ARTICLE IN PRESS

Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

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

Mechanisms by which organic fertilizer and effective microbes mitigate peanut continuous cropping yield constraints in a red soil of south China

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ARTICLE INFO

Keywords:
Red soil
Continuous cropping contraint
Peanut
Chemical and microbial mechanisms
Long-term fertilization

ABSTRACT

Red soils are acid soils which occupy about one fifth of the total area of tropical and subtropical soils in China. Peanut (*Arachis hypogaea* L.) is regarded as the main oil and cash crop in the red soil region. However, yields of peanut have gradually but significantly declined under continuous cropping. We conducted a field experiment in Jiangxi province, south China from 1996 to investigate the mitigation of the peanut continuous cropping constraint. Three types of fertilizer (chemical, organic, and organic fertilizer with effective microbes) were applied in the field. After 20 years of cropping the continuous cropping constraint was found to be more severe where chemical fertilizers were applied and was mitigated where the fields were amended with organic fertilizer or organic fertilizer with effective microbes. Soil properties and the rhizosphere bacterial community structure were determined in 2016. Long-term application (20 years) of organic fertilizer increased the plant available phosphorus in the rhizosphere soil. Phosphorus is known to be one of the most limiting factors for peanut growth in red soils and peanut yield was promoted as the soil phosphorus status increased. Organic fertilizer and effective microbes also optimized the rhizobacteria of peanut to mitigate the continuous cropping obstacle. The bacterial wilt pathogen (*Ralstonia*) decreased in abundance in the rhizosphere and bacterial wilt disease of peanut declined significantly. Moreover, beneficial bacteria in the rhizosphere capable of promoting peanut growth increased in abundance.

1. Introduction

Red soils are distributed widely in tropical and subtropical areas covering 45.2% of the total land area of the Earth (Zhao et al., 2002). They occupy about one fifth of the total tropical and subtropical area of south China and are is widespread in 15 provinces to the south of the Yangtze River, especially in Jiangxi and Yunnan provinces (Zhou et al., 2005, 2014). The acidic nature of red soils leads to deficiencies in numerous essential plant nutrients, especially N, P, K, Ca, Mg, S, Zn, B, and Cu (Wilson et al., 2004; Fan et al., 2009). Peanut (*Arachis hypogaea* L.) is one of the four most important oil crops and is an important economic crop in the hilly red soil region of south China (Li et al., 2012). However, the production of monoculture peanut in China has declined significantly. The production of *Arachis hypogaea* L. cv. Ganhua 5 in Jiangxi province decreased by over 60% compared to the 1989 production year (Teng et al., 2015). Continuous monoculture may explain the continuing declines in yield and quality (Larkin, 2003; Dai et al., 2009). The need for containment of pests and diseases as a consequence of continuous cropping is a worldwide issue and various strategies consisting of chemical, genetic and agricultural management approaches have been devised to increase crop yields and mitigate disease in peanut (Pavlou and Vakalounakis, 2005; Gil et al., 2008; Mbarga et al., 2012; Jordan et al., 2016).

Peanut continuous cropping constraints (often termed ‘continuous cropping obstacle’) are particularly widespread in the hilly red soil region of southeast China due to the increasing popularity of continuous monocropping without any crop rotation (Wang and Chen, 2005). Plant root exudates, rhizosphere microorganisms and the use of identical field management can create a specific soil microenvironment (Demarco et al., 2011). Consequently, as the fields are continuously monocropped, shifts in soil microbial communities may contribute to the observed decline in yield and increased disease incidence (Huang et al., 2013a,b; Li et al., 2012). The nutrient requirements of peanut are higher than the nutrient status of the soil. This nutrient imbalance may

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https://doi.org/10.1016/j.apsoil.2018.03.018
Received 8 December 2017; Received in revised form 16 March 2018; Accepted 30 March 2018

lead to deficiencies of some essential elements in the soil including plant available phosphorus and potassium (Li et al., 2014). As a result, plants cultivated after long-term continuous cropping cannot take up adequate nutrients from the soil, leading to restricted growth and susceptibility to environmental change (Li et al., 2014; Malhi et al., 1998). In addition, root exudates of peanut accumulate in the rhizosphere soil. Some components of the root exudates may be harmful to rhizosphere microorganisms and to the plant itself. The accumulation in the soil of plant allelochemicals including phenolic acids, fatty acids, alcohols, aldehydes, and ketones, may result in phytotoxicity and disruption of key physiological processes in plants (Huang et al., 2013a,b). Long-term fertilization has been found to be an efficient method of counteracting the harmful effects of continuous cropping. Zhong et al. (2010) found that long-term application of organic manure plus balanced fertilization with N, P and K promoted the soil microbial biomass, activity and diversity and thereby enhanced crop growth and production. Another long-term field experiment showed that long-term application of manures effectively prevents red soil acidification and increases crop productivity (Zhai et al., 2011). Xu et al. (2015) used peanut shell biochar to improve peanut kernel quality by influencing soil organic C and nutrient availability on a red ferrosol. However, the role of microbes, which are altered by long-term fertilization in the peanut rhizosphere in the mitigation of peanut continuous cropping constraint, remains unknown.

