Bioresource Technology 243 (2017) 294–303

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Effect of *Phanerochaete chrysosporium* inoculation on bacterial community and metal stabilization in lead-contaminated agricultural waste composting

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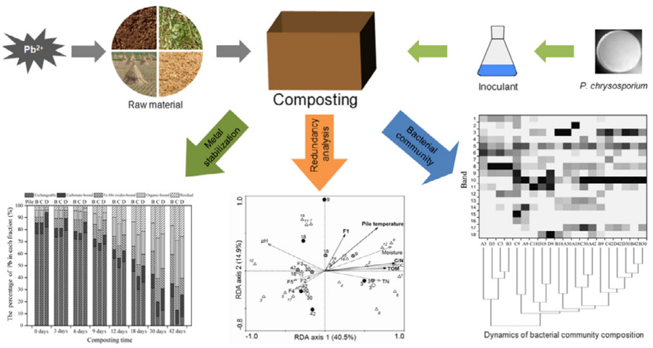
HIGHLIGHTS

- The toxicity of lead was reduced during composting by *Phanerochaete chrysosporium*.
- Inoculum with fungus increased the diversity of bacterial community under Pb stress.
- Key factors accounting for bacterial community dynamics were identified by RDA.
- Main species related to TOM degradation and Pb stabilization were analyzed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 April 2017
Received in revised form 19 June 2017
Accepted 22 June 2017
Available online 26 June 2017

Keywords:
Composting
Phanerochaete chrysosporium
Bacterial community
Lead
Redundancy analysis

ABSTRACT

The effects of *Phanerochaete chrysosporium* inoculation on bacterial community and lead (Pb) stabilization in composting of Pb-contaminated agricultural waste were studied. It was found that the bioavailable Pb was transformed to stable Pb after composting with inoculum of *P. chrysosporium*. Pearson correlation analysis revealed that total organic carbon (TOC) and carbon/nitrogen (C/N) ratio significantly ($P < 0.05$) influenced the distribution of Pb fractions. The richness and diversity of bacterial community were reduced under Pb stress and increased after inoculation with *P. chrysosporium*. Redundancy analysis indicated that C/N ratio, total organic matter, temperature and soluble-exchangeable Pb were the significant parameters to affect the bacterial community structure, solely explained 14.7%, 11.1%, 10.4% and 8.3% of the variation in bacterial community composition, respectively. In addition, the main bacterial species, being related to organic matter degradation and Pb stabilization, were found. These findings will provide useful information for composting of heavy metal-contaminated organic wastes.

1. Introduction

Composting is currently considered as an effective strategy to keep sustainable development of agricultural ecosystems (Zeng et al., 2015), which can converts agricultural wastes into valuable organic products for reusing (Jiang et al., 2015). Agricultural wastes are mainly comprised of cellulose, hemicellulose and lignin, among which lignin is a highly insoluble and irregular polymer of phenylpropane units that provides strength and rigidity to wood, protecting most of the cellulose and hemicellulose against enzymatic hydrolysis (Sharma et al., 2004). In consequence, the

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http://dx.doi.org/10.1016/j.biortech.2017.06.124
0960-8524/

biodegradation process of agricultural wastes is often slow in nature (Tuomela et al., 2000). Alternatively, considerable attentions have been focused on the development of composting with the inoculation of fungi for the efficient process of agricultural wastes, whereby a variety of fungi aroused great interest for their potential lignin degradation ability (Ferreira et al., 2016; Zeng et al., 2010). *Phanerochaete chrysosporium* (*P. chrysosporium*), the representative species of white-rot fungi that are the most efficient lignin degrading microorganism (Huang et al., 2008), has been widely studied owing to its powerful degradation ability against many kinds of organic substrates (Cheng et al., 2014; Zhao et al., 2015).

Composting has been considered as an alternative to the bioremediation of soils contaminated with polycyclic aromatic hydrocarbons (Tang et al., 2008; Zeng et al., 2013b), petroleum, pesticides (Zeng et al., 2013a), chlorophenols (Gong et al., 2009; Lai et al., 2016) and heavy metals (Xu et al., 2012). However, the efficiency of inoculation composting systems is affected by various factors such as the microbe, processing time, environmental condition and raw material (Xi et al., 2015). It has been reported that the composting system with inoculation do not always exhibit the superior performance, empirically caused by the competition between indigenous microbes and inoculated microbes (Nakasaki et al., 2013). Obviously, understanding the dynamic changes of microbial communities during the composting process becomes the key for successful application of inoculation in composting. To acquire a comprehensive knowledge on microbial communities during the composting, many molecular techniques including single-stranded conformation polymorphism (SSCP), terminal restriction fragment length polymorphism (t-PFLP) and polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) have been developed (Egert et al., 2004). Among these methods, PCR-DGGE has been widely used in many fields to monitor microbial community dynamics (Cahyani et al., 2003; Huang et al., 2017a). Redundancy analysis (RDA) and canonical correspondence analysis (CCA) based on the PCR-DGGE profiles were widely applied to reveal the correlation between microbial community composition and environmental factors (Aydin et al., 2015; Wang et al., 2015), and to identify the primary factors affecting microbial communities (Chen et al., 2014; Zhang et al., 2014).

Heavy metal pollution is a common environmental problem in the world (Abdolali et al., 2017; Hu et al., 2011). Lead (Pb), as a non-essential element, is a toxic heavy metal and widely distributed contaminant in the environment (Huang et al., 2008). The agricultural wastes derived from the Pb-contaminated sites will affect public health through the food chain and thus need proper disposal. Previous study confirmed that biosorption was an effective technology to remove Pb(II) by *P. chrysosporium* in aqueous medium (Yetis et al., 2000). However, there is limited information on the remediation of Pb-contaminated agricultural wastes by composting. Since the growth and activity of the indigenous microorganism and fungal inocula can be affected by heavy metals to some extent, for instance by Pb^{2+} (Cheng et al., 2014). Therefore, the understanding of agricultural wastes composting with fungal inoculum in the presence of Pb^{2+} is critical and necessary.

