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Linking plant ecological stoichiometry with soil nutrient and bacterial communities in apple orchards

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ABSTRACT

Soil microbial community and biodiversity maintains ecosystem stability through a variety of biotic and abiotic processes. However, the relationship among environmental factors, soil bacterial community, soil nutrient content and plant ecological stoichiometry has not been clearly confirmed at a regional scale, especially in orchard ecosystems. Here we collected 36 soil and plant samples from major apple-producing areas in three climate zones (humid, semi-humid and semi-arid) to link plant ecological stoichiometry with soil nutrient and bacterial communities in apple orchards. Soil bacterial diversity and community from next-generation high-throughput sequencing, soil texture and aggregates, soil nutrient content, and plant ecological stoichiometry were determined in this study. Structural equation modelling (SEM) was used to establish the relationships among the driving factors, soil bacterial community, soil nutrient content and plant ecological stoichiometry. The results indicated that soil bacteria diversity increased and community composition varied from humid to semi-arid zones at a regional scale, showing a positive correlation with soil organic matter and soil pH. The increases in the diversity and community composition of soil bacteria could improve soil nutrient availability, which would in turn increase nutrient absorption by leaves and alter the ecological stoichiometry of trees. We demonstrated that the same vegetation showed differences in ecological stoichiometry, mainly because of the key driving factors affecting soil bacterial diversity, and also altered soil nutrient availability and absorption of nutrients by trees. Therefore, the results of this study are of great significance in elucidating the belowground and aboveground feedback in an orchard ecosystem.

1. Introduction

Ecological stoichiometry represents the balance of multiple chemical elements in ecological interactions (Elser et al., 2000) spanning different levels of biological organization, from cellular metabolism to ecosystem structure (Li et al., 2014b). Many studies have examined the changes in stoichiometry caused by environmental factors in forest ecosystems (Han et al., 2005) and grassland ecosystems (Gong et al., 2011). These studies have improved our understanding of the biogeochemistry of carbon and other key nutrients in natural ecosystems. However, few studies have been conducted on the stoichiometry of agro-ecosystems, specifically, orchard ecosystems (Han et al., 2005; Gong et al., 2011). An extensive understanding of ecological stoichiometry in orchards is important for nutrient management of orchard trees (Buckley and Schmidt, 2003; Burns et al., 2016), which is conducive to ecological stability and fruit yields.

Ecological stoichiometry is particularly useful for establishing links between different ecosystem compartments (Elser et al., 2007; Yu et al., 2010), which are primarily divided into belowground and aboveground components (De Deyn and Van der Putten, 2005). Belowground soil microbial diversity and community composition are essential components that contribute to soil health (Brussaard et al., 2007). The soil microbial community, including the bacterial community (Bell et al., 2005; Fitter et al., 2005), plays an important role in many soil processes such as nutrient cycling (Mau et al., 2015), pest and disease suppression (Zhang et al., 2017), and the mitigation of adverse situations. It is widely recognized that beneficial bacteria in agricultural systems is important in nitrogen fixation and nutrient mineralization (Revillini et al., 2016), which promote the nutrient availability and thereby increase the uptake of plants (Weidner et al., 2015). However, excessive nutrients can reduce bacterial diversity (Farrer and Suding, 2016; Shen et al., 2010; Zhong and Cai, 2007) as well as the abundance of

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beneficial bacteria that contribute to agricultural production (Buckley and Schmidt, 2003; Singh et al., 2011). An important feedback mechanism exists between microorganisms and nutrients in orchard ecosystems, thus, it is necessary to further study the relationship among microbial function, nutrient availability and nutrient uptake of trees.

At the regional scale, stoichiometry follows a gradient that is related to environmental factors such as climate (Elser et al., 2010) and soil (Fan et al., 2015). The understanding of the impacts of these factors has been increasing based on recent studies, which have shown that leaf N and P stoichiometry exhibit characteristic patterns in different regions. Leaf N and P stoichiometry are correlated with mean annual precipitation (MAP) in tropical regions (Santiago et al., 2004), but these patterns can vary between regions, such as the negative correlation observed with MAP in the US (Sandel et al., 2010) and the positive correlation with mean annual temperature (MAT) observed in China (Han et al., 2005). However, at the global scale, leaf stoichiometry is inversely correlated with MAT (Reich and Oleksyn, 2004) and positively correlated with increases in MAP (Yuan and Chen, 2009). Therefore, climatic factors must be considered in orchard ecosystems. On the other hand, a change in soil properties can result in changes in the bacterial community and its functions (Bell et al., 2005; Zhou et al., 2012). Therefore, soil microbial diversity and community composition differ at the regional scale because of changes in climate and soil hydrological conditions (Bardgett and van der Putten, 2014). However, the factors responsible for the variations in bacterial diversity require further study. The factors driving changes in the microbial community might be an important contributor to changes in plant stoichiometry at a regional scale, such as alterations in soil nutrient availability and nutrient uptake, although few studies have addressed this topic. Therefore, it is necessary to elucidate the relationship among soil microbial diversity, soil nutrient availability, and plant stoichiometry, which is of great significance for sustainable development of farmland.

