Original Paper

Influences of a vermicompost application on the phosphorus transformation and microbial activity in a paddy soil

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Abstract: A pot experiment was conducted to investigate the effects of a vermicompost (VC) application on the phosphorus (P) transformation and microbial activity in a paddy soil. Changes in the following P forms were investigated: resin-P, concentrated HCl extracted inorganic ($C.HCl\text{-}P_i$) and organic P ($C.HCl\text{-}P_o$), diluted HCl extracted inorganic P ($D.HCl\text{-}P_i$), $NaHCO_3$ extracted inorganic ($NaHCO_3\text{-}P_i$) and organic P ($NaHCO_3\text{-}P_o$), NaOH extracted inorganic ($NaOH\text{-}P_i$) and organic P ($NaOH\text{-}P_o$), and residual P. The results showed that the vermicompost application significantly ($P < 0.05$) affected the pH, redox potential (Eh), water soluble Fe(II), HCl-extractable Fe(II), microbial biomass carbon (MBC), microbial biomass P (MBP), MBC/MBP ratio, and acid phosphatase activity (APA) of the paddy soil. In particular, the HCl-extractable Fe(II) increased by 25–56% with the vermicompost application when compared to the control (CK). With the exception of $C.HCl\text{-}P_i$, the vermicompost application greatly increased the contents of the various P forms in the soil. In particular, the labile P (resin-P, $NaHCO_3\text{-}P_i$, and $NaHCO_3\text{-}P_o$) and moderately stable P ($NaOH\text{-}P_i$ and $NaOH\text{-}P_o$) were significantly ($P < 0.01$) increased. The correlation analyses showed that $NaHCO_3\text{-}P_i$ was significantly and positively related to the MBC, MBP, and APA, while $NaHCO_3\text{-}P_o$ was significantly and negatively related to the MBC, MBP, and APA. Both $NaOH\text{-}P_i$ and $C.HCl\text{-}P_i$ were significantly and negatively related to the APA. Both $NaOH\text{-}P_o$ and $C.HCl\text{-}P_o$ were significantly and positively related to the MBP, while $NaOH\text{-}P_i$ was significantly and negatively related to the MBP. These results indicated that a vermicompost application could effectively enhance the dissolution and reduction of Fe and the consequent mobilisation of $NaOH\text{-}P_i$. In addition, the vermicompost application significantly ($P < 0.01$) increased the APA and effectively mobilised the $NaOH\text{-}P_o$.

Keywords: iron; microbial biomass phosphorus; phosphorus fractions; pot experiment

An essential macronutrient for plant growth, phosphorus (P) directly or indirectly participates in many metabolic processes in plants. Therefore, the P supply greatly influences the global agricultural productivity (Nishigaki et al. 2018). Although P is the 11th most abundant element in the earth's crust, the P supply by soil is often insufficient for crop growth (Von Sperber et al. 2017). Application of chemical P fertilisers can address soil P deficiency, but a large proportion of the added P is quickly fixed by soil minerals and organic

matter (OM) and not utilised by plants (Richardson et al. 2011; Zhou et al. 2018). Studies show that the use efficiency of P fertilisers is merely 10–25% by the first crop (Xu et al. 2012). The low use efficiency of P fertilisers has led to the ever-increasing application of P fertilisers worldwide. The over-application of P fertilisers is a waste of resources and causes huge economic loss. In addition, it results in soil hardening and acidification or salinization, consequently posing a serious threat to the eco-environment (Bouwman et al. 2017; Li et al. 2018). Therefore, it is of significance for agriculture production and ecological protection to effectively improve the P availability in soil. In natural conditions, most P in the soil is stable or moderately stable (Maranguit et al. 2017). In addition, the composition of the soil P pool differs greatly due to the differences in the climate, soil parent material, paedogenic process, and soil tillage (Yokoyama et al. 2018). Generally, P transforms between different forms under certain conditions, and the desorption of P from the soil particle surface, the mineralisation of organic P, and the dissolution of sparingly soluble P under acidic conditions are the major mobilisation means of P. Many studies have shown that adsorption and precipitation of P by soil particles and metal oxides has a great effect on the short-term fixation of P (de Brito Neto et al. 2018). Generally, a large proportion of inorganic P is fixed in agricultural soils. Consequently, it is hard to maintain the optimal soluble P level even for an agricultural production cycle (Syers et al. 2008). The phosphorus desorption rate differs greatly with the soil pH, mineral composition, and OM content (De Araújo et al. 2015). Organic P takes up 30–60% of the soil's total P (Lidbury et al. 2017). Therefore, inorganic phosphates generated from organic P mineralisation are important P sources for plants. Phosphatase plays an important role in the hydrolysis of organic P. While the form and solubility of the organic P are important factors influencing its mineralisation (Giles et al. 2017), the types and activity of the phosphatase are significantly affected by the microbial species and quantity (Menezes-Blackburn et al. 2018). Vermicompost is an organic fertiliser produced *via* fermentation of organic wastes under the effect of enzymes from the digestive system of earthworms. It is rich in small molecule organic acids and plant-essential microelements and has a high level of microbial activity and a good water holding capacity, obviously different in its physical, chemical, and biological properties from common

composting products (Bekele et al. 2018; Bhat et al. 2018). Vermicompost applications can improve the soil's fertility and, in turn, the agricultural productivity (Arancon et al. 2006). Studies have demonstrated soil P mobilisation can be directly or indirectly affected by vermicompost. According to Guppy et al. (2005), vermicompost reduces the P fixation by the soil *via* its rich organic acids which compete for binding sites with orthophosphates and complexes with metals. Other studies demonstrated that the organic acids in vermicompost effectively slow down the formation of sparingly soluble phosphates (Moghimi et al. 2018). Vermicompost is cheap compared with mineral fertilisers and can be quantitatively produced by recycling organic waste from agriculture and everyday life. Therefore, it is also environmentally friendly (Lim et al. 2015). Much research has been conducted on the role of vermicompost in plant nutrition and yield increase, plant disease resistance, and heavy metal toxicity mitigation (Li et al. 2012; Kumar et al. 2018; Pierart et al. 2019). However, few studies have been performed to investigate the effects of vermicompost on the transformation of the nutrient elements and the related mechanisms. In this study, a pot experiment was conducted to investigate the changes in the crop biomass, soil microorganisms, and P forms in a paddy soil applied with vermicompost. The synergic effect of the microbial activity and iron (Fe) reduction on the P mobilisation during crop growth was discussed. The findings from this study are expected to provide scientific support for improving the P fertiliser utilisation rate in arable soils using vermicompost.

MATERIAL AND METHODS

Material. The soil used in this study was collected from an experimental field (22°32'N, 112°24'E) of the Chinese Academy of Agricultural Sciences at the Sanshui District of Foshan City, Guangdong Province. The parent material is river alluvium and is loamy clay in texture. The soil had a $pH(H_2O)$ of 7.08, an organic matter (OM) content of 23.7 g/kg, a total nitrogen (TN) of 1.84 g/kg, a total phosphorus (TP) of 1.78 g/kg, a total potassium (TK) of 3.64 g/kg, an alkali-hydrolysable N of 86.0 mg/kg, an Olsen-P of 42.6 mg/kg, and an available K of 215 mg/kg. After plant roots, gravel, and any dead animal bodies were manually removed, the soil sample was air-dried, ground, and sieved to pass through a 2-mm sieve for later use in the pot experiment.

