Tracking organic matter and microbiota dynamics during the stages of lignocellulosic waste composting

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HIGHLIGHTS

- Lignocellulosic wastes (forest and horticultural wastes) were composted.
- The dynamics of biologically meaningful carbon pools were evaluated.
- The first thermophilic phase is crucial for the further evolution of the process.
- Multivariable analysis reveals parameters that characterize each stage.

ABSTRACT

The dynamics of biologically meaningful soluble and polymeric carbon fractions and the combined relationships between physical, chemical and biological parameters during composting of lignocellulosic waste were evaluated. The first thermophilic stage is crucial in determining the further evolution of soluble and polymeric carbon fractions but the dynamics of carbon is still important at the maturation stage. Multivariate data analysis showed that not only are all parameters interrelated but also influence one another's variability. To discern completion of bio-oxidative stage other parameters in addition to temperature should be measured. Evaluation of soluble organic carbon, microbial biomass carbon, pH and inorganic nitrogen can be of great use in detecting the composting stage. This study offers new insights into the mechanisms involved in the biodegradation of organic matter and help to prioritize the parameters that contribute at critical stages of the process.

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1. Introduction

Composting is one of the best choices for agricultural waste treatment because it is an environmentally friendly process, adds value to a wide variety of organic waste, and the compost obtained is suitable as an amendment for soils and thus enables waste to be recycled (Guardia et al., 2010; Vargas-García et al., 2010). Organic matter is transformed during composting as a result of complex interactions among chemical, physical and biological processes (Tejada et al., 2009).

Microorganisms are the driving force behind the transformation and stabilization of organic matter (Tang et al., 2004). Throughout the stages of the process there is a succession of different dominant microbial groups, as certain groups adapt to conditions of this changing environment better than the other groups (Kuok et al., 2012). Soluble organic compounds are easily biodegradable and they are used as the primary nutrient source for microbial growth (Said-Pullicino et al., 2007). However, the main nutrient sources in organic waste are polymers such as cellulose, lignin and proteins that are included in the solid fraction. These polymers are hydrolyzed by microbial extracellular enzymes releasing and solubilizing their basic monomers that are further metabolized (Vargas-García et al., 2010). Polymeric organic matter is thus transformed into a more stable form called humic-like fraction (López et al., 2002; Huang et al., 2010). Therefore, through the action of microorganisms, transformation occurs modifying an initial waste into humified material. As a consequence, organic matter partitions between liquid and solid fractions in composting. Several works focus on the study of soluble (Sánchez-Monedero et al., 1999; Said-Pullicino et al., 2007) and solid (Ciavatta et al., 1991; López et al., 2002) fractions but detailed knowledge on the mechanisms underlying the dynamics between both pools during the process is yet to be attained.

The most evident effect of microbial activity on organic matter is the increase in temperature inside the composting pile. For this reason, this parameter is used to easily monitor performance of the process at initial stages (bio-oxidative or self-heating stage) and it

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also helps to decide management operations such as turnings (Kuok et al., 2012). After completion of the bio-oxidative stage, several chemical and biological tests are needed to establish maturity or stabilization of organic matter and hence accomplishment of the process (Tejada et al., 2009). The selection of the most suitable maturity indexes (Iglesias and Pérez, 1992; Bustamante et al., 2008), the effect of different organic waste blends and management practices on the quality of final compost, and process duration has been the focus of extensive research (Guardia et al., 2010; Kuok et al., 2012; Lashermes et al., 2012; Yousefi et al., 2013). However, composting cannot be described satisfactorily analyzing one or several parameters independently. Physical, chemical and biotic variables are interrelated and each influences variability of another. Thus a multivariate data analysis is required to process information in a meaningful fashion (Böhm et al., 2013). As some examples, multivariate analysis were used to relate changes in community composition to changes in the environment (Cayuela et al., 2009; Huang et al., 2010; Zhang et al., 2011), to reveal the significant parameters that best describe the process or the maturity of compost (Bustamante et al., 2009) or to classify composted materials (Campitelli and Ceppi, 2008). Most of those works analyzed the whole data set obtained from the full composting period. Nevertheless, each composting stage may have its own critical parameters that should be identified.

The aim of this work was to study the dynamics of biologically meaningful soluble and polymeric carbon fractions and the combined relationships between physical, chemical and biological parameters during composting of lignocellulosic waste. A detailed evaluation of the changes in chemical and biochemical characteristics of lignocellulosic waste could offer a deeper insight into the mechanisms involved in the biodegradation during composting. Moreover a multivariate approach could help prioritize some of the parameters that contribute at critical stages of lignocellulosic waste composting so that the proper management practices could be performed driven by the most suitable parameter.

2. Methods

2.1. Composting procedure and sampling

Composting was carried out in the pilot plant of the University of Almeria (Almeria, Spain) that is equipped with a forced aeration system and automated controls of temperature and flux air. The starting materials were prepared by mixing (50:50 w/w) sun-dried tomato plant waste and pine woodchips, both ground to <3 cm, to give a final C/N ratio of 25. Three forced-aerated and trapezoidal piles (1.5 m width × 3 m length × 1 m height) of about 500 kg each were built up. The moisture content was kept between 45% and 50% by watering. The process was monitored on-site for temperature with long-handled (50 cm) thermometer probes PT100 model MPT2 (Lexitron-Guemisa, Madrid, Spain). The duration of the bio-oxidative stage and turning operations were determined by the temperature profile inside the pile. At this stage forced air was supplied from the bottom of the pile through three perforated PVC tubes (5 cm diameter × 5 m length) connected to a pump Lowarda CEAM-7013 (Montecchio Maggiore, Italy). Forced air (0.6 m s^{-1}) was supplied for 5 min every 4 h. This aeration regime ensured keeping a minimum of 10% oxygen (Vargas-García et al., 2010).

Samples were collected throughout the process at 19 critical periods driven by temperature changes. Sampling strategy was designed to guarantee a representative sample of the pile. Sub-samples were taken from nine different locations of each pile as follows: 3 surface samples (1–10 cm depth), 3 samples at 45 cm depth and 3 samples at 90 cm depth. Sub-samples were mixed in equal amounts to give a final sample weight of about 1 kg and then split into three parts for analytical replicates. Samples for most chemical analyses were air-dried at 40 °C overnight and ground to <1 mm, while those for microbial quantification and pH were freshly processed. Samples for analysis of total protein, reducing sugars (RS), phenolic acids, N–NH$_4$, N–NO$_3$, soluble organic carbon (SOC) and microbial biomass C (C$_{mic}$) were stored in plastic bags at −20 °C.

