



Differential soil microbial community responses to the linkage of soil organic carbon fractions with respiration across land-use changes



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ABSTRACT

Land-use change can modify terrestrial ecosystem processes with potentially important effects on below-ground carbon dynamics. Soil microbes are considered the rate-limiting factor in carbon decomposition. However, the effect of land-use change on soil microbial community and the mechanism of soil carbon dynamics remain unclear. In this study, soil samples were collected during four periods (April, June, August, and October) at sites in the Loess Plateau in China with different land-use types: *Robinia pseudoacacia* L. (RP) and abandoned land (AL); these areas were converted 40 years ago from similar farmlands, while the millet (*Setaria italica*) farmlands (FL) were selected as a control in our study. Quantitative PCR and Illumina sequencing of the 16S rRNA and ITS genes were performed to analyze the abundance, diversity, and compositions of the soil microbes (bacteria and fungi). Additionally, soil organic carbon fractions (soil organic carbon: SOC, dissolved organic carbon: DOC, microbial biomass carbon: MBC) and soil respiration components (soil respiration: SR, heterotrophic respiration: HR, autotrophic respiration: AR) were evaluated. The results showed that SOC fractions and soil respiration increased after land-use change, with significant correlation being observed. In particular, DOC was more related to SR and HR than to the other fractions. Moreover, the abundance and diversity of the microbes (bacteria and fungi) were greatly affected by the land-use change; both of them were significantly and positively correlated with soil organic carbon fractions and soil respiration components. For dominant bacterial phyla, both *Proteobacteria* and *Bacteroidetes* were significantly more abundant in the afforested soil than in the FL, while the abundances of *Actinobacteria* and *Chloroflexi* ranked as FL > AL > RP. For dominant fungal phyla, *Ascomycota* responded positively to land-use changes, whereas *Basidiomycota* responded negatively. Such changes in the abundances of microbial phyla were significantly correlated with the linkage of soil organic carbon fractions and respiration components. Altogether, these results suggest that the changes in components of soil respiration may be highly susceptible to soil organic carbon fractions, especially to DOC, and this linkage is largely modulated by microbial community across land-use changes.

1. Introduction

As the largest carbon pool in the terrestrial biosphere, soil contains much more carbon than that in either the vegetation or the atmosphere or their combination (Tarnocai et al., 2009). Thus, even a small shift in the soil organic carbon (SOC) pool can lead to significant changes in atmospheric CO₂ concentration, and further influence future climate change (Davidson and Janssens, 2006; Piao et al., 2012). Land-use change, particularly for afforestation, has the potential to alter the

terrestrial carbon cycle (Davidson and Janssens, 2006). Despite the wealth of studies focusing on below-ground soil carbon dynamics across land-use changes (Li et al., 2012; Garcia-Franco et al., 2015), there is uncertainty in the magnitude or direction of carbon fluxes in the SOC pool (Post and Kwon, 2000; Tardy et al., 2015). Numerous studies have mainly focused on the theory that changes in soil carbon stocks are influenced by plant decomposition and physical protection (Davidson and Janssens, 2006; Lange et al., 2015). However, the microbial regulation for carbon cycling towards land-use change remains unclear.

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This uncertainty has greatly hindered the identification of soil carbon balances in afforested ecosystem. Therefore, to improve our predications of soil carbon balances, there is an urgent need for a better understanding of microbial community responses.

Soil microbes respond differently to soil carbon dynamics as a result of changing plant diversity and organic matter quality across land use changes (Li et al., 2012; Deng et al., 2016). However, the directions and magnitudes of these responses are uncertain. For instance, large amounts of plant residue inputs may decrease the efficiency with which microbes use carbon or decompose soil organic matter, and ultimately reduce soil carbon storage (Fontaine et al., 2007). Conversely, several studies have shown that high plant diversity or high amounts of plant residue inputs can increase the storage of soil carbon from fresh carbon by altering the growth of soil microbes (Lange et al., 2015; Tardy et al., 2015). Nevertheless, the contradicting results have suggested the critical role of microbial decomposition in regulating soil carbon dynamics in a changing environment. Moreover, some microbial taxa participate in the decomposition of soil organic matter, causing changes in SOC fractions, ultimately affecting soil CO₂ efflux (Tardy et al., 2015; Goldfarb et al., 2011). However, how changes in microbial compositions effectively explain the alterations in SOC fractions and soil respiration remains unclear, especially in afforested ecosystems (Deng et al., 2016; Xiao et al., 2017). Several recent studies of advanced microbial models showed that the incorporation of soil microbes into biogeochemical models substantially improved the model prediction of soil carbon dynamics (Allison et al., 2010; Hararuk et al., 2015). Therefore, understanding microbial carbon dynamics across land-use changes is likely not only to shed light on the ecological carbon cycle in afforested ecosystems, but also to improve the predication of the carbon balances when assessing the effects of land-use change on the SOC pool.

