Science of the Total Environment 789 (2021) 147975

Contents lists available at ScienceDirect

Science of the Total Environment

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

Involvement of the metabolically active bacteria in the organic matter degradation during olive mill waste composting

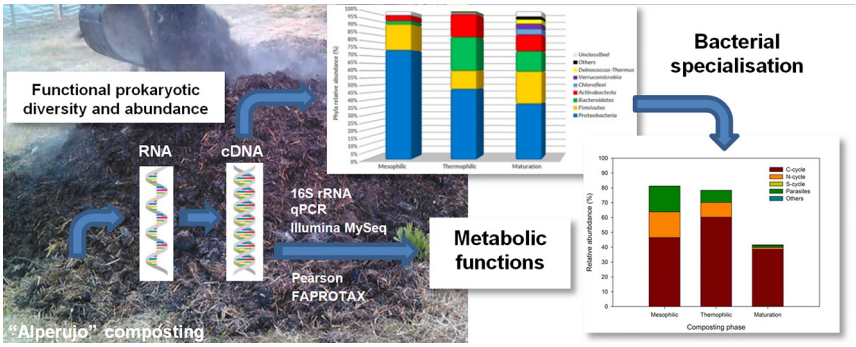
Germán Tortosa [a,*,1], Antonio J. Fernández-González [a,1], Ana V. Lasa [a,1], Elisabet Aranda [b,c], Fernando Torralbo [d,2], Carmen González-Murua [d], Manuel Fernández-López [a], Emilio Benítez [e], Eulogio J. Bedmar [a]

[a] Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín (EEZ), Agencia Estatal CSIC, c/ Profesor Albareda, 1, 18008 Granada, Spain
[b] Institute of Water Research, University of Granada, Edificio Fray Luis, c/ Ramón y Cajal, 4, 18071 Granada, Spain
[c] Department of Microbiology, Faculty of Pharmacy, University of Granada, Campus de Cartuja, s/n, 18071 Granada, Spain
[d] Department of Plant Biology and Ecology, University of the Basque Country (UPV/EHU), Barrio Sarriena, s/n, 48940 Leioa, Bizkaia, Spain
[e] Department of Environmental Protection, Estación Experimental del Zaidín (EEZ), Agencia Estatal CSIC, c/ Profesor Albareda, 1, 18008 Granada, Spain

HIGHLIGHTS

- RNA-based metabarcoding is a helpful tool to assess active bacteria during composting.
- qPCR confirmed to themophilic as the most active phase of the process.
- Differential abundance analysis revealed distinct dynamics for some bacterial genera.
- Statistical correlation suggested that active bacteria had metabolic specialisation.
- C and N cycles, and pathogen reduction were the most prominent functions predicted.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 March 2021
Received in revised form 5 May 2021
Accepted 19 May 2021
Available online 25 May 2021

Editor: Daniel CW Tsang

Keywords:
Active bacteria
"Alperujo"
cDNA
qPCR
Illumina MiSeq
FAPROTAX

ABSTRACT

RNA-based high-throughput sequencing is a valuable tool in the discernment of the implication of metabolically active bacteria during composting. In this study, "alperujo" composting was used as microbial model for the elucidation of structure-function relationships with physicochemical transformation of the organic matter. DNA and RNA, subsequently retrotranscribed into cDNA, were isolated at the mesophilic, thermophilic and maturation phases. 16S rRNA gene was amplified by quantitative PCR (qPCR) and Illumina MiSeq platform to assess bacterial abundance and diversity, respectively. The results showed that the abundance of active bacteria assessed by qPCR was maximum at thermophilic phase, which confirm it as the most active stage of the process. Concerning diversity, Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria were the main phyla presented in composts. Concomitantly, three different behaviours were observed for bacterial dynamics: some genera decreased during the whole process meanwhile others proliferated only at thermophilic or maturation phase. Statistical correlation between physicochemical transformations of the organic matter and bacterial diversity revealed bacterial specialisation. This result indicated that specific groups of bacteria were only involved in the organic matter degradation during bio-oxidative phase or humification at maturation. Metabolic functions predictions confirmed that active bacteria were mainly involved in carbon (C) and nitrogen (N) cycles transformations, and pathogen reduction.

* Corresponding author.
E-mail address: german.tortosa@eez.csic.es (G. Tortosa).
[1] Contributed equally to this article.
[2] Current address: Division of Plant Sciences, University of Missouri, Columbia, MO 65211, USA.

https://doi.org/10.1016/j.scitotenv.2021.147975
0048-9697/

1. Introduction

Composting is a low-cost technology which transforms organic wastes and by-products into biologically stable materials that can be used as soil amendments, fertilisers or substrates for soilless cultivation, reducing their environmental impact and enabling their organic matter and nutrients to be more productive (de Bertoldi et al., 1983; Diaz et al., 2007). An example is the olive oil industry, which yearly generates a huge amount of organic wastes such as "alperujo" or "alpeorujo" (AL), and humid and acidic pomace (Alburquerque et al., 2004; Roig et al., 2006; Dermeche et al., 2013). It has been demonstrated that composting is a feasible methodology to transform AL into commercial organic amendments and fertilisers (Tortosa et al., 2012), and this process is characterised by its humification process (Alburquerque et al., 2009).

Composting can be defined as a controlled bio-oxidative process, in which heterogeneous organic substrates are degraded by the sequential activity of a myriad of microorganisms present in the raw organic materials (Ryckeboer et al., 2003; López-González et al., 2015a, 2015b). During composting, chemical and physical changes occur in the organic substrates due to the multitude of reactions derived from the succession of different microbial populations, mainly bacteria and fungi (Diaz and Savage, 2007; Insam and de Bertoldi, 2007). The microbial development depends on the conditions and characteristics of the raw materials (water content, temperature, pH, nutrients, etc.) because their capacities to degrade the organic components of the starting materials are different. For that, the study of the microbial diversity and evolution, and its relationship with physicochemical changes during composting is one of the main challenges for compost scientists and technicians. In recent years, molecular techniques applied to the study of compost microbial communities have significantly increased the knowledge on their abundance, diversity and evolution during the process (Vivas et al., 2009; Tortosa et al., 2017, 2020; Jurado et al., 2020). DNA-based high-throughput sequencing studies like those performed through Illumina MiSeq/HiSeq platforms, or quantitative polymerase chain reaction (qPCR) provide relevant information on the microbial composition and relative abundance during composting (Tortosa et al., 2020), while RNA-based studies describe microbial communities that are metabolically active at each stage of the process (Meng et al., 2020). Unfortunately, transcriptional analyses applied to study the microbial ecology in environmental samples (metatranscriptomics) can lead to some methodological issues due to the instability of microbial mRNA (McGrath et al., 2008). An effective approach for avoiding this problem is to convert RNA into retrotranscribed DNA (cDNA), and then, amplify and sequence specific genes which can aid in identifying bacteria by using next generation sequencing technologies (metabarcoding) (Antunes et al., 2016; Ding et al., 2020).

Bacterial populations are recognised as the principal microbial actors during composting due to their abundance and their active role during organic matter decomposition (Insam et al., 2010; López-González et al., 2015a). It is considered essential to study the dynamics of bacterial communities along the process, owing to them being the keystone members for the optimization of the process itself. Recent studies have evaluated the involvement of bacteria on the physicochemical transformation of the organic substrates during several organic wastes composting, technologies and recipes (Tortosa et al., 2017; Wang et al., 2018; Wang et al., 2019; Ding et al., 2020; Kong et al., 2020; Sun et al., 2020; Zhang et al., 2020). Complementary, bioinformatic tools based on 16S rRNA sequences identification such us Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (Langille et al., 2013), Tax4Fun (Asshauer et al., 2015) or Functional Annotation of Prokaryotic Taxa (FAPROTAX) (Louca et al., 2016), can be used to predict bacterial metabolic involvement in the transformation of organic substrates during composting (Zhang et al., 2020; Zhu et al., 2020; Xu et al., 2021).

Some authors have already implemented metabarcoding techniques to address bacterial dynamics of composts and vermicomposts, enriching their studies through the study of the effect of physicochemical parameters on the structure of bacterial communities. For instance, Srivastava et al. (2021) found that there are correlations between the relative abundance of certain bacteria and physicochemical parameters of the vermicompost, being bacterial populations highly influenced by the substrate nature, environmental parameters and the activity of the earthworms. However, very little is known about the specific metabolic capabilities of bacterial populations of AL compost, based on amplicon sequencing.

According to this fact, the aims of this research were i) to explore new insights into the composition and dynamics of the active bacterial populations using Illumina MiSeq sequencing and qPCR technologies, and ii) to study the role of active bacteria and its metabolic functions contribution on the organic matter degradation during AL composting.

