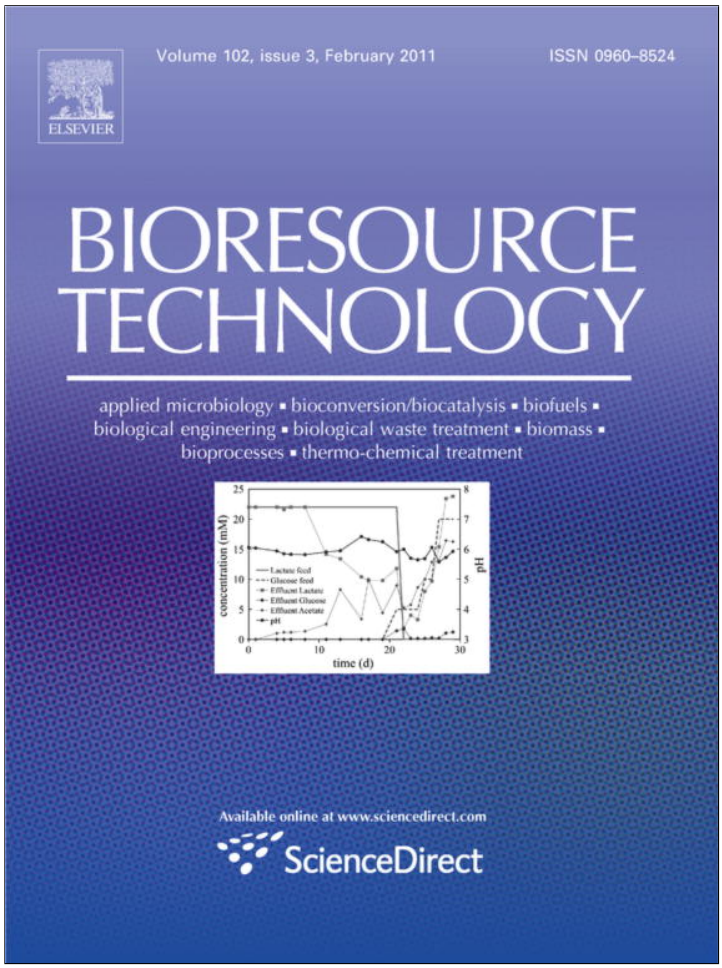

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Effects of physico-chemical parameters on the bacterial and fungal communities during agricultural waste composting

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ABSTRACT

The goal of this study was to identify and prioritize some of the physico-chemical parameters that contributed to bacterial and fungal community compositions during agricultural waste composting. Relationships between those parameters and microbial community compositions determined by PCR-DGGE were simultaneously evaluated by redundancy analysis (RDA). The results showed that the temporal variation of bacterial community composition was significantly related to water soluble carbon (WSC), ammonium and nitrate ($P < 0.05$), while the most variation in distribution of fungal community composition was statistically explained by pile temperature, WSC, and moisture content ($P < 0.05$). Significant amounts of the variation (54.9% and 56.0% for bacterial and fungal species data, respectively) were explained by those parameters, suggesting that those parameters were the most likely ones to influence, or be influenced by the bacterial and fungal species. Variation partitioning analyses indicated that WSC and pile temperature showed predominant effect on the bacterial and fungal community composition, respectively.

1. Introduction

Currently, composting is used as a major process of stabilizing agricultural organic wastes through the degradation of biodegradable components by microbial communities under controlled conditions. Bacteria and fungi species play significant roles in the decomposition and mineralization of agricultural organic wastes. Their community compositions are likely to be influenced by several physico-chemical parameters in composting systems. Substantial research efforts have been carried out to evaluate the impacts of various parameters on composting performance and microbial community composition (Cahyani et al., 2003; Eiland et al., 2001; Lei and VanderGheynst, 2000; Liang et al., 2003; Tang et al., 2007; Tang et al., 2009). In most cases, the effects of pile temperature and water soluble carbon (WSC) were greater than that of others (Cahyani et al., 2003; Ishii and Takii, 2003; Tang et al., 2007). While in study by Liang et al. (2003), moisture content even had an influence on the microbial activity greater than the temperature. The adjustment of initial pH had a significant effect on the microbial community structure throughout the composting process (Lei and VanderGheynst, 2000). Microbial community structure was affected by the initial C/N ratio treatment. Higher fungal/bacterial ratios and microbial biomass were found in the high C/N ratio treatments compared to the low ones (Eiland et al., 2001). Moreover, the physico-chemical parameters which affected the bacterial or fungal species were different (Griffin, 1985; Herrmann and Shann, 1997; Klamer and Bååth, 1998). Those contrasting reports clouded our understanding on which composting parameters drived the actual compositions of bacterial and fungal community. So far, the physico-chemical parameters have not been evaluated simultaneously with the bacterial and fungal composition changes to separate out their relative importance. It is of interest to conduct such research to determine to what extent of differences in bacterial and fungal communities are influenced by these parameters, respectively.

