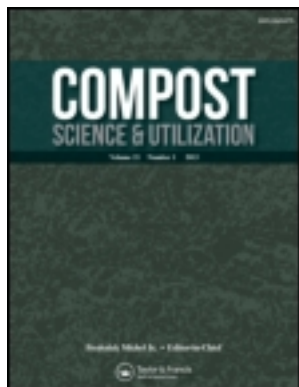


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Peanut Straw Decomposition Products Promoted by Chemical Additives and Their Effect on Enzymatic Activity and Microbial Functional Diversity in Red Soil

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ABSTRACT. The objectives of the present study were to determine the promotional effect of chemical additives on quality of peanut straw decomposition products and to evaluate the influence of the resulting products on soil biological properties. Straw was mixed with or without chemical additives, such as iron(II) sulfate (FeSO_4), alkali slag, or FeSO_4 combined with alkali slag, and decomposed for 50 days. The decomposition products were used as organic fertilizer and added to red soil for an incubation experiment. The chemical additives increased total organic carbon (C), total nitrogen (N), and available N content but decreased the C:N ratios in decomposition products compared to controls. Adding FeSO_4 gave the highest humic acid content (HA, 30.34 g kg^{-1}) and ratio of humic to fulvic acid (HA/FA, 0.53) and the lowest ratio of HA absorption value at 465 nm to that at 665 nm (E_4/E_6 , 6.05), suggesting high humification of decomposition products. Application of the resulting products to soil increased soil urease and invertase activities. BIOLOG analysis showed that microbial C utilization ability, Shannon–Weaver diversity, and McIntosh evenness indexes were improved by the organic fertilizer promoted by chemical additives. Principal component analysis indicated that microbial community structures were also influenced by different amendments in decomposition products. Our study provides a reference point for acquiring high quality straw compost and improving soil biological functions by organic fertilizer.

INTRODUCTION

Crop straw is an important organic resource and represents 1.94×10^3 Mt of total carbon (C), nitrogen (N), and phosphorus (P) in China (Li et al. 2003). Peanut is a common dryland crop and its straw biomass is vast in the red soil region of subtropical China. However, this crop residue is commonly discarded or burned

in this region—an obvious resource waste and a severe environmental problem. Composting is one approach for utilizing organic residue that has a long history in China. When composting is carried out, some additives are also added for their ability to accelerate decomposition or alleviate nutrient loss (Kithome et al. 1999; Mou and Wang 2008). For instance, Kithome et al. (1999) discovered that adding zeolites could reduce

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ammonia volatilization in decomposing straw. Mou and Wang (2008) found that total N and ammonium N were increased in compost with the addition of iron(II) sulfate (FeSO_4). However, the effects of additives on quality of compost, especially on quality of humic substances in decomposition products, have rarely been studied.

When organic fertilizer is applied, soil physical and chemical changes are often slow and may not be suitable for evaluating quick shifts in soil ecosystems. Microbiological properties, however, are very responsive and can provide accurate information when slight changes occur in soil (Dick and Tabatabai 1993). For example, soil enzymes as an index of soil microbial activity were immediately affected by the addition of organic matter (Albiach et al. 2000; Crecchio et al. 2004; Tejada et al. 2008). Albiach et al. (2000) demonstrated that municipal solid waste (MSW) compost most enhanced soil enzymatic activity compared to other amendments. Crecchio et al. (2004) showed a significant increase in dehydrogenase, β -glucosidase, urease nitrate reductase, and phosphatase activities with MSW compost in a field experiment. It was also reported that enzymatic activities in soil amended with fresh wastes were higher than those in soil amended with compost during a short incubation (Pascual et al. 2002). Community level physiological profiles (CLPPs) analysis was also used to detect rapid changes of soil microbial functional diversity with organic application (Carrera et al. 2007; Gomez et al. 2006; Nair and Ngouajio 2012)—organic amendments led to increases in C utilization ability, richness, and Shannon–Weaver diversity index (H') of soil microorganisms according to BIOLOG analysis (Gomez et al. 2006). Principal component analysis (PCA) of the BIOLOG data revealed that different organic amendments also affected soil microbial community structure (Gomez et al. 2006; Nair and Ngouajio 2012). The difference in soil biological properties was assumed to be due to quality and stability of organic matter in the different organic amendments (Nair and Ngouajio 2012; Pascual et al. 2002).

In subtropical China, retention of organic matter in red soils is low due to abundant wa-

ter and hot conditions. Dryland soil fertility is low and soil biological functions are relatively weak in this region. Increasing the retention of organic input and improving the biological function in red soils is an urgent problem. In view of the above, the aim of this study was to (1) determine the promotional effect of chemical additives, such as alkali slag or FeSO_4 , on the quality of peanut straw decomposition products and (2) analyze changes of enzymatic activity and microbial functional diversity in red soil amended with the resulting decomposition products. The practical purpose of this work is to provide a reference point for improving and utilizing straw compost.

