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Effects of long-term fertilization on *phoD*-harboring bacterial community in Karst soils

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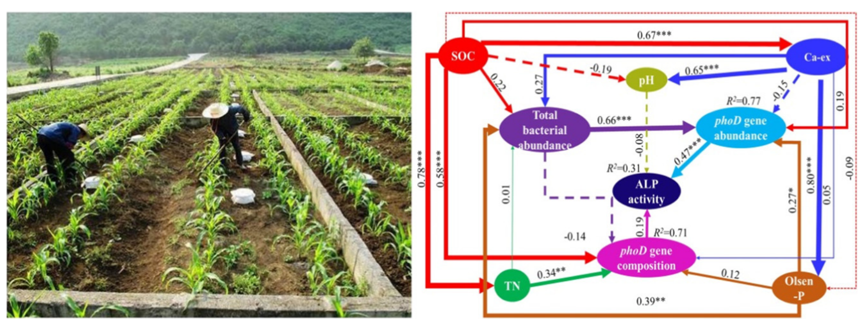
HIGHLIGHTS

- High-level organic inputs significantly changed the *phoD* community structure in Karst soils.
- The positive correlation was found between available P, ALP activity and *phoD* gene abundance.
- The P availability was also associated with soil Ca^{2+} concentration.

GRAPHICAL ABSTRACT



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ABSTRACT

Phosphorus (P) acquisition by plants from soil organic P mainly relies on microorganisms. Examining the community of functional microbes that encode phosphatases (e.g. PhoD) under different fertilization managements may provide valuable information for promoting soil organic P availability. Here, we investigated how the abundance and community diversity of *phoD*-harboring bacteria responded to long-term fertilization in Karst soils. Six fertilization treatments were designed as follows: non-fertilized control (CK), inorganic fertilization only (NPK), and inorganic fertilization combined with low- and high amounts of straw (LSNPK and HSNPK), or cattle manure (LMNPK and HMNPK). We found that soil available phosphorus (AP) content and the activity of alkaline phosphatase (ALP) were significantly higher in all combined inorganic/organic fertilization treatments, while the abundance of the *phoD* gene was only higher in the HMPNK treatment, compared to NPK. The combination of inorganic/organic fertilizations had no effect on the diversity of *phoD* genes compared to NPK alone, but the *phoD* gene richness was greater in these treatments as compared to the control. Only organic fertilization combinations with high amounts of organic matter (both HSNPK and HMNPK) significantly affected the *phoD* community structure. A structure equation model demonstrated that soil organic carbon (SOC), rather than P, greatly affected the *phoD* community structure, suggesting that organic P mineralization in soils is decoupled from C mineralization. Our results suggested that optimized combinations of inorganic/organic fertilizations could promote P availability via regulating soil *phoD*-harboring bacteria community diversity and ALP activity.

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1. Introduction

Phosphorus (P), one of key macronutrients for plants, is mostly limiting in high-yield agricultural ecosystems (Richardson and Simpson, 2011). P fertilizer is often applied beyond the requirement of crop growth due to soil P fixation, resulting in its unavailability for crops (Waldrip et al., 2011). However, accumulated P in soil could cause potential environmental risks, such as eutrophication, once the P mobilizes into water bodies. Additionally, the P rock resource is non-renewable, and could only be sustained over a limited time frame for mining (Cordell et al., 2009). Therefore, it is necessary to improve P use efficiency for both food security and environmental health using soil management practices.

Application of organic matter (e.g. straw and cattle manure) is considered an important soil management practice, which not only contributes to supplying plants with nutrients, but also maintains soil fertility via replenishing organic matter content (Steiner et al., 2007; Zhu et al., 2010). Many studies have documented the benefits of organic matter amendment for the soil nutrient cycle (Diacono and Montemurro, 2010). For example, soil available P was enhanced by inputs of organic manures (Damon et al., 2014; Garg and Bahl, 2008). Increasing available P by organic inputs commonly addresses two processes: (1) organic inputs mobilized the native inorganic P by release of organic acids during their mineralization, because of competitive adsorption between organic acids and phosphate by clay minerals and oxides which delaying P adsorption on active surfaces (Bolan et al., 1994), (2) organic inputs promoted organic P mineralization by increasing activity of P-hydrolyzing enzymes (Garg and Bahl, 2008). The carbon resource contained in organic matter as an energy supply was demonstrated to promote the size of the microbial community, which resulted in increased enzyme activity (e.g. phosphatase activity) (Dhull et al., 2004). However, little is known about whether organic matter amendment could also change the composition of corresponding functional genes to promote enzyme activity.

Phosphatases, consisting of alkaline phosphatase (ALP) and acid phosphatase (ACP), are enzymes that catalyze the hydrolysis of ester-phosphate bonds (with the exception of inositol phosphates such as phytate) to release the phosphate (Juma and Tabatabai, 1978; Nannipieri et al., 2011). Both ACP and ALP activities have been widely studied for the evaluation of organic P mineralization under different agricultural management regimes in many types of soils (Nannipieri et al., 2011). Previous studies have demonstrated that ALP was exclusively derived from soil microbes (Romanyà et al., 2017), while ACP was mainly synthesized and released by plants (Dinkelaker and Marschner, 1992; Krämer and Green, 2000). Three homologous ALP-encoding genes (*phoA*, *phoX* and *phoD*) in prokaryotic organisms have been identified based on sequence similarity and substrate specificity (Tan et al., 2013; Kathuria and Martiny, 2011). PhoA uses zinc and magnesium as cofactors, and hydrolyzes phosphomonoesters, whereas both PhoX and PhoD use calcium, and hydrolyze both phosphomonoesters and diesters (Kageyama et al., 2011; Majumdar et al., 2005; Ragot et al., 2015). Interestingly, the distribution of these three homologous genes varied with ecosystems. For example, *phoA*, *phoD* and *phoX* were widely distributed in aquatic ecosystems such as seawater and freshwater (Luo et al., 2009; Dai et al., 2014), while *phoD* genes are more frequently present in terrestrial ecosystems than *phoA* and *phoX* as observed from soil metagenomic datasets (Tan et al., 2013). Therefore, the *phoD* gene could be used as a key molecular marker to provide insight into soil organic P transformations.

