



Increased abundance of nitrogen fixing bacteria by higher C/N ratio reduces the total losses of N and C in cattle manure and corn stover mix composting

Wenming Zhang^{a,b,*}, Chenxu Yu^b, Xujie Wang^a, Long Hai^a, Juan Hu^c

^a College of Resources and Environmental Sciences, Gansu Agricultural University, Lanzhou 730070, PR China

^b Department of Agriculture and Biosystem Engineering, Iowa State University, Ames 50010, United States

^c Jilin Provincial Laboratory of Grassland Farming, Northeast Institute of Geography and Agroecology Chinese Academy of Sciences, Changchun 130102, PR China

ARTICLE INFO

Article history:

Received 7 October 2019

Revised 3 January 2020

Accepted 4 January 2020

Keywords:

N-loss

C-loss

C/N ratio

Nitrogen fixing bacteria

Denitrifying bacteria

ABSTRACT

The aim of this work was to investigate the effects of initial C/N ratio on the total losses of N and C during composting of cattle manure/corn stover mix, and to analyze how the initial C/N ratio impacts the successive progression of the microbiota to affect losses of N and C during the composting. Results indicated that the least of C-loss and N-loss occurred in 35:1 of initial C/N ratio. The succession of predominant bacteria in the composting mix was significantly affected by the temperature and the initial C/N ratio. Redundancy analysis showed that significant negative correlations existed between nitrogen fixing bacteria and N- and C-losses, and significant positive correlations existed between denitrifying bacteria and N- and C-losses. Higher initial C/N ratio appeared to promote growth of nitrogen fixing bacteria while compress the denitrifying bacteria. These results indicated that adjustment of the initial C/N ratio is an important way to control the losses of N and C in the compost. In addition, beneficial nitrogen fixing bacteria could potentially be screened and used as composting supplements to improve the quality of the compost.

© 2020 Published by Elsevier Ltd.

1. Introduction

Composting is one of the most effective methods for disposal of organic wastes. Using composts as organic fertilizer or soil amendments to improve soil fertility and biological activity and agricultural productivity is gaining popularity (Yang et al., 2019). Composting is a complex biological process which is profoundly affected by properties of the organic materials and environment factors, such as C/N ratio, nutrient content, particle size, moisture content, temperature, pH, aeration, etc. (Jain et al., 2018). Among these parameters, the initial C/N ratio is regarded as a key factor affecting the composting process as well as the properties of the products (Huang et al., 2004; Kumar et al., 2010). Lower C/N ratio facilitates the release of more soluble basic salts (Awasthi et al., 2014) and expedites nitrogen loss (Ren et al., 2010), while higher C/N ratio may lead to slower decomposition rates and longer composting cycle due to inadequate supply of N to support optimal microbial growth (Qiao et al., 2019), the most suitable initial C/N

ratio for composting has been found to be within 20:1–30:1 (Gao et al., 2010).

Losses of nitrogen (N) and carbon (C) are inevitable during the composting process due to the release of gaseous metabolic products such as NH₃, N₂O, CO₂ and CH₄ (Chen et al., 2019a; Ren et al., 2019). This not only is an environmental concern, but also leads to the decline of compost quality (Hao et al., 2004; Lim et al., 2017). Moreover, the microbial diversity and activity of the compost are also of great importance as they could affect microbial diversity and activity of the soil. During the composting process, the microbiota of the compost inevitably undergoes species succession (Bernal et al., 2009; Collins et al., 2006; He et al., 2013; Kumar, 2011). Previous studies have reported the effect of the initial C/N ratio on N-loss and C-loss (Eghball et al., 1997; Ekinci et al., 2019; Ogunwande et al., 2008) and correlations between dominant microbiota and environmental factors (e.g., TN, TOC, pH, NH₄⁺-N, NO₃⁻-N, C/N) (Meng et al., 2018; Yin et al., 2019). However, how the initial C/N ratio impacts the successive progression of the microbiota in different raw materials to affect losses of N and C during composting remains unclear. Understanding this connection would be very useful to improve the efficiency of composting and the quality of composting products.

* Corresponding author at: College of Resources and Environmental Sciences, Gansu Agricultural University, Lanzhou 730070, PR China.

E-mail address: zhangwm@gsau.edu.cn (W. Zhang).

Therefore, the primary objective of this study was to investigate the change of total losses of N and C during regular composting of cattle manure/corn stover mix with different initial C/N ratios, to characterize the succession of dominant species of microorganism during these processes using 16S rRNA gene sequencing throughout the four main composting stages, and to identify potential beneficial bacteria in composts with different initial C/N ratios. By analyzing the correlations between dominant bacterial species and the total losses of N and C, we aimed to better understand their roles in determining the total losses of N and C, and to identify possible means to ameliorate appropriate C/N ratio and/or inoculate beneficial bacteria in the composting mix to improve the quality of composting products, and to reduce the negative environmental footprint of the operation.

2. Materials and methods

2.1. Raw materials

The cattle manure (CM) and corn stover (CS) were collected from the farm of Zhongcha village, Huining county of Gansu province. Selected characteristics of the raw materials are shown in Table 1.

2.2. Composting and sampling

The CS was cut into pieces about 10 mm in size. Cattle manure was mixed with the CS to adjust the moisture content of the mixture materials to around 60% (The initial moisture content of the cattle manure was ~74.4%, it was dried to below 60% under natural condition, and then CS and water was added into the mixture to adjust the moisture content to around 60%) and C/N ratio of 20:1, 25:1, 30:1 and 35:1 to produce four types of composting materials (referred to as four “treatments”, A, B, C and D). The composting materials were packed loosely in an open box (60 × 60 × 45 cm, with a 90% fill) with colorful steel plate ceiling and composted under outdoor conditions (from Oct. 6th to Nov. 6th of 2018, Lanzhou, Gansu province) for 30 days. Three batches for each treatment were made as replicates. Each composting pile was turned once every three days. The temperature at the center of each pile as well as ambient temperature were measured twice a day at 9 am and 3 pm, respectively, and averaged and recorded. Day 1, 3, 12 and 30 were the time points representing the initial stage, the high-temperature stage, the decreasing-temperature stage and the maturity stage according to the center temperature, denoted by I, II, III and IV, respectively. All the piles were weighed and then blended before sampling. Samples were collected on days 1, 3, 6, 12, 15 and 30 with 1 kg (wet mass). Every sample was divided into three equal parts. One part was stored at 4 °C and used for pH, electronic conductivity (EC) and the seed germination index (GI) analysis, and another was air-dried and passed through a 0.1 mm sieve for total organic carbon (TOC) and total nitrogen (TN) analyses. The third part was vacuum-packed, frozen at –80 °C, and sent to Shanghai BIOZERON Co., Ltd for the 16S rRNA

gene sequencing and analysis (only samples collected on day 1, 3, 12 and 30 were sent for 16S rRNA gene sequencing).

2.3. Analyses of physico-chemical parameters

The chemical composition of the raw materials as well as each fresh sample collected during the composting process was determined as follows: Moisture content was measured with oven drying method, total organic carbon (TOC) and total nitrogen (TN) were measured by potassium dichromate and sulphuric acid method (Gao et al., 2010) and Kjeldahl digestion method (Gao et al., 2010), respectively, and the C/N ratio was calculated as C/N = TOC/TN. At each stage the loss of dry matter (DM, %), the nitrogen loss (N-loss, %) and the carbon loss (C-loss, %) of each pile were calculated from the pile mass (PM, dry basis), moisture content (MC, %), TOC and TN on days 1, 3, 6, 12, 15 and 30 as follows:

$$DM_{loss(i)} = [PM_{i+1} \times (1 - MC_{i+1}) - PM_i \times (1 - MC_i)] / DM_{initial}$$

$$N - loss_{(i)} = [PM_{i+1} \times (1 - MC_{i+1}) \times TN_{i+1} - PM_i \times (1 - MC_i) \times TN_i] / TN_{initial}$$

$$C - loss_{(i)} = [PM_{i+1} \times (1 - MC_{i+1}) \times TOC_{i+1} - PM_i \times (1 - MC_i) \times TOC_i] / TOC_{initial}$$

where i = 1, 2, 3, 4, 5 were with respect to the dates of the sampling.

