Journal of Environmental Management 265 (2020) 110528

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: http://www.elsevier.com/locate/jenvman

Research article

Integral approach using bacterial microbiome to stabilize municipal solid waste

Macarena M. Jurado *, Anny J. Camelo-Castillo, Francisca Suárez-Estrella, Maria J. López, Juan A. López-González, Maria J. Estrella-González, Ana B. Síles-Castellano, Joaquín Moreno

Unit of Microbiology, Department of Biology and Geology, CITE II-B, Agrifood Campus of International Excellence ceiA3, CIAMBITAL, University of Almeria, 04120, Almeria, Spain

ARTICLE INFO

Keywords:
Ecology
Compost
Microbial
Biological processes
Waste management

ABSTRACT

Biological transformation of municipal solid waste is an environment-friendly management strategy against recalcitrant residues. The bacterial biome that inhabit said residues are responsible of decomposing both simple and complex materials. For this reason, processes such as composting, which favor the acceleration of the transformation of organic matter, can contribute to the degradation of municipal solid waste. Not only as mere fertilizer for crops, but also as methods for the recovery of solid waste. However, the control of the conditions necessary to achieve an optimal process on an industrial scale is a great concern. Thus, the aim of this work focuses on the characterization of the bacterial microbiome on three municipal solid waste facilities in order to deepen the role of microorganisms in the state of the final product obtained. For it, an intensive metagenomic analysis as well as a battery of physicochemical determinations were carried out. The lack of adequate thermophilic phases was decisive in finding certain bacterial genera, such as *Lactobacillus*, which was significant through these processes. Biodiversity did not follow a common pattern in the three processes, neither in abundance nor in richness but, in general, it was greater during the bio-oxidative stage. Despite the different trend in terms of the degradation of carbon fractions in these wastes, at the end of the biodegradation treatments, a sufficient degree of bioestabilization of the organic matter was reached. The results offer the opportunity to obtain a level of detail unprecedented of the structure, dynamics and function of the bacterial community in real conditions, without the control offered by laboratory conditions or pilot plants.

1. Introduction

Management of Municipal Solid Waste (MSW) is one of the greatest concerns that governments must assume, both in developed and developing countries. These residues are among those recognized to pose the risk of biological hazard, getting to affect the health of people and cause negative impact to the environment. Additionally, MSW disposal in landfill can result in the loss of large amounts of potential valuable resources, if adequate separation is not previously made. It includes a significant part of the organic fraction, which conventionally is treated by anaerobic digestion and/or composting, allowing the production of energy and the stabilization of organic matter, respectively. Thus, leading the valorization of all the material (Rodríguez et al., 2019).

Of the two alternatives mentioned above, composting is considered one of the most successful biotechnological strategies for a sustainable recycling of organic matter. It has gained importance in European legislation as the method of choice for the treatment of biodegradable waste (Neher et al., 2013). In fact, the updated European Union (EU) Waste Law (2018) recommends the composting process as the first and most appropriate alternative for the treatment and revaluation of biowaste.

Microorganisms can colonize a wide variety of natural and anthropogenic environments, due to their metabolic diversity. For example, the production of metabolites such as proteins, carbohydrates or humic substances during the biological transformation of organic matter. This gives the varied microbial communities, and their metabolites, the ability to impact on physicochemical properties of the niches they occupy (Sepehri and Sarrafzadeh, 2019). For this reason, composting microbiome deserves special attention and deepen on its knowledge, since it will help to comprehend and optimize the process itself, especially necessary on a large scale.

The current development of '-omics' approaches has provided the

* Corresponding author.
E-mail address: mjr956@ual.es (M.M. Jurado).

https://doi.org/10.1016/j.jenvman.2020.110528
Received 30 July 2019; Received in revised form 25 March 2020; Accepted 28 March 2020
Available online 13 April 2020

opportunity to obtain a complete picture of the composting microbiome directly from samples (Wang et al., 2016). Using these techniques, the variety of microorganisms retrieved is enormous, including some not previously described for these habitats (de Gannes et al., 2013).

Despite the general efficiency of composting it is not always feasible to carry out an optimal process at the industrial level, usually due to the low qualification of personnel, insufficient space, inadequate resources, or simply for economic issues. Nonetheless, the activity of the indigenous microbiota itself usually allows to reach a level of biotransformation that results in obtaining a stabilized product. Bacteria are the most influential group of microbes due to their metabolic versatility. Therefore, understanding the dynamics of the bacterial community would be useful for the effective management and improvement of large scale composting processes of MSW (Brown et al., 2008).

In this work, three different large scale facilities dedicated to composting of MSW were sampled. Differences between physicochemical and microbial composition throughout the processes were evaluated. The specific objectives considered were: (i) to analyze the evolution of physicochemical parameters, basic and related to simple carbon transformation, through the processes, (ii) to evaluate and identify bacteria through an intensive sampling protocol in order to establish the community structure at different stages by using metagenomic approach, and finally (iii) to assess organic matter transformation and bacterial microbiome composition in order to establish the relationship between microorganisms and stability achieved in final products.

2. Material and methods

2.1. Sampling strategy and experimental set-up

The processes studied were carried out in three municipal waste treatment plants, here named Urban Solid Wastes (USW). They were located in the Southeast of Spain, specifically: Almeria (USW1), Albox (USW2), and Murcia (USW3). The first two plants facilities had similar operation conditions, that is, in-vessel turned windrows in bays, whereas the third was in-vessel tunnel composting with automated turning by augers. All the plants process municipal solid waste by composting, to obtain stabilized organic matter. The final destination is landfill sealing. Total duration of processes were (in months): 3.5 (USW1), 4.5 (USW2) and 3 (USW3).

Samples were collected at 6 critical stages of composting: Raw Materials (RM), Mesophilic phase (MES), Thermophilic phase (THER), Cooling phase (COOL), Maturation phase (MAT) and Final Product (FPR). At each stage, sub-samples were taken from nine different locations of the pile, covering several depths and surface. They were mixed in equal amounts, in order to achieve a homogeneous and representative sample. Each sample was divided in three parts for analytical replicates.

2.2. Analytical methods used for characterization of the composting processes

The moisture content was determined by drying at 105 °C for 24 h pH and Electrical conductivity (EC) were analyzed in a 1:10 (w/v) water extract. Bulk density (BD) was measured according to the US Composting Council (USCC, 2001). Total carbon (C) and nitrogen (N) were determined in solid samples using a LecoTruSpec C–N Elemental Analyzer (Leco Co., St. Joseph, MI, USA), then calculated C/N ratio. Organic matter (OM) content was determined by weight loss on ignition at 550 °C. Soluble organic carbon (SOC) and reducing sugars (RS) were analyzed in an extract of 10 g sample in 40 mL 0.5 M K_2SO_4 shaken at 200 rpm for 30 min and filtered through filter paper. SOC was measured using a total organic carbon (TOC) analyzer TOC–VCSN (Shimadzu Co., Kyoto, Japan), while RS, in a UV-1800 Spectrophotometer (Shimadzu Co., Kyoto, Japan) according to method described by Somogyi (1951). Carbon biomass (CBIO) was determined using the fumigation–extraction method according to Vance et al. (1987). Briefly, 10 g sample were fumigated in a desiccator with ethanol-free $CHCl_3$ for 24 h at 25 °C. After removal of chloroform vapor by evacuation of the desiccators. The carbon in fumigated and non-fumigated samples was extracted with 40 mL 0.5 M K_2SO_4 shaken at 200 rpm for 30 min. Extracts were filtered and total organic carbon was analyzed using a TOC–VCSN. CBIO was calculated by subtracting TOC of the non-fumigated samples from that of the fumigated ones. Total sugars (TS) were analyzed in solid samples (25 mg) after hydrolysis with 0.1 mL of 12 M H_2SO_4 for 16 h at room temperature, followed by addition of 2.4 mL of distilled water and heating in boiling water for 8 h (Safařík and Šantrůčková, 1992). TS in the hydrolyzate were spectrophotometrically quantified according to Dubois et al. (1956).

2.3. Bacterial DNA isolation, library preparation and sequencing

DNA extraction was performed using DNeasy PowerSoil DNA isolation kit (Qiagen). A total of 54 samples were processed corresponding to 3 treatment plants x 6 samplings x 3 repetitions. Bacterial DNA for sequencing was amplified by PCR, using the primers Bakt 341F (5′CCT ACG GGN GGC WGC AG 3′) and Bakt 805R (5′ GAC TAC HVG GGT ATC TAA TCC 3′), by the Supreme NZYTaq 2x Green Master Mix (NZYTech) and with the following cycle conditions: 5 min at 95 °C, continued by 25 cycles of 30 s at 95 °C, 30 s at 50 °C, 30 s at 72 °C, and a final step of 10 min at 72 °C. These primers amplified the variable regions 3 to 4 (V3–V4) of 16s rRNA gene with an expected size of 530 pb. A secondary amplification was made to join the index sequences which were required for multiplexing different libraries. It was carried out with identical conditions, but only 5 cycles and 60 °C as annealing temperature. Negative controls were included in primary and secondary amplifications using ultrapure water and the purified PCR product from the primary amplification, respectively.

The libraries products previously obtained were run on agarose gels stained with GreenSafe (NZYTech) to confirm the absence of nonspecific amplification and verify the library size. Then, these products were purified using the Mag-Bind RXNPure Plus magnetic beads (Omega Biotek). The final concentration of DNA per sample was measured with a Qubit dsDNA HSAssay (Thermo Fisher Scientific). The samples were mixed in equimolar amounts and the sequencing was performed on a MiSeq PE300 run (Illumina) at AllGenetics & Biology SL (La Coruña, Spain).

