Response of Soil Microbial Communities to Warming and Clipping in Alpine Meadows in Northern Tibet

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Abstract: In order to explore responses of soil microbial communities among different alpine meadows under warming and clipping, soil microorganisms of three alpine meadow sites (low altitude: 4313 m, alpine steppe meadow, 30°30′ N, 91°04′ E; mid-altitude: 4513 m, alpine steppe meadow, 30°31′ N, 91°04′ E; and high altitude: 4693, alpine Kobresia meadow, 30°32′ N, 91°03′ E) were measured using the phospholipid fatty acid (PLFA) method. Both warming and clipping significantly reduced PLFA content and changed the community composition of soil microbial taxa, which belong to bacterial and fungal communities in the alpine Kobresia meadow. Warming significantly reduced the soil total PLFA content by 36.1% and the content of soil fungi by 37.0%; the clipping significantly reduced the soil total PLFA content by 57.4%, the content of soil fungi by 49.9%, and the content of soil bacteria by 60.5% in the alpine Kobresia meadow. Only clipping changed the total fungal community composition at a low altitude. Neither clipping nor warming changed the microbial community composition at a moderate altitude. Soil temperature, soil moisture, and pH were the main factors affecting soil microbial communities. Therefore, the effects of warming and clipping on soil microbial communities in alpine meadows were related to grassland types and soil environmental conditions.

Keywords: soil microbes; alpine meadow; warming; clipping; phospholipid fatty acid method (PLFA); community composition

1. Introduction

Microbes, as decomposers of ecosystems, participate in the carbon, nitrogen, and phosphorus cycle and energy flow in various types of ecosystems around the world, which are important drivers of biogeochemical cycles [1,2]. As an important part of the soil system, soil microorganisms play an important role in the element balance of terrestrial ecosystems by regulating the decomposition of soil organic matter, plant litter, and the availability of plant nutrients [3]. The grassland ecosystem is one of the most common types of terrestrial ecosystems, and grazing is an important usage of land [4,5]. The composition of soil microbial communities in grassland ecosystems can reflect not only the changes in soil ecological environment under the influences of grazing and human activities but also the changes in the composition and function of the grassland ecosystem. The composition and function of the grassland ecosystem are often affected by human activities and climate change [6–9]. Therefore, studying the change mechanism of soil microbial communities under experimental warming, grazing, and their interaction can provide a theoretical basis for grazing management of grassland ecosystems and the construction of a multi-functional ecological security barrier [10,11].
To date, through controlled experiments, previous studies have been carried out on the effects of climate warming on the composition of soil microbial communities in ecosystems such as *Stipa* grassland, tundra, and forest. The effects of experimental warming on the soil microbial community composition varied between being inconsistent, showing no changes [12,13] and showing significant changes [3,14]. Previous studies examining the effects of grazing on the composition of soil microbial communities were carried out in the *Stipa* grassland and *Leymus chinensis* grassland. In one situation, the effects of grazing on the composition of a soil microbial community caused a significant change [15], while in another, no change was observed [3,16]. These inconsistent results of previous studies may be related to their different ecosystem types, community composition, and climatic conditions.

The Qinghai-Tibet Plateau is an ideal area to study the impact mechanism of global change on the ecosystem. The Qinghai-Tibet Plateau, as the highest altitude region in the world, with an average elevation of more than 4000 m, is known as the “third pole” of the world [17]. It is one of the most sensitive areas to global climate change and is the regulator and opener of the northern hemisphere climate [18]. Alpine meadow is an important grassland type on the Tibetan Plateau, which is showing an increase of 0.4 °C every ten years, twice the rate of global warming [19]. To date, some experimental studies have been carried out on the composition of soil microbial communities in the alpine meadow area of the Qinghai-Tibet Plateau [11,13]. Nonetheless, previous studies have mainly focused on the same type of alpine meadow. Therefore, there is a need for further exploration regarding whether soil microbial communities of various dominant species in different types of alpine meadows, as well as those in the same type of alpine meadow but under different environmental conditions, respond differently to warming and clipping. We hypothesized that the response of soil microbial communities of different alpine meadows to warming and clipping would be different. In order to test our hypothesis, this study deployed three experimental warming and clipping experimental platforms (two alpine steppe meadows and one alpine *Kobresia* meadow) at the grassland station in Damxung County in the Tibet Autonomous Region. The main objective of this study was to explore the response mechanism of soil microbial community compositions to the experimental warming and clipping treatment in alpine meadows in northern Tibet. It is expected to provide basic research data and a theoretical basis for how to maintain the soil quality and stability of soil microbial communities in the alpine meadows of northern Tibet in the context of global change.