NH₄⁺-N and NO₃⁻-N were extracted with 2 M KCl (1:10 fresh sample to KCl, w/v, dry mass basis) and analyzed in triplicate using a segmented flow analyzer (Technicon Autoanalyzer system, Germany) (Guo et al., 2012). pH, EC and GI of each sample were determined following Huang et al. (2004). Briefly, the aqueous compost extract was obtained by mechanically shaking the fresh sample mixed with double distilled water (DDW) at a solid: DDW ratio of 1:10 (w/v, dry mass basis) for 1 h. The suspension was then centrifuged at 12,000 rpm for 20 min and filtered through 0.45 μm membrane filters. The filtrate was then used for the following analyses: pH was determined using an Orion 920 ISE pH meter (Fisher Scientific, Pittsburgh, PA), EC was measured by an Orion 160 conductivity meter (Fisher Scientific, Pittsburgh, PA). For GIs determination, 5.0 mL of each extract were pipetted into a sterilized petri dish lined with 2 layers of filter paper (distilled water was used as control), twenty Chinese cabbage (*Brassica campestris* L.SPP. *Pekinensis*) seeds were evenly placed on the filter paper and incubated at 25 °C in the dark for 72 h, five replicates were analyzed for each sample and the GI was determined by the following formula (Zucconi et al., 1981).

$$GI (\%) = [\text{Seed germination of treatment} (\%) \times \text{Root length of treatment} (\text{cm})] \times 100 / [\text{Seed germination of control} (\%) \times \text{Root length of control} (\text{cm})]$$

2.4. DNA extraction and high-throughput sequencing

Microbial DNA was extracted from all samples of days 1, 3, 12 and 30 using the E.Z.N.A.[®] Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer's protocols. The V4-V5 region of the bacteria 16S ribosomal RNA gene were amplified by PCR (95 °C for 5 min, followed by 27 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s and a final extension at 72 °C for 10 min) using primers 515F 5'-barcode-GTCCAGCMGCCGCGG-3' and 907R 5'-CCGTAATTCMTTTRAGTTT-3' (Si et al., 2015). PCR products were purified with the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manu-

Table 1
Properties of raw materials.

Parameters	Cattle manure	Corn stover
Moisture content (%)	74.39 ± 0.85	11.05 ± 0.19
TOC (g/kg)	386.9 ± 5.2	486.19 ± 8.7
TN (g/kg)	19.15 ± 0.32	6.09 ± 0.18
C/N	20.30 ± 0.27	80.96 ± 0.42

Note: Values indicate mean ± standard deviation based on the samples with three replications. TOC: total organic carbon, TN: total nitrogen, C/N = TOC/TN.

facturer's instructions and quantified using QuantiFluor™-ST (Promega, U.S.). Then the amplicon library was paired-end sequenced (2×250) on an Illumina HiSeq platform (Shanghai BIOZERON Co., Ltd) according to the standard protocols. The raw sequence data were processed in QIIME 1.7.0 and deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SUB6064585).

2.5. Sequence data processing

All analyses were repeated three times. Statistical analysis was performed by SPSS 18.0 software using one-way ANOVA at significance $P < 0.05$ level to separate treatment means for the mass data. Operational Taxonomic Units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 <http://drive5.com/uparse/>) and chimeric sequences were identified and removed using UCHIME (Edgar et al., 2011). Community-scale multivariate analysis of non-metric multidimensional scaling (nMDS) was performed in R (Price et al., 2009). Multiple variations of correlations between selected influenced factors (N-loss and C-loss) and bacterial community composition were analyzed by redundancy analysis (RDA) with RDA function, and the factors were fitted to ordination plots by the vegan package in R software, as described by Liu et al. (2018).

3. Results and discussions

3.1. Effect of different C/N ratio on maturity indexes

Temperature determines the degradation process of organic matter during composting, and the succession of microbial communities inside the piles (Liu et al., 2014). As shown in Fig. 1a, in this study, throughout the entire composting process the ambient temperature was within the range of 9.5–15 °C. In all four treatments, the high temperature threshold (>50 °C) (Guo et al., 2012) was reached at day 2, and lasted 8 days. The highest temperature of 67.83 °C, 67.33 °C, 68.58 °C and 68.08 °C were reached at day 3, for 20:1, 25:1, 30:1 and 35:1 treatment, respectively. After day 10, all piles entered the decreasing temperature stage until day 16, and subsequently the maturation stage from day 16 to day 30, respectively (Fig. 1a). The temperature of treatment group D (35:1 C/N) piles increased and decreased slightly faster, and the mean temperature was slightly lower than that of the others. These results were consistent with Erickson et al. (2009), which was attributed to insufficient of nitrogen source of the high C/N piles. All four treatments satisfied the sanitary requirement for pile center temperature to reach over 50 °C for at least 7 days (Li et al., 2017; Zhang & Sun, 2014).

GI is regarded as a reliable parameter to evaluate the maturity of a compost (Li et al., 2012; Mao et al., 2018). As shown in Fig. 1b, at day 1, the GIs for the four treatments were 42.8%, 64.5%, 60.2% and 54.2%, respectively, and further dropped to the lowest value of 30.7%, 36.5%, 34.3% and 37.1% at day 6, respectively (Fig. 1b), which could be attributed to the release of ammonia (Fig. 1e), and the decompose of organic matter to organic acids (pH declined from day 3 in Fig. 1d), both inhibited the germination of the cabbage seeds. These change tendencies were in line with Zhong et al. (2018). Furthermore, The GIs of all four treatments increased to about 60% at day 15, and eventually reached 93.1%, 93.9%, 93.5% and 84.1% at day 30, respectively. It has been reported that $GI > 80\%$ is an indication of a mature compost (Zucconi et al., 1981). Hence, at day 30 all of the composts have reached maturity.

Change of pH could prompt the release of ammonia from ammonification and mineralization of organic nitrogen during the initial phase of composting (Wong et al., 2001). The pH values of

the four treatments increased from initial pH 7.35, 7.29, 7.05 and 7.02 to maximum pH 7.89, 7.53, 7.38 and 7.26 on day 3, respectively; and then gradually decreased to 7.35, 7.08, 7.09 and 6.85 at the mature stage, respectively (Fig. 1d). These change tendencies were in line with Zhong et al. (2018). The pH values of treatment D (initial C/N 35:1) piles were significantly lower than those of the others, possibly due to higher amount of corn stover was used in the initial C/N 35:1 piles to reach the designated C/N ratio, which released less ammonia (Fig. 1e) and produced more organic and inorganic acids.

The value of EC increased unavoidably with organic matter mineralization in composting. The ECs of four treatments increased rapidly during the first 3 days, and then increased much slower until the end of the composting, the final average ECs of the four treatments reached 4.48 mS/cm, 3.87 mS/cm, 3.63 mS/cm and 3.45 mS/cm, respectively (Fig. 1c). The EC of the treatment A piles (20:1 C/N ratio) was higher than 4 mS/cm due to the release of more soluble basic salts in low C/N ratio composting (Awasthi et al., 2014).

The $\text{NH}_4^+\text{-N}$ concentration in the four treatments increased at the beginning, and then gradually decreased (Fig. 1e). The highest $\text{NH}_4^+\text{-N}$ concentration occurred at day 6, which was in line with Huang et al. (2004). The $\text{NH}_4^+\text{-N}$ concentration was always lower with the increase of C/N ratio, this result was consistent with Huang et al. (2004). The $\text{NO}_3^+\text{-N}$ concentration in the four treatments increased continuously (Fig. 1f). The $\text{NO}_3^+\text{-N}$ concentrations of the 20:1 and 25:1 piles were significantly higher than those of 30:1 and 35:1 piles, similar to previous report (Guo et al., 2012). Overall, the inorganic nitrogen represented by the combined $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^+\text{-N}$ concentration were lower with the increase of C/N ratio due to the large amount of corn stover added.

3.2. Effect of different C/N ratio on DM-loss, C-loss and N-loss

The DM-loss, C-loss and N-loss have prominent effects on compost quality (Larney et al., 2006). In this study, the cumulative DM-loss, C-loss and N-loss across all piles showed similar tendencies (Fig. 2): A rapid loss stage in the first 6 days, followed by a relatively slow loss stage during day 6–15, and a stable stage in day 15–30. The DM-loss, C-loss and N-loss, especially N-loss of the 35:1 and 30:1 piles were always lower than those of the 20:1 and 25:1 piles, which may be attributed to more nitrogen fixing bacteria in 35:1 and 30:1 piles as shown below (Fig. 5). This observation was in line with earlier observation that a low C/N ratio is an important reason for nitrogen loss (Ren et al., 2010). The cumulative DM-loss, C-loss and N-loss at the end of the composting were listed in Table 2. They were lower than the result of Lim et al. (2017), which was a lab-scale operation that lasted much longer (~100 days). In this study, the composting operation was designed to mimic trough and windrow composting, both were widely used in industrial composting operation in china.

