Long-term warming impacts grassland ecosystem function : Role of diversity loss in conditionally rare bacterial taxa

Science of the Total Environment

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https://doi.org/10.1016/j.scitotenv.2023.164722

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Science of the Total Environment 892 (2023) 164722

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Long-term warming impacts grassland ecosystem function: Role of diversity loss in conditionally rare bacterial taxa

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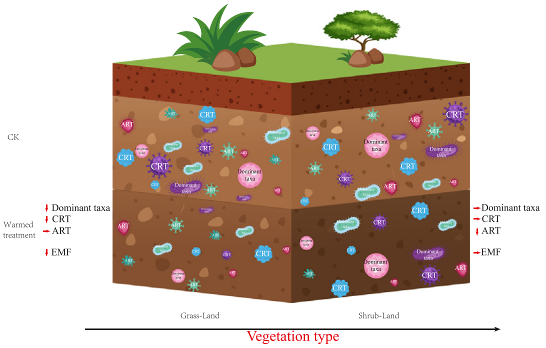
HIGHLIGHTS

- Simulated warming significantly lowered *ecosystem multifunctionality* in grassland.
- The negative response to the climate warming of various microbial taxa was different.
- The key role of conditionally rare taxa in regulating ecosystem multifunctionality

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Hai Guo

Keywords:
Climate change
Ecosystem function
Grassland
Microbe
Functional redundancy
Trade-off

ABSTRACT

The impact of microbial communities on ecosystem function varies due to the diverse biological attributes and sensitivities exhibited by different taxonomic groups. These groups can be classified as always rare (ART), conditionally rare (CRT), dominant, and total taxa, each affecting ecosystem function in distinct ways. Thus, understanding the functional traits of organisms within these taxa is crucial for comprehending their contributions to overall ecosystem function. In our study, we investigated the influence of climate warming on the biogeochemical cycles of the ecosystem in the Qinghai-Tibet Plateau, utilizing an open top chamber experiment. Simulated warming significantly lowered ecosystem function in the grassland but not in the shrubland. This discrepancy was due to the diverse responses of the various taxa present in each ecosystem to warming conditions and their differing roles in determining and regulating ecosystem function. The microbial maintenance of ecosystem function was primarily reliant on the diversity of bacterial dominant taxa and CRT and was less dependent on ART and fungal taxa. Furthermore, bacterial CRT and dominant taxa of the grassland ecosystem were more sensitive to changing climatic conditions than grassland ART, resulting in a more pronounced negative diversity response. In conclusion, the biological maintenance of ecosystem function during climate warming is dependent on microbiome composition and the functional and response characteristics of the taxa

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http://dx.doi.org/10.1016/j.scitotenv.2023.164722
Received 9 April 2023; Received in revised form 29 May 2023; Accepted 5 June 2023
Available online 10 June 2023

present. Thus, understanding the functional traits and response characteristics of various taxa is crucial for predicting the effects of climate change on ecosystem function and informing ecological reconstruction efforts in alpine regions of the plateau.

1. Introduction

Soil ecosystems perform numerous essential functions, such as primary production and nutrient cycling (Philippot et al., 2013; Wagg et al., 2014; Delgado-Baquerizo et al., 2016a, 2016b; Liu et al., 2017), which play a vital role in facilitating sustainable development. The impact of climate warming on ecosystem function is substantial, influencing aspects such as disease suppression, carbon sequestration, and plant growth (Rustad et al., 2001; Altizer et al., 2013; Lu et al., 2013). However, previous studies have predominantly focused on individual functions, such as net primary productivity and carbon emission, without encompassing the intricate nature of ecosystem performance. It is crucial to recognize that ecosystem performance entails synergies and trade-offs among multiple functions, highlighting the need for a comprehensive assessment. The study of multiple ecosystem functions can facilitate the development of comprehensive predictions concerning soil ecological functions under climate warming. Ecosystem function is an integrated approach that considers trade-offs among various functions, evaluating their ability to concurrently perform multiple functions within the soil (Li et al., 2021; Maestre et al., 2012). Numerous studies have demonstrated the impact of global change on ecosystem function, including degradation (Xu et al., 2021), fertilization (Cui et al., 2020), drought (Lozano et al., 2021), and elevated temperature (Valencia et al., 2018) of grassland systems. However, our understanding of how increased temperatures impact soil ecosystem function and how these changes may manifest at the mesocosm or field scales remains limited. Therefore, evidence from both controlled experiments and field-based studies is required to fully elucidate the influences of climate warming on ecological function and to determine potential driving factors.

Climate change has a profound impact on ecosystem dynamics by altering the composition and diversity of soil microbial communities, which exhibit high sensitivity to environmental changes (Castro et al., 2010; Maestre et al., 2015). Soil microbiomes play a pivotal role in fundamental ecological processes, including primary productivity and nutrient cycling, which are driven by the presence of diverse organisms (Hooper and Vitousek, 1998; Hector et al., 1999). It has also been reported that changes in plant communities, which also occur in response to temperature changes, affect the quality of organic matter and the allocation of carbon patterns could indirectly mediate the effects of climate warming on the ecological functions by affecting soil microbial biomass, community composition, and microbial turnover (Bragazza et al., 2015; Ward et al., 2015). The concurrent changes in climate warming and plant community dynamics make it challenging to determine their precise impacts on the diversity of microorganisms in the soil and their associated ecological functions.

Bacteria and fungi, as soil microbial organisms, have many differences in terms of their genetic, physiological, and functional characteristics. Consequently, their respective roles in soil ecosystem functioning are expected to differ (Sun et al., 2017). In alpine meadows, bacteria exert a greater influence on soil functions as compared to fungi due to their heightened ability to adapt to barren soils (Schmidt et al., 2014; Wang et al., 2019a). Moreover, bacteria preferentially utilize decomposable substrates, while fungi primarily decompose recalcitrant carbon compounds. This divergence in substrate preferences highlights the distinct roles bacteria and fungi play in the breakdown of organic matter and helps explain their differing prominence in various ecosystems (Boer et al., 2005; Kaisermann et al., 2015; Treseder et al., 2016; Eldridge and Delgado-Baquerizo, 2018). While the dominant role of bacteria in the alpine meadows of the Qinghai-Tibet Plateau has been investigated in previous studies, further investigation is needed to understand the relative significance of fungal and bacterial communities in the context of the impact of rising temperatures on soil ecosystem functioning. Soil microbial organisms have shown evidence of phylogenetic conservation in their responses to external disturbances such as nitrogen enrichment and increased temperatures (Martiny et al., 2015; Oliverio et al., 2017; Isobe et al., 2019), however, it is important to note that diverse responses to changes in environmental conditions have been observed among microbial taxa, including both rare and abundant taxa (Jiao et al., 2017; Jiao et al., 2019). According to Jiao and Lu (2020), rare taxa exhibit narrower environmental adaptation due to weaker phylogenetic signals of ecologically preferred traits and narrower response thresholds compared to abundant taxa. Similarly, the diversity of microbial subcommunities can lead to varying effects on soil ecosystem function. For example, studies by Campbell et al. (2011), Jousset et al. (2017), and Wan et al. (2021) have demonstrated that abundant taxa typically exhibit higher functional redundancies compared to rare taxa due to their greater competition potential and faster growth rates. It is commonly observed that functions provided by individual species are often limited (Hector and Bagchi, 2007; Gamfeldt et al., 2008; Isbell et al., 2011). Thus, it has been proposed that the critical driving force for ecosystem function comes from the rare microbial taxa characterized by high diversity, rather than the dominant taxa. This would suggest that rare taxa disproportionately contribute to essential biological processes (Chen et al., 2020b). Rare taxa can be subdivided into conditionally rare taxa (CRT) and always rare taxa (ART). Taxa that are usually rare, but could become an abundant members in communities when conditions become favorable, are regarded as CRT and account for more than half of rare taxa; while a few always remain rare regardless of conditions and are denoted ART (Shade et al., 2014; Campbell et al., 2011). Thus, CRT not only contributes to the high alpha diversity, like ART but also contributes to the dissimilarity among communities at different time points. By investigating the drivers of microbial dynamics, ecological research on CRT aims to enhance our understanding of the ecological resilience of microbial communities under disturbance conditions and the nuanced temporal variability they exhibit (Shade et al., 2014). To accurately predict the individual contributions of different taxa to ecosystem function it is necessary to separately quantify the influences of their diversity, given their important roles and distinctions. To investigate how climate warming regulates natural ecosystems and understand the underlying mechanisms, it is important to explore the correlations between the diversity of different components (below-ground vs. above-ground, bacteria vs. fungi, and rare vs. abundant taxa) and ecosystem functions across various environmental contexts.

