Fungal pathogens pose a potential threat to animal and plant health in desertified and pika burrowed alpine meadow on the Tibetan Plateau

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Fungal pathogens pose a potential threat to animal and plant health in

desertified and pika burrowed alpine meadow on the Tibetan Plateau

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Abstract

Intact Tibetan meadows provide significant defense against soil-borne pathogen dispersal. However, dramatic meadow degradation has been observed due to climate change and pika damage and their impacts on soil-borne pathogens are still unclear. With approximately 40% of the world’s population living in Tibetan Plateau and its downstream watersheds, this lack of knowledge should be of great concern. Here, we used Illumina amplicon sequencing to characterize the changes in potential human, domestic animal, plant, and zoonotic bacterial and fungal pathogens in non-degraded, desertified and pika burrowing meadows. The relative abundance of bacterial domestic animal pathogens and zoonotic pathogens were significantly increased by desertification. Pika burrowing significantly increased the relative abundance of bacterial human pathogens and zoonotic pathogens. The species richness and relative abundance of fungal pathogens was significantly increased by desertification and pika burrowing. Accordingly, fungal plant and animal pathogens categorized by FUNGuid significantly increased in desertified and pika burrow meadows. Soil chemical and plant properties explained 38 and 64% of the bacterial and fungal pathogen community variance, respectively. Therefore, our study indicates for the first time that both alpine meadow desertification and pika burrowing could potentially increase infectious disease risks in the alpine ecosystem, especially for fungal diseases.

**Key words:** Tibetan meadow desertification, pika behavior, microbial pathogens, richness and abundance
Introduction

Mounting evidence has shown that land use changes often drive a range of infectious disease outbreaks and emergence events (Martin and Watson 2016, Morris, et al. 2016, Patz, et al. 2004, Patz, et al. 2008, Zhang, et al. 2017). For example, tropical deforestation coincides with rises of a number of infectious diseases, such as malaria (Patz, et al. 2000) and Buruli ulcer (Morris, et al. 2016). The Tibetan Plateau, with 1.5 million km$^2$ of natural alpine grasslands, is experiencing dramatic grassland degradation and desertification due to climate warming, overgrazing, and rodent damage (Akiyama and Kawamura 2007; Gao, et al. 2010, Zhou, et al. 2005). There is currently about half a million km$^2$ of degraded grassland, among which severely degraded (including desertified) grasslands account for approximately 16% (Wang, et al. 2006), which was expected to stimulate soil-borne pathogenic communities. Changes in vegetation and soil properties during the Tibetan grassland degradation have been well studied (Gao, et al. 2005; Harris 2010; Wu, et al. 2014), however, there has been no research report on the response of soil-borne pathogenic microorganisms during this process.

Previous studies showed that Tibetan alpine grassland degradation significantly decreased plant diversity and altered plant composition, and in particular dramatically increased annual forbs (Li, et al. 2016), which has been found to favor the dispersal of plant pathogens and increase the risk of microbial pathogen infection and transmission (Keesing, et al. 2010, Malmstrom, et al. 2005; Maron, et al. 2011).
addition, the plant rhizosphere is a natural reservoir of many opportunistic human pathogens (Berg, et al. 2005, Kumar, et al. 2013; Mendes, et al. 2013), the dispersal of which is limited by the tight turf and root adherence in non-degraded grasslands (Berg, et al. 2005). However, their dispersal may be enhanced when turf is destroyed during grassland degradation (Harris 2010). Also, the enhanced damage caused by plant pathogens may also increase plant colonization and invasion by human pathogens (Mendes, et al. 2013; Teplitski, et al. 2011). On the other hand, soil physical and chemical deterioration during grassland degradation and desertification processes could also favor the growth of soil-borne pathogenic microorganisms (Huber and Haneklaus 2007; Zhang, et al. 2017). Therefore, we hypothesized that the Tibetan alpine meadow desertification could increase the richness and abundance of soil-borne pathogens.

