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Microbiological parameters as indicators of compost maturity

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ABSTRACT

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Aims: The objectives of this study were to determine the changes of microbial properties of pig manure collected from pens with different management strategies and composted using different turning and moisture regimes; relate their association with humification parameters and compost temperature; and identify the most suitable microbial indicators of compost maturity.

Methods and Results: Six different microbial parameters, including total bacterial count, oxygen consumption rate, ATP content, dehydrogenase activity, and microbial biomass C and N, along with humification parameters [humic acid (HA), fulvic acid (FA) and HA : FA ratio] and compost temperature were monitored during composting. Significant positive correlations were found between temperature and microbial properties, including O_2 consumption rate, ATP content, dehydrogenase activity, and microbial biomass N. The humification parameters also showed significant correlations with microbial properties of the manure compost. For instance, HA contents of pig manures was positively correlated with total aerobic heterotrophs, and microbial biomass N and C; and negatively correlated with O_2 consumption rate, ATP content, and dehydrogenase activity. Among the six microbial parameters examined, dehydrogenase activity was the most important factor affecting compost temperature and humification parameters. Composting strategies employed in this study affected the speed of composting and time of maturation. If the moisture content is maintained weekly at 60% with a 4-day turning frequency, the pig manure will reach maturity in 56 days.

Conclusions: The composting process went through predictable changes in temperature, microbial properties and chemical components despite differences in the initial pig manure and composting strategies used. Among the six microbial parameters used, dehydrogenase activity is the most suitable indicator of compost maturity. Compared with respiration rate, ATP content and microbial biomass procedures, dehydrogenase activity is the simplest, quickest, and cheapest method that can be used to monitor the stability and maturity of composts.

Significance and Impact of the Study: The results presented here show that microbial parameters can be used in revealing differences between composts and compost maturity. The statistical relationship established between humification parameters and microbial parameters, particularly dehydrogenase activity, demonstrates that it is possible to monitor the composting process more easily and rapidly by avoiding longer and more expensive analytical procedures.

Keywords: ATP content, decomposition, deep litter system, dehydrogenase activity, humic acid, microbial activity.

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INTRODUCTION

Compost is the product of aerobic process during which micro-organisms play an important role. Essentially, the micro-organisms decompose the organic matter into a stable amendment for improving soil quality and fertility (Rynk *et al.* 1992; Borken *et al.* 2002). During composting, the micro-organisms use the organic matter as a food source. The process produces heat, CO_2, H_2O vapour, and humus as a result of the growth and activities of micro-organisms (Epstein 1997). The success of the composting process, however, relies on the ability of the microbial community to sustain with its basic needs for moisture, oxygen, temperature control, and nutrient availability (Epstein 1997). As the majority of the micro-organisms responsible for the formation of mature compost are aerobes, they require or work best in the presence of oxygen.

In this study, spent pig litter disposed from the deep litter system was used for composting. The deep litter system is a pig raising method, where pigs are raised on a litter bedding material (sawdust mixed with a commercial bacterial product) (Tiquia *et al.* 1996). The pig excreta, once deposited, are quickly mixed with the litter bedding (sawdust) and are decomposed *in situ*. Previous studies indicated that the decomposition of the pig manure within the deep litter system is incomplete, hence the litter requires further composting in windrows to achieve maturity. Due variations in the number of pigs, types of commercial bacterial products, amounts of sawdust, and management strategies in the deep litter system, the properties of the spent pig litter from different pig pens are likely to be different. Such differences in the spent litter would affect the time of maturation and quality of the mature compost. The acceptance of the spent pig litter compost as a fertilizer and/or conditioner depends on the consistent composition of the mature compost. It has been reported that composting leads to homogeneity in the composition of the product irrespective of the initial material and the composting process (Garcia *et al.* 1993; Serra-Wittling *et al.* 1996). Such homogeneity would be useful in establishing maturity indices, which can be quantified numerically.

The operating strategies used during composting of spent pig litter would influence the composting process and time of maturation. Many difficulties associated with composting can be traced to insufficient oxygen levels to support the decomposition of compost. Compost microbes also require a moist environment because they live in the water films surrounding composting organic matter particles (Richard *et al.* 2002). The optimal moisture and turning frequency however vary significantly depending on type of initial composting material used (Serra-Wittling *et al.* 1996; Tiquia *et al.* 1996, 2001; Epstein 1997; Liang *et al.* 2003).

The evaluation of composts has focused on compost maturity to determine the completion of the composting process (Forster *et al.* 1993; Grebus *et al.* 1994; Tiquia 2003); and the assessment of composting strategies to optimize the process and produce good quality end product (Ouedraogo *et al.* 2001; Borken *et al.* 2002; Tiquia 2003). Most of the criteria used in the evaluation of the composting process and compost stability (maturity) were based on physical and chemical parameters of the organic material, whose behaviour reflects the metabolic activity of micro-organisms involved in the composting process. These parameters include a drop in compost temperature (Flynn and Wood 1996), degree of self-heating capacity (Rynk *et al.* 1992), oxygen consumption (Tiquia *et al.* 1996), phytotoxicity assays (Tiquia and Tam 1998; Contreras-Ramos *et al.* 2004), cation exchange capacity (Harada and Inoko 1980), organic matter and nutrient contents (Tiquia 2003; Baddi *et al.* 2004; Grigatti *et al.* 2004) and C : N ratio (De Bertoldi *et al.* 1983; Kaushik and Garg 2004).

The microbial activities, numbers and biomass are key parameters that can also be used to elucidate the composting process (Tiquia *et al.* 2002a). While changes in physico-chemical properties during the composting process have been extensively studied (Harada and Inoko 1980; Garcia *et al.* 1991; Mathur *et al.* 1993; Flynn and Wood 1996; Day *et al.* 1998; Tiquia *et al.* 1998a). The respiratory activities, nitrification potential, ATP content, enzyme activities, and microbial counts have also been successfully used to assess compost maturity (Forster *et al.* 1993; Iannotti *et al.* 1994; Tiquia 2002; Tiquia *et al.* 2002b; Ryckeboer *et al.* 2003). Most of these studies have been restricted to monitoring the changes in microbial activities during composting. Attempts have not been made to link these activities with humification parameters. Humification is widely considered as an important process during composting of organic materials, where humic substances are formed and nonhumic substance decompose (Schnitzer *et al.* 1993; Bernal *et al.* 1996; Hsu and Lo 1999; Baddi *et al.* 2004). As composting progresses, the percentage of humic substances is expected to increase relative to the total dry mass on the total organic matter (Wu and Ma 2002). As a result, humification-related parameters have been examined to represent compost stability and maturity (Sequi *et al.* 1986; Senesi 1989; Veeken *et al.* 2000; Provenzano *et al.* 2001; Baddi *et al.* 2004).

