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Precipitation changes, warming, and N input differentially affect microbial predators in an alpine meadow: Evidence from soil phagotrophic protists

Zhengkun Hu[a,b], Junneng Yao[a,b], Xiaoyun Chen[a,b], Xin Gong[a,b], Yi Zhang[a], Xianhui Zhou[c], Hui Guo[a], Manqiang Liu[a,b,*]

[a] College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, 210095, China
[b] Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, Nanjing, 210014, China
[c] State Key Laboratory of Grassland and Agro-ecosystems, School of Life Sciences, Lanzhou University, Lanzhou, 730000, China

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ABSTRACT

Soil phagotrophic protists are highly abundant and play a vital role in nutrient cycling through feeding on microbes. Global change factors, individually or in combination, often affect soil bacteria and fungi, but whether and how the resulting changes may cascade to affect phagotrophic protists remain largely unknown. Combining direct microscopic counting and high-throughput sequencing of 18s rRNA gene, we examined effects of precipitation changes, warming and nitrogen (N) input on soil phagotrophic protists in a 3-yr manipulation experiment with a Tibetan alpine meadow. Precipitation addition (+30%) enhanced but precipitation reduction (−30%) and warming decreased the alpha diversity of phagotrophic protists, primarily through altering soil moisture. However, N input (12 g N m^{-2} y^{-1}) increased protist abundance, and in particular, offset the negative effect of precipitation reduction on the relative abundance of phagotrophic protists through increasing the microbial biomass, implying a bottom-up trophic control. Together, these findings indicate that interactions of multiple global change drivers may affect soil protist communities directly by modifying the soil physiochemical environment and indirectly through trophic cascading, which have implications for the potential changes in their ecosystem functions in alpine meadow under future global change scenarios.

1. Introduction

The average Earth surface temperature has increased by 0.8 °C since the era of the industrial revolution, accompanied by significant changes in precipitation patterns (IPCC, 2013). In parallel, anthropogenic activities have dramatically enhanced soil nutrient availability by global N deposition and excessive fertilization (Galloway et al., 2008). Alterations in these global change factors can strongly affect plant productivity and carbon (C) allocation belowground, modifying the growth and activities of soil microbes (Eisenhauer et al., 2012; García-Palacios et al., 2015; Jansson and Hofmockel, 2020). Changes in microbial biomass could cascade to higher trophic levels, however, direct evidence illustrating the cascading effect from field is scarce.

Soil protists are highly abundant, ranging from 10^4 to 10^7 individuals per gram of soil (Adl and Gupta, 2006). With hundreds for more species, soil protists occupy various positions in the soil food web and support multiple ecosystem functions (Geisen et al., 2016; Seppey et al., 2017; Xiong et al., 2018). Although they can be phototrophs and parasites, the vast majority of soil protists (*c.* 80%) are composed of phagotrophs that feed on soil microbes (Oliverio et al., 2020; Xiong et al., 2021). Accordingly, phagotrophic protists play a critical role in soil nutrient cycling and C dynamics through affecting the population dynamics of microbes and their activities (Geisen et al., 2021; Saleem et al., 2012; Zhao et al., 2020). Phagotrophic protists live in the water film around soil particles, and their activities such as movement and predation depend on water availability in soil pore space (Adl and Gupta, 2006; Clarholm, 1981). Many studies in controlled microcosms and field experiments have revealed that reduced soil moisture often suppresses soil protists (Geisen et al., 2014; Krashevska et al., 2012; Song et al., 2021; Tsyganov et al., 2013). In this respect, alterations in precipitation could directly affect phagotrophic protist communities through changing soil water availability. However, effects of water availability on protists may interact with other environmental factors. For example, although precipitation reduction and warming may suppress phagotrophic protists by decreasing soil moisture, enhanced nutrient availability resulted from N input may promote phagotrophic protists through stimulating

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* Corresponding author. College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, 210095, China.
E-mail address: liumq@njau.edu.cn (M. Liu).

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plant productivity and subsequent microbial growth (Z. Hu et al., 2017; Krashevska et al., 2014, 2010). Therefore, alterations in environmental conditions (e.g., soil moisture) and microbial biomass induced by global change factors may significantly and interactively affect the abundance and composition of phagotrophic protist communities, particularly in environmental fragile systems such as alpine ecosystems.