As a natural host of many human and livestock pathogenic microorganisms, the behavior of plateau pika (*Ochotona curzoniae*), a small mammal, can directly affect the dispersal of soil microbial pathogens and spread diseases like salmonellosis, plague, tularemia, tick-borne rickettsiosis and pseudotuberculosis (Yu, et al. 2014; Zhang, et al. 2003, Zhou, et al. 2009). It can also indirectly affect the dispersal of pathogens through causing or enhancing meadow degradation (Zhou, et al. 2005; Zhou, et al. 2004). There are about 1.5 billion plateau pikas in the alpine meadow ecosystem and their density substantially increases with meadow degradation (Zhang, et al. 2003). Thus, the intensity pika behavior was hypothesized to increase soil pathogenic species richness and relative abundance as well.
The potential changes in soil pathogenic abundances may caused considerable health risks. A combination of poverty and remoteness on the Tibetan Plateau results in poor medical support and education systems, which may further increase the health risk resulted from microbial pathogen. In addition, many of China and Asia’s major rivers originate from Tibetan plateau, such as the Yangtze, Yellow, Indus, Mekong, and Ganges Rivers, sustaining life and agricultural water usage for nearly 40% of the world’s population (Eamer, et al. 2007). Therefore, the lack of knowledge on pathogenic response to Tibetan grassland degradation and pika behavior is of significant concern because any changes in pathogenic risk in Tibetan Plateau may cause significant effects on downstream water system and population.

Here, to investigate the impacts of Tibetan alpine meadow desertification and pika burrowing on soil pathogenic communities and the main environmental factors linked to their changes, we collected soil samples from non-degraded (ND), desertified (DM) and pika burrowed (PB) meadows and analyzed the richness and relative abundance of pathogens using 16S rRNA and ITS gene sequencing. The sequences annotation was done by blasting against pathogenic databases.

Materials and methods

Site description

This study was conducted during summer 2013 at the Naqu Ecological and Environmental Observation and Research Station, China (31° 17' N, 92° 06' E; 4501 m a.s.l.), which is located in the center of the major distribution of *Kobresia pygmaea*
on the Tibetan Plateau (Miehe, et al. 2008) and is about 22 km in the southeast of Naqu City and 270 km northeast of Lhasa (Fig. S1). The dominant plant species is *Kobresia pygmaea* with more than 85% canopy coverage for non-degraded meadow (Miehe, et al. 2008). The local climate is characterized by strong solar radiation with long, cold winters, and short, cool summers. The annual mean air temperature and annual mean precipitation are -2.1°C and 406 mm, respectively. Most of the precipitation falls between June and September.

Firstly, to assess the status of the alpine meadow here, we measured the relative coverage of vegetation, sand patches, and pika burrow exits and channels using a 100 m × 100 m quadrat that was divided into 10,000 1×1 m grid cells in August, 2013. Coverage was computed by the frequency of their occurrence in each of the 10,000 grid cells. In addition, all pika burrow exits within 1×1 m grid cells were also recorded.

**Soil sampling**

Non-degraded (ND) and desertified meadows (DM) were chosen based on plant community coverage and proportion of *Kobresia pygmaea*. For ND, the dominant plant species is *Kobresia pygmaea* with intact turf and more than 85% canopy coverage. Complete desertification occurs in DM with annual forbs as dominant species (e.g. *Heteropappus hispidus*, *Przewalskia tangutica Maxim*, *Chenopodium glaucum L.*, and *Axyris prostrate*). Soils from active pika burrow channels were collected to serve as pika burrowing samples (PB, Fig. S2). Active pika burrows were
marked randomly through observing pika behavior one day before taking soil samples. Seven quadrats (10×10 cm) with a pairwise distance of more than 50 m were randomly selected for ND and DM. Twenty quadrats (10×10 cm) were selected for PB along the pika burrow channels (Fig. S2). Plant species richness, coverage and composition of each quadrat were measured using point intercept sampling with 100 sampling points spaced equidistantly within the 10×10 cm frame. Above- and below-ground plant biomass was measured by taking the weights of all living plants above ground and 0-10 cm below ground roots in each quadrat.

The upper 10 cm soil was collected from 3 locations within each quadrat of ND, and DM and composited into a single bulk sample. For PB sampling, soils upper the pika burrow channel in each PB quadrat, i.e. from soil surface to pika burrow channel, were collected along the pika burrow channels (Fig. S2). All samples were shipped to lab a few days of collection where they were sieved to 2 mm and thoroughly homogenized. Parts of the soils were dried in natural condition for soil chemical and physical analyses and other parts were frozen under -80°C for molecular analysis.

