



Livestock grazing-exclusion under global warming scenario decreases phosphorus mineralization by changing soil food web structure in a Tibetan alpine meadow

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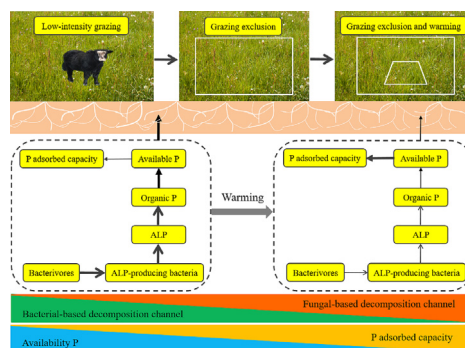
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HIGHLIGHTS

- Grazing exclusion and warming decreased the bacterial-based energy channels.
- Bacterivores had a positive effect on alkaline phosphomonoesterase.
- Fungal biomass had no effect on the acid phosphomonoesterase.
- Bacterial-based energy channels is a determinant of grazing optimization.

GRAPHICAL ABSTRACT



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ABSTRACT

The exclusion of grazing has been used extensively in alpine meadows on the Tibetan Plateau. Studies, however, have shown reported recent trends of decreasing concentrations of soil nutrients because of grazing exclusion and climate change. The effects of excluding grazing on the soil biogeochemical process of phosphorus cycling in alpine meadows are unclear, especially under climatic warming. We conducted a 5-year grazing-exclusion and warming-manipulation experiment to examine the effects of excluding grazing on fractions of soil phosphorus, microbial and nematode communities and enzymatic activities in treatments of low grazing intensity, grazing exclusion, and combined grazing exclusion and warming. Our results indicated that excluding grazing significantly decreased bacterivore and omnivore-predator densities, *phoD* gene abundance and alkaline phosphomonoesterase activity (in the 0–5 cm layer by –34, –41, –38 and –42 %) at altitudes of 3850 m, 4000 m, 4150 m and 4250 m, respectively. Structural equation modeling indicated that bacterivores positively affected *phoD* gene abundance, alkaline phosphomonoesterase activity and inorganic-phosphorus fractions. Combined grazing exclusion and warming significantly decreased bacterivore and omnivore-predator densities but significantly increased fungivore density (in the 0–5 cm layer by 238, 172, 119 and 65 %) at altitudes of 3850, 4000, 4150 and 4250 m, respectively. Structural equation modeling also indicated that the combined grazing-exclusion and warming treatment increased the soil fungi and fungivores, but the higher abundances of fungi and fungivores did not significantly affect acid phosphomonoesterase activity or

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inorganic-phosphorus fractions. Alternatively, the combined grazing-exclusion and warming treatment significantly increased the concentrations of amorphous and free aluminum, which were positively correlated with the maximum adsorption of phosphorus. The combined grazing-exclusion and warming treatment thus significantly decreased the availability of resin phosphorus (−63, −51, −81 and −67 %) in the 0–5 cm layer at altitudes of 3850, 4000, 4150 and 4250 m, respectively. Our results suggested that light grazing (0.5 yak ha^{−1} year^{−1}) could increase phosphorus mineralization and the activity of soil enzymes in alpine meadows under global warming. An adequate load of livestock pressure at each altitude can be an effective management technique, mainly under warming, to maintain an adequate, sustainable and equilibrated phosphorus cycle in the plant-soil system.

1. Introduction

Alpine meadows in Tibetan Plateau's cover an area of approximately 1.2×10^6 km², accounting for approximately 48 % of the land area, and they playing an important role in maintaining biodiversity, soil nutrients, and the livelihoods of herders (Wang et al., 2020a, 2020b). Alpine grazing lands, however, have been increasingly degraded for decades due to various factors, such as overgrazing and climate change (Zheng et al., 2020). Researchers have been looking for effective management techniques to stabilize communities and functions against overgrazing to restore degraded alpine meadows.

The exclusion of grazing is one such management technique that can potentially stabilize the community functions of degraded alpine meadows. Stabilizing effects have mostly been attributed to the exclusion of grazing, significantly increasing plant diversity, coverage and aboveground biomass (Wang et al., 2021a) and shifting plant dominant functional groups (Yao et al., 2019). Previous studies have mostly focused on relationships between grazing exclusion and plant communities, but little is known about the effects of grazing exclusion effects on the stability of soil food webs. Soil nematode food webs comprise major trophic consumers, including bacterivorous, fungivorous, herbivorous, omnivorous and predatory species (Ferris et al., 2001). Depending on their food, nematodes have a close link with other soil microorganisms and plants, and by regulating microbial communities and thus their functionality, nematodes play an important role in the decomposition of organic matter and regulation of nutrient cycling (Bardgett and van der Putten, 2014).

The availability of soil phosphorus (P) availability is controlled by biological and geochemical processes (Sun et al., 2020). Alpine meadows are characterized by a cold climate and short growing season and thus have low availabilities of soil P for use by plants, which could limit plant growth (Yang et al., 2021). A previous study identified P limitations on soil microbial metabolism in the alpine ecosystem of Gongga Mountain, especially at high altitudes (Cui et al., 2019). Grazing exclusion is widely regarded as a critical factor affecting soil P pools in alpine meadows, for three reasons. First, grazing exclusion can substantially improve the concentrations of soil total and available P due to higher community coverage, productivity and rate of litter decomposition (Yao et al., 2019). Second, the higher above- and belowground biomasses under grazing exclusion in alpine meadows provides more resources for soil microbes to secrete more soil phosphatases, which can greatly increase the decomposition of soil organic P (Wang et al., 2020a, 2020b). Third, long-term grazing exclusion may decrease soil P concentrations by reducing inputs of yak urine and dung, which substantively contribute to the maintenance of soil P nutrients by decomposition (Liang et al., 2019).