MATERIALS AND METHODS

Tested materials

The test soil was red soil (*Udic Ferrisol*) derived from quaternary red clay and sampled from a typical dryland crop field in Yujiang County, Jiangxi Province in subtropical China ($116^\circ 55' 30''$ E; $28^\circ 15' 30''$ N). Peanut straw was collected from the same plot after harvest. Total C and N contents of the straw were 359.86 and 10.95 g kg^{-1} , respectively. Following the research of Mou and Wang (2008), FeSO_4 was selected as one of the chemical additives in this experiment. Instead of lime, which is commonly used, alkali slag (from China Petrochemical Corporation, Nanjing Chemical Industrial Co. Ltd., Lianyungang Soda Ash Plant) was selected as another additive for composting. The first reason for choosing alkali slag was that, similar to lime, it was alkaline (pH 11.48) and rich in calcium (179.4 g kg^{-1}). Second, we considered the practical prospect of resource recycling since alkali slag is an industrial waste. In addition to calcium, alkali slag had 79.17 g kg^{-1} of magnesium (Mg), 0.29 g kg^{-1} of P, 12.03 mg kg^{-1} of copper, and 79.47 mg kg^{-1} of zinc. The decomposing microorganisms were from a commercial effective microorganism agent (EM agent; Nanchang Probiotics Biological Technology Co. Ltd., China) mainly including photosynthetic bacteria, yeast, lactic acid bacteria, actinomycetes, and *Bacillus* spp.

Straw decomposition experiment

Peanut straw was dried, ground, and sieved through a 0.42-mm mesh. Of the sieved straw, 80 g was used for preparation of organic fertilizer with the following treatments of three replicates:

1. Straw mixed with 2% EM agent and no chemical additive (N);
2. Straw mixed with 2% EM agent and 5% alkali slag (A);
3. Straw mixed with 2% EM agent and 3% FeSO₄ (F);
4. Straw mixed with 2% EM agent, 5% alkali slag, and 3% FeSO₄ (AF).

The appropriate C:N ratio was 25–35 at the beginning of composting, thus C:N ratios of the above mixtures were regulated to 25 by adding urea. Moisture was maintained at 65%. After 50 d of incubation under ventilation at 30°C, the resulting decomposition products were collected and dried or stored at 4°C for subsequent measurement and experiments.

Soil incubation experiment

Test soil was dried and passed through a 2-mm sieve. Of sieved soils, 100 g was mixed with 1% of the above resulting organic fertilizer (equivalent to 22.5 t ha⁻¹ of compost) with the following treatments of three replicates:

1. Red soil mixed without fertilizer (CK);
2. Red soil mixed with no chemical-promoted organic fertilizer (NO);
3. Red soil mixed with 1% alkali slag-promoted organic fertilizer (AO);
4. Red soil mixed with 1% FeSO₄-promoted organic fertilizer (FO);
5. Red soil mixed with 1% alkali slag combined with FeSO₄-promoted organic fertilizer (AFO).

Mixtures were adjusted to 65% of water holding capacity with distilled water and incubated in a 250-ml flask. The flasks were put in a growth chamber in darkness for 90 d at 28°C, and samples were collected and measured after 30, 60, and 90 d of incubation.

Sample measurements and statistical analysis

The pH of the decomposition products was determined by the potentiometric method, and total organic C was determined by the Tyurin method (Lu 1999). After H₂SO₄–H₂O₂ digestion, total N of decomposing matter was measured by the alkali-hydrolyzation-diffusion method, and available N by indophenol blue colorimetry.

Humic substances (HS), humic acid (HA), and the ratio of HA absorption value at 465 nm to that at 665 nm (E₄/E₆) were measured by the following method. Of air-dried decomposition products, 5 g was suspended with 25 ml of 0.1 M Na₄P₂O₇ and 0.1 M NaOH solution and shaken. The mixture was settled at room temperature for 16–20 h. After adding 5 ml of saturated Na₂SO₄ solution, this HS solution was filtered for the next analysis. Total C in HS solution was determined by a Multi N/C[®] 3100 analyzer (Analytik Jena AG, Germany). Then 25-ml aliquots of HS solution were incubated at room temperature overnight and filtered. HA solution was acquired by dissolving the resulting precipitate with 0.05M NaHCO₃ hot solution. Then the C content of HA was analyzed by a Multi N/C[®] 3100 analyzer, and E₄/E₆ were values determined by measuring the absorption value of HA at 465–665 nm by spectrophotometry.

