



# Long-term effects of legume mulching on soil chemical properties and bacterial community composition and structure



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## ARTICLE INFO

### Keywords:

Legume mulching  
*Chamaecrista rotundifolia*  
*Arachis pintoii*  
 Persimmon  
 Bacteria community composition  
 Soil properties

## ABSTRACT

Applying legumes as green manure has been reported to improve soil fertility and alter soil bacterial community structure. Few studies have been conducted to determine the effects of *Chamaecrista rotundifolia* and *Arachis pintoii* mulching on soil bacterial communities in orchards. In this study, we described the bacterial community in the 0–20 cm soil profile under long-term (approximate 20 years) *C. rotundifolia* and *A. pintoii* mulching of persimmon orchards in subtropical and tropical China. The experiment included three treatments: (1) terraced persimmon orchard with *A. pintoii* mulching (TPA), (2) terraced persimmon orchard with *C. rotundifolia* mulching (TPC), and (3) terraced persimmon orchard with no vegetation mulching (TPN, CK). Soil chemical properties were significantly altered by long-term *A. pintoii* and *C. rotundifolia* mulching. Concentrations of total phosphorus (TP), available nitrogen (AN), soluble organic carbon (SOC), total soluble carbon (TSC), and organic matter (OM) were significantly higher in TPA and TPC soils than in TPN soils; TN and  $\text{NH}_4^+$ -N were highest in TPA soils and  $\text{NO}_3^-$ -N was highest in TPC soils. Soluble organic nitrogen (SON) and total soluble nitrogen (TSN) concentrations were significantly lower in mulched soils than in TPN soil. Illumina-based sequencing showed that soil bacterial community composition and structure were significantly altered by *C. rotundifolia* and *A. pintoii* mulching (TPC and TPA), and the changes in the dominant soil bacterial communities were demonstrated as reductions in the Simpson and Shannon indices. *Proteobacteria* (relative abundance 37.55%–50.94%) and *Acidobacteria* (relative abundance 9.47%–12.02%) were enriched in TPA and TPC soils, while *Firmicutes* (relative abundance 15.74%–22.00%) were higher in TPN soil. Further taxonomic analyses of *Proteobacteria* revealed that the relative abundance of *Gammaproteobacteria* and *Deltaproteobacteria* in TPA and TPC were significantly higher than in TPN, while the *Betaproteobacteria* in TPA and TPC were significantly lower than in TPN. Genus *Phenylobacterium* and *Escherichia* were enriched in TPC, *Rhodoplanes* and *Escherichia* were enriched in TPA, while, *Kaistobacter*, *Sphingomonas*, *Burkholderia*, and *Enterobacter* were enriched in TPN. Family *Koribacteraceae* classified as *Acidobacteria* was significantly higher in TPA than in TPN and TPC, and the genus *Candidatus Koribacter* of *Acidobacteria* was significantly higher in TPA and TPC than in TPN. In-depth taxonomic analyses of *Firmicutes* showed the relative abundance of family *Bacillaceae*, and *Streptococcaceae* and genus *Bacillus* and *Lactococcus* were lower in TPA and TPC than in TPN, while those of family *Leuconostocaceae* and *Lactobacillaceae* and genus *Pediococcus* were higher in TPA and TPC than in TPN. The genus *Weissella* was only observed in TPC and TPA. Our results indicate that long-term *C. rotundifolia* and *A. pintoii* mulching significantly influences the soil chemical properties and bacterial communities of persimmon orchards in subtropical and tropical China.

## 1. Introduction

Cover plant intercropping in orchards is a common soil management practice, wherein legumes are planted between rows or throughout the orchard (Hartwig and Ammon, 2002). In recent decades, many studies have demonstrated the advantages of intercropping forage legumes in

orchards, including reduced soil and water losses (Prosdocimi et al., 2016; Zhu and Zhu, 2012), improved fruit yields (Srivastava et al., 2007), greater soil stability and nutrition content (Bénédicte et al., 2016; Cui et al., 2015; Qian et al., 2015; Ramos et al., 2011; Reeve et al., 2017), as well as higher soil organic carbon (SOC) stocks and soil organic matter (SOM) content (Bénédicte et al., 2016; Breulmann et al.,

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<https://doi.org/10.1016/j.agee.2018.09.001>

Received 24 November 2017; Received in revised form 23 August 2018; Accepted 1 September 2018

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2012; Cui et al., 2015; Liu et al., 2013). Ramos et al. (2011) reported that legume cover in orchards improved the wet aggregate stability of the soil and enhanced its chemical and biological properties, including SOC, total nitrogen (TN), available potassium, and enzyme activities. Similar results were also reported by Qian et al. (2015), who showed that leguminous mulching increased total organic carbon (TOC), TN, and invertase, urease, and alkaline phosphatase activity levels. Although Ângelo Rodrigues et al. (2013) reported that soil nitrogen was affected by *Lupinus albus* L. and *Vicia villosa* Rothl cover, the effect was slight and short-lived (Ângelo Rodrigues et al., 2013). Moreover, many studies have confirmed that various types of living mulch perform differently in different soil systems (Haney et al., 2010; Qian et al., 2015; Wu et al., 2011). Therefore, more research is needed to identify the benefits of mulching for orchards with different types of soil systems.

