Bioresource Technology 224 (2017) 101–111

Contents lists available at ScienceDirect

Bioresource Technology

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

Evolution of bacterial diversity during two-phase olive mill waste (“alperujo”) composting by 16S rRNA gene pyrosequencing

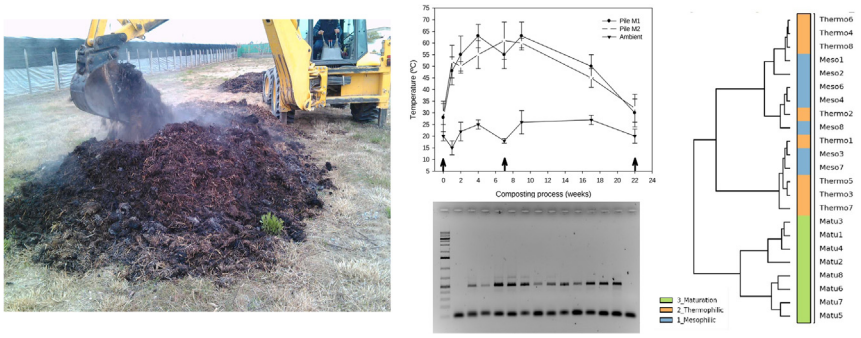
Germán Tortosa *, Antonio Castellano-Hinojosa, David Correa-Galeote, Eulogio J. Bedmar

Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín (EEZ), Agencia Estatal CSIC, E-419, 18080-Granada, Spain

HIGHLIGHTS

- Actinobacteria, Bacteriodetes, Firmicutes and Proteobacteria were the main phyla detected.
- Mesophilic and thermophilic phases did not affect bacterial population.
- Maturation increased bacterial diversity, especially due to new bacterial population were detected.
- *Planomicrobium* and *Ohtaekwangia* are proposed as biomarkers of AL composting maturation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 October 2016
Received in revised form 22 November 2016
Accepted 23 November 2016
Available online 29 November 2016

Keywords:
“Alperujo” composting
454-Pyrosequencing
Planomicrobium
Ohtaekwangia
Maturation biomarkers

ABSTRACT

Microorganisms are the main contributing factor responsible for organic matter degradation during composting. In this research, the 454-pyrosequencing of the 16S rRNA gene was used to elucidate evolution of bacterial diversity during mesophilic, thermophilic and maturation composting stages of the two-phase olive mill waste (“alperujo”), the main by-product of the Spanish olive oil industry. Two similar piles were performance composting AL with sheep manure as bulking agent. Actinobacteria, Bacteriodetes, Firmicutes and Proteobacteria were the main phyla found in genomic libraries from each composting phase. Shannon and Chao1 biodiversity indices showed a clear difference between the mesophilic/thermophilic and maturation phases, which was mainly due to detection of new genera. PCA analysis of the relative number of sequences confirmed maturation affected bacterial population structure, and Pearson correlation coefficients between physicochemical composting parameters and relative number of genera sequences suggest that *Planomicrobium* and *Ohtaekwangia* could be considered as biomarkers for AL composting maturation.

1. Introduction

To promote sustainable development, the European Union has adopted a legislative initiative called the Circular Economy (de Man and Friedge, 2016) whose objectives focus on reducing the use of raw materials, minimizing waste production, and encouraging reuse and recycling. In Spain, the olive oil industry generates a large amount of a solid by-product called “alperujo” (AL), a highly contaminative organic waste that needs to be treated for its revalorisation (Alburquerque et al., 2004). In order to achieve that, composting can be performed due to it being a simple, inexpensive and effective method for transforming organic waste as AL into organic amendments and fertilisers (Tortosa et al., 2012; Muktadirul Bari Chowdhury et al., 2013).

* Corresponding author.
E-mail address: german.tortosa@eez.csic.es (G. Tortosa).

http://dx.doi.org/10.1016/j.biortech.2016.11.098
0960-8524/

Composting is a biological process by which the organic matter from the raw materials is degraded by native microbial population due to its metabolic activity. This transformation is carried out by a complex temporal succession of a large number of microorganisms included bacteria, actinobacteria and fungi (López-González et al., 2015). Indeed, these population developments are directly affected by the evolution of several environmental factors of composting substrates like moisture, nutrient availability (especially carbon and nitrogen), oxygen rate, physical structure (particular size), pH, salinity, etc. (Insam et al., 2010). One of the main selective parameters in microbial evolution during composting is probably temperature, which defines the four phases of the process: mesophilic (25–40 °C), thermophilic (35–65 °C), cooling and maturation (Insam et al., 2010).

Bacterial diversity in composting has been investigated for decades using different approaches (culture and cultured-independent methods). Molecular methods like clone library sequencing, DNA fingerprinting, diagnostic microarrays or qPCR can be used successfully to monitor microbial community composition in detail (Hultman et al., 2010). Recent molecular tools based on DNA high-throughput sequencing technologies are considerably increasing the knowledge of microbial communities involved in composting. Until now, there are few examples of microbial ecology studies using this promising technology. Bibby et al. (2010) composted biosolids from wastewater treatment plants with sawdust, woodchips, or green waste, using a full scale windrow composting system. Partanen et al. (2010) worked in a pilot-scale compost plant, as well as a full-scale composting system using municipal biowaste mixed with wood chips. de Gannes et al. (2013) worked in a in-vessel rotary composting reactors (200L), using rice straw, sugar cane bagasse and coffee hulls which were amended with either cow or sheep manure. Neher et al. (2013) carried out the most complete study of microbial evolution during composting until now. They studied different compost recipes, composting systems (windrow, aerated piles and vermicomposting) and composting time. Tkachuk et al. (2014) used a static composting method for carcass, beef and cattle mortalities. Storey et al. (2015) worked in an industrial windrow composting plant, using shredded green waste and spent brewery grains with calcium ammonium nitrate or sludge from a wastewater plant as nitrogen amendments. Zhang et al. (2016) investigated in a 90-m^3 industrial-scale fermentor cornposting corn cobs mixed with fresh cow dung. Currently, no report using high-throughput sequencing applied to olive mill waste composting has been published. So, the information about bacterial communities evolution and dynamics during AL composting remains scarce.

In Tortosa et al. (2012), we demonstrated that composting is a feasible biotechnology for AL treatment and its revalorisation. Indeed, the physicochemical evolution of the organic matter degradation was studied in detail. In this research, we have focused on microbial aspects. We have used 454-pyrosequencing to study how microbial population and its evolution could be affected by the composting process. Also, we aimed at finding specific biomarkers for AL composting, with emphasis on the maturation process.

2. Materials and methods

2.1. Composting performance, raw material characterization and sampling procedure

Two trapezoidal piles, M1 and M2, of about 10 t each were arranged by mixing two-phase olive mill waste (“alperujo”, AL) and sheep manure (SM) (1:1, AL:SM, fresh weight) using a backhoe loader as previously indicated (Tortosa et al., 2012). The composting system was open and seven mechanical turnings were applied according to bioxidative phase and temperature evolution (Fig. 1), being more frequently during thermophilic phase (four turnings within the first two months). The substrate moisture was controlled by an aspersion system and kept above 40%.

To control and monitor the composting process, several parameters were analysed. Moisture content and temperature, pH, and electrical conductivity (EC), total nitrogen (T_N), total organic carbon (T_{OC}), total organic matter (OM), fat content, water-soluble carbon (WSC), water-soluble phenolic substances (WSPH), water-soluble carbohydrates (WSCH), lignin, cellulose and hemicellulose, and phytotoxicity by germination index (GI) tests with cress (*Lepidium sativum*) were determined as previously referred (Tortosa et al., 2012). The humification indices, humification ratio (HR), humification degree (HD) and percentage of humic acids (P_{AH}), were calculated according to Alburquerque et al. (2009).

Thirty (30) subsamples were randomly taken from the 0–25 cm layer of each pile after the mechanical turnings at the 1st, 7th and 22nd weeks, corresponding to the mesophilic, thermophilic and maturation phases, respectively (Fig. 1). Then, the samples were mixed, kept in a portable fridge, brought to the laboratory within 24 h of collection and maintained at −80 °C until DNA extraction and agrochemical characterization. For all experiments, a sample was defined as a composite of 30 subsamples collected at random depths from a given pile that were mixed to be representative of a pile. For each M1 and M2 piles, 4 analytical replicates were obtained from each composite sample corresponding to the mesophilic, thermophilic and maturation phases. A total of 24 replicates, 12 per pile, were then analysed.

