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Plateau pika burrowing and yak grazing jointly determine ecosystem greenhouse gas emissions of alpine meadow

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Abstract

Domestic yak (Bos grunniens) have coexisted with plateau pika (Ochotona curzoniae) for thousands of and they play irreplaceable roles in shaping the structure and function of alpine meadow ecosystem. However, the mechanisms whereby the greenhouse gas (GHG) emissions change in response to the interactive effects between yak grazing and plateau pika burrowing remain unclear. In this study, we examined the response of ecosystem GHG emissions (CO₂, CH₄, and N₂O flux) to yak grazing and pika burrowing in alpine meadow in Zoige County, China. The GHG emissions was measured with a static opaque chamber method. Our results revealed that CO₂, CH₄, N₂O flux and CO_2 -eq were significantly influenced by yak grazing and pika burrowing independently, and in conjunction. Crucially, the relative importance of pika burrowing was higher than yak grazing on a pastoral scale. Specifically, high pika burrowing led to an increase of 440.29%, and 110.72% for CO₂-eq relative to low pika burrowing under moderate and heavy yak grazing situations, respectively. The value of CO₂-eq with low pika burrowing was negative, especially under light yak grazing conditions. Furthermore, we found that GHG emissions were sensitive to plant species richness, soil temperature, soil moisture, soil organic carbon, and soil microbial factors. Structural equation modeling indicated that pika burrowing can affect CO₂-eq though altering soil temperature and belowground biomass under heavy yak grazing conditions and changing soil moisture and soil microbe under light yak grazing. The results of this study enrich our understanding of the role of small burrowing mammals in the carbon sequestration of alpine meadow. In the context of the carbon neutrality of alpine grassland ecosystem, small mammals' activities and their interactions with domestic livestock-induced changes in microtopography on GHG emissions should not be neglected.

KEYWORDS

driving mechanism, GHG emissions, plateau pika, soil properties, yak grazing

1 | INTRODUCTION

As the largest group of mammals, small mammals play irreplaceable roles in regulating ecological processes and functions of alpine grassland together with livestock (Davidson et al., 2012; Wang et al., 2020). Compared to the existed conflicts between small mammals and livestock, the mutualistic relationship due to their complementary effects is usually neglected (Martínez-Estévez et al., 2013; Smith et al., 2019; Tchabovsky et al., 2016). Indeed, plateau pika (*Ochotona curzoniae*) and domestic yak (*Bos grunniens*), Tibetan sheep and Hequ horse are well adapted to the hypoxic environment, and they have synergistic effect on plant and soil properties (e.g., plant composition, soil carbon accumulation, and soil nitrogen cycling) of alpine grassland (Li et al., 2019; Pech et al., 2007).

Plateau pika is a small diurnal and non-hibernating lagomorph, which live in subterranean, and its burrowing activity is seen to affect soil nutrient recycling in alpine grassland (Pang et al., 2021; Yu et al., 2017). At present, the ecological relationship between plateau pika and livestock has been further recognized (Li et al., 2019; Su et al., 2016; Wang et al., 2020). The plateau pika has a competitive relationship with livestock for food resources when they are both at high population densities (Badingqiuying et al., 2016; Pang et al., 2021). The physiological traits of plateau pika were altered by resource fluctuations, ultimately leading to population changes (Feijó et al., 2020). In contrast, plateau pika at low to moderate density has beneficial effects on livestock by increasing forage quality and productivity (Arthur et al., 2008). For example, the liveweight gain of sheep peaked at about 110 and 70 burrows/ha of pika density in warm and cold seasons, respectively (Wang et al., 2020). Furthermore, plateau pika can exploit yak feces to supplement their food intake and survive severe winter, which was demonstrated by the identification of yak DNA in pika stomach contents (Speakman et al., 2021). Many studies have examined the individual and distinctive effects of pika and livestock activities on grassland structure and function (Sun et al., 2015; Zhang et al., 2020). In alpine meadows, clipping and burrowing behaviour of plateau pika decreased aboveground biomass and plant height (Zhang et al., 2020), but significantly increased plant diversity, soil organic carbon, and soil nutrients (Pang et al., 2020; Yu et al., 2017). The changes in plant and soil properties can further affect the coupling and linkages between above and belowground of ecosystems (Wang et al., 2022). In the central grasslands of North America, the strong influences of domestic cattle (Bos taurus) and prairie dogs (Cynomys spp.) on structure and function of grassland were, where they co-occurred, compared to where each appeared alone (Davidson et al., 2010). However, such research to study the synthetic effects of domestic yak and plateau pika on the plant-soil feedbacks, especially their interactions on greenhouse gases (GHG) emissions of alpine meadow are currently lacking.