**Soil chemical and physical analyses**

Soil structure was measured by the hydrometer method (Ashworth, et al. 2001). Soil total carbon (TC), total nitrogen (TN), total phosphorous (TP) and total potassium (TK) were analyzed using an isotope ratio mass spectrometer with a Eurovector Elemental Analyzer (Isoprime-EuroEA 3000, Milan, Italy). Total organic carbon (TOC) was measured by a TOC-5000A analyzer (Shimadzu Corp., Kyoto,
Japan). The pH value was measured by using 5 g soil and 10 ml of deionized water. Soil available nitrogen (AN), available phosphorous (AP), and available potassium (AK) content were measured by the methods of Wu et al. (2011).

**DNA extraction, PCR and Illumina MiSeq sequencing analysis**

Total soil DNA was extracted from 0.5 g soil (dry weight) using FastDNA spin kit for soil (MP Biomedical, Carlsbad, CA, USA), according to the manufacturer’s instructions. DNA quality assessment and quantification was conducted using a Nano-Drop ND-1000 Spectro-photometer (NanoDrop Technologies Inc., Wilmington, DE, USA). Then the DNA extractions were diluted to 10 ng/μl to serve as PCR templates. For Illumina MiSeq sequencing analysis, bacterial 16S rRNA and fungal ITS genes were amplified from each soil sample. For bacterial 16S rRNA, the forward primer used was F515 combined with the reverse primer R907, as described by Zhou et al. (2011), which targeted the region V4/V5 of the 16S rRNA (Table S1). Fungal ITS2 region was amplified with a nested PCR approach as recommended by Berry et al. (2012). The entire ITS region of fungal DNA was first amplified using the fungal-specific primers ITS1-F and ITS4, and then a second PCR using the primers ITS3 and ITS4 targeted the ITS2 region (Table S1). Primers contained barcodes to allow subsequent position assignment of all amplicons. A PCR mix of 25 μl containing 1×PCR buffer (Fermentas, Vilnius, Lithuania), 0.2 μM of each primer, 0.2 mM deoxynucleoside triphosphates (dNTPs), 2.75 mM MgCl₂, 300 ng/μl BSA, 10 ng of template, and 2.5 Units of the Pfu polymerase (BioVision, Mountain View, CA, USA) was prepared for each amplification. Reactions, performed in triplicate, were
combined and purified using gel electrophoresis followed by the QIAquick gel
extraction kit and the Qiagen PCR purification kit and quantified using
QuantiFluorTM-ST (Promega, USA). Purified barcode tagged amplicons were pooled
in equimolar and paired-end sequenced on an Illumina MiSeq platform (Majorbio,
Shanghai) according to the standard protocols. These sequence data have been
submitted to the National Center for Biotechnology Information (NCBI) Sequence
Read Archive (SRA) databases under accession number SRP068608.

**Sequence analysis**

All sequence processing and diversity estimates were performed using QIIME
(Caporaso, et al. 2010). Raw sequences were quality-filtered with the following
criteria: (i) sequence reads were truncated at any site that obtained an average quality
score of <25 over a 10-bp sliding window, and the truncated reads shorter than 250 bp
were discarded; (ii) exact barcode and primer matching, and reads containing
ambiguous characters or containing more than 10 homopolymers were removed; and
(iii) only overlapping sequences longer than 20 bp were assembled according to their
overlapped sequence. Reads that could not be assembled were discarded. The
resulting reads were subject to denoising using DENOISER v. 0.91(Reeder and
Knight 2010) and chimera analysis using UCHIME and ChimeraSlayer as
implemented in QIIME. High-quality non-chimeric sequences were clustered into
operational taxonomic units (OTUs) using a 99% similarity threshold with the uclust
algorithm. The singleton OTUs (with only one read) were removed, and the remaining
sequences were sorted into each sample based on unique sample tags. For all 34
samples, a total of 616,808 effective 16S rRNA sequences were obtained and the
number of sequence reads in each sample ranged from 10,334 to 29,336. For ITS
sequences, 208,674 effective sequences were obtained and the number of sequence
reads in each sample ranged from 5,032 to 7,422. The most abundant sequence from
each OTU was selected as a representative sequence for that OTU. Bacterial OTU
representative sequences were classified taxonomically through blasting against the
Silva SSU Ref NR Database (version:132, Quast, et al. 2013), and fungal OTUs were
blasted against the UNITE fungal ITS Database (Abarenkov, et al. 2010) (both
prefiltered at 99% identity). Then the sequences were rarefied to 8,000 and 4,500
sequences per sample for bacterial and fungal sequences, respectively, and submitted
to further analysis. Bacterial α-diversity was estimated using species richness and
Faith’s phylogenetic diversity (Faith’s PD) (Faith 1992). Fungal α-diversity was
estimated using species richness.

