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Short-Term Simulated Warming Changes the Beta Diversity of Bacteria in Taiga Forests' Permafrost by Altering the Composition of Dominant Bacterial Phyla

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Abstract: Permafrost is widely degraded in the context of global warming. The spatial distribution of soil microbes in these cold habitats has received a lot of attention. However, knowledge on the changes in permafrost microbial communities following permafrost thaw is still limited. We used permafrost soil from a taiga forest for indoor experiments using pristine soil as a control (CK, −2 °C), simulating warming for 15 days at temperatures of 0 °C (T_1), 2 °C (T_2), and 4 °C (T_3). Amplicons of the hypervariable V4 region of the bacterial 16S rRNA gene were sequenced to identify bacterial communities present in the soils of pristine and warming treatments. Warming increased the average relative abundance of Proteobacteria (5.71%) and decreased that of Actinobacteriota (7.82%). The Beta diversity changed ($p = 0.001$) and significantly correlated with the pH, microbial biomass carbon (MBC), and available potassium (AK) of the soil ($p < 0.05$). Warming further increased the Alpha diversity (Simpson index), changing the functional pathways of the bacterial communities, whereby secondary functional pathways produced significant correlations with bacterial phyla ($p < 0.05$). Combined, the results indicated that short-term warming altered the Beta diversity of soil bacteria in a taiga forest's permafrost soil by decreasing the abundance of Actinobacteria and increasing that of Ascomycetes, while pH, MBC, and AK were identified as the soil factors influencing the structure and diversity of the bacterial communities.

Keywords: short-term simulated warming; permafrost; soil bacteria; composition and diversity

Citation: Jiang, Y.; Wu, S.; Yang, L.; Liu, Y.; Gao, M.; Ni, H. Short-Term Simulated Warming Changes the Beta Diversity of Bacteria in Taiga Forests' Permafrost by Altering the Composition of Dominant Bacterial Phyla. *Forests* **2024**, *15*, 693. https://doi.org/10.3390/f15040693

Academic Editors: Aurelia Onet and Paola Grenni

Received: 8 March 2024
Revised: 9 April 2024
Accepted: 10 April 2024
Published: 11 April 2024

1. Introduction

Global warming has emerged as a major topical issue, resulting in the melting of glaciers and permafrost, higher sea levels, an increased frequency of extreme weather events, and an increased loss of biodiversity [1]. The average world temperature has risen by 1.1 °C since 1880 and is predicted to reach a maximum of 5.7 °C by 2100 [2]. Permafrost located in high-latitude and high-altitude areas is extremely sensitive to climate change [1,3]. The global average temperature over the last 40 years has increased by 0.17 °C/decade, while warming rates of 0.63 °C/decade in the Arctic [4] and 0.34 °C/decade in the Qinghai–Tibet Plateau [5] have resulted in notable permafrost degradation [4,5]. Previous research found that melting permafrost in the Arctic releases large amounts of the greenhouse gases CO_2 and CH_4 into the atmosphere [6], and these gases produce effects conducive to climate warming [7,8]. The permafrost region of the Qinghai–Tibet Plateau will turn into a net atmospheric carbon source when the thickness of its permafrost layer decreases, releasing earlier fixed soil carbon in the form of potent greenhouse gasses [9].

Microorganisms are the main driving force for the turnover of soil organic matter, and the structure and composition of microbial communities is vital for soil carbon and nitrogen cycling; permafrost thawing can induce changes in these communities that affect greenhouse gas emissions and vegetation succession [10]. Therefore, research on how ecological processes in permafrost are changing as a result of climate change is important.

Soil microorganisms are widely involved in biogeochemical cycling processes. Bacteria are the primary biological markers of permafrost deterioration in permafrost soils because they are highly responsive to external environmental impacts [11,12]. In recent years, research on the response of soil bacteria in permafrost soils to warming has received increasing attention. However, the reported findings are not always consistent because of variations in warming times and experimental methodologies. For example, Xue et al. [13] found that warming significantly changed the functional structure of microorganisms in a permafrost active layer in Alaska by using snow fences for 1.5 years, but significant changes in the bacterial community structure were not observed. Perez-Mon et al. [14] simulated three years of warming that brought soil microbial communities from a permafrost layer to the surface, causing significant changes to their Beta diversity while reducing bacterial richness and diversity. Dong et al. [15] found that experimental indoor warming of the active layer soil from a permafrost region of the Greater Khingan Mountains for 45 days increased bacterial Alpha diversity and changed its Beta diversity. Obviously, the way bacteria respond to increasing temperatures varies across studies, due to variations in soil types, warming techniques, and geographic locations. Furthermore, research on how permafrost bacteria respond to global climate change is primarily focused on the active surface layer soil, while relatively little research on bacteria associated with deeper permafrost has been carried out [13,15,16]. This is because the surface layer of permafrost soil is more vulnerable to external interference than deeper layers. However, Mackelprang et al. [17] described that a bacterial community composition responds quickly to the thawing of permafrost soil, and these changes are more pronounced in the deeper permafrost layer than in the active surface layer. Due to the rapid replacement and extreme diversity of bacteria after years of permafrost degradation, our understanding of the impact of changes in bacterial community structure in permafrost remains limited [12]. Thus, studying how permafrost bacteria react to warming could increase our understanding of the potential impact of permafrost degradation on microbial communities and provide a valuable source of information for predicting soil carbon cycling following thawing.

The Greater Khingan Mountains are located at the southern edge of the Eurasian permafrost zone and are highly sensitive to climate change [18]. In the past few decades, significant permafrost deterioration has occurred in the area, mainly evidenced by the reduction in permafrost-covered areas, associated with a shift of the southern boundary towards the north, and by the increased thickness of the active layer [19]. This situation provides an ideal opportunity to explore changes in microbial communities in permafrost soil due to warming. Most studies conducted in this area have documented the community changes in soil bacteria in the field during natural warming [20,21] or by indoor microcosm simulations [15,22] performed with different types of permafrost from various regions [23]. However, most of these studies have focused on the active layer instead of the deeper permafrost layer [24,25]. Since the change in bacteria in response to warming is more rapid in the permafrost layer than in the active layer [17], the paucity of data on the deeper layer limits our understanding of the changes in the microbial community following permafrost thaw. In this study, the soil of the permafrost layer in a taiga forest was studied to analyze the changes in the structural composition and diversity of the bacterial community in response to warming by simulated indoor experiments. For this, high-throughput sequencing technology was applied to assess the composition and diversity of the soil bacteria. These were analyzed together with soil physicochemical properties in order to provide more in-depth insights about how increasing temperature can change the bacterial community of the permafrost layer in the context of climate warming.

2. Materials and Methods

2.1. Study Site

The study site is located in the Huzhong National Nature Reserve in the Greater Khingan Mountains, China (51°49′01″~51°49′1″ N, 122°59′33″~123°00′03″ E) (Figure S1). This area experiences less than 80 days per year without frost, the average annual temperature is −4 °C, and the average precipitation is 458.3 mm. Under these cold climate conditions, permafrost is widely developed. In the research region, *Larix gmelinii* and *Betula platyphylla* are the most common trees, *Rhododendro dauricum* and *Ledum palustre* are the most common shrubs, and *Maianthemum bifolium* is the most common herb [26].

2.2. Sampling Procedures and Incubation Experiments

Soil samples had been collected for a previous study that concentrated on fungal communities [27]. Briefly, the soil was collected from three different locations at a depth of 80 to 100 cm to ensure the sampling of the permafrost layer. The measured average in situ temperature of the soil samples was −2 °C. The samples were stored at −20 °C immediately after sampling and used for indoor warming experiments.

Based on the prediction that temperatures will rise by 1.0–5.7 °C towards the end of this century [2], we conducted simulated studies using three temperature gradients of 0 °C (raised by 2 °C), 2 °C (raised by 4 °C), and 4 °C (raised by 6 °C), using −2 °C (the measured average temperature of soil samples) as a control. Before the experiment, the soil column was cut into small sections of approximately 3–4 cm^3 in the freezer room, and these were mixed evenly to form a composite sample. This composite soil (approximately 100 g of dry weight) was added to sterile wide-mouth bottles (1 L) and pre-incubated at 0 °C (T_1), 2 °C (T_2), and 4 °C (T_3) for 3 days and then anaerobically incubated at 0 °C (T_1), 2 °C (T_2), and 4 °C (T_3) for 15 days, and all treatments were performed in triplicate [24].

