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Changes in microbial communities and respiration following the revegetation of eroded soil

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ABSTRACT

It is necessary to assess the responses of microbial communities and respiration to the revegetation of eroded soils for understanding the dynamics of soil carbon (C) pools and fluxes. In this study, three typical abandoned croplands (CL1, CL2 and CL3) and three secondary grasslands planted with *Coronilla varia* (GL1, GL2 and GL3) on the Loess Plateau of China were selected for sampling, and quantitative polymerase chain reaction (qPCR) and high-throughput sequencing were applied to intuitively discern differences in the soil bacteria and fungi. Our results showed that bacterial abundance in the abandoned croplands was 57 times higher than that of the secondary grasslands ($P < 0.05$), but no obvious changes ($P > 0.05$) in fungal abundance and microbial diversity were observed after 31 years of revegetation. We observed positive responses in *Actinobacteria*, *Firmicutes*, *Zygomycota* and *Ciliophora* and negative responses in *Bacteroidetes* and *Planctomycetes* to revegetation. In addition, the maximum soil microbial respiration was observed in the GL3 site (20.86 ± 0.69 mg CO_2-C kg^{-1} soil d^{-1}) followed by the GL1 site (19.97 ± 0.65 mg CO_2-C kg^{-1} soil d^{-1}), so revegetation significantly improved ($P < 0.05$) soil microbial respiration. Multiple stepwise regression analysis showed that dissolved organic carbon (DOC) explained up to 68.5% of the variation in soil microbial respiration, which indicated that the effects of changes in microbial properties in response to revegetation on soil microbial respiration were likely to be smaller than the potential effects of changes in the quality of organic matter. Labile organic matter is the primary rate-limiting factor for soil microbial respiration.

1. Introduction

Globally, soils are the largest terrestrial carbon (C) pool, storing approximately 2344 Pg C (1 Pg = 10^{15} g) in the top three meters (Van Hemelryck et al., 2011). Thus, even a small change in soil C concentration due to natural or human-caused disturbances, such as soil erosion, land use change and fertilization, may contribute to a significant net exchange of C between the pedosphere and the atmosphere (Olson et al., 2016). Recently, the terrestrial ecosystem C cycle has received increasing attention worldwide as the emission of carbon dioxide (CO_2) into the atmosphere is a key driver of global warming.

Soil erosion is the dominant soil degradation process and represents one of the most important but poorly quantified environmental problems (Nie et al., 2014). In past decades, intensive erosion promoted the emergence of a large area of degraded lands in China, especially in the hilly-gully region of the Loess Plateau, and high temperatures and evapotranspiration rates associated with high-intensity storms and fragile soils accelerated this degradation (Zhang et al., 2011a). Soil erosion strongly affects the distribution of sediments and the associated organic carbon within a landscape, but it also dramatically impacts the exchange of C between soils and the atmosphere (Bajracharya et al., 2000). Previous studies demonstrated that serious water erosion can accelerate the mineralization of soil organic carbon (SOC) by disrupting soil aggregates and organic-mineral complexes (Balesdent et al., 2000). To restore the degraded soil and control soil erosion on the Loess Plateau, the government began implementing the 'Grain-for-Green' program in 1999, and many degraded croplands were returned to grasslands or forestlands (Deng et al., 2014). For example, *Coronilla varia* with developed root systems was extensively planted in the Qiaozi-East watershed of the third sub-region of the Loess Plateau to

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control soil and water loss (Gustine and Moyer, 1990).

Revegetation can directly or indirectly impact the biological characteristics of the soil, particularly soil microorganisms, via shifts in soil properties such as moisture and porosity as well as the amount and quality of organic matter (Andriamananjara et al., 2016; Xiao et al., 2016; Zhang et al., 2016). As an important component of the terrestrial biosphere, soil microorganisms play crucial roles in humification, mineralization and nutrient cycling, so the variations in the activity, abundance and composition of soil microorganisms may significantly influence SOC mineralization and the size of the soil C pool. Revegetation usually increases soil C inputs by enhancing plant root exudation and litter production (Deng et al., 2014), but the observed responses of soil microbial communities and respiration to increased substrate during revegetation have been highly variable due to diverse experimental methods, soil properties and plant species (An et al., 2013; Lanza et al., 2016). Much of the previous research has indicated that revegetation processes enhance microbial community diversity and respiration (Cleveland et al., 2014; Zhang et al., 2016), but some studies have found the increases in soil microbial biomass and community diversity following revegetation to be associated with declines in soil microbial respiration (Dube et al., 2009; Deng et al., 2016). Therefore, a systematic and complete understanding of the response patterns of the microbial community and respiration to revegetation is still lacking. Revegetation can improve soil C content through abundant plant residue input, while C sequestration in soil following revegetation mainly depends on the trade-off between biomass input and microbial respiration. Microbial respiration is regulated by the interactions between soil microbial properties and micro-environments (Cleveland et al., 2014), so further understanding the response characteristics of microorganisms to revegetation and their link with SOC mineralization is very important for clarifying the mechanism governing soil C cycling.

