Agriculture, Ecosystems and Environment 268 (2018) 124–132

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Effects of organic substitution for synthetic N fertilizer on soil bacterial diversity and community composition: A 10-year field trial in a tea plantation

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ARTICLE INFO

Keywords:
Long-term fertilization
Tea plantation soil
Organic substitution ratio
Bacterial diversity
Community composition

ABSTRACT

Substitution of chemical nitrogen (N) with organic fertilizers in agricultural ecosystems has been promoted to sustain crop yield and soil quality. Soil microbes play key roles in soil nutrient cycling after organic matter addition. However, there is limited information about the effect of the organic substitution ratio (OSR) on soil bacterial communities, which are considered as a good indicator of soil quality in a tea plantation. In this study, a long-term field experiment with six treatments was established to study the effect of different OSRs of N, from pure synthetic fertilization (NPK) to 100% N substituted with organic fertilizer (OM100), on tea yield and soil bacterial communities. The soil bacterial community composition was measured using a high-throughput sequencing technique. The results showed that as the OSR increased, the soil bacterial diversity increased and the community structure shifted significantly. However, 25% N substituted with organic fertilizer (OM25) produced the highest yield. Additionally, the soil pH and organic carbon (SOC) were the predominant soil characteristics that accounted for the soil bacterial community structural change. With more chemical N being substituted with organic fertilizer, the soil pH, available potassium, SOC, total N, and microbial biomass C and N, were elevated; however, the yield of fresh tea leaves decreased. These results indicated the trade-off effect between tea yield and soil bacterial diversity under different OSRs, which could also alter the soil bacterial communities by changing soil characteristics.

1. Introduction

Tea (*Camellia sinensis*) is an important cash crop in subtropical and tropical areas, which is widely planted in southern China. Unlike cereal crops that are harvested for grain yield, tea plants are perennial and are always plucked for young shoots and leaves. Tea cultivation demands more N for high yield and quality components, e.g. the amino acid content (Kamau et al., 2008; Mudau et al., 2007; Ruan et al., 2010). Therefore, heavy synthetic N fertilization is common in field management; however, this causes environment problems, such as nitrate pollution of the waterbody and soil acidification (Maghanga et al., 2013; Oh et al., 2006; Saraswathy et al., 2007; Yang et al., 2018). Thus, substitution of synthetic N by organic fertilizer has been promoted in tea plantations. Compared with synthetic N fertilization, combined inorganic and organic fertilization results in higher yield, and better soil quality, e.g. better soil fertility, mitigated soil acidification, and better soil physical properties (Bedada et al., 2014; He et al., 2015; Liu et al., 2010; Martínez et al., 2017). Although the advantages of organic fertilization are generally recognized, the optimized substitution ratio remains unclear. It was reported that a higher substitution ratio resulted in higher rice yield in southern China (Bi et al., 2009). However, Huang et al. (2010) observed that 50% organic substitution produced higher corn yield compared with that produced from either pure NPK or 100% organic fertilization in the Northern China Plain.

Soil bacteria play a key role in the function and sustainability of agro-ecosystems by influencing soil nutrient cycling and soil structure (McGuire and Treseder, 2010; Singh et al., 2011; Soman et al., 2016). The structure of the soil bacterial community could be driven by soil

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https://doi.org/10.1016/j.agee.2018.09.008
Received 7 April 2018; Received in revised form 3 September 2018; Accepted 8 September 2018
0167-8809/

management, e.g. N fertilization and tillage (Chávez-Romero et al., 2016). Several studies reported that combined long-term inorganic and organic fertilization significantly increased soil bacterial diversity and changed the soil bacterial community in paddy fields (Ding et al., 2016) and wheat-rice rotation fields (Tian and Niu, 2015). Nevertheless, other reports showed that long-term fertilization slightly increased the soil bacterial abundance but had no significant effects on soil microbial structure in paddy soils (Daquiado et al., 2016; Li et al., 2015).

Response of soil properties, microbial community, and crop yields to various kinds of fertilization regimes have been studied for decades. However, little research has been carried out to investigate the effect of fertilization on soils in perennial crop plantations. Different types of vegetation can affect soil bacteria diversity and richness (Singh et al., 2014). Previous studies found that soil fertility, stand age, and plantation density had a strong impact on the soil microbial community structure, biomass, and its function (Han et al., 2007; Sun and Liu, 2004). However, there is a need to investigate these changes using longer-term field experiments to account for the instability of soil microbes over shorter periods. Furthermore, the effect of the organic substitution ratio (OSR) on soil microbial diversity and community structure is also unclear.

The objective of the present study was to test the hypothesis that different OSRs could affect the soil bacterial diversity, community composition, and fresh tea leaf yield. Therefore, a long-term field experiment on tea plantation with different OSRs was conducted in a typical tea plantation region of China to investigate (1) whether and how different OSRs can affect soil properties and shift the soil bacterial community structure; (2) which soil properties contributed to the change in soil bacterial diversity and community composition; and (3) what is the effect on fresh tea leaf yield of different OSRs?