2.3. Analyses of Soil Physicochemical Properties

The physiochemical properties of the soil had already been determined in a previous study [27]. Briefly, the pH of the soil was measured after mixing it with water (2 g soil in 20 mL) with a pH meter (PB-10, Sartorius, Gottingen, Germany) [28]. Microbial biomass carbon (MBC) was determined after chloroform fumigation extraction, carried out as previously described [29]. The concentrations of total nitrogen (TN) and of soil organic carbon (SOC) were measured using an elemental analyzer (Elementar Vario EL III) [30] and a TOC analyzer (Vario TOC cube, both from Elementar Analysensysteme GmbH, Hanau, Germany) [31], respectively. The available phosphorus (AP), available nitrogen (AN), and available potassium (AK) levels were analyzed by sodium bicarbonate extraction–molybdenum antimony colorimetry, alkali hydrolysis diffusion, and ammonium acetate extraction with flame photometry, respectively, as previously described [32].

2.4. DNA Extraction, Amplification, and MiSeq Sequencing

Total DNA was extracted from the soil with the PowerMax® Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA), and the DNA concentration was assessed using NanoDrop 2000 (Termo Fisher Scientific, Wilmington, DE, USA). The primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′) were used to amplify the V4 region of the bacterial 16 rRNA gene [33] with the following PCR conditions: 3 min pre-denaturation at 94 °C followed by 35 cycles with 45 s denaturation at 94 °C, 60 s annealing at 50 °C and 60 s extension at 72 °C, with a final extension at 72 °C for 10 min. The amplicons were recovered from 2% agarose gels, purified with the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and quantified by Quantus™ Fluorometry (Promega, Madison, WI, USA). The amplicons were sequenced on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) using PE300 chemicals at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The raw sequence data were uploaded to the SRA database at NCBI (https://submit.ncbi.nlm.nih.gov/subs/sra/SUB12215666/overview, accessed on 20 March 2023) with number PRJNA894956.

2.5. Bioinformatic and Statistical Analyses

Amplicon sequencing variants (ASVs) were obtained after quality control and the splicing of the original sequences, followed by a sequence noise reduction step, as previously described [27]. The obtained ASVs were attributed to taxa based on the Silva138/16s_bacteria species annotation database v138 (https://www.arb-silva.de/, accessed on 1 May 2022) using the naive Bayes classifier in Qiime2. Alpha diversity indices were calculated using Mothur v1.30.2 (https://www.mothur.org/wiki/Download_mothur, accessed on 1 May 2022). A relative abundance above 10% was considered dominant [34]. Given the large proportion of relative abundance of 'norank' taxa (sequences that were successfully aligned with a hit in the database that did not have any specific taxonomic information) and 'unclassified' taxa (sequences that failed to align with any hits in the database), these members were divided into two separate categories. Additionally, sequences with a relative abundance below 1% were combined as 'others'.

Any differences in the determined soil parameters, the relative abundance of bacterial phyla and genera, the functional prediction results, and the Alpha diversity were analyzed in SPSS 25.0 software (SPSS Inc., Chicago, IL, USA) using a one-way analysis of variance (ANOVA). Significant findings ($p < 0.05$) were found based on least significant difference (LSD) tests and are reported as means ± standard errors. The differences of relative abundance of bacteria at the phylum and genus level were plotted using Origin 2018 (Origin Software Inc., Northampton, MA, USA).

The difference in Beta diversity was determined, and a principal coordinates analysis (PCoA) based on a Bray–Curtis distance matrix was performed for all samples, together with three non-parametric tests of a permutational multivariate analysis of variance (Adonis), an analysis of similarities (ANOSIM), and a multi-response permutation procedure (MRPP) based on the distributed data of the ASVs. The Pearson correlation coefficient was used to identify relationships between bacterial phyla and Alpha diversity index or soil properties, with results displayed in a heatmap. Distance-based redundancy analysis (db-RDA) based on the Bray–Curtis distance matrix was conducted to determine the relationships between the bacterial communities and the physicochemical parameters of the soil. Figures and tables were prepared using the vegan and ggplot packages in R-3.3.1.

Lastly, using R-3.3.1, KEGG functional prediction was completed based on the Tax4Fun package, and the Spearman correlation coefficient between bacterial phyla and function was calculated using the "psych" package. Data with $|r| \geq 0.6$ and $p < 0.05$ were retained, and a network diagram was constructed using Gephi v0.10 (https://gephi.org/users/download/, accessed on 6 June 2023) [35].

3. Results

3.1. Effect of Warming on Soil Physicochemical Properties

The effects of the applied warming on the physical and chemical properties of soil have already been described in a previous publication [27]. The data that were used for statistical analyses in this study are summarized in Table S1.

3.2. Heating Results in Differences in Bacterial Community Composition

The sequencing data obtained from the control (CK) and from T_1, T_2, and T_3 reached saturation, according to the minimum number of required sample sequences after noise reduction processing using the DADA2 plugin of Qiime2 software. A total of 271,896 optimized sequences were obtained, and these clustered into 1620 ASVs. The highest number of ASVs was detected in T_3 (750), followed by T_2 (708) and CK (708), with 684 present in T_1. From the comparison among the four groups (Figure 1), we identified 260 common ASVs. The T_3 group contained the most unique ASVs (314), and this group's fraction of unique ASVs (41.9%) was higher than that for the other groups (32.8%, 32.5%, and 37.1% for CK, T_1, and T_2, respectively). Thus, both the total number of ASVs and the number and fraction of unique ASVs were the highest following T_3 treatment.

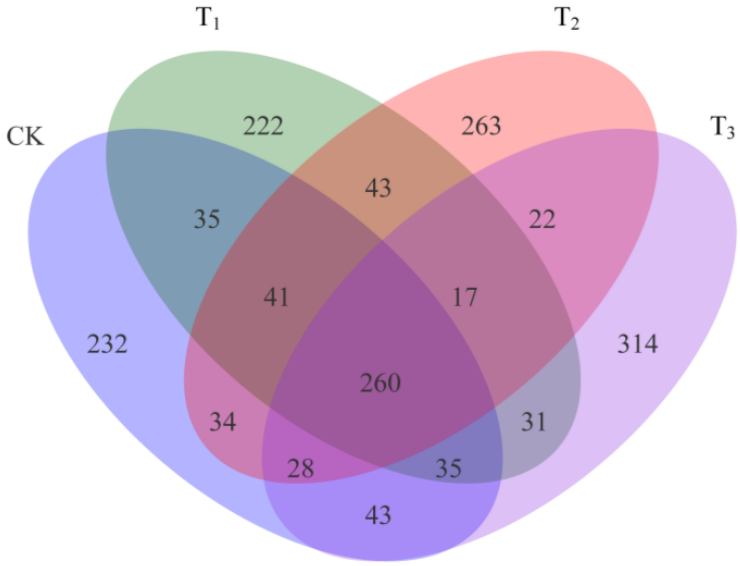

Figure 1. Venn diagram of ASVs identified in soil following incubation at different temperatures with CK: pristine permafrost soil (−2 °C); treatments T_1 (0 °C), T_2 (2 °C), and T_3 (4 °C).

A total of 32 phyla, 96 classes, 202 orders, 298 families, 411 genera, and 543 bacterial species were identified from the partial rDNA sequences. The bacterial community composition at the phylum level is presented in Figure 2a. The dominant phyla were Actinobacteriota (31.56%) and Proteobacteria (24.62%). Compared with CK, warming decreased the relative abundance of Actinobacteriota by, on average, 7.82% and increased the abundance of Proteobacteria by 5.71%. The relative abundance of Chloroflexi in T_2 was lower than in CK, and that of Bacteroidota was higher in the T_1 and T_2 samples than in CK and T_3. Differences in relative abundance between the groups were also observed for Desulfobacterota, Gemmatimonadota, and, in particular, Firmicutes, as the latter was quite strongly increased in the T_3 group compared to the other groups, while the relative abundance of Patescibacteria was strongly reduced. For the less abundant bacterial phyla collectively reported as 'other', a general trend was visible, as their relative abundance gradually decreased with increasing temperature, with the lowest values belonging to the T_3 group ($p < 0.05$).

