



Effects of amendments on phosphorous status in soils with different phosphorous levels

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ABSTRACT

Phosphate fertilizers are used in horticultural production to increase yields, but overuse can result in inefficient crop uptake and large loss of phosphorus (P). Soil amendments could potentially reduce the risk of P loss while maintaining high crop yield. The objective of this study was to investigate the effect of organic and inorganic amendments on P fractions in soils with different P levels (low, medium and high), and the underlying mechanisms. Five treatments were included: no amendment (CK), chemical fertilizers (CF), rice straw (FS), chicken manure compost (CM), and vermicompost (VM). Counterintuitively, the proportion of organic phosphorus (OP) tended to increase in the CF treatment, especially in soil with low P level. Results indicated that the change in the proportion of OP was mainly controlled by soil acid phosphomonoesterase, alkaline phosphomonoesterase and phosphate-solubilizing microorganisms. The proportion of available P increased in soil with low P level, but decreased in soil with high P level in the VM treatment. Non-labile P was preferentially formed in the VM treatment because of the high calcium content in vermicompost. Phosphates associated with aluminum (Al-P) were significantly higher in the CM treatment than in other treatments. This treatment also had a large presence of *Pseudomonas* and *Streptomyces* bacteria, which are capable of promoting the formation of Al-P. Our results indicated that mechanisms causing changes in soil P fractions were distinctly different among various amendments, and that vermicompost had the greatest potential to increase P supply in soil with low P level, and to decrease the risk of P loss in soil with high P level.

1. Introduction

Phosphorus (P) is one of the essential nutrients for plant growth (Raghothama, 2005). About 43% of the soils around the world are P-deficient (Liu et al., 2012). Farmers all over the world rely on chemical P-fertilizers to alleviate P-deficiency and make the agricultural production system more sustainable. However, due to the low plant use efficiency, intensive application of P fertilizer always leads to substantial accumulation of P in the surface soil, and increases the risk of P to be lost with subsurface flow (leaching) in some areas of the world (Haygarth et al., 2013). Accordingly, it is of high importance to choose appropriate amendments to maintain suitable P availability in soils with different P levels.

The availability of soil P is controlled to a large extent by the distribution of P fractions. According to the sequential extraction method

developed by Chang and Jackson (1957) and Hedley et al. (1982), soil P can be divided into seven fractions as organic P (OP), monocalcium phosphate ($\text{Ca}_2\text{-P}$), $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ ($\text{Ca}_8\text{-P}$), aluminum phosphates (Al-P), iron phosphates (Fe-P), occluded P and $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ($\text{Ca}_{10}\text{-P}$). Furthermore, according to the plant accessibility, these seven fractions can be regarded as labile P ($\text{Ca}_2\text{-P}$, $\text{Ca}_8\text{-P}$ and Al-P) and non-labile P (OP, Fe-P, occluded P and $\text{Ca}_{10}\text{-P}$) (Shen et al., 2004). $\text{Ca}_2\text{-P}$ can be readily used by plants, and $\text{Ca}_8\text{-P}$ and Al-P can be easily transformed into $\text{Ca}_2\text{-P}$, but the non-labile P fractions are relatively stable.

Redistribution and transformation of P fractions with P containing fertilizer applications have been frequently reported. For instance, inorganic P application increased the fraction of labile $\text{Ca}_2\text{-P}$ and $\text{Ca}_8\text{-P}$, whereas the fraction of non-labile $\text{Ca}_{10}\text{-P}$ decreased (Yang et al., 2012). A number of long-term experiments suggested that addition of chemical P fertilizers raised soil inorganic P content, but did not affect or

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decrease the soil organic P fraction (Takahashi and Anwar, 2007; Vu et al., 2008). Transformation of P fractions is to a large extent regulated by the enzyme phosphomonoesterase, while activities of phosphomonoesterase are under the control of soil P levels (DeForest et al., 2012). However, most of those studies were conducted in soils with low P levels. It remains unclear how P fertilizer application affect transformation of phosphorus fractions in soils with high P levels.

Manure is an important source of P, which has been widely used in agricultural production. The contribution of manure to the different fractions of soil P depends on its source. Compared to cattle and goat manure, poultry manure is richer in P concentration. However, a relatively higher amount of P was fixed or immobilized in soil with cattle and goat manure due to their higher content of Al and Fe compared to poultry manure (Azeez and Van Averbek, 2010). In addition, manure application can also increase the phosphomonoesterase activities by increasing the microbial abundance, and as a consequence, decrease soil organic P (Zohar et al., 2014).

Vermicompost is compost made with earthworms, and in contrast to conventional compost, it contains higher concentrations of available nutrients such as nitrates, orthophosphate, potassium (K), and Ca (Bhat et al., 2018; Hussain et al., 2018). Moreover, enzymes inside the intestinal tract of earthworms play an essential role in the breakdown of organic matter (Ghosh et al., 2018). This suggests that the application of vermicompost could decrease the concentration of OP, but increase the availability of P in soil. However, Jiménez et al. (2003) found that vermicompost application increased all P fractions, and the increase in OP was mainly ascribed to the reduced phosphomonoesterase activity.

Straw application has been used world-wide as a key agricultural management tool to increase soil organic matter content. Combined application of straw and chemical fertilizer decreased the concentration of soil available P because of microbial immobilization (Iqbal, 2009). However, Alamgir et al. (2012) reported that the application of straw increased the concentration of available P. These conflicting results could be due to differences in the contents of P in straw and soil.

