Canadian Journal of Microbiology
Revue canadienne de microbiologie

Soil microbial communities of three grassland ecosystems in the Bayinbuluke, China

Journal:	<i>Canadian Journal of Microbiology</i>
Manuscript ID	cjm-2017-0585.R1
Manuscript Type:	Note
Date Submitted by the Author:	21-Nov-2017
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Is the invited manuscript for consideration in a Special Issue? :	N/A
Keyword:	Bayinbuluke alpine grassland, Illumina amplicon sequencing, microbial community composition, degradation, soil

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1 **Soil microbial communities of three grassland ecosystems in the Bayinbuluke,**
2 **China**

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Abstract: The microbial community plays an important role in soil nutrient cycles and energy transformations in alpine grassland. In this study, we investigated the composition of soil microbial community collected from alpine cold swamp meadow (ASM), alpine cold meadow (AM), and alpine cold desert steppe (ADS) within the Bayinbuluke, China using Illumina amplicon sequencing. Of the 147,271 sequences obtained, 36 microbial phyla/groups were detected. The results showed that the ADS had lower microbial diversity than the ASM and AM, as estimated by the Shannon index. The *Verrucomicrobia*, *Chloroflexi*, *Planctomycetes*, *Proteobacteria*, and *Actinobacteria* were the predominant phyla in all three ecosystems. Particularly, *Thaumarchaeota* was only abundant in ASM, *Bacteroidetes* in AM, and *Acidobacteria* in ADS. Additionally, the predominant genus also differed with each ecosystem. *Candidatus Nitrososphaera* was predominant in ADS, the *Pir4* lineage in ASM, and *Sphingomonas* in AM. Our results indicated that the soil microbial community structure were different for each grassland ecosystem in the Bayinbuluke.

Key words: Bayinbuluke alpine grassland, Illumina amplicon sequencing, microbial community composition, degradation, soil

Alpine grassland ecosystems are considered valuable not only for their functions in carbon sequestration but also for biodiversity maintenance and soil and water conservation (Wen et al. 2013). In the past 50 years, many of these grasslands have degraded (Dong et al. 2012; Li et al. 2014). During the degradation process, alpine cold swamp meadow (ASM), alpine cold meadow (AM), and alpine cold desert steppe (ADS) become the three main subsystems in alpine grassland (Zhang et al. 2014). Because of the important role microorganisms play in the soils of alpine grassland, the number and relative abundance of microbes are fundamental to quantitative description of the system, as is true for any complex system (Gans et al. 2005). Consequently, a more comprehensive study of soil microbial diversity and community structure of the different ecosystems in alpine grassland are highly relevant.

Bayinbuluke alpine grassland (42°10'–43°30'N, 82°32'–86°15'E), a typical alpine grassland in temperate arid zone of China, is located in the middle of the southern slope of the Tianshan Mountains in Xinjiang Uygur autonomous region, China (He et al. 2014). Overgrazing during the last decades resulted in grassland vegetation deterioration and herbage yield decline in the alpine grassland. The regional climate is a typical alpine climate, and the annual average precipitation reaches 265.7 mm, with 78.1% occurring during the growing season. The annual average temperature is –4.8 °C, with a lowest monthly value of –27.4 °C in January and a highest monthly value of 11.2 °C in July (He et al. 2014). Previous study has investigated fluxes of methane, carbon dioxide, and nitrous oxide in the grassland (He et al. 2014); however, the soil microbial communities in the grassland have not been previously investigated. Therefore, the comprehensive investigation of the soil microbial community in the alpine grassland is important for controlling the development and succession of grasslands at the microcosmic level.