The characteristics of the bacterial community composition at the genus level are presented in Figure 2b. Notably, the relative abundance of *norank* (34.65%), *unclassified* (31.51%), and '*other*' genera (collective low-abundant genera, 18.68%) accounted for the highest relative abundance. Together, they accounted for approximately 85% of the sequence reads. The *norank* and *unclassified* taxa, together, reached over 60% and were ranked as CK (68.77%) > T_1 (66.56%) > T_2 (64.71%) > T_3 (64.61%), indicating that warming reduced their fraction and favored an increase in the percentage of identifiable and classifiable bacterial genera. At the same time, the relative abundance of '*other*' genera increased with increasing temperature and reached the highest levels in the T_3 group. The relative abundance of *Conexibacter* was higher, and the relative abundance of *Paludibacter* was lower in CK than in the other three groups. The relative abundances of *Candidatus_Solibacter* and *GOUTA6* were lower in the T_3 group than in the other three groups, while that of *Geobacter* was higher in T_2, and that of *Rhodoferax* was higher in both T_1 and T_2 than in the other groups ($p < 0.05$).

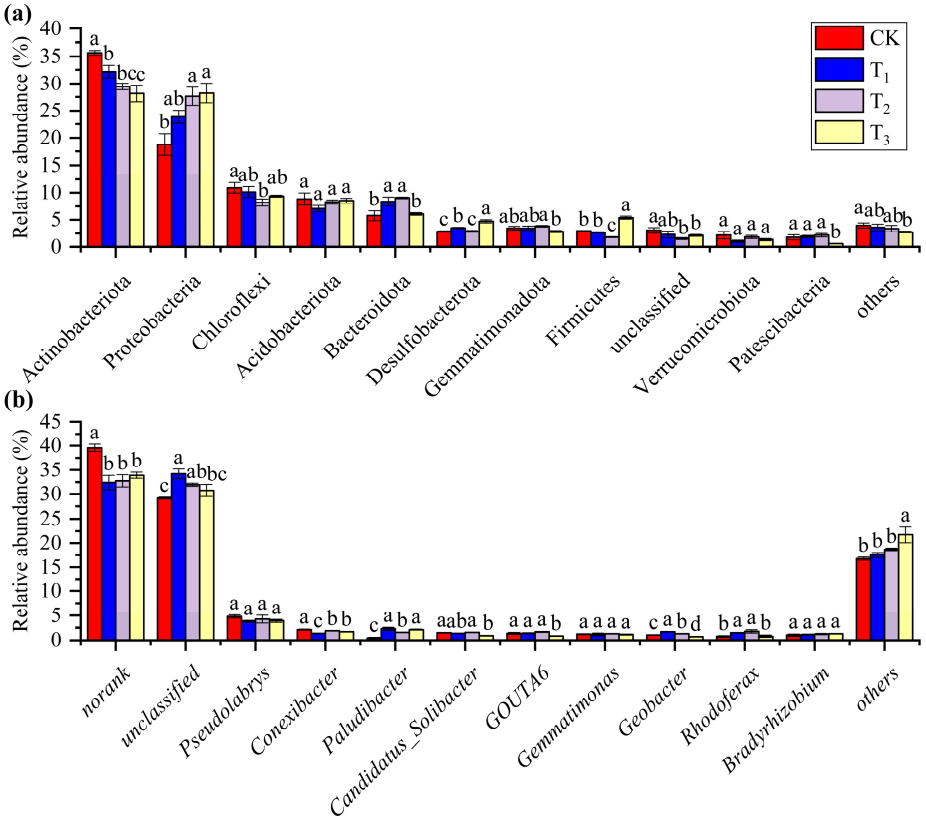

Figure 2. Distribution of bacterial phyla (**a**) and genera (**b**) identified following the different temperature treatments. Different lowercase letters above each bacterial classification represent a significant difference between groups, and having the same letter indicates a non-significant difference, where a > b, b > c, c > d ($p < 0.05$).

3.3. Effects on Bacterial Community Diversity

3.3.1. Alpha Diversity

As Table 1 shows, the coverage index of all groups was higher than 0.99, suggesting that the sequence data adequately address the bacterial diversity of the samples. Compared with the CK group, the Sobs and ACE indices did not change as a result of heat treatment ($p > 0.05$). No significant changes were observed for these indices between any of the warming treatments (T_1, T_2, and T_3) and the CK, despite the fact that the Shannon index was much lower in T_1 than in T_3. The Simpson index gradually decreased with the increase in temperature and was significantly lower in T_3 than in CK ($p < 0.05$). Therefore, it can be concluded that warming resulted in a higher Alpha diversity for the bacterial communities.

Table 1. Analysis of bacterial diversity indices following different temperature treatments.

Treatment	Coverage	Sobs	ACE	Shannon	Simpson ($\times 10^{-2}$)
CK	1.00 ± 0.00 a	392.67 ± 14.88 a	392.78 ± 14.90 a	5.22 ± 0.04 ab	1.19 ± 0.09 a
T_1	1.00 ± 0.00 a	380.00 ± 16.86 a	380.12 ± 16.97 a	5.16 ± 0.06 b	1.15 ± 0.13 ab
T_2	1.00 ± 0.00 a	382.33 ± 22.28 a	382.39 ± 22.30 a	5.27 ± 0.08 ab	0.89 ± 0.07 ab
T_3	0.99 ± 0.01 a	438.33 ± 17.02 a	438.91 ± 17.36 a	5.36 ± 0.05 a	0.86 ± 0.10 b

Data are represented as mean ± standard error ($n = 3$); different lowercase letters in the same column indicate significant differences ($p < 0.05$).

3.3.2. Beta Diversity

As shown in the PCoA plot (Figure 3), the bacterial communities of all samples were well separated and divided into two clusters along the horizontal axis. The T_1 and T_2 clusters were distributed in the third quadrant, whereas the CK cluster was distributed

in the second quadrant, and all three were clustered with negative values for the first component. The T_3 cluster on the right side of the plot was distributed in the first and fourth quadrants. With a cumulative explanation rate of 47.18%, PC1 and PC2 contributed 31.98% and 15.20% of the degree of explanation, respectively. Further analysis by the non-parametric test techniques Adonis, ANOSIM, and MRPP demonstrated that the bacterial Beta diversity was dramatically affected by the warming (Table 2, $p = 0.001$).

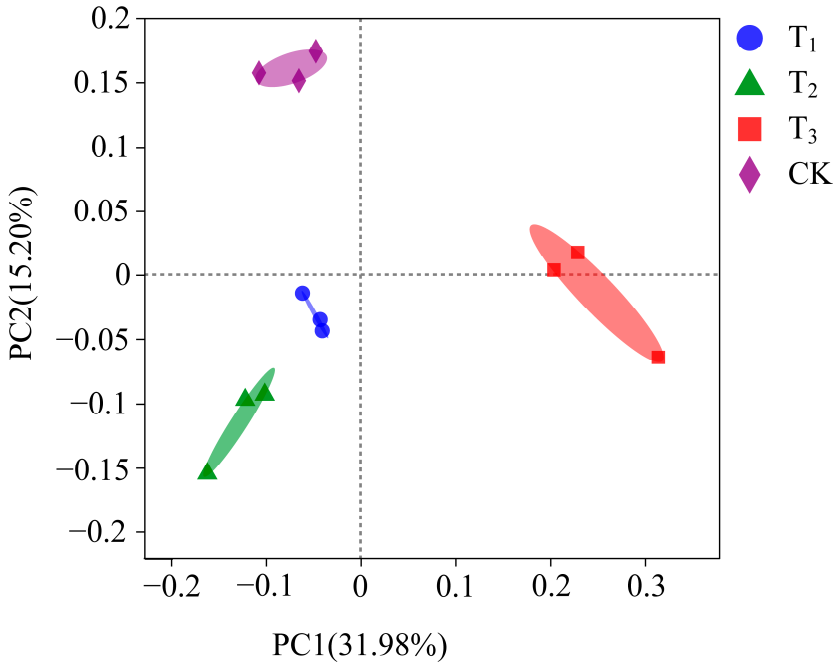

Figure 3. PCoA of the soil bacterial communities.