Recently advanced molecular biological techniques (e.g., PCR-DGGE) have been applied to composting system. The PCR-DGGE method has enabled the researchers to discern the dynamic development of microbial communities and to regulate the effects of different treatment regimes on their successions at molecular level (Cahyani et al., 2003; Gelsomino et al., 1999; Ishii and Takii, 2003; Nakasaki et al., 2009; Vivas et al., 2009). Those works were mainly restricted to a visual interpretation, neglecting the

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analytical potential of the method in terms of statistical significance and ecological interpretation (Fromin et al., 2002). Direct multivariate analyses, including redundancy analysis (RDA) and canonical correspondence analysis (CCA), were widely used to relate changes in community composition to changes in the environment, and provide statistical tests for those correlations. Nowadays, combinations of such high quality genetic fingerprinting methods with multivariate analysis techniques have been used in an increasing number of publications dealing with microbial communities in different ecosystems (Gilbride et al., 2006; Marschner et al., 2001; Roesti et al., 2006). These methods provide us the appropriate statistical tools for identifying composting factors that influence the diversity of microbial community composition among large sets of physico-chemical parameters. On the other hand, multicollinearity and complex interaction between composting parameters, make it difficult to distinguish between the separate effects of these parameters by studying each of them as fully independent variables. Variation partitioning, as an extension of direct multivariate analysis in a way analogous to partial correlation analysis, provides a solution for these problems (Borcard et al., 1992; Marschner et al., 2001; Roesti et al., 2006). Significant proportion of shared variation can be assigned to particular parameter by variation partitioning analysis after elimination of possible effects due to other composting parameters.

To achieve the deeper understanding of the process characteristic of agricultural waste composting, this study was carried out to determine whether changes in bacterial and fungal community compositions could be associated with physico-chemical parameters measured simultaneously. Those parameters included ambient temperature, pile temperature, pH, C/N ratio, moisture content, WSC, ammonium (NH_4^+-N) and nitrate (NO_3^--N). Meanwhile it was also operated to make sure which parameter(s) would be the critical one(s) affecting the distribution of bacterial and fungal species, respectively. Quantifying those relationships between parameters and microbial community compositions would lead to a new insight toward better control of commercial agricultural waste composting.

2. Methods

2.1. Experimental set-up and sample collection

The typical agricultural wastes were collected from the suburb of Changsha, China. Rice straw which was used as organic materials difficult to be decomposed, was air-dried and cut into 10–20 mm lengths. Air-dried bran was used to adjust the initial C/N ratio of composting materials. Several kinds of air-dried vegetables chopped into 10–20 mm pieces were used as easy-degradable materials. Soil collected from the Yuelu Mountain, Changsha, were sieved through a 40-mesh screen to remove coarse plant debris, and added to increase microbial population and offer some necessary nutrients. The common characteristics of those materials are shown in Table 1.

An experimental composting system with a dry weight of about 10 kg materials was set up indoors in this study. Rice straw, vegetables, soil and bran were homogenized at a ratio of 11:3:8:2 (wet weight) to obtain a mixture with an organic matter content of about 60% (dry weight) and a C/N ratio of about 30:1 (Zeng et al., 2010). The mixture was packed loosely in an open box with dimension of 0.5 m × 0.4 m × 0.45 m (length × width × height). The final thickness of the mixture was 0.4 m. The mixture had good heat preservation. The experiment was conducted for 36 days. Samples were collected daily at 8 AM during the first 12 days, and every 3 days afterwards. At each sampling occasion, three subsamples for parameter analysis were taken from different places of the composting material (about 0.2 m in depth). Samples for total DNA extraction were pooled, mixed and stored immediately at −20 °C before used. After sampling, moisture content was monitored and adjusted to about 55–60% during the first fermentation phase and about 45–50% during the second fermentation phase by the addition of sterile deionized water. To provide some aeration, the mixture was turned twice a week during the first 2 weeks and once a week afterwards.

Table 1
The common characteristics of the composting materials.