The aim of this study was to characterize the soil microbial community composition of the three grassland ecosystems (ASM, AM, and ADS) in the Bayinbuluke, China, using Illumina amplicon sequencing. We worked at 3 sampling sites in the grassland, representing each ecosystem (ASM, AM, and ADS) were

56 located about 0.5 km apart (Fig. S1). At each site, three replicated soil cores (0–20
57 cm), located about 2 m apart, were collected on 15 June 2014 using an 8 cm diameter
58 soil auger, as were surface soil samples (0–5 cm) for each core using a slicing disc.
59 After removing plant residues and gravels, the samples were gently stored in ice bags
60 and transported to the laboratory where each sample was divided into two. One
61 subsample was stored at –80 °C for DNA extraction, and the remainder was air-dried
62 and used for determination of physicochemical properties. The vegetation coverage
63 and dominant species investigated in the three subsystems (ASM, AM, and ADS;
64 Table S1) show that the community diversity of ASM and AM was higher than that of
65 ADS, and the coverage was 100%, 60%, and 10%, respectively. Geochemical and
66 field data for the 3 sampling sites are presented in Table S2. Then, the microbial DNA
67 was extracted using the MO BIO Power Soil DNA Extraction Kit (MO BIO
68 Laboratories, Carlsbad, CA). The V5–V6 hypervariable regions of the 16S rRNA gene
69 were amplified using the universal forward primer 789F
70 (5'-TAGATACCCSSGTAGTCC-3') and the reverse primer 1068R
71 (5'-CTGACGRCRGCCATGC-3') (Wang and Qian 2009; Lee et al. 2011;
72 Bougouffaet et al. 2013). V5–V6 amplicons of the 16S rRNA gene were sequenced
73 using paired-end method by Illumina Miseq at Personal Biotechnology Co., Ltd
74 (Shanghai, China). Pyrosequencing reads (further referred to as “reads”) were
75 processed using the Quantitative Insights Into Microbial Ecology (QIIME) v. 1.8.0
76 pipeline (Caporaso et al. 2010). After demultiplexing, quality filtering, denoising and
77 chimera removal, bacterial phylotypes were identified and assigned to operational
78 taxonomic units (OTUs, 97% cutoff) using Uclust algorithm (Edgar 2010) to generate
79 stable OTUs (He et al. 2015). The longest sequence in each cluster was chosen to be
80 the representative sequences, which were annotated by the Silva rRNA database
81 project (SILVA VERSION SSU11; <http://www.arb-silva.de/>). Microbial diversity was
82 measured by a series of OTU-based analyses of alpha-diversity implemented in the
83 QIIME pipeline. The generated nucleotide sequences have been deposited in the
84 NCBI Sequence Read Archive (SRA) database with accession number SRP068890.

85 Next-generation sequencing, such as Illumina amplicon sequencing, of the 16S

86 rRNA genes has allowed the discovery of novel lineages and the detection of much
87 higher microbial diversity than previously detected (Bik et al. 2012; Zinger et al. 2012;
88 Santoferrara et al. 2014). The OTU richness and diversity indices of the three soil
89 samples were estimated (Table 1). After quality control, 147,271 validated sequences
90 were obtained, classified into different OTUs based on the identity level at 97%.
91 The Chao values for ASM, AM, and ADS was 4193, 3609 and 4256, and the Shannon
92 index was 6.479, 6.437 and 4.777, respectively (Table 1).

93 All reads were aligned against the SILVA rRNA database project to derive the
94 phyla-level community taxonomic profiles using the software package ARB. The
95 relative abundance of microbial phyla in the ASM, AM, and ADS soils (Fig. 1) show
96 that, in total, 36 phyla/groups at the phylum level were detected. Of these,
97 *Thaumarchaeota*, *Acidobacteria*, *Proteobacteria*, *Chloroflexi*, *Actinobacteria*,
98 *Planctomycetes*, *Verrucomicrobia*, and *Bacteroidetes* were abundant at more than 5%,
99 and a low proportion of some phyla and candidate divisions had abundances lower
100 than 1% (Fig. 1). This were in line with several other studies demonstrating that these
101 microbial phyla are dominant soil microbial taxa using 16S rRNA gene clones or
102 pyrosequencing in many environments (Chow et al. 2002; Dunbar et al. 2002; He et al.
103 2006; Janssen 2006; Fierer et al. 2007; Brochier-Armanet et al. 2008; Lauber et al.
104 2008; Xu et al. 2014; Zhang et al. 2016; Zhang et al. 2017). Specifically,
105 *Thaumarchaeota* was only abundant in ASM, *Bacteroidetes* in AM, and *Acidobacteria*
106 in ADS (Fig. 1), indicating that the main taxa in the communities are constant despite
107 the very different relative abundances among soils and the fact that each ecosystem
108 soil has its own rare taxa. Three main reasons may account for this phenomenon. First,
109 *Thaumarchaeota* was abundant only in ASM soil, possibly related to the the high total
110 nitrogen (TN) and total organic carbon (TOC) concent of the ASM environment.
111 *Thaumarchaeota* is the third phylum of the Archaea, and all organisms of this lineage
112 are chemolithoautotrophic ammonia-oxidizers and may play important roles in
113 nitrogen and carbon cycles (Brochier-Armanet et al. 2008). Second, the highest TOC
114 content of the AM soil may explain the higher percentage of *Bacteroidetes* only in
115 AM. *Bacteroidetes* are a diverse heterotrophic bacterial division, and its aerobic