Table 2. Nonparametric test analysis based on Bray–Curtis distance.

Method	Adonis		ANOSIM		MRPP	
All groups	R^2	*p*	R	*p*	A	*p*
	0.56	0.001 ***	0.91	0.001 ***	0.27	0.001 ***

Adonis: permutational multivariate analysis of variance; ANOSIM: analysis of similarities; MRPP: multi-response permutation procedure. Significance is indicated as *** for $p \leq 0.001$.

3.4. Correlation Analyses

3.4.1. Relationships between Community Composition and Alpha Diversity and Soil Properties

A heatmap of identified correlations between the relative abundance of particular bacterial phyla and the characteristics of the soil is shown in Figure 4. The relative abundance of Actinobacteriota were negatively correlated with the levels of microbial components detected in the soil (MBC), and this phylum was highly strongly positively correlated with pH ($p < 0.001$). A reversed correlation was observed for Proteobacteria abundance, which were strongly positively correlated with MBC and negatively with pH ($p < 0.01$). Bacteroidota were also positively correlated with MBC. Desulfobacterota were negatively correlated with AN and pH, but Verrucomicrobiota correlated positively with AN. Unclassified bacteria were positively correlated with pH and strongly negatively correlated with MBC. Correlations for the diversity indices (Coverage, Sobs, ACE, and Shannon) with mean soil physical and chemical properties were also calculated, but none were significant ($p > 0.05$), except for the Simpson index, which was positively correlated with pH ($p < 0.05$).

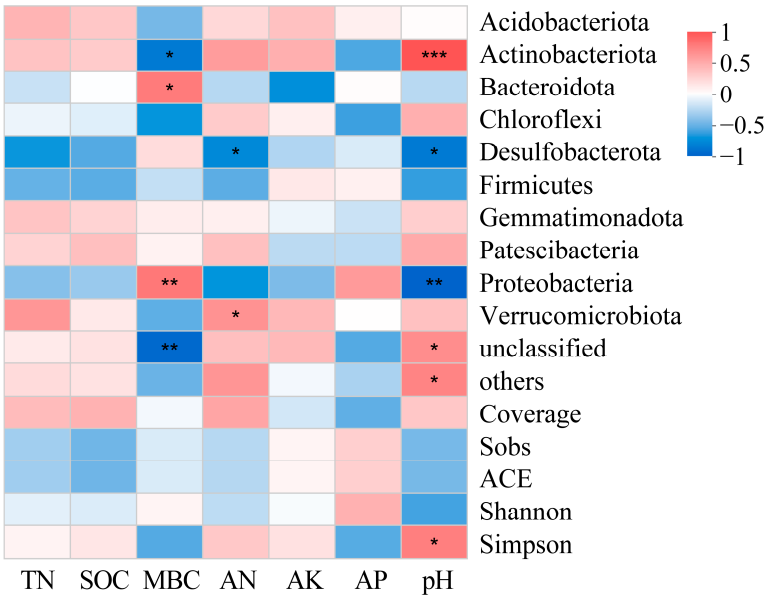

Figure 4. Correlation heatmap of bacterial phylum and Alpha diversity index and soil physicochemical properties. TN: total nitrogen; SOC: soil organic carbon; MBC: microbial biomass carbon; AN: available nitrogen; AK: available potassium; AP: available phosphorous. Significance is indicated as * for $0.01 < p \leq 0.05$, ** for $0.001 < p \leq 0.01$, *** for $p \leq 0.001$.

3.4.2. Relationship between Beta Diversity and Soil Physiochemistry

Relationships between the Beta diversity and soil characteristics were assessed by db-RDA, as illustrated Figure 5. The two axes accounted for 33% of the variations in the bacterial community structure observed, with CAP1 and CAP2 accounting for 20.33% and 12.67% of the differences, respectively. The MBC, pH, and AK of the soil produced longer arrows than the other characteristics, indicating that that they had a stronger effect on the bacterial population. Furthermore, Table 3 summarizes that among the analyzed soil environmental parameters, bacterial Beta diversity was substantially linked with pH, MBC, and AK ($p < 0.05$).

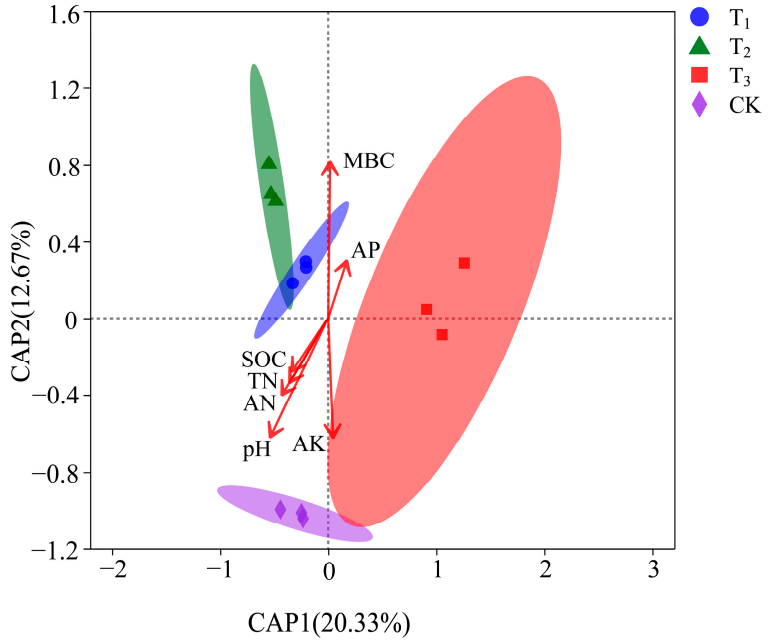

Figure 5. db-RDA of the soil bacterial communities.

Table 3. Significance analysis of the correlations between the soil physicochemical properties and the Beta diversity of the bacterial communities.

Soil Factors	R^2	p
pH	0.96	0.001 ***
TN	0.33	0.161
SOC	0.25	0.272
MBC	0.96	0.001 ***
AN	0.47	0.059
AK	0.56	0.032 ***
AP	0.16	0.494

Significance is indicated as *** for $p \leq 0.001$; the other findings were not significant ($p > 0.05$).

3.5. Bacterial Function Prediction

Table S2 displays the results of functional prediction based on the partial 16S rRNA gene sequences. On the first level of KEGG classification, metabolism (62.33%), genetic information processing (10.40%), environmental information processing (18.39%), cellular processes (5.56%), and human diseases (2.27%) were the primary functions identified that reached a relative abundance greater than 1%. At KEGG level 2, the relative abundance of eighteen functional pathways exceeded 1%. Among them, the relative abundance of amino acid metabolism (12.25%), membrane transport (9.77%), and carbohydrate metabolism (13.16%) was higher. Compared with the CK, simulated warming resulted in a significant change in the relative abundance of carbohydrate metabolism; lipid metabolism; nucleotide metabolism; xenobiotics biodegradation and metabolism; the metabolism of other amino acids; glycan biosynthesis and metabolism; cell motility, cell growth, and death; and infectious disease. Thus, several functional pathways were considerably affected by warming.

To further clarify the relationship between the functions of the identified bacterial communities, a network diagram was created, based on the results of the functional predictions at the phylum level (Figure 6; Table S3) This illustrated that, among the bacterial phyla, Patescibacteria (a candidate phylum) produced the largest node and also produced the most functional connections: this phylum was negatively correlated with glycan biosynthesis and metabolism, amino acid metabolism, metabolism of other amino acids, and xenobiotics biodegradation and metabolism. The second most functionally determining phylum was Proteobacteria, being positively correlated with cell motility and negatively correlated with energy metabolism and with nucleotide metabolism. Both Proteobacteria and Desulfobacterota were positively correlated with cell motility, while the group encompassing other phyla ('others') was strongly negatively correlated with this function. Firmicutes were the only phylum producing a positive correlation with glycan biosynthesis and metabolism, a function that more strongly negatively correlated with 'other' phyla and with Patescibacteria ($p < 0.05$). The thickest arrows in the figure representing negative correlations connected 'others' with cell motility and with glycan biosynthesis and metabolism, while Patescibacteria had the highest number of connections, which were also relatively strong. Fewer positive correlations than negative correlations were identified, and of the positive correlations, those between Proteobacteria and cell motility and between Acidobacteriota and amino acid metabolism were the strongest, as indicated by their thicker lines.