2. Material and methods

2.1. Field site and experiment design

The field experiment was initiated in 2007 at Longhu mountain tea garden, Fujian province, south-east China (119°34′ E, 27°14′ N), which is affiliated with the Tea Research Institute of Fujian Academy of Agricultural Sciences (TRI-FAAS). The altitude of this field was 163 m. This region could be characterized as a warm temperature, subtropical monsoon climate. The annual mean temperature and precipitation are 19.3 °C and 1646 mm, respectively. The soil type is a granite weathered red soil. At the beginning of the experiment, the soil properties were pH 4.15, total carbon (soil organic carbon (SOC)) 1.41%, total nitrogen (TN) 0.10%, available phosphorus (AP) 18.43 mg kg^{-1}, and available potassium (AK) 132.10 mg kg^{-1}.

The field experiment included six treatments. Except for the control treatment (CK, without N addition), the rest of the treatments were applied as follows: pure synthetic fertilization (NPK, without any organic fertilization), 25% N substituted with organic fertilizer (OM25), 50% N substituted with organic fertilizer (OM50), 75% N substituted with organic fertilizer (OM75), and 100% N substituted with organic fertilizer addition (OM100). CK also received the same amount of P and K as the NPK treatment.

All treatments were replicated three times. Each plot was 30 m^2 and arranged randomly. The tea trees were planted in the winter of 2006 with a row spacing of 1.5 m and plant spacing of 0.3 m (~40,000 trees ha^{-1}), using the tea tree variety “purple peony”. The N, P, and K application rates were 300 kg N ha^{-1}, 32.75 kg P ha^{-1}, and 62.23 kg K ha^{-1}, respectively. Urea, calcium superphosphate, and potassium sulfate were applied as synthetic N, P, and K fertilizers, respectively. Organic substitution was calculated according to the N application rate. The insufficient P and K in the organic substitution treatments were supplemented with synthetic P and K fertilizers.

Around the tea gardens, there are many housed livestock farms, especially pig farms. The pig-on-litter system (POL) was recommended as an efficient method to treat pig house waste, i.e., feces and urine, in China. Therefore, in this study, the pig manure fermented in the bedding materials, e.g. chaff and straw, was selected as the organic fertilizer because of these local conditions. The applied pig manure was produced by fermentation of pig litter under the POL system with a pH of 8.14. The oven-dried base nutrient contents of nitrogen, phosphorus, and potassium in the organic fertilizer are shown in Table 1. The ammonium and nitrate contents in the pig manure were 2.5 and 1.3 g kg^{-1}, respectively.

Table 1
Nutrient and heavy metal element contents (oven-dried) of the applied organic fertilizer.

Element	Content
Nutrient (g kg^{-1})	
C	399 (22)
N	13.3 (0.64)
P	2.64 (0.06)
K	4.19 (0.18)
Heavy metal (mg kg^{-1})	
As	8.86 (2.73)
Cd	1.03 (0.21)
Cr	4.15 (0.67)
Cu	777.93 (21.71)
Pb	5.23 (0.42)

Values are presented as mean with standard error (in brackets).

In each treatment, fertilizers were applied three times annually. Total organic fertilizer and calcium superphosphate were applied as base fertilizers in late November simultaneously because of their weak mobility and slow soil release characteristics. To reduce the nutrition loss and increase the use efficiency, the synthetic N fertilizer and potassium sulfate were split into three applications, namely 30% in early March, 30% in the middle of August, and 40% in late November, respectively, because they are easily lost through leaching in the studied subtropical regions. All fertilizers were applied in furrows that were ~10 cm wide and ~10 cm deep to reduce the nutrient loss and increase use efficiency, following the recommendation of good practice in China.

2.2. Soil sampling and preparation

Soil samples were collected in late February of 2017 before the spring fertilization. Ten soil cores (2 cm in diameter) about 10 cm away from the fertilization ditch were collected in each plot to a depth of 10 cm. Soils from the same plot were mixed as one sample. The collected fresh soil samples were divided into two parts. One part was air-dried and the other part was sieved through a 2-mm mesh, and also divided into two parts. One part was stored at −80 °C for DNA extraction, and the other part was immediately analyzed for mineral nitrogen (NH_4^+-N and NO_3^--N) and microbial biomass (microbial biomass C and N (MBC and MBN)).

2.3. Soil and organic fertilizer analysis

Soil pH was measured in a 1:2.5 soil-water solution using a pH meter (Orion 3 Star, Thermo Ltd., USA). SOC and TN were measured using a C/N elemental analyzer (Vario Max, Elementar, Germany). Soil AP and AK were extracted using the Mehlich 3 method (Mehlich, 2008), and then measured using Inductive Coupled Plasma (Thermo Jarrell Ash Ltd., Franklin, MA, USA).

Soil mineral N was extracted using 2 moL L^{-1} of KCl and analyzed using a Flow Injection Analyzer (SAN++, SKALAR Ltd., Breda, Netherlands). For the MBC and MBN, the samples were fumigated with chloroform (ethyl alcohol removed) in an airtight and dark vessel, extracted with 0.5 moL L^{-1} K_2SO_4, and then measured using a Total Organic Carbon (TOC) analyzer (Multi N/C 2100/1, Analytic JENA ag.,