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Soil bacterial community dynamics reflect changes in plant community and soil properties during the secondary succession of abandoned farmland in the Loess Plateau

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ABSTRACT

The effects of natural succession on plant communities and soil variables have been established, but changes in microbial communities and their response to plants and soils have not been well characterized in secondary succession. We investigated the changes in soil properties and plant and soil microbial communities during the secondary succession on abandoned cropland in the Loess Plateau of China using high-throughput sequencing of the 16S rRNA gene. The study analyzed a chronosequence of farmland undergoing spontaneous succession after being abandoned for 0 (farmland), 5, 10, 15, 20 and 30 years(y). Plant community metrics including percent cover, and above/belowground biomass, first decreased in the initial stage (<10 y) and then increased during the succession. *Proteobacteria*, *Acidobacteria*, *and Actinobacteria* were the dominant phyla of soil bacteria across all succession. Bacterial communities transitioned from *Acidobacteria*-dominant to *Proteobacteria*-dominant communities during the 30 years of succession. Levels of soil organic carbon (C), total nitrogen (N), nitrate N and bacterial diversity were lower soon (<5 years) after abandonment compared to the farmland, but they could recover to farmland levels after 15–20 years and were much improved after continued succession. Plant and bacterial community diversities (Shannon index and species richness) changed along successional time, but they showed different patterns, suggesting an incongruous process between plant and microbial succession. Organic C, total N, available N, and available P contents were significantly correlated with the abundance of most bacterial groups and the Shannon index, indicating the dependence of bacterial community diversity on soil nutrient supply.

1. Introduction

Plant secondary succession without human disturbance is an effective way to improve soil conditions and restore degraded environments (Walker et al., 2007; An et al., 2009; Zhang et al., 2015). Several studies have suggested that plant secondary succession occurs in these extreme environments (Urbanová et al., 2011; Xu et al., 2012), however, our understanding of secondary processes in arid and semiarid ecosystems is still poor, especially in the more temperate regions (Abella, 2010) where species diversity and composition usually changed rapidly (Lozano et al., 2014). Plant growth during the establishment of vegetation community in degraded ecosystems is most commonly limited by the shortage of mineral nutrients. Specifically, since soil microorganisms transform organic substrates, release mineral elements, and they may strongly influence the growth of plants during secondary succession. Plants, in turn, import carbon (C) and nitrogen (N) to the soil subsystem in the form of litter and root exudates, and specifically select for heterotrophic microbial communities (Singh et al., 2004). Plant succession is essentially the interaction between aboveground plants and belowground microorganisms (Kardol et al., 2006; Kuramae et al., 2011).

A variety of culture based studies have examined shifts in the structure and activity of microbial communities in and across vegetated stages of secondary succession (Chan et al., 2008; Banning et al., 2011; Kuramae et al., 2011; Zhang et al., 2015). These studies have partly characterized microbial succession across successional time but were not able to fully and accurately identify

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specific functional microbial groups, because many microbes are not easily isolated from environmental soils by traditional approaches (Prosser, 2002; Bailly et al., 2007). The structure and diversity of belowground microbial communities during ecological restoration have thus remained essentially a gray box. Next-generation sequencing technologies have recently offered new opportunities for studying belowground communities by profiling microbial composition at the species level. Microbial community composition is affected by soil factors such as pH, C, N and phosphorus (P) contents (Koranda et al., 2011; Shen et al., 2013; Li et al., 2014a), plant factors such as vegetation type (Oh et al., 2012), plant–microbial interactions (Knelman et al., 2012), and land-use history and restoration time (Jangid et al., 2013). Plant community structure and soil variability are partly responsible for the changes to soil microbial communities (Mahnert et al., 2015), but the relationships among plants, soils and microorganisms remain unknown.

The Loess Plateau, a temperate semiarid area, in the upper and middle region of the Yellow River in China has an area of 6.2×10^5 km^2. The loessial soil is prone to erosion by wind and water, and this region has suffered serious soil erosion where most of the topsoil has been lost. Abandoning sloped farmland to allow secondary succession is a traditional practice widely used for preventing soil erosion and for the rehabilitation of ecological environments on the plateau. Many croplands with slopes >15° have been abandoned for natural recovery without anthropogenic interference. The effects of succession on soil physicochemical properties, microbial dynamics, and enzymatic activities have been reported (An et al., 2008; Wang et al., 2009; Liang et al., 2010; Zhang et al., 2015), but information on the relationship between soil variables and plant and soil microbial communities is still scarce. This information is essential for understanding the essence of secondary succession and for the appropriate management and conservation of the ecological environment.