In this framework, our understanding of the responses of soil biotic communities and P availability to grazing exclusion is still limited and warrants investigation. Previous studies have mainly focused on the effects of grazing or the exclusion of grazing on soil microbial communities and subsequently on soil P dynamics (Yao et al., 2019; Wang et al., 2021a). Nematodes, as organisms with high trophic levels, however, can modify soil microbial activity and biomass by feeding on the microbes and subsequently affecting the processes and rates of decomposition of soil organic matter. For example, previous studies in other regions reported that bacterivores could increase the abundance of P-solubilizing bacteria, the decomposition of organic P and the concentrations of P in plant tissues

(Jiang et al., 2017; Ranoarisoa et al., 2018). Due to the slow rate of decomposition and the deposition of dung from grazing livestock, alpine meadow soil is rich in organic matter, which is an important factor regulating the soil nematode community (Wang et al., 2019). Bacterivorous colonizer-persister-1 (cp-1) nematodes have short life spans and can quickly recolonize fertile habitats (Ferris et al., 2001). A previous study also suggested that adding pig manure significantly increased the abundance of the bacterivore genus *Protorhabditis* (cp-1), which was positively correlated with bacterial abundance (Jiang et al., 2017). Alternatively, the activities of these nematodes can promote soil transport and produce channels, thereby greatly enhancing the diffusion of O₂ to deeper soil layers and eventually influencing Fe and Al oxides.

Moreover, we must also take into account that grazing is now occurring under increasingly warming conditions, but no studies have evaluated the effects of grazing and the exclusion of grazing on the soil nematode communities, organic P decomposition, or the adsorption and availability of P, especially under atmospheric warming induced by anthropogenic activity.

Human activity has caused global climatic warming, particularly in alpine regions (Zheng et al., 2020). Alpine meadows are thus simultaneously affected by grazing exclusion and climatic warming. Warming can strongly affect the soil P cycle by altering soil biological and geochemical variables (Rui et al., 2012). For example, climatic warming can stimulate microbial and soil phosphatase activity, thereby accelerating P cycling and plant growth in alpine meadows (Zheng et al., 2020), which together with grazing or the exclusion of grazing, could strongly influence microbial biomass, enzymatic activity and nutrient cycling (Wang et al., 2012). For example, a treatment of warming combined with grazing significantly increased the activity of soil phosphatase, suggesting that this combined could magnify the effects of warming or grazing (Rui et al., 2012). Another study suggested that warming significantly increased aboveground net primary production regardless of grazing, whereas grazing reduced the response of aboveground net primary production to warming (Wang et al., 2012). The effects of interactions between grazing exclusion and warming on the soil P cycle and plant growth in alpine meadows, however, remain to be elucidated.

Gongga Mountain, at the southeastern edge of the Tibetan Plateau, is a suitable area for grazing livestock due to the high precipitation. Many herders prefer their traditional practice of free grazing, which has a capacity of about one yak ha^{−1} year^{−1}. The grazing intensity at this site is about 0.5 yak ha^{−1} year^{−1}, which is categorized as low grazing intensity. We conducted an experiment with low grazing intensity, grazing exclusion and combined grazing-exclusion and warming at different altitudes in an alpine meadow on Gongga Mountain. Organic P represents the majority of total P in most alpine soils, so its biological cycling becomes increasingly important for the availability of P (He et al., 2018). We were thus the first time to conduct a field study combining grazing intensity with warming conditions to investigate their impacts on the soil trophic web. We studied the changes in the structure and functionality of the nematode and bacterial communities, focusing mainly on their impacts on the soil P cycle. We hypothesized that (1) grazing exclusion would decrease bacterial activity and projected decreases in alkaline phosphomonoesterase activity and P availability, because bacteria are strongly associated with the availability of soil nutrients, (2) a combined grazing-exclusion and warming treatment would increase fungal activity, acid phosphatase activity and P availability, because warming increases fungal biomass (Wang et al., 2017),

counteracting the negative effects of grazing on bacteria activity; and (3) the effects of grazing exclusion on soil P availability would be more negative at high than at low altitudes by the worse climate conditions hindering the system recovery after the grazing exclusion disturbance.

2. Materials and methods

2.1. Study site and experimental design

The experiment was conducted in the Gongga Mountain Observation and Experimental Station of Alpine Ecosystem (29.33–30.33°N, 101.50–102.25°E), Chinese Academy of Sciences. The research site was located in the Yajiageng Canyon on the eastern slope of Gongga Mountain, at the eastern edge of the Tibetan Plateau. This region has hot wet summers and cold dry winters. The average annual temperature is 3.8–4.1 °C, the average annual rainfall is 1100–1300 mm, and 70 % of the rainfall occurs during the growing season between June and October. The parental rocks are mainly composed of granitoids, the soil is rich in Al oxides, and the soil type is classified as an Udic Luvisol (Li et al., 2022). The initial soil P fractions sampled in May 2015, are described in Table S1. The dominant herbaceous plants are *Potentilla stenophylla*, *Polygonum viviparum*, *Carex laeta*, *Saussurea ceterach* and *Kobresia uncinoides*.

The alpine meadows in this region are mainly distributed from a low altitude of 3600 m to a high altitude of 4600 m (Li et al., 2022). The carrying capacity of grazing is about one yak ha⁻¹ year⁻¹, and the grazing intensity is about 0.5 yak ha⁻¹ year⁻¹, representing a low grazing intensity. We thus established three treatments, Grazing exclusion (GE), combined grazing exclusion and warming (GEW), and Low grazing intensity (G) as the control at altitudes of 3850 m, 4000 m, 4150 m and 4250 m a.s.l in April 2015. The experimental design was a completely randomized block with three replicates for each treatment at each altitude. Each plot had an area of 400 m² (20 × 20 m). To exclude livestock at each site, fences were constructed using wire netting to mainly exclude yaks and goats. Translucent synthetic glass was used to construct open-top chambers (OTCs) as a passive warming device to warm the alpine meadows (Li et al., 2022). Each OTC was 40 cm high and covered an area of 0.98 m² (Fig. S1). The plots for low grazing intensity were established next to the plots for grazing exclusion. Soil temperature was measured (depth of 5 cm) at 30-min intervals during the experimental period using automatic recording thermometers and thermistor sensors (CS616, USA). The pattern of changes in soil temperature and accumulated temperature in 2019 are presented in Figs. S2–3. The results indicated that the mean soil temperature increased by 1–1.5 °C (Campbell AR5, Avalon, USA).