2.4. Metagenomic data analysis

The raw data set were separated based on the sample-specific barcodes and deleted the indices and sequencing primers. The quality of the demultiplexed FASTQ files was verified by FastQC software (Andrews, 2010). Paired-end assembly of the R1 and R2 reads was performed with FLASH (Magoč and Salzberg, 2011), with a minimum length overlap of 30 base pairs. CUTADAPT software 1.3 was used to eliminate sequences below 300 nt which did not contain the PCR.

FASTQ files were analyzed using “Quantitative Insights into Microbial Ecology” (QIIME) v1.9.0 software (Caporaso et al., 2010). Sequence were quality-filtered with a Phred score of 20 quality threshold and the Chimeric sequences were eliminated using the UCHIME algorithm (Edgar et al., 2011) implemented in VSEARCH, with a reference based on chimera detection using Silva Database (Quast et al., 2013). It resulted in 8,141,350 total readings with a mean of 150,765 sequences per sample.

The sequences were taxonomically assigned using the open-reference approach in QIIME. The OTU picking was run using the Silva Database and each OTU was assigned to a microbial taxon using the UCLUST algorithm (Edgar, 2010) with a confidence threshold of 97%. Singletons and OTUs with less sequences than 0.005% of the total were excluded from the analysis. Assignments to photosynthetic bacteria such as Cyanobacteria were removed, since they are known to correspond to chloroplast DNA derived from plants, which was

amplified by universal primers. Low abundance of OTUs (below 0.1%) of each sample was removed to correct the index jumping phenomenon.

Rarefaction curves and alpha diversity index were calculated from rarefied samples, using Shannon (1948) and Simpson (1949) indices for diversity and Chao1 (Chao, 1984) and Abundance-based Coverage Estimator (ACE) (Chazdon et al., 1998) indices for richness. Beta diversity was calculated using weighted and unweighted UniFrac (Lozupone et al., 2006) distance matrices, and the principal coordinate analysis (PCoA) generated 2D and 3D plots for all mapping fields. In addition, samples were clustered using UPGMA (Unweighted Pair Group Method with Arithmetic mean, also known as average linkage) (Michener and Sokal, 1957). BLASTn was compared to the Ribosomal Database Project (Cole et al., 2014) 16s rRNA database for taxonomically assigning reads at the species level. Only top hits with >97% similarity and >380bp alignment length were considered, which would encompass the 16s hypervariable region v3-v4.

2.5. Statistical analysis

Analytical data obtained were subjected to statistical analysis using Statgraphics Centurion XVI.I (StatPoint Technologies Inc., Virginia). Analyses of variance (ANOVA) and a multiple comparison test (Fisher's Least Significant Difference) were performed to compare mean values for different factors and variables analyzed ($P < 0.05$). Microbial community comparisons and other statistical analysis from the metagenomics section were performed using the statistical software R v2.15.2 with the packages for Community Ecology (vegan), Euclidean Methods in Environmental Sciences (ade4) and gplots packages R (The R Foundation for Statistical Computing) (R Core Team, 2013). Other statistical analysis and graphs were performed using MicrobiomeAnalyst (Dhariwal et al., 2017). Calypso software version 8.84 was used with normalized via Cumulative Sum Scaling (CSS) in order to generate heatmaps for the Spearman's correlations between the bacterial genera and physicochemical parameters.

2.6. Data availability

16S rRNA gene sequences are stored in the MG-RAST public repository, available by Accession Number mgp92493. The whole datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

3. Results and discussion

3.1. Trend of physicochemical parameters during composting

Characteristic parameters used in composting monitoring followed known general trends and adequate from the legislative point of view, although not strictly optimal. Temperature is one of the main indicators of correct evolution of composting process. Thus, temperatures higher than 55 °C, are related to an adequate biotransformation of organic matter. The accomplishment of a thermophilic phase maintained during time is usually considered necessary to obtain a stabilized and sanitized product (Onwosi et al. 2017). In this work, the temperature profile did not exactly fulfilled the evolution typically recommended for composting processes. That is, a thermophilic phase in which the composting piles are kept above 55 °C for 3 consecutive days (EPA, 2003). Therefore, treatment used in the plants could not be considered strictly composting from the scientific point of view, although it allows the enough stabilization of organic waste.

The pH values were quite different between facilities and sampling stages (Table 1). The metabolic activity of microorganisms influences the evolution of pH and viceversa. Thus, pH values below 6 can hinder the performance of microorganisms, especially for bacteria that require pH between 6 and 7.5. Besides, it is important to keep in mind that pH evolution during composting also depended on the pH of the starting raw materials. Thus, Sundberg et al. (2004) found that the inhibition of thermophilic microorganisms, and the delay in the transition from mesophilic to thermophilic conditions might be justified by the decrease in pH at temperatures below 45 °C. This generates conditions that lead to a very low rate of nutrient degradation that lasts for long periods of

Table 1
Physicochemical parameters related to the control and monitoring of the processes studied (three Urban Solid Wastes treatment plants: USW1, USW2 and USW3). Sample code: Raw Materials mixture (RM), Mesophilic stage (MES), Thermophilic stage (THER), Cooling stage (COOL), Maturation phase (MAT), and Final Product (FPR).

Facility	Sample Code	Sampling days	T[a] (°C)	pH	EC[b] (mS/cm)	BD[c] (g/cm3)	M[d]%	C[e]%	N[f]%	C/N ratio[g]	OM[h]
USW 1	RM	0	22.0	5.2 ± 0.0	3.8 ± 0.6	0.1 ± 0.0	78.2 ± 4.7	40.3 ± 0.1	1.4 ± 0.0	28.5 ± 0.5	74.7 ± 0.1
	MES	1	22.0	7.8 ± 0.2	3.8 ± 0.5	0.1 ± 0.0	17.7 ± 4.2	32.3 ± 1.0	1.6 ± 0.1	20.2 ± 1.1	66.3 ± 2.0
	THER	14	40.0	8.4 ± 0.0	3.1 ± 0.8	0.1 ± 0.0	36.7 ± 6.0	27.1 ± 1.0	1.9 ± 0.1	14.0 ± 0.4	54.1 ± 1.8
	COOL	42	22.0	8.1 ± 0.3	5.6 ± 0.3	0.1 ± 0.0	21.1 ± 8.6	31.9 ± 1.9	2.0 ± 0.1	15.8 ± 1.4	57.6 ± 1.3
	MAT	78	45.0	8.6 ± 0.0	6.4 ± 0.3	0.1 ± 0.0	13.0 ± 0.3	27.6 ± 0.5	2.3 ± 0.0	12.1 ± 0.2	51.9 ± 1.6
	FPR	107	22.0	8.7 ± 0.1	7.2 ± 0.6	0.1 ± 0.0	11.3 ± 0.3	28.0 ± 0.6	2.4 ± 0.0	11.8 ± 0.3	53.9 ± 0.7
USW 2	RM	0	22.0	3.9 ± 0.2	13.0 ± 0.4	0.1 ± 0.0	84.8 ± 0.2	42.8 ± 0.8	1.5 ± 0.1	28.0 ± 1.4	75.1 ± 0.6
	MES	1	22.0	5.9 ± 0.1	4.0 ± 0.7	0.2 ± 0.1	43.0 ± 0.3	33.3 ± 0.3	1.1 ± 0.0	29.4 ± 0.6	70.8 ± 0.8
	THER	16	44.0	5.5 ± 0.1	6.0 ± 0.3	0.3 ± 0.0	53.9 ± 3.2	30.8 ± 0.4	1.6 ± 0.0	19.7 ± 0.6	66.6 ± 0.8
	COOL	28	22.0	7.9 ± 0.2	2.7 ± 0.3	0.5 ± 0.0	27.8 ± 2.7	32.5 ± 2.4	1.5 ± 0.1	22.4 ± 2.5	55.8 ± 0.2
	MAT	119	22.0	8.1 ± 0.1	5.9 ± 0.7	0.4 ± 0.0	8.8 ± 1.0	28.6 ± 0.7	1.5 ± 0.0	19.1 ± 0.4	52.2 ± 1.5
	FPR	138	22.0	7.5 ± 0.0	5.1 ± 0.8	0.6 ± 0.0	5.6 ± 0.2	23.9 ± 1.1	1.5 ± 0.1	15.6 ± 0.6	38.0 ± 1.4
USW 3	RM	0	22.0	7.2 ± 0.0	2.8 ± 0.1	0.2 ± 0.0	61.1 ± 5.2	37.0 ± 0.7	1.8 ± 0.1	20.1 ± 1.3	71.7 ± 0.9
	MES	1	22.0	5.3 ± 0.0	2.1 ± 0.2	0.2 ± 0.0	62.9 ± 2.6	35.4 ± 0.5	1.7 ± 0.0	21.4 ± 0.2	65.1 ± 2.6
	THER	7	49.0	5.6 ± 0.0	2.3 ± 0.1	0.2 ± 0.0	53.3 ± 3.8	34.9 0.3	1.6 ± 0.1	21.7 ± 1.0	67.7 ± 0.5
	COOL	26	22.0	5.9 ± 0.0	3.2 ± 0.5	0.3 ± 0.0	52.6 ± 0.7	32.5 ± 1.3	1.6 ± 0.1	20.9 ± 0.2	66.6 ± 0.7
	MAT	65	22.0	5.8 ± 0.0	3.1 ± 0.2	0.3 ± 0.0	53.1 ± 0.7	35.0 ± 0.4	1.6 ± 0.0	21.8 ± 0.1	66.4 ± 0.9
	FPR	93	22.0	6.0 ± 0.0	2.7 ± 0.3	0.3 ± 0.0	50.9 ± 0.7	34.7 ± 0.2	1.5 ± 0.1	22.4 ± 0.7	63.7 ± 1.1

[a] Temperature (T).
[b] Electrical Conductivity (EC).
[c] Bulk Density (BD).
[d] Moisture (M).
[e] Total Carbon (C).
[f] Total Nitrogen (N).
[g] Total Carbon/Total Nitrogen ratio.
[h] Organic Matter (OM).

time. This would explain the insufficient temperatures recorded during the processes studied. At the far side, pH values close to or higher than 9 promote the conversion of nitrogen to ammonia, which negatively affects the growth and activity of microorganisms (Moreno et al., 2013). In this work, the pH values increased throughout the process, reaching final values that oscillated around alkaline values, except in USW3 where no significant changes were recorded (Table 1). The same occurred for electrical conductivity (EC) and bulk density (BD) (Table 1). This could be due to an unfinished process. On the contrary, the data obtained for EC and BD in USW1 and USW2 support the correctness of the process, in comparison with optimal composting processes (López-González et al., 2013).