2. Materials and Methods

2.1. Experiment Design

The research plots are located at the grassland station in Damxung County in the Tibet Autonomous Region (30°30′–30°32′ N, 91°03′–91°04′ E). In July 2008, three representative plots of approximately 20 m × 20 m were established along the elevation gradient (4313–4693 m) on the southern slope of the Nyainqentanglha Mountain. In detail, the three 20 m × 20 m plots were located at 4313 m, 30°30′ N, 91°04′ E; 4513 m, 30°31′ N, 91°04′ E; and 4693 m, 30°32′ N, 91°03′ E, respectively [20]. The 4313 m and 4513 m sites were alpine steppe meadows, and the dominant species were *Stipa capillacea*, *Carex montis-everestii*, and *Kobresia pygmaea*, while the 4693 m site was an alpine *Kobresia* meadow, and the dominant species was *Kobresia pygmaea* [21]. Six open-top chambers (OTCs) devices, which were used to simulate the temperature increase, were set up with the design of completely randomized in each fence plot, and a fixed paired square was established for each OTC. The specifications of OTCs in this study were a 1.45-m bottom diameter, a 1.00-m opening diameter, and a height of 0.40 m. The material was 3-mm thick polycarbonate, which has the ability of transmittance of visible light and ultraviolet light of about 90%, while the near-infrared light transmittance is less than 5%. The experimental warming increased the growing season average soil temperature (Ts) by 1.23, 1.33, and 1.24 °C at the low, middle, and high altitudes in 2017, respectively [22]. In June 2009, three OTCs quadrats and three corresponding paired quadrats were selected as clipping treatment quadrats in each fenced plot according to the random zone group design, respectively, and clipping was
carried out. The aboveground part of the plant was mowed three times (June, July, September) each year during the growing season, with a stubble of 1 cm on the ground. In this study, four treatments were set in each plot (no warming and no clipping, C; warming and no clipping, W; clipping and no warming, CL; warming and clipping, W + CL). Each treatment had three replicates. The four treatments of C, W, CL, and W + CL at each altitude constituted a complete two-factor (warming and clipping) two-level factorial experimental design.

2.2. Soil Sampling and Analysis

In August 2013, surface soil samples (0–10 cm) were collected using a soil auger with a diameter of 3.7 cm. Three soil samples were taken from each quadrat and uniformly mixed to form a soil sample, which was immediately stored in an icebox, brought back to the laboratory, and stored in a refrigerator at −20 °C. A sieve with a hole diameter of 1 mm was used to screen the soil and pick out roots that were visible. Fresh soil samples were used for the determination of soil nitrate nitrogen (NO$_3^-$-N) and ammonium nitrogen (NH$_4^+$-N). The NO$_3^-$-N and NH$_4^+$-N were measured using a flow analyzer (LACHAT Quickchem Automated Ion Analyzer, LACHAT instrument, Milwaukee, USA). Some soil samples were air-dried to determine soil organic carbon (SOC) and total nitrogen (TN). Soil organic carbon was determined by the external heating of potassium dichromate [23]; The TN was determined by a carbon-nitrogen analyzer (vario MAX CN, Elementar Analysensysteme GmbH, Hanau, Germany). The pH of the soil was measured with a pH meter [24].

Phospholipid fatty acid method (PLFA) is a constant component of the membrane of living microorganisms, and the PLFA quantity of specific microbiota can reflect the in-situ soil fungi, bacterial biomass, and community composition [25]. The PLFA-based method has several advantages, being both reproducible and sensitive [26]. However, changes in environmental conditions and cell activity may affect the composition of cell membranes, and we currently have no way to distinguish between changing membrane composition and changing species composition [27]. Compared with the nucleic acid method, the PLFA method is more sensitive in detecting changes in microbial community composition [28], which also is an effective method to quickly determine whether soil fungi or bacteria are affected by treatment [26]. Some fresh soil samples were taken for the determination of the soil microbial community, and the PLFA method was adopted [29]. Soil microbial fatty acid extraction method was used with reference to Bossio and Kong [30,31]. The detailed steps for the separation and extraction of soil microbial fatty acids were detailed in the study of Fu et al. [11]. The samples were used for qualitative and quantitative analysis of PLFA by a gas chromatography-mass spectrometer [30]. Fatty acids were named using Frostegard’s method [32]. Specific soil microbial phospholipid fatty acid markers are shown in Table 1 [26,29,33–35].