2. Materials and methods

2.1. Composting performance, sampling and nucleic acids (total DNA, RNA and cDNA) obtention

Two trapezoidal piles (M1 and M2) of 10 tons each were made as biological replicates as earlier described by Tortosa et al. (2012). Physicochemical parameters such us moisture, temperature, pH, electrical conductivity (EC), organic matter (OM), lignin, cellulose and hemicellulose content, total organic carbon (T_{OC}), total nitrogen (T_N), fat content, water-soluble carbon (WSC), water-soluble carbohydrates (WSC), humification ratio (HR), humification degree (HD), percentage of humic acids (P_{AH}) and germination index (GI) were previously analysed in Tortosa et al. (2017). Samples were collected at the mesophilic (1st week), thermophilic (7th week) and maturation (22nd week) phases (Tortosa et al., 2020). Four independent technical replicates (1 and 2 for M1, and 3 and 4 for M2 piles, respectively) per each phase were individually extracted (Fig. S1). Total DNA, RNA extraction and cDNA synthesis were performed as previously described by Tortosa et al. (2020).

2.2. Quantitative PCR (qPCR)

The copy numbers of 16S rRNA gene present in total DNA and cDNA were quantified by qPCR as described previously (Castellano-Hinojosa et al., 2018). The qPCR analyses were carried out by amplifying the hypervariable V3 region primers 341F (5′-CCTACGGGAGGCAGCAG -3′) and 534-R (5′-ATTACCGCGGCTGCTGG −3′) (Muyzer et al., 1993). The PCR amplification conditions were: 95 °C for 7 min; 40 cycles consisting of 95 °C for 30 s, 60 °C for 40 s and 72 °C for 30 s, and a final step of 72 °C for 7 min. The melting curve was obtained at the end of each run by increasing temperature from 60 °C to 95 °C.

2.3. Illumina MiSeq sequencing and data processing

The hypervariable V3-V4 regions of the 16S rRNA gene from cDNA were amplified by using Illumina MiSeq technology as previously described (Takahashi et al., 2014). Sequencing was performed at the facilities of the Genomic Unit of the Institute of Parasitology and Biomedicine "López-Neyra" (IPBLN-CSIC, Granada, Spain), after checking cDNA quality and quantity by using a Nanodrop 1000 Spectrophotometer (Thermo Scientific). Data quality screening, trimming and overlapping were performed with the FastQC (v.0.11.5 release; http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), FASTX-Toolkit (v.0.014; release; http://hannonlab.cshl.edu/fastx_toolkit/index.html) and fastq-join (v.1.3.1 release; https://github.com/ExpressionAnalysis/ea-utils) bioinformatic utilities, respectively. Low-quality sequences with lower quality values than Q20 were discarded, and paired reads with >20 bp and < 15% of difference in the overlapping region were merged.

After that, the 16S rRNA gene sequences obtained were trimmed by the SEED2 software (v.2.1.05 release; Větrovský et al., 2018) and the

specific primers, sequences showing ambiguities, sequences <300 bp or sequences with quality <Q30 were removed. Chimeric reads were also eliminated using SILVA gold reference fasta and high-quality sequences were clustered into Operational Taxonomical Units (OTUs) at 97% of genetic similarity using *Vsearch dgc* approach with MOTHUR (v.1.40.5 release; Schloss et al., 2009). OTUs representing less than 0.005% of the total high-quality sequences were removed (Bokulich et al., 2013) and were classified by using Ribosomal Database Project (RDP-II) 16S rRNA reference database with an 80% cut-off (Cole et al., 2014). Mitochondria, chloroplast and unknown (unclassified at kingdom level) identifications were removed from the dataset. Rarefaction curves, alpha diversity indices (Observed and Chao1 richness; Shannon and Inverse Simpson, InvSimpson), and beta diversity assessed by the Non-metric MultiDimensional Scaling analysis (NMDS) based on Bray-Curtis dissimilarities were performed as previously described by Fernández-González et al. (2019).

2.4. Metabolic functions

Functional Annotation of Prokaryotic Taxa (FAPROTAX) bioinformatic tool (v.1.2.2 release) was employed as described by Louca et al. (2016). In brief, the taxonomical assignment of each OTU was compared with the annotations included in the FAPROTAX database by running the script *collapse_table.py*, both supplied by the developers (available at http://www.loucalab.com/archive/FAPROTAX/). This version of FAPROTAX accounted for 90 functional groups, comprising 7820 assignments.

2.5. Statistical analyses

Inferential statistics were applied to the bacterial abundance (qPCR), alpha diversity indices, taxonomical relative abundances and functional assignments at each composting phase by using the Statistical Analysis of Taxonomical and Functional Profiles (STAMP) open-source software v.2.1.3 release (Parks et al., 2014). One-way ANOVA and Tukey-Kramer *post-hoc* test ($p < 0.05$), considering Storey's FDR and eta-squared multiple and sample size corrections were also used. For beta diversity analysis, Permutational Multivariate Analysis of Variance (PERMANOVA) was performed as previously indicated (Fernández-González et al., 2019). Pearson correlation matrix was calculated with the GNU-PSPP open-source software v0.9.0 release (available in https://www.gnu.org/software/pspp/) as previously described (Tortosa et al., 2017).

2.6. Accession numbers

Raw sequences are available at the NCBI Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra) under PRJNA683198 accession number.

3. Results

3.1. Bacterial abundance (qPCR)

The total abundance of bacteria increased significantly during composting (Fig. 1a). At the thermophilic phase, 16S rRNA copy numbers amplified from total DNA reached values of $7.45 \times 10^{10} \pm 1.20 \times 10^{10}$ (g CDW)$^{-1}$, 2.5-fold higher than those found at mesophilic phase. At the end of the process, these copies were reduced to values of $5.35 \times 10^{10} \pm 7.42 \times 10^{9}$ (g CDW)$^{-1}$, while they were still significantly higher than mesophilic counterparts.

Similar behaviour in terms of abundance in the active bacteria (cDNA) and total bacterial community (DNA) was observed (Fig. 1b). The maximum values were obtained at the thermophilic phase, the most active stage, showing $1.07 \times 10^{8} \pm 2.30 \times 10^{7}$ (g CDW)$^{-1}$, 5-fold higher than the copy numbers found at both mesophilic and maturation phases.

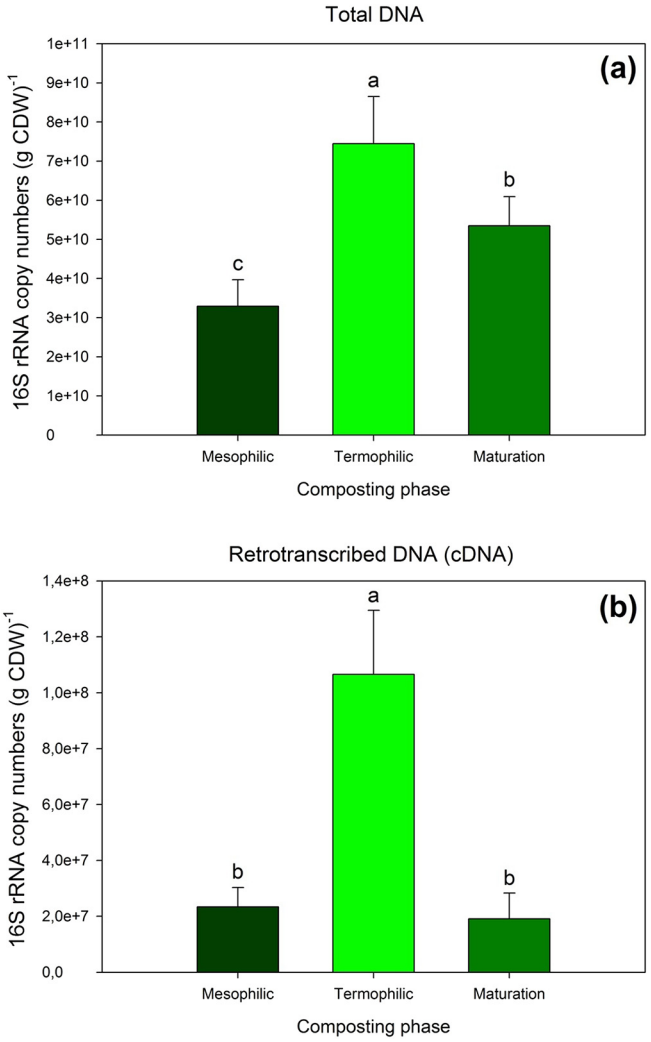


Fig. 1. 16S rRNA copy numbers per compost dry weight (g CDW)$^{-1}$ obtained from 16S rRNA gene amplification of total DNA (a) and cDNA (b) at mesophilic, thermophilic and maturation phases, respectively. Different letters indicate statistical difference ($p < 0.05$) according to one-way ANOVA with Tukey-Kramer *post-hoc* test.

3.2. Bacterial diversity

The number of sequences obtained by Illumina MiSeq amplification and sequencing of the 16S rRNA gene at mesophilic, thermophilic and maturation phases is shown in Table 1. Total sequences identified ranged from 80,867 to 104,575, and the percentage of the sequences identified at each composting phase was relevant (>80%), with the exception of maturation libraries at genus level (44.3%). Rarefaction curves (Fig. S2) and Good's coverage indices (from 99.93% to 99.98%) showed that the sequencing effort was enough to obtain a representative sample of each bacterial community collected.