Pathogenic species identification

The Silva 16S rRNA and UNITE ITS Database classify corresponding sequences
down to genus level, which was insufficient for the purposes of our study. To enable
the most accurate species level classification, a blast database containing all the 16S
rRNA and ITS sequences of the pathogenic species included in the enhanced
infectious disease database (Wardeh, et al. 2015) and in Taylor et al. (2001) was built
using BLAST+ (Camacho, et al.). The corresponding 16S rRNA and ITS sequences
were downloaded from Greengenes (Isolated_named_strains_16S_aligned.fasta)
(DeSantis, et al. 2006) and NCBI databases, respectively. Then, the 16S rRNA and
ITS sequences were aligned against the database using BLAST+ (Camacho, et al.) and identities higher than 99% were accepted.

Moreover, to further detect the response of microbial pathogenic traits to meadow degradation and pika effects, we used PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States, http://picrust.github.com (Langille, et al. 2013b)) and FUNGuild (Nguyen, et al. 2016) to annotate bacterial and fungal sequences, respectively. We followed the suggested methods for bacterial OTU picking with Greengenes 13.5 (http://huttenhower.sph.harvard.edu/galaxy/). We focused our analysis only on the functional category for infectious diseases and only the “highly probable” and “probable” confidence rankings were accepted.

Data analysis

Data analysis was conducted by using the packages vegan (Dixon 2003) and picante (Kembel, et al. 2010) with the statistical platform R (version 3.3.2). Tukey's HSD tests were used to compare environmental and microbial characteristics among the three meadow types. Non-parametric multivariate analysis of variance (ADONIS) and analysis of similarities (ANOSIM) based on Bray-Curtis distance matrices were used to test the differences in microbial community composition among different meadows. To evaluate possible linkages between microbial community and environmental variables, a total of 13 soil variables were analyzed (Table 1). Mantel tests were performed to find the important factors influencing microbial composition. Collinearity was tested using the variance inflation factor (VIF) test and recorded as
VIF>20. To better understand how much each environmental variable influences the microbial community structure, variation partitioning analysis (VPA) (Ramette and Tiedje 2007) was performed. The VPA resulted in three groups of soil and plant attributes for microbial pathogenic community. For bacterial pathogens, soil chemical attributes were pH value, TOC, AP, TK, and AK, soil physical attributes included soil clay and silt content, and plant attributes included plant above- and below-ground biomass, species richness, Shannon-Wiener diversity, and forbs coverage. Soil chemical attribute groups for fungal pathogenic community were pH value, TC, TOC, TN, TP, AP, TK, and AK, soil physical attributes included soil clay and silt content, and plant attributes included plant above- and below-ground biomass, species richness, and Shannon-Wiener diversity.

Results

Status of the investigated alpine meadow

The alpine meadow of the investigation area was under significant degradation and pika effects. Based on the 1×1 m grid investigation within a 100 m×100 m quadrat, the coverage of ND, DM, and PB (i.e. pika burrow entrances and channels) accounted for 72, 22 and 6% of the total experimental area, respectively (Fig. S3). Altogether, there were 3,944 pika burrow entrances (including active and inactive) within the 10,000 m² investigation area.

Response of plant and soil variables to meadow desertification and pika burrowing
We found a significant change in plant variables by meadow desertification and pika burrowing. Above- and below-ground plant biomass and coverage were significantly decreased for DM and PB relative to ND (Table 1). DM significantly decreased plant species richness and Shannon-Wiener diversity, while PB had no significant effect on them (Table 1). The relative coverage of forbs (i.e. *Heteropappus hispidus*, *Przewalska tongutica* Maxim, *Chenopodium glaucum* L., and *Axyris prostratus*) were 15% in ND, and significantly increased to 96 and 23% in DM and PB, respectively (Table 1).

Soil nutrition content was significantly decreased by meadow desertification and pika burrowing. Compared with ND, total soil carbon and soil organic carbon, total soil nitrogen and available nitrogen, total soil phosphorous and available phosphorous, total soil potassium and available potassium, and silt content significantly decreased, but soil sand content significantly increased in DM and PB meadows (Table 1). DM significantly increased while PB significantly decreased soil pH value relative to ND (Table 1).

