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Variations and Mutual Relations of Vegetation–Soil–Microbes of Alpine Meadow in the Qinghai-Tibet Plateau under Degradation and Cultivation

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Abstract: Artificial cultivation had been applied to recover the meadow suffering from serious degradation in the Qinghai–Tibet Plateau. Studies focusing only on the changes in vegetation, soil and microbes along the meadow degradation were insufficient, and artificial cultivation as an important part of succession was always neglected. Here, the variables of vegetation, soil, and soil bacteria are surveyed in four types of alpine meadow in the protected lands of the Qinghai–Tibet Plateau: intact alpine meadow (IAM), moderate degradation alpine meadow (MDAM), extreme degradation alpine meadow (black soil beach (BSB)), and artificial alpine grassland (AAG). The results indicated that degradation and cultivation significantly changed the characteristics of the vegetation community, physicochemical features of the soil, and soil bacterial community diversity. Soil bacteria took a considerably longer time to adapt to degradation and cultivation than vegetation and soil. Compared to IAM and BSB, ADAM and AAG had more specific bacteria identified by ANOVA and LEfSe analysis, implying an unstable state. Combined with vegetation and soil variables, it was speculated that the unstable AAG was not significantly improved from the degraded meadow, and also lagged significantly compared to IAM. Correlation analysis revealed that aboveground biomass, species richness, vegetation coverage, SOC, C/N, BD, WC, and pH were significantly associated with bacterial diversity under community level. Aboveground biomass was an effective indicator for soil bacterial gene copies. Redundancy analysis demonstrated that the soil bacterial community is mainly regulated by the vegetation coverage, Gleason index, Simpson index, TN, TP, and pH under phylum and genus level. Partial mantel test analysis indicated that the physicochemical features of the soil were the most important factor correlating with the soil bacterial community along the degradation and cultivation, compared to other environmental factors.

Keywords: alpine meadow; the Qinghai–Tibet Plateau; vegetation community characteristics; soil physicochemical features; soil bacterial community; degradation; cultivation

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1. Introduction

As the zonal vegetation types, alpine meadows and steppe cover more than 70% of the total area of the Qinghai–Tibet Plateau (QTP) [1]. They not only support livelihoods for the local people, but also provide a significant mass of ecosystem services, such as carbon storage, erosion control, and climate regulation from local to regional scales [2]. Sustained by global climate change and intense human activity, alpine meadows and steppe have been subjected to severe soil degradation [1,3]. The alpine meadows accounting for 38%

of the grassland area and covering more than 700,000 km^2 in the QTP provide greater biodiversity, productivity and are more sensitive to the soil degradation [4,5]. Therefore, the degradation of the alpine meadow can cause a greater loss of biomass and productivity, which will produce a profound effect on local livestock and the regional climate. It is worth noting that more than 30% of alpine meadows have experienced degradation or severe degradation in recent decades, which has been attracting a lot of interest [6].

The changes in vegetation community characteristics directly mirror the degradation of the alpine meadow. Species composition changes, a decline in diversity, and biomass decrease always accompany soil degradation [4,7]. Reduced vegetation coverage resulted in increasing availability of light [8], which decreases soil moisture and causes a ripple effect, such as increases in soil pH and bulk density [9,10], organic carbon and total nitrogen loss [1,11]. Soil microorganisms facilitate the release of nutrients, decomposition of organic matter, and participation in the cycle of carbon and nitrogen [12]. Their short life cycle produces extraordinary sensitivity to the microenvironment, which results in their terrific response to environmental stress and ecological change, especially in the alpine meadow [9,13]. Previous studies have proven that microbial community composition changes accompany soil degradation or degraded patch formation [13,14].

The changes in vegetation community characteristics, soil properties and multifunctionality with the degradation of the alpine meadow have been fully explored in recent decades [5,7,15–20], while variations in soil microorganisms under soil degradation gained preliminary recognition [21]. The extremely degraded alpine meadows, known as "black beach" are always transformed into artificial grassland to improve grass productivity and relieve the grazing pressure on the natural meadow [22,23]. However, as a significant improvement for alpine meadow degradation artificial cultivation was always neglected in previous studies [6,9]. This is not conducive to fully and accurately understanding the changing rules of soil factors in the degradation and cultivation of alpine meadow. Additionally, literature has emerged that offers contradictory findings about similar studies; for example, Luo et al. [24] believed that meadow degradation had no effect on the alpha diversity of soil bacteria, whereas Li et al. [13] found that there was significant change in the alpha diversity during meadow degradation; it was suggested that both vegetation and soil environmental factors affected the composition of the soil microbial community during alpine meadow degradation [9], while vegetation factors were proven to have no effect on the soil bacterial community in alpine meadow [13]. Furthermore, the relationships between soil microbes and factors under different taxonomic levels were always neglected in previous studies. Therefore, more research needs to be executed to thoroughly clarify the change and coupling of environmental factors relating to alpine meadow degradation and artificial cultivation.

This study aimed to determine the changes and paired relations of vegetation–soil–microbes during the degradation and artificial cultivation of alpine meadow. Specifically, we: (1) ascertained the variations in vegetation community characteristics and soil features relating to alpine meadow degradation and artificial cultivation; (2) explored the changes in composition, predicted function, and diversity of soil bacteria; (3) evaluated the paired relations of vegetation–soil–microbes and compared the importance of different environmental factors to regulate the bacterial community under different taxonomic levels. This study provides an important opportunity to advance the understanding of vegetation–soil–microbes relationships in the alpine meadow during degradation and cultivation, and offers some insights for the restoration and management of degraded alpine meadow on the QTP.

2. Materials and Methods

2.1. Sampling Area

The sampling sites were scattered in Dalag County (98°15′29″~100°32′41″ E to 32°36′42″~34°15′20″ N, 4200 m ASL), and situated on the southern part of the source area of the Yellow River and the hinterland of the Qinghai–Tibet Plateau, China (Figure 1).

The area is characteristic of the alpine sub-humid plateau climate, with an annual mean precipitation and temperature of 540.6 mm and −1.3 °C, respectively. Dalag County is one of the typical alpine meadow regions on the Qinghai–Tibet Plateau and is one of the seriously degraded meadow areas on the Yellow River source [25]. Typical vegetation types, including intact alpine meadow (IAM), moderately degraded meadow (MDAM), extremely degraded meadow (black soil beach (BSB)), and artificial alpine grassland (AAG), were adopted. According to the field conditions and previous study [1], the intact alpine meadow (IAM) featured >90% vegetation coverage, averages 5 cm tall, and >80% edible forage coverage; characteristics of approved MADM included 50–70% vegetation coverage and 20–50% edible forage coverage; the vegetation and edible forage coverage of the BSB were below 50% and 5%, respectively. Selected AAG was mainly covered by *Elymus nutans*, and distributed flat land (Table S1).