The present study investigated the dynamics of soil properties, plant communities and the structure of soil bacterial communities at sites representing 30 years of secondary succession on abandoned cropland in the Loess Plateau using 16S rRNA high-throughput sequencing. Our objective was to (i) evaluate the patterns of change in soil properties, plant communities, and soil bacterial communities during secondary succession after agricultural abandonment, (ii) to determine if changes between the plant and microbial communities are congruous, and (iii) determine the response soil bacterial communities to plant community and soil properties along the chronosequence.

2. Material and methods

2.1. Study site and sampling

A field experiment was carried out in natural grassland ecosystems. These sites were in the Zhifanggou Ecological Restoration Watershed (109°16′E, 36°46′N), near the Ansai Soil and Water Conservation Research Station on the Loess Plateau, where the mean annual temperature is 8.8 °C, with a mean minimum temperature in January of −6.2 °C and a mean maximum temperature in August of 37.2 °C. The area has a temperate semiarid climate. Mean annual precipitation is 510 mm, with over 60% falling from July to September. The soil, derived from wind-blown deposits, is classified as a Huangmian soil (a Calcaric Cambisol in the FAO classification). This area has been used as an experimental field base by the Chinese Academy of Sciences to monitor vegetation restoration.

The substitution of space for time, a common method in ecosystem research, is an effective way to investigate the changes in soil conditions and plant communities during natural succession (Felske et al., 2000; Tscherko et al., 2004; An et al., 2009; Walker et al., 2010; Jangid et al., 2013; Williams et al., 2013). We used this method to study the effect on soil bacterial communities of abandoning cropland for natural recovery. Five sloped farmlands abandoned for 5, 10, 15, 20, and 30 years were chosen as experimental sites based on their well-dated successional chronosequence. These sites had similar slope gradients, slope aspects, elevations, and previous framing practices. The main crops grown in these farmlands before abandonment were millet (*Setaria italica*) and soybean (*Glycine max*) in rotation. An active sloped farmland growing millet and soybean was used as a reference. The crops were manually harvested, and the plots at this site were manually plowed to a depth of 20 cm each year after harvesting. The sloped farmland was fertilized each year with 6.0 t ha^{-1} goat manure, 60 kg ha^{-1} N and.45 kg ha^{-1} phosphorus pentoxide (P_2O_5). Properties of the experimental sites are presented in Table 1.

Three 20 m × 20 m plots were established at each site in September 2014. Six 1 m × 1 m quadrats were randomly selected in each plot for measuring the characteristics of the vegetation. Plant coverage, aboveground/belowground biomass, and maximum/mean height were separately measured for each species in each quadrat. The plant was dug up and the aboveground parts were clipped and dried to obtain the aboveground biomass, and the roots were washed with tap water and dried at 70 °C for 48 h to obtain belowground biomass. Shannon diversity index of plant community (H_{Plant}) was calculated using the equation by Tscherko et al. (2004), where P_i is the relative abundance of each species in total sum, and n is the number of species. The number of species was used to estimate the richness (S_{Plant}).

Soil samples were collected from the top 20 cm of the soil profiles by a stainless steel corer 5 cm in diameter after the litter horizons were removed. All selected sampling points were free of lichens, biological crusts, and/or any other vegetation within a radius of 0.75 m. Twelve soil cores were collected along an S-shaped pattern from each plot and then mixed to form one sample. Visible plant roots, stones, litter, and debris were removed from each soil sample, which was then divided into two subsamples. One subsample was immediately stored at −80 °C for DNA analysis, and the other sample was air-dried for physicochemical analysis.

2.2. Soil chemical parameters

Soil organic carbon (OC) content was determined using the Walkley-Black method (Nelson and Sommers, 1982), and total nitrogen (TN) content was determined using the Kjeldahl method (Bremner and Mulvaney, 1982). Total phosphorus (TP) content was determined by melt-molybdenum, antimony, and scandium colorimetry, and available phosphorus (AP) was measured by the Olsen method (Olsen and Sommers, 1982). Ammonium nitrogen ($NH_4^+ - N$) and nitrate nitrogen ($NO_3^- - N$) were determined following extractions of fresh soil with 2 M KCl for 18 h and were analyzed colorimetrically on an Alpkem Autoanalyzer (OI Analytical, College Station, USA). Soil pH was determined by an automatic titrator (Metrohm 702, Swiss) in 1:2.5 soil: water suspensions.

2.3. DNA extractions and Illumina MiSeq high-throughput sequencing

DNA was extracted from 0.5 g of soil using a FastDNA spin kit for soil (MP Biomedicals, Cleveland, USA). The DNA extracts were diluted tenfold and assessed for quality and quantity using a spectrophotometer (NanoDrop ND-1000, NanoDrop Technologies, Wilmington, USA). The integrity of the DNA extracts was confirmed by 2% agarose gel electrophoresis. DNA was amplified by PCR in