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Full Length Article

Accumulation of Soil Available Phosphorus under Pig Manure Application Limits Microbial Community Function in Upland Soils

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Abstract

In this study, a two-year pot experiment with a peanut (*Arachis hypogaea* L.) and radish (*Raphanus sativus* L.) rotation system was conducted to investigate changes in soil chemical properties and microbial characteristics and to determine the internal relationship of these variables after pig manure application. The selected soil types include Agri-Udic Ferrosols, Ali-Udic Argosols and Ali-Udic Cambisols, which are widely distributed throughout subtropical China. The annual pig manure application amounts were 25, 50, 100, 200, 400 and 800 kg P ha^{-1}. The results of our experiments showed that the soil pH (R^2=0.57–0.87, P<0.01), organic carbon (R^2=0.73–0.91, P<0.01), total nitrogen (R^2=0.51–0.74, P<0.01), total phosphorus (R^2=0.81–0.94, P<0.01), available nitrogen (R^2=0.71–0.92, P<0.01) and available phosphorus (R^2=0.89–0.97, P<0.01) were positively and linearly correlated with the microbial biomass carbon/nitrogen in the three soils. The relationship between the functional diversity of the microbial community and the available phosphorus was parabolic. According to the most pronounced changes (i.e., the highest values), we deduced that the rational application rate of pig manure should be 100–200 kg P ha^{-1} in the three soils, which was verified by a lack of significant increase in peanut pod yield when the deduced fertilizer level was exceeded. In brief, our results indicated that excessive accumulation of soil available phosphorus under pig manure application reduced microbial community function, implying that the excessive application of pig manure could reduce soil quality.

Keywords: Pig manure; Microbial biomass; Microbial functional diversity; Available phosphorus; Parabolic equation

Introduction

As one valuable soil amendment, the field application of pig manure increases the soil pH, organic matter and available nutrients for crop growth (Kotzerke *et al*., 2008). The soil microbial biomass and enzyme activities show significant increases under the application of pig manure compared with chemical fertilizers (Lalande *et al*., 2000; Rochette *et al*., 2000; Peacock *et al*., 2001). The soil microbial properties are more sensitive and can rapidly respond to changes in the soil conditions and environmental disturbances compared with the chemical or physical properties, as key participators in the soil biochemical properties and ecosystem function (Joergensen and Emmerling, 2006; Verdenelli *et al*., 2013). To a greater extent, potentially important impacts may be observed in the microbial properties before they were reflected in other soil properties (Larkin *et al*., 2006), which are considered potentially sensitive, early and effective indicators of changes in the soil quality (Clemente *et al*., 2007).

The responses of soil microbial properties to pig manure amendments have been related to variations in the chemical properties of the soil. Microbial activity has typically been constrained by soil organic matter, which is the main source for soil microorganisms (Demoling *et al*., 2007; Senesi *et al*., 2007). Furthermore, soil pH is likely to be at least as important as soil organic matter in influencing the size of the microbial biomass (Wardle, 2002; Pietri and Brookes, 2008). Studies investigating the nutrient limitation of microbes in long-term field experiments emphasized the important role of phosphorus in increasing the biomass and activity of soil microorganisms (Chu *et al*., 2007), although nitrogen frequently limits soil microbial communities (Gilliam *et al*., 2011). However, it remains unclear whether the microbial properties had a quantitative dose-effect relationship with soil chemical properties upon manure application.

Several approaches used to characterize soil microorganisms, such as soil microbial biomass, Biolog carbon source substrate utilization, soil phospholipid fatty

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acid (PLFA) and PCR-denaturing gradient gel electrophoresis (PCR-DGGE) have been developed to evaluate the soil quality. Biolog carbon source substrate utilization is often used to analyze the community functional aspects of soil microbes (Zak *et al*., 1994), while PLFA profiles and PCR-DGGE are used to analyze the soil microbial structure (Bossio *et al*., 1998). Microbial biomass is one of the most classical and traditional used parameters in soil biology (Araújo and Monteiro, 2006), and the use of carbon substrates in Biolog Eco-Plate analysis is a reliable and sensitive method for detecting short-term changes in microbial functional diversity in response to amendment applications (Gomez *et al*., 2006). Moreover, microbial biomass and Biolog Eco-Plate methods are commonly used for quantitative analyses, while other approaches mostly analyze the effects of soil environmental change on microbial properties from a qualitative perspective.