2.2. DNA extraction and quantification

Frozen compost samples (~5 g) were homogenized with pestle and mortar under liquid nitrogen according to the recommendations by Neher et al. (2013). Then, DNA was extracted from 250 mg using the commercial PowerSoil® DNA isolation kit (MO-BIO) after mechanical breakage using a minibead beater cell disrupter (Mikro-Dismembrator S; Sartorius Stedim Biotech) for 30 s at 1600 rpm. Quality and size of DNAs were checked by electrophoresis on 1% agarose gel (40 min at 80 mV) stained with GelRed™ (Biotium) under UV light. DNAs were also quantified by spectrophotometry at 260 nm using a Nanodrop 1000 Spectrophotometer (Thermo Scientific).

2.3. Amplification and pyrosequencing of DNA

PCR amplification of the hypervariable V4-V5 regions of the 16S rRNA gene was performed over each individual DNA extraction using the coded-primer approach to multiplex pyrosequencing (Parameswaran et al., 2007). PCR was performed with an 8 bp key-tagged sequence joined to universal primers U519F and U926R (Baker et al., 2003). PCR reactions (50 µl) were done using a Master Taq kit 5 PRIME 1000U (http://www.5prime.com/) and contained 0.4 µM of each primer, 2.5 mM $MgCl_2$, 0.4 mM dNTPs mix (0.1 mM each), 1× MasterTaq buffer (1 mM Mg^{2+}), 1× Taq Master PCR Enhancer, 0.75 U of Taq DNA Polymerase, molecular-biology-grade water and 1–150 ng of the DNA template. The PCR program consisted of an initial denaturation step at 94 °C for 3 min, 25 cycles with denaturation at 94 °C for 15 s, primer annealing at 55 °C for 45 s and extension at 72 °C for 1 min, and finally, a heating step at 72 °C for 8 min for final extension. For each sample, only one amplicon per PCR reaction was generated and amplicon bands were quantified directly from agarose gel using BIO-RAD Quantity One software. All bands were equilibrated to the same concentration and checked again in 1% agarose gel. After that, samples were combined in equimolar amounts in one sample to reduce

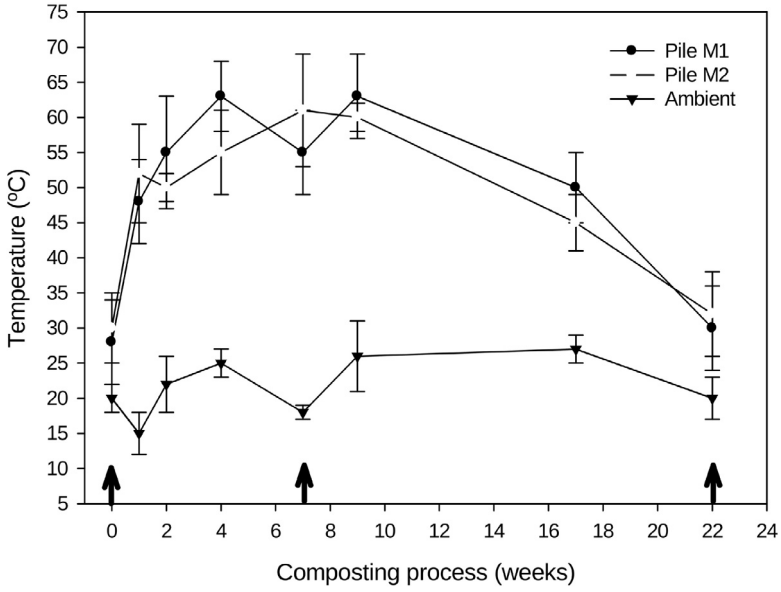


Fig. 1. Ambient and M1 and M2 pile temperatures during composting. Arrows show the time at which samples were taken: mesophilic (1st week), thermophilic (7th week) and maturation (22nd week) composting phases. Values are the mean of ten determinations and bars represent the standard deviation.

per-PCR variability and purified directly from agarose gel using Thermo Scientific GeneJET Gel Extraction Kit (k0691) according to the manufacturer's instructions. Finally, in order to check the presence of only one band, another agarose gel using three dilutions of the sample was done. After DNA quantification by Qubit fluorimetric, pyrosequencing of samples were carried out using a GS Junior Genome Sequencer 454 FLX System Roche at the facilities of Estación Experimental del Zaidin (EEZ-CSIC) sequencing service.

2.4. Taxonomic assignment of sequence reads and diversity indices

The 16S rDNA raw sequences were processed through the Ribosomal Data Project (RDP, release 11.3, http://pyro.cme.msu.edu) pyrosequencing pipeline supervised method (Cole et al., 2009). Sequences were trimmed for primers, quality filtered and assigned to DNA libraries according to their tags. For each M1 and M2 piles, 4 replicates were obtained from each one of the 3 composite samples corresponding to the mesophilic, thermophilic and maturation phases. DNA was independently extracted, pyrosequenced and RDP processed. That way, a total of 24 DNA libraries, 12 per pile, were obtained and their nomenclature is as follows:

Pile M1:
Meso1-4 (Meso1, Meso2, Meso3 and Meso4)
Thermo1-4 (Thermo1, Thermo2, Thermo3 and Thermo4)
Matu1-4 (Matu1, Matu2, Matu3 and Matu4)
Pile M2:
Meso5-8 (Meso5, Meso6, Meso7 and Meso8)
Thermo5-8 (Thermo5, Thermo6, Thermo7 and Thermo8)
Matu5-8 (Matu5, Matu6, Matu7 and Matu8)

Sequences shorter than 150 base pair, with quality scores <20 or containing any unresolved nucleotides were removed from the data set. Chimeras were identified using the Uchime tool from FunGene database and removed from the dataset. The RDP Classifier, a Bayesian rRNA classifying algorithm, was used to assign phylogenetic groups based on sequence similarity (80% of confidence level), and their relative abundances calculated. Matches with the RDP confidence estimate below 60% were designated as unclassified bacteria.

Sequences were also aligned using the Infernal alignment tool in RDP and clustered into operational taxonomic units (OTUs), defined at 97% similarity cut off using Complete Linkage Clustering RDP tool (unsupervised method). The number of sequences in each OTU was employed to calculate the Good's coverage index and Shannon (H') and Chao1 diversity indices respectively using RDP web tools available.

2.5. Statistical analyses

Descriptive statistical analyses (mean, absolute and relative errors) of relative sequence abundance were calculated for each pile and composting phase. Inferential statistical analyses were performed assuming normal distribution and homoscedasticity of the data using Statistical Analysis of Taxonomical and Functional Profiles (STAMP) open-source software v2.0.9 release (Parks et al., 2014). According to user's guide recommendations, multiples groups were analysed using one-way ANOVA with Tukey-Kramer *post-hoc* test at $p < 0.05$ to analyse statistical differences among obtained data during composting phases. Also, Storey's FDR for multiple correction and eta-squared for sample size correction tests were applied, respectively. The relationships among phyla found in M1 and M2 piles during substrate composting phases were studied using Principal Component Analysis (PCA) and the unweighted pair-group method with arithmetic average (UPGMA) plotted as a dendrogram. Pearson correlation coefficients and linear regressions were performed using GNU-PSPP open-source software v0.9.0 (available in https://www.gnu.org/software/pspp/) in order to calculate relationships between composting physico-chemical parameters and the genera identified during the process.

2.6. Accession numbers

Pyrosequencing reads are available at the EMBL-EBI European Nucleotide Archive Database (http://www.ebi.ac.uk/ena) under the accession number PRJEB15249.

3. Results and discussion

3.1. Composting process

A previous study by Tortosa et al. (2012) has shown that mechanical turning methodology applied to open windrow composting of AL with sheep manure as bulking agent results in the production of commercial organic amendments that can be used as plant fertilisers. Because the initial mixture used ensured appropriate porosity and aeration (Tortosa et al., 2012), the temperature increased rapidly from the beginning to reach the mesophilic phase in 1 week, followed by a thermophilic phase between the 2nd and the 17th weeks and a further maturation phase from the 17th to the 22nd weeks (Fig. 1). The composting substrate in each pile showed alkalinization along the process with initial pH values of 6.60 and 6.49 for piles M1 and M2, respectively, and of 7.83 and 7.51, respectively, at the end of the maturation phase (Table 1). The substrate of the piles also showed a clear reduction in salt content varying from 4.39 and 4.12 in the mesophilic phase to 1.16 and 1.05 dS m^{-1} in the maturation phase for piles M1 and M2, respectively (Table 1). Similar behaviour was found in Tortosa et al. (2012) and this reduction can be due to salt leaching produced by the irrigation system used for watering the piles, a process that has been discussed in the literature reviewed by Muktadirul Bari Chowdhury et al. (2013).