Overall, the alpha diversity indices were increased during composting (Fig.2). Richness, assessed by means of Observed OTUs and Chao1 index, was statistically increased ($p < 0.001$) during the process according to the one-way ANOVA test. Inverse of Simpson index also showed a similar tendency but not Shannon's, which only showed differences between the mesophilic and maturation phases ($p = 0.022$). The Permutational Analysis Of Variance (PERMANOVA) explained 20.67% of the total variance and showed that the bacterial communities from the two piles used as biological replicates were only significantly different ($p = 0.007$) at the beginning of the process (Fig. 3). Further, both piles showed clearly different bacterial communities ($p = 0.003$) among

Table 1
Taxa (N) and sequences (S) number obtained from 16S rRNA gene amplification of cDNA at mesophilic (Meso1-4), thermophilic (Thermo1-4) and maturation (Matu1-4) libraries, respectively.

	cDNA libraries					
	Meso1-4		Thermo1-4		Matu1-4	
	N	S	N	S	N	S
Phylum	7 ± 1	78,296 ± 17,119 (96.8%)	11 ± 1	104,231 ± 10,626 (99.7%)	14 ± 2	96,180 ± 16,847 (96.4%)
Class	14 ± 4	77,137 ± 16,364 (95.4%)	22 ± 2	104,038 ± 10,494 (99.5%)	31 ± 2	93,885 ± 18,238 (94.1%)
Order	25 ± 6	76,608 ± 16,193 (94.7%)	38 ± 4	103,230 ± 9993 (98.7%)	52 ± 2	85,682 ± 14,236 (85.9%)
Family	58 ± 17	73,167 ± 16,657 (90.5%)	92 ± 5	100,803 ± 9060 (96.4%)	104 ± 3	78,527 ± 15,285 (78.7%)
Genus	74 ± 29	68,470 ± 19,805 (84.7%)	141 ± 17	85,020 ± 5561 (81.3%)	157 ± 7	44,233 ± 14,122 (44.3%)
Total sequences identified	80,867 ± 19,374 (100%)		104,575 ± 10,803 (100%)		99,739 ± 17,325 (100%)	

Note: Values are expressed as the mean of the sequences from each library and its standard deviation. Numbers in brackets represent the percentage of identified sequences respect total sequences identified.

the composting phases (mesophilic, thermophilic and maturation), explaining 39.74% of the total variance.

Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria were the main phyla observed during the process, accounting for more than 84% of the number of sequences identified (Fig. 4). Phylum Proteobacteria decreased its relative abundance during composting, while Firmicutes, Bacteroidetes, Actinobacteria, Chloroflexi, Verrucomicrobia and Deinococcus-Thermus did the opposite, maturation being the phase with most phyla identified, and more evenly distributed.

The list of all the genera detected and their relative abundance is shown in Table S1. Three different behaviours were accounted for at genus level during the process (Table 2). *Stenotrophomonas*, *Halotalea* and *Pseudomonas* represented close to 50% of the sequences identified at the mesophilic phase, which are commonly found in olive mill wastes (Vivas et al., 2009; Muktadirul Bari Chowdhury et al., 2013; Tortosa et al., 2017). These genera, as well as *Acinetobacter, Planifilum, Delftia, Pelomonas, Komagataeibacter, Gluconobacter* and *Cohnella*, statistically decreased ($p < 0.05$) their relative abundance during the process. As expected, most of these

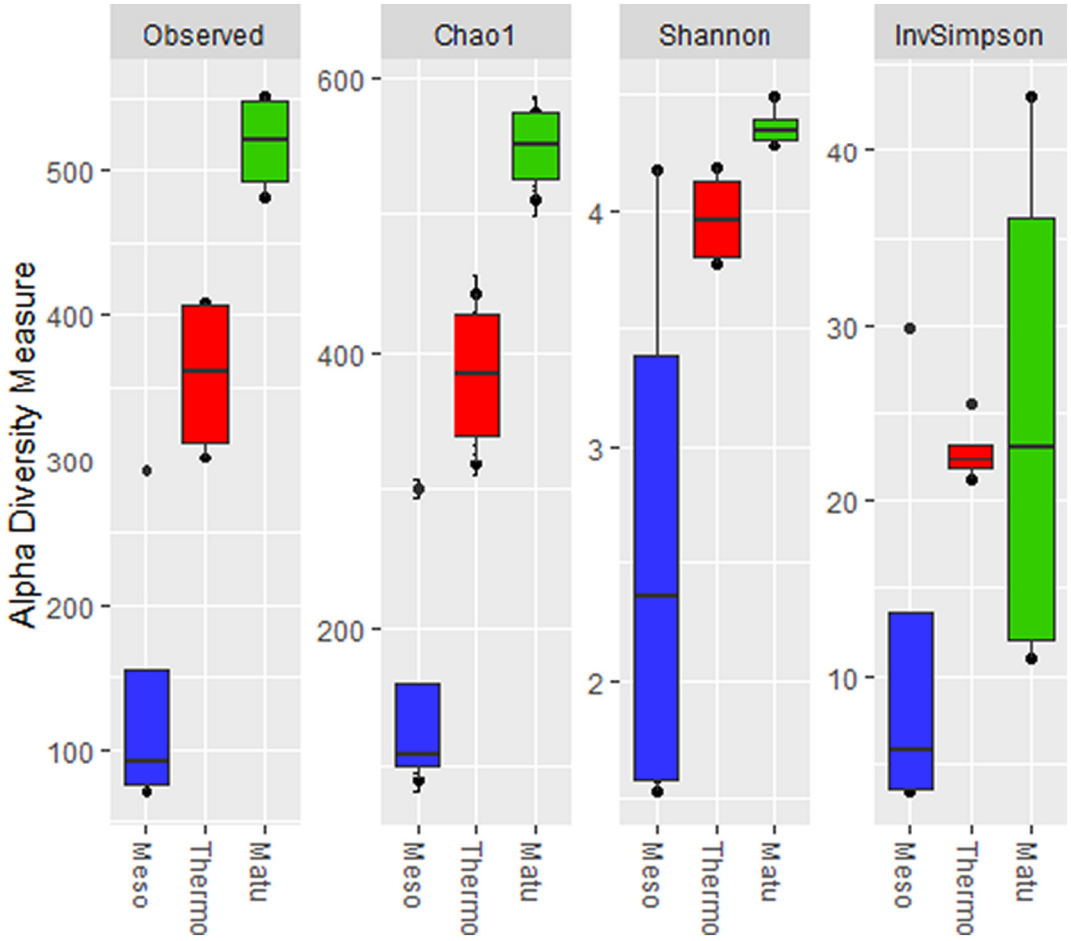


Fig. 2. Evolution of alpha diversity indices (observed OTUs and Chao1 richness; Shannon and inverse of Simpson diversity) obtained from 16S rRNA gene amplification of cDNA at mesophilic (Meso, green colour), thermophilic (Thermo, red colour) and maturation (Matu, blue colour) composting phases, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

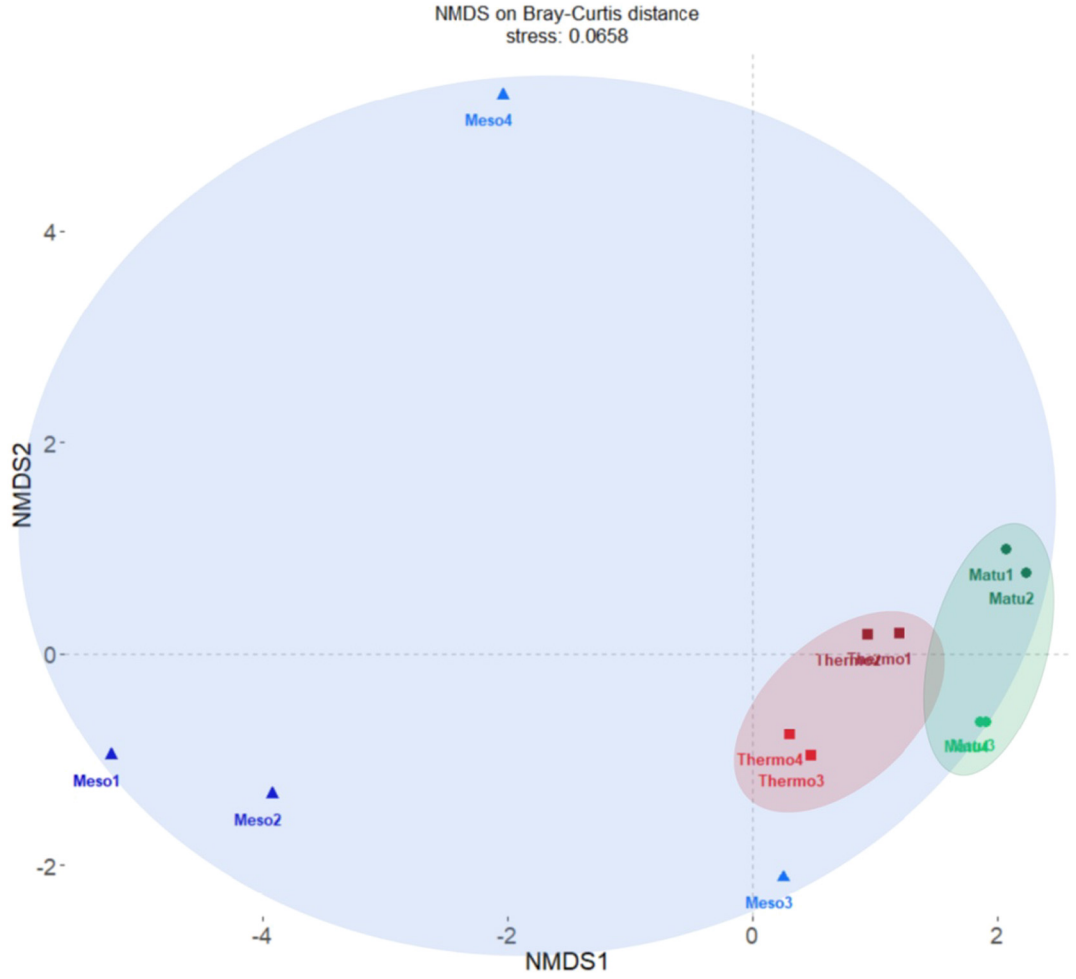


Fig. 3. Non-metric Multidimensional Scaling analysis (NMDS) based on Bray-Curtis dissimilarities of the libraries obtained by 16S rRNA gene amplification from cDNA at mesophilic (Meso1-4), thermophilic (Thermo1-4) and maturation (Matu1-4) phases respectively.

