed that the testicular weight of plateau pikas was significantly different in the density ($F_{1,33} = 4.945$， $p = 0.0284$). The litter size of female adult plateau pikas had no significant difference in the treatment, density and interaction between treatment and density (Supplementary Table 2). Simple effect analysis result showed testicular weights of male adult plateau pikas in low-density plot was significantly lower in the predation risk group than in control ($p = 0.0053$), and had no significant effects in high-density plot (Figure 4). In conclusion, predation risk has the most significant effect on the fertility of plateau pikas with low population density plot, but had no significant effect in high population density plot.

4. Discussion

4.1. Predation risk has no significant effect on the weight of plateau pika

Ecological factors such as food resources, population density and predation risk have great effects on the body weight and somatotype of small mammals (Batzli et al., 1983). A large number of field studies have shown that under the predation risk, the foraging activity of animals is inhibited, the food intake decreases sharply or the metabolic process changes, resulting in the loss of animal weight, so as to make a trade-off between current and future resources (Lima and Dill, 1990; Mcnamara and Houston, 1990; Wei et al., 2004). Our research showed that under the predation risk, the body weight of female plateau pikas increased, while that of males decreased. This is mainly due to the fact that reproduction is an activity with high energy consumption of animals, and male plateau pikas increase their reproductive investment in order to find more mating opportunities or food sources, resulting in weight loss. However, due to the pregnancy during the breeding period, the weight change of the female was significantly higher than that of the male. We also found that predation risk had no significant difference in the effect of predation risk on the weight of plateau pika.

We can explain the result from the following two aspects. Firstly, we consider that it is mainly related to the type of predation risk. Predators are usually divided into three functional categories: resident specialist, nomadic specialist and generalist (Andersson and Erlinge, 1977). *Mustela altaica* and *Mustela nivalis* belong to resident specialist, *Vulpes ferrilata* belong to nomadic specialist, and *Gyps himalayensis* and *Falco cherrug* belong to generalist. Klemola et al. (1998) found that generalist predators had no significant effect on the growth rate of small mammals such as *Clethrionomys glareolus* and *Microtus agrestis*. Wei et al. (2004) carried out a series of studies on the actual effects of generalists on the behavior and physical condition of plateau pikas. The results showed that the generalist predator did not change the reproduction and bodyweight of the plateau pikas. On the contrary, the resident specialist predators such as *Mustela altaica* had significant effects on the plateau pika. These predators search, capture and kill prey according to the olfactory signals. In our study, the use of UAV as a predation risk to interfere with plateau pikas is actually a way of interfering with predators such as *Gyps himalayensis*. Generalists circulate in the sky before predation, which makes plateau pikas alert in advance and rely on complex burrow systems to avoid risks. Secondly, we speculate that the intensity of predation risk in our research had not reached the level that affects the bodyweight of plateau pikas. Like most small mammals, plateau pikas can weigh the risk of predation and make appropriate behavioral decisions. Although UAV increase the risk of plateau pikas, its strength may be within the bearable range of plateau pikas. Plateau pikas can compensate for the increased risk of predation by changing the time allocation of behaviors such as foraging and vigilance.

4.2. The behavior of plateau pika varied greatly among different density populations under the risk of predation

A large number of studies have shown that predation risk will also increase the cost of anti-predator behavior, even when direct killing is not apparent (Preisser and Deegan, 2005; Schmitz, 2008). In many cases, prey respond to high predation risk by reducing their foraging activity, general mobility and by increasing vigilance and their use of safer microhabitats (Apfelbach et al., 2005). When the prey is exposed to the risk of predation for a long time, the prey will still maintain moderate foraging activity in order to meet the minimum energy needs (Lima and Bednekoff, 1999). While the prey is avoiding the predator, it is necessary to increase the intensity of anti-predator. The behavior change induced by the predator increases the energy demand of the prey and seriously affects its foraging efficiency. The lack of energy intake eventually leads to the change of prey fecundity (Creel et al., 2007; Zanette et al., 2011). The results of this study show that under high density pressure, the plateau pika responded to the predation risk by reducing the foraging time and increasing the vigilance time outside the burrows, while the plateau pika in the low population density pressure responded to the predation risk by increasing concealing time in the burrows. And the foraging, vigilance