The evolution of moisture observed in USW3 (Table 1) coincides with the range of values considered optimal for most of the mixtures used in composting, between 50 and 60% (Liang et al., 2003). Water is required for a suitable microbial activity. Therefore, moisture levels below 40–45% result in a decrease of the microbial activity, especially bacterial. Values below 20% can severely restrict microbial growth, which would imply stopping or slowing down (Moreno et al., 2013). On the other hand, excess moisture (>65%) negatively affects the availability of oxygen because water displaces air in the spaces between the particles and generates anaerobic conditions and nutrients lixiviation (Gajalakshmi and Abbasi, 2008). This was observed in USW1 and USW2 plants (Table 1). Nevertheless, although the raw materials started with an excess of humidity, the moisture in the final material decreased dramatically, difficulting the bacterial activity.

Regarding C/N ratio (Table 1), USW1 and USW2 followed a similar trend, starting with values of 28.5 and 28.0, respectively. These values are considered optimal for composting (between 25 and 30) (Jurado et al., 2014a). Subsequently, C/N values declined abruptly after mesophilic phase, and reached values far below 20 in final products. This was suggested by Golueke (1981) as indicative of composts sufficiently stable and mature. In the case of USW3, C/N ratio remained around 20 during the whole process, without significant changes. Even the highest result was reached in the last sample (FPR). This could be due to a premature finalization of the process or more likely to the accidental incorporation of fresh materials during the last stage. This incorporation could affect the balance between total carbon and total nitrogen, incorporating partially biotransformed organic matter that would increase the C/N ratio (Estrella-González et al., 2019). Finally, the

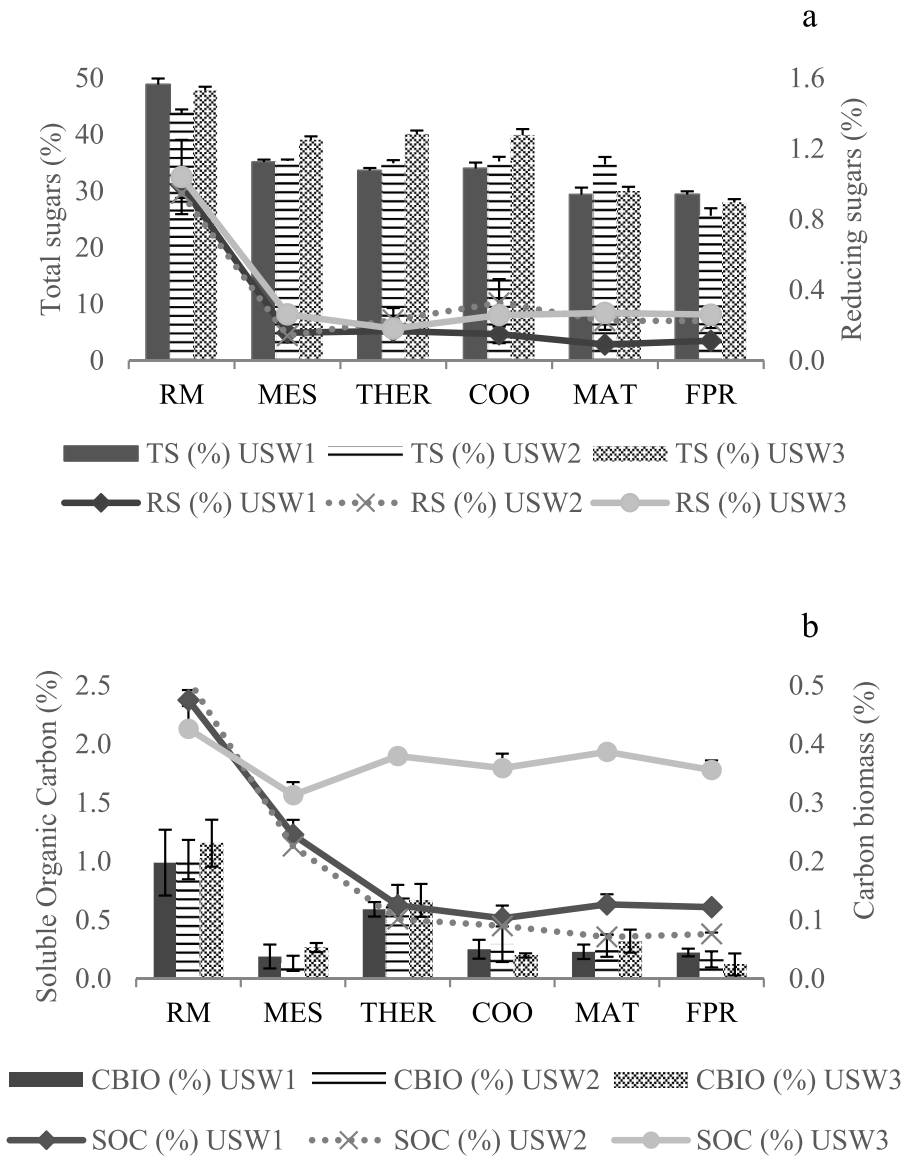


Fig. 1. Trends in parameters related to the biotransformation of organic matter during the processes studied (three Urban Solid Wastes treatment plants: USW1, USW2 and USW3). Y axes: (a) Total Sugars (TS) and Reducing Sugars (RS), and (b) Soluble Organic Carbon (SOC) and Carbon Biomass (CBIO). X axes: Raw Materials mixture (RM), Mesophilic stage (MES), Thermophilic stage (THER), Cooling stage (COOL), Maturation phase (MAT), and Final Product (FPR). Results are means (n = 3) ± SD (vertical bars). ($LSD_{TS\_USW1} = 24{,}1661^{b}$; $LSD_{TS\_USW2} = 18{,}6587^{a}$; $LSD_{TS\_USW3} = 24{,}3403^{b}$; $LSD_{RS\_USW1} = 1{,}75296^{b}$; $LSD_{RS\_USW2} = 0{,}671128^{a}$; $LSD_{RS\_USW3} = 0{,}3775^{a}$). ($LSD_{CBIO\_USW1} = 0{,}0822395^{a}$; $LSD_{CBIO\_USW2} = 0{,}0864252^{a}$; $LSD_{CBIO\_USW3} = 0{,}0907128^{a}$; $LSD_{SOC\_USW1} = 0{,}996598^{b}$; $LSD_{SOC\_USW2} = 0{,}894785^{a}$; $LSD_{SOC\_USW3} = 1{,}84919^{c}$). Different letters show significant differences.

declining evolution of OM in USW1 and USW2 (Table 1) matches with C/N values obtained, as it is for the scarce variation of OM values in USW3 that implies the lack of degradation during composting (Table 1). In any case, results for OM evolution were clearly indicative of microbial activity in the piles, with greater or lesser intensity according to the USW facility.

3.2. Evolution of carbon fractions during the composting processes of municipal solid waste at industrial scale

In addition to monitoring parameters, other factors are frequently used to assess organic matter transformation. Some of these are those related to carbon fraction such as Total Sugar (TS), Reducing Sugar (RS), Soluble Organic Carbon (SOC) and Carbon biomass (CBIO). The evolution of TS content had a similar pattern in all the plants, with a consecutive decrease throughout the process. The initial values were the highest (45–50%) and the lowest were obtained in the final products (25–30%) (Fig. 1a). The evolution of RS content followed a similar trend to that of TS, also for all the three plants (Fig. 1a). These results are reasonable considering that sugars represent the best available carbon source for most microbial species associated to composting. In contrast, other authors have reported an increase-decrease pattern during bio-oxidative phase in function of temperature changes (Jurado et al., 2014b). Taking this into account, the evolution of TS and RS in current processes was logical, since sugars represent the available carbon source for most microbial species associated with composting.

Evolution of SOC (Fig. 1b) followed the same decreasing trend in USW1 and USW2, as it also occurred for other parameters previously described such as RS, TS and OM, whereas in USW3 the lowest values were registered at mesophilic stage and maintained around 2% during the other stages. SOC of immature compost mainly consists of total sugars (including hemicellulose or cellulose), phenolic substances, amino acids, peptides and other easily biodegradable compounds (Jurado et al., 2014b). Thus, the lower values at the end of composting reveal that the biodegradation of the material is being accomplished because part of them are used exclusively by microorganisms for their own growth (Hsu and Lo, 1999). Regarding CBIO (Fig. 1b), material from all plants followed the same pattern. The highest values were obtained at the beginning, while the lowest were registered at mesophilic phase and in the final product. CBIO provides information about the extent to which microbial biomass evolves during composting. In fact, other studies based on composting of similar residues than those studied here have revealed the relationship between the decline in microbial biomass, from the maturation phase, with a slow and incomplete stabilization of organic matter (Villar et al. 2016).