The Qinghai-Tibetan alpine grasslands provide numerous vital ecological functions and services (Li et al., 2016; Che et al., 2018; Dang et al., 2020). According to Klein et al. (2004), alpine meadows are highly vulnerable to the ongoing impacts of climate change and land use development. Large-scale soil degradation in this region is primarily caused by human activities, and this is worsened by climate change (Harris, 2010; Wang et al., 2014b; Zhou et al., 2019). Assessing ecosystem function in this region aids in predicting responses to human activities and climate warming. It is essential to determine the contributions of biodiversity components to ecosystem function and predict the response of key microbial taxa. Field control experiments provide an optimal approach to test the influence of these factors. This study investigated the influence of prolonged climate warming on ecosystem function in two representative ecosystems (shrubland and grassland) within the Qinghai-Tibet plateau. It explored potential driving factors, such as plant species diversity and soil microbial diversity (bacteria vs. fungi, abundant vs. rare taxa), using an open top chamber (OTC) for a simulated warming experiment. We hypothesized that 1) based on the profound impact of vegetation on soil characteristics and microorganisms, the extent to which long-term warming negatively impacts ecosystem function is likely to vary across different ecosystems due to the varying responses of above-ground vegetation to climate change; and 2) functional effects would vary among the taxon with diverse biodiversity and biomass, the bacteria would contribute more than fungi in maintaining ecosystem function, and soil dominant microbial taxa or CRT, which have the potential to become dominant, are expected to play a more significant role in maintaining ecosystem function than rare taxa.

2. Materials and methods

2.1. Study area and experimental design

This experiment was conducted at the Haibei Alpine Meadow Ecosystem Research Station, in the northeastern Qinghai-Tibet plateau, China. The station is situated at 37°36 N, 101°19 E. The valley bottom has an average elevation of 3200 m and the mean annual temperature is -2 °C, ranging from -15.2 °C in January to 9.9 °C in July. The area experiences an average annual precipitation of 561 mm, with most rainfall occurring during the brief, cool summer. The growing season typically spans from May to September, and the soil type in the area has been identified as Mollic-cryic cambisol. For further information, refer to Zhang et al. (2017). Two types of ecosystems were established in the region, characterized by the dominant plant species of *Potentilla fruticosa* in the alpine shrubland and *Kobresia humilis* in the alpine meadow. A study site measuring 30 m × 30 m was established for each vegetation type, and protective fencing was installed to prevent animal interference. In 1997, six replicates of control and warming plots were established, which were randomly distributed, totaling 12 experimental plots per ecosystem site. Climate warming was simulated using OTCs. A 2 m gap was maintained between every plot. We select three sunny days in the first, second, and third ten days of each month, and measure the temperature every two hours between 10:00 and 18:00. Air temperature will be obtained through the installation of a thermometer, while soil temperature will be measured using a borehole thermometer. The mean temperature of the soil, measured at a depth of 12 cm, and the mean temperature of the air, measured at a height of 10 cm, were both increased by a range of 0.3 to 1.9 °C and 1.0 to 2.0 °C, respectively. During the vegetation growth season, the loss of soil moisture by OTCs was limited to a maximum of 3 % (Klein et al., 2004; Zhang et al., 2017).

2.2. Soil sample collection

In August 2018, soil samples were collected after subjecting the experimental plots to simulated climate warming treatment for a period of 21 years. From each treatment in each ecosystem, six replicates were selected as plots. Within each plot, a 50 cm × 50 cm area was randomly chosen, and five soil cores with a diameter of 5 cm were collected from the 0–5 cm depth after removing litter and other surface materials. These soil cores were then sieved through a 2.0 mm sieve to eliminate impurities such as stones and plant parts. The six soil core samples from plots of the same ecosystem were carefully combined into a single composite sample. Then, the composite sample representing either control or warming treatment conditions for each ecosystem was separated into three sub-samples. One sub-sample was stored at -20 °C for microorganism analysis, another sub-sample was stored at 4 °C for enzyme activity measurements, and the third sub-sample was sieved through 0.25 mm and 1 mm sieves for analysis of soil physicochemical properties.

2.3. Soil and plant properties

Soil water content was determined by calculating the difference in weight before and after drying the soil samples at 105 °C for 48 h. Before property analyses, all soil samples were dried in the air and ground to a fine powder. The concentrations of soil organic carbon (SOC), pH, total phosphorous (TP), total nitrogen (TN), and dissolved organic carbon (DOC) were analyzed using the methods previously described by Nelson et al. (1982). The concentration of TN in soil was evaluated using the Kjeldahl method (Bremner and Mulvaney, 1982). The concentration of TP in soil was assessed through colorimetric analysis after digestion with perchloric acid ($HClO_4$) and sulfuric acid (H_2SO_4). Soil pH was measured using a pH meter (Metrohm 702, Herisau, Switzerland) with water to soil ratio of 5:1. Soil nitrate-nitrogen (NO_3^--N) and ammonium (NH_4^+-N) were assessed using a continuous flow auto-analyzer (AutAnalyel, Bran + Luebbe GmbH, Norderstedt, Germany), after extraction with 1 M KCl, following the method described by Wu et al. (2020). The concentration of DOC in fresh soil was measured using a Liquid TOC II analyzer (Elementar Analyses System, Hanau, Germany) after distillation-based extraction. The activity of extracellular enzymes (CBH: cellobiohydrolase; BG: β-1,4-glucosidase; XYL: β-1,4-xylosidase; LAP: leucine aminopeptidase; NAG: *N*-acetyl-β-D-glucosaminidase; ALA: L-alanine aminopeptidaseand AP: phosphatase) was measured using a modified version of standard fluorometric techniques. Further details can be found in Appendix S2 (Marx et al., 2001; German et al., 2011).

In August 2018, during the peak of plant biomass, we randomly placed a permanent quadrat (0.25 m × 0.25 m) in the experimental plots. Samples of the plants living above ground were collected, and their biomass was estimated by weighing them after oven-drying at 65 °C for 48 h.

