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Response of soil aggregate-associated microbial and nematode communities to tea plantation age

Shengqiang Wang, Tingxuan Li*, Zicheng Zheng

College of Resources, Sichuan Agricultural University, Chengdu 611130, Sichuan, China

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ABSTRACT

Understanding the changes of soil microbial and nematode communities within aggregate fractions is essential for maintaining the stability of soil biological communities and soil health in agricultural ecosystems. However, the short- and long-term influence of converting abandoned land to tea (*Camellia sinensis* L.) plantations on soil aggregate-associated biological communities is still poorly understood. In this study, soil microbial and nematode communities were analyzed in soil fractions of different sizes collected from 0 to 20 cm depth in four tea plantations with the same plants (cultivar was "*Sichuan tea*") of various ages (16-, 23-, 31-, and 53-years) in the hilly area of western Sichuan in China. The aggregates were separated by a dry-sieving procedure into four fractions: large (> 2 mm), medium (2–1 mm), small (1–0.25 mm) macro-aggregates, and micro-aggregates (< 0.25 mm). Although soil microbial biomass (bacteria, fungi, and actinomycetes) was not correlated ($P > 0.05$) with most nematode abundance (bacterivores, plant-parasites, fungivores, and omnivores and predators) across aggregate fractions in different tea plantation ages, not only microbial biomass and nematode abundance but also their diversity was highest in the large macro-aggregates, indicating that these fractions had a complex biological community structure with more connections in soil food web, thus providing biological buffering and prohibiting the dominance of individual organisms via competition or predation. In the process of tea cultivation, the abundance of soil nematodes was largely dependent on microbial biomass in the macro-aggregates where pore space was large enough for nematodes to prey on microbes, while there were no correlations ($P > 0.05$) between microbial biomass and nematode abundance in the micro-aggregates. The 23-year-old tea plantation provided a relatively stable soil environment for the development of microbial and nematode communities, and subsequently induced an increase of soil microbial biomass, nematode abundance, and their diversity. Notably, the degradation of soil biological communities after 23-years underscores the need for sustainable soil management practices that would maintain the stability of soil biological communities and soil health after 23-years of tea cultivation in the hilly area of western Sichuan in China.

1. Introduction

Poor land-use practices resulted from increasing human population pressure over the last century, including deforestation, over-pasturing, and over-cultivation, have drastically increased the fragmentation of ecological environments in the hilly area of western Sichuan in China. This has led to not only the intense degradation of natural ecosystems, but also the reduction in the current and future soil productivity capacity as a result of wind and water erosion, declines in fertility, alterations in moisture and aeration levels, or shifts in soil fauna and flora. To ameliorate the situation, a series of nationwide conservation projects dedicated to rehabilitate these ecosystems have been implemented by the Chinese government (Li and Pang, 2010). Among them, one focus for the sustainable agricultural development of the hilly area of western Sichuan is to convert the abandoned land to tea (*Camellia sinensis* L.) plantations. As an important cash crop in many developing countries, tea is widely cultivated in Sri Lanka, India, and China. Particularly, as the largest tea producer all over the world, China had the area of 2.93 million hectares used for tea plantations in 2016 (International Tea and Committee, 2017), which has been continuously increasing.

As the primary regulators of decomposition, soil bacteria and fungi form key linkages between detritus and soil fauna, and comprise the principal resource base for soil food web (Briar et al., 2011). Maintaining the composition and diversity of the soil microbial community is critical to sustain soil ecosystem function and to mediate soil organic

* Corresponding author.
E-mail address: litinx@263.net (T. Li).

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matter (SOM) decomposition and nutrient cycling (Marschner et al., 2003). As a central role in the soil food web, soil nematodes are believed to be tightly associated with microbes both functionally and nutritionally (Ferris, 2010). In consideration of the trophic interplays, nematodes are assigned as bacterivores, plant-parasites, fungivores, and omnivores and predators (Yeates et al., 1993). Assessment of nematode community composition can provide unique insights into soil biological processes because different feeding groups of nematodes are specialized with respect to their food sources and play essential roles in functioning of soil ecosystems (Yeates et al., 1993; Ferris, 2010; Papatheodorou et al., 2012). Thus, measurement of soil microbial and nematode communities together is helpful to not only complement our understanding of the dynamics of soil food web, but also indicate the effectiveness of management intervention as well as the developmental status of restored ecosystems (Chen et al., 2015; Li et al., 2016).

To understand their variations in agricultural soils, the communities of soil microbes and nematodes should be recognized at different spatial scales (Briar et al., 2011; Jiang et al., 2013; Zhang et al., 2013). As the basic unit of the soil structure, aggregates serve as the heterogeneous assemblages of the mineral and organic particles. In the meanwhile, they are classified according to size such as micro- ($<$ 0.25 mm) and macro- ($>$ 0.25 mm) aggregates (Tisdall and Oades, 1982). The micro-aggregates are produced through the microbial mediated processes in the macro-aggregates, which depend intensively on the structural stability's persistent organic binding agents (Six et al., 2004). Conversely, the temporary associations of particulate organic matter, minerals, and micro-aggregates finally lead to the production of macro-aggregates, which are achieved mainly through the enmeshment by fungal hyphae and plant roots (Six et al., 2004). Besides access to food sources, a fundamental requirement for soil organisms is adequate space to accommodate their growth and movement and to allow for water and gas exchange (Queneherve and Chotte, 1996). For example, soil bacteria can occupy pore $<$ 3 μm in diameter, whereas larger organisms, such as soil nematodes, are limited to larger pores (Hassink et al., 1993). As a result, the concentration and availability of organic substances as well as the chemical and physical protection mechanisms dominate the distribution of soil biological communities within different aggregate fractions (Briar et al., 2011; Jiang et al., 2013; Zhang et al., 2013).

