Effect of *Geobacillus toebii* addition on composition transformations and microbial community in thermophilic bean dreg composting

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Research Article

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Abstract

A thermophilic bacterium, *Geobacillus toebii* GT-02, was successfully isolated from horse dung. The effectiveness of *Geobacillus toebii* GT-02 addition on composition transformations and the microbial community in bean dreg composting at 70°C for 5 days was investigated (T1), and the control consisted of bean dreg compost without GT-02 (CK). After 5 days, T1 presented higher total nitrogen contents (2.77%) and lower carbon-to-nitrogen ratios (14.4) than CK (2.34% and 16.9, respectively). The germination index (GI) of T1 was 135%, which was 56% higher than that of CK (86%). The dry matter loss in T1 was 54.16% (26.82% higher than the CK value (42.82%)). The mean microbial number in T1 was $4.94x10^7$ CFUs/g dry matter, which was 5.37 times that in CK. 16S rDNA sequencing identified *Bacillus*, *Geobacillus* and *Thermobacillus* as dominant in CK, while *Bacillus*, *Ammoniibacillus* and *Geobacillus* were dominant in T1. A canonical correspondence analysis showed that *Geobacillus* and *Ammoniibacillus* were positively correlated with the GI. Thus, composting with GT-02 can promote the maturity of bean dregs, and GT-02 has potential commercial application in thermophilic composting.

1 Introduction

Bean, as an important oil crop and food resource, has a long history in China. Bean dregs are byproducts of bean production, and 15 million tons of bean dregs are generated in China each year (Yang et al. 2020a). Bean dregs enriched with carbohydrates, protein and other nutritional components were easily decomposed and produced malodorous substances, which caused serious environmental contamination (Yang et al. 2020b; Zhang and Sun 2018).

Bean dregs can be used to produce active carbon, feed and organic fertilizer. To produce active carbon, bean dregs need more energy for pyrolysis (Wang et al. 2019). Bean dregs contain trypsin inhibitors, phytic acid, tannins and other anti-nutrition factors, which restrict their use as feed additives (Adeyemo and Onilude 2013). Due to their high contents of protein, minerals and other nutrients (Ying et al. 2013), bean dregs can be decomposed through composting into humus, which is considered a good fertilizer for plants (Yang et al. 2019). Furthermore, fertilizer is green and safe due to its low pathogenicity.

The traditional composting process involves a low temperature, which could result in a long composting period and low maturity. The traditional compost of bean dregs requires 56 days to reach standard maturity, but the germination index is only 80% (Yang et al. 2020c). Temperature is considered one of the most important variables affecting composting efficiency (Liang et al. 2003). High temperature can increase bioconversion efficiency, and superior composting quality and efficiency can be achieved by enhancing compost maturity and shortening the composting period (Du et al. 2021; Oshima and Moriya 2008). The traditional compost of sewage sludge needs 24 days to achieve maturity, whereas hyperthermophilic compost only needs 12 days (Yu et al. 2019). In addition, exposure to a temperature higher than 70°C for more than 25 min could inactivate pathogenic microorganisms in organic waste (Chang et al. 2019).

Microbes play a dominant role during composting because they have the capacity to mineralize nutrients by producing enzymes to decompose organic waste. Most microorganisms cannot live at high temperatures. Therefore, it is important to screen thermophilic microbes for the thermophilic compost of bean dregs.

Geobacillus toebii, which can grow on a rich complex medium at temperatures between 55 and 75°C (Poli et al. 2006), was initially isolated from hay compost in Korea (Rhee et al. 2002). This bacterium can produce amylase, protease, cellulase, xylanase, and mannanase, which are active at temperatures higher than 70°C (Thebti et al. 2016). At present, few studies have used *Geobacillus toebii* in compost, and the effect of *Geobacillus toebii* on the bean dreg composting process, particularly at a high temperature (70°C), has not been reported. To obtain a better understanding of *Geobacillus toebii* and to improve the composting efficiency at high temperatures, the purposes of this study were (i) to identify the dynamics of the composition and microbial community during the thermophilic aerobic composting of bean dregs with *Geobacillus toebii* and (ii) to investigate the relationship between the microbial community and composting parameters after adding *Geobacillus toebii*.

2 Materials And Methods

2.1 Enrichment and Isolation of bean dregs degrading bacteria

The samples collected from horse manure, pig manure and other organic fertilizers were inoculated in bean dreg medium (1 L water containing 10% bean dregs (v:w)) at 70°C for 48 h. The final enriched media were diluted serially and spread on bean dreg agar plates (1 L water containing 10% bean dregs and 1.5% agar (v:w:w)). The plates were incubated at 70°C for 12 h, and single colonies with morphological differences were selected and streaked on new plates. Single colonies were cultured for 12 h at 70°C and then centrifuged for 10 min at 6000 rpm to collect the bacteria. The collected bacteria was cultured in 50 g bean dregs for 5 days at 70°C. The degradation rate of dry bean dregs was investigated. Among the nine obtained isolates, GT-02 had the highest bean dreg degradation rate and was stored in a 15% glycerin tube at -80°C for further study.

2.2 16S rDNA sequencing and phylogenetic analysis

GT-02 DNA was extracted and purified by a Rapid Bacterial Genomic DNA Isolation Kit (Sangon Biotech, China). The extracted DNA was amplified (16S rDNA gene) using the primers 27F (5'-AGAGTTTGATCMTGGCTCAG – 3') and 1492R (5'- GGTTACCTTGTTACGACTT-3'). The PCR mixture contained 10.0 μL 2 x PrimeSTAR HS DNA Polymerase (Premix), 0.5 μL of each primer, 1 μL DNA template and water to 20 μL. The thermocycling conditions were as follows: 1 cycle of 5 min at 98°C; 30 cycles of 10 s at 98°C, 5 s at 55°C, and 1 min 30 s at 72°C; and a final extension step of 10 min at 72°C. The PCR products were purified by gel electrophoresis and then sequenced by Sanger sequencing (Sangon Biotech, China). The 16S rDNA gene sequences were compared in a BLAST search to the NCBI database. Phylogenetic analysis was performed using MEGA-X software. The relationships between

sequences were analysed using the neighbour-joining method. Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by analysing 1,000 randomized data sets.

2.3 Microorganism and inoculant preparation

The GT-02 strain was activated on 50 mL CYS liquid medium (Moriya et al. 2011) and shaken (150 rpm) at 70°C for 12 h. Then, all bacterial liquid was transferred into new 1 L CYS liquid medium and shaken (150 rpm) at 70°C for 12 h before use. The bacteria were centrifuged for 10 min at 6000 rpm, and the supernatant was discarded. The sediments were diluted in sterile distilled water and then adjusted to a concentration of 2.2×10^8 colony-forming units (CFUs)/mL.

2.4 Collection of composting materials and experimental design

The fresh bean dregs used in this experiment were obtained from Shanghai Tramy Green Food Company (Pudong, Shanghai, China). The bean dregs were dehydrated to approximately 70% by a solid-liquid separator, and the pH was adjusted to approximately 7.5 using NaOH. These bean dregs had not been sterilized. Forty millilitres (2.2×10^8 CFU/mL) of inoculant was added to 1 kg of bean dregs (T1), and 40 mL of sterile water was added to another 1 kg of bean dregs as a control (CK). The physicochemical properties of the raw stocks are shown in Table 1.

Table 1
Physicochemical properties of the raw materials used in this study.

Parameters	CK	T1
Moisture (%)	78.51 ± 0.09	78.84 ± 0.15
pH	7.50 ± 0.23	7.52 ± 0.10
EC (μS/cm)	639.60 ± 45.46	608.95 ± 36.06
TOC (%)[a]	38.74 ± 0.48	39.43 ± 0.41
TN (%)[a]	1.76 ± 0.05	1.84 ± 0.03
C/N ratio [a]	21.96 ± 0.44	21.43 ± 0.19
NH_4^+-N (mg/kg) [a]	2207.69 ± 331.70	2289.95 ± 187.84
NO_2^--N (mg/kg) [a]	712.85 ± 64.15	837.91 ± 121.80
NO_3^--N [a]	/	/
Protein (%)[a]	9.64 ± 0.14	9.63 ± 0.06
Fat (%)[a]	12 ± 0.84	12.1 ± 0.07
Fibre (%)[a]	43 ± 0.12	43 ± 0.42
CK (bean dregs), T1 (bean dregs + 10% Geobacillus toebii), EC (electrical conductivity), TOC (total organic carbon), TN (total nitrogen), C/N ratio (carbon/nitrogen ratio), NH4+-N (ammonium nitrogen), NO2–N (nitrite nitrogen), NO3–N (nitrate nitrogen). The data represent the means ± standard deviations from three measurements.		
[a]: Dry weight.		

The bench-scale compost system (Fig. 1) used in this study was designed to simulate the thermophilic phase of the composting process. Composting reactors (capacity 7.5 L, material weight 1 kg) were used in the experiment, set at 70°C, and forced ventilation (0.5 L/min). The experimental period was 5 days. The bean dregs were poured out every 24 h, fully mixed with 100 mL sterile water to controlled approximately 75% moisture, and collected. Twenty grams of sample was collected on days 0, 1, 2, 3, 4 and 5 from every reactor. One part of each sample was air dried and fined through a 0.1-mm sieve for physicochemical parameter determination, and the other part was used for microbiological analysis. Each treatment repeated 3 times.

2.5 Analytical methods

The weight of each compost pile was recorded using an electronic balance, and the dry matter weight was then calculated based on the pile weight and moisture content. The moisture content of samples was determined after drying at 105°C for 24 h. The moisture content of samples was adjusted to 75%, and then per gram sample was mixed with 10 mL of sterile water, and the mixture was oscillated for 10 min. One hundred microlitres of the liquid mixture was diluted and cultivated on CYS liquid medium at 70°C for 24 h to observe the number of colonies on the plate. Another liquid mixture was centrifuged for 10 min at 6000 rpm, and the supernatant was used to determine pH, electrical conductivity (EC) and the germination index (GI). The germination test was run using seeds of carrot, and the GI was calculated as described by (Li et al. 2020). The suspensions were filtered through a 0.45 μm membrane, and the filtrates were used to analyse dissolved organic matter (DOM) characterization by a fluorescence spectrometer (F-7000, Hitachi). Spectra were recorded at a scan rate of 2400 nm/min using Ex and emission (Em) slits. Wavelengths were set from 200 to 450 nm for Ex and from 250 to 550 nm for Em. A photomultiplier tube voltage of 400 V was applied for low-level light detection. The Fourier transform infrared (FTIR) spectra of dry matter were measured from 4000 to 600 cm^{-1} at 2 cm^{-1} by a Spectrometer II (PerkinElmer, USA). Total nitrogen (TN) and total organic carbon (TOC) were determined as described by (Yu et al. 2018). The protein content was estimated by multiplying the content of organic nitrogen (TN minus NH_4^+-N) by 6.25 (Chen et al. 2018). The ammonium (NH_4^+-N), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), fat content and crude fibre content were measured using the standard methods established by the International Standardization Organization (ISO 2000; ISO 2005; ISO 2009).