2.2. Soil sampling and analyses

Samples were collected from the topsoil and subsoil (0–5 and 5–10 cm layers) using an auger 5-cm in diameter and the five-point method at each altitude in August 2020. For the GEW treatment, the five cores were combined to form one composite sample. For the G and GE treatments, each of the five cores were divided into five subcores, and the 25 subcores (Fig. S4) were combined to form one composite sample to make the samples represent the plots. Sample collection avoided dung patches and bare soil when possible. Fresh soil was used for extracting nematodes as soon as possible in the laboratory. One subsample soil was used to measure microbial biomass P and enzymatic activity another subsample was used to determine the phoD gene abundance, and air-dried soil was used to determine the chemical properties.

Exchangeable cations were extracted using 0.1 M barium chloride (Hendershot and Martin, 1986). Free oxides of Fe and Al were extracted using the dithionite-citrate-bicarbonate solution by the method of Mehra and Jackson (1960). NH₄-oxalate (0.2 M) and Na₂P₂O₇ (0.1 M) solutions were used to extract amorphous Fe/Al oxides and organically bound Fe/Al (Fep, Alp) (Loveland and Digby, 1984; Mckeague et al., 1971). We used ICP-OES to determine the concentrations of cations and metals. The extraction of inorganic- and organic-P fractions was based on Hedley

et al. (1982), and total P was the sum of all P fractions. Microbial biomass P was extracted using chloroform fumigation (Sun et al., 2020).

A series of KH₂PO₄ concentrations (10, 25, 50, 100, 200, 300, and 500 mg L⁻¹) was used to determine the P adsorption capacity, of P, and these data were then used to fit the Langmuir adsorption isotherm: $C/X = C/X_{max} + 1/KX_{max}$ (Moughli et al., 1993), where X is adsorbed P, C is the P concentration, X_m is maximum P adsorption and K is a constant for the binding strength.

We used 50 g of fresh soil from each plot to extract nematodes using the cotton-wool filter method (Townshend, 1963). At least 150 nematodes in the sample were identified to the genus level following standard identification keys (Bongers, 1988). These nematodes were mainly bacterivores, fungivores, omnivores-predators, and herbivores. We calculated the nematode Shannon-Weaver diversity index (H) as $H' = -\sum p_i (\ln p_i)$, where p_i is the proportion of individuals in the taxon i . The enrichment index (EI), structure index (SI), basal index (BI) and channel index (CI) were also calculated as in Ferris et al. (2001). A high EI and SI value indicate that the soil is enriched and the soil food web is stable, respectively. A high BI and CI indicate that the soil food web has been diminished due to stress. Soil decomposition involves bacterial and fungal-based channels. The nematode channel ratio (NCR) indicates the relative weight of the soil decomposition channels and is calculated as $NCR = B/(B + F)$, where B and F are the relative weights of bacterivores and fungivores respectively, to total nematode abundance. NCR varies between 1 (totally bacterial decomposition) and 0 (totally fungal decomposition) (Yeates, 2003).

The phoD gene encodes alkaline phosphomonoesterase and can be used to analyze bacteria that produce this enzyme (Sakurai et al., 2008). We extracted soil DNA using a Power Soil® DNA Isolation Kit (MoBio, California, USA) following to the manufacturer's instructions. The primers ALPS-F730 and ALPS-R1101 were used to analyze phoD gene abundance (Sakurai et al., 2008). We determined the activities of acid phosphomonoesterase (ACP) and alkaline phosphomonoesterase (ALP) using the procedure in Tabatabai and Bremner (1969).

2.3. Plant community

One subplot (0.5 × 0.5 m) was randomly established in each plot to investigate the plant community. The aboveground biomass of each plant species was collected and dried at 70 °C for 48 h to estimate biomass dry matter.

2.4. Statistical analysis

Statistical analysis was performed using SPSS v. 17.0 (SPSS Inc., Chicago, USA). The effects of grazing exclusion and combined grazing exclusion and warming on soil properties, enzymatic activity, nematode density and the ecological indices were tested using one-way analyses of variance and Fisher's least significant difference post hoc. A correlation analysis was used to quantify the associations between the soil properties and nematode density. AMOS 7.0 software was applied to obtain a causal understanding of the soil food web. In this structural equation modeling, the χ^2 -test ($P > 0.05$), the Bentler-Bonnet normed fit index (NFI) and the goodness-of-fit index (GFI) were used to assess the model fit.

3. Results

3.1. Abiotic responses to grazing exclusion and combined grazing exclusion and warming

GE significantly decreased the concentrations of Fe and Al, which were almost half of those in G (Table 1). GE had no impact on the concentrations of free, organic and amorphous Al but significantly decreased the concentration of organic Fe in the 0–5 cm and 5–10 cm layers at altitudes of 3850 and 4000 m, respectively (Fig. S5). GE significantly ($P < 0.05$) decreased the concentration of resin-P, NaHCO₃ Pi, and NaOH Pi in the

Table 1

Responses of soil chemical properties at the study site. Values are the means \pm SEs, $n = 3$. Different letters indicate significant differences among treatments. G, Grazing; GE, Grazing exclusion; GEW, Grazing exclusion and Warming.

