

Effect of pig manure amendments on microbial biomass and functional diversity in three agricultural soils

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Abstract. Understanding the effects of pig manure integrated with chemical fertilizers on soil microbial properties can provide valuable insights into developing more sustainable agro-ecosystems. This study conducted six combinations of pig manure treatments (6, 12, 24, 48, 96 and 192 t hm⁻²). Fertilization methods include: 30% of conventional chemical fertilizers, conventional fertilization (CF) and an unfertilized control (CK). By using three soils in subtropical China, investigated the impact of different manure applications on microbial activity and functional diversity in a peanut-oilseed rape cropping system. The results indicated that appropriate amounts of pig manure integrated with chemical fertilizer may increase microbial activity and functional diversity compared to the chemical fertilizer treatment in the three soils. For the improvement of soil microbial activities, the pig manure application should be no more than 48 t hm⁻² in Ferrosols and 96 t hm⁻² in Acrisols and Cambisols.

1. Introduction

In recent years, organic fertilizer application is one of the most active fields and rapid fields of research progress in fertilizer application. Organic fertilizer application not only affects the growth and yield of crops, but also affects soil fertility (Dinesh et al., 2006; Tejada et al., 2008). The addition of organic manure has been proposed not only as one method of maintaining levels of nutrients in agricultural soils, but also for increasing the biological activity (Widmer et al. 2006; Denef et al. 2009). Microorganisms play an important role in soil, as biochemical transformations of major elements, being the source and sink of nutrients for plants, or in physical properties of soils, such as aggregation (Anderson, 2003; Pan et al. 2006). Researches indicate that microbiological characteristics may respond more rapidly to environmental changes and are more sensitive to changes in the environment, compared to chemical and physical properties (Hao et al. 2008; Hai et al. 2010). This requires accurate prediction and assessment of the effects of these amendments under varying soil and environmental conditions (Zhong et al. 2010). There are many indexes reflecting soil fertility, among which the microbial biomass (MBC) and functional diversity have been used to describe soil qualities under different agricultural practices (Mäder et al. 2002; Badalucco et al. 2010; Vallejo et al. 2010). Therefore, the objectives of this study were to investigate changes of microbiological properties after different quantities of manure were applied to the three soils in southern China and to search for safe application rate of pig manure for this system.

2. Materials and methods

2.1 Site description



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The experiment was conducted at the Ecological Experimental Station of Red Soil, Chinese Academy of Agricultural Science in Qiyang County, Hunan Province (26°45.12' N, 111°52.32' E). This research site is characterized by a typical subtropical humid monsoon climate with an annual air temperature ranging from 18.5°C to 41°C, a mean annual rainfall of 1296 mm and frost - free period of 292 days.

2.2 Experimental design

Three dominating upland soils in subtropical China (Ferrosols, Acrisols and Cambisols) were collected to set up the experimental plot according to the original layers in the field, respectively. The initial soil properties were listed in Table 1. The fertilization experiment was performed in eight treatments with three replicates per treatment, the annual application rates of pig manure were 6, 12, 24, 48, 96 and 192 t hm⁻² (P1~P6), with 30% of the local conventional chemical fertilization rate (N: 80 kg hm⁻², P₂O₅: 60 kg hm⁻²; K₂O: 60 kg hm⁻²). Chemical fertilizer application at the local conventional fertilization rate was termed “CF”. The control treatment “CK” had no allochthonous organic amendments or chemical fertilizers. The pig manure was collected from pig farms near the experimental station.

Prior to sowing, the surface soil samples (0-20 cm) were mixed with fertilizers as a basic fertilizer in each growing season. Peanut-oilseed rape rotations were conducted during this experiment and peanuts were sown in the middle of May and harvested in the middle of September. Oilseed was then sown in the beginning of October and harvested in the beginning of May. The experiments were conducted under the condition of natural rainfall.

