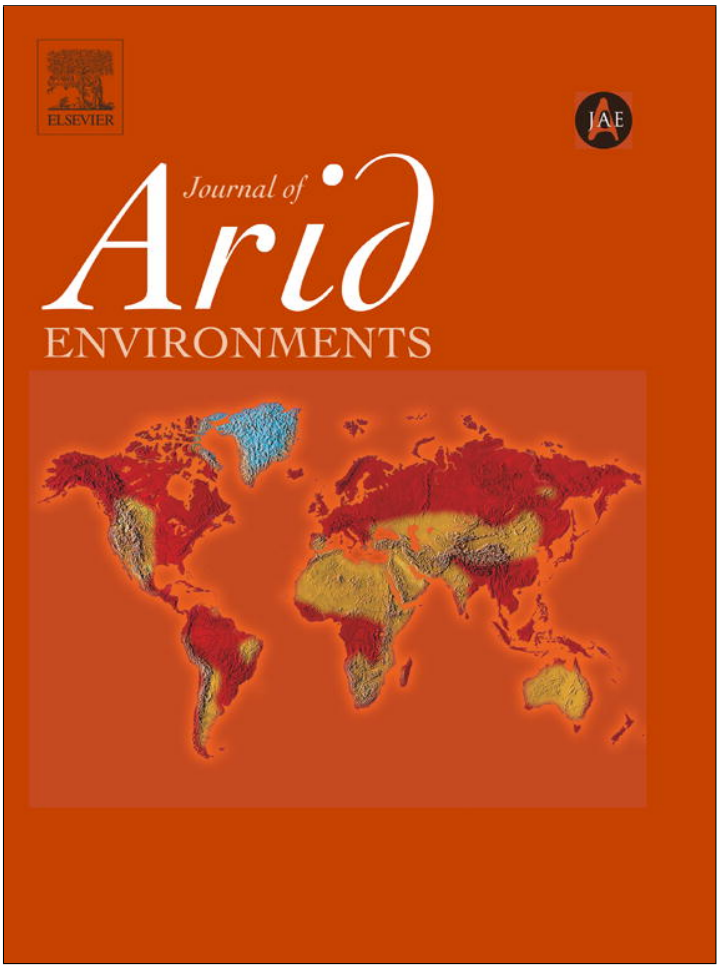

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Structure of soil bacterial communities in relation to environmental variables in a semi-arid region of Mongolia

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ABSTRACT

Patterns in soil bacterial communities, and the factors that determine them, have been little explored in arid and semi-arid environments. It is unclear to what extent the diversity and community composition of arid-land soil bacterial communities follow vegetation habitats, or conversely other relatively independent soil variables. It is also unclear whether the factors (e.g. pH) that contribute to variation in bacterial communities in some moister environments also operate on a local scale in semi-arid environments. To identify the main factors in shaping bacterial community structure in semi-arid environments, we sampled a mosaic of habitats under different vegetation, landscape and edaphic conditions in central Mongolia, including steppe, forest-steppe, and abandoned wheat field. Soil DNA was extracted and pyrosequenced for 16S rRNA gene identification. NMDS results showed that bacterial community structures are slightly different from one habitat to another. However, the similarity between communities both within and between habitats is determined more strongly by soil texture than by vegetation type and drainage conditions. Moreover, the relative abundances of certain phyla are correlated with specific soil properties such as salinity and soil texture, in ways that have not previously been found in semi-arid environments. Actinobacteria, for example, show a negative correlation with salinity and Bacteroidetes display a positive relationship with percentage silt and clay. It also appears that the most important environmental variables (soil texture and salinity) affecting the bacterial community within this semi-arid environment are different from those found in moister environments, with no detectable effect of pH.

1. Introduction

The description and understanding of bacterial diversity in nature is currently in its early stages. Until about ten years ago, only around 9747 species had been identified through culturing (http://www.bacterio.cict.fr/number.html). Culture-independent approaches using molecular techniques, such as DGGE, T-RFLP, cloning, etc., have revealed many thousands of previously unknown species in various environments around the world for decades. Now, metagenetic analysis together with massive parallel sequencing techniques have further revolutionized microbial ecology by making it possible to identify an order of magnitude more microbial taxa (Costello et al., 2009; Delmotte et al., 2009; Sogin et al., 2006)

As taxonomic resolution has increased due to the rapid development of molecular techniques, microbiologists have started to explore patterns in the community structure and diversity of microbial biota. Despite several preliminary reviews, these patterns are still poorly understood (Martiny et al., 2006; Ramette and Tiedje, 2007). In particular, it is not clear to what extent microbial communities are variable within and between habitats, whether there is a spatial pattern in these communities, and what environmental factors explain the greatest part of the observed variation in community structure. One possibility is that the composition of the plant community itself might primarily determine the soil bacterial community. It has been reported that different plant species have distinctive bacterial communities in their rhizosphere (Garbeva et al., 2008), and different vegetation types are known to harbor distinct sets of species of other groups of organisms such as birds (Borges, 2004), insects (Sugiura et al., 2008) and macrofungi (Zhang et al., 2010). Hence there are reasons for expecting that the same could be true of soil bacteria in semi-arid vegetation. However, it has been reported in studies of other biomes that combinations of biotic and abiotic factors, i.e. vegetation, land-use, geographic distance, and soil characteristics,

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affect bacterial species diversity and community composition (Fierer and Jackson, 2006; Hansel et al., 2008; Yergeau et al., 2007). Recently, it has been clearly shown that soil pH is one of the major drivers controlling soil bacterial community structure on a regional scale (Fierer and Jackson, 2006; Lauber et al., 2009; Tripathi et al., 2012). Soil texture has also been noted as having a strong association with below-ground microbial communities (Girvan et al., 2003; Lauber et al., 2008). Despite such findings, sampling across the world's land environments is still patchy and incomplete, so it is generally unclear to what extent soil texture, pH or other factors influence the composition of soil bacterial communities.

Arid and semi-arid regions occupy about 41% of the total land surface on Earth (Reynolds et al., 2007). Bacterial community structure in arid lands is relatively poorly understood compared to moister environments despite their vast areal extent. In dry environments in other parts of the world outside Mongolia, several environmental variables have previously been reported to be important in shaping soil microbial communities. Precipitation, vegetation cover, and pH have so far been found to be main factors in controlling bacterial community structure in arid and semi-arid environments (Angel et al., 2010; Fierer and Jackson, 2006). Microbial community composition also tends to be sensitive to alterations in local water content and N (Liu et al., 2009; Zhang et al., 2008). We set out here to study whether the same identifiable patterns hold true in Mongolia.

