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Biodiversity and succession of mycobiota associated to agricultural lignocellulosic waste-based composting

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HIGHLIGHTS

- Dynamics of fungal community in composting of agricultural residues were analyzed.
- According to its influence fungi were classified as Resident or Transient mycobiota.
- Fungal diversity decreases as the lignocellulose-based composting proceeds.
- Biological activity degree and chronology determine the structure of the mycobiota.
- Cooling phase acts as a transitional period between bio-oxidative and maturity stages.

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ABSTRACT

A comprehensive characterization of the culturable mycobiota associated to all stages of lignocellulose-based composting was achieved. A total of 77 different isolates were detected, 69 of which were identified on the basis of the 5.8-ITS region sequencing. All the isolates were assigned to the phyla Ascomycota and Basidiomycota, with prevalence of the Sordariomycetes (19) and Eurotiomycetes (17) classes. *Penicillium* was the most represented genus (11 species), while the species *Gibellulopsis nigrescens* and *Microascus brevicaulis* were detected at all the composting stages and showed the highest relative abundances. Fungal diversity decreased as the process proceed, while similarity between fungal communities associated to different samples were maximal for those phases closely connected chronologically and showing similar biological activity degree. Thus, the structure of the lignocellulose-based composting mycobiota can be divided into two major stages corresponding to bio-oxidative phase and maturation phase together with the final product, with a transitional cooling stage joining both of them.

1. Introduction

Organic wastes have become a major environmental and health concern in modern society. Different strategies have been proposed in order not just to minimize their negative effects but to turn them into resources. Composting fulfill these requirements and represents one of the most successful methodology for a sustainable management of organic waste. In fact, it is gaining importance in legislation as the method of choice for the treatment of biodegradable waste (Neher et al., 2013).

Successful composting depends on the action of different microbial populations, which in turn are influenced by physico-chemical conditions and nutritional characteristics of material being composted. The proper management of factors such as moisture, oxygen content or temperature determines the correct sequence of microorganisms and, therefore, the generation of a suitable final compost (Partanen et al., 2010). On the other hand, the nutritional properties of the raw material being composted are major responsible for the identity of the microbial communities associated to specific processes, as they selectively affect their composition and, consequently, the metabolic capabilities for transforming main organic substrates (Song et al., 2014; Wei et al., 2012).

In line with their outstanding role in composting, microorganisms have attracted the interest of researchers and articles published in this subject are numerous (Albrecht et al., 2010; Franke-White et al., 2014; Zhang et al., 2014). Nevertheless, there are still many different aspects that remain unclear, especially if we consider the uniqueness of the composting process. The

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complexity of the process on the basis of both physico-chemical and nutritional factors, as well as interactions between them, makes every single process unique in terms of microbial populations and their distribution according to composting chronology (López González et al., 2014), despite of the presence of microorganisms associated to these biotransformations whatever the conditions. A deeper understanding of the microbial communities associated to processes with specific conditions would significantly contribute to improving their efficiency.

Many different microorganisms are involved in the composting process, with bacteria and fungi being the most prominent (MacCready et al., 2013; Neher et al., 2013). Bacteria have been at the centre of attention as they are supposed to dominate the transformation processes because of their higher thermal tolerance (Bonito et al., 2010). However, fungi also play a key role in composting, not only because of the extracellular enzymes they produce which allow them to decompose different polymeric compounds (De Gannes et al., 2013), but on account of the ability of some species to grow at more severe environmental conditions (Langarica-Fuentes et al., 2014). Nevertheless, the relative importance of fungi in relation to prokaryotic organisms is depending on the conditions of the process, specially the nutritional properties of the materials being composted (Huang et al., 2010; Zhang et al., 2014).

The study of the microbiota associated to composting can be approached from two different methodological perspectives: culture-dependent and culture-independent protocols. Methods based on molecular techniques have become popular nowadays because of their potential to provide a more comprehensive picture of the composting microbiome, as they avoid the problems associated to cultivation (Hultman et al., 2010). However, results from fungal DNA analyses are not as good as expected, since a great number of studies reveal low levels of diversity (De Gannes et al., 2013). The extensive use of techniques that provide limited information from a taxonomic point of view, not at the species level, may be responsible for such unexpected results (Neher et al., 2013). Taking this into account, and considering that the information generated by both methodologies may vary greatly (Chroni et al., 2009), and culturability of microorganisms in composting environments is usually high (Hiraishi et al., 2003), molecular approaches should not be seen as substitutes for traditional culture-dependent methods but as complementary technologies to improve our knowledge on composting microorganisms.

In accordance with the above mentioned, the main objective of this study was to identify the culturable fungal community associated to the composting of agricultural waste and their structure at the major stages of the process. The establishment of links between specific species or groups and phases in composting, or the identification of dominant species throughout the process (Resident Mycobiota) in contrast to microorganisms which occur only occasionally (Transient Mycobiota) (López González et al., 2015), can contribute to perform more efficient processes and generate improved final products.