This study aimed to evaluate the impact of *P. chrysosporium* inoculation on the indigenous bacterial community and metal toxicity in composting of Pb-contaminated agricultural wastes. The distribution of Pb fractions during composting and the correlation analysis between Pb fractions and the physico-chemical parameters were investigated. The dynamics of bacterial communities were revealed by heatmap and cluster analysis. RDA was performed to clarify the correlation between the physico-chemical parameters and bacterial community compositions. Variation partitioning analysis was used to distinguish the effect of each significant parameter as well.

2. Materials and methods

2.1. Microorganism and inoculant preparation

The inoculant strain *P. chrysosporium* (BKM-F-1767) was purchased from China Center for Type Culture Collection (Wuhan, China). The strain was maintained on potato dextrose agar (PDA) slants at 4 °C and transferred to PDA plates at 37 °C for 48 h before use. The fungal spores from plates were diluted in sterile distilled water and then adjusted to a concentration of 2.0×10^6 CFU/mL according to our previous work (Huang et al., 2016).

2.2. Composting materials and experimental set-up

The representative agricultural wastes were obtained from the suburb of Changsha, China. The rice straw was air-dried and cut into a length of about 1 cm, used as the natural substrate rich in lignocellulose. The uncontaminated soil collected from the Yuelu Mountain in Changsha was sieved to pass a 40-mesh screen to remove the coarse plant debris, and was then used to enrich the microbial species and populations and supply nutrients. The vegetables including cabbage, celery leaves and beetroot tops were collected from a local market and used as the easy metabolizing substrate after cutting them into small pieces (about 1 cm × 1 cm). Bran was used to adjust the initial C/N ratio of the composting materials. The chemical characteristics of composting materials are shown in Table 1.

The composting materials including rice straw, soil, vegetables and bran were thoroughly homogenized at a ratio of 11:8:3:2 (wet weight). Four experimental composting systems (piles A, B, C and D) with each about 15 kg of composting materials (wet weight) were set up indoors in 90-L open polystyrene boxes with the external and internal dimensions of $0.55 \times 0.45 \times 0.50$ m and $0.50 \times 0.40 \times 0.45$ m (length × width × height). The organic matter content of the mixture was about 60% and the initial C/N ratio was about 30:1. The $Pb(NO_3)_2$ solutions were added to the pile B, C and D with the final Pb^{2+} content of 30, 30 and 400 mg/kg, respectively. The pile A was free from Pb contamination and used the equal amount of sterile water as a replacement. The pile C and D were inoculated with 2% of *P. chrysosporium* spore suspensions, and the pile A and B used as the control without the inoculants. After inoculation, the piles were turned over thoroughly. The experiment was performed in triplicates and conducted for 42 days. Moisture content was kept at about 60% by adding sterile water every 6 days during the first 12 days. To aerate and mix the compost material, the composting piles were turned manually twice a week during the first 2 weeks and once a week afterwards. During the composting, three subsamples from the upper, middle and lower layers of the compost pile were collected on day 0, 3, 6, 9, 12, 18, 30 and 42. The subsamples were mixed and divided into two parts. One part was used for physico-chemical parameter determination and Pb analysis, another was kept at −20 °C for total DNA isolation.

2.3. Physico-chemical parameter analysis

The pile temperature in each compost pile was recorded by a thermometer. The moisture content was estimated based on weight loss at 105 °C. The total organic matter (TOM) was measured by weight loss on ignition at 540 °C. Total organic carbon (TOC) was determined using a Shimadzu TOC-V analyzer (Shimadzu Corporation, Kyoto, Japan). The total nitrogen (TN) was measured according to the Kjeldahl digestion. After mixing the fresh samples with distilled water at a ratio of 1:10 (w/v) and mechanically shaking at 150 rpm for 20 min, the suspension was used for pH determination.

Table 1
The physico-chemical characteristics of the raw composting materials.

Materials	Moisture content (%)	TOC[a] (g/kg)	TN[b] (g/kg)	C/N[c] ratio	pH
Rice straw	10.32	410.2	8.7	47.15	n.d.
Soil	25.27	50.5	2.3	21.96	5.02
Vegetables	81.52	95.1	4.9	19.41	n.d.
Bran	15.62	480.5	45.1	10.65	n.d.

n.d., not determined.
[a] TOC, total organic carbon.
[b] TN, total nitrogen.
[c] C/N, TOC/TN.

2.4. Chemical speciation analysis

About 5 g (wet weight) of sample was dried at 105 °C for 6 h and ground in a mortar, then 1 g of dry sample (power) was used for subsequent analysis. The five fractions of Pb were measured by a sequential extraction procedure reported by Tessier et al. (1979). After each extraction, the supernatant extract was obtained from the mixture by centrifuging and used for Pb content analysis by flame atomic absorption spectrometry (AAS700, PerkinElmer, USA). The fraction distributions of Pb were expressed by the ratio of Pb in each fraction to the total content of Pb in compost samples. In this paper, F1 to F5 represents the soluble-exchangeable, carbonates-bound, Fe-Mn oxides-bound, organic matter-bound and residual Pb, respectively.

2.5. DNA extraction and PCR-DGGE analysis

The total genomic DNA was isolated from 0.5 g (wet weight) of each compost sample on day 3, 9, 18, 30 and 42 using the MOBIO PowerSoil DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA). The fragment of 16S rDNA gene was amplified with bacterial universal primer 338F/518R with a GC clamp (LaPara et al., 2000). The PCR mixture contained 20 μL 2.5 × HotMaster PCR Mix (Eppendorf), 1 μL each primer (10 μM), and 1 μL template DNA (≈20 ng) and diluted to a final volume of 50 μL with sterile Milli-Q water. The PCR program involved an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 40 s and elongation at 72 °C for 40 s, and a final step of elongation at 72 °C for 7 min before holding at 4 °C.