Outwardly, Mongolian steppe resembles semi-arid grasslands in other parts of the world. Grazing effects on microbial communities and physicochemical soil properties have been a principal area of study in the inner Mongolian steppe (Qi et al., 2011; Steffens et al., 2008; Zhao et al., 2009). However, little information is available on the detailed patterns of the soil bacterial community structure and diversity in either Mongolia or Inner Mongolia. Up until recently, correlations between environmental variables and bacterial community structure were explored at a broader empirical level – selected culturable species or major phyla – due to limitations in the analytical precision available. Due to next generation sequencing technologies, it is now possible to investigate bacterial community composition at a much finer scale of taxonomic resolution (Claesson et al., 2010).

In this paper, we set out to assess the patterns of bacterial diversity and community composition in a region that is previously unexplored from a metagenetic viewpoint: the semi-arid steppe region of the state of Mongolia. In particular, we assessed the overall pattern of bacterial diversity on both a within-habitat (alpha) and between-habitat (beta) scale. We then used these patterns of diversity to determine first, whether there is evidence of habitat specificity in the bacterial community within this environment, and second, to what extent it follows identifiable environmental gradients, at both the total bacterial community and different taxonomic levels such as phylum, class, and order.

2. Materials and methods

2.1. Sampling locality and field sampling strategy

Samples were taken from the buffer and core protection area of Hustai National Park in Mongolia, a reserve which is widely recognized for its successful reintroduction program of Przewalski horses. The park is located 100 km southwest of Ulaanbaatar, the capital city of Mongolia. Although it is included in the Mongol Daurian steppe province by phytogeographical classification (Hilbig, 1995), the core area of Hustai National Park constitutes a southwestern spur of the Hentii mountains (Wallis de Vries et al., 1996). The landscape is dominated by a central mountain range of granitic rocks with valleys creating varying habitat types.

The climate of the sampled area is dry continental with a mean annual temperature of +0.2 °C and a mean annual precipitation of 270 mm (Wallis de Vries et al., 1996). Samples were taken in fall, on 10 October 2009. Elevation ranges between 1100 and 1840 m above sea level. The nature reserve's buffer zone contains abandoned agricultural fields in the north and the broad valley of the River Tuul, one of Mongolia's major rivers. Running water originates as springs and mostly flows only in the upper part of a gully as it gradually seeps through the soil. Until November 1993 when the area was designated a nature reserve, the area was heavily grazed by livestock including sheep, goats, cattle and horses roughly estimated at 15,000 cattle or horse equivalents (Wallis de Vries et al., 1996).

The area is located beyond the southern fringe of discontinuous permafrost in Mongolia (Ishikawa et al., 2005; Sharkhuu, 2003). Forest cover is relatively low (~5%) whereas grassland and shrubland constitute the largest portion (88%) of the area (Wallis de Vries et al., 1996). A recent study has shown that the forested area has become more fragmented while the abandoned agricultural fields have become similar to mountain steppe (Bayarsaikhan et al., 2009).

We sampled sets of three sites per habitat, from each of the following habitat types: abandoned wheat field, mountain steppe, dry river valley (at the lower range of a gully where running water has seeped down to the subsoil), birch (*Betula platyphylla*) forest, the rocky south-facing slope of a mountain, and a wet river valley (upper part of a gully where water is moving at or near the soil surface) (Fig. 1). From each site, we took five equal-sized samples (about 300 g of the top 5 cm of soils underneath leaf litter) from four edges and one center of each hectare using a soil corer. These five samples were homogenized to make a single spatially integrated sample for each hectare. Samples were frozen on the day of sampling and stored at −80 °C for two weeks before DNA was extracted. Details on sample locations, climate, vegetation information, and chemical/physical properties (e.g. soil texture, salinity, humus, etc.) are shown in Table S1. Scattered soil samples from Southern China ($n = 35$) and Malaysia ($n = 28$) (Tripathi et al., 2012) were collected and pyrosequenced by authors and the overall bacterial diversity in soil were compared with that of Mongolian soil.

2.2. Pyrosequencing and data processing

DNA was extracted from the collected soil samples using the Power Soil DNA extraction kit (MO BIO Laboratories, Carlsbad, CA, USA) as directed by the manufacturer's instructions. Isolated soil DNAs were stored at below −20 °C. DNAs isolated from each sample were amplified using primers targeting the V1 to V3 regions (27F-518R) of the bacterial 16S rRNA and PCR reactions were carried out as described previously (Chun et al., 2010). The DNA sequencing was performed by Chunlab Incorporation (Seoul, Korea) using 454 GS Junior Sequencing System (Roche), according to the manufacturer's instructions. Sequences were denoised, processed, and analyzed following the mothur pipeline (Schloss et al., 2009). All sequences were classified using the EzTaxon-extended database (Kim et al., 2011).

2.3. Statistical analyses

To compare community-level bacterial diversity, we used both non-parametric and parametric diversity indices (e.g. Chao1, ACE, and Shannon index) based on the number of OTU's (operational taxonomic units). The OTU was defined at a 97% sequence similarity

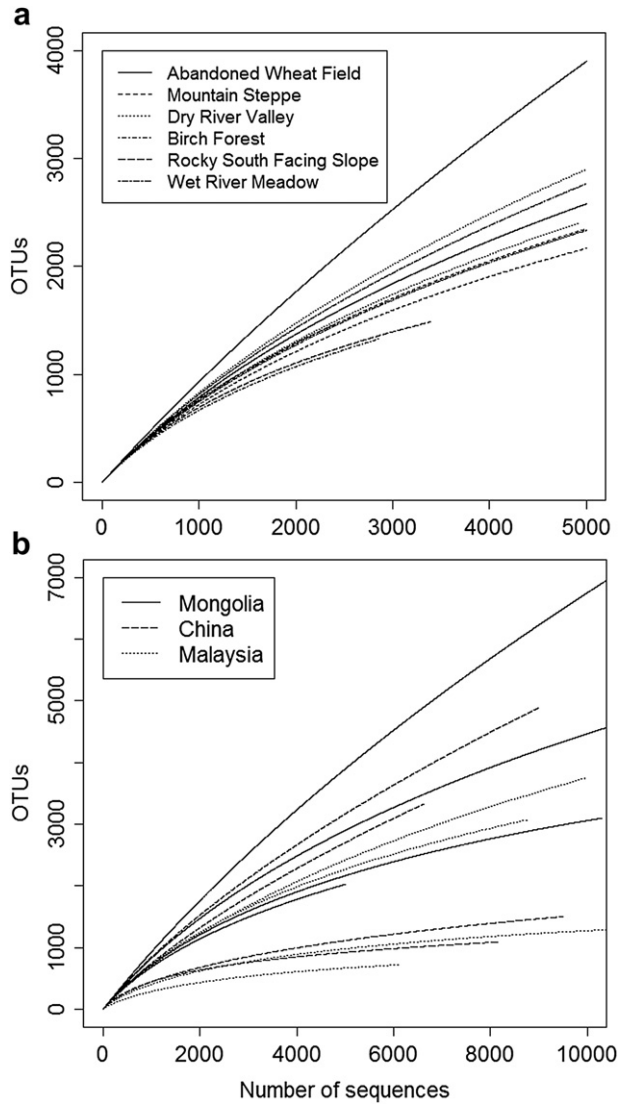

Fig. 1. Rarefaction results for the comparison (a) between six different habitats from Mongolian soils, (b) between other biomes, subtropical (China) and tropical area (Malaysia). The highest and lowest levels of diversity from each habitat are shown in (a). Two high and low levels of diversity among 18 Mongolian soils, 35 forest soils in Southern China, and 28 Malaysian forest and grassland soils are represented in (b).

