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The combined effects of cover crops and symbiotic microbes on phosphatase gene and organic phosphorus hydrolysis in subtropical orchard soils

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ABSTRACT

P deficiency is a major obstacle for crop production in subtropical red soils in South China, and the hydrolysis of organic P (Po) is of great significance in these soils due to the immobilization of P by Fe and Al. Cover cropping in orchards and symbiotic microbial inoculation are considered to improve soil quality, including P status, however, their effects on the hydrolysis of Po is little known. In this study, five soil managements were established in a guava orchard in South China for two and a half years, including clean culture (CC), cover cropping with *Paspalum natatu* (PN), PN with arbuscular mycorrhizal fungal inoculation (PNA), cover cropping with *Stylosanthes guianensis* (SG), SG with rhizobial inoculation (SGR). Soil chemical, biochemical and microbial properties were analyzed. Results indicate that soil pH and SOM content tended to increase following cover cropping alone or with microbial inoculation. Po content was significantly elevated in PNA. Po fractionation revealed that cover cropping alone or with microbial inoculation significantly affected the contents of moderately labile Po (MLPo) and moderately resistant Po (FAPo). Enzyme assay indicated that cover cropping with microbial inoculation increased the activities of acidic phosphomonoesterase (ACP), neutral phosphomonoesterase (NP) and alkaline phosphomonoesterase (ALP), with ALP the most sensitive, although ACP activity dominated in red soils. Correlation analysis suggested a significantly positive relationship between ALP activity and MLPo or FAPo. PCR-DGGE profile of the *alp*-harboring bacterial community showed that cover cropping with *S. guianensis* and mycorrhizal inoculation to *P. natatu* promoted the bacterial diversity and/or species richness. For almost all the measured parameters, PN and SG were comparable, however, PNA was superior to SGR, indicating the stronger additive effect of arbuscular mycorrhizal fungus than that of rhizobia. Cat-PCA indicated that MLPo was the most influential factor on phosphomonoesterase. In general, this study suggests that, in subtropical orchards with red soil, cover cropping with microbial inoculation can improve the Po hydrolysis via the promoted *alp*-harboring bacterial community and then ALP activity. Our results also suggest that the combination of *P. natatu* and arbuscular mycorrhizal fungus is better than *S. guianensis* and rhizobia, which possesses practical significance for sustainable production in these orchards.

1. Introduction

Red soil, classified as Ultisols and Oxisols in the USDA soil taxonomy system, is a typical soil type in tropical and subtropical areas (FAO-Unesco, 1974). In China, red soil accounts for nearly 20% of total terrestrial land and mainly distributes in South China (FAO-Unesco, 1974; Li, 1983). Due to high temperature and heavy precipitation, desilicification and allitization of soils dominate the soil genesis, and thus red soil is typically rich in Al and Fe with low pH while deficient in soil organic matter (SOM) (Xu et al., 2003; Wang et al., 2014). Among the macronutrients essential for plant growth, P is the most limiting element as a result of immobilization by high

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levels of Al and Fe (Wei et al., 2010). Therefore, it is a huge challenge to alleviate the P deficiency.

Raising the SOM content is a multifaceted strategy to improve soil quality (see review by Reeves, 1997), including the increase of bioavailability of soil nutrients, e.g. P. Laird and Chang (2013) reported that removal of 90% above ground residue for 19 years led to a decrease in SOM by 12% and thus a substantial degradation of soil quality. Cover cropping is one of the most important orchard soil managements in South China, where *Stylosanthes guianensis* (a tropical legume species) and *Paspalum natatu* (a tropical gramineae species), with ability to increase the soil fertility or to alleviate the potential soil erosion, are two widely-used cover crops in hilly orchards (Guodao et al., 1997; Yao et al., 2005; Yang et al., 2012). Meanwhile, cover cropping can also increase the SOM content via foliage littering (shoot-derived carbon) and root turnover, exudation (root-derived carbon) (Atucha et al., 2013; Liu et al., 2013a, b). In a simulated experiment with ^{13}C labeling, the shoot-derived soil organic carbon (SOC) and the root-derived SOC were quantified as 178–364 and 122–347 mg C/kg soil, respectively (Comeau et al., 2013). With cover cropping for ten successive years, the SOM content significantly increased in sweetsop, litchi, longan, guava orchards (Liu et al., 2013a, b). Soil P exists in two forms, namely inorganic P (Pi) and organic P (Po), with the latter accounting for 30%~65% (Harrison, 1987). In red soil, most Pi is immobilized by Al and Fe (Wei et al., 2010), and thus Po is the important potential P source for plant growth (Chen et al., 2002; Steffens et al., 2010). Po in the SOM can release P via phosphatase hydrolysis (Tabatabai, 1994; Turner and Haygarth, 2005).