Olive mill wastes are characterized by an important organic matter composition, especially AL (Alburquerque et al., 2004). The initial content of OM for piles M1 and M2 was 77.3% and 80.3%, respectively, and, regardless of the pile analysed, lignin and hemicellulose were the most important fraction (Table 1). For each pile, an almost 30% and a 50% decrease in OM and hemicellulose content was found during the composting process, respectively (Table 1). These results agree with those published by Alburquerque et al. (2009), who composted AL with cotton waste, grape stalk, olive leaf and fresh cow bedding as bulking agents. Transformation of the organic matter by the microbial community during composting was also evident from the decreases in fat content, WSC, WSCH and WSPH throughout the process for each of the M1 and M2 piles (Table 1). The water-soluble organic matter fraction (WSC, WSCH and WSPH) is recognized as the most easily-bioassimilable by microbial communities, and are often used as indices to measure stability and maturation during composting (Bernal et al., 1998). On the other hand, transformation of the organic matter during composting increases humification (HD and P_{AH} close to 80% respectively) which, in turn, would result in reduction of the water soluble organic matter fraction, especially in WSCH and WSPH content (Sánchez-Monedero et al., 1999) (Table 1). The mature composts obtained were non-phytotoxic as their GI values were 92% and 94% for piles M1 and M2, respectively (Table 1). Other agrochemical characteristics of composts like T_{OC}, T_N and the T_{OC}/T_N ratio were similar to other previously published (Muktadirul Bari Chowdhury et al., 2013 and references therein).

3.2. Pyrosequencing and sequencing analysis

When compared to Sanger methods, high-throughput sequencing technologies such as 454-pyrosequencing provide a more complete, efficient and effective DNA amplification of bacterial population in environmental samples. Pyrosequencing of the V1-V9 hypervariable regions of 16S rRNA gene is a powerful tool to estimate prokaryotic biodiversity (Baker et al., 2003). In this study, a total of 129477 and 168950 sequences were obtained from the twelve 16S rDNA samples sent to pyrosequencing of each M1 and M2 piles, respectively, of which 76832 and 93281 were retained after filtering and removing chimeras (Table 2). Of those sequences, 4714 ± 1894, 3914 ± 564 and 4534 ± 1117 were found in Meso1-4, Thermo1-4 and Matu1-4 libraries and 6249 ± 1233, 7926 ± 1131 y 6305 ± 768 sequences in Meso5-8, Thermo5-8 and Matu5-8 libraries, respectively (Table 2). The libraries also contained 99 ± 55 (2.1%), 138 ± 82 (3.5%), and 242 ± 120 (5.3%) unclassified sequences for M1 pile and 137 ± 51 (2.2%), 214 ± 91 (2.7%), and 665 ± 233 (10.5%) for M2 pile respectively (Table 2). For a given pile, statistical differences ($p > 0.05$) in the number of sequences in the libraries corresponding to each composting phase were not found. The number of sequences obtained in this study is similar to that found by de Gannes et al. (2013) after composting different plant wastes mixed with manures (2695–7277) and

Table 1
Main physicochemical characteristics of composting substrates during the mesophilic, thermophilic and maturation phases in M1 and M2 composting piles.

Parameters[a]	M1 pile			M2 pile		
	Mesophilic	Thermophilic	Maturation	Mesophilic	Thermophilic	Maturation
Moisture	51.41c	43.18b	40.05a	54.84c	30.50a	36.91b
pH[b]	6.60a	7.06b	7.83c	6.49a	6.82b	7.51c
EC[b] (dS m^{-1})	4.39a	4.07a	1.16b	4.12a	3.62b	1.05c
OM (%)	77.3a	65.1b	55.7c	80.3a	61.2b	53.2c
Lignin (%)	31.1a	38.2b	35.1ab	29.4a	36.1b	28.7a
Cellulose (%)	16.2b	13.4a	14.2ab	14.1a	16.9b	17.3ab
Hemicellulose (%)	29.2c	17.2b	9.2a	28.4c	18.1b	12.2a
T_{OC} (%)	41.31c	39.03b	25.29a	42.08c	37.74b	25.25a
T_N (%)	1.51a	1.65b	1.71c	1.33a	1.42b	1.29c
T_{OC}/T_N ratio	27.40c	24.64b	14.75a	31.69c	28.58b	19.57a
Fat content	4.5c	2.1b	0.4a	4.8c	1.9b	0.3a
WSC (%)	5.6c	2.1b	1.8a	6.1c	2.5b	1.6a
WSCH (%)	1.9c	1.1b	0.2a	1.7c	0.9b	0.4a
WSPH (%)	0.9a	0.6a	0.1b	0.8a	0.7a	0.2b
HR	25.1a	27.3ab	30.5b	28.2ab	29.1b	27.2a
HD	65.5a	74.1b	80.4c	59.4a	76.7b	78.9c
P_{AH} (%)	39.9a	60.8b	80.7c	41.5a	65.4b	75.9c
GI	0a	30b	92c	0a	41b	94c

Note: Data represent the mean of four replicates with less than 5% of relative error. For each pile, 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 $p < 0.05$.
EC: electrical conductivity, OM: total organic matter, T_{OC}: total organic carbon, T_N: total nitrogen, WSC: water-soluble organic carbon, WSCH: water-soluble carbohydrates, WSPH: water-soluble phenols, HR: humification ratio, HD: humification degree, P_{AH}: percentage of humic acids, GI: Germination index by Zucconi test.
[a] Data based on dry weight.
[b] Water extract 1:10.

Table 2
Number of taxa (N) and of 16S rRNA sequences (S) in genomic libraries from the mesophilic, thermophilic and maturation phases of M1 and M2 composting piles, respectively.

M1 pile	Genomic libraries					
	Meso1-4		Thermo1-4		Matu1-4	
	N	S	N	S	N	S
Phylum	14 ± 2	4615 ± 1763 (97.9%)	14 ± 2	3776 ± 566 (96.5%)	16 ± 1	4292 ± 1062 (94.7%)
Class	26 ± 6	4444 ± 1745 (94.3%)	25 ± 3	3682 ± 559 (94.1%)	32 ± 3	4137 ± 1025 (91.2%)
Order	40 ± 9	4099 ± 1549 (86.9%)	38 ± 3	3387 ± 480 (86.5%)	47 ± 5	3766 ± 907 (83.1%)
Family	106 ± 15	3582 ± 1452 (76.0%)	101 ± 9	2898 ± 293 (74.0%)	95 ± 12	3481 ± 856 (76.8%)
Genus	183 ± 37	2426 ± 918 (51.5%)	165 ± 17	2026 ± 205 (51.8%)	164 ± 16	2141 ± 574 (47.2%)
Total sequences identified	4714 ± 1894 (100%)		3914 ± 564 (100%)		4534 ± 1117 (100%)	
Unclassified sequences	99 ± 55 (2.1%)		138 ± 82 (3.5%)		242 ± 120 (5.3%)	
M2 pile	Genomic libraries					
	Meso5-8		Thermo5-8		Matu5-8	
	N	S	N	S	N	S
Phylum	15 ± 3	6112 ± 1104 (97.8%)	14 ± 3	7712 ± 996 (97.3%)	24 ± 1	5640 ± 676 (89.5%)
Class	28 ± 3	5939 ± 1096 (95.0%)	27 ± 5	7420 ± 999 (93.6%)	39 ± 1	4799 ± 513 (76.1%)
Order	45 ± 7	5645 ± 892 (90.3%)	49 ± 9	7031 ± 819 (88.7%)	59 ± 2	3728 ± 397 (59.1%)
Family	109 ± 17	5039 ± 409 (80.6%)	119 ± 19	6146 ± 309 (77.5%)	127 ± 7	3626 ± 358 (57.5%)
Genus	191 ± 33	3670 ± 172 (58.7%)	225 ± 47	4398 ± 113 (55.5%)	229 ± 11	2629 ± 259 (41.7%)
Total sequences identified	6249 ± 1233 (100%)		7926 ± 1131 (100%)		6305 ± 768 (100%)	
Unclassified sequences	137 ± 51 (2.2%)		214 ± 91 (2.7%)		665 ± 233 (10.5%)	

Note: Values represent the mean of the sequences in each library followed by the standard deviation. For each taxon, numbers in brackets show the percentage of identified sequences.

lower than those published by Storey et al. (2015) when composting shredded green waste and spent brewery grains (average of 15361), and those obtained by Zhang et al. (2016) (8971–12962) in compost from corn cobs mixed with fresh cow dung, respectively.