Soil bacteria—regarded as important indicators of soil quality and health—participate in many stages of nutrient cycling, such as the fixation of nitrogen and the decomposition of dead organisms (Anderson, 2003; Burns et al., 2013; Forbes, 2008; Pignataro et al., 2012). Many studies have focused on legume/cereal intercropping systems, which have been shown to be beneficial for bacteria, fungi, and innumerable other organisms, and are capable of affecting the structure of bacterial communities, thus increasing bacterial diversity (Alvey et al., 2003; Duchene et al., 2017; Toda and Uchida, 2017). However, few studies have focused on legume/fruit systems. For instance, Qian et al. (2015) reported that living mulches consisting of *Coronilla varia* L., *Trifolium repens* L., and *Lolium perenne* L. in apple orchards significantly improved soil bacterial activity, increased community diversity, and changed the structure and function of the soil bacterial community (Qian et al., 2015). In guava (*Psidium guajava* Linn.) orchards located on subtropical red soils in South China, the bacterial diversity and/or species richness of the soil was promoted by *Stylosanthes guianensis* mulches (Cui et al., 2015). The plant species used are important factors affecting soil microbial community structure (Breulmann et al., 2012). Therefore, the various types of living mulch have different influences on bacterial communities (Breulmann et al., 2012; Haney et al., 2010; Qian et al., 2015).

Red soils, classified as Ultisols according to the U.S Soil Taxonomy System, span an area of approximately 1.14 million km<sup>2</sup> in subtropical and tropical China, accounting for nearly 12% of the total national area (Cui et al., 2015; Shi et al., 2010). The orchards built in these regions are severely affected by water and soil erosion because of the specific natural conditions (high temperature, runoff caused by intense rainstorms, hilly with slopes > 15°) in these locations and intensive tillage (Wilson et al., 2004). In recent decades, living mulches have been used to reduce soil erosion in these orchards. The legumes *Chamaecrista rotundifolia* and *Arachis pintoi*, which were introduced to China from Australia in 1996, are commonly intercropped in subtropical or tropical orchards to reduce soil erosion. Moreover, many studies of *C.*

*rotundifolia* and *A. pintoi* intercropping systems have been carried out, including studies of soil properties and soil ecological benefits (Duchene et al., 2017; Huang et al. 2004; Weng et al., 2004). However, the effects of *C. rotundifolia* and *A. pintoi* mulches on soil bacterial communities are not well documented.

We hypothesize that the bacterial community composition and structure of orchard soils vary under long-term *C. rotundifolia* and *A. pintoi* mulching conditions and may contain unique bacterial communities with different compositions, which have a significant effect on soil properties. Thus, in this study, we examine differences in bacterial community diversity, richness, and composition (using the Illumina-based sequencing method), and differences in soil properties caused by long-term use of *C. rotundifolia* and *A. pintoi* mulch in persimmon orchards. Previous studies on the effects of legume mulches on soil microbes were based on polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) profiles (Cui et al., 2015; Sharma et al., 2005) and restriction fragment length polymorphism (T-RFLP) (Qian et al., 2015). High-throughput sequencing analysis on the Illumina MiSeq platform is an effective technique for studying soil bacterial communities, which can analyze the V3 or V4 conserved regions from microbial 16S rRNA (Magoc and Salzberg, 2011).

## 2. Materials and methods

### 2.1. Site description

The research site was located in Yuchi village, Sanming City, Fujian Province, China (26°25'N, 117°57'E), which is a long-term experimental field station built in 1996. The climate of the study area is typical humid subtropical, with high temperatures and humidity in the summer and cool temperatures in the winter. The mean annual air temperature is 19.2 °C, and the maximum temperature in July ranges from 26.6 °C to 28.9 °C; minimum temperatures in January range from 9.0 °C to 12.0 °C. The annual precipitation fluctuates from 1254.5 mm to 2171.9 mm, with more than 80% falling between February and October. The soil type is clay.

The study area (approximately 1000 m<sup>2</sup>) consisted of 6 plots in a completely randomized design with 3 treatments and 2 replicates. Each experimental plot (4 m wide and 25 m long) was divided into 8 artificial terraces where eight fruit trees were planted and forage legumes (*Chamaecrista rotundifolia* and *Arachis pintoi*) were cultivated around the trees for more than 20 years. Three treatments were set up: terraced persimmon orchard with *A. pintoi* mulching (TPA), terraced persimmon orchard with *C. rotundifolia* mulching (TPC), and terraced persimmon orchard with no vegetation mulching (TPN; control treatment) (Figs. 1 and 2). The management of each treatment was different over the 20-year period:

1) Legume management. *A. pintoi*, which is a perennial legume, was



Fig. 1. Picture of peaches with *C. rotundifolia* and *A. pintoi* mulching.

Note: Peaches were planted in 1996 at a long-term field experimental station and were replaced by persimmons in 2012.

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