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Ant nests increase litter decomposition to mitigate the negative effect of warming in an alpine grassland ecosystem

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Warming can decrease feeding activity of soil organisms and affect biogeochemical cycles. The ant *Formica manchu* is active on the nest surface and prefers a hot, dry environment; therefore, warming may provide a favourable environment for its activities. We hypothesized that *F. manchu* benefit from warming and mitigate the negative effects of warming on litter decomposition. We examined the effects of ant nests (nest absence versus nest presence) and warming (+1.3 and +2.3°C) on litter decomposition, soil properties and the plant community in alpine grassland. Decomposition stations with two mesh sizes were used to differentiate effects of microorganisms (0.05 mm) and macroinvertebrates (1 cm) on decomposition. Ant nests increased litter decomposition with and without macroinvertebrates accessing the decomposition station when compared to plots without ant nests. Only litter decomposition in ant nests with macroinvertebrates having access to the decomposition station was not affected negatively by warming. Plots with ant nests had greater soil carbon, nutrient contents and plant growth than plots without ant nests, regardless of warming. Our results suggest that ant nests maintain ecosystem processes and functions under warming. Consequently, a management strategy in alpine grasslands should include the protection of these ants and ant nests.

1. Introduction

Litter decomposition, driven by invertebrates and microorganisms, is important for nutrient cycling in terrestrial ecosystems [1,2]. It also acts as a link between above- and below-ground ecosystems by providing resources for soil organisms and plant growth [3]. Climate warming is a main driving factor for ecosystem processes and functions, as it affects litter decomposition by influencing the feeding activity of ectothermic organisms [4,5]. For example, when precipitation was sufficient, the decomposition activity of soil organisms increased with warming because of increased metabolic demands [6,7]. Studies demonstrated that warming could impede litter decomposition in alpine ecosystems because it decreases soil water content [2,8]. However, most studies examined only how microbial activity responded to climate warming, without considering the responses of macroinvertebrates [6,9]. Due to differences in physiology between microorganisms and macroinvertebrates, their responses to warming are often asynchronous, and the decomposition activity of macroinvertebrates might be more sensitive to warming than that of microorganisms [10,11]. More studies are warranted to compare their responses to climate warming, as knowledge of the effects of climate warming on ecosystem functions is important for the conservation and management of alpine ecosystems.

Macroinvertebrates promote litter decomposition through litter breakdown, metabolism and interactions with microorganisms [12,13]. Some ecological engineers, such as termites [14,15] and ants [16,17], have particularly strong impacts on ecosystem processes and functions. Ants play a key role in decomposition of organic matter, and this may explain the greater soil nutrient contents in ant nests [18–20]. In addition, greater microbial biomass and unique microorganisms associated with high N content and cycling in ant nests were also correlated with ecosystem function of ant nests [21,22]. This is particularly important for alpine ecosystems where plant growth is limited mainly by N, and consequently, an increase in N can promote plant growth [23]. However, the role of ants in ecosystem processes and functions has rarely been quantified [20,24]. Given the high biomass and abundance of ants (biomass of ants exceeds the combined biomass of wild birds and mammals) in many ecosystems [25], a general knowledge of how ants affect litter decomposition, soil nutrition pools and plant community could improve our understanding of conservation of ants and insect biodiversity [1,26,27], and secure sustainable functions of grassland ecosystems.

The effects of warming on feeding activity of ectothermic organisms are usually complex because the magnitude and directionality of the effects depend on the sensitivity to temperature, tolerance to warming, and behaviour of ectothermic organisms. Response of ants to warming is related to latitude and elevation. For example, in areas where seasonality is pronounced, ants in high latitudes and elevations are less susceptible to the negative effect of warming than ants in tropical regions [28,29]. With studies on the effect of warming, Del Toro *et al.* [24] reported a decrease in feeding activity of ants, litter decomposition and soil nutrition contents in ant nests, whereas Diamond *et al.* [30] reported an increase in the abundance of heat-tolerant ants in the nests. However, it is still unclear how warming affects the ecosystem functions provided by ants [29].

The surface temperature of the Tibetan Plateau has increased by 1.8°C over the past 50 years [31], a rate which is faster than many other regions of the world, and this has had an impact on the alpine ecosystem [32]. The Tibetan Plateau has low soil moisture, and moisture limitation caused by warming is an important inhibiting factor in litter decomposition [7,8]. Therefore, in alpine ecosystems, warming usually reduces water availability to limit the feeding activity of soil organisms and litter decomposition, which may affect soil nutrients and plant growth. Worker ants (*Formica manchu*) forage on the nest surface, which is hotter and drier than the surrounding soil surface, therefore, these ants may be more adapted to a hot and dry environment than other soil organisms. Solitary soil organisms are often affected negatively by warming; however, ants are eusocial, and are less affected or may even benefit from warming by thermally buffering their nests and avoiding extreme temperatures behaviourally [29]. Thus, ecosystem processes provided by ants may respond differently to warming from those provided by other soil organisms.

Formica manchu is the dominant ant species and is distributed widely on the Tibetan Plateau, and builds its nest above ground [33]. The wide distribution and aggressive behaviour of *F. manchu* cause substantial soil turnover, which can provide new insights in the understanding of biogeochemical cycles in response to warming in alpine ecosystems. How ants regulate litter decomposition and ecosystem function to enhance the robustness of the ecosystem under climate warming is a key scientific issue for future management of the alpine ecosystem. We carried out an *in situ* study with ant nests and experimental warming to address the question: do ants have the adaptability to alleviate the negative effect of warming on ecosystem functions? The *in situ* study employed meshes of different sizes to permit or deny macroinvertebrates access to a decomposition station, which enabled us to assess the effects of macroinvertebrates and soil microorganisms on litter decomposition under warming. We hypothesized that: (i) litter decomposition will be faster with than without ant nests; (ii) since moisture is the key inhibiting factor on the Tibetan Plateau, warming will reduce litter decomposition with the absence of ant nests, but due to the high tolerance to warming and the nesting behaviour of ants, litter decomposition will not be affected negatively by warming with the presence of ant nests; and (iii) ant nests will increase soil carbon, nutrients and plant growth, and these effects will not be affected by warming. This study will provide a better understanding of the effects of ants on ecosystem functions, and the results will be helpful in formulating management decisions to conserve the sensitive alpine ecosystem.

2. Materials and methods

(a) Study area and the ant, *Formica manchu*

This study was conducted in Maqin county, Qinghai province (34° 27′45″ N, 100°12′37″ E; 3738 m above sea level), southeast Tibetan Plateau, China. We selected a typical plain meadow that had been the subject of numerous studies [34,35]. The mean annual air temperature ranges between −0.1°C and −1.2°C, and mean annual rainfall, which occurs mainly from May to September, ranges between 463 and 602 mm. The regional vegetation and soil are alpine meadow. The dominant plant species are sedges, including *Kobresia humilis* and *K. pygmaea*, and grasses, including *Elymus nutans*, *Poa crymophlia*, *P. pratensis* and *Festuca sinensis*.

The site is characterized by numerous *F. manchu* ant nests (figure 1). We surveyed the site prior to the study and recorded 61 *F. manchu* nests in 50 m × 50 m, and each ant nest covered an area of 0.2–0.3 m^2. The main foraging range of the ants was within a radius of 1 m around the ant nest.