2.6 DNA extraction and high-throughput 16S rDNA pyrosequencing

The compost samples were subjected to DNA extraction, 16S rDNA gene amplification, and subsequent microbial community analysis by Illumina sequencing. Total genomic DNA was extracted from 0.5-g compost samples using the CTAB method and used as the template for PCR (Zhao et al. 2013). The V4-V5 region of the 16S rDNA gene was amplified using the primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3'). After amplification, the PCR products were purified using AMPure Beads (Beckman Coulter, USA). The application mixtures were used for pyrosequencing with a MiSeq platform (Shanghai Majorbio Technology, China).

2.7 Statistical analysis

SPSS v.19.0 was used for the statistical analyses, and Origin 2020 was used to prepare the figures. CCA was performed using the Canoco 5.0 software package.

3 Results And Discussion

3.1 Isolation and Characterization of bean dreg-degrading strains

The samples collected from horse manure, pig manure and other organic fertilizers were inoculated in a medium enriched with bean dregs at 70°C for 48 h. Among the nine obtained isolates, GT-02 had the highest bean dreg degradation rate of 54%. Therefore, we focused further investigation on GT-02.

A 1362-bp amplification fragment of 16S rDNA was obtained by PCR (GenBank accession number MW406939). This sequence was compared with others in the GenBank database, aligning the 16S rDNA sequences with several *Geobacillus sp.* strains and constructed a phylogenetic tree (Fig. 2a). The phylogenetic tree clearly showed that strain GT-02 belongs to the *Geobacillus toebii* branch and was similar to *Geobacillus toebii* R-32652, *Geobacillus toebii* NBRC 107807, and *Geobacillus toebii* SK-1 with 99.78%, 99.63% and 99.05% similarities, respectively. *Geobacillus toebii* was a gram-positive, aerobic rods and motile bacterial (Sung et al. 2002).

The growth characteristics of strain GT-02, such as temperature and pH values, were investigated. The bacterial strain could grow within a range of 40–75°C and pH 6.5–9.5, and the optimum temperature and pH were 65°C and 7.5, respectively (Fig. 2b and Fig. 2c). Compared to other *Geobacillus toebii* strains, the maximum growth temperature and pH of strains R-32652 and SK-1 were 70°C and 9.0 (Coorevits et al. 2012; Sung et al. 2002), respectively. These results showed that strain GT-02 was more resistant to high temperature and alkalinity.

3.2 Changes in the composition of bean dregs during composting

3.2.1 Changes in TOC and TN of bean dregs during composting

TOC is usually used as an energy source by microorganisms (Chan et al. 2016), and the mineralization of TOC causes a loss of dry matter. The variations in TOC of dry bean dregs are presented in Fig. 3a. In this study, a slow increase in TOC was observed in CK and T1 during composting, but only a slight difference was found between the two groups after 5 days of composting ($p < 0.05$, ANOVA). During composting, the TOC content in CK increased from 38.74% (day 0) to 39.48% (day 5) and that in T1 increased from 39.43% (day 0) to 39.99% (day 5). The initial TOC content was higher in T1 than in CK, and this increase was likely due to the presence of additional bacteria with some organic matter (OM). However, the changes in TOC content observed in this study differed from those reported by (Yu et al. 2018), who found that the TOC gradually decreased. The reason for the differences between this and the previous study might be that dry bean dregs contain 43% crude fibre, which is difficult to degrade, while pig manure contains less crude fibre. In addition, the pile volume decreased during the composting of bean dregs, which resulted in a similar accumulation of TOC at the end of the composting period.

Figure 3b shows the dynamic changes in the TN content in the dry matter of bean dregs, and the TN content of CK and T1 exhibited a clear upward trend during composting. The TN content in CK increased from 1.76–2.34% during the composting period. The reason for this increase is likely that other substances were lost during composting, which led to nitrogen enrichment (Wang et al. 2017). However, the content of TN in CK exhibited a slight downward trend on the third day, which was likely due to the promotion of NH_3 volatilization by high temperatures (Ogunwande et al. 2008). The TN content in T1 increased from 1.84–2.77% during composting. Further analysis showed that the TN content in T1 on the last day of composting was significantly different ($P < 0.05$, ANOVA) and 18.38% higher than that in CK, and this difference was attributed to the growth of *Geobacillus toebii* and the rapid degradation of bean

dregs. The above results indicated that the addition of *Geobacillus toebii* accelerated the composting process and the degradation of bean dregs, which led to a relatively higher TN content.

3.2.2 Changes in C/N and GI of bean dregs during composting

The carbon-to-nitrogen ratio (C/N) is usually used to evaluate the degree of compost maturity (Kauser et al. 2020; Lei et al. 2021). Compost with a C/N ratio less than 20 is considered mature, and values of 15 or less are superior (P et al. 2009). As shown in Fig. 3c, the C/N ratio of CK increased from 21.96 to 22.50 over the first 3 days, which indicated that the rate of TN loss was faster than that of OM loss during the thermophilic compost and then declined to 16.86 by the end of the composting period. In fact, the C/N and GI (Fig. 3d) of CK after 5 days indicated that the compost had reached the maturity standard, and the bean dregs were considered to have been decomposed. The C/N ratio of T1 increased from 21.43 to 23.01 over the first 2 days, which corresponded to rapid growth of *Geobacillus toebii* (Fig. 8a). During this period, the microorganisms consume part of the nitrogen-containing compounds, resulting in a decrease in the total amount of nitrogen and an increase in the C/N ratio. Starting on the third day, the C/N ratio of T1 exhibited a similar trend as that observed in CK. After 4 days, both CK and T1 met the criteria (C/N ratio < 20) of mature compost. On day 5, the C/N ratio (14.4) of T1 was less than 15, and this value was significantly lower ($P < 0.05$) than that of CK (16.9). This result indicated that the bean dregs in T1 were more mature than those belonging to the CK group. The results indicated that thermophilic composting can contribute to the maturity of bean dregs and that the inoculation of *Geobacillus toebii* could accelerate this process.

The GI is traditionally used to evaluate the phytotoxicity and maturity of compost (Chang et al. 2019). As shown in Fig. 3c, the GI values of CK and T1 were 51.85% on day 2 and 41.98% on day 1. Phytotoxicity, which is usually caused by various heavy metals and low-molecular-weight substances, such as NH_3 and organic acids, can reduce seed germination and inhibit root development (Liu et al. 2020). During composting, bean dregs might produce NH_3, organic acids and other substances, which could trigger a decrease in the GI. The GI of T1 showed a clear decrease, which was likely due to the production of toxic organic acids and might also explain the decrease in pH observed in T1 (Fig. 3e). Due to the degradation of organic acids, the GI of T1 increased to 95.06% on the third day and continued to increase to more than 100%, whereas in CK, the GI only reached 86.4% at the end of the composting period. These results revealed that the maturity of T1 on day 3 was markedly higher than that of CK on day 5 and thus suggest that *Geobacillus toebii* can significantly enhance the composting efficiency by accelerating the maturation process and thus reducing the thermophilic composting period from 5 to 3 days.

3.2.3 Changes in pH and EC of bean dregs during composting

The variation in pH observed during composting is due to the interaction between inorganic nitrogen and organic acids produced by the decomposition of organic matter (Nie et al. 2020). As shown in Fig. 3e, the pH changes in CK and T1 were different. The pH of CK was initially 7.5 and then gradually increased to reach a value of 8.72 at the end of the composting period. The ammonification process and the release of free NH_3 during OM degradation lead to increases in pH (Dias et al. 2010). The pH of T1 decreased during the initial phase, which was due to the formation of organic acids by *Geobacillus toebii*, and then

increased to reach 8.76 on day 2 due to acid consumption and ammonia formation. The pH of T1 slowly decreased to 8.1 due to ammonia volatilization or ammonia conversion. These study findings showed that the pH value of the compost was significantly affected by the addition of *Geobacillus toebii*. *Geobacillus toebii* can produce abundant high-temperature enzymes, such as amylase, protease, cellulase, xylanase, and mannanase (Thebti et al. 2016), which explains why the ammonification process was faster in T1 than in CK and thus the higher pH was found in T1.

The EC, which is a measure of the total ion concentration, describes changes in the levels of organic and inorganic ions such as SO_4^{2-}, Na^+, NH_4^+, K^+, Cl^-, and NO^{3-} during the composting process (Zhang et al. 2014). As shown in Fig. 3f, the EC of the two groups increased significantly during composting. The increase in EC observed in this study was due to the decomposition of a fraction of OM into mineral salts and ammonium ions, and these results showed that thermophilic composting can effectively promote the degradation of bean dregs. Bean dregs inoculated with *Geobacillus toebii* exhibit enhanced degradation. At the end of the composting period, the EC of T1 was higher than that of CK, and the higher ion concentration of T1 could provide more nutritional factors for plant growth.

3.3 Nitrogen transformation process of bean dregs during composting

The TN content in compost samples generally includes organic nitrogen, ammonia nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N) and nitrate nitrogen (NO_3^--N), and the changes in the TN content in compost are mainly caused by ammonification and nitrification (Zhang and Sun 2014). As shown in Fig. 4a, a rapid increase in the NH_4^+-N concentration was observed during the initial composting period until a peak was reached after 2 days in CK, which could be ascribed to the mineralization of organic nitrogen in bean dregs and weak nitrification at high temperature (Hu et al. 2007; Jiang et al. 2013). The NH_4^+-N content decreased rapidly from days 2 to 5 in CK, which was due to nitrification to NO_2^--N and the volatilization of NH_3. The increase in the NH_4^+-N content detected in T1 during the first 2 days was faster than that in CK, and the NH_4^+-N content in T1 then decreased rapidly until the end of the composting period. A possible reason for this finding is that *Geobacillus toebii* rapidly decomposed the nitrogenous organic compounds to generate a large amount of NH_4^+-N. At the end of the composting period, the concentration of NH_4^+-N in T1 was lower than that in CK, which was due to the enhancement of nitrification obtained with *Geobacillus toebii* inoculation.