A variety of biotic and abiotic processes directly lead to GHG emissions in alpine meadows (Cai et al., 2017; Zhu et al., 2015). Specifically, soil respiration contained soil microbial, soil fauna and root respiration are the main sources of CO_2 emissions; CH_4 and N_2O are emitted through the combination of microbial processes (e.g., methanogenesis, nitrifier-denitrification and denitrification) in soil (Luo et al., 2020). In the meantime, these emission processes are also driven by change in environmental factors due to climate change (Eskander & Fankhauser, 2021; Luo et al., 2020), grazing management (You et al., 2021) and land degradation (Abdalla et al., 2018). To the best of our knowledge, most of the field trials on GHG emissions of Tibetan grassland were conducted in controlled small plots where livestock and small mammals were both excluded (Wang et al., 2019).

and interactive impacts on ecosystem GHG emissions through their foraging, burrowing behaviours, and excrement (Qin et al., 2015; Tang et al., 2021). For example, the contents of soil organic carbon and nitrogen were increased by excreta decomposition of livestock, then led to more CH₄ emissions by stimulating microbial activities such as methanogens (Cai et al., 2017; Maljanen et al., 2007). Likewise, Plateau pika can enhance GHG emissions by triggering a decline in plant photosynthetic tissue, and loss in soil nutrients with burrowing activities (Liu et al., 2013; Peng et al., 2015; Qin et al., 2020). Furthermore, plateau pika activities could offset the positive effects of warming on soil organic carbon pool (Yuan et al., 2021). Contrarily, the positive impacts of plateau pika disturbance on soil carbon stock were also observed in few studies in alpine grassland (Yu et al., 2017: Zhao et al., 2019). Yet, there is still considerable debate on the ecosystemlevel effects of plateau pika burrowing on GHG emissions in alpine meadows, especially, no direct measurements of CO2, CH4, and N2O emissions related to soil carbon stock responding to the combined disturbance of pika and vak.

Hence, we established an experiment with two conditions/situations/'treatments' (pika burrowing and yak grazing) in an alpine meadow of Zoige County on the east QZP. In this study, we aim to investigate the effects of plateau pika and yak grazing on GHG emissions in the pasture for grazing in the warm season. In detail, we address that: (1) What are the distinctive and synthesize effects of pika burrowing and yak grazing on GHG emissions? (2) What are the driving mechanisms regulating these effects? We hypothesize that: () GHG emissions will be higher in the plot with heavy yak grazing intensity and high pika burrow density; (ii) On a pastoral scale, GHG emissions probably are governed by pika burrowing more than yak grazing; and (iii) Pika burrowing and yak grazing directly or indirectly affect GHG emissions through their synergistic impacts on plant, soil properties, and soil microbe diversity (Figure 1). The results of this study



FIGURE 1 Conceptual framework of the mechanistic pathways of combination of livestock and small mammals on GHG emissions

enrich our understanding of the role of small burrowing mammals in the carbon sink of the alpine meadows.

2 | MATERIALS AND METHODS

2.1 | Site description

The field experiment was conducted in a typical warm-season yak grazing farm pasture (latitude $33^{\circ}40'11''$ N, longitude $102^{\circ}56'51''$ E, elevation ca. 3490 m) located 20 km northern of Zoige County (Figure S1A). The mean annual precipitation (MAP) is 640 mm, and mainly occurs in warm season (from May to September). The mean annual temperature (MAT) is 0.9°C. The minimum temperature is -9.6° C in January and the maximum temperature is 11.1° C in July (Liu et al., 2022). During the sampling period (in August 2018 and 2019), the monthly total precipitation was 99.41 and 130.57 mm, and the monthly mean temperature was 12.28 and 10.55°C. Vegetation type is alpine meadow, dominated by *Kobresia humilis, Elymus nutans, Saussurea* spp. and *Anemone* spp. The soil is classified as Mat-Cryic Cambisols (Chinese Soil Taxonomy Research Group, 1995). Prior to carrying out the experiment, the study area was freely grazed with yak during warm season each year.