		3850 m		4000 m		4150 m		4250 m	
		0–5 cm	5–10 cm	0–5 cm	5–10 cm	0–5 cm	5–10 cm	0–5 cm	5–10 cm
Ca (cmolc kg ⁻¹)	G	6.68 \pm 1.1	4.93 \pm 0.36	5.93 \pm 0.91	2.27 \pm 0.32	7.61 \pm 0.45	6.25 \pm 0.67	9.46 \pm 0.75	6.89 \pm 0.49
	GE	5.57 \pm 0.86	3.84 \pm 0.43	4.09 \pm 0.34	1.39 \pm 0.17	7.07 \pm 0.63	5.06 \pm 0.24	9.31 \pm 0.13	9.87 \pm 0.88
	GEW	9.13 \pm 1.38	4.22 \pm 0.52	5.04 \pm 0.76	1.93 \pm 0.24	7.74 \pm 0.79	5.26 \pm 0.36	9.03 \pm 0.78	8.36 \pm 1.13
Fe (cmolc kg ⁻¹)	G	22.41 \pm 2.44a	31.16 \pm 5.65a	14.64 \pm 0.21a	12.59 \pm 2.04a	17.39 \pm 0.73a	27.67 \pm 1.59a	17 \pm 0.75a	22.67 \pm 1.51a
	GE	12.09 \pm 2.71b	13.05 \pm 1.99b	7.7 \pm 0.95b	6.7 \pm 1.3b	13.63 \pm 1.28b	21.97 \pm 0.78b	14.11 \pm 0.64b	14.93 \pm 0.43b
	GEW	14.73 \pm 1.13b	12.64 \pm 1.13b	9.37 \pm 0.49b	7.2 \pm 0.24b	14.56 \pm 0.52ab	21.71 \pm 0.97b	13.88 \pm 0.46b	16.53 \pm 0.24b
K (cmolc kg ⁻¹)	G	9.4 \pm 0.69a	11.78 \pm 1.31a	11.82 \pm 0.82a	14.73 \pm 0.49a	9.18 \pm 0.82a	9.86 \pm 0.51	8.09 \pm 0.99a	6.78 \pm 0.3a
	GE	4.22 \pm 0.77b	3.77 \pm 0.71b	6.39 \pm 0.85b	10.04 \pm 1.33b	7.02 \pm 0.5b	9.6 \pm 0.85	5.31 \pm 0.26b	5.72 \pm 0.3ab
	GEW	5.29 \pm 0.35b	3.65 \pm 0.67b	6.3 \pm 0.62b	12.36 \pm 0.51ab	6.53 \pm 0.34b	10.55 \pm 0.45	4.76 \pm 0.18b	4.89 \pm 0.33b
Mg (cmolc kg ⁻¹)	G	7.77 \pm 0.43	9.7 \pm 1.31	7.14 \pm 0.25a	4.69 \pm 1.28	7.85 \pm 0.29	9.59 \pm 0.44	7.45 \pm 0.17	8.11 \pm 0.97
	GE	6.41 \pm 0.96	7 \pm 0.72	3.26 \pm 0.22b	2.23 \pm 0.27	9.39 \pm 0.69	8.68 \pm 0.94	6.56 \pm 0.22	6.67 \pm 0.51
	GEW	7.98 \pm 0.33	7.33 \pm 0.66	4.03 \pm 0.3b	2.6 \pm 0.09	9.65 \pm 0.67	8.75 \pm 0.43	6.66 \pm 0.56	7.82 \pm 1.01
Mn (cmolc kg ⁻¹)	G	0.62 \pm 0.09	0.43 \pm 0.08	0.35 \pm 0.07	0.13 \pm 0.08	0.66 \pm 0.06	0.74 \pm 0.1	1.01 \pm 0.15a	0.45 \pm 0.15
	GE	0.54 \pm 0.14	0.42 \pm 0.04	0.15 \pm 0.04	0.03 \pm 0.01	0.71 \pm 0.08	0.58 \pm 0.01	0.42 \pm 0.04b	0.37 \pm 0.01
	GEW	0.84 \pm 0.1	0.51 \pm 0.04	0.17 \pm 0.06	0.04 \pm 0.01	0.87 \pm 0.12	0.71 \pm 0.07	0.45 \pm 0.02b	0.42 \pm 0.05
Al (cmolc kg ⁻¹)	G	111.45 \pm 7.82a	149.85 \pm 26.01a	93.96 \pm 13.18a	65.72 \pm 6.9a	67.81 \pm 5.26a	85.6 \pm 3.82a	50.01 \pm 2.08a	68.98 \pm 10.12a
	GE	53.84 \pm 13.64b	65.06 \pm 10.24b	45.38 \pm 1.45b	38.64 \pm 5.64b	48.96 \pm 2.91b	66.37 \pm 3.18b	35.74 \pm 3.19b	35.06 \pm 0.48b
	GEW	73.58 \pm 5.4b	60.56 \pm 9.46b	50.97 \pm 6.04b	42.5 \pm 3.33b	52.84 \pm 1.99b	65.49 \pm 4.41b	37.25 \pm 2.86b	49.31 \pm 2.16ab

0–5 cm layer at all altitudes but did not affect the concentration of NaOHs Pi (Table S2).

The concentrations of exchangeable Al and Fe in GEW were almost half of those in G (Table 1). GEW significantly decreased the concentrations of exchangeable K in the 0–5 cm at all altitudes (Table 1). GEW significantly ($P < 0.05$) increased the concentration of amorphous Al and decreased the concentrations of organic and amorphous Fe in the 0–5 and 5–10 cm layers at all altitudes (Fig. S5). The initial P fractions were similar among G, GE and GEW (Table S1). After five years of warming, however, GEW significantly ($P < 0.05$) decreased the concentrations of resin-P, NaHCO₃ Pi, and NaOH Pi in the 0–5 cm layer at all altitudes but did not affect the concentrations of NaOHs Pi (Table S2). GEW significantly increased maximum P adsorption (Fig. 1), which was positively correlated with the concentrations of amorphous and free Al (Fig. 2).

3.2. Biotic responses to grazing exclusion and combined grazing exclusion and warming

GE significantly decreased the densities of total nematodes, bacterivores and omnivores-predators in the 0–5 cm and 5–10 cm layers at all altitudes but had no effect on the densities of fungivores and herbivores (Table S3). The relative abundance of fungivores in the 0–5 cm layer was significantly higher in GE than G at all altitudes (Fig. 3). GE significantly decreased the density of *Glauxinema*, with densities of only 5, 23, 18 and 21 % in G in the 0–5 cm layer at 3850, 4000, 4150, and 4250 m, respectively. (Table S3).