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

Soil type	pH (2.5:1)	Total C (g kg ⁻¹)	Total N (g kg ⁻¹)	Total P (g kg ⁻¹)	Available N (mg kg ⁻¹)	Available P (mg kg ⁻¹)	Available K (mg kg ⁻¹)
Ferrosols	4.49	4.97	1.58	1.22	57.59	31.25	37.69
Acrisols	4.63	3.12	0.85	0.89	39.88	14.47	31.36
Cambisols	5.21	4.02	1.03	0.67	45.62	22.51	40.38

3. Sample collection and analysis

After harvesting the oilseed rape, topsoil (0-20 cm depth) samples from all treatments were collected. After sampling, large pieces of plant material and animal residue were removed, the soil samples were milled with a gavel and passed through a 2-mm sieve. The sieved soil samples were air-dried to approximately 40% of their maximum water-holding capacity (MWHC) and incubated at 25°C for 1 week in an airtight container for microbial analysis. Soil microbial biomass was measured using the chloroform-fumigation extraction method.

Microbial biomass carbon (MBC) was estimated from the difference in extractable organic C between the fumigated and unfumigated soil as follows (Vance et al. 1987):

$$\text{MBC} = E_C \times 2.64$$

where E_C refers to the difference in extractable organic C between the fumigated and unfumigated treatments, 2.64 is the proportionality factor for biomass C released by fumigation extraction. Microbial biomass carbon (MBN) was calculated using the equation (Brookes et al. 1985):

$$\text{MBN} = F_N / 0.54$$

where F_N = (total N from fumigated soil) - (total N from unfumigated soil).

The patterns of potential carbon source utilization by soil microbial communities with different fertilizer treatments were assessed using a Biolog Ecoplate system containing 31 ecologically relevant sole carbon sources (organic substrate) and a water control and provided three within-plate replications of those substrates in each plate (Biolog Inc., Hayward, CA, USA). Functional microbial diversity was analyzed with the Shannon-Weaver index (H) using the equation $H = -\sum P_i \ln P_i$ and dominant and evenness were analyzed with the Simpson (D) and McIntosh (U) indices using the equations $D = 1 / \sum (P_i)^2$ and $U = \sqrt{\sum n_i^2}$, respectively, where P_i is the ratio of the activity on each substrate (OD_i) to the sum of the activities on all substrates ($\sum OD_i$), N is the total number of carbon sources (31), n_i is

the relative absorbance of the well (Preston-Mafham et al. 2002).

The significant differences among the fertilization treatments were assessed using a one-way ANOVA followed by the LSD test at a 5% probability level.

3.1 Soil microbial biomass

There were significant differences in MBC and MBN between the control (CK) and fertilization treatments (Fig. 1). In general, MBC and MBN in the Ferrosols were the highest among the three red soils with the corresponding fertilization, the Cambisols was the lowest. There was no significant increase when the pig manure application reached 96 t hm⁻² in the Ferrosols and 48 t hm⁻² in the red Acrisols and Cambisols. Compared with the conventional chemical fertilizer application treatment (CF), MBC and MBN significantly increased in the P3 treatment in the three soils.

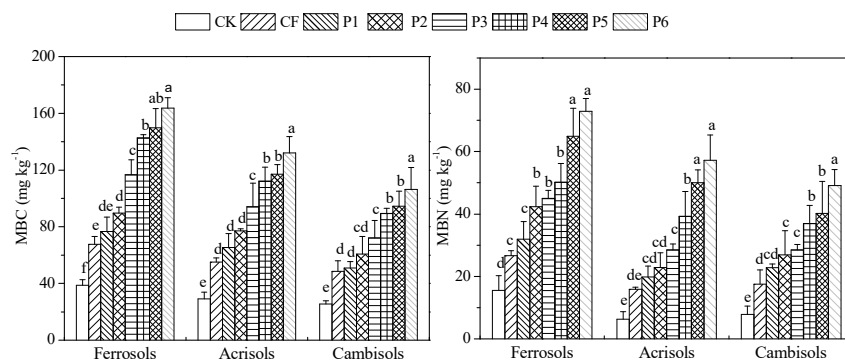


Fig. 1 Effects of fertilization on the soil microbial biomass carbon (MBC) and microbial biomass nitrogen in the different soils (MBN)