DGGE analysis was performed according to Zhang et al. (2011). The PCR samples (30 μL) were loaded onto the 1-mm-thick 8% (w/v) polyacrylamide gels with gradient of 35–65% denaturants. Electrophoresis was performed at 60 °C for 12 h at 90 V. After electrophoresis, the DGGE gels were stained with SYBR Green I for 30 min and scanned by a gel imaging system.

2.6. Statistical analysis

The results were present as the means and standard deviation of three replicates. The differences between the means were analyzed by one-way analysis of variance (ANOVA) and Tukey's multiple-comparison test. Correlation analysis was carried out to clarify the relationship between physico-chemical parameters and Pb fractions. All the above analyses were performed using SPSS (version 18.0, SPSS, Chicago, IL). The significance level of differences was kept at $P < 0.05$.

DGGE banding profiles were detected and digitized after average background subtraction for each lane using Quantity One software (version 4.5, Bio-Rad Laboratories, USA). The position and relative intensity data of DNA bands for each sample were recorded according to our previous researches (Huang et al., 2017a,b). Shannon-Wiener diversity index (H) for the bacterial community was calculated using Quantity One V4.5. As the longest gradient from detrended correspondence analysis (DCA) based on bacterial DGGE profiles was 2.307, redundancy analysis (RDA) was performed to compare species–environment correlations using CANOCO version 4.5 and the significance was evaluated by Monte Carlo permutation test (499 permutations). Prior to analysis, all environmental variables were centered with unit variance. Manual forward selection was performed to test whether the species composition was independent of the environmental variables. Variation partitioning analysis was carried out to distinguish the effect of each significant variable.

3. Results and discussion

3.1. Changes of physico-chemical characteristics during composting

The changes of physico-chemical parameters during the composting process are presented in Fig. 1. Temperature is considered as an important indicator of the composting process performance. As shown in Fig. 1a, the ambient temperature varied from 25 to 29 °C during the whole composting process. The pile temperature increased rapidly in the first 3 days (the mesophilic phase, day 0 to day 3) and maintained above 50 °C for 9 days (the thermophilic phase, day 3 to day 12), then gradually decreased to about 40 °C (the cooling phase, day 12 to day 18) and finally maintained around 30 °C (the maturation phase, day 18 to day 42). The thermophilic phase with the temperature above 55 °C for more than 3 days is considered to be sufficient to produce sanitary compost by destroying many pathogens (Rashad et al., 2010). In this study, this phase lasted for more than 3 days for all piles and longer for pile A (control) and C (treatment with 30 mg/kg Pb^{2+} and *P. chrysosporium*) during composting process. The significant difference was found between the temperature of pile A and B (treatment with 30 mg/kg Pb^{2+}) from day 3 to day 30, between pile A and C on day 3, between pile A and D (treatment with 400 mg/kg Pb^{2+} and *P. chrysosporium*) from day 3 to day 18, indicating the inhibition effect of Pb on microbial activities which mostly associated with the consumption of nutrient. However, there was no significant difference between that of pile A and C from day 6 to day 42 except on day 18, it was suggested that inoculation with *P. chrysosporium* could protect microorganisms against Pb^{2+} toxicity. This is likely the result of the defense mechanisms of *P. chrysosporium* in response to Pb^{2+}, including extracellular chelation and intracellular uptake (Petr, 2003). However, in high concentration of Pb^{2+}, the pile temperature was significantly lower ($P < 0.05$) than the control without Pb^{2+} (pile A) during the first 18 days of composting and no significant difference was observed after 18 days. This may be attributed to the inhibition of high Pb concentration to *P. chrysosporium* in the early exposure period which delayed the stabilization of toxic Pb^{2+} ions (Huang et al., 2010b).

pH is an important parameter in composting process for its role in microbial growth and metabolism. As shown in Fig. 1b, the pH in all piles increased fast in the first 9 days and maintained about 8.0–