**Response of bacterial and fungal communities to meadow desertification and pika burrowing**

Bacterial community properties were little changed by meadow desertification and pika burrowing, except for the OTU level composition. *Proteobacteria* (ND 29 ± 0.6%, DM 30 ± 1%, PB 30 ± 1%) were the most abundant bacterial phyla in the three meadow soils, followed in decreasing order of relative abundance by *Actinobacteria*...
(ND 25 ± 1.3%, DM 24 ± 1.6%, PB 27 ± 1%) and Acido bacteria (ND 25 ± 0.7%, DM 23 ± 0.8%, PB 22 ± 1%) (Fig. S4A). No significant differences in bacterial phylum composition, species richness, and phylogenetic diversity (Faith’s PD) were found among these meadow soils (Fig. S4), while significant shifts in bacterial OTU composition were found (Table S2).

Fungal composition and diversity were dramatically changed by meadow desertification and pika burrowing. DM and PB significantly changed the fungal phylum (Fig. S4 B) and OTU composition (Table S2) and increased their species richness by 162 and 164%, respectively, relative to ND (Fig. S4 D). The fungal community was dominated by Ascomycota, which accounted for 96 ± 8%, 82 ± 9%, and 88 ± 7% of the total reads for ND, DM, and PB meadows, respectively (Fig. S4B).

For ND, the Ascomycota mainly consisted of Sordariomycetes (77 ± 6%), followed by the class Leotiomycetes, Dothideomycetes, and Eurotiomycetes (Fig. S4B). The relative abundance of Sordariomycetes significantly decreased, while Dothideomycetes and Eurotiomycetes significantly increased in DM and PB meadows relative to ND. The two increased groups were notable for their content of major plant (Hane, et al. 2007) and animal and human opportunistic pathogens (Gueidan, et al. 2008).

Response of pathogenic communities to meadow desertification and pika burrowing

Relative abundance of certain bacterial pathogens increased by meadow
desertification and pika burrowing. In total, 203 bacterial pathogenic species were detected in the three meadow soils according to the sequence alignment, which accounts for 2.3% ± 0.3%, 2.5% ± 0.3%, and 2.1% ± 0.1% of ND, DM, and PB bacterial community, respectively. There were 71 species shared among the three soils (Fig. S5). MD and PB had no significant impacts on species richness (Fig. 1A) and relative abundance (Fig. 1C) of overall bacterial pathogens. Then, the pathogens were further classified into human, plant, domestic animal and zoonotic pathogens based on the database assignment. MD significantly increased the relative abundance of bacterial pathogens for domestic animals and bacterial zoonotic pathogens by 180 and 81%, respectively, relative to ND (Fig. 1C). PB significantly increased the relative abundance of bacterial human pathogens by 61% and bacterial zoonotic pathogens by 256% relative to ND (Fig. 1C). Additionally, we found that *Ralstonia solanacearum*, which is probably the most destructive soil-borne plant pathogenic bacteria worldwide (Mansfield, et al. 2012), significantly increased in relative abundance by 72 and 77% in DM and PB meadows compared to ND (Fig. S6). The rodent bacterial pathogens *Ralstonia pickettii* and *Staphylococcus saprophyticus* were only detected in pika burrows (Fig. S6).

PICRUSt prediction did not show significant effects of DM and PB on overall human infectious disease. The Nearest Sequenced Taxon Index (NSTI), which indicates the accuracy of PICRUSt, were 0.2±0.003, 0.2±0.004, and 0.2±0.003 for ND, DM, and PB soil bacterial communities, respectively, indicating reasonable predictions (Langille, et al. 2013a). Though significant changes in overall human
infectious disease were not found, the predicted pathogenic *Escherichia coli* infection, shigellosis, *Staphylococcus aureus* infection, and *Vibrio cholerae* infection, which belong to human infectious disease pathways, were significantly increased by DM compared with ND (Fig. 2A). PB was predicted to significantly increase amoebiasis, pathogenic *Escherichia coli* infection, shigellosis, and *Vibrio cholerae* infection pathways compared with ND (Fig. 2B).

Fungal pathogens were significantly stimulated by meadow desertification and pika burrowing. There were 33 fungal pathogenic species detected in the three meadow soils, which accounts for $0.1 \pm 0.02$, $5.7 \pm 0.4$, and $10.2 \pm 2.3\%$ of ND, DM, and PB fungal community, respectively. 5 fungal pathogenic species were shared among the three soils (Fig. S5). Both the species richness and relative abundance of all kinds of fungal pathogens increased significantly by DM and PB (Fig. 1 B and D). The fungal zoonotic pathogens *Scopulariopsis brevicaulis* and *Fusarium solani* significantly enriched in DM and PB meadows compared to ND (Fig. S6). In alignment with blast results, FUNGuild prediction results showed that the overall fungal pathogens, plant pathogens and animal pathogens were significantly increased by DM and PB (Fig. 2).