cut-off and all diversity analysis and rarefaction curves were generated using this cut-off. We also measured phylogenetic diversity (Faiths' PD) to interpret branch lengths in each phylogenetic tree and make inferences about diversity, because the indices mentioned above do not describe the evolutionary history or phylogeography of each bacterial community (Faith, 1992). All diversity indices were generated based on a randomly selected subset of 881 sequences (the minimum number of sequences across all samples) without replacement per sample in order to avoid incomparability of measurements resulting from different numbers of sequences per sample.

To determine the extent to which separate samples share bacterial species in common, the species co-occurrence pattern was investigated within and between habitats. Taxonomic information of each OTU was inferred from the EzTaxon-e database and then used to show the relative distribution of shared OTU's.

Non-metric multidimensional scaling (NMDS) was generated to investigate whether there is a significant difference in bacterial community composition between samples using Bray–Curtis index. The difference in species (OTU) composition between samples both within and between habitats was tested using the analysis of similarity (ANOSIM) with 999 permutations in PRIMER v6 (Clarke and Gorley, 2006). Possible correlations between environmental variables vs. species (OTU) composition of bacterial community and bacterial species (OTU) diversity vs. soil properties were determined using the Mantel test. In case of a strong association between two matrices, Spearman's rank correlation between soil properties and the relative abundance of lower taxonomic groups (e.g. phylum, class, and order) was investigated. A subset of the environmental variables that best correlate to bacterial community similarities was determined using the BEST function in PRIMER v6. Salinity, humus, and water content were log-transformed and soil texture (% silt and clay) was square root-transformed to normalize the values for statistical analysis. Colinearity was checked by the pairwise comparison between all environmental variables.

Pyrosequencing produced a large number of novel sequences, which are not affiliated with known taxa in public databases. Lineage novelty was first determined by identifying the closest relatives of each sequence in SILVA (Pruesse et al., 2007) and EzTaxon-e database. 20–30 representative sequences of each bacterial phylum were retrieved from EzTaxon-e database, and then aligned manually based on rRNA secondary structure using jPhydit software (Jeon et al., 2005) with masking hypervariable regions. Sequences which did not fit the secondary structure and are less than 300 bp in length were excluded to avoid the effects of sequencing errors. A phylogenetic tree was built, based on the neighbor joining (NJ) algorithm with a maximum composite likelihood method using MEGA 5 (Kumar et al., 2008). All graphs were generated using R packages (http://www.R-project.org).

3. Results

3.1. General description of sequencing result and soil bacterial community composition

In total, we obtained 152,839 quality sequences of the V1–V3 region of the 16S rRNA gene, with an average read length of 446 bp. On average, there were over 8491 sequences per sample with a range of 881 to 20,184 reads between samples. Sequencing results reveal a high bacterial diversity across 18 samples, as compared to subtropical (China) and tropical rain forest (Malaysia) (Fig. 1b). Rarefaction curves together with diversity indices reveal that amongst the Mongolian samples, abandoned wheat field has the highest diversity, whereas birch forest is the least diverse (Fig. 1a and Table 1).

At higher taxonomic levels, the samples taken in this study are generally similar to one another. Most samples are dominated by the phyla or subclasses Bacteroidetes, Acidobacteria, Alphaproteobacteria, Actinobacteria, Betaproteobacteria, and Gemmatimonadetes, accounting for 19.7%, 17.5%, 11.7%, 7.4%, 7.0%, and 4.1%, respectively (Fig. S1). Pyrosequencing also reveals the consistent presence of rare candidate phyla in the soil, an aspect which was not usually considered in past studies of arid environments due to the difficulty of detecting these phyla. Candidate divisions TM7, OP10, OD1, SM2F11, GN02, OP3, and WS5 (all previously reported in literature from other habitats and/or other parts of the world) are consistently found across all habitats here despite their low abundance. Interestingly, certain candidate phyla are confined to or absent from specific habitats; GN04, ANW, LD1, OMAN, and SAR406 are found only in wet river meadow, while TM6 and BRC1 (Bacteria rice cluster1), which have been frequently found in rice paddy soil (Derakshani et al., 2001), are absent from abandoned wheat field and rocky south facing slope, respectively. The most abundant

Table 1
Sequencing result and diversity indices for soil bacterial communities from the studied sites.

Sample	Habitat	Total reads	OTU's[a]	Chao1[b]	Shannon[b]	Simpson 1/*D*[b]	Phylogenetic Diversity[b]
AWF1	Abandoned wheat field	14238	8843	**4434.9**	**6.54**	**1976.3**	**71.3**
AWF2	Abandoned wheat field	19518	6639	2227.9	6.27	632.5	55.5
AWF3	Abandoned wheat field	20184	7300	3029.2	6.36	751.9	56.7
MST1	Mountain steppe	10122	3892	1885.6	6.15	471.5	**49.7**
MST2	Mountain steppe	9708	4020	1948.9	6.25	649.4	52.2
MST3	Mountain steppe	8393	3888	1726.2	6.24	689.7	53.8
DRV1	Dry river valley	3522	2292	2107.1	6.29	715.3	57.3
DRV2	Dry river valley	4634	2697	2028.8	6.33	909.1	59.1
DRV3	Dry river valley	19353	7591	2617.0	6.40	1053.7	61.0
BFO1	Birch forest	881	690	2662.4	6.29	645.2	70.2
BFO2	Birch forest	2633	1414	**1397.5**	**6.11**	474.4	56.1
BFO3	Birch forest	4801	2272	1778.4	6.11	398.9	57.3
RSF1	Rocky south facing slope	2392	1739	2214.5	6.39	1111.1	61.2
RSF2	Rocky south facing slope	3207	1761	1463.8	6.20	663.6	57.3
RSF3	Rocky south facing slope	2746	1890	2065.6	6.29	800.0	54.5
WRM1	Wet river meadow	16212	6155	2492.9	6.37	1030.9	67.9
WRM2	Wet river meadow	6523	3083	2417.8	6.24	**372.0**	63.7
WRM3	Wet river meadow	3772	2377	2108.6	6.38	1007.0	65.7

[a] The number of OTU's was generated at the similarity cutoff of 97%.
[b] Diversity indices represent the mean of three randomly selected subsets ($n = 881$) for each sample. The highest and lowest estimates are in bold faces.