Urease activity was measured by the indophenol colorimetric method using urea as substrate, and invertase activity was measured by the 3, 5-dinitrosalicylic colorimetric method using sucrose as the substrate (Guan 1986). Soil microbial functional diversity was assessed by CLPPs analysis using 96-well BIOLOG Eco-plates[™] (Biolog Inc., CA, USA). Each 96-well plate contained three replicates of 31 soil C sources and the water blank control. Of soil samples, 5 g was suspended in 50 ml of 0.85% NaCl solution. The mixture was shaken for 30 min and settled for another 30 min at room temperature. The supernatant was diluted at 1:20 with NaCl solution—100-μl aliquots were inoculated into a 96-well Eco-plate and incubated at 25°C. Light absorbance of each well was measured at regular 24-h intervals. Average well color development (AWCD), H', and McIntosh evenness index (U)

TABLE 1. Nutrient changes in peanut straw decomposition products promoted by different chemical additives

	pH	TC (g kg ⁻¹)	TN (g kg ⁻¹)	AN (g kg ⁻¹)	C:N
N	8.03 ± 0.22 a	336.82 ± 14.57 a	18.25 ± 1.75 b	2.14 ± 0.44 c	18.46
A	7.88 ± 0.01 a	341.29 ± 5.61 a	23.82 ± 0.77 a	5.74 ± 0.03 a	14.33
F	8.10 ± 0.04 a	359.25 ± 9.47 a	22.03 ± 0.01 ab	2.74 ± 0.37 c	16.31
AF	7.84 ± 0.01 a	370.54 ± 11.74 a	22.04 ± 0.84 ab	4.37 ± 0.02 b	16.81

Note. N: no additive control; A: alkali slag-promoted decomposition products; F: FeSO₄-promoted decomposition products; AF: alkali slag combined FeSO₄-promoted decomposition products; TC: total carbon; TN: total nitrogen; AN: available nitrogen; C:N: ratio of TC to TN. Different letters on each column indicate significant difference at $P < 0.05$.

were calculated as described by Zhong and Cai (2007).

Data were analyzed using SPSS 13.0 for Windows (SPSS, Chicago, IL, USA). Significant differences among treatments were determined by one-way ANOVA with Duncan's multiple range test method at $P < 0.05$. Color development data of BIOLOG was further analyzed by PCA. Graphics were constructed using Microsoft Office Excel 2003.

RESULTS AND DISCUSSION

Effect of chemical additives on properties of straw decomposition products

Effect of Chemical Additives on Nutrient of Decomposition Products

The pH of all treatments was approximately 7.84–8.10 (table 1)—slightly different to other studies, which showed pH 8–9 at the end of the decomposition process (Rajbanshi et al. 1998). Some studies reported that organic acids accumulated in later stages of composting (Eklind and Kirchmann 2007; Mathur 1991). Therefore, lower pH in treatments A or AF might be due to more production of organic acids.

Compared to the no additive control, chemical additives increased the total C by 1.3%–10.01%, total N by 20.7%–30.5%, and available N by 28.0%–168.2% (table 1). The highest content of total C (370.54 g kg⁻¹) was in treatment AF; however, the highest total N (24.64 g kg⁻¹) and available N (5.74 g kg⁻¹) were in treatment A (table 1). The organic material in straw when

decomposed is transformed to water, mineral substances, and volatile matter. In the process, the weight loss of straw caused increases in organic and mineral nutrients per unit weight in the decomposition products. The above results indicated that chemical additives could accelerate the decomposition process and contribute to nutrient concentration.

The C:N ratio, an indicator of straw decay, was 14.33–18.36 in all treatments at the end of the decomposition process (table 1). Generally, C:N ratios decrease and gradually reach an equilibrium value, which indicates complete decomposition of organic matter (Diaz et al. 1993). Some researchers demonstrated that the C:N ratio of mature compost is <16 (Chen 1990; Garcia et al. 1992). In the present study, C:N ratios of decomposition products promoted by chemical additives were usually approximately 16. In treatment A, the C:N ratio was 14.33, indicating well-decayed straw.

Effect of Chemical Additives on Characters of Humic Substances

Structure of HS is considered an indicator of compost maturity (Sequi and Benedetti 1995) and influences nutrient flux and availability (Nardi et al. 2002). Total HS, HA, ratio of humic to fulvic acid (HA/FA), and E₄/E₆ ratios of decomposition products were also measured in the present study. When alkali slag was applied, total HS content was highest—3.7% higher than in controls (table 2). However, when FeSO₄ was used, the highest HA content (30.34 g kg⁻¹), highest HA/FA ratio (0.53), and lowest E₄/E₆ (6.05) were detected in decomposing

FIGURE 1. Dynamic changes of urease (A) and invertase (B) activities in red soil amended with chemical additive-promoted decomposition products. CK: no fertilizer control; NO: no chemical additive-promoted organic fertilizer; AO: alkali slag-promoted organic fertilizer; FO: FeSO_4 -promoted organic fertilizer; AFO: alkali slag combined FeSO_4 -promoted organic fertilizer.