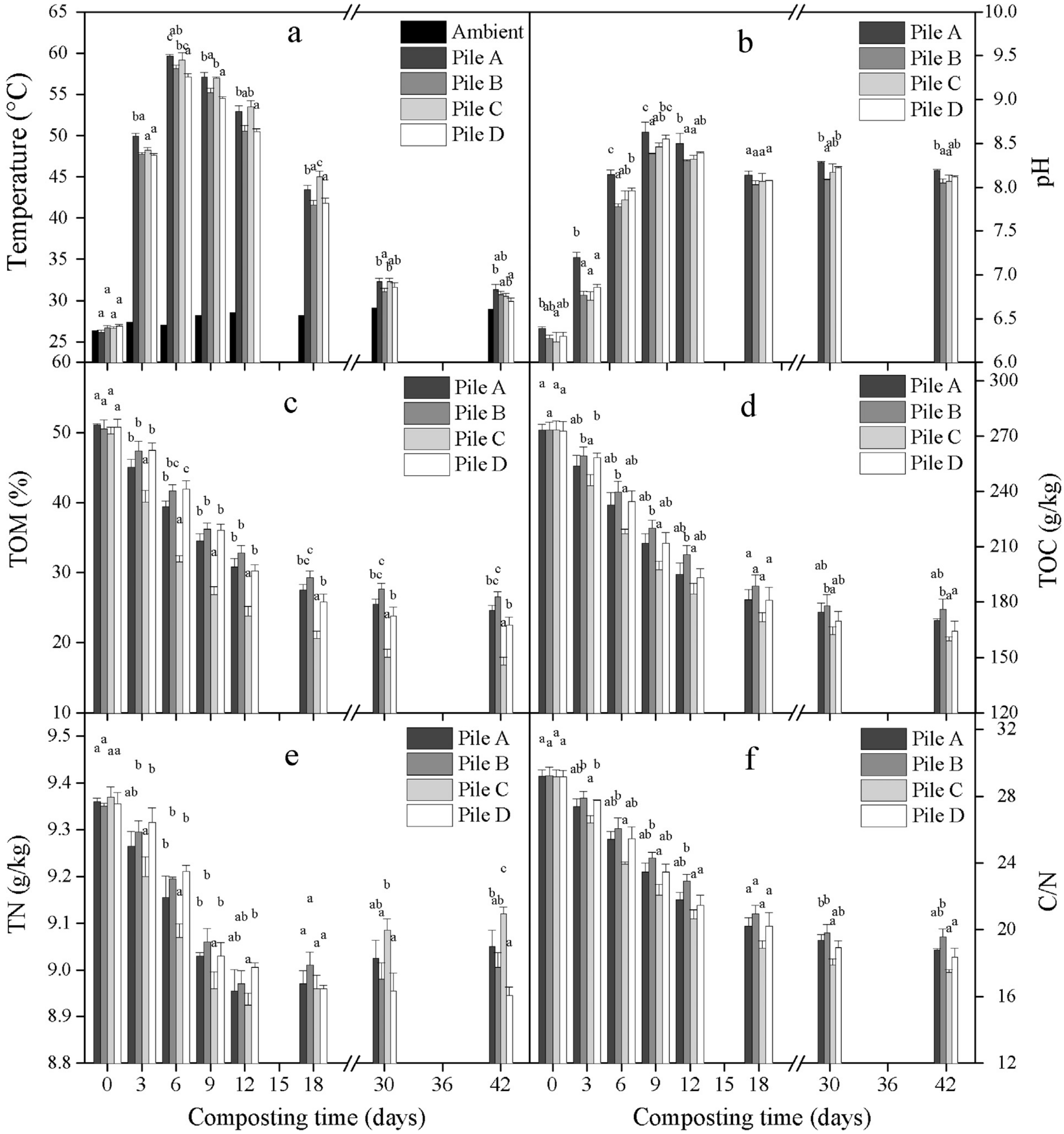

Fig. 1. Changes of physico-chemical parameters during the composting process. Error bars indicate standard deviation ($n = 3$). For each day, the means on the four columns followed by different letters are significant different at $P < 0.05$ according to Tukey's multiple-comparison test.

8.5 during the last stage of composting process. pH in pile A was significantly higher ($P < 0.05$) than pile B and C from day 3 to day 12, higher than pile D from day 3 to day 6. No significant difference was found between the pH in pile B and C, between in pile C and D. The previously study confirmed that the rise of pH at the initial stage lay in the production of ammonia matters (such as NH_4^+) during organic matter catabolism by microorganism (Hattori et al., 1999). Under the stress of Pb^{2+}, microorganism can secrete low molecular weight organic acids like oxalic and citric acid to chelate with Pb^{2+}, which may be account for the lower pH in Pb^{2+} treated piles than that without Pb^{2+} addition (Li et al., 2011). The slightly alkaline pH (about 8.0) at the end of composting for all piles indicated that the compost was mature (Lin, 2008).

The variations in TOM and TOC were presented in Fig. 1c and d. Composting of agricultural waste is an aerobic process to decomposition of organic matter into humus which used as a good fertilizer for plants (Cahyani et al., 2003). In this study, the rapidly decrease of the organic matter content was observed for all piles in mesophilic and thermophilic phases (the first 12 days) and slowly for cooling and maturation phases (day 18 to day 42). The TOM content was significantly lower ($P < 0.05$) in pile C than the others during the whole process except on day 0, which is 24.6%, 26.6%, 16.8% and 22.6% for pile A–D after 42 days of decomposition, suggesting that the organic matter was effectively degraded by *P. chrysosporium* under Pb^{2+} concentration of 30 mg/kg. The decrease of TOM for pile B and D was restrained (although insignificant) when compared to pile A in the first 9 days, whereas it was reactivated for pile D after 9 days. This implied that high concentration of Pb^{2+} could delay the decomposition of organic matter and the effect would disappear with time shift, the probable reason may be the limited bioavailable fraction of Pb during the later phase due to nonspecific binding (Petr, 2003). As presented in Fig. 1d, the tendency of TOC was similar with TOM for all piles, the initial TOC content was about 270 g/kg and the final was 159–176 g/kg with the degradation ratio range from 35.5% to 41.8%. The TOC is usually used as the energy source for the microorganisms (Chan et al., 2016), and the mineralization of TOC will cause the main loss of TOM which results in a similar trend.

Fig. 1e presents the dynamic changes of TN concentrations, a loss of nitrogen was observed during the first 9 days for all piles. After 9 days, the TN concentration in pile C (8.96 g/kg) was significantly lower than that in pile A (9.05 g/kg), pile B (9.00 g/kg) and pile D (8.95 g/kg) ($P = 0.045$, 0.017, 0.045, respectively). However, a greater increase of TN concentration was observed after 12 days for pile C than the others. The NH_4^+-N is accumulated by the degradation and mineralization of organic nitrogen through ammonification and can be volatilized as NH_3 thus resulting in nitrogen loss in the initial stage of composting (Sommer, 2001). Whereas the little loss of TN during the later stage may result from the less emission of NH_3 due to low temperature and the decomposition of biomass to organic nitrogen (Zhang et al., 2017). The ratio of C/N is usually used to evaluate the degree of compost maturity (Mathur et al., 1993). In this work, the C/N ratio presented in Fig. 1f showed an obvious decline from initial value of about 30 to final values of 18.8, 19.6, 17.4 and 18.4 for pile A–D, respectively. The final values for pile A–D all met the criteria (<20) of mature compost (Huang et al., 2010a). The C/N ratio was significantly lower ($P < 0.05$) in pile C and D than in pile B, indicating extensive decomposition of organic matter under Pb stress by inoculation with *P. chrysosporium*, which was in accordance with our results in Fig. 1c.