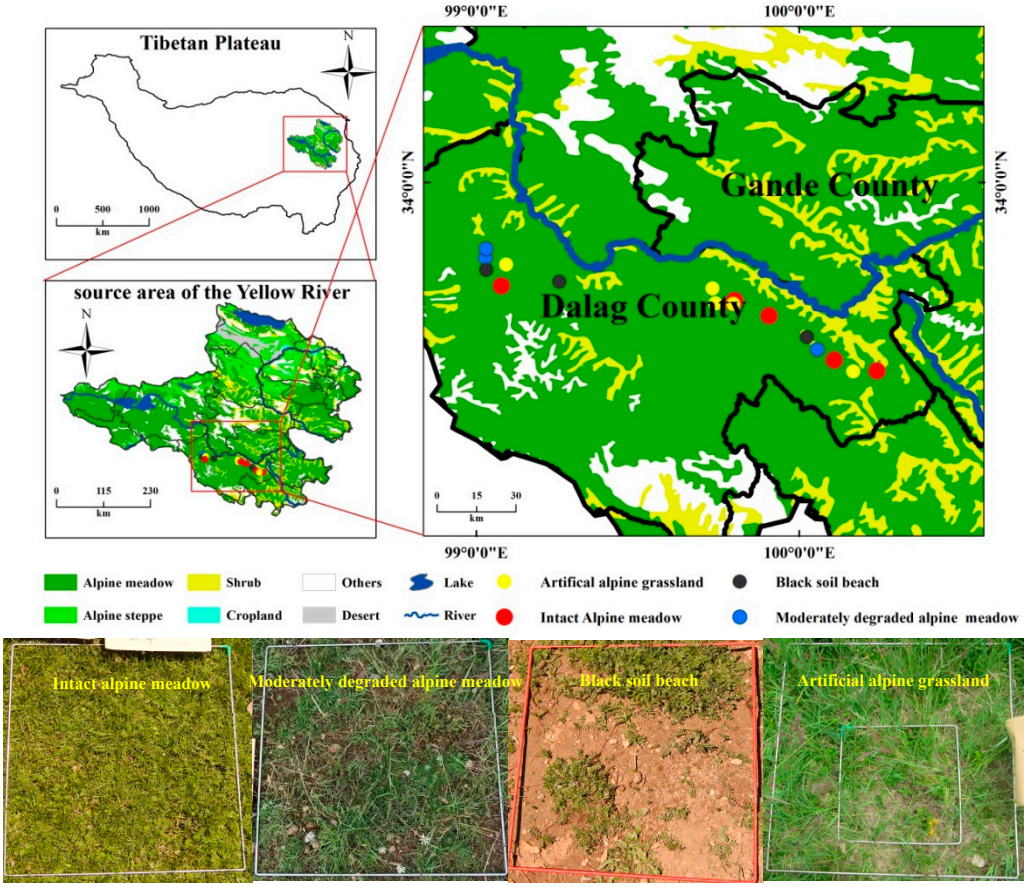

Figure 1. Study sites in the three-river source area of the Qinghai–Tibet Plateau. The border of the Qinghai–Tibet plateau quoted from Zhang et al. [26]; the border of the Three-River-Source National Park quoted from Wei (2018) [27].

2.2. Vegetation Investigation and Soil Sampling

In August 2019, 6–11 typical plots (50 × 100 m) were applied to each style with different degradation and cultivation levels, and three random quadrats (50 × 50 cm) (replicates) about 20 m apart were arranged within each plot. Information including the slope and altitude in each plot was also recorded. In addition, the vegetation coverage, species name, coverage, richness, and height in each quadrat were investigated. Aboveground biomass was also collected and weighted after drying at 65 °C for 24 h. Furthermore, the Shannon–Wiener index, Simpson index, Gleason index, and Pielou index were calculated in terms of the established protocol [28]. After the vegetation investigation, two steel cutting rings (5 cm diameter, 100 cm^3 volume) were employed to determine the soil bulk density and moisture in each quadrat (0–10 cm). One homogenized soil sample from each quadrat

was collected by soil anger with 3.5 cm diameter (0–10 cm). The homogenized samples were sieved (<2 mm) to remove stones, roots and other materials. The sieved sample was divided into two parts; one part was air-dried for physicochemical analysis, and the other part was stored at −20 °C. Three soil samples from three quadrats in the same plot were homogenized to one sample for DNA extraction.

2.3. Soil Physicochemical Analyses

The collected soil was air-dried and texture analyses (>63 mm (sand), 4–63 mm (silt), <4 mm (clay)) were performed using a Mastersizer 2000 laser diffractometer that was capable of analyzing particles with sizes between 0.02 and 2000 mm [29]; soil pH and electrical conductivity (EC) were measured with a pH and conductivity meter in a soil-to-water ratio of 1:2.5; soil organic carbon (SOC; g kg^{-1}) was determined using the dichromate oxidation method [30]; total nitrogen (TN, g kg^{-1}) was measured by the Kjeldahl method [31]; soil total phosphorus (TP) and potassium were determined colorimetrically after wet digestion with H_2SO_4 and $HClO_4$ [6]; soil total potassium (TK) was determined by the flame emission method with a flame photometer [9].

2.4. Soil DNA Extraction and Sequencing

Total genomic DNA in the soil was extracted with the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's instructions. The metagenomic DNA was utilized as a template for quantitative PCR using the Applied Biosystems 7500 sequence detection equipment, in which SYBR green is used to identify the amplicons quantitatively. To check DNA extract 1% agarose gel was used. The universal primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were adopted to amplify the hypervariable region V3-V4 of the bacterial 16S rRNA gene [32]. The PCR amplification was executed as follows: denaturizing for 3 min at 95 °C, followed by 27 cycles of denaturing at 95 °C for 30 s, annealing 30 s at 55 °C, extension 45 s at 72 °C, single extension for 10 min at 72 °C, and ending at 4 °C. After triplicate PCR reactions, the product was extracted from 2% agarose gel, and purified with the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300 bp) on an Illumina MiSeq platform (Illumina, San Diego, CA, USA), and sequenced following the standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The raw reads were deposited into the NMDC Sequence Read Archive (SRA) database (Accession Number: NMDC10017903).

2.5. MiSeq Processing and Bioinformatics Analysis

The original DNA fragments were demultiplexed, quality-filtered by Trimmomatic and FLASH-merged as follows: reads less than 50 bp and containing ambiguous characters were eliminated; reads that could not be assembled were also discarded. Operational taxonomic units (OTUs) with ≥97% similarity were clustered using UPARSE version 7.1 software [33]. Each OTUs was examined by RDP Classifier according to the SSU-rRNA SILVA database with a confidence threshold of 0.7 [9]. The samples were normalized by standardizing with the least data. The QIIME program was used to calculate microbial community diversity indices. Ultimately, a total of 1,530,555 high-quality, chimera-free 16S gene sequences ranged from 41,054 to 61,867 per sample were obtained.

2.6. Statistical Analysis

The descriptive statistics were compiled to form a multi-element database using PASW (IBM Inc., Armonk, NY, USA). A one-way analysis of variation (ANOVA) with Duncan's method was used to compare the data concerning the vegetation and soil bacterial community characteristics and soil physicochemical features among different degradations and under cultivation. Analyses of paired relationships among vegetation community characteristics, soil bacterial community characteristics, and soil physicochemical features were performed using Pearson correlations, which were visualized by Originpro 21 (Origin-

Lab Corporation, Northampton, MA, USA). A Soil Texture Triangle coordinate plot was also performed in Originpro 21. The ANOVA with the least significant difference (LSD) multiple comparisons post-hoc test was applied to characterize the differences in the relative abundance of the same bacteria among different degradations and cultivation. Bacterial data were analyzed by the principal coordinate analysis (PCoA) with Bray–Curtis dissimilarity. Specific species among different groups were identified by using a linear discriminant analysis effect size (LEfSe) method [9]. The effects of vegetation community characteristics and soil physicochemical features on the soil bacterial composition based on different ecological scales were tested by redundancy analysis (RDA) and the Monte Carlo Permutation test, and executed in the vegan package of R [33]. Independent variables with VIF < 10 were analyzed by RDA [11]. Furthermore, the correlations between soil bacterial community Unweighted or Weighted uniFrac distance matrices and the environmental variables distance matrices were measured by using a partial Mantel test in R (vegan package). The bacterial community functions were analyzed and predicted using FAPROTAX (http://www.zoology.ubc.ca/louca/FAPROTAX (accessed on 5 November 2021).