The objectives of this study were to investigate the effects of different amendments on the form of P in soils with different P levels, so as to increase our understanding about which practice is optimal to meet the need of P for crop growth, and at the same time reduce the risk of P loss, and the underlying mechanisms.

2. Materials and methods

2.1. Study sites and experimental design

Three soils with different phosphorous levels, low (L), medium (M) and high (H) were collected from three adjacent sites which had different durations of tomato cultivation in the Guanghui town of Yuhong District, Shenyang City, Liaoning Province. The soil is classified as a Hapli-Udic Cambisol according to FAO classification. Soil P content increased with the increase in duration of tomato cultivation. Selected chemical properties of these soils are listed in Table 1. Collected soils

Table 1
Selected soil properties of collected soils with different P levels.

P level	TP	OP	Ca10-P	Fe-P	Al-P	Ca8-P	Ca2-P	pH
	mg kg ⁻¹							
L	518	260	183	43.2	8.24	52.1	2.63	8.14
M	1209	775	167	89.9	71.3	97.6	15.8	7.85
H	2999	1377	757	244	130	484	29.8	6.17

TP, total phosphorus; OP, organic phosphorus; Ca10-P, represents a group of phosphates with chemical structure similar to $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$; Fe-P, Fe phosphates; Al-P, Al phosphates; Ca8-P, a group of phosphates with chemical structure similar to $\text{Ca}_8\text{H}_2(\text{PO}_4)_6\cdot 5\text{H}_2\text{O}$; Ca2-P, monocalcium phosphate. L, soil with low P level; M, soil with medium P level; H, soil with high P level.

were used to fill polyethylene plastic pots with a diameter of 30 cm and a height of 28 cm (20 pots for each soil) in 2015. Before filling each pot with 15 kg of soil, soils were mixed homogeneously with fertilizers. Five treatments were established: no fertilization (CK), chemical fertilizer addition (CF), fresh rice straw addition (FS), chicken manure compost addition (CM) and vermicompost addition (VM). Application rates of chemical fertilizers in the CF treatment were: 0.4 g N, 0.11 g P, and 0.33 g K kg⁻¹ soil in the form of urea, superphosphate and potassium sulfate, respectively. The added N, P and K were equal to 900 kg N, 248 kg P and 743 kg K ha⁻¹, which are common levels for the production of tomato in this area. Addition rates of rice straw, chicken manure compost and vermicompost were 40.8, 13.1 and 8.3 g kg⁻¹ soil, respectively. In order to ensure that all fertilizers were applied on an equal N, P and K basis, extra chemical N, P or K were added to FS, CM and VM treatments according to the nutrient content of added organic materials. Selected chemical properties of all fertilizers are shown in Table 2. Tomato (*Solanum lycopersicum*) variety, 'Gold Crown No. 9', was cultured one plant per pot. A complete randomized block design was applied, and all treatments were replicated four times.

2.2. Soil sampling and chemical analysis

On May 26 (fruiting stage) of 2016, a composite soil sample was taken from each pot. Fresh soils were passed through a 2.0 mm sieve and subsamples were stored at -80 °C for further DNA extraction. For chemical characterization, soil samples were air dried at room temperature.

The fractions of inorganic P were measured by the sequential extraction procedure according to Chang and Jackson (1957) and Hedley et al. (1982). Soil organic P in soil was measured according to the NaOH-EDTA extraction method described by Cade-Menun and Preston (1996).

Soil acid phosphomonoesterase (AcP) (EC 3.1.3.2) was measured according to the protocol as described by Tabatabai (1994). Briefly, about 1.0 g fresh soil was incubated with 0.2 ml methylbenzene, 1.0 ml 0.05 M *p*-nitrophenyl phosphate and 4.0 ml modified universal buffer of pH 6.5 at 37 °C for 1 h. Then 1.0 ml 0.5 M CaCl_2 and 4.0 ml 0.5 M NaOH were added to terminate the reaction. Absorbance of the filtrate was measured using a spectrophotometer. To measure alkaline phosphomonoesterase (AlP) (EC 3.1.3.1), the universal buffer of pH 6.5 was replaced by universal buffer of pH 11.

The soil pH (soil:water, 1:5) was determined using a Thunder Magnetic SJ-3F pH Meter (INESA, Shanghai, China). Soil exchangeable Ca was extracted with $\text{CH}_3\text{COONH}_4$ according to the method by Ochoa-Hueso et al. (2014). Soil available Fe was determined using the diethylenetriaminepentaacetic acid (DTPA) extraction method according to Wang et al. (2017). Soil organic matter (SOM) was measured by the method of dichromate oxidation and titration with ferrous ammonium sulfate (Bao, 2000). Soil microbial biomass carbon (MBC) was measured by chloroform fumigation extraction method (Vance et al., 1987).

2.3. Soil DNA extraction and 16S rRNA gene amplification and sequencing

Soil total DNA was extracted from 0.500 g of each sample using the Fast DNA® SPIN Kit for Soil (MP Biomedicals, California, US), according to the manufacturer's protocol, then stored in duplicate at -20 °C for sequencing.

To amplify the bacterial fragments of the appropriate size for MiSeq, primers F341 (5'-ACTCCTACGGGSGCAGCAG-3') and R806 (5'-GGA-CTACVGGGTAT CTAATC-3') targeting V3-V4 hypervariable regions of bacterial 16S rRNA genes were selected (Sun et al., 2017). The 16S rRNA gene sequencing procedure was performed using the Illumina HiSeq2500 PE250 platform by the Shanghai Realbio Technology Institute (China). The quality control (QC) was performed on the basis of raw data.

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