Appropriate application of pig manure increased soil nutrient content and microbial activity (Zhang *et al*., 2012; Lupwayi *et al*., 2014); however, its excessive application had no significant positive effect on crop production (Liu *et al*., 2009). Large amounts of fertilizers are required to maintain crop production, even in soil with a higher nutrient content (He *et al*., 2013; Zhao *et al*., 2014); thus, it has been speculated that the excessive retention of fertilizers within the soil is likely to decrease nutrient efficiency due to decreased microbial community function. Therefore, we conducted a two-year pot experiment to investigate the correlation between soil chemical and microbial properties in response to manure application in soils under peanut and radish rotation systems.

The objectives of this study were to (i) investigate the effects of pig manure application on the properties of upland soil in subtropical China, (ii) determine the internal correlations between the chemical and microbial characteristics upon the application of pig manure and (iii) evaluate the factors influencing the decrease in microbial community function.

Materials and Methods

The experiments were conducted at the Ecological Experimental Station of Red Soil, Chinese Academy of Soil Science, Yujiang County, Jiangxi. Three dominating upland soils in subtropical China, Agri-Udic Ferrosols (Oxisols, US ST) from red clay, Ali-Udic Argosols (Ultisols, US ST) from red sandstone and Ali-Udic Cambisols (Inceptisols, US ST) from granite soil were collected to fill plastic buckets (55 cm height and 34 cm diameter) according to the original layers in the field. The original 0–20 cm and 20–50 cm layers of soil were used to fill separate buckets from 0–20 cm and 20–50 cm, respectively (Fig. 1). The initial soil properties are listed in Table 1.

The pot experiment was performed with eight treatments: the annual application rates of pig manure were 25 (P1), 50 (P2), 100 (P3), 200 (P4), 400 (P5) and 800 (P6) kg P ha^{-1}, with half of the local conventional chemical fertilization rate. Chemical fertilizer application at the local conventional fertilization rate was termed “CF”. The control treatment “CK” had no allochthonous organic amendments or chemical fertilizers. Conventional chemical fertilization involved the application of 100 kg ha^{-1} nitrogen, 50 kg ha^{-1} phosphorus and 100 kg ha^{-1} potassium and the fertilizer varieties applied were urea, calcium magnesium phosphate and potassium chloride, respectively. Pig manure with an average total phosphorus content of 12.55 g kg^{-1}, total nitrogen content of 28.8 g kg^{-1}, total carbon content of 304.5 g kg^{-1} (dry matter basis) and water content of 69% was collected from pig farms near the experimental station. Each treatment included three replicates arranged randomly.

Peanut-radish (*Arachis hypogaea* L.–*Raphanus sativus* L.) rotation, a common upland farming system in the hilly red soil region of subtropical China, was used in this experiment in 2012 and 2013. Prior to peanut sowing, surface soil samples (0–20 cm) were mixed with fertilizers and used as a basic fertilizer during each growing season. No fertilizers were applied during the peanut and radish growing periods. Peanuts were sown in mid-April and harvested in mid-August. Radish, as a catch crop, was then sown in the beginning of September and harvested in the beginning of December. December to March of the following year was a leisure period. The peanuts were planted at a density of two plants per pot and radishes were planted at a density of four plants per pot. The experiments were conducted under natural rainfall conditions and the fields were managed in the style of the local farmers with the exception of weeding by hand.

Soil Sample Collection

After harvesting the radish plants in 2013, soil samples (0–20 cm depth) from every treatment condition were collected and divided into two groups. One group was naturally air-dried at room temperature and passed through a 0.149-mm sieve to analyze the soil organic carbon, total nitrogen (total N) and total phosphorus (total P). Soil samples sifted through a 2 mm sieve were used to analyze the available nitrogen (available N) and available phosphorus (available P). The second group was directly milled with a gavel and passed through a 2 mm sieve for immediate water content and microbial analysis.

Soil Sample Analysis

The soil pH was measured with a glass electrode using a soil-to-water ratio of 1:2.5 (Lu, 1999). The soil organic carbon was determined using the potassium dichromate oxidation and semi-micro Kjeldahl nitrogen methods (Lu, 1999). The total N was determined via the oxidation of sulfuric acid and the semi-micro Kjeldahl nitrogen method (Lu, 1999). The total P was analyzed by acid digestion and determined via the colorimetric method (Lu, 1999). The available N was analyzed via the alkaline hydrolysis diffusion method and the available P was extracted with 0.5 M $NaHCO_3$ and determined via the colorimetric method. Soil water content was determined by oven drying (105°C for 8 h) (Lu, 1999).