2.2. Biological analysis

The cultivable mesophilic and thermophilic bacteria, actinobacteria and fungi were estimated using a standard dilution-plating procedure. Ten grams of compost were suspended in 90 mL of sterile saline solution (NaCl, 0.9% w/v) and shaken for 30 min at room temperature. Ten-fold dilutions were made in sterile saline solution and 0.1 mL was spread out in appropriate medium for each microbial group. Medium and incubation time used were as follow: bacteria were cultured in APHA (Cultimed, Spain) for 48 h; actinobacteria were cultured in sodium caseinate agar (in g L^{-1}: sodium caseinate 2.0, asparagine 0.1, sodium propionate 4.0, K$_2$HPO$_4$ 0.5, MgSO$_4$ 0.1, FeSO$_4$ 0.001, glycerol 5, agar 15) for 96 h; and fungi were cultured in Rose Bengal agar (Cultimed, Spain) for 96 h. Mesophilic and thermophilic microorganisms were incubated at 30 and 50 °C, respectively. Plate counts were expressed as logarithm of colony forming units per gram of compost dry weight (Log CFU/g dw).

Microbial biomass C (C$_{mic}$) was determined using the fumigation–extraction method according to Vance et al. (1987). Compost sample (10 g) was fumigated with ethanol-free CHCl$_3$ for 24 h at 25 °C in a desiccator. After removal of chloroform vapor by evacuation of the desiccators, the carbon in fumigated samples was extracted with 40 mL 0.5 M K$_2$SO$_4$ shaken at 200 rpm for 30 min. Clear extracts were obtained by filtration and total organic carbon (TOC-fumigated) was analyzed using a TOC–VCSN (Shimadzu Co., Kyoto, Japan). In parallel, a non-fumigated sample (10 g) was K$_2$SO$_4$ extracted, filtered and total organic carbon (TOC-non fumigated) was analyzed as above. Microbial biomass C (C$_{mic}$) was calculated by subtracting TOC of the non-fumigated samples from TOC of the fumigated samples.

2.3. Chemical and physical analysis

The moisture content was determined by drying at 105 °C for 24 h. 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 loss on ignition at 550 °C to a constant weight.

Several soluble fractions were analyzed in extracts obtained from compost samples. Soluble organic carbon (SOC), reducing sugars (RS) and total proteins were analyzed in an extract of 10 g compost in 40 mL 0.5 M K$_2$SO$_4$ shaken at 200 rpm for 30 min and filtered through filter paper. SOC was analyzed using a total organic carbon (TOC) analyzer TOC–VCSN (Shimadzu Co., Kyoto, Japan). Reducing sugars and total proteins were analyzed in a UV-1800 Spectrophotometer (Shimadzu Co., Kyoto, Japan) according to the methods described by Somogyi (1951) and Herber et al. (1971), respectively. N–NH$_4$ and N–NO$_3$ were determined in aqueous extracts by Kjeldahl distillation in an Auto-Titration Kjeldahl Distiller (Pro-Nitro A 4002430) (Selecta S.A., Spain). The aqueous extracts were obtained by filtration of a mixture of 5 g sample and 100 mL distilled water after shaking at 200 rpm for 30 min.

Polymers (lignocellulose, total sugars, fats–oil–waxes and resins) along with humic-like and phenolic compounds were analyzed in solid compost samples. Lignocellulose fractions (cellulose, hemicellulose and lignin) were analyzed by using a fiber analyzer

Ankom (Ankom Tech., Macedon, NY, USA). For total sugars analysis, compost samples (25 mg) underwent 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 (Šafařík and Šantrůčková, 1992). Total sugars in the hydrolyzate were spectrophotometrically quantified according to Dubois et al. (1956). Fats–oils–waxes (FOW) and resins fractions were determined gravimetrically by weighing diethyl ether (FOW) and alcohol (resins) Soxhlet extracts obtained from 25 g compost sample (Stevenson, 1994). Phenolic acids and humic substances were analyzed in pyrophosphate ($Na_4P_2O_7$) extracts. Phenolic acids were extracted from solid samples according to the method described by Morita (1980) and quantified as described by Marambe and Ando (1990). The humic-like fractions were determined according to the method described by Ciavatta et al. (1991). In brief, a pyrophosphate alkaline-extract was obtained by shaking at 120 rpm for 24 h two-grams of sample with 200 mL of pyrophosphate–NaOH solution (0.1 M $Na_4P_2O_7$, 10 H_2O + 0.1 M NaOH [pH 13]). The dark-colored extract was centrifuged and filtered and used for TOC analysis of pyrophosphate alkaline-extractable carbon (Cext). The pyrophosphate–NaOH extract was further fractionated according to Ciavatta et al. (1991) into humic acid-like (HA) and fulvic acid-like (FA) fractions. Humic acid-like carbon (HA) and fulvic acid-like carbon (FA) were analyzed by measurement of TOC in the corresponding fraction.

2.4. Data analysis

All analyses were conducted in triplicate and data are presented as the mean. The normality of data was tested using the Shapiro–Wilk test ($p > 0.05$). A one way analysis of variance (ANOVA) and multiple comparison tests of Fisher's least significant difference (LSD) at a 95% confidence level were used to test for significant differences between sampling times. Multivariate statistical analyses were performed on the standardized matrices of the physical, chemical and biotic parameters. The presence of categories within samples collected at different composting times was investigated using stepwise linear discriminant analysis (LDA). The method used for variable selection was to minimize the Wilks' lambda statistics. Principal component analysis (PCA) was used for data reduction of the selected physical, chemical and biotic parameters at critical intervals of the composting. All data analysis was performed using Statgraphics Centurion XVI version 16.1.17 (Stat-Point, Inc., Virginia).

3. Results and discussion

3.1. Evolution of physico-chemical parameters

Temperature is one of the main parameters used to monitor composting. Fig. 1 and Table 1 show respectively the evolution of this parameter and sampling timing which was planned according to temperature values. The thermal profile was typical of turned piles and allowed to distinguish three stages: bio-oxidative, cooling and maturation. Bio-oxidative stage lasted for 42 days, similar to results obtained by Vargas-García et al. (2010). Temperature rose soon after the beginning of the composting process and reached 65 °C within 2 days. Three thermophilic stages were accomplished (TER1, TER2 and TER3), each reaching lower temperature than the previous one. Turnings were performed each time temperature steadily decreased for two consecutive days. After the third turning, at day 26, temperature increased slowly up to 40 °C but did not exceed this thermal level afterwards. Samples were collected before reaching 40 °C, i.e. at mesophilic stages (MESA), also at thermophilic stages (TER), and right after

temperature fell down and turning was performed (MESD). During thermophilic stages, samples were taken every three days, provided temperature kept constant or increased. The first two thermophilic stages were longer (about 6 days) and reached higher thermal values (above 50 °C) than the third one (5 days above 40 °C). This behavior is related to the gradual depletion of easily biodegradable material along the bio-oxidative stage. This was confirmed later by chemical analysis performed in samples. The third thermophilic stage exhibited random fluctuations in temperature and it was followed by a prolonged cooling stage (77 days) in which temperature was around 40 °C. This trend can be explained by the high lignocellulose content of materials that provide a continuous carbon source to sustain microbial activity thus preventing full cooling, but it is not enough to reach a true thermophilic stage. Yousefi et al. (2013) also noted this behavior in composting municipal solid waste. Maturation extended for 70 days, and final compost was obtained after 189 days. Several samples were taken at cooling (MES) and maturation (MAD) stages as well as at final compost (PRF).