This paper reports the study of six different microbial parameters, including total bacterial count, oxygen consumption rate, ATP content, dehydrogenase activity, and microbial biomass C and N during composting. The changes of these parameters and their association with humification parameters [humic acid (HA), fulvic acid (FA) and HA : FA ratio] and compost temperature were evalu-

ated to identify the most suitable microbial indicators of compost maturity. The effects of initial material and composting strategies on the microbial properties and maturation of the spent litter were also investigated.

MATERIALS AND METHODS

Composting set-up and sampling

Pig manures were collected from different pens where the deep litter system was employed (Table 1). In the present study, six pens, which differed in the number of pigs, amount of sawdust, type of bacterial inoculum [i.e. Elimexal (Horiba, Kyoto, Japan), Vitacogen (Snow Brand Seed Ltd, Sapporo, Japan), Biogreen (Agrimix BV, Landgraaf, the Netherlands), odour control-organic fertilizer (C.J. Rockwell, Nebraska, USA)] and litter management (i.e. layering, mixing) were set-up (Table 1). After 10–13 weeks of pig raising, the pig manure was removed from the pens and stacked in heaps. A total of 12 pyramidal heaps, about 2 m in diameter at the base and 1·5 m in height were built from the six pens (pens A–F; Table 1). The piles were composted in a composting shed. The composting strategies used in these study (Table 2) were based on those used by several compost operators and livestock farmers, and on that reported in the literature (Rynk *et al.* 1992; Tiquia *et al.* 1996, 1997; Epstein 1997).

During composting, air temperature and pile temperatures at a depth of 60 cm were monitored, after turning. Temperatures were taken twice a week during composting. Five temperature readings were taken from each pile. The piles were turned using a front-end truck loader. Pile 1 was turned every 2 days and the rest were turned every 4 days during the process (Table 2). At the beginning of composting, the moisture content of the piles was adjusted to 50–70%. Moisture contents of piles 4, 5 and 6 were adjusted to 50, 60 and 70%, respectively, on days 15, 32 and 63, whereas those of piles 9 and 11 were adjusted to 60% weekly until the end of composting. Water was added to these piles to adjust the moisture to their designed values (Table 2). The weight, density and moisture content of each pile were considered in calculating the amount of water needed to adjust the moisture content of pig manure to the desired values. No further adjustment in moisture content was carried out in piles 1–3, 7–9 and 11 after the initial moisture adjustment at day 0.

The composting trial was terminated between 74 and 126 days, when compost temperatures declined to ambient level (Table 2). Phytotoxicity test was also performed on Chinese spinach (*Amaranthus espinosus*) at the end of the trial to ensure that the damaging effects and toxicity of the spent pig litter were eliminated (Table 3). Phytotoxicity assays have been used to evaluate compost maturity (Zucconi *et al.* 1981; Tiquia 2000). It has been suggested that a germination index (a factor of relative seed germination and root elongation) of ≥80 indicates the disappearance of phytotoxicity in composts (Zucconi *et al.* 1981). Such a value was reached in all piles at the end of composting (Table 3).

Samples were collected at five random locations in each pile at day 0 and then weekly until the end of composting. These five samples were combined and mixed to generate a composite sample. Triplicate composite samples were taken from each pile for chemical and microbial analyses.

Table 1 Description of the pigpens during pig rearing under the pig-on-litter system

	Pig pens					
Pens	A	B	C	D	E	F
Size of pig pen (m^2)	32	30	30	40	40	30
No. of pigs	32	30	30	40	40	30
Age of pigs at start (days)	99	82	78	113	72	78
Raising period (days)	92	115	117	91	123	117
Litter management	Mixing	Layering	Layering	Layering	Layering	Layering
Total feed consumption* (kg)	6545	8308	9619	8196	11 910	9619
Total sawdust consumption (bags)	60	70	90	82	91	90
Bacterial product used	Elimexal	Vitacogen	Biogreen	Biogreen	No bacterial product added	Odour control-organic fertilizers
Composting pile†	1–3	4–6	7	8	9, 10	11, 12

*The feed contained a mixture of corn yellow, soya bean meal, fish meal, fat, and small amount of dicalcium phosphate, lysine, Tasmix 77 [with premixed Cu and Zn) (Feed World, Christchurch, New Zealand)], Tylan (Elanco, Greenfield, IN, USA) and Hygromix (Elanco).
†Spent litter in piles 1–3 were collected from pen A; spent litter in piles 4–6 were collected from pen B; spent litter in pile 7 was collected from pen C; spent litter in pile 8 was collected from pen D; spent pig litter in piles 9 and 10 was collected from pen E; spent pig litter in piles 11 and 12 was collected from pen F.

Table 2 Composting treatments and temperature characteristics of spent pig litter

		Composting treatments			
Piles	Composting duration (days)	Initial moisture (%)*	Moisture adjustment	Moisture adjustment frequency	Turning frequency†
1	74	50	No	–	Every 2 days
2	74	50	No	–	Every 4 days
3	126	50	No	–	Every 7 days
4	91	50	Yes	Days 15, 32 and 63	Every 4 days
5	91	60	Yes	Days 15, 32 and 63	Every 4 days
6	91	60	Yes	Days 15, 32 and 63	Every 4 days
7	91	60	No	–	Every 4 days
8	91	60	No	–	Every 4 days
9	91	60	Yes	Weekly	Every 4 days
10	91	60	No	–	Every 4 days
11	91	60	Yes	Weekly	Every 4 days
12	91	60	No	–	Every 4 days

*Moisture content was determined by drying the of pig manure samples at 105°C for 24 h. Water was added to piles 4–5, 9 and 11 to adjust the moisture to their designed values.
†The piles were turned using a front-end truck loader.