3.2 Soil community functional diversity

The substrate Shannon-wiener index (H), Simpson index (D) and McIntosh index (U) for the different pig manure applications in the three soils at 72 h are shown in Table 2, these indices represent the richness, dominance and evenness, respectively. Compared with the conventional chemical fertilizer treatment (CF), the Shannon-wiener index did not significantly increase with pig manure application in the Ferrosols; However, it significantly increased in the pig manure treatments in Acrisols and Cambisols. The Simpson indices were not significantly different between CK, conventional chemical fertilizer treatment (CF) and pig manure treatments in the three soils. The McIntosh index significantly increased in the pig manure treatments when the amount of pig manure reached 12 t hm⁻² in the Ferrosols and 6 t hm⁻² in the Acrisols and Cambisols.

Table 2 Effects of pig manure on the microbial community Shannon-wiener index (H), Simpson index (D) and McIntosh index (U) in the soils

Treatme nt	Shannon-wiener (H)			Simpson (D)			McIntosh (U)		
	Ferros ols	Acrisol s	Cambiso ls	Ferroso ls	Acrisol s	Cambis ols	Ferroso ls	Acrisols	Cambisols
CK	2.06b	2.64c	2.01b	0.87a	0.91a	0.65a	1.10b	0.75c	0.51c
CF	2.39a	2.68c	2.00b	0.87a	0.92a	0.66a	1.19b	0.82c	0.53c
P1	2.44a	2.77 b	2.09a	0.90a	0.93a	0.66a	1.20b	1.00b	0.81b
P2	2.54a	2.85ab	2.10a	0.88a	0.94a	0.65a	1.35a	1.18b	1.02ab
P3	2.62a	2.93a	2.11a	0.90a	0.92a	0.65a	1.37a	1.10b	1.00ab
P4	2.68a	2.91a	2.11a	0.91a	0.93a	0.66a	1.35a	1.19b	1.09a
P5	2.64a	2.94a	2.11a	0.90a	0.94a	0.66a	1.37a	1.43a	1.20a
P6	2.68a	2.99a	2.15a	0.93a	0.93a	0.66a	1.45a	1.52a	1.21a

3.3 Principal component analysis

To further understand the differences in the microbial communities with the different fertilization treatments, Biolog data at 72 h in the three soils were subjected to principal component analysis (PCA), as is shown in Fig. 2. The microbial community characteristic variations induced by the different fertilizer applications in the three soils could be explained by the first two principal components (PCs). The major two-dimensional components, PC1 and PC2, explained 42.3% and 19.9%, respectively, of the total variance in the Biolog data in the Ferrosols, 41.2% and 24.6% in the red Acrisols, 46.6% and 21.1% in the Cambisols, indicating large differences in the catabolic capability of the soil microbial communities with the various treatments. In the Ferrosols, the PCA showed a differentiation of the higher pig manure treatments (P4, P5 and P6) compared to the conventional chemical fertilizer application (CF) and control treatments (CK). In the Acrisols, there were significant differences in the PC1 values for the P5 and P6 treatments compared to the CK and CF treatments. There was a similar effect on the Cambisols with the different fertilization treatments. PCA separation of the community-level physiological profiles suggests that pig manure application has a significant influence on the microbial community activity.

