Journal of Soil Science and Plant Nutrition (2022) 22:4587–4597
https://doi.org/10.1007/s42729-022-00928-3

ORIGINAL PAPER

Succession of Bacterial Community Function in a Continuous Composting System with Spent Mushroom Substrate and Sawdust as Bulking Agents

Qianqian Chen[1] · Bo Liu[1] · Jianmei Che[1] · Guohong Liu[1] · Huai Shi · Jieping Wang[1] · Lulong Zhong[2]

Received: 17 February 2022 / Accepted: 5 July 2022 / Published online: 31 August 2022

Abstract
Composting is a reliable way for manure treatment and nutrient recycle, but the accumulation of pig manures is not effectively treated with the limited available lands. In this study, a continuous composting system was designed to increasing the treatment capacity by adding pig manure three times at the beginning of each composting cycle. The aim of the present study was to assess the composting parameters and bacterial community structure in this system. Physicochemical factors, bacterial community, and functional prediction of each composting cycles were evaluated. The result indicated that content of total nitrogen and NH_4^+-N increased, while the amount of carbon, cellulose, and hemicellulose decreased during the composting process. At the same time, the amount of lignin increased firstly, and then decrease to 17.3% after the last composting cycle. Furthermore, bacterial community structures were different among the three composting cycles. The correlation heatmap between environment factors and microbial community indicated that C/N ratio and pH were the main factors affecting the community structure. Additionally, bacteria participated in nitrogen cycles were found in the composting materials. *Paracoccus denitrificans*, *Jonesia denitrificans*, and *Geobacillus thermodenitrificans* were the main denitrifiers, and became the most abundance after the second composting cycle. The numbers of KEGG Orthology (KO) associated with ammonification, nitrification, and denitrification increased after the composting. The present study illustrated that more composting cycles might reduce the ability of nitrogen assimilation, but increases the degradation of lignin, cellulose, and hemicellulose in continuous composting system.

Keywords Agricultural waste · Bacterial community · Continuous composting system · Functional KOs · Functional prediction · Solid manure

1 Introduction

The intensive swine industry for human food purposes produces a considerable amount of pig manures (Zhang et al. 2021). However, the utilization of pig manures was limited by their transport and the available lands (Wang et al. 2018). Inefficient application of manure had shown negative impacts on environment, including agricultural soil pollution, aquatic eutrophication, and ozone depletion (Pardo et al. 2017; Zahraet al. 2021). Manure treatment became an important issue associated with environmental quality, human health, and resource recycle (Pandey and Chen 2021). Furthermore, the direct application of pig manures as fertilizers on the lands is also limited under strict regulatory supervision (Varma et al. 2021). Therefore, the considerable amount of manures must be treated efficiently and effectively for addressing the urgent concerns of pig raising wastes.

Composting is the most common strategy for minimizing the negative impact of manure dispose, and recycling organic wastes into fertilizers economically (Costa and Akdeniz 2019; Bernal et al. 2017). The process of composting entails a series of complex chemical and microbiological transformations (Cáceres et al. 2018; Sánchez et al. 2017). Microbial activities are the critical drivers in the composting process, which can improve the mineralization and

✉ Bo Liu
fzliubo@163.com

[1] Agricultural Bio-Resource Research Institute, Fujian Academy of Agriculture Sciences, Fuzhou 350003, China

[2] Ningde Institute of Agricultural Sciences, Ningde 352000, China

humification of organics (Onwosi et al. 2017). In the composting system, agricultural residuals, for instance spent mushroom substrates, rice husks, straws, and sawdusts, are used as the bulking agent for composting. It was reported that adding of bulking agents increases germination index and humic acid of the final compost, and also reduces nitrogen loss, NH_3, and N_2O emissions (Jolanun and Towprayoon 2010; Li et al. 2018). However, the climatic and seasonal changes impact their costs and yield levels. Furthermore, the storage and transportation of biomass produced in agriculture also increase greatly the composting operation costs. Therefore, a continuous composting system was developed to overcome these problems by reducing the consumption of bulking agents and extending their service life.

In the traditional composting system, the organic material was mixed wholly for a period of composting, without extra manure addition during the whole composting process. However, the continuous composting was carried out with adding manure twice on the 30th and 60th day, respectively. At the end of a composting period of 30 days, fresh solid pig manure (SPM) was added for the next cycle. Finally, the composting residues could be used as semi-finished fertilizer. More importantly, the continuous composting was designed as an effective approach to deal with PM and reduce operation costs. It is confirmed by previous studies that the C/N ratio, pH, moisture, and bulking agents influence the composting process (Zhou et al. 2014; Akdeniz 2019; Li et al. 2021). Therefore, the changes of physicochemical properties and enzymatic activities during the continuous composting need to be further investigated to optimize the composting conditions. Nitrogen is an essential component for organisms, and NH_4^+-N and NO_3^--N can be easily taken and assimilated as fertilizer by plants (Canfield et al. 2010; Kuypers et al. 2018). Meanwhile, nitrogen is a key factor influencing microbial communities (Cáceres et al. 2018). Therefore, it is necessary to reveal the profiles of nitrogen content and their effects on the microbial succession during the composting processes.

The main objective of this study was to evaluate the response of the physicochemical factors and succession of bacterial communities to the continuous composting process. Therefore, the physicochemical factors including of carbon, nitrogen contents, and enzyme activities were analyzed. The succession of bacterial community in continuous composting system was characterized by MiSeq sequencing. In addition, the impact of environmental factors on bacterial community structure was also investigated. Furthermore, to identify the related microbes and their function in nitrogen cycle, KEGG Orthology (KO) associated with ammonification, nitrification, and denitrification was analyzed by PICRUSt predicting.

2 Materials and Methods

2.1 Composting Materials

The composting system is located in a farm at Jiaocheng District, Fujian Province, China. The width, depth, and length of composting tanks were 4, 1.2, and 20 m, respectively. Spent mushroom substrates and sawdust (SMSS) were mixed at a 1:1 ratio (v/v) to be used as composting bulking agents. The characteristics of substrates used in composting construction are shown in Table 1. The solid fraction of pig manure (PM) was collected by a solid–liquid separation technology. Finally, the humidity of solid pig manure (SPM) was maintained at 54.5%. As the bulking agents, the SMSS was mixed with SPM. The final humidity and pH were 52.5% and 6.8, respectively. The separated liquid waste was fermented for agricultural irrigation.