2. Methods

2.1. Composting procedure and sampling

The composting process was carried out using agricultural residues (sun-dried tomato plants waste and pine woodchips at a 1:1 (w:w) ratio. Procedure and sampling was performed as indicated by López-González et al. (2013). Briefly, the mixed materials, about 500 kg, were disposed in trapezoidal piles (1.5 m width × 3 m length × 1 m height) and the process was extended over 189 days. According to results reported by López-González et al. (2013), 42 days accounted for the bio-oxidative stage and the remaining 147 days were cooling and maturation stages. During the bio-oxidative stage, piles were aerated by means of forced air supplied ($0.6\ m^3\ s^{-1}$ during 5 min every 4 h) from three perforated PVC tubes located at the bottom of the pile and connected to a pump Lowarda CEAM-7013 (Montechhio Maggiore, Italy). This regime guarantees the maintenance of O_2 concentration over 10% (Vargas-García et al., 2010).

A total of 19 samples were collected at different composting times (Table 1). Composite samples of 1 kg were constructed collecting material from nine different points strategically placed on the pile for accurate homogeneity and representation. Samples were split into three replicates after thoroughly mixing, fractionated and treated according to further specific protocols: freshly processed for microbial analyses and moisture and pH determination, or air-dried at 40 °C and grounded to <1 mm for chemical analyses.

2.2. Chemical and physical analyses

The moisture content was determined by drying at 105 °C for 24 h. The pH was analyzed in a 1:10 (w/v) water extract. Total carbon (C) and nitrogen (N) were determined in solid samples by dry combustion at 950 °C using a LecoTruSpec C–N Elemental Analyzer (Leco Co., St. Joseph, MI, USA). Organic-matter content was assessed by determination of weight loss on ignition at 550 °C.

2.3. Microbial analyses

Mesophilic and thermophilic fungi were estimated from a suspension obtained by adding 10 g of fresh material to 90 mL of sterile saline solution (0.9% NaCl in distilled water). The suspension was shaken (150 rpm) at room temperature for 30 min. Afterward, a 10-fold serial dilution method was performed and 100 μL aliquots of appropriated dilutions were spread out in Rose Bengal Chloramphenicol Agar plates (Cultimed, Spain). Fungi were allowed to grow for 96 h at 30 °C (mesophilic) or 50 °C (thermophilic). Results were expressed as colony-forming units (CFU) per gram of compost dry weight. All different morphotypes identified were isolated in the same conditions as above, and regularly transferred to fresh agar slant and preserved for long-term conservation at −80 °C in cryoballs (Cryoinstant™, Deltalab, Barcelona, Spain).

In order to minimize the influence of potential replicates, isolates were photographically registered with a Canon EOS 450D Camera provided with lens Zoom EF-S18–55 mm f/3.5–5.6 IS. An accurate and in-depth study of the photographs allowed the reassignment of preliminary segregations.

2.4. Molecular identification of isolates

An ITS region-based protocol was used for isolates identification. Genomic DNA was extracted from the sedimented biomass of a fresh culture, using the Plant DNAzol® Reagent (Invitrogen, Carlsbad, CA, USA), according to manufacturer instructions. The amplification reaction was carried out using MyCycler thermal cycler (BioRad, Hercules, CA, USA) and ITS1 (5′-TCCGTAGGTGAAC CTGCGG-3′)/ITS4 (5 0 -TCCTCCGCTTATTGATATGC-3 0) primers. The reaction mixture (20 μL) contained: 4 μL AptaTaq Fast PCR Master (Roche, Mannheim, Germany), 0.06 μM each primer, and 2 μL of DNA template. The amplification program was as follows: 94 °C for 10 min, 30 cycles (94 °C for 1 min, 51 °C for 1 min, and 72 °C for 3 min) and 72 °C for 10 min. Amplified PCR products were checked by gel electrophoresis on 1% agarose gel in SB buffer supplemented with 0.002% GelRed (Biotium Inc., Hayward, CA, USA) for DNA visualization, and purified using the Diffinity Rapid Tips

Table 1
Sampling chronology and physico-chemical parameters evolution throughout the process.

Sample code	Sampling days	Phase	Temperature (°C)	Moisture (%)	pH	C/N ratio	Organic matter (%)
S1	0	Raw materials	24.0	49.9 ± 1.2	7.0 ± 0.0	27.7 ± 2.4	65.9 ± 1.4
S2	1	1st rising mesophilic	43.2	48.4 ± 1.1	8.4 ± 0.0	25.7 ± 1.2	62.9 ± 1.7
S3	2	1st early thermophilic	64.9	51.1 ± 0.7	8.4 ± 0.1	24.8 ± 1.8	62.9 ± 3.3
S4	5	1st late thermophilic	65.1	44.8 ± 0.7	8.3 ± 0.1	25.4 ± 2.2	58.5 ± 3.6
S5	7	1st decreasing mesophilic	59.8	49.4 ± 1.4	8.6 ± 0.0	24.7 ± 0.7	58.3 ± 2.5
S6	8	2nd rising mesophilic	40.4	49.9 ± 0.2	8.6 ± 0.0	24.5 ± 1.4	59.0 ± 3.2
S7	9	2nd early thermophilic	59.4	45.7 ± 2.1	8.6 ± 0.0	24.5 ± 0.9	64.8 ± 0.5
S8	12	2nd late thermophilic	54.8	44.6 ± 0.8	8.7 ± 0.0	22.9 ± 1.9	61.9 ± 1.6
S9	14	2nd decreasing Mesophilic	45.9	41.0 ± 0.5	8.7 ± 0.0	23.5 ± 1.8	63.7 ± 2.1
S10	15	3rd rising mesophilic	44.2	47.4 ± 0.5	8.7 ± 0.0	23.5 ± 1.7	62.7 ± 2.8
S11	16	3rd early thermophilic	48.2	46.5 ± 0.8	8.7 ± 0.0	23.8 ± 1.3	62.5 ± 1.2
S12	26	3rd decreasing mesophilic	31.3	48.7 ± 0.6	8.5 ± 0.1	22.7 ± 1.1	62.0 ± 0.3
S13	28	4th rising mesophilic	34.8	47.4 ± 0.7	8.5 ± 0.1	20.6 ± 0.6	63.8 ± 4.6
S14	42	Mesophilic (Cooling)	39.4	49.7 ± 0.3	8.7 ± 0.0	20.7 ± 1.9	59.5 ± 1.3
S15	56	Mesophilic (Cooling)	40.0	41.0 ± 0.3	8.7 ± 0.0	21.6 ± 0.8	59.3 ± 2.6
S16	63	Mesophilic (Cooling)	33.9	46.6 ± 2.5	8.6 ± 0.0	19.7 ± 0.8	59.2 ± 2.2
S17	119	Early maturation	38.5	50.9 ± 1.5	8.6 ± 0.0	17.3 ± 0.3	53.8 ± 1.6
S18	168	Late maturation	26.5	42.5 ± 1.7	8.5 ± 0.0	14.4 ± 0.5	48.0 ± 3.5
S19	189	Final product	20.6	40.8 ± 1.8	8.5 ± 0.1	13.3 ± 0.7	43.9 ± 2.4