2.2 | Experimental design

In early July 2018, two sampling areas (2-hectare each) were established 0-100 m and 900-1000 m from the entrance to the pasture where vak were released and subsequently dispersed over the pasture (Figure S1B). Since yak tends to concentrate close to the entrance of pasture to forage, rest, ruminate, drink, and excrete (Du et al., 2017), the distance away from the entrance to pasture was deemed to represent a surrogate for grazing intensity. In general, grazing intensity near the herder's house was higher, and herbage utilization gradually diminished with a greater distance away from the entrance to pasture (Guo et al., 2020). To evaluate the grazing intensity, we recorded the number of yaks in each sampling area every hour between 08:00 am and 18:00 pm each day in July 2018 and 2019. The grazing intensity was calculated with a published evaluation method (see Zhang et al., 2015). The grazing intensity was estimated as 9.10 and 0.80 yaks/ha respectively, corresponding to the abovementioned distances from the entrance. Hence, the two yak grazing conditions in this study were heavy stocking at 9.10 yak ha⁻¹ and light stocking at 0.80 yaks/ha.

In each sampling area (grazing conditions), three plots were randomly confined for light and heavy disturbance by plateau pika, respectively, for a total of 12 plots (Figure S1B). The size of each plot was 25 m \times 25 m, which is about the mean home range of plateau pika (Pang et al., 2020; Smith, 2008). In this study, the total pika burrows per plot (pika burrowing density) was used to represent the disturbance of plateau pika. We investigated pika burrows per plot by using aerial photography with light unmanned aerial vehicles (Qin et al., 2020) to gain two pika burrowing situations (low/high pika burrowing and heavy/light yak grazing). The density of light and high pika burrowing conditions were about 600 and 1800 burrow/ha, respectively. In addition, within each plot, we randomly set 4 quadrats (0.5 m \times 0.5 m) to obtain the GHG emissions, plant and soil samples (Figure S1B). Also, the pika burrow density of each quadrat was investigated.

For detailed main parameters of each yak grazing condtons and pika burrowing condtons, see Table S1. The terminology used in the paper is the internationally accepted standard terms for grazing lands (Allen et al., 2011).

2.3 | Field sampling and analyzing

2.3.1 | GHG emissions measurement

GHG emissions (CO₂, CH₄, and N₂O flux) was measured with a static opaque chamber and the gas analyzer method following the guideline in the previous research (Wei et al., 2020). In each aforementioned quadrat, the air samples were collected in each chamber at four-time intervals for each sampling event (0, 10, 20, and 30 min) from 09:00 to 11:00 am, and from 15:00 to 17:00 pm to represent daily average flux. Gas samples were collected on six consecutive days each year (13th to 18th August in 2018, 15th to 20th August in 2019). Gas samples were drawn through a three-way stopcock, using a 60 ml syringe, and then transferred for storage into 500 ml aluminum foil gascollecting bags (Luo et al., 2020). In the laboratory, a CH₄/CO₂ analyzer with syringe injection (DLT-100, Model No. 908-0011-0001) was used for simultaneous CH₄ and CO₂ analysis, and an N₂O/CO analyzer (Model No. 908-0015-0000) was used for simultaneous N₂O analysis. The fluxes were calculated based on the equation with modifications made for QTP conditions (Liu et al., 2017) as follows:

$$F = \rho \cdot \frac{V}{A} \cdot \frac{P_s}{P_0} \cdot \frac{T_0}{T} \cdot \frac{dC_t}{D_t}$$

Where: F is GHG flux (mg m⁻² hr⁻¹), ρ is gas density under standard conditions (1.977 and 0.717 kg m⁻³ for CO₂ and CH₄, respectively), V is chamber volume (m³), A is the base area of the chamber (m²), Ps is the atmospheric pressure (KPa) of the sampling sites, P₀ is the atmospheric pressure under standard conditions (101.325 kPa), T₀ is the temperature under standard conditions (273.15 K), T is the temperature inside the chamber (K), and dCt/dt is the average rate of concentration change with time.

2.3.2 | CO_2 equivalents (CO_2 -eq.)

 N_2O and CH_4 have global warming potentials that are 310- and 25-times higher than CO_2 (Sperow, 2020). The $CO_2\text{-}eq$ was calculated as

$$CO_2 - eq = CO_2$$
 flux + 25 × N₂O flux + 310 × CH₄ flux

2.3.3 | Plant and soil properties determination

After GHG emissions measurements in each quadrat, we measured plant surveys and collected soil samples. In the 0.5 m \times 0.5 m quadrat under each chamber, plant height and total number of plant species (SR) were measured. All on-ground plants were harvested and bagged to bring back the indoor. The dry weight after oven-dried at 65°C for 48 hr represented the aboveground biomass (AGB).