Table 3 Temperature characteristics at different stages of composting, and germination index (GI) of the mature product

	Temperature characteristics*							
Piles	Initial temperature (°C)	Time to reach 55°C (days)	Time to reach >55°C (days)	Peak temperature (°C)	Time to reach peak temperature (°C)	Duration of thermophilic phase (°C)	Time to drop to ambient level (30–35°C days)	GI†
1	48	4	5	63	15	22	60	83
2	51	2	4	65	21	28	64	81
3	48	2	4	64	21	28	126	80
4	50	2	4	69	4	45	91	85
5	44	2	4	69	7	45	91	84
6	48	2	4	59	7	17	64	86
7	40	4	4	57	14	14	91	82
8	31	4	4	63	4	14	64	85
9	31	4	5	62	17	20	91	80
10	31	4	9	64	10	20	56	96
11	32	4	9	58	14	24	56	95
12	31	4	9	63	10	20	56	96
Range	31–51	2–4	4–9	57–69	4–21	20–45	56–126	80–96

*Pile temperatures were taken at a depth of 60 cm in each pile twice a week. Five temperature readings were taken from each pile.
†Data were obtained from Tiquia (2000). Data shows the germination index at the time composting was terminated.

Chemical characteristics

Pig manure samples were analysed for: moisture content (105°C for 24 h); Kjeldahl N (Bremmer 1996), organic matter (O.M.) and total organic C contents by loss on ignition (550°C for 5 h) (Nelson and Sommers 1996). The O.M. concentration of the pig manure was computed from the ash content:

$$\text{Ash content (g kg}^{-1}) = \frac{\text{Ash weight of compost (g)}}{\text{Dry weight of compost (kg)}},$$

$$\text{O.M. content (g kg}^{-1}) = 1000 - \text{Ash content of compost (g kg}^{-1}).$$

The total organic C was estimated from the O.M. value using the conventional 'Van Bemmelem factor' of 1·724. This factor is based on the assumption that soil O.M. contains 58% C (Allison 1965). The theoretical N concentration of the spent pig litter was calculated by adding Kjeldahl N concentrations with $(NO_3^- + NO_2^-)$ – N

concentrations. The C : N ratio was then based on the concentration of total organic C and total N.

HA and FA fraction of the pig manure was analysed using a precipitation method (Swift 1996). Spent pig litter samples were diluted with NaOH (0·1 mol l^{-1}) in a conical flask and the air inside was displaced with N_2. The conical flask was stoppered and shaken at 200 *g* at 23°C for 24 h. After 24 h, the supernatant, which is the humic material fraction or humus extract, was separated by centrifugation at 2000 *g* for 10 min. The precipitate was washed with distilled water and separated by centrifugation. The supernatant fraction, which is a combination of alkaline extracts and the washings, was acidified to pH 2 using HCl. The sample was allowed to stand at room temperature for 24 h, and then acidified. The supernatant was the FA fraction and the precipitate was the HA. Both fractions were dried and weighed. HA and FA substances were calculated based on gram HA or FA per kg O.M.

Enumeration of total aerobic heterotrophs

Estimation of total aerobic heterotroph numbers of the pig manure samples was quantified by direct plating on appropriate media (Zuberer 1994). The serially diluted manure suspension was inoculated onto the agar using plate frequency technique (Tiquia *et al.* 1998b). Each agar was divided onto eight sections and 0·1 ml of the compost suspension was dropped on each of the sections. After incubation, any visible growths observed on any of the eight sections were scored positive. The total number of sections with growth at each dilution was counted and the populations of total aerobic heterotrophs in the pig manure sample were estimated using the Most Probable Number (MPN) method (Woomer 1994).

ATP content

An ATP detection kit (Analytical Luminescence Laboratory, Baltimore, MD, USA) and a luminometer (Monolight 1500, Analytical Luminescence Laboratory) were used to determine the microbial ATP of the pig manure samples. One gram of each sample was extracted with 49 ml Tris–EDTA buffer for 1 min, then 100 μl if the mixed solution was transferred to a cuvette. An aliquot (100 μl) of Extralight ATP releasing reagent (Analytical Luminescence Laboratory) was added to the cuvette and mixed gently. The cuvette was place into the counting chamber of the instrument and after a 20-s waiting period, 100 μl Firelight reagent (Analytical Luminescence Laboratory) was added to initiate the reaction. The readings were taken and converted from relative light units to concentration of ATP using the ATP standard supplied by the manufacturer.

O_2 consumption rate

The substrate-induced respiration rate of the pig manure samples was measured according to the method described by West *et al.* (1986). A glucose solution was used as a substrate to maximize respiratory responses. The amounts of O_2 consumed by the micro-organisms were determined using a Gilson Differential Respirometer. The instrument has 14 flasks of 19 ml nominal volume. The centre well of each flask contained 0·3 ml of 5 mol l^{-1} KOH to absorb CO_2, and a small fan of fibreglass filter paper to increase the surface area of absorptive surface. The samples and glucose solution were transferred to each of the flasks. The flasks were shaken throughout the assay at 100 strokes min^{-1} in a 25°C water bath. The volume changes were measured every 30 min subsequently using directly calibrated micromanometers.

Dehydrogenase activity

The dehydrogenase activity of the pig manure samples was measure using colorimetric method (Tabatabai 1994). Manure samples were extracted with $CaCO_3$ and 2,3,5-triphenyltetrazolium chloride in a screw-capped test tube, and then incubated at 37°C for 24 h. After incubation, methanol was added to the sample, and then it was shaken for 1 min. The suspension was filtered through a glass funnel plugged with absorbent cotton, into a volumetric flask. The intensity of the red colour was measured using a spectrophotometer set at 485 nm. The amount of triphenyl formazan (TPF) produced was calculated with reference to calibration curve prepared from TPF standard.

Microbial biomass C and N

The chloroform ($CHCl_3$) fumigation-extraction technique described by West *et al.* (1986) and Vance *et al.* (1987) was used to determine microbial biomass C and N of the pig manure samples. The samples were fumigated with alcohol-free $CHCl_3$ in a vacuum desiccator lined with moistened paper, with a beaker containing $CHCl_3$ and anti-bumping granules. After fumigation, the desiccator was kept in the dark at 25°C for 24 h. The beaker of $CHCl_3$, and the moistened filter paper were removed and $CHCl_3$ was repeatedly evacuated in the vacuum desiccator. After evacuation, the samples were extracted with 0·5 mol l^{-1} K_2SO_4 (1 : 5 sample : solution) for 30 min then filtered. An unfumigated control sample was prepared at the time the fumigation started and was extracted with 0·5 mol l^{-1} K_2SO_4. The microbial biomass C was extracted using a TOC analyser (Shimadzu TOC analyser; Shimadzu, Kyoto, Japan) whereas the microbial biomass N was measured using distillation method (Mulvaney 1996).