Soil microbes are key drivers of P cycling in soils (Marschner, 2008). Under P limitation condition, available P can be released from Po through the processes of mineralization by soil microbes, and thus the processes of Po mineralization are greatly important for the bioavailability of P in soils (Stewart and Tiessen, 1987). Diesters in soils can be hydrolyzed by phosphodiesterase (PD) to release monoesters, and then Pi is released through hydrolysis of monoesters by alkaline phosphomonoesterase and acid phosphomonoesterase (Tabatabai, 1994; Turner and Haygarth, 2005). It is accepted that plants secrete only acid phosphomonoesterase while microbes produce both alkaline and acid phosphomonoesterase (Spohn and Kuzyakov, 2013). Clearly, soil microbes are greatly involved in the hydrolysis of Po and therefore contribute much to plant P nutrition (Marschner, 2008). Many experiments point out that inoculation of selected microbial strains can effectively improve the P nutrition of plants, among which arbuscular mycorrhizal fungi (AMF) are of special importance. AMF are symbiotic soil fungi and can greatly promote P uptake by host plants, especially in P limiting soils (Smith and Read, 2008). Much evidence has been accumulating to indicate that AMF greatly improve the soil quality, especially of stressed soils, mainly via soil aggregation with glomalin (Rillig, 2004). Moreover, AMF can interact with other microbes to enhance the promotive effects (Barea et al., 2002). For example, dual inoculation with *Glomus mosseae and Rhizobium* interactively promoted the quality of weathered soft rock soils (as revealed by soil enzymes) and the plant performance of black locust seedlings (Gong et al., 2012).

To alleviate the P limitation in hilly orchards in South China, we have established cover cropping system in a guava orchard for two and a half years and symbiotic microbial inoculants were also employed. We hypothesized that cover cropping can improve P cycling in soil via increasing the hydrolysis of Po, and that microbial inoculation may mediate the improvement depending on microbial species. In this study, we monitored the phosphatase activities and Po fractions, and further detected the diversity of *alp*-harboring bacterial community. We aimed to reveal how cover crops and symbiotic microbes jointly affect the Po hydrolysis and finally the P status in red soil in orchards.

2. Materials and methods

2.1. Site description and experimental design

A guava (*Psidium guajava* Linn.) orchard at Heshan Hilly Land Interdisciplinary Experimental Station (E112°54′, N22°41′), Chinese Academy of Science in Guangdong province was selected as experimental site, where a subtropical monsoon climate was described previously (Chen et al., 2012). The terraced orchard was established on a hilly slope.

Five soil managements were practiced in this orchard, including clean culture (CC, namely regular weeding and leaving bare soil), cover cropping with *P. natatu* (PN), cover cropping with *P. natatu* plus AMF inoculation (PNA), cover cropping with *S. guianensis* (SG), cover cropping with *S. guianensis* plus rhizobial inoculation (SGR). Each management consisted of six plots representing six replicates, with twelve guava plants in each plot (about 48 m^2). *P. natatu* is tropical perennial gramineae plant commonly used in hilly orchards for the protection from soil erosion, and easy to establish symbiosis with AMF (Ishii et al., 2007), while *S. guianensis* is tropical perennial legume plant widely used in orchards to increase soil fertility (Guodao et al., 1997). In CC plots, all native weeds were manually removed every half month to leave bare soil during the experiment, while in the cover cropping plots, *P. natatu* or *S. guianensis* were grown in respective plots after weeding. In addition, mowing was conducted at an interval of about 45 days from April to September during the experiment. The cover crops almost did not grow due to low temperature and soil drought from October to March, and thus mowing was not necessary. *Rhizophagus irregularis* BGC BJ09 (AMF) from the Bank of Glomales in China (BGC) and *Bradyrhizobium vigna* ACCC 14150 (rhizobia) from Agricultural Culture Collection of China (ACCC) were used for symbiotic inoculation. For the inoculant production in laboratory, AMF was propagated with sorghum and clover as host plants and rhizobia was propagated using yeast extract–mannitol medium (YM). Symbiotic microbes were inoculated twice, e.g. one and six months after the emergence of cover crops. For AMF inoculation, trench of about 10 cm deep was prepared and a dose of about 3.2×10^4 spores per square meter was applied. For rhizobial inoculation, bacterial suspension was diluted and applied at the rate of about 7.5×10^{11} cells per square meter. A total of thirty plots were randomly arranged on the hill slope.

2.2. Soil sampling and preparation

Soils were sampled after the establishment of cover crops for two and a half years. In each plot, two guava plants were randomly selected and three soil cores with a depth of 20 cm were sampled around each plant. Six soil cores were well mixed by sieving through 2-mm mesh to produce a composite sample for each plot. All soil samples were transported to the laboratory on ice within 2 h.

In laboratory, each soil sample was divided into several aliquots. One aliquot was stored at 4 °C for enzyme activity assay within three days. Another aliquot was air-dried and stored at 4 °C for the analysis of pH, SOM and total P (TP). The left aliquots were stored at −80 °C for the analysis of Po and PCR-DGGE within two weeks.

2.3. Phosphomonoesterase activity assay

Phosphomonoesterase activity was estimated by measuring the release of *p*-nitrophenol (PNP) from *p*-nitrophenyl phosphate (PNPP) following exposure to soil as described by Tabatabai and Bremner (1969). The assay of acid phosphomonoesterase (ACP), neutral phosphomonoesterase (NP), alkaline phosphomonoesterase (ALP) activity was conducted at pH of 4.9, 7.0, 9.4,