3. Results

3.1. Variations in Vegetation and Soil Variables under Degradation and Cultivation

With the degree of meadow degradation increasing, the frequency and importance value of *Cyperaceae* and *Gramineae* are decreasing, *Asteraceae* and other toxic and noxious species are gradually occupying a principal position, and Gramineae plants such as *Elymus nutans Griseb*, *Poa pratensis* L., and *Puccinellia tenuiflora (Griseb.) Scribn. et Merr*. are the preferred species for artificial cultivation (Table S1). Specially, the vegetation characteristics varied among different levels of degradation and cultivation (Table 1). The coverages are significantly decreased from IAM to BSB, and the AAG coverage is equivalent to the MDAM coverage. The Gleason index is gradually and significantly decreasing in the order of IAM (2.01), MDAM (1.76), BSB (1.21), and AAG (0.89). In terms of Shannon–Wiener index, there is no significant difference between IAM (1.90) and BSB (1.83), and it is significantly higher in MDAM (2.10) and lower in AAG (1.56), while the opposite result is found for the Simpson index. The Pielou indexes do not significantly differ among MDAM (0.80), BSB (0.83), and AAG (0.83). The above-ground biomass of IAM is 253.02 g m^{-2}, which is 3.80, 5.34, 2.87 times higher than that of MDAM (66.51 g m^{-2}), BSB (47.38 g m^{-2}), and AAG (88.10 g m^{-2}).

As for soil physiochemical features, what stands out in the table is the difference between IAM and other types of meadow. The pH in IAM is significantly lower than other types of meadow, while EC, BD, WC, TOC, TN, TP are significantly higher. As two stages of degradation, the levels of all soil physicochemical features in MDAM and BSB do not show significant differences. Closer inspection of the Table 1 shows that some soil physicochemical features, such as EC, WC and TP in AAG, present certain advantages over the degraded meadows (MDAM and BSB). In addition, although the content of TOC in AAG does not show significant differences from the degraded meadows, its mean value (4.09 g kg^{-1}) is slightly higher. It is noteworthy that the content of TP (821.63 mg kg^{-1}) is comparable to the content of IAM (811.58 mg kg^{-1}), and significantly higher than that of MDAM (643.40 mg kg^{-1}) and BSB (697.55 mg kg^{-1}). The content of TK maintains homogeneity among different degradations and cultivation. The results, as shown in Figure S1, indicate that the soil particle size distributions of IAM and AAG are more concentrated than those of MDAM and BSB, being imprisoned in the frames of sandy loam, loam, and silty loam as classified by the international soil classification system.

Table 1. Calculated statistics for vegetation–soil–bacteria variables of the alpine meadow under degradation and cultivation (means ± the standard deviation).

	Variables	IAM	MDAM	BSB	AAG
Vegetation	Coverage (%)	96.42 ± 2.34 a	44.21 ± 15.22 b	31.67 ± 12.13 c	55.56 ± 10.97 b
	Gleason index	2.01 ± 0.31 a	1.76 ± 0.30 b	1.21 ± 0.29 c	0.89 ± 0.27 d
	Shannon–Wiener index	1.90 ± 0.20 a	2.10 ± 0.17 b	1.83 ± 0.18 a	1.56 ± 0.16 c
	Simpson index	0.16 ± 0.04 a	0.11 ± 0.02 b	0.17 ± 0.05 a	0.26 ± 0.05 c
	Pielou index	0.74 ± 0.05 a	0.80 ± 0.03 b	0.83 ± 0.09 b	0.83 ± 0.08 b
	Aboveground biomass (g m^{-2})	253.02 ± 53.80 a	66.51 ± 78.43 b	47.38 ± 30.93 c	88.10 ± 23.39 b
Soil	pH	5.65 ± 0.27 a	6.81 ± 0.58 b	6.67 ± 0.68 b	6.58 ± 0.66 b
	EC (μS cm^{-1})	192.87 ± 112.48 a	100.03 ± 72.68 c	136.11 ± 55.72 bc	144.53 ± 79.13 abc
	BD (g cm^{-3})	0.68 ± 0.20 a	1.20 ± 0.14 b	1.08 ± 0.16 b	1.13 ± 0.19 b
	WC	0.86 ± 0.26 a	0.26 ± 0.09 c	0.37 ± 0.10 bc	0.43 ± 0.15 b
	TOC (g kg^{-1})	9.12 ± 3.04 a	3.17 ± 0.70 b	3.74 ± 1.19 b	4.09 ± 1.04 b
	TN (%)	0.74 ± 0.25 a	0.30 ± 0.06 b	0.40 ± 0.22 b	0.40 ± 0.07 b
	TP (mg kg^{-1})	811.58 ± 147.31 a	643.40 ± 143.75 b	697.55 ± 130.62 b	821.63 ± 226.64 a
	TK (%)	1.95 ± 0.22 a	2.24 ± 0.34 a	2.13 ± 0.34 a	2.08 ± 0.31 a
Soil bacteria	Sobs ($\times 10^{-3}$)	2.70 ± 0.20 a	2.77 ± 0.11 ab	3.07 ± 0.12 c	3.04 ± 0.21 bc
	Shannon	6.28 ± 0.22 a	6.50 ± 0.05 ab	6.62 ± 0.06 b	6.67 ± 1.15 b
	Simpson ($\times 10^{-3}$)	7.65 ± 6.09 a	3.87 ± 0.22 a	3.93 ± 0.35 a	3.49 ± 1.02 a
	Ace ($\times 10^{3}$)	3.74 ± 0.30 a	3.87 ± 0.15 ab	4.24 ± 0.15 b	4.06 ± 0.26 ab
	Chao ($\times 10^{3}$)	3.73 ± 0.29 a	3.87 ± 0.12 a	4.25 ± 0.11 b	4.04 ± 0.24 ab
	Coverage (%)	97.40 ± 0.23 a	97.32 ± 0.11 ab	97.04 ± 0.09 b	97.27 ± 0.21 ab
	Shannon even	0.79 ± 0.02 a	0.82 ± 0.00 b	0.82 ± 0.00 b	0.83 ± 0.01 b
	Simpson even	0.06 ± 0.02 a	0.09 ± 0.01 ab	0.08 ± 0.01 ab	0.10 ± 0.03 b
	Quantity ($\times 10^{9}$ copies g^{-1})	27.30 ± 8.02 a	17.58 ± 1.03 b	23.01 ± 10.17 b	4.32 ± 0.97 c

IAM, intact alpine meadow; MDAM, moderately degraded alpine meadow; BSB, black soil beach; AAG, artificial grassland; EC, electrical conductivity; BD, bulk density; WC, water content; TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium. Different lowercase letters in the same row indicate significant differences ($p < 0.05$).

3.2. Changes in Soil Bacterial Diversity, Composition, Structure, and Predicted Functions

In general, the response of soil bacterial alpha diversity indexes to degradation and cultivation lags behind soil and vegetation variables. Most indexes, except for Simpson's diversity index in IAM, are significantly lower than those of other types of alpine meadow. Similarly to the soil physicochemical features, most of the indexes, except for the Sobs and Chao richness index between MDAM and BSB, do not show significant differences. Sobs and Chao richness in MDAM is comparable to that in IAM, but significantly lower than that in BSB. There is a remarkable result that the alpha diversity indexes in AAG have no significant difference from the degraded meadows (MDAM and BSB). The gene copy number in IAM (2.73×10^{10} copies g^{-1}) is observed to be significantly higher than other types of alpine meadow, and the gene copy number in AAG is the lowest (4.32×10^{10} copies g^{-1}). The result, as presented in Figure 2, demonstrate that the four types of alpine meadow significantly differed from each other based on PCoA with the Bray–Curtis distance (ANOSIM: R = 0.670, $p = 0.001$; Adonis: $R^2 = 0.550$, $p = 0.001$). It can also be seen that the bacterial communities in MDAM and BSB have greater similarity, separately from AAG and IAM.

