European Journal of Soil Biology 86 (2018) 34–41

Contents lists available at ScienceDirect

European Journal of Soil Biology

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

Changes in the soil bacterial community structure and enzyme activities after intercrop mulch with cover crop for eight years in an orchard

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ARTICLE INFO

Handling editor: Christoph Tebbe

Keywords:
Clostridiales
Firmicutes
Grass cover
Mulch
Soil enzyme
Soil organic matter

ABSTRACT

Bacteria are major decomposers of plant residues in soil and play important roles in nutrient cycling. However, in apple orchards, little is known about the responses of soil bacterial communities to intercrop mulch of the cover crop. Thus, two treatments were tested in this study: 1) conventional approach (CT), where no cover crop was planted and natural weeds were controlled every month by a farmer; 2) intercrop-mulch of the cover crop (GC), where the cover crop grew from late March to early July and it was mowed in early July, August, and September, and residues were left on the soil surface as mulch. Intercrop mulch of the cover crop enhanced the total nitrogen (TN), soil organic carbon (SOC), and soil water content (SWC) by approximately 19%, 15%, and 28%, respectively, compared with CT. The activities of β-glucosidase (BG), β-xylosidase (BXYL), and cellobiohydrolase (CBH) were also increased by the intercrop-mulch of the cover crop, where the activities after the GC treatment were 12.3%, 22.0%, and 14.7% higher than CK, respectively. The relative abundances of the phylum *Firmicutes*, class *Clostridia*, order *Clostridiales*, and families *Ruminococcaceae* and *Lachnospiraceae* were enhanced greatly by GC. The relative abundances of some genera (relative abundance > 0.05%) in the families *Ruminococcaceae* and *Lachnospiraceae* differed significantly under GC and CT (GC > CT, $p < 0.05$). Some of these genera had positive correlation ($p < 0.05$) with TN, SOC, BG, BXYL, and CBH. However, PICRUSt and Tax4Fun analyses indicated that the relative abundances of potential genes encoding BG and BXYL were not increased by GC, and the relative abundance of the potential gene encoding CBH was higher in GC according to PICRUSt analysis. We conclude that intercrop mulch with the cover crop can increase the BG, BXYL, CBH activities and the relative abundances of some bacteria which were related to plant biomass degradation.

1. Introduction

The use of cover crops is a widely applied soil improvement and conservation technique in agriculture e.g. in rotation with intercropping systems [1,2], and many benefits of cover crops have been reported, including modification of the temperature [3], control of weeds [4], decreased runoff and soil erosion [5], reduced nitrogen leaching [6], improved soil phosphorus (P) retention and cycling [7], increased soil organic carbon (SOC) stock [8], and maintenance of soil aggregate stability [9].

In an intercropping system, cover crops replace the bare inter-row soil and are ploughed under as green manure after harvest of the main crops [10,11] or mowed to obtain residues, which are left on the ground as mulch materials [12–15]. Generally, this will impact the soil microbial community and enzyme activities by changing soil characteristics (e.g., pH, temperature, soil water content) [16–18]. Moreover, cover crops offer more organic substrates for microbial growth by input of plant residues and rhizodeposition (root material and exudation) [19–21].

Soil microorganisms are significant for the sustainability of an agricultural production system and are linked with the cycling and stability of soil organic matter. They are also related to soil health and are sensitive to changes in soil attributes and management [22–24]. In the soil ecosystem, soil bacteria and fungi are crucial decomposers that metabolize and turn over organic matter by secreting specific extracellular enzymes (e.g., cellulase, xylanase, phosphatase) to break down

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https://doi.org/10.1016/j.ejsobi.2018.01.009
Received 11 June 2017; Received in revised form 31 January 2018; Accepted 31 January 2018

large organic molecules into monomers, which are then available for plant uptake. Thus, studying the soil microbial community and its response to the application of organic materials should increase our understanding of the turnover and utilization of substances and organic matter in soil.

In recent years, several studies have focused on the effects of cover crops on soil microorganisms in the intercropped orchard. Intercropping grass with Chinese hickory (*Carya cathayensis* Sarg.) improved the microbial community functional diversity [25]. Cover crops can promote the bacterial diversity and species richness in subtropical orchard soils [16]. The application of cover crops was beneficial for the development of soil enzyme activities and microbial diversity in apple orchards [26].

With the development of fast and efficient DNA sequencing technologies and the improvement of databases for sequence deposition and phylogenetic analyses, taxonomic assignment of individual microbial taxa from environmental samples has now become possible and attractive for ecological studies [27]. It has been shown that *Acidobacteria* and *Cyanobacteria* are related to the high carbon and nitrogen contents present in soils following cover crop treatments [1]. Furthermore, some plant-growth-promoting rhizobacteria *Acinetobacter* and *Pseudomonas* may accumulate in the rice rhizosphere when rotated with cover crops [28]. These researches, however, mainly focused on the rotation system, in which the cover crops acted as a “green manure”, and little research has focused on the effect of cover crops on the bacterial community structure in the intercropping system, especially in orchards treated with intercrop mulch.

The objective of this study was to gain a more comprehensive understanding of the effects of intercrop mulch of cover crop on soil bacterial communities using high-throughput sequence for studying the changes of the soil bacterial community structure due to the application of intercrop mulch of cover crop in an apple orchard. Soil characteristics and soil enzyme activities were also analyzed as indicators for functional changes of the soil microbial communities. We hypothesized that intercrop mulch with cover crop would: 1) increase the soil enzyme activities which contribute to plant biomass breakdown; and 2) enhance the abundances of some soil bacteria related to plant biomass degradation due to the input of plant residues.