| Microbial Type                          | PLFA Markers                                      |
|----------------------------------------|---------------------------------------------------|
| Fungi                                  | 16:1ω5c, 18:1ω9c, 18:2ω6c                        |
|                                        | 14:0, i14:0, i15:0, a15:0, i16:0, a16:0, 10Me16:0, |
|                                        | 16:1ω7c, 17:0, 10Me17:0, i17:0, a17:0, cy17:0ω7c, |
|                                        | 17:1ω8c, 10Me18:0, 18:1ω7c and cy19:0ω7c          |
| Bacteria                               | i14:0, i15:0, a15:0, i16:0, a16:0, i17:0, a17:0   |
| Gram-positive bacteria (G⁺)            | 16:1ω7c, cy17:0ω7c, 17:1ω8c, 18:1ω7c and cy19:0ω7c|
| Gram-negative bacteria (G⁻)            | 10Me16:0, 10Me17:0 and 10Me18:0                   |
| Actinomycetes                          |                                                  |
| Arbuscular mycorrhizal fungi (AMF)     | 16:1ω5c                                          |
| Others                                 | 6:0, 18:0, 22:0, 24:0                             |

Table 1. The phospholipid fatty acid (PLFA) markers for soil microbial communities.
2.3. Statistical Analysis and Plotting

The indices of soil environmental factors, soil microbial biomass, and its community composition were analyzed by the two-way analysis of variance (ANOVA). The soil environmental factors in the three experimental plots were analyzed by the principal components analysis (PCA). The principal coordinate analysis (PCoA) was used to rank the 21 markers of soil PLFA, and the two-way ANOVA was performed on the first two axes to determine the changes in the composition of soil microbial community. The adonis multivariate analysis of variance was used to test whether there were significant differences between groups under different treatments. The variance partition analysis (VPA) and the mantel tests were used to explore the relationship between soil microorganisms and environmental factors. Correlation analysis was used to test relevant relationships between soil PLFA content and environmental factors. All statistical analyses were performed by the R 3.5.2; mapping software involved the R 3.5.2 and the Sigmaplot 12.5.

3. Results

3.1. Impact of Warming and Clipping on Environmental Factors

Warming significantly reduced soil moisture (SM) in the three experimental sites and significantly increased $T_s$ in the three experimental sites but had no significant effect on TN, SOC, C: N (the ratio of SOC and TN), NO$_3^-$-N, NH$_4^+$-N, and pH. Clipping significantly reduced the content of NO$_3^-$-N in the low altitude plot but had no significant effect on the other soil indices of the three plots. The interaction between warming and clipping only had a significant effect on TN and SOC in the moderate altitude plot (Figure A1, Table 2).

Table 2. The two-way analysis of variance of three sites’ environmental factors under warming and clipping treatment.

| Variables | Low   | Mid   | High  |
|-----------|-------|-------|-------|
|           | W     | CL    | W × CL| W     | CL    | W × CL|
| TN        | 0.11  | 0.1   | 2.27  | 2.94  | 2.36  | 9.61 * |
| SOC       | 0.64  | 0.82  | 0.01  | 3.21  | 0.21  | 5.68 * |
|          |       |       |       |       |       | 1.18  | 0.24  | 0.03  |
| SOC/TN    | 1.07  | 4.63  | 3.05  | 2.44  | 1.27  | 0.98  | 0.17  | 0.33  | 2.71  |
| NH$_4^+$-N| 0.64  | 0.33  | 2.83  | 0.42  | 0.72  | 0.01  | 0.02  | 0.91  | 1.29  |
| NO$_3^-$-N| 1.63  | 0.03  | 0.75  | 0.73  | 10.73 *| 0.33  | 0.21  | 2.95  | 0.07  |
| pH        | 0.07  | 1.49  | 0.84  | 1.16  | 0.17  | 2.86  | 0.14  | 1.67  | 1.67  |
| $T_s$     | 15.42 **| 0.22 | 0.02  | 35.61 ***| 0.10  | 0.00  | 70.24 ***| 0.05  | 0.02  |
| SM        | 30.46 ***| 0.67 | 0.07  | 27.66 ***| 2.51  | 0.01  | 16.29 **| 0.02  | 0.00  |

Note: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. TN: Total nitrogen, SOC: soil organic carbon, SOC/TN: the ratio of SOC to TN, NH$_4^+$-N: ammonium nitrogen, NO$_3^-$-N: nitrate nitrogen, $T_s$: soil temperature, and SM: soil moisture.

Three-way ANOVA was performed on soil environmental factors with the three variables of plots, warming, and clipping. There were significant differences between the three plots. It can be seen from the PCA analysis that compared with the two alpine steppe meadows, the alpine Kobresia meadow had higher SM, SOC, TN, C: N, and NH$_4^+$-N, while NO$_3^-$-N, $T_s$, and pH were lower (Figure A2).