The bacterial composition and their significant differences at the phylum and genus level (relative abundance > 0.01%) are visually presented in Figure 3. At the phylum level, Proteobacteria is the most abundant in all samples, and the average abundance is not significantly different among different degradations and cultivation, but its average abundance in MDAM (21.38%) is significantly lower than that in IAM (29.10%), BSB (26.93%), and AAG (34.93%). The other phyla with relative abundance >0.01% follow the order: Actinobacteria (10.30–30.47%), Acidobacteria (18.10–24.99%), Chloroflexi (7.72–12.59%), Gemmatimonates (2.44–6.31%), Bacteroidetes (1.80–4.79%), Firmicutes (0.70–6.18%), Rolubacteria (1.40–3.11%), Verrucomicrobia (1.13–2.14%), Nitrosporea (0.76–1.47%), and Patescibacteria (0.27–1.47%). The abundance of some phyla such as Actinobacteria, Chloroflexi, Gem-

matimonates, Bacteroidetes among different groups present significant differences, and higher abundances are identified in AAG and MDAM (Figure 3c). At the genus level, representatives of 44 common genera (56.89–64.71%) are ranked based on the average abundance >0.01. The most interesting aspect is that most genera among the four groups exhibit significant variability in their abundance (Figure 3b). In addition, genera abundance in IAM and MDAM present more significant differences, whereas genera abundance in BSB and MDAM demonstrate less significant variations (Figure 3d).

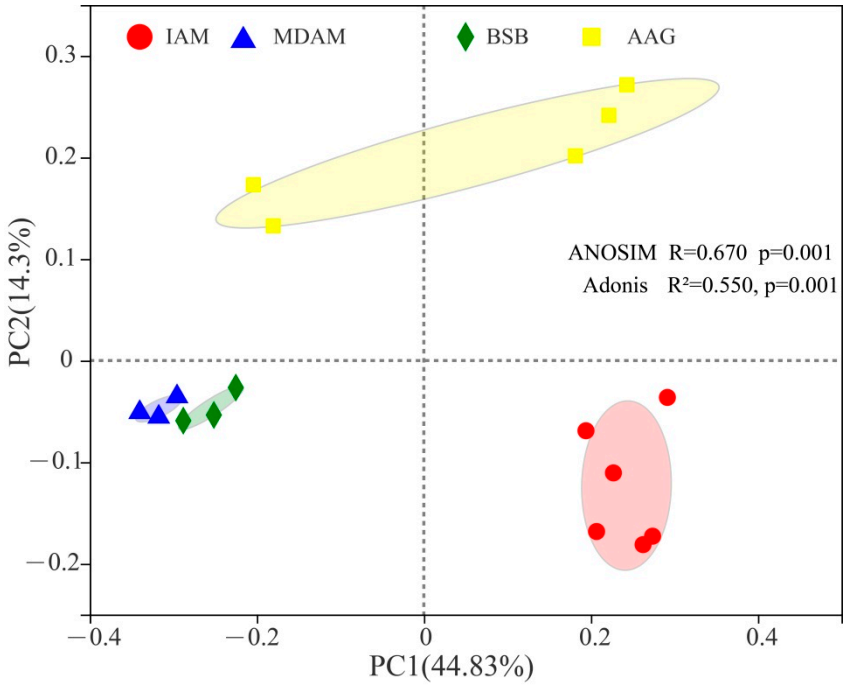

Figure 2. Principal coordinates analysis (PCoA) of bacterial communities in the alpine meadows with different groups based on the Bray–Curtis distances. IAM, intact alpine meadow; MDAM, moderately degraded alpine meadow; BSB, black soil beach; AAG, artificial grassland.

In terms of bacterial structure, IAM has more unique bacteria, followed by AAG, BSB, and MDAM from OTU level to phylum level (Table 2). Moreover, IAM and AAG have more common bacteria, accounting for 59.95%, 26.89%, 19.59%, 14.57%, 12.57%, 7.32, and 3.33% of the total common bacteria, respectively, from OTU level to phylum level. The abundance of common bacteria in different groups is also different. The linear discriminant analysis effect size (LEfSe) is applied to further identify the characterization of the different abundances and their associated categories among different groups (Figure 4). Forty-five bacteria are identified with LDA (linear discriminant analysis) scores over 4.0, exhibiting significant differences among different groups. Most specialized bacteria are significantly numerous in MDAM (15 bacteria) and AAG (17 bacteria), while the minority are numerous in IAM (7 bacteria) and BSB (6 bacteria). More specifically, Gemmatimonadetes (phylum, class, order, family), norank_f_Gemmatimonadaceae (genus), Bacteroidetes (phylum, class), Solibacterales (order), Solibacterales_Subgroup_3 (family), Subgroup_7 (order), and norank_o_Subgroup_7 (family, genus) are numerous in AAG; Gaiellales (order), norank _o_Gaiellales (family, genus), norank_c_Actinobacteria (family, order, genus), Chloroflexi (phylum, class), KD4-96 (class), norank_c_KD4-96 (order, family, genus) are abundant in BSB; Bacilli (class), Bacillales (order), Bacillaceae (family), Bacillus (genus) have the maximum abundance in IAM; Actinobacteria (phylum, class) concentrate in BSB.

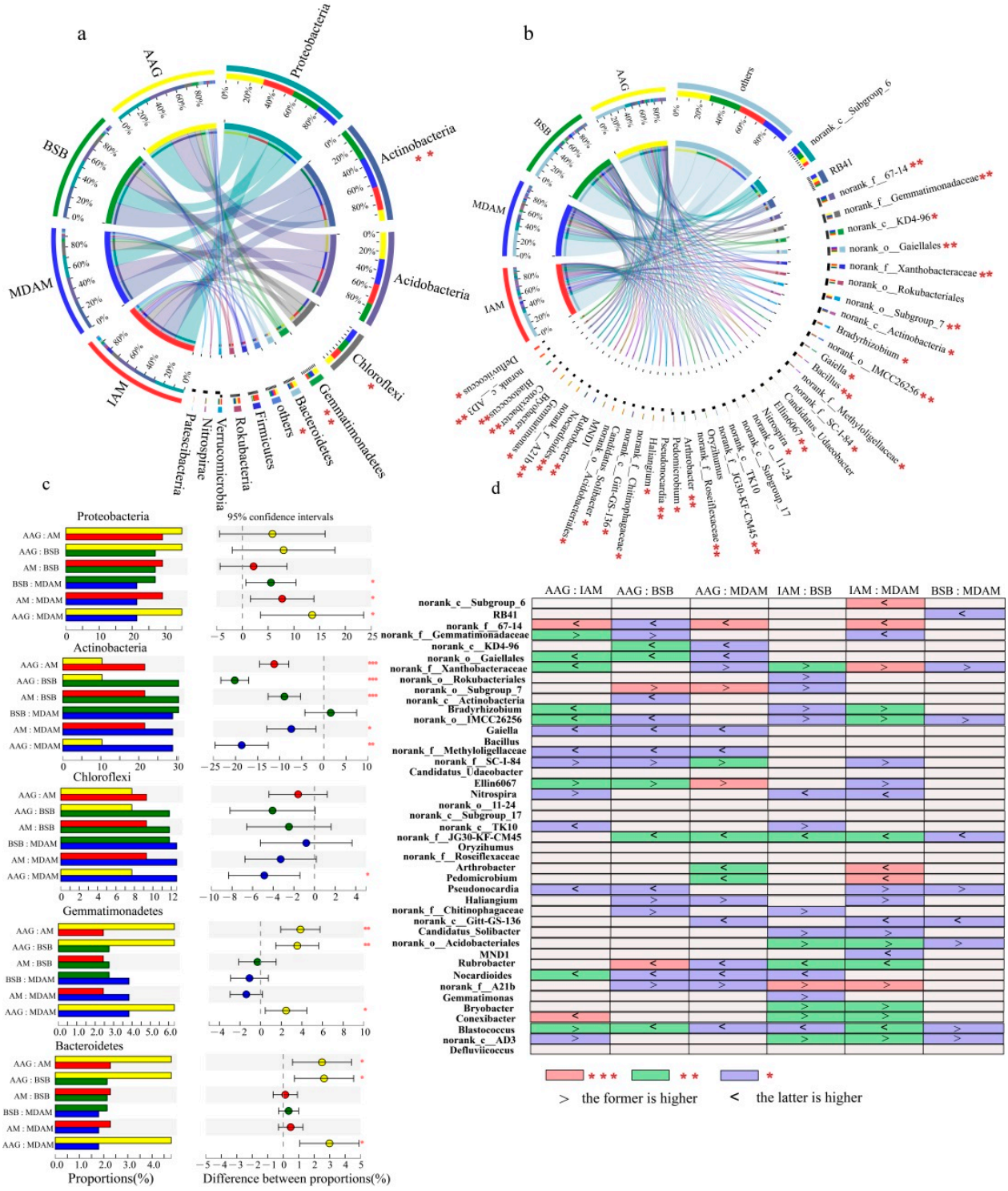

Figure 3. The relative abundance of the dominant bacterial (**a**,**b**) at the phyla and genus level and their composition variations (**c**,**d**) in alpine meadows with different groups. Phyla or genera with a relative abundance of less than 0.01 in all samples were classified as others. *** <0.001; ** <0.01; * <0.05. IAM, intact alpine meadow; MDAM, oderately degraded alpine meadow; BSB, black soil beach; AAG, artificial grassland.