116 members are able to degrade biopolymers that could be present in the high-molecular
117 weight fraction of the dissolved organic material, such as cellulose, chitin, and
118 proteins (Kirchman 2002). Third, sequences affiliated with *Acidobacteria* were
119 abundant, possibly related to the ADS environment, characterized by low diversity of
120 plant communities and low TOC content. Previous study reported that the members of
121 the phylum *Acidobacteria* form the abundant population in non-rhizosphere soil
122 samples (Rohini-Kumar et al. 2013). Observation by Fierer et al. showed the relative
123 abundance of *Acidobacteria* members in the bulk of the soil region with low available
124 carbon (Fierer et al. 2007).

125 The relative abundance of genus for each ecosystem was also determined (Fig. 2).
126 *Candidatus Nitrososphaera*, *Roseiflexus*, *Sphingomonas*, *Candidatus*
127 *Xiphinematobacter*, *Chthoniobacter*, *Pir4* lineage, *Pirellula*, *Ferruginibacter*,
128 *Blastocatella*, and a large proportion of unclassified genera were detected (Fig. 2).
129 The results showed that the relative abundance of each genus was different in each
130 sample. Particularly, *Candidatus Nitrososphaera* was predominant in the ADS soil,
131 the *Pir4* lineage in ASM, and *Sphingomonas* in AM. These differences may reflect
132 different vegetation types, nutrient availability, and soil moisture, which could
133 influence microbial community composition at local scales (Fierer and Jackson 2006),
134 consistent with the significant variations of vegetation types, nutrient availability, and
135 soil moisture among ASM, AM, and ADS in our study (Table S1 and S2).

136 In conclusions, this is among the first reports on the soil microbial communities of
137 three grassland ecosystems in the Bayinbuluke, China using Illumina amplicon
138 sequencing. The 36 microbial phyla/groups were detected, and *Thaumarchaeota*,
139 *Acidobacteria*, *Proteobacteria*, *Chloroflexi*, *Actinobacteria*, *Planctomycetes*,
140 *Verrucomicrobia*, and *Bacteroidetes* were the predominant phyla in all three
141 ecosystems. At genus level, *Candidatus Nitrososphaera*, *Roseiflexus*, *Sphingomonas*,
142 *Candidatus Xiphinematobacter*, *Chthoniobacter*, *Pir4* lineage, *Pirellula*,
143 *Ferruginibacter*, *Blastocatella* were detected. Our results showed that the soil
144 microbial community structures were different for three grassland ecosystems in the
145 Bayinbuluke.

146

147 **Acknowledgements**

148 We thank staff at the Institute of Lake Bosten of the Environmental Protection Bureau
149 of Bayingolin Mongolia Autonomous Prefecture for helping with sample collection.
150 We are grateful to the editor and anonymous reviewers for their constructive
151 comments and helpful suggestions. This study was supported by the Special
152 Environmental Research Funds for Public Welfare of the State Environmental
153 Protection Administration, China (grant 201309041).

154

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240 majority of aquatic microbial diversity. *Mol Ecol.* **21(8)**: 1878–1896.