As shown in Fig. 4b, the NO_2^--N content in CK continued to increase during composting, which was likely due to the nitrification activity of thermophilic bacteria, such as *Ammoniibacillus*. The NO_2^--N content of T1 was higher than that of CK at the initial stage, and this increase was due to the high content of NO_2^--N in *Geobacillus toebii*. The NO_2^--N concentration of T1 rapidly increased to 4608 mg/kg on the fourth day, and a possible reason for this finding is that *Geobacillus toebii* was growing on bean dregs and secreted nitrifying enzymes that could accelerate the transformation of NH_4^+-N to NO_2^--N. Subsequently, the NO_2^--

N concentration of T1 decreased from 4608 to 4165 mg/kg at the end of the composting period, and this decrease can be mainly attributed to the transformation of NO_2^--N to other substances.

NO_3^--N was not detected during composting because nitrification was limited by the high temperatures and a lack of nitrifying bacteria. (Yang et al. 2019) clearly confirmed negative correlations between NO_3^--N and both the temperature and pH. In this high-temperature and alkaline environment, which restricts the activity of nitrifying bacteria, NO_3^--N is not easily generated.

3.4 Changes in the organic composition of bean dregs during composting

Dry matter loss is an important parameter reflecting the degradation of bean dregs. As shown in Fig. 5a, the dry matter loss showed similar trends during the degradation of bean dregs in both T1 and CK. Without the participation of microbes, dry bean dregs were hardly degraded at 70°C for 5 days. Dry bean dregs were degraded by 42.82% after 5 days at 70°C without the addition of exogenous thermophilic bacteria. This meant that fresh bean dregs contained a few thermophilic microbes. In T1, the dry matter loss was 54.16% at the end of the composting period. The degradation rate of dry matter in T1 was 26.48% higher than that in CK. These results revealed that *Geobacillus toebii* could grow on bean dregs and significantly contributed to the degradation of OM.

The dry weight changes were influenced by the organic composition, including protein, fat and fibre. The protein, fat and fibre losses in both experiments are shown in Fig. 5. As shown in Table 1, the dry matter of bean dregs contained 9.64% protein, which could provide nutrition for microbes. As illustrated in Fig. 5b, the protein loss in CK gradually increased, which indicated that the growth of microbes consumed the protein in bean dregs. T1 contained more microbes than CK, and the protein loss in T1 exceeded that in CK on the first day. On the third day, the protein loss in T1 reached the maximal value (49.8%), whereas the value in CK was only 36.5%. This result indicated that the available protein in T1 was basically completely consumed by the third day. After 3 days, the protein loss in T1 decreased. The possible reason was that more enzyme proteins were synthesized by microorganisms.

The fat loss in both CK and T1 increased during composting (Fig. 5c). The fat loss in T1 clearly increased faster than that in CK. Moreover, the fat in T1 was basically consumed completely at the end of the composting period, whereas the fat loss in CK was only 58.3%. These results indicated that *Geobacillus toebii* could secrete high-temperature-resistant enzymes and thereby accelerate the degradation of fat.

During the initial period of composting, the fibre loss in T1 was basically the same as that in CK, as shown in Fig. 5d. A possible reason for this finding was that fibre was difficult to degrade. Starting on the third day, the difference in fibre loss between T1 and CK was more obvious. Notably, the fibre loss in T1 was 27.75% higher than that in CK at the end of the composting period. The increased fibre loss in T1 illustrated that more cellulases were synthesized and secreted. These enzymes are likely associated with proteins, which might explain the increase in the protein content in T1 starting on the fourth day.

The above-described results indicated that *Geobacillus toebii* tends to utilize easily degradable OM, such as proteins and fats, and has the ability to degrade fibres. According to the above-described degradation rates of OM, the ability of *Geobacillus toebii* to degrade OM in bean dregs was in the order fat > protein > fibre. Therefore, during the thermophilic composting of bean dregs, the addition of *Geobacillus toebii* could accelerate the degradation of OM.

3.5 Spectral analysis of the composition during bean dreg composting

3.5.1 Analysis of the effects of *Geobacillus toebii* on the DOM of bean dregs by EEM fluorescence spectra

According to the study conducted by (Chen et al. 2003), the EEM fluorescence spectrum can be delineated into five Ex/Em regions. The fluorescence peaks that fall in Regions I and II (Ex < 250 nm, Em < 380 nm) are associated with simple aromatic proteins such as tyrosine, whereas peaks that fall in Regions III (Ex < 250 nm, Em > 380 nm), IV (250 < Ex < 280 nm, Em < 380 nm) and V (Ex > 250 nm, Em > 380 nm) are related to fulvic acid-like substances, soluble microbial byproducts and humic acid-like substances, respectively. The EEM spectrum of DOM from bean dregs is shown in Fig. 6a. At the beginning of composting, the similar EEM profiles of CK and T1 indicated that the DOM of bean dregs consisted of soluble microbial byproducts and small amounts of humus substances. Moreover, the fluorescence intensity of T1 was higher than that of CK due to the addition of *Geobacillus toebii*. As the composting process proceeded, the peak of CK in Region I became weaker until it disappeared on day 3, whereas a peak was detected in Region V. This finding indicated that during the thermophilic composting process, soluble microbial byproducts are degraded or used for the synthesis of humic acid-like substances, which is similar to the results obtained by (Yu et al. 2019). As the fluorescence peak of humic acids increased, the corresponding peak fluorescence intensity of soluble microbial byproducts gradually disappeared. According to the study conducted by (Jouraiphy et al. 2005), the peak in Region IV disappeared, reflecting a degradation of labile OM, and microbial activity is favoured by high concentrations of labile OM. Therefore, microbial activity would cause the disappearance of the peak in Region IV as the composting process proceeds.

However, for T1, the fluorescence peak in Region IV disappeared on the first day, and a new peak appeared in Region V. This finding might be due to the addition of *Geobacillus toebii*, an exogenous bacterium that significantly degrades soluble DOM in microorganisms or uses it to synthesize humic acids. Moreover, on the third day, the fluorescence intensity of Region V obtained for T1 reached the maximal value, and this value was higher than that found for CK. This finding indicated that *Geobacillus toebii* can promote the synthesis of humic acids and improve the maturity of composting products. Subsequently, at the end of the composting period, the fluorescence intensity in Region V obtained for T1 showed a slight decrease, which might be because the produced humic acids decompose easily at high temperatures. The thermophilic composting process should be terminated as soon as possible after the compose reaches maturity. *Geobacillus toebii* significantly accelerated the degradation of labile OM and then promoted the formation of humus substances, which suggests that the humification process in T1 was faster than that in CK.

3.5.2 Analysis of the effects of *Geobacillus toebii* on the composition of bean dregs by FTIR spectroscopy

The FTIR spectra in the 4000-to-600-cm^{-1} region were evaluated in this study. Figure 6b shows the FTIR bands of the bean dregs in the CK and T1 groups at the start of and after composting. The FTIR spectra of the bean dregs exhibited nine predominant absorption peaks at 3355, 2926, 2857, 1743, 1632, 1415, 1373, 1234 and 1047 cm^{-1} and two small peaks at 1155 and 892 cm^{-1}. The FTIR spectra depict a broad band centred at 3355 cm^{-1} corresponding to the O-H stretching vibration, whereas the peak at 1743 cm^{-1} was attributed to strong C = O stretching of carboxylic acids or ketones (Hagemann et al. 2018). The band at 1632 cm^{-1} was generated by the asymmetric COO^- stretching of carboxylic acids, whereas the peak at 1415 cm^{-1} was generated by the symmetric COO^- stretching of deprotonated carboxylic acid (Huang et al. 2018). The peak at 1234 cm^{-1} was attributed to C-O stretching and O-H deformation of carboxyl groups and C-O stretching of aryl ethers (Yu et al. 2019). These peaks suggested that organic acids existed in the raw bean dreg materials. Compared with the results obtained for CK, the disappearance of the peak at 3350 cm^{-1} and the greater decreases in the peaks at 1715, 1632, 1415 and 1234 cm^{-1} observed in the T1 spectra illustrated that organic acids were significantly decomposed during composting. Above all, these results indicated that the addition of *Geobacillus toebii* accelerated the degradation of organic acids.

The change at 1373 cm^{-1} could be attributed to the C-N stretching of amines (He et al. 2014; Lv et al. 2013), and the peak at 892 cm^{-1} was caused by the strong C-H stretching of aromatics and the strong and broad N-H stretching of amines I and II (Malik et al. 2018). The peaks at 1373 and 892 cm^{-1} decreased in the T1 spectra during composting but remained basically unchanged in the CK spectra. These peaks suggested that crude proteins were rapidly degraded by *Geobacillus toebii*. Another peak at 1155 cm^{-1}, which was obviously decreased in T1, was attributed to the C-N stretch of aliphatic amines (Malik et al. 2018). This result showed that aliphatic amines might be partly transformed into ammonia nitrogen by *Geobacillus toebii*. Additionally, the peaks at 2926 and 2857 cm^{-1} were attributed to symmetric and asymmetric stretching vibrations of aliphatic C-H bonds in CH_3 and CH_2 groups, whereas the peak at 1047 cm^{-1} was attributed to C-O stretching of polysaccharides (Hagemann et al. 2018; Yu et al. 2019). These peaks gradually decreased, which indicated that microbes consumed part of the polysaccharides and fats in both CK and T1 during the composting period. Moreover, the peaks in the T1 spectra decreased faster than those in the CK spectra during composting, which demonstrated that the addition of *Geobacillus toebii* accelerated the degradation of polysaccharides and fats. (Wu et al. 2017) confirmed that polysaccharides, their degradation products and nitrogenous compounds are the main precursors for the formation of humus substances. Therefore, protein and polysaccharides might constitute the key structures that lead to the acceleration of the formation of humus substances in T1, which could explain the higher GI obtained with T1 compared with CK.