(b) Experimental design

We studied the effect of three temperature treatments (no warming, NW; low warming, LW; and high warming, HW) in areas with ant nests and without ant nests (figure 1). There were 10 replicates per treatment, and a total of 60 treatment plots: 2 areas × 3 temperature treatments × 10 replicates of each. Decomposition measurements were done in two seasons and with two mesh sizes, which prevented or permitted macroinvertebrates access to a decomposition station, and thus, there

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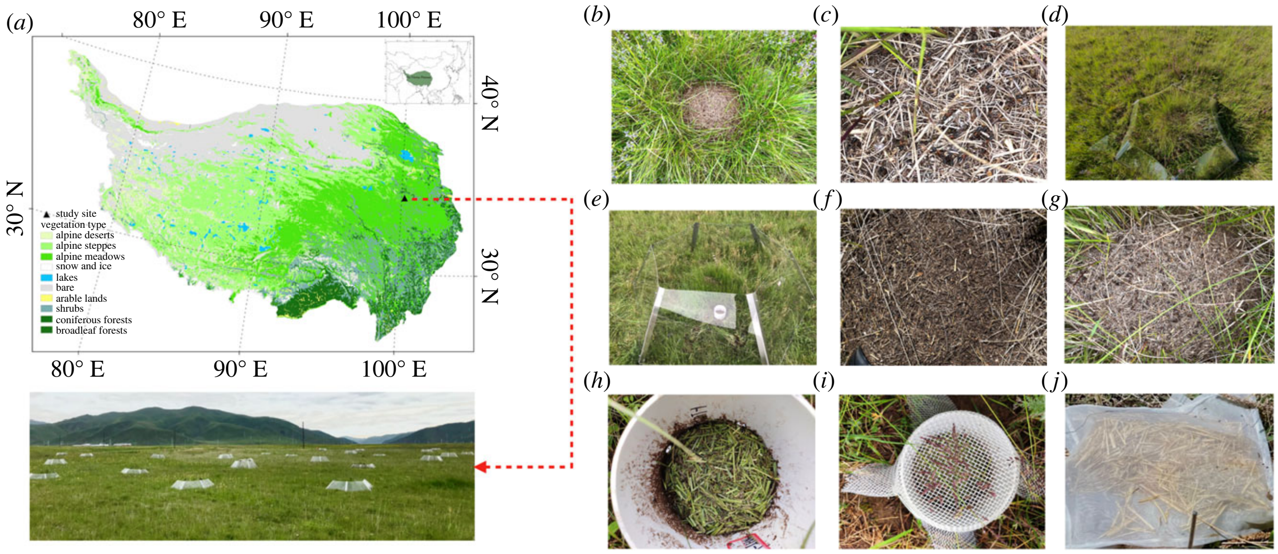

Figure 1. Study site in alpine grassland (*a*), ant nest (*b*), worker ants and litter on the nest (*c*), low warming treatment (*d*), high warming treatment (*e*), surface of ant nest in cold season (*f*), surface of ant nest in warm season (*g*), decomposition stations of 1 cm mesh (*h*,*i*) and decomposition stations of 0.05 mm mesh (*j*).

were 240 decomposition measurements. To avoid the influence of ants on plots without ant nests, the distance between plots with and without ant nests was at least 3 m (figure 1*a*).

Warming was started in August 2019 by using open top chambers (OTCs; top edge = 60 cm, bottom edge = 95 cm, height = 40, 60 cm, coverage area = 2.3 m^2; figure 1*d*,*e*), following the standard method described in the International Tundra Experiment (ITEX) [36]. The gap between the bottom of the OTCs and the surface was 5 cm (figure 1*e*), and therefore, the foraging activities of ants were not restricted by the edges of the OTCs. The level of warming was manipulated by adjusting the height of the OTCs and keeping the top and bottom size unchanged [37]. Sensors (PH-TWS, Xinpuhui Technology, Wuhan, China) were used to monitor and record the soil surface (0–5 cm) temperature without ant nests and soil temperature below the nest refuse pile with ant nests from October 2019 to August 2020. The soil temperature increased by an average of 1.59°C (LW) and 2.36°C (HW) without ant nests (electronic supplementary material, figure S1*a*) and 1.02°C (LW) and 2.32°C (HW) with ant nests (electronic supplementary material, figure S1*b*). Soil and vegetation were sampled and surveyed in late August 2020.

(c) Decomposition measurement

Litter decomposition was measured in two seasons: (i) cold, from October 2019 to May 2020 and (ii) warm, from May 2020 to late August 2020. No forager ants were observed on an ant nest at the beginning of the study in October 2019, which indicated that the ants had entered the overwintering stage. Decomposition stations were composed of polyvinylchloride (PVC) pipes (1 cm mesh size) and nylon bags (0.05 mm mesh size). The 1 cm mesh allowed all soil organisms while the 0.05 mm mesh allowed only microorganisms to enter the decomposition station [1]. In general, we followed the method used by Veldhuis *et al.* [14] in which the PVC pipes measured 10 cm in height and 10 cm in diameter, with four 1 cm holes at the bottom of the pipe to allow ants and other macroinvertebrates to enter the decomposition station (figure 1*h*). PVC pipes were installed above the ground after removing all plants and litter, and ants were found in the PVC pipe shortly after it was installed. The top of the PVC pipe was covered with a 1-mm mesh to prevent litter from entering (figure 1*i*). To compare the differences in microbial-mediated litter between with and without ant nest treatments, nylon mesh bags of 0.05 mm were used to allow only microorganisms to enter (figure 1*j*), and the bags were spread out above the ground in the without ant nest treatment but under the nest refuse pile in the with ant nest treatment.

Two dominant Gramineae (*E. nutans* and *P. crymophlia*) were used as substrates for decomposition. The degree of dominance (importance value) of the plants was more than 25% in both the plots with and without ant nests, and there was much gramineous litter on the surface and inside the ant nest. Fresh grasses were collected in August 2019, brought to the laboratory, dried at 65°C to a constant mass, and stored. To facilitate the mixing of the litter, the grasses were cut into 1 cm pieces and mixed thoroughly. The PVC pipe (1 cm) and nylon mesh bag (0.05 mm) were filled with 6 g of substrates (3 ± 0.01 g *E. nutans* and 3 ± 0.01 g *P. crymophlia*) in October 2019, and the remains were collected in May 2020, and then re-filled with 6 g substrate and the remains were collected in late August 2020. The collected litter was washed with clean water and dried at 65°C to a constant mass. Fences were erected around the warming and decomposition equipment to prevent damage from large mammals such as yaks.

(d) Plant community survey and soil sampling

The plant community was surveyed in late August 2020, following standard field vegetation survey methods [34]. Three quadrats (0.25 m × 0.25 m) were examined in each treatment plot, and the three quadrats were combined for statistical analysis. To maintain the integrity of the ant nests, we did not cut plants to measure above-ground biomass.

In late August 2020, 3 soil core (diameter: 3 cm) samples, at a depth of 0–10 cm, were collected in each treatment and combined into one [35]. Soil was sampled from the area where plants grew in the outer ring of the ant nest of plots with ant nests and random areas of plots without ant nests. Soil pH was measured in deionized water solution (soil:water = 1 : 10) using an electrode (PB-10, Sartorius, Göttingen, Germany). Soil total nitrogen (TN) was determined by an organic elemental analyser (Vario EL III, Elementar, Langenselbold, Germany), soil organic carbon (SOC) was measured by colorimetry with a mixture of sulfuric acid and potassium dichromate, and soil nitrate nitrogen (NO_3^-–N) and ammonium nitrogen (NH_4^+–N) were measured using a flow analyser (Auto Analyzer 3, Evisa, Ludwigshafen, Germany).