3.2. Transformation of Pb fractions

As shown in Fig. 2, Pb was mainly existed in the fraction of soluble-exchangeable at the beginning of composting process, which was 76.4%, 76.3% and 82.0% for pile B–D, respectively. The fraction distribution of Pb transformed from soluble-exchangeable to organic-bound and residual with the composting time, while the fraction of carbonate-bound and Fe-Mn oxides-bound did not exhibit an obvious change compared with the other fractions. The Pb fraction of soluble-exchangeable and residual did not show obvious changes until day 9, which might be ascribed to the acidic or neutral condition (Fig. 1b) that was unfavorable to the stabilization of Pb. It was suggested by Singh and Kalamdhad (2012) that weakly alkaline condition ($pH < 11$) was advantageous to passivate Pb by forming Pb-organic matter complex. In this study, the pH was 8.0–8.5 after 9 days and the Pb stabilization was observed at the same time. After 42 days of composting, the Pb-fraction of soluble-exchangeable in pile B–D was in the order: pile B (24.0%) > pile D (7.2%) > pile C (1.5%), and that of residual was in the order: pile C (29.1%) > pile D (25.8%) > pile B (16.6%). The results suggested that the bioavailable Pb had been transformed to stable Pb through composting, especially in pile C and D, which indicated that Pb toxicity was reduced after inoculation with *P. chrysosporium*. It might be because *P. chrysosporium* could secrete extracellular metal chelators to immobilize Pb^{2+} ions and reduce the bioavailability of Pb. Li et al. (2011) reported that the accumulation of oxalate was induced in *P. chrysosporium* under Pb^{2+} stress to reduce Pb^{2+} toxicity by chelation. In addition, *P. chrysosporium* could promote the production of humic substances by enhancing the degradation of organic matter, and the formation of organo-metallic complexes contributed to the passivation of Pb (Zeng et al., 2007).

3.3. Influence of the compost properties on Pb distribution

The distribution and bioavailability of metal mainly rely on the metal itself and the properties of the medium during the composting of metal-contaminated agricultural wastes (Zeng et al., 2007). Correlation matrices of Pb fractions with pile temperature, pH, TOM, TOC, TN, C/N ratio and Shannon-Wiener index (H) of bacteria were performed to investigate the influence of these selected variables on Pb distribution during composting (Table 2). Interestingly, both TOC and C/N ratio had a significant positive correlation with F1 ($P < 0.01$), and negative correlation with the other fractions ($P < 0.05$). TOM and TN were significantly positive correlated with F1 ($P < 0.01$), and negative correlated with F3, F4 and F5 ($P < 0.01$). A similar result was found in sewage sludge and swine manure composting that the evolution of heavy metal distributions and bioavailability depended on both the total metal concentrations and the other properties such as pH and the degradation of organic matter (He et al., 2009). The results suggested that the stabilization of Pb was accompanied by the decomposition of agricultural waste, and composting is an alternative method for the remediation of Pb pollution. However, no significant correlation was found between the Pb distribution and diversity of bacteria.

3.4. Bacterial community variation

Samples from pile A, B, C and D on day 3, 9, 18, 30 and 42 (represent the different stages of composting) were used to evaluate the dynamics of bacterial community by PCR-DGGE in the present study. As shown in Fig. 3, most bands were distributed in the thermophilic phase (day 3 to day 9), which could be attributed to the fast degradation of organic matter in this period (Fig. 1c). There were 17, 13, 17, 13 dominant bands detected in samples from pile A, B, C and D during the whole composting process, respectively. The decline in band numbers for pile B and D could be ascribed to the toxicity of Pb. Unlike pile B and D, the band numbers in pile C did not decrease. Band 7 and 11 appeared in pile A, C and D other than in pile B during the mesophilic and thermophilic phase, band 17 was mainly detected during the last phases in pile A, C and D but not detected in pile B, in addition, band 2, 15 and 16 only exist during the thermophilic phase in pile A and C, band 13 and 14 appeared in all trials except the in pile D. The results suggested that band 7, 11, 17 species were sensitive to Pb stress but could survive under Pb stress with *P. chrysosporium* inoculation, whereas band 2, 15, 16 species were more sensitive to Pb stress and could not survive under 400 mg/kg Pb stress even in treatment with *P. chrysosporium*. It was suggested that the richness of bacteria would be reduced by Pb toxicity and *P. chrysosporium* inoculation could increase the amounts and types of dominant bacteria species during composting under low Pb stress (30 mg/kg). However, band 9 disappeared during the cooling and maturation phase after exposure to Pb, regardless the inoculation or not. Band 5 appeared during the whole composting process for all groups, indicating its strong adaptability to Pb stress and *P. chrysosporium* inoculation. Interestingly, band 12 emerged only in piles with the treatment of Pb, it was suggested that this species might be Pb dependent and had resistance to Pb.

The Shannon-Wiener index (H), an indicator of species richness as well as equitability in ecology (Dilly et al., 2004), was calculated to evaluate the diversity of bacterial community composition during the composting process. As shown in Table 3, H of bacteria was significantly lower in pile D than pile A during the whole process, lower in pile B than pile A except on day 9, indicating the negative impact of Pb on the diversity of bacterial community. Whereas the diversity of bacteria in pile C maintained stable on day 3, increased on day 9, decreased on day 18 and showed no significant difference thereafter when compared with in pile A. The results revealed that

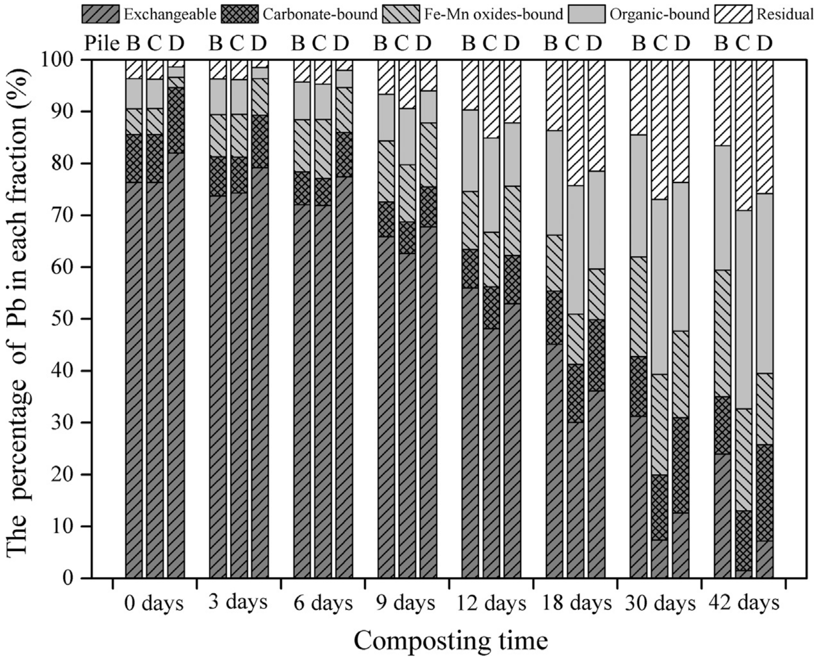

Fig. 2. The distribution of Pb in different fractions after sequential extraction of the compost samples.