3.3. Coverage and diversity indices

A wide range of diversity indices are commonly used in bacterial biodiversity studies, especially using high-throughput sequencing approaches. In this study, the evolution of the microbial diversity was estimated by richness (number of OTUs), Shannon (H') and Chao1 indices as well as the Good's coverage (Table 3). At 97% similarity, a total of 2519 and 2763 distinct OTUs were present in piles M1 and M2, respectively. In both piles, no statistical differences between mesophilic and thermophilic phases were found, but during maturation, the number of OTUs increased to almost double (Table 3). At 90% confidence interval, the Good's coverage index was higher than 90% for each of the 24 genomic libraries analysed in this study (Table 3), which indicates that the sequences obtained represent the entire bacterial population well. The Shannon index of 5.38 and 5.32 for the OTUs found in libraries from the mesophilic and thermophilic phases of pile M1, and of 5.31 and 5.40 for those of pile M2 were statistically similar, which suggests that bacterial richness was not affected by heat increases during the thermophilic phase. For both Matu1-4 and Matu5-8 libraries, corresponding to the maturation phase of piles M1 and M2, respectively, the Shannon indices of 5.81 and 5.99 were statistically higher than those determined during the mesophilic and thermophilic phases, respectively (Table 3). For piles M1 and M2, the Chao1 indices also showed the absence of significant differences between the mesophilic and thermophilic phases and a clear increase in the indices corresponding to the maturation phase (Table 3). All these data confirmed an increase in the bacterial diversity during maturation, especially evident in the detection of new bacterial populations. de Gannes et al. (2013) also found differences between mesophilic and maturation bacterial communities, contrary to the commonly accepted postulate related to recolonization of the mesophilic flora during maturation. It should be noted, however, that the newly appeared genera in the maturation phase were poorly represented as their relative abundances were lower than 0.1% of the total identified sequences, and most of them corresponded to singletons (Table 3).

3.4. Bacterial diversity

Valid sequences in Meso1-4, Thermo1-4 and Matu1-4 libraries distributed into 14, 14, and 16 phyla, respectively, and those in Meso5-8, Thermo5-8 and Matu5-8 libraries did in 15, 14 and 24 phyla, respectively (Table 2). Numbers of classes, orders, families

Table 3
Richness (number of OTUs), singletons, Good's coverage index, Shannon (H') and Chao1 indices in genomic libraries from M1 and M2 piles during composting process.

	Richness (OTUs)	Singletons	Good's coverage* (%)	Shannon (H′)	Chao1
M1 pile					
Meso1-4	626a	350a	90.5b	5.38a	1034a
Thermo1-4	631a	275a	93.0b	5.32a	967a
Matu1-4	1262b	792b	82.0a	5.81b	2885b
M2 pile					
Meso5-8	730a	287a	95.5b	5.31a	1095a
Thermo5-8	860a	342a	95.8b	5.40a	1313a
Matu5-8	1173b	487b	92.2a	5.99b	1762b

* Good's coverage index was calculated as [(1 − singletons/total number of sequences) × 100].
Note: For each pile, 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 $p < 0.05$.

and genera corresponding to each mesophilic, thermophilic and maturation phases during composting of piles M1 and M2 are also shown in Table 2.

Phyla Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria were the most abundant in the DNA libraries from piles M1 (Fig. 2a) and M2 (Fig. 2b), and represented more than 90% of the total identified sequences. Previous work has shown that structure of microbial communities is greatly influenced by the specific cultivar from which the olive mill waste is generated (Tsiamis et al., 2012); however, dominance of Actinobacteria, Firmicutes and Proteobacteria in olive mill wastes was revealed after analysis of a database survey containing 585 16S rRNA gene sequences (Ntougias et al. (2013). Moreover, Actinobacteria, Firmicutes and Proteobacteria were also among the most abundant phyla during the composting processes using different organic wastes as the high-throughput sequencing has revealed (de Gannes et al., 2013; Neher et al., 2013; Storey et al., 2015).

It is well known that the composting process affects bacterial population (Insam and de Bertoldi, 2007). In general, differences in structure and composition of the phyla identified in the

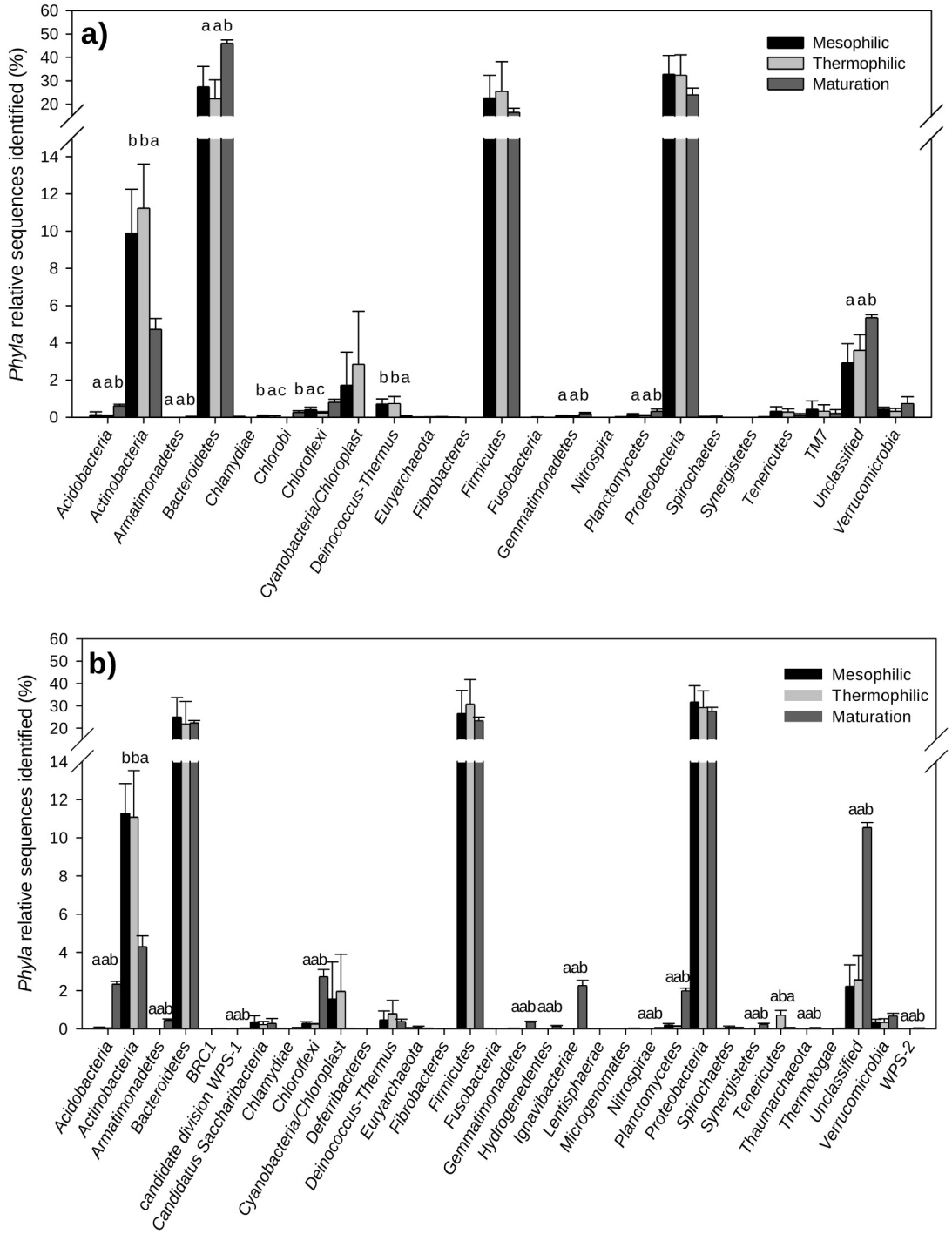


Fig. 2. Relative abundance (%) of the sequences corresponding to phyla identified in piles M1 (a) and M2 (b) during the composting process. Data represent the mean of the sequences in libraries Meso1-4, Thermo1-4 and Matu1-4 from pile M1 and libraries Meso5-8, Thermo5-8 and Matu5-8 form pile M2, respectively. Bars represent the standard deviation. For each phylum, values with the same lower-case are not statistical different according to one-way ANOVA with Tukey-Kramer *post-hoc* test at $p < 0.05$.

mesophilic and thermophilic phases in pile M1 were not found (Fig. 2a), but significant ($p < 0.05$) changes occurred during the maturation process. Based on the phyla composition found in the mesophilic/thermophilic phase, the relative abundance of Acidobacteria, Armatimonadetes, Bacteroidetes, Chlorobi, Chloroflexi, Gemmatimonadetes and Planctomycetes increased during the maturation phase, and Actinobacteria and Deinococcus-Thermus decreased (Fig. 2a). Similarly, in relation with pile M2, statistical differences ($p > 0.05$) were not found in the relative abundance of phyla detected in the mesophilic and thermophilic phase. In this pile, Acidobacteria, Armatimonadetes, candidate division WPS-1, Chloroflexi, Gemmatimonadetes, Hydrogenedentes, Ignavibacteriae, Nitrospirae, Planctomycetes, Synergistetes, Thaumarchaeota and WPS-2 increased significantly ($p > 0.05$) during the maturation phase while phylum Actinobacteria showed a significant decrease (Fig. 2b).