3.2. Effect of Warming and Clipping on Soil Microbial Biomass and Community Composition

Warming significantly reduced the contents of total PLFA, fungi, actinomyces, and AMF in the soil of the alpine Kobresia meadow, but had no significant effect on the soil microbial biomass of the two alpine steppe meadows. The contents of soil total PLFA, fungi, bacteria, G$^-$, actinomyces, and AMF in the alpine meadow were significantly reduced by clipping. The interaction between warming and clipping only had a significant effect on the content of soil fungi in the low altitude plot and the content of G$^+$ in the moderate altitude plot (Figure A3, Table 3).
### Table 3. The two-way analysis of variance of soil microbial content under warming and clipping in three alpine meadow sites.

| Taxa          | Low       | Mid       | High       | Low × CL  | Mid × CL | High × CL |
|---------------|-----------|-----------|------------|-----------|-----------|-----------|
|               | W CL W CL | W CL W CL | W CL W CL | W CL W CL | W CL W CL | W CL W CL |
| PLFA          | 0.36      | 2.37      | 1.91       | 0.14      | 4.79      | 5.52      |
| Fungi         | 0.39      | 4.91      | 6.66 *     | 0.17      | 2.32      | 9.30 *    |
| Bacteria      | 0.45      | 2.18      | 1.48       | 0.18      | 4.99      | 3.27      |
| G⁺            | 0.63      | 0.82      | 0.90       | 0.59      | 1.71      | 5.34 *    |
| G⁻            | 0.54      | 3.55      | 1.53       | 0.01      | 0.79      | 4.32      |
| Actinomycetes | 0.01      | 2.75      | 2.36       | 0.20      | 2.23      | 3.66      |
| AMF           | 0.01      | 1.31      | 1.58       | 1.55      | 0.40      | 0.05      |
| F/B           | 0.05      | 0.74      | 5.07       | 0.59      | 1.02      | 1.45      |
| G⁺/G⁻         | 0.37      | 0.63      | 2.10       | 0.15      | 3.23      | 0.40      |

Note: * p < 0.05, ** p < 0.01, *** p < 0.001.

The PCoA ranking analysis in Figure 1 showed that the contribution rates of the first two axes were 37.97% and 26.51%, respectively; four different experimental treatments of the alpine *Kobresia* meadow were clearly separated in the ranking diagram.

![Figure 1](image_url)

**Figure 1.** The PCoA of soil PLFA based on the Bray–Curtis distance. C: control; W: only warming; CL: only clipping; W + CL: warming and clipping.

The adonis analysis of soil microbial community influenced by warming, clipping, and their interaction is shown in Table A2. Warming changed the composition of G⁻, actinomycete, and fungi communities at the high altitude plot. Clipping changed the composition of soil fungi communities at the low altitude plot and the composition of soil bacteria, actinomycetes, G⁺, G⁻, and fungi communities at the high altitude plot. Clipping and warming had interactions on the composition of soil fungi communities at the low altitude plot, soil bacteria, G⁺, and G⁻ communities at the moderate altitude plot (Table A2).

#### 3.3. Relationship between Soil Microbial Community and Environmental Factors

The contents of total PLFA, fungi, bacteria, G⁺, G⁻, and actinomycetes were positively related to NH₄⁺-N (Table A1). Mantel test showed that the composition of soil bacteria was correlated...
with pH at $p < 0.05$ and $T_s$ at $p < 0.10$. The composition of soil fungi was correlated with pH at $p < 0.05$ and with NO$_3$-$\text{N}$ at $p < 0.10$. The G$^+$/G$^-$ was correlated with soil pH at $p < 0.10$ (Figure 2a, Table A3). The composition of G$^+$ was correlated with soil TN and pH at $p < 0.10$. The composition of actinomycetes was correlated with pH at $p < 0.05$ and with NO$_3$-$\text{N}$ and SM at $p < 0.10$. The content of AMF was correlated with NH$_4^+$-$\text{N}$ at $p < 0.10$ (Figure 2b, Table A3).

The $T_s$ alone explained 14.6% variance of the soil total PLFA community, and interaction with other factors explained 10.9% variation of the soil total PLFA community. That is, $T_s$ combined explained 25.5% variation of the soil total PLFA community. The SM and pH explained 12.4% variance of the soil total PLFA community without interaction with other factors; interacting with other factors explained 9.0% variation of the soil total PLFA community. That is, the SM and pH combined explained 21.4% variance of the soil total PLFA community. The soil available nitrogen content (NH$_4^+$-$\text{N}$ and NO$_3$-$\text{N}$) alone explained 3.8% variance of the total PLFA community; interacting with other factors explained 10.6% variation of the soil total PLFA community. That is, the soil available nitrogen explained 14.4% variation of the soil total PLFA community. Soil carbon and nitrogen (TN, SOC, C: N) and other factors interacted to explain 3.5% variance of the PLFA community (Figure 3a). The $T_s$ alone explained 13.7% variance of the soil bacterial community, and interaction with other factors explained 10.6% variation of the soil bacterial community. That is, the $T_s$ combined explained 24.3% variation of the soil bacterial community. The SM and pH explained 10.2% variance of the soil bacterial community without interaction with other factors; interacting with other factors explained 8.8% variation of the