Table 2
Pearson correlation coefficients of selected variables and Pb fractions.

Pb fraction	pH	TOM[a]	TOC[b]	TN[c]	C/N[d] ratio	Shannon-Wiener index (H) of bacteria
Soluble-exchangeable (F1)	−0.539**	0.876**	0.906**	0.629**	0.924**	0.285
Bound to Carbonates (F2)	0.076	−0.389	−0.484*	−0.237	−0.510*	0.092
Bound to Fe-Mn Oxides (F3)	0.646**	−0.751**	−0.801**	−0.603**	−0.808**	0.207
Bound to Organic Matter (F4)	0.509*	−0.870**	−0.885**	−0.607**	−0.904**	0.342
Residual (F5)	0.541**	−0.898**	−0.900**	−0.646**	−0.916**	0.574

[a] TOM, total organic matter.
[b] TOC, total organic carbon.
[c] TN, total nitrogen.
[d] C/N, TOC/TN.
* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

there was an obvious shift in the composition of the bacterial community due to Pb toxicity and *P. chrysosporium* inoculation could increase the bacterial diversity.

Heatmap of the bacterial community composition based on the DGGE bands and relative band intensities (Chen et al., 2016), as well as the cluster analysis of DGGE profiles, are shown in Fig. 4. As reported by Marschner et al. (2001), the variations of the relative intensity of a specific band in different lanes indicated the changes in the abundance of this species. It was obvious that the bacterial communities changed sharply with the composting time. Cluster analysis (UPGMA) separated the DGGE fingerprints of bacterial community into two major clusters based on the time of day 3 and the others with the similarity of 27%. The homology coefficient of four piles on day 9 and 18 was 0.38 and 0.41, respectively, which showed that both the Pb stress and inoculation with *P. chrysosporium* had an impact on the bacterial community composition. With the exception of a few samples, the patterns of different samples at the same sampling period tend to cluster together (Fig. 4), such as the group of A3, D3, C3 and B3 (the mesophilic phase) with the similarity of 52%, the group of D42, D30, B42 and B30 (the maturation phase) with the similarity of 72%, suggesting that the pile temperature was another important factor in bacterial community succession.

3.5. Correlation between bacterial community with environmental parameters

The physico-chemical parameters will influence or be influenced by the changes and distribution of bacteria community during agricultural waste composting (Wang et al., 2015). To identify the degree of influence of the environmental parameters on the bacterial community composition and which parameters were most influential, RDA was performed to clarify the correction between bacterial community composition and environmental variables. The results were shown in Table 4. The Monte-Carlo test with 499 permutations showed that the correction between the bacterial species data and environmental variables was statistically significant for both the first canonical axis ($P = 0.006$, $F = 1.361$) and all canonical axes ($P = 0.008$, $F = 2.550$). The first two canonical axes explained 40.5% and 14.9% of the variation in the species data, respectively, and 93.9% of the variation was explained by all selected environmental variables. The manual forward selection using a Monte Carlo permutation test ($P < 0.05$) indicated C/N ratio was the primary parameters driving the succession of the bacterial species, followed by TOM, temperature and F1 (soluble-exchange Pb). According to the variation partitioning analysis shown in Table 5, C/N ratio solely explained 14.7% ($P = 0.004$) of the variance

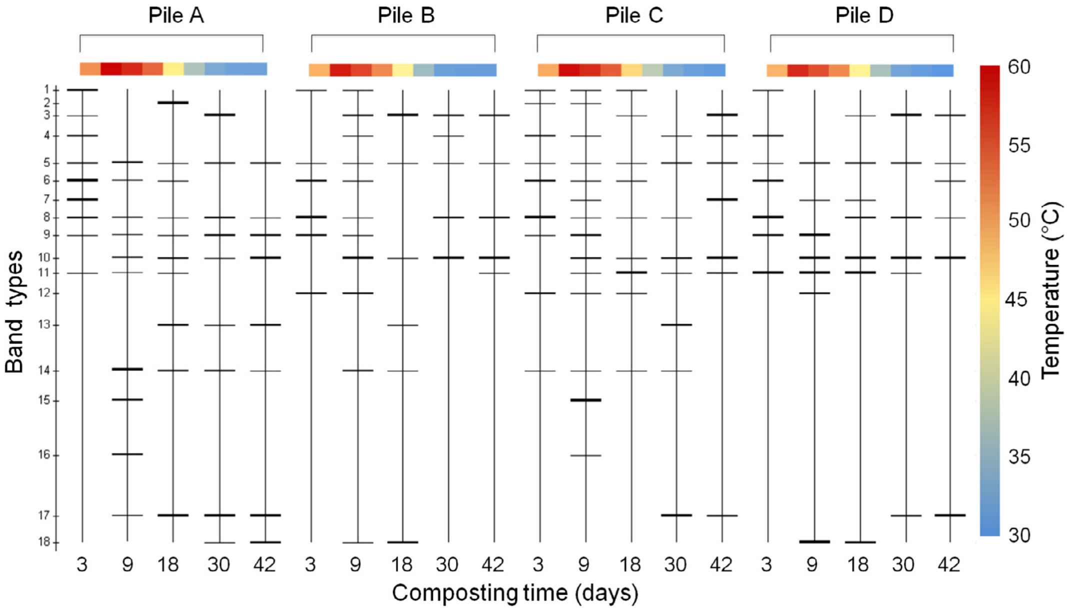

Fig. 3. The diagram of dynamic changes in bacterial community by DGGE fingerprint during the composting process. The color bar above represents the temperature dynamics from day 3 to day 42.