2.2 Composting Experiment Designs and Sampling

The composting experiment was carried out by three composting cycle of 30 days (d), generating a 90-day process. The new material was added at the beginning of the second and third composting cycles, respectively. After the first composting cycle, the solid pig manure (SPM) was mixed with spent mushroom substrates and sawdust (SMSS) for static composting. After 30 days, SPM was added undergoing the second composting cycle. After another 30 days, the third composting cycle was operated by added SPM as the abovementioned manner. In each composting cycle, the composting mixture was turned every 3 days in the first 2 weeks and then turned every 5 weeks thereafter. The air was blown through fans (0.5–2 $m^3 \cdot h^{-1}$) by longitudinal ventilation when the ambient temperature was over 35 ℃. SMSS was added to the composting materials when the humidity was over 70%. Five hundred grams of materials from surface layer (i.e., 20 cm) and deep layer (50 cm) was collected at

Table 1 Characteristics of physicochemical characteristics of substrates used in composting construction (dry-weight based)

Sample	pH	Moisture (%)	Total C ($g \cdot kg^{-1}$)	Total N ($g \cdot kg^{-1}$)	Total P ($g \cdot kg^{-1}$)	C/N
Solid pig manure	6.27 ± 0.08	54.5 ± 1.24	104.9 ± 3.02	11.0 ± 0.39	5.58 ± 0.47	9.54 ± 1.01
Mushroom residue and sawdust	6.97 ± 0.06	17.6 ± 0.29	509.7 ± 5.76	2.33 ± 0.21	2.60 ± 0.32	218.8 ± 4.27

the end of each cycle, and named as SFC (surface materials of the first composting cycle), DFC (deep materials of the first composting cycle), SSC (surface materials of the second composting cycle), DSC (deep materials of the second composting cycle), STC (surface materials of the third composting cycle), and DTC (deep materials of the third composting cycle), respectively.

2.3 Physicochemical Characteristics Analyses of the Composting Materials

For all samples, the basic parameters such as pH, total nitrogen (TN), and carbon (TC) content were determined as reported previously (Chen et al. 2021). The pH was measured in a 1:10 water soluble extract (weight:weight, w:w) obtained after shaking the fresh samples in water suspension. TN and TC were determined by Kjeldahl and combustion methods, respectively. The hemicellulose, cellulose, and lignin contents were measured according to previously method (Van Soest et al. 1991). Additionally, the concentrations of ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were measured by the colorimetric method, with pretreatment of KCL (Liu et al. 2019). Protease and urease activities of the all composting materials were also measured, respectively. Protease activity was determined by measuring the amount of the released amino acids after reaction with casein for 10 min at 40 ℃, based on the method of Ladd and Butler (1972). The content of NH_3-N produced by urease hydrolysis of urea was by the method of Kandeler (1996). The reaction was performed under the conditions of 37 ℃ for 24 h, using Comin UE Kit (Suzhou, China).

2.4 DNA Extraction and Sequencing

Microbial community genomic DNA (gDNA) was extracted from 100 mg of each composting sample using the FastDNA™ PowerSoil kit (MoBio, CA). After concentrated and purified, the gDNA was used to amplify the V3–V4 regions of 16S rDNA by PCR to analyze bacterial community. The PCR was carried out by using the TruSeq® DNA Sample Pre kit according to the manufacturer's instructions (www.illumina.com). Sequencing was performed using the Illumina Miseq at Majorbio, Inc., Shanghai, China. Paired-end reads from the original DNA fragments were merged by FLASH (V1.2.11) to get raw tags, and then qualified by QIIME (V1.9.1). All the raw datasets had been deposited into NCBI's Sequence Read Achieve (accession number: SRS7139815-SRS71398649).

2.5 Bioinformatics Analysis

Sequences with ≥ 97% similarity were assigned to the same operational taxonomic units (OTUs) by Uparse (Uparse v7.0.1090). Taxonomic information for each representative sequence of each OTU was annotated by the RDP classifier (Version 2.11). The taxon abundance of each sample was generated into the phylum, class, order, family, and genera levels (Ma et al. 2016). All of the analyses from clustering to alpha (within sample) and beta diversity (between samples) were performed with Mothur (Version 1.30.2). The correlations between enzyme activities and N content were conducted in SPSS 16.0 with Pearson correlation coefficient. The differences of microbial community in the three composting cycles were analyzed by Kruskal–Wallis *H* test. The correlation heatmap of environmental parameters and OTUs was performed by R pheatmap package (Version 3.3.1). Taxonomic information of each sample was treated by PICRUSt for functional prediction (Douglas et al. 2018). The functional KOs and bacteria were extracted to calculate the relative contents of functional groups in nitrogen transformation.

3 Results

3.1 Physicochemical Properties of the Composting Materials

Physicochemical properties of the compost samples are shown in Table 2. The carbon concentration in the deep layers remained constant along the whole composting

Table 2 Characteristics of physicochemical properties of composting materials (dry-weight based)

Sample	TN (%)	TC (%)	pH	C/N	NH_4^+-N (mg·g^{-1})	NO_3^--N (mg·g^{-1})	Lignin (%)	Cellulose (%)
SFC	2.34 ± 0.18c	47.08 ± 0.52c	7.05 ± 0.11c	20.12 ± 0.21b	3.13 ± 0.15d	1.26 ± 0.08c	10.99 ± 0.61c	22.41 ± 1.52a
DFC	2.21 ± 0.10d	48.09 ± 0.36b	6.94 ± 0.16d	21.76 ± 0.29a	4.09 ± 0.08c	1.21 ± 0.09c	7.20 ± 0.59d	19.46 ± 1.35b
SSC	3.86 ± 0.21a	50.84 ± 0.27a	7.04 ± 0.08c	13.176 ± 0.38d	3.62 ± 0.10c	1.30 ± 0.07b	25.58 ± 1.72a	11.53 ± 0.68d
DSC	3.24 ± 0.14b	47.31 ± 0.28	8.30 ± 0.17a	14.605 ± 0.57c	7.07 ± 0.21a	1.86 ± 0.07a	15.44 ± 0.75	15.25 ± 1.06c
STC	3.20 ± 0.16b	44.12 ± 0.41d	7.72 ± 0.11b	13.79 ± 0.26c	5.59 ± 0.18b	1.05 ± 0.05d	19.62 ± 1.32b	12.74 ± 0.90d
DTC	3.25 ± 0.29b	47.55 ± 0.20b	7.46 ± 0.05b	14.633 ± 0.33c	6.01 ± 0.19b	1.16 ± 0.06c	17.31 ± 0.89b	14.55 ± 1.26c

TN, total nitrogen; *TC*, total carbon; *SFC*, surface layer of first cycle; *DFC*, deep layer of first cycle; *SSC*, surface layer of second cycle; *DSC*, deep layer of second cycle; *STC*, surface layer of third cycle; *DTC*, deep layer of third cycle

processes. In the surface layers, carbon contents increased slightly to 50.8% after the second cycle, and then decreased to 44.1% after the third cycle. The amount of lignin increased from 7.2 to 25.6% after the second composting period, and decreased to 17.3% after the third cycle. As the soluble carbon was consumed, the lignin content increased. After the second composting, contents of cellulose rapidly decreased from 22.4 to 11.5%, and hemicellulose content decreased from 33.7 to 18.0%. After then, their contents remained constant after the third composting. The highest total nitrogen (TN) concentration was found in the surface layer of the second composting cycle. At the end of the second composting cycle, the relative contents of nitrogen and carbon were the highest in the surface layer among all samples. The C/N ratios decreased rapidly after the second cycle till 13.1, and remained stable at 13.8 after the third cycle. During the composting cycles, the C/N values decreased from 21.9 to 13.8, which represented satisfactory maturation. Compared with composting materials after the first composting cycle, the adding of solid pig manure (SPM) significantly increased the concentrations of total nitrogen (TN) in the materials from the second composting cycle. However, little increasing of TN was observed after the third composting cycle. Along the three composting cycles, the NH_4^+-N concentrations in the composting materials increased from 3.13 to 7.07 $mg \cdot g^{-1}$, and the deep layers had higher concentration than the surface layers. The NO_3^--N concentrations increased to the peak after the second composting cycle (1.85 $mg \cdot g^{-1}$) and then decreased after the third composting cycle. Meanwhile, the pH value of the all composting materials increased from 7.1 to 8.3. The correlation analysis revealed that the NO_3^--N concentration was negative with the pH value (Fig. 1).

