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Effects of tea plantation age on soil aggregate-associated C- and N-cycling enzyme activities in the hilly areas of Western Sichuan, China

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ABSTRACT

Research on the changes of soil carbon (C)- and nitrogen (N)-cycling enzyme activities within aggregate fractions is essential for improving our understanding of soil organic matter (SOM) dynamics in agricultural ecosystems. However, the short- and long-term implications of the conversion of abandoned croplands to tea (*Camellia sinensis* L.) plantations on enzyme activities associated with aggregate fractions remains poorly understood. In the present study, we hypothesized that (i) the concentrations of soil organic C and total N would increase as the tea plantations continued to age, and (ii) the potential of SOM decomposition would differ with the stage of tea cultivation, as there would be significant changes in the activities of soil C- and N-cycling enzymes over time. The activities of β-glucosidase, invertase, urease, and protease were analyzed in different size fractions of soil aggregates that were collected from 0 to 20 cm depth in four tea plantations with the same cultivar (*Sichuan tea*) of various ages (16-, 23-, 31-, and 53-years) in the hilly areas of Western Sichuan, China. The aggregates were separated using a dry-sieving procedure into four fractions, namely, large (> 2 mm), medium (2–1 mm), small (1–0.25 mm) macro-aggregates, and micro-aggregates (< 0.25 mm). In the early stage (in the first 23-years), tea cultivation contributed to a significant increase in organic C and total N stocks in the whole-soil, and these changes were primarily reflected in the increases of such elements stocks in the large macro-aggregates. For example, the increase in organic C stock associated with large macro-aggregates in the early stage accounted for 99.64% of the increase in organic C stock in the whole-soil. In the later stage (after 23-years), however, the increases in organic C and total N stocks in the whole-soil were primarily reflected in the increases of such elements stocks in the small macro-aggregates. Meanwhile, soil C- and N-cycling enzyme activities associated with the large macro-aggregates were significantly increased in the first 23-years of tea cultivation. These results indicated that young tea plantations in the early stage had limitations with respect to the potential for SOM sequestration; however, as the tea plantations matured under the present system of management, the SOM sequestration has improved.

1. Introduction

Poor land-use practices resulted from growing human population pressure over the last century, including excess reclamation, overgrazing, deforestation, have drastically exacerbated the fragmentation of ecological environments in the hilly areas of Western Sichuan, China. This has led to not only the intense degradation of natural ecosystems, but also the reduction of current and future soil productivity capacities. To ameliorate this situation, a series of nationwide conservation projects dedicated to the rehabilitation of these ecosystems have been implemented by the Chinese government (Li and Pang, 2010). Among them, one focus for sustainable agricultural development in the hilly areas of Western Sichuan was the conversion of abandoned croplands to tea (*Camellia sinensis* L.) plantations (Zheng et al., 2011). 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 dedicated an area of 2.93 million ha for use as tea plantations in 2016 (International Tea Committee, 2017), which has been continuously increasing.

The maintenance of soil organic matter (SOM) is of great importance to guarantee the productivity of agricultural ecosystems (Oades, 1984). However, it is difficult to directly quantify SOM gains and losses within a short time due to their very small natural variability and annual changes, as well as the generally high background stocks (Sollins et al., 1996). Conversely, more dynamic characteristics such as soil hydrolase activities, which play an important role in the

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degradation of polymeric materials and the supply of low molecular weight substrates to microorganisms (Nannipieri et al., 2012), have quicker responses to the changes occurring in environmental stresses or agricultural management practices than SOM (Burns, 1982). In general, the dynamics of SOM are estimated by the changes of soil carbon (C) and nitrogen (N) cycles (Sollins et al., 1996). Since the release of C and N from SOM is regulated through enzyme activities, soil C- and N-cycling enzymes are of interest at the ecosystem scale (Burns, 1982). For instance, in terms of the enzymes that are related to the C cycle, β-glucosidase and invertase play critical roles in the release of the low molecular weight sugars that are important as energy sources for microorganisms (Liang et al., 2014; Zhang et al., 2015; Michele et al., 2017). On the other hand, urease and proteases are of great importance in the N cycle, since urea is hydrolyzed by urease into ammonium, while proteins are hydrolyzed by proteases to amino acids (Qin et al., 2010; Li et al., 2017; Xie et al., 2017). Therefore, it is crucial to determine the activities of C- and N-cycling enzymes for evaluating the potential of SOM sequestration and decomposition (Tisdall and Oades, 2012).