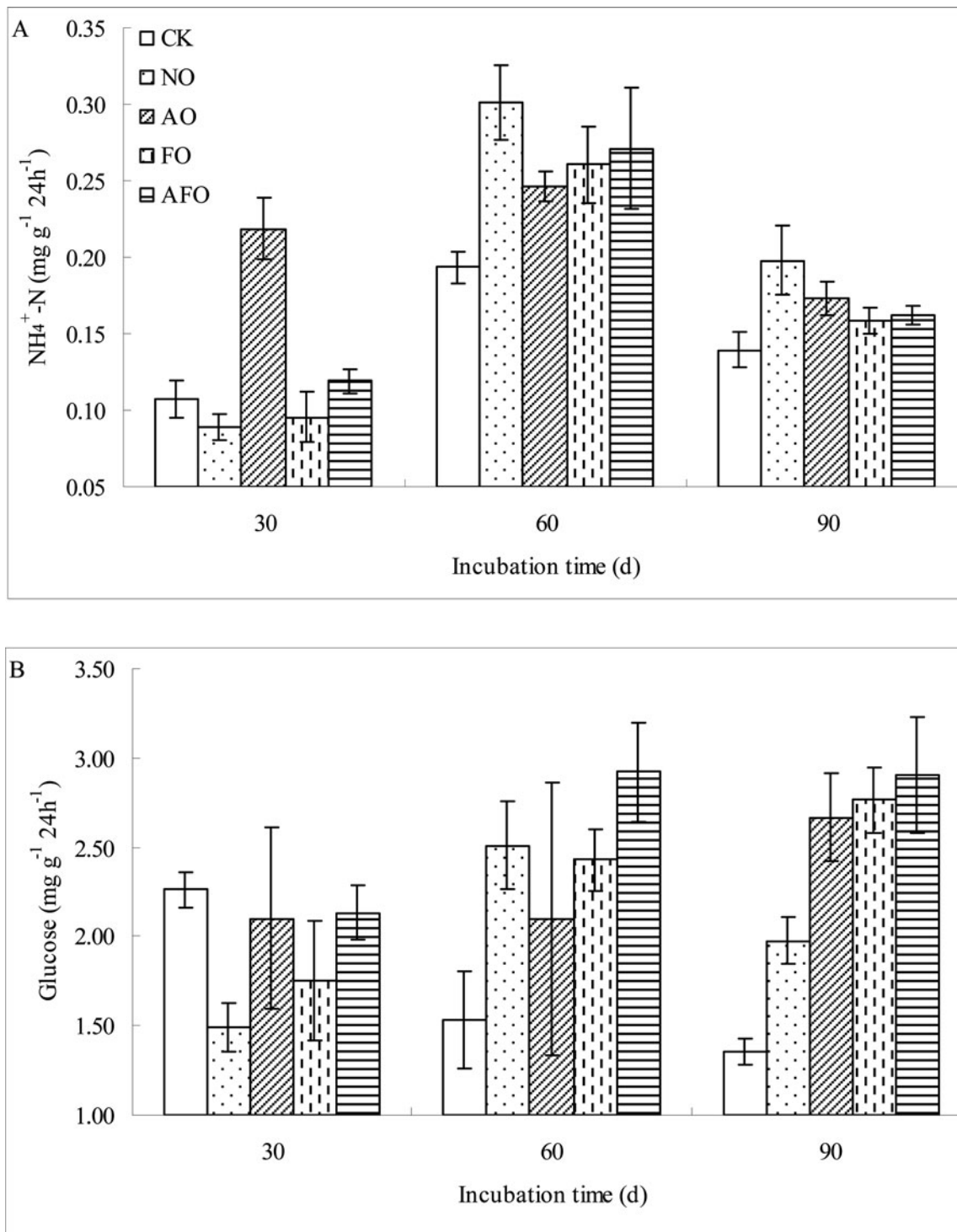


FIGURE 2. Dynamic changes of average well color development (A), Shannon index (B), and McIntosh index (C) in red soil amended with chemical additive-promoted decomposition products. CK: no fertilizer control; NO: no chemical additive-promoted organic fertilizer; AO: alkali slag-promoted organic fertilizer; FO: FeSO_4 -promoted organic fertilizer; AFO: alkali slag combined FeSO_4 -promoted organic fertilizer. (Continued)