The soil microbial biomass was measured using the chloroform-fumigation extraction method and determined using a multi N/C 3100 TOC analyzer (Analytik Jena, Germany). The microbial biomass carbon (MBC) was estimated from the difference in the extractable organic C of fumigated and non-fumigated soil (Vance *et al*., 1987)

$MBC = E_C \times 2.64$,

Where E_C refers to the difference in the extractable organic C between the fumigated and non-fumigated treatments and 2.64 is the proportionality factor for the biomass C that is released via fumigation extraction. The microbial biomass nitrogen (MBN) was calculated using the following equation (Brookes *et al*., 1985): $MBN = F_N/0.54$, where F_N = (total N from fumigated soil) - (total N from non-fumigated soil).

The patterns of potential carbon source utilization by the soil microbial communities with different fertilizer treatments were assessed using a Biolog Eco-Plate system (Biolog Inc., Hayward, CA, USA). The microbial community functional richness, diversity and evenness were analyzed using the Shannon-Wiener, Simpson and Pielouindices, respectively (Preston-Mafham *et al*., 2002).

Crop Yield Measurements

The peanut pods and straws with roots were separately harvested by hand at maturity. After being cleaned with water and air-dried to a constant weight.

Statistical Analysis

The means (n=3) and standard deviations (S.D.) of the soil pH, soil organic C, total N and P, available N and P, microbial biomass carbon/nitrogen and peanut yield are presented. Significant differences among the fertilization treatments were assessed using one-way analysis of variance (ANOVA) followed by the least significant difference test at a 5% probability level. The associations between the microbial properties and soil chemical properties were tested using a redundancy discrimination analysis (RDA) (CANOCO 4.5). The correlation between the available P and microbial community function was determined by Origin version 8 software.

Results

Variability of Peanut Pod Yield

Fertilization significantly increased peanut pod yield (P<0.05) compared to the control treatment (CK) (Fig. 2). The peanut pod yield was significantly increased in treatments P2-P6 in Agri-Udic Ferrosols and Ali-Udic Cambisols and in treatments P3-P6 in Ali-Udic Argosols compared to the yield obtained with chemical fertilizer treatment (CF). However, there was no significant yield increase when the application level was increased beyond P3 in Agri-Udic Ferrosols and beyond P4 in Ali-Udic Argosols and Ali-Udic Cambisols.

Variability of the Soil Chemical Properties

Application of pig manure at different rates in Agri-Udic Ferrosols significantly increased the soil pH (P<0.05) compared to the CK and CF treatments (Table 2). The pH significantly increased when the application level was increased beyond P4 in Ali-Udic Argosols and beyond P2 in Ali-Udic Cambisols. The more highly concentrated manure treatments caused a significant increase in the soil organic carbon, total N/P and available N/P compared to the CF treatment. However, the rates of increase differed, with available P showing the highest rate of increase. For example, compared to the CF treatment, the highly concentrated manure treatments (P3-P6) caused 3.66–29.54 fold increases in the available P in the three soils, while available N increased by 0.40–1.06 fold.

Variability in the Soil Microbial Biomass and Community Function

Fertilization caused significant increases in the soil microbial biomass C (MBC) compared to the CK treatment (Fig. 3). When fertilization was increased beyond the levels of P4 in Agri-Udic Ferrosols, P3 in Ali-Udic Argosols and P3 in Ali-Udic Cambisol, the MBC was significantly increased compared to its level under CF treatment. The microbial biomass N (MBN) was less affected than MBC under low pig manure application in the three soils, while the MBN increased significantly with treatments P4-P6 in Agri-Udic Ferrosols, P6 in Ali-Udic Argosols and P6 in Ali-Udic Cambisol compared to the CK and CF treatments. The Shannon-Wiener, Simpson and Pielou indices gradually increased from P1 to P4 in Agri-Udic Ferrosols and Ali-Udic Argosols and from P1 to P3 in Ali-Udic Cambisol; however, as pig manure application increased beyond these levels, these indices significantly decreased (Fig. 4).