The Tibetan alpine meadow is one of the largest alpine ecosystems in the world (total at 2.5 million km^2) (Chen et al., 2013). Over the past 60 years, the Tibetan plateau has experienced faster climate warming (c. 2.7 °C) than the global average (c. 0.8 °C) (Kuang and Jiao, 2016), coupled with increasing frequency of extreme weather events and greater variation in precipitation patterns (Chen et al., 2013). Concurrent with climate change, unprecedented human perturbations including increasing grazing intensity, N fertilization have profoundly enhanced the N availability in the alpine ecosystems of the Tibetan plateau (Liu et al., 2015; Zhang et al., 2020). The Tibetan alpine ecosystems are characterized with high soil C and low soil N availability, largely due to constraints of low temperature on microbial decomposition of soil organic matter (SOM) (Yang et al., 2008). Warming and N input can thus alleviate the limitations on SOM decomposition in this system, and promote plant growth, productivity and litter input to soil, providing new resources for microbial growth and subsequent grazers (J. Hu et al., 2017; Wang et al., 2014; Zhang et al., 2020). Previous studies have shown that climate warming, precipitation changes, and N input significantly affected soil moisture, nutrient availability, and microbial (mainly bacteria and fungi) biomass and diversity in the Tibetan alpine meadow (Shen et al., 2018; Zeng et al., 2018; Zhang et al., 2016), and such changes have been linked to the abundance of microbial predators such as microbivorous and carnivorous nematodes (J. Hu et al., 2017; Wu et al., 2021). However, whether and how these global change factors affect the diversity and abundance of phagotrophic protists on alpine ecosystem are largely unknown.

Despite the tremendous abundance of phagotrophic protists, molecular investigation of their diversity is rare compared with those of soil bacterial and fungal communities (Bates et al., 2013). Based on morphological method, previous studies have shown that environmental changes, such as alterations in soil nutrient and water availability, could significantly affect the density of phagotrophic protist, but their effects on the diversity of phagotrophic protists were negligible (Eisenhauer et al., 2012; Geisen et al., 2014; Krashevska et al., 2010, 2012). However, the low resolution of morphological approaches may underestimate protist diversity and possibly explain why these studies failed in detecting any changes in the protist alpha diversity (Geisen et al., 2018).

Using both morphological method and high-throughput sequencing of 18s rRNA gene, we assessed the responses of abundance and diversity of soil phagotrophic protist to precipitation changes, warming and N input in a 3-yr manipulation experiment with a Tibetan alpine meadow. We hypothesized (H1) that decreases in soil water availability induced by precipitation reduction and warming would suppress the diversity and abundance of phagotrophic protists, and precipitation addition would likely alleviate water stress on phagotrophic protists. We also hypothesized that (H2) N-enhancement of microbial biomass would stimulate the abundance of phagotrophic protists via the bottom-up effect, offsetting the negative effects of precipitation reduction and warming.

2. Materials and methods

2.1. Study site and experimental design

This study was conducted in an alpine meadow at Alpine Research Station of Lanzhou University, at Maqu county, Gansu Province (33°59′N, 102°00′E, c. 3538 m a.s.L.) on the eastern Tibetan Plateau. During last forty years, the mean annual rainfall is 615 mm and mean annual temperature is 1.2 °C at the experimental site. The soil was classified as Cambisol (FAO taxonomy), with a pH of 6.0, and total soil C and N at 98.2 g C kg^{-1} and 6.9 g N kg^{-1} soil in the top soil (10 cm). The plant community is dominated by *Elymus nutans, Kobresia capillifolia, Carex kansuensis* and *Anemone rivularis.*

