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Revegetation as an efficient means of improving the diversity and abundance of soil eukaryotes in the Loess Plateau of China

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ABSTRACT

Different revegetation types have been used to restore degraded soil in the Loess Plateau, China. Most studies have focused on the impact of revegetation types on soil properties. However, we understand little about how different revegetation types influence the diversity and abundance of soil eukaryotic communities. Artificial revegetation types and natural grasslands were selected in semiarid areas, and slope farmland was as the control, and pyrosequencing of the 18S rRNA gene region was used to analyze changes in soil eukaryotic diversity and composition. A total of 41,965 valid sequences were generated using 454 pyrosequencing. The number of sequences per sites ranged from 5488 to 6288, with an average of 5967. At a genetic distance of 3%, the highest eukaryotic diversity was found in artificial forestry, while the lowest in natural fenced grassland. The dominant sampled taxa mainly included fauna, fungi, and flora. At the phylum level, the dominant phyla were: (faunal) *Craniata*, *Nematoda*, *Arthropoda*, *Rhizaria*; (fungi) *Ascomycota*, *SAR*, *Mucoromycotina*; and (flora) *Streptophyta*. pH, SWC, TP, SOC, and AP significantly influenced the abundance of some fauna, such as *Craniata*, *Arthropoda*, *Annelida*, *Rhizaria*, and *Amoebozoa*. Artificial revegetation has a beneficial impact in improving the diversity and abundance of soil eukaryotes, but long-term grazing exclusion of natural grassland may have a negative impact on the diversity and abundance of soil eukaryotes. Revegetation by afforestation is an ecologically efficient practice for eukaryotes recovery of degraded soil in the Loess Plateau.

1. Introduction

Soil degradation is a widespread phenomenon in arid and semi-arid areas, and can result in the loss of ecological function and services (Wei et al., 2006; Zhu et al., 2010). The Loess Plateau of China is widely known for its complex terrain and loess formations. Severe soil erosion has caused sustained deterioration of the ecosystem of this area (Jing et al., 2013; Wei et al., 2006).

Revegetation is considered to be an effective tool for improving the quality of soil and rehabilitating degraded environments (Wang et al., 2012; Zhang et al., 2011). Revegetation is a traditional practice widely used for soil and water conservation in the Loess Plateau of China (An et al., 2013a, 2013b). Since the 1950s, to solve problems related to soil erosion, the Chinese government initiated an artificial afforestation project, and many areas have been planted with shrubs and trees in the Loess Plateau (Zhang et al., 2011; Wang et al., 2012). For example, Korshinsk peashrub (*Caragana Korshinskii* Kom.), black locust (*Robinia pseudoacacia* L.), and alfalfa (*Medicago sativa* L.) are widely used during afforestation (Jiang et al., 2006; Jia et al., 2010; Qiu et al., 2010; Zhang et al., 2011). In recent years, the effect of different revegetation types on soil physicochemical properties, soil aggregate stability, soil microbial, rhizosphere soil microbial and enzyme activities have been reported in the Loess Plateau (Jia et al., 2005; Jiang et al., 2009; Zhu et al., 2010; Zhang et al., 2011; Wang et al., 2012; An et al., 2013a, 2013b; Zhang et al., 2013). For example, An et al. (2013a, 2013b) concluded that revegetation as an efficient ways of increase soil aggregate stability on the Loess Plateau. However, composition and structure of communities of soil micro-organisms are frequently ignored because of their small size and the limited methods available for analyzing these organisms.

Soil eukaryotes play important roles in the maintenance of soil nutrients and in biogeochemical cycles. For example, the fragmentation and homogenization of plant organic matter are completed by soil fauna, and hydrolysis of the degraded organic material is performed by fungi (Damon et al., 2012). In addition, ecological

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Table 1
Description of six major sampling sites in the Loess Plateau areas.

Vegetation types	Main vegetation	Code	Soil type	Minor herbaceous
Natural fencing grassland	*Stipa przewalskyi* + *Artemisia sacrorum*	NFG	Loessal soil	*Stipa grandis* + *Potentilla bifurca*
Natural grazing grassland	*Stipa bungeana* + *Thymus mongolicus*	NGG	Loessal soil	*Carex aridula* + *Heteropappus altaicus*
Artificial grasslands	*Medicago Sativa* L.	AG	Loessal soil	*Medicago Sativa* L.
Artificial forestry	*Robinia pseudoacacia*	AF	Loessal soil	*S. bungeana* + *Artemisia gmelini*
Artificial shrublands	*Caragana korshinskii*	AS	Loessal soil	*S. bungeana* + *Agropyron cristatum*
Slope farmland	Grain	SF	Loessal soil	*Zea mays* + *Linum usitatissimum*

studies of soil protozoa point to possible major roles for protozoa in carbon and nitrogen cycles and in energy transmission; sarcodina, in particular, can affect nutrient mineralization, energy flow, and soil fertility (Li et al., 2010a, 2010b). Based on traditional approaches including analysis of morphological, physiological and biochemical properties, many eukaryotic microbes cannot easily be isolated from environmental soil and/or cannot be grown *in vitro* (Prosser, 2002; Bailly et al., 2007). Pyrosequencing is a powerful alternative to traditional approaches based on *in vitro* experiments (Orgiazzi et al., 2012). For example, Tian et al. (2009) demonstrated the eukaryotic diversity in Arctic sediment using 18S rRNA gene clone library analysis. Baldwin et al. (2013) reported impacts of inundation and drought on eukaryotic biodiversity in semi-arid floodplain soils. Different biogeographical patterns of microbial eukaryotes in epilithic biofilms were studied by a small-subunit (SSU) rRNA-based method (Ragon et al., 2012). To our knowledge, there is little information on eukaryotic communities in soils that have been investigated under different revegetation types in semi-arid areas of China. In addition, little is known about eukaryotic diversity in soils, and the relationships between revegetation types and soil eukaryotes.