3.6 Change in the bacterial community during bean dreg composting

Microbes play a dominant role in the degradation of dry matter during composting (Zhang et al. 2020), and CFUs are used to count living bacteria. There were some thermophilic bacteria in fresh bean dregs. As shown in Fig. 7a, the concentration of bacteria in CK increased from 4.75 x 10^4 to 1.33 x 10^7 CFUs/g dry matter during the first 3 days of composting and then decreased to reach a value of 8.53 x 10^6 CFU/g at the end of the composting period. According to (Poli et al. 2006), *Geobacillus toebii* can grow at a high temperature between 55 and 75°C, which meant it could coexist with endogenous thermophilic bacteria in bean dregs at 70°C. In T1, the number of living microbes was initially 1.65 x 10^7 CFUs/g dry matter, which was nearly 348-fold higher than that in CK, reached the maximal value (1.09 x 10^8 CFUs/g dry matter) on the second day, which was 8-fold higher than that in CK and then declined over time until the end of the composting period. During the 5-day composting period, the mean number of living microbes in T1 was 4.94x10^7 CFUs/g dry matter, which was 5.37 times that in CK due to the addition of *Geobacillus toebii*. Therefore, due to a high concentration of thermophilic bacteria, the degradation of bean dregs in T1 was faster than that in CK.

The V4-V5 regions of the 16S rDNA gene were determined by Illumina MiSeq sequencing to characterize the microbial community structure during composting. The genus-level phylogenetic characteristics of CK and T1 are compared in Fig. 7b, and the figure displays the relative abundances of various microbes in the communities during thermophilic composting. The composition of the bacterial communities of CK and T1 showed obvious differences. Prior to composting, *Leuconostoc* (39%), *Brevibacillus* (18%) and *Weissella* (19%) were the dominant genera in bean dregs. At the end of the composting period, the microbial community in CK was significantly changed. Specifically, the percentages of *Leuconostoc*, *Brevibacillus* and *Weissella* markedly decreased to 0.03%, 2.03% and 0.01%, respectively, after five days of composting, and these populations were replaced by *Bacillus* (42%), *Thermobacillus* (11%), *Geobacillus* (12%) and *Ammoniibacillus* (7%). However, the original content of *Bacillus* in bean dregs was 7%, and the initial abundances of *Thermobacillus*, *Geobacillus* and *Ammoniibacillus* were basically 0%. Previous studies have shown that these four genera contain many thermophilic bacteria, such as *Bacillus* sp. *PPS-52*, *Thermobacillus xylanilyticus*, *Ammoniibacillus agariperforans* and *Geobacillus toebii* (Sakai et al. 2015; Touzel et al. 2000). Thermophilic composting significantly increased the proportion of these bacteria, which indicated that these four genera played an important role in the degradation of bean dregs. *Acinetobacter* (0.04%) was found in fresh bean dregs, which could cause various infections and survive exposure to various common disinfectants (Munoz-Price and Weinstein 2008). After thermophilic composting, this species was not detected in CK and T1, which indicated that a high temperature could kill pathogenic bacteria in bean dregs.

Geobacillus toebii was added to T1 at the beginning of the composting period, and *Geobacillus* significantly increased in T1 at the end of the composting period, which indicated that *Geobacillus toebii* exhibits good compatibility with bean dregs. After thermophilic composting, the microbial community structure of T1 was similar to that of CK, and the main genera were *Bacillus* (26%), *Ammoniibacillus* (20%), *Geobacillus* (16%) and *Thermobacillus* (12%), but the abundances were changed. During the thermophilic composting of bean dregs, the abundances of microorganisms belonging to *Geobacillus*

remained high, which may explain why the rate of composition transformation in T1 was faster than that in CK. At the end of the composting period, the proportion of *Ammoniibacillus* in T1 was markedly higher than that in CK. *Ammoniibacillus gen. nov.* and *Ammoniibacillus agariperforans* sp. *nov*. can utilize ammonium but not nitrate, nitrite, urea or glutamate for growth (Sakai et al. 2015), which might explain why the T1 group showed a faster decrease in the ammonia nitrogen content and a faster increase in the nitrite nitrogen content than the CK group. Therefore, the effect of *Geobacillus toebii* on the microbial community structure of bean dregs was significant and might both improve the growth of *Ammoniibacillus* and promote the N transformation process.

3.7 Canonical correlation analysis of the relationship between the microbial community and bean dreg composition

A CCA can identify the relationships among environmental factors and the microbial community. The first two axes in the CCA plot shown in Fig. 8 explain 94.4% of the cumulative variance between the species data and environmental variables. The results indicated that the GI and C/N ratio were significantly correlated with the CFUs. The CFUs effectively promoted the maturity of bean dregs during thermophilic composting; thus, the maturity of T1 was higher than that of CK. *Geobacillus*, *Ammoniibacillus*, *Symbiobacterium*, *norank_f_Bacillaceae* and *unclassified_f_Paenibacillaceae* were positively related to the GI. Moreover, *Geobacillus* and *Ammoniibacillus* were found at high abundances in bean dregs after composting and were predicted to be the dominant genera responsible for thermophilic composting. Therefore, enhancing the populations of *Geobacillus* and *Ammoniibacillus* could improve the composting efficiency and accelerate the maturation of bean dregs.

Additionally, *norank_o_Chloroplast*, *Weissella*, *Leuconostoc* and *Lactococcus* were positively correlated with the C/N ratio and negatively correlated with the GI. These microorganisms were not conducive to compost maturation, and the main reason for this finding was their inability to grow at high temperature.

Ammonia nitrogen was only slightly related to the GI, whereas nitrite nitrogen was positively correlated with the GI. However, the formation of nitrate nitrogen required ammonia nitrogen as a precursor. *Bacillus* and *norank_c_Bacilli* played an important role in the formation of ammonia nitrogen, and *Geobacillus* and *Ammoniibacillus* were positively correlated with nitrite nitrogen, which could promote the transformation of ammonia nitrogen to nitrite nitrogen.

The types of OM could be ranked based on the degree of their positive correlation with the GI as fat > fibre > protein. This finding indicated that changes in the protein content had only a slight effect on the maturity of bean dregs, which might be due to the production of various enzymes during microbial metabolism. However, fat and fibre degradation could enhance the GI and improve the maturation of compost. *Geobacillus* and *Ammoniibacillus* were positively correlated with fat loss, whereas *Thermobacillus* can promote fibre loss due to degradation.

These findings suggested that *Geobacillus* and *Ammoniibacillus* improved the maturation of compost by promoting fat degradation and nitrite nitrogen formation, and this study provides theoretical guidance for the management of bean dregs during thermophilic composting.

4 Conclusions

This study screened a thermophilic bacterial strain of *Geobacillus toebii* GT-02 at 70°C for bean dreg composting. The addition of GT-02 promoted the degradation of fats, proteins, fibre and other organic matter and the generation of humus during composting. Strain GT-02 also enhanced the conversion of organic nitrogen to ammonia nitrogen and then ammonia nitrogen to nitrous nitrogen.

The results of microbial diversity showed that the proportion of thermophilic bacteria of *Ammonibacillus* and GT-02 increased at the end of the compost, and it was further found that these thermophilic bacteria were positively correlated with the compost maturity index of GI. Therefore, the addition of GT-02 was the key factor accelerating the transformation of composition during bean dreg composting. It is suggested that GT-02 may play an important role in the composting of other fibre-rich materials, such as bean dregs.

Declarations

Acknowledgments

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

XJC analyzed the data and wrote the manuscript; ZJZ & JPS obtained funding and designed the experiments; CJW, CYW, QYL, PZ & WD were responsible for composting experimental process, sampling, and physicochemical indexes detection; All authors reviewed the manuscript.

References

Adeyemo SM, Onilude AA (2013) Enzymatic Reduction of Anti-nutritional Factors in Fermenting Soybeans by Lactobacillus plantarum Isolates from Fermenting Cereals Nigerian Food Journal 31:84-90 doi:10.1016/s0189-7241(15)30080-1

Chan MT, Selvam A, Wong JW (2016) Reducing nitrogen loss and salinity during 'struvite' food waste composting by zeolite amendment Bioresour Technol 200:838-844 doi:10.1016/j.biortech.2015.10.093

Chang R, Guo Q, Chen Q, Bernal MP, Wang Q, Li Y (2019) Effect of initial material bulk density and easily-degraded organic matter content on temperature changes during composting of cucumber stalk J Environ Sci (China) 80:306-315 doi:10.1016/j.jes.2017.10.004

Chen SS, He J, Wang HY, Dong B, Li N, Dai XH (2018) Microbial responses and metabolic pathways reveal the recovery mechanism of an anaerobic digestion system subjected to progressive inhibition by ammonia Chem Eng J 350:312-323 doi:10.1016/j.cej.2018.05.168

Chen W, Westerhoff P, Leenheer JA, Booksh K (2003) Fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter Environ Sci Technol 37:5701-5710 doi:10.1021/es034354c

Coorevits A, Dinsdale AE, Halket G, Lebbe L, De Vos P, Van Landschoot A, Logan NA (2012) Taxonomic revision of the genus Geobacillus: emendation of Geobacillus, G. stearothermophilus, G. jurassicus, G. toebii, G. thermodenitrificans and G. thermoglucosidans (nom. corrig., formerly 'thermoglucosidasius'); transfer of Bacillus thermantarcticus to the genus as G. thermantarcticus comb. nov.; proposal of Caldibacillus debilis gen. nov., comb. nov.; transfer of G. tepidamans to Anoxybacillus as A. tepidamans comb. nov.; and proposal of Anoxybacillus caldiproteolyticus sp. nov Int J Syst Evol Microbiol 62:1470-1485 doi:10.1099/ijs.0.030346-0

Dias BO, Silva CA, Higashikawa FS, Roig A, Sanchez-Monedero MA (2010) Use of biochar as bulking agent for the composting of poultry manure: effect on organic matter degradation and humification Bioresour Technol 101:1239-1246 doi:10.1016/j.biortech.2009.09.024

Du G et al. (2021) Exogenous enzyme amendment accelerates maturity and changes microflora succession in horse and wildlife animal manure co-composting Environmental Science and Pollution Research doi:10.1007/s11356-020-11568-4