genera (with the exception of *Planifilum* and *Cohnella*) belong to Proteobacteria, the most abundant phylum detected, which decreased during the process. By contrast, *Carnobacterium, Olivibacter, Flavobacterium, Rhodococcus, Sphingobacterium, Pseudoxanthomonas, Bordetella, Cellvibrio, Desemzia* and *Actinotalea* proliferated mostly at the thermophilic phase. Finally, *Luteimonas, Parapedobacter,*

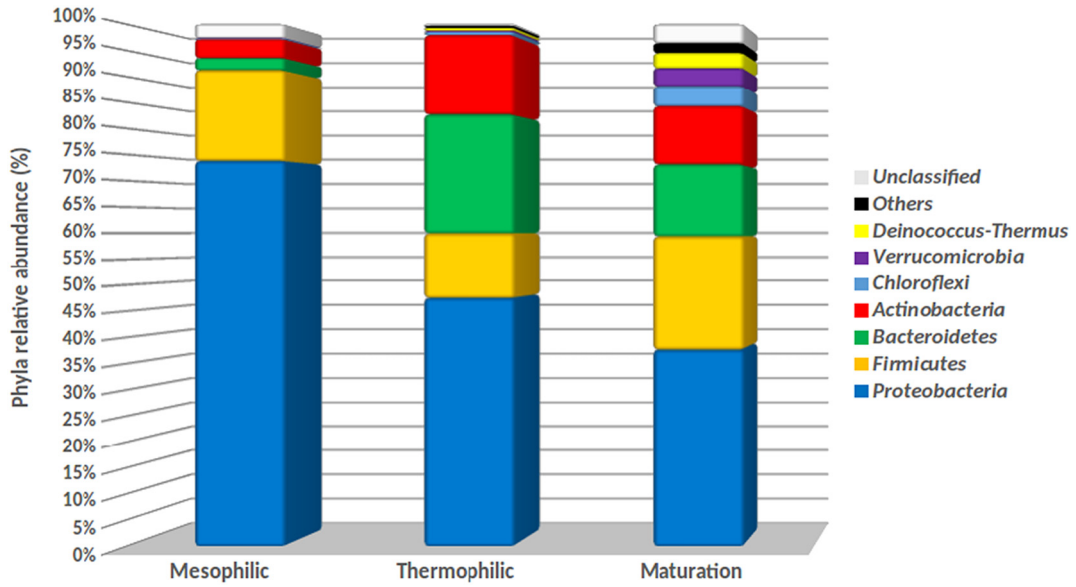


Fig. 4. Relative abundance of phyla identified from 16S rRNA gene amplification of cDNA during mesophilic, thermophilic and maturation phases.

Table 2
Main identified genera obtained from 16S rRNA gene amplification of cDNA at mesophilic (Meso1-4), thermophilic (Thermo1-4) and maturation (Matu1-4) libraries, which represented >1% of the total sequences obtained.

Phylum	Family	Genus	Meso1-4	Thermo1-4	Matu1-4
Decreasing during composting					
Proteobacteria	Xanthomonadaceae	*Stenotrophomonas*	20.9 ± 23.2	6.8 ± 7.6	0.2 ± 0.2
Proteobacteria	Halomonadaceae	*Halotalea*	16.1 ± 18.0	6.5 ± 4.6	0.0 ± 0.0
Proteobacteria	Pseudomonadaceae	*Pseudomonas*	12.7 ± 9.9	10.0 ± 2.0	3.0 ± 1.0
Proteobacteria	Moraxellaceae	*Acinetobacter*	8.5 ± 16.8	0.9 ± 1.1	0.6 ± 0.7
Firmicutes	Thermoactinomycetaceae_2	*Planifilum*	3.4 ± 6.4	0.1 ± 0.1	0.0 ± 0.0
Proteobacteria	Comamonadaceae	*Delftia*	2.6 ± 4.8	0.0 ± 0.0	0.0 ± 0.0
Proteobacteria	Comamonadaceae	*Pelomonas*	2.3 ± 4.2	0.0 ± 0.0	0.0 ± 0.0
Proteobacteria	Acetobacteraceae	*Komagataeibacter*	2.0 ± 4.1	0.0 ± 0.0	0.0 ± 0.0
Proteobacteria	Acetobacteraceae	*Gluconobacter*	1.4 ± 2.9	0.0 ± 0.0	0.0 ± 0.0
Firmicutes	Paenibacillaceae_1	*Cohnella*	1.1 ± 1.4	0.0 ± 0.0	0.0 ± 0.0
Increasing only at thermophilic phase					
Firmicutes	Carnobacteriaceae	*Carnobacterium*	0.3 ± 0.2	7.7 ± 7.4	1.1 ± 1.3
Bacteroidetes	Sphingobacteriaceae	*Olivibacter*	0.0 ± 0.0	5.7 ± 3.0	0.0 ± 0.0
Bacteroidetes	Flavobacteriaceae	*Flavobacterium*	1.3 ± 2.4	4.6 ± 5.0	0.9 ± 0.3
Actinobacteria	Nocardiaceae	*Rhodococcus*	0.2 ± 0.3	3.2 ± 0.9	0.3 ± 0.2
Bacteroidetes	Sphingobacteriaceae	*Sphingobacterium*	0.0 ± 0.0	3.8 ± 3.8	0.1 ± 0.0
Proteobacteria	Xanthomonadaceae	*Pseudoxanthomonas*	0.0 ± 0.0	2.9 ± 3.1	0.2 ± 0.1
Proteobacteria	Alcaligenaceae	*Bordetella*	0.0 ± 0.0	1.8 ± 0.8	0.0 ± 0.0
Proteobacteria	Pseudomonadaceae	*Cellvibrio*	0.0 ± 0.1	1.5 ± 1.7	0.3 ± 0.3
Firmicutes	Carnobacteriaceae	*Desemzia*	0.0 ± 0.1	1.4 ± 1.1	0.6 ± 0.6
Actinobacteria	Cellulomonadaceae	*Actinotalea*	0.0 ± 0.1	1.3 ± 0.9	0.2 ± 0.2
Actinobacteria	Nocardioidaceae	*Aeromicrobium*	0.0 ± 0.0	1.2 ± 1.2	0.3 ± 0.1
Actinobacteria	Nocardiopsaceae	*Nocardiopsis*	0.1 ± 0.2	1.1 ± 1.2	0.0 ± 0.0
Increasing at maturation phase					
Proteobacteria	Xanthomonadaceae	*Luteimonas*	0.2 ± 0.3	1.2 ± 0.4	6.4 ± 5.5
Bacteroidetes	Sphingobacteriaceae	*Parapedobacter*	0.0 ± 0.1	3.7 ± 1.2	3.7 ± 4.3
Deinococcus-Thermus	Trueperaceae	*Truepera*	0.0 ± 0.0	0.5 ± 0.2	2.9 ± 2.2
Firmicutes	Planococcaceae	*Planomicrobium*	0.0 ± 0.0	0.0 ± 0.0	2.1 ± 1.9
Bacteroidetes	Cyclobacteriaceae	*Algoriphagus*	0.0 ± 0.0	0.0 ± 0.0	1.9 ± 2.0
Proteobacteria	Nannocystaceae	*Nannocystis*	0.0 ± 0.1	0.0 ± 0.1	1.3 ± 1.5
Chloroflexi	Caldilineaceae	*Litorilinea*	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.0

Truepera, Planomicrobium, Algoriphagus, Nannocystis and *Litorilinea* proliferated exclusively at the maturation phase.

3.3. Correlations between active bacteria and physicochemical parameters

Pearson correlation matrix between physicochemical parameters and metabolically active bacteria (Table 3) showed positive correlations between *Stenotrophomonas, Halotalea, Pseudomonas* or *Cohnella* with hemicellulose, fat and WSCH contents, and also, negative correlations with HR or P_{AH}. By contrast, genera like *Luteimonas, Parapedobacter, Truepera, Planomicrobium, Algoriphagus, Nannocystis* or *Litorilinea* showed the opposite tendency, showing negative correlations with temperature, EC and the organic matter parameters (OM, hemicellulose, T_{OC}, T_{OC}/T_N, fat WSC and WSCH) and positive with pH, T_N and the humification's counterparts (lignin content, HR, HD or P_{AH}).