Table 3
Shannon–Wiener index (H) for DGGE profile of bacterial communities. Means with different letters in the same column are significantly different ($P < 0.05$) based on Tukey's multiple comparison test.

	Composting time (days)				
	3	9	18	30	42
Pile A	1.96 ± 0.02c	1.94 ± 0.03b	2.07 ± 0.02c	1.95 ± 0.04c	1.93 ± 0.05c
Pile B	1.58 ± 0.05a	1.93 ± 0.03b	1.53 ± 0.06a	1.43 ± 0.05a	1.38 ± 0.06a
Pile C	1.97 ± 0.01c	2.53 ± 0.05c	1.79 ± 0.04b	1.97 ± 0.03c	1.87 ± 0.03c
Pile D	1.76 ± 0.03b	1.81 ± 0.06a	1.80 ± 0.05b	1.59 ± 0.02b	1.66 ± 0.04b

in bacterial community composition, TOM 11.1% ($P = 0.006$), temperature 10.4% ($P = 0.010$) and F1 8.3% ($P = 0.020$), respectively. The variance shared by C/N, TOM, temperature and F1 was 11.2%. Four variables together explained up to 55.7% of the variance ($P = 0.002$). These results suggested that above four parameters and the interactions among them played an important role in bacterial community dynamics. TOM and C/N ratio represent the process of organic matter degradation and compost maturity, are also associated with the carbon and nitrogen sources for microbes, and are considered as the important parameters in composting (Huang et al., 2016). The pile temperature and substrates are reported to be the main two factors driving the bacterial community (Cahyani et al., 2003). Microbial metabolism is highly dependent on temperature and the dynamics of microbial community composition are greatly affected by temperature (Zhang et al., 2011). As the most bioavailable and toxic fraction of Pb, F1 greatly influences microorganism growth and metabolism due to its high mobility (Huang et al., 2010b). However, it does not imply that the other parameters do not have impacts although the significance level was low ($P > 0.05$) in our study. Each species is influenced by different levels of physico-chemical parameters during the composting process (Wang et al., 2015).

To assess the relationship between bacterial community and physico-chemical parameters, ordination triplot of RDA was performed to display the distribution of bacterial species to environmental variables (Fig. 5). Since the arrow length represented the importance of the variable, and its orientation reflected the correlation with the axis, it was evident that F1 had a more important influence on the distribution of bacterial community when compared to the other fractions of Pb and showed positive correlation with both axis 1 and 2. Moreover, among five factions of Pb, F1 was the only significant environmental variable, indicating the propable toxicity of bioavailable Pb to bacterial community. F1 was positive correlated to pile temperature, moisture, C/N, TOC and TOM, while the other Pb fractions (F2 to F5) were positive correlated to pH and highly negative correlated to the other parameters. It is reasonable to speculate that inoculation with *P. chrysosporium* resulting in a lower C/N, TOC and TOM (Fig. 1) may contribute to the transformation of mobile and toxic fraction of Pb (F1) to insoluble and non-toxic fraction of residual Pb (F5), which is consistent with our results in Fig. 2. Band 15 and 16 bacteria, that only appeared in pile A and C on day 9 (thermophilic phase) as presented in Fig. 3, were significantly affected by F1 and pile temperature, indicating both species were sensitive to Pb toxicity and adapted to high temperature conditions. Band 1, 2, 4, 6, 8, 9, 12, 14, 15 and 16 bacteria showed positive correlation to TOM, TOC and C/N, suggested their potential role in decomposition of organic matter. Band 3, 5 and 17 bacteria had a highly correlation to F4 and F5, which might contribute to the stabilization of Pb.

Microorganisms are the drivers for the biodegradation of agricultural wastes during composting. Composting process could be accelerated by inoculating with functional microbes (Zhang and Sun, 2014). Since heavy metals such as Pb can influence the microbial growth and activity thus leading to inhibit the biodegradation process, *P. chrysosporium* was inoculated in this study due to its tolerance and assistance to Pb as reported previously (Huang