To understand their variations in agricultural soils, the activities of soil enzymes should be elucidated over different spatial scales (Bach and Hofmockel, 2014). As the fundamental units of soil structures, aggregates serve as heterogeneous assemblages of mineral and organic particulates. In the meanwhile, they are classified according to size such as micro- (< 0.25 mm) and macro- (> 0.25 mm) aggregates (Tisdall and Oades, 2012). Micro-aggregates are generated through microbially-mediated processes within macro-aggregates, which depend extensively on the structural stability's persistent binding agents (Six et al., 2004). Conversely, the associations of particulate organic matter, minerals, and micro-aggregates finally lead to the production of macro-aggregates, which are achieved mainly through the temporary (i.e., plant roots and fungal hyphae) and transient (i.e., plant- and microbial-derived polysaccharides) binding agents (Six et al., 2004). As a result, the concentration and availability of organic substances, as well as the chemical and physical protection mechanisms drive the distribution of soil enzyme activities within different aggregate fractions (Allison and Jastrow, 2006; Nannipieri et al., 2012).

Our previous studies have found that long-term tea cultivation had a significant influence on the microbial biomass and activity within soil aggregates (Wang et al., 2017). However, changes in soil enzyme activities remain unclear, despite the fact that these parameters reflect SOM dynamics. Therefore, the primary goals of the present study were to ascertain the short- and long-term impacts of the conversion of abandoned croplands to tea plantations on the (i) concentrations of organic C and total N, and (ii) activities of C- and N-cycling enzymes (β-glucosidase, invertase, urease, and protease) associated with aggregate fractions. It was hypothesized that (i) the concentrations of soil organic C and total N would increase as the tea plantations continued to age, and (ii) the potential of SOM decomposition would differ with the stage of tea cultivation, as there would be significant changes in the activities of soil C- and N-cycling enzymes over time.

2. Materials and methods

2.1. Experimental site

The field site selected for the research is located at the Zhongfeng long-term agricultural experimental site of Sichuan Agricultural University in Ya'an, Sichuan, China (Wang et al., 2016). 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, of which 72.6% precipitates between July and September. The layer being exposed belongs to sedimentary rock, which was predominantly formed since the Mesozoic age, with the soil type being Typic Haplic–Perudic Argosols having the texture of clay-loam (FAO, 1990). Since the 1950s, "*Sichuan tea*" has been continuously cultivated in the upper streams of Yangtze River, as part of a reforestation project. Therefore, no-tillage "*Sichuan tea*" constitutes the predominant cultivar in the region, with the plantations differing in age.

The cultivation density of the tea plants (narrow row = 35 ± 15 cm wide, broad row = 150 ± 15 cm wide, and in each two plants had a distance of 16 ± 4 cm) was set at approximately 80,000 plants per hectare. As the basal fertilizer, a mixed fertilizer (granular, N-P_2O_5-K_2O: 20%-8%-8%), and swine manure (liquid, N-P_2O_5-K_2O: 0.17%-0.05%-0.19%) at a rate of 750 kg ha^{-1} and 15,000 kg ha^{-1}, respectively, were applied annually along the vertical edges under the tree canopy in mid-October. Moreover, replenishing top dressing was required three times annually. In mid-February of the following year, 1500 kg ha^{-1} of mixed fertilizer and 600 kg ha^{-1} of urea were used. Meanwhile, in late-May and July, 750 kg ha^{-1} of mixed fertilizer and 300 kg ha^{-1} of urea were administered to the soil surface, where the replenishing top dressing was at the same position as that of the basal fertilizer application. These fertilization practices were practiced since the beginning of the land use change. All the tea plantations were deployed with yellow papers instead of chemical pesticides to prevent pests. Herbicides were not utilized during the tea planting process. A light pruning of the tea plants was carried out in February of each year.

2.2. Experimental design

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

Wang et al. (2016) explained the experimental design in detail. Briefly, four tea plantations with the same cultivar (*Sichuan tea*) of various ages (16-, 23-, 31-, and 53-years) were situated on similar geomorphologic units with similar soil parent material, slope direction and gradient, and fertilization practices. The land had been abandoned prior to tea cultivation (Zheng et al., 2011). 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 randomly collected with plastic bags prior to soil sampling from five subplots (0.5 m × 0.5 m) at the soil surface, which were 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 analyzed for organic C and total N concentrations (Table 1). The position of soil sampling was the same as for the litter sampling. In each plot, five soil samples (at the 0–20 cm depth) were obtained between the rows of tea plants using a spade to maintain the soils, relatively, in their natural aggregates, and subsequently combined into a composite soil sample. Different sterile containers were used to seal the 20 composite soil samples, which were 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. On the other hand, an additional five soil samples from each plot were randomly collected to determine the whole-soil properties, including pH, bulk density, organic C, total N, and enzyme activities (Table 1).