241

242 Figure Legend:

243 Fig. 1 Relative abundances of microbial community composition at phylum level for
244 the ASM, AM, and ADS.

245

246 Fig. 2 Relative abundances of microbial community composition at genera level for
247 the ASM, AM, and ADS.

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Relative abundances of microbial community composition at phylum level for the ASM, AM, and ADS.

95x71mm (300 x 300 DPI)

Relative abundances of microbial community composition at genera level for the ASM, AM, and ADS.

77x56mm (300 x 300 DPI)

Table 1. Estimated OTU richness and diversity indices for the ASM, AM, and ADS.

Sites	OTUs (97%)	Chao	Shannon
ASM	2189	4,193	6.479
AM	2364	3,609	6.437
ADS	2697	4,256	4.777

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Table S1. The vegetation coverage and dominant species of the alpine cold swamp meadow (ASM), alpine cold meadow (AM), and alpine cold desert steppe (ADS) in the Bayinbuluke, China.

	Type of grassland		
	ASM	AM	ADS
Coverage	100%	60%	10%
Species	<i>Leymussecalinus</i>	<i>Leymussecalinus</i>	<i>Artemisia caespitosa</i>
	<i>Artemisia caespitosa</i>	<i>Artemisia caespitosa</i>	<i>Stipasubsessiliflora</i>
	<i>Stipasubsessiliflora</i>	<i>Stipasubsessiliflora</i>	
	<i>Hordeumbrevisubulatum</i>	<i>Potentillarupestris</i>	
	<i>Ranunculus</i>		
	<i>trautvetterianus</i>	<i>Allium korolkowii</i>	
	<i>Allium korolkowii</i>	<i>Astragalusnivalis</i>	
	<i>Astragalusnivalis</i>	<i>Equisetum ramosissimum</i>	
	<i>Equisetum ramosissimum</i>	<i>Agropyroncristatum</i>	
	<i>Agropyroncristatum</i>		
	<i>Astragalusnanjiangianus</i>		

Table S2. The soil physicochemical properties in the ASM, AM, and ADS. Abbreviations include TN (total nitrogen), TP (total phosphorus), TOC (total organic carbon), and $\text{NH}_4^+\text{-N}$ (ammonia nitrogen).

Sites	Moisture (%)	pH	TN (mg/kg)	TP (mg/kg)	TOC (%)	$\text{NH}_4^+\text{-N}$ (mg/kg)
ASM	51	8.05	4002.50	1019.21	29.50	1.50
AM	33	8.06	4009.13	794.66	25.29	1.24
ADS	4	8.10	3105.42	599.07	5.46	0.17

Draft

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2 meadow (ASM), alpine cold meadow (AM), and alpine cold desert steppe (ADS) in
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	Type of grassland		
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	<i>Stipasubsessiliflora</i>	<i>Stipasubsessiliflora</i>	
	<i>Hordeumbrevisubulatum</i>	<i>Potentillarupestris</i>	
	<i>Ranunculus trautvetterianus</i>	<i>Allium korolkowii</i>	
	<i>Allium korolkowii</i>	<i>Astragalusnivalis</i>	
	<i>Astragalusnivalis</i>	<i>Equisetum ramosissimum</i>	
	<i>Equisetum ramosissimum</i>	<i>Agropyroncristatum</i>	
	<i>Agropyroncristatum</i>		
	<i>Astragalusnanjiangianus</i>		

4

- 5 Table S2. The soil physicochemical properties in the ASM, AM, and ADS.
6 Abbreviations include TN (total nitrogen), TP (total phosphorus), TOC (total organic
7 carbon), and $\text{NH}_4^+\text{-N}$ (ammonia nitrogen).

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AM	33	8.06	4009.13	794.66	25.29	1.24
ADS	4	8.10	3105.42	599.07	5.46	0.17

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8 Fig.S1. Geographic locations of 3 soil sampling sites in the Bayinbuluke alpine
9 grassland. Sites are indicated by labels ASM, AM, and ADS.

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112x50mm (300 x 300 DPI)

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