Statistical analyses

Box plots were constructed to provide a visual summary of the distribution of microbial data collected from 12 different piles at various stages of composting. The graphs were plotted using Sigma Plot 2000 (SPSS, Inc., Chicago, IL, USA). Pearson product–moment correlation coefficient were calculated to show relationship between microbial parameters (total aerobic heterotrophs, O_2 consumption rate, ATP content, and microbial biomass N and C) and compost maturity parameters (temperature, HA, FA and HA : FA ratio). To determine the most important microbial parameters affecting compost maturity and humification process, a stepwise multiple regression analysis was performed. Statistical analyses were computed using SigmaStat version 1.0 (Jandel Corporation, San Rafael, CA, USA).

RESULTS

Temperature profiles

The temperature characteristics of each pile were unique for each composting treatment (Table 3). Initial compost temperatures ranged from 31 to 51°C. As composting proceeded, these temperatures increased rapidly. By days 2–4, compost piles reached thermophilic temperature (55°C). Highest peak temperature (69°C) was observed in piles 4 and 5, and this peak temperature was reached within a shorter period of time (4–7 days) compared with the rest of the piles, which took 10–21 days. Temperatures in piles 4 and 5 approached >65°C and had the longest (45 days) duration of thermophilic phase (>55°C) (Table 3). The time to reach ambient level varied significantly among different piles. Pile 3 (50% moisture, no moisture adjustment, turned once a week) took 126 days to reach ambient temperature. Piles 10–12 (60% moisture, turned twice a week) took 56 days to reach ambient level (Table 3).

Chemical profiles

Total N, C, C : N ratio, HA and FA were influenced by differences in the components of the initial compost material (Table 1) and composting strategy (Table 2). At the beginning of composting, there was a wide variation in the concentration of these parameters among different piles (Table 4). The C contents varied between 505 and 525 g kg^{-1}; the N contents between 18 and 30·5 g kg^{-1}; the C : N ratios between 16·7 and 27·4, HA contents between 20·7 and 37·2 g kg^{-1} O.M.; FA contents between 70·0 and 114·6 g kg^{-1} O.M.; and HA : FA rations between 0·22 and 0·46 (Table 4). At the end of composting, C concentrations and C : N ratios decreased, whereas N, HA and FA concentrations, and HA : FA ratios increased (Table 4).

During composting HA contents of the pig manure evolved and became predominant over FA. HA contents

Table 4 Mean concentrations of total organic C, ash, N, P and K of the initial and composted spent litter

	Chemical properties*											
	C (g kg^{-1})		N (g kg^{-1})		C : N ratio		HA (g kg^{-1} O.M.)		FA (g kg^{-1} O.M.)		HA : FA ratio	
Pile	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
1	505	496	19·3	19·4	26·2	25·6	31·6	41·1	109·2	109·9	0·29	0·37
2	506	494	19·2	19·3	26·4	25·6	34·7	42·8	113·5	103·9	0·31	0·41
3	505	497	23·9	23·6	21·1	22·0	37·2	37·9	118·0	105·0	0·32	0·36
4	525	508	19·5	27·1	26·5	18·8	27·0	45·0	105·1	97·6	0·26	0·46
5	523	507	20·2	26·8	24·6	18·9	27·4	42·8	114·0	107·9	0·24	0·40
6	523	509	18·0	24·8	27·4	20·6	25·2	37·3	112·4	114·6	0·22	0·33
7	517	500	24·7	20·9	20·9	23·9	34·0	60·0	75·0	79·0	0·45	0·76
8	519	492	19·0	20·0	27·3	24·6	24·0	60·0	73·0	100·0	0·33	0·60
9	516	496	25·6	31·3	20·2	15·9	30·8	77·5	72·2	71·8	0·43	1·08
10	508	497	30·5	31·9	16·7	11·9	29·8	89·7	69·7	80·3	0·43	1·12
11	516	496	25·6	31·3	20·1	15·9	33·1	83·5	72·2	70·9	0·46	1·18
12	513	495	27·4	34·5	18·8	14·4	20·7	85·7	70·8	70·9	0·29	1·21
Range	505–525	492–508	18·0–30·5	19·3–34·5	16·7–27·4	11·9–25·6	20·7–34·7	37·3–89·7	69·7–118·0	70·9–114·6	0·22–0·46	0·33–1·21

HA, humic acid; FA, fulvic acid; O.M., organic matter.

*Samples were collected at five random locations of the piles after turning. These five samples were combined and mixed to generate one composite sample. Mean values of three composite samples from each pile are shown. Final data refer to that time point mentioned in Table 3. Data shown are based on 105°C dry weight.

increased from initial values of 20·7–34·7 to 37·3–89·7 g kg^{-1} O.M. at the end of composting, whereas FA had very little change (Table 4). Greatest increase in HA contents was observed in piles 10–13 (Table 4). HA : FA ratios in piles 9–12 increased to over 80% at the end of composting, whereas piles 1–5 increased only between 28 and 50%. These results suggest that the degree of humification in piles 9–12 was higher than piles 1–5 (Table 4).

Total aerobic heterotrophs and O_2 consumption rate

Differences in initial pig manure composition (Table 1) did not significantly affect the changes in total aerobic heterotroph counts (Fig. 1a). The populations of these microorganisms were similar in all piles at the beginning of composting. During the thermophilic stage, populations of total aerobic heterotrophs increased to between 8·2 and 10 Log_{10} MPN g^{-1} and then fluctuated at these levels during the cooling and mature stages of composting (Fig. 1a).