Contribution of Soil Chemical Properties to the Changes in Microbial Properties

The RDA was used to further establish links between the soil chemical variables and microbial properties (Fig. 5). Soil available P had the strongest effect (longest vector length) on the properties of microbes (R^2=0.89–0.97, P<0.01) in the three soils. As shown by the vector bio-plot, soil chemical variables such as soil pH (R^2=0.57—0.87, P<0.01), organic carbon (R^2=0.73—0.91, P<0.01), total

Table 1: Physical and chemical properties of surface soils (0－20 cm)

Soil type	pH (H_2O)	Organic C (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Available N (mg kg^{-1})	Available P (mg kg^{-1})	Silt (0.002-0.02 mm) (%)	Clay (<0.002 mm) (%)
Agri-Udic Ferrosols	4.64	5.16	0.50	0.40	59.50	28.34	20.68	45.92
Ali-Udic Argosols	4.51	2.82	0.30	0.11	48.50	5.82	7.88	15.44
Ali-Udic Cambisols	5.44	3.92	0.41	0.28	58.48	20.40	11.61	6.79

Table 2: Soil chemical properties after two years of pig manure application in the three soils (0－20 cm)

Soil type	Treatment	pH	Organic C (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Available N (mg kg^{-1})	Available P (mg kg^{-1})
Agri-Udic Ferrosols	CK	4.52±0.05e	5.23±0.30d	0.64±0.05d	0.55±0.07c	32.88±2.61e	1.12±0.41g
	CF	4.73±0.01d	5.43±0.54d	0.64±0.05d	0.58±0.08c	51.55±5.61d	9.97±1.89f
	P1	4.84±0.02d	5.30±0.67d	0.63±0.04d	0.58±0.04c	50.43±3.68d	21.54±1.61e
	P2	4.74±0.04d	5.41±0.69d	0.70±0.06d	0.60±0.08c	54.10±5.81cd	24.03±3.28e
	P3	5.17±0.04c	5.59±0.09d	0.75±0.01cd	0.77±0.03c	62.13±6.68c	49.21±4.44d
	P4	5.28±0.12c	7.24±0.68c	0.92±0.18bc	0.89±0.21b	81.38±7.91b	83.57±8.04c
	P5	5.88±0.14b	8.87±0.03b	0.99±0.05b	0.87±0.18b	89.83±5.20b	112.4±10.8b
	P6	6.38±0.02a	10.95±1.02a	1.20±0.18a	1.05±0.09a	106.6±11.84a	185.1±12.0a
Ali-Udic Argosols	CK	4.46±0.05c	3.82±0.18b	0.44±0.05b	0.21±0.01d	29.20±2.12d	0
	CF	4.59±0.06c	3.96±0.25b	0.41±0.06b	0.25±0.01d	45.33±2.12c	4.06±0.86e
	P1	4.68±0.13c	4.23±0.30b	0.46±0.02b	0.24±0.02d	47.78±6.37c	9.88±1.65e
	P2	4.87±0.09bc	4.25±0.41b	0.42±0.07b	0.26±0.05d	52.68±5.74c	11.64±1.59e
	P3	4.88±0.11bc	4.65±0.48b	0.45±0.06b	0.28±0.05d	53.90±5.61c	28.75±3.14d
	P4	5.21±0.06b	5.58±0.73a	0.51±0.10b	0.40±0.02c	63.48±3.68b	42.33±7.84c
	P5	5.86±0.14a	5.69±0.50a	0.56±0.04b	0.47±0.03b	65.83±9.72b	69.14±9.56b
	P6	6.02±0.02a	6.15±0.07a	0.72±0.11a	0.64±0.06a	77.18±6.37a	124.0±16.7a
Ali-Udic Cambisols	CK	5.37±0.05e	4.34±0.41c	0.45±0.08d	0.23±0.01c	31.42±6.46d	0
	CF	5.42±0.03de	4.17±0.29c	0.47±0.02d	0.24±0.02c	42.26±3.87c	5.64±1.05e
	P1	5.45±0.13de	4.80±0.90c	0.50±0.08d	0.30±0.05c	45.64±4.39c	8.38±1.06e
	P2	5.61±0.09cd	5.17±0.68c	0.56±0.06cd	0.34±0.04c	48.72±2.12c	11.38±1.27e
	P3	5.67±0.03c	5.14±0.55c	0.62±0.06cd	0.36±0.03c	51.41±5.91c	26.33±2.06d
	P4	6.19±0.16b	7.52±0.10b	0.69±0.08bc	0.59±0.06b	61.70±6.97b	40.79±5.63c
	P5	6.31±0.08b	7.53±0.98b	0.72±0.14b	0.61±0.11b	62.55±2.93b	61.54±5.44b
	P6	6.61±0.03a	8.97±0.89a	0.88±0.16a	0.82±0.07a	74.70±3.87a	94.38±10.8a