The Loess Plateau, which is located at the boundary of the arid and semiarid areas in China, has suffered from severe soil erosion and is characterized by low vegetation coverage (Bai and Dent, 2009). To rehabilitate the degraded lands, the Chinese government has implemented a series of environmental protection policies; one such policy is the Grain to Green Program (GTGP) (Fu et al., 2000). These policies result in increased net primary productivity and reduce the degree of soil disturbance; these effects have led to changes in above-ground and below-ground ecosystems (Ren et al., 2016a,b, 2017a). Numerous studies have been conducted to investigate the changes in soil carbon and nitrogen dynamics associated with the soil microbial community across land-use changes in an afforested ecosystem (Deng et al., 2016; Xiao et al., 2017; Ren et al., 2016a,b). However, most of these studies ignore the fact that below-ground carbon dynamics consist of a continuum of organic carbon fractions and CO₂ efflux (Garcia-Franco et al., 2015; Liang et al., 2015). Furthermore, traditional methods such as PLFA analysis or PCR-DGEE make it difficult to identify the effects of land-use changes on microbial communities at the species level and show limited phylogenetic or taxonomic resolution (Grayston et al., 2004; Frostegård et al., 2011). In contrast, the advent of next-generation sequencing has provided detailed information for soil microbial communities, and helped to clearly describe the soil microbial diversity and the processes structuring compositions (Biddle et al., 2008). Through using this method, we can gain a comprehensive understanding of the trends that characterize microbial community responses to land-use change, or the mechanisms governing the below-ground carbon cycling to disturbance in afforested ecosystems.

In this study, we hypothesized that soil respiration components may be changed synchronously with SOC fractions across land-use changes, and this alteration may be stimulated by the diversity and abundance of soil microbes (bacteria and fungi). In addition, we predicted that alterations in specific microbial taxa would account for the changing SOC fractions and components of soil respiration in an afforested ecosystem. Therefore, we focused on the following: (i) the changes in SOC fractions and soil respiration components, and (ii) the response of the abundance, diversity, and compositions of soil microbes to the linkage of

SOC fractions with respiration components across land-use changes.

2. Method and material

2.1. Study area description

The study area is located in the Wuliwan watershed of Ansai County, Shaanxi Province, China (36°51'41.23"–36°52'50.87"N, 109°19'49.20"–109°21'46.46"E), which is located in middle area of the Loess Plateau. This region is a temperate semiarid area with an average annual temperature of 8.8 °C and average annual precipitation of 510 mm (mainly from July to September). On average, there are about 157 frost-free days and 2415 h of total yearly sunshine. The present soil type is classified as Huangmian soil (Calcic Cambisols, FAO) and is particularly susceptible to erosion.

Since 1973, the area has been used as a field experimental base to control soil erosion through wind and water by the Institute of Soil and Water Conservation, Chinese Academy of Sciences. The integrated management of vegetation restoration and soil and water conservation has been carried out gradually. In particular, all of the farmlands (FL) with slopes higher than 25° were converted to forestlands and abandoned lands. Currently, the forestlands with *Robinia pseudoacacia* L. (RP) and abandoned land (AL) have become the main vegetation areas for rehabilitating ecology in the region. Moreover, RP is leguminous and has higher rates of net primary productivity than the former FL. AL was generated from previous FL due to their extremely low productivity and far distance from farmers' residences; thus, many farmlands have been abandoned for natural recovery without anthropogenic interference (i.e., cultivation, fertilization). For these three land-use types, the important values of each species in RP and AL were shown in Table S1. The averages of soil bulk density (BD) and clay content ranged from 1.1 to 1.2 and 8.9% to 9.6%, respectively. During the four periods, the averages of soil water contents and pH ranged from 7.1% to 20.2% and from 8.4 to 8.5, respectively (Table S2).

2.2. Experimental design, plant investigation and trenching

Sampling was carried out in April, June, August, and October of 2014. Two land-use types were selected based on the afforestation of former farmlands in the area: RP and AL, both of which had been afforested for 40 years. An adjacent FL was used as a reference site. All the study sites were located within the same area. These three land-use types have similar elevations and have been subjected to similar farming practices in the past. Moreover, their soils have developed from the same parent materials. Before afforestation, the main crops grown in these sites were millet (*Setaria italica*) and soybean (*Glycine max*), and their farming practices were similar. Experimental designs were described previously in detail (Ren et al., 2016a,b; Fig. S1). In brief, for each land-use type, we chose three 25 m × 50 m sites, which were considered the three independent replicates, since the distance between any two sites exceeded the spatial dependence (< 13.5 m) of most soil variables (Marriott et al., 1997); two 25 m × 25 m plots were established in each replicate site.

In addition, six quadrats (0.5 m × 0.5 m) (three trenched quadrats and three untrenched quadrats) were randomly established in each plot, and the trenches (0.5 m wide and 0.8 m deep) were excavated in October 2013. After covering the trenches with a 2-mm thick plastic sheet, we refilled them with soil, and the above-ground plants were carefully removed without any disturbance.

2.3. Soil respiration measurement and soil sampling

A total of six polyvinyl chloride (PVC) collars (16 cm inner diameter, 12 cm deep) were inserted to a depth of 10 cm. Three PVC collars of trenched quadrats were used to determine the soil HR, and another three PVC collars in untrenched quadrats were used to determine the

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