3.3. Metagenomic analysis of municipal solid waste composting processes on industrial scale

After the sequencing of the samples, a total of 808,226 16S rRNA reads were obtained. The average number of reads per sample was 44,901 (range between 32,558 and 65,729 reads), after sequence length, quality filtering and chimera removal. All this information came from the 54 total samples analyzed in this work. After observing the biological heterogeneity between the replicas, it was decided to mix and treat them as a single sample. For this reason, the rarefaction curves, α and β diversity and taxonomic classification were calculated from subsampling with 32,000 sequences for each sampling stage, where each replica provided the same information. The end results were based on 18 samples, corresponding to the 6 stages sampled in each treatment plant studied.

The differences in diversity and richness between USW1, USW2 and USW3, were compared with the help of Chao1, ACE, Shannon and Simpson indices (Table 2) and rarefaction curves (Fig. 2). USW1 showed the greatest diversity and richness during COOL stage, and the lowest in RM. In regards to USW2, bacterial diversity was greater at the beginning of the process (RM) and bacterial richness in COOL phase. On the other hand, MAT was the phase with the lowest diversity and richness. Finally, in USW3 the highest and lowest bacterial diversity were observed in RM and THER phases, respectively. Meanwhile, the greatest richness was observed in MES stage and the lowest in COOL. Due to the differences among the three facilities, regarding the mixture of raw materials and the operating techniques used, diversity and richness followed completely different trends. López-González et al. (2015) reported maximum bacterial diversity consistently found at the mesophilic and thermophilic stages during lignocellulosic waste-based composting. This affirmation about the biodiversity of composting has been attributed mainly to the restrictiveness of the thermophilic phase, since it requires changes in the composition of resident microbiota. At any rate, the explanation of the great differences found between the highest and lowest values of diversity and richness recorded in this work can be supported by the conclusions of other studies that did not indicate temperature as the selective force that drives microbial diversity. In this sense, Adams and Frostick (2009) suggested the participation of other factors as critical to determine biodiversity in composting environments. Throughout this work it could be observed a bacterial succession not very different from processes with a higher level of optimization. Although the conditions were less controlled and, despite a clear absence of sustained thermophilic phase during the minimum time necessary, the bacterial diversity and richness were significant, coinciding with previous works (Tkachuk et al., 2014).

Table 2
Diversity and richness indexes in each phase sampled during the processes studied (three Urban Solid Wastes treatment plants: USW1, USW2 and USW3). Sample code: Raw Materials mixture (RM), Mesophilic stage (MES), Thermophilic stage (THER), Cooling stage (COOL), Maturation phase (MAT), and Final Product (FPR).

Company	Sample Code	Observed OTUs	Chao 1	ACE	Shannon	Simpson
USW 1	RM	140	181	192	0,960	0,320
	MES	276	313	316	3,337	0,924
	THER	273	290	291	4,103	0,966
	COOL	316	331	334	4,208	0,971
	MAT	293	317	315	4,124	0,971
	FPR	315	327	328	4,196	0,969
USW 2	RM	312	318	318	4,407	0,978
	MES	284	313	308	3,824	0,958
	THER	320	350	354	3,631	0,925
	COOL	369	398	401	4,307	0,975
	MAT	232	267	279	2,882	0,885
	FPR	278	316	310	3,656	0,951
USW 3	RM	364	388	392	4,873	0,986
	MES	377	406	410	2,679	0,711
	THER	245	270	270	1,608	0,470
	COOL	228	257	260	2,532	0,869
	MAT	263	314	320	2,682	0,878
	FPR	261	305	313	2,376	0,820

The assignment of the 16S rRNA reads showed significant differences in the composition of the bacterial groups between all samples of different plants (USW1, USW2 and USW3). However, some genera coincide in the different processes and phases, mainly belonging to the phyla *Firmicutes, Proteobacteria, Actinobacteria* and *Bacteroidetes*. These results coincide with previous work carried out exclusively from the cultivable microbiota, isolated from composting processes (López-González et al., 2015; Tortosa et al., 2017).

In this work, bacterial succession was studied at different levels. At the level of bacterial order, in USW1 treatment plant, the most frequent order detected in RM phase was *Enterobacteriales* (83.7%), and in MES phase, *Lactobacillales* (35.4%) and *Bacillales* (27.3%). In the THER, COOL, MAT and FPR phases, the taxonomic orders with greater abundance were included in "Other" group with reads lower than 10% (37.8%, 32.1%, 38.4%, and 37.5%, respectively). The most abundant order in USW2 was *Lactobacillales* 34.1%, followed by taxonomic orders

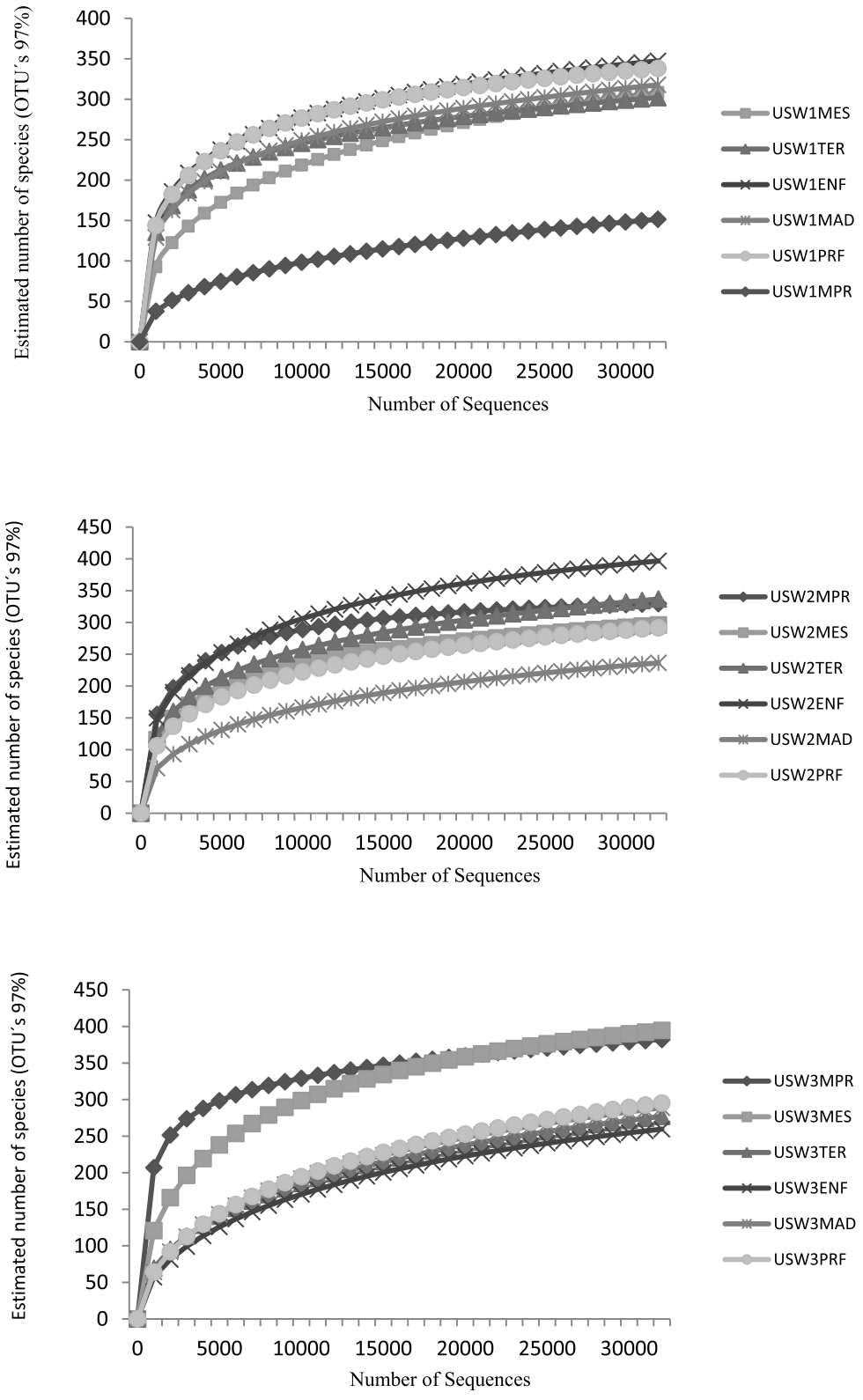


Fig. 2. Rarefaction curves divided into three graphs, corresponding to the tree Urban Solid Waste (USW) treatment plants studied, during the different phases sampled:(Raw Materials mixture-RM, Mesophilic stage-MES, Thermophilic stage-THER, Cooling stage-COOL, Maturation phase-MAT, and Final Product-FPR). The rarefaction curves related the sequencing effort to the estimated number of species, determined by Operational Taxonomic Units (OTUs) at 97% of sequence identity. Y axes shows the number of reads (sequencing effort) obtained by sequencing the 16S rRNA gene. X axes shows the number of operational taxonomic units (OTUs) at a level of 97% (estimated number of bacterial species). Curves code: Plant (USW1, USW2 and USW3) and Phase (RM, MES, THER, COOL, MAT and FPR).

<10% (32.1%). Specifically, in MES phase stood out *Lactobacillales* (30.1%) and *Corynebacteriales* (28.5%); in THER, *Lactobacillales* (50.2%) and *Corynebacteriales* (13.0%); in COOL phase orders under 510% (46.9%), *Lactobacillales* (12.5%) and *Corynebacteriales* (12.0%); in MAT phase, *Bacillales* (33.4%), and a 27.0% assigned to *Actinobacteria* class with an order unidentified. In the last phase, FRP, *Bacillales* (33.3%) was the most abundant order, as well as a 25.7% assigned to orders <10%. Finally, in USW3, the most common order observed in the raw materials was represented by those under 5% (52.9%), followed by *Pseudomonadales* order (30.8%). In the next stages, MES, THER, COOL, MAT and FPR, the most frequent order was *Lactobacillales* with a representation of 83.1%, 90%, 35.9%, 65.3% and 46.2%, respectively. It was followed by order *Bacillales* with a relative abundance of 38.6% and 41.7% during the COOL and FPR stages.