	Moisture content (%)	TOC ($g\ kg^{-1}$)	TN ($g\ kg^{-1}$)	C/N ratio	pH
Rice straw	11.73	428.0	8.8	48.8	–[a]
Vegetables	79.06	97.2	5.0	19.6	–[a]
Soil	26.13	55.1	2.4	22.7	4.73
Bran	14.06	474.1	41.2	11.5	–[a]

[a] Sample not quantified.

2.2. Physico-chemical parameter analyses

When sampling, the ambient temperature and pile temperature in the center of the composting material were recorded by a temperature meter. The pH was determined with a digital pH meter by mechanically shaking the fresh sample in water suspension at a ratio of 1:10 (w/v) at 200 rpm for 40 min. Ammonium and nitrate were extracted with 2 M KCl and determined by a Continuous Flow Analyzer (FIAstar 5000, FOSS, Denmark). The moisture content was determined by drying the fresh sample at 105 °C for 24 h. The dried sample was ground and analyzed for total organic carbon (TOC) by dry combustion at 550 °C, and total nitrogen content (TN) by Kjeldahl digestion analysis (nitrate value was added), respectively. C/N ratio was calculated from the values of TOC and TN. WSC concentration was measured with a compost extract (water to wet compost ratio of 10:1) in a Total Organic Carbon Analyzer (TOC-5000A, Shimadzu, Japan) after centrifuging at 12,000 rpm for 15 min and filtering through a 0.45 μm membrane filter.

2.3. DNA extraction and PCR-DGGE

Bacterial and fungal community genomic DNA was extracted according to the method described previously (Yang et al., 2007). The extracted DNA was purified, then dissolved in 100 μl of TE buffer and stored at −20 °C before used. The 16S rDNA and 18S rDNA genes were amplified with bacterial universal primers 338F/518R (Li et al., 2008) and fungal universal primers NS1/Fung (Hoshino and Morimoto, 2008), respectively. A GC clamp was attached to forward primers to prevent complete separation of the strands during DGGE.

The PCR mixture was prepared with 1 μl of template DNA, 5 μl of 10× PCR buffer, 1 μl of dNTPs (10 mM each), 1 μl of each primer (20 μM), 2 μg of bovine serum albumin, 2 U of Taq polymerase (TaKaRa, Japan) and adjusted to a final volume of 50 μl with sterile deionized water. The PCR amplification of 16S rDNA was performed as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturing at 94 °C for 45 s, annealing at 55 °C for 40 s and extension at 72 °C for 40 s, and single extension at 72 °C for 7 min, and end at 4 °C. The 18S rDNA PCR program was carried out with an initial denaturation step at 94 °C for 5 min, followed by 35 cycles consisting of denaturation at 94 °C for 45 s, annealing at 55 °C for 50 s and elongation at 72 °C for 1 min, followed by a final elongation step at 72 °C for 7 min, and end at 4 °C.

DGGE was carried out using a Dcode Universal Mutation Detection System (Bio-Rad, USA). PCR samples (30 μl) containing approximately equal amounts of PCR amplicons were loaded onto the 1-mm-thick 8% (w/v) polyacrylamide gels in 1× TAE buffer

using a denaturing gradient ranging from 35% to 70% for bacterial and 20% to 50% for fungal PCR samples (100% denaturant is defined as 7 M urea and 40% deionized formamide). Electrophoreses were performed at 60 °C and 120 V for 12 h. After stained with SYBR Green I nucleic acid gel stain (Molecular Probes, Carlsbad, CA, USA), the gels were scanned and analyzed with the QuantityOne software (version 4.5, Bio-Rad, USA).

2.4. Data analysis

Three replicates were used in all parameter analysis. Data presented as the mean values of triplicates and the maximum difference among triplicate results was 5%. One way analysis of variance (ANOVA) was performed to compare the mean values for different sampling time, and to test whether there were any significant differences among the means at the 95% confidence level.

DGGE banding profiles both for bacterial and fungal community were digitized after average background subtraction for the entire gel. Band position and intensity data for each sample were exported to an excel spreadsheet prior to further statistical analyses. The relative intensity of a specific band was transformed according to the sum of intensities of all bands in a pattern (Roesti et al., 2006). The bands with relative contribution below 1% were discarded from the analysis. Canoco (version 4.5, Centre for Biometry, Wageningen, The Netherlands) was used for determination of multivariate relationships between bacterial and fungal community compositions and physico-chemical parameters. Detrended correspondence analysis (DCA) was carried out first to decide between linear or unimodal response model for these microbial data. The length of the first DCA ordination axis was 2.115 for bacterial and 3.175 for fungal species data, respectively, which did not indicate clear unimodal species responses. Therefore, redundancy analysis (RDA) was performed to ordinate the spatial and temporal compositions of the bacterial and fungal community to the measured composting parameters (Lepš and Šmilauer, 2003).