2.4. Extraction, amplification, and sequencing of DNA and processing of sequencing data

Microbial DNA was extracted from 0.25 g frozen soil samples using the Power Soil DNA Isolation Kit provided by MO BIO Laboratories (Carlsbad, CA, USA). To characterize microbial populations, a fragment of the 16S rRNA gene for bacteria, and the ITS1 region for fungi, were amplified using the primer pairs ITS1F/ITS2-2043R and 338F/806R, respectively. Detailed amplification protocols for the internal transcribed spacer can be found in Appendix S1. High-throughput sequencing analysis was performed on the Illumina Hiseq 2500 platform with 2250 paired ends.

The collected sequences underwent quality control using quantitative insights into microbial ecology (QIIME), following previously reported methods (Bokulich et al., 2013), to ensure high-quality, clean tags. Sample sorting was performed using a barcode sequence, and the unassigned chimera check (UCHIME) method was employed to eliminate chimeric sequences (Edgar et al., 2011). Subsequently, the sequences were clustered using UPARSE software with a 97 % similarity threshold for assignment to operational taxonomic units (OTUs). Taxonomic lineages of the representative sequences of bacteria and fungi were determined using the Ribosomal Database Project (RDP) Classifier, Basic Local Alignment Search Tool (BLAST), and QIIME tool, and were assigned to the SILVA and UNITE and International Nucleotide Sequence Database Collaboration (INSDC) databases (Chen et al., 2020c).

The quantification of functional genes associated with the cycles of carbon, nitrogen, phosphorus, and sulfur was performed by using the Quantitative Microbial Ecology Chip (QMEC), a high throughput quantitative-PCR-based chip designed for assessing the functional potential of microorganisms. All primers used in the analysis were verified and validated (Zheng et al., 2018; Chen et al., 2020a). The Wafergen SmartChip Real-time PCR system (Wafergen, Fremont, CA, USA) was used for amplification in a 100 nL reaction system. Each qPCR reaction was performed in triplicate per primer set, with a non-template negative control included in each run. The enzyme was initially activated at 95 °C for 5 min, followed by denaturation at the same temperature for 30 s. Subsequently, annealing was carried out at 58 °C for 30 s, and elongation was performed at 72 °C for 30 s. The melting curve analysis was conducted using the Smart Chip qPCR Software. Amplification efficiencies and peak values that fell outside the range of 0.9–1.1 were not considered. The limit of detection was set at a cycle threshold (CT) of 31.

2.5. Definition of abundant versus rare, and generalist versus specialist taxa

The overall microbial community was partitioned into sub-communities based on a threshold of relative abundance, with rare taxa at 0.05 % and

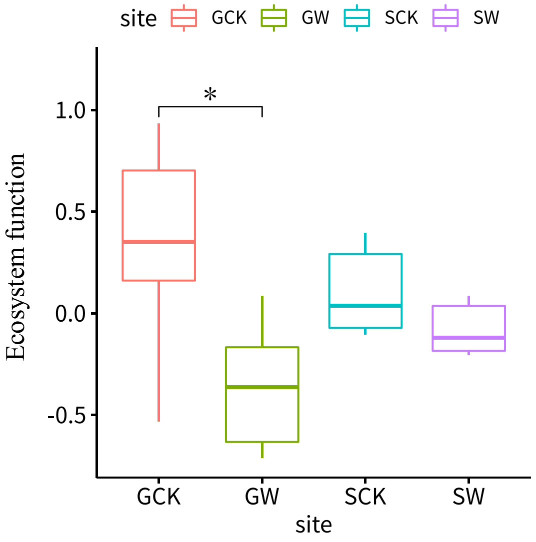

Fig. 1. The response of average ecosystem function index to global warming. P < 0.05 was indicated by the * (n = 6). Control Shrub Land, Warmed Shrub Land, control Grass Land, warmed Grass Land simplified to SCK, SW, GCK, GW respectively.

abundant taxa at 0.5 %. The groupings used to categorize taxa were established in previous research (Liu et al., 2015; Dai et al., 2016) (Table S1 and Appendix S3). These categories included conditionally rare and abundant taxa (CRAT), conditionally abundant taxa (CAT), always abundant taxa (AAT), conditionally rare taxa (CRT), always rare taxa (ART), and moderate taxa (MT). The analysis of OTU niche differences showed a positive correlation with phylogenetic distance at short distances, as illustrated in Fig. S1, indicating the conservation of OTU environment preferences based on phylogeny. Taxonomic classification at the phylum level can be found in Table S2.

2.6. Data analysis

We evaluated ecosystem function using two approaches: single-threshold and multiple-threshold ecosystem function. First, we analyzed 21 ecosystem functions associated with services essential for the production of other ecosystem services, including primary production, soil properties, fertility, and nutrient cycling (Garland et al., 2021). We standardized the following functions using z-scores: above-ground biomass, SOC, DOC, TN, TP, available nitrogen (nitrate nitrogen and ammoniacal nitrogen), extracellular enzyme activities associated with carbon, nitrogen, and phosphorus cycles, and functional genes associated with methane metabolism, carbon degradation and fixation, and the cycling processes for nitrogen, phosphorus, and sulfur. The ecosystem function was calculated as the average value of each function. The average level of multiple functions can be determined using an ecosystem function index that standardizes each function to a common scale and averages their standardized values. This approach, as noted in studies by Maestre et al. (2012) and Chen et al. (2020b), ensures consistency and objectivity in measuring ecosystem function. To standardize the data on ecosystem function, a z-score transformation method was utilized, resulting in a standard deviation of 1 and a mean of 0 (Chen et al., 2020b). Specifically, in the single-threshold approach, we established a linear relationship to predict the number of functions performed above or at thresholds of 20 %, 40 %, 60 %, and 80 %. For all calculations, we utilized the “multifunc” package (Byrnes et al., 2014) in R.

Our study on biodiversity encompassed various measurement dimensions, including Shannon diversity, the Chao1 index, the Abundance-based Coverage Estimator (ACE), and species richness. To assess the comprehensive microbial diversity of the soil, we applied a z-score transformation method which was denoted as soil biodiversity (z-score). The quantification of microbial alpha diversity was conducted using the “vegan” package.

We conducted Wilcoxon rank-sum tests to investigate the effects of long-term warming, single functions, and microbial diversity on ecosystem function. A Pearson correlation heatmap was used to analyze the associations between microorganism diversity, single functions, and ecosystem function. The R package, relaimpo, was used to determine the relative importance of CRT, dominant taxa, and ART for both bacteria and fungi, in explaining the variations in ecosystem function (Grömping, 2006).

3. Results

3.1. Impact of warming on ecosystem function and individual functions

In the grassland, ecosystem function was significantly decreased by warming ($p < 0.05$) (Fig. 1). Among the individual functions, warming resulted in increased above-ground biomass, while SOC, carbon fixation,