For both, piles M1 and M2, the relative abundance of bacterial genera found during the composting process varied with the composting phases, with 12 genera increasing (Table 4a), 25 decreasing in their relative abundance (Table 4b). These results show that bacterial communities had a similar evolution during composting, which resulted in two composts with alike bacterial structure and composition. In fact, regardless of the pile studied, the most abundant genera in the mesophilic/thermophilic bioxidative phases were *Bordetella, Flavobacterium, Halomonas, Halotalea, Olivibacter, Parapedobacter*, *Planifilum, Pseudomonas, Pseudoxanthomonas* and *Sphingobacterium*, whereas *Planomicrobium, Flavobacterium, Chryseobacterium, Pseudomonas, Ohtaekwangia, Sphingobacterium, Pedobacter* and *Luteimonas* were the predominant genera in the maturation phase. Many of those genera are widespread in natural and anthropogenic environments, such as soil, fresh and marine waters, sediments, plant rhizospheres, activated sludge and waste-water treatment systems, and even have directly been isolated from different composts (Weon et al., 2006; Wang et al., 2008; Lee et al., 2009; Kim et al., 2010, 2012; Gibello et al., 2011; Han et al., 2013; Yabe et al., 2013). *Flavobacterium*, *Chryseobacterium* and *Pedobacter* were the dominant genera at the maturation phase. These genera have the ability to produce carboxymethylcellulose, an enzyme involved in polysaccharide and lignocellulosic compounds degradation (Kim et al., 2012; de Gannes et al., 2013). Some spore-forming genera like *Bacillus* and *Planifilum* (Sung et al., 2002; Han et al., 2013; Poudel et al., 2014) and non-spore-forming bacteria like *Microbacterium* (Vaz-Moreira et al., 2009), *Pseudoxanthomonas* (Weon et al., 2006), *Olivibacter* (Wang et al., 2008) and *Parapedobacter* (Kim et al., 2010), which were predominant during the thermophilic phase, have also been isolated from

Table 4
Relative number of sequences (%) of genera identified in piles M1 and M2 during composting phases which showed statistical differences (a: increasing; b: decreasing).

Phylum	Family	Genus	M1 pile			M2 pile		
			Meso1-4	Thermo1-4	Matu1-4	Meso5-8	Thermo5-8	Matu5-8
a								
Acidobacteria	–	*Gp6*	0.16a	0.05a	0.89b	0.03a	0.03a	2.00b
Bacteroidetes	Marinilabiaceae	*Alkaliflexus*	0.34a	0.45a	1.38b	0.04a	0.03a	8.12b
Bacteroidetes	Flavobacteriaceae	*Chryseobacterium*	0.82a	0.09a	4.50b	0.32a	0.13a	2.92b
Bacteroidetes	Flavobacteriaceae	*Flavobacterium*	4.05b	1.44a	15.84c	2.20b	0.79a	3.57c
Bacteroidetes	Cytophagaceae	*Adhaeribacter*	0.34a	0.01a	1.90b	0.00a	0.01a	0.58b
Bacteroidetes	–	*Ohtaekwangia*	0.41a	0.18a	2.12b	0.00a	0.06a	1.89b
Bacteroidetes	Sphingobacteriaceae	*Pedobacter*	2.21b	0.13a	13.80c	0.06a	0.06a	1.42b
Bacteroidetes	Cytophagaceae	*Pontibacter*	0.24a	0.00a	1.36b	0.01a	0.01a	0.42b
Chlorobi	Ignavibacteriaceae	*Ignavibacterium*	0.05a	0.00a	0.55b	0.01a	0.01a	5.39b
Firmicutes	Planococcaceae	*Planomicrobium*	1.31b	0.06a	7.73c	0.07a	0.09a	6.62b
Proteobacteria	Hyphomicrobiaceae	*Devosia*	0.86a	0.68a	1.86b	0.25a	0.16a	1.49b
Proteobacteria	Sinobacteraceae	*Steroidobacter*	0.16a	0.12a	0.80b	0.17a	0.09a	3.25b
		Sum of sequences (%)	10.95	3.21	52.73	3.16	1.47	37.67
b								
Actinobacteria	Microbacteriaceae	*Microbacterium*	1.74b	1.74b	0.66a	2.88b	1.98b	0.44a
Actinobacteria	Pseudonocardiaceae	*Saccharomonospora*	1.34b	2.01c	0.01a	0.97b	1.23c	0.06a
Actinobacteria	Corynebacteriaceae	*Corynebacterium*	1.32b	1.36b	0.00a	1.32b	1.32b	0.05a
Actinobacteria	Brevibacteriaceae	*Brevibacterium*	1.01b	1.09b	0.00a	1.70b	1.56b	0.02a
Actinobacteria	Bogoriellaceae	*Georgenia*	0.73b	0.69b	0.08a	0.83a	1.09c	0.01a
Bacteroidetes	Sphingobacteriaceae	*Olivibacter*	1.79b	2.26b	0.27a	6.39b	4.57b	0.14a
Bacteroidetes	Sphingobacteriaceae	*Parapedobacter*	6.53b	5.85b	1.68a	6.34b	5.11b	1.19a
Bacteroidetes	Chitinophagaceae	*Gracilimonas*	0.65b	1.47c	0.04a	0.51b	0.84b	0.08a
Firmicutes	Clostridiales_Incertae Sedis XI	*Tepidimicrobium*	0.78b	1.30c	0.02a	0.87b	1.20c	0.11a
Firmicutes	Thermoactinomycetaceae	*Planifilum*	3.70b	5.38c	0.26a	2.83b	3.71b	0.59a
Firmicutes	Bacillaceae	*Bacillus*	0.92b	0.63b	0.14a	0.94b	0.76ab	0.55a
Firmicutes	Halanaerobiaceae	*Halocella*	0.32b	0.81c	0.04a	2.17b	3.32c	0.46a
Firmicutes	Thermoactinomycetaceae	*Thermoactinomyces*	1.24b	1.01b	0.03a	0.56b	0.91b	0.17a
Firmicutes	Staphylococcaceae	*Jeotgalicoccus*	0.83b	1.22b	0.00a	0.84b	1.36b	0.00a
Firmicutes	Leuconostocaceae	*Weissella*	0.18b	0.13b	0.01a	2.50b	1.73b	0.00a
Firmicutes	Carnobacteriaceae	*Atopostipes*	0.71b	1.19c	0.00a	0.57b	1.10c	0.02a
Proteobacteria	Halomonadaceae	*Halomonas*	2.35b	2.30b	0.04a	0.96b	1.35c	0.03a
Proteobacteria	Halomonadaceae	*Halotalea*	1.69b	1.54b	0.02a	3.42c	2.27b	0.03a
Proteobacteria	Rhodobacteraceae	*Paracoccus*	1.46b	1.56b	0.22a	0.87b	1.03c	0.55a
Proteobacteria	Moraxellaceae	*Acinetobacter*	1.45b	1.25b	0.32a	2.45b	1.92b	0.70a
Proteobacteria	Alcaligenaceae	*Bordetella*	2.01b	1.95b	0.13a	1.63b	1.38b	0.17a
Proteobacteria	Acetobacteraceae	*Gluconacetobacter*	0.65b	0.95c	0.00a	0.98b	1.69c	0.01a
Proteobacteria	Idiomarinaceae	*Idiomarina*	1.13b	1.27b	0.04a	0.63b	0.83b	0.00a
Proteobacteria	Xanthomonadaceae	*Pseudoxanthomonas*	3.53b	4.37b	0.24a	4.16b	3.17b	0.14a
Tenericutes	Acholeplasmataceae	*Acholeplasma*	0.65b	0.55b	0.20a	0.79b	1.28c	0.09a
		Sum of sequences (%)	39.44	45.21	4.45	49.12	48.23	5.71
		Total sequences (%)	50.39	48.42	57.18	52.28	49.70	43.38

Note: For each genus and pile, 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 $p < 0.05$.