Table 2. Distribution of total bacteria, unique, and common in different groups.

Levels	IAM	MDAM	BSB	AAG	Common	AAG&IAM
Phylum	35 (2)	32 (0)	34 (0)	37 (1)	30	1
Class	98 (3)	88 (1)	94 (1)	104 (2)	82	6
Order	251 (16)	214 (2)	232 (4)	268 (11)	191	24
Family	429 (34)	347 (4)	379 (4)	439 (19)	302	44
Genus	748 (60)	589 (12)	654 (13)	773 (37)	485	95
Species	1643 (138)	1271 (26)	1408 (45)	1713 (89)	978	263
OTU	5477 (997)	4092 (254)	4567 (283)	6072 (636)	2070	1241

The number in brackets indicates the unique bacteria in different groups; IAM, intact alpine meadow; MDAM, moderately degraded alpine meadow; BSB, black soil beach; AAG, artificial grassland.

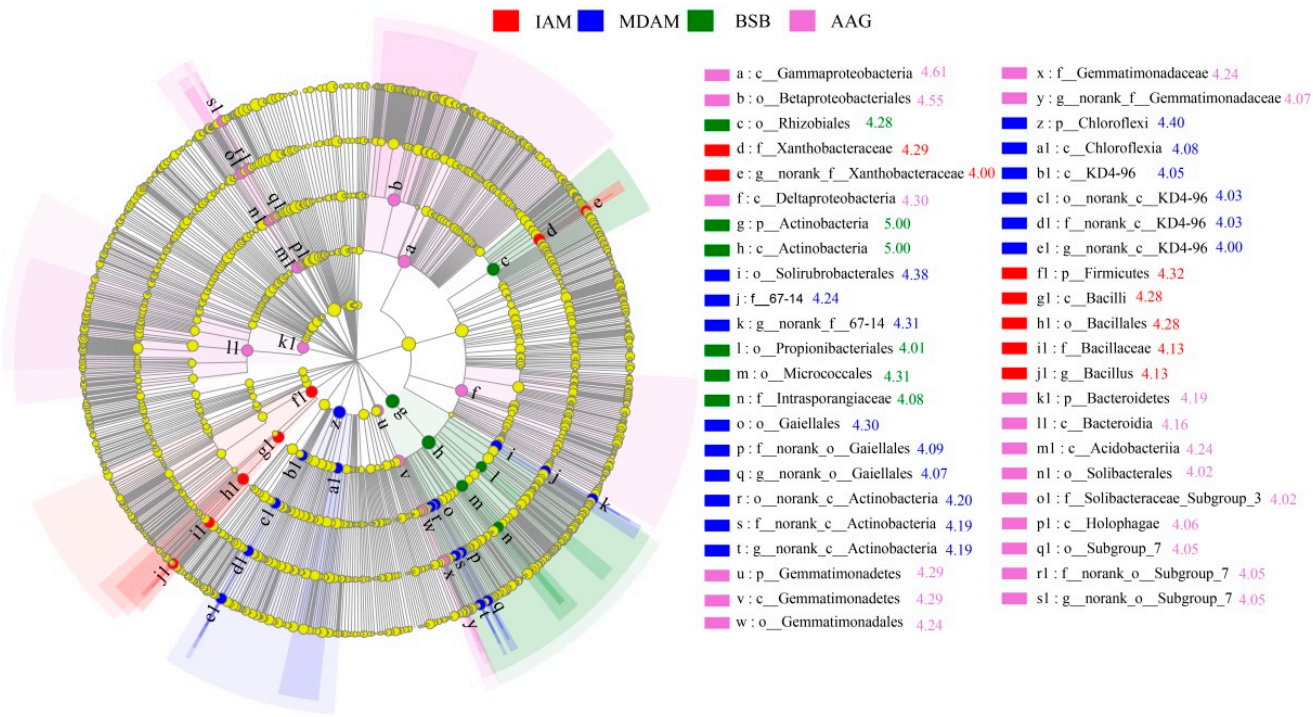

Figure 4. Linear discriminant analysis effect size (LEfSe) analysis of microbial abundance in different types of alpine meadow, identified with a threshold value of 4.0. The LDA scores are listed after the legend. IAM, intact alpine meadow; MDAM, moderately degraded alpine meadow; BSB, black soil beach; AAG, artificial grassland.

The function proportions of the bacteria in soils among different groups are predicated based on the Functional Annotation of Prokaryotic Taxa (FAPROTAX 1.2.4) database. The functions related to the carbon and nitrogen cycle, human pathogens, and plant pathogens are main interests in this study. The proportions of all selected functions except 'plant_pathogen', 'human_pathogens', 'nitrate_denitrification' are significantly different among different groups (Figure 5). With the degradation of the alpine meadow, chemoheterotrophy and aerobic chemoheterotrophy show a significant upward trend, and nitrate reduction shows a trend of first increasing then decreasing. AAG shows more remarkable advantages in nitrification and aerobic ammonia oxidation, and IAM exhibits great strength in nitrogen fixation. AAG is roughly comparable to IAM in aerobic ammonia oxidation, phototrophy, photoautotrophy, nitrate respiration, and methanotrophy, but has significant advantages over degraded meadows.