3.4. Prediction of metabolic functions of active bacteria during composting

The assignment of the functional profiles could only be ascribed to 25.31% of the total OTUs identified at the mesophilic, thermophilic and maturation phases (Table 4). The most prominent functions were related to C cycle (organic matter degradation), N cycle and Parasites (or pathogen reduction).

In the former category, the proportion of "chemoheterotrophy" and "aerobic chemoheterotrophy" predicted functions (with *Pseudomonas*, *Flavobacterium* and *Acinetobacter* as the main contributors) reached to 41%, 46% and 34% at the mesophilic, thermophilic and maturation phases, respectively (Table S2). As expected, the abundance of most of the metabolic functions detected like "aerobic chemoheterotrophy", "aromatic compound degradation" or "chemoheterotrophy" decreased during composting. This transformation decreased during the process, as well as the relative abundance of *Flavobacterium, Pseudomonas, Acinetobacter* or *Halocella,* among others. By contrast, *Rhodococcus* contributed most to metabolic functions such as "hydrocarbon degradation", "aromatic hydrocarbon degradation" and "aliphatic non-methane hydrocarbon degradation" during thermophilic phase, when the organic matter degradation was more relevant.

N cycle functions such as "nitrate reduction", "nitrate respiration" and "nitrogen respiration" had a relevant role during composting, decreasing during the process. The principal contributors to these functions were *Stenotrophomonas*, *Achromobacter* and *Paracoccus*, as well as *Opitutus*, *Hydrogenispora* and *Nitrospira*, which also decreased their abundance during the process.

Especially remarkable was the reduction ($p < 0.05$) registered during the process for functions related to pathogens such us "animal parasites" or "symbionts" and "human pathogen all", with the well-known human pathogens *Stenotrophomonas*, *Acinetobacter and Roseomonas* as the principal contributors.

4. Discussion

qPCR is a valuable and reproducible method for quantification of the total microbial abundance in organic wastes and composts (Galitskaya et al., 2017; Meng et al., 2020; Sun et al., 2020; Tortosa et al., 2020). Also, it can be applied for studying growing kinetics and decay of viable bacteria during organic waste treatments (Li et al., 2014; Ge et al., 2019). DNA amplification methodologies provide reliable information about total microbial abundance of an environmental sample but do not differentiate between inactive (dormant or dead) and metabolically active soil microbiome members (Steven et al., 2017). For that, the amplification of cDNA from retrotranscribed RNA seems to be a valuable approach for the estimation of metabolically active members of bacterial communities (Steven et al., 2017; Meng et al., 2020). According to results, the abundance of total (DNA) and active (cDNA) bacteria

Table 3
Pearson correlation matrix ($n = 12$) between physicochemical parameters of compost and the main genera identified from the 16S rRNA gene amplification of cDNA during mesophilic, thermophilic and maturation phases, respectively. Green and red folders represent positive and negative correlations.

Genus	Moisture	Temp.	EC	OM	Hemi	T_{OC}	T_{OC}/T_N	Fat	WSC	WSCH	pH	T_N	Lig.	HR	HD	P_{AH}	GI
Decreasing during composting																	
Stenotrophomonas					0.62*			0.59*		0.71**				-0.85**		-0.62*	
Halotalea					0.63*			0.57*		0.67*				-0.71**		-0.59*	
Pseudomonas			0.66*		0.62*	0.60*		0.58*		0.71**				-0.79**		-0.62*	-0.63*
Planifilum															-0.55*		
Cohnella					0.61*			0.57*		0.67*				-0.72**			
Increasing only at thermophilic phase																	
Carnobacterium	-0.75**																
Olivibacter		0.56*															
Flavobacterium																	
Rhodococcus	-0.67*	0.69**															
Sphingobacterium												0.57*					
Pseusoxanthomonas	-0.71**																
Bordetella	-0.70**	0.68*															
Cellvibrio												0.70**					
Desemzia	-0.84**																
Aeromicrobium	-0.75**											0.59*					
Nocardiopsis		0.56*										0.57*					
Increasing at maturation phase																	
Luteimonas		-0.66*	-0.70**		-0.70**	-0.68*	-0.82**	-0.60*		-0.55*	0.80**		0.75**	0.73**	0.60*	0.68**	0.74**
Parapedobacter					-0.64*		-0.59*	-0.55*	-0.61*		0.62*	0.82**		0.63*	0.61*	0.61*	
Truepera		-0.69**	-0.73**	-0.59*	-0.72**	-0.72**	-0.85*	-0.64*		-0.59*	0.83**		0.78**	0.73**	0.63*	0.71**	0.78**
Planomicrobium		-0.66*	-0.73**	-0.61*		-0.74**		-0.58*	-0.46	-0.63*			0.63*				0.66*
Algoriphagus		-0.65*	-0.63*		-0.60*	-0.61*	-0.79**				0.75**	0.56*	0.69**	0.67*		0.59*	0.67*
Nannocystis		-0.62*	-0.59*		-0.58*	-0.57*	-0.76**				0.72**	0.59*	0.65*	0.67*		0.56*	0.64*
Litorilinea		-0.62*	-0.69**	-0.58*		-0.70**		-0.55*		-0.61*			0.58*				0.62*

Temp.: temperature, EC: Electrical conductivity, OM: organic matter, Hemi.: Hemicelloluse, T_{OC}: total organic carbon, WSC: water-soluble carbon, WSCH: water-soluble carbohydrates, T_N: total nitrogen, Lig.: Lignine, HR: humification ratio, HD: humification degree, P_{AH}: percentage of humic acids, GI: germination index.
* Significant at $p < 0.05$. ** Significant at $p < 0.01$.

increased during AL composting. These findings agree with the statement that composting is a biological process, in which the autochthonous microbial community proliferates during organic matter degradation, especially during the bio-oxidative phase (de Bertoldi et al., 1983; Ryckeboer et al., 2003; López-González et al., 2015a, 2015b). Indeed, several reports have found similar tendencies in bacterial abundance during industrial sewage sludge composting (Galitskaya et al., 2017) or compost bedding for dairy cows (Sun et al., 2020). Our data confirmed that the thermophilic phase is the most active stage during composting, in which the most recalcitrant organic matter compounds are degraded and the microbial pathogens are eliminated (Ryckeboer et al., 2003; Alburquerque et al., 2009; López-González et al., 2015a).

DNA metabarcoding based on high-throughput sequencing technology is nowadays becoming a fast and cost-effective tool to assess microbial diversity in natural and man-made environments, providing valuable and supplemental information to cultured-based methods (Deiner et al., 2017; Garlapati et al., 2019). Illumina Miseq is one of the most prominent sequencing technologies applied for studying microbial diversity of samples subjected to organic waste treatments like anaerobic digestion, composting or vermicomposting (Tortosa et al., 2017; Ruiz-Sánchez et al., 2019; Jurado et al., 2020; Kolbe et al., 2019). In this study, the alpha diversity indices of active bacteria (cDNA) increased during AL composting. These data agreed with those found by Wang et al. (2019) and Ding et al. (2020), who analysed cDNA from different organic wastes (tomato stalk, lawn grass, cow dung and anaerobic digested residue) composting. In addition, a similar trend was observed in alpha diversity when DNA-based sequencing was performed (Tortosa et al., 2017; Wang et al., 2019). These findings confirmed that composting increases microbial succession and bioavailability over time (Fierer et al., 2010), especially during bio-oxidative phase, when the degradation of the organic matter and humification takes place (Alburquerque et al., 2009). As mentioned before, cDNA sequencing can provide reliable information about active microbial populations at each phase of composting (Steven et al., 2017; Wang et al., 2019; Ding et al., 2020). Results found Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria as the main phyla contributors. These phyla seem to play a prominent role during the process since they are ubiquitous in soils, plant-associated microbiota and composts (Wang et al., 2019; Ding et al., 2020).

The implication of bacterial populations on physicochemical transformation during composting is nowadays becoming an interesting issue for the optimization of the process itself (Wang et al., 2018, 2019; Ding et al., 2020; Jurado et al., 2020; Kong et al., 2020; Meng et al., 2020; Sun et al., 2020; Zhang et al., 2020). During AL composting, several fractions of the raw organic matter were transformed, being especially remarkable the degradation of lignin, hemicellulose and cellulose, and the easily-degradable fractions (fats, WSC and WSCH, respectively). Also, an increase in pH, T_N or humification parameters (HR, HD, P_{AH}) and a reduction in EC and phytotoxiciy (GI) were commonly observed (Alburquerque et al., 2009; Tortosa et al., 2012, 2017). In this research, the Pearson correlation matrix between physicochemical parameters and the abundance of metabolically active bacteria (Table 3) showed positive correlations between *Stenotrophomonas, Halotalea, Pseudomonas* or *Cohnella* with hemicellulose, fat and WSCH contents, and also, negative correlations with HR or P_{AH}. That means that these genera, which decreased their relative abundance during composting, could have an important role during the organic matter degradation at bio-oxidative stage (*meso* and thermophilic phases) and their

Table 4
Functional annotation of taxa assigned by FAPROTAX from 16S rRNA gene amplification of cDNA at mesophilic, thermophilic and maturation phases respectively, and relative abundance of each identified function.