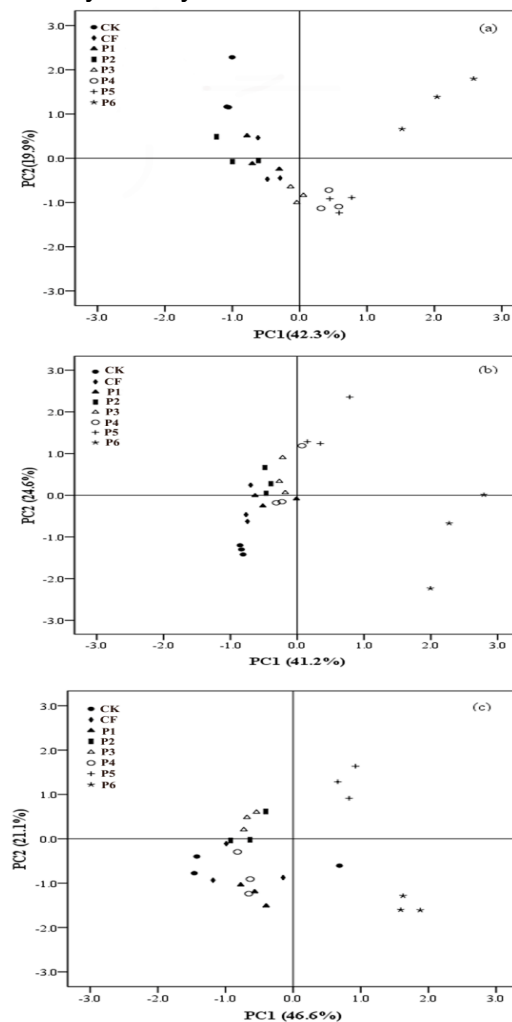


Fig. 2 Principal component analysis of the community-level physiological profiles with fertilization treatment in the three soils (a: Ferrosols, b: Acrisols, c: Cambisols)

4. Discussion

Previous research studies have reported that fertilization has a profound impact on microbial activity. Liu et al. (2009) and Chakraborty et al. (2011) determined that microbial biomass carbon and nitrogen were significantly higher with pig manure in combination with a chemical fertilizer compared to the

chemical fertilizer alone. In this study, we reached a similar conclusion. The effects of the soil type on the microbial biomass were quite remarkable, which may be partially explained by the difference in physical and chemical properties (Table 1). The Ferrosols soil has considerably higher clay fractions and total C and N than the other soils, which generally tend to support greater microbial activity (Calbrix et al. 2007, Tu et al. 2006).

In our study, distinct difference in the soil microbial community structure was directly associated with manure amendments. This result was primarily due to pig manure application altering the soil microbial community metabolism of the carbon source model and improving the carbon source utilization ability of the microbial community in the short term (Gagliardi et al. 2001; Gomez et al., 2006; Toyota et al. 2006). Generally, substrate diversity and evenness increased with manure application compared to the chemical fertilizer treatment (CF), implying the species richness in the manure treatments was higher compared to the chemical fertilizer treatment or some species in these treatments had stronger carbon source utilization abilities in the microbial community (Parham et al. 2003). The Simpson indices displayed no significant differences among these treatments, indicating that the common species in the microbial community were basically the same. Studies have demonstrated that the balanced application of organic and inorganic supplements have substantial incremental effects on the soils microbial functional diversity and activity (Böhme et al. 2005; Pengthamkeerati et al. 2011). Overall, the Ferrosols had a higher level of microbial biomass and activity compared to the Acrisols and Cambisols. However, the substrate richness and diversity indices of the Ferrosols were lower than the Acrisols, indicating that the greater biomass is likely due to the increased numbers of select organism groups rather than increases in the numbers and types of organisms with different functional capabilities, also demonstrated by Larkin et al. (2006). The PCA results suggest that the microbial community structure changed when the pig manure amount was greater than 48 t hm⁻² for the Ferrosols and 96 t hm⁻² for the Acrisols and Cambisols.

5. Conclusion

In conclusion, appropriate amounts of pig manure integrated with a chemical fertilizer may improve microbial activity compared to the use of only chemical fertilizer in three soils. With regarding to the soil microbial activities, the pig manure application rate should be no more than 48 t hm⁻² in Ferrosols and 96 t hm⁻² in Acrisols and Cambisols.

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