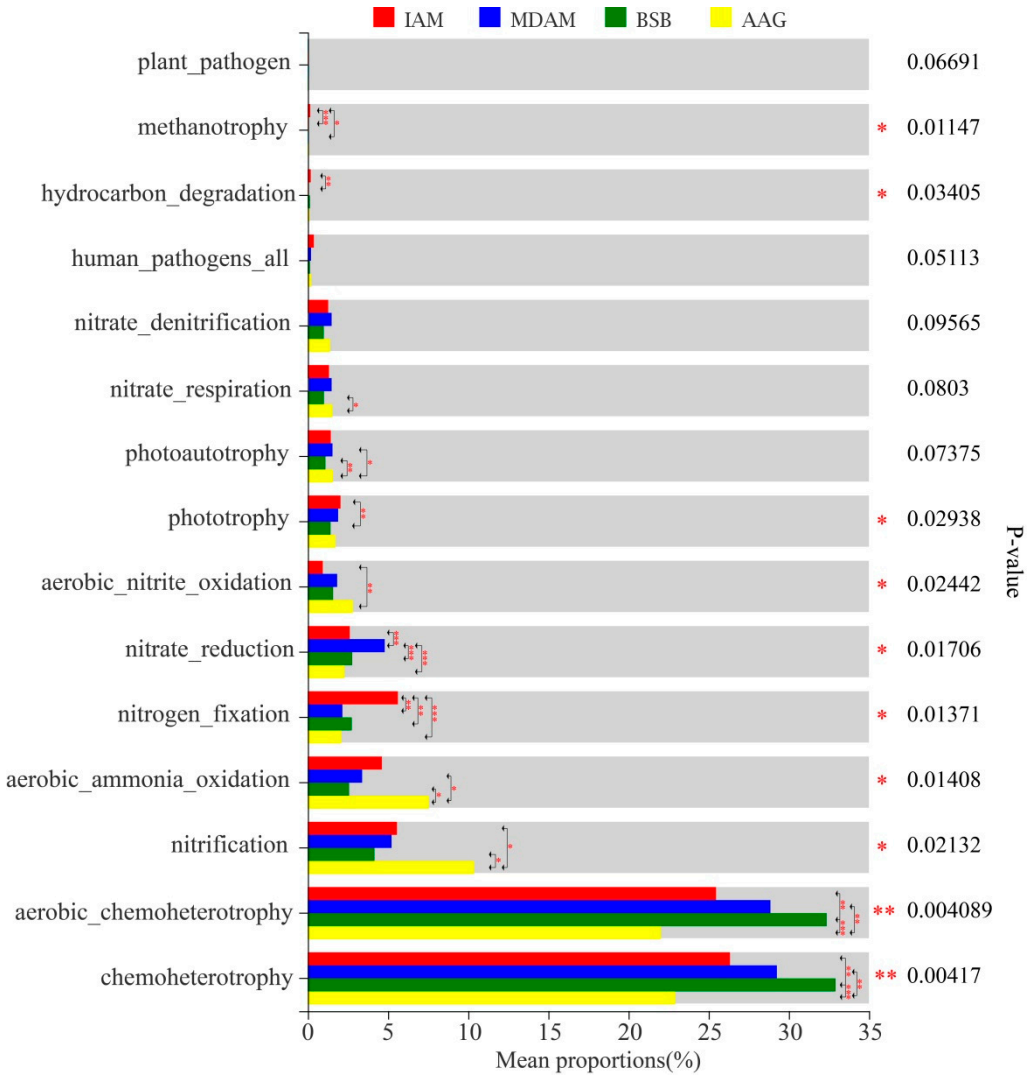

Figure 5. Comparison of the predicted microbial functions among different types of alpine meadows based on the FAPROTAX database (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$); IAM, intact alpine meadow; MDAM, moderately degraded alpine meadow; BSB, black soil beach; AAG, artificial grassland.

3.3. Relationships between Microbial Community and Environmental Variables under Different Taxonomic Level

The inter- and intra-relationships among vegetation–soil–bacteria variables are shown in Figure 6. It is apparent that intra-group correlations are stronger than inter-group correlations. The inter-relationships should be more concerned. The vegetation diversity indexes do not show significant correlation with the corresponding microbial alpha diversity indexes, except richness indexes. A significant negative correlation was found between species richness, the Gleason index for vegetation community, and the Sobs and Chao richness index for microbial communities. In addition, there is a significant correlation between vegetation above-ground biomass, and most microbial alpha diversity indexes, indicating that it is an important impact factor at the microbial community scale. Closer inspection of the figure shows that gene copy number has a significant positive correlation with vegetation above-ground biomass. Further analysis reveals that soil pH, WC, and C/N are important factors significantly correlating with microbial alpha diversity indexes. Meanwhile, the Shannon and Simpson diversity index and the Shannon evenness index are the most sensitive factors significantly correlating with the soil physicochemical features. Gene copy number has a significant positive correlation with TN, TOC and WC, and a negative correlation with soil BD and pH. Vegetation coverage and above-ground biomass

are closely related to the majority of soil physicochemical features. Together, these results provide important insights that soil pH, WC, TN, TOC, C/N, BD, vegetation coverage, and above-ground biomass are the important factors closely associated with microbial diversity and biomass at the community level.

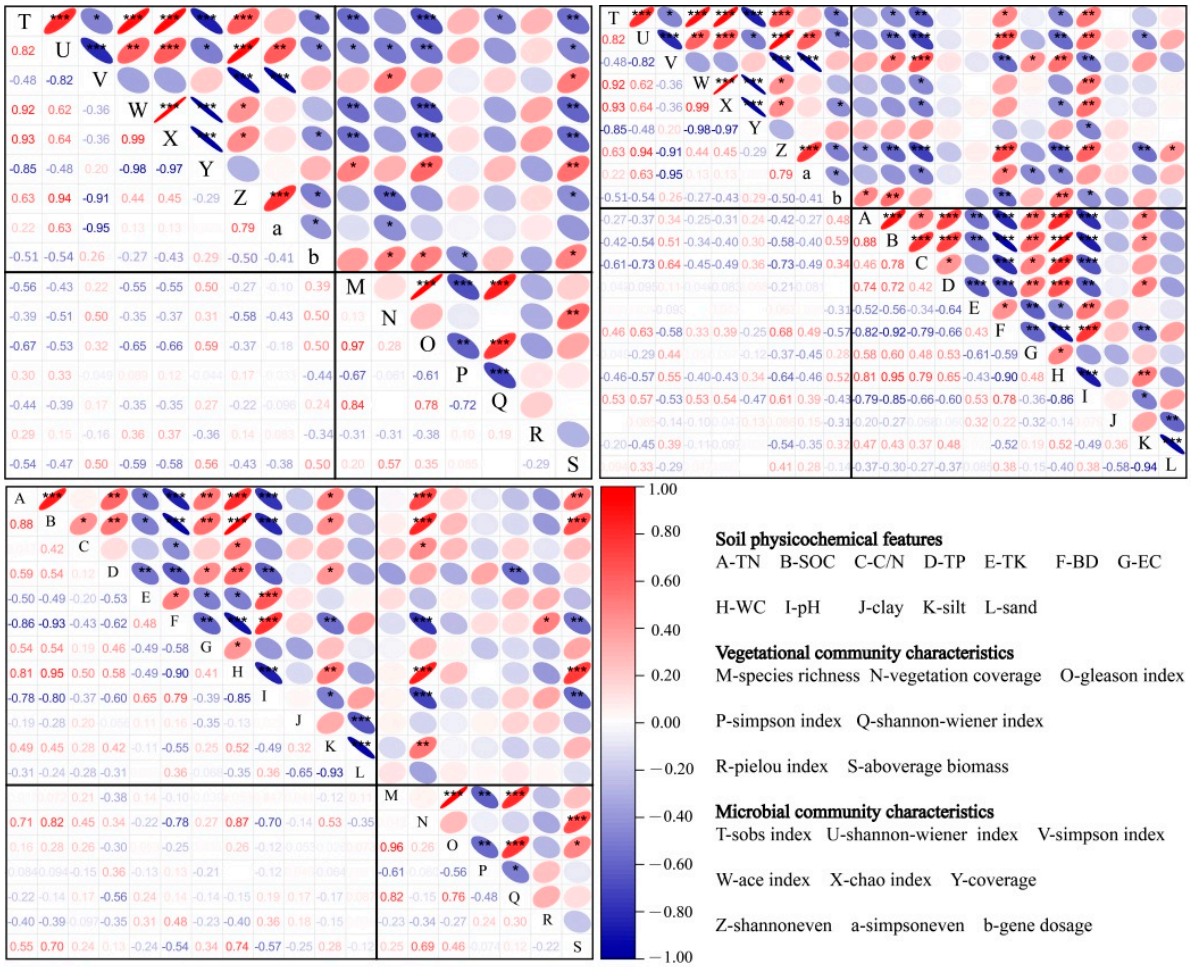

Figure 6. Correlation analysis among soil physicochemical features, vegetation community characteristics, and microbial community characteristics of the alpine meadow. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. IAM, intact alpine meadow; MDAM, moderately degraded alpine meadow; BSB, black soil beach; AAG, artificial grassland; EC, electrical conductivity; BD, bulk density; WC, water content; TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium.