(e) Data analysis and statistics

Decomposition rates were calculated as: $\%$ mass loss month^{-1} = $(1-(M_t/M_0))\times 100/t$, with M_0 the initial mass of litter, M_t the mass of remaining litter after a given period t and t the incubation time of litter in months. The Shannon–Wiener and Simpson indices and richness were used to describe the diversity of plant communities. Shannon–Wiener index (H) was calculated as: $H = -\sum_{i=1}^{S} P_i \times (\ln P_i)$, and the Simpson index (C) as: $C = 1 - \sum_{i=1}^{S} P_i^2$, where S = number of species in a sample plot. The importance value of plants P_i was calculated as [38]: P_i = Relative height × Relative coverage/2.

To test for plant composition differences between plots with and without ant nests, non-metric multidimensional scaling was used to ordinate dissimilarity plant community matrices in a two-dimensional ordination space [39]. Two-way analysis of variance and *post hoc* Tukey pairwise comparisons were used to test the effects of mesh (0.05 mm and 1 cm) and season (cold and warm season), and their interaction on litter decomposition. To examine the impact of the warming treatments and the ant nest presence on soil, vegetation and litter decomposition, we used linear mixed effect models with the warming treatment, ant nest and their interactions as fixed effects and measurement plot as the random effect. To meet the assumptions of normality, data were log-transformed. A $p < 0.05$ was accepted as significant, and Tukey's test in the LSMEANS package was used to make multiple comparisons [40].

To determine how ant nests/activity affect ecosystem functions, we generated a piecewise structural equation model (SEM) to analyse the effects of ant nest and warming on litter decomposition, soil properties, plant growth and plant diversity. In the piecewise SEM, we fitted multiple separate linear mixed models with plot as a random factor. In the analysis, χ^2 above the significance level ($p > 0.05$) indicated the model fits the data and could be accepted. All statistical analyses and figures used R software 4.1.1 with LSMEANS [40], piecewise SEM [41], nlme [42], vegan [39] and lme4 packages [43].

3. Results

(a) Effect of ant nest and warming on litter decomposition

Decomposition rate was faster in the warm than the cold season, regardless of the presence of ant nests (figure 2*a*,*b*). In the warm season, decomposition of substrate was significantly greater with than without macroinvertebrates having access to the decomposition station (figure 2*a*,*b*). Litter decomposition with ant nests that denied access to macroinvertebrates was 199% and 141% greater than without ant nests in the cold and warm seasons, respectively (figure 2*c*,*e*; table 1). At stations which permitted access to macroinvertebrates, litter decomposition with ant nests was 52% greater in the cold season and 170% greater in the warm season than without ant nests (figure 2*d*,*f*; table 1). In the cold season, warming significantly increased litter decomposition that prevented macroinvertebrate access (figure 2*c*; table 1). In the warm season, warming significantly increased decomposition at stations which permitted access to macroinvertebrates with ant nests, but decreased decomposition at stations that denied access to macroinvertebrates with ant nests (figure 2*e*,*f*), and decreased decomposition at stations which denied and permitted access to macroinvertebrates without ant nests (figure 2*e*,*f*; table 1).

(b) Effect of ant nest and warming on soil and vegetation

The content of SOC, TN, NO_3^-–N and NH_4^+–N with ant nests increased by 156%, 77%, 274% and 252%, respectively, when compared to plots without ant nest (figure 3*a*; table 1). The height and coverage of grasses and the total coverage were greater ($p < 0.05$) with than without ant nests (figure 3*a*; table 1). Plots with ant nests had lower richness, and Shannon and Simpson diversities (figure 3*a*) than without ant nests, and plant species composition differed between plots with and without ant nests (figure 3*b*). The importance values of grass species were greater ($p < 0.05$), but of forbs were lesser ($p <$ 0.05) in plots with than without ant nests (figure 3*c*). Ant nests increased the soil nutrient pool and plant growth both with and without warming, while warming had no effect on the contribution of ant nests (figure 3*a*; table 1).

(c) Role of ants in ecosystem functions

We examined the potential pathways for ant nests to influence ecosystem function by SEMs, and the pathways presented in figure 4 were all significant ($p < 0.05$). Ant nests promoted litter decomposition with and without access of macroinvertebrates to the decomposition station, and increased the SOC and NH_4^+–N indirectly. The increase of decomposition with access of macroinvertebrates to the decomposition station promoted SOC content, and the increase of decomposition without access of macroinvertebrates to the decomposition station promoted NH_4^+–N content. In forage production, ant nests/activity increased the height of grasses directly and also indirectly by increasing soil NH_4^+–N content. In plant community diversity, ant nests/activity decreased the Shannon diversity of plant communities directly. Warming decreased decomposition at stations which denied access to macroinvertebrates but increased height of grasses.

4. Discussion

(a) Ant nest effects on ecosystem process

Microorganisms with ant nests decomposed litter faster than without ant nests in both cold (199%) and warm (141%) seasons. These marked differences might be related to the interaction between ants and microorganisms. Ants move litter to the nest, and the more suitable environment and greater microbial biomass in the nest may promote litter decomposition and nutrient cycling [13,14]. The present study is the first to quantify the process in an alpine ecosystem. In this way, ants produce a favourable environment in the nests, and the interactions between ants and nest microorganisms are beneficial for nutrient cycling in the grassland ecosystem.

Ants affect soil turnover and resource removal directly, and soil nutrient dynamics indirectly [18,20,24]. In one such study, ants removed 61% of ground resources, while other invertebrates removed the remaining 39% [20]. In the present study, when all soil organisms were involved in litter decomposition, ant nests increased litter decomposition by 52% in the cold season and 170% in the warm seasons. The increased litter decomposition not only affected nutrient cycling, but also reduced the accumulation of litter on the ground and increased the penetration of photosynthetically active radiation and, hence, promoted plant growth [44]. We believe that these effects were caused mainly by ants. Although there were other macroinvertebrates in ant nests (e.g. *Platyrhopalopsis* spp., *Heteropaussus* spp., Staphylinidae