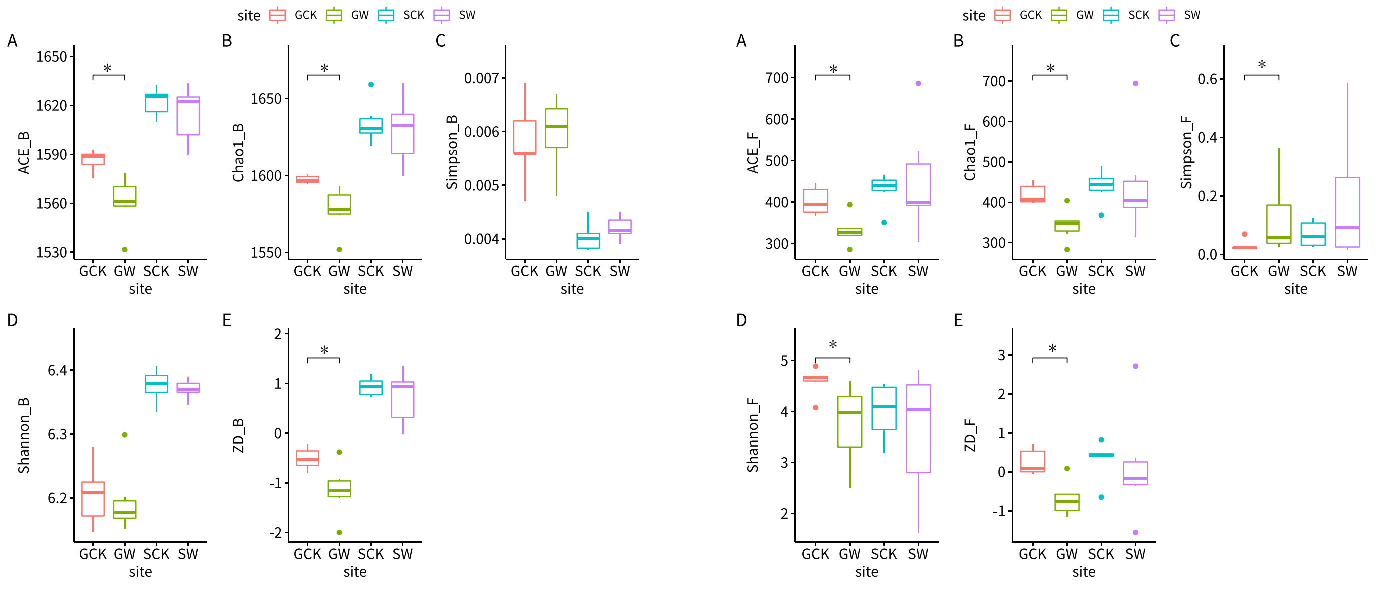

Fig. 2. Diversity of bacteria and fungi community in response to global warming. P < 0.05 was indicated by the * (n = 6). Control Shrub Land, Warmed Shrub Land, control Grass Land, warmed Grass Land simplified to SCK, SW, GCK, GW respectively. B: bacteria; F: fungi.

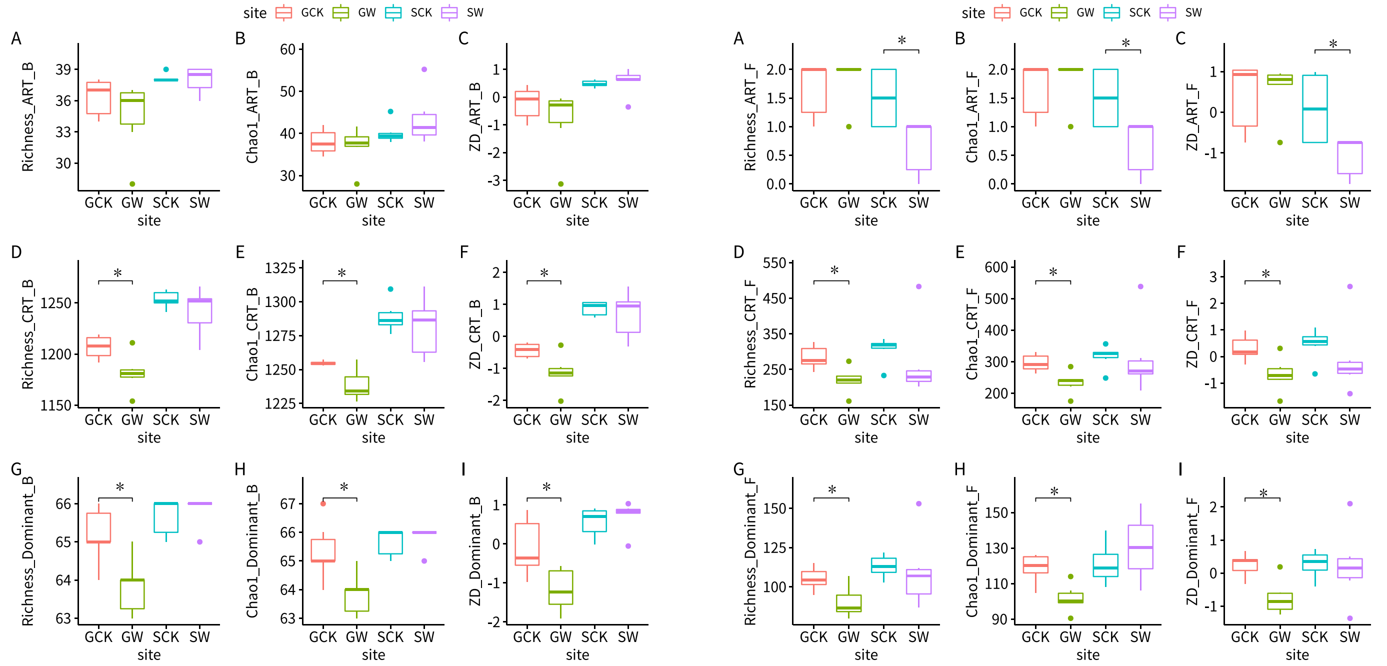

Fig. 3. The influences of global warming on diversity of bacterial and fungal subcommunity. P < 0.05 was indicated by the * (n = 6). Control Shrub Land, Warmed Shrub Land, control Grass Land, warmed Grass Land simplified to SCK, SW, GCK, GW respectively. B: bacteria; F: fungi. ART, always-rare taxa; Dominant, Dominant taxa; CRT, conditionally rare taxa.

methane metabolism, carbon degradation, and sulfur, phosphorus, and nitrogen cycling processes were decreased ($p < 0.05$) (Fig. S2). Warming of the shrubland ecosystem resulted in a decrease in BG, NAG, and XXL ($p < 0.05$) (Fig. S2).

3.2. Impact of warming on soil microbial diversity

Warming significantly reduced overall fungal and bacterial diversities in the grassland ecosystem ($p < 0.05$) (Fig. 2). Warming also resulted in a decrease in the diversity of microbial dominant taxa and CRT, while the diversity of ART remained unaffected in grassland samples (Fig. 3). Of note, warming did result in a decrease in diversity of fungal ART in the shrubland ecosystem ($p < 0.05$) (Fig. 3).

3.3. Relationship between bacterial or fungal diversity and ecosystem function

The individual functions SOC, TP, CBH, carbon fixation, methane metabolism, carbon degradation, and nitrogen and phosphorus cycling processes were all positively associated with increased diversity of bacterial dominant taxa ($p < 0.05$) (Fig. 4). Most of these functions were also positively correlated with the diversity of bacterial CRT and bacterial dominant taxa ($p < 0.05$) (Fig. 4). The overall diversity of fungi, as well as fungal