Physico-chemical parameters were analyzed in samples collected (Table 1). Moisture was always around 50% because of periodic watering. The initial pH was high (7.9) and it increased from the beginning of composting, reaching a plateau after completion of the first thermophilic stage with pH of 8.6–8.7, that was maintained up to the start of maturation. Only two significant pH decreases were noticed throughout this period, at the first early thermophilic stage (TER1B) and after completion of the third thermophilic stage (MESD3) which are likely to be related to the production of organic acids. Total C moderately declined during the bio-oxidative stage (around 2–3%) and sharply in cooling and maturation stages (up to 7%). This trend was similar to that detected by Tejada et al. (2009). The evolution of total N followed an almost constant trend along the bio-oxidative stage, and subsequently increased during the maturation stage. Consequently, there were not N losses in the whole material and the final increase can be attributed to a concentration effect as a consequence of degradation of organic C compounds, which reduced the dry mass (Said-Pullicino et al., 2007). C/N ratio was gradually descending, and reached a final value of 13 that shows stabilization of composted material (Bustamante et al., 2008).

3.2. Microbial communities

The microbial communities underwent sharp modifications as illustrated in Fig. 2a–d. The counts of mesophilic bacteria and actinobacteria (around 10^8 CFU/g dw) in raw materials (MPR) were higher than fungal community (10^6 CFU/g dw) and they also dominated during the process (Fig. 2d). The thermophilic bacteria and actinobacteria levels in MPR were around one order of magnitude lower than their mesophilic counterparts, whereas initial counts of thermophilic fungi were quite low (2×10^2 CFU/g dw) (Fig. 2c).

Mesophilic bacteria reached their maximum number (2×10^9 CFU/g dw) in just one day after initiation of composting (MESA1), then levels steadily decreased up to the beginning of maturation stage (MES7) in which counts fell down from 1×10^8 CFU/g dw to 5.5×10^7 CFU/g dw and maintained this level thereafter (Fig. 2a). These findings coincide with those of Kuok et al. (2012). Fluctuations in thermophilic bacteria were clearly driven by temperature changes (Fig. 2a). The number of thermophilic bacteria increased significantly after the start of the first mesophilic stage (MESA1) and reached two maximum peaks at the beginning of the second (MESA2) and third (MESA3) thermophilic stages. After completion of bio-oxidative stage, thermophilic bacteria followed further similar trend to the ones reported for mesophilic bacteria (Fig. 2c).
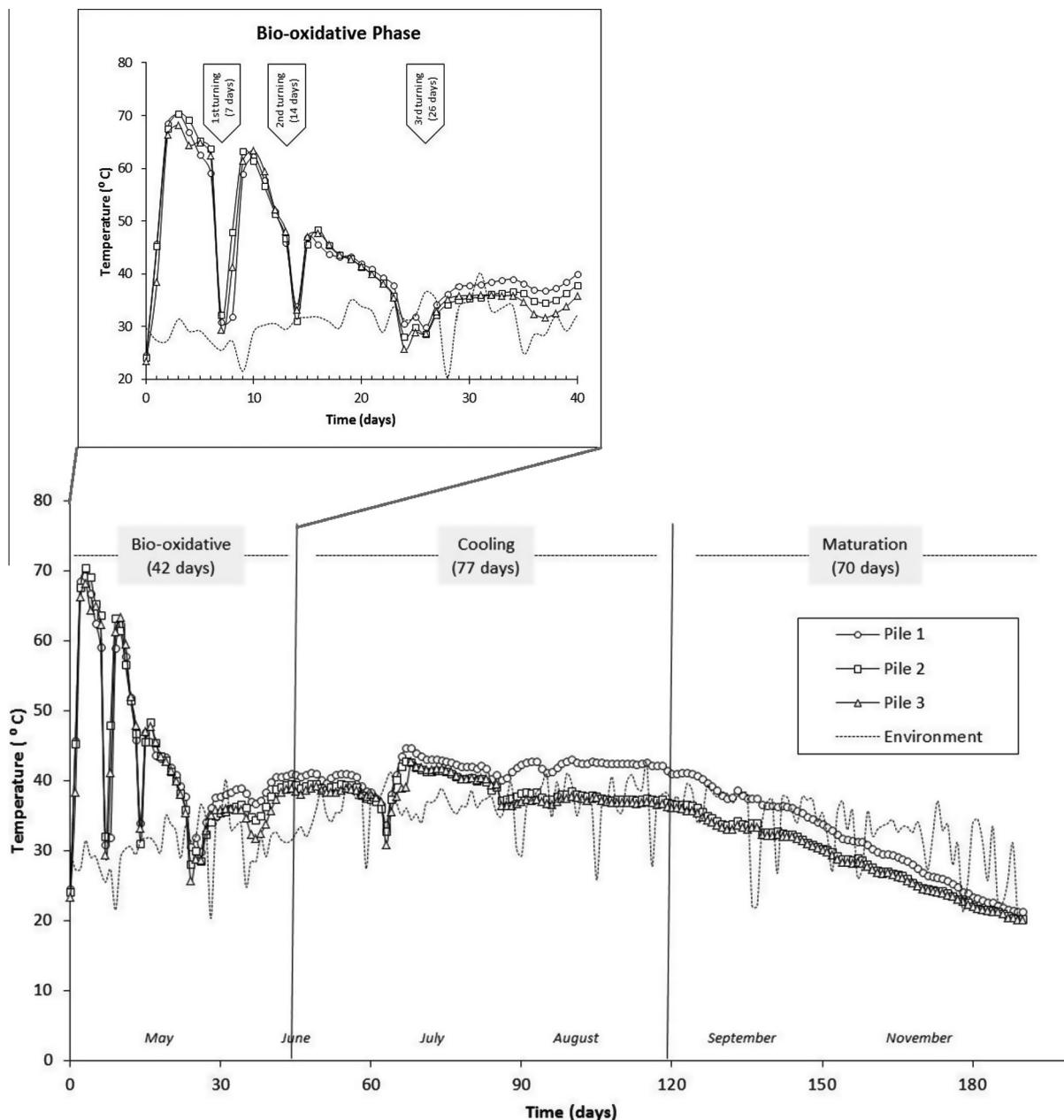
Fig. 1. Changes in temperature of the three composting piles (bottom). Detail of temperature evolution and times of windrow turnings during bio-oxidative stage (top).