The multi-factorial field experiment was established in May 2015 on a slight northeastern-facing slope. The manipulative experiment had a randomized block design with three treatment factors and their combinations. It included three precipitation levels (control, precipitation reduction (control - 30% precipitation), and precipitation addition (control + 30% precipitation)), two temperature levels (control and warming (W)), and two N levels (control and N input (12 g N m^{-2} year^{-1})), resulting in 12 treatments (three precipitation levels × two warming levels × two N levels). Each treatment had four replicates, that is, four blocks, leading to a total of 48 plots (4 m × 4 m each). For precipitation reduction treatment, rain shelters made of v-shaped transparent plexiglass were used to block 30% precipitation. The blocked precipitation from each precipitation reduction plot was collected by a plastic container and then manually sprayed to the nearest precipitation addition plot within 24–48 h after the rainfall ended. Consequently, each precipitation addition plot received an addition of 30% natural rainfall without changing precipitation frequency. The open top chambers (OTC) were used to establish warming treatment. The OTC had a hexagonal design, made of transparent plexiglass, each with area of 1.5 m^2 and height in 0.5 m. Each warming plot has four chambers. For N input treatment, urea was applied in mid-May and late-June at a rate of 6 g N m^{-2} year^{-1} each time. Considering the increasing N deposition in future and potential N fertilization to support the yield of forage grass, we selected a N input level at 12 g N m^{-2} year^{-1} (Zhang et al., 2020).

2.2. Soil sampling and analysis

Soil samples (0–10 cm) were collected and composited from three random locations in each plot using a 50 mm diameter core in September 2017. Soil samples from each plot replicate were mixed into a composited sample and the kept in a plastic bag before being transported by express main to laboratory in Nanjing. Soil samples were then passed through a 2 mm sieve; all visible plant materials were carefully removed during this process. A subsample was also immediately frozen at −20 °C for molecular analysis. The remaining soil samples were stored at 4 °C and analyzed within 7–10 days after sampling.

Dissolved organic C (DOC) were extracted from 10.0 g fresh soil using 50 ml ultrapure water and then centrifuged at 5152 *g* for 10 min. The filtrate that passed through a 0.45 mm filter membrane was analyzed using a total C analyzer (Elementar, Germany). Mineral N was extracted with 2 M KCl in a1:5 ratio of soil to water, and then filtered through ashless filter paper and determined by an auto analyzer (Seal Analytical, Germany). Soil pH was measured in a 1:2.5 ratio of soil to water.

Soil microbial biomass was measured by analyzing soil phospholipid fatty acid (PLFA). Soil PLFAs were measured according to (Bligh and Dyer, 1959) with slight modifications. Briefly, 8.0 g freeze-dried soil was extracted with a chloroform–methanol–citrated buffer mixture (25 ml at a 1 : 2: 0.8 v/v). Lipid classes were separated into phospholipid, neutral, and glycolipid by solid phase extraction) tube (ANPEL Laboratory Technologies Inc., Shanghai, China) containing 0.5 g anhydrous sodium sulfate. The phospholipids were transesterified to a mild alkaline methanolysis (Bossio et al., 1998) and the resulting fatty acid methyl esters were extracted in hexane and dried under N_2. Samples were then dissolved in hexane and analyzed on an Agilent 6850 series gas chromatograph with MIDI peak identification software (v.4.5; MIDI Inc., Newark, DE, USA).

The density of phagotrophic protists (mainly flagellates, amoebae and ciliates) were enumerated using a modified most-probable number method (Darbyshire et al., 1974), Briefly, 3.0 g fresh soil was suspended in 30 mL sterile Neff's modified amoebae saline (NMAS) and gently shaken (180 rpm) for 30 min on a vertical shaker. Threefold dilution series with tryptic soy broth (TSB) and NMAS at 1:9 v/v were prepared in 96-well microtiter plates in quadruplicates. The microtiter plates were incubated at 15 °C in darkness, and the wells were inspected for presence of protists using an inverted microscope at × 100 to × 400 magnification after 7, 14 and 21 days. The density of protists was expressed as the number of individuals per gram of dry soil.

For molecular quantification of protists, soil DNA was extracted from 0.50 g soil samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer's protocols. DNA quality and quantity were determined using a NanoDrop 2000 Spectrophotometer (Bio-Rad Laboratories Inc., USA.). The V4 region of the 18S rRNA gene were amplified by PCR (95 °C for 5 min, followed by 27 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s and a final extension at 72 °C for 10 min) using primers TAReuk454FWD1 (5′-CCAGCASCYGCGGTAATTCC-3′) and TAReukREV3 (5′-ACTTTCGTTCTTGATYRA-3′), where barcode is an eight-base sequence unique to each sample. PCR reactions were performed in triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions.