The mineral fertilisers used in this study were urea (46.1% N), monopotassium phosphate (KH_2PO_4; 20.0% P and 16.9% K), and potassium chloride (KCl; 50.1% K). The vermicompost used was provided by the Fogang Fatsoil Agricultural S&T Co. Ltd. at Qingyuan City, Guangdong Province. The vermicompost feedstock was livestock and poultry faeces and agricultural biomass waste. The vermicompost was air-dried with the gravel removed and ground to pass through a 2-mm sieve so that homogeneous properties were obtained. The physicochemical properties of the vermicompost were: $pH(H_2O)$ of 7.05, OM content of 273 g/kg, TN of 1.86 g/kg, TP of 3.39 g/kg, TK of 10.1 g/kg, alkali-hydrolysable N of 436 mg/kg, Olsen-P of 461 mg/kg, and available K of 300 mg/kg.

The pakchoi (*Brassica rapa* L. ssp. *chinensis*) seeds were provided by Hangcheng Seeds Co. Ltd. of Fengcheng City, Jiangxi Province. The pakchoi variety was Youxuan Chinese Cabbage. The seeds were germinated in a 128-well seedling plate and the seedlings were transplanted after about 14 days when the 3rd true leaf was observed.

Experimental design. Each of the 40 plastic pots with a diameter of 13 cm and height of 11 cm was filled with 1 kg of soil. To guarantee sufficient N, P, and K for the pakchoi growth, mineral fertilisers of urea, KH_2PO_4, and KCl were applied at 332, 192, and 182 mg/pot, respective to all the pots. That is, mineral fertilisers were applied at an N : P : K ratio of 1.5 : 0.4 : 1.2. The pots were divided into two groups: the vermicompost application group (VC) and the no vermicompost application group (CK). For the 20 pots in the VC, 80 g of vermicompost was added to each pot, and no vermicompost was added for those in CK. The mineral fertilisers and the vermicompost were mixed thoroughly with the soil and water was added to 60% field capacity. Three pakchoi seedlings were transplanted into each pot. The pots were watered every day to maintain the soil moisture during the experiment. At day 0, 7, 14, 21, and 35, four pots were sacrificed from each group for soil sampling and each of these four pots were treated as a replicate of the specific sampling. From the pots taken at day 14, 21, and 35, the pakchoi plants were also collected for the belowground and aboveground biomass determination.

Chemical analyses. Soil P fractionation was conducted according to Tiessen et al. (1983), and the following P fractions were obtained: resin-P, concentrated HCl extracted inorganic (C.HCl-P_i) and organic P (C.HCl-P_o), diluted HCl extracted inorganic P (D.HCl-P_i), $NaHCO_3$ extracted inorganic ($NaHCO_3$-P_i) and organic P ($NaHCO_3$-P_o), NaOH extracted inorganic (NaOH-P_i) and organic P (NaOH-P_o), and residual P. Of these P fractions, resin-P, $NaHCO_3$-P_i, and $NaHCO_3$-P_o are considered labile P forms, NaOH-P_i and NaOH-P_o moderately stable P forms, and C.HCl-P_i, C.HCl-P_o, D.HCl-P_i, and residual stable P forms. The microbial biomass C (MBC) and P (MBP) were determined by the fumigation-extraction method (Wei et al. 2018). The soil HCl-extractable Fe(II) and water-soluble Fe(II) were extracted with 0.5 mol/L HCl and ultrapure water, respectively, and determined using the 1,10-phenanthroline photometric method (Yu et al. 2016). The acid phosphatase activity (APA) was determined by the disodium phenyl phosphate colorimetric method (Ge et al. 2018). The pakchoi biomass and soil and vermicompost physicochemical properties, *i.e.*, redox potential (Eh), pH, organic matter (OM), TN, TP, TK, Olsen-P, alkali-hydrolysable N, and available K, were determined according to Lu (2000).

Statistical analyses. Statistical analyses were performed with SPSS statistics 20 (IBM, USA). The Pearson correlation between the various factors was performed. The *t*-test was performed to examine the statistical significance of the independent variables.

RESULTS

Changes in the pakchoi biomass. Both the belowground and aboveground biomass of the pakchoi increased with time, it was especially rapidly from day 14 to 21, and did not differ significantly between the VC and CK at all the sampling times (Figure 1).

Changes in the soil pH, Eh, and Fe(II). The soil pH in the CK obviously decreased in the first 21 days and then it slowly changed (Figure 2A). In contrast, the soil pH in the VC did not change much from day 0 to 35. Consequently, the soil pH was significantly ($P < 0.01$) higher in the VC than in the CK from day 14 to 35, with an approximate 0.6-unit difference. The soil Eh in the CK obviously increased during the first 21 days and then it slowly changed (Figure 2B). In contrast, the soil Eh in the VC increased in the first 7 days, decreased rapidly afterwards, and then increased again from day 14. Consequently, the soil Eh was significantly ($P < 0.01$) lower in the VC from day 14 to 35.

The water soluble Fe(II) decreased rapidly from day 0 to 14 and then stabilised in both the VC and the CK (Figure 2C). From day 0 to 35, the water-
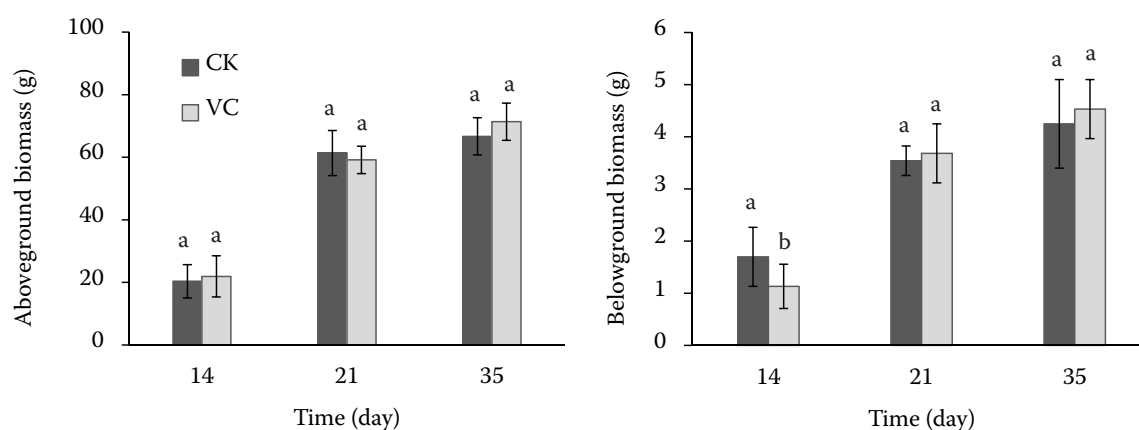
Figure 1. The pakchoi aboveground and belowground biomass changes in the CK and VC; the different lowercase letters indicate significant (*P* < 0.05) differences in the pakchoi biomass between the CK and the VC at the same sampling time
CK – control; VC – vermicompost

soluble Fe(II) was 4.69–12.4 mg/kg higher in the CK than in the VC, and the differences were statistically significant (*P* < 0.01). the HCl-extractable Fe(II) decreased from day 0 to 7 and then increased in both the VC and the CK (Figure 2D). In addition, the HCl-extractable Fe(II) increased more rapidly in the VC than in the CK from day 21 to 35. From day 0

to 35, the HCl-extractable Fe(II) was 7.93–20.2 mg/kg higher in the VC than in the CK, and the differences were statistically significant (*P* < 0.01).