All physico-chemical parameters were centred with unit variance by SPSS (version 11.5), so that the outputs produced by the multivariate analyses were not influenced by the different magnitudes of the units of measurement of those parameters (Gilbride et al., 2006). The RDAs were performed with default settings. The community similarities were graphed by using ordination biplots with scaling focused on inter-species differences, so that the distances between centroid points for individual samples were easily interpreted. Forward selections were performed to test which parameters had a significant influence on the bacterial and fungal composition, respectively. The selection procedures were stopped when the parameters to be added was not significant anymore. In addition, following the forward selection, variation partitioning was conducted to discriminate the influence of each significant parameter using partial RDA (Borcard et al., 1992; Lepš and Šmilauer, 2003). The significant parameter was used as constraining variable while the other significant ones were used as covariables, which allowed us to estimate the proportion of variation solely explained by each of them separately. Monte Carlo reduced model tests with 499 unrestricted permutations were used to statistically evaluate the significance of the first canonical axis and of all canonical axes together. Statistical significance was kept at $P < 0.05$ for all analyses.

3. Results

3.1. Physico-chemical parameters

The changes of physico-chemical parameters are shown in Fig. 1. The pile temperature increased significantly to 56 °C during the first 4 days because of the quick degradation of organic compounds. The pile temperature higher than 55 °C was kept for at least 3 days, which is the minimum requirement for a proper disinfection of waste materials from animal and plant pathogens (Yu et al., 2007). After then, the pile temperature decreased gradually to the ambient level (Fig. 1a). The pH gradually increased from 6.74 to 9.05 during the first 3 weeks due to production of ammonia during ammonification and mineralization of organic nitrogen. Then the value decreased at the later stage because of the volatilization of ammoniacal nitrogen and the H^+ release resulting from microbial nitrification process. The C/N ratio decreased significantly from 31.15 to 16.62 due to the decomposition of the organic

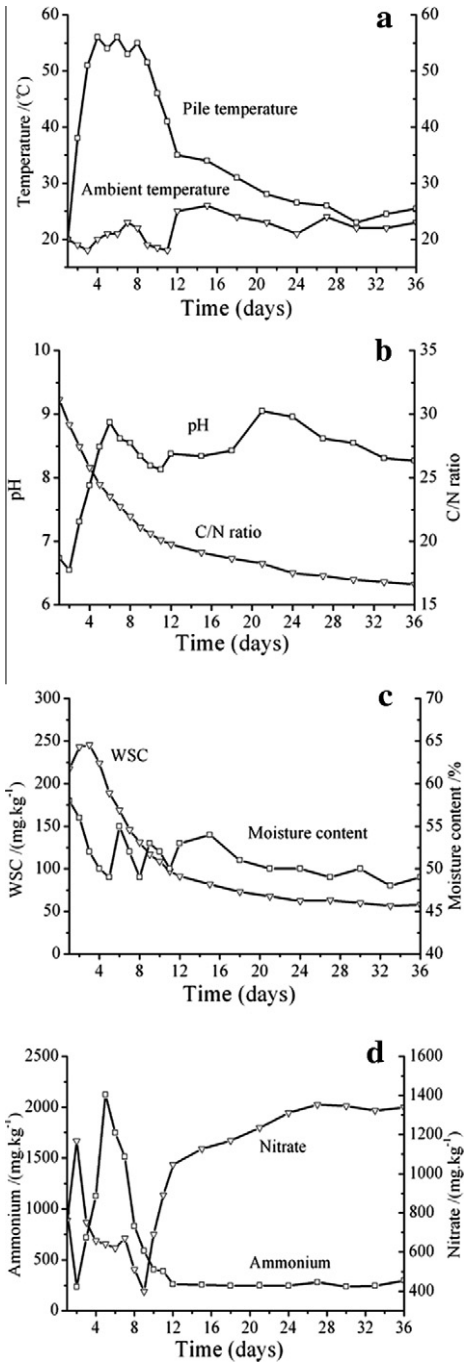

Fig. 1. Changes of physico-chemical parameters (a) ambient and pile temperature; (b) pH and C/N ratio; (c) WSC and moisture content; and (d) ammonium and nitrate during composting process.