Purified PCR products were quantified by Qubit®3.0 (Life Invitrogen) and every twenty-four amplicons whose barcodes were different were mixed equally. The pooled DNA product was used to construct Illumina Pair-End library following Illumina's genomic DNA library preparation procedure. Then the amplicon library was paired-end sequenced (2 × 250) on an Illumina MiSeq platform (Shanghai BIOZERON Co., Ltd) according to the standard protocols. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Project accession number: PRJNA751367). The QIIME software package (version 1.9.1) was used to analyze the raw Illumina HiSeq sequencing data. The sequences among these remaining reads were used to define operational taxonomic units (OTUs) using Usearch (version 7.1 http://drive5.com/uparse/) with a threshold of 97% similarity. The PR2 database was used as the reference for taxonomy assignments.

2.3. Data analyses

The alpha diversity of soil protists was determined using the numbers of observed OTUs, and beta diversity between treatments was calculated by Bray-Curtis dissimilarity index at OTU level. Protist OTU were assigned to phagotrophs according to (Adl et al., 2019). Linear mixed models (LMM) were used to analyze the effects of precipitation changes, warming, and N input on soil physiochemical properties and the alpha diversity, the density and relative abundance of phagotrophic protists and soil microbial biomass (block as a random effect and factors as fixed effects). To further interpret significant interactions, data were divided into subsets based on one of the factors of the interaction and post hoc comparisons were done for each level of the factor (García-Palacios et al., 2016). The Tukey's HSD test was used for post hoc comparisons of factors with more than two levels. Random forest analysis was employed to identify the most important predictors of alpha diversity of phagotrophic protists among soil physiochemical properties. Beta diversity of phagotrophic protists was correlated to the dissimilarity matrices from soil physiochemical properties using Mantel correlations. Linear regressions were used to test the relationships between soil microbial biomass and the relative abundance of phagotrophic protists. All statistical analyses were performed in R software (version 3.6.3).

3. Results

A total of 111367 high-quality protist sequence were generated from the 48 soil samples, with 76991 sequences being assigned to phagotrophic protists. A total of 724 OTUs (determined at 97% similarity) were identified across the sample set. Precipitation changes ($p < 0.001$) and warming ($p = 0.003$) significantly affected the alpha diversity of phagotrophic protist community. While precipitation addition increased protist alpha diversity, warming and precipitation reduction decreased this alpha diversity (Fig. 1A and B). Among all soil physiochemical properties measured in this study, soil moisture was the factor that best predicted the alpha diversity of phagotrophic protists (Fig. 1C). Also, beta diversity of phagotrophic protists (Bray-Curtis dissimilarity) was significantly correlated with changes in the soil moisture, rather than soil pH and nutrients, induced by global change (Mantel $R = 0.216$; $p < 0.001$; Figs. 1D and S1).

Precipitation changes significantly ($p < 0.001$) affected the density of soil flagellates, amoebae, and ciliates, with precipitation addition increasing but precipitation reduction decreasing the density of these protists (Fig. 2). N input significantly ($p < 0.01$) increased the density of flagellates and amoebae, and amplified the effect of increased precipitation on amoebae (N × P; $p = 0.026$; Fig. 2; Table 1). Without N input, decreased precipitation significantly decreased the relative abundance of phagotrophic protists (Fig. 3A). However, this inhibiting effect was attenuated by N input (N × P; $p = 0.042$; Fig. 3A). Similarly, there was a marginally significant interaction between precipitation changes and N input on soil microbial biomass as measured by PLFA analysis (N × P; $p = 0.067$; Fig. 3B). Under precipitation reduction, the relative abundance of phagotrophic protists was significantly related to soil microbial biomass ($p = 0.022$; Fig. 3C), while this positive relation was decoupled under ambient precipitation and precipitation addition (Fig. 3C).

4. Discussion

Terrestrial ecosystems are experiencing multiple global change factors such as altered precipitation, elevating temperature and nutrient enrichment. Ecologists have begun to explore their consequences for soil communities and ecosystem functions; our study, however, differs from few previous studies on this topic in three aspects. First, while most researches focused on the responses of belowground communities to a single global change factor (Krashevska et al., 2012; Song et al., 2021; Waldrop et al., 2004), the current study examined the effects of multiple factors including precipitations changes, warming and N input simultaneously. Second, our study was conducted on the Tibetan plateau, the world's largest and highest plateau, which is considered to be susceptible to global change and plays a critical role in global C cycle. Therefore, the present study represents a rare exploration of how multiple global change factors affect belowground communities in an ecologically important but fragile system. Last but not least, compared with most studies that focus on how soil bacteria and fungi respond to global change (Delgado-Baquerizo et al., 2017; Yuan et al., 2021; Zhang et al., 2020), our study revealed that not only the abundance, but also the diversity of phagotrophic protists, is sensitive and, respond differently to alterations in global change factors. Our study provided, to our knowledge, the first empirical evidence on the responses of soil protist to multiple global change factors in the Tibetan plateau.

