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Changes in the soil nutrient levels, enzyme activities, microbial community function, and structure during apple orchard maturation

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ABSTRACT

The quality of soil is affected by its microbiological composition, which may change over time. This study investigated differences in the soil quality during the maturation of apple orchards. Soil nutrients, soil enzymes, community-level physiological profiles (CLPP), and terminal restriction fragment length polymorphism (T-RFLP) profiles were determined in soil samples from four apple orchards (aged 3, 7, 15, and 23 years) and a neighboring wasteland. The orchard soil organic matter, total nitrogen, invertase, and urease levels increased initially as the apple orchards matured, before decreasing, while the soil total phosphorus accumulated. The total potassium levels were significantly lower in the orchards than the wasteland ($P < 0.05$). The 7-year-old orchard soil had the highest carbon source metabolic activity, Shannon diversity index, richness index, and evenness index, whereas the 23-year-old orchard soil had the lowest levels for all four indicators. T-RFLP indicated that the 62-bp and 136-bp T-RFs were enriched, whereas the 129-bp and 285-bp T-RFs declined during apple orchard maturation. This study indicates that the old orchard soil had an inferior micro-ecological environment compared with the younger orchards. Thus, measures such as balanced fertilization, intercropping, and green cover may improve the soil environment in apple orchards.

1. Introduction

The soil is the substrate for fruit growing and the soil quality has direct effects on fruit safety, fruit quality, and productivity. Thus, the management of soil and its evolution over time have become a major research focus. Many factors can affect the soil quality, which poses complex problems for researchers. In order to characterize the soil quality accurately, researchers are increasingly integrating data related to soil enzymes, soil nutrients, the microbial community, and its functional diversity to develop soil quality evaluation systems (Badiane et al., 2001; Gil-Sotres et al., 2005).

Soil enzymes are involved in most biochemical functions of soil, and they are very sensitive to changes in the environment, so they are considered as good indicators to predict changes of soil quality, and as indices of soil quality or productivity (Bergstrom et al., 1998). Zhang et al. (2004) demonstrated that some enzyme activities, such as invertase and peroxidase, could be applied to monitor the changes of soil condition during spruce forest restoration. Guo et al. (2011) compared the influence of mixed N fertilization on soil extracellular enzymatic activities and found that the highest enzyme activities were in the medium organic N fertilization treatment. Yuan et al. (2011) also showed that the soil enzyme activity levels were useful indicators when comparing the impact of different fertilization regimes.

Functional diversity and structure are widely used to give expression to microbial performance, especially in the influence of disturbances (Pignataro et al., 2012), and Biolog is a reliable method for evaluating these parameters (Stefanowicz, 2006). Biolog can reflect the changes in the microbial community in a sensitive manner. For example, Islam et al. (2011) investigated the effects of different fertilization regime on the CLPP of paddy soils. The results showed that soils receiving both organic and inorganic fertilizer had a significantly higher Shannon diversity index than soils received organic or inorganic fertilizer only. T-RFLP is a popular semi-quantitative, high-throughput, sensitive, and repeatable technique, which is applied to detect the microbial community composition (Sipos et al., 2007). T-RFLP reflects changes in microbial communities at the molecular level and is generally used as a complementary approach with Biolog and phospholipid fatty acid (PLFA) analysis (Chun et al., 2011; Tipayno et al., 2012; Trabelsi et al., 2012).

The physical, chemical, and biological properties of soil can be affected by management, fertilization, crops, and the duration of cultivation. Pankhurst et al. (2002) reported that changes in tillage

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practices had significant effects on the soil chemical and microbiological properties, and the latter was especially sensitive. Lupwayi et al. (1998) demonstrated that protective cultivation and crop rotation could preserve soil microbial functional diversity. Ge et al. (2008) found that long term application of organic manure had a positive influence on the soil microbial community diversity and structure. However, few researchers have studied the effects of the duration of cultivation on the physical, chemical, and biological properties of soil. Xu et al. (2010) showed that there was a positive correlation between the enhancement of soil biological condition and the revegetation time. Xue et al. (2006) showed that the soil net nitrification rate had an increasing tendency in tea orchards from 8 to 50 years, followed by a decreasing tendency from 50 to 90 years. Plassart et al. (2008) reported that the fungal genetic diversity strongly correlated with the ages of grasslands.

The Loess Plateau is a major apple production region in China (963,000 ha) and apple growing has developed into an important industry in this area. However, the effect of the duration of cultivation on the quality of apple orchard soil is poorly understood, especially the effects of age on the soil microbial community and its function. In this study, therefore, we sampled soils from the Loess Plateau and comprehensively analyzed the soil nutrients, enzyme activities, and the microbial community function and structure in apple orchards of four different ages (3, 7, 15, and 23 years). The results were analyzed to determine the changes in soil quality indicators over time, thereby facilitating a comprehensive evaluation of the quality of apple orchard soils of different ages. This study is important because it provides the basis for the appropriate management and fertilization of orchards of different ages, thereby facilitating soil remediation. Our data will also promote the sustainable development of the fruit industry, particularly in the Loess Plateau region of China.