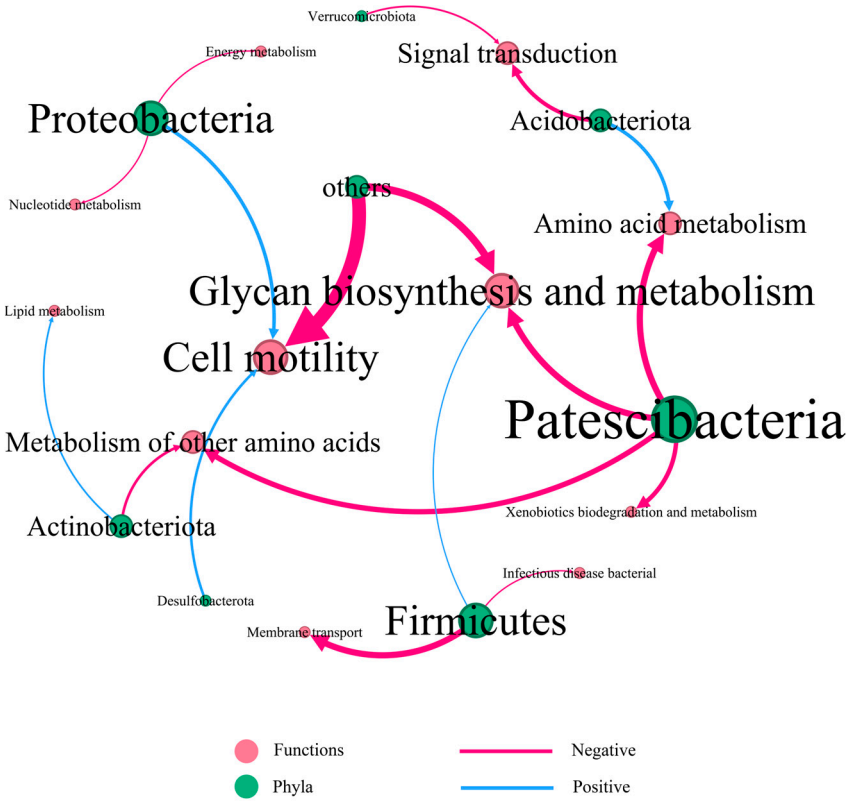

Figure 6. Network analysis of bacterial phyla and functional pathways. Node size represents connectivity, with larger nodes indicating more connections. Line thickness represents the absolute value of the correlation coefficient. The font size is proportional to the node size. Values are specified in Table S3.

4. Discussion

4.1. Short-Term Warming Affects the Composition and Structure of the Bacterial Communities

Because of functional and structural properties, different bacterial groups respond differently to changes in their environment [36,37]. In this study, Actinobacteriota and Proteobacteria were the dominant phyla in each treatment, which was similar to the results of a previous study on this permafrost soil [38]. Actinobacteriota had the highest average relative abundance, and its presence in these low-temperature soil environments is mainly attributed to its active DNA repair mechanism and strong metabolic capacity [39,40]. It was found that the relative abundance of Actinobacteriota was significantly reduced under warming conditions. Possibly, these bacteria are less capable of surviving acid conditions, as a significant decrease in soil pH resulted from the warming treatment [41]. Proteobacteria, on the other hand, underwent a vast increase in relative abundance as the temperature rose. These bacteria are typically classified into a co-nutrient category; and the phylum comprises a large number of bacterial species engaged in the initial metabolism of unstable organic compounds [42]. Furthermore, Proteobacteria exhibit resilience to temperature increases because of their superior capacity to break down resistant organic materials, which allows them to access more nutrients for growth in a restricted environment [43,44]. It is interesting to note that, at the bacterial genus level, the total relative abundance of unknown and unclassified bacteria decreased with increasing temperature. However, over 60% of the taxa were either *norank* or *unclassified*. That this fraction was so large may be because the most recent data stored in taxonomic databases poorly match the bacterial communities typically residing in permafrost soils [45].

4.2. Short-Term Warming Affects the Bacterial Community Diversity

Temperature is a key factor for microbial survival, and warming generally affects bacterial diversity [46,47]. Chen et al. [48] reported that warming significantly reduced bacterial Alpha diversity in permafrost soil, which is inconsistent with our findings. Microbial communities exhibit large biogeographic distribution variances related to the high geographical variability caused by different vegetation types, soil conditions, and climate [49]. Due to the different responses of different microbial groups to warming [50], the results of warming on the Alpha diversity of microorganisms in the forest soil of the Greater Khingan Mountains may differ from that of the grasslands of the Qinghai–Tibet Plateau, producing inconsistent results. During short-term warming, at first, soil nutrients become available for microbial utilization as a result of permafrost thawing, so that dominant populations do not immediately compete with and displace weaker populations [51,52]. Microbial communities react to warming with a certain delay, so that the short-term simulated warming applied here might have been too short to negatively affect the Alpha diversity of the bacterial communities [50,53]. In addition, Chen et al. [48] found that warming significantly changed the Beta diversity of bacteria, and herein, we report similar observations: changes in soil physicochemical properties after the thawing of the permafrost had an effect on the Beta diversity of the microorganisms [54]. In the permafrost regions of the Tibetan Plateau [31] and the Arctic [55], pH was revealed as the primary variable influencing changes in soil bacterial community diversity at the regional scale. Our study also found a substantial correlation between pH and Beta diversity. Members of soil bacterial communities have different pH tolerance limits, meaning that variations in pH can impact the bacterial communities' composition and structure [56]. Furthermore, Zhang et al. [57] discovered, through extensive sampling and incubation investigations, that nitrogen and phosphorus co-limited soil microorganisms in the permafrost regions of the high-elevation Tibetan Plateau. However, we found no correlations between TN, AN, and AP levels in the soil and bacterial Beta diversity, illustrating that the results obtained with various permafrost soils are not always consistent, possibly as a result of the different adaptive capacities of the present bacterial taxa to environmental changes in different areas during the formation of regional geology [58].

It is worth noting that AK correlated with bacterial Beta diversity in this study, which is similar to the results of a pairwise study of forest soil bacteria in the permafrost regions of the Tibetan Plateau [59]. This could be because some soil bacterial taxa can change the solubility and efficacy of K, which, in turn, influences the selection of these particular bacteria [60,61]. There is very limited literature on the potassium limitations of soil microorganisms. Mori et al. [62] found that K does not limit microbial activity. Moro's [63] field trial results showed that soil microorganisms were mainly limited by C and N but not by K, even in soils with low K effectiveness. It is still unclear whether K significantly influences the structure of bacterial communities in permafrost regions, as studies on the relationship between K and bacterial community Beta diversity are rare, especially in permafrost research.

4.3. Short-Term Warming Affects the Bacterial Community Functions

Microorganisms are important biological components of soils, and their functional characteristics can partially reflect fundamental ecological processes including the cycling of nutrients and carbon in the soil [48,64]. Carbohydrate metabolism is the secondary functional pathway with the highest relative abundance, and it is widely involved in the formation, transformation, and degradation of carbohydrates. Previous studies describe that Actinobacteriota, Proteobacteria, and Bacteroidota are all closely associated with carbohydrate metabolism [65,66]. However, we did not observe significant correlations between this functionality and particular phyla, so carbohydrate metabolism is absent from the network diagram presented in Figure 5. Functionality in the metabolism of carbohydrates is possibly the result of multiple phyla working together, as evidenced by the lack of a strong correlation ($r < 0.6$) with individual phyla. Amino acids are important carbon sources for

metabolic processes. Yadav et al. [67] concluded that Acidobacteriota can utilize a wide range of amino acids as substrates to complete respiration and fermentation processes. Consistent with another study [68], the present work identified a positive correlation between Acidobacteriota and amino acid metabolism. In addition, the network diagram showed that Proteobacteria have a strong correlation (represented by thicker lines) with cell motility [69], and that Acidobacteriota are correlated with amino acid metabolism [70], which corroborated our previous study. As for the negative correlations in the network diagram, Patescibacteria were negatively correlated with amino acid metabolism, glycan biosynthesis and metabolism, the metabolism of other amino acids, and xenobiotics biodegradation and metabolism, but there is a paucity of data on the role of these bacteria in these metabolic pathways, and further studies are needed.

