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Effects of regenerating vegetation on soil enzyme activity and microbial structure in reclaimed soils on a surface coal mine site

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ABSTRACT

The aim of this study was to evaluate the influence of reclaimed scenarios on soil enzyme activities and microbial community in a reclaimed surface coal mine on the Northwest Loess Plateau of China. Soil samples were collected from a bare land (CK), and a plantation (PL) and four mixed forests (MF1-4). Soil physicochemical characteristics, four enzyme activities and microbial abundance and T-RFLP (terminal restriction fragment length polymorphism) profiles were measured. Effects of reclaimed scenarios on soil nutrients content, enzyme activities and microbial community were pronounced. Soil organic carbon could be well used to predict the major differences in enzyme activities, and microbial abundance and composition. Soil enzyme activities were more significantly correlated with fungal abundance than bacterial and archaeal ones. The higher soil nutrient content, enzyme activities, and microbial abundance and diversity were from mixed forests and the lowest ones were from CK, which suggested mixed forests would be feasible scenarios in semi-arid Loess Plateau. Soil bacteria, archaea and fungi evolved with reclaimed process, but the influences of reclaimed scenarios on each domain of microbial abundance, diversity and composition were different. These findings suggested that soil bacteria, archaea and fungi play different ecological roles during restoration process.

1. Introduction

Soil enzymes play a fundamental role in nutrient mineralization, organic matter decomposition and plant nutrient cycling. Soil enzyme activities integrate information about microbial status and soil physicochemical conditions (Aon and Colaneri, 2001; Sinsabaugh et al., 2010), and are indicators of the functioning of soil ecosystems (Ciarkowska et al., 2014). They have been similarly used in studies on the effectiveness of reclaimed treatments on soil quality (Li et al., 2012; Schimann et al., 2012; Finkenbein et al., 2013; Ciarkowska et al., 2014). The activity of acid phosphatase may be a useful bioindicator for monitoring soil quality of acid, P-deficient substrates from subtropical surface coal mines (Finkenbein et al., 2013), and urease and invertase activities can be used as progress indicators for soil-rehabilitation processes in industrial areas (Ciarkowska et al., 2014). Soil enzyme activities were improved by applying weathered coals in reclaimed opencast-mining areas of the Loess Plateau of China (Li et al., 2012).

Enzymes are associated with proliferating soil microbial communities, which play a key role in many soil processes and the delivery of essential soil ecosystem services (Gomez-Sagasti et al., 2012). Soil microbial community is one of the most important issues for restoration of sustainable ecosystem in post-mining lands because most sites have unfavorable properties (Kaschuk et al., 2010). Soil microbial activities and structure varied with different rehabilitation types and time (Claassens et al., 2008; Dangi et al., 2012; Finkenbein et al., 2013). We applied T-RFLP (terminal restriction fragment length polymorphism) into analyzing the effects of reclaimed scenarios and fertilizer treatments on soil bacterial, archaeal and fungal communities during the initial recovery stage, and found that soil microbial communities were significantly influenced by reclaimed scenarios and T-RFLP was proven powerful in describing differences and changes in soil microbial community structures (Li et al., 2013b).

Mining activities have produced great damage to eco-environment, and legal requirements to reclaim highly disturbed lands are becoming increasingly common (Holl, 2002). Soil enzyme activities and microbial properties have increasingly been used indicators of soil quality to evaluate the success of reclamation efforts (Ciarkowska et al., 2014; Li et al., 2013b Schimann et al., 2012). For the Loess Plateau of China, there are few studies on the

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use of soil enzyme and microbe to assess the success of reclamation in surface coal mines treated with various amendments (Li et al., 2012; Li et al., 2012). In this study, we present soil enzyme activities and microbial abundance and diversity in six soil samples from reclaimed scenarios in surface coal mine, the Northwest Loess Plateau of China. The aim of this study was to describe and evaluate the effects of different reclaimed scenarios on soil properties development and microbe succession.

2. Materials and methods

2.1. Site description and soil sampling

This study was conducted on reclaimed mine spoils in antaibao opencast coal mining area (39°23′–39°23′N; 112°11′–112°30′E) in Northwest Plateau Loess, China. The altitude is 1300–1400 m. The climate is terrestrial temperate, and the area experiences semi-arid monsoons. Annual average precipitation is about 450 mm, with rainfall occurring mainly from June to September. The annual average air temperature is about 6.2 °C, and the frost-free season ranges in length from 115 to 130 days.

There were six reclaimed scenarios in the reclaimed spoils, including a control site (CK), a plantation (PL) and four mixed forests (MF1-4). The details of vegetation and time since reclamation are listed in Table 1. In September, 2011, the upper 10 cm of soil was collected from 6 random locations within a given plot of ≈00 m^2 and composited into a single bulk sample in triplicate at each site. After removing stones and large pieces of plant materials, soil samples were sieved to 2 mm and homogenized, and subsamples for microbial analysis were stored at 4 °C and the other ones for chemical analysis were air dried.