Table 2
Indices used for estimating fungal diversity throughout the composting process.

Index	Diversity	Type	Formula
Rarefaction curves	α	Species richness	$E(S) = \sum 1 - \frac{(N-N_i)/n}{N/n}$
Shannon–Wiener	α	Evenness (structure)	$H' = -\sum p_i \log_2 p_i$
Simpson	α	Dominance (structure)	$\lambda = \sum p_i^2$
Qualitative Sørensen–Dice	β	Similarity	$I_S = \frac{2c}{a+b}$
Quantitative Sørensen–Dice	β	Similarity	$I_{Squant} = \frac{2pN}{aN+bN}$

N = total number of individuals in the sample, N_i = number of individuals of the ith species, n = standardized size of the sample.
$p_i = n_i/N$, n_i = count of the ith species and $N = \Sigma n$.
a = number of species in sample 1, b = number of species in sample 2, c = number of species shared by the two samples.
pN = sum of the lower of the two abundances recorded for species found in the two samples, aN = number of individuals in sample 1, bN = number of individuals in sample 2.

(Sigma–Aldrich, St. Louis, MO, USA). Sequencing was performed using capillary sequencer ABI Hitachi 3500 Genetic Analyzer (Applied Biosystems, Waltham, MA, USA). The assembled sequences obtained by alignment of forward and reverse amplification product were compared for similar nucleotide sequences with the BLAST search of the National Center of Biotechnology Information (NCBI, http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the pairwise sequence alignment tool available at the International Mycological Association web (www.mycobank.org). Taxonomic assignment was retrieved from this last tool.

2.5. Data processing

Biodiversity indices for fungal species throughout the composting were estimated from the number of isolates and their counts, using α and β-diversity indices (Table 2). Rarefaction curves, Shannon–Wiener and Simpson indices were calculated using the free software BioDiversity Professional version 2.0 (McAleece et al., 1997). Phylogenetic relationships within ITS sequences (both isolates and those of closely related strains retrieved from database) were inferred using Mega version 6.0 software (Tamura et al., 2013).

3. Results and discussion

3.1. Composting process

Physico-chemical data in Table 1 were in accordance with expectations for a composting of lignocellulosic materials (López-González et al., 2013). Temperature course fit the typical evolution of composting processes operated with turning treatments. An initial thermal rising, which allowed maximal values over 65 °C was registered. Afterwards, two decreasing thermophilic peaks, ranging between 59 °C and 48 °C, were promoted by mechanical turning. The subsequent cooling stage registered values slightly higher than expected, which probably are ascribable to some residual microbial activity on remaining lignocellulosic materials (Wei et al., 2012). C/N ratio and organic matter profiles were also determined by the large proportion of lignocellulosic components, the recalcitrant nature of which is conducive to slow but steady decline during the bio-oxidative and cooling stages of the biotransformation process and sharp fall in maturation (Alburquerque et al., 2009). Together with pH, these results support the correctness of the process and, therefore, the representativeness of the fungal community profiles associated to lignocellulosic waste-based composting. More in-depth information about the physico-chemical aspects of the process that ensure its correct procedure is reported by López-González et al. (2013).

3.2. Fungal community composition

The analysis of the 5.8-ITS region allowed the identification of 69 different species (Fig. 1), eight remaining unidentified. ITS region has been proposed as the standard barcode for fungi molecular identification because of its higher species discrimination capability and a more clearly defined barcode gap in comparison to other DNA regions (Schoch et al., 2012). However, these markers are not equally satisfactory for different phyla (Bellemain et al., 2010). Successful results for the unidentified isolates might be obtained by using a combination of primers covering a broader range of species. Identified isolates were all placed in the phyla Ascomycota (59 isolates) and Basidiomycota (10 isolates). Ascomycetes were assigned to six different classes, Eurotiomycetes (17) and Sordariomycetes (19) being in the majority. The rest of strains were classified into Dothideomycetes (10), Leotiomycetes (1), Pezizomycetes (1) and Saccharomycetes (11) classes. Among basidiomycetes, isolates were equally distributed