The effects of the environmental variables on the microbial community are explained by a redundancy analysis (RDA) based on the phylum and genus level (Figure 7). At the phylum level, two coordinate axes explain 47.12% and 18.70% of the variation, respectively. Gleason and Simpson index of the vegetation community and TP are the key factors affecting the composition of bacterial communities significantly. At the genus level, two coordinate axes only represent 27.94% and 16.67% of the variation. Coverage and the Simpson index of the vegetation community and TN, TP, and pH in soils were significantly associated with bacterial communities. Furthermore, the partial Mental test was applied to explore the relationships between environmental matrixes and bacterial communities at the phylum and genus level (Table 3). Soil physicochemical features are significantly associated with bacterial community composition across all samples. In contrast, bacterial community distance is not significantly correlated with vegetation community characteristics, topographic factors, and the combination of vegetation community characteristics and topographic factors when soil physicochemical features are used as a control based on

Weighted UniFrac distance. Therefore, minor differences in bacterial community compositions are generally observed among pairs of samples with the close soil environment. It is also apparent that vegetation community characteristics have closer relationships with bacterial communities than the topographic factors (Table 3). Closer inspection of the table shows that the correlations between soil physicochemical features, vegetation community characteristics and bacterial community are more pronounced at the genus level than that at the phylum level, but the opposite result is detected from the correlation between the topographic factors and bacterial community.

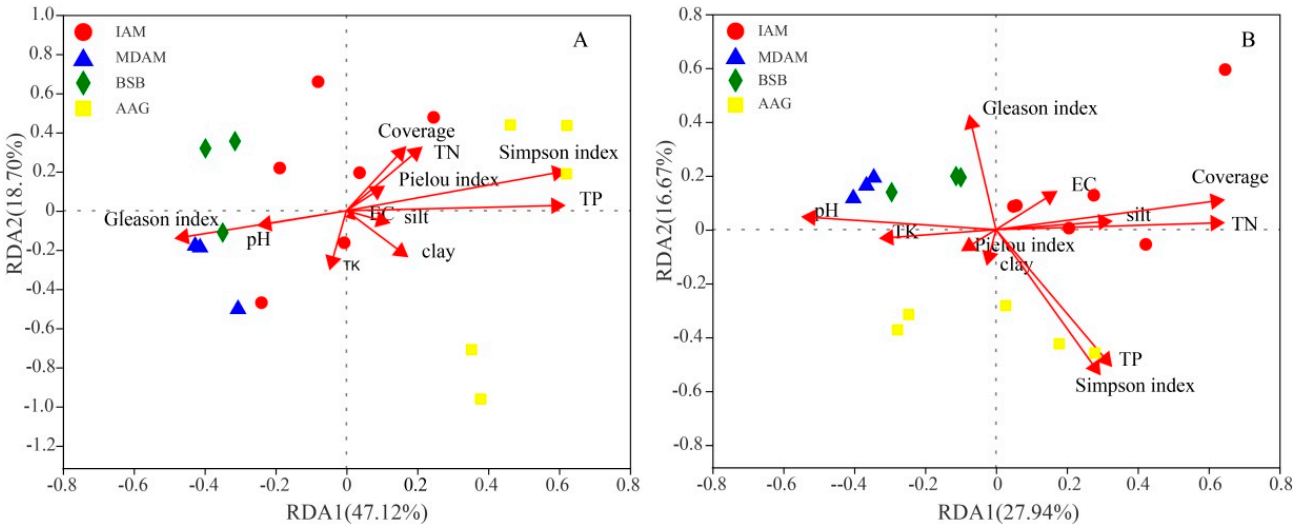

Figure 7. Redundancy analysis (RDA) of soil microbial communities and environmental factors based on phyla level (**A**) and genus level (**B**) in alpine meadow. The red arrows represent the direction and significance of environmental factors with microbial community structures. IAM, intact alpine meadow; MDAM, moderately degraded alpine meadow; BSB, black soil beach; AAG, artificial grassland; EC, electrical conductivity; TN, total nitrogen; TP, total phosphorus; TK, total potassium.

Table 3. The results of correlation between soil bacterial community UniFrac distance and environmental variables using partial Mantel test analysis under phyla level and genus level.

		Control Factor	Weighted UniFrac Distance		Unweighted UniFrac Distance	
			r	*p*-Value	r	*p*-Value
phyla	S	V + T	0.4515	0.004	0.2910	0.011
	V	S + T	0.1339	0.144	0.2246	0.033
	T	S + V	−0.0852	0.785	0.2543	0.008
	V + T	S	−0.0335	0.592	0.3211	0.005
	S + T	V	0.3103	0.011	0.3504	0.004
	S + V	T	0.4664	0.001	0.3420	0.002
Genus	S	V + T	0.5464	0.001	0.4636	0.001
	V	S + T	0.2189	0.068	0.3664	0.003
	T	S + V	−0.0567	0.654	−0.0186	0.531
	V + T	S	0.0403	0.334	0.2064	0.027
	S + T	V	0.4011	0.002	0.3781	0.003
	S + V	T	0.5826	0.001	0.5356	0.001

S, soil physicochemical features; V, vegetation community characteristics; T, topographic factors. *p*-values are based on 9999 permutations.

4. Discussion

4.1. Degradation and Cultivation Significantly Changes the Biotic and Abiotic Components

Changes in the dominant species and vegetation coverage were the preferred evidence for identifying vegetation degradation [1]. With the meadow degradation, the sedge was

gradually replaced by miscellaneous and toxic grasses (Table S1) [4]. It was found that the coverage, species richness, and aboveground biomass significantly decreased with meadow degradation [34], which was verified in our study (Table 1). However, the Shannon–Wiener index and Simpson index had the highest and lowest levels in MDAM. This result might be explained by the fact that the importance value of each vegetation species was equivalent and there was no absolute competitive ability of any vegetation species in the community. It had been suggested that the moderate degradation stage was a transitional point for meadow degradation, with this transition mainly mirrored in the soil physiochemical features (Table 1) [35,36]. The level of all investigated physiochemical features dramatically changed before the MDAM stage and remained stable after the MDAM, except TK (Table 1). This result might be explained by the fact that the increased sand content and decreased clay content in the soil reduced its moisture retention capacity and denitrification with the onset of degradation, resulting in leaching losses of soil nutrition (Table 1) [21,37]. Artificial cultivation was used as an available restoration strategy for improving plant and soil resilience in degraded meadow and alleviating the conflict of grass storage on the Qinghai–Tibet Plateau [38]. Xing et al. [39] showed that artificial cultivation significantly improved the vegetation and soil environment compared to BSB. In this study, the improvement was insignificant and mainly occurred in the soil environment. Gao et al. [40] believed that highly degraded grassland could be restored by revegetated grassland through 16–18 years' recovery. Moreover, the improvements were inconsistent between soil physicochemical features, vegetation community characteristics, and soil quality lagged behind the vegetation quality under restoration (Tables 2 and 3) [40].

Soil bacteria are important in the natural cycle of organic matter, soil formation, and soil fertility [41]. The composition and structure of soil bacteria will ultimately affect these functions [42]. In this study, similar dominant bacteria were also found as in the previous studies, regardless of relative abundance discrepancies [9,13]. The relative abundance of dominate soil bacteria including Proteobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, and Bacteroidetes significantly varied suffered from degradation and cultivation. Proteobacteria, widely recognized as copiotrophic bacteria, had the lowest abundance in MDAM possibly due to the low-fertility and alkaline soil in a cold environment (Figure 3c; Table 1) [14,43]. The relative abundance of Actinobacteria, which prefer to survive and reproduce in a neutral or alkaline environment, decreased with degradation and cultivation (Figure 3) [6,9,36]. The significant change in Chloroflexi mainly occurred between MDAM and AAG (Figure 3c). The reason for this was not clear, but it might have something to do with oxidation of inorganic carbon and degradation of macromolecules such as cellulose [44]. The variation of Gemmatimonadetes was consistent with Bacteroidetes and mainly concentrated in AAG (Figures 3 and 4). Deuruyn et al. [45] believed that Gemmatimonadetes preferred environments with low soil moisture, while Bacteroidetes preferred anaerobic environments with high moisture [46]. Therefore, we deduced that these two bacteria concentrated in AAG were possibly related to the specific nitrogen utilization in artificial grassland independent of soil moisture [47,48]. Other dominant bacteria such as Acidobacteria, Firmicutes, Rokubacteria, Verrucomicrobia, Nitrospire, and Patescibacteria, possibly characteristic of wider ecological amplitude and higher interference resistance, were stable in the process of succession [6,11]. At the genus level, the relative abundance of most of soil bacteria differed throughout the four alpine grasslands, indicating a filtering effect of the environmental conditions (Figure 3b) [41]. This also suggested that bacteria at the genus level were more sensitive to the environmental conditions compared to those at the phylum level. MDAM and AAG had more significantly differentiated species, possibly due to the unstable environment in these stages (Figures 3b and 4). Previous studies paid more attention to the differentiated species of shared bacteria among different environments [9,13,41]. The unique bacteria were also identified among different succession stages in our study. Although AAG had more differentiated species, it also had more unique bacteria regardless of different taxonomic units (Table 2). Epsilonbacteraeota was identified as a unique phylum in the AAG, which was