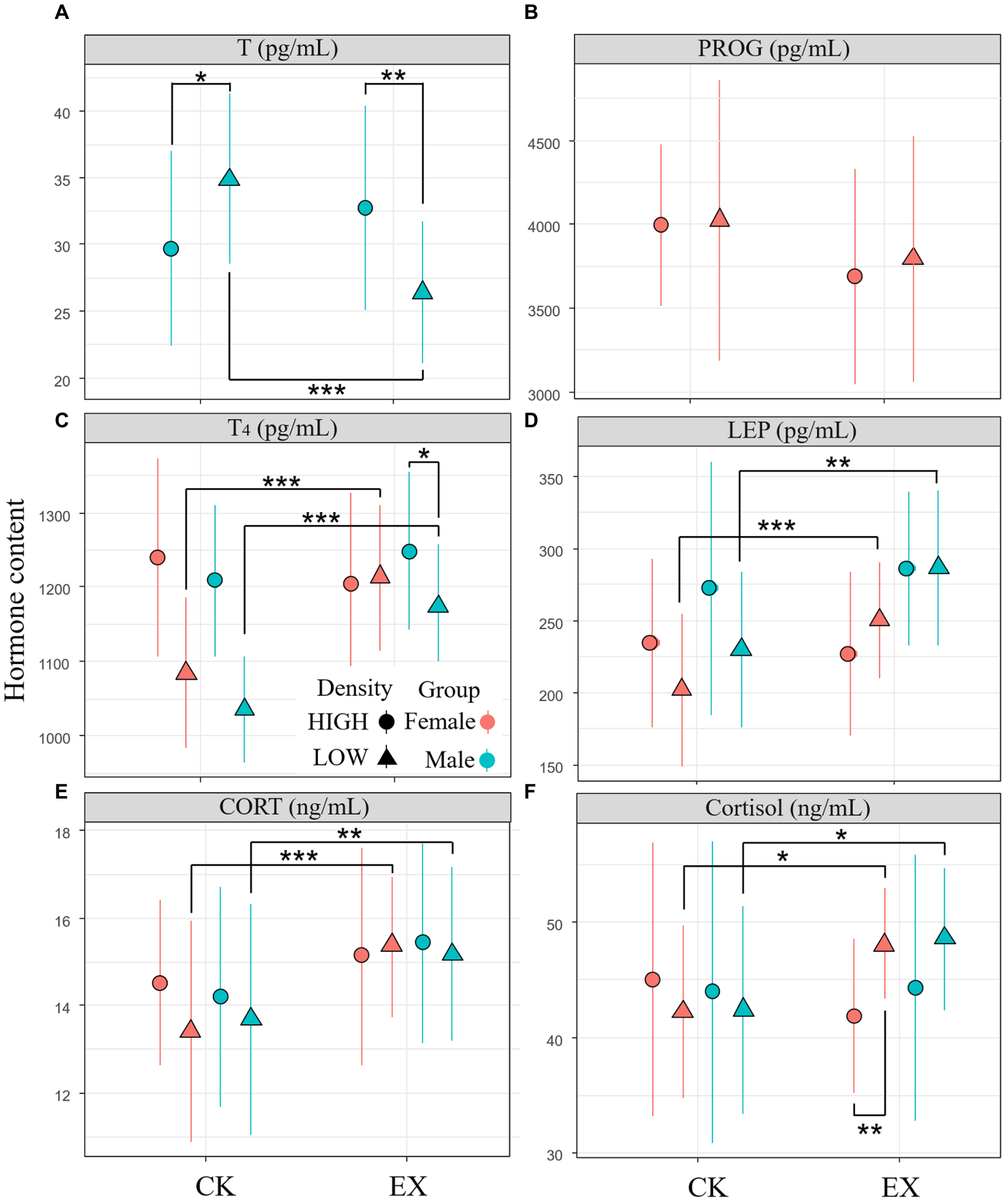

FIGURE 3
Mean concentration (± SE) of fences testosterone (T, *n*=89 individual) in Figure 1A, progesterone (PROG, *n*=89 individual) in Figure 1B, thyroid (T4, *n*=179 individual) in Figure 1C, leptin (LEP, *n*=179 individual) in Figure 1D, corticosterone (CORT, *n*=179 individual) in Figure 1E, cortisol (*n*=179 individual) in Figure 1F of adult plateau pikas between predation risk treatment group and control group. The blue solid point represents the predation risk treatment group (EX), red solid point represents the control (CK); dot represents the high population density area (HIGH), triangle represents the low population density area (LOW). *$p<0.05$; **$p<0.01$; ***$p<0.001$.

and concealing behavior of plateau pika in high density pressure were not significantly different between the predation risk treatment group and the control, while these behavior in low population density area were significantly different. The reason may be that the increase of population density leads to the decrease of individual predation risk, that is, the "many-eye effect" under predation risk. According to the theory of multi-eye effect, the risk of predation varies with the size of the population, and the increase of population density is beneficial for individuals to find predators earlier. Under the pressure of high population density, plateau pikas are easier to perceive predation risk through information transmission between individuals, and take corresponding measures. This has been confirmed by many related studies in small mammals, such as the earlier human are found with the increase in the population density of *Octodon degus*.