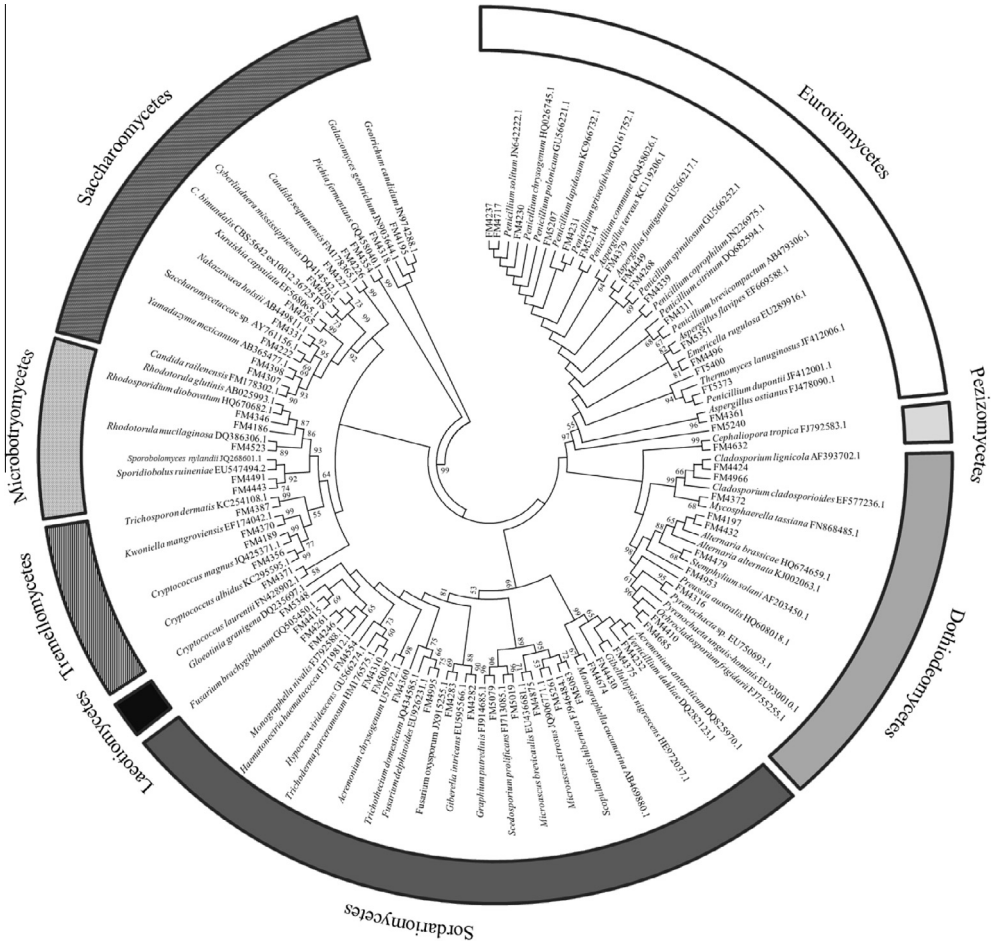


Fig. 1. Phylogenetic tree based on ITS sequences of isolates retrieved from agricultural waste composting process, paired with homologue sequences obtained from the NCBI and MycoBank database. The tree was constructed using the UPGMA method and the p-distance method for computing the evolutionary distances. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown (values > 50%).

in two classes, Microbotryomycetes and Tremellomycetes. Despite being one the most numerous in terms of species, the diversity at the genus level was really low in the Eurotiomycetes class. Up to 11 isolates were classified as *Penicillium*, while other four were ascribed to the genus *Aspergillus*. On the contrary, isolates belonging to Sordariomycetes class were distributed in 14 different genera, with *Acremonium*, *Fusarium*, *Microascus* and *Monographella* as the only ones hosting two or more species. Representatives of a high number of genera were also detected in Saccharomycetes class, in which the 11 isolated species were assigned to nine different genera.

Eurotiomycetes and Sordariomycetes led the composting process as they were predominant throughout all the stages (Fig. 2). Species like *Gibellulopsis nigrescens* and *Microascus brevicaulis* were detected at all the sampling times, and others like *Aspergillus fumigatus*, *Fusarium oxysporum* and *Monographella cucumerina* appeared on more than 65% of the samples. At the opposite, 18 species were isolated only at one sampling time, four of them belonging to the genus *Penicillium*. From a quantitative point of view, *G. nigrescens* and, in particular, *M. brevicaulis* were also prevalent throughout the process. During the maturation phase and in the final product, *Scopulariopsis hibernica* replaced both microorganisms as the dominant fungus, with relative frequencies higher than 60%.