matter by the microorganisms during the composting process (Fig. 1b). Moisture content was adjusted to about 60% during the first fermentation phase and about 45–50% during the second fermentation phase, which was considered to be the optimal range for bacterial and fungal species activity (Liang et al., 2003). WSC increased slightly in the first 3 days, and then decreased significantly to below 100 mg kg^{-1} (dry weight) within 12 days, and reached low levels afterwards (Fig. 1c). The ammonium increased significantly and reached peak value on the 5th day due to ammonification with an increase in pile temperature and pH, as well as the mineralization of organic-N compound. Its content decreased to low level by volatilization loss and immobilization by microorganism. During the rest of the experiment no significant change was found. The nitrate concentration showed a slight increase on the 2nd day. Then the content decreased significantly to 398.3 mg kg^{-1} (dry weight) on the 9th day as the high temperature and excessive amount of ammonia inhibited the activity and growth of nitrifying organisms, and gradually increased to as high as 1353.4 mg kg^{-1} (dry weight) afterwards (Fig. 1d).

3.2. Bacterial and fungal DGGE profiles

The fingerprint of the microbial community structure was obtained in the resulting DNA band pattern, in which each band represents a group of bacterial or fungal species having 16/18S rDNA sequences with a similar melting temperature (Ovreas et al., 1997). Species that are present at low population densities are not presented in the communities (Gelsomino et al., 1999). In this research, microbial communities were very dynamic during the composting process, as strong shifts in the DGGE profiles were observed between the different sampling times (Figs. 2 and 3). Fifty different 16S rDNA bands and 33 different 18S rDNA bands were detected in the DGGE profiles. Most of those bands appeared to be ubiquitous but differed in abundance in the different composting environment.

3.3. Redundancy analyses

In order to determine to what extent the eight parameters affected the microbial community compositions, both bacterial and fungal DGGE fingerprints were analyzed by redundancy analysis.

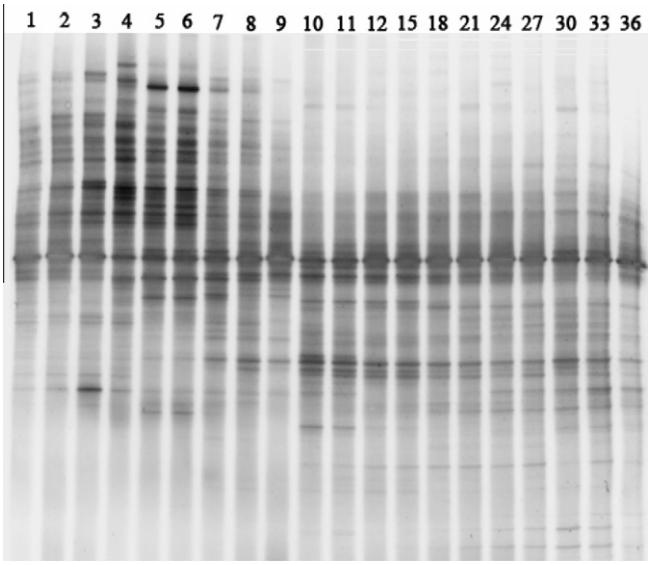

Fig. 2. DGGE profiles of amplified 16S rDNA fragments from the compost samples. The numbers refer to the sampling days.

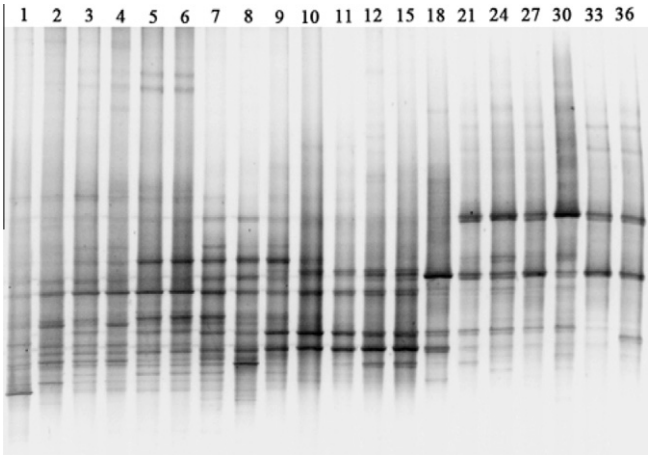

Fig. 3. DGGE profiles of amplified 18S rDNA fragments from the compost samples. The numbers refer to the sampling days.

These results are shown in Table 2. Monte Carlo tests for the first and all canonical axes were highly significant ($P = 0.002$), indicating that these composting parameters may be important in explaining microbial community compositions. The first two canonical axes for bacterial DGGE fingerprints explained 32.2% and 16.3% of the variation in the species data, respectively. For fungal species data, 43.5% and 12.7% of the variation were explained by the first two canonical axes. Approximate 2/3 of the variation both in the bacterial and fungal species data (67.1% and 68.6%) were explained by all four significant canonical axes. The amount rose to 73.2% and 75.5% of the variation in the species data were explained by all canonical axes, respectively.