The respiration rates, in terms of the oxygen consumption (Fig. 1b), were affected by differences in composition of the initial pig manure (Table 1). The oxygen consumption rates of the 12 piles varied from 0 to 2·4 μl min^{-1} g^{-1} and as composting proceeded, the variability among these piles narrowed (see box plot in Fig. 1b). As expected, the oxygen consumption rates were highest (1·2–2·6 μl min^{-1} g^{-1}) during the thermophilic stage of composting. The rates decreased rapidly to 0·2–0·8 μl min^{-1} g^{-1} during the cooling stage and were

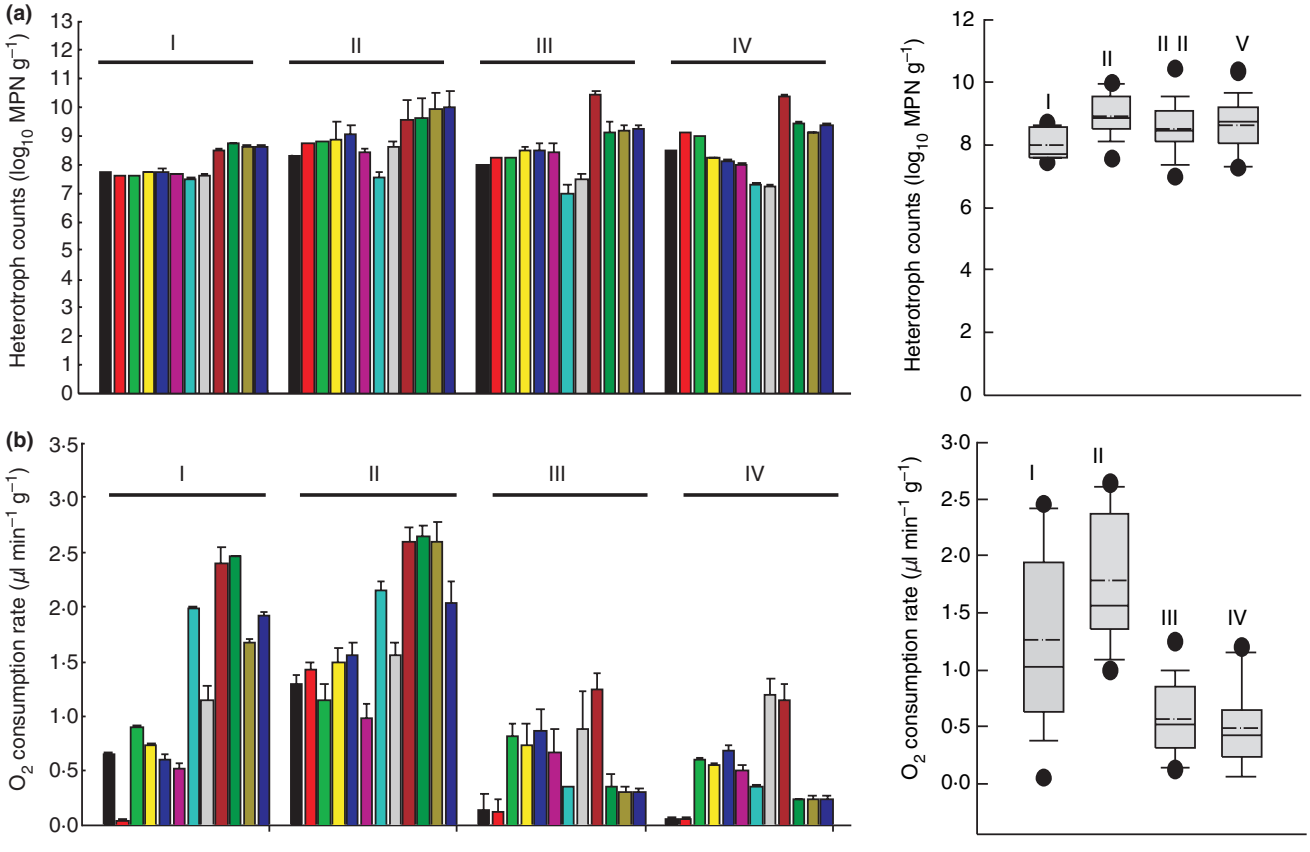

Fig. 1 Total aerobic heterotroph counts (a) and O_2 consumption rates (b) of pig manure, and box plots showing the distribution of aerobic heterotroph counts and O_2 consumption rate data from the 12 piles at different phases of composting. Mean of three composite samples are shown. Initial phase (I) = day 0, pile temperature ranged from 31 to 51°C; thermophilic phase (II) = days 20–45, pile temperature ranged from 55 to 69°C; cooling phase (III) = days 21–120, pile temperature ranging from 40 to 50°C; mature phase (IV) = days 56–126, pile temperature ranged from 28 to 35°C. Box plots next to the bar graphs show a visual summary of the distribution of total aerobic heterotrophs and oxygen consumption data collected from 12 different piles at various stages of composting. The lines inside the rectangle indicate the median of the sample distribution. The upper and lower boundaries of each rectangle indicate the upper quartile and lower quartile respectively. The upper and lower whickers are the upper and lower extreme values respectively. (■) Pile 1; (■) pile 2; (■) pile 3; (■) pile 4; (■) pile 5; (■) pile 6; (■) pile 7; (■) pile 8; (■) pile 9; (■) pile 10; (■) pile 11; (■) pile 12