3.2 Relationship of Protease, Urease Activities, and N Content

The highest protease activities were observed after the first composting cycle, and then declined to 0.6 $U \cdot g^{-1}$ through the other composting cycles. The urease activities remained at approximately 3.0 $U \cdot g^{-1}$, and increased slightly till 3.3 $U \cdot g^{-1}$ along the composting process. The relation between protease and urease activities and N content of the composting samples are shown in Fig. 1. Protease had positive relationships with nitrate concentration and moisture, while urease had positive relationships with pH, nitrogen, and ammonium concentrations.

3.3 Microbial Diversity

3.3.1 Taxonomic Composition

Numbers of microorganisms entail material transformations in composting. In the continuous composting system, a total of 2361 bacterial OTUs were found, including 36 phyla. The indices including Sobs, Shannon, and Simpson indicated the bacterial richness and diversity decreased along the composting process (Table 3).

Bacterial diversity analysis found the most abundant taxa throughout 36 phyla were *Firmicutes*, *Chloroflexi*, *Actinobacteriota*, *Proteobacteria*, *Bacteroidota*, and *Gemmatimonadota* (Fig. 2). *Firmicutes*, *Chloroflexi*, *Proteobacteria*, and *Actinobacteriota* contributed more than 80% of all identified sequences. *Firmicutes* was the most dominant in the surface layers, with the relative abundance of 60.1%, 44.1%, and 50.9% after the first, second, and third composting cycles,

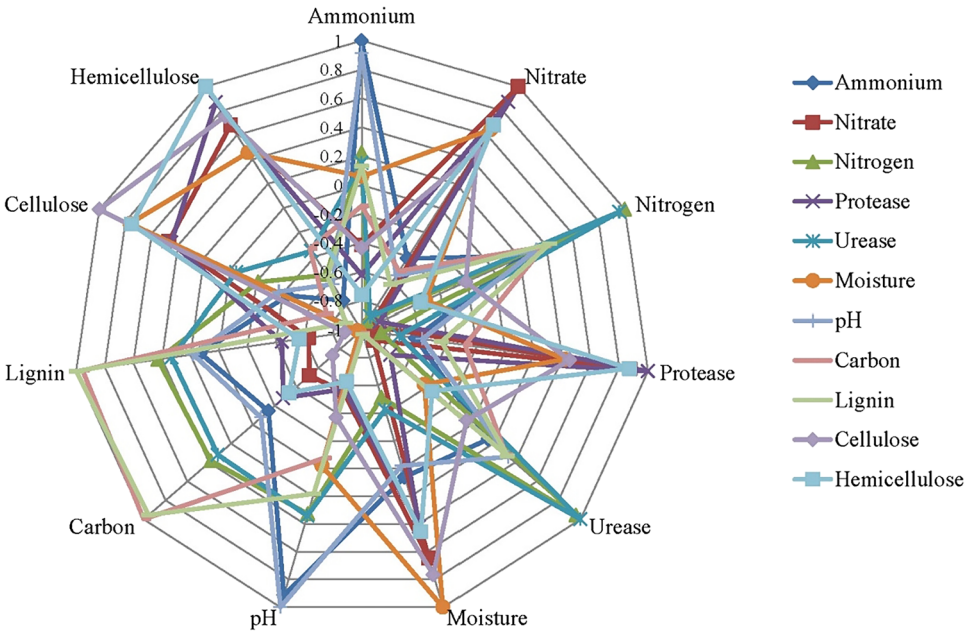

Fig. 1 The relationships between activities of protease, urease, and composting parameters of the composting samples

Table 3 The values of biodiversity indices along the composting cycles

Sample	Shannon	Simpson	Ace	Chao	Coverage
SFC	4.75 ± 0.18a	0.03 ± 0.01d	1476.92 ± 110.5a	1477.23 ± 118.5a	0.994 ± 0.0d
DFC	4.21 ± 0.32c	0.05 ± 0.01b	1116.59 ± 197.4b	1092.42 ± 182.1c	0.995 ± 0.0c
SSC	4.38 ± 0.15b	0.05 ± 0.01b	1119.83 ± 43.4b	1143.16 ± 67.4b	0.995 ± 0.0c
DSC	4.31 ± 0.41b	0.04 ± 0.01c	879.57 ± 179.5d	875.29 ± 165.7d	0.997 ± 0.0a
STC	4.30 ± 0.07b	0.05 ± 0.0b	1070.01 ± 24.6c	1095.16 ± 57.87c	0.995 ± 0.0c
DTC	3.505 ± 0.18d	0.12 ± 0.02a	848.49 ± 11.7d	859.52 ± 26.4d	0.996 ± 0.0b