Values within the same column not followed by the same letter differ significantly (P<0.05)

nitrogen (R^2=0.51—0.74, P<0.01), total phosphorus (R^2=0.81—0.94, P<0.01) and available nitrogen (R^2=0.71—0.92, P<0.01) were all positively and linearly correlated with MBC and MBN. However, the selected soil chemical variables were not significantly linearly correlated with the functional diversity of the microbial community. Among these variables, the available P had a vertical relationship with the functional richness, diversity and evenness of the microbial community, indicating no linear correlation.

The Relationship between Available P and Microbial Community Function

Because the soil available P had the strongest effect on microbial properties in the three soils, the relationship between available P and microbial community function was further investigated. The richness (Shannon-Wiener), diversity (Simpson) and evenness (Pielou) indices all fit well to parabolic equations with the available P in Agri-Udic Ferrosols (R^2=0.52, 0.77 and 0.74, respectively, P<0.05), Ali-Udic Argosols (R^2=0.56, 0.70 and 0.63, respectively, P<0.05) and Ali-Udic Cambisol (R^2=0.52, 0.54 and 0.53, respectively, P<0.05) (Fig. 6). The fitting degree of diversity (Simpson) and evenness (Pielou) indices decreased in the following order: Agri-Udic Ferrosols > Ali-Udic Argosols > Ali-Udic Cambisol. This result was in accordance with the accumulation of available P in the three soils (Table 2). Before the apex of the parabola, the microbial community function exhibited significant increases as available P increased. However, there was a significant decrease in microbial community function after the apex. The critical available P concentrations were 66.2, 64.2 and 64.2 mg kg^{-1} for the Shannon-Wiener, Simpson and Pielou indices, respectively, in Agri-Udic Ferrosols; these values were 37.2, 38.9 and 37.2 mg kg^{-1} in Ali-Udic Argosols and 34.3, 38.9 and 37.2 mg kg^{-1} in Ali-Udic Cambisol, respectively.

Discussion

Microorganisms participate in the transformation and mineralization of organic matter and nutrient re-establishment; thus, soil nutrient cycling can affect microbial decomposition and metabolism (Bending *et al.*, 2002; Iqbal *et al.*, 2014). This study provided further evidence for the observed chemical properties of upland soil in subtropical China, including soil pH, organic C, total N/P and available N/P, which directly and positively affect microbial biomass C and N. This result agreed with those of other researchers who demonstrated a statistically significant relationship between the pH, organic C and soil

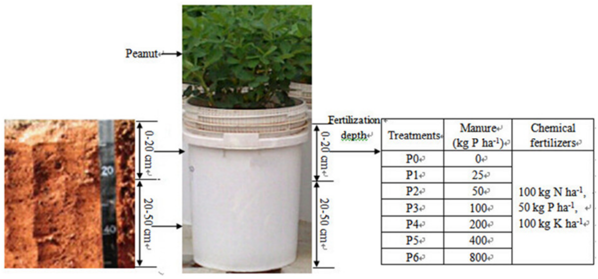

Fig. 1: Setup of the pot experiment

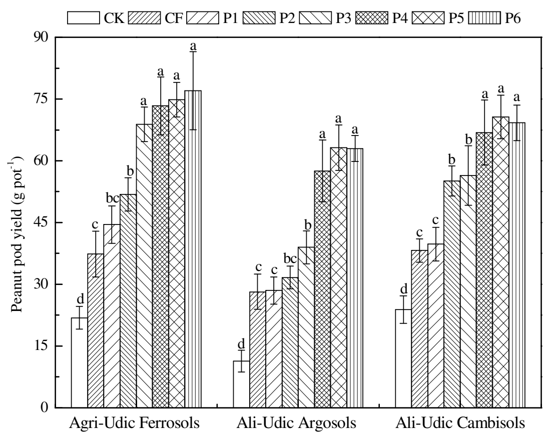

Fig. 2: Peanut pod yield in the three soils with pig manure application

For each soil, different letters indicate significant differences among the fertilization treatments at $P<0.05$.The error bars represent the standard deviation. CK: control treatment, CF: conventional chemical fertilization, P1－P6: pig manure at 25, 50, 100, 200, 400 and 800 kg P ha^{-1}with half the amount of conventional chemical fertilizer