Mesophilic and thermophilic actinobacteria (Fig. 2a) increased between the start of the process and the second thermophilic stage (TER2A), at which time both communities reached maximum levels. Afterwards, mesophilic actinobacteria followed a similar trend and levels to those described for mesophilic bacteria except for a wider decline in the maturation stage (MAD1-PRF) (Fig. 2d). Thermophilic actinobacteria also fell drastically in this late stage after displaying a continuous decrease from day 9 (TER2A). This result was also obtained by Vargas-García et al. (2010).

Mesophilic fungi (Fig. 2b) sharply declined at the first and second thermophilic stages (TER1 and TER2) showing different pattern of variation in both cases. The number of mesophilic fungi dropped from 9.1×10^5 CFU/g dw (day 0) to 4.7×10^4 CFU/g dw (day 2) in the early stage of the first thermophilic stage (TER1A). The counts increased just three days later even when temperature was still in the thermophilic range (TER1B) and kept this trend up to reach a value of 4.3×10^5 CFU/g dw at the second early

thermophilic stage (TER2A). A new decline was noticed at the second late thermophilic stage (TER2B) after three days being exposed to temperatures above 50 °C. High temperatures are known to inactivate fungi (Hassen et al., 2001) and the adverse effect of high temperature on mesophilic fungi was clearly demonstrated in this study, although fungi did not disappear at the thermophilic stage. This result suggests that mesophilic fungi adapt to thermal conditions as the bio-oxidative stage proceeds. In contrast, the numbers of thermophilic fungi exhibited low levels in the raw materials and steadily increased as temperature rose until the first thermophilic stage was accomplished (MESD1), then peaked at the second thermophilic stage (TER2A) and MESD2 and stabilized thereafter.

Results obtained demonstrated that the microbial communities adapt to temperature in bio-oxidative stage as noticed by the different growth levels pattern in each of the three thermophilic stages (Fig. 2c). Fungal community was particularly sensitive to temperature so that their dynamics depended on thermal values

Table 1
Samples collected and physicochemical parameters measured during composting.

Sample code	Sampling days	Phase	Temperature (°C)	Moisture (%)	pH	C (%)	N (%)	C/N
MPR	0	Raw materials	24.0	49.9 ± 1.2	7.0 ± 0.0	26.1 ± 1.4	0.9 ± 0.0	27.7 ± 2.4
MESA1	1	1st Rising mesophilic	43.2	48.4 ± 1.1	8.4 ± 0.0	24.5 ± 1.1	1.0 ± 0.0	25.7 ± 1.2
TER1A	2	1st Early Thermophilic	64.9	51.1 ± 0.7	8.4 ± 0.1	24.6 ± 2.4	1.0 ± 0.0	24.8 ± 1.8
TER1B	5	1st Late thermophilic	65.1	44.8 ± 0.7	8.3 ± 0.1	26.9 ± 0.7	1.1 ± 0.1	25.4 ± 2.2
MESD1	7	1st Decreasing mesophilic	59.8	49.4 ± 1.4	8.6 ± 0.0	25.4 ± 0.3	1.0 ± 0.0	24.7 ± 0.7
MESA2	8	2nd Rising mesophilic	40.4	49.89 ± 0.2	8.6 ± 0.0	25.7 ± 1.4	1.0 ± 0.0	24.5 ± 1.4
TER2A	9	2nd Early thermophilic	59.4	45.7 ± 2.1	8.6 ± 0.0	24.5 ± 0.6	1.0 ± 0.0	24.5 ± 0.9
TER2B	12	2nd Late thermophilic	54.8	44.6 ± 0.8	8.7 ± 0.0	24.7 ± 1.6	1.1 ± 0.0	22.9 ± 1.9
MESD2	14	2nd Decreasing mesophilic	45.9	41.0 ± 0.5	8.7 ± 0.0	25.0 ± 0.7	1.1 ± 0.1	23.5 ± 1.8
MESA3	15	3rd Rising mesophilic	44.2	47.4 ± 0.5	8.7 ± 0.0	26.2 ± 0.2	1.1 ± 0.1	23.5 ± 1.7
TER3A	16	3rd Early thermophilic	48.2	46.5 ± 0.8	8.7 ± 0.0	25.5 ± 1.5	1.1 ± 0.0	23.8 ± 1.3
MESD3	26	3rd Decreasing mesophilic	31.3	48.7 ± 0.6	8.5 ± 0.1	22.5 ± 0.1	1.0 ± 0.1	22.7 ± 1.1
MESA4	28	4th Rising mesophilic	34.8	47.4 ± 0.7	8.5 ± 0.1	22.2 ± 0.5	1.1 ± 0.0	20.6 ± 0.6
MES5	42	Mesophilic (cooling)	39.4	49.7 ± 0.3	8.7 ± 0.0	24.4 ± 1.6	1.2 ± 0.1	20.7 ± 1.9
MES6	56	Mesophilic (cooling)	40.0	41.0 ± 0.6	8.7 ± 0.0	23.2 ± 0.8	1.1 ± 0.0	21.6 ± 0.8
MES7	63	Mesophilic (cooling)	33.9	46.6 ± 2.5	8.6 ± 0.0	23.4 ± 0.9	1.2 ± 0.0	19.7 ± 0.8
MAD1	119	Early maturation	38.5	50.9 ± 1.5	8.6 ± 0.0	21.5 ± 0.1	1.2 ± 0.0	17.3 ± 0.3
MAD2	168	Late maturation	26.5	42.5 ± 1.7	8.5 ± 0.0	17.3 ± 1.0	1.2 ± 0.0	14.4 ± 0.5
PRF	189	End product	20.6	40.8 ± 1.8	8.5 ± 0.1	16.7 ± 1.6	1.2 ± 0.1	13.3 ± 0.7

and duration of the stage. Nevertheless, nutritional and environmental factors other than temperature also affect microbial community dynamics.

3.3. Organic matter fractions dynamics

During the composting of lignocellulose-rich substrates, the microorganisms release soluble organic intermediates from polymers. This causes the solid polymeric matrix to partially solubilize (soluble fraction), remain almost unchanged (polymeric fraction) or reorganize in such a way that it is transformed in humus-like substances (humic fractions). These pools continuously interchange substances during composting by means of microbiological transformations or chemical reactions. The dynamics of biologically meaningful components of soluble, polymeric and humic-like fractions during composting was analyzed in this work and the results are described below.

3.3.1. Soluble fractions

According to Hofman and Dusek (2003) organic matter extracted with 0.5 M K_2SO_4 can be used as a measure of the fraction of total organic matter, which is actually available for microorganisms. In this work potassium sulfate extract was used to analyze soluble organic carbon (SOC), reducing sugars (RS) and proteins along with microbial biomass C (C_{mic}). Ammonium and nitrate were also analyzed in order to determine the nitrogen mobilization pattern from solid matrix (mainly proteins). The results are presented as carbon and nitrogen soluble fractions (Fig. 3a and b).