**Pathogenic community changes were linked to soil and plant properties**

Soil chemical and plant properties were the most important factors shaping bacterial and fungal pathogenic community. The Mantel test showed that soil silt, sand content, plant above- and below-ground biomass, coverage, species richness, and relative abundance of forbs were significantly correlated with the changes in the
overall bacterial pathogenic communities (Table 1). In addition, plant above-ground biomass, coverage, species richness, and relative abundance of forbs were significantly correlated with changes in all types of bacterial pathogenic communities (Table 1). Regarding fungal pathogens, significant associations between the fungal pathogen shifts and all the tested soil physiochemical and plant properties were found (Table 1). Further variation partitioning analysis showed that all the tested soil physiochemical and plant properties explained more than 40% of bacterial pathogenic composition variance, which was mainly explained by soil chemical and plant properties (Fig. 3). More than 60% of fungal pathogenic community variations could be explained by the tested soil and plant attributes, except for plant pathogenic composition (Fig. 4). Soil chemical and plant properties were the most important factors shaping fungal pathogenic composition, and accounted for more than 40% of the variation (Fig. 4).

Discussion

With approximately 40% of the world’s population living in its downstream watersheds, the Tibetan Plateau is considered “Asia’s Water Tower” (Eamer, et al. 2007). However, grasslands of the Tibetan Plateau, where most of Asia’s great rivers originate, are becoming increasingly degraded and increasing in pika population densities, which were expected to stimulate the soil-borne pathogens. Thus, understanding the response of Tibetan meadow soil-borne pathogens to degradation and pika behavior is essential.
Here we demonstrated that DM and PB significantly stimulated soil pathogens, especially for fungal pathogens, using the 16S- and ITS-MiSeq method that has been found to be sensitive in detecting pathogens (Razzauti, et al. 2015, Srinivasan, et al. 2015). The variations in microbial pathogenic community were mainly explained by soil chemical and plant properties (Fig. 3 and 4). Meadow desertification and pika burrowing significantly decreased soil nutrient content (Table 1), which is consistent with previous reports (Li, et al. 2016; Sun, et al. 2015), and might favor the growth of pathogens due to their lower reliance on soil nutrients than other microbes (Huber and Haneklaus 2007; Zhang, et al. 2017). Compared to bacteria, higher sensitivity of fungi to soil nutrient limitation has been found before (Dooley and Treseder 2012; Hartmann, et al. 2014, Lauber, et al. 2008). Thus, pathogenic fungi may be more favored due to the greater depression of normal fungi resulting from decreased soil nutrients relative to bacterial communities. This might be one reason for the more significant change in fungal pathogens relative to bacterial pathogens (Fig. 1 and 2). Meanwhile, soil nutrients in many situations are the first and foremost line of defense against plant pathogens for plants (Huber and Haneklaus 2007). Thus, their decrease should be indirectly beneficial for the invasion and growth of plant pathogens. Decreased plant diversity and increased abundance of annual forbs in desertified and pika burrowed meadows (Table 1) have also been found to increase the prevalence of various plant pathogens and to cause negative effects on grassland plant production (Malmstrom, et al. 2005; Maron, et al. 2011). Many opportunistic human pathogens live in plant rhizospheres (Berg, et al. 2005, Kumar, et al. 2013; Mendes, et al. 2013),
which was considerably destructured by desertification and pika burrowing (Table 1). Thus, the increased human pathogens may partly result from the destruction of turf and plant rhizospheres in desertified and pika burrowed meadows. For example, the human pathogen *Stenotrophomonas maltophilia* (ND 0.002 ± 0.001%, PB 0.008 ± 0.004%), which is frequently detected in plant rhizospheres (Berg, et al. 2005), was increased by pika burrowing. In addition, as an important reservoir of pathogens responsible for numerous zoonotic diseases in humans and livestock, increased pika density and the corresponding tick vectors may also cause the increased microbial pathogens. For example, *Ralstonia pickettii* and *Staphylococcus saprophyticus*, both rodent pathogens, were only detected in the pika burrow channels (Fig. S6).