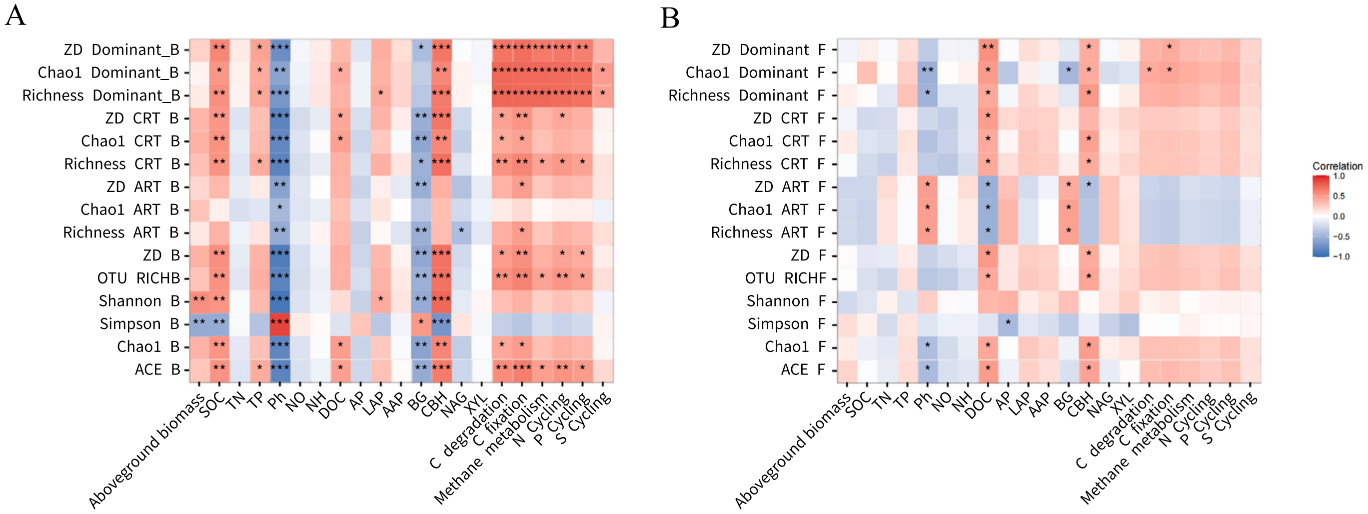

Fig. 4. Relationships between the diversity of bacterial (A) and fungal (B) community and each ecosystem function. The blue highlighted the negative correlation coefficients, while red highlighted positive correlation coefficients. The *, **, *** means P < 0.05, 0.01, 0.001. SOC: Soil total organic carbon; TN: Soil total nitrogen; TP: Soil total phosphorus; DOC: Soil dissolved organic carbon; NO: Soil nitrate nitrogen; NH: Soil ammonium nitrogen; pH: Soil acidity and alkalinity; CBH: cellobiohydrolase; BG: β-1,4-glucosidase; XYL: β-1,4-xylosidase; LAP: leucine aminopeptidase; NAG: *N*-acetyl-β-D-glucosaminidase; ALA: L-alanine aminopeptidaseand AP: phosphatase. B: bacteria; F: fungi.

dominant taxa and CRT, showed positive correlations with DOC and CBH. Negative correlations were observed between BG and pH with bacterial diversity ($p < 0.05$). Increased diversity of fungal dominant taxa was positively correlated with carbon fixation and degradation. However, the diversity of fungal ART exhibited negative correlations with both CBH and dissolved organic carbon ($p < 0.05$) (Fig. 4).

In the single-threshold approach, there were no significant positive correlations between plant diversity, species richness, and ecosystem function at each threshold ($p > 0.05$) (Fig. S3). However, ecosystem function showed a significant positive correlation with bacterial diversity at 20 %, 40 %, and 60 % as well as with fungal diversity at 20 % and 40 % thresholds in the single-threshold approach ($p < 0.05$) (Fig. 5). Ecosystem function exhibited significant correlations with the bacterial dominant taxa at 20 %, 40 %, 60 %, and 80 % thresholds, as well as with CRT at 20 % and 60 % thresholds. Additionally, ART showed a significant correlation with ecosystem function only at the 20 % threshold ($p < 0.05$) (Fig. 6). Furthermore, there were significant correlations between ecosystem function and the diversity of fungal dominant taxa and fungal CRT at both 20 % and 40 % thresholds ($p < 0.05$). However, no significant relationship was found between ecosystem function and the diversity of fungal ART at any of the four thresholds ($p > 0.05$) (Fig. 6).

At a threshold of 67 %, the inclusion of one bacterial species resulted in the highest positive effect on ecosystem function, with a realized maximum effect of diversity (Rmde) of 2.168 (Fig. 5). This means that the addition of a single bacterial species led to a 2.168 increase in ecosystem function. Similarly, the inclusion of one bacterial dominant taxa, CRT, or ART at 67 %, 67 %, and 47 % thresholds, respectively, resulted in increases of 3.399, 2.052, and 1.336 in ecosystem function (Fig. 6). For fungi, at a 47 % threshold, the addition of one fungal species yielded a Rmde of 1.890, or an increase of ecosystem function by 1.890 (Fig. 5). The inclusion of one fungal dominant taxon and one fungal CRT increased the ecosystem function by 2.170 and 1.650, respectively, at a 47 % threshold (Fig. 6).

3.4. Predictors of ecosystem function

The analysis carried out using the ‘relaimpo’ tool in R revealed a strong fit when integrating the diversity of various microbial taxa and plant diversity, with a modeled R^2 value of 58.36 %. The results showed that bacterial subcommunities played a crucial role in supporting ecosystem functions, whereas fungal subcommunities had a lesser impact (Fig. 7). Additionally, plant diversity had a secondary role in shaping ecosystem function (Fig. 7). When considering the diversity of different taxa, microbial dominant taxa, and CRT exhibited the highest contribution in shaping ecosystem function, with a greater power in explaining the overall variability compared to ART (Fig. 7).

