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# The response of soil bacterial communities to mining subsidence in the west China aeolian sand area

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ABSTRACT

Soil bacteria play a vital role in terrestrial ecosystems and are very sensitive to changes in the environment. Land subsidence due to underground coal mining could affect soil properties, but the extent of this effect on the soil bacterial community remains unclear. Here, we investigated the effect of land subsidence on soil bacterial communities and their response to changes in the soil environment in a control area and a land subsidence area in the West China Aeolian Sand Area. The results showed that electrical conductivity (EC), total carbon, (TC), total nitrogen (TN), available potassium (AK) and soil organic matter (SOM) at a soil depth of 20 cm were significantly decreased in the land subsidence area compared to the unexploited area, and Illumina MiSeq sequencing data revealed that the bacterial community at a soil depth of 0–180 cm was dominated by *Pseudomonas, Gp4, Gp6, Sphingomonas*, *Gemmatimonas, Arthrobacter*, *Aciditerrimonas* and *Gaiella*. Land subsidence decreased soil microbial richness and diversity. In addition, there was a significant decrease in the relative abundance of some core genera in the topsoil, such as *Sphingomonas, Nocardioides* and *Saccharibacteria_genera_incertae_sedis*, indicating that the dominant bacteria had strong anti-interference abilities and played important roles in the nutrient-poor soils of the mining area. Redundancy analysis (RDA) showed that the main factors driving the changes in the bacterial community structure were EC, water content (WC) and soil depth. The vertical leakage of water and nutrients was caused by subsidence and cracks in the ground, leading to decreased soil microbial richness and diversity. These results suggested that the soil nutrients and soil microbial community has yet to recover by self-healing after two years of land subsidence; thus, artificial restoration might be required.

## 1. Introduction

Coal is one of the major energy sources in China. As coal is excavated, the weight of the overlying strata originally supported by the coal is supported only by the remaining pillars or walls. Therefore, surface subsidence and cracks can occur due to subsidence of the overlying strata, which warps and breaks away to surface under the effect of gravity. Subsequently, soil water content and nutrients are lost, soil enzyme activities decrease, and soil microbial communities are altered, especially, the ecological environment in the West China Aeolian Sand Area has continuously deteriorated due to the combination of coal mining and water-wind erosion. The soil particles in the west China aeolian sand area was mainly composed of medium and fine sand, but land subsidence has resulted in an increase in coarse and medium sands and a decrease in fine sand (Zhao et al., 2015). In addition, soil nutrients can leach into deep layers in mine subsidence areas, resulting in a decrease in soil fertility (Chen et al., 2009). The effects of mining subsidence on nutrients in sandy soil were far greater than those on soil physical properties: water content could recover to approximately 75% 7 years after subsidence, soil porosity could be completed recovery, and nitrogen (N) and phosphorus (P) could gradually be restored 12–17 years after subsidence (Wang et al., 2014).

Soil microbial communities are an important part of an ecosystem, and their abundance, distribution and diversity are closely related to soil structure and function, e.g., nitrogen-fixing bacteria can combine N with oxygen or hydrogen to form ammonia or nitrates, which can be absorbed by plants (Baldani et al., 2014). In addition, phosphorus bacteria and potassium bacteria are capable of transforming soil P and potassium (K) into available P and available K to improve soil fertility (Ahmad et al., 2009). Recent studies have shown that soil microorganisms are sensitive to changes in environmental factors. For instance, the addition of N was shown to reduce soil microbial biomass

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carbon (C) and N in a semiarid temperate steppe in northern China, and the soil microbial biomass showed positive dependence on soil water content and dissolved organic C (Wei et al., 2010). In China, Li et al. (2015) found that N addition decreased the microbial biomass but did not affect the soil fungal biomass. In contrast, P addition increased the biomass of both bacteria and fungi, and the response of soil fungi was more sensitive to P addition than that of bacteria in a secondary tropical forest (Li et al., 2015). Therefore, soil microorganisms can be used as indicators to reflect changes in soil quality caused by natural or human disturbances.

A better understanding of soil bacterial diversity following surface subsidence due to underground coal mining could provide a theoretical foundation for the management and restoration of land subsidence and may allow analysis of the effect of mining on soil quality. Thus far, little research has been conducted on the effect of land subsidence on soil microbial diversity. Li et al. (2014a, 2014b) studied soil bacterial diversity at the Liuxin mining field in Xuzhou City, East China, and the results showed that the reclamation site presented higher bacterial diversity and a more complex community structure than an adjacent, coal-excavated subsidence site (Li et al., 2014). However, to our knowledge, the soil bacterial diversity of coal mining areas in the West China Aeolian Sand Area has not been reported.

In the present study, we investigated the effect of post-mining subsidence on soil bacterial diversity and its response to edaphic variables in the West China Aeolian Sand Area using Illumina MiSeq sequencing.