Although no significant increase in bacterial pathogen species richness was detected for each soil sample by DM and PB (alpha diversity, Fig. 1A), their overall species richness (gamma diversity) increased by 41 and 96%, respectively (Fig. S5). The alpha (Fig. 1B) and gamma (Fig. S5) diversity and relative abundance of fungal pathogens was dramatically increased by meadow desertification and pika burrowing (Fig. 1B and D). The increased pathogenic diversity and abundance were expected to increase infectious possibility for plant and animals in local and downstream ecosystems. Positive trends in the proportion of records of fungi infecting animals and plants, and fungal disease alerts were found to occur worldwide in recent years, which comprised the highest threat for both animal- and plant-host (Fisher, et al. 2012). In our study, the dramatically increased relative abundance of the fungal pathogen *Scopulariopsis brevicaulis* in pika burrowed meadows (Fig. S6), which is associated...
with a broad spectrum of clinical syndromes, such as onychomycosis, and which is multi-resistant to broad-spectrum antifungal agents available today (Cuenca-Estrella, et al. 2003, Kwon-Chung and Bennett 1992), may pose a threat to human health. The significantly increased relative abundance of *Fusarium solani* species in both desertified and pika burrowed meadows (Fig. S6) may cause infections in both humans and plants (Zhang, et al. 2006). *Fusarium solani* species associate with serious invasive mycoses in immunocompromised and immunosuppressed patients with a high mortality rate (Krcmery, et al. 1997). In addition, many of the *Fusarium* species have the potential to produce a range of toxic secondary metabolites that cause a potential health risk when contaminated grass is grazed by livestock (Doohan, et al. 2003). Notably, these potentially increased negative impacts of microbial pathogens may be further enhanced by strong climate warming (Altizer, et al. 2013), relatively poor socio-economic conditions, and dust storms on the Tibetan Plateau. Moreover, compared to the Han population, Tibetans seem to be less resistant to certain pathogens, such as *Mycobacterium tuberculosis* (Li, et al. 2011). Although the accuracy of amplicon sequencing methods on pathogenic species-level identification was about 87.5% (Srinivasan, et al. 2015) and these results should be further tested with more physiological evidences, our results suggest more attention should be paid on Tibetan grassland pathogenic response to desertification and pika behavior due to its significant effects on local and downstream water systems and populations.

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Figure 1 The species richness and relative abundance of overall, human, plant, domestic animal, and zoonotic bacterial (A and C) and fungal (B and D) pathogens in non-degraded meadows (ND), desertified meadows (DM), and pika burrowed meadow (PB), which were compared among three types of meadows using HSD tests. Different letters indicate significant difference at 0.05 level. Error bars represent standard error (n=7 for BD and DM, n=20 for PB).

Figure 2 Log response ratio (95% confidence interval) for human infectious disease KEGG pathways predicted by PICRUSt and fungal pathogens classified by FUNGuild in desertified meadows and non-degraded meadows (A), and in pika burrowed meadows and non-degraded meadows (B). Values significantly more than zero indicate that the corresponding pathway was significantly increased in desertified (A) or pika burrowed (B) meadows compared to non-degraded meadows, whereas values not different from zero indicate that the pathways were unchanged.

Figure 3 Partitioning of potential bacterial pathogenic composition variance, which shows the relative proportions of pathogenic composition variations that can be explained by different types of soil attributes. Soil chemical attributes (SA1) were pH value, soil total organic carbon, available phosphorous, total potassium, and available potassium; soil physical attributes (SA2) included soil clay and silt content; plant attributes (PA) included above- and below-ground biomass, species richness, Shannon-Wiener diversity, and forbs coverage. A, total pathogenic composition; B, human pathogenic composition; C, plant pathogenic composition; D, domestic animal
pathogenic composition; E, zoonotic pathogenic composition.