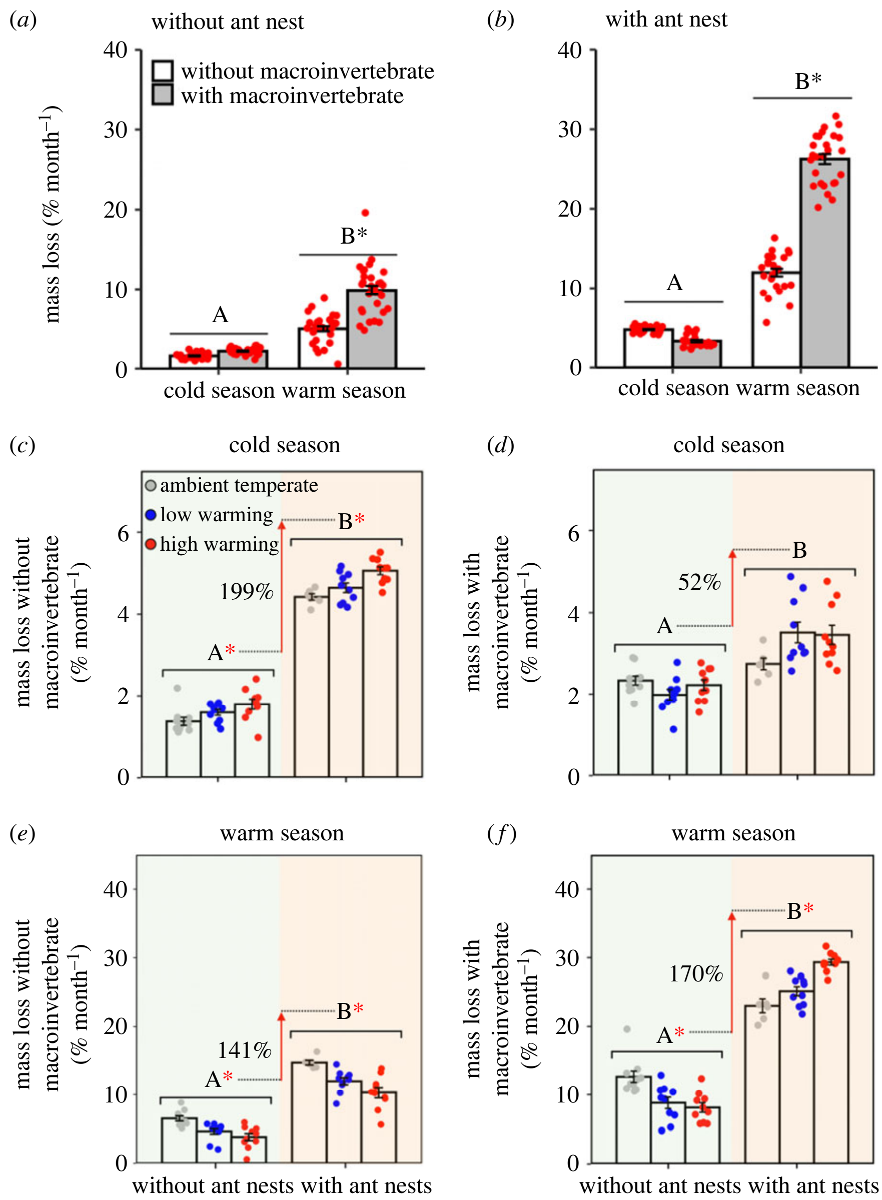

Figure 2. The effect of mesh size that permits access (0.05 mm) or denies access (1 cm) to macroinvertebrates to decomposition stations on litter decomposition at plots without (*a*) and with (*b*) ant nests in different seasons (cold and warm). Effects of ant nest and warming on litter decomposition in each mesh size (*c,e*: decomposition at 0.05 mm; *d,f*: decomposition at 1 cm). Bars represent means ± s.e. In (*a,b*), $^*p < 0.05$ (between mesh sizes), capital letters represent significant differences between seasons ($p < 0.05$). In (*c–f*), $^*p < 0.05$ (between warming levels with and without ant nests; multiple comparisons in linear mixed model), capital letters represent significant differences between with and without ant nests ($p < 0.05$).

and Myrmecophilidae), their abundance was very low compared to ants, and therefore, we reasoned that it was unlikely that they caused such a large impact. Due to the large difference in decomposition capacity between ants and other soil organisms, the contribution of ants could not be compensated by other soil organisms [20]. This also highlights the importance of the protection of ants and ant nests.

(b) The response of ecosystem process to warming

In the litter decomposition mediated only by microorganisms, warming increased litter decomposition in the cold season, but decreased litter decomposition in the warm season. Warming in the alpine ecosystem usually mitigates temperature limitation of microbial activity [45]. The decrease of microbial activity in the warm season may be due to the increase in evapotranspiration and the decrease of water availability, especially in OTC warming experiments, as OTC warming is more likely to cause a greater decrease in air and soil moisture than an electric heater [2,8]. Ants can create optimal environments for decomposition, which is vital for nest microorganisms as they are vulnerable to external disturbances, such as high temperature and low moisture in the warm season [14]. But in the present study, nest microorganisms were still affected negatively by warming in the warm season. Perhaps the short time of warming in this study did not allow the ants to respond fully to the effects of warming on nest microorganisms, such as building deeper nests to protect nest microorganisms from extreme temperatures.

Our results demonstrated that ants appeared to be more active in the warm environment than other soil organisms. In the warm season, the decomposition of substrate decreased without ant nests but increased with ant nests under warming treatment when macroinvertebrates had access to the decomposition station. The negative effect of warming on litter decomposition was mitigated when ants had access to the decomposition station in plots with ant nests. In the warm season, temperatures can be extremely

Table 1. Linear mixed model for the effects of ant nest and warming on soil, vegetation and litter decomposition. Significant differences are in italics ($p < 0.05$).

	ant nest		warming		ant nest × warming	
terms	*F* value	*p*-value	*F* value	*p*-value	*F* value	*p*-value
soil						
soil organic carbon	223.50	*<0.001*	0.35	0.71	0.16	0.85
total nitrogen	58.68	*<0.001*	0.03	0.97	0.22	0.80
NO_3^-—N	245.73	*<0.001*	0.07	0.82	0.32	0.51
NH_4^+—N	184.34	*<0.001*	0.02	0.82	0.08	0.77
pH	0.18	0.67	24.51	*<0.001*	3.48	*<0.05*
vegetation						
coverage (grass)	229.01	*<0.001*	0.72	0.49	1.76	0.18
height (grass)	245.47	*<0.001*	5.04	*<0.01*	0.31	0.18
coverage (total)	19.44	*<0.001*	0.85	0.43	0.07	0.93
richness	58.67	*<0.001*	1.85	0.17	2.29	0.11
Shannon	92.85	*<0.001*	1.54	0.22	3.07	0.05
Simpson	115.90	*<0.001*	0.62	0.54	2.58	0.09
litter decomposition						
decomposition without macroinvertebrates						
cold season	1620.98	*<0.001*	5.58	*<0.01*	1.05	0.36
warm season	325.90	*<0.001*	8.88	*<0.001*	1.41	0.25
decomposition with macroinvertebrates						
cold season	53.91	*<0.001*	1.18	0.32	5.03	*<0.01*
warm season	761.08	*<0.001*	13.013	*<0.001*	27.82	*<0.001*

high with a concomitant decrease in water availability under climate warming [6]. In contrast with other soil organisms in plots without ant nests, ants collected litter on the nest surface at high temperature and low humidity, which indicated that ants are better adapted to a hot, dry environment than other soil organisms, so they tend to be more active under climate warming. When the temperatures are very high under climate warming, ants can cope by thermally buffering their nests or behaviourally by avoidance [29]. A mesocosm study reported that warming by 3.5 and 5.0°C inhibited litter decomposition by ants [24]. This difference between Del Toro *et al.* [24] and the current study may be due to the lower warming level and environmental differences between *in situ* and mesocosm studies. For example, the shade from vegetation in the present study could protect the ants from the negative effects of high temperature [5,46]. Furthermore, the present study site was located in a high-altitude alpine ecosystem, where the differences in air temperature and soil moisture between the cold and warm seasons are large. Ants in this area are more likely to benefit from warming because of their wide ecological amplitude [28,29]. Consequently, ants can increase the litter decomposition even when other soil organisms are affected negatively by warming.