In this study, we hypothesized that (H1) the abundance and diversity of the microbial community in the revegetated lands is significantly higher due to the enhancement of soil nutrients and that (H2) the magnitude of SOC mineralization in the study sites is primarily controlled by soil microbial properties. To test our hypotheses, the abundance and community structure of soil bacteria and fungi were investigated in abandoned croplands and secondary grasslands (*Coronilla varia*) in the Qiaozi watershed of the Loess Plateau. Advanced molecular biology techniques, high-throughput sequencing and quantitative polymerase chain reaction (qPCR) were applied to intuitively discern the dynamics of the soil microorganisms. Therefore, the goals of this study were to (i) investigate the response patterns of microbial communities to revegetation and (ii) explore the difference in microbial respiration between secondary grasslands and abandoned croplands.

2. Materials and methods

2.1. Experimental site

The study was located in the Qiaozi watershed (34°36′–34°37′N, 105°42′–105°43′E) in the hilly-gully region of the Loess Plateau, which is situated in the vicinity of the city of Tianshui, Gansu Province, China. The Qiaozi watershed consists of the adjacent Qiaozi-East and Qiaozi-West sub-watersheds, which have similar terrain, rainfall conditions and soil categories. The major difference between them is that *Coronilla varia* was widely planted in the Qiaozi-East sub-watershed in 1985 to prevent soil and water loss, while no management actions were taken in the Qiaozi-West sub-watershed. The climate is semi-arid with mean annual precipitation of 496–628 mm, more than two-thirds of which occurs in the form of short-duration and high-intensity storms during the summer months (June to September). The mean annual temperature is 10.7 °C, and the hottest month is July with an average temperature of 22.6 °C. The dominant soil type is black cinnamonic (Calcic Cambisols, FAO). Three abandoned croplands, which were cultivated beginning in 1988 and abandoned after 2005, in the Qiaozi-West sub-watershed (CL group: CL1, CL2 and CL3) and three secondary grasslands, which were planted with *Coronilla varia* in 1985, in the Qiaozi-East sub-watershed (GL group: GL1, GL2 and GL3) were selected for study. The main crop grown in the cultivated areas before abandonment was maize (*Zea mays* L.), and *Heteropappus altaicus* and *Artemisia capillaries* were the dominated plants after 11 years of natural succession. The erosion rates in the abandoned croplands ranged from 1599.50 to 2877.76 t km^{-2} yr^{-1} (Table S1). The basic characteristics of the study sites are presented in Table S2.

2.2. Soil sampling and treatment

A 20 m × 40 m sampling plot was selected in each site. Before the soil samples were collected, the litter layer of the topsoil (approximately 1 cm) was removed, and five quadrats were randomly placed in each sampling plot. Each quadrat had a dimension of 1 m × 1 m, and samples were taken in each quadrat using a 5 cm diameter corer. In May 2016, nine soil core samples were collected from each quadrat at a depth of 0–10 cm and then mixed together so that each analyzed soil sample was a mixture of 9 sub-samples. After carefully removing the fine roots, stones and organic materials, each soil sample was divided into two parts, one of which was air dried to determine the soil physicochemical characteristics. The other part was sieved (2 mm) and immediately stored at −70 °C to analyze the soil microbial communities.

2.3. Measurement of soil physicochemical properties

Air-dried soil samples were crushed with a wood mallet to pass through a 2-mm sieve. Soil pH was determined with a digital pH meter (Woonsocket, RI, USA) using a soil-to-water ratio of 1:2.5 (w/v), and soil bulk density (BD) was determined using the cutting ring method. The distribution of soil particle sizes was analyzed with a laser particle size analyzer (MS-200, Malvern, UK). Additionally, subsamples were crushed in a mortar to pass through a 0.25 mm sieve and transported to the Institute of Subtropical Agriculture of the Chinese Academy of Sciences for SOC and total nitrogen (TN) analyses. SOC was analyzed using the dichromate oxidation method of Walkley and Black (1934), and TN was measured using the Kjeldahl (1883) method. The alkali N-proliferation method was used to measure the available N (Duan et al., 2016), and dissolved organic carbon (DOC) was determined as described by Li et al. (2015). Finally, microbial biomass carbon (MBC) was measured using the chloroform-fumigation extraction method (Vance et al., 1987). The soil physicochemical properties of the study sites are presented in Table 1.

2.4. Quantification of bacterial and fungal abundance

Soil DNA was extracted using a PowerSoil® DNA isolation kit (Omega Bio-Tek, USA), and the quantity and quality of the DNA extracts were assessed using a spectrophotometer (ND-1000, Isogen Life Science). The universal primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 518R (5′-ATTACCGCGGCT GCTGG-3′) were used to amplify the bacterial 16S ribosomal RNA gene (Huang et al., 2014a), and the primers 18S-F (5′-CGGCTACCACATCC-AAGGAA-3′) and 18S-R (5′-GCTGGAATTACCGCGGCT-3′) were used to amplify the 18S ribosomal RNA gene. PCR amplifications were performed in a PRISM® 7500 Fast Real-Time PCR System (Applied Biosystems, Italy). The PCR procedure to amplify the 16S rRNA gene were 95 °C for 10 min followed by 40 cycles consisting of 15 s at 95 °C, 60 s at 55 °C and 90 s at 72 °C. The 18S rRNA gene was amplified under the following PCR conditions: 95 °C for 10 min followed by 40 cycles consisting of 15 s at 95 °C, 60 s at 50 °C and 45 s at 72 °C (Zhang et al., 2011b). Amplification was carried out in a total volume of 20 μl containing 16.4 μl of 2 × Real SYBR Mixture, 0.8 μl of each primer