GE significantly ($P < 0.05$) decreased EI and SI in the 0–5 cm and 5–10 cm layers at all altitudes. Meanwhile, GE decreased the dominance of the bacterial-based pathway of decomposition, indicated by a lower NCR (Table 2). GE significantly ($P < 0.05$) decreased ALP activity and phoD gene abundance at all altitudes but significantly decreased ACP activity only at 4250 m (Fig. 4).

The relative abundance of fungivores in the 0–5 cm and 5–10 cm layers was significantly higher in GEW than G at all altitudes (Fig. 3). GEW significantly decreased the density of *Glauxinema* in the 0–5 cm layer, with densities of only 19, 13, 15 and 17 % in G at 3850, 4000, 4150, and 4250 m, respectively (Table S3). GEW significantly ($P < 0.05$) decreased H', EI, SI, BI and CI in the 0–5 cm and 5–10 cm layers at all the altitudes. GEW decreased the dominance of the bacterial-based pathway of decomposition, indicated by a lower NCR (Table 2).

GE and GEW significantly decreased plant diversity compared with G at 4000 and 4150 m but did not have significant effects at 3850 or 4250 m. GEW significantly increased the relative abundance of *Ligusticum scapiforme* at 4000, 4150, and 4250 m (Table S4). GEW significantly increased the relative abundance of *Polygonum viviparum* at 3850, 4000 and 4150 m (Table S4).

3.3. Structural equation modeling

Structural equation modeling indicated that GE and GEW had a pivotal effect on the soil food web, and that GE and GEW increased the fungal-

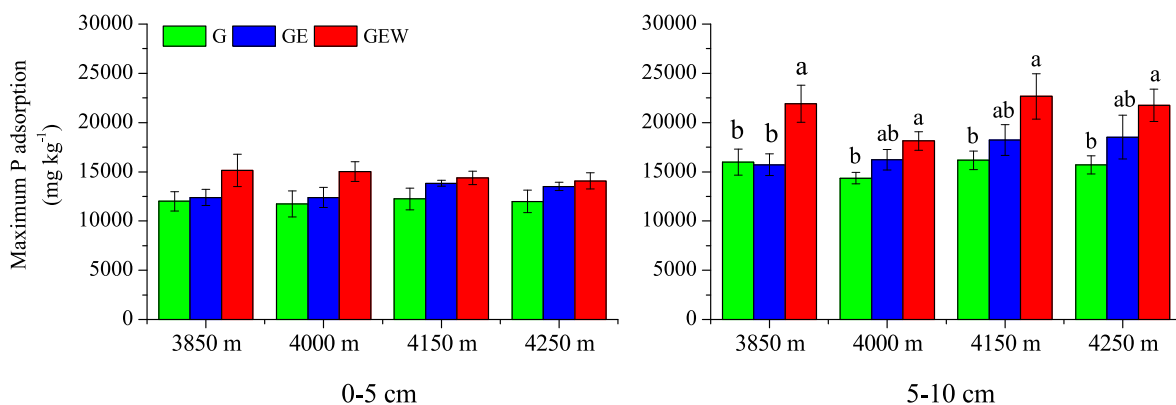


Fig. 1. Changes in maximum P adsorption in the treatments. Different letters for each altitude represent significant differences among treatments ($P < 0.05$). G, Grazing; GE, Grazing exclusion; GE + W, Grazing exclusion and Warming.

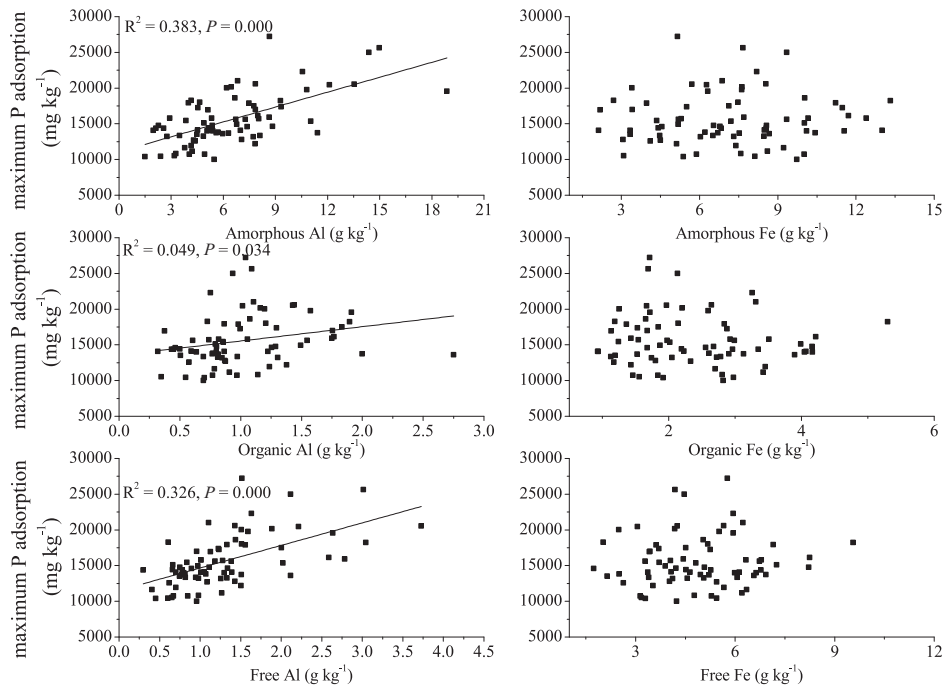


Fig. 2. Relationships between the concentrations of Fe and Al oxides and maximum P adsorption.

based energy channels but decreased the bacterial-based energy channels (Fig. 5a, b). GE and GEW did not significantly influence the abundance of herbivores.

4. Discussion

4.1. Abiotic responses to grazing exclusion and combined grazing exclusion and warming

The combined grazing-exclusion and warming treatment significantly increased the concentrations of amorphous Al and free Al, perhaps because warming can increase soil weathering (White and Blume, 1995). The correlation analysis suggested that the concentrations of Al oxides was positively correlated with maximum P adsorption (Fig. 2). The combined grazing-exclusion and warming treatment thus significantly increased maximum P adsorption and subsequently decreased the availability P. The geochemical adsorption by Al oxides was the key process in these soils, especially in the 5–10 cm layer.