To analyze the taxonomy at the level of bacterial genera (Fig. 3) Silva database was used. However, it was insufficient to recognize the genera in USW1, during first stage, since the family Enterobacteriaceae, which represents 81.0% of the total sequences, showed up classified as "ambiguous taxa". Thus, in order to find out the related genera, a reclassification was made using Ribosomal Database Project Classifier. Then, it was found out *Erwinia* (53.3%) and unclassified_Enterobacteriaceae (43.7%), as the most abundant genera in this family. In MES stage, the most common genera were *Lactobacillus* (33.9%), *Bacillus* (21.6%), *Limnochorda* (13.5%) and *Corynebacterium 1* (8.1%). In THER, COOL, MAT, and FPR phases most reads corresponded to genera by under 10%, (37.8%, 32.1%, 38.4%, 37.5%, respectively) followed by the genera *Nocardiopsis* with 12.0% and *Halomonas* 9.7% in THER, *Corynebacterium* 1 (20.7%) and *Lactobacillus* (94%) in COOL phase, *Brevibacterium* 9.6% in MAT phase, and finally *Planococcus* with a relative abundance of 8.7% and *Brevibacterium* 8.3% in the FRP phase. In USW2 treatment plant, the most abundant genus observed in RM sampling was represented by genera under 10% (32.1%), which collected all the "other" different bacterial genera that are represented in a large part, but in a small percentage each one, followed by *Lactobacillus* (18.4%.). In MES phase *Corynebaterium 1* (28.3%) and *Lactobacillus* (26.2%), in THER *Lactobacillus* (52%) and *Corynebaterium 1* (13%), in COOL phase genus <10% (46.9%) and *Corynebacterium 1* (12%), in MAD phase the most abundant genus was represented by genera unidentified in Actinobacteria class (27%) and *Melghirimyces* (13.0%) and, finally, in the FPR phase the most common genus was represented by genus under 10% and genus unidentified in Actinobacteria class (10.6%). Finally, in USW3 plant, RM phase was mostly represented by genera under 5% with a 52.9% of relative abundance, followed by genus *Pseudomonas* (16.2%) and *Acinetobacter* (14.6%), in MES, THER, COOL, MAT and FPR. Furthermore, a high abundance of reads assigned to *Lactobacillus* genus was observed, 81.6%, 90%, 35.8%, 64.7% and 45.7%, respectively. Followed by the *Bacillus* and *Corynebacterium 1* genera in the samples COOL (35.8%, 15.3%), MAT (64.7%, 13.3%) and FPR (45.7%, 6.8%).

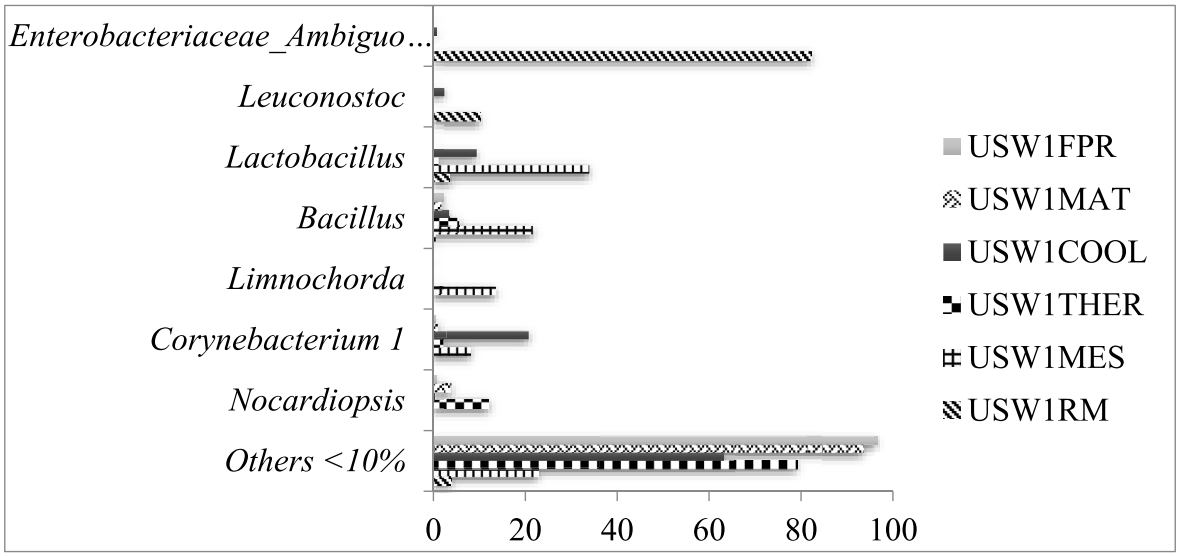
Most genera found are widespread in natural and anthropogenic environments, such as soil, fresh and marine waters, sediments, plant rhizospheres, activated sludge and waste-water treatment systems, and even have been isolated from different composts (Tortosa et al., 2017). On the other hand, *a priori* it seems unlikely to find bacteria in composting of the genus *Lactobacillus* due to its association with anaerobic growth conditions. Nevertheless, different authors, such as Wakase et al. (2008), reported that *Lactobacillus* was detected in many composting processes, mainly at the early stages. This bacterial genus is associated with the production of lactic and acetic acid, which leads to the lowering of pH values in the environment. Accurately, the high diversity of *Lactobacillus* bacteria observed in this work in different stages throughout the processes may be related to the fluctuation of pH, which sometimes reached low values (Song et al., 2018). Likewise, this type of facultative anaerobic microbes has been recognized as a valuable marker for the identification of anaerobic conditions in aerobic biotransformation processes, such as composting (Weglarz et al., 2018).

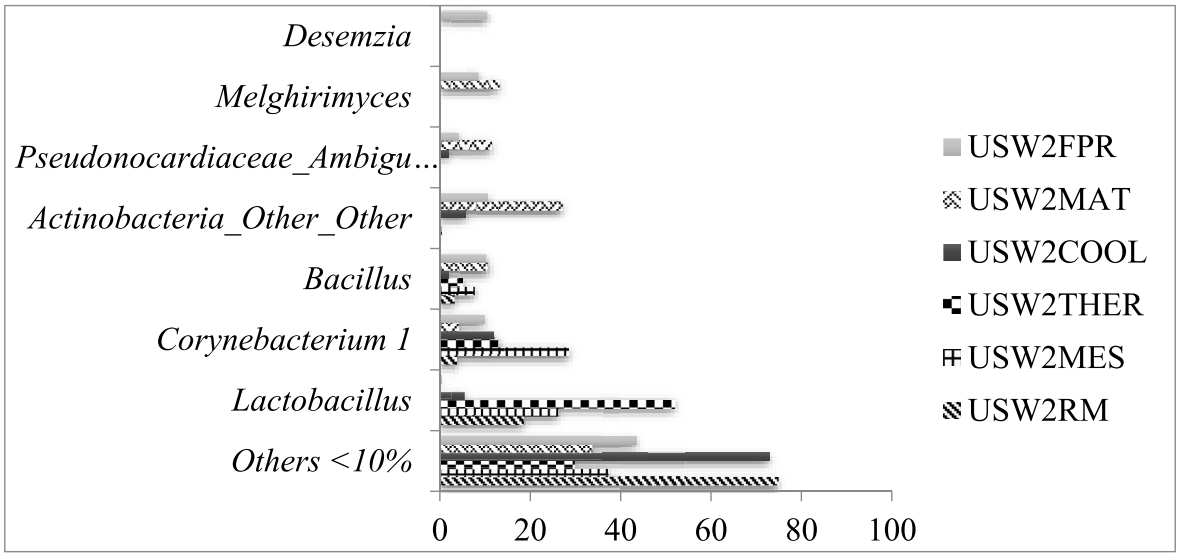
To identify up to species level some of the most interesting genera, as well as to look for the presence of common enteropathogen species that are interesting from the point of view of public health, the sequences were subjected to BLASTn. Although the use of the long amplicon of 16S rRNA gene has been reported as a useful tool for the identification of species and studies of microbial diversity, it is not as accurate as other specific genes. Computer simulations show that the accuracy of taxonomic allocation, especially at the species level, decreases drastically in short reads, such as those of current Illumina or Ion Torrent technologies (Claesson et al., 2010). Therefore, the assignment at the species level should be treated with caution. But, though the limitations of sequencing with Illumina are known, the use of short amplicon sequencing was tested in order to minimize errors in taxonomic allocation with the considerations described in the 2.4. section for BLASTn.