Soil moisture (SM) and soil temperature (ST) were measured at depth of 0–10 cm during gas sample collection using Field-Scout TDR-100. Soil bulk density (SBD) was measured with the cutting ring method. Soil was sampled at depth of 0–10 cm using an auger with 9 cm diameter, then root and soil fractions were separated. The root fractions were washed and weighed as belowground biomass (BGB) after drying. The soil fractions were sieved through a 0.2 mm mesh, then take sufficient samples for refrigerated storage (-80° C) for the determination of soil microorganisms. The remaining samples were stored in a refrigerator at 4°C for soil properties analysis. Soil total nitrogen (STN) was obtained from colorimetric method with the Element analyzer (Eelementar vario EL cube Germany) and soil

organic carbon (SOC) was measured with the traditional potassium dichromate oxidation method. The soil microbial community was analyzed by Illumina MiSeq sequencing analysis (Liang et al., 2021).

2.4 | Statistical analysis

Firstly, the distributions and normality were inspected by a goodnessof-fit test (Shapiro–Wilk test). We used a mixed linear model to assess the effects of yak grazing and pika burrowing and their interaction on GHG emissions, plant and soil properties. In this model, yak grazing and pika burrowing were used as two fixed factors, and the year was used as a random factor. The level of significance test set at p < 0.05. A Tukey's HSD test with the "*agricolae*" package was performed to evaluate differences in GHG emissions, plant and soil properties, and microbe diversity between two pika burrowing conditions, or between two yak grazing conditions, with significance at p < 0.05. Next, we conducted principal component analysis (PCA) through "*FactoMineR*", "*factoextra*" and "*corrplot*" packages in R software based on data of plant characteristics and soil properties to reveal the explanatory



FIGURE 2 Relative frequency of CO₂ flux (a), CH₄ flux (b), N₂O flux (c) and CO₂-equation (d) under low and high pika burrowing conditions

powers of variance in different condtons. Several principles should be taken into account: (a) The sampling adequacy of individual and set variables by the Kaiser-Meyer-Olkin measure (KMO value >0.50) and Bartlett's test of sphericity (p < 0.05); (b) Removal of variables with communality values <0.5 and (c) The selection of main components by the latent root criterion (eigenvalues >1.0). The relationships between GHG emissions with plant and soil properties and microbe diversity were explored with the "corrplot" package. Finally, structural equation modelling (SEM) with "sem" package was used to evaluate the direct and indirect effect of pika burrowing on CO₂-eq via changes in plant properties, soil properties, and microbe under light grazing and heavy grazing conditions. Prior to SEM analyses, the main components (PC1) of each plant, soil, and microbe properties group were selected via the PCA approach. Correlations among PC1 of each plant, soil, and microbe properties group with AGB, BGB, SR, SBD, STN, and soil bacteria diversity under light grazing and heavy grazing conditions were explored in "corrplot" package. Path coefficients and their significance

were estimated with the maximum likelihood method. The model fitting degree was expressed by *p* values of χ^2 test >0.05, root mean square error of approximation (RMSEA) < 0.05, standardized root mean square residual (SRMSR) < 0.08, comparative fit index (CFI) > 0.90, Tucker-Lewis iIndex (TLI) > 0.90. All statistical analyses were performed in R version 4.1.2 (R Core Team, 2021) and all figures were drawn using ORIGIN 2021b software.

3 | RESULTS

3.1 | Response of GHG emissions to pika burrowing and yak grazing

For the mean values, CO₂ flux, CH₄ flux, N₂O flux and CO₂-eq ranged from 606.61 to 940.12 mg m⁻² hr⁻¹ (Figure 2a), -44.27 to -29.48 mg m⁻² hr⁻¹ (Figure 2b), 0.30 to 0.37 mg m⁻² hr⁻¹ (Figure 2c)



FIGURE 3 Response of GHG emissions (CO₂ flux (a), CH₄ flux (b) and N₂O flux(c)) and CO₂-equation (d) to yak grazing and pika burrowing conditions. Significant differences (p < 0.05) between pika burrowing conditions under the same yak grazing conditions are indicated by different lower-case letters; significant differences between yak grazing conditions under same pika burrowing conditions are expressed by asterisk; *0.01 < p < 0.05, **0.001 < p < 0.01, ***p < 0.001; left half in grey represents heavy yak grazing, right half in white represents light yak grazing, HP, high pika burrowing; LP, low pika burrowing [Colour figure can be viewed at wileyonlinelibrary.com]

and -405.85 to 319.48 mg m⁻² hr⁻¹ (Figure 2d) under low and high pika burrowing conditions, respectively.