3.4. Forward selections and variation partitioning

In this research, the aim was to identify which of the parameters might be the drivers of changes occurring in the bacterial and fungal community composition, respectively. Parameters that best described the most influential gradients were identified by forward selection. Explanatory variables were added until addition of further parameters failed to improve significantly ($P < 0.05$) the model explanatory power. In this procedure, WSC, ammonium, and nitrate were found to statistically explain the variation ($P < 0.05$) in the distribution of bacterial species data. The RDA model (i.e., WSC, ammonium, and nitrate) statistically explained up to 54.9% of the variation ($P = 0.002$). For the fungal species data, the temporal variation was best related to pile temperature, WSC, and moisture content ($P < 0.05$) among samples. The RDA model (i.e., pile temperature, WSC, and moisture content) statistically explained up to 56.0% of the variation ($P = 0.002$). These results indicated that those parameters as well as the interactions among them had most impacts on bacterial and fungal community composition, respectively.

Variation partitioning analysis was performed to extract the variation in the bacterial and fungal community composition explained by each of the three significant parameters without the effects of others (Borcard et al., 1992). Percentages of variation explained by each of the significant parameters in Table 3 are those without the shared variation. WSC solely explained 19.5% ($P = 0.002$) of the variation in the bacterial DGGE profiles, ammonium 14.1% ($P = 0.004$) and nitrate 8.5% ($P = 0.002$), respectively. The variation shared by WSC, ammonium and nitrate was 12.8%. Pile temperature solely explained 17.0% ($P = 0.002$) of the variation of fungal community composition, whereas WSC, moisture content explained 11.8% ($P = 0.002$) and 8.5% ($P = 0.01$), respectively. The

Table 2
Redundancy analysis results of bacterial and fungal DGGE profiles.

Axis	Eigen value	Species–environment correlation	Cumulative % variation of species	Cumulative % variation of species–environment	Sum of all canonical Eigen values
Bacterial RDA					
Axis 1	0.322	0.984	32.2	43.9	0.732
Axis 2	0.163	0.959	48.5	66.3	
Axis 3	0.107	0.944	59.2	81.0	
Axis 4	0.078	0.870	67.1	91.7	
Fungal RDA					
Axis 1	0.435	0.981	43.5	57.7	0.755
Axis 2	0.127	0.858	56.2	74.5	
Axis 3	0.084	0.864	64.6	85.6	
Axis 4	0.040	0.830	68.6	90.9	

Monte Carlo significance tests for bacterial data: sum of all Eigen values, 1.000; significance of first canonical axis, F value = 5.215, P = 0.002; significance of all canonical axes, F value = 3.751, P = 0.002. Monte Carlo significance tests for fungal data: sum of all Eigen values, 1.000; significance of first canonical axis, F value = 8.483, P = 0.002; significance of all canonical axes: F value = 4.233, P = 0.002. F and P values were estimated using Monte Carlo permutations.

Table 3
Eigen values, F values and P values obtained from the partial RDAs testing the influence of the significant parameters on the bacterial and fungal community composition, respectively.

Parameters included in the model	Eigen value	% Variation explains solely	F value	P value
Bacterial RDA				
WSC	0.195	19.5	6.894	0.002
Ammonium	0.141	14.1	5.001	0.004
Nitrate	0.085	8.5	3.009	0.002
All the above together	0.549	54.9	6.481	0.002
Fungal RDA				
Pile temperature	0.170	17.0	6.174	0.002
WSC	0.118	11.8	4.298	0.002
Moisture content	0.085	8.5	3.071	0.010
All the above together	0.560	56.0	6.775	0.002

Partial RDAs based on Monte Carlo permutation (n = 499) kept only the significant parameters in the models. For each partial model, the other significant parameters were used as covariables. F and P values were estimated using Monte Carlo permutations. Sum of all Eigen values for both partial RDAs were 1.000.

shared variation was 18.7%. The positions of each of the sampling days with respect to the first two environmental axes are shown in Fig. 4a for bacterial data and Fig. 4b for fungal data. The composting condition of each sampling time can be visualized in these biplots.