3.3. The nMDS analysis of the bacterial communities during the composting processes with different treatments

The nMDS analysis is a nonparametric ordination-based method for reducing ecological community data complexity and identifying meaningful relationships/similarities amongst communities (Price et al., 2009). In an nMDS plot, distances between data points gave an indication of the degree of difference among them, and clustering of group of data points reflected their similarities (Chen et al., 2019b). As shown in Fig. 3, data points from the four treatments at different stages during the composting process formed into three clusters representing the initial stage (stage I), the high temperature stage (stage II) and the later stage (stages III and IV), suggesting that the progression of the composting and the resulted temperature changes were the most important determining factor for the characteristics of the microbiota. A closer look

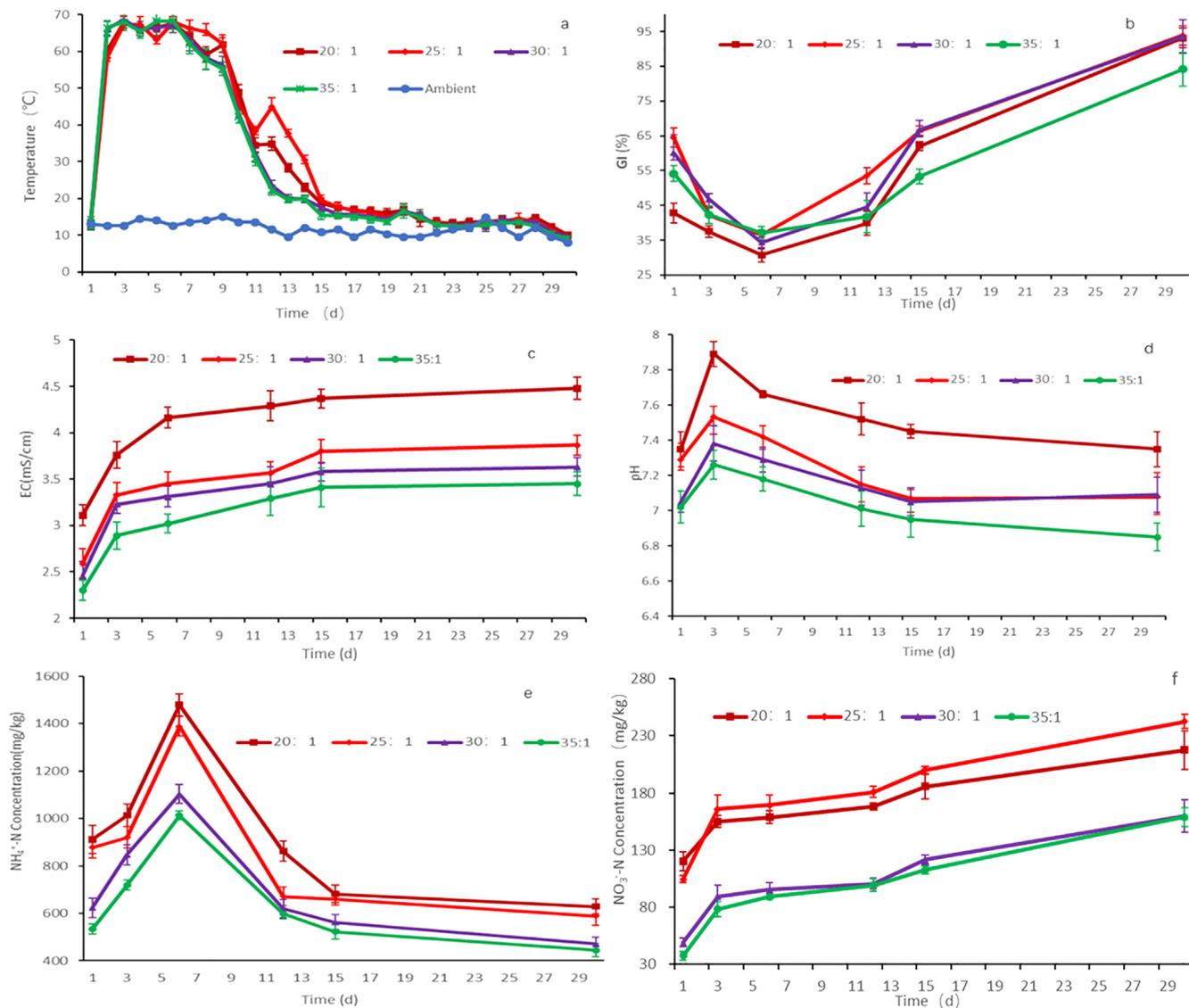


Fig. 1. Evaluation of temperature (a), GI (b), EC (c), pH (d), $\text{NH}_4^+\text{-N}$ concentration (e) and $\text{NO}_3^-\text{-N}$ concentration (f) of the four treatments. The data presented as means \pm SD, $n = 5$ for GI and $n = 3$ for others.

at the first cluster, which included all four treatments at the initial stage, revealed no clearly separated pattern, suggesting that the initial microbiota among all treatment groups showed significant similarity. As the composting process progressed to stage II (the 2nd cluster), a separation started to emerge among treatment groups (A: 20:1 C/N ratio, B: 25:1 C/N ratio, C: 30:1 C/N ratio and D: 35:1 C/N ratio), suggesting that the microbiota in the four treatments started to clearly differentiate from each other from day 10 on during the composting process. Same patterns were observed for the third cluster (stage III and IV), where the separation among treatments was clear. Apparently, the initial C/N ratio had a significant impact on the characteristics of the microbiota in the compost mix as the composting process progressed towards maturity.

3.4. Different succession patterns observed in the bacterial communities during the composting processes with different treatments

Data on bacterial abundance at genus level can help to explain the role of microbial activities in a composting system (Mao et al.,

2018). In this study, the predominant bacterial species in samples from the four treatment groups were found to be different. The succession of bacterial communities in composting mix of the four treatments during each of the four stages were showed in Fig. 4 (phylum level) and Fig. 5 (genus level).

The predominant bacteria at phylum level (Fig. 4) were *Proteobacteria*, *Bacteroidetes*, *Deinococcus-Thermus*, *Firmicutes*, *Actinobacteria* at the beginning, this was different from Sun et al. (2019), due to different source materials. During the composting process, the relative abundance of these microbes changed significantly. The relative abundance of *Proteobacteria* and *Bacteroidetes* decreased firstly, and then increased, while opposite trend was observed for the *Firmicutes* and *Actinobacteria*. These results were again different from previous reports (Ren et al., 2016; Sun et al., 2019), indicating that bacterial communities in the same type of manure (cattle) from different sources could be quite different, which would greatly affect the subsequent development of bacterial succession during the composting process. Meanwhile, the relative abundance of *Deinococcus-Thermus* plunged, while *Halanaerobiaeota* surged at the high temperature stage and then decreased continuously, and *Gemmatimonadetes* surged at the