In the cold season, warming increased decomposition of substrate in plots with ant nests when ants had access to the decomposition station, albeit the difference was not significant. Previous studies demonstrated a strong link between the timing of arthropod activity and snowmelt in alpine ecosystems under warming [47]. We reasoned that this may have been due to earlier foraging time of ants under the warming treatment [48], because the dispersion of data points increased in LW and HW, but most of the decomposition values were similar among NW, LW and HW treatments (figure 2*d*). Further studies are warranted to understand the effect of warming on the activity time of ants on the Tibetan Plateau.

(c) Ant nests and warming effects on ecosystem functions

Ant nests are often considered as fertility islands and hotspots of nutrient cycling [16]. Given the high biomass and abundance of ants in ecosystems [25], they have a substantial impact on ecosystem functions [29]. The present study demonstrated that the nest/activity of the ant, *F. manchu*, increased SOC, TN, NH_4^+–N and NO_3^-–N by promoting litter decomposition. The nest area was nutrient-rich and promoted the growth of palatable grass species, as was reported in other studies [49,50]. We reason that the better growth of grass species near ant nests may be related to the greater nutrient pool (especially N and available N contents) in ant nests (figure 4), since plant growth is limited mainly by N in alpine ecosystems [23]. More grass biomass increases the accumulation and decomposition of litter in the ant nest, and therefore, ant nests become hotspots for C and nutrient cycling. In addition, the present study demonstrated that the effects of ant nest/activity on soil and vegetation were

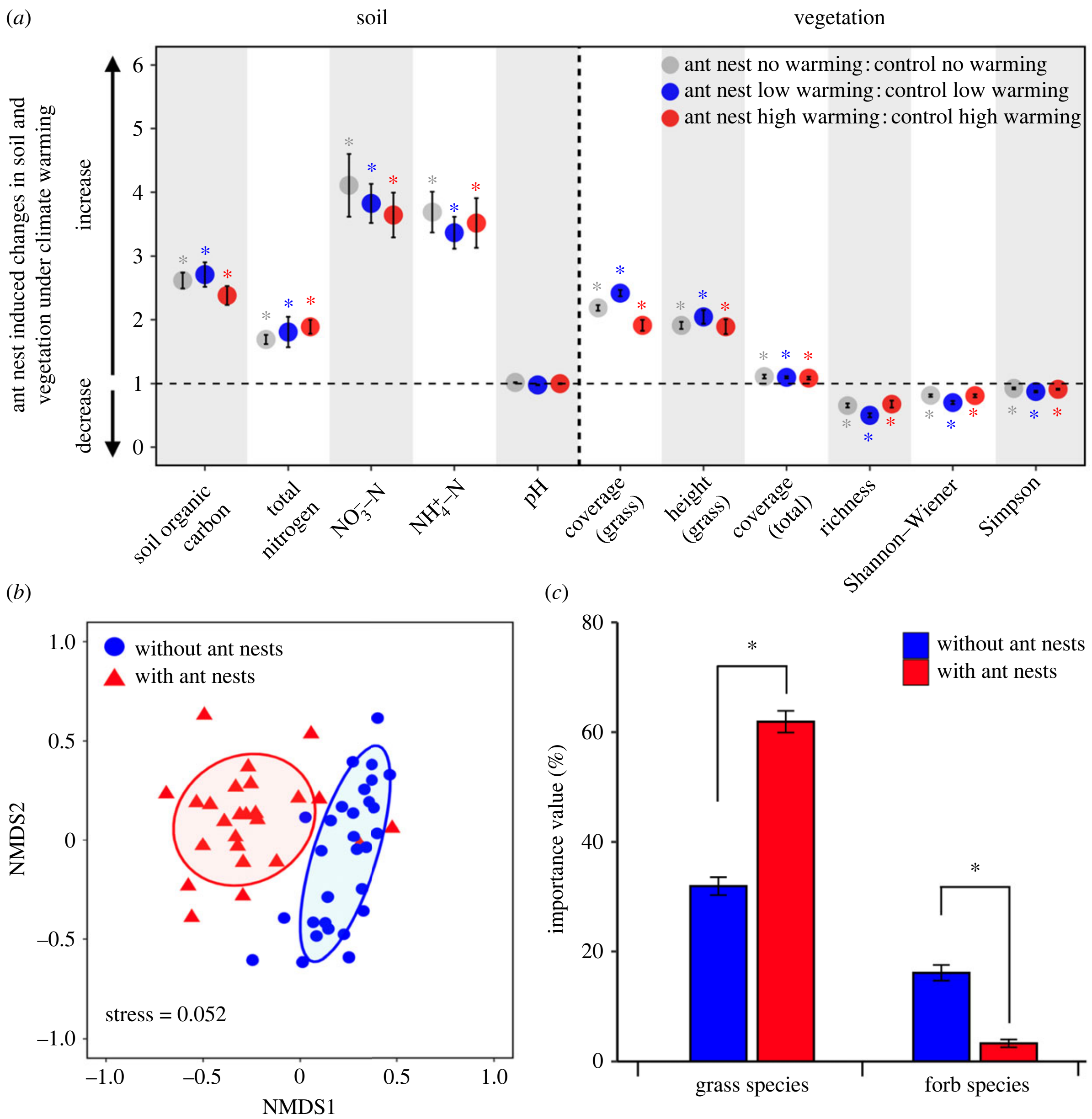

Figure 3. The effect of ant nests (ant nests/without ant nest means ± s.e.) on soil and vegetation variables at different warming levels (*a*). Treatments include control no warming, control low warming, control high warming, ant nest no warming, ant nest low warming and ant nest high warming. According to the results of multiple comparisons in linear mixed model, asterisk represents a significant increase (the ratio > 1) or decrease (the ratio < 1) with ant nests at different warming levels ($p < 0.05$). Non-metric multidimensional scaling (NMDS) ordination of vegetation composition with ant nests (red triangle) and without ant nests (blue dots) (*b*). Effect of ant nest on importance value of grass species and forb species (*c*). Bars represent means ± s.e. *$p < 0.05$ (paired *t*-test) between with and without ant nests.

not affected by warming. This suggests that ecosystem functions linked to ant nest/activity will likely be stable under future climate warming. As *F. manchu* are distributed widely on the Tibetan Plateau [33], these effects may have important impacts over large areas.

Ant activity reduced the diversity of the plant community, but altered the species composition, with more grasses and fewer forbs near the ant nests. The nests increased the habitat heterogeneity, which was also reported in a previous study [17]. According to the habitat heterogeneity hypothesis, an increase in habitat heterogeneity enables different species to coexist and increase the biodiversity by increasing the niche dimensionality [51]. Ant activity increased habitat heterogeneity to support the coexistence of more species (such as nest microorganisms) and increased biodiversity [21], which ultimately promoted soil nutrient pools and plant growth. Ants select and transport specific seeds [50], and as a result, these plants are dominant around the ant nest [52], which could explain our observations.