Hagemann N et al. (2018) Effect of biochar amendment on compost organic matter composition following aerobic composting of manure Sci Total Environ 613-614:20-29

doi:10.1016/j.scitotenv.2017.08.161

He XS, Xi BD, Zhang ZY, Gao RT, Tan WB, Cui DY (2014) Insight into the evolution, redox, and metal binding properties of dissolved organic matter from municipal solid wastes using two-dimensional correlation spectroscopy Chemosphere 117:701-707 doi:10.1016/j.chemosphere.2014.09.060

Hu TJ, Zeng GM, Huang DL, Yu HY, Jiang XY, Dai F, Huang GH (2007) Use of potassium dihydrogen phosphate and sawdust as adsorbents of ammoniacal nitrogen in aerobic composting process J Hazard Mater 141:736-744 doi:10.1016/j.jhazmat.2006.07.027

Huang M, Li Z, Huang B, Luo N, Zhang Q, Zhai X, Zeng G (2018) Investigating binding characteristics of cadmium and copper to DOM derived from compost and rice straw using EEM-PARAFAC combined with two-dimensional FTIR correlation analyses J Hazard Mater 344:539-548 doi:10.1016/j.jhazmat.2017.10.022

ISO (2000) Animal Feeding Stuffs–Determination of Crude Fibre Content. International Organization for Standardization Publ. Geneva, Switzerland,

ISO (2005) Soil Quality—Determination of Nitrate, Nitrite and Ammonium in Field-Moist Soils by Extraction with Potassium Chloride Solution–Part 2: Automated Method with Segmented Flow Analysis

ISO (2009) Oilseeds—Determination of oil content (Reference method).

Jiang T, Schuchardt F, Li GX, Guo R, Luo YM (2013) Gaseous emission during the composting of pig feces from Chinese Ganqinfen system Chemosphere 90:1545-1551 doi:10.1016/j.chemosphere.2012.08.056

Jouraiphy A, Amir S, El Gharous M, Revel JC, Hafidi M (2005) Chemical and spectroscopic analysis of organic matter transformation during composting of sewage sludge and green plant waste Int Biodeter Biodegr 56:101-108 doi:10.1016/j.ibiod.2005.06.002

Kauser H, Pal S, Haq I, Khwairakpam M (2020) Evaluation of rotary drum composting for the management of invasive weed Mikania micrantha Kunth and its toxicity assessment Bioresour Technol 313:123678 doi:10.1016/j.biortech.2020.123678

Lei L et al. (2021) Effects of phosphogypsum and medical stone on nitrogen transformation, nitrogen functional genes, and bacterial community during aerobic composting Sci Total Environ 753:141746 doi:10.1016/j.scitotenv.2020.141746

Li Y et al. (2020) Odor emission and microbial community succession during biogas residue composting covered with a molecular membrane Bioresour Technol 297:122518 doi:10.1016/j.biortech.2019.122518

Liang C, Das KC, McClendon RW (2003) The influence of temperature and moisture contents regimes on the aerobic microbial activity of a biosolids composting blend Bioresour Technol 86:131-137

doi:10.1016/s0960-8524(02)00153-0

Liu T, Kumar Awasthi M, Kumar Awasthi S, Ren X, Liu X, Zhang Z (2020) Influence of fine coal gasification slag on greenhouse gases emission and volatile fatty acids during pig manure composting Bioresour Technol 316:123915 doi:10.1016/j.biortech.2020.123915

Lv B, Xing M, Yang J, Qi W, Lu Y (2013) Chemical and spectroscopic characterization of water extractable organic matter during vermicomposting of cattle dung Bioresour Technol 132:320-326 doi:10.1016/j.biortech.2013.01.006

Malik SN, Ghosh PC, Vaidya AN, Mudliar SN (2018) Ozone pretreatment of biomethanated distillery wastewater in a semi batch reactor: mapping pretreatment efficiency in terms of COD, color, toxicity and biohydrogen generation Biofuels:1-9

Moriya T, Hikota T, Yumoto I, Ito T, Terui Y, Yamagishi A, Oshima T (2011) Calditerricola satsumensis gen. nov., sp. nov. and Calditerricola yamamurae sp. nov., extreme thermophiles isolated from a high-temperature compost Int J Syst Evol Microbiol 61:631-636 doi:10.1099/ijs.0.018416-0

Munoz-Price LS, Weinstein RAJNEJoM (2008) Acinetobacter infection 358:1271-1281

Nie E, Gao D, Zheng G (2020) Effects of lactic acid on modulating the ammonia emissions in co-composts of poultry litter with slaughter sludge Bioresour Technol 315:123812 doi:10.1016/j.biortech.2020.123812

Ogunwande GA, Osunade JA, Adekalu KO, Ogunjimi LA (2008) Nitrogen loss in chicken litter compost as affected by carbon to nitrogen ratio and turning frequency Bioresour Technol 99:7495-7503 doi:10.1016/j.biortech.2008.02.020

Oshima T, Moriya T (2008) A preliminary analysis of microbial and biochemical properties of high-temperature compost Ann N Y Acad Sci 1125:338-344 doi:10.1196/annals.1419.012

P BM, A AJ, R M (2009) Composting of animal manures and chemical criteria for compost maturity assessment. A review Bioresour Technol 100

Poli A et al. (2006) Geobacillus toebii subsp. decanicus subsp. nov., a hydrocarbon-degrading, heavy metal resistant bacterium from hot compost J Gen Appl Microbiol 52:223-234 doi:10.2323/jgam.52.223

Rhee SK et al. (2002) Characterization of Symbiobacterium toebii, an obligate commensal thermophile isolated from compost Extremophiles 6:57-64 doi:10.1007/s007920100233

Sakai M, Deguchi D, Hosoda A, Kawauchi T, Ikenaga M (2015) Ammoniibacillus agariperforans gen. nov., sp. nov., a thermophilic, agar-degrading bacterium isolated from compost Int J Syst Evol Microbiol 65:570-577 doi:10.1099/ijs.0.067843-0

Sung MH et al. (2002) Geobacillus toebii sp. nov., a novel thermophilic bacterium isolated from hay compost Int J Syst Evol Microbiol 52:2251-2255 doi:10.1099/00207713-52-6-2251

Thebti W, Riahi Y, Gharsalli R, Belhadj O (2016) Screening and characterization of thermo-active enzymes of biotechnological interest produced by thermophilic Bacillus isolated from hot springs in Tunisia Acta Biochim Pol 63:581-587 doi:10.18388/abp.2016_1271

Touzel JP, O'Donohue M, Debeire P, Samain E, Breton C (2000) Thermobacillus xylanilyticus gen. nov., sp. nov., a new aerobic thermophilic xylan-degrading bacterium isolated from farm soil Int J Syst Evol Microbiol 50 Pt 1:315-320 doi:10.1099/00207713-50-1-315

Wang B et al. (2019) Fabrication of bean dreg-derived carbon with high adsorption for methylene blue: Effect of hydrothermal pretreatment and pyrolysis process Bioresour Technol 274:525-532 doi:10.1016/j.biortech.2018.12.022

Wang X, Zhao Y, Wang H, Zhao X, Cui H, Wei Z (2017) Reducing nitrogen loss and phytotoxicity during beer vinasse composting with biochar addition Waste Manag 61:150-156 doi:10.1016/j.wasman.2016.12.024

Wu J et al. (2017) Identifying the key factors that affect the formation of humic substance during different materials composting Bioresour Technol 244:1193-1196 doi:10.1016/j.biortech.2017.08.100

Yang Y, Awasthi MK, Bao H, Bie J, Lei S, Lv J (2020a) Exploring the microbial mechanisms of organic matter transformation during pig manure composting amended with bean dregs and biochar Bioresour Technol 313:123647 doi:10.1016/j.biortech.2020.123647

Yang Y, Awasthi MK, Ren X, Guo H, Lv J (2019) Effect of bean dregs on nitrogen transformation and bacterial dynamics during pig manure composting Bioresour Technol 288:121430 doi:10.1016/j.biortech.2019.121430

Yang Y, Du W, Ren X, Cui Z, Zhou W, Lv J (2020b) Effect of bean dregs amendment on the organic matter degradation, humification, maturity and stability of pig manure composting Sci Total Environ 708:134623 doi:10.1016/j.scitotenv.2019.134623

Yang Y, Kumar Awasthi M, Du W, Ren X, Lei T, Lv J (2020c) Compost supplementation with nitrogen loss and greenhouse gas emissions during pig manure composting Bioresour Technol 297:122435 doi:10.1016/j.biortech.2019.122435

Ying Z, Jianchun H, Huanyu Z, Hui XU, Longfu XU (2013) Research of Further Processing and Comprehensive Utilization of Soybean Dregs Soybean ence 32:555-560

Yu Z et al. (2019) Hyperthermophilic composting accelerates the humification process of sewage sludge: Molecular characterization of dissolved organic matter using EEM-PARAFAC and two-dimensional correlation spectroscopy Bioresour Technol 274:198-206 doi:10.1016/j.biortech.2018.11.084

Yu Z et al. (2018) The distinctive microbial community improves composting efficiency in a full-scale hyperthermophilic composting plant Bioresour Technol 265:146-154 doi:10.1016/j.biortech.2018.06.011

Zhang C et al. (2020) Material conversion, microbial community composition and metabolic functional succession during green soybean hull composting Bioresour Technol 316:123823 doi:10.1016/j.biortech.2020.123823

Zhang J, Lu F, Shao L, He P (2014) The use of biochar-amended composting to improve the humification and degradation of sewage sludge Bioresour Technol 168:252-258 doi:10.1016/j.biortech.2014.02.080

Zhang L, Sun X (2014) Changes in physical, chemical, and microbiological properties during the two-stage co-composting of green waste with spent mushroom compost and biochar Bioresour Technol 171:274-284 doi:10.1016/j.biortech.2014.08.079

Zhang L, Sun X (2018) Effects of bean dregs and crab shell powder additives on the composting of green waste Bioresour Technol 260:283-293 doi:10.1016/j.biortech.2018.03.126

Zhao HY, Li J, Liu JJ, Lu YC, Wang XF, Cui ZJ (2013) Microbial Community Dynamics During Biogas Slurry and Cow Manure Compost J Integr Agr 12:1087-1097 doi:10.1016/S2095-3119(13)60488-8