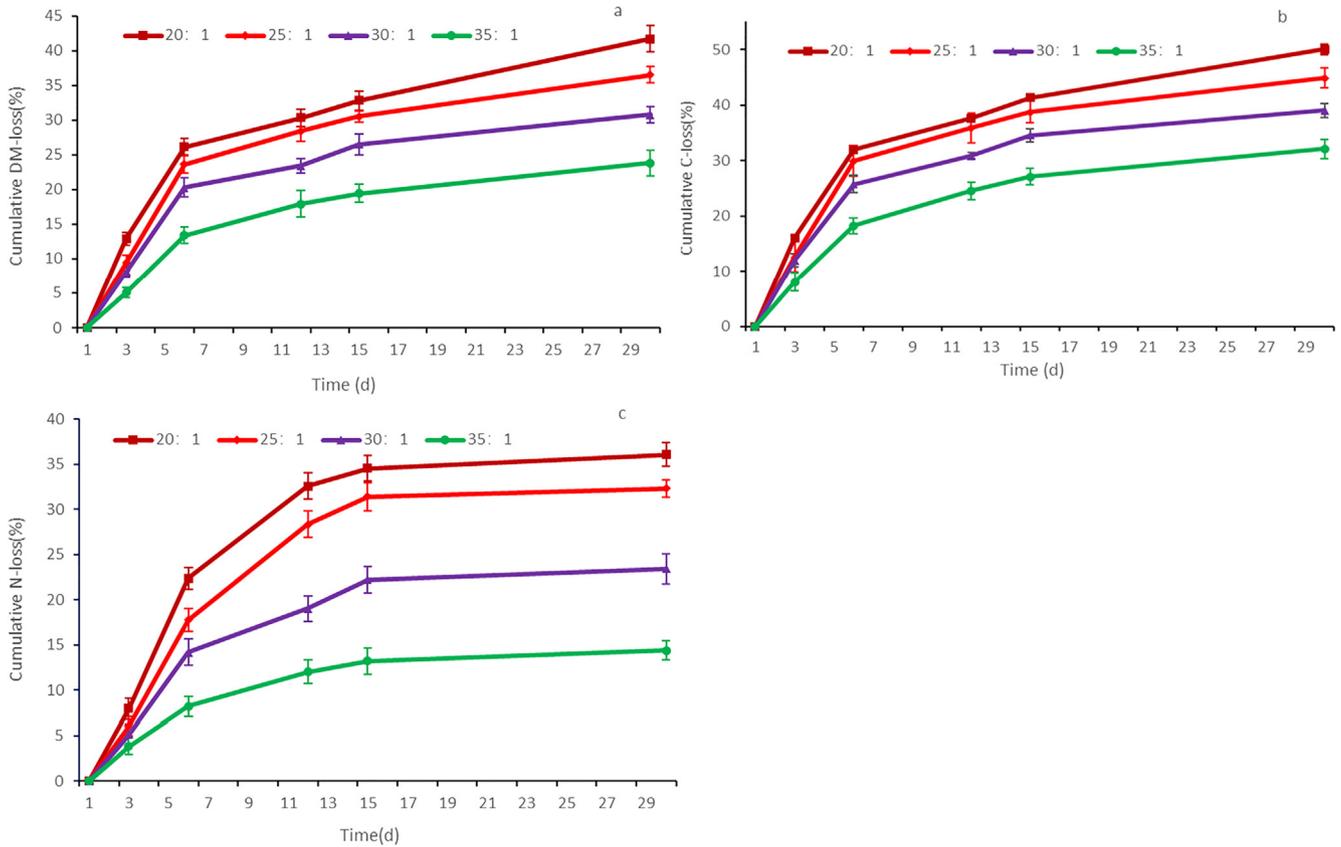


Fig. 2. Cumulative DM-loss (a), C-loss (b) and N-loss (c) of the four treatments. The data presented as means \pm SD, n = 3.

Table 2
Cumulative DM-loss, N-loss and C-loss of the four treatments at the maturity.

Treatments	Cumulative DM-loss (%)	Cumulative N-loss (%)	Cumulative C-loss (%)
20:1	41.70 \pm 1.81	36.06 \pm 1.33	50.04 \pm 0.93
25:1	36.5 \pm 1.24	32.31 \pm 0.98	44.89 \pm 1.81
30:1	30.80 \pm 0.98	23.44 \pm 1.65	39.04 \pm 1.23
35:1	23.8 \pm 0.80	14.39 \pm 1.06	32.10 \pm 1.73

decreasing temperature stage and then decreased slightly, suggesting *Deinococcus-Thermus* could not tolerate the high-temperature, and *Gemmatimonadetes* may play important roles in developing the compost. Furthermore, *Actinobacteria* significantly increased in the higher C/N ratio piles, *Actinobacteria* has excellent ability to decompose complex molecules, particularly lignocellulosic components, and they can survive at high temperature during composting and generate soluble carbohydrate (Kausar et al., 2011). The boost of their presence in the high C/N piles suggested that they played active roles in developing the compost as well, and could serve as beneficial compost supplements.

At genus level (Fig. 5), at the beginning of the composting the nine in top fifteen most abundant bacteria were same across the treatments as follows, *Pseudomonas*, *Fermentimonas*, *Xanthomonadaceae_uncultured*, *Flavobacterium*, *Marinilabiliaceae_norank*, *Halomonas*, *Sphingobacteriaceae_uncultured*, *Luteimonas* and *Pusillimonas*. While *Truepera*, *Cellvibrionaceae_uncultured*, *Sphingopyxis*, *Aliidiomarina*, *Proteiniphilum* and *Saprosiraceae_uncultured* decreased gradually with the increase of C/N ratio, and *Parapedobacter*, *Cellvibrio*, *Devosia*, *Pseudoxanthomonas*, *Acinetobacter* and *Sphingobacteriaceae_uncultured* surged in the 35:1 C/N ratio piles, indicating that the addition of high amount of corn stover

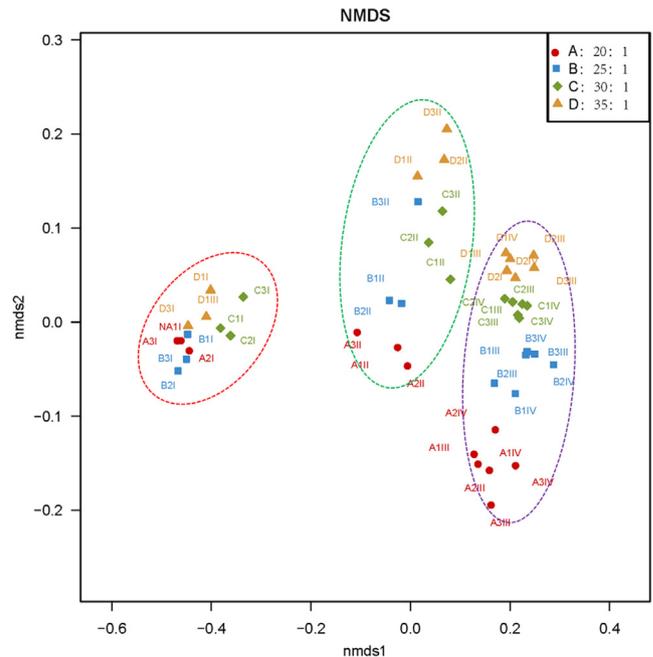


Fig. 3. The nMDS analysis of 16S rRNA gene sequencing of four treatments in different stage (Four stages: I, II, III and IV; three repeats for each measurement: 1, 2 and 3).

promoted the growth of these bacteria. *Parapedobacter* was reported to be able to hydrolyze polycyclic aromatic hydrocarbon and use pyrene as the sole carbon source for growth (Zhang

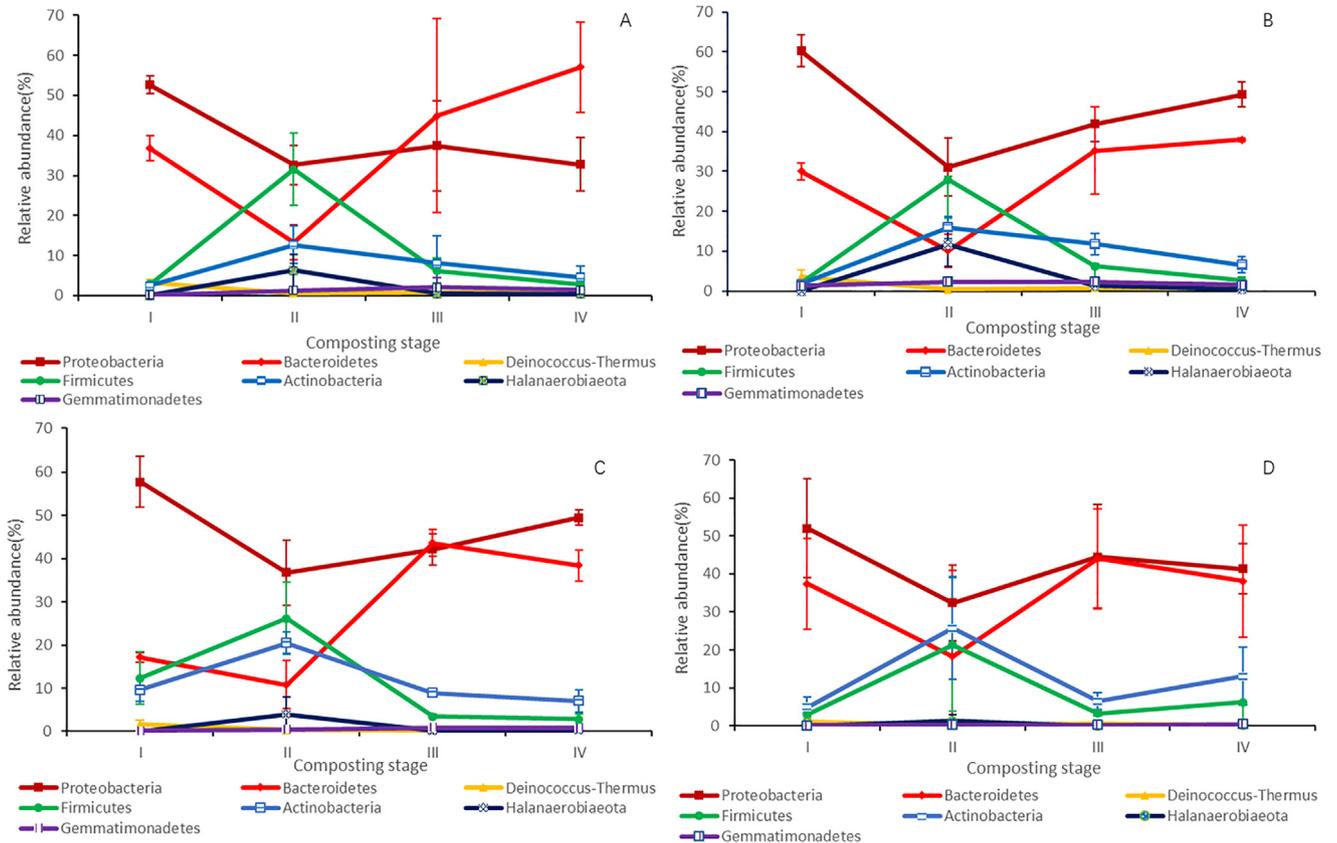


Fig. 4. Succession of the bacterial communities of the four treatments on the phylum level.