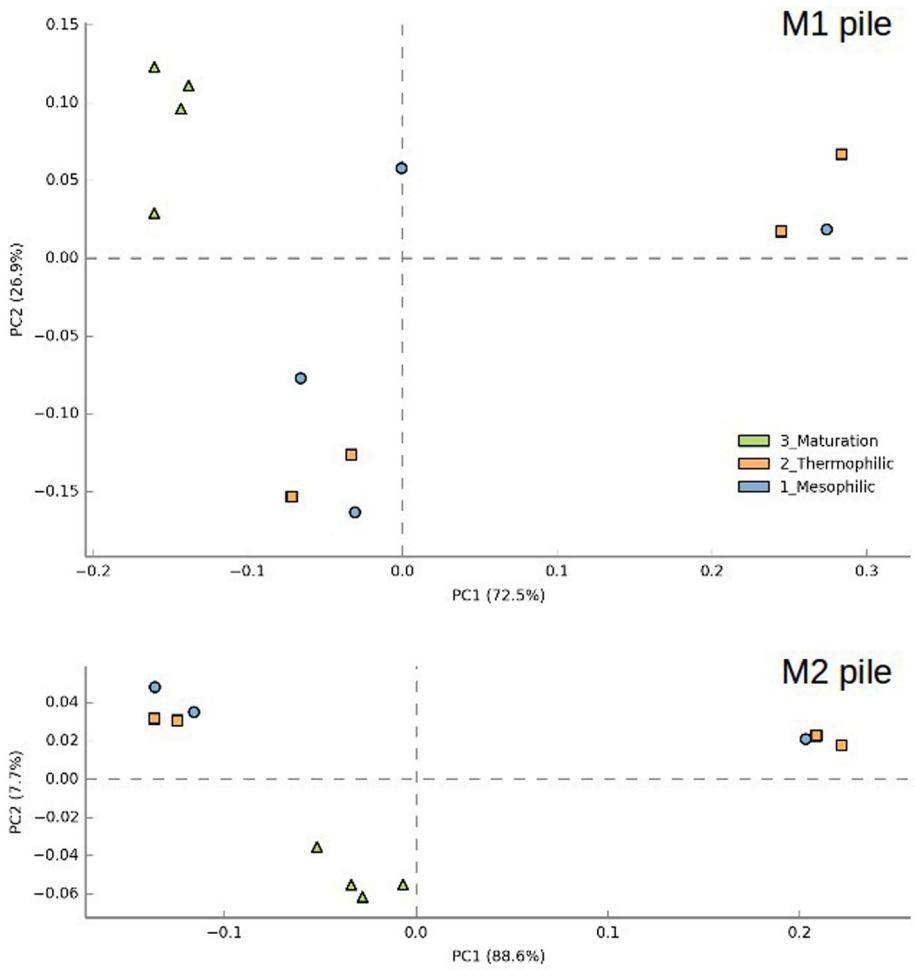


Fig. 3. Principal component analysis (PCA) of the sequences at phylum level included in libraries from M1 (above) and M2 (below) piles, respectively.

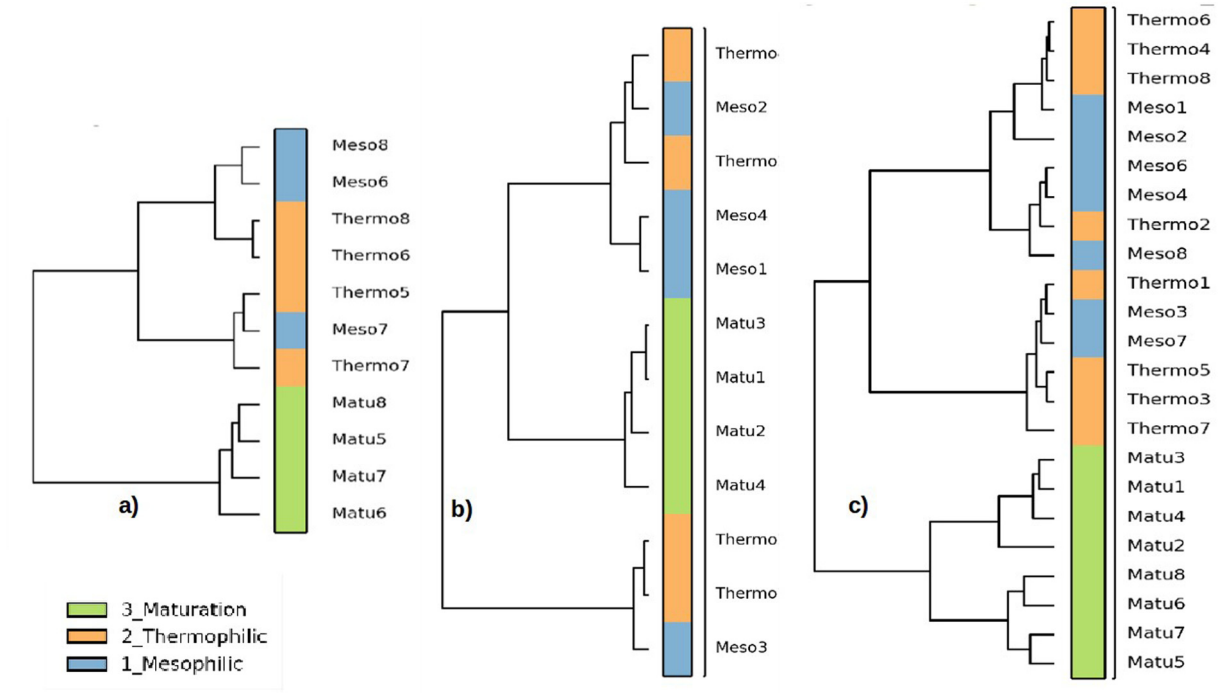


Fig. 4. Dendrograms based on average neighbour (UPGMA) with clustering threshold of 0.75 of M1 (a), M2 (b) and M1 + M2 piles (c) respectively.

Table 5
Pearson correlation matrix (n = 24) between composting parameters and genera identified during composting of M1 and M2 piles. Green and blue folders represent positive and negative correlations, respectively.