4.3. Hormones concentration and fecundity of plateau pika in low population density were more affected by predation risk

The adjustment in antipredator responses to cope with predation risk levels might be driven by stress response (Monclús et al., 2009).

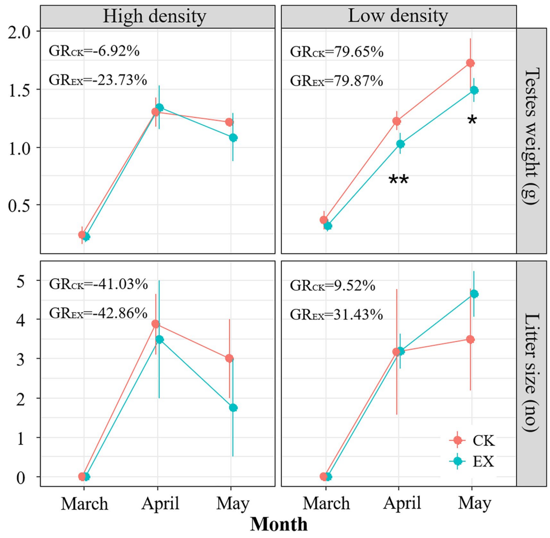

FIGURE 4
Changes in testicular weight and litter size in plateau pika under predation risk. The blue solid point represents the predation risk treatment group (EX), the red solid point represents the control (CK); dot represents the high population density area (HIGH), the triangle represents the low population density area (LOW). GR denotes the change rate. *$p<0.05$; **$p<0.01$.

If predation stress persists, higher glucocorticoid concentrations in chronic exposure can lead damage to mammals, including immunosuppression and weight loss (Mateo, 2007; Sheriff et al., 2009; Clinchy et al., 2011). Experimental data from laboratory studies confirm that predator-induced chronic stress will lead to the decrease of prey individual sex hormone content, and then affect their reproduction (Reeder and Kramer, 2005; Blas et al., 2007). In our research, it was found that under predation risk, the hormone levels of plateau pikas in the low population density were significantly different from those in the control, but the difference was not significant in the high population density. This indicated that predation risk did not affect the hormone secretion in high population density of plateau pika. There are two reasons for this result. One is that the increase of population density makes it easier for plateau pika to find predators and reduce the risk of predation, that is, the "many eyes effect." Therefore, the plateau pika in high population density avoids the abnormal increase of stress hormones caused by the risk of predation; secondly, due to the high competitive pressure in the high population density of plateau pika, the levels of hormones such as CORT and cortisol have been kept at a high level, predation risk has little effect on hormone levels. But the competition pressure is smaller in the low population density, and the response to predation risk is more obvious. Our results on the behavior patterns of the plateau pikas under predation risk indicated that the foraging and vigilance time of the plateau pikas in the high-density areas were not significantly affected by predation risk. This is also the main reason why there is no significant change in endocrine hormone in plateau pikas. In addition, from the average plant height of different plateau pika population density areas in the study sites, it can be inferred that the average plant height in the low density area is higher than that in the high density area, which increases the predation risk of the plateau pika and further increases the pressure level of the pika.

Predation risk has a significant impact on the reproductive, pregnancy rate and mating activities of prey (Ylönen, 1989). Reproductive activities can increase the risk of predation at all stages of reproductive events (Magnhagen, 1991). Therefore, the presence of predation risk will reduce its reproductive, whereas overwintered small mammals with low residual reproductive value may make their 'terminal investment' in the second (and probably last) summer of their life (Korpimaki et al., 1994). In our study, it was found that the predation risk had the most significant effect on the testes weight of male plateau pika in low population density, but not on high density population. We suggested that plateau pika could assess predation risks and make decision to avoid them. Under the low-density population, the plateau pika not only has to deal with the external predation risk, but also face the competitive pressure within the population, such as protecting territory, fighting for mating and food resources, especially in the breeding period. Under high density pressure, plateau pikas maybe increase their inclusive fitness by altruistic behavior to other individuals within the population when predation risk increases, and reduced the risk of individuals within the population. In the study of plateau pika behavior and the response of endocrine hormones to predation risk, we also found that in the face of predation risk, plateau pikas with low population density were more likely to increase concealing time in the burrows, while high-density plateau pikas population tended to increase the vigilance time, and endocrine hormones of plateau pika had no significant effect on the risk of predation under high population density. Therefore, the plateau pika with high population density can reduce the predation risk through the vigilance behavior among individuals within the population, and effectively reduce the inhibition of predation risk on reproduction.