According to this assignment, the genus *Lactobacillus* present in all the facilities, with high proportion (>24%) in 5 phases of USW3 was dominated by *uncultured: lactobacillus* and *uncultured compost bacterium*, with most than 40% of the total reads assigned to *Lactobacillus* genus. Moreover, in the phases COOL, MAT and FPR, more than 35% of reads corresponded to *Lactobacillus ingluviei*. This species has been considered to be nonpathogenic and used in a wide variety of foods and products for humans and animals. However, it has been reported to be resistant to antibiotic at levels that exceed those recommended by the European Food Safety Authority (EFSA) (Campedelli et al., 2018). The relative abundance of this genus, as well as the absence of thermophilic bacteria, coincides with the fact that high temperatures were not reached. In fact, *Lactobacillus* has an optimal growth between 30 and 45 °C (Partanen et al., 2010). The proportion of the relative abundance of *Lactobacillus* observed in bio-oxidative stage in USW2 and USW3, specifically during RM and MES phases, respectively, was described in other studies related to composting microbiota (Partanen et al., 2010). These authors revealed that both *Lactobacillus* spp. and *Acetobacter* spp appeared as the main bacterial groups at the beginning of the process. These same authors observed that gram-positive bacteria such as *Bacillus* spp. and strains of actinobacteria were dominant in thermophilic stage. Specifically, analyzing the species belonging to the genus *Bacillus* present in the three plants, during the mesophilic phase in USW1, 70% of the reads corresponded to *Bacillus* sp. This stood out in the last phases of USW2 (MAT and FPR), as well as in the transition after biooxidative stage in (COOL and MAT phases). More than 50% of sequences assigned to *Bacillus* genus matched with *uncultured bacillus* sp, *uncultured compost_bacterium* and *uncultured soil_bacterium*. In cooling phase of USW3 was present *Bacillus thermoamylovorans* with more than 30%, typically found in composting processes (López-González et al., 2015). Finally, in the final product of USW3, dominated *Bacillus alveayuensis* (61%), a thermophilic bacterium originally isolated from deep-sea sediments (Bae et al., 2005).

In addition, the sequences obtained in the USW1, USW2 and USW3 plants were compared with the enteropathogenic microorganism databases, specifically with species belonging to the genera *Campylobacter, Escherichia, Salmonella, Shigella, Yersinia* and *Vibrio*. This cross-checking of data can help verify the hygienization of the raw material after having undergone a biodegradation process. No sequences assigned to *Campylobacter, Escherichia coli* and *Shigella,* were found. In USW2 and USW3 treatment plants, the proportion of sequences matching to *Salmonella* and *Yersinia* genera was very low (<1.3%). But it is remarkable that in the USW1 composting plant, more than 10% of the readings corresponded to *Salmonella enterica* subsp. *arizonae*, but 90% belonged to the raw material phase. This strain is a common gut inhabitant of reptiles, with snakes as the most common reservoir. Though human cases due to this organism are exceedingly rare, it may infect young infants and immunocompromised persons (Mahajan et al., 2003). Accordingly, although municipal waste is an expected source of gastrointestinal microbiota (Yang et al., 2014), biological treatment of organic matter has demonstrated to be an efficient method to remove these pathogenic agents.

Below is shown a Two-dimensional Principal Coordinates Analysis





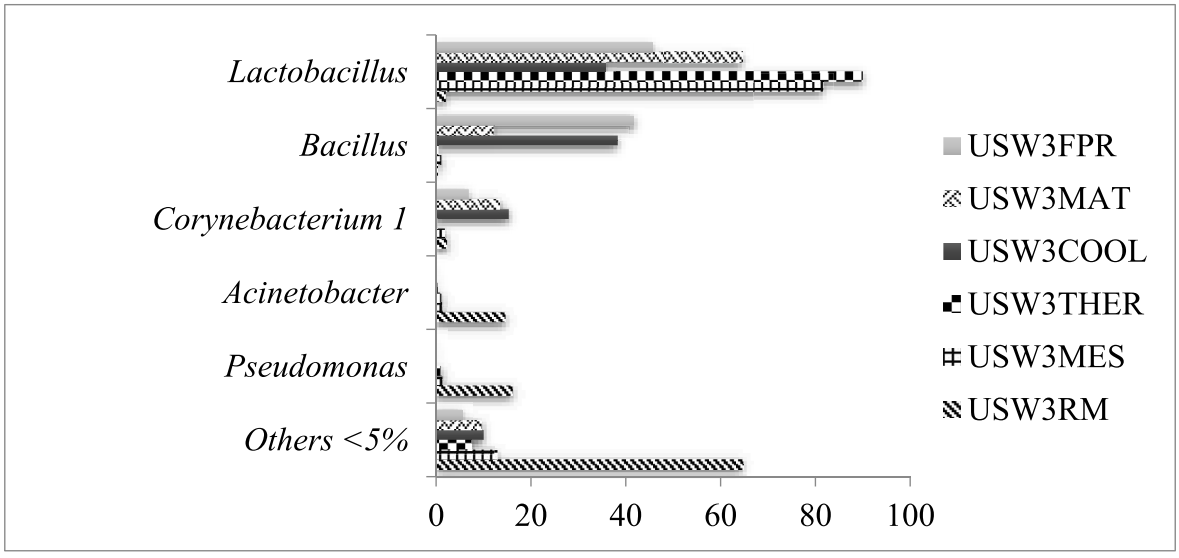


Fig. 3. Bacterial taxonomic composition of the processes studied (three Urban Solid Wastes treatment plants: USW1, USW2 and USW3). The bars show the relative abundance of the most representative bacterial genera (Y axes) as inferred by PCR amplification and pyrosequencing of the 16S rRNA gene in each phase (X axes). Bacterial genera that were under low percentage (‹10% in USW1 and USW2; ‹5% in USW3) were grouped in the "Others" category.

(PCoA) (Fig. 4) elaborated to analyze the differences in bacterial composition between samplings. The results of the 18 samples plotted show that 73% of the variability in the data could be explained by the first two components. Weighted and unweighted PCoA analyses produced similar results. The PCoA separated most of sampling stages, RM, MES, THER, COOL, MAT and FPR, occupying a different position in the 2D space, indicating that microbial composition was different ($p = 0.001$, PERMANOVA test with 1000 permutations). However, some samples came out grouped into two different small groups, one corresponding with most of the USW3 phases (MES, THER, COOL, MAT and FPR), and the other related to THER, MAT and FPR of USW1 treatment plant. That is, the evolution of the bacterial microbiome seems to be closely related to the nature of the material used and its transformation throughout the different stages except in USW2.

3.4. Overall correlation analysis of bacterial microbiome and physicochemical results

To explore the significant correlations between bacterial microbiota and physico-chemical parameters studied in the MSW facilities (USW1, USW2 and USW3), Spearman's rank correlations ($p < 0.05$) were determined and represented on a heatmap (Fig. 5). A group of physicochemical parameters were found to be positively correlated (dark shades) with some bacterial genera, for example; pH was correlated with *Saccharomonospora, Staphylococcus, Brevibacterium, Brachybacterium, Salinimicrobium, Halomonas, Oceanobacillus* and *Gracilibacillus* genera; *Staphylococcus* and *Brevibacterium*; C/N ratio with *Proteus, Weisella, Pediococcus* and *Leuconostoc*; Total Sugar with *Enterococcus, Weisella, Acinetobacter* and *Leuconostoc;* Organic Matter with *Proteus, Enterococcus, Weisella, Pediococcus, Leuconostoc, Lactococcus, Acinetobacter, Clostridium* sensu stricto 1 and *Lactobacillus*; Moisture with *Enterococcus, Weisella, Leuconostoc, Lactococcus, Acinetobacter, Pantoea, Clostridium* sensu stricto 1 and *Lactobacillus*; and, finally, Total Carbon with *Proteus, Weisella, Pediococcus, Leuconostoc* and *Acinetobacter*.

On the other hand, negative correlations (light shades) were found between physicochemical parameters and some bacterial genera. This is the case of pH with *Proteus, Enterococcus, Weisella, Pediococcus, Leuconostoc, Lactococcus, Lysinibacillus, Clostridium* sensu stricto 1 and *Lactobacillus* genera; C/N with *Halomonas, Salinimicrobium, Brevibacterium, Staphylococcus* and *Oceanobacillus;* Total Sugar with *Brachybacterium, Brevibacterium, Gracilibacillus, Oceanobacillus* and *Saccharomonospora;* Organic Matter with *Halomonas, Salinimicrobium, Brachybacterium, Brevibacterium, Gracilibacillus, Staphylococcus, Oceanobacillus* and *Saccharomonospora;* Moisture and Total Carbon with *Salinimicrobium, Brachybacterium, Brevibacterium, Gracilibacillus, Staphylococcus, Oceanobacillus* and *Saccharomonospora*; and, finally, Soluble Organic Carbon with *Brachybacterium, Brevibacterium, Gracilibacillus*, *Staphylococcus, Oceanobacillus* and *Saccharomonospora.*

With respect to bacterial division represented in cluster (Fig. 5), on one hand, a group of microorganisms related to low pH values appears at the same time related to the utilization of easily assimilable carbon fractions. This has been previously reported by some authors (Song et al., 2018) who described that one of the characteristics of biological waste, especially food waste, is the large amount of easily degradable substances such as sugars, fats, starches and grease, whose degradation implies the appearance of acidic intermediate by-products. In this sense,