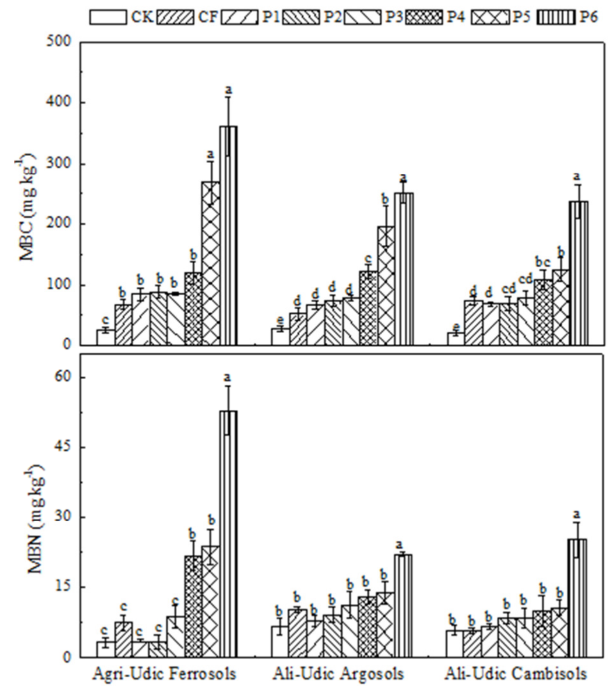

Fig. 3: Carbon and nitrogen levels in soil microbial biomass in the three soils with pig manure application

For each soil, different letters indicate significant differences among the fertilization treatments at $P<0.05$.The error bars represent the standard deviation. CK: control treatment, CF: conventional chemical fertilization, P1－P6: pig manure at 25, 50, 100, 200, 400 and 800 kg P ha^{-1}with half the amount of conventional chemical fertilizer

microbial biomass in arable soil (Brookes *et al*., 1985; Aciego Pietri and Brookes, 2008; Liu *et al*., 2009). Carbon and N are important factors governing the growth of microorganisms and differences in soil nutrients may cause variations in soil microbial activity, with higher levels supporting greater microbial biomass and activity (Vance and Chapin, 2001; Stark *et al*., 2007). Previous studies found that P is the most limiting element for biological activity in tropical forest soils. Declining P availability directly affects microbial processes and significantly restricts C/N cycling, eventually limiting soil microbial activity (Cleveland *et al*., 2002; Vitousek *et al*., 2010). A similar result was obtained in the current study, which determined that available P (R^2=0.89–0.97, $P<0.01$) was better correlated with microbial biomass than other soil chemical properties such as soil organic matter (R^2=0.73–0.91, $P<0.01$) and available N (R^2=0.71–0.92, $P<0.01$). This result suggests that the rapid accumulation of P with increasing manure application could greatly influence soil microbial biomass.

The functional diversity of microbial communities is widely used as a metric of microbial performance, especially in the influence of disturbances (Pignataro *et al*., 2012), and this functional diversity is affected by soil chemical properties. Zhong and Cai (2007) found that the functional diversity of microbial communities increased by 4.32–22% when P was applied (compared with no P application); nevertheless, the increase was only 0.49–3.39% under N application (compared with no N application) in red clay soil (Agri-Udic Ferrosols) in a long-term experiment. However, there is no evidence to support that the functional diversity of a microbial community consistently increases with increasing P concentration in soil. Our results demonstrated that the relationship between the microbial community function and the available soil P concentration was parabolic in the three soils. Excessive accumulation of P in the soils significantly decreases the functional diversity of the microbial community, partially because the excessive accumulation alters the microenvironment of the microbial habitat (Vitousek *et al*., 2010). Moreover, the activities of certain dominating microorganisms are likely enhanced under P eutrophication, and these microorganisms produce enough toxic metabolites to cause a decrease in the microbial diversity (Torsvik *et al*., 2002). In addition, excessive increases in soil available P can alter the sorption

