Archaea diversity of reclaimed soil in the coal gangue hill

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Abstract. Coal gangue is the most discharged solid waste in the process of coal mining. The coal gangue hill formed by stacking brings serious environmental pollution to the surrounding environment. The vegetation reconstruction of the coal gangue hill is one of the effective ways to solve the huge accumulation of coal gangue and environmental restoration. The growth of plants can affect the number and diversity of soil microorganisms, while microorganisms can promote soil nutrient cycling. Current reports have focused mainly on bacteria, actinomycetes and fungi, but little is known about the composition and function of archaea in the coal gangue reclamation soil. In this study, PCR-16S rRNA gene high-throughput sequencing technology was used to investigate the diversity of archaea in the coal gangue soil reconstructed by vegetation, to enrich the ecological assessment of coal gangue hill. The results showed that the species richness and diversity of archaea were great different in different sampling sites. Thaumarchaeota (>90%) and SCG (>90%) was the absolute dominant phylum and class of archaea in the gangue soil, respectively. Identified dominant genus was unidentified_SCG (5.5%), and more than 90% of archaea genera were unidentifiable, indicating a large number of unknown archaea resources in this soil environment. SCG, as a kind of chemoautotrophic ammonia oxidizing archaea, plays potential roles in nitrogen cycling of the coal gangue reclamation soil.

1. Introduction

Opencast coal mining is the main mining way in the world [1]. A large number of gangues are produced in the process of coal mining and washing, which are piled up for a long time to form gangue hills and occupy a large amount of land. The biodiversity of the mining area is damaged, resulting in environmental pollution of the mining area and its surrounding areas. To solve this problem, large-scale open-pit coal mines will adopt the way of mining and backfilling, and the soil environment is restored by plant reclamation. With the prolonging of phytoremediation time, the population and diversity of microorganisms increase. The secretions and apoptotic residues released during plant growth provide abundant food sources for microorganisms, and improve the population and diversity of microorganisms in the environment. At the same time, soil microorganisms can participate in mineralization and assimilation and oxidation-reduction reaction, decomposing insoluble substances in coal gangue, promoting weathering and nutrient release. They also participate in the formation of soil aggregates, and improve soil structure [2-5]. Currently, the researches on soil microorganisms in reclaimed land of mining area mainly focus on bacteria, actinomycetes and fungi [6-9]. Archaea account for a large proportion of soil microbial community and biomass, and participate in the biogeochemical cycle of carbon, nitrogen, hydrogen, sulfur, etc. However, little is known about the composition and function of
archaea in the coal gangue reclamation soil. In this study, 16S rRNA gene high-throughput sequencing technology was used to investigate the diversity of archaea in the coal gangue reclamation soil, to provide useful information for the ecological assessment of the reclamation soil.

2. Materials and methods
The samples were collected from the soil layer of coal gangue hill in the West Open-pit Coal Mine of Fushun, China. Torch trees were selected for planting, which have excellent environmental adaptability in reducing scour, enhancing infiltration, water and soil conservation, low nutrition requirements, drought tolerance and salt alkali. Three sampling sites (TR1, TR2 and TR3) were set up at least 500 meters away from each other. After removing 5cm topsoil from the roots of the torch trees with a small sterile shovel, random multi-point method was used for sampling. The soil samples were transported back to the laboratory in ice box for macrogenomic DNA extraction [10]. Using macrogenomic DNA as template, 16S rRNA V9 region was amplified by PCR with barcode. The primers for PCR amplification were 1106F (5'-TTWAGTCAGGCAACGAGC-3') and 1378R (5'-TGTGCAAGGAGCAGGGAC-3'). According to the concentration of PCR products, the samples were mixed equally. After 2% PCR agarose gel electrophoresis was used to detect the PCR products, the products were recovered from the target strip using the gel recovery kit (Qiagen Company). The TruSeq® DNA PCR-Free Sample Preparation Kit was used to construct the library. The constructed library was quantified by Qubit and Q-PCR. After the library was qualified, Hiseq 2500 PE250 was used to sequence by paired end sequencing, and the relevant data were processed.