(ranked number 1) OTU is *Novosphingobium capsulatum* (4.3%) in open birch forest subsample 1. The most abundant genus across all sites is *Ferruginibacter* (2.4% on average), a member of Bacteroidetes.

Overall, compared to work in other biomes and in other regions of the world, the samples analyzed in this study are unusually diverse in terms of phyla and contain a considerable number of sequences which do not seem to fall into any known phyla (Fig. S4). A total of 16 new lineages (designated as MoS1–MoS16) are present in our samples, at the phylum and subphylum levels. Most of these are more closely related to recently discovered candidate bacterial phyla, rather than to previously-described cultured phyla. When considering the tree topology supported by NJ trees with greater than 50% of bootstrap values, MoS group 1 is likely to be a new relative of candidate division WS5, MoS group 4 is related to Tenericutes, MoS group 7 to WS1, MoS group 14 to Gemmatimonadetes, and MoS group 16 to OD1.

3.2. The degree of bacterial species (OTU) overlap and similarity in bacterial community composition within and between habitats

There is a low degree of overlap between samples, both within and between habitat types. The proportion of overlapped OTU's within habitats ranges from 1.2% to 8.3%. Abandoned wheat field (7.7%) and mountain steppe (8.3%) seem to share more OTU's, whereas birch forest (1.2%) and rocky south facing slope (2.7%) revealed relatively low levels of OTU overlap (Fig. S3). The turnover of species composition varies across habitats. Only 0.45% of the total OTU's are shared across all habitats. There are two sets of samples with higher degrees of species overlap (where the proportion of overlapped species is greater than 15% in pairwise comparison). One set consists of abandoned wheat field, mountain steppe, and dry river valley, whereas the other comprises mountain steppe, dry river valley, and rocky south-facing slope. There is however a low level of overlap between mountain steppe and rocky south facing slope, indicating that different species must be shared between these two sets. On the other hand, birch forest and wet river meadow showed low levels of species overlap with other habitats, suggesting the presence of relatively distinct assemblages of habitat-specific bacteria. These two habitats shared less than 7% of OTU's with abandoned wheat field.

To determine whether there is a significant difference in bacterial community structure within and between habitats, we performed ANOSIM using the Bray–Curtis similarity index. Overall, each habitat harbored certain bacterial lineages that differentiated it from other habitats (Global $R = 0.66$, $P < 0.001$). A pairwise test revealed, however, that the level of similarity among habitats varies according to the particular pair of habitats being compared. Wet river meadow and abandoned wheat field soils are taxonomically more distinct from other habitats ($R = 1$, $P < 0.01$ and $0.78 < R < 0.96$, $P < 0.01$), and dry river valley, rocky slope, and mountain steppe samples are all relatively similar to one another ($0.07 < R < 0.41$, $P < 0.02$).

3.3. Relationship between bacterial community structure and measured environmental variables

An NMDS plot was generated to determine if the bacterial community variation is governed mainly by vegetation type or whether other environmental variables have more influence. When we calculated two commonly-used distance matrices, Bray–Curtis and Unifrac distance, the result was almost the same. Samples from the same habitat tend to cluster together (Fig. 2a). Interestingly, samples are also clustered according to soil texture irrespective of the geographical distance between them (Fig. 2b). Community structure does not cluster with other variables, such as pH, salinity, humus, and moisture levels, indicating that among the analyzed variables soil texture is the best predictor of community composition. The result is supported by finding the 'best' match between environmental variables and multivariate patterns of community assemblage using the BEST function in PRIMER v6. Soil texture is the single explanatory variable that best explains bacterial community structure ($\rho = 0.590$, $P < 0.01$), and the second best optimization is the combination of soil texture and salinity ($\rho = 0.589$, $P < 0.01$). At the phylum level, Actinobacteria reveal a strong relationship with salinity (Mantel $r = 0.539$, $P < 0.05$), and Bacteroidetes have a clear association with soil texture (Mantel $r = 0.724$, $P < 0.001$) (Table S2). We investigated these two phyla further to explore any significant association at lower taxonomic levels within them. The relative abundance of all three subgroups belonging to Actinobacteria reveals a negative correlation with salinity (Fig. S2). Amongst Bacteroidetes subgroups, Cytophagia and Flavobacteria abundances show a negative association with soil texture (% silt and clay), whereas Sphingobacteria display the opposite trend (Fig. S2).

Total species (OTU) richness per sample does not appear to depend upon any identifiable soil variables. No significant

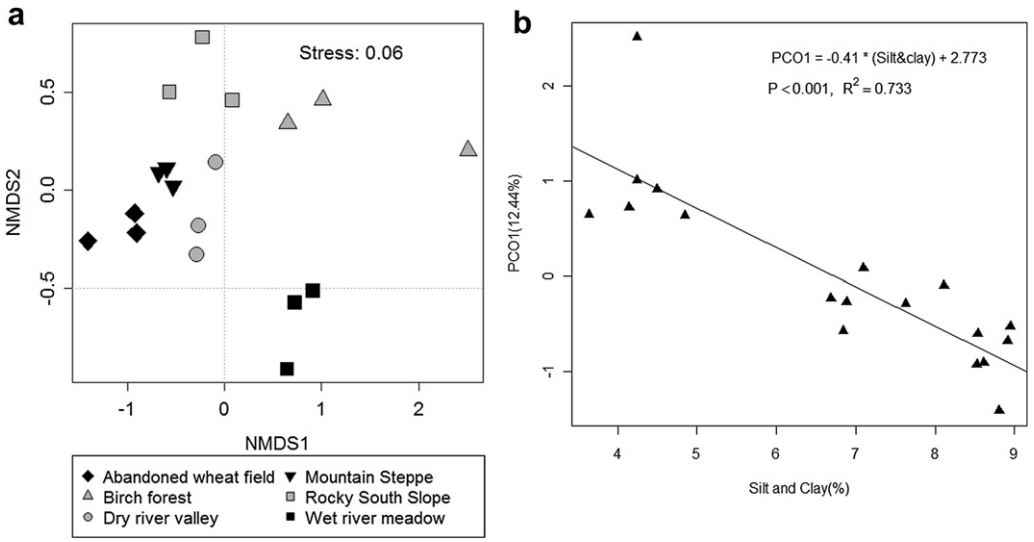

Fig. 2. NMDS plot showing (a) the phylogenetic similarity between samples based on pairwise Unifrac distance and (b) the first component from a principal coordinate analysis regressed against soil texture (Silt and clay % content) Pair-wise unweighted UniFrac distances between samples were calculated based on maximum likelihood (ML) tree of a randomly selected subset ($n = 881$) per sample.

relationship is present between OTU richness and pH, salinity, humus, and soil texture (data not shown).