SOC and RS in the soluble fraction are represented in Fig. 3a together with microbial biomass C (C_{mic}) and temperature, in order to show the relationship between carbon release and removal by microbial biomass and temperature effects. As expected, main changes in SOC fractions were detected at bio-oxidative stage and showed a general decreasing trend during composting. The first thermophilic stage (TER1) was the most critical for this carbon fraction. SOC and RS increased quickly after the start of the process and peaked at the beginning of the first thermophilic stage (TER1A) when temperature reached the highest value (65 °C). At the next sampling time (5 days), at which temperature was still in the thermophilic range, the values of these soluble fractions significantly decreased. In this period, microbial biomass C (C_{mic}) followed an opposite profile to SOC and RS. Afterwards, C_{mic} variations were similar to that of thermal tendency. This behavior clearly indicates that dynamics of carbon soluble fractions is strongly related to

microbial activity and temperature. SOC is expected either to be quickly metabolized by microorganisms as it is released from the bulk organic matter or to be eventually leached. Consequently, a decrease in microbial activity may lead to an accumulation of this fraction provided that a monomer releasing activity (e.g. enzymatic hydrolysis) is present. This was probably the cause of the increase in SOC at the early thermophilic stage that coincided with low C_{mic} levels because of the deleterious effect of the high temperature on biological survival. This initial increase has also been reported by Zmora-Nahum et al. (2005) during composting of biowaste. The recovery of C_{mic} levels above the initial ones when temperature was still in the thermophilic range and the concomitant decrease in SOC reflects a quick adaptation of microbial community to high temperature. This acclimatization effect was also observed for microbial counts as described in Section 3.2. Even though very few references can be found in the literature that can be compared with this study, other with the similar water soluble extracts report a continuous decrease on this fraction throughout composting (Zmora-Nahum et al., 2005; Said-Pullicino et al., 2007). This decrease depends on the continuous mineralization of soluble organic compounds and the re-polymerization and condensation pathways that lead to the formation of complex organic substances in the solid fraction (Said-Pullicino et al., 2007).

The dynamics of soluble inorganic (N–NH₄ and N–NO₃) and organic (soluble proteins) nitrogen are represented in Fig. 3b. Nitrogen is partitioned during composting between insoluble (mainly proteins) and soluble fractions that are released from the former by means of ammonification and proteolytic reactions. Ammonium can be further oxidized to NO_2^- or NO_3^- by nitrification. As a consequence, soluble nitrogen comprises a mixture of ammonium, NO_2^-, NO_3^-, aminoacids and peptides (Guardia et al., 2010). Initial stages of composting are usually dominated by proteolytic and ammonifying activities that give rise to the release of ammonium and soluble proteins while nitrification predominates during maturation (Guardia et al., 2010). This was the general profile found in the soluble nitrogen fraction during the composting process developed in this work (Fig. 3b). Soluble proteins and ammonium concentrations were higher in the bio-oxidative stage than in maturation and N–NO₃ was the main N component of nitrogen soluble fraction in maturation. Fluctuations of soluble proteins during bio-oxidative stage followed a profile similar to that reported for SOC with sharp differences between the first and further thermophilic stages. This fraction accumulated at the beginning of the first thermophilic stage (TER1) because of
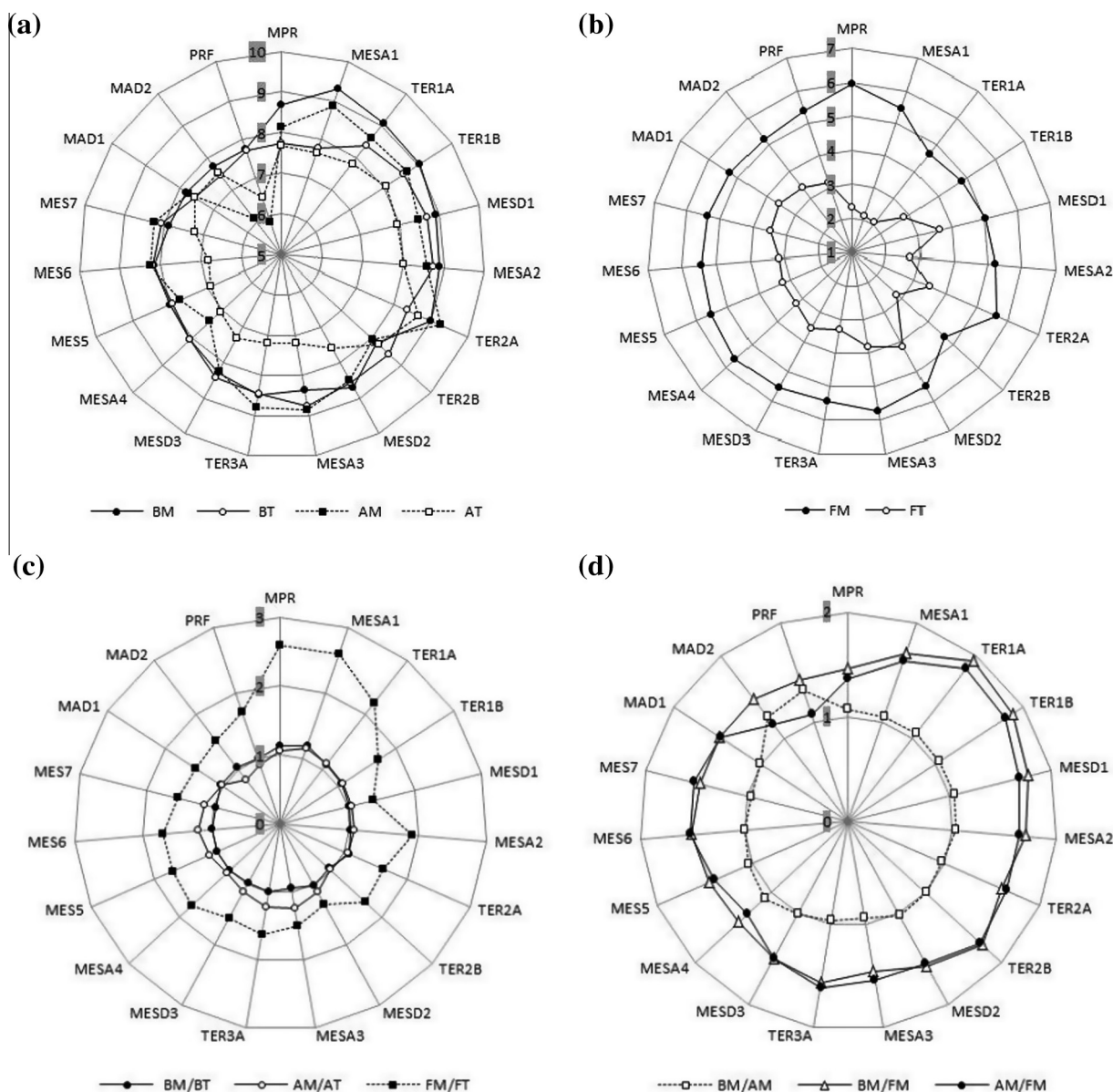
Fig. 2. Variation of microbial counts (mean Log CFU/g dw, n = 3, SD < 5%) of (a) mesophilic and thermophilic bacteria (BM, BT) and actinobacteria (AM, AT) and (b) mesophilic and thermophilic fungi (FM, FT); (c) ratio between mesophilic and thermophilic bacteria (BM/BT), actinobacteria (AM/AT) and fungi (FM/FT); (d) ratio between mesophilic bacteria, actinobacteria and fungi. The radial lines represent sample codes (see Table 1).