The mixed linear model analysis demonstrated that GHG emissions were significantly influenced by yak grazing (p < 0.05) and high pika burrowing (p < 0.05), each independently, and by yak grazing and pika burrowing (p < 0.05) in conjunction (Table S1). CO₂ flux, CH₄ flux, and CO₂-eq under pika burrowing condtons were significantly (p < 0.05) higher than that under low pika burrowing condtons regardless of yak grazing treatments (Figure 3a,b,d). Under heavy yak grazing condtons, high pika burrowing led to increases by 60.82%, 32.59%, 34.40%, and 440.29% for CO₂ flux, CH₄ flux, N₂O flux and CO₂-eq (Figure 3). Meanwhile, under light yak grazing condtons, high pika burrowing for CO₂ flux, CH₄ flux, N₂O flux and CO₂-eq (relative to low pika burrowing for CO₂ flux, CH₄ flux, N₂O flux and CO₂-eq, respectively (Figure 3).

3.2 | Response of plant, soil properties to pika burrowing and yak grazing

The PCA analysis demonstrated that 59% of the total variance for the plant and soil properties was explained by the first two axis (Figure 4). In detail, PC1 was more closely to SM, BGB, SOC and STN; PC2 was more closely to AGB and SR (Figure 4). SR, SM, SOC and STN under high pika burrowing conditions were significantly (p < 0.05) lower than that under low pika burrowing conditions regardless yak grazing conditions (Figure 5c,e,g,h). With heavy yak grazing, ST was significantly (p < 0.05) greater under high pika burrowing conditions than that under low pika burrowing conditions (Figure 5d). There was no



FIGURE 4 Principal component analyses of plant and soil properties. AGB, aboveground biomass; BGB, belowground biomass; SR, plant species richness; ST, soil temperature; SM, soil moisture; SBD, soil bulk density; SOC, soil organic carbon; STN, soil total nitrogen [Colour figure can be viewed at wileyonlinelibrary.com]

significant difference (p > 0.05) in soil microbial diversity between heavy and low pika burrowing conditions in both two yak grazing situations (Figure 5i-k).

3.3 | SEM mining the GHG emissions links to environmental factors induced by pika activities, and yak grazing

The CO₂ flux, CH₄ flux and CO₂-eq were negative closely associated with SR, SBD, SOC, and STN (p < 0.05; Figure 6a) under heavy yak grazing conditions. Similarly, Shannon-Winner index of soil microbe, STN, and SOC were the dominant factors that correlated with GHG emissions (i.e., CO₂ and CH₄ flux), followed by SBD and SR (Figure 6b) under light yak grazing conditions. Furthermore, significant positive relationships were found between the GHG emission with the ST (p < 0.05) as well as pika burrow density (p < 0.05; Figure 6b).

SEM was used to further reveal the driving mechanisms that affect the GHG emissions by pika burrowing under two yak grazing conditions (Figure 7). Under heavy yak grazing, the SEM analysis demonstrated that pika burrowing had significant negative influences on plant and soil properties with the effect coefficients of -0.52 and -0.44, respectively (Figure 7a). Contrarily, pika burrowing exerted significant positive effects on ST with the effect coefficient of 0.78 (Figure 7a). CO₂-eq was determined by ST, soil properties, and soil microbe with standard total effect values of 0.06, -0.33, and -0.22, respectively (Figure 7b). Under light yak grazing, pika burrow density had significant negative effects on plant, soil properties and SM with the effect coefficients of -0.68, -0.59, and -0.58, respectively (Figure 7c). There were significant positive effects of SM, and plant properties on CO₂-eq (standard total effect values are 0.16 and 0.26, respectively; Figure 7d). Nevertheless, soil microbe, and soil properties had significant negative effects on CO2-eq with effect coefficients of -0.36 and -0.10, respectively (Figure 7d).