The Karst region studied here is, characterized by limestone and sinkholes, covers 550,000 km^2 of southwest China, and is one of the largest Karst regions in the world (Jiang et al., 2014). Southwestern China is located in a subtropical monsoon climate zone and has abundant rainfall in the wet season (approximately 900–1400 mm). As such, Karst farmland in this region has high bedrock exposure with a very shallow soil. Moreover, the thin calcareous soil usually has a low nutrient buffering capacity (Zhang et al., 2007; Jarvie et al., 2014).Therefore, Karst farmland of southwest China is often perceived as highly vulnerable to P loss. Previous study indicated that organic P was more resistant to leaching than inorganic P (Berg and Joern, 2006; Kang et al., 2011), suggesting that organic P sources could be more sustainable than inorganic P sources for maintaining crop productivity. It is known that only orthophosphate anion (PO_4^{3-}) was utilized by crops after organic P mineralization, and $PO_4^{3-}$was released via activity of phosphomonoesterases (i.e., PhoD). Several recent studies have investigated the *phoD*-harboring bacterial community in soils under different fertilization treatments (Tan et al., 2013; Chen et al., 2017; Luo et al., 2017). However, the results were inconsistent, suggesting that the *phoD*-harboring microbes were sensitive, and responded differently to various fertilization applications in different soil types. Therefore, the *phoD*-harboring bacterial community response to application of organic matter in Karst soil could differ from that in other soil types due to its high calcium content and its pH.

In the current study, we aimed to reveal the impact of inorganic and organic fertilization combinations on soil available P, potential ALP activity, and *phoD* gene community structure based on a 10-year field experiment in Karst soils in southern China. We hypothesized that organic matter application could increase the level of available soil P, possibly by promoting microbial activity and changing community structure of *phoD*-harboring bacteria. Our results are expected to provide novel insights into how to enhance P availability in Karst soils by stimulating *phoD*-harboring microbial activity via organic matter amendment.

2. Materials and methods

2.1. Study site and experimental design

A field experiment was carried out under maize-soybean rotation in calcareous soil in a Karst region, located within the Huanjiang Observation and Research Station for Karst Ecosystem, Chinese Academy of Sciences, Huanjiang County, Guangxi Province, China (24°44N, 107°51E). The climate at this location is a subtropical monsoon climate, with an average annual air temperature of 18.5 °C and an average annual precipitation of 1389 mm. The field fertilization experiment was initiated in 2006. At the start of the trial, the main chemical properties of the surface soil (0–20 cm) were: organic C 24.96 g kg^{-1}, total N 2.24 g kg^{-1}, total P 1.35 g kg^{-1}, total K 13.39 g kg^{-1}, available N 153.85 mg kg^{-1}, available P 5.47 mg kg^{-1}, available K 64.02 mg kg^{-1}.

This experiment consisted of six fertilization treatments: (1) non-fertilized control (CK); (2) inorganic NPK fertilization (NPK); (3) inorganic NPK fertilization with low amount of straw (2700 kg ha^{-1} soybean straw in the maize cropping season, and 740 kg ha^{-1} maize straw in soybean cropping season, respectively) (LSNPK); (4) inorganic NPK fertilization with a high amount straw (5400 kg ha^{-1} soybean straw in the maize cropping season, and 1480 kg ha^{-1} maize straw in soybean cropping season, respectively) (HSNPK); (5) inorganic NPK fertilization with a low amount of cow manure (3730 and 420 kg ha^{-1} cow manure in maize and soybean cropping season respectively) (LMNPK); (6) inorganic NPK fertilization with a high amount of cow manure (7430 and 840 kg ha^{-1} cow manure in maize and soybean cropping season, respectively) (HMNPK). Total inorganic and organic N, P and K fertilization were applied at the rate of 200 kg N ha^{-1}, 90 kg P_2O_5 ha^{-1}, and 120 kg K_2O ha^{-1} during the maize season and 22.5 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}, and 67.5 kg K_2O ha^{-1} during the soybean season. Cow manure was applied at rate of 7430 or 840 kg ha^{-1}, that is, about 120 or 13.6 kg N ha^{-1}, 68 or 7.7 kg P_2O_5 ha^{-1}, and 87 or 9.8 kg K_2O ha^{-1}. Maize straw was applied at rate of 1480 or 740 kg ha^{-1}, that is, about 13.5 or 6.75 kg N ha^{-1}, 3.4 or 1.7 kg P_2O_5 ha^{-1}, and 4.3 or 2.15 kg K_2O ha^{-1}. Soybean straw was applied at rate of 5400 or 2700 kg ha^{-1}, that is, about 93 or 46.5 kg N ha^{-1}, 22 or 11 kg P_2O_5 ha^{-1}, and 72 or 36 kg K_2O ha^{-1}. The nutrients contained in the organic matter were included in the calculation