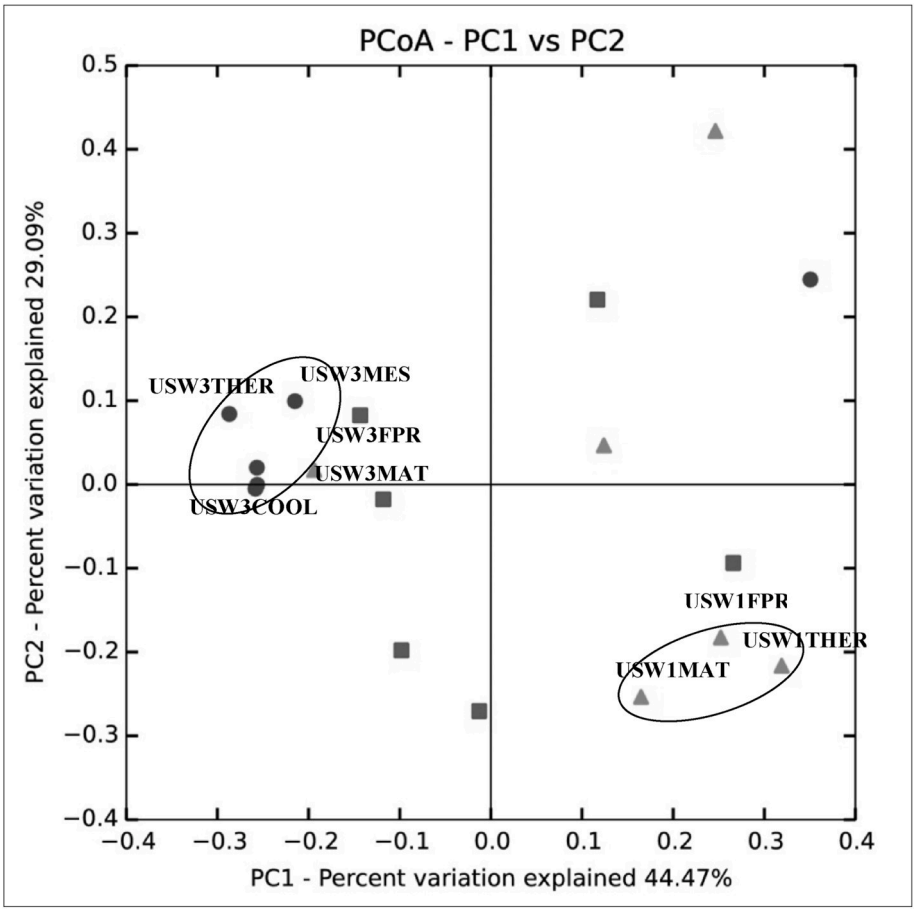


Fig. 4. Principal Coordinates Analysis (PCoA) according to bacterial composition estimated by 16S rRNA gene sequencing and distributed among samples. Unweighted Bray-Curtis distances were used (a weighted analysis provided very similar results). Samples coded in grayscale and shaped, according to the treatment plants studied. Urban Solid Wastes: USW1 (light gray triangles), USW2 (gray squares) and USW3 (dark gray circles).

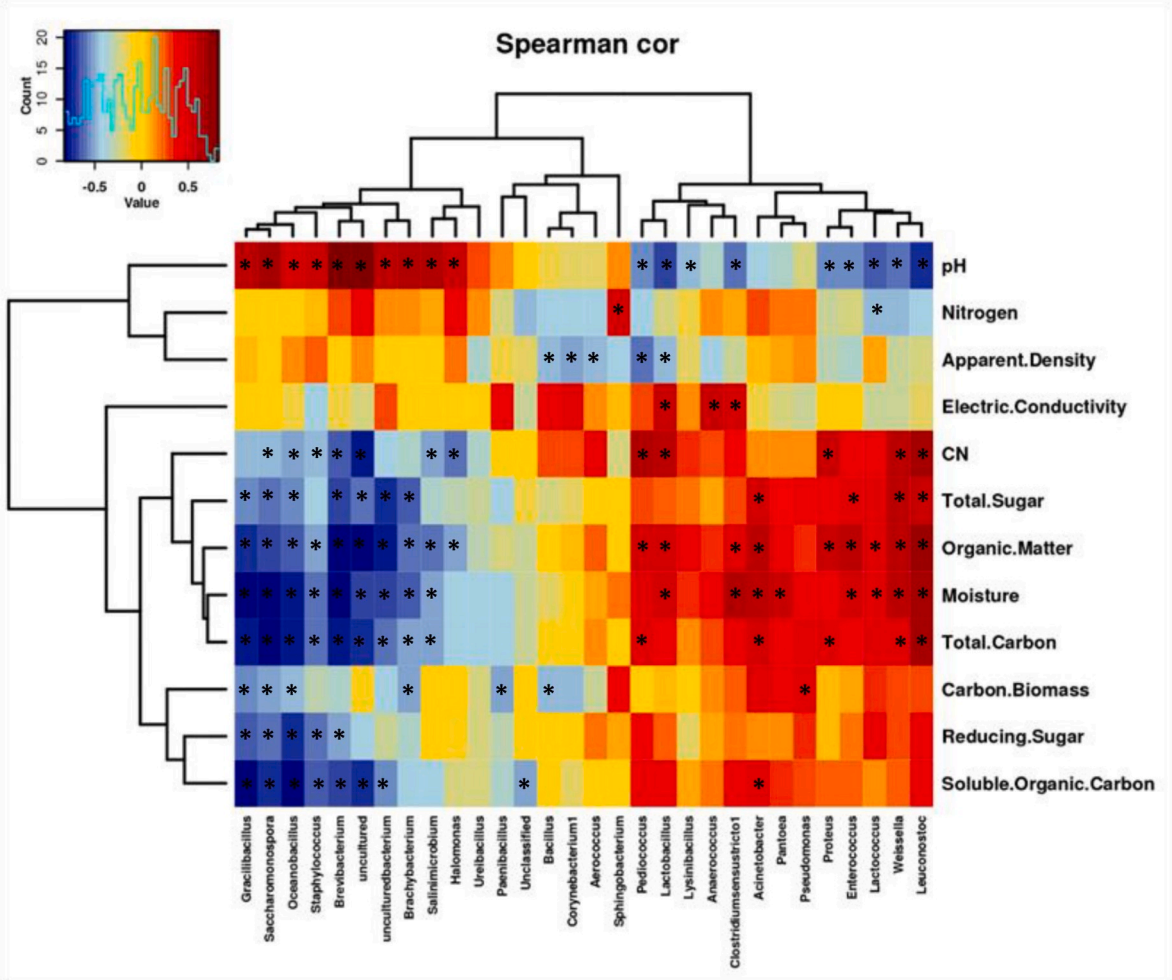


Fig. 5. Correlations between physicochemical parameters (see Table 1 and Fig. 1 for sample characteristics) and bacterial composition (see Fig. 2 for sample values of microbial diversity and richness) at genus level in samples of municipal solid waste subjected to composting (based Spearman's correlation). Positive and negative correlations are represented by shades between light gray and dark gray, respectively. Statistically most significant correlations at the level of $p < 0.05$ marked with an asterisk. Dendrograms represent different clustering among the different physicochemical parameters measured and the bacterial genera. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the presence of organic acids, like lactic and acetic, provokes acidity in the material. In addition, since anaerobic microenvironments can be found in large-scale composting piles due to the restriction of oxygen flow, which coincides with the high humidity values also indicated in this work, organic acids can be produced by obligate or facultative anaerobes such as *Lactobacillus* (Song et al., 2018), which could explain the results obtained in this work. Surely, it has been promoted by the insufficient temperature reached during the processes. It is very important to consider that most of the lactic acid bacteria have the capability to produce antimicrobial compounds and maybe it could explain the low proportion of other genera (Schnürer and Magnusson, 2005).

Conversely, the other well-defined group of bacteria, as it appears in the cluster, includes halotolerant representatives, such as *Salinimicrobium, Gracilibacillus* and *Halomonas*. It makes sense with the positive correlation with high pH values and EC, in contrast with the negative relation with low moisture. These types of microorganisms that inhabit the studied windrows, could have also a role in biodegradation processes. Precisely, *Gracilibacillus* and *Halomonas* have been described as degrading bacteria of polysaccharides associated with halophilic environments such as those generated in seaweed composting processes (Tang et al., 2011). Some authors have also indicated the ability of these halotolerant bacteria to produce some classes of enzymes (lipases, proteases, amylases and cellulases) as well as biopolymers, with an important presence in composting processes (Oliveira et al., 2015).

4. Conclusions

Although composting facilities on industrial-scale do not control optimal conditions so closely during biodegradation processes, a vibrant microbial community is present in the material with a fundamental role in its transformation. So, even after putting spoke in microbes wheels, they manage to provide the material with a degree of stabilization sufficient for the standards established in the legislation. Thus, this work serves to enhance the significance of bacterial microbiome. At the same time, it should encourage the corresponding entities and companies to try to optimize the composting processes, in order to avoid possible sanitary problems and, in addition, to achieve a higher degree of quality in the final product.

The absence of certain factors, such as sufficient thermal range of the windrows, have allowed to observe in this work the presence of facultative anaerobic and thermotolerant bacteria detected in the three piles, for example *Lactobacillus* and other genera of halotolerant bacteria closely related to this type of waste. It is necessary to take into account that, despite the lack of deep control in the processes, the physicochemical parameters studied demonstrated a sufficient degree of transformation and stabilization of organic matter. This supports the fact that the percentage of pathogens is low.

Credit author statement

Even though the corresponding author is responsible for ensuring that the descriptions are accurate and agreed upon by all authors, both the role of the corresponding author and the rest of the authors was relevant, contributing in multiple roles and in an equitable manner. However, due to the significant implications of the first two authors of the manuscript (M.M. Jurado and A.J. Camelo-Castillo), we would like them to have exactly the same consideration as the main authors.

Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgements

This work was supported by the Spanish "Ministerio de Economía y Competitividad", Project AGL2015-64512-R.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2020.110528.