In this study, we examined changes in soil eukaryotic communities under different revegetation types in the Loess Plateau, China. We hypothesized that the revegetation improved soil eukaryotic diversity, and different revegetation types have different effects on the restoration of the soil eukaryotic community. To test our hypothesis, we applied pyrosequencing of the 18S rRNA gene region to analyze the soil eukaryotic structure in six revegetation types. Our objective was to use these revegetation types to examine how soil eukaryotes may respond to vegetation change and to determine which revegetation type has the greatest capacity to facilitate soil recovery.

2. Methods

2.1. Study site and sampling

The sample sites were located in semiarid areas of the Loess Plateau of China near the city of Yuanzhou (Ningxia province). This area has a sub-arid climate that is characterized by the cycles of periodic floods and droughts (An et al., 2013a,b). The mean annual temperature is 7 °C. Mean annual rainfall is 425 mm, with about 60–75% of the annual rainfall falling in July to September.

Based on different revegetation types, six sites were selected including sites dominated by one of three species, korshinsk peashrub (AS), black locust (AG), alfalfa (AF), and three other types of sites described as grazing exclusion grassland (or natural fencing grassland, NFG), natural grazing grassland (NGG), and slope farmland (SF) (Table 1). AS, AG, and AF are the artificial revegetation site types and species, stands of these species had been in place for 20 years. Grazing had been excluded in the NFG for 20 years, and the dominant species is *Carex aridula* and *Artemisia frigida*. Grazing grassland is moderately grazed by sheep (about four sheep ha^{-1}), and the dominant species is perennial bunchgrasses by *Stipa bungeana*. SF is planted annually with crop rotation between corn and wheat, and SF is exposed to normal farming practices (with low fertilizer input) and represents the field conditions prior to afforestation (An et al., 2013a, 2013b). These six revegetation sites were similar for the slope aspect and gradient, altitude, and previous management practices.

In August 2012, according to previous sampling methods (Zhang et al., 2011; An et al., 2013a, 2013b), we established three 50 m × 100 m plots within each vegetation type (except the slope farmland plot was 50 m × 20 m), totaling 18 plots for six vegetation type sites. The litter layers of top soil were removed prior to soil sampling. According to three slope position, soil samples were taken along an "S" curve from five sampling points in each slope position by soil auger (5 cm diameter at 20 cm depth), and then six soil samples were mixed together to make one large sample for each slope position, and three soil samples were collected for each plot. A total of 54 fresh samples were collected for all vegetation sites. At the AS plot, soil samples were collected at a distance of 20–30 cm from the basal part of a stem; at the AG plot, soil samples were collected at a distance of 50–80 cm from the trees. All samples were sieved on-site through 1.4 mm mesh. The samples were then divided into two parts: three replicate samples of each plot were mixed to form a pooled sample of about 20 g was transported on dry-ice to the laboratory and then stored at −80 °C for DNA extraction; the other sample was air-dried and used to analyze the soil physical and chemical properties following the procedures of Wang et al. (2012).

2.2. DNA extraction and amplification of 16S rRNA genes and 454 pyrosequencing

Genomic DNA of nine soils was extracted at 0.5 g of nine mixed soils using an EZNA soil DNA Kit (OMEGA Bio-tek, Inc., Doravilla, GA, USA), according to the manufacturer's specifications. The quality and quantity of DNA was tested by 1% agarose gel electrophoresis and with a spectrophotometer (ND-1000, Thermo Scientific, Wilmington, DE, USA).

The 18S rRNA gene of eukaryotes was amplified using two sets primers 3NDF (5′-GGCAAGTCTGGTGCCAG-3′) (Cavalier-Smith et al., 2009) and V4_Euk_R2 (5′-ACGGTATCT (AG) ATC (AG) TCTTCG-3′) (Bråte et al., 2010). The 20 μl PCR reaction system contained 4 μl 5× FastPfu Buffer, 2 μl mM dNTPs, 0.8 μl of each primer (5 μM), 0.4 μl FastPfu polymerase (TransStart Fastpfu DNA Polymerase, TransGen) and 10 ng template DNA. The PCR amplification was completed using ABI GeneAmp® 9700. The amplification program consisted of an initial denaturation at 95 °C for 2 min, 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. All samples were amplified in triplicate. PCR products were tested by 2% agarose gel containing ethidium bromide. Next, all the PCR products of the same samples were pooled. The PCR products were purified with an AxyPrep DNA gel Extraction Kit (Axygen Bio-sciences) according to the manufacturer's specifications. The concentrations of amplicons were quantified by PicoGreen® dsDNA Quantitation Reagent and QuantiFluor™-ST (Promega) as recommended by the manufacturer. EmPCR (emulsion-based clonal amplification) was