As noted previously, it is not wise to presume the existence of a standard composting microbiota, since feedstock and operating procedure determine the qualitative and quantitative presence of specific microorganisms. It is however not unreasonable to assume that some of them might be active agents in different composting processes sharing similar characteristics and requiring similar transformations. Neher et al. (2013) reported evidences that support this hypothesis by comparing fungal communities associated to processes with different raw materials and operating system. In the case of lignocellulose material as feedstock, species belonging to the genus *Aspergillus* or *Fusarium* are usually described as prevalent in the fungal community (Hubbe et al., 2010), as in this study. On the contrary, *Gibellulopsis* and *Microascus*, which seem to be determinant in the process here described, play a minor role in this type of composting processes according to literature. These results suggest that what might be called the Resident Mycobiota, the fungal communities leading the events that take place during the composting process, could be grouped into two categories. On the one hand, some species are usually associated to lignocellulose-based composting no matter the nature of the lignocellulosic feedstock (General Resident Mycobiota). On the other hand, certain species which are generally considered to play secondary roles become primary, probably as a consequence of a better adaptation to the unique conditions of every specific process (Specific Resident Mycobiota). In this sense, both *G. nigrescens* and *M. brevicaulis* are generally recognized as saprophytic soil inhabitants associated to the transformation of organic matter from vegetal tissues (Zare et al., 2007; Jagielski et al., 2013). Thus, they have the metabolic capability for acting in composting processes with lignocellulose materials as feedstock,

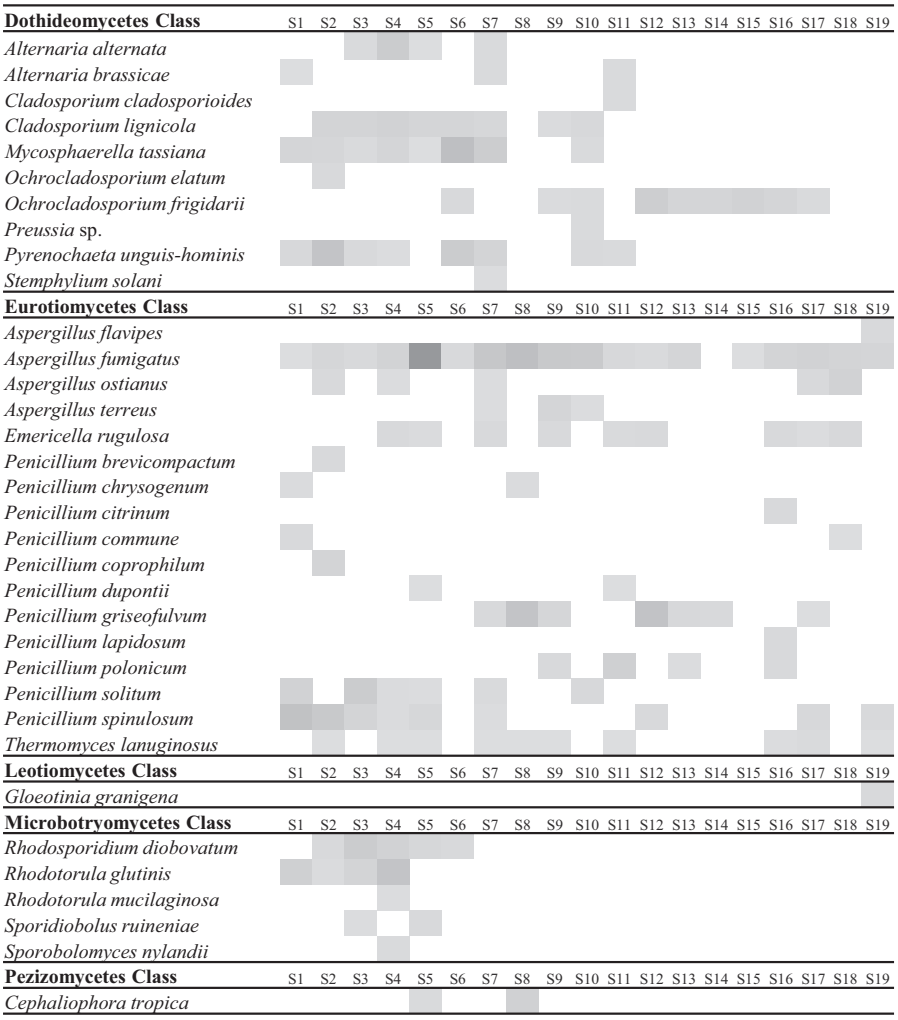


Fig. 2. Heat map representing distribution (expressed as percentage) of fungal isolates within sampling times. 0%- -100%.

and their predominance depend on their more successful adaptation to the particular environment created by the specific combination of nutritional material and operating conditions. This differentiation between fungal community structures has been previously reported by Zhang et al. (2008). They studied the mycobiota associated to the organic matter decomposition process on forest soils and, despite the distances, they obtained similar results when comparing the communities detected in two soils with different vegetation but similar environmental conditions.

Opposite to this so-called Resident Mycobiota, other fungi only occurred occasionally (Transient Mycobiota). These microorganisms, originally present in the feedstock or coming from the surroundings, probably are not competitive enough to remain permanently, whether for the lack of metabolic capabilities or due to the inability to adapt to the ever-changing environmental conditions of composting.