**Figure 2.** Relationship between the composition/content of soil microbial community and environmental factors. (a): Relationship between the composition/content of soil bacteria, fungi, F/B, and G$^+$/G$^-$ communities and environmental factors. (b): Relationship between the composition/content of soil G$^+$, G$^-$, actinomycetes, and AMF communities and environmental factors. Note: SM: soil moisture, $T_s$: soil temperature, TN: Total nitrogen, NH$_4^+$-$\text{N}$: ammonium nitrogen, NO$_3$-$\text{N}$: nitrate nitrogen, SOC: soil organic carbon, and C: N: the ratio of SOC to TN. PLFA: phospholipid fatty acid; G$^+$: gram-positive bacteria; G$^-$: gram-negative bacteria; AMF: arbuscular mycorrhizal fungi.
soil bacterial community. That is, SM and pH combined explained 19.0% variation. The soil available nitrogen content (NH$_4^+$-N and NO$_3^-$-N) alone explained 3.3% variance of the soil bacterial community; interacting with other factors explained 10.3% variation of the soil bacterial community. That is, the total soil available nitrogen content explained 13.6% variation of the soil bacterial community. Soil carbon and nitrogen (TN, SOC, C: N) and other factors interacted to explain 3.7% variance of the bacterial community (Figure 3b). The $T_s$ alone explained 26.7% variance, and interaction with other factors explained 28.4% of the variation of the soil bacterial community. That is, the $T_s$ combined explained 55.1% of the variation of the soil bacterial community. The SM and pH explained 34.1% variance of the soil fungi community without interaction with other factors; interacting with other factors explained 27.9% variation of the soil fungi community. That is, the SM and pH combined explained 62.0% variation of the soil fungi community. The soil available nitrogen content (NH$_4^+$-N and NO$_3^-$-N) alone explained 13.4% variance of the soil fungi community; interacting with other factors explained 27.2% variation of the soil fungi community. That is, the total soil available nitrogen content explained 40.6% variation of the soil fungi community. Soil carbon and nitrogen (TN, SOC, C: N) alone explained 4.1% variance of the soil fungi community, and interaction with other factors explained 16.2% variation of the soil fungi community. That is, the $T_s$ combined explained 17.3% variation of the soil fungi community (Figure 3c).

![Figure 3](image_url)

Figure 3. Variance partition analysis of the relative effects of different environmental variables on soil (a) microbial PLFA, (b) bacteria, and (c) fungi. Notes: SM: soil moisture, $T_s$: soil temperature, NH$_4^+$-N: ammonium nitrogen, NO$_3^-$-N: nitrate nitrogen, CN: soil organic carbon, total nitrogen, and the ratio of SOC to TN. C: control; W: only warming; CL: only clipping; W + CL: warming and clipping.

4. Discussion

Although neither warming nor clipping changed the soil $G^+/G^-$ and $F/B$ of the alpine meadow, clipping changed the composition of soil microbial community at the low and high altitude plots and the soil bacteria at high altitudes at $p < 0.10$. The interaction of warming and clipping changed the composition of soil bacteria at the moderate altitude plot, which was consistent with the previous research results [36]. For example, both Wang et al. and Ford et al. found that warming and clipping did not change the $F/B$ value, but the composition of soil microbial communities changed [36,37]. Furthermore, Wang et al. found that warming and clipping significantly reduced the value of $F/B$, while the composition of soil microbial communities did not change in alpine meadows in the Hongyuan County [38]. Therefore, in the context of global change, changes in the composition of soil microbial communities cannot be inferred just by changes in $G^+/G^-$ and $F/B$. 


The composition of soil bacteria and fungi was closely related to soil pH (Figure 2), which was consistent with previous research results [39]. Soil pH can directly affect the composition of soil microbial community by affecting the enzyme activity, metabolic function, and the ability to absorb nutrients of soil microorganisms [40,41], as well as indirectly by affecting the plant community [42].

The content of soil PLFA was significantly reduced by warming and clipping. The negative effect of warming may be related to the decrease in soil moisture (Figures 3 and A4). The inhibitory effect of soil drought on microorganisms was greater than the promoting effect of soil temperature on microorganisms, which was consistent with the previous research results [36]. Soil moisture is an important factor affecting soil biological activity [43]. Soil moisture can limit the transfer of soil nutrients in the soil and inhibit the metabolism of microorganisms. The decrease in soil moisture may lead to a reduction in microbial biomass [44]. The negative effect of clipping on the microbial community of alpine meadow may be due to the decrease in soil quality and the destruction of nutrient balance caused by clipping [45,46]. Sorensen et al. believed that clipping would reduce the conversion rate of soil nitrogen, and the utilization efficiency of biological nitrogen would have a negative effect [47].