4.2. Biotic responses to grazing exclusion and combined grazing exclusion and warming

Our results demonstrated that grazing exclusion and combined grazing exclusion and warming significantly decreased bacterivore density, especially the density of *Glauxinema*, which includes cp-1 bacterivores that have short life spans and respond rapidly to available nutrients (Ferris et al., 2001). This decrease may have been due to the decrease in the input of soil nutrients from the deposition of dung. Previous studies have reported that cp-1 bacterivores are positively affected by pig manure (Jiang et al., 2017). Grazing exclusion significantly decreased nematode EI, indicating that exclusion could reduce the input of soil nutrients from the deposition of dung (Wang et al., 2018). Grazing exclusion significantly decreased nematode NCR, mainly due to the decreased abundance of bacterivores, so grazing exclusion altered the nematode food web and decreased the bacterial-based energy channels (Fig. 5a).

Combined grazing exclusion and warming significantly increased fungivore density (Table S3), which have been due to the higher fungal

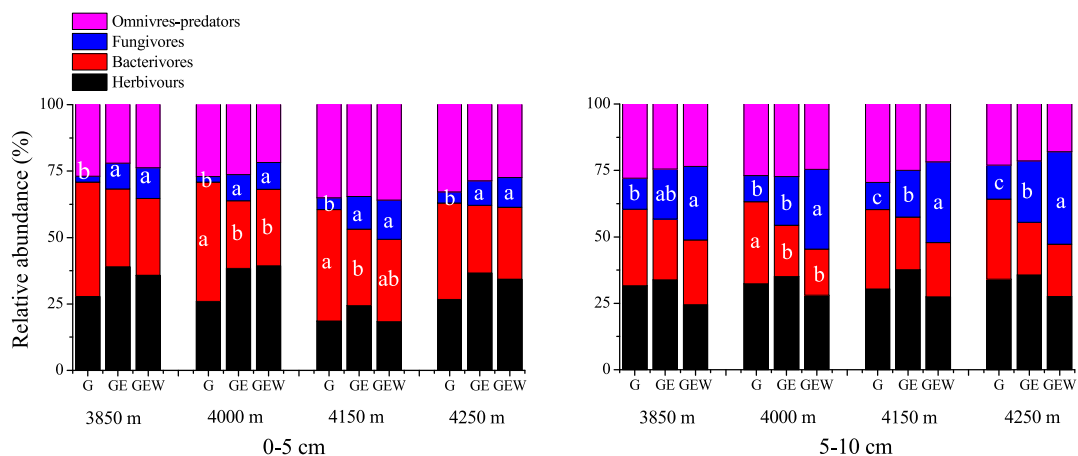


Fig. 3. Changes in taxonomic compositions of the nematode communities in the treatments. G, Grazing; GE, Grazing exclusion; GE + W, Grazing exclusion and Warming.

Table 2

Diversity and richness of the nematode communities at the study site. Values are the means ± SEs, n = 3. Different letters indicate significant differences among the treatments. G, Grazing; GE, Grazing exclusion; GE + W, Grazing exclusion and Warming.

		3850 m		4000 m		4150 m		4250 m	
		0–5 cm	5–10 cm	0–5 cm	5–10 cm	0–5 cm	5–10 cm	0–5 cm	5–10 cm
<i>H'</i>	G	3.28 ± 0.02a	2.82 ± 0.04a	3.15 ± 0.01a	2.77 ± 0.11a	3.12 ± 0.02a	2.88 ± 0.01a	3.17 ± 0.03a	2.87 ± 0.04a
	GE	3.09 ± 0.02b	2.57 ± 0.08ab	3.03 ± 0.03ab	2.61 ± 0.02ab	2.94 ± 0.04b	2.67 ± 0.08b	2.99 ± 0.03b	2.51 ± 0.03b
	GEW	3.07 ± 0.01b	2.48 ± 0.12b	3 ± 0.05b	2.45 ± 0.1b	2.85 ± 0.07b	2.49 ± 0.05b	2.98 ± 0.07b	2.35 ± 0.1b
NCR	G	0.95 ± 0.01a	0.71 ± 0.04a	0.95 ± 0.01a	0.76 ± 0.02a	0.906 ± 0.015a	0.74 ± 0.05a	0.9 ± 0.01a	0.7 ± 0.02a
	GE	0.74 ± 0.04b	0.56 ± 0.06ab	0.72 ± 0.04b	0.52 ± 0.05b	0.702 ± 0.004b	0.52 ± 0.03b	0.73 ± 0.04b	0.46 ± 0.02b
	GEW	0.72 ± 0.03b	0.47 ± 0.06b	0.73 ± 0.04b	0.37 ± 0.05c	0.677 ± 0.039b	0.4 ± 0.02b	0.7 ± 0.04b	0.36 ± 0.02b
EI	G	84.3 ± 3.1a	77.2 ± 3.7a	86.14 ± 2.58a	69.7 ± 6.17a	86.21 ± 2.93a	68.67 ± 5.92a	83.2 ± 4.2a	75.8 ± 2.1a
	GE	58.9 ± 0.8b	44.5 ± 2.6b	65.84 ± 6.26b	53.2 ± 1.62b	55.31 ± 3.32b	47.03 ± 1.93b	57.8 ± 3.9b	49 ± 4.1b
	GEW	53.4 ± 3.4b	41.5 ± 4.7b	56.83 ± 3.8b	48.29 ± 1.32b	47.33 ± 0.3b	44.75 ± 0.82b	53.2 ± 3.6b	47 ± 2.4b
SI	G	91.9 ± 2a	90.4 ± 1a	93.29 ± 0.55a	89.65 ± 1.69a	94.18 ± 0.86a	89.09 ± 2.09a	94.2 ± 0.8a	91.5 ± 0.8a
	GE	84 ± 1.2b	76.6 ± 2.6b	89.38 ± 0.96ab	82.14 ± 3.14ab	89.06 ± 0.8b	79.37 ± 3.43b	85.5 ± 2.5b	78.9 ± 3.2b
	GEW	79.5 ± 1.2b	72.7 ± 2.8b	83.89 ± 3.66b	77.02 ± 1.42b	85.44 ± 1.49b	73.21 ± 2.7b	84 ± 2.3b	72.6 ± 3.5b
BI	G	5.5 ± 1.3c	7.2 ± 0.9b	4.7 ± 0.55b	8.38 ± 1.51b	4.23 ± 0.7c	8.86 ± 1.81b	4.5 ± 0.8b	6.7 ± 0.6b
	GE	13 ± 0.8b	19.6 ± 1.7a	8.56 ± 0.3b	14.69 ± 2.13a	9.57 ± 0.46b	17.28 ± 2.51a	12.2 ± 2.1a	17.5 ± 2.3a
	GEW	16.5 ± 0.4a	22.7 ± 1.9a	12.79 ± 1.91a	18.9 ± 0.99a	12.85 ± 1.15a	21.93 ± 1.78a	13.3 ± 1.3a	22.1 ± 2.7a
CI	G	2.2 ± 0.7b	17.7 ± 2.8b	0.93 ± 0.09b	19.91 ± 3.14c	1.95 ± 1.03b	21.05 ± 7.61b	1.7 ± 0.6b	16.9 ± 3.2c
	GE	27.5 ± 3.6a	61 ± 2.7a	18.23 ± 4.51a	52.69 ± 2.7b	22.69 ± 5.08a	62.31 ± 6.25a	23.2 ± 1.2a	60.6 ± 3.1b
	GEW	30.7 ± 5.5a	73.8 ± 8.8a	21.43 ± 3.94a	72.05 ± 3.91a	33.99 ± 4.21a	77.46 ± 3.03a	31.7 ± 7.5a	74.2 ± 3.9a