Apples (*Malus pumila*) are widely planted in China, which is the world's largest producer of apples (Wang et al., 2016). The main apple production areas are distributed from the coastal to inland areas and across 3 precipitation lines and 3 major climatic zones, providing an advantage for the illumination of the relationship among soil microbial diversity, soil nutrient availability, and plant stoichiometry. Therefore, three typical production areas were chosen to represent different regional climate characteristics in this study, and the apple orchards in the different regions represented the same land-use type.

On the other hand, compared with traditional sequencing technology, the High-throughput sequencing approach allowed us to obtain the microbial diversity (Shannon index and Chao1 index) and community composition, including the abundance of major microbial communities (Hartmann et al., 2015). Therefore, the High-throughput sequencing analysis of 16S rRNA gene amplicons was used to obtain the bacteria diversity and community composition. And, the structural equation modelling (SEM) was used to reveal the correlation among soil bacteria diversity, soil nutrient availability, and plant stoichiometry. The results of this work are expected to contribute to an understanding of tree ecological stoichiometry and its driving factors and to maintaining the sustainable productivity of orchards.

2. Materials and methods

2.1. Study sites and sample collection

In the current study, three regions (Qixia, Quzhou and Luochuan) spanning three climate zones (humid, semi-humid and semi-arid, respectively) from the coastal to inland regions of mainland China were selected for investigation. Qixia represented the humid zone, Quzhou represented the semi-humid zone, and Luochuan represented the semi-arid zone. The MAP and MAT are shown in Fig. S1. The average annual temperature is 12.8 °C in Qixia, 14.7 °C in Quzhou and 10.5 °C in Luochuan (Fig. S1a), and the average annual precipitation is 691.6 mm in Qixia, 580.5 mm in Quzhou and 601.2 mm in Luochuan (Fig. S1b). The variety of apple is “Fuji”, which is the major variety planting in China. The age of apple trees is between 10–20 years, which is within the full fruit period. The apple trees were planted with a density of 400–500 plants per hectare.

There were 12 replicate soil samples from each region. The soil classification and location of the sampling sites in three regions were showed in Table S1. To eliminate disturbance, the sampling sites were located at least 10 meters apart. Five soil cores from surface soil (0–20 cm) collected within each sampling site were combined to a composite soil sample and sealed in a polythene wrapper, stored in an ice box and transported to the laboratory within 10 h. Soil samples were collected from each region (Fig. S2), on July 25th in Quzhou, July 30th in Luochuan and August 4th in Qixia in 2016. Each replicate soil sample was divided into two subsamples following the removal of any visible living plant materials, such as roots or leaves, in the laboratory. One subsample was stored at −80°C for microbial community analysis, and one sample was air-dried at room temperature for soil physicochemical property analysis. In addition, triplicate soil samples were collected from the surface within each sampling site using a ring cutter ($V = 100\ cm^3$) and subsequently combined in a single wrapper to determine soil bulk density (SBD). The same samples were placed in an aluminium box (diameter of 10 cm) to characterize the soil aggregates.

Additionally, plant samples (new roots and new leaves) were collected at the soil sampling sites. The new roots of a single apple tree were collected from four pits located approximately 1.5 m from the tree trunk in different directions, and samples from four trees of each sampling site were composited into one net. Similarly, the new leaves of one apple tree were collected from the middle-high portion of trees located in four different directions, and the samples from the four trees were combined into a new net for that orchard. After cleaning them with water, the plant (root and leaf) samples were oven-dried at 105 °C for 30 min and at 60 °C for 48 h. The dried samples were pulverized and sieved before analysis.

2.2. Soil and plant analysis

Soil available phosphorus (SAP) was extracted with 0.5 mol L^{-1} $NaHCO_3$ and measured via the molybdenum blue method using a spectrophotometer. Soil pH was determined at a soil-to-water ratio of 1:2.5 using a soil pH instrument after shaking the soil water for 30 min. Soil organic matter (SOM) was measured with 0.25 mm of sieved soil using the potassium dichromate-volumetric method, and soil total N (STN) and soil total C (STC) were analysed using an elemental analyser (EA1108, Carlo Erba, Turin, Italy). Soil texture (clay) was measured via the hydrometer method, and SBD was calculated from the difference in the gravimetric weight of the soil cores before and after oven drying at 105 °C for 12 h based on the individual core volume.

Soil aggregates were measured via the wet-sieving method, following the method of Wilson and Li (Li et al., 2015; Wilson et al., 2009). Four aggregate size classes were separated from each sample (> 2000 μm, 250–2000 μm, 53–250 μm and 20–53 μm in diameter). The 20–53 and 53–250 μm size fractions were defined as micro-aggregates, and the 250–2000 and > 2000 μm size fractions were defined as macro-aggregates (Wilson et al., 2009).

Plant (root and leaf) C and N were analysed using an elemental analyser. Plant (leaf and root) phosphorus content was measured using inductively coupled plasma-atomic emission spectrometry (ICP) after digestion with concentrated nitric acid.

2.3. High-throughput sequencing analysis of 16S rRNA gene amplicons

Microbial community DNA was extracted from 5 g of well-mixed soil from each sample using the PowerSoil™ DNA Isolation Kit and then frozen and stored at −80 °C. All the DNA extracted from the soil were checked for quality using agarose gel electrophoresis and a NanoDrop