Genus	Temperature	EC	OM	Hemi cellulose	T_{OC}	T_{OC}/T_N	Fat content	WSCH	pH	Lignine	HR	HD	P_{AH}
Alkaliflexus	-0.66**	-0.72**	-0.62**	-0.51**	-0.73**	-0.48*	-0.58**	-0.61**	0.55**	0.65**	0.46*	0.53**	0.67**
Planomicrobium	-0.92**	-0.97**	-0.75**	-0.79**	-0.96**	-0.88**	-0.76**	-0.78**	0.92**	0.94**	0.67**	0.79**	0.95**
Ignavibacterium	-0.63**	-0.70**	-0.6**	-0.48*	-0.70**	-0.43*	-0.55**	-0.59**	0.51**	0.62**	0.43*	0.50**	0.64**
Flavobacterium	-0.69**	-0.62**	NS	-0.51**	-0.61**	-0.75**	-0.41*	-0.42*	0.69**	0.64**	0.40*	0.49**	0.63**
Steroidobacter	-0.70**	-0.76**	-0.62**	-0.52**	-0.76**	-0.50**	-0.59**	-0.63**	0.57**	0.67**	0.46*	0.54**	0.70**
Chryseobacterium	-0.88**	-0.86**	-0.61**	-0.68**	-0.85**	-0.84**	-0.62**	-0.65**	0.83**	0.84**	0.57**	0.67**	0.84**
Gp6	-0.82**	-0.87**	-0.70**	-0.63**	-0.87**	-0.66**	-0.67**	-0.71**	0.71**	0.79**	0.55**	0.65**	0.81**
Ohtaekwangia	-0.91**	-0.92**	-0.71**	-0.73**	-0.91**	-0.87**	-0.71**	-0.71**	0.88**	0.90**	0.64**	0.73**	0.90**
Devosia	-0.86**	-0.78**	-0.59**	-0.62**	-0.82**	-0.85**	-0.59**	-0.58**	0.81**	0.80**	0.55**	0.61**	0.78**
Pedobacter	-0.66**	-0.61**	-0.40*	-0.53**	-0.61**	-0.75**	-0.43*	-0.44*	0.70**	0.60**	0.44*	0.52**	0.63**
Adhaeribacter	-0.75**	-0.71**	-0.49**	-0.60**	-0.71**	-0.79**	-0.51**	-0.52**	0.76**	0.72**	0.50**	0.58**	0.72**
Pontibacter	-0.76**	-0.72**	-0.50**	-0.60**	-0.72**	-0.80**	-0.52**	-0.53**	0.77**	0.73**	0.50**	0.59**	0.73**
Parapedobacter	NS	0.47*	0.42*	0.44*	0.50**	NS	0.41*	0.40*	-0.45*	-0.46*	-0.41*	-0.44*	-0.48*
Acinetobacter	NS	NS	0.41*	0.42*	0.45*	0.51**	0.43*	NS	-0.45*	-0.46*	-0.44*	-0.40*	-0.43*
Bacillus	NS	0.45*	0.42*	0.50**	0.48*	NS	0.43*	0.42*	-0.53**	-0.50**	-0.45*	-0.49**	-0.49*
Paracoccus	0.58**	0.67**	0.42*	0.52**	0.62**	0.43*	0.44*	0.53**	-0.55**	-0.56**	NS	-0.50**	-0.62**
Microbacterium	0.47*	0.53**	0.53**	0.51**	0.59**	0.52**	0.51**	0.45*	-0.55**	-0.57**	-0.54**	-0.51**	-0.55**
Bordetella	0.41*	0.51**	0.41*	0.45*	0.52**	NS	0.40*	0.42*	-0.46*	-0.47*	NS	-0.45*	-0.50**
Olivibacter	0.47*	0.44*	0.44*	0.42*	0.52**	0.57**	0.43*	NS	-0.51**	-0.51**	-0.47*	-0.41*	-0.47*
Pseudoxanthomonas	0.47*	0.53**	0.44*	0.45*	0.55**	0.45*	0.44*	0.42*	-0.49*	-0.50**	-0.43*	-0.45*	-0.53**
Corynebacterium	0.64**	0.67**	0.50**	0.51**	0.63**	0.58**	0.50**	0.55**	-0.61**	-0.63**	-0.40*	-0.52**	-0.64**
Halomonas	0.44*	0.52**	NS	NS	0.45*	NS	NS	0.46*	NS	-0.44*	NS	NS	-0.48*
Halotalea	0.45*	0.48*	0.51**	0.49*	0.55**	0.54**	0.50**	0.44*	-0.53**	-0.54**	-0.53**	-0.48*	-0.52**
Brevibacterium	0.82**	0.79**	0.65**	0.65*	0.82**	0.86**	0.65**	0.62**	-0.80**	-0.82**	-0.62**	-0.65**	-0.79**
Georgenia	0.61**	0.55**	NS	NS	0.53**	0.50**	NS	0.40*	-0.52**	-0.53**	NS	NS	-0.51**
Idiomarina	0.60**	0.67**	0.48*	0.52**	0.64**	0.50**	0.50**	0.58**	-0.54**	-0.59**	NS	-0.51**	-0.64**
Weissella	0.40*	NS	NS	NS	0.43**	0.55**	NS	NS	-0.46*	-0.45*	-0.44*	NS	NS

NS: not significant, EC: electrical conductivity, OM: total organic matter, T_{OC}: total organic carbon. T_N: total nitrogen, WSCH: water-soluble carbohydrates, HR: humification ratio, HD: humification degree, P_{AH}: percentage of humic acids.
* Significant at $p < 0.05$.
** Significant at $p < 0.01$.

different mature composts. All these findings mean that these genera could be well adapted to the composting environments and their evolution. By contrast, the role of most of them are not well understood but they have been identified using high-throughput sequencing in composting studies. de Gannes et al. (2013) found *Chryseobacterium, Flavobacterium, Pedobacter, Devosia* and *Steroidobacter* to be the most abundant genera in their study. Also, Storey et al. (2015) found *Chryseobacterium, Flavobacterium, Pseudoxanthomonas* or *Bacillus* to be the main genera during composting.

Based on culture-dependent methods, it has been assumed that mesophilic microbial populations could enter dormancy during the thermophilic phase and then re-colonize composting substrates during the maturation phase, thus explaining increases in bacterial richness in mature compost. Using 454 pyrosequencing, our data, however, indicate a clear difference in bacterial diversity between the mesophilic/thermophilic and maturation composting phases. de Gannes et al. (2013) also showed that microbial communities in the mesophilic and mature phases were distinct, with major components of the former being supplanted in the mature phase. It is possible that residual or partially degraded, thermally-resistant DNA molecules from the thermophilic phase, under the conditions used in this study, could be extracted and amplified. In fact, Partanen et al. (2010) have shown that rapid turnings of the organic material used for composting produced a fast bacterial cell denaturation. It is possible, that the composting process used in this study with only 3 mechanical turnings in 6 weeks between the mesophilic and thermophilic phases helped to slow down DNA degradation. Despite genomic analysis, transcriptome RNA sequencing (RNA-Seq) and/or RT-PCR approaches have to be carried out to elucidate the real active bacterial population present in each composting phase.

3.5. Statistical analysis and microbial biomarkers

Fig. 3 shows the Principal Component Analysis (PCA) calculated using the relative number of sequences corresponding to phyla included in the 12 libraries corresponding to pile M1 and those of the 12 libraries corresponding to pile M2, respectively (Fig. 3). The first principal component (PC1) explained an important percentage of the total variances of the data, being 72.5% and 88.6% for M1 and M2 piles, respectively. As it was shown before, maturation was the only phase that statistically affected bacterial biodiversity and evolution. Our data showed that sequences in libraries Matu1-4 grouped in a defined cluster while phyla in libraries Meso1-4 and Thermo1-4 were mixed in a wider group (Fig. 3). Similar result was found after PCA analysis of sequences in libraries from pile M2 (Fig. 3). In order to test reproducibility, the analysis was carried out in both piles separately but, similar behaviour was obtained if the M1 and M2 pile data were pooled (data not shown). An example of this fact can be seen in the phylogenetic trees shown in Fig. 4. Both M1 and M2 piles are shown separately and also, M1+M2, clustered maturation independently of meso and thermophilic phases, respectively.

It is well known that composting modulates the bacterial population ecology, mainly due to variations in some environmental factors (temperature, pH, aeration, moisture, etc.), composting substrates or availability of nutrients (Insam and de Bertoldi, 2007). In order to elucidate the effect of the composting process in the bacterial diversity, some statistical analyses were performed. Pearson correlation coefficients between the genera found in each composting phase and some of the composting parameters showed that *Alkaliflexus, Planomicrobium, Ignavibacterium, Flavobacterium, Steroidobacter, Chryseobacterium, Gp6, Ohtaekwangia, Devosia,*

Pedobacter, Adhaeribacter and *Pontibacter* were negatively correlated with temperature, EC, OM, hemicellulose content, T_{OC}, T_{OC}/T_N ratio, Fat content and WSCH and positively correlated with pH, lignine and the humification parameters HR, HD and P_{AH}. On the other hand, *Parapedobacter, Acinetobacter, Bacillus, Paracoccus, Microbacterium, Bordetella, Olivibacter, Pseudoxanthomonas, Corynebacterium, Halomonas, Halotalea, Brevibacterium, Georgenia, Idiomarina* and *Weissella* negatively correlated with pH, lignine and the humification parameters HR, HD and P_{AH}, and positively correlated with temperature, EC, OM, hemicelullose, T_{OC}, T_{OC}/T_N ratio, Fat content and WSCH. According to Pearson coefficients (Table 5), some of these genera could be selected as a biomarkers of composting maturation. A lineal regression study between the relative number of sequences in the 24 libraries from piles M1 and M2 and the composting parameters revealed that genera *Planomicrobium* and *Ohtaekwangia*, whose relative number of sequences increased during maturation, showed the best correlation ($R^2 > 0.80$) with most of the composting parameters studied like pH, EC or lignin content (data not shown). Thus, genera *Planomicrobium* and *Ohtaekwangia* could be considered as possible biomarkers indicative of the maturation process during AL composting. Despite that, more research is needed to confirm these results.

4. Conclusions

454-Pyrosequencing shows differences in bacterial diversity during AL composting. In general, no statistical difference between mesophilic and thermophilic phases was detected. Only maturation affected microbial diversity, containing higher bacterial richness than that found in the mesophilic and thermophilic phases. Actinobacteria, Bacteriodetes, Firmicutes and Proteobacteria were the main phyla in each one of the composting phases. Statistical analyses revealed relationships between composting physicochemical parameters and bacterial genera found during evolution of the process. Genera *Planomicrobium* and *Ohtaekwangia* correlated best with the main composting parameters during the composting process, so that they could be considered as biomarkers for AL composting maturation.