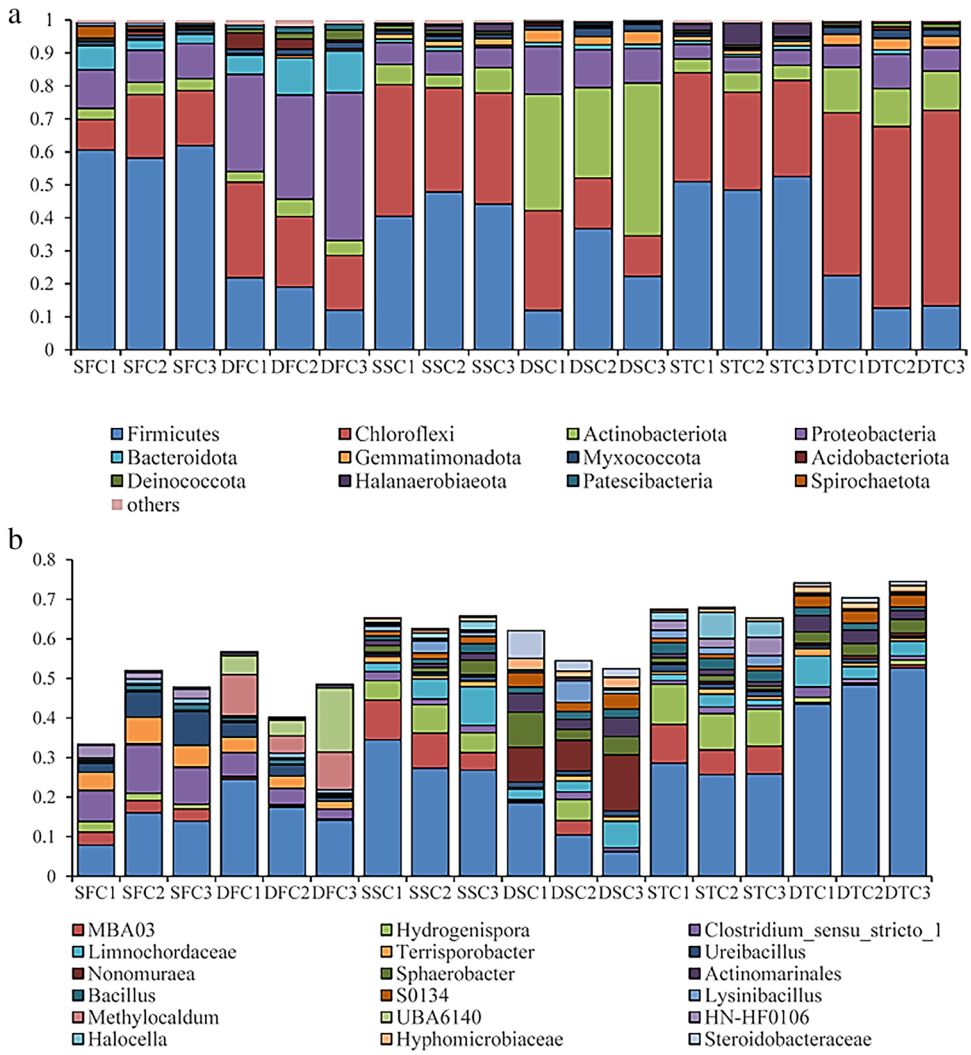

Fig. 2 Relative abundances of predominant bacterial compositions at the phylum (**a**) and genus (**b**) levels. SFC, surface materials of the first composting cycle; DFC, deep materials of the first composting cycle; SSC, surface materials of the second composting cycle; DSC, deep materials of the second composting cycle; STC, surface materials of the third composting cycle; DTC, deep materials of the third composting cycle

respectively. However, the deep layers from the three composting cycles had different dominant phyla. *Proteobacteria*, *Actinobacteriota*, and *Chloroflexi* were the main bacteria after the first, second, and third composting cycles, respectively. A genus of o_SBR1031 was the most dominant genus in the composting materials, and its relative content increased from 12.6% after the first composting cycle to 48.1% after the third cycle. In the surface layers, the genera of o_MBA03, *Hydrogenispora* and *Clostridium* sensu stricto had high abundance. The relative contents of g_o_MBA03 and *Hydrogenispora* increased along the composting process, while the contents of *C.* sensu stricto decreased. In the deep layers, the high abundance genera were *Methylocaldum* (7.7%) and UBA6140 (7.6%) after the first composting cycle and *Nonomuraea* (10.4%) and *Sphaerobacter* (5.3%) after the second composting cycle.

3.3.2 The Significant Difference of Microbial Community During Composting Process

In the phylum level, the relative contents of *Chloroflexi*, *Proteobacteria*, *Actinobacteriota*, *Bacteroidota*,

Gemmatimonadota, *Myxococcota*, *Halanaerobiaeota*, and *Bdellovibrionota* had significant correlations with the composting cycles (Fig. 3). The relative content of the phylum *Chloroflexi* increased from 18.7% after the first composting cycle to 42.6% in third composting cycle. In the composting, the relative content of the phylum *Proteobacteria* significantly decreased from 23.0 to 6.30%, and that of the phylum *Bacteroidota* reduced by 88.2% along the composting process.

The bacterial genera were also compared among the three composting cycles. The contents of *Clostridium* sensu stricto, *Terrisporobacter*, and *Ureibacillus* in phylum *Firmicutes* reduced by 80.6%, 74.2%, and 73.2%, respectively. However, the contents of g_*SBR1031* and *Sphaerobacter* in the phylum *Chloroflexi* showed an increasing trend, which increased from 15.7 and 0.1 to 37.5% and 2.2%, respectively.

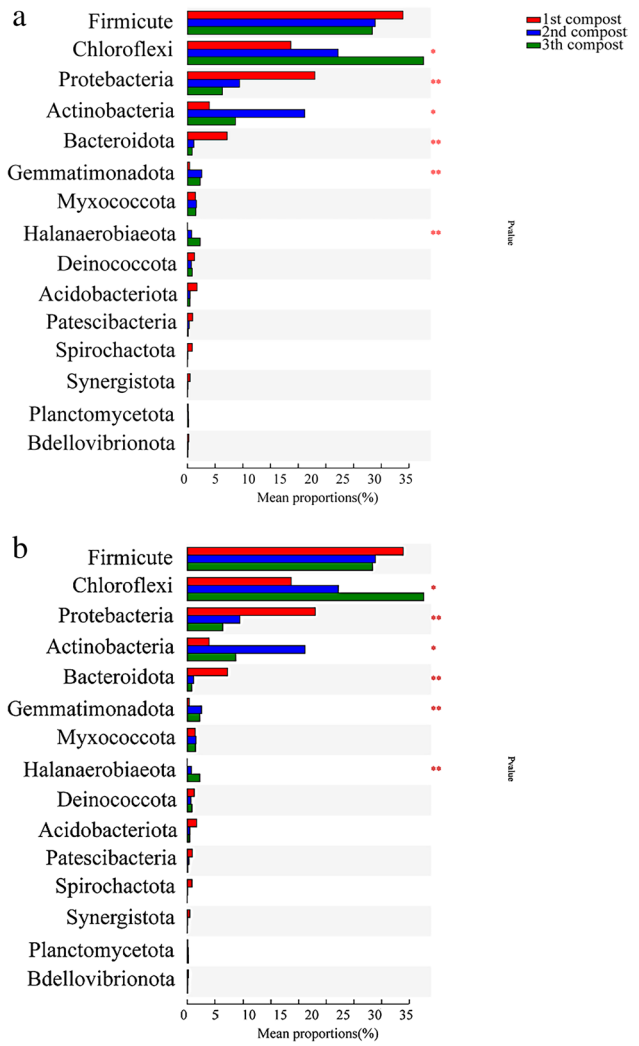

Fig. 3 Significant difference of bacteria in different composting cycles at phylum (**a**) and genus (**b**) levels. 1st compost, the first composting; 2nd compost, the second composting; 3rd compost, the third composting. *$P < 0.05$, **$P < 0.01$

3.4 Linking the Top Bacterial Communities with the Composting Cycles

In order to reveal the relationships between environment factors and microbial community, the correlation heatmap was derived based on the top 30 OTUs in the composting process. All those OTUs were classified into two groups. After the first group, OTU689, OTU269, OTU496, OTU380, OTU2110, OTU337, OTU428, and OTU1238 had positive correlations with nitrate, C/N ratio, and moisture, while the other OTUs had negative relationships with these parameters (Fig. 4). OTU689-*Methylocaldum szegediense* with the highest abundant in first composting period had a significantly positive relationship with the content of total carbon, while OUT496-UBA6140 in the family *Methylophilaceae* had a significantly positive relationship with the concentration of nitrate ($P < 0.001$). Relative abundance of OTU269-*Pseudomonas* was 1.2% and 1.4% in the surface and deep layers after the first period, respectively, and had the highest correlation with the concentration of nitrate in the all tested OTUs. As the compost developed, the OTU1238-*Terrisporobacter* and anaerobic OTU428-*Clostridium* sensu stricto 1 distributed mostly in the deep layers, and their relative contents decreased from 5.6% and 8.2% after the first composting cycle to 1.0% and 1.1% after the third compost cycle, respectively. OUT380-*Chelativorans composti*, OTU2110-*Turicibacter* sp. H121, and OTU337-*Ureibacillus* had significant negative relationships with the contents of ammonium ($P < 0.001$). The relative abundances of those OTUs also decreased from 1.1, 1.9, and 3.3 to 0.6%, 0.25%, and 0.4% at the end of this composting, respectively.