characteristic of autotrophic, motile, thermophilic and possibly absorbed nitrogen from ammonium taken up from the environment or created from ambient nitrate and nitrite by utilizing a range of function redox modules [49]. In addition, AAG and IAM covered more common bacteria regardless of taxonomic units, indicating a possibly improving microbial environment in the AAG (Table 2). Microbial functional genes are more sensitive to environmental disturbance [50,51]. Consistent with the previous studies, Chemoheterotrophy, aerobic chemoheterotrophy, and nitrification were the dominant functional bacteria groups under degradation and cultivation (Figure 5) [9,13]. With the degradation, the proportions of chemoheterotrophy, aerobic chemoherotrophy, and methanotrophy increased and carbon was reduced (Figure 6; Table 1). However, the drastic increase occurred between the IAM and MDAM stages and moderately increased subsequently, consistent with the variations in most of soil physicochemical features [13]. It was also found that IAM had the highest nitrogen fixation capacity (Figure 5). These findings suggested the superiority of IAM in carbon and nitrogen fixation [3,23,52]. The abundance of nitrification, aerobic ammonia oxidation, and aerobic nitrite oxidation increased at AAG, which facilitated the formation and accumulation of nitrogen assimilated by crops [52]. An implication of this was the possibility that artificial cultivation improved the geochemical cycling of nitrogen. Degradation had potential effects on bacteria involved in nitrate reduction and MDAM was an inflection point for this function (Figure 5). The high abundance of nitrogen reduction functional bacteria suggested soil nitrogen emissions [53]. Therefore, MDAM was possibly the key stage for nitrogen loss under meadow degradation and required more attention. Therefore, the restoration of degraded meadows should be arranged before the MDAM stage.

4.2. The Relationships among the Biotic and Abiotic Factors under Different Taxonomic Level

The correlation analysis suggested interactions among soil physicochemical features, vegetation community characteristics, and bacterial diversities at the community level (Figure 6). TN, SOC, BD, and WC interacted with the vegetation coverage and above-ground biomass. Degradation of vegetation coverage increased the loss of TN and SOC stocks [23]. Grazing or rodent activity reduced aboveground biomass and impeded root system growth, resulting in a reduction in SOM inputs [54]. BD was a necessary indicator for predicting TN and SOC, and was affected by vegetation coverage and above-ground biomass [23]. Degradation directly resulted in the synergistic variation of vegetation coverage and WC [55]. The above indicators, pH, and the Gleason index were also found to interact with soil bacterial diversities and biomass (Figure 6). Previous studies had verified that SOC, WC, BD, and pH had a high explanatory power for soil bacterial communities [33,41]. Above-ground biomass, species richness, and coverage of vegetation community also significantly affected the soil bacterial community [5,56]. Gene copy numbers, widely used to represent absolute microbial abundance, decreased accompanied by meadow degradation [57,58]. Interestingly, there was a positive correlation between the gene copy numbers of soil bacterial communities and vegetation above-ground biomass ($p < 0.05$). Correlation was also significantly positive between vegetation coverage, the Gleason index, TN, TOC, WC, and negative with the Simpson index, pH, and BD.

Correlations between environmental factors and soil bacterial compositions were changed at the phylum and genus level (Figure 7). This finding was also reported by Zhou et al. [6]. It was found that only the Gleason index, Simpson index, and TP were significantly related to the soil bacterial structure at the phylum level, while vegetation coverage, Simpson index, TN, TP, and pH were closely associated with the soil bacterial structure at the genus level ($p < 0.05$). Other environmental factors, such as SOC, BD, and WC, were not key environmental factors for the soil bacterial structure [2]. In accordance with the present results, previous studies have demonstrated that TP was the key factor of microbial growth [59,60]. Another implication from our result was that the distribution of plant species in different communities resulted in different soil bacterial compositions. More pronounced environmental factors were detected at the genus level, possibly due to greater variability of soil bacterial species among different habitats (Figure 5). These

driving factors identified in this study were not exactly consistent with other studies. This discrepancy might be due to the different study sites, sampling times, and study areas with complex interactions of environmental factors [6,9]. The composition of the soil microbial community was affected by vegetation, soil, and topographic characteristics [6,61]. Furthermore, the effect of soil physicochemical features on the soil microbial community was always considered to outweigh the effect of vegetation community characteristics [5,9]. In this study, topographic factors were also considered due to sampling sites with varied topographic factors. Soil physicochemical features had closer relations than vegetation community characteristics on soil bacterial communities, regardless of the phylum or genus level, which also broadly supported the work of other studies in this area [9,21]. Although vegetation destruction was the initial symptom of meadow degradation, soil nutrients varied more than other environmental variables and became the crucial driver of soil microbial community composition change [9,13]. Zhang et al. [62] also concluded that there was a lag effect of vegetation characteristics on soil bacterial communities. Moreover, topographic factors had the least relation with soil bacterial communities in comparison to soil and vegetation characteristics. The relationship between topographic factors and the soil bacterial community was equivalent to an indirect interaction. Topography modified soil bacterial communities predominantly through its effect on the soil's physicochemical features [63].

5. Conclusions

Meadow degradation and artificial cultivation greatly changed the vegetation, soil, and microbe variables. Synchrony existed between the deterioration of soil vegetation before the MDAM stage. Improved soil quality lagged behind the vegetation quality after cultivation. Soil bacteria response to degradation and cultivation lagged behind soil and vegetation variables. The soil bacterial community structures were altered dramatically with the levels of degradation and cultivation. The dominant bacteria among different degradation and cultivation levels were similar with significantly differentiated relative abundance. A one-way analysis of variation (ANOVA) and linear discriminate analysis (LDA) effect size (LEfSe) analysis indicated that MDAM and AAG were the transitional and unstable stages for meadow reverse succession, and had more specific bacteria. One of the more significant findings to emerge from this study was that AAG also had prominent unique bacteria and more bacteria in common with IAM. FAPROTAX prediction analysis suggested that bacterial function in MDAM had potential effects on nitrate reduction, while bacteria function in AAG facilitated the formation of nitrate easily assimilated by plants. The unstable AAG was not significantly improving from degraded meadow, and also lagged a large gap to IAM. The relationships among the biotic and abiotic factors under different taxonomic levels varied. Above-ground biomass, species richness, vegetation coverage, SOC, C/N, BD, WC, and pH were significantly correlated with soil bacterial diversities. Above-ground biomass was an effective indicator for predicting soil bacterial biomass. The soil bacterial compositions were mostly connected to the TP and Simpson index when both phylum and genus levels were considered. Moreover, soil factors had closer relations with soil bacterial communities than vegetation and topographical factors regardless of phylum or genus levels.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/land11030396/s1; Figure S1: Soil texture triangle coordinates of different types of alpine meadow, based on the international soil classification system; Table S1: Basic information of the alpine meadow under degradation and cultivation.

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