We also found that the effect of predation risk on the litter size of female plateau pikas had no significant difference under high and low population densities. We think that this may be related to the trade-off between reproduction and predation risk of female plateau pikas during breeding period. Sheriff et al. (2009) also found that compared with the control, the litter size of female snowshoe hare (*Lepus americanus*) under predation risk had no significant difference, but the stress hormone level and body weight of their offspring decreased significantly. In our study of progesterone in female plateau pikas, we also found that predation risk did not significantly inhibit progesterone secretion. This also indirectly indicates that female plateau pikas in the breeding period will not reduce their investment in reproduction under the risk of predation, so as to ensure the birth of offspring. However, this "maternal stress" can affect the entire life cycle of offspring through epigenetic modification (Sophie and McGowan, 2015). To sum up, we have come to the following conclusion. The body weight of the pikas was not significantly different between treatment and control plots. Under the high population density pressure, the plateau pika responded to the predation risk by reducing the foraging time and increasing the vigilance time outside the burrows, while the plateau pika under the low population density pressure responded to the predation risk by increasing concealing time in the burrows. Predation risk could significantly affect the behavior, endocrine hormones and fecundity of plateau pika with low population density, but not on high density population.

5. Application and prospect

In the recent years, due to overgrazing and the global climate change, the alpine meadow has degraded seriously. The degraded meadow provides the plateau pika with a better habitat and results in the overabundance of plateau pika (Liu et al., 2012). In order to manage pests, a variety of methods have been used. The commonly used one to control plateau pika is poisoning, which kills them with botulins of models C and D (Yang, 2017). These methods are limited to reduce the population density and the number of mammals and eliminate the species from the alpine rangeland ecosystem in a period of time and region. This pests control strategy "population elimination" ignores the function and position of the species in the ecosystem and eliminate the function of the species in the ecosystem. In addition, the high frequency and large-scale use of rodenticide has destroyed the integrity of the original food web of rangeland ecosystem, and the natural enemies of pests have plummeted or even become extinct. Thus, it creates more favorable habitat conditions for the occurrence of pests damage. The Chinese government has added these rodenticide types to the restricted list of pesticides, such as botulins of models C and D. Almost all of these rodenticides are prevention and control drugs for plateau pika. Therefore, it is essential to find an effective ecological control method. Over the past 30 years, the ecological control method of introducing natural enemies (eagles, Tibetan fox, etc.) into the field have also been widely used, and some results have been achieved. However, our study found that the ecological control method is limited to the low-density plateau pika population, and the effect on the population with high density is not obvious. Therefore, the prevention and control of pikas damage in alpine rangeland should have varying method of different population densities, that is the ecological control measures such as natural enemies were used in the low density area, and the artificial vegetation was restored in the high density area, which reduced the population density of plateau pika, and then use ecological control when the population density decreased. In this study, UAV was used as the source of predation risk, and the bionic principle was used to interfere with the plateau pika. This efficient, safe and environmental control method can not only control the population of plateau pika, but also achieve the purpose of protecting biodiversity, and has a broad application prospect.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

RZ and LH contributed to the conceptualization, formal analysis, funding acquisition, investigation, methodology and project administration, software, supervision, and the writing original draft. RH and ZT contributed to the data curation. RZ contributed to the writing review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1037377/full#supplementary-material

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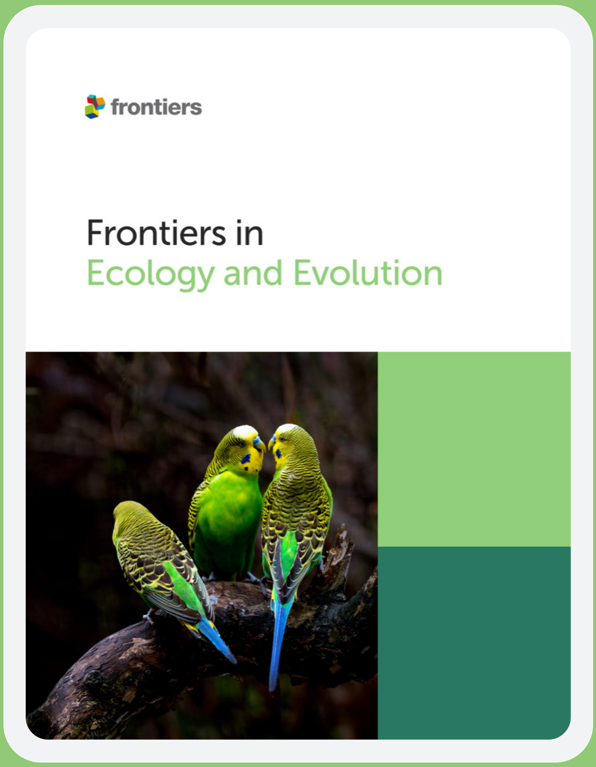

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