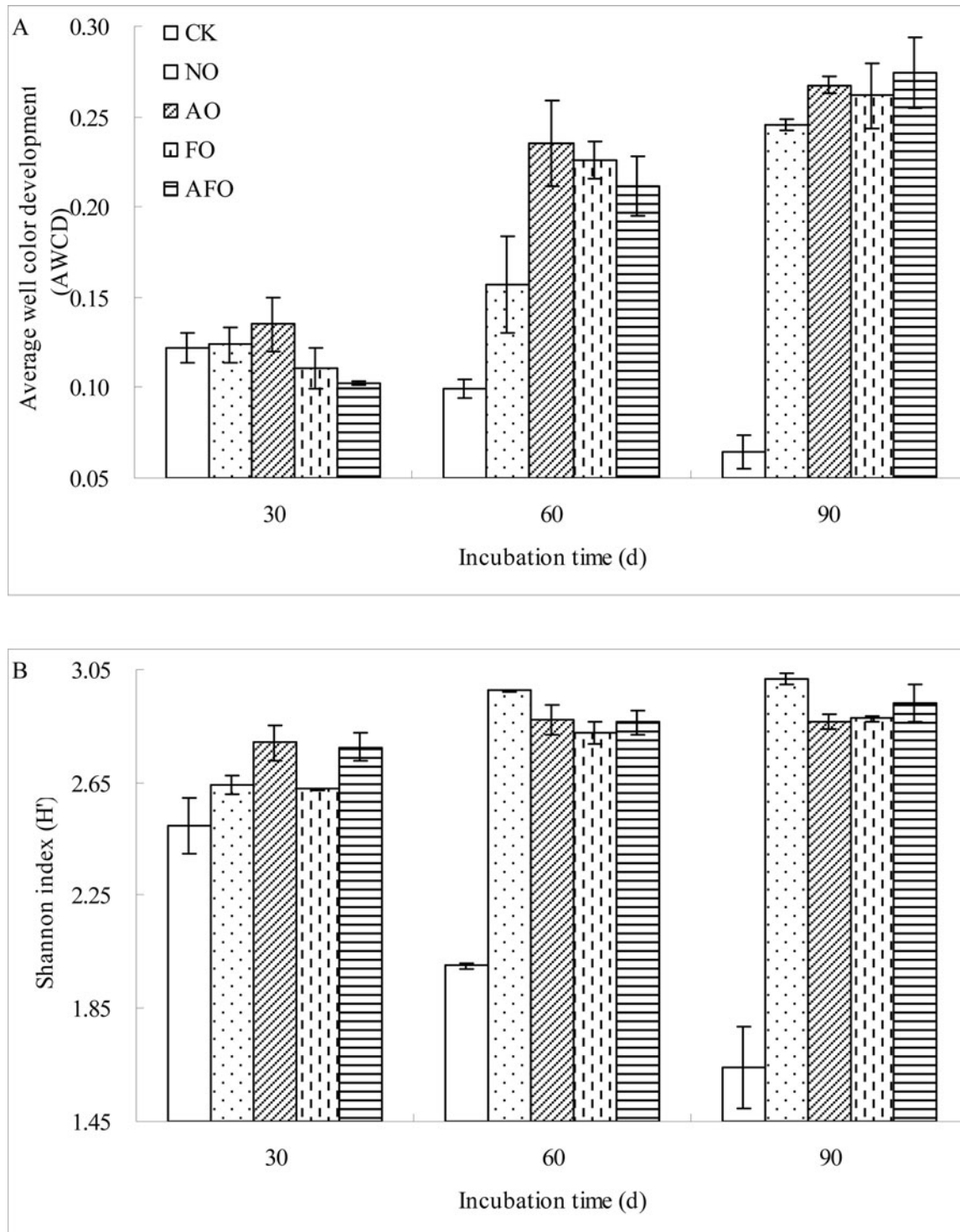
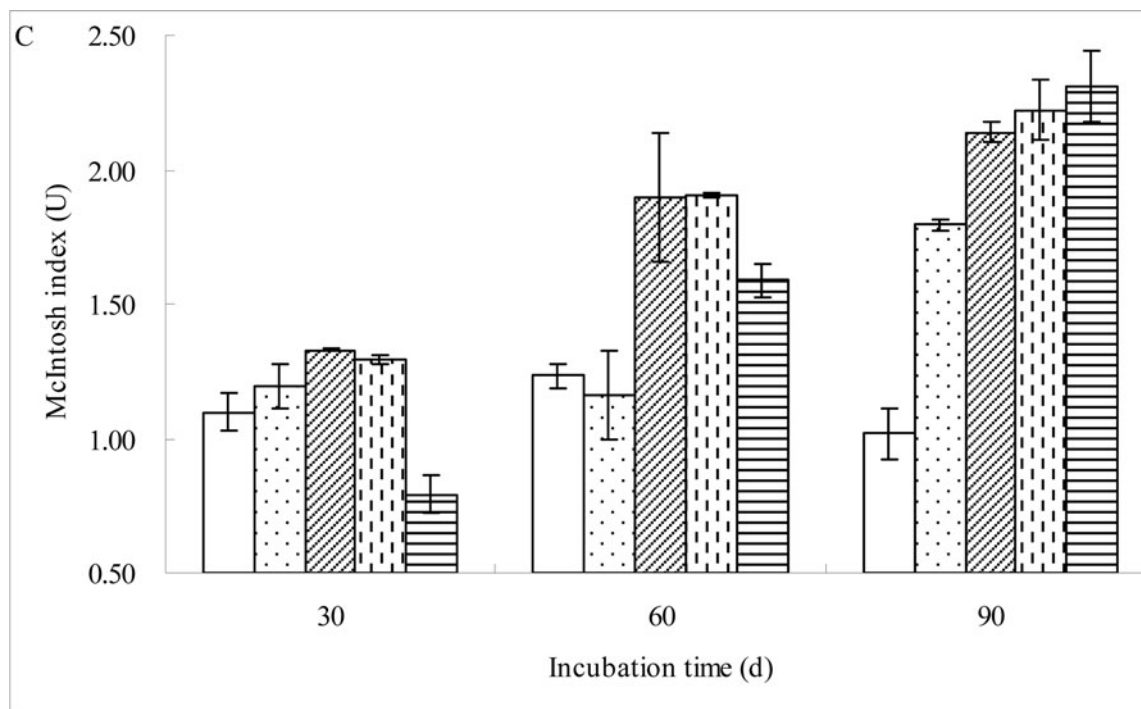