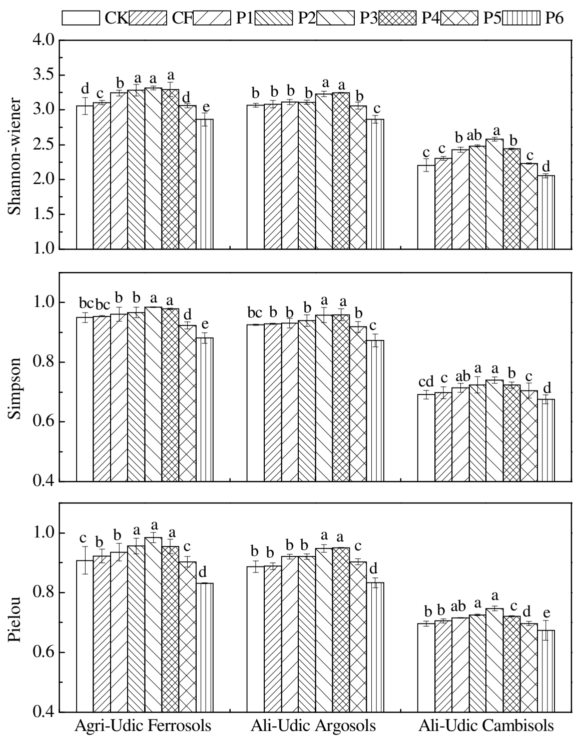

Fig. 4: Soil microbial community richness (Shannon-Wiener), diversity (Simpson) and evenness (Pielou) in the three soils with pig manure application

In each soil, different letters indicate significant differences among the fertilization treatments at P<0.05.The error bars represent the standard deviation. CK: control treatment, CF: conventional chemical fertilization, P1－P6: pig manure at 25, 50, 100, 200, 400 and 800 kg P $ha^{-1}$with half the amount of conventional chemical fertilizer

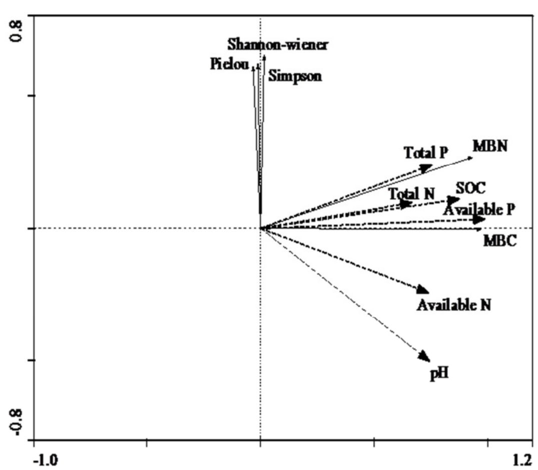

Fig. 5: Redundancy discrimination analysis bi-plot showing links between the soil chemical and microbial properties in the three soils

equilibrium, which may increase orthophosphate and decrease the activity of phosphatase, thereby weakening the use of carbon sources (Stone *et al*., 2013). To date, few studies have focused on the quantitative relationship between the available P and the functional diversity of microbial communities. The present study revealed that the functional diversity of microbial community decreased under excessive accumulation of available P, suggesting that the excessive retention of fertilizers within soil decreases microbial transformation of nutrients.

The critical value of soil P for optimal crop yield, soil fertility and environmental safety has been investigated (Bai *et al*., 2013); however, the critical value of soil P for optimal microbial properties has not yet been reported. In this study, the critical value of soil available P was considered to be that below which P addition had a positive impact on microbial properties and above which the response was unexpected. The critical value in the Agri-Udic Ferrosols was greater than that in the Ali-Udic Argosols and Ali-Udic Cambisol, which may be partially due to differences in the soil structure and parent materials. The high clay content in the more heavily textured Agri-Udic Ferrosols could result in greater inorganic P retention ability (Olson *et al*., 2010). Moreover, the high clay content in the Agri-Udic Ferrosol could greatly increase the substrate availability and its turnover rate (Sessitsch *et al*., 2001; Girvan *et al*., 2003; McLauchlan *et al*., 2006). In addition, the soil texture was among the most important abiotic determinants of soil microbial properties and it influenced microbial habitats in multiple ways (Garbeva *et al*., 2004; Bowles *et al*., 2014). Clay content can be linked to protection of soil microbes from predation, desiccation, heat and pH fluctuations; thus, microbes in clay soils are more strongly resistant to changes in external conditions (Bach *et al*., 2010).