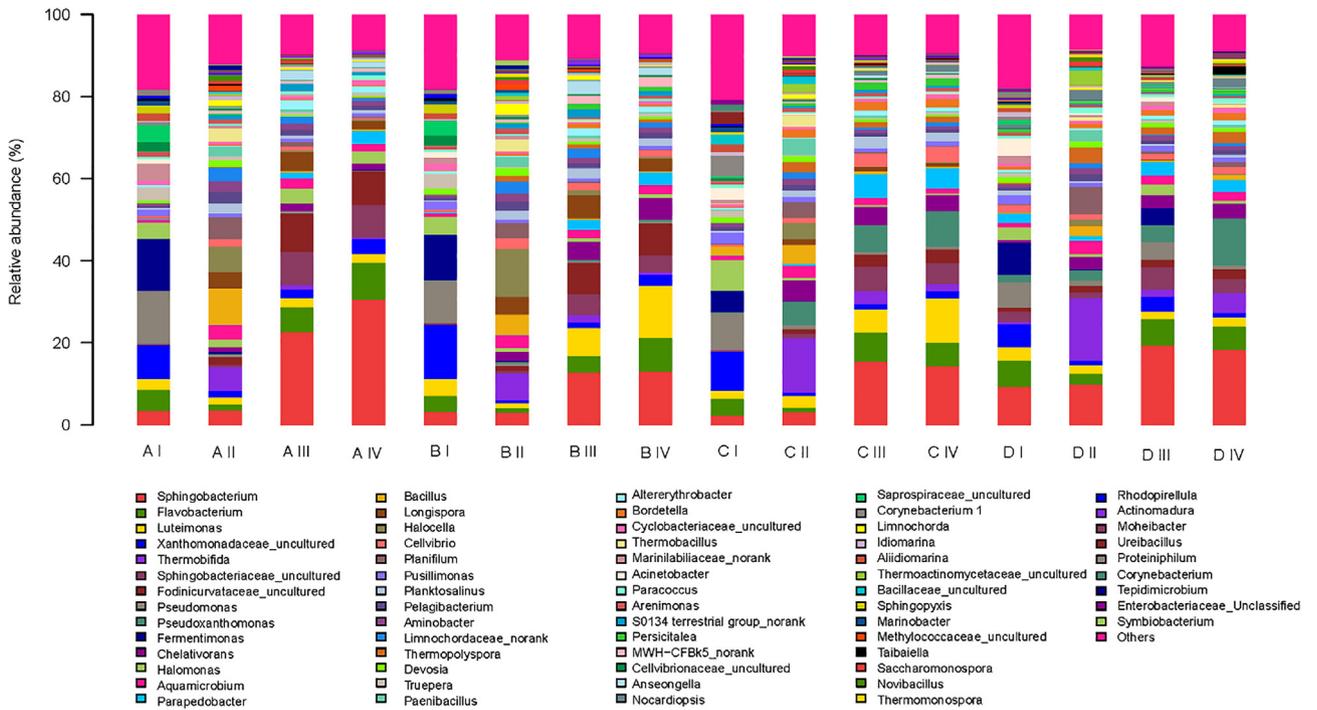


Fig. 5. Succession of the bacterial communities of the four treatments.

et al., 2015a). *Cellvibrio* was known for its ability to degrade plant cell wall (Forsberg et al., 2016). *Devosia* was reported to fix nitrogen (Rivas et al., 2002). *Pseudoxanthomonas* was capable of degrading benzenes compound and proteins which could be useful for bioremediation (Gore & Chakule, 2011). These bacteria played significant roles in the degradation of the lignocellulosic biomass (i.e., corn stover) in the composting mix, therefore high C/N ratio favored their growth and proliferation.

At the high-temperature stage, the top fifteen most abundant bacteria of the initial stage dropped rapidly to far below 1%. The top five most abundant bacteria in four treatments were *Bacillus* (8.9%), *Halocella* (6.3%), *Thermobifida* (5.8%), *Planifilum* (5.4%) and *Longispora* (3.9%) in 20:1 C/N ratio, *Halocella* (11.6%), *Thermobifida* (6.6%), *Bacillus* (5.0%), *Longispora* (4.3%) and *Planifilum* (3.8%) in 25:1 C/N ratio, *Thermobifida* (13.2%), *Pseudoxanthomonas* (5.8%), *Chelativorans* (5.2%), *Bacillus* (4.6%) and *Halocella* (3.9%) in 30:1 C/N ratio, *Thermobifida* (15.3%), *Sphingobacterium* (9.8%), *Planifilum* (6.7%), *Thermopolyspora* (3.8%) and *Thermoactinomyces_uncultured* (3.6%) in 35:1 C/N ratio, respectively. Meanwhile, *Planifilum*, *Thermobifida*, *Sphingobacterium*, *Thermopolyspora* and *Thermoactinomyces_uncultured* significantly increased with the increase of C/N ratio, suggesting that higher C/N ratio promoted the growth and reproduction of these bacteria. *Planifilum* and *Thermoactinomyces* belonged to the family of *Thermoactinomycetaceae*, which harbors thermophilic organisms with *Actinomyces*-like mycelial growth (Logan & Halket, 2011), *Sphingobacterium* has been reported to degrade organic compounds (Mohammad et al., 2006), *Thermopolyspora* was reported to degrade biomass, and can survive at high temperature during the composting (Antunes et al., 2016). The presence of these thermophilic bacteria might be the main reason that the core temperature of the 35:1 pile increased slightly faster than others (Fig. 1a).

More similarities were observed among the compositions of the bacterial communities of the decreasing-temperature stage and the mature stage, which were also affected significantly by the initial C/N ratios (Fig. 3). At the decreasing-temperature stage, the top five most abundant bacteria in four treatments were *Sphingobacterium* (22.7%), *Fodinicurvataceae_uncultured* (9.4%), *Sphingobacteriaceae_uncultured* (8.1%), *Flavobacterium* (6.0%) and *Longispora* (4.5%) in 20:1 C/N ratio, *Sphingobacterium* (12.8%), *Fodinicurvataceae_uncultured* (7.7%), *Luteimonas* (6.8%), *Longispora* (5.7%) and *Sphingobacteriaceae_uncultured* (5.1%) in 25:1 C/N ratio, *Sphingobacterium* (15.5%), *Flavobacterium* (7.0%), *Pseudoxanthomonas* (6.4%), *Sphingobacteriaceae_uncultured* (6.2%) and *Parapedobacter* (5.9%) in 30:1 C/N ratio, *Sphingobacterium* (19.4%), *Flavobacterium* (6.4%), *Sphingobacteriaceae_uncultured* (5.5%), *Pseudomonas* (4.3%) and *Fermentimonas* (4.2%) in 35:1 C/N ratio, respectively. The relative abundance of *Planifilum* and *Thermobifida* surged with the increase of C/N ratio. *Thermobifida* is a thermophilic actinomycete, which can degrade polyesters and polylactic acid, and can produce plastic-degrading cutinases (Kitadokoro et al., 2019). Compost with *Thermobifida* would be favored for certain soil remediation applications where ability to degrade plastics is important.

At the maturity stage, the top five most abundant bacteria in four treatments were *Sphingobacterium* (30.7%), *Flavobacterium* (8.9%), *Fodinicurvataceae_uncultured* (8.3%), *Sphingobacteriaceae_uncultured* (8.0%) and *Xanthomonadaceae_uncultured* (3.6%) in 20:1 C/N ratio, *Sphingobacterium* (13.3%), *Luteimonas* (12.7%), *Flavobacterium* (8.2%), *Fodinicurvataceae_uncultured* (7.8%) and *Chelativorans* (5.2%) in 25:1 C/N ratio, *Sphingobacterium* (14.3%), *Luteimonas* (10.7%), *Pseudoxanthomonas* (8.6%), *Flavobacterium* (5.9%) and *Sphingobacteriaceae_uncultured* (5.1%) in 30:1 C/N ratio, *Sphingobacterium* (18.4%), *Pseudoxanthomonas* (11.4%), *Flavobacterium* (5.6%), *Thermobifida* (4.8%) and *Chelativorans* (3.6%) in 35:1 C/N ratio, respectively. The relative abundance of

Sphingobacteriaceae_uncultured plunged while *Bordetella*, *Nocardiopsis*, *Cellvibrio* and *Thermobifida* surged with the increase of C/N ratio. *Bordetella* were positively correlated with $\text{NO}_3\text{-N}$ in cattle manure composting (Meng et al., 2019). *Nocardiopsis* could produce a variety of bioactive compounds and secrete extracellular enzymes (Bennur et al., 2015). In brief, these bacteria are all positive contributors to biomass degradation, and could play beneficial roles in soil amendment applications.