4 | DISCUSSION

4.1 | Responses of GHG emissions to pika burrowing and yak grazing

Response of GHG emissions to grazing regimes has been well known on the QZP (Liu et al., 2017; Luo et al., 2020), but the activities of small mammals (e.g., pika burrowing and zokor digging) on GHG emissions remains controversial and how yak grazing and pika burrowing jointly shape GHG emissions are still unclear. Our results of field trials emphasized that CO₂, CH₄, N₂O flux, and CO₂-eq were significantly influenced by pika burrowing and yak grazing (Table S2; Figure 3). GHG emissions were the highest in the site which disturbed heavily by yak and pika simultaneity (Figure 3). Crucially, our results found that the relative importance of pika burrowing was greater than yak grazing on a pastoral scale (Table S2). Generally, compared to plateau pika activities, domestic livestock have broader landscape-scale



FIGURE 5 Response of aboveground biomass (a), belowground biomass (b), plant species richness (c), soil temperature (d), soil moisture (e), soil bulk density (f), soil organic carbon (g), soil total nitrogen (h), and soil bacterial diversity Chao1 index (i), observed species (j), Shannon-Wiener diversity index (k), Pielou evenness index (l) to yak grazing and pika burrowing conditions. Significant differences (p < 0.05) between pika burrowing conditions under same yak grazing conditions are indicated by p values of t-test; significant differences between yak grazing conditions under same pika burrowing conditions are expressed by asterisk; *0.01 < p < 0.05, **0.001 < p < 0.01, ***p < 0.001; left half in gry represents heavy yak grazing, right half in white represents light yak grazing; HP, high pika burrowing; LP, low pika burrowing

effects, while plateau pika have more intensive, localized impacts due to their sedentary behaviour and burrowing activities (Wang et al., 2019). Grazing events (foraging, trampling and excrements) could change soil water content, soil temperature, and the substrates supply from plant part to soil biota and microorganism that mediate the progress of GHG emissions production (Dowhower et al., 2020). Recent studies also showed that GHG emissions were affected by stocking rate rather than grazing system in grassland ecosystem (Ma et al., 2021; Wang et al., 2019). For example, increased yak excrement decomposition under high stocking rate may promote CO₂ emission by microbes through stimulating soil organic matter mineralization (Tang et al., 2021). In comparing to suitable stocking rate, high stocking rates lead to progressive reductions in plant biomass, which eventually result in reducing soil carbon stock (Chen et al., 2011). In agreement with other studies (Liu et al., 2013; Zhou et al., 2020), our study also demonstrated the important roles of plateau pika on GHG emissions (Figure 3). Specifically, high pika burrowing led to an increase of 440.29% and 110.72% for CO2-eq under heavy and light

yak grazing conditions, respectively (Figure 3). GHG emissions are closely related to plant cover, soil attributes, and microbial activity (Cai et al., 2017) which are regulated by pika through their foraging and burrowing activities (Yuan et al., 2021). For example, soil attributes (e.g., SBD, SOC, and soil texture) can change water holding capacity, then affect gas diffusivity of soils and relevant microbial activities, thereby modifying GHG emissions (Cai et al., 2017; Zhao et al., 2019). More potential mechanisms GHG emission from the interactive effects of pika burrowing and yak grazing need further to be explored.

4.2 | The driving mechanisms regulating mutual effects between pika burrowing and yak grazing on GHG emissions

Livestock and small mammals produce CO_2 flux themselves directly and affect CO_2 flux indirectly through changing substrate availability



FIGURE 6 Relevance among variables of plant, soil properties, and microbe diversity with GHG emissions under heavy (a) and light (b) yak grazing conditions. The value within the square represents the value of the correlation coefficient. *, ** and *** show the significant correlations at 0.05, 0.01 and 0.001 levels. AGB, aboveground biomass; BGB, belowground biomass; Chao1, Chao1 diversity index of soil bacteria; Obs, observed species of soil bacteria; SR, plant species richness; ST, soil temperature; SM, soil moisture; SBD, soil bulk density; SOC, soil organic carbon; STN, soil total nitrogen; Shannon, Shannon-Wiener diversity index of soil bacteria [Colour figure can be viewed at wileyonlinelibrary.com]