H', Shannon-Weaver diversity index; NCR, nematode channel ratio; EI, Enrichment index; SI, Structure index; BI, Basal index; CI, Channel index.

biomass in this treatment (Li et al., 2022). Also, fungivores have previously been positively correlated with warming (Ruess et al., 1999). Other soil faunal groups, such as fungivorous mites, have also responded positively to

warming (Briones et al., 2009). Alternatively, warming has indirect effects on soil nematode communities due to changes in the plant community. Many species of *Ligusticum* are medical plants (Wang et al., 2021b), and

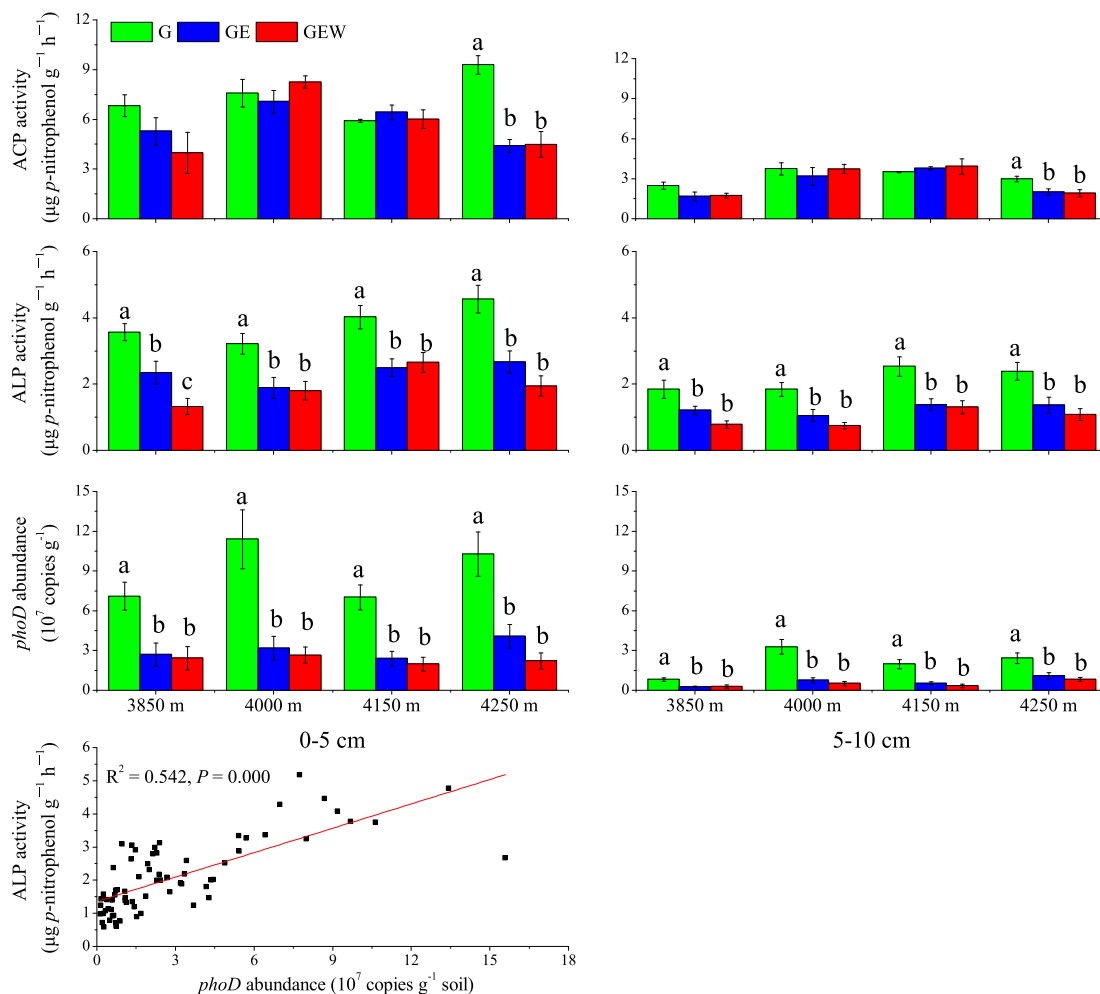


Fig. 4. Changes in acid and alkaline phosphomonoesterase activities and phoD abundance in the treatments. G, Grazing; GE, Grazing exclusion; GE + W, Grazing exclusion and Warming.