References

Adams, J.D., Frostick, L.E., 2009. Analysis of bacterial activity, biomass and diversity during windrow composting. Waste Manag. 29 (2), 598–605.
Andrews, S., 2010. FastQC: a quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc.
Bae, S.S., Lee, J.H., Kim, S.-J., 2005. *Bacillus alveayuensis* sp. *nov.*, a thermophilic bacterium isolated from deep-sea sediments of the Ayu Trough. Int. J. Syst. Evol. Microbiol. 55 (3), 1211–1215. https://doi.org/10.1099/ijs.0.63424-0.
Brown, S., Kruger, C., Subler, S., 2008. Greenhouse gas balance for composting operations. J. Environ. Qual. 37, 1396–1410.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7 (5), 335–336. https://doi.org/10.1038/nmeth.f.303.
Campedelli, I., Mathur, H., Salvetti, E., Clarke, S., Rea, M.C., Torriani, S., Ross, R.P., Hill, C., O'Toole, P., 2018. Genus-wide assessment of antibiotic resistance in lactobacillus spp. Appl. Environ. Microbiol. https://doi.org/10.1128/AEM.01738-18.
Chao, A., 1984. Nonparametric Estimation of the number of classes in a population. Scand. J. Stat. 11, 265–270.
Chazdon, R.L., Colwell, R.K., Denslow, J.S., Guariguata, M.R., 1998. Statistical methods for estimating species richness of woody regeneration in primary and secondary rain forests of Northeastern Costa Rica. Man and the Biosphere Series. In: Dallmeier, F., Comiskey, J.A. (Eds.), Forest Biodiversity Research, Monitoring and Modeling: Conceptual Background and Old World Case Studies. Parthenon Publishing Group, Pearl River, NY, pp. 285–309. Paris, UNESCO.
Claesson, M., Wang, Q., O'Sullivan, O., Greene-Diniz, R., Cole, J., Ross, R., O'Toole, P., 2010. Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. Nucleic Acids Res. 38 (22) e200-e200.
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 (Database issue), D633–D642. https://doi.org/10.1093/nar/gkt1244 [PMID: 24288368].
de Gannes, V.D., Eudoxie, G., Hickey, W.J., 2013. Prokaryotic successions and diversity in composts as revealed by 454-pyrosequencing. Bioresour. Technol. 133, 573–580.
Dhariwal, A., Chong, J., Habib, S., King, I., Agellon, L.B., Xia, J., 2017. MicrobiomeAnalyst - a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Res. 45, W180–W188. https://doi.org/10.1093/nar/gkx295.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26 (19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.
Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27 (16), 2194–2200. https://doi.org/10.1093/bioinformatics/btr381.
EPA (Environmental Protection Agency, USA), 2003. Environmental regulations and technology. In: Control of Pathogens and Vector Attraction in Sewage Sludge. EPA625-R-92-013.
Estrella-González, M.J., Jurado, M.M., Suárez-Estrella, F., López, M.J., Lópz-González, J. A., Moreno, J., 2019. Enzymatic profiles associated with the evolution of the lignocellulosic fraction during industrial-scale composting of anthropogenic waste: comparative analysis. J. Environ. Manag. 248, 109312. https://doi.org/10.1016/j.jenvman.2019.109312.
EU Waste Law, 2018. Directive (EU) 2018/851 of the European Parliament and of the Council, of 30 May 2018, Amending Directive 2008/98 on Waste.
Gajalakshmi, S., Abbasi, S.A., 2008. Solid waste management by composting: state of the art. Environ. Sci. Technol. 38, 311–400.
Golueke, C.G., 1981. Principles of biological resource recovery. Biocyele 22, 36–40.
Hsu, J.H., Lo, S.L., 1999. Recycling of separated pig manure: characterization of maturityand chemical fractionation of elements during composting. Water Sci. Technol. 40, 121–212.
Jurado, M.M., Suárez-Estrella, F., Vargas-García, M.C., López, M.J., López-González, J. A., Moreno, J., 2014a. Evolution of enzymatic activities and carbon fractions throughout composting of plant waste. J. Environ. Manag. 133, 355–364.
Jurado, M.M., Suárez-Estrella, F., Vargas-García, M.C., López, M.J., López-González, J. A., Moreno, J., 2014b. Increasing native microbiota in lignocellulosic waste composting: effects on process efficiency and final product maturity. Process Biochem. 49, 1958–1969.
Liang, C., Das, K.C., McClendon, R.W., 2003. The influence of temperature and moisture contents regimes on the aerobic microbial activity of a biosolids composting blend. Bioresour. Technol. 86 (2), 131–137.
López-González, J.A., López, M.J., Vargas-García, M.C., Suárez-Estrella, F., Jurado, M., Moreno, J., 2013. Tracking organic matter and microbiota dynamics during the stages of lignocellulosic waste composting. Bioresour. Technol. 146, 574–584.
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.
Lozupone, C., Hamady, M., Knight, R., 2006. UniFrac: an online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinf. 7, 371. https://doi.org/10.1186/1471-2105-7-371.
Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27 (21), 2957–2963.
Mahajan, R.K., Khan, S.A., Chandel, D.S., Kumar, N., Hans, C., Chaudhry, R., 2003. Fatal case of *Salmonella enterica* subsp. *arizonae* gastroenteritis in an infant with microcephaly. J. Clin. Microbiol. 41 (12), 5830–5832.
Michener, C.D., Sokal, R.R., 1957. A quantitative approach to a problem in classification. Evolution 11, 130. https://doi.org/10.2307/2406046.
Moreno, J., López, M.J., Vargas-García, M.C., Suárez-Estrella, F., 2013. Recent advances in microbial aspects of compost production and use. Acta Hortic. 1013, 443–457.
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, e79512.
Oliveira, L.C.G., Locosque-Ramos, P., Marem, A., Kondo, M.Y., Rocha, R.C.S., Bertolini, T., Silveira, M.A.V., Batista da Cruz, J., Pantaroto de Vasconcellos, S., Juliano, L., Okamoto, D.N., 2015. Halotolerant bacteria in the São Paulo Zoo composting process and their hydrolases and bioproducts. Braz. J. Microbiol. 46 (2), 347–354. https://doi.org/10.1590/S1517-838246220130316.
Onwosi, C.O., Igbokwe, V.C., Odimba, J.N., Eke, I.E., Nwankwoala, M.O., Iroh, I.N., Ezeogu, L.I., 2017. Composting technology in waste stabilization: On the methods, challenges and future prospects. J. Environ. Manag 190, 140–157. https://doi.org/10.1016/j.jenvman.2016.12.051.
Partanen, P., Hultman, J., Paulin, L., Auvinen, P., Romantschuk, M., 2010. Bacterial diversity at different stages of the composting process. BMC Microbiol. 10, 94. https://doi.org/10.1186/1471-2180-10-94.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41 (Database issue), D590–D596. https://doi.org/10.1093/nar/gks1219.
R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. http://www.R-project.org/.
Rodríguez, P., Cerda, A., Font, X., Sánchez, A., Artola, A., 2019. Valorisation of biowaste digestate through solid state fermentation toproduce biopesticides fromBacillus thuringiensis. Waste Manag. 93, 63–71.
Safařík, I., Šantrůčková, H., 1992. Direct determination of total soil carbohydrate content. Plant Soil 143, 109–114.
Sepehri, A., Sarrafzadeh, M.-H., 2019. Activity enhancement of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria in activated sludge process: metabolite reduction and CO2 mitigation intensification process. Appl. Water Sci. 9, 131. https://doi.org/10.1007/s13201-019-1017-6.
Shannon, C.E., 1948. A mathematical theory of communication. Bell Syst. Tech. J. 27, 379–423. https://doi.org/10.1145/584091.584093.
Simpson, E.H., 1949. Measurement of diversity. Nature 163–688.
Somogyi, M., 1951. Notes on sugar determination. J. Biol. Chem. 73, 599–612.
Song, C., Zhang, Y., Xia, X., Qi, H., Li, M., Pan, H., Xi, B., 2018. Effect of inoculation with a microbial consortium that degrades organic acids on the composting efficiency of food waste. Microb. Biotechnol. 11, 1124–1136. https://doi.org/10.1111/1751-7915.13294.

Sundberg, C., Smårs, S., Jönsson, H., 2004. Low pH as an inhibiting factor in the transition from mesophilic to thermophilic phase in composting. Bioresour. Technol. 95 (2), 145–150. https://doi.org/10.1016/j.biortech.2004.01.016.

Schnürer, J., Magnusson, J., 2005. Antifungal lactic acid bacteria as biopreservatives. Trends Food Sci. Technol. 16, 70–78. https://doi.org/10.1016/j.tifs.2004.02.014.

Tang, J., Wang, M., Zhou, Q., Nagata, S., 2011. Improved composting of *Undaria pinnatifida* seaweed by inoculation with *Halomonas* and *Gracilibacillus* sp. isolated from marine environments. Bioresour. Technol. 102, 2925–2930. https://doi.org/10.1016/j.biortech.2010.11.064.

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.

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

USCC, 2001. Test Methods for the Examination of Composting and Compost, second ed. U.S. Composting Council, Bethesda, MD.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.

Villar, I., Alves, D., Garrido, J., Mato, S., 2016. Evolution of microbial dynamics during the maturation phase of the composting of different types of waste. Waste Manag 54, 83–92. https://doi.org/10.1016/j.wasman.2016.05.011.

Wakase, S., Sasaki, H., Itoh, K., Otawa, K., Kitazume, O., Nonaka, J., Satoh, M., Sasaki, T., Nakai, Y., 2008. Investigation of the microbial community in a microbiological additive used in a manure composting process. Bioresour. Technol. 99, 2687–2693.

Wang, C., Dong, D., Wang, H., Müller, K., Qin, Y., Wang, H., Wu, W., 2016. Metagenomic analysis of microbial consortia enriched from compost: new insights into the role of Actinobacteria in lignocellulose decomposition. Biotechnol. Biofuels 9, 22. https://doi.org/10.1186/s13068-016-0440-2.

Weglarz, T.C., Holsen, L.K., Ribbons, R.R., Hall, D.J., 2018. Microbial diversity and nitrogen-metabolizing gene abundance in backyard food waste composting systems. J. Appl. Microbiol. 125, 1066–1075.

Yang, Y., Li, B., Zou, S., Fang, H.H., Zhang, T., 2014. Fate of antibiotic resistance genes in sewage treatment plant revealed by metagenomic approach. Water Res. 62, 97–106.