FIGURE 2. Continued.



matter. Usually, high HA/FA ratios indicate possible further humification and stabilization of decomposition products. E_4/E_6 value is also an important indicator reflecting the condensation and aromatization degree of HA (Zuconi et al. 1981). E_4/E_6 decreases with increasing molecular weight and with condensing of aromatic rings (Lguirati et al. 2005). Brunetti et al. (2008) reported that metal ions could catalyze humification and contribute to the quality of decomposing matter. The present results suggested that Mg, Fe, or other metal ions in alkali slag or $FeSO_4$

not only promoted the humification of decomposition products but also induced formation of macromolecular aromatic substance in HA.

Effect of straw decomposition products on biological properties of red soil

Effect of Straw Decomposition Products on Enzymatic Activity

Urease is an important enzyme involved in N cycles (Pascual et al. 1998). In the present

TABLE 2. Characters of humic substances in peanut straw decomposition products promoted by different chemical additives

	HS ($g\ kg^{-1}$)	HA ($g\ kg^{-1}$)	HA/FA	E_4/E_6
N	98.58 ± 1.35 ab	23.09 ± 6.88 a	0.31	8.77 ± 1.47 a
A	102.25 ± 1.00 a	30.12 ± 0.57 a	0.42	6.77 ± 1.35 a
F	87.49 ± 3.54 b	30.34 ± 0.99 a	0.53	6.05 ± 0.40 a
AF	92.79 ± 4.12 ab	22.33 ± 1.41 a	0.32	6.10 ± 0.41 a

Note. N: no additive control; A: alkali slag-promoted decomposition products; F: $FeSO_4$ -promoted decomposition products; AF: alkali slag combined $FeSO_4$ -promoted decomposition products; HS: humic substances; HA: humic acid; HA/FA: ratio of humic to fulvic acid; E_4/E_6 : ratio of HA absorption value at 465 nm to that at 665 nm. Different letters on each column indicate significant difference at $P < 0.05$.

study, soil urease activity in the AO treatment was highest and was 83.7%–145% higher than in other treatments at 30 d (figure 1A). It is generally acknowledged that organic fertilizer affects soil enzymatic activity directly according to their composition as well as by stimulation of soil microbial activity (Goyal et al. 1993; Hattori 1988). For example, sewage sludge amendment contributed many nitrogenated compounds and raised urease activities in soil (Pascual et al. 2002). AO treatment had the highest total and available N content of all treatments and possibly explained the relevant high urease activity in the present study. The results also showed that urease activities in all treatments increased

to peak value at 60 d but decreased at 90 d. A high concentration of mineralized NH_4^+ in organic matter was likely to inhibit urease activity in soils (García-Gil et al. 2000). Therefore, it was assumed that mineralization of decomposition products and resulting excessive nitrogenated metabolites led to the decline in urease activity with incubation in the present study.

Invertase participates in metabolic processes of soil organic matter. Unlike urease activity, there was no significant difference in invertase activities among the different treatments at 30 d. However, the activities in treatments AO, FO, or AFO were 35.7%–47.0% higher than in NO at 90 d (figure 2B). It was said that soil invertase

FIGURE 3. Treatment scatter plot (A) and substance loading variables (B) on PC1 and PC2 axes by principal component analysis of BIOLOG data. CK: no fertilizer control; NO: no chemical additive-promoted organic fertilizer; AO: alkali slag-promoted organic fertilizer; FO: FeSO_4 -promoted organic fertilizer; AFO: alkali slag combined FeSO_4 -promoted organic fertilizer. The numbers 30, 60, and 90 indicate different numbers of days of incubation. (Continued)