2. Materials and methods

2.1. Site description and experimental design

The experiment was conducted at the Weibei Dryland Experimental Station at Northwest A&F University (109°56′E, 35°21′N; altitude of 838 m), Baishui County, Shaanxi Province, China. The soil of the apple orchard was silt loam (with 8% sand, 67% silt and 25% clay) and classified as Haplustalfs (USDA textural classification system). The chemical characteristics of topsoil (0–20 cm) before the experiment (2008) were as follows: total nitrogen 1.03 g kg^{-1}, organic matter content 13.02 g kg^{-1}, nitrate nitrogen 22.40 mg kg^{-1}, ammonium nitrogen 2.5 mg kg^{-1}, available phosphorus 15.94 mg kg^{-1}, pH 8.3. The rainfall distribution is dominated by a monsoon climate. In this region, the summer is hot and moist (daily maximum temperatures can reach 39.4 °C in July) whereas the winter and early spring are always cold and dry (daily minimum temperatures can reach −16.7 °C in January). The average rainfall at the experimental site was 570 mm, where 60% occurred in the summer (July–September). On average, the total annual radiation at this site is 5360 MJ m^{-2} and the number of sun hours is 2477 h. There are usually 171 frost-free days each year. Agriculture in this region is completely dependent on natural precipitation.

The experiment was established in a Fuji apple (*Malus pumila* Mil.) orchard in 2008. Apple trees were planted in 2005 on M.26 (rootstock) with a density of 1200 plants per hectare. Two treatments were included: conventional approach, CT; and intercrop of cover crop treatment, GC. Each treatment was replicated three times. Each replicate contained two rows, with 12 apple trees in each row. The surface area of each replicate was approximately 200 m^2. For CT, no cover crop was planted and natural weeds were controlled monthly by farmers (strimmer combined with hoeing and manual weed control). The residues were removed to leave bare soil during the experiment. For GC, crown vetch (*Coronilla varia* L.) was sown in each inter-row of apple trees at a depth of 1.5 cm and a sowing rate of 6.0 kg per hectare of orchard. Crown vetch sprouted in late March each year and was mowed in early July, August and September, and the residues were left on the soil surface as mulch. Goat manure (35.1% dry organic matter, 0.53% N, 0.31% P_2O_5 and 0.47% K_2O) was applied at a rate of 36 t ha^{-1} in each treatment.

2.2. Soil sampling and characteristic analyses

2.2.1. Soil sampling

In mid-September 2016, approximately ten days after the last mulching, six 20-cm-deep soil cores were randomly collected using an open-faced bucket probe (5 cm diameter) in each of the three replicate plots. The six soil cores were well mixed and combined into a single sample. Each soil sample (six soil samples in total = two treatments x three replicates) was passed through a 2-mm sieve and divided into four parts: the first was dried in an oven (105 °C) to measure the soil water content (SWC), the second was used for soil chemical analysis, the third was stored at 4 °C to await soil enzyme activities analysis and the fourth was stored at −80 °C until DNA extraction.

2.2.2. Soil characteristics analyses

Nitrate (NO_3^--N) and ammonium (NH_4^+-N) nitrogen were extracted at a ratio of 5 g fresh soil to 50 mL 1 M KCl. After shaking for 1 h, the extracts were filtered and analyzed by a continuous flow analytical system (AA3, SEAL Company, Germany). The soil organic carbon (SOC) was determined using the K_2CrO_7-H_2SO_4 oxidation method. The soil total nitrogen (TN) was measured by acid digestion according to the Kjeldahl method. The soil available P (AP) was extracted with 0.5 M $NaHCO_3$ (pH 8.5) at a soil-to-solution ratio of 1:20 (weight/volume) for 30 min and measured using a colorimetric procedure. Soil pH was assayed with a pH meter (Mettler-Toledo FE 20; Switzerland) at a soil-to-water ratio of 2:5 (weight/volume). The soil enzyme activities (Table 1) were determined using a microplate fluorimetry method [29]. Soil suspensions were prepared (1.0 g dry mass of fresh soil with 125 mL deionized water as the buffer) and 200 mL of each soil suspension was then pipetted into a 96-well black plate. The assay well contained 200 mL of the sample suspension and 50 mL of the substrate solution. The negative control well contained 200 mL of the buffer and 50 mL of the substrate. The sample control well contained 200 mL of the sample suspension and 50 mL of the buffer. The reference standard well contained 200 mL of the buffer and 50 mL of the standard (10 mM 4-MUB). The quench control well contained 200 mL of the sample suspension

Table 1
Enzymes and corresponding substrates, abbreviations, and enzyme commission number.

Enzyme	Substrate	Abbreviation	EC
β-glucosidase	4-MUB-β-D-glucopyranoside	BG	3.2.1.21
β-xylosidase	4-MUB-β-D-xylopyranoside	BXYL	3.2.1.37
Cellobiohydrolase	4-MUB-β-D-cellobioside	CBH	3.2.1.91
β-*N*-acetylglucosaminidase	4-MUB-*N*-acetyl-β-D-glucosaminide	NAG	3.2.1.52
Phosphatase	4-MUB-phosphate	PHOS	3.1.3.1

EC, Enzyme Commission number describing enzymatic function at increasing level of detail (the first number distinguishes 1-oxireductases, 2-transferases, 3-hydrolases, 4-lyases, 5-isomerases, and 6-ligases).
4-MUB is 4-methylumbelliferyl.