3.5. Effect of the succession of dominant bacterial communities on N-loss and C-loss

Redundancy analysis (RDA) is usually used to identify the relationships between environmental factors and bacteria community (Li et al., 2017). Fig. 6 shows an RDA to investigate the correlations between the N-loss, C-loss and the top 15 genera in each treatment group at each stage during the composting process. Significantly negative correlations were found between *Thermopolyspora* ($p < 0.001$), *Pseudoxanthomonas* ($p < 0.001$), *Nocardiopsis* ($p < 0.001$), *Bordetella* ($p < 0.01$), *Pseudomonas* ($p < 0.01$), *Thermoactinomyces_uncultured* ($p < 0.01$), *Thermobifida* ($p < 0.01$) and the N-loss, as well as C-loss. *Thermopolyspora* is well-known for its capacity to produce xylanases and thermostable hemicellulases (Zhang et al., 2015b), and eventually convert organic waste to humus (Pan et al., 2012).

Pseudoxanthomonas was capable of promoting plant growth and degrading proteins and benzene compound for bioremediation (Gore & Chakule, 2011). *Bordetella* could assimilate nitrogen (Moura et al., 2018). *Nocardiopsis* are well known for diazotrophic activity and production of indole-3-acetic acid (IAA) (Dashti et al., 2018; Swarnalakshmi et al., 2016). *Thermoactinomyces* was reported to play important roles in non-symbiotic N fixation, $\text{NO}_3\text{-N}$ reduction, plant growth and biocontrol of plant pathogens (Baraniya et al., 2016). *Thermobifida* can degrade all major plant cells and fix nitrogen (Bhatia & Sharma, 2010; Tania et al., 2018). *Pseudomonas* were reported as nitrogen fixation bacteria as well (Han et al., 2019). It is clear that the nitrogen fixation capabilities of these microbes are the main reason behind their negative correlations to N-loss. Promoting their presence in the composting mix would hence have a beneficial effect on the quality of the compost.

Meanwhile significantly positive correlations were found between *Anseongella* ($p < 0.001$), *Fodinicurvataceae_uncultured* ($p < 0.001$), *Longispora* ($p < 0.01$), *Pelagibacterium* ($p < 0.01$), *Limnochordaceae_norank* ($p < 0.01$), *S0134_terrestrial_group_norank* ($p < 0.01$), *Limnochorda* ($p < 0.05$), *MWH - CFBk5_norank* ($p < 0.05$), *Aminobacter* ($p < 0.05$), *Halomonas* ($p < 0.05$), *Halocella* ($p < 0.05$), *Fermentimonas* ($p < 0.05$) and the N-loss, as well as C-loss. *Halomonas* was reported to have denitrification activity (Guo et al., 2013). *Pelagibacterium* have been reported to be highly halotolerant (Preena et al., 2017). *Aminobacter* was reported to have both denitrification and denitrification activities (Kostytsia et al., 2018). *Fermentimonas* were identified as protein-degrading bacteria (Bi et al., 2020). Their abilities for nitrogen removal could negatively affect the quality of the compost.

Nonetheless, as shown in Table S1, *Thermopolyspora*, *Pseudoxanthomonas*, *Nocardiopsis*, *Bordetella*, *Pseudomonas*, *Thermoactinomyces_uncultured* and *Thermobifida* were all beneficial to nitrogen and carbon fixing and their relative abundance increased with the C/N ratio, while *Anseongella*, *Fodinicurvataceae_uncultured*, *Longispora*, *Pelagibacterium*, *Limnochordaceae_norank*, *S0134_terrestrial_group_norank*, *Limnochorda*, *MWH-CFBk5_norank*, *Aminobacter*, *Halomonas*, *Halocella*, *Fermentimonas* were all adverse to nitrogen and carbon fixing, and their relative abundance decreased with the C/N ratio. Promoting presence and activities of nitrogen fixing bacteria while reducing presence

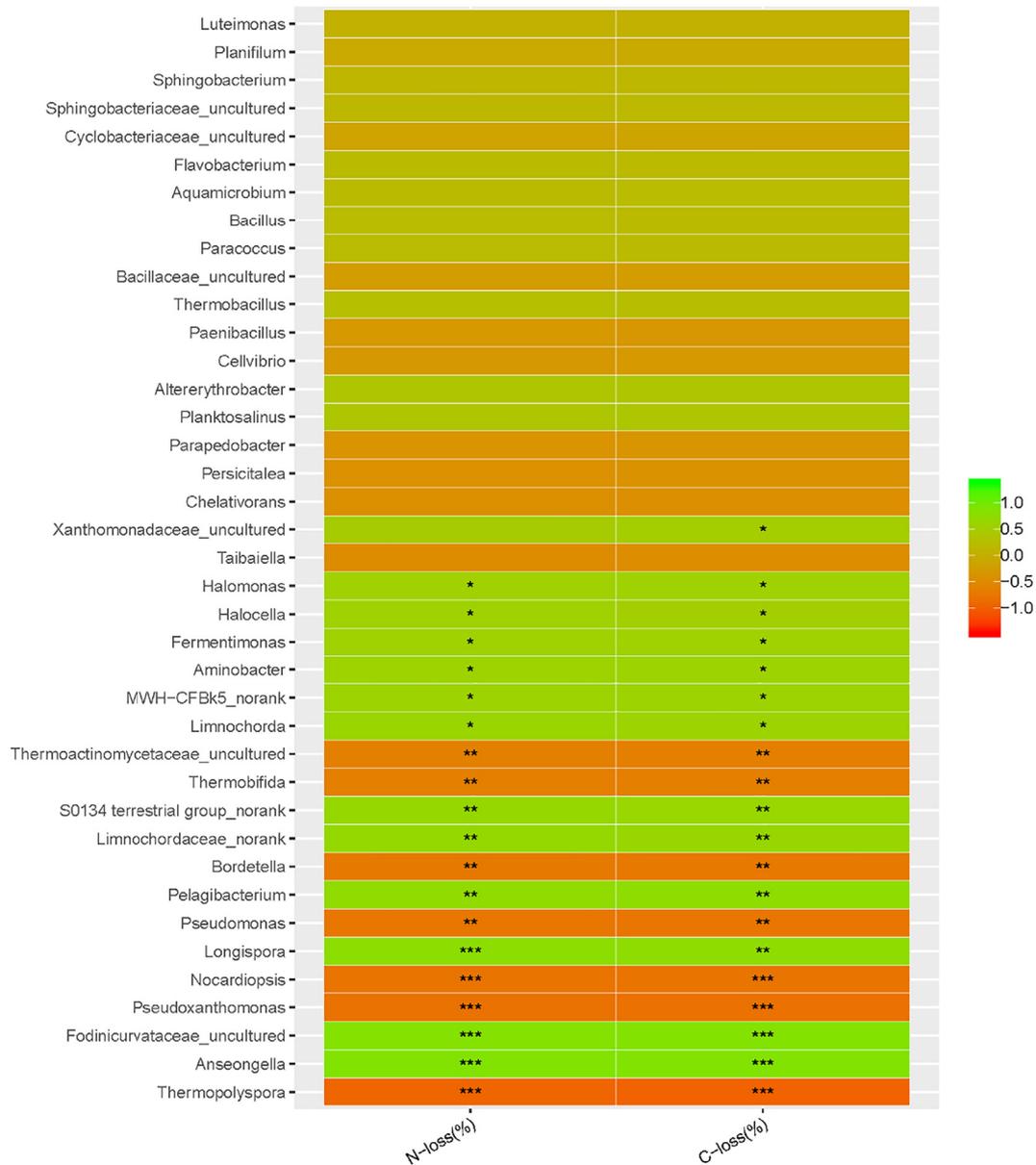


Fig. 6. Redundancy analysis (RDA) of each stage N-loss, C-loss and top 15 genera (related abundances exceed 1%) in each stage of each treatment of composting samples (***) represents for $P < 0.001$, ** represents for $P < 0.01$, * represents for $P < 0.05$).

and activities of denitrifying bacteria throughout the composting processes should be explored as means to improve quality of composting products.

4. Conclusion

In this study, the effect of initial C/N ratio on cattle mature/corn stove mix on the total losses of N and C and succession of dominant bacteria were analyzed. The results showed that the cumulative C-loss and N-loss were affected by the initial C/N ratio, and the least of C-loss and N-loss was observed for 35:1 C/N ratio. Microbiota analysis during the composting process indicated that the makeup of the bacterial communities experienced significant change throughout the different stages of the composting process, and the dominant species could differ greatly due to the differences in the initial C/N ratio. Redundancy analysis showed that appropriate selection of higher C/N ratio could promote the relative abundance of nitrogen fixing bacteria while reduce the relative abundance of denitrifying bacteria, which could lead to reduction

in N- and C-losses. These results also suggested that beneficial bacteria could be screened and selected from compost that could be used as composting supplements to improve the quality of the compost.