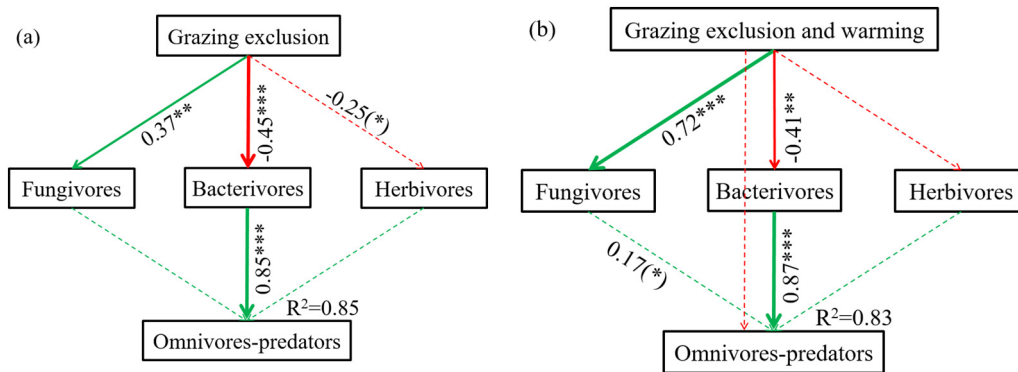


Fig. 5. Structural equation model of the effects of grazing exclusion (a) and combined grazing exclusion and warming (b) on the soil nematode food web. Green arrows indicate positive effects, red arrows represent negative effects, and dashed arrows indicate non-significant path coefficients. Arrow thickness represents the magnitude of the path coefficients. (*), $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Values associated with arrows represent standardized path coefficients. R^2 indicate the proportion of variation explained by relationships with other variables. Results of model fitting: (a) $\chi^2 = 7.353$, $P = 0.061$, NFI = 0.954, GFI = 0.945, RMSEA = 0.176; (b) $\chi^2 = 2.807$, $P = 0.246$, NFI = 0.983, GFI = 0.977, RMSEA = 0.093.

will inevitably affect soil biota. Our results also suggested that the biomass of *Ligusticum scapiforme* was significantly positively correlated with the abundance of fungivores in the 0–5 and 5–10 cm layers (Fig. S6). Our results thus stress the importance of plant composition for structuring below-ground faunal communities. Our results suggest that fungal-dominated energy and nutrient channels in food webs may increase in alpine meadow soil under warming conditions. The low NCRs also indicated that soil function shifted from bacterial-energy channels to the fungal-energy channels. We therefore concluded that warming would aggravate the effect of the exclusion of grazing on the soil food web, leading to more severe degradation of the food web. Supported by the low SI.

4.3. Path analyses: a general overview

Structural equation modeling indicated that the exclusion of grazing decreased the bacterial-based energy channel (Fig. 5). Bacterivores can increase the abundance and activity of bacteria that produce alkaline phosphomonoesterase by selective feeding (Ranoarisoa et al., 2018), so reducing the abundance of bacterivores can decrease the mineralization of organic P and the availability of P. The lower concentration of resin P in the GE plots were partly due to the lower ALP activity and the subsequently slower decomposition of organic P in the soils of the alpine meadows. These results thus supported our first hypothesis that excluding grazing would decrease the bacterial-based energy channel, alkaline phosphomonoesterase activity, the mineralization of organic P and availability of P. Thus, ALP activity thus played an important role in regulating the availability of soil P.

The combined grazing-exclusion and warming treatment increased the fungal-based energy channel (Fig. 5). The higher abundance of fungi and fungivores, however, had few effects on ACP activity and the fractions of inorganic P, for two potential reasons. First, the combined grazing-exclusion and warming treatment did not significantly increase ACP activity, inconsistent with a previous study reporting that phosphatase activity and P availability increased with temperature (Shaw and Cleveland, 2020), perhaps because the combined grazing-exclusion and warming treatment decreased the amount of soil moisture and subsequently decreased microbial activity. These results partly supported our second hypothesis that the combined grazing-exclusion and warming treatment would increase the fungal-based energy channels but not the mineralization of organic P or the availability of P.

The exclusion of grazing did not significantly affect ACP activity at 3850, 4000 and 4150 m but significantly decreased it at 4250 m. Excluding grazing would thus aggravate soil P deficiency more at high than low altitudes, perhaps because excluding grazing was previously reported to significantly decrease fungal biomass at 4250 m (Li et al., 2022). Low grazing intensity is important for maintaining fungal biomass and activity in alpine meadows at high altitudes. The exclusion of grazing would thus aggravate

soil P deficiency more at high than low altitudes, perhaps because excluding grazing in our study significantly decreased fungal biomass at high altitudes, supporting our third hypothesis.

5. Conclusion

Grazing increased soil nematode abundance and diversity, mainly due to the large increase in bacterivore abundance, which in turn may have been due to an increase in available resources for nematodes from the deposition of dung and the decomposition of litter (Banegas et al., 2015). Alternatively, cattle dung contains many microbes, which could increase food resources for nematodes (Lovell and Jarvis, 1996). Grazing increased bacterivore abundance, which could increase the abundance and activity of bacteria that produce alkaline phosphomonoesterase and increase P availability. Grazing can thus potentially affect plant production because it alleviates P limitation in alpine meadows. Exclusion grazing, though, significantly decreased the density of omnivores-predators, which may have been due to the decrease in bacterivore abundance, emphasizing the stronger bottom-up effects on the higher nematode trophic levels. Our results generally suggested that the exclusion of grazing would be disadvantageous for restoring alpine meadows, especially at high altitudes. Low grazing intensity can be an effective management technique, mainly under warming, to maintain and adequately use the P cycle in the plant-soil system.

CRedit authorship contribution statement

RYC and NL designed the study. FS, HJ, XMZ and NL carried out laboratory work. FS performed the experiments, analyzed the data and wrote the manuscript. All authors read and approved the paper.

Data availability

Data will be made available on request.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.162313>.

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