Metabolic functions	Mesophilic	Thermophilic	Maturation	Main taxa contribution
C cycle				
Chemoheterotrophy	21.19ab	27.05a	18.21b	*Flavobacterium, Pseudomonas, Acinetobacter, Nocardioides, Euzebya, Halocella, Hyphomicrobium, Cellvibrio, Opitutus*
Aerobic chemoheterotrophy	19.75	18.66	15.54	*Pseudomonas, Flavobacterium, Acinetobacter, Nocardioides, Hyphomicrobium, Euzebya*
Fermentation	1.38	6.10	2.09	*Halocella, Opitutus, Verrucomicrobium*
Aromatic compound degradation	3.10ab	2.56a	0.73b	*Acinetobacter, Nocardioides, Rhodococcus*
Hydrocarbon degradation	0.29b	1.66a	0.32b	*Rhodoccocus*
Aromatic hydrocarbon degradation	0.12b	1.66a	0.21b	*Rhodoccocus*
Aliphatic non-methane hydrocarbon degradation	0.12a	1.66b	0.21a	*Rhodoccocus*
Cellulolysis	0.13	0.63	0.24	*Halocella, Cellvibrio*
Methylotrophy	0.08	0.03	0.17	*Paracoccus*
Methanol_oxidation	0.08	0.03	0.06	*Paracoccus*
Methanotrophy	0.00	0.00	0.11	*Methylopila, Methylobacter, Methylocaldum*
Phototrophy	0.01	0.00	0.22	*Rhodobacter*
Photoheterotrophy	0.00	0.00	0.22	*Rhodobacter*
C and N cycles				
Ureolysis	0.26	0.24	0.29	*Verrucomicrobium, Roseomonas*
Chitinolysis	0.01	0.00	0.05	*Lysobacter*
N cycle				
Nitrate reduction	5.90	3.17	0.29	*Opitutus, Stenotrophomonas*
Nitrate respiration	5.53	3.14	0.16	*Stenotrophomonas, Achromobacter, Paracoccus*
Nitrogen respiration	5.53	3.14	0.16	*Stenotrophomonas, Achromobacter, Paracoccus*
Nitrogen fixation	0.04	0.11	0.08	*Hydrogenispora, Bradyrhizobium, Azotobacter*
Nitrate denitrification	0.01	0.02	0.02	*Paracoccus*
Nitrite denitrification	0.01	0.02	0.02	*Paracoccus*
Nitrous oxide denitrification	0.01	0.02	0.02	*Paracoccus*
Denitrification	0.01	0.02	0.02	*Paracoccus*
Nitrite respiration	0.01	0.02	0.02	*Stenotrophomonas, Achromobacter, Paracoccus*
Aerobic ammonia oxidation	0.00	0.00	0.01	*Nitrosospira*
Nitrification	0.00	0.00	0.01	*Nitrosospira*
S cycle				
Sulfate respiration	0.02	0.00	0.03	*Desulfomicrobium, Desulfovibrio, Syntrophobacter*
Respiration of sulfur compounds	0.02	0.00	0.03	*Desulfomicrobium, Desulfovibrio, Syntrophobacter*
Dark oxidation of sulfur compounds	0.00	0.00	0.02	*Bosea*
Parasites				
Animal parasites or symbionts	8.49a	4.54a	0.68b	*Stenotrophomonas, Acinetobacter, Roseomonas*
Human pathogens_all	8.47a	3.58ab	0.65b	*Stenotrophomonas, Acinetobacter, Roseomonas*
Human gut	0.19	0.01	0.00	*Citrobacter, Escherichia/Shigella*
Mammal gut	0.19	0.01	0.00	*Citrobacter, Escherichia/Shigella*
Predatory or exoparasitic	0.01	0.00	0.13	*Bdellovibrio, Phaselicystis*
Other				
Dark hydrogen oxidation	0.01	0.02	0.23	*Paracoccus, Hydrogenispora*
Total contribution (%)	81.0	78.1	41.3	

For each function, values followed by the same lower-case letter among the mesophilic, thermophilic and maturation composting phases are not statistically different according to one-way ANOVA with Tukey-Kramer post-hoc test at $p < 0.05$.

contribution to the composting humification could be scarce By contrast, genera like *Luteimonas, Parapedobacter, Truepera, Planomicrobium, Algoriphagus, Nannocystis* or *Litorilinea* showed the opposite tendency, showing negative correlations with temperature, EC and the organic matter parameters (OM, hemicellulose, T_{OC}, T_{OC}/T_N, fat WSC and WSCH) and positive with pH, T_N and the humification's counterparts (lignin content, HR, HD or P_{AH}). These findings suggest that these genera, which increased their relative abundance only during maturation, could have a relevant role during the composting humification.

As mentioned before, active bacterial specialisation found during the process and their metabolic function predictions confirmed that composting is a complex biological process (Jurado et al., 2014; López-González et al., 2015a, 2015b; Jurado et al., 2020). C and N cycle transformation, and pathogen reduction were the main metabolic functions detected in AL composting. *Pseudomonas*, *Flavobacterium* and *Acinetobacter,* which are commonly found in composts (Tortosa et al., 2017; Zhang et al., 2020; Ding et al., 2020) and possess a versatile metabolism which can be leveraged for organic matter degradation and bioremediation (Filonov et al., 2020; Ma et al., 2020; Phulpoto et al., 2021), were mainly involved in C cycle transformations. Also, predictions of C functions confirmed composting as an aerobic process, in which several bacteria transform the organic matter to obtain energy and carbon substances (Insam et al., 2010; López-González et al., 2015a). It is well known that AL is a lignocellulosic material (Alburquerque et al., 2009; Muktadirul Bari Chowdhury et al., 2013) and can be properly degraded by *Rhodococcus* (Xu et al., 2018), which is considered as a promising actinobacteria for environmental biotechnologies (Krivoruchko et al., 2019). *Stenotrophomonas,* a ubiquitous gamma-proteobacteria commonly found in composts (Yang et al., 2006), can be used to enhance the composing process (Nevita et al., 2018). *Stenotrophomonas* and *Achromobacter* have a relevant role in N cycle during mesophilic composting of agroindustrial wastes (Pepe et al., 2013). *Paracoccus,* a gram-negative bacteria with an important denitrifier capacity (Yang et al., 2020), is also commonly found in cow manure composts (Meng et al., 2020). As expected, *Stenotrophomonas*, *Acinetobacter* and *Roseomonas* decreased during the process, which confirm to composting as a reliable procedure for the organic waste disinfection and pathogen reduction of the end-product (Bustamante et al., 2008).

5. Conclusions and future prospects

RNA-based high-throughput sequencing analyses are a valuable tool to discern the involvement of the active bacterial communities in the organic matter degradation during composting. The abundance of active bacteria assessed by qPCR of 16S rRNA gene from cDNA confirmed to thermophilic as the most active phase of the process. During composting, a different evolution of some specific groups of bacteria was clearly detected. Statistical correlation between bacterial diversity and physicochemical transformations during composting suggested a bacterial specialisation, with some genera specifically involved in the organic matter degradation during the bio-oxidative phase, meanwhile other were only related to maturation and the humification process. These findings were confirmed with the prediction of a sizeable amount of metabolic functions of the active bacteria, being those related to C cycle (organic matter degradation), N cycle and Parasites (or pathogen reduction) the most prominent functions.

According to our results it can be concluded that some microorganisms such as *Luteimonas, Parapedobacter or Planomicrobium* among others, which increased their relative abundance during maturation, could have a relevant role during the composting humification. In order to confirm this hypothesis, a future composting experiment based on microbial bioaugmentation should be carried out.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147975.

CRediT authorship contribution statement

Germán Tortosa: Conceptualization, Methodology, Investigation, Writing – original draft, Visualization. **Antonio J. Fernández-González:** Conceptualization, Methodology, Investigation, Writing – original draft, Visualization. **Ana V. Lasa:** Conceptualization, Methodology, Investigation, Writing – original draft, Visualization. **Elisabet Aranda:** Resources, Writing – review & editing. **Fernando Torralbo:** Methodology, Investigation, Writing – review & editing. **Carmen González-Murua:** Resources. **Manuel Fernández-López:** Resources. **Emilio Benítez:** Resources, Writing – review & editing. **Eulogio J. Bedmar:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgements

This work was supported by the ERDF-co-financed projects from Junta de Andalucía (Spain) [P12-AGR-1968], Spanish MINECO [AGL2015-64582-CO3-02, AGL2017-84745-R] and MINECO-CSIC RECUPERA 2020 [20134R070]. G. Tortosa thanks the farmer J. González Almendros for his financial and technical assistance during AL composting. Support by Basque Country Government [IT-583 932-16] and the Spanish Red Sirena [AGL2015-68881-REDT] are also acknowledged. E. Aranda thanks MINECO-ERDF for supporting her Ramón y Cajal project [RYC-2013-12481] and [CTM2017-84332-R]. Finally, Francis Lewis is also acknowledged for the written English revision.