the decline in microbial activity at high temperature, but it decreased afterwards. Bustamante et al. (2008) also observed this stabilization of organic nitrogen. Ammonium (N–NH$_4$) concentrations were rather low in comparison to values reported in nitrogen-rich substrates, such as manure and household waste (Cayuela et al., 2009). This low ammonification rate was expected for lignocellulose-rich substrates due to lower substrate degradation. The changes in N–NH$_4$ during the bio-oxidative stage strongly depended on temperature. Ammonium decreased at the beginning of the first (TER1A) and the second thermophilic (TER2A) stages because of high temperature along with alkaline pH (Table 1) led to volatilization. These conditions still prevailed at the late sampling times of each thermophilic stage (TER1B and TER2B) but ammonia increased instead. This result supports the idea that factors other than protein hydrolysis, nitrification, alkaline pH, leaching and volatilization are involved in the dynamics of this nitrogen species. Thus, the shift to microbial capabilities

that promote N–NH$_4$ uptake as a consequence of thermal acclimatization may partially explain this behavior. Nitrate (N–NO$_3$) was detected throughout the composting process. The concentrations of this N fraction steadily decreased during bio-oxidative stage except for the second thermophilic stage (TER2B) in which a sharp increase was detected. Nitrification occurred mainly during the maturation stage due to the elevated temperature and intense biodegradation of organic matter in the active stage of composting. Both factors, temperature and organic matter content, are known to have a negative impact on the ammonia oxidation, the first step of nitrification (the ammonia oxidation (nitritation) to NO$_2^-$) (Zeng et al., 2013). Although temperature is reported to negatively influence nitrification, N–NO$_3$ content increased in the second thermophilic stage (55 °C). Recent studies revealed the existence of thermophilic ammonium oxidizers (Maeda et al., 2010) whose activity could account for the obtained result.
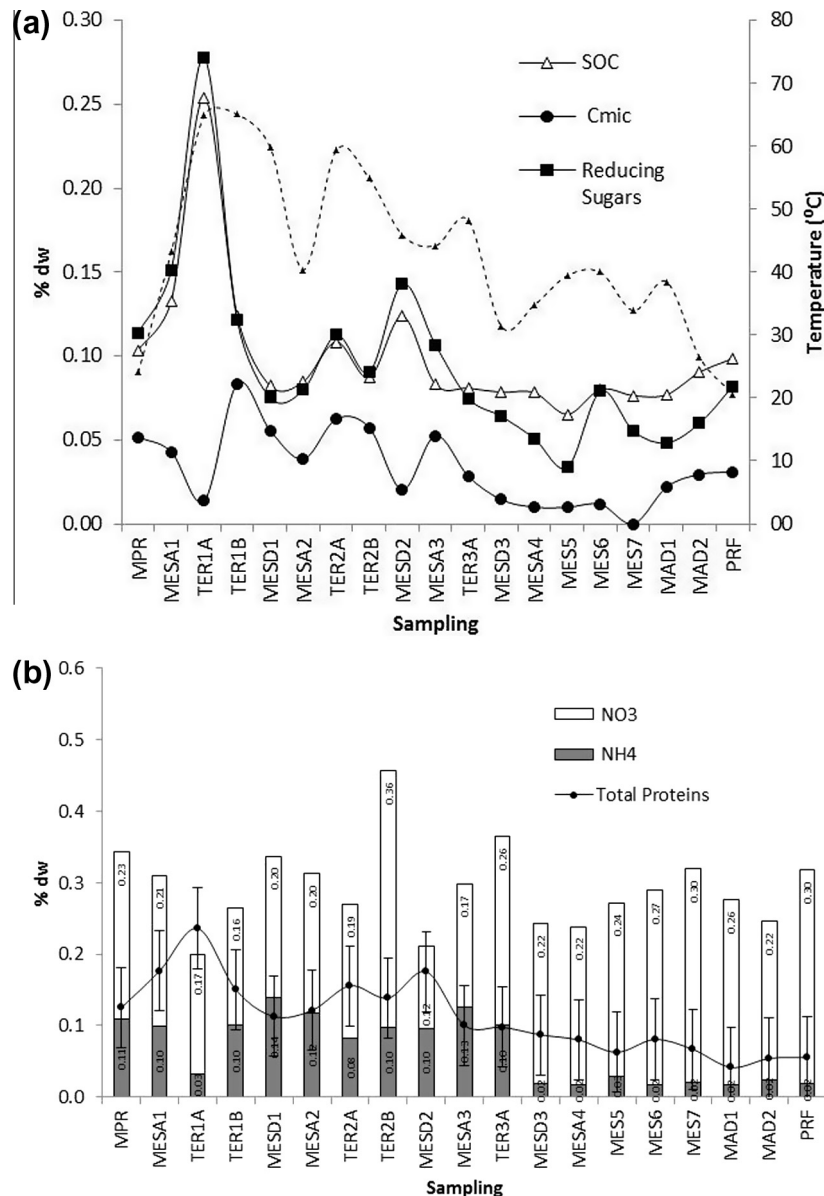
Fig. 3. Evolution with composting time of (a) soluble carbon fractions [soluble organic carbon (SOC) (Δ) and Reducing sugars (■)] along with microbial biomass C (C$_{mic}$) (●) and Temperature (▲); (b) Soluble nitrogen fractions (N–NO$_3$, N–NH$_4$) and Total proteins (●). Results are expressed as mean (n = 3), error bars for total proteins represent LSD, other data SD < 5%. *X*-axis represents sample codes (see Table 1).

3.3.2. Polymeric fractions and humification

The most slowly-degradable fractions during composting of lignocellulosic waste comprise polymers (mainly lignocellulose) and the organic carbon fractions that are extracted with pyrophosphate under alkaline conditions (Cext), also known as humic-like extractable carbon (Iglesias and Pérez, 1992). This humic-like carbon originates mainly from lignocellulose as a consequence of humification (Stevenson, 1994). The changes undergone in components of the polymeric and humic-like fraction during composting are shown in Fig. 4a and b.