Figure 4 Partitioning of potential fungal pathogenic composition variance, which shows the relative proportions of pathogenic composition variations that can be explained by different types of soil attribute. Soil chemical attributes (SA1) were pH value, soil total carbon, total organic carbon, total nitrogen, total phosphorous, available phosphorous, total potassium, and available potassium; soil physical attributes (SA2) included soil clay and silt content; plant attributes (PA) included above- and below-ground biomass, species richness, and Shannon-Wiener diversity. A, total pathogenic composition; B, human pathogenic composition; C, plant pathogenic composition; D, domestic animal pathogenic composition; E, zoonotic pathogenic composition.
Table 1 Summary of soil attribute measurements for non-degraded (ND), desertified (DM), and pika burrowed meadow (PB) soils and their correlation with microbial pathogen composition (r values) as shown by Mantel tests. Values are mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>ND</th>
<th>DM</th>
<th>PB</th>
<th>bacterial pathogens</th>
<th>fungal pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall</td>
<td>Human</td>
<td>Domestic</td>
<td>Plant</td>
<td>Overall</td>
</tr>
<tr>
<td>pH</td>
<td>7.6±0.1b</td>
<td>8.8±0.2a</td>
<td>7.2±0.1c</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>TC(%)</td>
<td>6.5±0.1a</td>
<td>3.5±0.1b</td>
<td>3.8±0.1b</td>
<td>&lt;0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>TOC(%)</td>
<td>4.2±0.1a</td>
<td>2±0.1c</td>
<td>2.3±0.1b</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>TN(g/kg)</td>
<td>3.4±0.1a</td>
<td>0.6±0.1c</td>
<td>1.3±0b</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>AN(mg/kg)</td>
<td>171.6±3.8a</td>
<td>46.8±1.7c</td>
<td>64.9±1.8b</td>
<td>&lt;0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>TP(g/kg)</td>
<td>0.8±0a</td>
<td>0.2±0b</td>
<td>0.2±0b</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>AP(mg/kg)</td>
<td>6.1±0.2a</td>
<td>2.1±0c</td>
<td>2.4±0b</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>AK(mg/kg)</td>
<td>14.7±0.7a</td>
<td>7.6±0.2b</td>
<td>6.9±0.1b</td>
<td>&lt;0.1</td>
<td>0.1</td>
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<tr>
<td>Clay(&lt;2μm %)</td>
<td>17.1±0.5a</td>
<td>6.4±0.4b</td>
<td>16.3±1.4a</td>
<td>0.1</td>
<td>0.1</td>
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<tr>
<td>Silt(2-50μm %)</td>
<td>46.9±0.9a</td>
<td>33.3±0.5b</td>
<td>35.6±1.2b</td>
<td>0.2*</td>
<td>0.1</td>
</tr>
<tr>
<td>Sand(5-2000μm %)</td>
<td>35.9±1.2c</td>
<td>60.3±0.4a</td>
<td>48.1±2.4b</td>
<td>0.2*</td>
<td>0.1</td>
</tr>
<tr>
<td>AGB g/m2</td>
<td>1831.9±65.6a</td>
<td>868.3±26.3c</td>
<td>1325.2±52.4b</td>
<td>0.2*</td>
<td>0.2*</td>
</tr>
<tr>
<td>BGB g/m2</td>
<td>4312.3±206.8a</td>
<td>326.3±20.4c</td>
<td>3521.2±409b</td>
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<td>0.1*</td>
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<tr>
<td>Coverage %</td>
<td>93.2±1.4a</td>
<td>43.2±6.1c</td>
<td>80.3±1.9b</td>
<td>0.2*</td>
<td>0.2*</td>
</tr>
<tr>
<td>Species richness</td>
<td>6.4±0.3a</td>
<td>2.6±0b</td>
<td>6.2±0.4a</td>
<td>0.2*</td>
<td>0.2*</td>
</tr>
<tr>
<td>Plant Shannon diversity</td>
<td>0.9±0.1a</td>
<td>0.6±0b</td>
<td>1±0.1a</td>
<td>&lt;0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Forbs %</td>
<td>15.1±2.5c</td>
<td>95.6±2a</td>
<td>22.8±2.2b</td>
<td>0.2*</td>
<td>0.2*</td>
</tr>
</tbody>
</table>

Abbreviations: Soil total carbon (TC), total organic carbon (TOC), total nitrogen (TN), available nitrogen (AN), total phosphorous (TP), human pathogens, domestic pathogens, plant pathogens, zoonotic pathogens.
available phosphorous (AP), total potassium (TK), available potassium (AK), clay content (Clay), silt content (Silt), sand content (Sand), extractable DNA (DNA). In a row, if the lower case letter is the same, there is no significant difference, and different letters indicate significant difference at 0.05 level. For Mantel test **P<0.01, *P<0.05
Figure 1

289x202mm (300 x 300 DPI)
Figure 2

289x202mm (300 x 300 DPI)
Figure 3