4. Discussion

4.1. Environmental relationships with overall species richness

At the overall bacterial species (OTU) richness level, there are no obvious local-scale patterns in species richness amongst the samples in this study, and no observable correlates with environmental factors such as texture, pH or salinity – for the whole community at least. Between samples there is also a substantial turnover in bacterial species composition, both between vegetation habitats, and between replicate samples from the same habitat. Thus, when samples from the same or different habitat types are compared, the proportion of overlapping species between any two samples is usually well under 10%. In general it seems that a very large pool of bacterial species is being sampled in this landscape, for there is no sign of an overall asymptote being reached as more samples are added.

Several previous studies have provided evidence that soil bacterial richness is higher in the clay-sized fraction of soil than in the sand-sized fraction (Ranjard et al., 2000; Sessitsch et al., 2001). However, it is not clear yet whether there is a close correlation between soil texture and overall bacterial species (OTU) diversity. Carson et al. (2010) recently reported that low pore connectivity caused by low water potential can increase soil bacterial diversity – and suggested that this may be more important than particle size difference. Our work however showed no differences in overall bacterial species (OTU) diversity across the range of soil textures.

It is unclear why no relationship between pH and soil bacterial diversity has been detected in this study, given that the relationship is so widespread in the world (Lauber et al., 2009; Tripathi et al., 2012). This may be partly because the pH range in the sampled environment is not very broad (pH 5.9–8.2), lacking the most acidic pH values. Nevertheless, many previous studies have shown a strong peak around neutral and then a decline in bacterial diversity towards pH values around or greater than pH 8 (Fierer and Jackson, 2006; Lauber et al., 2009; Tripathi et al., 2012) – whereas in our samples no such decline is evident.

4.1.1. Vegetation/habitat type and soil bacterial community composition

To some extent, it is evident that soil bacterial communities in the environment we studied do relate to habitat types as would traditionally be perceived by ecologists. Despite the high turnover in species composition between samples, bacterial community members are shared more often *within* than *between* habitats. The extent to which bacterial communities are similar to one another also varies between particular pairs of habitats, with dry river valley and rocky south facing slope clustering together more closely. On an ordination diagram, there is a noticeable tendency for samples from within the same habitat to cluster together, although there is considerable overlap in the scatter between different habitats. However, a more thorough statistical analysis of community composition reveals that both at a whole community level and at the level of selected phyla, vegetation/habitat type is of secondary importance compared to soil texture and salinity. In an ordination, samples and habitats of similar soil texture fall closely into three main clusters on an ordination, and with certain habitats clustering next to one another despite their very different vegetation ecology.

4.1.2. Soil salinity vs. relative abundance of particular bacterial phyla

Although overall OTU diversity does not correlate with any identifiable environmental variables, there is a clear relationship between abundance of selected phyla, and salinity and soil texture.

Other researchers have studied the effect of salinity on microbial community structures in several habitats such as estuaries, lakes, and solar salterns (Baati et al., 2010; Crump et al., 2004; Foti et al., 2008). It has previously been reported that salinity is a major factor in controlling microbial diversity, function, and community composition in aquatic environments (Crump et al., 2004). However, there have been few studies of salinity effects on bacterial communities in terrestrial soil, as salinity was not commonly measured for investigating soil microbial communities (Lozupone and Knight, 2007).

Most phyla that we studied here show no significant relationship to salinity, but Actinobacteria do show a significant relationship at the $P = 0.01$ level ($r = 0.539$). This study is the first in the literature to demonstrate a clear relationship between actinobacterial abundance

and a salinity gradient in a dry soil environment. A similar pattern, a decrease in the relative abundance of Actinobacteria with an increase of salinity, was reported recently with a salinity gradient in Baltic Sea floor sediments (Herlemann et al., 2011). Interestingly, four different subgroups of Actinobacteria at the order level all show the pattern of decreasing abundance with increasing salinity, suggesting that a shared trait across Actinobacteria plays an important role in their response to salinity. However, currently little is known about underlying mechanisms that might cause the abundance of Actinobacteria to decrease as salinity level increases, although it has been hypothesized that metabolic diversity of prokaryotes reduces with increasing salinity, for thermodynamic reasons.

4.1.3. Relation to soil texture

A Mantel test reveals a significant correlation between overall bacterial community composition and soil texture (% silt and clay). This is in accordance with previous studies (Girvan et al., 2003; Lauber et al., 2008). However, a close association between abundance of specific phyla and soil texture, has not previously been reported. Among the several dominant phyla in our samples, Bacteroidetes showed the strongest correlation with soil texture ($r = 0.724$, $P < 0.001$). Bacteroidetes are categorized ecologically as copiotrophs and are more abundant in soils with high carbon availability (high C mineralization), indicating that this phylum plays an important role in decomposing organic materials in soil (Fierer et al., 2007). Soil texture is one of the essential properties affecting the ability of microbes to physically access organic substances and it significantly determines soil moisture, nutrient availability, and retention (Vanveen and Kuikman, 1990). It is possible that particles of a particular texture and ionic surface conditions offer conditions that favor this group of bacteria over others. In a moist temperate soil environment, Sessitsch et al. (2001) reported that large particles (sand) were dominated by Alphaproteobacteria, whereas small particles (silt and clay) harbored more Acidobacteria. However, we did not find any correlation between these two groups and soil texture. It has been reported that there is no striking correlation between Bacteroidetes and soil texture (Fierer et al., 2007). However, our observation revealed that there is a strong positive correlation between relative abundance of Bacteroidetes and content of clay and silt, indicating that abundance patterns of certain phyla can vary between habitats or geographic locations. More interestingly, at the lower taxonomic level, different subgroups of Bacteroidetes showed quite distinct patterns. Cytophagia and Flavobacteria showed a decrease in abundance with increasing silt and clay, while Sphingobacteria showed the opposite trend. Sphingobacteria was dominated by Chitinophagaceae (on average 76%) suggesting that this family has a link with abundant silt and clay particles, although the relationship is as yet unknown.

Unusual and distinctive aspects of the soil biota we sampled in Mongolia also include an abundance of apparently novel phyla, whose ecological role is unknown. We found 16 phylogenetically distinct groups of bacteria from Mongolian soils which belong to novel lineages at the phylum or subphylum level with no close relatives in the currently available 16S rRNA gene databases. Many of these are candidate subphyla, most closely related to members of recently identified candidate bacterial phyla, rather than to previously cultured phyla. For example, MoS1 is most closely related to WS5, MoS6 to OMAN, MoS7 to WS1, MoS10 and 11 to SM2F11, and MoS15 to OD1, respectively. Some entirely novel phyla may be present: for example there were no close relatives of MoS15 and MoS16 in public databases. Despite their novelty, members of the new candidate phyla MoS3 and MoS4 are widespread within this study area, being found across all habitats in our sampling. The extent to which the lineages we have found are endemic to Mongolian steppe environments is unclear, given the lack of comprehensive geographic databases of bacterial lineages.