Declaration of Competing Interest

The authors claim no conflict of interest.

Acknowledgements

This work was supported by the Natural Science Fund of Gansu Province (18JR3RA180) and the Subject Fund of Gansu Agricultural University (GAU-XKJS-2018-212).

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.wasman.2020.01.006>.

References

- Antunes, L.P., Martins, L.F., Pereira, R.V., Thomas, A.M., Barbosa, D., Lemos, L.N., Silva, G.M.M., Moura, L.M.S., Epamino, G.W.C., Digiampietri, L.A., Lombardi, K.C., Ramos, P.L., Quaggio, R.B., de Oliveira, J.C.F., Pascon, R.C., da Cruz, J.B., da Silva, A. M., Setubal, J.C., 2016. Microbial community structure and dynamics in thermophilic composting viewed through metagenomics and metatranscriptomics. *Sci. Rep.* 6.
- Awasthi, M.K., Pandey, A.K., Khan, J., Bundela, P.S., Wong, J.W.C., Selvam, A., 2014. Evaluation of thermophilic fungal consortium for organic municipal solid waste composting. *Bioresour. Technol.* 168, 214–221.
- Baraniya, D., Puglisi, E., Ceccherini, M.T., Pietramellara, G., Giagnoni, L., Arenella, M., Nannipieri, P., Renella, G., 2016. Protease encoding microbial communities and protease activity of the rhizosphere and bulk soils of two maize lines with different N uptake efficiency. *Soil Biol. Biochem.* 96, 176–179.
- Bennur, T., Kumar, A.R., Zinjarde, S., Javdekar, V., 2015. Nocardiosis species: Incidence, ecological roles and adaptations. *Microbiol. Res.* 174, 33–47.
- Bernal, M.P., Albuquerque, J.A., Moral, R., 2009. Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour. Technol.* 100 (22), 5444–5453.
- Bhatia, S., Sharma, D.K., 2010. Mining of genomic databases to identify novel biodesulfurizing microorganisms. *J. Ind. Microbiol. Biotechnol.* 37 (4), 425–429.
- Bi, S., Qiao, W., Xiong, L., Mahdy, A., Wandra, S.M., Yin, D., Dong, R., 2020. Improved high solid anaerobic digestion of chicken manure by moderate in situ ammonia stripping and its relation to metabolic pathway. *Renew. Energy* 146, 2380–2389.
- Chen, L., Jiang, Y., Liang, C., Luo, Y., Xu, Q., Han, C., Zhao, Q., Sun, B., 2019a. Competitive interaction with keystone taxa induced negative priming under biochar amendments. *Microbiome* 7 (1), 77.
- Chen, X.M., Zhao, X.Y., Ge, J.P., Zhao, Y., Wei, Z.M., Yao, C.H., Meng, Q.Q., Zhao, R., 2019b. Recognition of the neutral sugars conversion induced by bacterial community during lignocellulose wastes composting. *Bioresour. Technol.* 294, 122153.
- Collins, G., Kavanagh, S., McHugh, S., Connaughton, S., Kearney, A., Rice, O., Carrigg, C., Scully, C., Bhreathnach, N., Mahony, T., Madden, P., Enright, A.M., O'Flaherty, V., 2006. Accessing the black box of microbial diversity and ecophysiology: Recent advances through polyphasic experiments. *J. Environ. Sci. Health, A: Tox. Hazard. Subst. Environ. Eng.* 41 (5), 897–922.
- Dashti, N., Ali, N., Salamah, S., Khanafer, M., Al-Shamy, G., Al-Awadhi, H., Radwan, S. S., 2018. Culture-independent analysis of hydrocarbonoclastic bacterial communities in environmental samples during oil-bioremediation. *Microbiolopen* 8, (2) e00630.
- Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27 (16), 2194–2200.
- Eghball, B., Power, J.F., Gilley, J.E., Doran, J.W., 1997. Nutrient, carbon, and mass loss during composting of beef cattle feedlot manure. *J. Environ. Qual.* 26 (1), 189–193.
- Ekinci, K., Tosun, I., Bitrak, B., Kumbul, B.S., Sevik, F., Suluk, K., 2019. Effects of initial C/N ratio on organic matter degradation of composting of rose oil processing solid wastes. *Int. J. Environ. Sci. Technol.* 16 (9), 5131–5140.
- Erickson, M.C., Liao, J., Ma, L., Jiang, X., Doyle, M., 2009. Inactivation of *Salmonella* spp. in cow manure composts formulated to different initial C: N ratios. *Bioresour. Technol.* 100 (23), 5898–5903.
- Forsberg, Z., Nelson, C.E., Dalhus, B., Mekasha, S., Loose, J.S.M., Crouch, L.I., Rohr, A.K., Gardner, J.G., Eijsink, V.G.H., Vaaje-Kolstad, G., 2016. Structural and functional analysis of a lytic polysaccharide monooxygenase important for efficient utilization of chitin in *cellulibrio japonicus*. *J. Biol. Chem.* 291 (14), 7300–7312.
- Gao, M., Liang, F., AnYu, Li, B., Yang, L., 2010. Evaluation of stability and maturity during forced-aeration composting of chicken manure and sawdust at different C/N ratios. *Chemosphere* 78 (5), 614–619.
- Gore, D., Chakule, A., 2011. Homology modeling and function prediction in uncharacterized proteins of *Pseudoxanthomonas spadix*. *Bioinform.* 1, 23–32.
- Guo, R., Li, G., Jiang, T., Schuchardt, F., Chen, T., Zhao, Y., Shen, Y., 2012. Effect of aeration rate, C/N ratio and moisture content on the stability and maturity of compost. *Bioresour. Technol.* 112, 171–178.
- Guo, Y., Zhou, X.M., Li, Y.G., Li, K., Wang, C.X., Liu, J.F., Yan, D.J., Liu, Y.L., Yang, D.H., Xing, J.M., 2013. Heterotrophic nitrification and aerobic denitrification by a novel *Halomonas campisalis*. *Biotechnol. Lett.* 35 (12), 2045–2049.
- Han, S., Li, J., Zhou, Q., Liu, G., Wang, T., 2019. Harmless disposal and resource utilization of wastes from the lake in China: dewatering, composting and safety evaluation of fertilizer. *Algal Res.* 43, 101623.
- Hao, X.Y., Chang, C., Larney, F.J., 2004. Carbon, nitrogen balances and greenhouse gas emission during cattle feedlot manure composting. *J. Environ. Qual.* 33 (1), 37–44.
- He, Y., Xie, K., Xu, P., Huang, X., Gu, W., Zhang, F., Tang, S., 2013. Evolution of microbial community diversity and enzymatic activity during composting. *Res. Microbiol.* 164 (2), 189–198.
- Huang, G.F., Wong, J.W.C., Wu, Q.T., Nagar, B.B., 2004. Effect of C/N on composting of pig manure with sawdust. *Waste Manage.* 24 (8), 805–813.
- Jain, M.S., Jambhulkar, R., Kalamdhad, A.S., 2018. Biochar amendment for batch composting of nitrogen rich organic waste: effect on degradation kinetics, composting physics and nutritional properties. *Bioresour. Technol.* 253, 204–213.
- Kausar, H., Sariah, M., Saud, H.M., Alam, M.Z., Ismail, M.R., 2011. Isolation and screening of potential actinobacteria for rapid composting of rice straw. *Biodegradation* 22 (2), 367–375.
- Kitadokoro, K., Kakara, M., Matsui, S., Osokoshi, R., Thumarat, U., Kawai, F., Kamitani, S., 2019. Structural insights into the unique polylactate-degrading mechanism of *Thermobifida alba* cutinase. *FEBS J.* 286 (11), 2087–2098.
- Kostrystia, A., Papiro, S., Morrison, L., Ijaz, U.Z., Collins, G., Lens, P.N.L., Esposito, G., 2018. Biokinetics of microbial consortia using biogenic sulfur as a novel electron donor for sustainable denitrification. *Bioresour. Technol.* 270, 359–367.
- Kumar, M., Ou, Y.-L., Lin, J.-G., 2010. Co-composting of green waste and food waste at low C/N ratio. *Waste Manage.* 30 (4), 602–609.
- Kumar, S., 2011. Composting of municipal solid waste. *Crit. Rev. Microbiol.* 31 (2), 112–136.
- Larney, F.J., Sullivan, D.M., Buckley, K.E., Eghball, B., 2006. The role of composting in recycling manure nutrients. *Can. J. Soil Sci.* 86 (4), 597–611.
- Li, H., Duan, M., Gu, J., Zhang, Y., Qian, X., Ma, J., Zhang, R., Wang, X., 2017. Effects of bamboo charcoal on antibiotic resistance genes during chicken manure composting. *Ecotoxicol. Environ. Saf.* 140, 1–6.
- Li, R., Wang, J.J., Zhang, Z., Shen, F., Zhang, G., Qin, R., Li, X., Xiao, R., 2012. Nutrient transformations during composting of pig manure with bentonite. *Bioresour. Technol.* 121, 362–368.
- Lim, S., Park, H., Hao, X., Lee, S., Jeon, B., Kwak, J., Choi, W., 2017. Nitrogen, carbon, and dry matter losses during composting of livestock manure with two bulking agents as affected by co-amendments of phosphogypsum and zeolite. *Ecol. Eng.* 102, 280–290.
- Liu, J., Zhang, X., Wang, H., Hui, X., Wang, Z., Qiu, W., 2018. Long-term nitrogen fertilization impacts soil fungal and bacterial community structure in a dryland soil of Loess Plateau in China. *J. Soil. Sediment.* 18 (4), 1632–1640.
- Liu, W., Wang, S., Zhang, J., Xu, T., 2014. Biochar influences the microbial community structure during tomato stalk composting with chicken manure. *Bioresour. Technol.* 154, 148–154.
- Logan, N.A., Halket, G., 2011. Developments in the taxonomy of aerobic, endospore-forming soil bacteria. In: Logan, N.A., Vos, P. (Eds.), *Soil Biology*. Springer, Berlin, Heidelberg, pp. 1–29.
- Mao, H., Lv, Z., Sun, H., Li, R., Zhai, B., Wang, Z., Awasthi, M.K., Wang, Q., Zhou, L., 2018. Improvement of biochar and bacterial powder addition on gaseous emission and bacterial community in pig manure compost. *Bioresour. Technol.* 258, 195–202.
- Meng, Q.X., Wang, W., Men, M.Q., Bello, A., Xu, X.H., Xu, B.S., Deng, L.T., Jiang, X., Sheng, S.Y., Wu, X.T., Han, Y., Zhu, H.F., 2019. Microbial community succession and response to environmental variables during cow manure and corn straw composting. *Front Microbiol.*, 10
- Meng, X., Liu, B., Xi, C., Luo, X., Yuan, X., Wang, X., Zhu, W., Wang, H., Cui, Z., 2018. Effect of pig manure on the chemical composition and microbial diversity during co-composting with spent mushroom substrate and rice husks. *Bioresour. Technol.* 251, 22–30.
- Mohammad, B.T., Wright, P.C., Bustard, M.T., 2006. Bioconversion of isopropanol by a solvent tolerant *Sphingobacterium mizutae* strain. *J. Ind. Microbiol. Biotechnol.* 33 (12), 975–983.
- Moura, R.B., Santos, C.E.D., Okada, D.Y., Martins, T.H., Ferraz, A.D.N., Damianovic, M. H.R.Z., Foresti, E., 2018. Carbon-nitrogen removal in a structured-bed reactor (SBRRA) treating sewage: operating conditions and metabolic perspectives. *J. Environ. Manage.* 224, 19–28.
- Ogunwande, G., Osunade, J., Adekalu, K., Ogunjimi, L.A.O., 2008. Nitrogen loss in chicken litter compost as affected by carbon to nitrogen ratio and turning frequency. *Bioresour. Technol.* 99 (16), 7495–7503.
- Pan, I., Dam, B., Sen, S.K., 2012. Composting of common organic wastes using microbial inoculants. *3 Biotech* 2 (2), 127–134.
- Preena, P.G., Manju, N.J., Deepesh, V., Thomas, A., Singh, I.S.B., 2017. Genetic diversity of nitrate reducing bacteria in marine and brackish water nitrifying bacterial consortia generated for activating nitrifying bioreactors in recirculating aquaculture systems. *Aquac. Res.* 48 (12), 5729–5740.
- Price, L.B., Liu, C.M., Melendez, J.H., Frankel, Y.M., Engelthaler, D., Aziz, M., Bowers, J., Rattray, R., Ravel, J., Kingsley, C., Keim, P.S., Lazarus, G.S., Zenilman, J.M., 2009. Community analysis of chronic wound bacteria using 16S rRNA gene-based pyrosequencing: impact of diabetes and antibiotics on chronic wound microbiota. *PLoS One* 4, (7) e6462.
- Qiao, C.C., Penton, C.R., Liu, C., Shen, Z.Z., Ou, Y.N., Liu, Z.Y., Xu, X., Li, R., Shen, Q.R., 2019. Key extracellular enzymes triggered high-efficiency composting associated with bacterial community succession. *Bioresour. Technol.* 288.
- Ren, G., Xu, X., Qu, J., Zhu, L., Wang, T., 2016. Evaluation of microbial population dynamics in the co-composting of cow manure and rice straw using high throughput sequencing analysis. *World J. Microbiol. Biotechnol.* 32 (6), 101.
- Ren, L.M., Schuchardt, F., Shen, Y.J., Li, G.X., Li, C.P., 2010. Impact of struvite crystallization on nitrogen losses during composting of pig manure and cornstalk. *Waste Manag.* 30 (5), 885–892.
- Ren, X.N., Wang, Q., Awasthi, M.K., Zhao, J.C., Wang, J.C., Liu, T., Li, R.H., Zhang, Z.Q., 2019. Improvement of cleaner composting production by adding diatomite: from the nitrogen conservation and greenhouse gas emission. *Bioresour. Technol.* 286.
- Rivas, R., Velazquez, E., Willems, A., Vizcaino, N., Subba-Rao, N.S., Mateos, P.F., Gillis, M., Dazzo, F.B., Martinez-Molina, E., 2002. A new species of *Devosia* that forms a unique nitrogen-fixing root-nodule symbiosis with the aquatic legume *Neptunia natans* (L.f.) Druce. *Appl. Environ. Microbiol.* 68 (11), 5217–5222.