4. Discussion

Community profile data obtained by PCR-DGGE are useful for assessing the effects of different environmental factors on microbial community composition, which can be statistically evaluated using multivariate analyses (Yang and Crowley, 2000). Usually, band intensity differences between species cannot be used as an indicator for species abundance (Muyzer and Smalla, 1998). However, if the intensity of a given band increases or decreases in different samples, it does indicate a relative increase or decrease in abundance of this species (Marschner et al., 2001). Therefore, the relative band intensities were used to investigate the relationships between microbial community compositions and physico-chemical parameters in this research. The relative importance of each significant parameter was then calculated through the variation partitioning analysis. Conforming how physico-chemical parameters affect the microbial community compositions is an important step to better interpret changes that are seen during waste composting system.

The microbial community composition was substantially influenced by WSC during the composting process, as WSC explained up to 19.5% of the variation in the bacterial DGGE profiles and 11.8% in the fungal DGGE profiles. It was reported that the concentration of dissolved organic carbon was an important factor affecting microbial community structure and metabolic type (Ishii and Takii, 2003; Maeda et al., 2009), as different microbial strains are adapted to different nutritional conditions. Pile temperature induced a significant modification (17.0% of the variation, P = 0.002) in the fungal community composition over WSC (11.8% of the variation, P = 0.002) in the compost samples, while no significant relationship has been obtained between pile temperature and bacterial community composition. The importance of pile temperature fluctuations for the development of microbial activity and biomass has been highlighted by several previous studies (Herrmann and Shann, 1997; Klamer and Bååth, 1998; Xiao et al., 2009). Not only is microbial metabolism highly temperature dependent, but also the community composition dynamics are dramatically influenced by pile temperature. In agreement, Cahyani et al. (2003) commented that pile temperature and substrates available to bacteria seemed to be the main factor determining the bacterial community. As an important parameter, moisture content of the materials provides a medium for the transport of dissolved nutrients required for the metabolic and physiological activities of microorganisms. The enhancement of composting activities induced by temperature increment could be realized by increasing moisture content alone (Liang et al., 2003). The significant correlation between moisture content and fungal community composition was obtained in this study, while moisture content had no significant effect on bacterial community composition. These results indicated that fungal community was likely to be more sensitive to temperature fluctuations and moisture content adjustments than bacterial community in this research.

Significant relationships have been found between ammonium and nitrate with the bacterial ($P < 0.05$) but not fungal species, suggesting that these two parameters were likely to influence, or be influenced by bacterial species. Ammonia and its unincorporated form ammonium are the most important nitrogenous compounds available in composting materials. The ammonium can be oxidized to nitrate via nitrite (NO_2^-) by ammonia oxidizers during the composting process (Bernal et al., 2009; Kowalchuk et al., 1999). Ammonia-oxidizing bacteria (AOB) may play an important role in nitrification during composting (Yamamoto et al., 2010). In recent study, *Nitrosomonas*-like ammonia oxidizers and *Nitrosopira*-related nitrite oxidizers of the β subdivision of the class *Proteobacteria* have been detected in almost all of the compost materials (Yamamoto et al., 2010; Kowalchuk et al., 1999). As nitrate accumulated in the later phase of the composting process