Figures

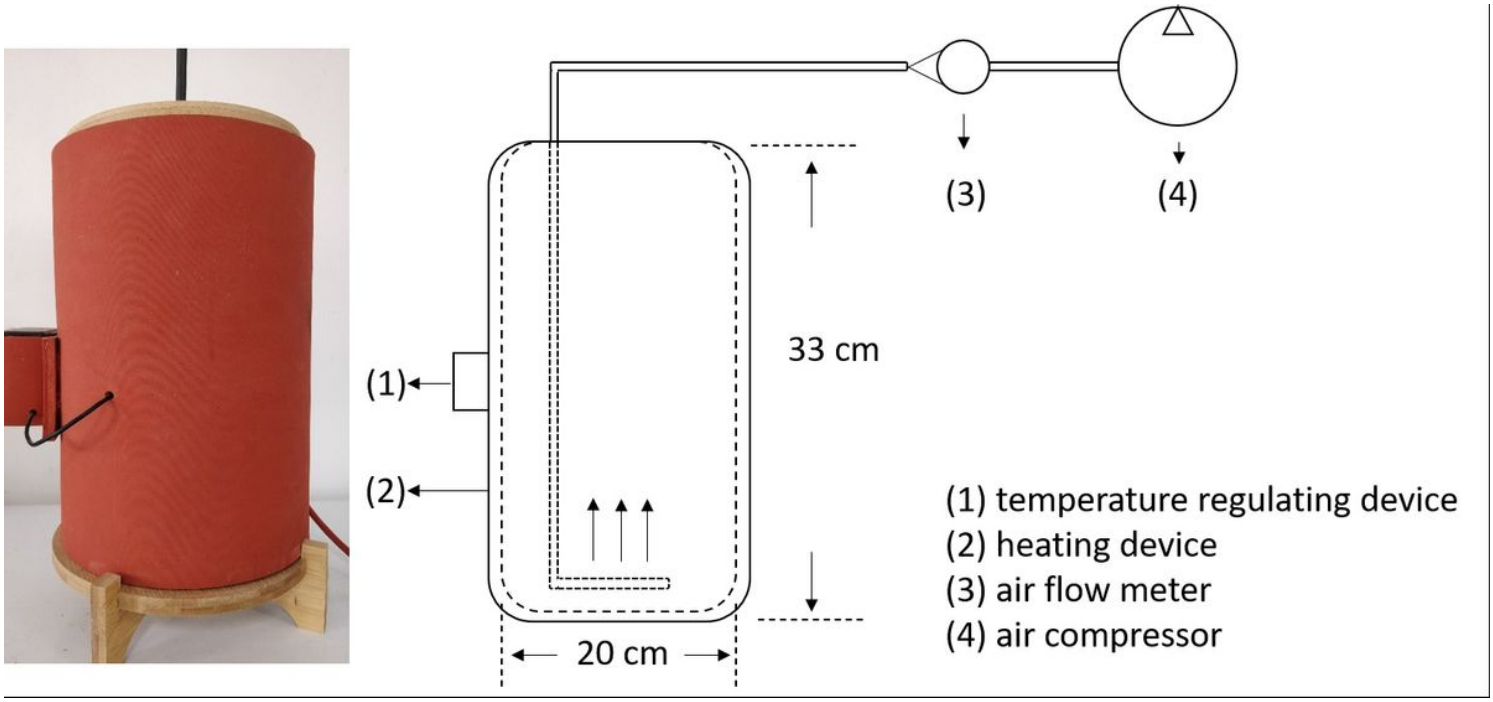

Figure 1

Bench-scale compost system.

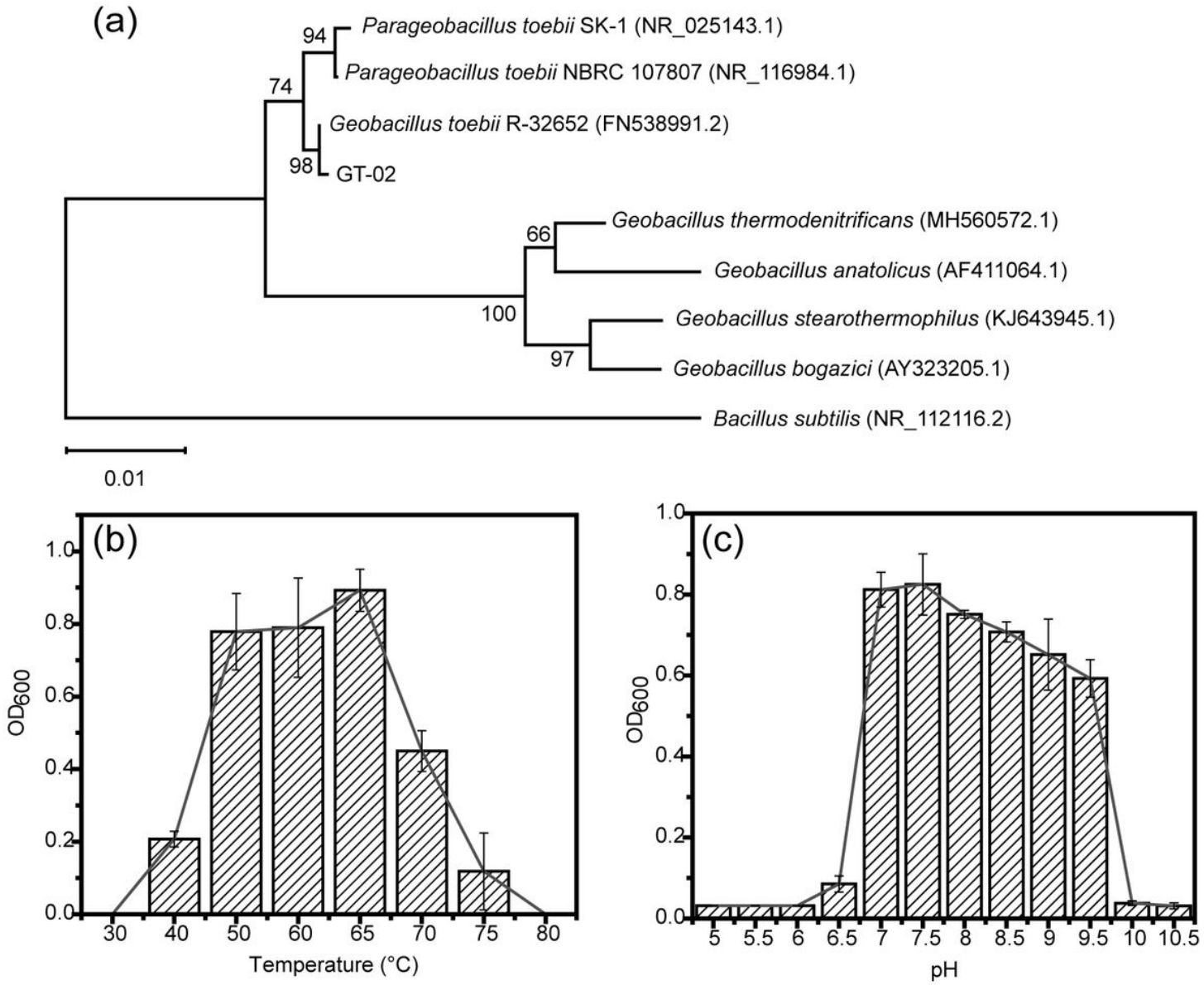

Figure 2

(a) Phylogenetic tree based on 16S rDNA gene sequences from related species of the genus Geobacillus constructed using the neighbour-joining method with 1,000 bootstrap replicates. Branch length is indicated at each node. (b) The growth curve of strain GT-02 with temperature. (c) The growth curve of strain GT-02 with pH.

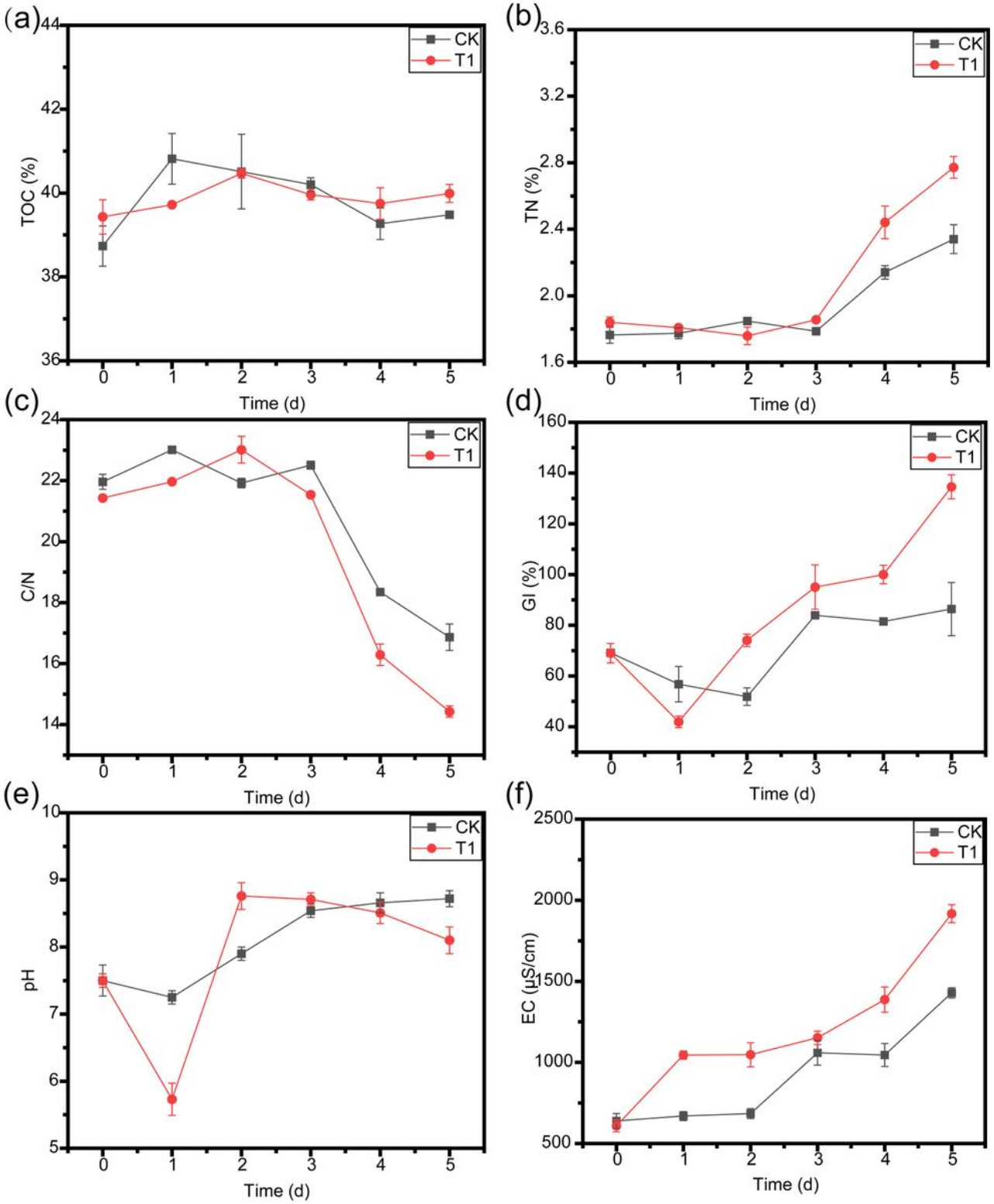

Figure 3

Profiles of TOC (a), TN (b), C/N (c), GI (d), pH (e) and EC (f) during the composting of CK and T1. The data represent the means ± standard deviations from three measurements.