Polymeric fractions analyzed included cellulose, hemicellulose, lignin and fats–waxes–oil and resins (FWO + resins) whose values are shown in Fig. 4a along with other related carbon fractions (organic matter, Cext and total sugars) that would explain their dynamics. The organic matter content decreased around 6% during the bio-oxidative stage and 44% in cooling and maturation stages. In line with the above are results obtained for total carbon (Table 1) and total sugars trends (Fig. 4a). This behavior is explained by the mineralization of the organic matter, mainly total sugars, that are

lost as CO$_2$. Polymer fractions (FWO + Resins and lignocellulose) along with total humic-like extractable carbon (Cext) accounted for most of total organic matter at any stage of composting (Fig. 4a). This is reasonable considering that main components of bulk materials are insoluble polymers and humic-like substances. Furthermore, organic matter dynamics during composting followed the same change pattern as total sugars content (Fig. 4a). The procedure used for total carbohydrate determination quantifies not only soluble mono-, oligo- and polysaccharides but also insoluble polysaccharides such as cellulose (Šafařík and Šantrůčková, 1992). Accordingly, most carbon and organic matter losses were linked to the mineralization of total carbohydrates and therefore the evolution of this latter fraction was also related to modification of holocellulose (cellulose plus hemicellulose) as explained below.

The starting material was composed of 28.2% cellulose, 4.4% hemicellulose and 13.3% lignin. Hemicellulose did not change significantly during composting. In contrast, cellulose steadily decreased from the start up to the end of composting and its decline
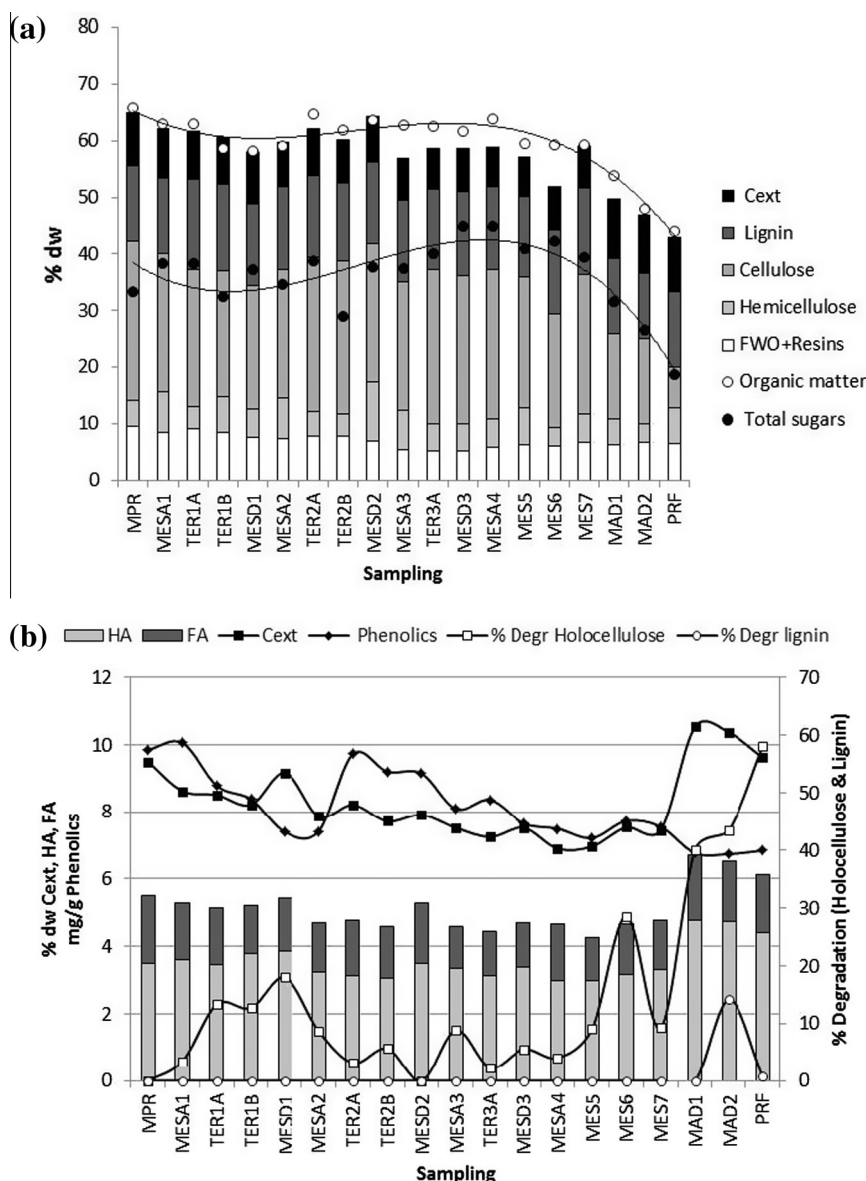
Fig. 4. Variation with composting time of (a) polymeric fractions [pyrophosphate alkaline-extractable carbon (Cext), lignin, cellulose, hemicellulose and fats–waxes–oils and resins (FWO + resins)], organic matter (○) and total sugars (●); (b) humic-like fractions [Humic acids-like (HA), fulvic acids-like (FA) and pyrophosphate alkaline-extractable carbon (cext) (■)], phenolic acids (♦) and percentage of degradation of lignin (○) and holocellulose (□). Results are expressed as mean (*n* = 3, SD < 5%). X-axis represents sample codes (see Table 1).

was particularly sharp during maturation stage. Cellulose decrease coincided with the above mentioned decline of total sugars content. It is usual that cellulose does not degrade during the early stage of composting because there are other readily biodegradable compounds, as it was also evident by soluble carbon fraction results discussed in Section 3.3.1 (Fig. 3a). This behavior has been reported in other composting process, for example, Huang et al. (2010) detected biodegradation of cellulose only after 30 days of lignocellulosic waste composting.

Lignin levels remained unchanged until the start of maturation stage at which period content significantly decreased. The lowest lignin content (11.3% dw) was obtained at MAD2 sampling (168 days). It is noteworthy that lignin degradation coincided with the period of the highest cellulose degradation, as the presence of the former limits the biodegradation of the latter because both are bounded fractions. Therefore, as previously hypothesized, the prolonged cooling stage obtained

in composting (Fig. 1) could relate to the presence of large amount of nutrients retained by this recalcitrant fraction that are slowly released from the polymeric matrix. In composting processes, lignin is either not degraded (Lashermes et al., 2012) or degraded during the maturation stage (Paradelo et al., 2013), as obtained in this work.

Resins, fat, waxes and oils (FWO + resins) accounted for a small fraction of starting material (10% dw). This hydrophobic fraction dropped during bio-oxidative stage up to reach a value of 6% dw at MESA3 sampling time (15 days) and remained unchanged afterwards (Fig. 4a). Thus, degradation of this fraction is highly related to temperature changes. It mainly occurred during the first two thermophilic stages (TER1 and TER2) at which temperature was much higher than that of the third thermophilic stage (TER3A) when FWO + resins stabilized. This decreasing trend has been also reported in composting of oily materials such as olive mill waste (Cayuela et al., 2009).