2. 2. Soil chemical properties and enzyme activities

Soil pH in dH_2O was measured using a glass combination electrode in subsets of air-dried soils at a soil: solution ratio of 1:2.5. Soil organic carbon (SOC) was determined by the dichromate oxidation method. Total, available and ammonium nitrogen (TN, AVN and AMN) were determined by the Kjeldahl method, alkaline hydrolysis diffusion method and indophenol-blue colorimetry, respectively (Lu, 2000). We used 3,5-dinitrosalicylic acid colorimetry, 2,3,5-triphenyl tetrazolium colorimetry, pyrogallol colorimetry and indophenol-blue colorimetry to measure soil invertase, dehydrogenase, polyphenol oxidase and urease activities, respectively (Guan, 1986).

2.2. PCR assay for real-time quantification and T-RFLP

DNA was extracted from 0.5 g soil using the Ultra-clean TM soil DNA Isolation Kits (MoBio Laboratory, USA) according to the manufacturer's protocol. The DNA extracts were 100-fold diluted and used as template with a final content of 1–10 ng in each reaction mixture to amplify soil microbial rRNA genes.

Real-time PCR was performed on an iCycler iQ-5 thermocycler (Bio-Rad). We use the probe TM189F and Premix Ex Taq™ (Takara Biotechnology, Japan) to analysis bacterial 16S rRNA gene quantitative assay. SYBR® Premix Ex TaqTM with Green I (Takara Biotechnology, Japan) was applied into the reaction mixture for quantifying archaeal and fungal rRNA gene. We use primers labeled at the 5′-end with the reporter dye FAM (6-carboxy-fluorescein) for T-RFLP analysis. The detailed information on primer, probe, and PCR condition were described in our previous study (Li et al., 2013a).

2.3. T-RFLP analysis

We used 1% agarose gel electrophoresis and purified with Wizard® SV Gel and PCR Clean-Up System (Promega, USA) to verify PCR products. Restriction endonucleases *Hha*I and *Msp*I (Takara Biotechnology, Japan) digested purified products, respectively, and the reactions including 4 U of enzyme and about 500 ng of DNA were incubated at 37 °C for 3 h in the manufacturer's recommended reaction buffer. Further purified digestion products was mixed with deionized formamide and the internal standard GeneScan-ROX1000 (bacteria)/LIZ 500 (archaea and fungi) (Applied Biosystems) and denatured for 3 min at 95 °C. The DNA fragments were size separated using a 3130xl Genetic Analyzer (Applied Biosystems).

2.4. Statistical analysis

The effects of regenerating vegetation on soil chemical properties, enzyme activities and microbial characteristics were examined through one-way analysis of variance (ANOVA) with Duncan test. We used Person linear correlation to determine whether there was significant correlation among tested abiotic and biotic characteristics. Spearman rank correlation was examined microbial RFs-pairs association. These statistical analyses were performed using SPSS 13.0 for Windows.

We analyzed soil microbial richness, diversity and evenness according to restricted fragments (RFs). Richness, Shannon–Wiener diversity, Simpson diversity and Pielou evenness indices were computed according to the number and relative ratios of RFs (Li et al., 2013a).

The weighted value of bacteria, archaea and fungi were ranked 4, 3 and 3, respectively, according to the ratios of logarithm the bacterial, archaeal and fungal rRNA gene copies. The matrices of RFs ratios for bacteria, archaea, fungi and total microbe were used as the basis of the community analysis. A matrix of environmental factors was applied into analyzing the relationships between the reclaimed scenarios and environmental factors using canonical correspondence analysis (CCA). CCA was performed using the CANOCO (Ter Braak and Šamilauer 2002), and we used the Monte Carlo permutation test to test the significant level ($P < 0.05$) between environmental data and microbial matrices.

Table 1
The reclaimed vegetation composition and time.

	Vegetation	Reclaimed time (*a*)
CK	Reclaimed area covered with soil in 2009	1
PL	*Robinia pseudoacacia*	18
MF1	*R. pseudoacacia*,*Pinus tabuliformis*	18
MF2	*Hippophae rhamnoides*, *Caragana Korshinskii*	18
MF3	*C. Korshinskii*, *Elaeagnus angustifolia*, *Ulmus pumila*	18
MF4	*H. rhamnoides*, *C. Korshinskii*, *E. angustifolia*	18

Table 2
Soil pH, organic carbon (SOC), total, available and ammonium nitrogen (TN, AVN and AMN) from different reclaimed scenarios in the reclaimed mining area.

	pH	SOC (g/kg)	TN (g/kg)	AVN (mg/kg)	AMN (mg/kg)
CK	7.47 ± 0.14a	6.00 ± 0.62a	4.27 ± 0.81a	38.27 ± 7.05a	6.62 ± 0.69a
PL	7.33 ± 0.05a	16.61 ± 0.94c	5.93 ± 0.75b	73.73 ± 4.28b	15.46 ± 0.42c
MF1	7.33 ± 0.15a	33.00 ± 3.37e	10.80 ± 0.26d	123.20 ± 4.85d	12.62 ± 1.34b
MF2	7.50 ± 0.12a	11.55 ± 0.86b	4.30 ± 0.20a	31.73 ± 3.23a	13.57 ± 1.17b
MF3	7.38 ± 0.14a	25.80 ± 1.56d	7.93 ± 0.12c	80.73 ± 3.52b	16.68 ± 0.58c
MF4	7.45 ± 0.09a	27.67 ± 2.15d	7.27 ± 1.10c	92.40 ± 4.85c	15.23 ± 0.83c

Data are means ± standard deviations. The different case letters indicate that the means are significantly different among reclaimed scenario ($P < 0.05$) with Duncan test.