2. Materials and methods

2.1. Study site

This study was conducted in Yan'an Fruit Bureau at Luochuan (35°47′54″N, 109°28′36″E), which is located in the middle of Shaanxi Province, China. Luochuan has a semi-humid, continental climate. The average annual temperature is 9.2 °C, average annual rainfall is 620 mm, and average attitude is 1072 m. Four apple orchards were selected as study sites to investigate the differences in the soil quality of apple orchards with different ages. The apple orchards were constructed on wasteland during 2009, 2005, 1997, and 1989, so they were 3, 7, 15, and 23 years old, respectively, when the soil samples were collected. A neighboring wasteland covered with sparse grasses was also used as the control. All of the apple orchards received the same fertilization regime, with an average of *c.* 4000 kg organic fertilizer and 400 kg N ha^{-1} $year^{-1}$. Clean tillage was used in all orchards.

2.2. Soil sampling

Soil samples were collected from apple orchards aged 3, 7, 15, and 23 years during June, 2012. A neighboring wasteland soil was also sampled as a reference soil. Five trees were randomly chosen in each orchard, and soils from the depth of 0–20 cm underground at a distance of 1.0 m from each tree in four different positions were collected to make the subsamples. Then the five subsamples were mixed together to constitute the sample. The samples were transported in a car refrigerator to the lab, and divided into three parts. The first part was air-dried to determine the chemical properties. The second part was stored at +4 °C before the analysis of the CLPP (on the next day) and soil enzymes (within four days). The third portion was stored at –20 °C for the T-RFLP analysis.

2.3. Determination of the soil chemical properties

Total nitrogen (TN) was estimated as described by Kjeldahl (1883), and available phosphorus (P) was estimated according to Homer and Pratt (1961). Organic matter (OM) was assayed by the dichromate oxidation method (Lu, 2000). Mineral N was extracted with 2 M KCl, and assayed using a segmented flow analyzer (Skalar). The available potassium (K) was extracted using 1 N ammonium acetate and analyzed by flame photometry.

The soil urease activity was determined using the colorimetric method described by Kandeler and Gerber (1988). The invertase activity was determined using the method described by Guan et al. (1986) with a modification. The soil catalase activity was determined using the potassium permanganate titration method (Guan et al., 1986). The soil alkaline phosphatase activity was determined as described by Tabatabai and Bremner (1969).

2.4. CLPP

The catabolic diversities of the microbial communities were estimated by Biolog ECO-microplates (Biolog Co., Hayward, CA, USA), which comprised three replications containing 31 carbon (C) sources and a control well without a C source. Three replicates of fresh soil samples weighing 5 g (dry weight) were each dissolved in 45 mL of sterile 0.85% NaCl and shaken for 30 min at 200 rpm. The suspension was stepwise diluted to 10^{-3} using sterile 0.85% NaCl. Then 150 μL diluted suspensions were inoculated to each well of the ECO-microplate and incubated at 28 °C for 240 h. The C sources utilization was measured with a Biolog Microstation™ (BIO-TEK Instruments Inc., Winooski, VT, USA) at 590 nm every 24 h.

2.5. T-RFLP profiles

DNA extraction was performed using an EZNA™ Soil DNA kit (Omega Bio-tek Inc., USA) following the manufacturer instruction. PCR amplification was carried out with the primer 5′-6-fluorescein-phosphoramidite-labeled 8f (AGAGTTTGATCCTGGCTCAG) and 1492r (GGTTACCTTGTTACGACTT) (Tipayno et al., 2012). The 50 μL reaction mixtures contained 1 μL DNA template, 0.5 μL of each 20 pM primer (Takara Bio, China), 25 μL 2× Taq MasterMix (Cwbiotech, China), 23 μL RNA-free water. The PCR was performed with a denaturation at 94 °C for 4 min, followed by 29 cycles of 94 °C for 45 s, 56 °C for45 s, 72 °C for 60 s, and a final extension at 72 °C for 7 min. DNA was amplified using an PTC-200 DNA Engin Cycler (Bio-Rad, USA).The PCR products' quality was confirmed by 1% (w/v) agarose gel electrophoresis.

The PCR products were purified using an EZgene™ Gel/PCR Extraction Kit (Biomiga, USA), and then digested for 12 h with Msp I and Hha I enzymes (Takara Bio, China) as described by the protocols. The digested samples were analyzed by Shanghai GeneCore Biotechnologies Co. Ltd (Shanghai, China) using an ABI 3730 DNA Analyzer.

2.6. Statistical analyses

For Biolog analysis, the average well color development (AWCD) was calculated to reflect the microbial activity: $AWCD = \Sigma(Ci - R)/31$, where Ci is the absorbance of 31 C sources and R is the absorbance of the control well. The Shannon diversity index (H') and the evenness index (E') were calculated using the data at 96 h, and the calculations were performed as described by Liu et al. (2012). The richness index (S) was the number of substrates utilized at 96 h.