According to the critical value of soil available P for optimal microbial properties, pig manure should generally be applied at less than 200 kg P ha^{-1}. In fact, there was no significant increase in the peanut yield above this application level in the three soils (Fig. 2), indicating that the decreased microbial community function may prevent the increase in peanut yield, although soil nutrient levels increased with different manure application rates. Further verification of these results in long-term field experiments will be required before they can be widely applied to field production.

Conclusion

Pig manure amendments enhanced soil nutrient contents and microbial biomass; however, excessive application (>200 kg P ha^{-1}) could significantly decrease microbial community function. Soil properties, including soil pH, organic carbon, N and P, were positively correlated with microbial biomass carbon and nitrogen in the three soils after two growing seasons with pig manure application. Among the selected chemical properties, the soil available P was the most significant factor influencing soil microbial properties and the relationship between microbial community function and available P can be

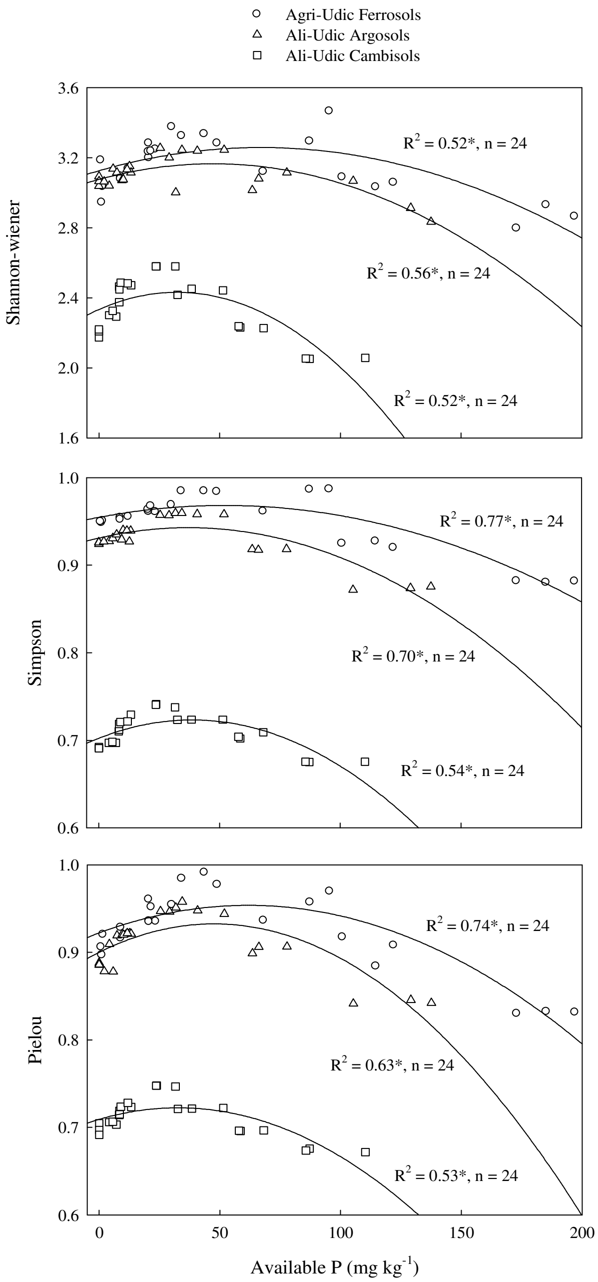

Fig. 6: The response of microbial community richness (Shannon-Wiener), diversity (Simpson) and evenness (Pielou) to the available P in the three soils.* Indicates the significance level between microbial community indices and available P at $P<0.05$

described by a parabolic equation. According to the parabolic equations, we deduced that the rational application rate of pig manure should be 100–200 kg P ha^{-1} with half the amount of conventional chemical fertilizer in the three soils. The present study demonstrated that the accumulation of available P within the soils resulted in decreased microbial functional diversity and less effect on peanut yield, suggesting that appropriate controls for pig manure application are necessary to ensure the quality of the soil environment and stable crop production.

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