In the second group, the distributions of OTUs had negative relationships with moisture, nitrate concentrations, and C/N ratio. OTU1260-*Thermomonospora*, OTU1251-*Nonomuraea*, OTU954-*Sphaerimonospora*, OTU1690-*Sphaerobacter thermophilus*, OTU1904-*Hyphomicrobiaceae*, and OTU992-*Steroidobacteraceae* had significantly positive relationships with ammonium concentrations and pH. Those OTUs had the highest relative abundance in the deep layer of the second composting cycle, expecting OTU1260-*Thermomonospora* after the third composting cycle. OTU578-*Ureibacillus thermophiles* had a negative relationship with contents of carbon and nitrate, but positive with those of nitrogen and pH ($P < 0.001$), and decreased along the composting process. OUT1188-SBR1031, OTU1125-*Halocella*, OTU788-MBA03, OTU286-*Hydrogenispora*, OTU1293-*Limnochordaceae*, and OTU1153-MBA03 had positive relationships with nitrogen concentrations, but negative with moisture and C/N ratio ($P < 0.001$). The relative abundant of these OTUs increased along the compost process, and became the dominant bacteria at the end of composting. Those OTUs were prevalent in the anaerobic deep layer of the third composting cycle.

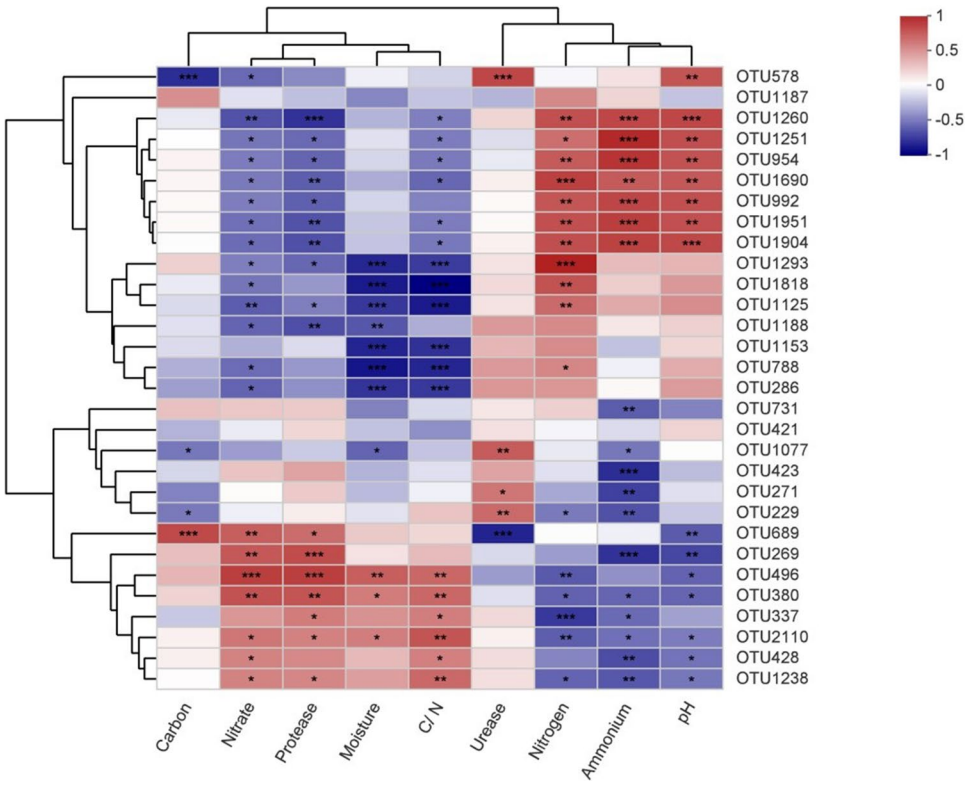

Fig. 4 The correlation heatmap of environmental parameters and OTUs. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

3.5 Succession of Functional Groups in Nitrogen Transformation

To reveal bacterial function in the transformation of nitrogen, the related bacteria and KEGG Orthology (KO) in nitrogen cycles were analyzed. Throughout the composting process, many bacteria participated in nitrogen cycles were found in the composting materials. *Azospirillum* and *Klebsiella* were found as the main nitrogen-fixing bacteria in the composting system. In ammonification, *Streptomyces* (42.4%), *Pseudomonas* (22.0%) and *Mycobacterium* (10.3%) were the main genera. *Nitrosomonas* in the third composting cycle is confirmed as ammonia-oxidation bacteria (AOB) (Campanaro et al. 2020). The main nitrite oxidation bacteria (NOB) during the composting process were *Nitrospira* and *Nitrolancea*, which are confirmed as the main NOB in nitrification (Kuypers et al. 2018). *Paracoccus denitrificans*, *Jonesia denitrificans*, and *Geobacillus thermodenitrificans* are the main denitrifiers (Huang et al. 2018a, b). They were found to have the most abundance after the second composting cycle. They are all at a high relative abundance during the composting process.

The KOs assigned to nitrogenase was the most abundant after the first composting cycle, and decreased along the composting process (Fig. 5). In the deep layers, the numbers of KOs associated with urease (Ure) in ammonification increased along the composting process. However, those KOs in the surface layer increased after the second cycle, and then decreased after the third cycle. This result indicated that the ammonification was more intensive in the deep layer, and more content of NH_4^+-N accumulated in the deep layer, actually. Generally, the decrease of KOs contents in nitrogen fixation and increase of those in ammonification led to the changes of NH_4^+-N concentration through the composting.

Compared with KOs in other processes, the KOs assigned to nitrification were at a very low level. In our composting system, relative abundances of ammonia monooxygenase (Amo) and hydroxylamine oxidase (Hao) firstly decreased after the second composting cycle, and then increased after the third composting cycle, while the relative content of nitrite oxidoreductase (Nxr) increased along the composting process. In sum, the contents of KOs assigned to nitrification increased after the second composting cycle, and decreased slightly after the third composting cycle. The change of their content was coincident with the changes of NO_3^--N concentration.

The relative abundances of KOs associated with denitrification also increased after the second composting cycle, and decreased after the third composting cycle. The relative contents of membrane-bound nitrate reductase (Nap) and nitric oxide reductase (Nor) were the highest after the first composting cycle, and then decreased. However, those of both nitrite reductase (Nir) and nitrous oxide reductase (Nos) were the highest after the second and third composting cycles, respectively.