Changes in the MBC, MBP, and APA. The soil MBC increased from day 0 to 21 in both the CK and the VC with that in the CK increasing more rapidly (Figure 3A). From day 21 to 35, the MBC decreased
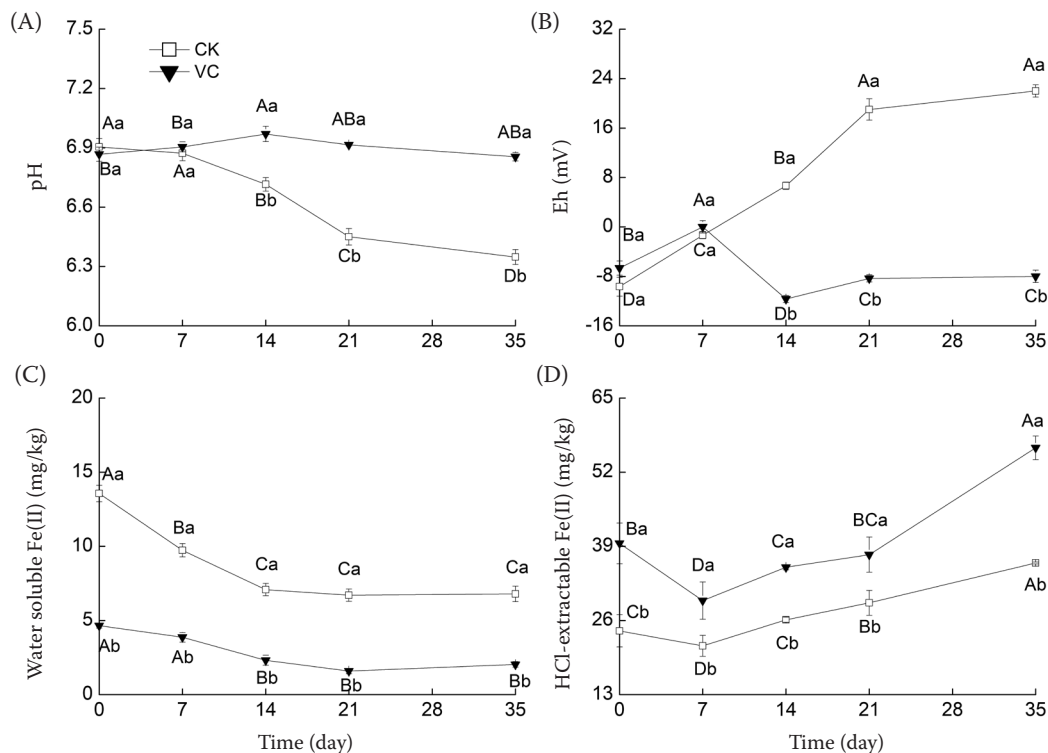

Figure 2. The soil pH (A), redox potential (Eh) (B), water soluble Fe(II) (C) and HCl-extractable Fe(II) (D) changes in the control (CK) and the vermicompost (VC)
Different capital letters indicate significant (*P* < 0.05) differences between different sampling times for a same treatment, and different small letters indicate significant (*P* < 0.05) differences between different treatments for a same sampling time
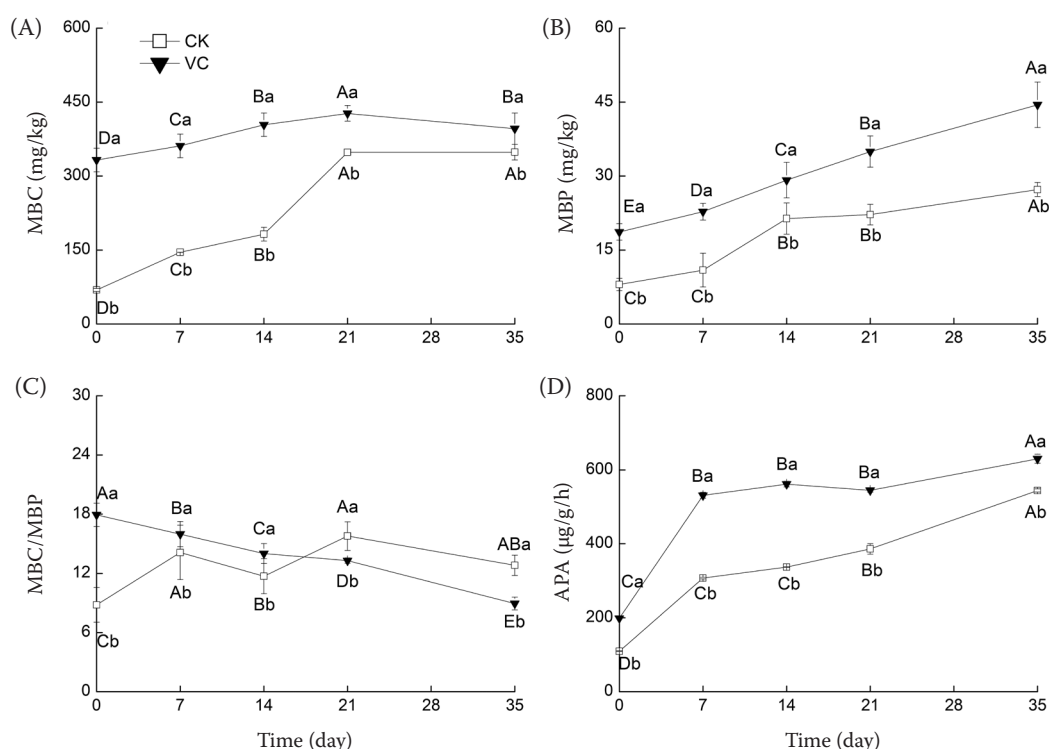
Figure 3. Changes in the soil microbial biomass C (MBC) (A), microbial biomass P (MBP) (B), MBC/MBP (C) and acid phosphatase activity (APA) (D) in the control (CK) and the vermicompost (VC)

Different capital letters indicate significant ($P < 0.05$) differences between different sampling times for a same treatment, and different small letters indicate significant ($P < 0.05$) differences between different treatments for a same sampling time

in the VC and stabilised in the CK, and it was 18% higher on average ($P < 0.01$) in the VC than in the CK at all the sampling days. The soil MBP in the VC basically increased linearly from day 0 to 35, and that in the CK first increased rapidly from day 0 to 14 and then slowly changed from day 14 to 35 (Figure 3B). Similarly, the MBP was 37–133% higher ($P < 0.01$) in the VC than in the CK at all the sampling days. The soil MBC/MBP ratio was significantly ($P < 0.05$) higher from day 0 to 14, but significantly ($P < 0.05$) lower from day 21 to 35 in the VC than in the CK (Figure 3C). The acid phosphatase activity increased from day 0 to 35, with a rapid increase for the first 7 days, especially in the VC (Figure 3D). In addition, the APA was significantly ($P < 0.01$) higher in the VC than in the CK from day 0 to 35, with a 16% difference at day 35.