3.3. Evolution of the mycobiota structure throughout the composting process

Fungi are critical to the correct development of the composting process. According to this predominant role, the establishment of the composition of the fungal community on the basis of the process conditions and chronology can contribute to optimize the transformation of organic wastes into valuable products. However, the many factors influencing the process make difficult the selection of the most appropriate criterion. Temperature is widely recognized as one of the most significant parameter in composting (Tang et al., 2007; Hosseini and Aziz, 2013), but other physico-chemical parameters are equally determinant in assessing the evolution of the process (Raj and Antil, 2011). In order to ascertain the existence of differences in the mycobiota structure evolution in response to the criterion of analysis used, data were evaluated according to both physico-chemical and thermal stages occurring in the process. Physico-chemical stages were established on the basis of previous results of a discriminant analysis conducted using a comprehensive pool of data including moisture, pH, total carbon, total nitrogen, organic matter content, soluble organic carbon, reducing sugars, total proteins, N-NH_4, N-NO_3, lignocellulose fractions, total sugars, fats–oil–waxes and resins content, humic-like substances, phenolic compounds and microbial biomass carbon (López-González et al., 2013), while thermal stages grouped together sampling times with similar thermal values. Thus, four physico-chemical phases were established coinciding with the chronology of the process (SI: S1–S5; SII: S6–S11; SIII: S12–S16; SIV: S17–S19). For its part, the thermal differentiation of the process lead to the establishment of five stages (Ms: S1, S2, S5, S6, S9, S10, S12, S13; T: S3, S4, S7, S8, S11; C: S14–S16; Mt: S17–S18; FP: S19). According to both criteria, fungi belonging to Sordariomycetes class were prevalent no matter the stage

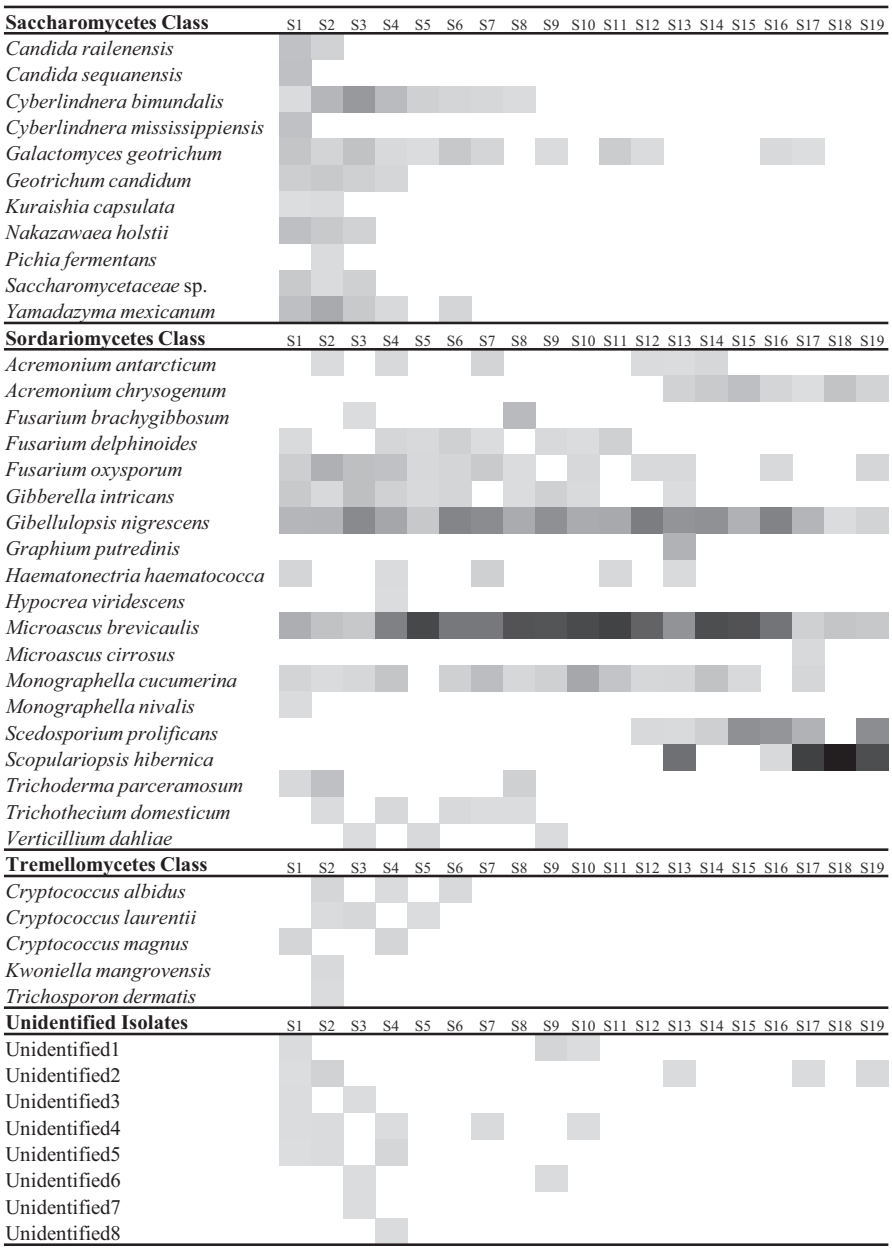
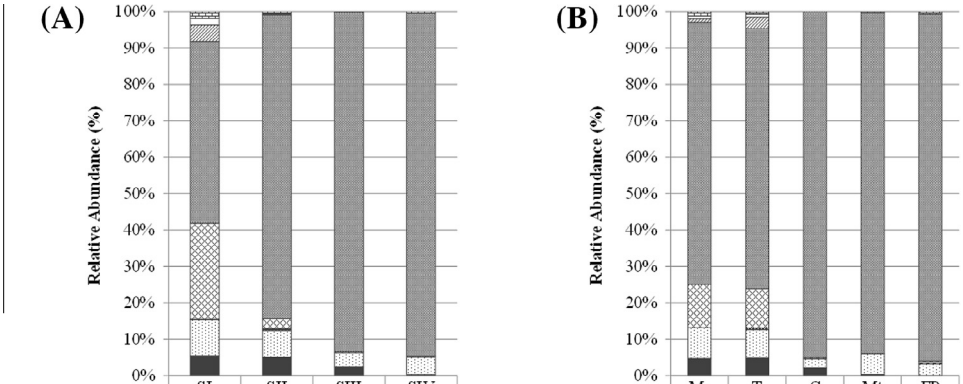