Most soil protists are phagotrophs that feed on bacteria, fungi and other small protists, thereby regulating soil biodiversity and ecosystem functions such as nutrient cycling and plant growth (Bonkowski, 2004; Geisen et al., 2020; Saleem et al., 2012). However, most protist-related studies have so far focused on how their abundance and community structure respond to environmental changes (Eisenhauer et al., 2012; Z. Hu et al., 2017), whereas their diversity has long been ignored. Using high-throughput sequencing of 18S rRNA gene, our results showed that precipitation addition increased but precipitation reduction and warming decreased the alpha diversity of phagotrophic protists, which consistent with our first hypothesis, indicating that alterations in soil water availability may exert a dominant control over the phagotrophic protist diversity. This result is consistent with some studies (Song et al., 2021; Zhao et al., 2019), but contrasts with the results of a study conducted in tropical forests, where altered precipitation regimes have negligible effects on the species richness of testate amoebae, a major

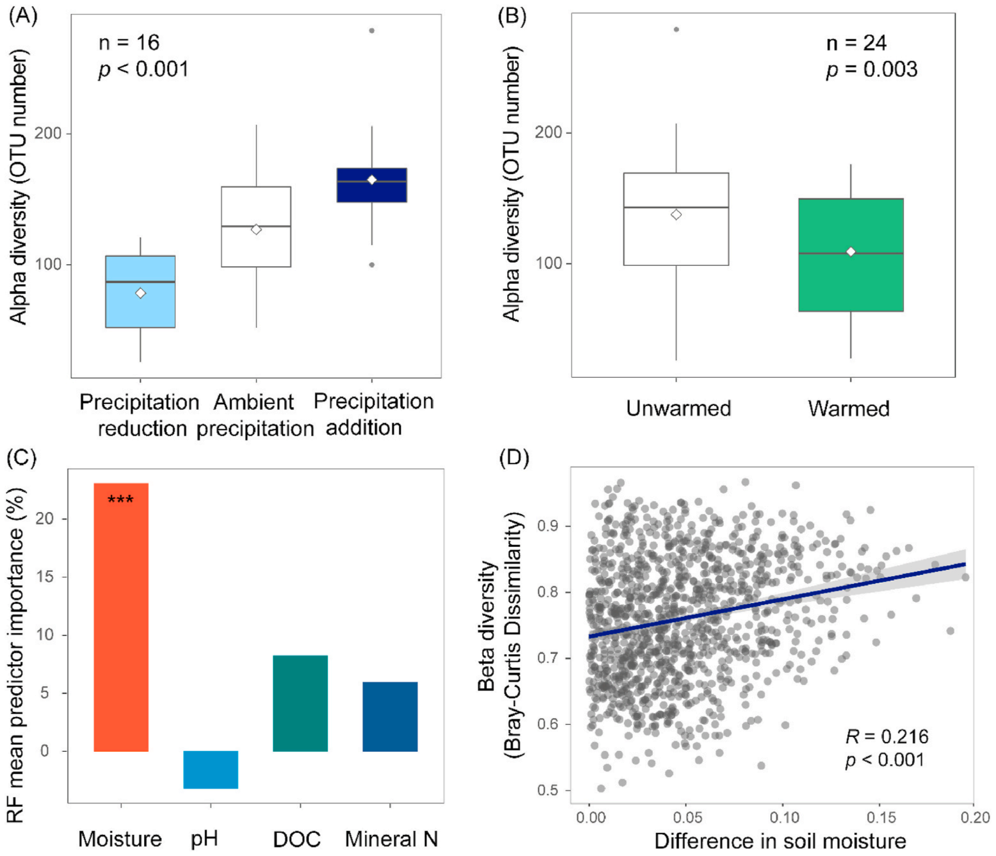

Fig. 1. Effects of global change factors on the diversity of soil phagotrophic protist. (A–B) Main effects of precipitation changes and warming on the alpha diversity of phagotrophic protists. (C) Random forest (RF) mean predictor importance of soil physiochemical properties as predictors of alpha diversity of phagotrophic protists. (D) Correlations between beta diversity of phagotrophic protist (Bray-Curtis dissimilarity) and difference in soil moisture. For simplification, the non-significant treatments are combined and only significant ($p < 0.05$) or marginally significant ($p < 0.1$) treatments and interactions are shown. Detailed results showing the effects of each treatment are listed in supplementary file (see Fig. S3).