Our previous studies have found that long-term tea cultivation significantly influences the distribution and stability of soil aggregates (Wang et al., 2017). However, the changes of soil biological communities still remain unclear. Therefore, the proposed study is designed to determine the short- and long-term influence of converting abandoned land to tea plantations on the dynamics of soil microbial and nematode communities associated with aggregate fractions. It is hypothesized that (i) the microbial and nematode communities within soil aggregates would differ with the size of aggregates due to resource and space constraints within each fraction, (ii) soil microbial and nematode communities would differ with the age of tea plantations because there would be significant changes in the distribution and stability of soil aggregates during the tea planting process in the hilly area of western Sichuan in China.

2. Materials and methods

2.1. Experimental site

Research began in January 2014 at the Zhongfeng long-term agricultural experimental site of Sichuan Agricultural University in Ya'an, Sichuan, China (Wang et al., 2017). The prevailing climate surrounding the study site is a subtropical monsoon climate. The annual mean temperature is 15.4 °C while the highest and lowest daily mean temperatures are 35.2 °C and 4.3 °C, respectively. The annual mean precipitation is 1500 mm, 72.6% of which precipitates between July and September. The layer being exposed belongs to sedimentary rock predominantly formed since the Mesozoic age, with the soil being Luvisols with a texture of clay loam (IUSS Working Group WRB, 2014). "*Sichuan tea*" has been continuously cultivated in the upper streams of Yangtze River as part of a reforestation project ever since 1950s. Therefore, no-tillage "*Sichuan tea*" constitutes the predominant cultivar in the region, with plantations differing in age.

The cultivation density of tea (narrow row = 35 ± 15 cm wide, broad row = 150 ± 15 cm wide, and each two plants had a distance of 16 ± 4 cm) was set at approximately 80,000 plants per hectare. As the basic fertilizer, 750 kg ha^{-1} of complex fertilizer (solid, N-P_2O_5-K_2O: 20%–8%–8%) and 15,000 kg ha^{-1} of swine manure (liquid, N-P_2O_5-K_2O: 0.17%–0.05%–0.19%) were annually applied along the vertical edges under the tree canopy in mid-October. Moreover, it requires replenishing the top dressing three times annually. In mid-February of the following year, 1500 kg ha^{-1} of complex fertilizer and 600 kg ha^{-1} of urea were used. Meanwhile, in late-May and July, 750 kg ha^{-1} of complex fertilizer and 300 kg ha^{-1} of urea were administered to the soil surface, where the top dressing was replenished at the same position as that of the basic fertilizer application. All the tea plantations are deployed with yellow papers instead of chemical pesticides to prevent pests. Herbicides were not utilized during the tea planting process. Tea leaves were picked in March, June, and August each year. There was a light pruning just after spring harvest in each year, and the prunings were left in situ as surface mulch.

2.2. Experimental design

Soil changes in a tea plantation chronosequence with the similar climate and soil status were monitored through space-for-time substitution, which is a valid method in assessing the variations over time (Sparling et al., 2003). The different ages of the tea plantations provide an opportunity for elucidating the changes occurring during the tea planting process since the geomorphologic processes can determine the soil conditions to a great extent prior to tea cultivation.

Wang et al. (2017) explained the experimental design in details. Briefly, litter and soil samples from four tea plantations with the same plants (cultivar was "*Sichuan tea*") of various ages (16-, 23-, 31-, and 53-years) were collected in September 2014. All studied tea plantations were situated on similar geomorphologic units with similar soil parent material, slope direction and gradient, and fertilization practices. The land had been abandoned before the tea cultivation. A complete random design with five replicates was employed for each tea plantation, generating a total of 20 plots (15 m × 15 m). The distance between any two plots in each plantation was about 50 m.

2.3. Litter and soil sampling

In each plot, five litter samples were collected with plastic bags prior to soil sampling from five subplots (0.5 m × 0.5 m) at the soil surface and subsequently combined into a composite litter sample. A total of 20 composite litter samples undertook the oven-dried process under 80 °C to a constant weight, weighed, and analyses were conducted to confirm their organic C and total N concentrations. The position of soil sampling was the same as that of litter sampling. Five undisturbed soil samples were obtained in each plot at the soil depth of 0–20 cm and subsequently combined into a composite soil sample. Different sterile containers have been used to seal the 20 composite soil samples, which are subsequently used for laboratory analysis. Afterwards, the composite soil samples were carefully fragmented along the natural planes of weakness to natural aggregates and sieved (mesh size 5 mm) to eliminate macrofauna, stones, and large roots. The litter and soil properties of the four tea plantations are listed in Table 1.

2.4. Soil aggregate separation

Soil aggregates were separated by a dry-sieving procedure as described by Schutter and Dick (2002). The field-moist clods ($<$ 5 mm)