4.1.4. Seasonal timing

Our samples were taken during the onset of autumn at this locality, the end of growing season with nighttime temperatures still several degrees above freezing due to warmer than average prevailing weather. It is unclear to what extent sampling during the much hotter, drier summer or the much colder winter would reveal a different set of bacterial communities. Seasonal dynamics of microbial communities and biogeochemical consequences of species turnover in terrestrial ecosystems have long been investigated (Bardgett et al., 1999; Smit et al., 2001). However, there have been few detailed investigations on seasonal fluctuations of bacterial community composition in arid and semi-arid environments (Bell et al., 2009). It would be interesting and instructive to sample the same localities at other times of year, and in multiple years, for understanding community functional changes in Mongolian semi-arid environments.

5. Conclusions

Our observations show that soil bacterial communities in the Mongolian semi-arid environment are to some extent related to habitat type (where habitat is defined in terms of vegetation type and topographic situation). Salinity also appears to play a pivotal role in variation in community composition in this environment. However, the overall differences and similarities between the bacterial communities across the different habitat types can much more effectively be explained by soil texture, with habitats that have similar soil texture falling closely into three main clusters on an ordination, despite their very different vegetation ecology.

Overall, it seems that subtle details of soil characteristics – texture and salinity – influence the bacterial ecology in this system, and that the plant community or overall drainage conditions are only of secondary importance. It will be interesting to investigate whether the same relationships found here between phylum relative abundance and environmental variables occur in other semi-arid soil environments around the world. The finding of the important role of soil texture may ultimately give important clues to the ecological role and trophic needs of each of these groups, most of whose members remain uncultured. As well as revealing novel relationships with environmental factors, this study discovered many new subgroups of bacteria, including new candidate phyla. Further work is necessary to reveal the true microbial diversity of Mongolian soils, and the factors which control it.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jaridenv.2012.09.014.

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<Supplementary Materials>

Table S1 Correlations between bacterial community composition and soil properties

Bacterial taxa	Mantel's correlation†				
	% Silt and clay	Salinity (μS/cm)	pH	Water content	Humus
Total community	0.608***	0.381	0.143	0.438*	0.288
Acidobacteria	0.531***	0.258	0.108	0.34	0.246
Actinobacteria	0.491***	**0.539****	0.158	0.343	0.237
Bacteroidetes	**0.724*****	0.397	0.143	0.445*	0.284
Alphaproteobacteria	0.347***	0.223	0.054	0.297	0.295
Betaproteobacteria	0.552***	0.28	0.077	0.382	0.233
Deltaproteobacteria	0.354***	0.246	0.094	0.2	0.283
Gammaproteobacteria	0.290	0.151	0.124	0.267	0.164
Gemmatimonadetes	0.578***	0.379	0.187	0.393*	0.191
Verrucomicrobia	0.355***	0.325	0.189	0.317	0.197
Planctomycetes	0.551***	0.246	0.073	0.235	0.303

†Significance level was shown as *** ($P<0.001$), ** ($P<0.01$), and * ($P<0.05$). Bacterial phyla, which were further investigated at lower taxonomic level, are presented in bold.

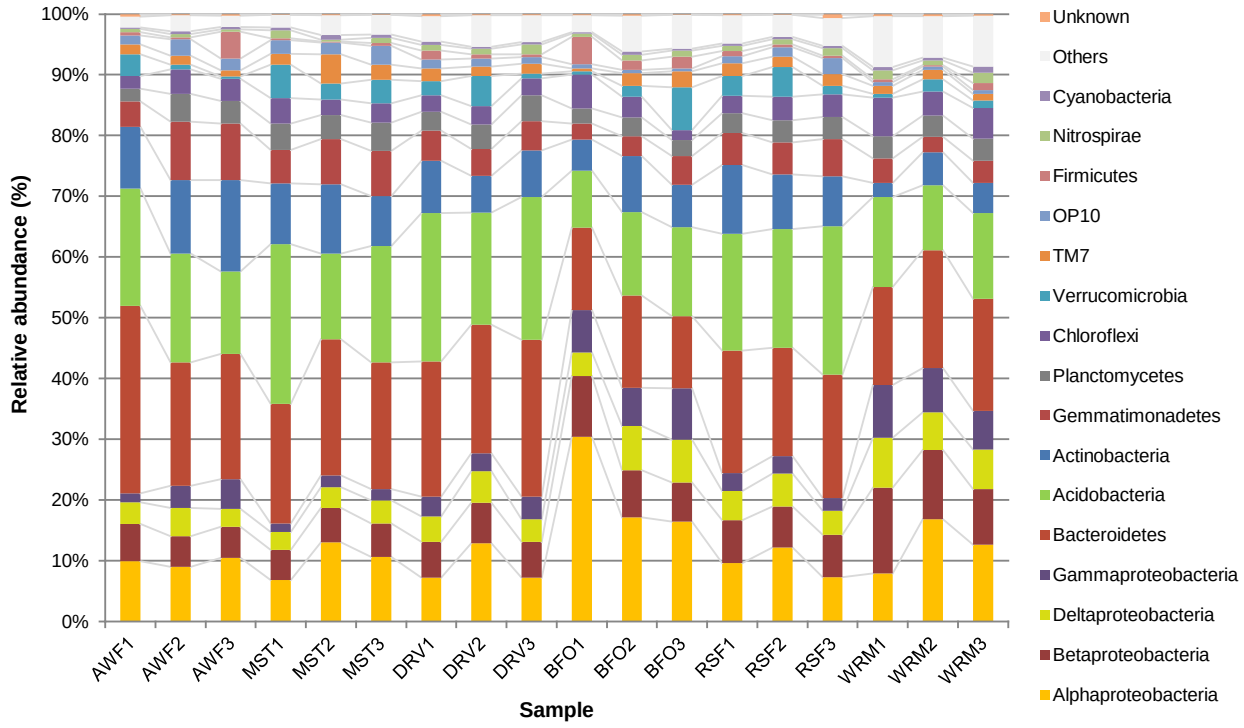


Fig S1 Taxonomic composition of bacterial phyla in six habitats. AWF, abandoned wheat field; MST, Mountain steppe; DRV, dry river valley; BFO, birch forest; RSF, rocky south facing slope; WRM, wet river meadow.

Fig S2 Co-occurrence of bacterial species within each habitat. Values represent the number of shared OTUs defined at the 97% sequence similarity. AWF, abandoned wheat field; MST, Mountain steppe; DRV, dry river valley; BFO, birch forest; RSF, rocky south facing slope; WRM, wet river meadow.