Changes in the soil labile P. Of the labile P fractions of resin-P, $NaHCO_3-P_i$, and $NaHCO_3-P_o$, resin-P is the most labile fraction and important part of the labile P in the soil. The $NaHCO_3-P_i$ and $NaHCO_3-P_o$ are those inorganic and organic P adsorbed on the soil surface, respectively, including the soil MBP (taking up a small proportion). As can be seen from

Figure 4, the vermicompost application greatly increased the labile P forms with the largest increase in the resin-P. The average resin-P in the VC was 332 mg/kg higher than (or 2.10 times) that in the CK. The resin-P decreased slowly with time in both the VC and the CK, with that in the VC decreasing more slowly than that in the CK. $NaHCO_3-P_i$ showed a clear increase from day 0 to 14 and then a clear decrease from day 21 to 35 in both the VC and the CK, and it increased more rapidly in the VC than in the CK in the first 14 days with a daily difference of 12.1 mg/kg. $NaHCO_3-P_o$ decreased by 4.44 and 1.32 mg/kg in the VC and the CK, respectively after 35 days.

Changes in the moderately stable P forms. $NaOH-P_i$ and $NaOH-P_o$ are moderately stable inorganic and organic P forms, respectively, in soils. These two P fractions are mainly the part of the P strongly binding to the surfaces of the Fe and aluminium (Al) oxides *via* chemisorption. They are slowly mobilised and available for plant growth. As shown in Figure 5, $NaOH-P_i$ took up a major proportion of the total moderately stable P in the soil while $NaOH-P_o$ took up a small portion. $NaOH-P_i$ increased for the first 7 days, decreased rapidly from day 7 to 14, and then
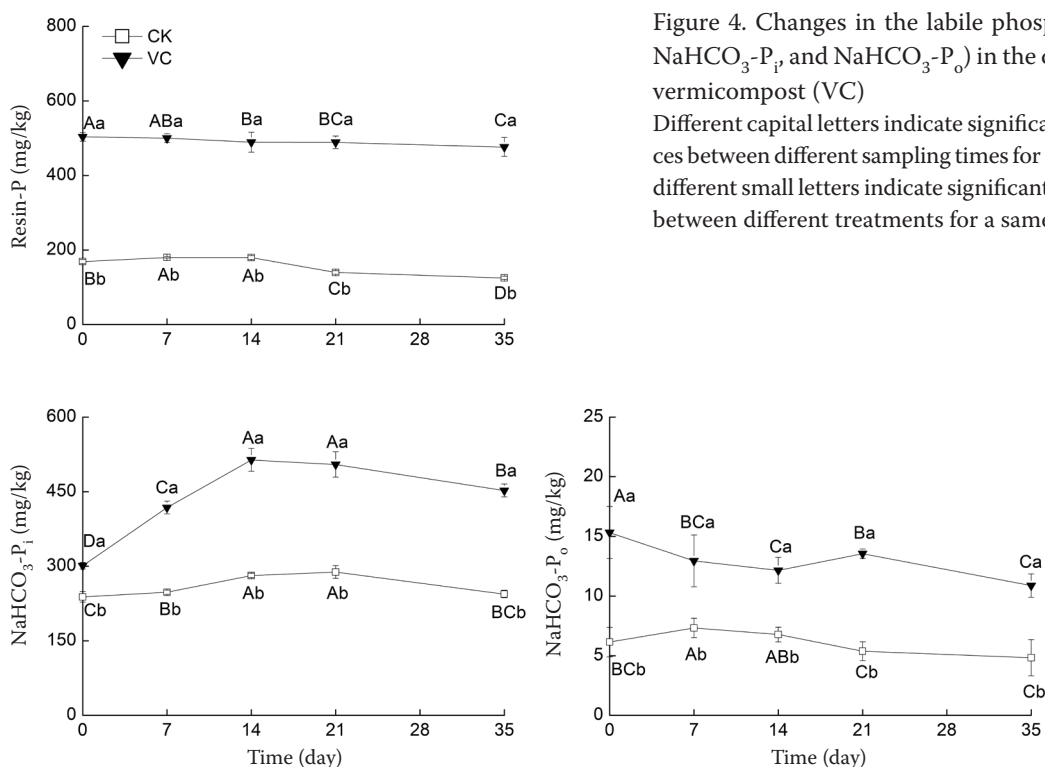
Figure 4. Changes in the labile phosphorus (*i.e.*, resin-P, $NaHCO_3$-P_i, and $NaHCO_3$-P_o) in the control (CK) and the vermicompost (VC)

Different capital letters indicate significant ($P < 0.05$) differences between different sampling times for a same treatment, and different small letters indicate significant ($P < 0.05$) differences between different treatments for a same sampling time

stabilised in both the VC and the CK. At the end of the experiment, NaOH-P_i decreased by 18% and 10% in the VC and the CK, respectively. NaOH-P_o showed a trend of increasing in both the VC and the CK. Both NaOH-P_i and NaOH-P_o were significantly ($P < 0.01$) higher in the VC than in the CK, with differences of 20.7–60.5 mg/kg.

Changes in the stable P forms. D.HCl-P_i, C.HCl-P_i, C.HCl-P_o, and residual-P are stable P forms in soils. D.HCl-P_i is mainly the part of the P associated with apatite. C.HCl-P_i, C.HCl-P_o, and residual-P are hardly

available to plants. As shown in Figure 6, D.HCl-P_i and C.HCl-P_i were the major stable P forms in the soil and changed little with time (Figure 6A, B). D.HCl-P_i was about 338 and 437 mg/kg in the CK and the VC, respectively, and the difference was significant ($P < 0.01$). There was no significant ($P > 0.05$) difference in the C.HCl-P_i between the VC and the CK. C.HCl-P_o increased in the VC, with a rapid increase from day 14 to 21, and it increased from day 0 to 21 and then deceased slightly in the CK (Figure 6C). As a result, C.HCl-P_o was 66% higher on average in
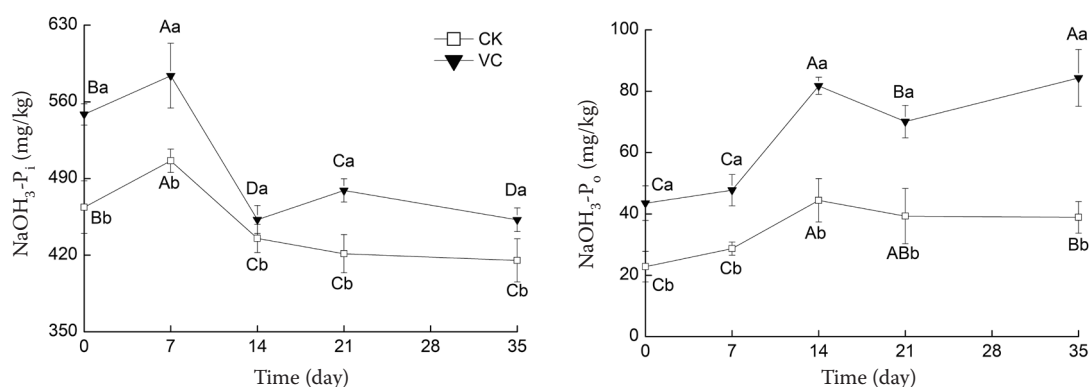