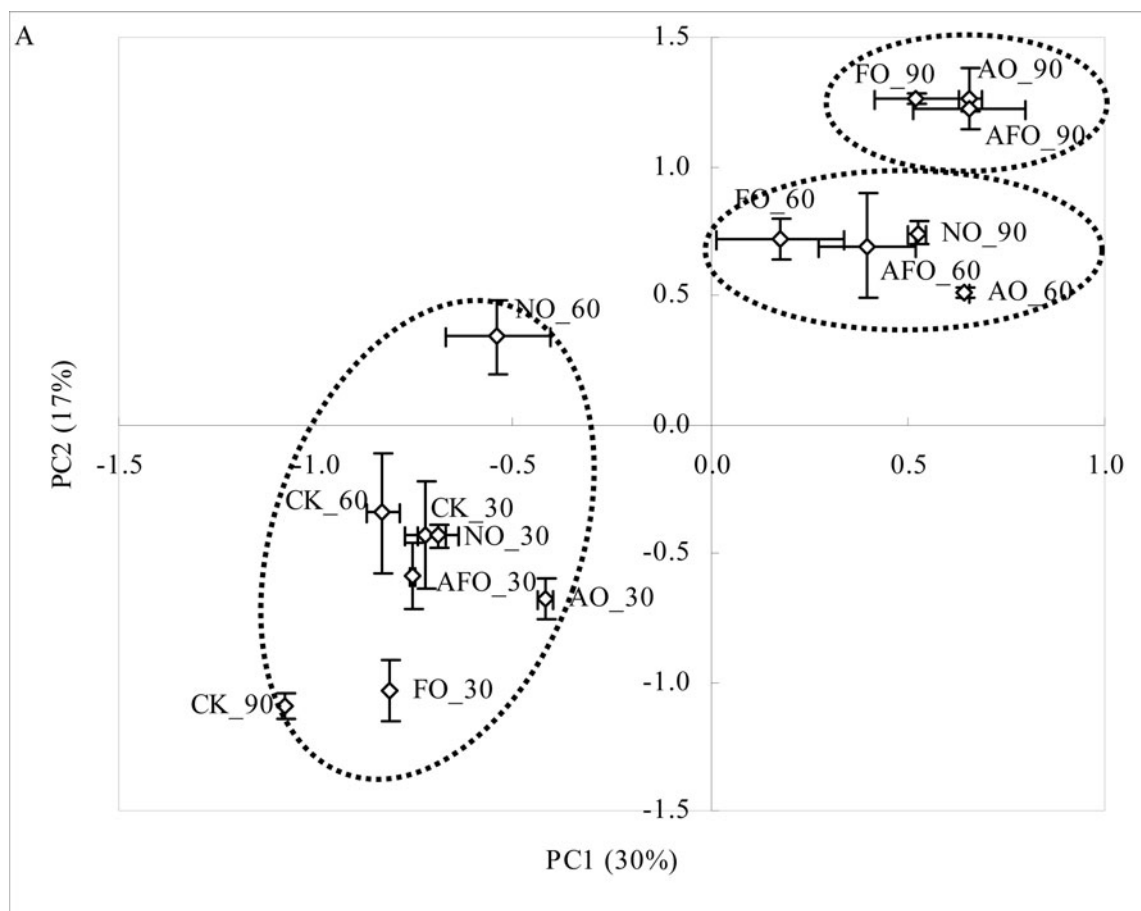
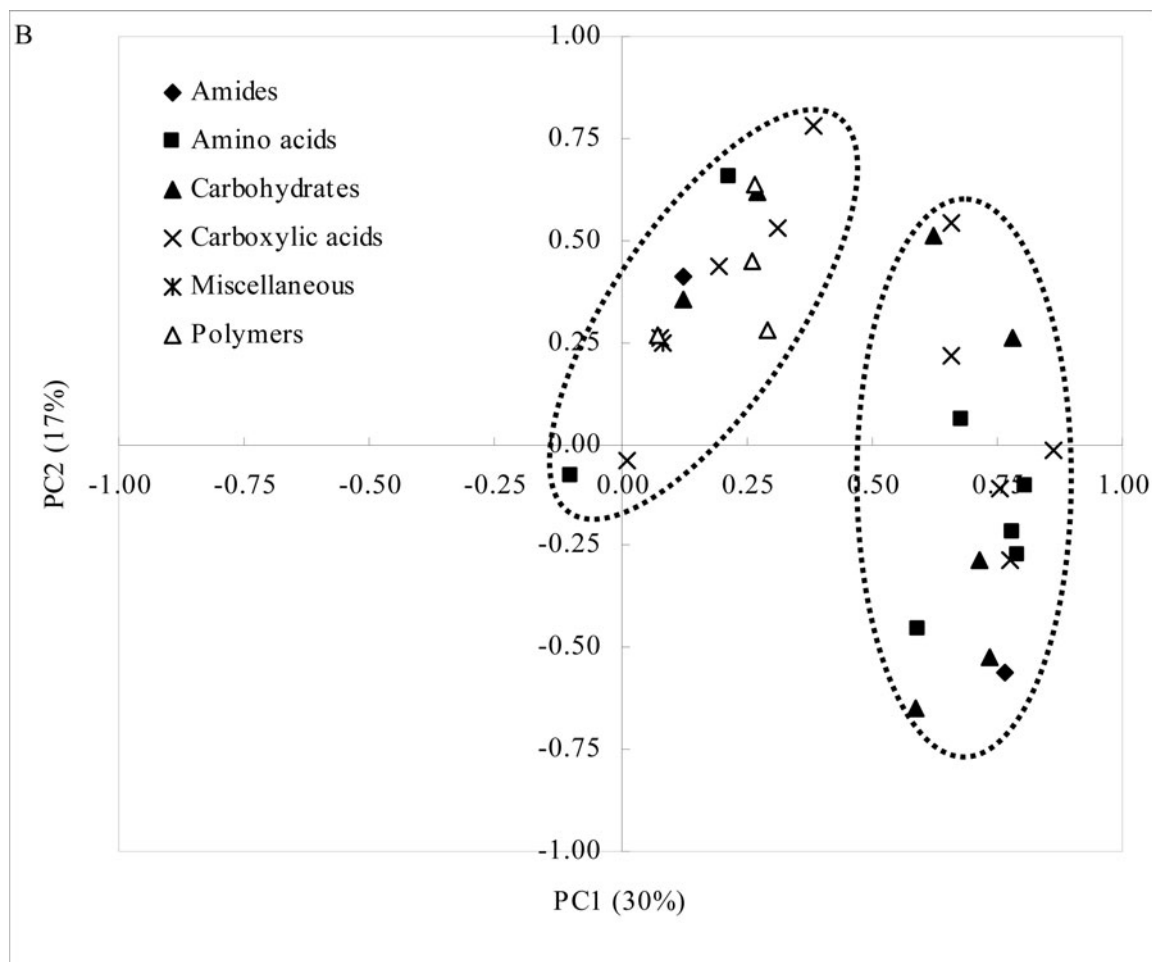


