International Journal of Mining Science and Technology 24 (2014) 701–706

Contents lists available at ScienceDirect

International Journal of Mining Science and Technology

journal homepage: www.elsevier.com/locate/ijmst

# Pyrosequencing-based assessment of bacterial community structure in mine soils affected by mining subsidence

Li Yuanyuan [a], Chen Longqian [a,*], Wen Hongyu [b], Zhou Tianjian [a], Zhang Ting [a]

[a] Jiangsu Key Laboratory for Resources and Environment Information Engineering, School of Environment and Spatial Informatics, China University of Mining & Technology, Xuzhou 221116, China
[b] School of Life Science, Jiangsu Normal University, Xuzhou 221116, China

ARTICLE INFO

*Article history:*
Received 12 December 2013
Received in revised form 13 February 2014
Accepted 10 March 2014
Available online 17 August 2014

*Keywords:*
Mining subsidence land
Soil physicochemical properties
454 pyrosequencing
Bacterial diversity

ABSTRACT

Based on the 454 pyrosequencing approach, this research evaluated the influence of coal mining subsidence on soil bacterial diversity and community structure in Chinese mining area. In order to characterize the bacterial community comparatively, this study selected a field experiment site with coal-excavated subsidence soils and an adjacent site with non-disturbed agricultural soils, respectively. The dataset comprises 24512 sequences that are affiliated to the 7 phylogenetic groups: proteobacteria, actinobacteria, bacteroidetes, gemmatimonadetes, chloroflexi, nitrospirae and unclassified phylum. Proteobacteria is the largest bacterial phylum in all samples, with a marked shift of the proportions of alpha-, beta-, and gammaproteobacteria. The results show that undisturbed soils are relatively more diverse and rich than subsided soils, and differences in abundances of dominant taxonomic groups between the two soil groups are visible. Compared with the control, soil nutrient contents decline achieves significant level in subsided soils. Correlational analysis showed bacterial diversity indices have significantly positive correlation with soil organic matter, total N, total P, and available K, but in negative relation with soil salinity. Ground subsidence noticeably affects the diversity and composition of soil microbial community. Degeneration of soil fertility and soil salinization inhibits the sole-carbon-source metabolic ability of microbial community, leading to the simplification of advantage species and uneven distribution of microbial species. This work demonstrates the great potential of pyrosequencing technique in revealing microbial diversity and presents background information of microbial communities of mine subsidence land.



## 1. Introduction

Disturbance of soil ecosystems that impacts normal functioning of microbial community structure is potentially detrimental to soil formation, energy transfers, nutrient cycling, plant re-establishment and long-term stability. To date, damage to the soil caused by surface subsidence in mining areas has reached about 600 ha in China [1]. Mining alters soil physical and structural properties, and the effect of coal mining collapse on the soil microbial ecosystem is very obvious [2]. However, as a sensitive means of assessing soil quality, diversity of microorganisms is critical to the maintenance of soil health and quality [3]. Bacteria, the most abundant and diverse group of soil organisms, play a key role in the nutrient cycle, decomposition of organic matter, and purification of polluted soils [4]. Being significantly impacted by coal mining collapse, bacterial diversity can serve as an effective indicator of soil quality [5]. While subsidence in coal-mining areas is known to alter species and associated soil properties, to the author's knowledge, the influencing mechanism of coal mining collapse on soil microbial community remains relatively unknown.

In recent years, many studies reported the mechanisms of soil physicochemical property changes in mining subsidence areas. Some studies focused on the variation law of the soil physics features, soil moisture, soil density, porosity, and structure [6–8]. Other studies have examined changes in soil chemical property affected by mining subsidence, with a focus on soil organic matter, pH, and nutrient content [9–11]. In fact, compared to soil physicochemical properties, soil microbial diversity could be more sensitive to environmental changes, which, to a large extent, remains poorly understood [12].

Molecular cultivation-independent methods have successfully provided relatively deeper insight into the ecological processes that affect the bacterial structure to help identify unknown bacteria [13–15]. However, these tools still underestimate the overall diversity of a microbial community and are unable to detect rare

* Corresponding author. Tel.: +86 13952115869.
*E-mail address:* longqianchencumt@163.com (L. Chen).

http://dx.doi.org/10.1016/j.ijmst.2014.07.002
2095-2686/

species in a complicated environmental system, with primary drawbacks including intense labor requirements and expense as well [16]. The 454 pyrosequencing technology has gained increasing attention as a novel tool for studying the microbial diversity, which can investigate the microbial community more completely and accurately, offering us comprehensive insights into the biogeography of bacterial communities. Pyrosequencing approaches have already been applied to a range of field of studies [17–19]. However, there is no report applying 454 pyrosequencing techniques to examine the bacterial diversity in coal mine soils.

The objective of this study is to investigate the effects of coal mining collapse on soil bacterial communities, species diversity, and to find out the main factors that influence these parameters. This study may offer data and scientific guidance for the state to conduct land reclamation programs in mining areas. Moreover, high-throughput pyrosequencing analysis will potentially provide an integrated and relevant vision of soil ecological system on molecular level.