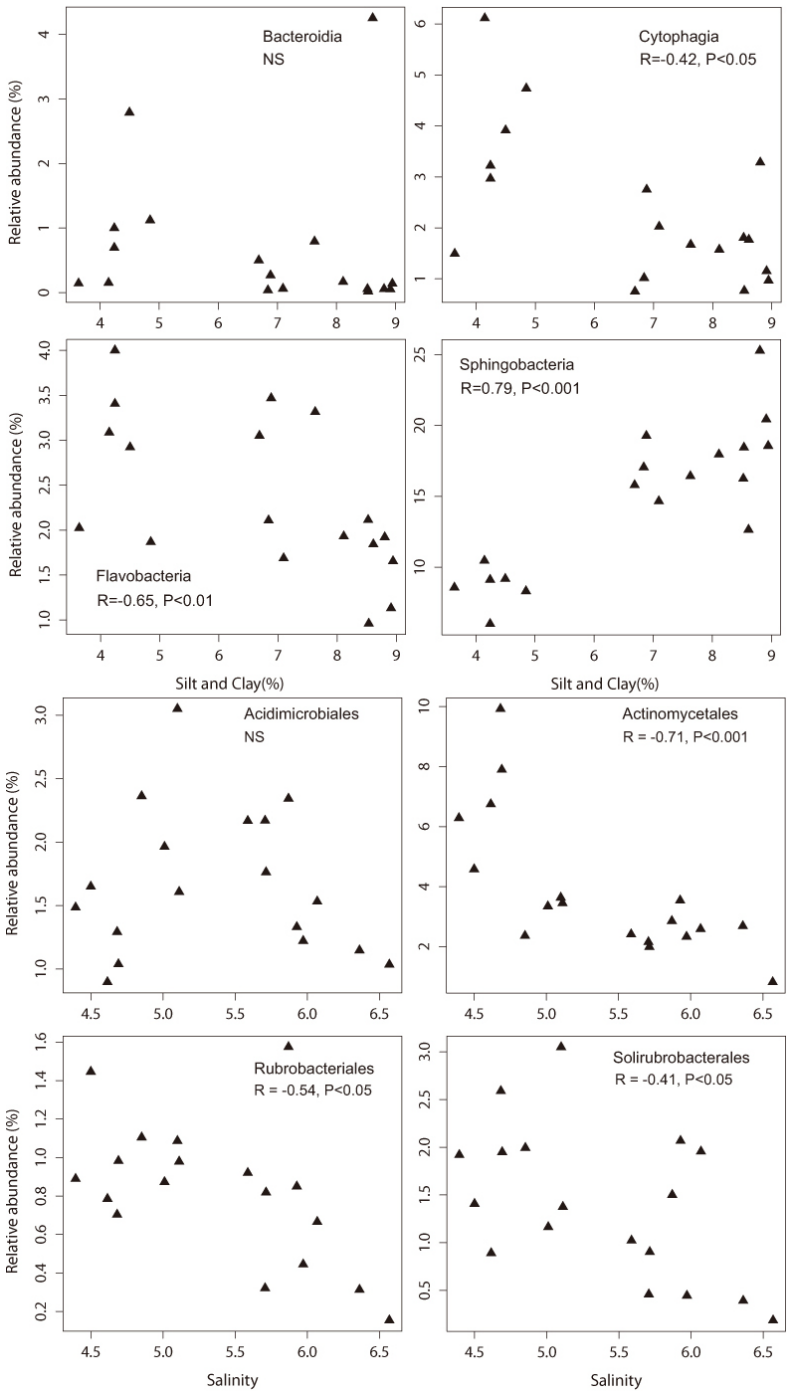
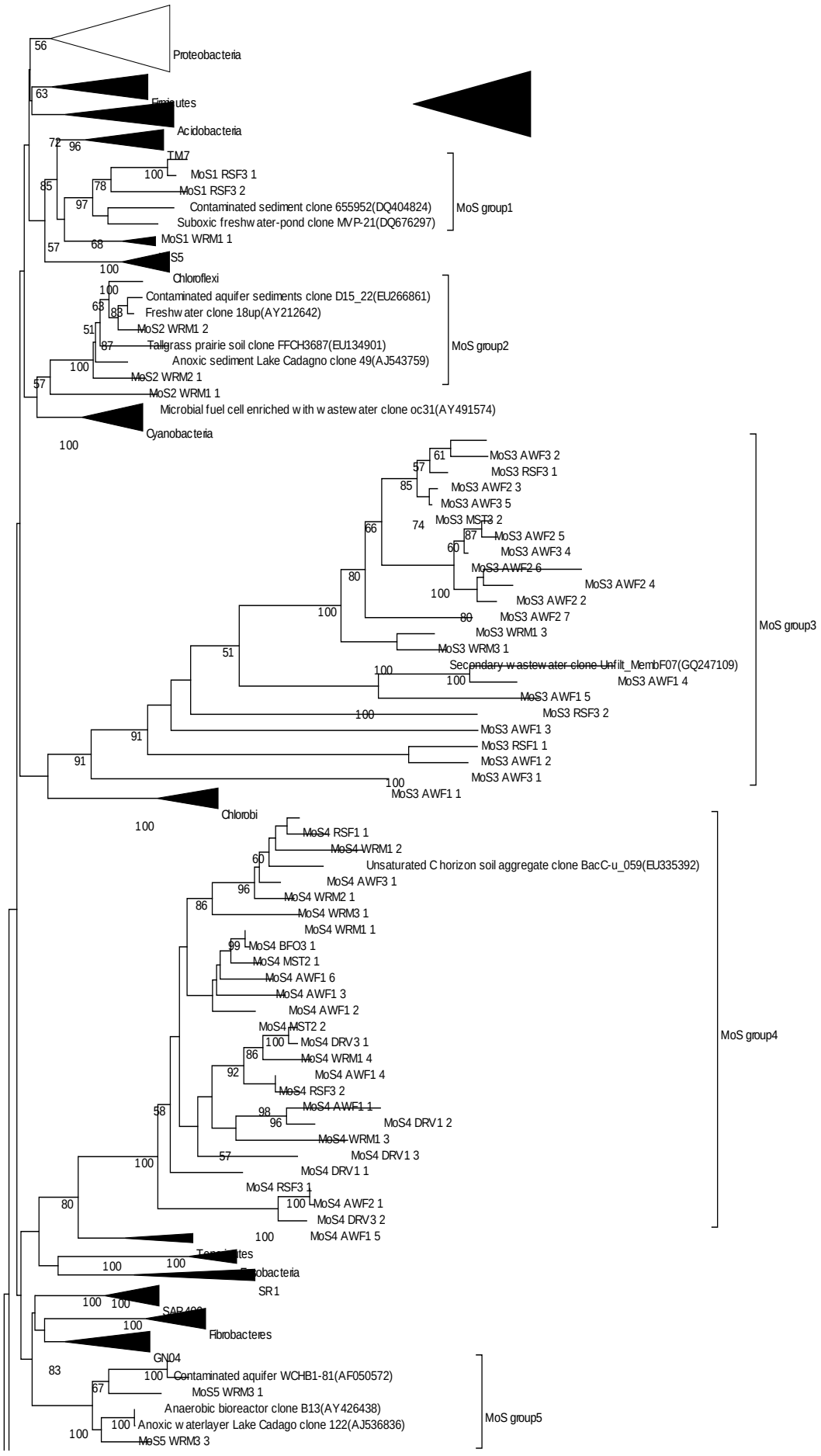