- Si, B.C., Liu, Z.D., Zhang, Y.H., Li, J.M., Xing, X.H., Li, B.M., Duan, N., Lu, H.F., 2015. Effect of reaction mode on biohydrogen production and its microbial diversity. *Int. J. Hydrog. Energy* 40 (8), 3191–3200.
- Sun, Y., Men, M., Xu, B., Meng, Q., Bello, A., Xu, X., Huang, X., 2019. Assessing key microbial communities determining nitrogen transformation in composting of cow manure using illumina high-throughput sequencing. *Waste Manag.* 92, 59–67.
- Swarnalakshmi, K., Senthilkumar, M., Ramakrishnan, B., 2016. Endophytic actinobacteria: nitrogen fixation, phytohormone production, and antibiosis. In: *Plant Growth Promoting Actinobacteria*. Springer, pp. 123–145.
- Goyal, Tania, M., Baranwal, M., 2018. In silico approach to analyze the biochemical pathways of bacterial metabolite synthesis. Springer Singapore, Singapore, pp. 141–159.
- Wong, J.W.C., Mak, K.F., Chan, N.W., Lam, A., Fang, M., Zhou, L.X., Wu, Q.T., Liao, X.D., 2001. Co-composting of soybean residues and leaves in Hong Kong. *Bioresour. Technol.* 76 (2), 99–106.
- Yang, X.T., Song, Z., Zhou, S.H., Guo, H., Geng, B., Peng, X.W., Zhao, G.Z., Xie, Y.J., 2019. Insights into functional microbial succession during nitrogen transformation in an ectopic fermentation system. *Bioresour. Technol.* 284, 266–275.
- Yin, Y.A., Gu, J., Wang, X.J., Zhang, Y.J., Zheng, W., Chen, R., Wang, X.C., 2019. Effects of rhamnolipid and Tween-80 on cellulase activities and metabolic functions of the bacterial community during chicken manure composting. *Bioresour. Technol.* 288, 121507.
- Zhang, J., Cai, X., Qi, L., Shao, C., Lin, Y., Zhang, J., Zhang, Y., Shen, P., Wei, Y., 2015a. Effects of aeration strategy on the evolution of dissolved organic matter (DOM) and microbial community structure during sludge bio-drying. *Appl. Microbiol. Biotechnol.* 99 (17), 7321–7331.
- 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.
- Zhang, L.L., Ma, H.X., Zhang, H.Q., Xun, L.Y., Chen, G.J., Wang, L.S., 2015b. *Thermomyces lanuginosus* is the dominant fungus in maize straw composts. *Bioresour. Technol.* 197, 266–275.
- Zhong, X., Ma, S., Wang, S., Wang, T., Sun, Z., Tang, Y., Deng, Y., Kida, K., 2018. A comparative study of composting the solid fraction of dairy manure with or without bulking material: performance and microbial community dynamics. *Bioresour. Technol.* 247, 443–452.
- Zucconi, F., Pera, A., Forte, M., Debertoldi, M., 1981. Evaluating toxicity of immature compost. *Biocycle* 22 (2), 54–57.