Acknowledgements

This work was supported by the ERDF-cofinanced project P12-AGR1968 from Junta de Andalucía. Financial and technical support by J. González is also acknowledged. G. Tortosa strongly thanks A. J. Fernández-González, J. F. Cobo-Díaz, P. J. Villadas and F. Martínez-Abarca for helpful discussions. Finally, D. Francis Lewis is also acknowledged for improvement of the written English.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.11.098.

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.
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.
Baker, G.C., Smith, J.J., Cowan, D.A., 2003. Review and re-analysis of domain-specific 16S primers. Microbiol. Methods 55, 541–555.
Bernal, M.P., Paredes, C., Sánchez-Monedero, M.A., Cegarra, J., 1998. Maturity and stability parameters of compost prepared with a wide range of organic wastes. Bioresour. Technol. 63, 91–99.
Bibby, K., Viau, E., Peccia, J., 2010. Pyrosequencing of the 16S rRNA gene to reveal bacterial pathogen diversity in biosolids. Water Res. 44, 4252–4260.
Cole, J.R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R.J., Kulam-Syed-Mohideen, A.S., McGarrell, D.M., Marsh, T., Garrity, G.M., Tiedje, J.M., 2009. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res. 37, 141–145.
de Gannes, V., Eudoxie, G., Hickey, W.J., 2013. Prokaryotic successions and diversity in composts as revealed by 454-pyrosequencing. Bioresour. Technol. 133, 573–580.
de Man, R., Friedge, H., 2016. Circular economy: European policy on shaky ground. Waste Manag. Res. 34, 93–95.
Gibello, A., Vela, A.I., Martín, M., Mengs, G., Alonso, P.Z., Garbi, C., Fernánndez-Garayzábal, J.F., 2011. *Pseudomonas composti* sp. nov., isolated from compost samples. Int. J. Syst. Evol. Microbiol. 61, 2962–2966.
Han, S.I., Lee, J.C., Lee, H.J., Whang, K.S., 2013. *Planifilum composti* sp. nov., a thermophile isolated from compost. Int. J. Syst. Evol. Microbiol. 63, 4557–4561.
Hultman, J., Kurola, J., Rainisalo, A., Kontro, M., Romantschuk, M., 2010. Utility of molecular tools in monitoring large scale composting. In: Insam, H. et al. (Eds.), Microbes at Work. Springer-Verlag Berlin Heidelberg, pp. 135–151.
Insam, H., de Bertoldi, M., 2007. Microbiology of the composting process. In: Diaz, L. F., de Bertoldi, M., Bildingmaier, W., Stentiford, E. (Eds.), 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. et al. (Eds.), Microbes at Work. Springer-Verlag Berlin Heidelberg, pp. 1–34.
Kim, S.J., Weon, H.Y., Kim, Y.S., Yoo, S.H., Kim, B.Y., Anandham, R., Kwon, S.W., 2010. *Parapedobacter luteus* sp. nov. and *Parapedobacter composti* sp. nov., isolated from cotton waste compost. Int. J. Syst. Evol. Microbiol. 60, 1849–1853.
Kim, J.J., Kanaya, E., Weon, H.Y., Koga, Y., Takano, K., Dunfield, P.F., Kwon, S.W., Kanaya, S., 2012. *Flavobacterium compostarboris* sp. nov., isolated from leaf-and-branch compost, and emended descriptions of *Flavobacterium hercynium*, *Flavobacterium resistens* and *Flavobacterium johnsoniae*. Int. J. Syst. Evol. Microbiol. 62, 2018–2024.
Lee, H.G., Kim, S.G., Im, W.T., Oh, H.M., Lee, S.T., 2009. *Pedobacter composti* sp. nov., isolated from compost. Int. J. Syst. Evol. Microbiol. 59, 345–349.
López-González, J.A., Suárez-Estrella, F., Vargas-García, M.C., López, M.J., Jurado, M. M., Moreno, J., 2015. Dynamics of bacterial microbiota during lignocellulosic waste composting: studies upon its structure, functionality and biodiversity. Bioresour. Technol. 175, 406–416.
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.
Neher, D.A., Weicht, T.R., Bates, S.T., Leff, J.W., Fierer, N., 2013. Changes in bacterial and fungal communities across compost recipes, preparation methods and composting times. PLoS One 8 (11), e79512. http://dx.doi.org/10.1371/journal.pone.0079512.
Ntougias, S., Bourtzis, K., Tsiamis, G., 2013. The microbiology of olive mill wastes. BioMed Res. Int., 1–16 http://dx.doi.org/10.1155/2013/784591 784591.
Parameswaran, P., Jalili, R., Tao, L., Shokralla, S., Gharizadeh, B., Ronaghi, M., Fire, A. Z., 2007. A pyrosequencing-tailored nucleotide barcode design unveils opportunities for large-scale sample multiplexing. Nucleic Acids Res. 35, e130.
Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G., 2014. STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics. http://dx.doi.org/10.1093/bioinformatics/btu494.
Partanen, P., Hultman, J., Paulin, L., Auvinen, P., Romantschuk, M., 2010. Bacterial diversity at different stages of the composting process. BMC Microbiol. 10, 1–11.
Poudel, P., Miyamoto, H., Miyamoto, H., Okugawa, Y., Tashiro, Y., Sakai, K., 2014. Thermotolerant *Bacillus kokeshiiformis* sp. nov. isolated from marine animal resources compost. Int. J. Syst. Evol. Microbiol. 64, 2668–2674.
Sánchez-Monedero, M.A., Roig, A., Cegarra, J., Bernal, M.P., 1999. Relationships between water-soluble carbohydrate and phenol fractions and the humification indices of different organic wastes during composting. Bioresour. Technol. 70, 193–201.
Storey, S., Chualain, D.N., Doyle, O., Clipson, N., Doyle, E., 2015. Comparison of bacterial succession in green waste composts amended with inorganic fertiliser and wastewater treatment plant sludge. Bioresour. Technol. 179, 71–77.
Sung, M.H., Kim, H., Bae, J.W., Rhee, S.K., Jeon, C.O., Kim, K., Kim, J.J., Hong, S.P., Lee, S. G., Yoon, J.H., Park, Y.H., Baek, D.H., 2002. *Geobacillus toebii* sp. nov., a novel thermophilic bacterium isolated from hay compost. Int. J. Syst. Evol. Microbiol. 52, 2251–2255.
Tkachuk, V.L., Krause, D.O., Knox, N.C., Hamm, A.C., Zvomuya, F., Ominski, K.H., McAllister, T.A., 2014. Targeted 16S rRNA high-throughput sequencing to characterize microbial communities during composting of livestock mortalities. J. Appl. Microbiol. 116, 1181–1194.
Tsiamis, G., Tzagkaraki, G., Chamalaki, A., Xypteras, N., Andersen, G., Vayenas, D., Bourtzis, K., 2012. Olive-mill wastewater bacterial communities display a cultivar specific profile. Curr. Microbiol. 64, 197–203.
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.
Vaz-Moreira, I., Lopes, A.R., Faria, C., Spröer, C., Schumann, P., Nunes, O.C., Manaia, C. M., 2009. *Microbacterium invictum* sp. nov., isolated from homemade compost. Int. J. Syst. Evol. Microbiol. 59, 2036–2041.

Wang, L., Ten, L.N., Lee, H.G., Im, W.T., Lee, S.T., 2008. *Olivibacter soli* sp. nov., *Olivibacter ginsengisoli* sp. nov. and *Olivibacter terrae* sp. nov., from soil of a ginseng field and compost in South Korea. Int. J. Syst. Evol. Microbiol. 58, 1123–1127.

Weon, H.Y., Kim, B.Y., Kim, J.S., Lee, S.Y., Cho, Y.H., Go, S.J., Hong, S.B., Im, W.T., Kwon, S.W., 2006. *Pseudoxanthomonas suwonensis* sp. nov., isolated from cotton waste composts. Int. J. Syst. Evol. Microbiol. 56, 659–662.

Yabe, S., Aiba, Y., Sakai, Y., Hazaka, M., Kawahara, K., Yokota, A., 2013. *Sphingobacterium thermophilum* sp. nov., of the phylum *Bacteroidetes*, isolated from compost. Int. J. Syst. Evol. Microbiol. 63, 1584–1588.

Zhang, L., Zhang, H., Wang, Z., Chen, G., Wang, L., 2016. Dynamic changes of the dominant functioning microbial community in the compost of a 90-m^3 aerobic solid state fermentor revealed by integrated meta-omics. Bioresour. Technol. 203, 1–10.