5. Conclusions

The experimental simulated warming of permafrost soil changed the composition and structure of the bacteria present in the soil, in particular by altering the relative abundance of the dominant phyla of Actinobacteriota and Proteobacteria, and affected the Beta diversity. Among the changed soil chemical and physical characteristics, pH, MBC, and AK were found to be the important factors influencing Beta diversity. Alpha diversity increased and functional pathways changed after warming, and some predicted bacterial functions were affected by shifts in the bacterial taxa. Empirical results obtained by our group and by others have shown that the structure and diversity of bacterial communities are significantly affected by short-term simulated warming. However, this research field is still developing, and the existing data are not yet sufficient to address the following questions: (1) How does the microbial community change in situ as permafrost soil is exposed to warming? (2) How do the microbial communities of the permafrost layer and their functional genes respond to long-term warming? (3) How do different permafrost ecosystems regulate the processes of carbon and nitrogen cycling, possibly affecting greenhouse gas emissions? These research subjects remain to be further studied.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f15040693/s1, Figure S1: Map of the field sampling sites on the Greater Khingan Mountains; Table S1: Soil physicochemical properties under different temperature treatments; Table S2: Relative abundance (>1%) of potential functional pathways at level 2 in different treatments; Table S3: Network diagram data.

Author Contributions: Y.J. and L.Y. completed the experiment and wrote the manuscript. S.W. helped analyze the data and completed the literature search and analysis. L.Y. and H.N. helped revise the manuscript and editing its language. Y.L. and M.G. helped complete field sample sampling. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by The Financial Special Project of Heilongjiang Province (CZKYF2024-1-A008).

Data Availability Statement: Relevant data materials are available from the corresponding author on request.

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

References

1. Sunday, J.M. The pace of biodiversity change in a warming world. *Nature* **2020**, *580*, 460–461. [CrossRef] [PubMed]
2. Masson-Delmotte, V.; Zhai, P.M.; Pirani, A.; Connors, S.L.; Péan, C.; Berger, S.; Caud, N.; Chen, Y.; Goldfarb, L.; Gomis, M.I.; et al. IPCC, 2021: Summary for Policymakers. In *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*; Cambridge University Press: New York, NY, USA, 2021; pp. 14–17. [CrossRef]
3. Schuur, E.A.G.; McGuire, A.D.; Schaedel, C.; Grosse, G.; Harden, J.W.; Hayes, D.J.; Hugelius, G.; Koven, C.D.; Kuhry, P.; Lawrence, D.M.; et al. Climate change and the permafrost carbon feedback. *Nature* **2015**, *520*, 171–179. [CrossRef]
4. Rantanen, M.; Karpechko, A.Y.; Lipponen, A.; Nordling, K.; Hyvärinen, O.; Ruosteenoja, K.; Vihma, T.; Laaksonen, A. The Arctic has warmed nearly four times faster than the globe since 1979. *Commun. Earth Environ.* **2022**, *3*, 168. [CrossRef]

5. You, Q.L.; Cai, Z.Y.; Pepin, N.; Chen, D.L.; Ahrens, B.; Jiang, Z.H.; Wu, F.Y.; Kang, S.C.; Zhang, R.N.; Wu, T.H.; et al. Warming amplification over the Arctic Pole and Third Pole: Trends, mechanisms and consequences. *Earth-Sci. Rev.* **2021**, *217*, 103625. [CrossRef]
6. Lyon, S.W.; Destouni, G.; Giesler, R.; Humborg, C.; Morth, M.; Seibert, J.; Karlsson, J.; Troch, P.A. Estimation of permafrost thawing rates in a sub-arctic catchment using recession flow analysis. *Hydrol. Earth Syst. Sci.* **2009**, *13*, 595–604. [CrossRef]
7. Knoblauch, C.; Beer, C.; Schuett, A.; Sauerland, L.; Liebner, S.; Steinhof, A.; Rethemeyer, J.; Grigoriev, M.N.; Faguet, A.; Pfeiffer, E.M. Carbon dioxide and methane release following abrupt thaw of Pleistocene permafrost deposits in Arctic Siberia. *J. Geophys. Res.-Biogeosci.* **2021**, *126*, e2021JG006543. [CrossRef]
8. Tanski, G.; Wagner, D.; Knoblauch, C.; Fritz, M.; Sachs, T.; Lantuit, H. Rapid CO_2 release from eroding permafrost in seawater. *Geophys. Res. Lett.* **2019**, *46*, 11244–11252. [CrossRef]
9. Wang, J.F.; Wu, Q.B. Annual soil CO_2 efflux in a wet meadow during active layer freeze-thaw changes on the Qinghai-Tibet Plateau. *Environ. Earth Sci.* **2013**, *69*, 855–862. [CrossRef]
10. Jansson, J.K.; Tas, N. The microbial ecology of permafrost. *Nat. Rev. Microbiol.* **2014**, *12*, 414–425. [CrossRef]
11. Zhou, H.; Zhang, D.G.; Jiang, Z.Z.; Sun, P.; Xiao, H.L.; Wu, Y.; Chen, J.G. Changes in the soil microbial communities of alpine steppe at Qinghai-Tibetan Plateau under different degradation levels. *Sci. Total Environ.* **2018**, *651*, 2281–2291. [CrossRef]
12. Zhang, B.G.; Wu, X.K.; Zhang, G.S.; Zhang, W.; Liu, G.X.; Chen, T.; Qin, Y.; Zhang, B.; Sun, L.K. Response of soil bacterial community structure to permafrost degradation in the upstream regions of the Shule River Basin, Qinghai-Tibet Plateau. *Geomicrobiol. J.* **2017**, *34*, 300–308. [CrossRef]
13. Xue, K.; Yuan, M.M.; Shi, Z.J.; Qin, Y.J.; Deng, Y.; Cheng, L.; Wu, L.; He, Z.L.; Van Nostrand, J.D.; Bracho, R.; et al. Tundra soil carbon is vulnerable to rapid microbial decomposition under climate warming. *Nat. Clim. Chang.* **2016**, *6*, 595–600. [CrossRef]
14. Perez-Mon, C.; Stierli, B.; Plötze, M.; Frey, B. Fast and persistent responses of alpine permafrost microbial communities to in situ warming. *Sci. Total Environ.* **2021**, *807*, 150720. [CrossRef] [PubMed]
15. Dong, X.F.; Liu, C.; Ma, D.L.; Wu, Y.F.; Man, H.R.; Wu, X.W.; Li, M.; Zang, S.Y. Organic carbon mineralization and bacterial community of active layer soils response to short-term warming in the Great Hing'an Mountains of Northeast China. *Front. Microbiol.* **2021**, *12*, 802213. [CrossRef] [PubMed]
16. Liu, F.T.; Kou, D.; Chen, Y.L.; Xue, K.; Ernakovich, J.G.; Chen, L.Y.; Yang, G.B.; Yang, Y. Altered microbial structure and function after thermokarst formation. *Glob. Chang. Biol.* **2021**, *27*, 823–835. [CrossRef] [PubMed]
17. Mackelprang, R.; Waldrop, M.P.; DeAngelis, K.M.; David, M.M.; Chavarria, K.L.; Blazewicz, S.J.; Rubin, E.M.; Jansson, J.K. Metagenomic analysis of a permafrost microbial community reveals a rapid response to thaw. *Nature* **2011**, *480*, 368–371. [CrossRef]
18. Jin, H.J.; Yu, Q.H.; Lü, L.Z.; Guo, D.X.; He, R.X.; Yu, S.P.; Sun, G.Y.; Li, Y.W. Degradation of permafrost in the Xing'anling Mountains, Northeastern China. *Permafr. Periglac. Process.* **2007**, *18*, 245–258. [CrossRef]
19. Gao, W.F. Emission Characteristics and Influencing Factors of Greenhouse gas in the Continuous Permafrost Region of Daxing'an Mountains, Northeast China. Ph.D. Thesis, Northeast Forestry University, Harbin, China, 2019.
20. Song, Y.Y.; Jiang, L.; Song, C.C.; Wang, X.W.; Ma, X.Y.; Zhang, H.; Tan, W.W.; Gao, J.L.; Hou, A.X. Microbial abundance and enzymatic activity from tussock and shrub soil in permafrost peatland after 6-year warming. *Ecol. Indic.* **2021**, *126*, 107589. [CrossRef]
21. Feng, J.J.; Wang, C.; Lei, J.S.; Yang, Y.F.; Yan, Q.Y.; Zhou, X.S.; Tao, X.Y.; Ning, D.L.; Yuan, M.T.M.; Qin, Y.J.; et al. Warming-induced permafrost thaw exacerbates tundra soil carbon decomposition mediated by microbial community. *Microbiome* **2020**, *8*, 3. [CrossRef]
22. Dong, X.F.; Liu, C.; Wu, X.D.; Man, H.R.; Wu, X.W.; Ma, D.L.; Li, M.; Zang, S.Y. Linking soil organic carbon mineralization with soil variables and bacterial communities in a permafrost-affected tussock wetland during laboratory incubation. *Catena* **2023**, *221*, 106783. [CrossRef]
23. Dong, X.F.; Man, H.R.; Liu, C.; Wu, X.D.; Zhu, J.J.; Zheng, Z.C.; Ma, D.L.; Li, M.; Zang, S.Y. Changes in soil bacterial community along a gradient of permafrost degradation in Northeast China. *Catena* **2023**, *222*, 106870. [CrossRef]
24. Jiang, L.; Song, Y.Y.; Sun, L.; Song, C.; Wang, X.; Ma, X.Y.; Liu, C.; Gao, J.L. Effects of warming on carbon emission and microbial abundances across different soil depths of a peatland in the permafrost region under anaerobic condition. *Appl. Soil Ecol.* **2020**, *156*, 103712. [CrossRef]
25. Ren, J.S.; Song, C.C.; Hou, A.X.; Song, Y.Y.; Zhu, X.Y.; Cagle, G.A. Shifts in soil bacterial and archaeal communities during freeze-thaw cycles in a seasonal frozen marsh, Northeast China. *Sci. Total Environ.* **2018**, *625*, 782–791. [CrossRef] [PubMed]
26. Yang, L.B.; Jiang, Y.B.; Zhou, T.; Cui, F.X.; Zhu, D.G.; Xu, F. Effects of litter fall on soil fungal diversity under snow cover in the Greater Xing'an Mountains. *Res. Environ. Sci.* **2022**, *35*, 1037–1044. [CrossRef]
27. Jiang, Y.B.; Yang, L.B.; Wu, S.; Zhou, T. Warming changes the composition and diversity of fungal communities in permafrost. *Ann. Microbiol.* **2023**, *73*, 7. [CrossRef]
28. Kim, H.M.; Jung, J.Y.; Yergeau, E.; Hwang, C.Y.; Hinzman, L.; Nam, S.; Hong, S.G.; Kim, O.S.; Chun, J.; Lee, Y.K. Bacterial community structure and soil properties of a subarctic tundra soil in Council, Alaska. *FEMS Microbiol. Ecol.* **2014**, *89*, 465–475. [CrossRef] [PubMed]
29. Shang, W.; Wu, X.D.; Zhao, L.; Yue, G.Y.; Zhao, Y.H.; Qiao, Y.P.; Li, Y.Q. Seasonal variations in labile soil organic matter fractions in permafrost soils with different vegetation types in the central Qinghai-Tibet Plateau. *Catena* **2016**, *137*, 670–678. [CrossRef]