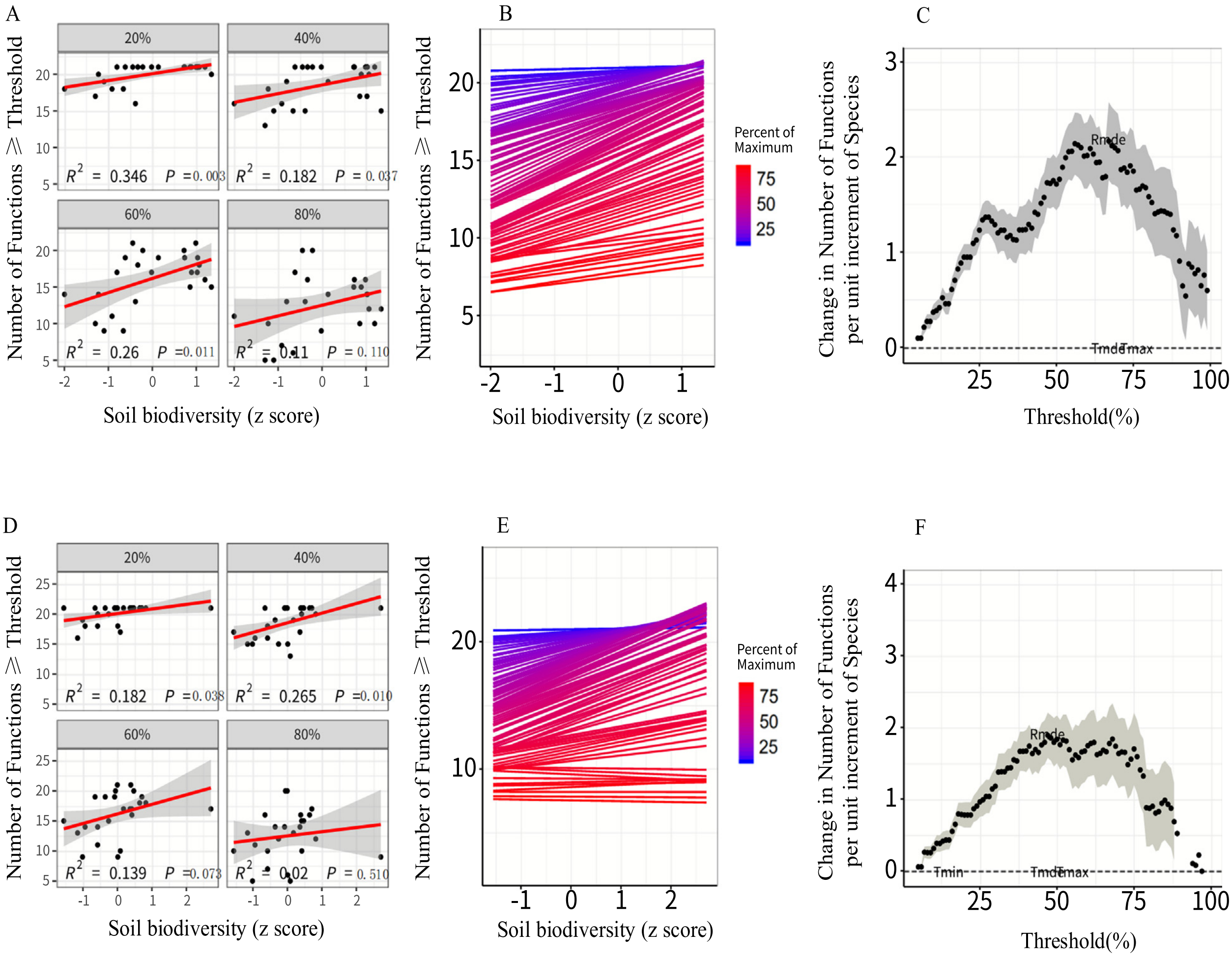

Fig. 5. The relationship between ecosystem function and microbial diversity (bacteria: (A); fungi: (D)) at four different thresholds 20 %, 40 %, 60 %, and 80 % of maximum. The significant linear relationship fitted the ordinary least-squares (OLS) regression model was indicated by the red solid line, while the 95 % confidence interval of the fit was represented by gray shaded area. A variety of ecosystem function thresholds are impacted by diversity influences. The impact of bacterial and fungal diversity (B and E) on functions exceeding thresholds. The slope between soil microbial diversity and the number of functions above threshold (ranging from 5 to 99 % of maximum for each function) was represented by lines. The dotted curves illustrated the changes in the number of functions per unit increase of bacterial (C) and fungal (F) diversity.

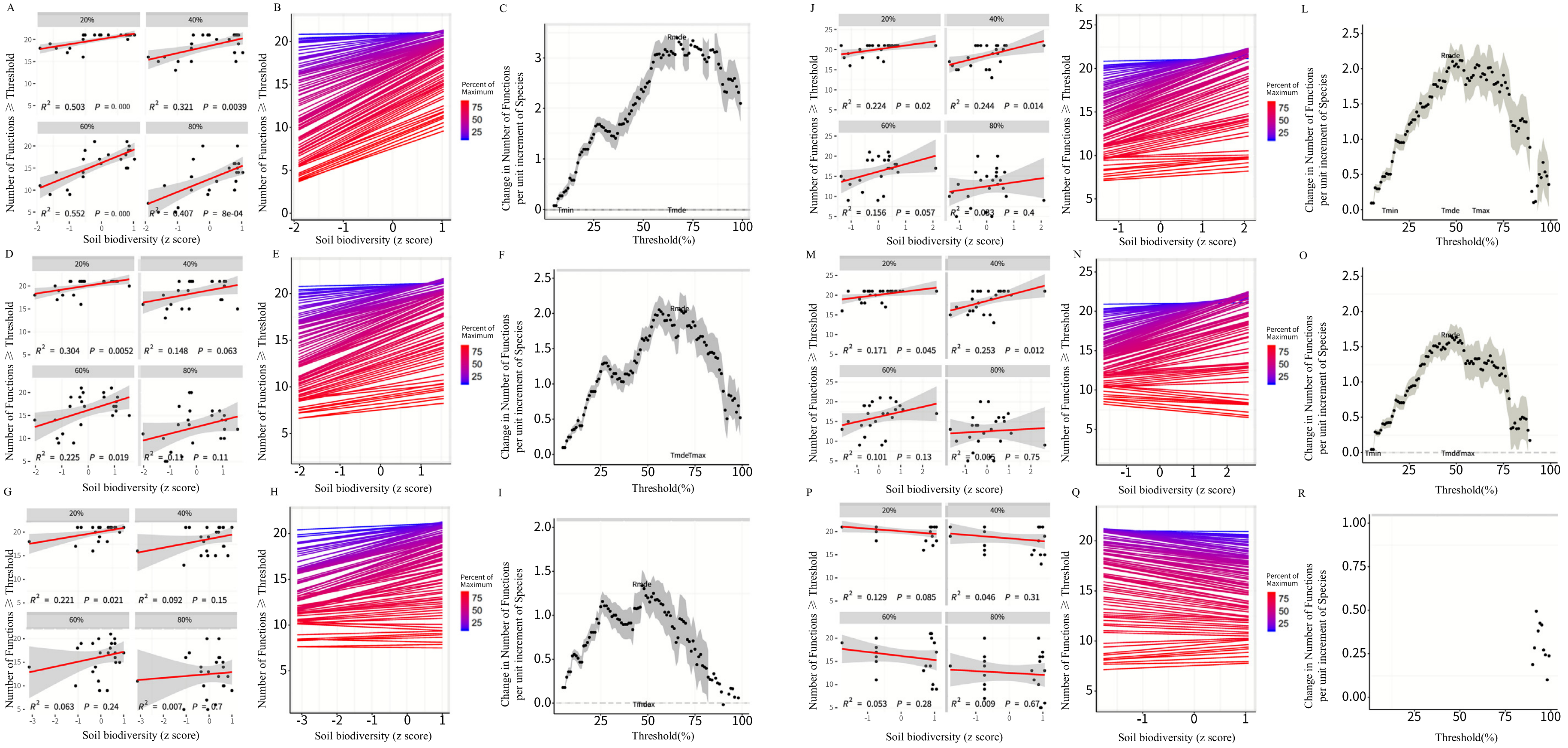

Fig. 6. The relationship between microbial diversity (bacterial dominant taxa (A), CRT(D), ART(G); fungal dominant taxa (J), CRT(M), ART(P)) and ecosystem function, at four different thresholds 20 %, 40 %, 60 %, and 80 % of maximum. The significant linear relationship fitted the OLS regression model was indicated by the red solid line, while the 95 % confidence interval of the fit was represented by gray shaded area. A variety of ecosystem function thresholds are impacted by diversity influences. The impact of bacterial dominant taxa (B), CRT (E), ART (H) and fungal dominant taxa (K), CRT (N), ART (Q) diversity on functions exceeding thresholds. The slope between soil microbial diversity and the number of functions above threshold (ranging from 5 to 99 % of maximum for each function) was represented by lines. The dotted curves illustrated the changes in the number of functions per unit increase of bacterial dominant taxa (C), CRT(F), ART(I) and fungal dominant taxa (L), CRT(O), ART(R) diversity.

