



Changes in rhizospheric microbial community structure and function during the natural recovery of abandoned cropland on the Loess Plateau, China



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ABSTRACT

This study examined the structure and function of rhizospheric microbial communities of two successional dominant species (*Artemisia capillaries* and *Artemisia sacrorum*) during the natural recovery of abandoned cropland on the Loess Plateau in China. Soil samples were collected from the roots of *A. capillaries* from cropland abandoned for 1, 3, 5, 10, and 13 years and from the roots of *A. sacrorum* from cropland abandoned for 13, 15, 20, and 30 years. The levels of phospholipid fatty acids (PLFA) were determined to characterize microbial community structure, and community-level physiological profiles for identifying the patterns of use of carbon (C) substrates were determined to describe the functional diversity of the microbial communities. The rhizospheric microbial biomass (total PLFA), Gram-negative bacterial PLFA, fungal PLFA, and the carbon use (carbohydrates, amino acids, carboxylic acids, and polymers) of the pioneering successional species, *A. capillaries*, tended to decrease during the first 10 years and thereafter increased. The middle-late successional species, *A. sacrorum*, exhibited increasing Gram-negative bacterial, Gram-positive bacterial, bacterial, total PLFA, and use of carbohydrates, amino acids, carboxylic acids, phenolic acids, and polymers as the abandonment time increased. The higher microbial biomass, Gram-negative bacterial and bacterial PLFAs was found in the rhizospheric soil than that in the bulk soil for *A. capillaries*. Redundancy and correlation analyses indicated that the rhizospheric microbial PLFAs and C use for *A. capillaries* were more closely correlated with its coverage and aboveground biomass than with soil organic C and total nitrogen (N), while those for *A. sacrorum* were correlated more with organic C and total N contents than with coverage and aboveground biomass. Our study demonstrated that the structure and function of the rhizospheric microbial communities of different successional species generally changed differentially over time. Rhizospheric microbial communities were initially determined primarily by the host plant, but the soil C and N supplies selected for specific microfloras during the middle-late stage.

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1. Introduction

Natural recovery without further anthropogenic disturbance is an effective means of restoring ecosystems with degraded soil (Walker et al., 2007). This process is characterized by an initial dominance of one species, subsequent co-existence with other species, substitution by another dominant species and finally the development of a climax community adapted to the local conditions (Zhang et al., 2012). Plant growth during the establishment of vegetation communities in degraded ecosystems

is most commonly limited by the availability of mineral nutrients. Specifically, soil microorganisms transform organic substrates, release mineral elements and hence strongly influence the establishment of plants during primary succession. Plants, in turn, add energy to the soil subsystem in the form of litter and root exudates and specifically select for heterotrophic microbial communities (Miethling et al., 2000). Natural succession is thus an interactive process between aboveground plants and belowground microorganisms.

Studies of natural revegetation have focused on individual distinguishable aboveground indicators and soil parameters, with little attention being given to the link between the aboveground plant community and the soil microbial community, although some studies have explored the dynamics of microbial properties

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such as microbial biomass (An et al., 2009), metabolic status (Rutigliano et al., 2004), enzymatic activities (Shillam et al., 2008) and the composition of the microbial community (Tscherko et al., 2004; Zornoza et al., 2009). A lack of knowledge of the connection between plants and soil microbial communities may lead to an incomplete understanding of the recovery of revegetated ecosystems, considering the role of microorganisms in soil energy transfer, nutrient cycling, vegetation reestablishment and the long-term stability of ecosystems.

As the zone where energy flow and nutrient cycling mostly frequently occur in soils, the rhizosphere has chemical and biological characteristics distinct from those in other parts of the soil. In this microsphere, actively growing roots release organic compounds into the soil such as sloughed cells, secretions, lysates and exudates (Buee et al., 2009). These compounds support the growth of the microbial community in the rhizosphere and will lead to increased population densities and to community structure distinct from those in the bulk soil (Steer and Harris, 2000). Rhizospheric microbial communities are important for the functioning of the ecosystem, both for their direct interactions with plants (both beneficial and deleterious to growth) and for the cycling of nutrients and organic matter (Söderberg et al., 2004). Information about the microbial communities in the rhizospheres of plants is much needed for elucidating the interaction between plants and soil microorganisms.

The Loess Plateau covers an area of approximately 9.6×10^6 ha in the upper and middle catchment area of the Yellow River (An et al., 2009). Loess is highly prone to erosion by wind and water; the soil of this region has been referred to as the “most highly erodible soil on earth”. The Chinese government launched a series of conservation projects focusing on the ecological restoration by reconverting cropland to forests and grassland. One important project involves abandoning croplands with slopes $>15^\circ$ to produce green land by natural restoration without human disturbance. This conversion has contributed to the improvement of soil quality and the effective protection against erosion. Most studies, however, have focused mainly on changes in the aboveground vegetation (Guo et al., 2010), soil nutrient status (Liang et al., 2010) and the physical (Jiao et al., 2013) and microbial (Jiang et al., 2009) properties of the soil. Although some researchers have investigated the soil microbial community structure during the vegetation succession of Loess Plateau (Huang et al., 2011; Xiao et al., 2013), they mainly focused on the bulk soil and only determined the microbial PLFAs, the information on the rhizospheric soil microbial function is lacking.