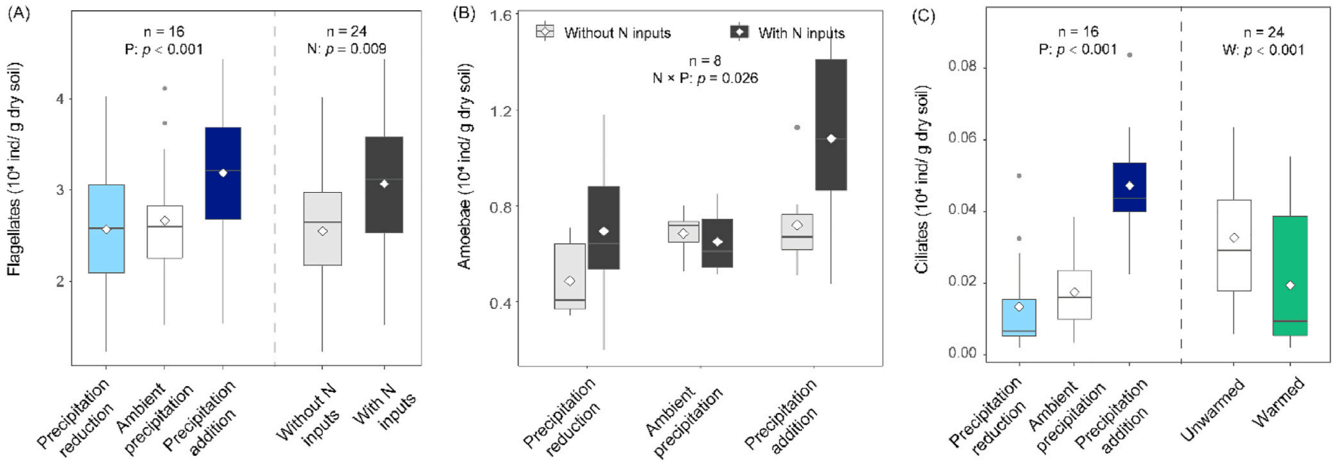

Fig. 2. Effects of warming, N inputs and altered precipitation on the density of flagellates (A) and amoebae (B), and ciliates (C). For simplification, the non-significant treatments are combined and only significant ($p < 0.05$) treatments and interactions are shown. Detailed results showing the effects of each treatment are listed in supplementary file (see Fig. S4). W represents warming, N represents N input and P represents precipitation changes.

group of phagotrophic protists in soils (Krashevska et al., 2012). Increases in soil moisture could alleviate the dispersal limitations on soil protists, but the stimulation of protist richness may depend on local climate type. In humid areas such as tropical rainforest, alterations in precipitation regimes may have limited effects on soil moisture as a result of high annual rainfall and frequent rainfall events (Krashevska et al., 2012). However, precipitation changes and warming resulted in nonnegligible changes in soil moisture in the alpine ecosystem of this study (Fig. S2), where annual rainfalls (c. 615 mm) are far less than those in tropical rainforests (usually more than 2000 mm). Therefore, the negative effects of precipitation reduction and warming on protist diversity may be more prolonged in xeric than in mesic ecosystems. Considering the pivotal role of soil microbial predators in regulating rates of ecosystem processes (Delgado-Baquerizo et al., 2020; Wagg et al., 2014), the rapid response of alpha diversity of phagotrophic protists to short-term fluctuations in soil moisture suggests that climate change may have larger impacts on the stability and functions of alpine ecosystems than expected.