(d) Asynchrony of ants and nest microorganisms in response to warming

In the warm season, in plots with ant nests, warming increased litter decomposition with ants but reduced litter decomposition with only microorganisms. The difference in responses indicated that the interaction between ants and nest microorganisms could be affected by warming. Ants build nests, which create a favourable environment for microorganisms, and transport large amounts of litter to the nests for microbial decomposition, which demonstrates the close relationship between ants and nest microorganisms [13]. Since ants were not affected negatively by warming, it is possible that in long-term warming treatment, ants build

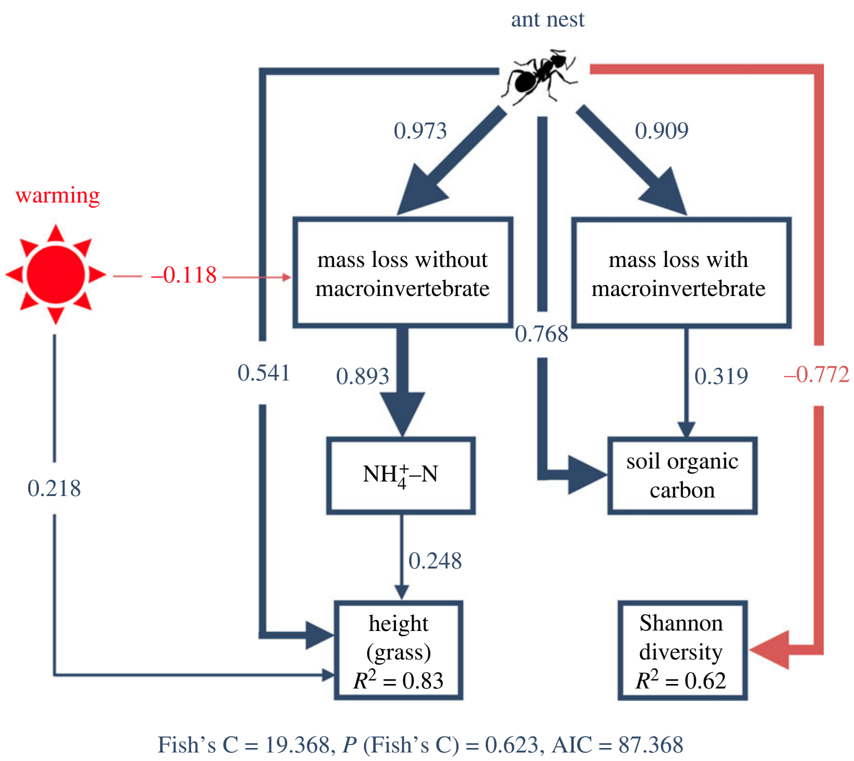

Figure 4. Piecewise structural equation model describing the effects of ant nest and warming on ecosystem process and function. Red lines indicate negative effects, and blue lines indicate positive effects. Only significant paths are shown, and the width of lines represents the standardized effect size. The p (χ^2) > 0.05 indicates the model is consistent with the data.

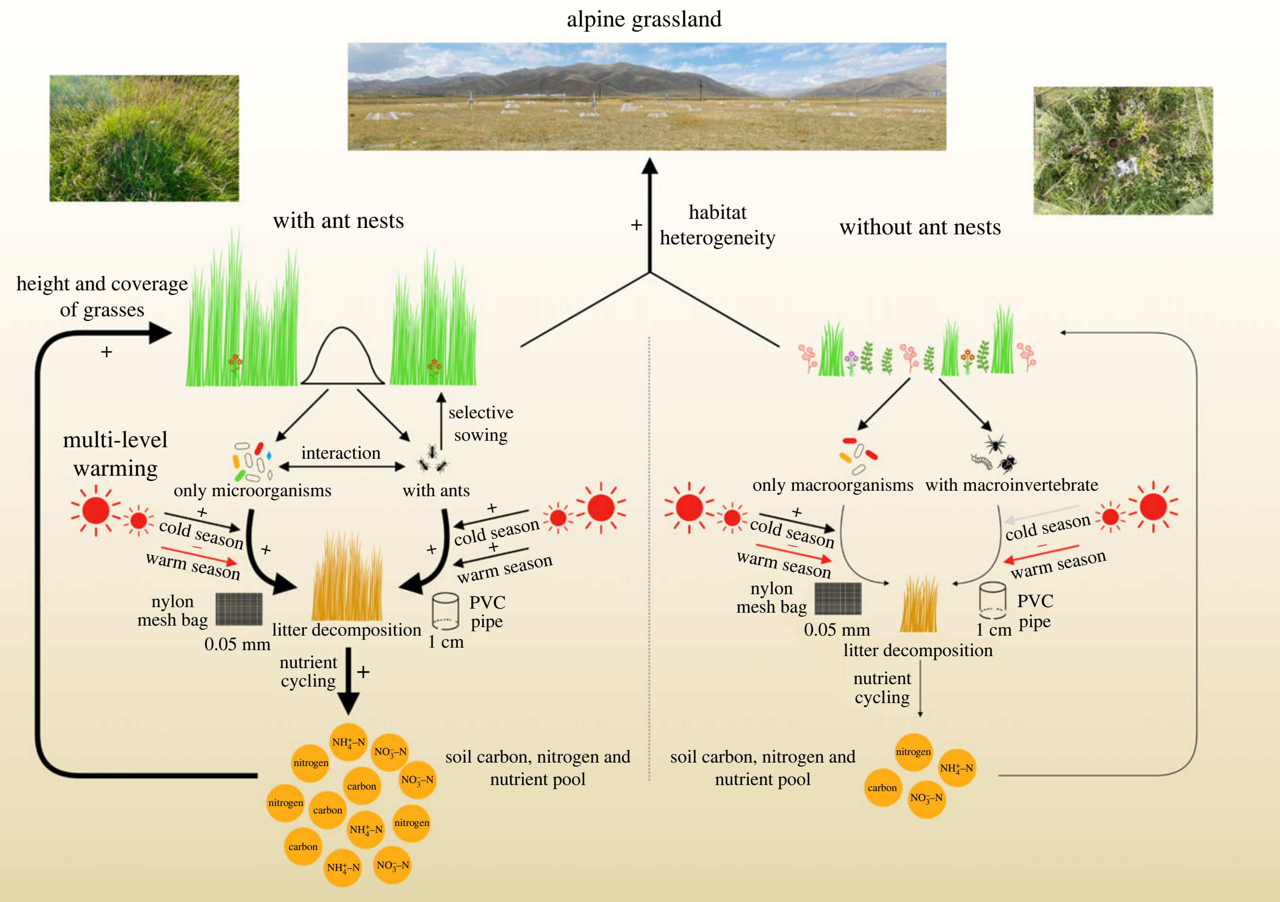

Figure 5. Summary of the effect of ant activity on ecosystem functions under climate warming. Ants build nests by soil manipulation and select seeds, which alter the surrounding vegetation composition and increase the habitat heterogeneity of the site. Increase of habitat heterogeneity provides more niches for soil organisms (nest microorganisms). Ants and nest microorganisms promote litter decomposition of substrates, and then increase the soil carbon and nitrogen pools by enhancing nutrient cycling. Greater content of soil nutrients, in turn, stabilizes the plant community structure near ant nests. A greater contribution of ants to litter decomposition under climate warming in the warm season mitigates the negative effects of climate warming on litter decomposition.

larger and deeper nests to protect microorganisms from the negative effects of warming [24], as biological regulation is one of the important functions of ants in ecosystems [13].