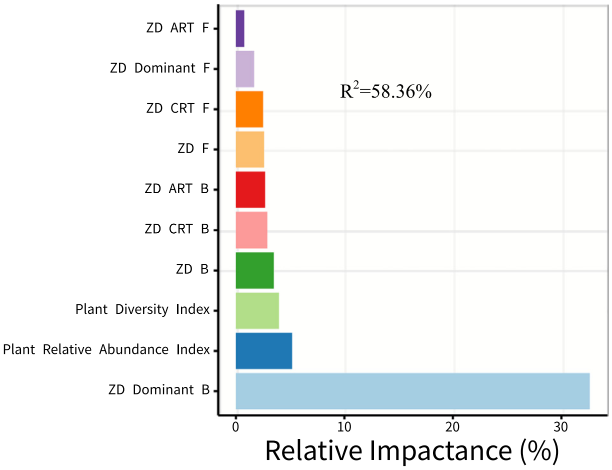

Fig. 7. Calculation of the relative importance of each variable using dominance analysis. The predictor importance (Relative Impactance (%)) of microbial and plant community diversity on soil ecosystem function is depicted in the figure.

4. Discussion

4.1. Maintenance mechanisms of ecosystem function under long-term global warming

The findings of this study support our initial hypothesis that global warming negatively impacted the ecosystem function of the grassland, while no significant effect was observed in the shrubland. Climate warming likely led to a reduction in soil moisture levels at the Qinghai-Tibet Plateau (Wang et al., 2014a), which in turn restricted certain functions associated with the soil biogeochemical cycle. This limitation in soil moisture availability may have resulted in a decline in ecosystem function through the modulation of microbial metabolism (Jing et al., 2015; Xue et al., 2015; Xu et al., 2021). The prolonged period of warming on the Qinghai-Tibet Plateau may have resulted in a substantial decrease in soil microbial diversity as highlighted in a previous study (Chen et al., 2021). Microbial diversity is crucial for the maintenance of ecosystem function, thus the observed reduction in microbial diversity may have contributed to the decline in ecosystem function observed in our study. Alterations in the number of species within a community have been recognized as a significant factor influencing ecosystem function (Cardinale et al., 2011a, 2011b; Cardinale et al., 2012; Tilman et al., 2014; Gamfeldt et al., 2015). Over the past two decades, empirical research has provided compelling evidence of the profound influence of species loss on various ecosystem processes, including primary production and nutrient cycling (Balvanera et al., 2006; Cardinale et al., 2006a, 2006b; Cardinale et al., 2011a, 2011b). Notably, a field experiment conducted across a broad range of sites, encompassing subarctic to tropical regions, consistently demonstrated that reductions in decomposer functional diversity led to decreased rates of litter decomposition and carbon and nitrogen cycling (Whittingham, 2011). Key species, positive interactions, and niche complementarity influence function increase as species richness rises. A well-established positive correlation between species richness and ecological functions has been observed, however, this correlation tends to diminish in highly diverse communities (Cardinale et al., 2006a, 2006b; O'Connor et al., 2017). Specifically, the relationship between species richness and ecological function exhibit a steep incline at low richness levels, but this rate of increase diminishes as richness continues to rise. It is important to note that not all biodiversity and ecosystem function relationships yield positive outcomes (Soliveres et al., 2016b), thus, the positive effects of microbial diversity on ecosystem function can be masked by negative relationships. In such systems, the negative interactions, driven by competition and predation, tend to be more pronounced in diverse communities.

However, it is important to consider that the observed effects may be influenced by the presence of different plant types at the two study sites. Soil microbes are vital for the maintenance of various ecosystem functions, including litter decomposition, nutrient cycling, and primary productivity (Peter et al., 2011; Jing et al., 2015; Delgado-Baquerizo et al., 2016b). The relationship between microbial diversity and ecosystem function has been extensively studied, consistently revealing a positive correlation between enhanced ecosystem function and increased microbial diversity (Jing et al., 2015; Chen et al., 2020b). These functions encompass important processes such as nutrient cycling, climate regulation, litter decomposition, and primary production (Van Der Heijden et al., 2008; Bodelier, 2011; Bardgett and Van Der Putten, 2014; Wagg et al., 2014). Several studies have emphasized the significance of above-ground diversity in preserving various ecosystem functions, highlighting its significance as compared to the role of soil microbes (Jing et al., 2015; Soliveres et al., 2016a). Plant traits, such as productivity and phylogenetic distance, have been identified as key driving factors influencing the recovery of soil microbial diversity under climate warming (Deng et al., 2018; Yang et al., 2019; Liu et al., 2020). These plant traits likely influence litter quality and inputs, as well as abiotic factors, which in turn impact soil microbial diversity (de Vries et al., 2012; Diaz et al., 2016). The impact of warming on ecosystem functions can be mediated through a chain reaction involving the diversities of the plant and microbial communities across multiple trophic levels. In our study, we observed that the diversity of plants and microorganisms in the shrubland rebounded to pre-warming levels after a long period of recovery, whereas the same recovery did not occur in the grassland. The rate of recovery of plant diversity from global warming can vary depending on the underlying mechanisms driving community reassembly (Zhang et al., 2017). The transmission of the impact of plant species diversity on ecosystem function is likely achieved through the alteration of soil microbial diversity.

Abiotic factors, such as soil pH and moisture, can also influence the negative effects of elevated temperature on ecosystems. The discrepancies in soil pH observed between the two ecosystems in our investigation may be attributed to the plant species present, as supported by studies conducted by Scheibe et al. (2015) and Dawud et al. (2017) on spatial variations. There is compelling evidence demonstrating that changes in both soil bacterial diversity and community structure are associated with varying soil pH gradients (Fierer and Jackson, 2006; Lauber et al., 2009; Griffiths et al., 2011; Shen et al., 2013; Liu et al., 2018). Additionally, Griffiths et al. (2011) identified distinct groups of dominant bacteria at threshold pH levels of 6.9 and 5.2 in soil. A recent empirical study conducted in a semi-arid grassland on the Loess Plateau demonstrated that soil acidification inhibited 15 out of 19 individual ecosystem functions (Wei et al., 2022). Overall, the variation in pH among different plant communities may induce diverse responsive changes in ecosystem function to global warming by influencing the composition of the microbial community. Further studies in different ecosystems and environments are necessary to validate the generalizability of our findings. By conducting similar investigations, we can establish the representativeness of our results and gain a comprehensive understanding of the ecology of large-scale biodiversity loss. It is crucial to recognize the significance of both above-ground and below-ground biotic components to obtain a holistic understanding of ecosystem dynamics and functioning.

While few studies have specifically examined the roles of below-ground and above-ground ecosystem components separately, existing research has revealed that their responses to disturbance and their impacts on ecosystem functions can vary (Allan et al., 2014; Balvanera et al., 2014; Jing et al., 2015). These findings suggest that the diversity of microbes in soil is positively associated with ecosystem function at higher levels, whereas the diversity of above-ground plant communities may not directly influence ecosystem function. However, other studies have emphasized the importance of maintaining ecosystem versatility, which is dependent on above-ground diversity (Jing et al., 2015; Soliveres et al., 2016a). In our study, we found that soil microorganisms made a greater contribution to ecosystem functions compared to the above-ground plant community. This difference in contribution can be attributed to the specific ecosystem functions that were investigated, which included many soil properties. Similar studies have also considered functions related to soil microbial activities, such as nutrient cycling (Jing et al., 2015; Delgado-Baquerizo et al., 2016a, 2016b; Soliveres et al., 2016b). Interestingly, we observe that plant diversity did not have a direct impact on ecosystem functioning through cascade effects on microorganisms. Instead, the combined diversity of the above-ground and below-ground components significantly influenced the variation in ecosystem function, primarily through the indirect effects of plants on the below-ground microbial community. It is important to note that conducting similar studies in other ecosystems and environments will provide further insights and validate the representativeness of our results. Irrespective of the underlying mechanisms involved, the contrasting relationships observed between ecosystem functioning and the above-ground and below-ground biotic components highlight the importance of considering both components to gain a comprehensive understanding of the ecology of biodiversity loss across a wide range of ecosystems.