## 2. Materials and methods

### 2.1. Study site and sample collection

Soil sampling was performed in June, 2012, at the Liuxin mining subsidence area, comprising subsidence land and nearby undisturbed land, located in the north of Xuzhou city, Jiangsu province, China. A north temperate zone monsoon climate dominates this area, with a mean annual rainfall of 868.6 mm, 2390 sunshine hours, an average frost-free period of 216 days, and mean annual air temperature of 13.8 °C.

Samples were collected from two selected sites representing two sample groups: (1) TXD (a mining subsidence site); and (2) ZH (an adjacent undisturbed site). The mining subsidence site is adjacent to farmland (the undisturbed site as control), which has not been cultivated for at least the last 20 years. Three plots (30 m × 30 m) were established on both the subsided site and control site for soil sampling. From each plot, nine subsamples were collected to a depth of 20 cm along an S-shaped transect, using a 3 cm soil corer in diameter. Soil samples were pooled into one composite sample, yielding three replicates per site. After removal of the forest litter and roots (>2 mm), samples were stored in polyethylene bags and transported to the laboratory. Finally, samples were stored at 4 °C prior to soil properties determination and microbial analyses.

### 2.2. Edaphic properties of the soil samples

According to *Soil Agricultural Chemistry Analysis*, soil organic matter (OM) was determined using the oil bath heating-potassium dichromate volumetric method; pH was tested on air-dried subsamples (sieved to < 5 mm) using a glass combination electrode with a soil water ratio of 1:2.5; total nitrogen (total N) was determined by using the semi-micro Kjeldahl method [20]. After soil samples were digested with melting NaOH, the total amount of phosphorus (total P) was determined by the Mo-Sb colorimetric method; contents of available potassium (available K) and available phosphorus (available P) were measured by 1 mol/L neutral $NH_4OAC$ method and the Olsen method, respectively. A TZS-EC-I conductivity meter was used for the determination of soil salinity.

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

DNA was extracted from approximately 1 g of soil per sample by employing the E.Z.N.A.®Soil DNA Kit for soil. The concentration and purification of the extracted DNA (2 μL) were determined by agarose gel electrophoresis (1%) and microspectrophotometry (NanoDropÒND-2000, NanDrop Technologies, Wilmington, DE).

The bacterial primer set of forward primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer 533R (5′-TTACCGCGGCTGCTGGCAC-3′) were used for amplifying the 500 bp DNA fragment of the 16S rRNA gene. Products were confirmed by electrophoreses of 2 μL of each reaction on 2% agarose gel. The PCRs were carried out in triplicate 20 μL reactions with 4 μL 5-fold FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 5 μM of each primer, 0.4 μL diluted DNA sample, 0.4 μL of TransStart FastPfu DNA Polymerase and approximately 10 ng of DNA template by operating the PCR Gene Amp 9700.

The amplification program consisted of an initial denaturation step of 95 °C for 2 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s with a final extension step at 72 °C for 5 min. Replicate PCR products of the same sample were assembled within a PCR tube. Then they were visualized on agarosegels (2% in TBE buffer) containing ethidium bromide, and purified with Axy Prep DNA gel extraction kit. Prior to sequencing, the DNA concentration was determined using a PicoGreen® dsDNA Quantitation Reagent and passed the quality control on a QuantiFluor™-ST Real-time PCR System according to the manufacturer's protocol. Following quantitation, the amplicons from each reaction mixture were pooled in equimolar ratios based on concentration and subjected to emulsion PCR to generate amplicon libraries. Amplicon pyrosequencing was performed on a Roche Genome Sequencer GS FLX Titanium platform at Majorbio Bio-pharm Technology Co., Ltd., Shanghai, China.

### 2.4. Data analysis

Quality-trimmed sequencing reads were confirmed by using the comprehensive bioinformatics software package-Mothur. Sequences from each OTU (operational taxonomic units) were taxonomically assigned with a bacterial 16S rRNA Silva reference alignment using a naïve Bayesian classifier. OTUs were used to calculate community diversity (Shannon diversity indices), evenness (Shannon equitability index) and richness (abundance based coverage estimator-ACE, bias-corrected Chao1) to a cutoff of 0.03. Taxonomic identities of sequences were assigned using the RDP-II Classifier program at a confidence level of 97%. Experimental data for statistics and correlations analysis were conducted by using Excel and SPSS software.

## 3. Results and discussion

### 3.1. Soil parameters in mining subsided and undisturbed soils

The two analyzed soil groups showed differences with respect to soil nutrient indicators such as soil total N, total P, available K, and soil salinity (Table 1). Most of the soil nutrient contents were generally higher in site ZH, when compared with those in site TXD. The results of correlation analysis indicated that the difference in soil salt and nutrient between TXD and ZH reached the significant level ($p < 0.05$).

Coalfield exploitation results in ground subsidence in large areas and decline of soil fertility, and thus has significant effects on soil factors. Several studies have documented that long-term ground subsidence leads to soil erosion and nutrients loss [7,10,21]. Land subsidence and deformation result in changes of soil physicochemical properties, causing decomposition, eluviation and deposition of organic matter and minerals [21–23]. Recent studies have shown that total *N* and total *P* of subsided soils were either significant or high significant ($P < 0.01$) compared to control