and soil physico-chemical characteristics (Han & Zhu, 2020; Li et al., 2019; Pelster et al., 2016). The CO₂ flux measured in this study consisted of root and soil respiration. The major factor determining the root respiration rate is root biomass (Lubbers et al., 2013). In this study, foraging and consumption of plants by pika reduced the biomass of root, especially under light yak grazing conditions (Figure 5b), then decreased carbon fixation via promoting root respiration (Girkina et al., 2018). Soil respiration is mainly determined by soil properties and microbial activities (Jian et al., 2018). Under heavy yak grazing, CO₂ flux was significantly positively related to ST and negatively correlated with SR, , and STN (Figure 6a). High pika burrowing may increase CO₂ flux by promoting ST and reducing SR, SOC and STN (Figure 5d,e,g,h). Pika burrowing can promote ST due to creating tunnel and turn deep soil to the surface and expose to the air (Qin et al., 2015). Meanwhile, loosening the surface soil led to more oxygen in soil, then made more soil carbon releases, which may decrease the soil's organic carbon stock (Zhang et al., 2015; Zhou et al., 2020). In the sampling plot which was heavily disturbed by pika and yak , the possible reason for higher CO₂ emissions was competition among microorganisms induces the microbes to use more C energy for cell

integrity and maintenance and led to higher soil respiration (Jacotot et al., 2018).

CH₄ flux is affected by many factors such as soil water content and soil temperature (Pelster et al., 2016), These factors influence CH₄ emission by altering the quantity and activity of methaneoxidizing bacteria and methanogens (Aghdam et al., 2017; Cai et al., 2017). CH₄ flux in this study showed good consistency with soil temperature and negative relations with soil moisture (Figure 6a). Soil temperature under high pika burrowing conditions was significantly higher than that under low pika burrowing conditions (Figure 5d). Soil temperature was increased with more fresh soil was exposed to the sunlight due to pika burrowing contained excavating, cleaning, and maintaining the underground cavern (Chen et al., 2017; Qin et al., 2021). Some studies showed that CH₄ flux in grassland is closely related to microbial composition, abundance, and function of methanotrophs (e.g., Proteobacteria and Verrucomicrobia) and methanogens (Görres et al., 2013; Zhang et al., 2015). Our results also found that the alpha diversity of soil bacteria has a significant negative correlation with CH₄ flux, particularly under light yak grazing conditions (Figure 6b). And that, the alpha diversity of soil bacteria under heavy



Structural equation model (SEM) fitted to linking pika burrow density to plant, soil properties, ST, SM, and soil microbial factors to FIGURE 7 GHG emissions under heavy (a) and light (c) vak grazing conditions. Significant paths are expressed by black and gry arrows. The thickness of the solid arrows reflects the magnitude of the standardized SEM coefficients. Standardized coefficients are listed beside each significant path."-"values respect negative effects. Standardized total effects bar graph (direct plus indirect effects) derived from the SEM depicted above (B and D). AGB, aboveground biomass; BGB, belowground biomass; Chao1, Chao1 diversity index of soil bacteria; Obs, observed species of soil bacteria; SR, plant species richness; ST, soil temperature; SM, soil moisture; SBD, soil bulk density; SOC, soil organic carbon; STN, soil total nitrogen; Shannon, Shannon-Wiener diversity index of soil bacteria

yak grazing was significantly higher than that under light yak grazing (Figure 5k,m). These differences in soil microbes probably provide an explanation for CH₄ flux was increased with heavyigh grazing intensities. Besides, high-intensity fecal addition of yak could stimulate CH₄ release. Anaerobic conditions formed by wetting of urine deposition can stimulate methanogenic activity (Cai et al., 2017). Also, the decrease of redox potential and increase of soil pH caused by urea hydrolysis are beneficial to methanogenic activity (Wang et al., 2021).

N₂O flux is mainly dependent on soil nitrification, denitrification, and mineralization processes, which could offer the substrates for N₂O production (Xu et al., 2008). Indeed, soil mineralization, nitrification, and denitrification rate are all sensitive to soil temperature, soil moisture, and contents of soil organic matter (Thilakarathna & Hernandez-Ramirez, 2021). Under heavy yak grazing conditions, N₂O emissions from high pika burrowed area was up to 1.3 times higher than from low pika burrowed areas (Figure 3c). The promotion of soil aeration and permeability due to plateau pika digging enhanced the microbial nitrification, which possibly was the main process (reduction of NO_3^- to NO_2^- , then to N_2O) of N_2O emission (Wang et al., 2021). Meanwhile, more yak dung deposition by heavy grazing pressure would enhance the abundance of ammonia-oxidizing bacteria (AOB), which are

functionally more important in nitrification in soil (Cai et al., 2017). Additionally, changes in oxidation-reduction condition of soil through reducing soil moisture and organic matter in mixed subsoil with topsoil (Yu et al., 2017) might be another explanation for N_2O emission.