Compared with warming, clipping had a greater impact on the microbial biomass and community composition of bacteria (Tables 3 and A2). This is probably related to the following reasons. (1) Our previous study found that clipping had a greater impact on the productivity of alpine meadow communities in the Northern Tibet than warming [48]. Community productivity is closely related to the composition of soil microbial communities [49–51]. (2) Compared with warming, clipping has a more materiality influence on the composition of plant communities in alpine meadows [52]. The composition of the plant community can change the quality and quantity of litters, thus causing changes in the microbial population and community composition [53]. The reduction of litter mass caused by clipping is an important reason for the decrease in microbial quantity. (3) Clipping will reduce plant leaf area and photosynthesis [54]. Plants meet the demand for nutrients and energy of plants by increasing the absorption of nutrients from the underground part of the soil, thus increasing the competition between plants and microorganisms. (4) Compared with warming, clipping may have a greater impact on soil NO$_3^-$-N. Soil NO$_3^-$-N is an important factor affecting bacterial biomass and community composition [55–57].

This study found that soil microbial communities of different types of meadows have different responses to warming and clipping, which was consistent with previous studies [16,58]. Compared with alpine meadows, the soil microbial biomass and community composition of typical $Kobresia$ meadows in the alpine region may be more sensitive to short-term (<6 years) warming and mowing. This may be due to the following reasons. Firstly, the climate conditions of the two types of meadows were different. Compared with the alpine steppe meadow, the alpine meadow was located at a higher altitude, with lower air temperature and soil temperature [59]. Joseph et al. [60] found that microbial communities were more susceptible to interference from external environmental factors and more sensitive to climate change at lower temperatures. Fu et al. found that the effect of simulated warming on soil PLFA content and G$^{-}$ content increased with the increase of altitude [58]. Zhang et al. also found that the positive effects of warming on soil microbial biomass carbon and nitrogen decreased with increasing mean annual temperature [61]. Secondly, warming had a stronger promoting effect on soil respiration in the alpine $Kobresia$ meadow, and the temperature sensitivity of soil respiration increases with the elevation [62]. The increase of soil respiration reduced the available substrates of soil [63], and the decrease of soil nutrients may increase the competition among microorganisms. Thirdly, compared with the alpine $Kobresia$ meadow, the composition of soil bacteria community, fungal community composition, and species alpha diversity of alpine steppe meadow based on high-throughput sequencing technology may have a more intense response to experimental warming (>7 years) [20]. Short-term simulated warming (<2 years) had no significant effect on the soil microbial carbon and the nitrogen content and carbon-nitrogen ratio of the alpine meadow and alpine $Kobresia$ meadow [64]. Therefore, the response of soil microorganisms to experimental warming in the alpine
*Kobresia* meadow and alpine steppe meadow may also be related to the duration of the experimental warming, the adopted soil microbial indices, and the observation techniques. Fourthly, the alpine steppe meadow and alpine *Kobresia* meadow had different sensitivity to grazing. Grazing significantly reduced the soil microbial biomass of the alpine *Kobresia* meadow but had no significant effect on the soil microbial biomass of the alpine steppe meadow [11,59].

5. Conclusions

The biomass and composition of soil microbial communities in different grassland types respond differently to warming and clipping. The alpine *Kobresia* meadow in the high altitude was more sensitive to the effect of warming and clipping. Soil temperature, soil moisture, and pH can better explain the changes in the biomass and composition of microbial communities. Furthermore, clipping had stronger negative effects on soil bacterial communities. Therefore, in the context of global changes, the management and utilization of alpine meadows in the Northern Tibetan should adopt appropriate measures according to different meadow types and environmental conditions.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

![Figure A1](image-url)

*Figure A1*. Warming and clipping effects on (a) SM, (b) Ts, (c) pH, (d) TN, (e) NH$_4^+$-N, (f) NO$_3^-$-N, (g) SOC and (h) C/N. Note: SM: soil moisture, $T_s$: soil temperature, TN: Total nitrogen, NH$_4^+$-N: ammonium nitrogen, NO$_3^-$-N: nitrate nitrogen, SOC: soil organic carbon and C/N: the ratio of SOC to TN. C: control; W: only warming; CL: only mowing; W + CL: warming and mowing. Error bars represent standard errors ($n = 3$).
Figure A2. PCA analysis of soil environmental factors.