References

Alburquerque, J.A., Gonzálvez, J., García, D., Cegarra, J., 2004. Agrochemical characterisation of "alperujo", a solid by-product of the two-phase centrifugation method for olive oil extraction. Bioresour. Technol. 91, 195–200. https://doi.org/10.1016/S0960-8524(03)00177-9.

Alburquerque, J.A., Gonzálvez, J., Tortosa, G., Baddi, G.A., Cegarra, J., 2009. Evaluation of "alperujo" composting based on organic matter degradation, humification and compost quality. Biodegradation. 20, 257–270. https://doi.org/10.1007/s10532-008-9218-y.

Antunes, L., Martins, L., Pereira, R., Thomas, A., Barbosa, D., Lemos, L., Silva, G., Moura, L., Epamino, G., Digiampietri, L., Lombardi, K., Ramos, P., Quaggio, R., de Oliveira, J., Pascon, R., Cruz, J., da Silva, A., Setubal, J., 2016. Microbial community structure and dynamics in thermophilic composting viewed through metagenomics and metatranscriptomics. Sci. Rep. 38915. https://doi.org/10.1038/srep38915.

Asshauer, K.P., Wemheuer, B., Daniel, R., Meinicke, P., 2015. Tax4Fun: predicting functional profiles frommetagenomic 16S rRNA data. Bioinformatics 31, 2882–2884. https://doi.org/10.1093/bioinformatics/btv287.

Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knigh, R., Mills, D.A., Caporaso, J.G., 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat. Methods 10, 57–59. https://doi.org/10.1038/nmeth.2276.

Bustamante, M.A., Moral, R., Paredes, C., Vargas-García, M.C., Suárez-Estrella, F., Moreno, J., 2008. Evolution of the pathogen content during co-composting of winery and distillery wastes. Bioresour. Technol. 99, 7299–7306. https://doi.org/10.1016/j.biortech.2007.12.051.

Castellano-Hinojosa, A., Maza-Márquez, P., Melero-Rubio, Y., González-López, J., Rodelas, B., 2018. Linking nitrous oxide emissions to populations dynamic of nitrifying and denitrifying prokaryotes in four full-scale wastewater treatment plant. Chemosphere. 200, 57–66. https://doi.org/10.1016/j.chemosphere.2018.02.102.

Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., Brown, C.T., Porras-Alfaro, A., Kuske, C.R., Tiedje, J.M., 2014. Ribosomal database project: data and tools for high throughput rRNA analysis. Nucleic Acids Res. 42, 633–642. https://doi.org/10.1093/nar/gkt1244.

de Bertoldi, M., Vallini, G., Pera, A., 1983. The biology of composting: a review. Waste Manag. Res. 1, 157–176. https://doi.org/10.1016/0734-242X(83)90055-1.

Deiner, K., Bik, H.M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D.M., de Vere, N., Pfrender, M.E., Bernatchez, L., 2017. Environmental DNA metabarcoding: transforming how we survey animal and plant communities. Mol. Ecol. 26, 5872–5895. https://doi.org/10.1111/mec.14350.

Dermeche, S., Nadour, M., Larroche, C., Moulti-Mati, F., Michaud, P., 2013. Olive mill wastes: biochemical characterizations and valorization strategies. Process Biochem. 48, 1532–1552. https://doi.org/10.1016/j.procbio.2013.07.010.

Diaz, L.F., Savage, G.M., 2007. In: Diaz, L.F., de Bertoldi, M., Bildingmaier, W., Stentiford, E. (Eds.), Factors that Affect the Process. Compost Science and Technology. Elsevier, Amsterdam, pp. 25–48.

Diaz, L.F., de Bertoldi, M., Bildingmaier, W., Stentiford, E., 2007. Compost Science and Technology. Elsevier, Amsterdam.

Ding, J., Wei, D., An, Z., Zhang, C., Jin, L., Wang, L., Li, Y., Li, Q., 2020. Succession of the bacterial community structure and functional prediction in two composting systems viewed through metatranscriptomics. Bioresour. Technol. 313, 123688. https://doi.org/10.1016/j.biortech.2020.123688.

Fernández-González, A., Villadas, P.J., Gómez-Lama, C., Valverde-Corredor, A., Belaj, A., Mercado-Blanco, J., Fernández-López, M., 2019. Defining the root endosphere and rhizosphere microbiomes from the world olive germplasm collection. Sci. Rep. 9, 20423. https://doi.org/10.1038/s41598-019-56977-9.

Fierer, N., Nemergut, D., Knight, R., Craine, J.M., 2010. Changes through time: integrating microorganisms into the study of succession. Res. Microbiol. 161, 635–642. https://doi.org/10.1016/j.resmic.2010.06.002.

Filonov, A., Delegan, Y., Puntus, I., Valentovich, L., Akhremchuk, A., Evdokimova, O., Funtikova, T., Zakharova, M., Akhmetov, L., Vetrova, A., Titok, M., 2020. Complete genome sequence of *Pseudomonas putida* BS3701, a promising polycyclic aromatic hydrocarbon-degrading strain for bioremediation technologies. Microbiol. Resour. Announc. 9 (40). https://doi.org/10.1128/MRA.00892-20 e00892-20.

Galitskaya, P., Biktasheva, L., Saveliev, A., Grigoryeva, T., Boulygina, E., Selivanovskaya, S., 2017. Fungal and bacterial successions in the process of co- composting of organic wastes as revealed by 454 pyrosequencing. PLoS One 12 (10), e0186051. https://doi.org/10.1371/journal.pone.0186051.

Garlapati, D., Charankumar, B., Ramu, K., Madeswaran, P., Ramana-Murthy, M.V., 2019. A review on the applications and recent advances in environmental DNA (eDNA) metagenomics. Rev. Environ. Sci. Biotechnol. 18, 389–411. https://doi.org/10.1007/s11157-019-09501-4.

Ge, J., Huang, G., Sun, X., Yin, H., Han, L., 2019. New insights into the kinetics of bacterial growth and decay in pig manure–wheat straw aerobic composting based on an optimized PMA–qPCR method. Microb. Biotechnol. 12, 502–514. https://doi.org/10.1111/1751-7915.13380.

Insam, H., de Bertoldi, M., 2007. In: Diaz, L.F., de Bertoldi, M., Bildingmaier, W., Stentiford, E. (Eds.), Microbiology of the Composting Process. Compost Science and Technology. Elsevier, Amsterdam, pp. 25–48.

Insam, H., Franke-Whittle, I., Goberna, M., 2010. Microbes in aerobic and anaerobic waste treatments. In: Insam, H., Franke-Whittle, I., Goberna, M. (Eds.), Microbes at Work. Springer-Verlag, Berlin Heidelberg, pp. 1–34.

Jurado, M., López, M.J., Suárez-Estrella, F., Vargas-García, M.C., López-González, J.A., Moreno, J., 2014. Exploiting composting biodiversity: study of the persistent and biotechnologically relevant microorganisms from lignocellulose-based composting. Bioresour. Technol. 162, 283–293. https://doi.org/10.1016/j.biortech.2014.03.145.

Jurado, M.M., Camelo-Castillo, A.J., Suárez-Estrella, F., López, M.J., López-González, J.A., Estrella-González, M.J., Síles-Castellano, A.B., Moreno, J., 2020. Integral approach using bacterial microbiome to stabilize municipal solid waste. J. Environ. Manag. 265, 110528. https://doi.org/10.1016/j.jenvman.2020.110528.

Kolbe, A.R., Aira, M., Gómez-Brandón, M., Pérez-Losada, M., Domínguez, J., 2019. Bacterial succession and functional diversity during vermicomposting of the white grape marc *Vitis vinifera* v. Albariño. Sci. Rep. 9, 7472. https://doi.org/10.1038/s41598-019-43907-y.

Kong, Z., Wang, W., Wang, M., Chai, L., Wang, X., Liu, D., Shen, Q., 2020. Bacterial ecosystem functioning in organic matter biodegradation of different composting at the thermophilic phase. Bioresour. Technol. 317, 123990. https://doi.org/10.1016/j.biortech.2020.123990.

Krivoruchko, A., Kuyukina, M., Ivshina, I., 2019. Advanced *Rhodococcus* biocatalysts for environmental biotechnologies. Catalysts. 9, 236. https://doi.org/10.3390/catal9030236.

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Thurber, R.L.V., Knight, R., Beiko, R.G., Huttenhower, C., 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat. Biotechnol. 31, 814–821. https://doi.org/10.1038/nbt.2676.

Li, D., Tong, T., Zeng, S., Lin, Y., Wu, S., He, M., 2014. Quantification of viable bacteria in wastewater treatment plants by using propidium monoazide combined with quantitative PCR (PMA-qPCR). J. Environ. Sci. 26, 299–306. https://doi.org/10.1016/S1001-0742(13)60425-8.

López-González, J., Suárez-Estrella, F., Vargas-García, M., López, M., Jurado, M., Moreno, J., 2015a. Dynamics of bacterial microbiota during lignocellulosic waste composting: studies upon its structure, functionality and biodiversity. Bioresour. Technol. 175, 406–416. https://doi.org/10.1016/j.biortech.2014.10.123.