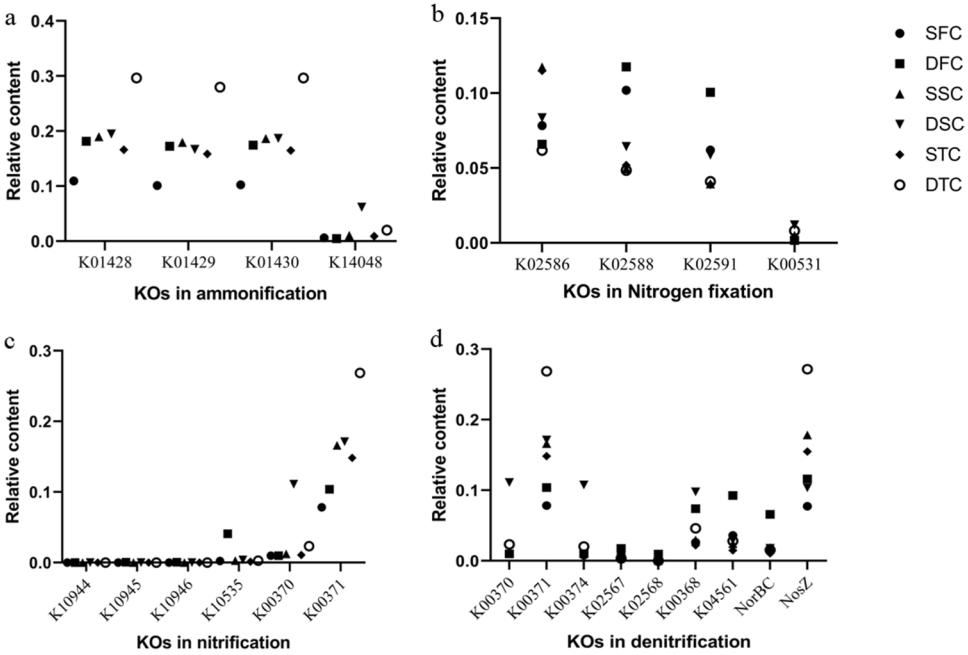

Fig. 5 Distribution of KOs in ammonification (**a**), nitrogen fixation (**b**), nitrification (**c**), and denitrification (**d**) processes. SFC, surface materials of the first composting cycle; DFC, deep materials of the first composting cycle; SSC, surface materials of the second composting cycle; DSC, deep materials of the second composting cycle; STC, surface materials of the third composting cycle; DTC, deep materials of the third composting cycle

4 Discussion

The addition of bulking agents can provide degradable organic carbon, improving the composting efficiency (Zhou et al. 2014). In the continuous composting system, the lignin, cellulose, and hemicellulose was utilized by microorganisms which accelerated their degradation. The low biodegradation rate of lignin fraction and the high degradation efficiency of cellulose and hemicellulose were detected in manure composting, in accordance with previous results (Qiao et al. 2019). Therefore, the continuously composting is benefit for lignin, cellulose, and hemicellulose degradation.

The C/N ratio is confirmed as one of the most critical factors influencing the composting, and is also an important index of composting maturity (Qiao et al. 2021). The C/N ratio decreases along the composting process. It is reported that the higher loss rate of organic C than that of N led to the decrease of C/N ratio (Zhang et al. 2021). The pH values increase during composting process. The result in our research also indicated that the changes in pH values are coincident with the NH_4^+-N concentration (Cáceres et al. 2015). It is stated that the nitrification is dominant process when the pH value is within the range of 6.0–7.0, and the release of H^+ during nitrification leads to the decrease of pH value (Cáceres et al. 2018; Onwosi et al. 2017). Meanwhile, the higher pH value could also inhibit the nitrification and lead to NH_3 emission (Akdeniz et al. 2019). As the highest content of NH_4^+-N after the third composting cycle, decrease of NO_3^--N concentrations indicated excessive amount of NH_4^+-N would inhibit the nitrification (Cáceres et al. 2018).

Protease hydrolyzes proteins into amino acids which are related to the mineralization of nitrogen from proteins in composting process (Qiao et al. 2019). Furthermore, urease in ammonification could catalyze the inorganic nitrogen to NH_4^+-N (Liu et al. 2018). The cooperation of protease and urease increased the content of available N for microorganism. Overall, the urease activity had shown opposite trends to that of protease, which was also observed in the composting process at ectopic fermentation bed system (Chen et al. 2021).

The changes of microbial community composition are found to be associated with composting parameters, such as C/N ratio, pH, and humidity (Zhou et al. 2014; Li et al. 2021). *Firmicutes*, *Chloroflexi*, *Proteobacteria*, and *Actinobacteriota* contributed more than 80% of all identified sequences, which distribute widely in composting system and contribute to the degradation of organic substances (Li et al. 2018; Liu et al. 2021). The phylum *Chloroflexi* including many anaerobic thermophiles becomes the predominant phylum in later period of composting (Li et al. 2018). As the most dominant genus in the composting materials, genus of o_SBR1031 has been identified as the major microbial populations in anaerobic digesters (Xia et al. 2016). *Hydrogenispora* in the surface layers has been confirmed to decompose chitin in composting systems (Uua et al. 2020). *Methylocaldum* and UBA6140 in the deep layers are methanotrophs which had been provided to be effective in reducing methane emission (Li et al. 2018). OTU689-*Methylocaldum szegediense* with the highest abundant in the first composting period had a significantly positive relationship with the content of total carbon, while OUT496-UBA6140 in the family *Methylophilaceae* had a

significantly positive relationship with the concentration of nitrate ($P < 0.001$). As a thermophilic methanotroph, *M. szegediense* and UBA6140 could transform CH_4 to CO_2, which playing important roles in carbon period (Koo and Rosenzweig 2021). Meanwhile, methane oxidation coupled with nitrate/nitrite reduction might lead to a correlation between methanotroph and nitrate (Raghoebarsing et al. 2006). Species of the genus *Pseudomonas* are also dominant in other pig manure composting, and are powerful in degradation of hydrocarbons and phenol (Wilkes and Aristilde 2017). *Clostridium* sensu stricto 1 and *Terrisporobacter* from animal intestine are the dominant group at the beginning of manure composting, which is supported by other study (Huang et al. 2018a, b). OTU578-*Ureibacillus thermophiles* had a negative relationship with contents of carbon and nitrate, but positive with those of nitrogen and pH ($P < 0.001$), and decreased along the composting process. *U. thermophiles* has been confirmed as the major species for enhancing soluble organic materials in a thermophilic sewage sludge digestion (Jang et al. 2013). Thus, the higher content of *U. thermophiles* could improve the composting process by providing soluble organics for microorganism. The *Anaerolineae* (*Chloroflexi*), MBA03, and *Hydrogenispora* have been identified as the major microbial populations in anaerobic digesters (Xia et al. 2016; Uua et al. 2020; Sardar et al. 2021). *Halocella* and *Limnochordaceae* have been found in the anaerobic denitrification of saline wastewater (Guo et al. 2013). Those OTUs were prevalent in the anaerobic deep layer of the third composting cycle.

The microorganism in nitrogen cycles played pivotal roles in the composting process, and finally transformed organic materials into the high-quality bio-fertilizers (Qiao et al. 2019). Both of nitrogen fixation and ammonification processes are related with NH_4^+-N production (Kuypers et al. 2018). Nitrogen fixation is biologically carried out by microorganisms that carry nitrogenase and thus can fix N_2 into NH_4^+-N (Lindström and Mousavi 2020). *Klebsiella* and *Azospirillum* participated in biological nitrogen fixation were found in composting materials (Lindström and Mousavi 2020). In ammonification process, urease could catalyze the hydrolysis of organic nitrogen to NH_4^+-N, leading to NH_3 emission and nitrogen loss (Liu et al. 2018). The changes of KOs associated with urease (Ure) indicated that the ammonification was more intensive, and more content of NH_4^+-N accumulated in this layer, actually.