Fig. 2 (*continued*)

(Fig. 3), with relative abundancies at levels exceeding 90% during the second half of the process, which corresponds to the period of stabilization of the organic matter. As a result of the dominance of this class, some of the other communities detected during the first part of the process disappeared or drastically decreased. That was the case for Dothideomycetes, Tremellomycetes and, specially, Saccharomycetes classes. This chronological pattern seems to be typical for yeasts, that are usually associated to the early stages of composting because of environmental conditions (Langarica-Fuentes et al., 2014). Only fungi belonging to Eurotiomycetes class were detected at considerable levels during late stages of composting. These results suggest the existence of a selective effect of the process on the structure of the fungal communities, which further supports conclusions already reported in previous studies in relation to the metabolic capabilities of the mycobiota associated to the process and its chronological evolution (López González et al., 2014).

On the other hand, the clustering of samplings according to thermal criterion showed the almost complete absence of differences in the structure of the fungal community when comparing mesophilic and thermophilic samplings at the level class. Such result is surprising taking into account that temperature is considered as the most important factor affecting the structure of the microbial community. De Gannes et al. (2013), who described similar results, pointed out that the mere existence of a thermophilic phase is not enough to promote this influence. A combined threshold level of both heat and time is necessary to significantly affect the composition of the mycobiota. Moreover, the lignocellulosic character of the feedstock might promote the persistence of fungal species during the thermophilic phase in contrast to what happens in low lignocellulosic content-based



	SI	SII	SIII	SIV
Unidentified Fungi	1,91	0,64	0,10	0,44
Tremellomycetes	1,87	0,16	0,00	0,00
Microbotryomycetes	4,43	0,16	0,00	0,00
Sordariomycetes	50,00	83,43	93,33	94,20
Saccharomycetes	26,31	2,79	0,30	0,02
Pezizomycetes	0,12	0,45	0,00	0,00
Leotiomycetes	0,00	0,00	0,00	0,26
Eurotiomycetes	9,96	7,36	3,91	4,86
Dothideomycetes	5,40	5,02	2,36	0,23

	Ms	T	C	Mt	FP
Unidentified Fungi	1,24	0,80	0,00	0,27	0,77
Tremellomycetes	0,80	0,77	0,00	0,00	0,00
Microbotryomycetes	0,96	3,09	0,00	0,00	0,00
Sordariomycetes	71,88	71,45	94,96	93,71	95,17
Saccharomycetes	11,91	10,69	0,34	0,03	0,00
Pezizomycetes	0,07	0,54	0,00	0,00	0,00
Leotiomycetes	0,00	0,00	0,00	0,00	0,77
Eurotiomycetes	8,38	7,76	2,55	5,65	3,28
Dothideomycetes	4,76	4,89	2,15	0,35	0,00

Fig. 3. Distribution of fungi (class level) isolated at the different stages of the composting process. (A) Relative abundance differentiated according to physico-chemical stages (SI: S1–S5; SII: S6–S11; SIII: S12–S16; SIV: S17–S19); (B) relative abundance differentiated according to thermal stages (Ms: mesophilic; T: thermophilic; C: cooling; Mt: maturation; FP: final product).

processes (Insam and de Bertoldi, 2007). In addition, not all studies point to temperature as the selective force that drives microbial diversity in composting. In this sense, Adams and Frostick (2009) suggested the involvement of factors different from thermal values as critical for determining biodiversity in composting environments.

The indices used to assess biodiversity (Table 3) clearly confirmed the scenario described above. The α diversity, Shannon–Wiener and Simpson indices evidenced the steady decrease of fungal diversity, as well as the increasing homogeneity of the mycological community when the evolution of the process was considered by clustering samplings on the basis of physico-chemical parameters. In terms of thermal stages, biodiversity values corresponding to mesophilic and thermophilic stages were almost the same and different than those associated to late stages characterized by less biological activity (cooling and maturation stages, and final product). Thus, biodiversity according to Shannon–Wiener was clearly lower for these later stages, while community homogeneity estimated by Simpson index was higher. Rarefaction analysis (Fig. 4) also pointed to the same direction. Although the saturation zone was approached in most of the cases, this phenomenon occurred much earlier in inverse proportion to the chronology of the physico-chemical stages. Thus, the recovering of the mycobiota associated to every process stage demanded a lower number of samplings as the composting proceeds. In the same way, the thermal stages showed clear differences between samplings representing varying levels of biological activity. The lesser the level of activity (cooling, maturation and final product samplings), the earlier the saturation zones is approached. This pattern of decreasing diversity as the process evolves has been previously described (Agnolucci et al., 2013; Langarica-Fuentes et al., 2014). Although there is no such thing as a unique community composition and dynamic, a more complex and diverse fungal community is usually found at the earlier phases of composting, while later stages use to be characterized by more homogeneous communities.