Figure 5. Changes in the moderately stable phosphorus forms (NaOH-P_i and NaOH-P_o) in the control (CK) and the vermicompost (VC)

Different capital letters indicate significant ($P < 0.05$) differences between different sampling times for a same treatment, and different small letters indicate significant ($P < 0.05$) differences between different treatments for a same sampling time

Figure 6. Changes in the stable phosphorus forms D.HCl-P$_i$ (A), C.HCl-P$_i$ (B), C.HCl-P$_o$ (C), and residual-P (D) in the control (CK) and the vermicompost (VC)

Different capital letters indicate significant ($P < 0.05$) differences between different sampling times for a same treatment, and different small letters indicate significant ($P < 0.05$) differences between different treatments for a same sampling time
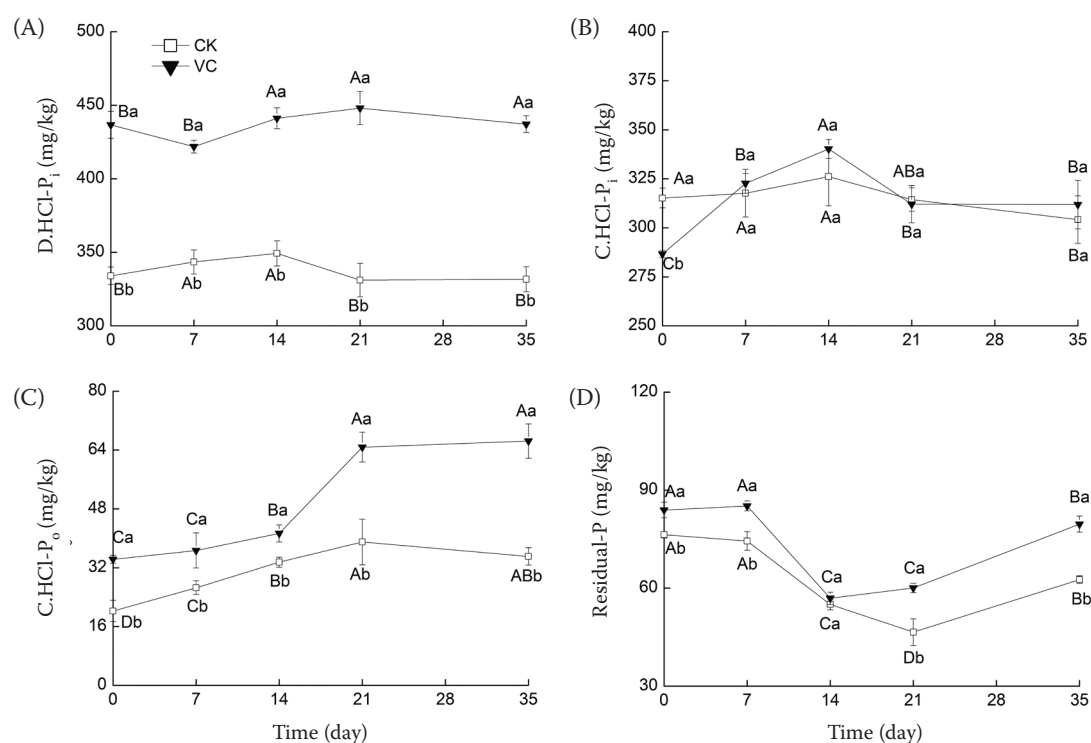
the VC than in the CK for the last two weeks. The vermicompost application significantly ($P < 0.01$) increased the C.HCl-P$_o$ in the soil. The residual-P did not change much in the first 7 days in both the VC and the CK, it decreased rapidly from day 7 to 14 in the VC and from day 7 to 21 in the CK, and then it increased (Figure 6D). The residual-P was significantly ($P < 0.05$) higher in the VC than in the CK at all the sampling days except at day 14.

Correlation between the APA, MBC, MBP, Fe(II), and various P forms. As shown in Table 1, the labile P forms of NaHCO$_3$-P$_i$ and NaHCO$_3$-P$_o$ were significantly ($P < 0.05$) related to the HCl-extractable Fe(II) in the CK while they were significantly ($P < 0.05$) related to the APA, MBC, and MBP in the VC. In addition, they were significantly ($P < 0.01$) related to each other in the VC. The moderately stable P form NaOH-P$_i$ was significantly related to the APA, MBC, MBP, and HCl-extractable Fe(II), while NaOH-P$_o$ was significantly related to the MBP and NaHCO$_3$-P$_i$. In addition, the two P forms were significantly ($P < 0.01$) and negatively related to each other. The residual-P was significantly related to NaHCO$_3$-P$_i$, NaOH-P$_i$, and C.HCl-P$_o$.

DISCUSSION

The soil pH directly influences the forms and bioavailability of the nutrients and the quantity, composition, and activity of the microorganisms. In this study, the pH decreased from day 0 to 21 in the CK (Figure 2). As can be seen from Figure 1, the pakchoi grew rapidly in this period. Consequently, more protons and organic acids might have been excreted by the plants to obtain enough nutrients for the rapid growth, which led to the decrease in the soil pH. The soil pH in the VC was relatively stable. This differs from manure applications, which often lead to a rapid increase in the soil pH. The major reason might be that although the dried vermicompost contains a large amount of small molecule organic acids and humic acids which continuously dissolve and release protons (Muruganandham & Parimala 2018), the produced protons might be consumed by such processes as nitrate reduction, OM decomposition, and Fe(III) reduction. The soil Eh indicates the soil redox intensity and greatly influences the elemental transformation and nutrient availability in the soil. A one-time application of a large amount

Table 1. The correlation between the acid phosphatase activity (APA), microbial biomass carbon (MBC), microbial biomass phosphorus (MBP), hydrochloric acid (HCl)-extractable Fe(II), and various P forms in the CK (without the vermicompost application) and in the VC (with the vermicompost application)