In the two-step nitrification, the NH_4^+ is oxidized by ammonia monooxygenase (Amo) to hydroxylamine, and then catalyzed to NO_2^- and NO_3^- by hydroxylamine oxidase (Hao) and nitrite oxidoreductase (Nxr), sequentially (Cáceres et al. 2018). In sum, the contents of KOs assigned to nitrification increased after the second composting cycle, and decreased slightly after the third composting cycle. The change of their content was coincident with the changes of NO_3^--N concentration. Furthermore, the relative abundances of KOs associated with denitrification also increased after the second composting cycle, and decreased after the third composting cycle. Nitrate reduction by membrane-bound nitrate reductase (Nap) or periplasmic nitrate reductase (Nar) is the first step in denitrification, providing nitrite for other nitrogen processes (Tsementzi et al. 2016). Nitrite is reduced to NO by nitrite reductase (Nir), and finally transformed to N_2O or N_2 by nitric oxide reductase (Nor) or nitrous oxide reductase (Nos) (Kuypers et al. 2018). The relative contents of Nor were the highest after the first composting cycle, while those of both Nir and Nos were the highest after the second and third composting cycles. Thus, it is speculated that the emission of N_2O might decrease with increasing of the composting cycles. It is also reported previously that the N_2O emission was remarkably affected by composting duration and bulking agent, and the N_2O emission declined with the increase of the composting time (Zhang et al. 2021). The conversion of NH_4^+-N to NO_3^--N is benefit for nitrogen retain and increasing the fertility of composting materials, so that more composting cycles might reduce the ability of nitrogen assimilation (Sánchez et al. 2017).

5 Conclusions

This study provided an assessment of physicochemical characteristics, bacterial community, and functional properties of a continuous solid pig manure composting system. After the composting process, nitrogen and NH_4^+-N concentrations increased. Simultaneously, the C/N ratios decreased after the second composting cycle, and stabilized after the third cycle. During the whole composting process, the relative contents of *Clostridium* sensu stricto, *Terrisporobacter*, and *Ureibacillus* reduced significantly, while those of *SBR1031* and *Sphaerobacter* increased. The C/N ratio and pH were the main factors for the changes of bacterial community. In summary, more composting cycles might reduce the ability of nitrogen assimilation, but increase the degradation of lignin, cellulose, and hemicellulose.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42729-022-00928-3.

Author Contribution Qianqian Chen and Liu Bo conceived and designed the experiments; Qianqian Chen, Jianmei Che, Guohong Liu, Huai Shi, and Lulong Zhong performed the experiments; Qianqian Chen and Jieping Wang analyzed the data; Qianqian Chen and Liu Bo wrote the paper. All authors reviewed and edited the manuscript.

Funding This research was supported by the grants from the Youth Science and Technology Talent of Fujian Academy of Agricultural Sciences (no. YC2019002).

Data Availability The availability of data and materials is on the base of personal request.

Declarations

Ethics Approval and Consent to Participate The manuscript was reviewed and ethically approved for publication by all authors. The manuscript was reviewed and consents to participate by all authors.

Consent for Publication The manuscript was reviewed and consents to publish by all authors.

Competing Interests The authors declare no competing interests.

References

Akdeniz N (2019) A systematic review of biochar use in animal waste composting. Waste Manag 88:291–300. https://doi.org/10.1016/j.wasman.2019.03.054

Bernal MP, Sommer SG, Chadwick D, Qing C, Guoxue L, Michel FC Jr (2017) Current approaches and future trends in compost quality criteria for agronomic, environmental, and human health benefits. Adv Agron 144:143–233. https://doi.org/10.1016/bs.agron.2017.03.002

Cáceres R, Magrí A, Marfà O (2015) Nitrification of leachates from manure composting under field conditions and their use in horticulture. Waste Manag 44:72–81. https://doi.org/10.1016/j.wasman.2015.07.039

Cáceres R, Malińska K, Marfà O (2018) Nitrification within composting: a review. Waste Manag 72:119–137. https://doi.org/10.1016/j.wasman.2017.10.049

Campanaro S, Raga R, Squartini A (2020) Intermittent aeration of landfill simulation bioreactors: effects on emissions and microbial community. Waste Manag 117:146–156. https://doi.org/10.1016/j.wasman.2020.08.010

Canfield DE, Glazer AN, Falkowski PG (2010) The evolution and future of Earth's nitrogen cycle. Science 330:192–196. https://doi.org/10.1126/science.1186120

Chen Q, Wang J, Zhang H, Shi H, Liu G, Che J, Liu B (2021) Microbial community and function in nitrogen transformation of ectopic fermentation bed system for pig manure composting. Bioresour Technol 319:124155. https://doi.org/10.1016/j.biortech.2020.124155

Costa T, Akdeniz N (2019) A review of the animal disease outbreaks and biosecure animal mortality composting systems. Waste Manag 90:121–131. https://doi.org/10.1016/j.wasman.2019.04.047

Douglas GM, Beiko RG, Langille MGI (2018) Predicting the functional potential of the microbiome from marker genes using PICRUSt. Methods Mol Biol 1849:169–177. https://doi.org/10.1007/978-1-4939-8728-3_11

Guo Y, Zhou X, Li Y, Li K, Xing J (2013) Heterotrophic nitrification and aerobic denitrification by a novel Halomonas campisalis. Biotechnol Lett 35(12):2045–2049. https://doi.org/10.1007/s10529-013-1294-3

Huang S, Chen C, Jaffé PR (2018) Seasonal distribution of nitrifiers and denitrifiers in urban river sediments affected by agricultural activities. Sci Total Environ 642:1282–1291. https://doi.org/10.1016/j.scitotenv.2018.06.116

Huang YN, Wang XH, Cao Q, Fu XC, Fu WY, Ma J, Zhang M (2018) Analysis of microbial community changes in pig excrement during compost process based on high-throughput sequencing technology. J Microbiol 38(5):21–26. https://doi.org/10.3969/j.issn.1005-7021.2018.05.004

Jang HM, Park SK, Ha JH, Park JM (2013) Microbial community structure in a thermophilic aerobic digester used as a sludge pretreatment process for the mesophilic anaerobic digestion and the enhancement of methane production. Bioresour Technol 145:80–89. https://doi.org/10.1016/j.biortech.2013.01.094

Jolanun B, Towprayoon S (2010) Novel bulking agent from clay residue for food waste composting. Bioresour Technol 101(12):4484–4490. https://doi.org/10.1016/j.biortech.2010.01.116

Kandeler E (1996). In: Schinner F, Ohlinger R, Kandeler E, Margesin R (eds) Methods in soil biology. Springer-Verlag, Heidelberg, New York, pp 168–170

Koo CW, Rosenzweig AC (2021) Biochemistry of aerobic biological methane oxidation. Chem Soc Rev 50(5):3424–3436. https://doi.org/10.1039/D0CS01291B