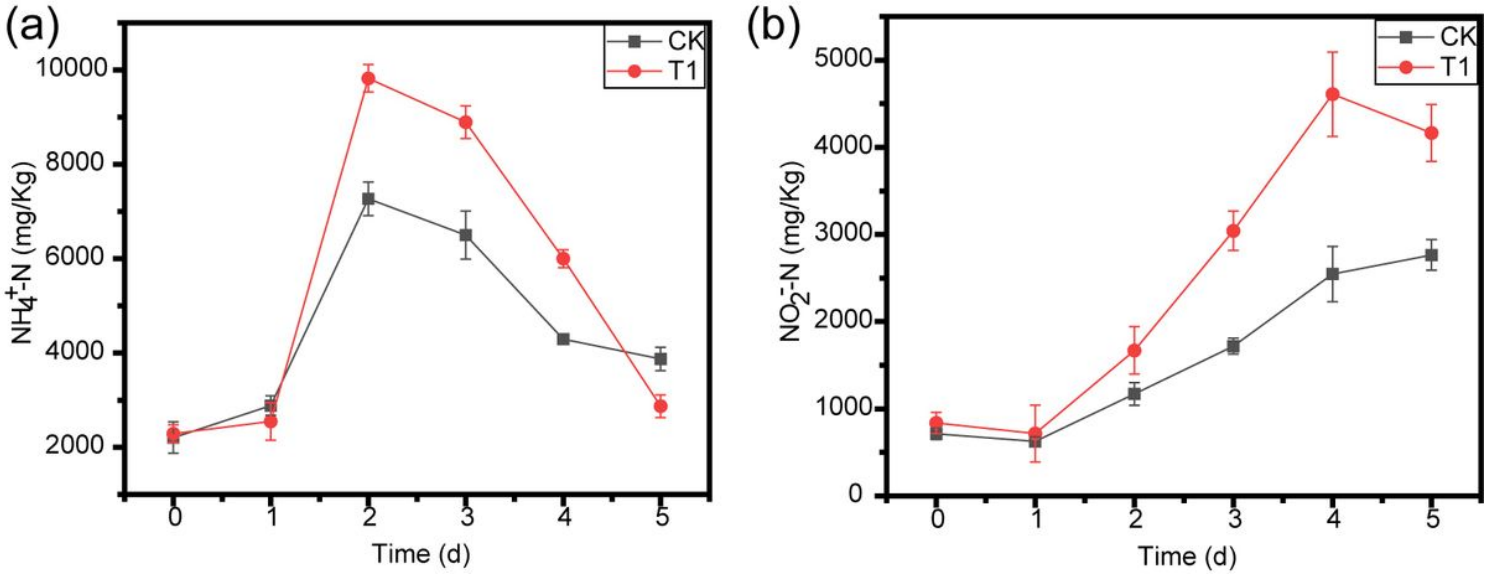

Figure 4

Profiles of NH4+-N (a) and NO2–N (b) during the composting of CK and T1. The data represent the means ± standard deviations from three measurements.

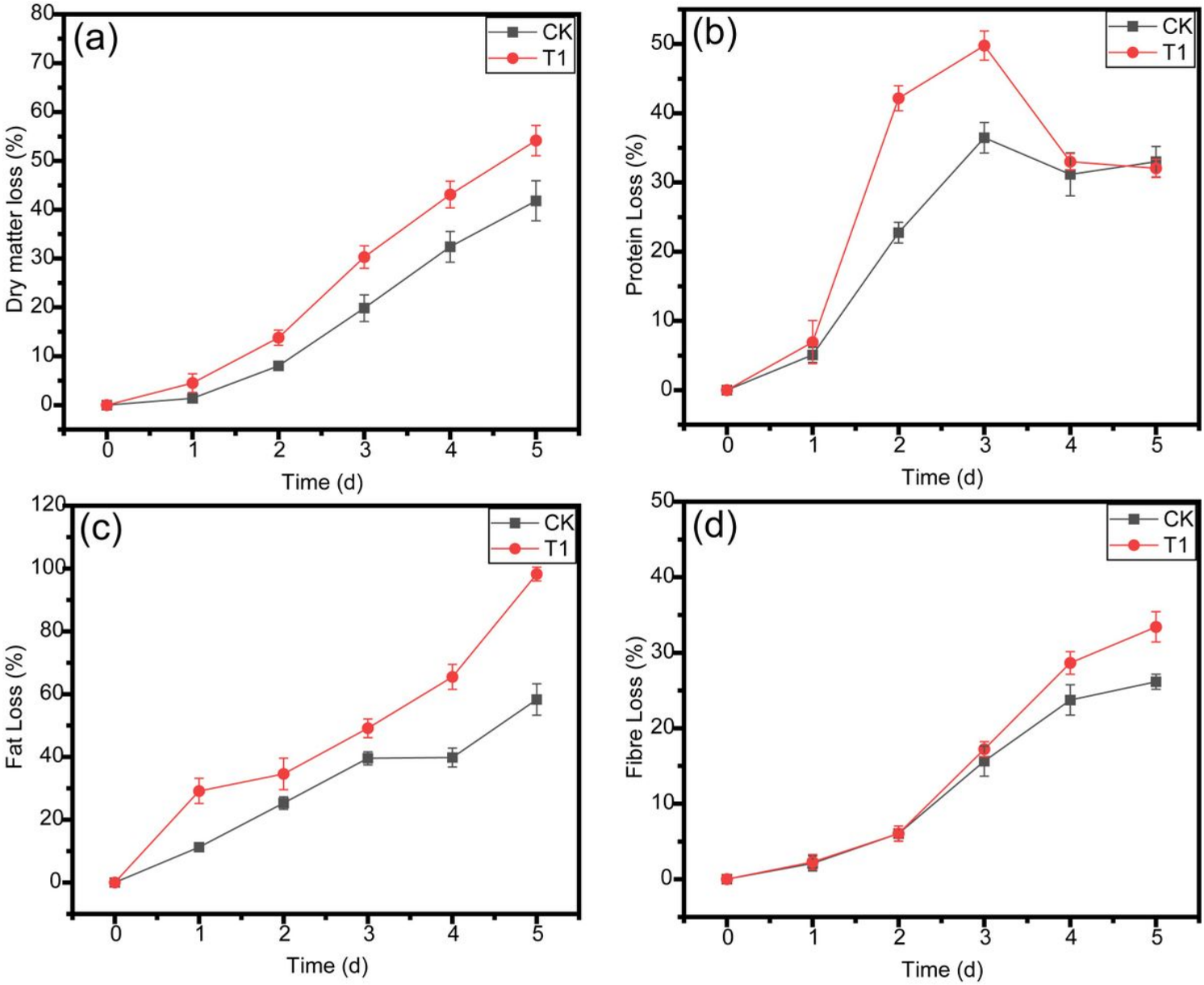

Figure 5

Profiles of dry matter loss (a), protein loss (b), fat loss (c) and fibre loss (d) in CK and T1 during composting. The data represent the means ± standard deviations from three measurements.