Fig S3 Relationship between salinity and the relative abundance of subgroups of Actinobacteria, and between silt and clay (%), and the relative abundance of subgroups of Bacteroidetes Spearman's rank correlations between each subgroup and soil properties are shown. Salinity values (μS/cm) were log-transformed and the percentage of silt and clay was square root-transformed.


Proteobacteria
Firmicutes
Acidobacteria
TM7
MoS1 RSF3 1
MoS1 RSF3 2
Contaminated sediment clone 655952(DQ404824)
Suboxic freshwater-pond clone MVP-21(DQ676297)
MoS group1
MoS1 WRM1 1
S5
Chloroflexi
Contaminated aquifer sediments clone D15_22(EU266861)
Freshwater clone 18up(AY212642)
MoS2 WRM1 2
Tallgrass prairie soil clone FFCH3687(EU134901)
Anoxic sediment Lake Cadagno clone 49(AJ543759)
MoS group2
MoS2 WRM2 1
MoS2 WRM1 1
Microbial fuel cell enriched with wastewater clone oc31(AY491574)
Cyanobacteria
MoS3 AWF3 2
MoS3 RSF3 1
MoS3 AWF2 3
MoS3 AWF3 5
MoS3 MST3 2
MoS3 AWF2 5
MoS3 AWF3 4
MoS3 AWF2 6
MoS3 AWF2 4
MoS3 AWF2 2
MoS3 AWF2 7
MoS3 WRM1 3
MoS3 WRM3 1
Secondary wastewater clone Unfilt_MembF07(GQ247109)
MoS3 AWF1 4
MoS3 AWF1 5
MoS3 RSF3 2
MoS3 AWF1 3
MoS3 RSF1 1
MoS3 AWF1 2
MoS3 AWF3 1
MoS3 AWF1 1
MoS group3
Chlorobi
MoS4 RSF1 1
MoS4 WRM1 2
Unsaturated C horizon soil aggregate clone BacC-u_059(EU335392)
MoS4 AWF3 1
MoS4 WRM2 1
MoS4 WRM3 1
MoS4 WRM1 1
MoS4 BFO3 1
MoS4 MST2 1
MoS4 AWF1 6
MoS4 AWF1 3
MoS4 AWF1 2
MoS4 MST2 2
MoS4 DRV3 1
MoS4 WRM1 4
MoS4 AWF1 4
MoS4 RSF3 2
MoS4 AWF1 1
MoS4 DRV1 2
MoS4 WRM1 3
MoS4 DRV1 3
MoS4 DRV1 1
MoS4 RSF3 1
MoS4 AWF2 1
MoS4 DRV3 2
MoS4 AWF1 5
MoS group4
SR1
Fibrobacteres
GN04
Contaminated aquifer WCHB1-81(AF050572)
MoS5 WRM3 1
Anaerobic bioreactor clone B13(AY426438)
Anoxic waterlayer Lake Cadago clone 122(AJ536836)
MoS5 WRM3 3
MoS group5


Bacteroidetes
Aquificae
MoS6 BFO3 1
MoS6 WRM3 2
MoS6 MST2 1
Tallgrass prairie soil clone FFCH13262(EU132808)
MoS6 AWF3 1
MoS6 DRV3 2
MoS6 DRV1 1
MoS6 MST1 1
Australian arid soil clone 0425-2M17(AF234118)
MoS6 MST3 1
MoS6 DRV3 1
MoS6 AWF1 2
MoS6 MST2 2
MoS6 WRM3 1
MoS6 MST3 2
MoS6 DRV1 2
MoS6 AWF1 1
MoS group6
Actinobacteria
WS1
Hypersaline microbial mat clone 05D2Z18(DQ330736)
MoS group7
Caldisericа
MoS8 BFO3 1
MoS8 WRM3 1
MoS8 MST3 1
MoS8 WRM1 2
MoS8 AWF3 1
MoS8 MST1 1
Tallgrass prairie soil clone FFCH17416(EU134961)
MoS8 WRM2 1
MoS8 MST2 1
Mineral spring clone BB35(AM167972)
MoS8 WRM1 1
MoS8 RSF1 1
MoS8 WRM2 2
MoS8 BFO2 1
MoS8 WRM1 3
MoS8 RSF2 1
MoS group8
WRM3 2
WS6
MoS9 AWF2 1
Heavy metal polluted soil clone G19-235(GQ487975)
MoS group9
MoS9 DRV3 1
SM2F11
Hot spring Yellowstone clone SM2F11(AF445733)
MoS10 AWF3 1
Hypersaline microbial mat clone MAT-CR-P6-E01(EU246293)
MoS10 WRM1 1
MoS group10
MoS11 AWF2 1
MoS11 MST2 1
MoS group11
Trembling aspen rhizosphere clone Elev_16S_998(EF019807)
MoS12 WRM3 1
MoS12 WRM2 1
MoS12 WRM1 1
MoS12 RSF2 1
MoS group12
OP11
Elusimicrobia
MoS13 WRM1 1
MoS13 WRM2 1
MoS group13
Activated sludge clone B28(FJ660517)
MoS group14
MoS14 AWF1 1
Gemmatimonadetes
AD3
Verrucomicrobia
Deinococcus-Thermus
DSMV
MoS group15
OD1
MoS16 MST3 1
MoS16 WRM1 2
MoS16 DRV3 1
MoS16 WRM1 1
MoS16 AWF3 1
MoS16 DRV2 1
MoS16 DRV1 1
MoS16 WRM2 1
MoS group16
Planctomycetes
Natrialba asiatica(D14123)
Methanobacterium bryantii(M59124)
Pyrolobus fumarii(X99555)
100

Fig S4 Phylogenetic position of 'novel' sequences identified in Mongolian soil samples The tree was constructed using neighbor-joining method after masking hypervariable region of 16S rRNA gene sequences. Three archaeal sequences were used as outgroup for tree construction. Bootstrap values are based on 100 replicates and scale bar represents changes per nucleotide position.