Overall, our results indicated that through the driver of pika burrowing, SEM indicated soil microbe, and soil properties demonstrated significant negative effects on CO₂-eq under two grazing conditions (Figure 7). Whereas plant properties showed different effects on CO₂-eq. Pika burrowing can affect CO₂-eq though altering ST in the site near to entrance and changing SM in the site far from entrance (Figure 7). The lack of plant insulation is associated with greater temperature in bare soil due to heavy yak grazing (Liu et al., 2017; Qin et al., 2020), which can lead to more soil organic carbon being mineralized (Pang et al., 2021; Yu et al., 2017). In addition, higher water infiltration generated more dissolved organic carbon to leach into deep soil, thus resulting in carbon loss of topsoil (Pang et al., 2020). These different driving mechanisms further verify pika burrowing and yak grazing may interact synergistically to facilitate carbon loss. However, much remains to be explored about how these linkages between domestic yak and plateau pika and their context dependencies translate into synthetic effects on GHG emissions of alpine meadows.

Limitations of this study

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and Junker Most of the regions in the alpine meadow are undergoing carbon loss due to degradation (Zhang et al., 2014). Increasing efforts have been carried out to research the internal mechanism and consequences of carbon budget induced by small mammals (Tang et al., 2021; Zhao et al., 2019). Our study has two main aspects distinguish from other few studies in the past on this subject. First, we comprehensively consider the impacts of pika burrowing, yak grazing and their reciprocal actions on GHG emissions. Second, our results indicated that GHG emissions governed by pika burrowing more than yak grazing on a pastoral scale. Given that our experiment was carried out in the summer pasture, the seasonal variation in GHG emissions was not considered. Moreover, we indicated grazing intensity as the distance to the entrance of the pasture. This could limit our ability to make the quantitative evaluation of the effects of yak grazing on GHG emissions because of the lack of GHG emissions from rumination of yak (Lin et al., 2009). To get a better understanding of the responses of GHG

et al., 2009). To get a better understanding of the responses of GHG emissions to pika burrowing and yak grazing, further research is needed to: (1) quantify the combined effects of plateau pika and domestic yak on seasonal variation of GHG emissions, especially in cold seasons; (2) large-scale, long-term experiments are essential to be established in other areas of QZP.

5 | CONCLUSIONS

In the context of carbon neutrality of QZP, it is vitally important to explore the effects of small mammals and their interactions with livestock on GHG emissions. Our findings indicated that CO₂, CH₄, N₂O flux, and CO₂-eq were significantly influenced by pika burrowing and yak grazing independently, and in conjunction. Crucially, the relative importance of pika burrowing was higher than yak grazing on a pastoral scale. A notable observation in this study was that the value of CO₂-eq with low pika burrowing was negative, especially under light yak grazing conditions. CO2-eq was more sensitive to community structure, soil properties, and soil microbial factors. Furthermore, our results suggest that pika burrowing can affect CO₂-eq though altering ST and BGB in the site with heavy yak grazing and changing SM and soil microbe in the site with light yak grazing. In the context of carbon neutrality of grassland ecosystem of QZP, pika's activities and their interactions with livestock-induced changes in microtopography on GHG emissions should not be neglected. Moreover, as the climate crisis is getting serious, natural-based solutions (NbS) offer a no-regret route for tackling climate change and reducing carbon loss. Our study demonstrates that appropriate pika populations are beneficial for soil carbon sequestration in alpine meadow. Hence, policymakers and scientists should re-examine the plateau pika poisoning programmes that have the objective of eradicating populations in future.

AUTHOR CONTRIBUTIONS

Yingxin Wang and Fujiang Hou conceived the ideas and designed the methodology; Yingxin Wang, Shuaijun Hou, Yuhui Tian, and Zhaofeng

Wang collected the data; Yingxin Wang, Jian Sun, Shenghua Chang, and Junhe Chen analyzed the data; Yingxin Wang, Yongqiang Qian, Jianmin Chu, and Fujiang Hou wrote the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

No conflict of interest exists in the submission of this manuscript.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

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