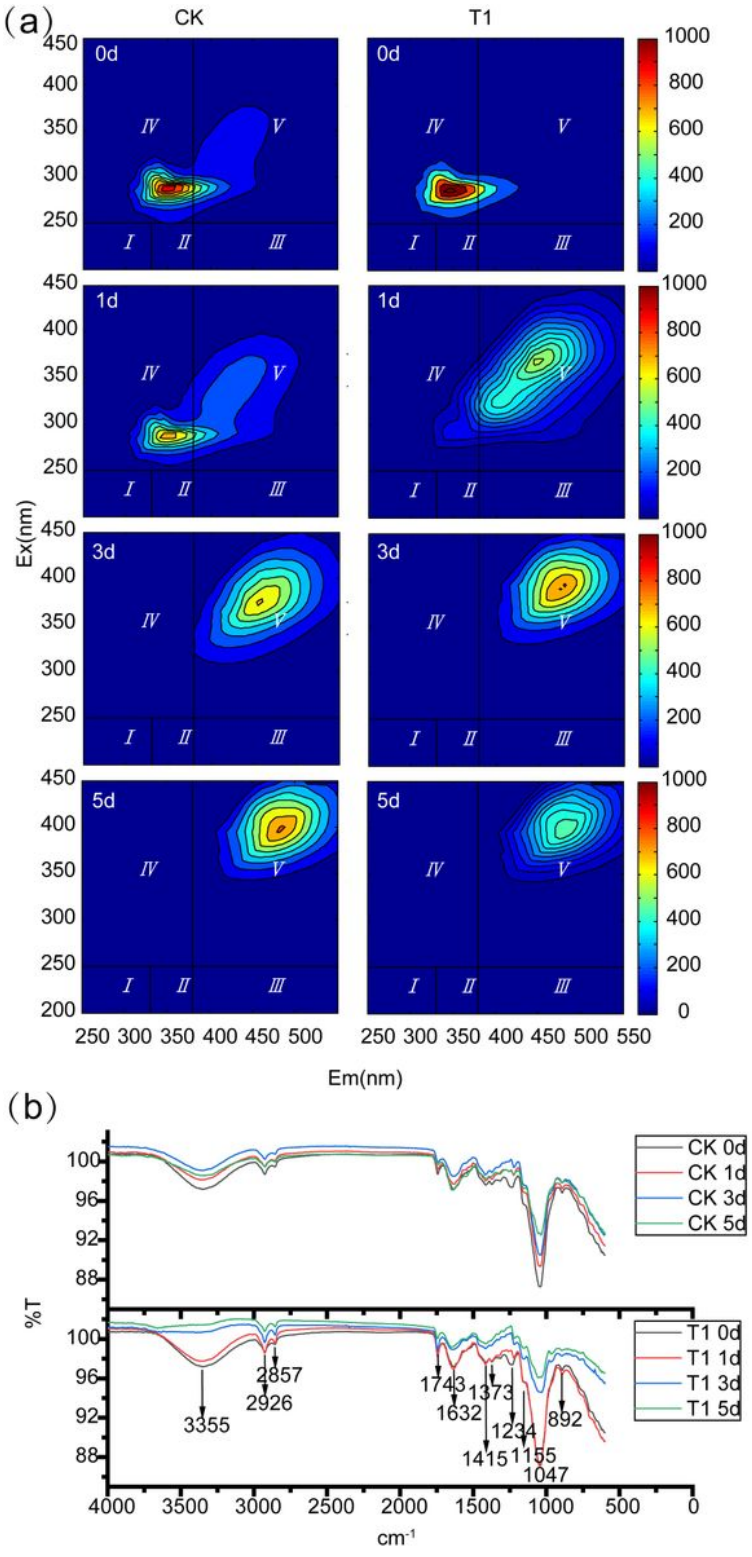

Figure 6

(a) Fluorescence excitation-emission matrix spectra of dissolved organic matter in CK and T1. Em (nm): emission wavelength (nm); Ex (nm): excitation wavelength (nm). Regions I and II: simple aromatic proteins; Region III: fulvic acid-like substances; Region IV: soluble microbial byproducts; and Region V: humic acid-like substances. (b) The 4000-to-600-cm-1 regions of the Fourier transform infrared spectra of CK and T1.

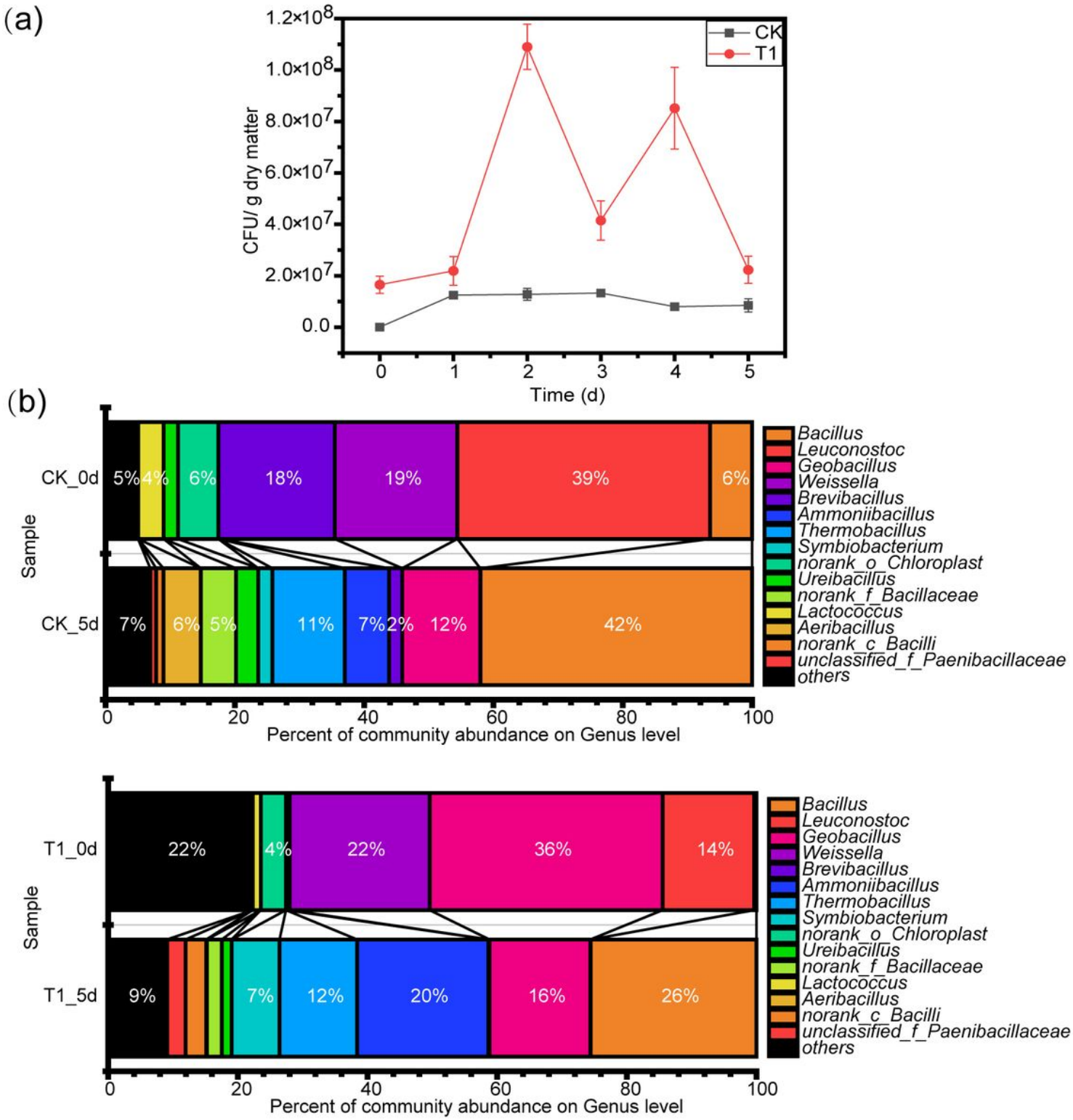

Figure 7

(a) Profiles of CFUs in CK and T1 during composting. (b) Taxonomic classification at the genus level of predominant 16S rDNA gene sequences (relative abundance, top 15) in CK and T1. The data represent the means ± standard deviations from three measurements.

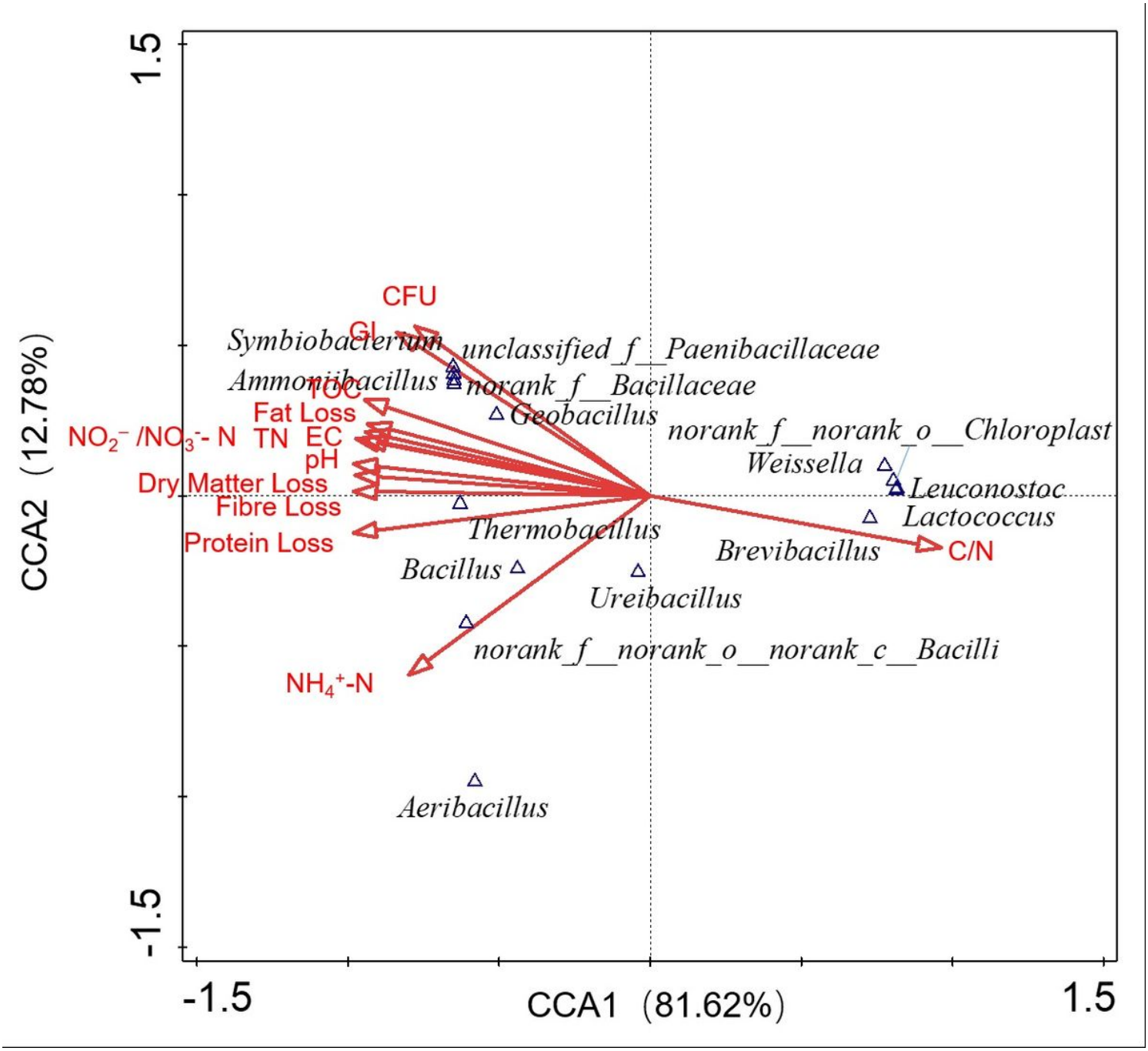

Figure 8

CCA between the composition and microbial community of CK and T1.