30. Hu, W.G.; Zhang, Q.; Li, D.Y.; Cheng, G.; Mu, J.; Wu, Q.B.; Niu, F.J.; An, L.Z.; Feng, H.Y. Diversity and community structure of fungi through a permafrost core profile from the Qinghai-Tibet Plateau of China. *J. Basic Microbiol.* **2015**, *54*, 1331–1341. [CrossRef]
31. Wu, X.D.; Zhao, L.; Liu, G.M.; Xu, H.Y.; Zhang, X.L.; Ding, Y.J. Effects of permafrost thaw-subsidence on soil bacterial communities in the southern Qinghai-Tibetan Plateau. *Appl. Soil Ecol.* **2018**, *128*, 81–88. [CrossRef]
32. Ade, L.J.; Hu, L.; Zi, H.B.; Wang, C.T.; Lerdau, M.; Dong, S.K. Effect of snowpack on the soil bacteria of alpine meadows in the Qinghai-Tibetan Plateau of China. *Catena* **2018**, *164*, 13–22. [CrossRef]
33. Bates, S.T.; Berg-Lyons, D.; Caporaso, J.G.; Walters, W.A.; Knight, R.; Fierer, N. Examining the global distribution of dominant archaeal populations in soil. *ISME J.* **2011**, *5*, 908–917. [CrossRef] [PubMed]
34. Uroz, S.; Buée, M.; Murat, C.; Frey-Klett, P.; Martin, F. Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. *Environ. Microbiol. Rep.* **2010**, *2*, 281–288. [CrossRef]
35. Wang, X.S.; Guan, X.Y.; Zhang, X.J.; Xiang, S.Z.; Zhang, R.R.; Liu, M.Z. Microbial communities in petroleum-contaminated seasonally frozen soil and their response to temperature changes. *Chemosphere* **2020**, *258*, 127375. [CrossRef] [PubMed]
36. Zhang, N.; Chen, K.L.; Wang, H.S.; Yang, Y. Effect of simulated warming on soil microorganism of bird island in Qinghai Lake. *Microbiol. China* **2021**, *48*, 722–732. [CrossRef]
37. Gianoulis, T.A.; Raes, J.; Patel, P.V.; Bjornson, R.; Korbel, J.O.; Letunic, I.; Yamada, T.; Paccanaro, A.; Jensen, L.; Snyder, M.; et al. Quantifying environmental adaptation of metabolic pathways in metagenomics. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 1374–1379. [CrossRef] [PubMed]
38. Steven, B.; Pollard, W.H.; Greer, C.W.; Whyte, L.G. Microbial diversity and activity through a permafrost/ground ice core profile from the Canadian high Arctic. *Environ. Microbiol.* **2008**, *10*, 3388–3403. [CrossRef] [PubMed]
39. Yergeau, E.; Hogues, H.; Whyte, L.G.; Greer, C.W. The functional potential of high Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray analyses. *ISME J.* **2010**, *4*, 1206–1214. [CrossRef] [PubMed]
40. Johnson, S.S.; Hebsgaard, M.B.; Christensen, T.R.; Mastepanov, M.; Nielsen, R.; Munch, K.; Brand, T.; Thomas, M.; Gilbert, P.; Zuber, M.T.; et al. Ancient bacteria show evidence of DNA repair. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 14401–14405. [CrossRef] [PubMed]
41. Spohn, M.; Klaus, K.; Wanek, W.; Richter, A. Microbial carbon use efficiency and biomass turnover times depending on soil depth—Implications for carbon cycling. *Soil Biol. Biochem.* **2016**, *96*, 74–81. [CrossRef]
42. Axelrood, P.E.; Chow, M.L.; Radomski, C.C.; McDermott, J.M.; Davies, J. Molecular characterization of bacterial diversity from British Columbia forest soils subjected to disturbance. *Can. J. Microbiol.* **2002**, *48*, 655–674. [CrossRef]
43. Maestre, F.T.; Delgado-Baquerizo, M.; Jeffries, T.C.; Eldridge, D.J.; Ochoa, V.; Gozalo, B.; Quero, J.L.; García-Gómez, M.; Gallardo, A.; Ulrich, W.; et al. Increasing aridity reduces soil microbial diversity and abundance in global drylands. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 15684–15689. [CrossRef]
44. DeAngelis, K.M.; Allgaier, M.; Chavarria, Y.; Fortney, J.L.; Hugenholtz, P.; Simmons, B.; Sublette, K.; Silver, W.L.; Hazen, T.C. Characterization of trapped lignin-degrading microbes in tropical forest soil. *PLoS ONE* **2011**, *6*, e19306. [CrossRef]
45. DeSantis, T.Z.; Hugenholtz, P.; Larsen, N.; Rojas, M.; Brodie, E.L.; Keller, K.; Huber, T.; Dalevi, D.; Hu, P.; Andersen, G.L. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. *Environ. Microbiol.* **2006**, *72*, 5069–5072. [CrossRef]
46. Wu, L.W.; Zhang, Y.; Guo, X.; Ning, D.L.; Zhou, X.S.; Feng, J.J.; Yuan, M.M.; Liu, S.; Guo, J.J.; Gao, Z.P.; et al. Reduction of microbial diversity in grassland soil is driven by long-term climate warming. *Nat. Microbiol.* **2022**, *7*, 1054–1062. [CrossRef]
47. Sheik, C.S.; Beasley, W.H.; Elshahed, M.S.; Zhou, X.H.; Luo, Y.Q.; Krumholz, L.R. Effect of warming and drought on grassland microbial communities. *ISME J.* **2011**, *5*, 1692–1700. [CrossRef]
48. Chen, Y.L.; Liu, F.T.; Kang, L.Y.; Zhang, D.Y.; Kou, D.; Mao, C.; Qin, S.Q.; Zhang, Q.W.; Yang, Y.H. Large-scale evidence for microbial response and associated carbon release after permafrost thaw. *Glob. Chang. Biol.* **2020**, *27*, 3218–3229. [CrossRef]
49. Griffiths, R.I.; Thomson, B.C.; James, P.; Bell, T.; Bailey, M.; Whiteley, A.S. The bacterial biogeography of British soils. *Environ. Microbiol.* **2011**, *13*, 1642–1654. [CrossRef]
50. Weedon, J.T.; Kowalchuk, G.A.; Aerts, R.; van Hal, J.; van Logtestijn, R.; Tas, N.; Röling, W.F.M.; van Bodegom, P.M. Summer warming accelerates sub-arctic peatland nitrogen cycling without changing enzyme pools or microbial community structure. *Glob. Chang. Biol.* **2012**, *18*, 138–150. [CrossRef]
51. Rocca, J.D.; Simonin, M.; Blaszczak, J.R.; Ernakovich, J.G.; Gibbons, S.M.; Midani, F.S.; Washburne, A.D. The microbiome stress project: Toward a global meta-analysis of environmental stressors and their effects on microbial communities. *Front. Microbiol.* **2019**, *9*, 3272. [CrossRef]
52. Jousset, A.; Bienhold, C.; Chatzinotas, A.; Gallien, L.; Gobet, A.; Kurm, V.; Küsel, K.; Rillig, M.C.; Rivett, D.W.; Salles, J.F.; et al. Where less may be more: How the rare biosphere pulls ecosystems strings. *ISME J.* **2017**, *11*, 853–862. [CrossRef]
53. Rinnan, R.; Michelsen, A.; Bååth, E.; Jonasson, S. Fifteen years of climate change manipulations alter soil microbial communities in a subarctic heath ecosystem. *Glob. Chang. Biol.* **2007**, *13*, 28–39. [CrossRef]
54. Siciliano, S.D.; Palmer, A.S.; Winsley, T.; Lamb, E.; Bissett, A.; Brown, M.V.; van Dorst, J.; Ji, M.K.; Ferrari, B.C.; Grogan, P.; et al. Soil fertility is associated with fungal and bacterial richness, whereas pH is associated with community composition in polar soil microbial communities. *Soil Biol. Biochem.* **2014**, *78*, 10–20. [CrossRef]
55. Feng, Y.Z.; Grogan, P.; Caporaso, J.G.; Zhang, H.Y.; Lin, X.G.; Knight, R.; Chu, H.Y. pH is a good predictor of the distribution of anoxygenic purple phototrophic bacteria in Arctic soils. *Soil Biol. Biochem.* **2014**, *74*, 193–200. [CrossRef]