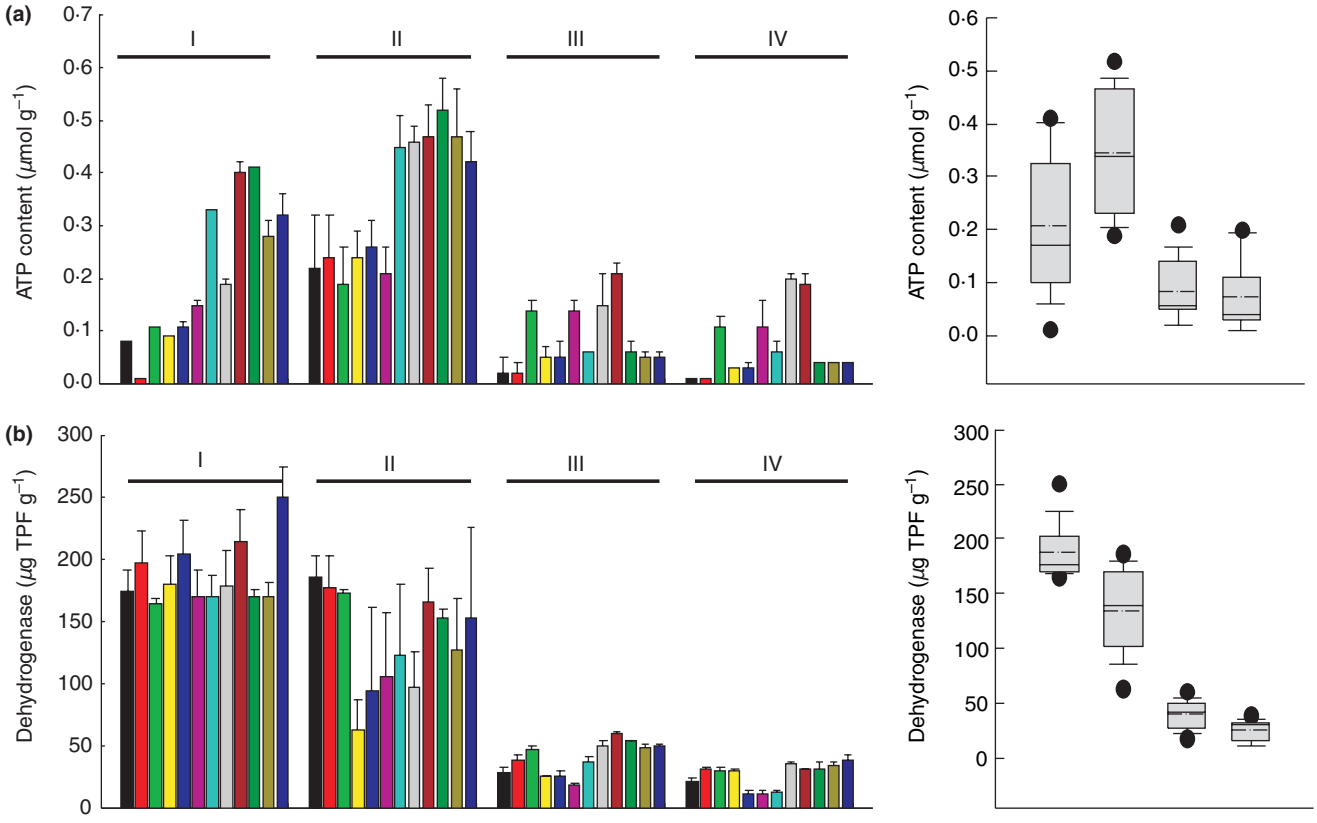

Fig. 2 ATP contents (a) and dehydrogenase activities (b) of pig manure, and box plots showing the distribution of ATP content and dehydrogenase activity data from the 12 piles at different phases of composting. Mean of three composite samples are shown. Initial phase (I) = day 0, pile temperature ranged from 31 to 51°C; thermophilic phase (II) = days 20–45, pile temperature ranged from 55 to 69°C; cooling phase (III) = days 21–120, pile temperature ranging from 40 to 50°C; mature phase (IV) = days 56–126, pile temperature ranged from 28 to 35°C. Box plots next to the bar graphs show a visual summary of the distribution of ATP content and dehydrogenase activity data collected from 12 different piles at various stages of composting. The lines inside the rectangle indicate the median of the sample distribution. The upper and lower boundaries of each rectangle indicate the upper quartile and lower quartile respectively. The upper and lower whickers are the upper and lower extreme values respectively. (■) Pile 1; (■) pile 2; (■) pile 3; (■) pile 4; (■) pile 5; (■) pile 6; (■) pile 7; (■) pile 8; (■) pile 9; (■) pile 10; (■) pile 11; (■) pile 12

maintained around this level during the mature stage of composting (Fig. 1b).

ATP content and dehydrogenase activity

The trend in ATP contents (Fig. 2a) of the pig manure samples was similar to that of respiration rates (Fig. 1b). The initial ATP contents rose slightly from initial values of 0·1–0·3 to 0·2–0·5 μmol g^{-1} during the thermophilic stage of composting. The ATP contents then dropped to 0·15–0·29 μmol g^{-1} and remained at these levels at the mature stage of composting (Fig. 2a). Differences in composition of the initial pig manure (Table 1) and composting strategies affected the ATP contents (Fig. 2a) of the litter piles as shown by the large variability among 12 piles during composting. However, as composting proceeded, variability decreased (see box plot in Fig. 2a).

Dehydrogenase activity has been used as a measure of total biological activity in soils and composts (Scheafer 1963; Alef 1995; Tiquia *et al.* 1996). In this study, the dehydrogenase activity of the pig manure compost was highest (160–250 μg TPF g^{-1}) at the beginning of composting (Fig. 2b). The activity decreased as composting proceeded and began to level-off (12–39 μg TPF g^{-1}) towards the end of composting.

Microbial biomass C and N

At the beginning of composting, the microbial biomass varied between 0·1 and 2·6 mg g^{-1} (Fig. 3a). As composting proceeded, the microbial biomass C contents of all piles increased to a level between 4·3 and 6·9 mg g^{-1} and were maintained at this level until the end of composting.