Although N input significantly promoted soil acidification and nutrient availability (Tables S1 and S2), our study indicated that soil pH and mineral N content were the poor predictors for the alpha and beta diversity of phagotrophic protists (Figs. 1 and S1). In line with our

Table 1

Linear mix model showing the effects of altered precipitation, warming and N inputs and all possible interactions on the alpha diversity and relative abundance of phagotrophic protists, and density of flagellates, amoebae, and ciliates, and total PLFA.

Source of variation	df	Alpha diversity of phagotrophic protist		Relative abundance of phagotrophic protist		Density of flagellates		Density of amoebae		Density of ciliates		Total PLFA	
		χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p
P	2	**54.70**	**<0.001**	4.719	0.094	**27.28**	**<0.001**	**19.07**	**<0.001**	**120.0**	**<0.001**	**27.16**	**<0.001**
W	1	**9.793**	**0.002**	0.029	0.865	1.936	0.164	3.341	0.068	**23.20**	**<0.001**	2.419	0.12
N	1	1.261	0.261	**11.73**	**<0.001**	**6.859**	**0.009**	**8.751**	**0.003**	0.054	0.817	**25.39**	**<0.001**
P*W	2	1.443	0.486	0.896	0.639	2.240	0.326	1.524	0.467	0.931	0.628	3.773	0.120
P*N	2	4.044	0.132	**6.326**	**0.042**	0.699	0.705	**7.307**	**0.026**	3.094	0.213	5.381	0.067
W*N	1	1.006	0.316	0.619	0.431	0.058	0.809	0.278	0.598	0.135	0.714	1.025	0.311
P*W*N	2	1.904	0.386	3.308	0.191	0.115	0.944	0.348	0.840	3.455	0.178	1.761	0.415

W represents warming, N represents N input and P represents precipitation changes.

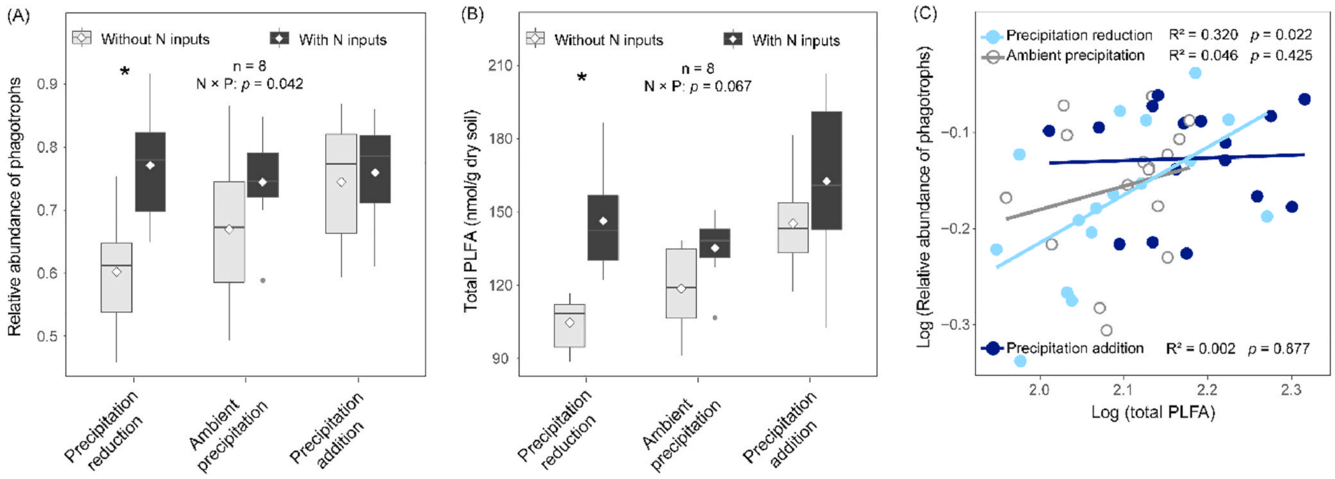

Fig. 3. Effects of warming, N inputs and altered precipitation on the relative abundance of phagotrophic protists (A) and total PLFA (B), and the relationships between phagotrophic protists and total PLFA (C). For simplification, the non-significant treatments are combined and only significant ($p < 0.05$) or marginally significant ($p < 0.1$) treatments and interactions are shown. The stars indicate significant differences between N inputs treatments after simple main effects test (n = 8). Detailed results showing the effects of each treatment are listed in supplementary file (see Fig. S5). W represents warming, N represents N input and P represents precipitation changes.