FIGURE 3. Continued.



activities were related to organic C (Stemmer et al. 1998). In the present study, products that included chemical additives had higher contents of organic C—this would contribute to the higher enzymatic activity during longer incubations.

Effect of Straw Decomposition on Soil Microbial Functional Diversity

Similar to the study of Gomez et al. (2006), AWCD, H' , and U increased with incubation time for all organic amendments (figure 2). Particularly, AWCD and U were higher in treatment AO or FO at 30 and 60 d; however, they were highest for AFO at 3.8%–350% and 4.1%–126% higher than in other treatments at 90 d (figures 3A, 3C). H' was highest in treatment AO and

0.4%–11.6% higher than for other amendments at 30 d. However, H' was highest in treatment NO at 60 and 90 d of incubation (figure 3B). This convergence in microbial functional diversity with use of different types of organic matter was also found by Nair and Ngouajio (2012). This was possibly because different microorganisms grew when different organic matter was applied (Fließbach and Mäder, 1997).

PCA results showed that principal component 1 (PC1) explained 30% and PC2 explained 17% of variation (figures 3A, 3B). Two distinct groups of substrate utilization were identified along PC1 and contributed to segregation of different organic inputs. Specifically, five of seven amino acids, five of seven carbohydrates, and five of nine carboxylic acids had original

loading variables that were significantly correlated with PC1 (data not shown). In other words, when chemical-promoted products were applied for more than 60 d, soil microorganisms tended to use amino acids, carbohydrates, and part of the carboxylic acids (figures 3A, 3B). However, using specific substrates, such as L-threonine, D-mannitol, α -D-lactose 4-hydroxy benzoic acid, D-glucosaminic acid, α -ketobutyric acid, and Tween 80, determined the separation of different incubation times along PC2 (figures 3A, 3B). Different composts and incubation times had obvious impacts on substrate utilization pattern and soil microbial community structure in other studies also (Bucher and Lanyon 2005; Nair and Ngouajio 2012). Bucher and Lanyon (2005) reported different soil microbial communities in treatments with and without dairy manure. Recently, Nair and Ngouajio (2012) found that microbial communities were difficult to distinguish among different treatments initially, but community structure separated clearly with compost or no-compost after a long period. They also supposed that PCA separation of microbial communities was primarily due to differences in substrate utilization in their treatments (Nair and Ngouajio 2012). For instance, the 'r' selected bacteria—i.e., those with faster reproduction and death under disturbance—were affected by high levels of organic C and N and grew rapidly with organic amendments (Nair and Ngouajio 2012). In the present study, quantity and quality of organic C differed for the different straw decomposition products. Chemical-promoted products usually had higher contents of organic C and higher condensation of humic substances. These factors may have determined the different growth strategies and development of microorganisms in the incubation experiment.

CONCLUSION

Our results showed that adding chemical additives could increase the nutrient content and promote maturity of peanut straw decomposition products. Chemical additives, especially FeSO_4 , improved the humification and condensation of the decomposing matter. When the resulting

products were used as organic fertilizer in red soil they could increase urease and invertase activities with incubation. BIOLOG analysis indicated that microbial functional diversity and community structure were influenced by different organic amendments and incubation time. The present study contributes to improvement of straw biomass utilization and soil biological function by optimizing composition of compost.

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