## 2. Materials and methods

### 2.1. Site description

This study was performed in Daliuta Town, Shenmu County, Shaanxi Province, Northwest China (110° 00′ −110° 24′E, 39° 11′−39° 29′N). This area is located along the southern margin of the Mu Us Desert in the north of the Loess Plateau and is characterized by low precipitation, high evaporation, semi-fixed dunes, and an arid or semiarid climate that forms a fragile ecosystem (Lei et al., 2010). The Aeolian Sand Area covers an area of 3 $km^2$ and is covered by aeolian sandy land with a depth of 5–50 m; the soil is sandy soil, which is seriously affected by soil erosion and desertification (Tai et al., 2016). The mean annual temperature in this area is 7.3 °C; mean annual precipitation is 395.26 mm, with the main period of rainfall occurring from June to September; annual evaporation is 1788.4 mm; and the annual average wind speed is 3.2 m $s^{-1}$. The soil particles are mainly composed of medium and fine sand (Zhao et al., 2015). Underground mining is the main coal-mining method in this area. The average depth of the coal seam is approximately 200 m, and the thickness of the coal seam is approximately 4–10 m in the study area. Sparse poplars, *Caragana korshinskii* and *Artemisia desertorum* grow around the study area.

### 2.2. Soil sample collection

The land subsidence area was mined in September 2013. Two years after subsidence (November 2015), soil sampling was performed in the subsidence area and in an adjacent unexploited area (control). There was a distance of approximately 500 m between the neighboring sites, which had the same soil type and soil texture. We sampled soils in a vertical direction, at 20, 100 and 180 cm, in the control area (C1, C2, and C3, respectively) and in the subsidence area (S1, S2, and S3, respectively). In each area, three quadrats (3 m × 3 m per quadrat) were established as triplicate sampling sites. In each quadrat, three composite samples were randomly collected at each soil depth. After carefully removing fine roots, each sample was divided into two portions. One portion was naturally air-dried and sieved through a 2-mm mesh for the evaluation of soil properties. The other portion was stored at −80 °C for subsequent high-throughput Illumina sequencing analysis.

### 2.3. Determination of soil properties

The soil pH and electrical conductivity (EC) of the air-dried samples were measured using a pH meter and a conductivity meter at 1:2.5 and 1:5 soil/water ratios (w/v) (Gao et al., 2015; Zhang et al., 2013), respectively. The soil water content (WC) was determined through gravimetric analysis, in which 10.0 g of fresh soil from each sample was dried overnight at 105 °C, and WC was obtained from the difference between the fresh weight and dry weight of the soil (Torres-Cortes et al., 2012). Soil organic matter (SOM) was determined according to the method reported by Kalembasa and Jenkinson (2006), and total carbon (TC) and total nitrogen (TN) were analyzed following the methods of Schumacher (2002) and Sparks et al. (1996), respectively. Available potassium (AK) and available phosphorus (AP) were extracted using the methods described by Mitchell et al. (2010).

### 2.4. DNA extraction, PCR amplification and pyrosequencing

Total genomic DNA was extracted from 500 mg of soil per sample using the E.Z.N.A.™ Soil DNA Kit (Omega Biotek, USA) according to the manufacturer's instructions. The extracted DNA was purified and then quantified using a NanoDrop 2000 spectrophotometer (Thermo, USA). The V3-V4 regions of the 16S rRNA gene from each extract were PCR amplified using the following program: 2 min at 95 °C followed by 25 cycles of 30 s at 95 °C for denaturation, 30 s at 55 °C for annealing and 45 s at 72 °C for extension with a final extension at 72 °C for 10 min; the primers 341F 5′-barcode-CCTACGGGNGGCWGCAG-3′ and 805R 5′-GACTACHVGGGTATCTAATCC-3′ were used (Yang et al., 2014). The barcode is an eight-base sequence unique to each sample. The PCR mixture consisted of 5 μL of 10 × PCR buffer, 0.5 μL of 10 mM dNTPs, 10 ng of template DNA, 0.5 μL of each primer (50 mM) and $ddH_2O$ to a total volume of 50 μL. The PCR products were confirmed through 2% agarose gel electrophoresis. Next, amplicons extracted from the 2% agarose gels were purified using a PCR purification kit (Agencourt AMPure XP, Beckman) and quantified using a Qubit®2.0 fluorimeter (Invitrogen, USA) according to standard protocols. The purified amplicons were pooled in equimolar ratios and paired-end sequenced (2 × 300) on the Illumina MiSeq platform (Sangon Biotech, Shanghai) according to standard protocols.

### 2.5. Statistical and bioinformatics analysis

Prior to the bioinformatics analysis, the raw reads were quality filtered using QIIME (version 1.8.0) and were truncated at any sites. Short reads less than 200 bp in length and those with a low quality score for the tail sequence, i.e., less than 20 over a 10-bp sliding window, were removed. Next, the primers and one of the barcode sequences were matched, and reads with an ambiguous character were discarded. Additionally, chimeric sequences were excluded using Uchime (version 4.2.4). After completing the above procedures, the quality-trimmed sequencing reads were aligned using the RDP database. Operational taxonomic units (OTUs), defined as sharing > 97% similarity, were clustered using Usearch (version 5.2.236). Richness and diversity indices, including the Chao, ACE, Coverage, Simpson and Shannon indices, were calculated based on the OTUs with a cutoff value of 0.03 using Mothur (version 1.30.1). Significant differences in the relative abundance of bacteria between samples were determined through Fisher's exact test using STAMP (version 2.1.3). Principal component analysis (PCA), heatmap analysis and redundancy analysis (RDA) were performed using the vegan packages in R (version 2.0-1.0), and similarity contours overlaid on PCA ordinations were based on groupings developed with the Cluster routine. Venn diagrams were created using the Venn diagram package in R (version 1.6.16).

All the soil property data and the Pearson correlation coefficients were analyzed using SPSS 19.0. The data were analyzed as the means of three replicates, and the difference was considered significant when the