4.2. Ecosystem function response to bacterial and fungal community diversity

In terrestrial ecosystems, the presence of diverse bacteria in the soil exerts a significant impact on ecosystem function, particularly affecting specific ecological processes such as nutrient cycling and decomposition. Numerous studies have demonstrated the pivotal role of soil bacteria in these processes (Van Der Heijden et al., 2008; Wagg et al., 2014; Delgado-Baquerizo et al., 2016a, 2016b; Wang et al., 2019b; Yuan et al., 2020; Delgado-Baquerizo et al., 2020). The involvement of soil bacteria in nitrogen and carbon cycling and ammonium oxidation and nitrogen fixation has been proposed as a potential explanation for their impact on ecosystem function (Moore and de Ruiter, 1991; Eaton et al., 2012; Pajares and Bohannan, 2016). This phenomenon can be attributed to the metabolic versatility of bacteria which encompass various modes of nutrition such as photoautotrophy, chemoautotrophy, and heterotrophy, enabling them to play a dominant role in regulating ecosystem function (Schmidt et al., 2014; Wang et al., 2019a). After disturbances, there is an increased demand for nitrogen in both above-ground and below-ground ecosystems. This leads to an abundance of bacteria that are capable of carrying out important processes such as ammonium oxidation and nitrogen fixation (Nasto et al., 2014). As a result, bacteria play a key role in regulating the stability and resilience of ecosystem functions. Compared with bacteria, fungi generally exhibit higher tolerance to infertile soil conditions (Austin et al., 2004). Consequently, the large effect of superior fungal diversity on ecosystem function may not be as pronounced in soils with optimal conditions (such as during the growing season in our study).

4.3. Functional role of abundant versus rare taxa diversity

Our findings indicate that the diversity of bacterial dominant taxa and bacterial CRT exhibited a significant and positive correlation with ecosystem function, suggesting their vital role in driving ecosystem processes. This contrasts with recent studies that have highlighted the importance of rare microbial taxa in supporting ecosystem function. In reality, natural ecosystems consist of species with varying abundances, fitness levels, and niche utilization. The interactions between abundant and rare taxa can lead to distinct functional characteristics and response mechanisms. However, the numerical advantage of abundant taxa may enhance their contribution to ecosystem function. Recent research has also demonstrated that rare microbial taxa exhibit higher metabolic activity compared to their abundant counterparts, further emphasizing the complexity of microbial dynamics in ecosystem functioning (Lynch and Neufeld, 2015; Xue et al., 2018). Small groups of microorganisms often possess specialized functions related to nitrogen metabolism, sulfur metabolism, and the biodegradation and metabolism of xenobiotics. These functional capacities are not taxonomically redundant, meaning their response to environmental change depends heavily on the specific taxa performing these functions. Studies have shown that specific taxa are responsible for carrying out these specialized functions, and their loss or decline can result in the loss of these narrow processes, such as the degradation of specific substrates (Singh et al., 2014; Delgado-Baquerizo et al., 2016a; Xun et al., 2019). In contrast, broad processes such as overall respiration, metabolic potential, and cell yield are carried out by a wider array of taxa and are therefore more resistant to loss (Schimel and Gulledge, 1998; Rivett and Bell, 2018).

Additionally, because of the many metabolically active lineages (Logares et al., 2014), some microbial rare taxa serve as seed banks, functioning as a source of insurance during global warming (Yachi and Loreau, 1999). However, the rare taxa members may by a persistent functional pool by acting as an active microbial seed bank with high biodiversity, which to some extent represent a substantial amount of ecological potential (Pedros-Alio, 2012; Lynch and Neufeld, 2015). Their contribution to community function would remain relatively small because the biomass of rare taxa is negligible in comparison to the abundant taxa among members microbial community (Pedros-Alio, 2012). Because the diversity of functional gene is a key indicator for functional redundancy, abundance of gene may represent the capacity of function (Xun et al., 2019). Ignoring the shifts in taxa that have the potential of successful transformation from rare to abundant species may underestimate the important role of CRT in structuring microbial communities and affecting community functions over time and inadvertently overrate the role of ART when evaluating the community in a specific environment. Our findings suggest that the diversity of ART has a greater impact on ecosystem function than bacterial CRT taxa. This conclusion differs from previous assumptions that rare taxa play a disproportionate role in community function. However, it should be noted that rare taxa have the potential to transform into dominant taxa, which can promote ecosystem function and contribute to stability during climate changes. Thus, ignoring shifts in taxa from rare to abundant species may underestimate the role of CRT in structuring microbial communities and overrate the role of ART when evaluating communities in specific environments.

5. Conclusions

In this study, we developed an integrated framework to investigate the combined effects of below-ground and above-ground plant biodiversity on ecosystem function in alpine meadow and shrub ecosystems. These ecosystems were exposed to simulated warming for a period of 21 years on the Qinghai-Tibet Plateau. Our findings indicate that long-term warming led to a decline in ecosystem function in the grassland. Specifically, the warming treatment resulted in a reduction in the diversity of bacterial dominant taxa and CRT, which was the primary factor driving the decline in ecosystem function. The significant contribution of bacterial dominant taxa and CRT to ecosystem function highlights their importance in maintaining specific ecological processes. Functions carried out by these taxa may be lost if their abundance decreases or if they become extinct. Therefore, it is crucial to understand the attributes and responses of these influential taxa to predict the impacts of biodiversity loss on ecosystem service provision under future climate change scenarios. Furthermore, it is necessary to extend our investigations to other ecosystems and environments to gain a broader understanding of the attributes and responses of dominant taxa and CRT. By doing so, we can improve our predictions of how biodiversity loss will affect ecosystem function and the provision of ecosystem services in the face of climate change.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.164722.

CRediT authorship contribution statement

Wenjing Chen: Conceptualization, Methodology, Software, Investigation, Formal analysis, Data curation, Writing – original draft. **Huakun Zhou:** Data curation, Writing – original draft, Resources, Supervision. **Yang Wu:** Visualization, Investigation. **Jie Wang:** Visualization, Investigation. **Ziwen Zhao:** Visualization, Investigation. **Yuanze Li:** Software, Validation. **Leilei Qiao:** Software, Validation. **Kelu Chen:** Software, Validation. **Guobin Liu:** Visualization, Writing – review & editing. **Coen Ritsema:**

Visualization, Writing – review & editing. **Violette Geissen:** Visualization, Writing – review & editing. **Xinrong Guo:** Visualization, Writing – review & editing. **Sha Xue:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

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 might have influenced the work reported in this paper.

Acknowledgements

Funding

This work was financially supported by the National Natural Science Foundation of China Joint Fund Project, Grant/Award Numbers: U21A20186; Natural Science Foundation of Qinghai Province Innovation Team Project, Grant/Award Number: 2021-ZJ-902; Second Qinghai[1]Tibet Plateau Comprehensive Scientific Research Project, Grant/Award Number: 2019QZKK0302-02; the Science Fund for Distinguished Young Scholars of Shaanxi Province, China (2021JC-50); the Shaanxi Creative Talents Promotion Plan-Technological Innovation Team (2023-CX-TD-37).

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