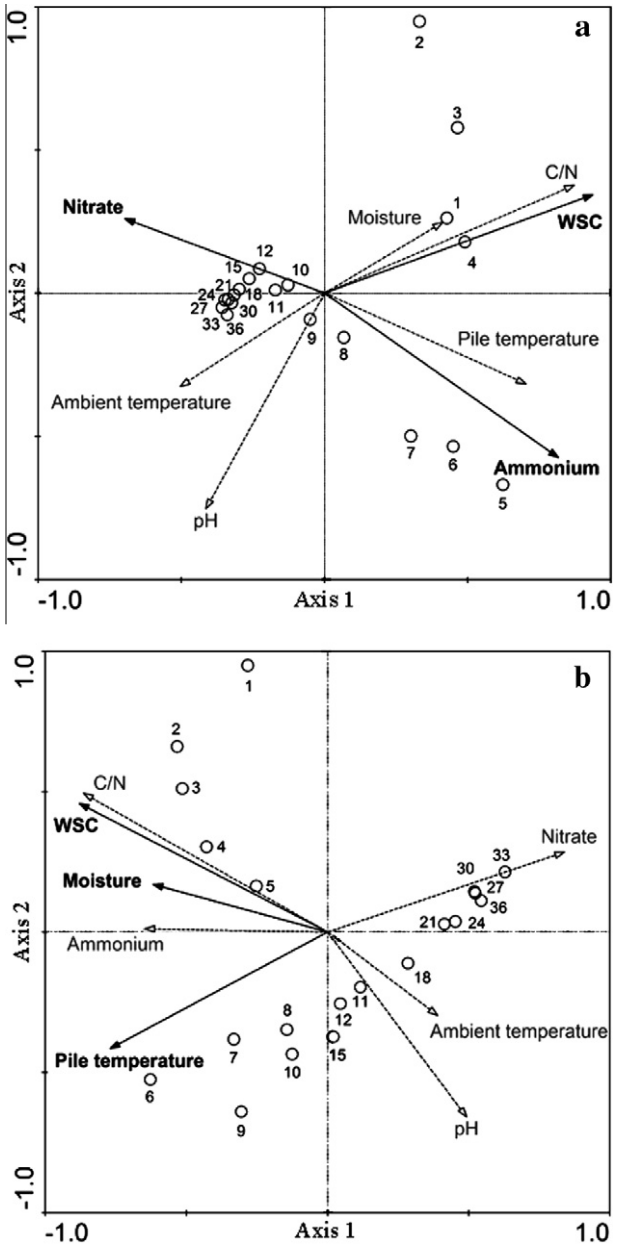

Fig. 4. DGGE band data redundancy analysis for (a) bacterial and (b) fungal species. Significant composting parameters are indicated by solid lines with filled arrows while supplementary parameters are shown using gray dotted lines with unfilled arrows. Samples are represented by open circles and sample numbers refer to the sampling days.

(Fig. 1d), it is obvious that nitrite oxidizers are active in the present compost pile (Maeda et al., 2010). Conditions which are favorable for activity of AOB may affect the net nitrogen balance during the composting process (Kowalchuk et al., 1999).

It has been recognized that ammonia-oxidizing archaea (AOA) played an even more important role than AOB in nitrification under many conditions, such as soil (He et al., 2007; Leininger et al., 2006). AOA may also be key community for ammonia oxidation in composting systems. Maeda and co-workers have not detected any AOA sequences in the cow manure composting samples, even in soil samples as a positive control (Maeda et al., 2010). While other researchers have reported that AOA sequences were present after the pile temperature decreased. The number of the archaeal *amoA* gene copies was significantly higher than that of bacterial *amoA* gene copies (Yamamoto et al., 2010). To our knowledge, the abundance and composition of AOB and AOA, and their roles in nitrification and to what extent these ammonia oxidizers might affect nitrogen transformation balance during the composting process are not well understood yet. To determine the relationships between nitrification and AOB and/or AOA populations is critically important to our understanding and management of the nitrogen cycle in composting system. Therefore, a further study should be carried out to determine the ecology of these ammonia oxidizers and their functions in the agricultural waste composting system.

There was no significant relationship between ambient temperature and microbial community composition because of less direct interaction. The importance and effects of C/N ratio, pH on composting activity and microbial community composition have been reported by several researchers (Eiland et al., 2001; Lei and VanderGheynst, 2000). However, no significant correlation with neither bacterial nor fungal community was found in this study. Notwithstanding, it did not imply that those parameters were of no importance in determining microbial community compositions. It can be only concluded that those parameters were not significantly related to temporal changes in the compositions of the bacterial and fungal community in this research.

It was shown that the eight chosen physico-chemical parameters accounted for a significant amount of the variation in the community composition by RDA. Some variation remained unexplained (26.8% and 24.5% for bacterial and fungal species data, respectively). Perhaps part of the unexplained temporal variations could relate to other microbial species (e.g., actinomycete and archaea) and physico-chemical parameters (e.g., aeration conditions) which were not measured here. Despite not having been assessed, the presence and importance of those microbial species and parameters were probably related to those eight parameters. Such factors could reinforce the importance of nutrient concentrations and pile temperature on regulating bacterial and fungal community compositions during this agricultural waste composting. In addition, physico-chemical parameters that appear most important in a given study appear to be somewhat site-specific, depending on the special composting materials, conditions and the parameters that are measured. These may explain the contrasting reports on the relative importance of some parameters in the determination of microbial community composition. We suggest that the next work in quantifying the impact of different physico-chemical parameters will be to link the observed changes in microbial community composition to quantification of microbial community function. Interpreting the changes in microbial community composition in terms of the statistical analyses will be a valid way to prioritize the complex factors influencing microbial community function.

5. Conclusions

The temporal variation of bacterial community composition was significantly related to WSC, ammonium and nitrate. Pile temperature, WSC, and moisture content explained the most variation in distribution of fungal species data. Those physico-chemical parameters were the most likely ones to influence, or be influenced by the bacterial and fungal species. WSC and pile temperature showed predominant effect on the bacterial and fungal community composition, respectively. In addition, fungal community was likely to be more sensitive to temperature fluctuations and moisture content adjustment than bacterial community.

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