results, N addition (5 g N m^{-2} year^{-1}) had minor effects on the species richness of testate amoebae in a tropical forest (Krashevska et al., 2014). However, N fertilization (over 20 g N m^{-2} year^{-1}) dramatically reduced soil protist diversity and altered protist community in agroecosystems (Zhao et al., 2019). One possible explanation of inconsistences among the aforementioned studies may be the differences in N doses as high N input in agroecosystems prone to suppress soil biodiversity (Dai et al., 2018; de Graaff et al., 2019). Importantly, these results highlight the context dependency of how N input influence protist diversity and the associated mechanisms, emphasizing the need to explore the effects of N input in diverse ecosystems.

Many studies, including field investigations (Eisenhauer et al., 2012; Thakur et al., 2019) and incubation experiments (de Nijs et al., 2019; Geisen et al., 2014), have shown that enhanced N and water availability tend to increase the abundance and/or biomass of soil microorganisms. In this study, we also found positive effects of N input and increased precipitation on the density of phagotrophic protist, particularly those of flagellates and amoebae. However, the density of ciliates was not affected by N input, but significantly decreased by warming (Fig. 2). Given that the cell size of ciliates was much larger than flagellates and amoebae (Adl and Gupta, 2006; Geisen et al., 2018), these results suggest that in alpine system, changes in community composition of protistan consumers may be related to cell size with smaller species being more sensitive to nutrient additions, and larger species being more sensitive to water losses. In a microcosm study manipulating moisture gradient, Geisen et al. (2014) also found large taxa of soil protists were more strongly affected by drought than small organisms.

Interestingly, our results showed that N input interacted with precipitation to influence the relative abundance of phagotrophic protists (Fig. 3). Although N input did not significantly increase phagotrophic protists across all treatments, they did offset the negative effects of precipitation reduction on the proportion of phagotrophic protists. This result suggests that predictions on changes of belowground communities and functions based on single-factor experiments might be misleading (Reich et al., 2020; Rillig et al., 2019). N-stimulation of phagotrophic protists under reduced precipitation could be attributed to the bottom-up effect resulting from N-induced increases in prey abundance, as shown by the positive relationship between relative abundance of phototrophs and soil total PLFAs (Fig. 3). These results support our second hypothesis. Surprisingly, the prey-predator relationship was decoupled with increasing precipitation, suggesting that trophic interactions could be altered by climate change. These changes in predator-prey interactions may then cascade to affect community and ecosystem-level processes. For example, a recent study examined how drought-induced shifts in predator-prey interactions affect decomposition rates (Amundrud and Srivastava, 2016). Future work is therefore needed to understand how alterations in biotic interactions induced by global change factors may influence ecosystem processes under future climate change scenarios (Crowther et al., 2015; Laws, 2017; Saleem et al., 2013).

5. Conclusions

In conclusion, our study provides new empirical evidence that soil protist communities are sensitive to alterations in global change factors in the Tibetan alpine meadow. Global change factors could directly affect the diversity of phagotrophic protists via altering soil moisture, and indirectly affect their abundance through trophic cascading. Importantly, N input offset the negative effect of precipitation reduction on the relative abundance of phagotrophic protists. This result indicates the interactive effects of global change factors on microbial consumer communities and call for caution in extrapolating results from single-factor experiments to predict the impacts of global change on the soil food web. Our results have several implications for ecosystem productivity and C storage in the Tibetan alpine meadow. Given that phagotrophic protists form a major component of detritus food web responsible for nutrient cycling, their negative responses to precipitation reduction and warming may limit plant productivity and C storage in the Tibetan alpine meadow in a warmer and drier world. Most importantly, increasing demands for meats have prompted local nomads to increase grazing intensity. However, low grass productivity as a result of low temperature and soil N availability cannot support high animal density, leading to the suggestion to increase plant productivity via N fertilization. Positive effect of N input on phagotrophic protists may in turn stimulate microbial activities, accelerating decomposition and soil C losses. Together, our results highlight the need for better assessment of the responses of soil microbial predators to the ongoing global changes and their potential impacts on functioning of Tibetan alpine ecosystems.

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.soilbio.2021.108521.

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