56. Meyer, S.T.; Ebeling, A.; Eisenhauer, N.; Hertzog, L.; Hillebrand, H.; Milcu, A.; Pompe, S.; Abbas, M.; Bessler, H.; Buchmann, N.; et al. Effects of biodiversity strengthen over time as ecosystem functioning declines at low and increases at high biodiversity. *Ecosphere* **2016**, *7*, e1619. [CrossRef]
57. Zhang, D.Y.; Wang, L.; Qin, S.Q.; Kou, D.; Wang, S.Y.; Zheng, Z.H.; Peñuelas, J.; Yang, Y.H. Microbial nitrogen and phosphorus co-limitation across permafrost region. *Glob. Chang. Biol.* **2023**, *29*, 3910–3923. [CrossRef]
58. Zhang, H.M.; Anders, P.; Samuel, F.; Elberling, B.; Jia, Z.J. Soil microbiomes modulate distinct patterns of soil respiration and methane oxidation in arctic active layer and permafrost. *Acta Microbiol. Sin.* **2017**, *57*, 839–855. [CrossRef]
59. Wang, X.J.; Zhang, Z.C.; Yu, Z.Q.; Shen, G.F.; Cheng, H.F.; Tao, S. Composition and diversity of soil microbial communities in the alpine wetland and alpine forest ecosystems on the Tibetan Plateau. *Sci. Total Environ.* **2020**, *747*, 141358. [CrossRef]
60. Pereira, L.B.; Vicentini, R.; Ottoboni, L.M.M. Changes in the bacterial community of soil from a neutral mine drainage channel. *PLoS ONE* **2014**, *9*, e96605. [CrossRef]
61. Miransari, M. Soil microbes and the availability of soil nutrients. *Acta Physiol. Plant.* **2013**, *35*, 3075–3084. [CrossRef]
62. Mori, T.; Wang, S.H.; Wang, Z.H.; Wang, C.; Mo, H.; Mo, J.M.; Lu, X.K. Testing potassium limitation on soil microbial activity in a sub-tropical forest. *J. For. Res.* **2019**, *30*, 2341–2347. [CrossRef]
63. Moro, H.; Kunito, T.; Saito, T.; Yaguchi, N.; Sato, T. Soil microorganisms are less susceptible than crop plants to potassium deficiency. *Arch. Agron. Soil Sci.* **2014**, *60*, 1807–1813. [CrossRef]
64. Li, H.; Yang, S.; Xu, Z.W.; Yan, Q.Y.; Li, X.B.; van Nostrand, J.D.; He, Z.L.; Yao, F.; Han, X.G.; Zhou, J.Z.; et al. Responses of soil microbial functional genes to global changes are indirectly influenced by aboveground plant biomass variation. *Soil Biol. Biochem.* **2017**, *104*, 18–29. [CrossRef]
65. Pitta, D.W.; Pinchak, W.E.; Indugu, N.; Vecchiarelli, B.; Sinha, R.; Fulford, J.D. Metagenomic analysis of the rumen microbiome of steers with wheat-induced frothy bloat. *Front. Microbiol.* **2016**, *7*, 689. [CrossRef]
66. Shanks, O.C.; Kelty, C.A.; Archibeque, S.; Jenkins, M.; Newton, R.J.; McLellan, S.L.; Huse, S.M.; Sogin, M.L. Community structures of fecal bacteria in cattle from different animal feeding operations. *Appl. Environ. Microbiol.* **2011**, *77*, 2992–3001. [CrossRef]
67. Yadav, A.; Borrelli, J.C.; Elshahed, M.S.; Youssef, N.H. Genomic analysis of family uba6911 (group 18 *Acidobacteria*) expands the metabolic capacities of the phylum and highlights adaptations to terrestrial habitats. *Appl. Environ. Microbiol.* **2021**, *87*, e94721. [CrossRef]
68. Li, Y.Y.; Xu, T.T.; Ai, Z.; Wei, L.L.; Ma, F. Diversity and predictive functional of Caragana jubata bacterial community in rhizosphere and non-rhizosphere soil at different altitudes. *Environ. Sci.* **2023**, *44*, 2304–2314. [CrossRef]
69. Poncin, K.; Gillet, S.; De Bolle, X. Learning from the master: Targets and functions of the CtrA response regulator in *Brucella abortus* and other alpha-*proteobacteria*. *FEMS Microbiol. Rev.* **2018**, *42*, 500–513. [CrossRef]
70. Peng, T.S. Effects of Phosphorus Addition on Soil Nutrient Elements and Microorganisms in Young *Phoebe bournei* Plantations. Master's Thesis, Central South University of Forestry and Technology, Changsha, China, 2022.