Figure A3. Warming and clipping effects on (a) PLFA, (b) fungi, (c) bacteria, (d) G⁺, (e) G⁻, (f) actinomycetes, (g) AMF, (h) the ratio of bacteria and fungi and (i) the ratio of G⁺ and G⁻. Note: PLFA: phospholipid fatty acid; G⁺: gram-positive bacteria; G⁻: gram-negative bacteria; AMF: arbuscular mycorrhizal fungi; C: control; W: only warming; CL: only mowing; W + CL: warming and mowing. Error bars represent standard errors (n = 3).
Figure A4. Variance partition analysis of the relative effects of different environmental variables on soil (a) G\(^+\): gram-positive bacteria; (b) G\(^-\): gram-negative bacteria; (c) AMF: arbuscular mycorrhizal fungi Notes: SM: soil moisture, \(T_s\): soil temperature, NH\(_4^+\)-N: ammonium nitrogen, NO\(_3^-\)-N: nitrate nitrogen, CN: soil organic carbon, total nitrogen and the ratio of SOC to TN.

Table A1. Correlation analysis between soil PLFAs content and environmental factors.

| PLFA | Fungi | Bacteria | G\(^+\) | G\(^-\) | Actinomycetes | AMF | F/B | G\(^+\)/G\(^-\) |
|------|-------|----------|--------|--------|---------------|-----|-----|----------------|
| TN   | 0.33  | 0.24     | 0.33   | 0.39   | 0.23          | 0.20| 0.18| 0.04           |
| SOC  | 0.25  | 0.22     | 0.25   | 0.33   | 0.14          | 0.11| 0.10         | 0.13           |
| SOC/TN | 0.30  | 0.30     | 0.30   | 0.38   | 0.18          | 0.16| 0.19         | 0.20           |
| NH\(_4^+\)-N | 0.62 ** | 0.48    | 0.63 ** | 0.70 *** | 0.51 *        | 0.51 * | 0.36 | 0.01 | 0.19 |
| NO\(_3^-\)-N | -0.38 | -0.42    | -0.38 | -0.39 | -0.38         | -0.32| -0.31 | -0.12 | -0.18 |
| pH   | -0.29 | -0.20    | -0.30 | -0.38 | -0.17         | -0.17| -0.23 | -0.05 | -0.32 |
| \(T_s\) | -0.40 | -0.37    | -0.40 | -0.48 | -0.31         | -0.27| -0.30 | -0.09 | -0.24 |
| SM   | 0.39  | 0.36     | 0.40   | 0.47   | 0.33          | 0.27| 0.27         | 0.04           |

Note: * \(p < 0.05\), ** \(p < 0.01\), *** \(p < 0.001\). TN: Total nitrogen, SOC: soil organic carbon, SOC/TN: the ratio of SOC to TN, NH\(_4^+\)-N: ammonium nitrogen, NO\(_3^-\)-N: nitrate nitrogen, Ts: soil temperature, and SM: soil moisture. PLFA: phospholipid fatty acid; G\(^+\): gram-positive bacteria; G\(^-\): gram-negative bacteria; AMF: arbuscular mycorrhizal fungi; F/B: the ratio of fungi and bacteria; G\(^+\)/G\(^-\): the ratio of gram-positive bacteria and gram-negative bacteria.

Table A2. Adonis multivariate analysis of variance on soil microbial composition under warming and clipping treatments in three alpine meadow sites, Northern Tibet.

| Taxa | Treatment | Low Altitude | | Middle Altitude | | High Altitude | |
|------|-----------|--------------|-----------------|-------------------|-----------------|----------------|
|      | \(R^2\) | \(F\) | \(p\) | \(R^2\) | \(F\) | \(p\) | \(R^2\) | \(F\) | \(p\) |
| PLFA | W       | 0.06 | 0.80 | 0.457 | 0.06 | 0.80 | 0.424 | 0.06 | 0.80 | 0.433 |
|      | CL      | 0.20 | 2.72 | 0.097 | 0.20 | 2.72 | 0.100 | 0.20 | 2.72 | 0.086 |
|      | W \(\times\) CL | 0.17 | 2.35 | 0.129 | 0.17 | 2.35 | 0.118 | 0.17 | 2.35 | 0.117 |
|      | W       | 0.10 | 1.11 | 0.354 | 0.06 | 0.81 | 0.403 | 0.05 | 0.56 | 0.571 |
| G\(^+\) | CL     | 0.08 | 0.86 | 0.423 | 0.11 | 1.65 | 0.216 | 0.21 | 2.45 | 0.02 * |
|      | W \(\times\) CL | 0.09 | 0.98 | 0.365 | 0.28 | 4.15 | 0.043 * | 0.04 | 0.49 | 0.629 |
|      | W       | 0.09 | 1.33 | 0.272 | 0.01 | 0.17 | 0.798 | 0.19 | 7.19 | 0.021 * |
| G\(^-\) | CL     | 0.21 | 3.15 | 0.068 | 0.06 | 0.77 | 0.433 | 0.37 | 22.03 | 0.001 *** |
|      | W \(\times\) CL | 0.16 | 2.35 | 0.132 | 0.35 | 4.92 | 0.039 * | 0.03 | 1.27 | 0.302 |
### Table A2. Cont.