Properties	APA	MBC	MBP	HCl-extractable Fe(II)	Resin-P	$NaHCO_3\text{-}P_i$	$NaHCO_3\text{-}P_o$	$NaOH\text{-}P_i$	$NaOH\text{-}P_o$	$D.HCl\text{-}P_i$	$C.HCl\text{-}P_i$	$C.HCl\text{-}P_o$	Residual-P
CK													
APA	1												
MBC	0.883**	1											
MBP	0.871**	–0.850**	1										
HCl-extractable Fe(II)	0.023	0.508*	–0.171	1									
Resin-P	–0.533*	–0.306	0.001	–0.078	1								
$NaHCO_3\text{-}P_i$	0.216	–0.411	0.384	0.551*	–0.541*	1							
$NaHCO_3\text{-}P_o$	–0.539*	0.242	0.203	–0.550*	0.484	–0.301	1						
$NaOH\text{-}P_i$	–0.574*	–0.655**	–0.758**	–0.599*	0.157	–0.339	–0.223	1					
$NaOH\text{-}P_o$	0.460	0.555*	0.711**	–0.491	–0.210	0.606*	0.038	–0.859**	1				
$D.HCl\text{-}P_i$	–0.122	–0.368	–0.121	–0.729**	0.375	0.134	–0.339	0.365	–0.094	1			
$C.HCl\text{-}P_i$	–0.371	–0.366	–0.318	–0.334	–0.142	0.283	–0.337	0.285	–0.094	0.576*	1		
$C.HCl\text{-}P_o$	–0.179	0.025	0.875**	0.353	–0.664**	0.300	–0.443	0.085	0.198	0.238	0.514*	1	
Residual-P	–0.524*	–0.729**	–0.705**	–0.063	0.529*	–0.873**	0.197	0.595*	–0.782**	–0.008	–0.047	–0.554*	1
VC													
APA	1												
MBC	0.673**	1											
MBP	0.727**	0.628*	1										
HCl-extractable Fe(II)	–0.206	0.526*	0.286	1									
Resin-P	–0.239	0.239	0.200	0.139	1								
$NaHCO_3\text{-}P_i$	0.717**	0.828**	0.566*	–0.449	0.290	1							
$NaHCO_3\text{-}P_o$	–0.859**	–0.570*	–0.522*	0.342	0.309	–0.711**	1						
$NaOH\text{-}P_i$	–0.527*	–0.595*	–0.715**	–0.755**	–0.406	–0.578*	0.347	1					
$NaOH\text{-}P_o$	0.325	0.425	0.878**	0.099	0.130	0.512*	–0.538*	–0.834**	1				
$D.HCl\text{-}P_i$	0.395	0.641**	0.376	–0.487	0.287	0.447	–0.378	–0.430	0.570*	1			
$C.HCl\text{-}P_i$	–0.701**	0.416	0.200	–0.655**	–0.476	0.522*	–0.421	–0.374	0.357	0.527*	1		
$C.HCl\text{-}Po$	–0.189	0.384	0.732**	–0.394	–0.560*	0.499	0.044	–0.031	–0.290	0.287	–0.107	1	
Residual-P	–0.321	–0.628*	–0.229	0.678**	–0.455	–0.775**	0.365	0.668**	–0.381	–0.862**	–0.478	–0.555*	1

$NaHCO_3\text{-}P_i$ – $NaHCO_3$ extracted inorganic P; $NaHCO_3\text{-}P_o$ – $NaHCO_3$ extracted organic P; $NaOH\text{-}P_i$ – NaOH extracted inorganic P; $NaOH\text{-}P_o$ – NaOH extracted organic P; $D.HCl\text{-}P_i$ – diluted HCl extracted inorganic P; $C.HCl\text{-}P_i$ – concentrated HCl extracted inorganic P; $C.HCl\text{-}P_o$ – concentrated HCl extracted organic P; *, ** indicate a significant difference at 0.05 and 0.01, respectively

of organic fertiliser often leads to a rapid decrease in the Eh due to the anaerobic decomposition of the OM. However, only a slight decrease in the Eh was observed in the VC. The reason might be that the vermicompost has a high porosity, which improves the soil aeration. In addition, vermicompost is rich in OM, which greatly stimulates the soil microbial activity and enhances the microbial respiration intensity (García-Sánchez et al. 2017). Iron activity is sensitive to environmental changes and mainly governed by the soil pH and Eh. The soil pH influences the Fe solubility and Fe(III) reduction (Li et al. 2016). In this study, the water-soluble Fe(II) in the VC decreased in the first 14 days and was lower than that in the CK. This might be explained by the fact that the vermicompost application led to a high C/Fe ration and, in turn, the coprecipitation in the VC. Riedel et al. (2013) found that a large amount of dissolved OM (DOM) coprecipitated with the Fe ions on the aeration of anoxic fen pore waters with a C/Fe ration of 10–30. Colombo et al. (2012) reported that Fe ions are readily chelated by the carboxyl and phenolic hydroxyl groups of humic acids to form large aggregates. In addition, the Fe(II) ions in the soil solution were adsorbed by the negatively charged vermicompost particles which have a large specific area. Many studies have demonstrated that Fe mainly exists as Fe oxides in soils and the variable changes in the Fe oxides can strongly adsorb and fix the P (Lü et al. 2017). The higher HCl-extractable Fe(II) in the VC and its clear increase with time might be partly explained by that the aggregates in the VC were rich in OM and provided ubiquitous anaerobic microenvironments, and the reducing products of the OM anaerobic decomposition stimulated the reduction of the Fe oxides in the aggregates. The significantly positive correlation between the HCl-extractable Fe(II) and the microbial biomass in the VC indicated that the rapid reproduction of soil microorganisms after the vermicompost application had stimulated the Fe reduction (Table 1). It might be because the vermicompost application had stimulated the growth of the Fe-reducing microorganisms, of which, Fe-reducing bacteria are the major microorganisms that can dissimilatory reduce Fe(III) to obtain energy for growth (Ding et al. 2014). In addition, the rich small-molecule organic compounds (*e.g.*, carbohydrates, organic acids) in the vermicompost are not only a growth medium for microbes, but also electron donors for the Fe reduction. Vermicompost is rich in humic acid as well, and the quinone moieties in humic acid

play an important role in the Fe reduction by acting as an electron shuttle between the microbes and Fe oxides (Burgos et al. 2003). The synergistic effect of the OM and microbes further enhances the Fe reduction. It is known that Fe reduction results in the activation of Fe oxides, and studies have shown that Fe oxide particles of different activities have different specific surface areas, and in turn different adsorption capacities for P (Zhang et al. 2018). The correlation analysis showed that the HCl-extractable Fe(II) was significantly and negatively related to NaOH-P_i (mainly the part of the inorganic P adsorbed on the surfaces of the Fe and Al oxides) in both the VC ($r = -0.755$) and the CK ($r = -0.599$), and the correlation coefficient was much larger for the VC. This was consistent with the findings of Linghu et al. (2016). The significantly negative correlation between NaHCO$_3$-P_i and NaOH-P_i in the VC indicates that NaOH-P_i (a moderately stable P form) was the major source of NaHCO$_3$-P_i (an available P form), which agrees with the above discussion on the reduction effect of the vermicompost on the Fe(III).