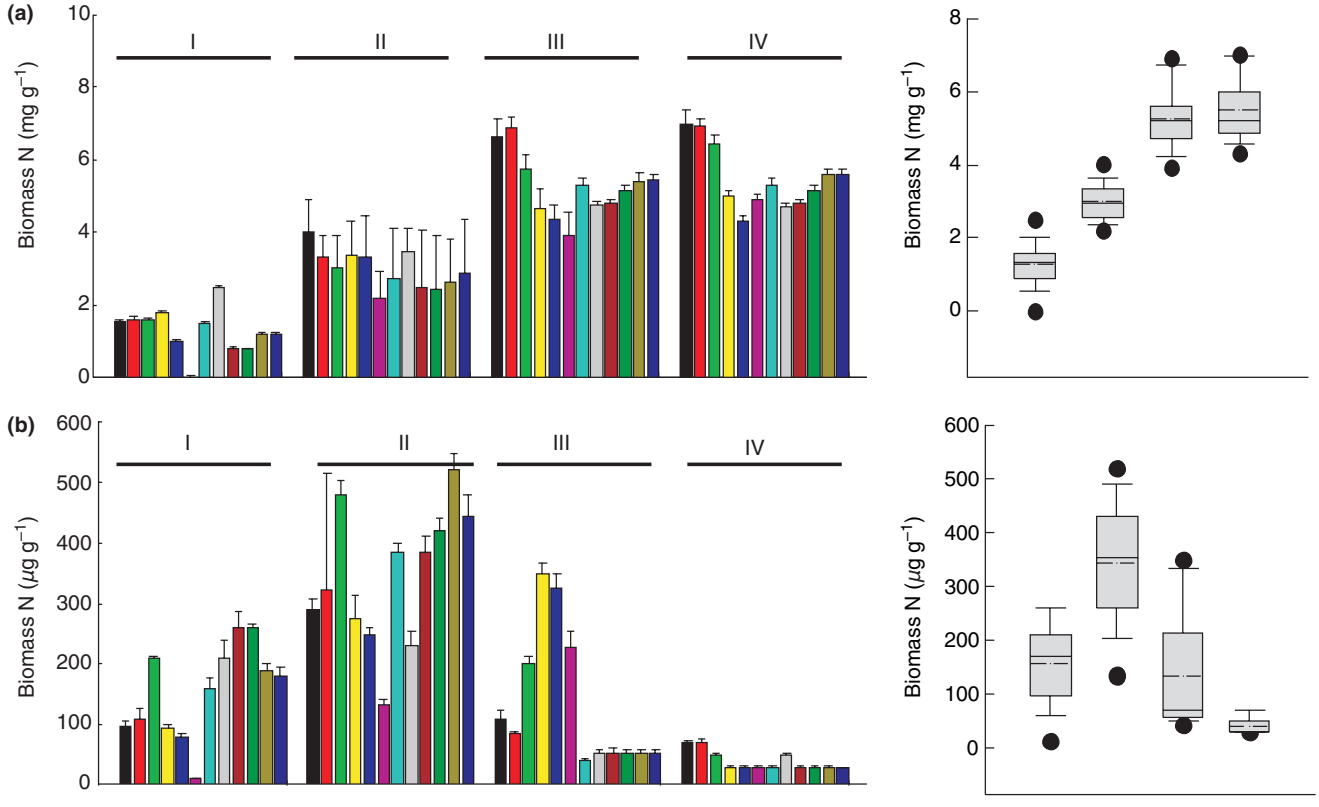

Fig. 3 Microbial biomass C (a) and N contents (b) of pig manure and box plots showing the distribution of microbial biomass C and N data from the 12 piles at different phases of composting. Mean of three composite samples are shown. Initial phase (I) = day 0, pile temperature ranged from 31 to 51°C; thermophilic phase (II) = days 20–45, pile temperature ranged from 55 to 69°C; cooling phase (III) = days 21–120, pile temperature ranging from 40 to 50°C; mature phase (IV) = days 56–126, pile temperature ranged from 28 to 35°C. Box plots next to the bar graphs show a visual summary of the distribution of microbial biomass C and N collected from 12 different piles at various stages of composting. The lines inside the rectangle indicate the median of the sample distribution. The upper and lower boundaries of each rectangle indicate the upper quartile and lower quartile respectively. The upper and lower whickers are the upper and lower extreme values respectively. (■) Pile 1; (■) pile 2; (■) pile 3; (■) pile 4; (■) pile 5; (■) pile 6; (■) pile 7; (■) pile 8; (■) pile 9; (■) pile 10; (■) pile 11; (■) pile 12

The microbial biomass N contents of all piles were between 98 and 260 μg g^{-1} during the initial stage of composting trial. These values increased rapidly between 110 and 490 μg g^{-1} during thermophilic phase. Thereafter, the microbial biomass contents in all piles declined and were maintained at low levels (20–40 μg g^{-1}) at the end of composting (Fig. 3b).

Correlation between microbial properties, temperature and humification parameters

The rise and fall of temperature has been reported to be correlated with microbial properties (Epstein 1997). Here, significant positive correlations were found between temperature and microbial properties such as O_2 consumption rate, ATP content, dehydrogenase activity and microbial biomass N (Table 5). This result shows that the rise and fall of temperature was paralleled with the changes of these four microbial properties. The humification parameters (HA, FA and HA : FA ratio) also showed significant correlations with microbial properties of the manure compost. For instance, HA was positively correlated with total aerobic heterotrophs, and microbial biomass N and C, and negatively correlated with O_2 consumption rate, ATP content and dehydrogenase activity (Table 5). FA was negatively correlated with microbial properties wit the exception of dehydrogenase activity and microbial biomass C and N, where no correlations were found. Significant positive correlations were found between HA : FA and total aerobic heterotrophs, dehydrogenase activity and microbial biomass N and C (Table 5).

Multiple regression analysis

The multiple regression analysis revealed that among the five microbial parameters (total aerobic heterotrophs, O_2

Table 5 Pearson product–moment correlation coefficient (r) and probability (P) values between different microbial, temperature and humification parameters

	Temperature		HA		FA		HA : FA	
Microbial parameters	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value
Heterotrophs	0·15	0·3060	0·40	0·0044	−0·49	0·0003	−0·38	0·0084
O_2 consumption rate	0·42	0·0024	−0·36	0·0013	−0·47	0·0007	−0·15	0·3010
ATP content	0·49	0·0004	−0·33	0·0205	−0·49	0·0004	−0·124	0·4000
Dehydrogenase activity	0·64	<0·0001	−0·53	<0·0001	−0·15	0·3150	0·53	<0·0001
Biomass C	−0·35	0·0141	0·57	<0·0001	−0·04	0·7700	0·42	0·0020
Biomass N	0·39	0·005	0·38	0·0067	−0·19	0·1760	−0·26	0·0780

Heterotrophs = total aerobic heterotrophs; HA, humic acid; FA, fulvic acid. Correlations were based on 48 averaged data from the manure compost piles.

Table 6 Multiple regression analyses between different microbial, temperature and humification parameters

Regression equation	Multiple r^2 value	F value	Significance of F
Temperature = 35·9 − (17·9 × dehydrogenase) + (85·6 × ATP content) + (0·07 × biomass N)	0·51	15·2	<0·0001
HA = −0·45 + (10·1 × dehydrogenase) + (3·61 × biomass C) − (0·049 × biomass N)	0·51	15·3	<0·0001
FA = 176·1 − (9·14 × heterotrophs) − (15·1 × O_2 consumption) + (0·05 × biomass N)	0·43	11·1	<0·0001
HA : FA ratio = −1·30 + (0·24 × dehydrogenase) − (0·0009 × biomass C)	0·44	17·7	<0·0001

Multiple regression analyses was calculated based on six microbial parameters (total aerobic heterotrophs, O_2 consumption rate, ATP content, dehydrogenase activity, biomass C, and biomass N) using STEPWISE method with PIN (probability of F-to-enter) = 0·050 limit. HA, humic acid; FA, fulvic acid; heterotrophs = total aerobic heterotrophs; O_2 consumption = oxygen consumption rate.

consumption rate, ATP content, dehydrogenase activity and microbial biomass N and C) examined, dehydrogenase activity was the most important factor affecting the changes in temperature. Dehydrogenase activity was also the most important parameter influencing the changes in humification parameters with the exception of FA where total aerobic heterotrophs counts was the most important parameter influencing dehydrogenase activity changes during composting (Table 6).