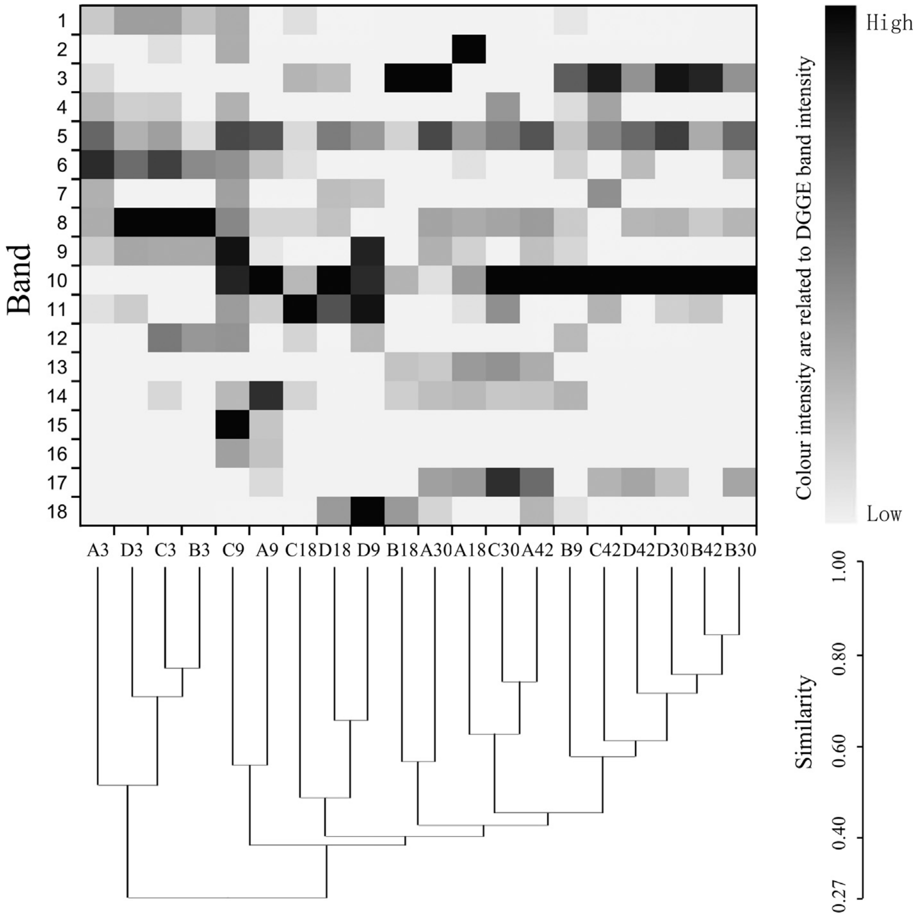

Fig. 4. Heatmap and dendrogram of DGGE profiles of the bacterial community. The cluster analysis was based on the unweighted pair-group (UPGMA) method with arithmetic averages by Quantity One. The labels above the dendrogram denote samples from pile A, B, C and D on day 3, 9, 18, 30 and 42.

Table 4
Redundancy analysis results of bacterial DGGE profiles.

Axis	Eigen value	Species-environment correlation	Cumulative % variation of species	Cumulative % variation of species–environment	Sum of all canonical Eigen values
Axis 1	0.405	1.000	40.5	43.1	0.939
Axis 2	0.149	0.980	55.3	59.0	
Axis 3	0.114	0.996	66.8	71.1	
Axis 4	0.080	0.999	74.7	79.6	

The results of Monte Carlo significance tests: sum of all Eigen values, 1.000; significance of first canonical axis, $F = 1.361$, $P = 0.006$; significance of all canonical axes, $F = 2.550$, $P = 0.008$. F and P values were estimated using Monte Carlo permutations ($n = 499$).

Table 5
Eigen values, *F* and *P* values obtained from the partial RDAs testing the impact of the significant variables on the bacterial community composition.

Parameters included in the model	Eigen value	% Variation explains solely	F value	P value
C/N	0.147	14.7	3.970	0.004
TOM	0.111	11.1	3.013	0.006
Temperature	0.104	10.4	2.812	0.010
F1	0.083	8.3	2.251	0.020
All the above together	0.557	55.7	3.147	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.

et al., 2010b). Previous work indicated that bacterial community was mainly influenced by environmental parameters (Zhang et al., 2011). In this work, the main environmental parameters affecting bacterial community under Pb stress were identified (Fig. 5). Based on the redundancy analysis of the bacterial community data and environmental variables, a clearly correlation between bacterial species and parameters associated to composting efficiency, as well as the main species related to the distribution of Pb fractions, were also presented in Fig. 5. However, further studies are needed to explore the detailed microbial

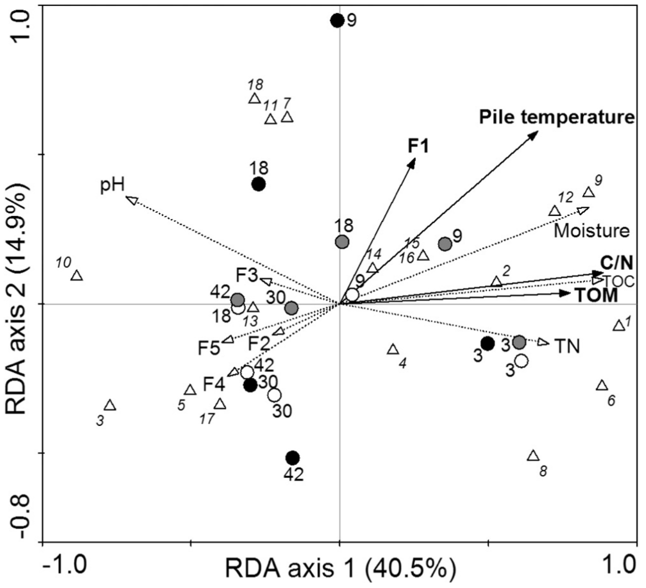

Fig. 5. Redundancy analysis of the correlation between bacterial community and environmental variables. F1 to F5 represents the soluble-exchangeable, carbonates-bound, Fe-Mn oxides-bound, organic matter-bound and residual Pb, respectively. Significant environmental variables are indicated by *solid lines with filled arrows* while supplementary variables by *dotted line with unfilled arrows.* Bacterial species are indicated by *empty triangles* and the *italic numbers* refer to the band number. Samples are represented by *empty circles* (pile B), *gray circles* (pile C), and *black circles* (pile D). *Sample numbers* refer to the sampling days.

community composition by high-throughput sequencing and the response of specific microbial species in heavy metal-contaminated agricultural waste composting. Then it will be possible to enhance the agricultural waste degradation and metal stabilization by regulating the corresponding environmental factors or by isolating the functional microbes and putting them into application.

4. Conclusion

Pb^{2+} significantly influenced the richness and diversity of bacterial community, and the inoculation with *P. chrysosporium* could contribute to the transformation of bioavailable Pb to inactive Pb thus reducing its toxicity to microorganisms. The distribution of Pb fractions was significantly related to TOC and C/N ratio ($P < 0.05$) according to Pearson correlation analysis. Based on RDA analysis, the bacterial species compositions were significantly affected by C/N ratio, TOM, temperature and soluble-exchangeable Pb. Moreover, the main species related to each composting factor was different, thus might supply a strategy to improve the composting process by adjusting relevant composting parameters.

Acknowledgements

The study is financially supported by the Program for the National Natural Science Foundation of China (51521006, 51378190, 51579098, 51408206, 51508175), the National Program for Support of Top-Notch Young Professionals of China (2014), the Program for New Century Excellent Talents in University (NCET-13-0186), the Program for Changjiang Scholars and Innovative Research Team in University (IRT-13R17), Scientific Research Fund of Hunan Provincial Education Department (521293050), and Hunan Provincial Science and Technology Plan Project (2016RS3026, 2015SK2001).

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