Microorganisms can enhance the P mobilisation. The soil MBC, MBP, and MBC/MBP reflect the activities of the P-solubilising and P-accumulating microorganisms and their P-mineralisation and P-accumulation rates. Phosphorus accumulation by microorganisms inhibits the fixation of the available P by the soil. In this study, the vermicompost application increased the MBP and MBC. In addition, MBC/MBP ratio after 21 days was lower in the VC than in the CK, indicating that the vermicompost application not only increased the MBP by increasing the number of microbes, but also increased the microbial P-fixation by increasing the reproduction of the P-accumulating microbes. Labile P is a direct P nutrition source for plants. However, as it is readily fixed by soil particles, its concentration generally deceases with time and is of a low level at equilibrium. The resin-P concentration was relatively stable in the VC during the 35 days, indicating that the vermicompost application enhanced the P mobilisation. Some researchers believe that under natural conditions, the soil available P is mainly decided by the relative rates of the organic P mineralisation and available P fixation, two processes that occur simultaneously (Ghosh 2018). Acid phosphatase is a protease mainly secreted by plants, but also by some fungi and bacteria. It catalyses the hydrolysis of organic P to produce the phosphate ions available to plants and its activity is affected by many

factors including the pH, available P, and microbes (Margalef et al. 2017). The significantly negative correlation between the APA and resin-P in the CK might be because compared with that in the VC, the inorganic P in the CK decreased significantly with time and its negative feedback inhibition effect on the APA was weakened (Huang et al. 2015). The acid phosphatase activity was significantly and negatively related to $NaHCO_3$-P_o in both the VC ($r = -0.859$, $P < 0.01$) and the CK ($r = -0.539$, $P < 0.05$), implying that the vermicompost application did not drive the $NaHCO_3$-P_o decomposition by stimulating the C and energy need of the microorganisms. In the VC, $NaOH$-P_o increased prominently. It might be explained by the rich organic P in the vermicompost. After the application of the vermicompost, the organic P adsorption by the mineral particles such as the Fe and Al oxides could be significantly enhanced. The soil MBP was significantly and positively related with the APA and $NaOH$-P_o. It is known that the chemical structure of organic P has a great effect on its hydrolysis catalysed by phosphatase. The vermicompost application increased the part of $NaOH$-P_o whose decomposition was more readily catalysed by AP, and consequently, $NaOH$-P_o became the major P source of the P-solubilising and P-accumulating microorganisms. At the end of the experiment, $C.HCl$-P_o rose significantly in both the VC and the CK and was significantly ($P < 0.01$) related to the MBP. The correlation coefficient between the $C.HCl$-P_o and MBP was much larger than those between the $C.HCl$-P_o and resin-P, $NaHCO_3$-P_o, and $NaHCO_3$-P_i, indicating that the formation of $C.HCl$-P_o in the vermicompost-applied soil was closely related to the MBP. It is known that nucleic acids and phospholipids are the major P-containing components of microorganisms. In vermicompost-applied environments, nucleic acids and phospholipids are released when microorganisms die, and the subsequent biochemical processes related to the MBP turnover might be related to the formation of $C.HCl$-P_o.

CONCLUSION

The vermicompost application helped to maintain a proton balance in the paddy soil system, continuously regulated the soil pH, and improved the soil redox environment. Vermicompost had a positive effect on the Fe dissolution and reduction in the paddy soil, raised the HCl-extractable Fe(II) to a

relatively high level, and stimulated the mobilisation of the moderately stable $NaOH$-P_i.

The vermicompost-applied soil was more favourable for microbial growth and reproduction. In addition, the P accumulation by the microorganisms was enhanced in the VC, which inhibited the fixation of labile P by the soil particles. The soil MBP was significantly ($P < 0.01$) and positively related with the APA and $NaOH$-P_o, indicating that the vermicompost application had a stimulating effect on the APA and could effectively enhance the $NaOH$-P_o mobilisation. In addition, the mineralisation of the rich $NaOH$-P_o in the VC enhanced the P accumulation by the soil's microorganisms.

The vermicompost application significantly raised the contents of the various P forms except for the stable $C.HCl$-P_i, with the increases in the labile P contents being the most prominent. The vermicompost application enhanced the formation of $C.HCl$-P_o while it enhanced the mineralisation of $NaHCO_3$-P_o and $NaOH$-P_o. A significantly ($P < 0.01$) positive correlation between $C.HCl$-P_o and MBP was found.

REFERENCES

Arancon N., Edwards C., Bierman P. (2006): Influences of vermicomposts on field strawberries: Part 2. Effects on soil microbiological and chemical properties. Bioresource Technology, 97: 831–840.

Bekele A., Kibret K., Bedadi B., Balemi T., Yli-Halla M. (2018): Effects of lime, vermicompost and chemical P fertilizer on yield of maize in Ebantu District, Western highlands of Ethiopia. African Journal of Agricultural Research, 13: 477–489.

Bhat S.A., Singh S., Singh J., Kumar S., Vig A.P. (2018): Bioremediation and detoxification of industrial wastes by earthworms: Vermicompost as powerful crop nutrient in sustainable agriculture. Bioresource Technology, 252: 172–179.

Bouwman A., Beusen A., Lassaletta L., Van Apeldoorn D., Van Grinsven H., Zhang J. (2017): Lessons from temporal and spatial patterns in global use of N and P fertilizer on cropland. Scientific Reports, 7: 40366.

Burgos W.D., Fang Y., Royer R.A., Yeh G.-T., Stone J.J., Jeon B.-H., Dempsey B.A. (2003): Reaction-based modeling of quinone-mediated bacterial iron(III) reduction. Geochimica et Cosmochimica Acta, 67: 2735–2748.

Colombo C., Palumbo G., Sellitto V.M., Rizzardo C., Tomasi N., Pinton R., Cesco S. (2012): Characteristics of insoluble, high molecular weight iron-humic substances used as plant iron sources. Soil Science Society of America Journal, 76: 1246–1256.

De Araújo L.M.C., Sampaio E.V.D.S.B., De Araújo M.D.S.B. (2015): Phosphorus desorption from Fe and Al oxides mediated by soil microorganisms. Communications in Soil Science and Plant Analysis, 46: 633–640.

de Brito Neto J. F., Bull L.T., da Silva A., Pereira e.L., Soares C.S., de Lima Junior J.A. (2018): Phosphorus adsorption and its relationship to the physical and chemical characteristics with different soil classes. African Journal of Agricultural Research, 13: 419–424.

Ding X., Zhang S., Wang R., Liao X., Li S. (2014): Exogenous labile C application enhances Fe-P utilization for mycorrhizal plants through iron-reducing bacteria in subtropical soil. Journal of Soil Science and Plant Nutrition, 14: 803–817.

García-Sánchez M., Taušnerová H., Hanč A., Tlustoš P. (2017): Stabilization of different starting materials through vermicomposting in a continuous-feeding system: Changes in chemical and biological parameters. Waste Management, 62: 33–42.

Ge Y., Wang Q., Wang L., Liu W., Liu X., Huang Y., Christie P. (2018): Response of soil enzymes and microbial communities to root extracts of the alien *Alternanthera philoxeroides*. Archives of Agronomy and Soil Science, 64: 708–717.

Ghosh A. (2018): Phosphorus mineralization in an alluvial soil as influenced by organic manure addition and time of incubation. International Journal of Chemical Studies, 6: 1727–1730.

Giles C.D., George T.S., Brown L.K., Mezeli M.M., Richardson A.E., Shand C.A., Wendler R., Darch T., Menezes-Blackburn D., Cooper P. (2017): Does the combination of citrate and phytase exudation in *Nicotiana tabacum* promote the acquisition of endogenous soil organic phosphorus? Plant and Soil, 412: 43–59.