These similarities were clearly reflected in the analysis of β diversity. Sørensen's–Dice index, both qualitative and quantitative,

Table 3
Biodiversity indices (evenness, H', and dominance, D, for α-diversity, and qualitative, I_S, and quantitative, I_{Squant}, similarity for β-diversity) for the different physico-chemical stages (SI: S1–S5; SII: S6–S11; SIII: S12–S16; SIV: S17–S19) and thermal stages (Ms: mesophilic; T: thermophilic; C: cooling; Mt: maturation; FP: final product).

Index	Physico-chemical stages					Thermal stages				
	SI	SII	SIII	SIV		Ms	T	C	Mt	FP
Shannon-Wiener (H')		4.40	2.69	2.52	1.73		3.71	3.56	2.10	1.58	1.78
Simpson (λ)		0.08	0.32	0.27	0.52		0.18	0.19	0.34	0.57	0.43
Sørensen's–Dice qualitative index (I_S)	SI	–				Ms	–				
	SII	0.66	–			T	0.77	–			
	SIII	0.31	0.47	–		C	0.37	0.31	–		
	SIV	0.30	0.39	0.67	–	Mt	0.40	0.26	0.71	–	
						FP	0.26	0.18	0.94	0.62	–
Sørensen's–Dice quantitative index (I_{Squant})	SI	–				Ms	–				
	SII	0.54	–			T	0.82	–			
	SIII	0.40	0.70	–		C	0.63	0.63	–		
	SIV	0.14	0.12	0.31	–	Mt	0.24	0.15	0.24	–	
						FP	0.17	0.24	0.25	0.80	–

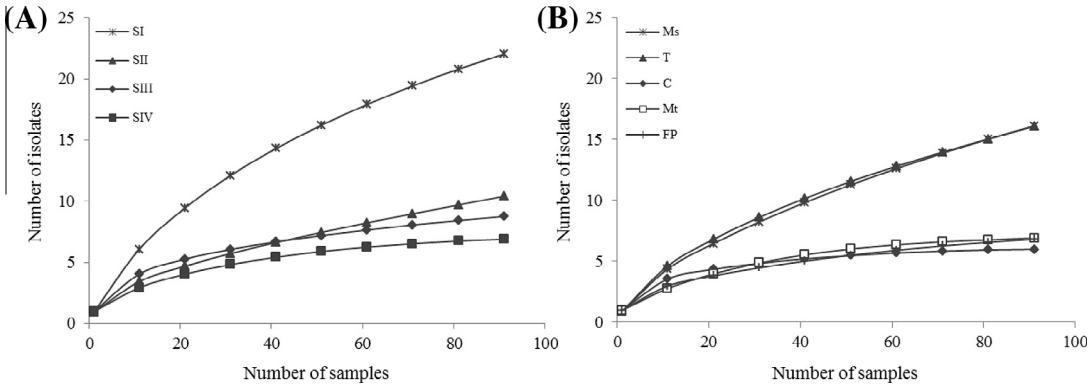


Fig. 4. Rarefaction curves of isolates richness for the compost samples. (A) Differentiation according to physico-chemical stages (SI: S1–S5; SII: S6–S11; SIII: S12–S16; SIV: S17–S19); (B) differentiation according to thermal stages (Ms: mesophilic; T: thermophilic; C: cooling; Mt: maturation; FP: final product).

informs about the level of similarity between paired combinations of the different stages. The comparison of pairs of stages established according to the evolution of physico-chemical properties led to higher values (higher similarity) when comparing chronologically subsequent samples. In the case of thermal stages, a high similarity was observed between mesophilic and thermophilic stages, on the one hand, and cooling, maturation and final product samplings, on the other hand. Nevertheless, some differences between qualitative and quantitative results may be emphasized, especially in the case of thermal stages. Most of these divergences focused on the cooling stage and the degree of similarity with the other samplings. While homogeneity between the cooling period and the previous stages corresponding to bio-oxidative phase was substantially higher when quantitative data were considered, the comparison with the following stages revealed a degree of similarity considerably greater for qualitative data. These results support the role of the cooling stage as a transition phase between the initial bio-oxidative period and the final stabilization stage as regards mycobiota. Thus, it would be during this time that the fungal species that are going to dominate the second part of the process begin to establish themselves. The nutritional and physico-chemical conditions at this stage of the process are probably suitable for the growth of species not competitive enough to play an important role during the bio-oxidative phase (Neher et al., 2013), coming from the surroundings or from spores or vegetative cells that survive this previous period (Hultman et al., 2010). In brief, the identity and population of the fungal community throughout the composting of lignocellulosic materials is depending on the greater or lesser degree of the biological activity of the stage. Thus, the mycobiota associated to the bio-oxidative phase is markedly different from that of maturity and stabilization stages, and the transition between both periods is mediated by the cooling phase. The prevalence and quantitative dominance of some fungal genera point out to these microorganisms as major players in the mycobiota of the composting process, since this dominance constitutes a sign of adaptation to the prevalent conditions at every stage and a better use of the nutritional resources. It remains to be known whether sample applies for other raw materials.

4. Conclusions

Fungi are decisive for the proper conduct of the composting procedure. By acquiring knowledge of the mycobiota associated to different stages and factors that are the major drivers of both qualitative and quantitative changes, a most effective control of the process can be achieved. Results obtained in this study support the hypothesis of the uniqueness of composting concerning mycobiota, with fungi belonging to different categories depending on their relevance in the process (Resident and Transient mycobiota). According to the structure of the community, two major phases corresponding to the bio-oxidative period and the maturation stage were established, with a transitional cooling phase between them.

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