The substitution of space for time is an effective way of studying the changes in soil conditions and plant communities during the natural recovery (Walker et al., 2010) and has been widely applied in ecosystemic research (An et al., 2009; Zhang et al., 2012; Tscherko et al., 2004, 2005; Cutler et al., 2014). To add to our understanding of the processes characteristic of natural

restoration, our study used this approach in a successional gradient of eight sloped croplands on the Loess Plateau abandoned for 1–30 years to investigate the response of rhizospheric microbial communities to the vegetation changes. Changes in the microbial community structure were measured by analyzing the levels of phospholipid fatty acids (PLFA), which can serve as biomarkers across several microorganism taxa and provide quantitative information of their relative biomasses (Li et al., 2013; Kaur et al., 2005). Microbial functional diversity was measured as community-level physiological profiles (CLPP) based on the use of carbon (C) sources using Biolog EcoPlates™. Based on these two approaches, we addressed the following hypotheses: (1) the changes in rhizospheric PLFA CLPP profiles during succession differ between the microbial communities of pioneering successional species and middle-late successional species and (2) the main factor influencing the rhizospheric PLFA and CLPP profiles varies with successional stage. These analyses will increase our knowledge of relationships among aboveground vegetation and belowground microbial community during natural recovery and have implication for the management of natural ecosystem.

2. Materials and methods

2.1. Study site

The study site is situated at Dunshan watershed ($109^\circ19'23''\text{E}$, $36^\circ51'30''\text{N}$), northern Loess Plateau of China. The area has a temperate semiarid climate: the mean annual temperature of 8.8°C , and the mean annual precipitation is 509 mm, with about 60–75% falling in July to September. The annual evaporation ranges from 1500 to 1800 mm. The soil type in the study area is classified as a loessal Cambisol (FAO), which was originated from wind deposits and characterized by a yellow color, absence of bedding, silty texture, looseness, macroporosity and wetness-induced collapsibility. In 1999, the Chinese government launched the program of reconverting the slope cropland to the forest land and grassland to solve the issue of the environment development. One important ecological project is abandoning slope croplands for natural recovery without human disturbance.

2.2. Experimental design and soil sampling

Eight abandoned croplands in the watershed were selected as experimental sites. The sites had been abandoned for 1, 3, 5, 10, 13, 15, 20, and 30 years. The abandonment years were determined by inquiries of local farmers and from relevant government land documents. These sites have similar gradients and elevations and had been subjected to similar farming practices. Before abandonment, the main crops of the eight croplands were millet (*Setaria italica*) and soybean (*Glycine max*). One crop was grown each year, and had been cultivated for over 40 years. After abandonment, the

Table 1
Geographic features and floristic compositions of the sampling sites (Zhang et al., 2012).

Abandoned cropland	Slope aspect	Slope gradient	Altitude (m)	Plant community	Minor species
1-year	E	25°	1274	<i>A. capillaries</i>	<i>S. collina</i> , <i>P. australis</i>
3-year	E10°N	22°	1273	<i>A. capillaries</i>	<i>H. altaicus</i> , <i>E. crusgalli</i> , <i>S. collina</i> , <i>P. australis</i>
5-year	W10°N	25°	1298	<i>A. capillaries</i> – <i>H. altaicus</i>	<i>L. davurica</i> , <i>E. crusgalli</i> , <i>P. annua</i> , <i>L. perenne</i>
10-year	E30°N	24°	1312	<i>H. Altaicus</i> – <i>A. capillaries</i>	<i>L. davurica</i> , <i>S. bungeana</i> , <i>L. perenne</i>
13-year	N30°E	22°	1282	<i>A. capillaries</i> – <i>A. sacrorum</i>	<i>S. bungeana</i> , <i>H. altaicus</i> , <i>V. sepium</i> , <i>P. annua</i> , <i>L. davurica</i>
15-year	N25°W	28°	1256	<i>A. sacrorum</i> – <i>S. bungeana</i>	<i>L. davurica</i> , <i>P. bifurca</i> , <i>S. collina</i>
20-year	N35°E	22°	1287	<i>S. bungeana</i> – <i>A. sacrorum</i>	<i>L. davurica</i> , <i>P. bifurca</i> , <i>C. squarrosa</i> , <i>S. collina</i>
30-year	E30°N	30°	1258	<i>A. sacrorum</i>	<i>S. bungeana</i> , <i>P. heterophylla</i> , <i>V. sepium</i>

The soil type for all the sites was Loessal soil. *A. capillaries*: *Artemisia capillaries*; *A. sacrorum*: *Artemisia sacrorum*; *H. altaicus*: *Heteropappus altaicus*; *S. Bungeana*: *Stipa bungeana*. *S. collina*: *Salsola collina*; *E. crusgalli*: *Echinochloa crusgalli*; *P. australis*: *Phragmites australis*; *L. perenne*: *Lolium perenne*; *L. davurica*: *Lespedeza davurica*; *P. bifurca*: *Potentilla bifurca*; *P. Annua*: *Poa annua*; *C. squarrosa*: *Cleistogenes squarrosa*; *V. sepium*: *Vicia sepium*; *P. bifurca*: *Potentilla bifurca*; *P. heterophylla*: *Patrinia heterophylla*.

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