(e) Implications in management of alpine ecosystem

Many studies have reported the different role of insects in ecosystem functions [4,16], and their worldwide decline under climate change [53]. There are few studies that examined the warming effects on ecosystem function provided by the ants [29]. The present study addressed the role of ants in maintaining ecosystem processes and functions under warming (figure 5). Ants play a key role in nutrient cycling and in increasing the growth of palatable grass species under climate warming, which is beneficial to grassland ecosystems because it mitigates nitrogen limitation of plant growth and increases grassland carrying capacity on the Tibetan Plateau. Warming provides a better and more stable environment for the activity of ants and increase their feeding activity to mitigate the negative effect of climate warming on nutrient cycling in the alpine ecosystem (figure 5). Although the current study demonstrated that ants could mitigate some of the negative effects of warming on ecosystem processes and functions, more research is still warranted to further clarify the role of ants in climate warming, such as the interaction between ants and other macroinvertebrates on a larger scale. The protection of ants and ant nests should be considered in grassland management options to cope with the impact of climate warming on the alpine ecosystem.

Data accessibility. All data are available in Dryad Digital Repository: https://doi.org/10.5061/dryad.8gtht76tg [54].

Additional information is provided in electronic supplementary material [55].

Authors' contributions. B.L.: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing—original draft and writing—review and editing; M.H.: investigation; W.W.: investigation; J.N.: investigation; M.S.: writing—review and editing; H.Z.: investigation; L.M.: investigation; A.A.D.: methodology, writing—review and editing; J.L.: investigation; T.Z.: investigation; Y.B.: investigation; J.Z.: methodology; L.H.F.: writing—review and editing; Z.S.: conceptualization, formal analysis, funding acquisition, methodology, project administration, supervision and writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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References

1. Handa IT *et al.* 2014 Consequences of biodiversity loss for litter decomposition across biomes. *Nature* **509**, 218–221. (doi:10.1038/nature13247)
2. Christiansen CT, Haugwitz MS, Priemé A, Nielsen CS, Elberling BO, Michelsen A, Grogan P, Blok D. 2017 Enhanced summer warming reduces fungal decomposer diversity and litter mass loss more strongly in dry than in wet tundra. *Glob. Change Biol.* **23**, 406–420. (doi:10.1111/gcb.13362)
3. Bardgett RD, van der Putten WH. 2014 Belowground biodiversity and ecosystem functioning. *Nature* **515**, 505–511. (doi:10.1038/nature13855)
4. Prather CM *et al.* 2013 Invertebrates, ecosystem services and climate change. *Biol. Rev.* **88**, 327–348. (doi:10.1111/brv.12002)
5. Yin R, Eisenhauer N, Auge H, Purahong W, Schmidt A, Schäedler M. 2019 Additive effects of experimental climate change and land use on faunal contribution to litter decomposition. *Soil Biol. Biochem.* **131**, 141–148. (doi:10.1016/j.soilbio.2019.01.009)
6. Thakur MP, Reich PB, Hobbie SE, Stefanski A, Rich R, Rice KE, Eddy WC, Eisenhauer N. 2018 Reduced feeding activity of soil detritivores under warmer and drier conditions. *Nat. Clim. Change* **8**, 75–78. (doi:10.1038/s41558-017-0032-6)
7. Liu H, Lin L, Wang H, Zhang Z, Shangguan Z, Feng X, He J. 2021 Simulating warmer and drier climate increases root production but decreases root decomposition in an alpine grassland on the Tibetan plateau. *Plant Soil* **458**, 59–73. (doi:10.1007/s11104-020-04551-y)
8. Hong J, Lu X, Ma X, Wang X. 2021 Five-year study on the effects of warming and plant litter quality on litter decomposition rate in a Tibetan alpine grassland. *Sci. Total Environ.* **750**, 142306. (doi:10.1016/j.scitotenv.2020.142306)
9. Hågvar S, Klanderud K. 2009 Effect of simulated environmental change on alpine soil arthropods. *Glob. Change Biol.* **15**, 2972–2980. (doi:10.1111/j.1365-2486.2009.01926.x)
10. Siebert J, Thakur MP, Reitz T, Schädler M, Schulz E, Yin R, Weigelt A, Eisenhauer N. 2019 Extensive grassland-use sustains high levels of soil biological activity, but does not alleviate detrimental climate change effects. *Adv. Ecol. Res.* **60**, 25–58. (doi:10.1016/bs.aecr.2019.02.002)
11. Yin R, Qin W, Zhao H, Wang X, Cao G, Zhu B. 2022 Climate warming in an alpine meadow: differential responses of soil faunal vs. microbial effects on litter decomposition. *Biol. Fertil. Soils* **58**, 509–514. (doi:10.1007/s00374-022-01639-8)
12. Hättenschwiler S, Tiunov AV, Scheu S. 2005 Biodiversity and litter decomposition in terrestrial ecosystems. *Annu. Rev. Ecol. Evol. Syst.* **36**, 191–218. (doi:10.1146/annurev.ecolsys.36.112904.151932)
13. Wills BD, Landis DA. 2018 The role of ants in north temperate grasslands: a review. *Oecologia* **186**, 323–338. (doi:10.1007/s00442-017-4007-0)
14. Veldhuis MP, Laso FJ, Olff H, Berg MP. 2017 Termites promote resistance of decomposition to spatiotemporal variability in rainfall. *Ecology* **98**, 467–477. (doi:10.1002/ecy.1658)
15. Ashton LA *et al.* 2019 Termites mitigate the effects of drought in tropical rainforest. *Science* **363**, 174–177. (doi:10.1126/science.aau9565)
16. Farji-Brener AG, Werenkraut V. 2017 The effects of ant nests on soil fertility and plant performance: a meta-analysis. *J. Anim. Ecol.* **86**, 866–877. (doi:10.1111/1365-2656.12672)
17. De Almeida T, Mesléard F, Santonja M, Gros R, Dutoit T, Blight O. 2020 Above- and below-ground effects of an ecosystem engineer ant in Mediterranean dry grasslands. *Proc. R. Soc. B* **287**, 20201840. (doi:10.1098/rspb.2020.1840)
18. Wardle DA, Hyodo F, Bardgett RD, Yeates GW, Nilsson MC. 2011 Long-term aboveground and belowground consequences of red wood ant exclusion in boreal forest. *Ecology* **92**, 645–656. (doi:10.1890/10-1223.1)
19. Kendrick JA, Ribbons RR, Classen AT, Ellison AM. 2015 Changes in canopy structure and ant assemblages affect soil ecosystem variables as a foundation species declines. *Ecosphere* **6**, 1–20. (doi:10.1890/ES14-00447.1)
20. Griffiths HM, Ashton LA, Walker AE, Hasan F, Evans TA, Eggleton P, Parr CL. 2018 Ants are the major agents of resource removal from tropical rainforests. *J. Anim. Ecol.* **87**, 293–300. (doi:10.1111/1365-2656.12728)