Guppy C.N., Menzies N.W., Moody P.W., Blamey F.P.C. (2005): Competitive sorption reactions between phosphorus and organic matter in soil: A review. Soil Research, 43: 189–202.

Huang L., Zhang Y., Shi Y., Liu Y., Wang L., Yan N. (2015): Comparison of phosphorus fractions and phosphatase activities in coastal wetland soils along vegetation zones of Yancheng National Nature Reserve, China. Estuarine, Coastal and Shelf Science, 157: 93–98.

Kumar A., Prakash C.B., Brar N.S., Kumar B. (2018): Potential of vermicompost for sustainable crop production and soil health improvement in different cropping systems. International Journal of Current Microbiology and Applied Sciences, 7: 1042–1055.

Lü C., Yan D., He J., Zhou B., Li L., Zheng Q. (2017): Environmental geochemistry significance of organic phosphorus: An insight from its adsorption on iron oxides. Applied Geochemistry, 84: 52–60.

Li H., Li X., Dou Z., Zhang J., Wang C. (2012): Earthworm (*Aporrectodea trapezoides*)-mycorrhiza (*Glomus intraradices*) interaction and nitrogen and phosphorus uptake by maize. Biology and Fertility of Soils, 48: 75–85.

Li S., Li Y., Huang X., Hu F., Liu X., Li H. (2018): Phosphate fertilizer enhancing soil erosion: Effects and mechanisms in a variably charged soil. Journal of Soils and Sediments, 18: 863–873.

Li X., Zhang W., Liu T., Chen L., Chen P., Li F. (2016): Changes in the composition and diversity of microbial communities during anaerobic nitrate reduction and Fe(II) oxidation at circumneutral pH in paddy soil. Soil Biology and Biochemistry, 94: 70–79.

Lidbury I.D., Fraser T., Murphy A.R., Scanlan D.J., Bending G.D., Jones A.M., Moore J.D., Goodall A., Tibbett M., Hammond J.P. (2017): The 'known'genetic potential for microbial communities to degrade organic phosphorus is reduced in low-pH soils. MicrobiologyOpen, 6: e00474.

Lim S.L., Wu T.Y., Lim P.N., Shak K.P.Y. (2015): The use of vermicompost in organic farming: Overview, effects on soil and economics. Journal of the Science of Food and Agriculture, 95: 1143–1156.

Linghu R.Y., Wang R.P., Liang J.W., Liao X.R., Zhan Z.S., Wu Y.G. (2016): Effects of iron reducing bacteria on the transformation of phosphorus in vegetable red soils. Journal of Agro-Environment Science, 35: 1742–1749.

Lu R.K. (2000): The Analytic Method of Soil and Agricultural Chemistry. Beijing, China Agricultural Science and Technology Press.

Maranguit D., Guillaume T., Kuzyakov Y. (2017): Land-use change affects phosphorus fractions in highly weathered tropical soils. Catena, 149: 385–393.

Margalef O., Sardans J., Fernández-Martínez M., Molowny-Horas R., Janssens I., Ciais P., Goll D., Richter A., Obersteiner M., Asensio D. (2017): Global patterns of phosphatase activity in natural soils. Scientific Reports, 7: 1337.

Menezes-Blackburn D., Giles C., Darch T., George T.S., Blackwell M., Stutter M., Shand C., Lumsdon D., Cooper P., Wendler R. (2018): Opportunities for mobilizing recalcitrant phosphorus from agricultural soils: A review. Plant and Soil, 427: 5–16.

Moghimi N., Hosseinpur A., Motaghian H. (2018): The effect of vermicompost on transformation rate of available P applied as chemical fertilizer in a calcareous clay soil. Communications in Soil Science and Plant Analysis, 49: 2131–2142.

Muruganandham M., Parimala S.A.G. (2018): Evolution of humic material in different organic waste during vermicomposring using *Perionyx ceylanensis*. International Journal of Zoology Studies, 3: 48–52.

Nishigaki T., Sugihara S., Kobayashi K., Hashimoto Y., Kilasara M., Tanaka H., Watanabe T., Funakawa S. (2018): Fractionation of phosphorus in soils with different geological and soil physicochemical properties in southern Tanzania. Soil Science and Plant Nutrition, 63: 291–299.

Pierart A., Maes A.Q., Dumat C., Sejalon-Delmas N. (2019): Vermicompost addition influences symbiotic fungi communities associated with leek cultivated in metal-rich soils. Environmental Science and Pollution Research, 26: 20040–20051.

Richardson A.E., Lynch J.P., Ryan P.R., Delhaize E., Smith F.A., Smith S.E., Harvey P.R., Ryan M.H., Veneklaas E.J., Lambers H. (2011): Plant and microbial strategies to improve the phosphorus efficiency of agriculture. Plant and Soil, 349: 121–156.

Riedel T., Zak D., Biester H., Dittmar T. (2013): Iron traps terrestrially derived dissolved organic matter at redox interfaces. Proceedings of the National Academy of Sciences, 110: 10101–10105.

Syers J., Johnston A., Curtin D. (2008): Efficiency of Soil and Fertilizer Phosphorus Use. Reconciling Changing Concepts of Soil Phosphorus Behaviour with Agronomic Information. FAO Fertilizer and Plant Nutrition Bulletin, Rome, FAO.

Tiessen H., Stewart J., Moir J. (1983): Changes in organic and inorganic phosphorus composition of two grassland soils and their particle size fractions during 60–90 years of cultivation. Journal of Soil Science, 34: 815–823.

Von Sperber C., Stallforth R., Du Preez C., Amelung W. (2017): Changes in soil phosphorus pools during prolonged arable cropping in semiarid grasslands. European Journal of Soil Science, 68: 462–471.

Wei K., Sun T., Tian J., Chen Z., Chen L. (2018): Soil microbial biomass, phosphatase and their relationships with phosphorus turnover under mixed inorganic and organic nitrogen addition in a *Larix gmelinii* plantation. Forest Ecology and Management, 422: 313–322.

Xu G., Shao H.B., Sun J.N., Chang S.X. (2012): Phosphorus fractions and profile distribution in newly formed wetland soils along a salinity gradient in the Yellow River Delta in China. Journal of Plant Nutrition and Soil Science, 175: 721–728.

Yokoyama D., Mori T., Wagai R., Hiradate S., Kitayama K. (2018): Characteristics of phosphorus fractions in the soils derived from sedimentary and serpentinite rocks in lowland tropical rain forests, Borneo. Soil Science and Plant Nutrition, 64: 218–221.

Yu H.Y., Liu C.P., Zhu J.S., Li F.B., Deng D.M., Wang Q., Liu C.S. (2016): Cadmium availability in rice paddy fields from a mining area: The effects of soil properties highlighting iron fractions and pH value. Environmental Pollution, 209: 38–45.

Zhang G.S., Xue J.X., Ni Z.W., Li J.C. (2018): Phosphorus accumulation and sorption characteristics of P-enriched soils in the Dian Lake basin, southwestern China. Journal of Soils and Sediments, 18: 887–896.

Zhou Y., Zhu H., Yao Q. (2018): Contrasting P acquisition strategies of the bacterial communities associated with legume and grass in subtropical orchard soil. Environmental Microbiology Reports, 10: 310–319.