The rhizosphere harbors a very large number of microorganisms and soil invertebrates and is considered to be one of the most dynamic interfaces on Earth (Philippot et al., 2013). Although microbes comprise a very small proportion of the soil mass they play essential roles in the cycling of elements such as nitrogen, phosphorus, sulfur, and iron in addition to performing other ecosystem functions (Lugtenberg and Kamilova, 2009; Miransari, 2014). Furthermore, plants nurture a vast community of commensal and mutualistic microbes that provide the plant with essential services such as enhanced nutrient uptake, nitrogen fixation, growth promotion, and protection from pathogens (Pieterse et al., 2014). The structure of the microbial community can also be altered by soil properties. Microbes are supposed to be one of the most sensitive biological indicators for monitoring changes in soil quality (Pulleman et al., 2012; Schloter et al., 2003). The deterioration of soil properties, variation in soil pH, and accumulation of root exudates including allelochemicals during long-term monocropping may have a substantial effect on the microbial community in the rhizosphere soil. In previous studies the total amounts of bacteria and actinomycetes declined and fungal abundance (especially pathogenic fungi) was found to increase with continuous monoculture of peanut (Li et al., 2012). Peanut growth and yield were increased by co-inoculation with *Bradyrhizobium* and some root microorganisms (Badawi et al., 2011). Sudini et al. (2011) found that the Proteobacteria, Acidobacteria, Firmicutes, Bacteroidetes, and Actinomycetes were the predominant bacterial phyla found in all peanut-cropping sequences.

The objectives of the current study were to evaluate changes in soil properties and the responses of the soil functional bacteria under the impact of long-term fertilization treatments that included chemical fertilizer, organic fertilizer, and effective microbes. As the patterns of change in functional bacteria were discovered, fertilization strategies were devised to deal with continuous cropping constraints, and finally, the mitigation patterns of peanut continuous cropping contstraints under different fertilization treatments applied for twenty years were investigated.

2. Materials and methods

2.1. Field experiment

The long-term experiment was performed in a peanut (Ganhua 5) field continuously monocropped from 1996 to 2016 at the Red Soil Ecological Experimental Station, Chinese Academy of Sciences, Yingtan, Jiangxi province, south China (28°12′ N, 116°55′ E). The soil type at the experimental site is Ferric Acrisols according to the FAO soil classification system.

The experimental field was uncultivated before 1996 and three different fertilization treatments were applied, namely chemical fertilizer (CF), organic fertilizer (OF) and organic fertilizer mixed with effective microbes (OF_EM). The treatments were arranged in a complete randomized block design in triplicate. Each plot was 33.3 m^2 in area with a concrete wall embedded 20 cm into soil between adjacent plots. Urea, calcium magnesium phosphate and potassium chloride were applied as the sources of N, P and K in the chemical fertilizer plot (CF) and the final concentration of N, P, K were 45, 45, and 135 kg ha^{-1}, respectively. Pig manure purchased from a nearby pig farm after fermentation every year was used as organic fertilizer in the OF plot. The average composition of pig manure was (in g kg^{-1}) OM 602, N 26.7, P 18.3, and K 53.1 with a pH of 8.5 and the dosage was 1.69 t ha^{-1} (dry weight basis). At the same time, 14 kg P ha^{-1} of calcium magnesium phosphate and 45 kg K ha^{-1} as potassium chloride were added to the OF plot as the P and K contents of pig manure were too small to reach the final rates of P and K in the chemical fertilization. Effective microbes were a mixed culture of naturally-occurring, beneficial microorganisms stored in our laboratory (comprising *Rhizobium* isolated from the nodules of peanut, photosynthetic bacteria, *Acetobacter*, *Lactobacillus*, *Bacillus*, and *Actinomycetes*) that has been used with considerable success to improve soil quality and increase the growth and yield of crops. In the organic fertilizer treatment combined with effective microbes, the amount of organic fertilizer with calcium magnesium phosphate and potassium chloride applied were the same as in thein organic fertilizer plot and 20.55 L ha^{-1} effective microbes (1×10^9 cfu mL^{-1}) were also added. All fertilizers were applied before sowing each year.

Peanut (Ganhua 5) was sown by hand on April 10th and harvested in the middle of August each year from 1996 to 2016. The peanut was sown by placing two seeds in each hole. The distance between two holes was 10 cm and the distance between two rows was 30 cm. The three plots received the same field management.

2.2. Soil and plant sampling

An initial soil sample was taken from each plot before the experiment started in 1996. The initial composite soil sample consisting of five cores to 15 cm depth was collected and mixed by sieving. A total of three composite samples were taken from the three treatments for determination of soil properties, Namely pH, OM content, total N, P, and K, hydrolyzable N and available P and K.

Rhizosphere soil samples were taken from each plot after harvesting during the long-term experiment in 2002, 2005, 2008, 2012, 2013, and 2016. One composite rhizosphere soil sample was taken from each plot consisting of roots of 10 randomly selected peanut plants. The roots were shaken vigorously to separate the loosely adhering soil and the soil remaining was collected as a rhizosphere soil sample (Smalla et al., 2001). Three composite samples of each treatment were obtained and a total of nine samples were taken. The soil samples (5 g of each) were stored at −80 °C for DNA analysis. The remaining soil was air-dried and sieved for determination of basic physicochemical properties (Lu, 1999), namely pH, OM content total N, P, and K, hydrolyzable N and available P and K.

When the peanut was harvested in 2016 all the plant biomass in each plot was collected to determine the yields. The plants were air-dried in the field for 4–6 d. The shelled peanuts were obtained and dried at 65 °C to remove natural moisture. The dry weights of the peanut plants and shelled peanuts were determined.

2.3. DNA extraction and PCR amplification

Rhizosphere soil collected in 2016 was used to investigate the