21. Delgado-Baquerizo M, Eldridge DJ, Hamonts K, Singh BK. 2019 Ant colonies promote the diversity of soil microbial communities. *ISME J.* **13**, 1114–1118. (doi:10.1038/s41396-018-0335-2)
22. Li X *et al.* 2021 A facilitation between large herbivores and ants accelerates litter decomposition by modifying soil microenvironmental conditions. *Funct. Ecol.* **35**, 1822–1832. (doi:10.1111/1365-2435.13812)
23. Chen H *et al.* 2022 Carbon and nitrogen cycling on the Qinghai-Tibetan plateau. *Nat. Rev. Earth Envion.* **3**, 701–716. (doi:10.1038/s43017-022-00344-2)
24. Del Toro I, Ribbons RR, Ellison AM. 2015 Ant-mediated ecosystem functions on a warmer planet: effects on soil movement, decomposition and nutrient cycling. *J. Anim. Ecol.* **84**, 1233–1241. (doi:10.1111/1365-2656.12367)
25. Schultheiss P, Nooten SS, Wang R, Wong MK, Brassard F, Guénard B. 2022 The abundance, biomass, and distribution of ants on Earth. *Proc. Natl Acad. Sci. USA* **119**, e2201550119. (doi:10.1073/pnas.2201550119)
26. Denmead LH, Darras K, Clough Y, Diaz P, Grass I, Hoffmann MP, Nurdiansyah F, Fardiansah R, Tscharntke T. 2017 The role of ants, birds and bats for ecosystem functions and yield in oil palm plantations. *Ecology* **98**, 1945–1956. (doi:10.1002/ecy.1882)
27. Basset Y, Lamarre GP. 2019 Toward a world that values insects. *Science* **364**, 1230–1231. (doi:10.1126/science.aaw7071)
28. Diamond SE, Sorger DM, Hulcr J, Pelini SL, Del Toro I, Hirsch C, Oberg E, Dunn RR. 2012 Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. *Glob. Change Biol.* **18**, 448–456. (doi:10.1111/j.1365-2486.2011.02542.x)
29. Parr CL, Bishop TR. 2022 The response of ants to climate change. *Glob. Change Biol.* **28**, 3188–3205. (doi:10.1111/gcb.16140)
30. Diamond SE *et al.* 2016 Climatic warming destabilizes forest ant communities. *Sci. Adv.* **2**, e1600842. (doi:10.1126/sciadv.1600842)
31. Wang B, Bao Q, Hoskins B, Wu G, Liu Y. 2008 Tibetan plateau warming and precipitation changes in East Asia. *Geophys. Res. Lett.* **35**, L14702. (doi:10.1029/2008GL034330)
32. Hopping KA, Knapp AK, Dorji T, Klein JA. 2018 Warming and land use change concurrently erode ecosystem services in Tibet. *Glob. Change Biol.* **24**, 5534–5548. (doi:10.1111/gcb.14417)
33. Xiong Z, Qian Y, Xu Z, Li B, Zhai J, Huang Z, He Q. 2020 Distribution patterns of ant species from Qinghai part of Qilian Mountain National Park. *J. Northeast For. Univ.* **48**, 92–104. (doi:10.13759/j.cnki.dlxb.2020.11.017)
34. Shang Z, Deng B, Ding L, Ren G, Xin G, Liu Z, Wang Y, Long R. 2013 The effects of three years of fencing enclosure on soil seed banks and the relationship with above-ground vegetation of degraded alpine grasslands of the Tibetan plateau. *Plant Soil* **364**, 229–244. (doi:10.1007/s11104-012-1362-9)
35. Bai Y *et al.* 2020 Long-term active restoration of extremely degraded alpine grassland accelerated turnover and increased stability of soil carbon. *Glob. Change Biol.* **26**, 7217–7228. (doi:10.1111/gcb.15361)
36. Marion GM *et al.* 1997 Open-top designs for manipulating field temperature in high-latitude ecosystems. *Glob. Change Biol.* **3**, 20–32. (doi:10.1111/j.1365-2486.1997.gcb136.x)
37. Zhu J, Zhang Y, Yang X, Chen N, Li S, Wang P, Jiang L. 2020 Warming alters plant phylogenetic and functional community structure. *J. Ecol.* **108**, 2406–2415. (doi:10.1111/1365-2745.13448)
38. Whittaker RH. 1972 Evolution and measurement of species diversity. *Taxon* **21**, 213–251. (doi:10.2307/1218190)
39. Oksanen AJ *et al.* 2016 Vegan: community ecology package. *J. Veg. Sci.* **14**, 927–930. (doi:10.4135/9781412971874.n145)
40. Lenth RV. 2016 Least-squares means: the R package lsmeans. *J. Stat. Softw.* **69**, 1–33. (doi:10.18637/jss.v069.i01)
41. Lefcheck JS. 2016 piecewiseSEM: piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods Ecol. Evol.* **7**, 573–579. (doi:10.1111/2041-210X.12512)
42. Pinheiro J. 2012 nlme: linear and nonlinear mixed-effects models. R package version 3.1-103.
43. Bates D, Maechler M, Bolker BM, Walker SC. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48. (doi:10.18637/jss.v067.i01)
44. Klein JA, Harte J, Zhao X. 2004 Experimental warming causes large and rapid species loss, dampened by simulated grazing, on the Tibetan Plateau. *Ecol. Lett.* **7**, 1170–1179. (doi:10.1111/j.1461-0248.2004.00677.x)
45. Koch O, Tscherko D, Kandeler E. 2007 Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils. *Glob. Biogeochem. Cycles* **21**, GB4017. (doi:10.1029/2007GB002983)
46. Kaspari M, Clay NA, Lucas J, Yanoviak SP, Kay A. 2015 Thermal adaptation generates a diversity of thermal limits in a rainforest ant community. *Glob. Change Biol.* **21**, 1092–1102. (doi:10.1111/gcb.12750)
47. Høye TT, Kresse JC, Koltz AM, Bowden JJ. 2020 Earlier springs enable high-Arctic wolf spiders to produce a second clutch. *Proc. R. Soc. B* **287**, 20200982. (doi:10.1098/rspb.2020.0982)
48. Yin R *et al.* 2023 Experimental warming causes mismatches in alpine plant-microbe-fauna phenology. *Nat. Commun.* **14**, 2159. (doi:10.1038/s41467-023-37938-3)
49. Wang S, Wang H, Li J, Zhang Z. 2017 Ants can exert a diverse effect on soil carbon and nitrogen pools in a Xishuangbanna tropical forest. *Soil Biol. Biochem.* **113**, 45–52. (doi:10.1016/j.soilbio.2017.05.027)
50. De Almeida T, Blight O, Mesléard F, Bulot A, Provost E, Dutoit T. 2020 Harvester ants as ecological engineers for Mediterranean grassland restoration: impacts on soil and vegetation. *Biol. Conserv.* **245**, 108547. (doi:10.1016/j.biocon.2020.108547)
51. Stein A, Gerstner K, Kreft H. 2014 Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. *Ecol. Lett.* **17**, 866–880. (doi:10.1111/ele.12277)
52. Chomicki G, Kadereit G, Renner SS, Kiers ET. 2020 Tradeoffs in the evolution of plant farming by ants. *Proc. Natl Acad. Sci. USA* **117**, 2535–2543. (doi:10.1073/pnas.1919611117)
53. Sánchez-Bayo F, Wyckhuys KA. 2019 Worldwide decline of the entomofauna: a review of its drivers. *Biol. Conserv.* **232**, 8–27. (doi:10.1016/j.biocon.2019.01.020)
54. Luo B *et al*. 2023 Data from: Ant nests increase litter decomposition to mitigate the negative effect of warming in an alpine grassland ecosystem. Dryad Digital Repository. (doi:10.5061/dryad.8ght76tg)
55. Luo B *et al*. 2023 Ant nests increase litter decomposition to mitigate the negative effect of warming in an alpine grassland ecosystem. Figshare. (doi:10.6084/m9.figshare.c.6699435)