| Taxa         | Treatment | Low Altitude |          |          |          |          | Middle Altitude |          |          |          | High Altitude |          |          |
|--------------|-----------|--------------|----------|----------|----------|----------|-----------------|----------|----------|----------|---------------|----------|----------|
|              |           | R²  | F   | p    | R²  | F   | p    | R²  | F   | p    |               |          |          |
| Actinomycetes| W         | 0.00 | 0.03 | 0.947 | 0.02 | 0.29 | 0.599 | 0.25 | 15.97 | 0.005 **   | W         | 0.00 | 0.03 | 0.947 | 0.02 | 0.29 | 0.599 | 0.25 | 15.97 | 0.005 ** |
|              | W × CL    | 0.13 | 1.50 | 0.242 | 0.28 | 4.15 | 0.057 | 0.06 | 4.15  | 0.088      | W         | 0.08 | 0.10 | 0.369 | 0.03 | 0.41 | 0.601 | 0.06 | 0.79  | 0.49   |
| Bacteria     | CL        | 0.17 | 2.15 | 0.130 | 0.09 | 1.34 | 0.288 | 0.25 | 3.04  | 0.007 **   | B         | 0.14 | 1.76 | 0.184 | 0.32 | 4.57 | 0.044 * | 0.04 | 0.54  | 0.655  |
|              | W         | 0.04 | 0.99 | 0.364 | 0.04 | 0.46 | 0.739 | 0.23 | 6.64  | 0.006 **   |            |      |      |      |      |      |      |      |      |      |
| Fungal       | CL        | 0.27 | 6.67 | 0.020 * | 0.07 | 0.80 | 0.446 | 0.47 | 13.83 | 0.001 ***  | F         | 0.36 | 8.92 | 0.010 ** | 0.14 | 1.56 | 0.217 | 0.03 | 0.92  | 0.391  |
|              | W × CL    | 0.13 | 1.50 | 0.242 | 0.28 | 4.15 | 0.057 | 0.06 | 4.15  | 0.088      | W × CL    | 0.25 | 15.97 | 0.005 **   | W         | 0.00 | 0.03 | 0.947 | 0.02 | 0.29 | 0.599 | 0.25 | 15.97 | 0.005 ** |

Note: * p < 0.05, ** p < 0.01, *** p < 0.001. W: warming; CL: clipping; W × CL: the interaction of warming and clipping.

### Table A3. Mantel test between the composition/content of soil microbial community and environmental factors.

|       | TN  | SOC | SOC/TN | NH₄⁺-N | NO₃⁻-N | pH  | Tₛ  | SM  |
|-------|-----|-----|--------|--------|--------|-----|-----|-----|
| G⁺    | 0.14 * | 0.11 | 0.06 | 0.07 | 0.00 | 0.17 * | 0.16 | 0.14 |
| G⁻    | −0.06 | −0.06 | 0.00 | 0.04 | 0.06 | 0.10 | 0.03 | 0.01 |
| Actinomycetes | 0.00 | −0.01 | −0.01 | 0.12 | 0.11 * | 0.16 ** | 0.07 | 0.16 * |
| AMF   | −0.01 | −0.01 | 0.00 | 0.17 * | 0.02 | 0.10 | 0.03 | 0.06 |
| Bacteria | 0.11   | 0.09 | 0.05 | 0.08 | 0.01 | 0.18 * | 0.16 * | 0.14 |
| Fungi | 0.00 | −0.01 | −0.03 | 0.12 | 0.13 * | 0.16 | 0.04 | 0.10 |
| F/B   | −0.13 | −0.13 | −0.14 | −0.10 | −0.08 | 0.08 | −0.10 | −0.14 |
| G⁺/G⁻ | 0.09 | 0.07 | 0.03 | 0.01 | −0.07 | 0.18 * | 0.12 | 0.06 |

Note: * p < 0.05, ** p < 0.01, *** p < 0.001. PLFA: phospho lipid fatty acid; G⁺: gram-positive bacteria; G⁻: gram-negative bacteria; AMF: arbuscular mycorrhizal fungi; F/B: the ratio of fungi and bacteria; G⁺/G⁻: the ratio of gram-positive bacteria and gram-negative bacteria.

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