DISCUSSION

Despite differences in the components of the initial pig manure samples (i.e. use of different bacterial inocula and different amounts of sawdust and pig manure) and composting strategies (i.e. turning frequency, moisture adjustment), the composting process went through predictable changes in temperature, microbial properties and chemical components. However, composting strategies employed in this study affected the speed of composting, and time of maturation (Table 3). For instance, it took 126 days for pile 3 to drop to ambient level; 91 days for piles 4, 5, 7 and 9; 67 days for piles 2, 6 and 8; 60 days for pile 1; and 56 days for piles 10–12 (Table 3). This study revealed that control of the composting process of pig manure demands an understanding of the interaction of environmental factors such as aeration (by turning) and moisture content. For efficient composting, moisture content must be maintained weekly at 60% with a 4-day turning frequency. Addition of bacterial inoculum onto the litter in the initial pig-on-litter system had no value on the rate of composting if moisture content was adjusted to 60% during windrow composting. The present study also demonstrated that if the above conditions are met, the pig manure reached maturity in 56 days.

The ability to tell whether or not a compost is mature, is important to compost makers, plant operators and end users. Unstable and immature compost can sustain high microbial activities. Thus, when such a compost is used as a soil amendment or plant growth medium, it may reduce oxygen concentration in the soil and immobilize nitrogen, thereby causing serious N-deficiencies in crops (Zucconi *et al.* 1981). Strictly speaking, stability of composts is often associated with microbial activity (Hue and Liu 1995). The numbers of total aerobic heterotrophs of pig manure compost were extremely high in all piles at the beginning of composting, due to the fact that the pig manure compost contained large amounts of partially decomposed pig faeces and urine, which contain high bacterial population (Tiquia *et al.* 1996). These indigenous bacteria decompose the organic matter and transform the N components through oxidation, nitrification

and denitrification (Atkinson *et al.* 1996), which generate heat during composting. After a period of elevation, the temperature gradually decreased to ambient levels. At this stage, the decomposition of organic matter in the pig manure became more stabilized, hence less heat was released and the microbial activities in terms of the O_2 consumption rate, ATP content, dehydrogenase activity, and microbial biomass N decreased to low levels.

The maturity of compost is related to the presence of HAs in composts. HAs are normally generated towards the last stage of composting (Veeken *et al.* 2000), which requires several weeks to a few months (Inbar *et al.* 1990). They are extremely important soil components because they constitute a stable fraction of carbon, thus regulating the carbon cycle and the release of nutrients, including nitrogen, phosphorus and sulfur (Stevenson 1984). Additionally, the presence of humic substances improves water-holding capacity, pH buffering and thermal insulation (Stevenson 1984), and stimulates the activities of microfloral and microfaunal organisms (Burns *et al.* 1986). This study demonstrated that composting strategies had an impact on the degree of humification. Composting in piles 10–12 (piles turned every 4 days and moisture contents adjusted to 60%) had higher HA contents than piles 1–9 (piles turned infrequently and without any moisture adjustment during composting), suggesting that the quality of compost in these three piles are better than the other piles in terms of organic matter. Besides the mineralization of organic matter, organic matter in composts can be converted to humic substances. The pathways for the formation of humic substances (the lignin theory, polyphenol theory and Maillard route) are extensively described by Stevenson (1984). It has been observed that the polyphenol theory (condensation route) has a significant contribution to the formation of HA during composting. Veeken *et al.* (2000) pointed that during the initial stage of composting, the organic matter is mineralized; hence, the respiration rate (microbial activity) is high. However, during the later (maturing) stage of composting, humic substances particularly HA increases and most of the organic matter is stabilized; thus, the respiration rate declines to low level. This trend was also seen in the present study. The increase in HA corresponded with decreases in O_2 consumption rate, ATP content and dehydrogenase activity to low levels (Table 5).

The stability of compost is connected to the microbial activities during composting. During composting, the degradable organic matter and nitrogenous compounds in the pig manure were broken down by micro-organisms. This process resulted in increases in microbial activities (O_2 consumption rate, ATP content, dehydrogenase activities and microbial biomass N). Towards the end of composting, no further decomposition is taking place as C and N became stabilized. Consequently, no more heat is released as a result of microbial activities, and so the O_2 consumption rate, ATP content, dehydrogenase activities and microbial biomass N dropped and stabilized to low levels.

In this study, dehydrogenase activity was found to be mostly associated with changes in temperature and humification parameters (HA and HA : FA ratio) (Table 5). The statistical relationship established between humification parameters and dehydrogenase activity demonstrates that it is possible to monitor the composting process more easily and rapidly by avoiding longer and more expensive analytical procedures. In unstable pig manure, the dehydrogenase activity can be as high as 250 μg TPF g^{-1} (Fig. 2b). This value dropped to as low as 12–35 μg TPF g^{-1} as the pig manure reached maturity. A dehydrogenase activity of 35 μg TPF g^{-1} is proposed as a predictor of compost maturity in this study. This value was similar to the value predicted by Tiquia *et al.* (2002b) during composting of poultry manure. Dehydrogenase activity is thought to reflect the total range of oxidative activity of soil microflora, and consequently may be a good indicator of microbiological activity (Tabatabai 1994). Tiquia *et al.* (1996) indicate that the oxygen uptake and dehydrogenase activity are closely related. Dehydrogenase therefore provides an information on the active portion of the compost microbial community – an information that cannot be obtained from assessing bacterial population density using viable plate count and total microbial biomass estimations. Here, it can be seen that dehydrogenase activity can be used to monitor the composting process and as a valid marker of compost maturity. The decrease in dehydrogenase activity to low levels towards the end of composting indicates that there was no more active decomposition going on, and that the pig manure compost reached maturity. Compared with respiration rate, ATP content and microbial biomass procedures, dehydrogenase activity is the simplest, quickest, and cheapest method that can be used to monitor the stability and maturity of composts.

The box plots provided excellent visual summary of the distribution and variability of the microbial data from the 12 piles at different stages of composting (Figs 1–3). The interquartile range (box plot) decreased with composting time, indicating that the variability among 12 piles was reduced as composting progressed. The median (shown as a line across the box) of the 12 piles during the cooling stage was closer to the median during the mature stage. Moreover, the outliers (solid circles outside the box plot) were also closer to the median at the end of the composting process. These results imply that although the composition of the initial pig manure are different because of variations in treatments under the deep litter system (i.e. number of pigs, amount of sawdust added, type of bacterial inocula added and litter management) (Table 1), when the pig manure compost piles become stabile and mature, their microbial properties reach similar values.

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