López-González, J., Vargas-García, M., López, M., Suárez-Estrella, F., Jurado, M., Moreno, J., 2015b. Biodiversity and succession of mycobiota associated to agricultural lignocellulosic waste-based composting. Bioresour. Technol. 187, 305–313. https://doi.org/10.1016/j.biortech.2015.03.124.

Louca, S., Parfrey, L.W., Doebeli, M., 2016. Decoupling function and taxonomy in the global ocean microbiome. Science. 353, 1272–1277. https://doi.org/10.1126/science.aaf4507.

Ma, Q., Liu, S., Li, S., Hu, J., Tang, M., Sun, Y., 2020. Removal of malodorant skatole by two enriched microbial consortia: performance, dynamic, function prediction and bacteria isolation. Sci. Total Environ. 725, 138416. https://doi.org/10.1016/j.scitotenv.2020.138416.

McGrath, K.C., Thomas-Hall, S.R., Cheng, S.T., Leo, L., Alexa, A., Schmidt, S., Schenk, P.M., 2008. Isolation and analysis of mRNA from environmental microbial communities. J. Microbiol. Methods 75, 172–176. https://doi.org/10.1016/j.mimet.2008.05.019.

Meng, Q., Han, Y., Zhu, H., Yang, W., Bello, A., Deng, L., Jiang, X., Wu, X., Sheng, S., Xu, Y., Xu, X., 2020. Differences in distribution of functional microorganism at DNA and cDNA levels in cow manure composting. Ecotox. Environ. Safe. 191, 110161. https://doi.org/10.1016/j.ecoenv.2019.110161.

Muktadirul Bari Chowdhury, A.K.M., Akratos, C.S., Vayenas, D.V., Pavlou, S., 2013. Olive mill waste composting: a review. Int. Biodeterior. Biodegrad. 85, 108–119. https://doi.org/10.1016/j.ibiod.2013.06.019.

Muyzer, G., De Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59, 695–700.

Nevita, T., Sharma, G.D., Pandey, P., 2018. Composting of rice-residues using lignocellulolytic plant-probiotic *Stenotrophomonas maltophilia*, and its evaluation for growth enhancement of *Oryza sativa* L. Environ. Sust. 1, 185–196. https://doi.org/10.1007/s42398-018-0017-z.

Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G., 2014. STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics https://doi.org/10.1093/bioinformatics/btu494.

Pepe, O., Ventorino, V., Blaiotta, G., 2013. Dynamic of functional microbial groups during mesophilic composting of agro-industrial wastes and free-living (N_2)-fixing bacteria application. Waste Manag. 33 (7), 1616–1625. https://doi.org/10.1016/j.wasman.2013.03.025.

Phulpoto, I.A., Hu, B., Wang, Y., Ndayisenga, F., Li, J., Yu, Z., 2021. Effect of natural microbiome and culturable biosurfactants-producing bacterial consortia of freshwater lake on petroleum-hydrocarbon degradation. Sci. Total Environ. 751, 141720. https://doi.org/10.1016/j.scitotenv.2020.141720.

Roig, A., Cayuela, M.L., Sanchez-Monedero, M.A., 2006. An overview on olive mill wastes and their valorization methods. Waste Manag. 26, 960–969. https://doi.org/10.1016/j.wasman.2005.07.024.

Ruiz-Sánchez, J., Guivernau, M., Fernández, B., Vila, J., Viñas, M., Riau, V., Prenafeta-Boldú, F.X., 2019. Functional biodiversity and plasticity of methanogenic biomass from a full-scale mesophilic anaerobic digester treating nitrogen-rich agricultural wastes. Sci. Total Environ. 649, 760–769. https://doi.org/10.1016/j.scitotenv.2018.08.165.

Ryckeboer, J., Mergaert, J., Vaes, K., Klammer, S., De Clercq, D., Coosemans, J., Insam, H., Swings, J., 2003. A survey of bacteria and fungi occurring during composting and self-heating processes. Ann. Microbiol. 53, 349–410.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09.

Srivastava, V., Squartini, A., Masi, A., Sarkar, A., Pratap Singh, R., 2021. Metabarcoding analysis of the bacterial succession during vermicomposting of municipal solid waste employing the earthworm *Eisenia fetida*. Sci. Total Environ. 766, 144389. https://doi.org/10.1016/j.scitotenv.2020.144389.

Steven, B., Hesse, C., Soghigian, J., Gallegos-Graves, L.V., Dunbar, J. 2017. Simulated rRNA/DNA ratios show potential to misclassify active populations as dormant. Appl. Environ. Microbiol. 83:e00696–17. doi:https://doi.org/10.1128/AEM.00696-17.

Sun, L., Han, X., Li, J., Zhao, Z., Liu, Y., Xi, Q., Guo, X., Gun, S., 2020. Microbial community and its association with physicochemical factors during compost bedding for dairy cows. Front. Microbiol. 11, 254. https://doi.org/10.3389/fmicb.2020.00254.

Takahashi, S., Tomita, J., Nishioka, K., Hisada, T., Nishijima, M., 2014. Development of a prokaryotic universal primer for simultaneous analysis of bacteria and archaea using next generation sequencing. PLoS One, e105592 https://doi.org/10.1371/journal.pone.0105592.

Tortosa, G., Alburquerque, J.A., Ait Baddi, G., Cegarra, J., 2012. The production of commercial organic amendments and fertilisers by composting of two-phase olive mill waste ("alperujo"). J. Clean. Prod. 26, 48–55. https://doi.org/10.1016/j.jclepro.2011.12.008.

Tortosa, G., Castellano-Hinojosa, A., Correa-Galeote, D., Bedmar, E., 2017. Evolution of bacterial diversity during two-phase olive mill waste ("alperujo") composting by 16S rRNA gene pyrosequencing. Bioresour. Technol. 224, 101–111. https://doi.org/10.1016/j.biortech.2016.11.098.

Tortosa, G., Torralbo, F., Maza-Márquez, P., Aranda, E., Calvo, C., González-Murua, C., Bedmar, E., 2020. Assessment of the diversity and abundance of the total and active fungal population and its correlation with humification during two-phase olive mill waste ("alperujo") composting. Bioresour. Technol. 295, 122267. https://doi.org/10.1016/j.biortech.2019.122267.

Větrovský, T., Baldrian, P., Morais, D., 2018. SEED 2: a user-friendly platform for amplicon high-throughput sequencing data analyses. Bioinformatics. 34, 2292–2294. https://doi.org/10.1093/bioinformatics/bty071.

Vivas, A., Moreno, B., Garcia-Rodriguez, S., Benitez, E., 2009. Assessing the impact of composting and vermicomposting on bacterial community size and structure, and microbial functional diversity of an olive-mill waste. Bioresour. Technol. 100, 1319–1326. https://doi.org/10.1016/j.biortech.2008.08.014.

Wang, K., Mao, H., Li, X., 2018. Functional characteristics and influence factors of microbial community in sewage sludge composting with inorganic bulking agent. Bioresour. Technol. 249, 527–535. https://doi.org/10.1016/j.biortech.2017.10.034.

Wang, T.T., Sun, Z.Y., Wang, S.P., Tang, Y.Q., Kida, K., 2019. Succession of total and active microbial community during the composting of anaerobic digested residue. Waste Biomass Valori. 11, 4677–4689. https://doi.org/10.1007/s12649-019-00779-7.

Xu, R., Zhang, K., Liu, P., Han, H., Zhao, S., Kakade, A., Khan, A., Du, D., Li, X., 2018. Lignin depolymerization and utilization by bacteria. Bioresour. Technol. 269, 557–566. https://doi.org/10.1016/j.biortech.2018.08.118.

Xu, Z., Ma, Y., Zhang, L., Han, Y., Yuan, J., Li, G., Luo, W., 2021. Relating bacterial dynamics and functions to gaseous emissions during composting of kitchen and garden wastes. Sci. Total Environ. 767, 144210. https://doi.org/10.1016/j.scitotenv.2020.144210.

Yang, H.C., Im, W.T., Kang, M.S., Shin, D.Y., Lee, S.T., 2006. *Stenotrophomonas koreensis* sp. nov., isolated from compost in South Korea. Int. J. Syst. Evol. Microbiol. 56, 81–84. https://doi.org/10.1099/ijs.0.63826-0.

Yang, J., Feng, L., Pi, S., Cui, D., Ma, F., Zhao, H.P., Li, A., 2020. A critical review of aerobic denitrification: insights into the intracellular electron transfer. Sci. Total Environ. 731, 139080. https://doi.org/10.1016/j.scitotenv.2020.139080.

Zhang, C., Gao, Z., Shi, W., Li, L., Tian, R., Huang, J., Lin, R., Wang, B., Zhou, B., 2020. Material conversion, microbial community composition and metabolic functional succession during green soybean hull composting. Bioresour. Technol. 316, 123823. https://doi.org/10.1016/j.biortech.2020.123823.

Zhu, L., Wei, Z., Yang, T., Zhao, X., Dang, Q., Chen, X., Wu, J., Zhao, Y., 2020. Core microorganisms promote the transformation of DOM fractions with different molecular weights to improve the stability during composting. Bioresour. Technol. 299, 122575. https://doi.org/10.1016/j.biortech.2019.122575.