Kuypers M, Marchant HK, Kartal B (2018) The microbial nitrogen-cycling network. Nat Rev Microbiol 16:263–276. https://doi.org/10.1038/nrmicro.2018.9

Ladd JN, Butler JHA (1972) Short-term assay of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biol Biochem 4:19–39. https://doi.org/10.1016/0038-0717(72)90038-7

Li S, Li D, Li J, Li Y, Li G, Zang B, Li Y (2018) Effect of spent mushroom substrate as a bulking agent on gaseous emissions and compost quality during pig manure composting. Environ Sci Pollut Res Int 13:12398–12406. https://doi.org/10.1007/s11356-018-1450-3

Li MX, He XS, Tang J, Li X, Zhao R, Tao YQ, Wang C, Qiu ZP (2021) Influence of moisture content on chicken manure stabilization during microbial agent-enhanced composting. Chemosphere 264(Pt2):128549. https://doi.org/10.1016/j.chemosphere.2020.128549

Lindström K, Mousavi SA (2020) Effectiveness of nitrogen fixation in rhizobia. Microb Biotechnol 13(5):1314–1335. https://doi.org/10.1111/1751-7915.13517

Liu Y, Dai Q, Jin X, Dong X, Peng J, Wu M, Liang N, Pan B, Xing B (2018) Negative impacts of biochars on urease activity: high pH, heavy metals, polycyclic aromatic hydrocarbons, or free radicals. Environ Sci Technol 52(21):12740–12747. https://doi.org/10.1021/acs.est.8b00672

Liu T, Wang M, Mukesh KA, Chen H, Sanjeev KA, Duan Y, Zhang Z (2019) Measurement of cow manure compost toxicity and maturity based on weed seed germination. J Clean Prod 245:118078. https://doi.org/10.1016/j.jclepro.2019.118894

Liu L, Wang T, Li S, Hao R, Li Q (2021) Combined analysis of microbial community and microbial metabolites based on untargeted metabolomics during pig manure composting. Biodegradation 32(2):217–228. https://doi.org/10.1007/s10532-021-09935-0

Ma J, Wang Z, Li H, Park HD, Wu Z (2016) Metagenomes reveal microbial structures, functional potentials, and biofouling-related genes in a membrane bioreactor. Appl Microbiol Biotechnol 100:5109–5121. https://doi.org/10.1007/s00253-016-7312-3

Onwosi CO, Igbokwe VC, Odimba JN, Eke IE, Nwankwoala MO, Iroh IN, Ezeogu LI (2017) Composting technology in waste stabilization: on the methods, challenges and future prospects. J Environ Manage 190:140–157. https://doi.org/10.1016/j.jenvman.2016.12.051

Pandey B, Chen L (2021) Technologies to recover nitrogen from livestock manure - a review. Sci Total Environ 784:147098. https://doi.org/10.1016/j.scitotenv.2021.147098

Pardo G, Moral R, Del PA (2017) SIMSWASTE-AD-A modelling framework for the environmental assessment of agricultural waste management strategies, anaerobic digestion. Sci Total Environ 574:806–817. https://doi.org/10.1016/j.scitotenv.2016.09.096

Qiao C, Ryan PC, Liu C, Shen Z, Ou Y, Liu Z, Xu X, Li R, Shen Q (2019) Key extracellular enzymes triggered high-efficiency composting associated with bacterial community succession. Bioresour Technol 288:121576. https://doi.org/10.1016/j.biortech.2019.121576

Qiao C, Penton CR, Liu C, Tao C, Deng X, Ou Y, Liu H, Li R (2021) Patterns of fungal community succession triggered by C/N ratios during composting. J Hazard Mater 2401:123344. https://doi.org/10.1016/j.jhazmat.2020.123344

Raghoebarsing AA, Pol A, van de Pas-Schoonen KT, Smolders AJP, Ettwig KF, Rijpstra WIC (2006) Amicrobial consortium couples anaerobic methane oxidation to denitrification. Nature 440(7086):918–921. https://doi.org/10.1038/nature04617

Sánchez ÓJ, Ospina DA, Montoya S (2017) Compost supplementation with nutrients and microorganisms in composting process. Waste Manag 69:136–153. https://doi.org/10.1016/j.wasman.2017.08.012

Sardar MF, Zhu C, Geng B, Huang Y, Abbasi B, Zhang Z, Song T, Li H (2021) Enhanced control of sulfonamide resistance genes and host bacteria during thermophilic aerobic composting of cow manure. Environ Pollut 275:116587. https://doi.org/10.1016/j.envpol.2021.116587

Tsementzi D, Wu J, Deutsch S, Nath S, Rodriguez-R LM, Burns AS, Ranjan P, Sarode N, Malmstrom RR, Padilla CC, Stone BK, Bristow LA, Larsen M, Glass JB, Thamdrup B, Woyke T, Konstantinidis KT, Stewart FJ (2016) SAR11 bacteria linked to ocean anoxia and nitrogen loss. Nature 536:179–183. https://doi.org/10.1038/nature19068

Uua B, Sb B, Au B, Aka B (2020) Symbiotic chitin degradation by a novel anaerobic thermophilic bacterium Hydrogenispora sp. UUS1–1 and the bacterium Tepidanaerobacter sp. GT38. Enzyme Microb Tech 144:109740. https://doi.org/10.1016/j.enzmictec.2020.109740

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Varma VS, Parajuli R, Scott E, Canter T, Lim TT, Popp J, Thoma G (2021) Dairy and swine manure management - Challenges and perspectives for sustainable treatment technology. Sci Total Environ 778:146319. https://doi.org/10.1016/j.scitotenv.2021.146319

Wang M, Ma L, Strokal M, Ma W, Liu X, Kroeze C (2018) Hotspots for nitrogen and phosphorus losses from food production in China: a county-scale analysis. Environ Sci Technol 52(10):5782–5791. https://doi.org/10.1021/acs.est.7b06138

Wilkes RA, Aristilde L (2017) Degradation and metabolism of synthetic plastics and associated products by Pseudomonas sp.: capabilities and challenges. J Appl Microbiol. 123(3):582–593. https://doi.org/10.1111/jam.13472

Xia Y, Wang Y, Wang Y, Chin F, Zhang T (2016) Cellular adhesiveness and cellulolytic capacity in anaerolineae revealed by omics-based genome interpretation. Biotechnol Biofuels 9(1):111. https://doi.org/10.1186/s13068-016-0524-z

Zahra MB, Fayyaz B, Aftab ZEH et al (2021) Mitigation of degraded soils by using biochar and compost: a systematic review. J Soil Sci Plant Nutr 21:2718–2738. https://doi.org/10.1007/s42729-021-00558-1

Zhang Z, Liu D, Qiao Y, Li S, Chen Y, Hu C (2021) Mitigation of carbon and nitrogen losses during pig manure composting: a meta-analysis. Sci Total Environ 783:147103. https://doi.org/10.1016/j.scitotenv.2021.147103

Zhou H, Chen T, Gao D, Zheng G, Chen J, Pan T, Liu H, Gu R (2014) Simulation of water removal process and optimization of aeration strategy in sewage sludge composting. Bioresour Technol 171:452–460. https://doi.org/10.1016/j.biortech.2014.07.006