3. Results and discussion
3.1. Sequencing results of 16s rRNA Gene
An average of 84966 original sequences, an average of 84864 effective sequences and an average DNA length of 282 bp were obtained from the soil samples of coal gangue. Among the three DNA samples, there are 108 (TR1 sample), 1740 (TR2 sample) and 320 (TR3 sample) of unique OTUs. 131 of OTUs are shared by the three samples (Figure 1). OTUs in TR2 sample are much more than that in the other two samples. This indicates that there are great differences in archaea species in different sampling sites, reflecting a great spatial heterogeneity between sampling sites.

![Figure 1. VENN image of three samples.](image-url)
3.2. Alpha diversity of archaea communities

Alpha diversity can reflect the richness and diversity of microbial communities in the samples. The diversity index is used to evaluate the diversity and species richness of microbial communities in different samples. Table 1 shows that the coverage rates of all samples are higher than 99%, indicating that the sequencing depth is reasonable and can basically represent the real situation of samples. The diversity and richness of archaea communities of the three samples are great different, especially TR2 sample. This indicates that there is a significant difference in the composition of archaea communities in the coal gangue soil planting torch trees. This may be related to the spatial heterogeneity of coal gangue soil environment.

3.3. Composition of Archaea communities

At the level of phylum, the archaea communities of TR samples are assigned to four phyla, namely Thaumarchaeota, Euryarchaeota, Dhev-6 and Crenarchaeota (Figure 2). Thaumarchaeota is the predominant archaea phylum with an average relative abundance of 90% (Figure 2). At the level of class, SCG belonging to Thaumarchaeota in TR samples is predominant, with an average relative abundance of 90% (Figure 3). At the level of genus, the unidentified genera in TR samples are predominant, with the relative abundance of more than 90% (Figure 4), indicating that a large number of unknown archaea exist in the coal gangue reclamation soil. The identified dominant genus is unidentified_SCG (5.5% on average) (Figure 4). From the level of phylum, class and genus, the community composition of TR2 sample is significantly different from that of the other two samples, which is consistent with the result in 3.2. Thaumarchaeota was proposed by Brochier-Armanet et al in 2008, which is divided from mesophilic archaea into a new phylum as the third major group of archaea [11]. A previous study has shown that SCG, as a chemoautotrophic ammonia oxidizing archaea, plays a potential role in soil energy cycle [12]. Nitrogen is an indispensable nutrient element for plant growth. Therefore, SCG may play an important role in the nitrogen cycle of this soil environment [13, 14]. In addition, the functions of a large number of unknown genera of SCG in this soil environment and their relationship with plant roots need further study.

Figure 2. Relative abundance of archaea communities of three samples at the level of phylum.
Table 1. Alpha diversity indexes of three samples.

| Number | Shannon | Simpson | Chao1   | ACE    | Coverage |
|--------|---------|---------|---------|--------|----------|
| TR1    | 0.808   | 0.143   | 465.739 | 474.054| 0.999    |
| TR2    | 3.345   | 0.545   | 2284.254| 2209.81| 0.997    |
| TR3    | 1.044   | 0.185   | 638.164 | 645.759| 0.999    |

Figure 3. Relative abundance of archaea communities of three samples at the level of class.

Figure 4. Relative abundance of archaea communities of three samples at the level of genus.
4. Conclusion
In the coal gangue reclamation soil of the mining area, there are great differences in the composition and diversity of archaea communities between different sampling sites, which is related to the spatial heterogeneity of the soil environment. SCG assigned to Thaumarchaeota is predominant in the coal gangue soil of vegetation reconstruction. As a kind of chemoautotrophic ammonia oxidizing archaea, SCG plays a potential role in the nitrogen cycle of the reclaimed soil. In addition, there are a large number of untapped archaea resources in the soil environment.

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