The changes in polymeric fractions during composting are expected to be linked to those of humic-like substances because the latter are the final stages of degradation of polymers (Stevenson, 1994). Fig. 4b shows the evolution of components of this fraction during composting (i.e. Cext, HA, FA) along with other parameters that could be related to its dynamics (phenolic acids and degradation of holocellulose and lignin). As expected, humic substances changes were found only in the maturation stage (MAD1 to PRF). In this period all humic-like substances significantly increased and this paralleled with the most active degradation of carbohydrate polymers (holocellulose, i.e. cellulose plus hemicellulose) and lignin. Phenolic compounds profile also might be linked to humic-like dynamics. These compounds exhibited a general decreasing trend except for the increase obtained at second thermophilic stage (TER2A). This evolution resembled to that obtained by Tejada et al. (2009) and Paradelo et al. (2013). These results suggest a polyphenol repolymerization due to abiotic factors during MAD1 that would contribute to humic-like substances formation. The ratio HA/FA increased at maturation stage due to increases in humic acids-like content, which is a high molecular fraction of humic-like extract (Iglesias and Pérez, 1992). This result reinforces the fact that there is a repolymerization over the substances in the maturation stage.

3.4. Interactions among physical, chemical and biotic composting parameters

Descriptive information previously discussed provided useful explanation on the dynamics of main variables involved in composting of lignocellulosic waste. On the basis of results obtained it was clear that composting evolved through different stages. In order to detect relevant parameters at each stage of composting, a multivariate analysis was performed. First, these stages were identified by means of a discriminant analysis (DA) and then a principal component analysis (PCA) was applied to the data matrix for each period to prioritize significant parameters.

DA was applied to all parameters determined in this study except those providing redundant information. The parameters not included in the model were selected according to information provided by descriptive analysis and to Pearson correlation coefficient (significant correlation $r > 0.8$ at $p < 0.001$) with another parameter. Thus, organic matter (correlated with C), total sugars (correlated with holocellulose), reducing sugars and soluble proteins (correlated with SOC) were avoided. The variables FWO + resins and phenolic acids were excluded in the stepwise DA because their tolerance level was below the established minimum ($p < 0.05$). The DA grouped samples according to the composting stage during which they were taken (Fig. 5). Two discriminant functions were obtained that explained 76.4% of the variation. The first discriminant function accounts for 51.5% of the variation and separates the samples of the various composting stages based on humification parameters (Cext and HA) and biotic plus soluble fraction and temperature (BM, C_{mic}, SOC, N–NH_4, T). Along the second function, which explains an additional 24.9% of the variation, pH, holocellulose and N were the most determining variables. The samples grouped in four clusters in the plot space that allowed to discriminate composting stages. Samples from the first thermophilic stage (MPR-MESD1) clustered in group I, and samples of following sampling times until the beginning of the third thermophilic stage (MESA3-TER3A) clustered in group II. This result confirms the differentiation between the first and further thermophilic stages as described earlier. Samples taken at cooling (MESD3-MES7) and at the maturation (MAD1-PRF) stages formed two clear homogeneous groups which were ascribed to stages III and IV, respectively. Consequently, DA analysis showed that the bio-oxidative stage was
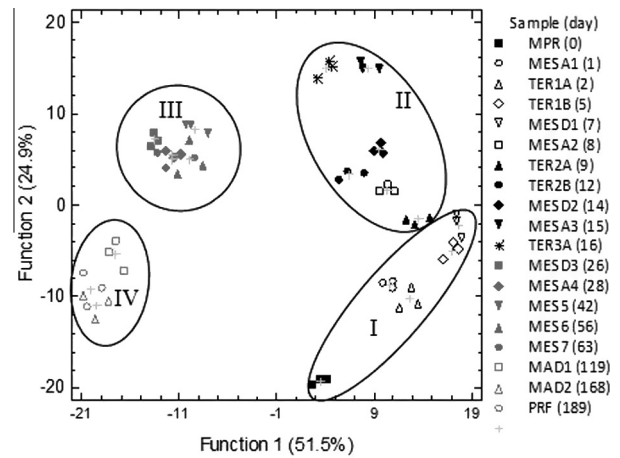

Fig. 5. Discriminant analysis loading plot of physical, chemical and biological data. Data are grouped in four classes: I, MPR-MESD1; II, MESA2-TER3A; III, MESD3-MES7; IV, MAD1-PRF. Sampling days are shown beside sample code.

shorter than previously suggested by the thermal profile (16 days in contrast to 42 days).

A PCA of the total variation was applied in the four periods and the relationships between each of the two principal components are shown in Fig. 6a–d. The cumulative contribution rate of the two principal components (PC1 and PC2) reached 51.8%, 43.6%, 42% and 54.2% for stage I, II, III and IV, respectively. The results indicated some interesting trends in the four periods. In the first period (stage I), the most influential variables in PC1 (34.9% of variance) were mesophilic fungi (FM), holocellulose, thermophilic bacteria (BT) and temperature (T) while SOC and N–NH_4 had the higher loads for PC2 (17.9% of variance) (Fig. 6a). This result reaffirms the significance of temperature in this critical stage, as it limits growth of fungi and stimulates thermophilic bacteria community (Zhang et al., 2011). It also shows that polymeric carbohydrates (holocellulose) play an important role while proteolysis and ammonification seem to be the other main processes that characterize this stage. These processes have also been previously reported in the early stages of composting (Guardia et al., 2010; Huang et al., 2010; Zeng et al., 2013). In the second period (stage II), SOC and mesophilic bacteria (BM) along with pH, N–NH_4 and N–NO_3 presented the highest loads on PC1 and the two soluble nitrogen forms (N–NO_3 and N–NH_4) accounted for the most significant parameters in PC2 (Fig. 6b). Consequently, the situation drastically changed with respect to the first thermophilic stage being the release of N–NH_4 and its mineralization to N–NO_3 the most influential processes along with pH effects. It is also noticeable to remark the shift of microbial community influence from FM and BT in stage I to BM in stage II. Zhang et al. (2011) also found significant relationships between ammonium and nitrate with the bacterial but not fungal species, suggesting that these two parameters were likely to influence, or be influenced by bacterial species. Lignin, SOC and mesophilic fungi (FM) were the most influential variables in PC1 in the third period (stage III) while N–NH_4 and C_{mic} gave the highest loads in PC2 (Fig. 6c). In the fourth period (stage IV) loads of many parameters were quite similar in PC1 being SOC, carbon (C) and mesophilic actinobacteria (AM) the most influential parameters while nitrogen (N) and lignin exhibited the highest loads in PC2 (Fig. 6d). These results confirm the important role that lignin plays in the final stages of composting as the core for humic-like substances formation that took place yet since the start of cooling stage at 26 days. This early influence of lignin content on the behavior of the process along with the notable role of actinobacteria was also reported by Huang et al. (2010). It is also
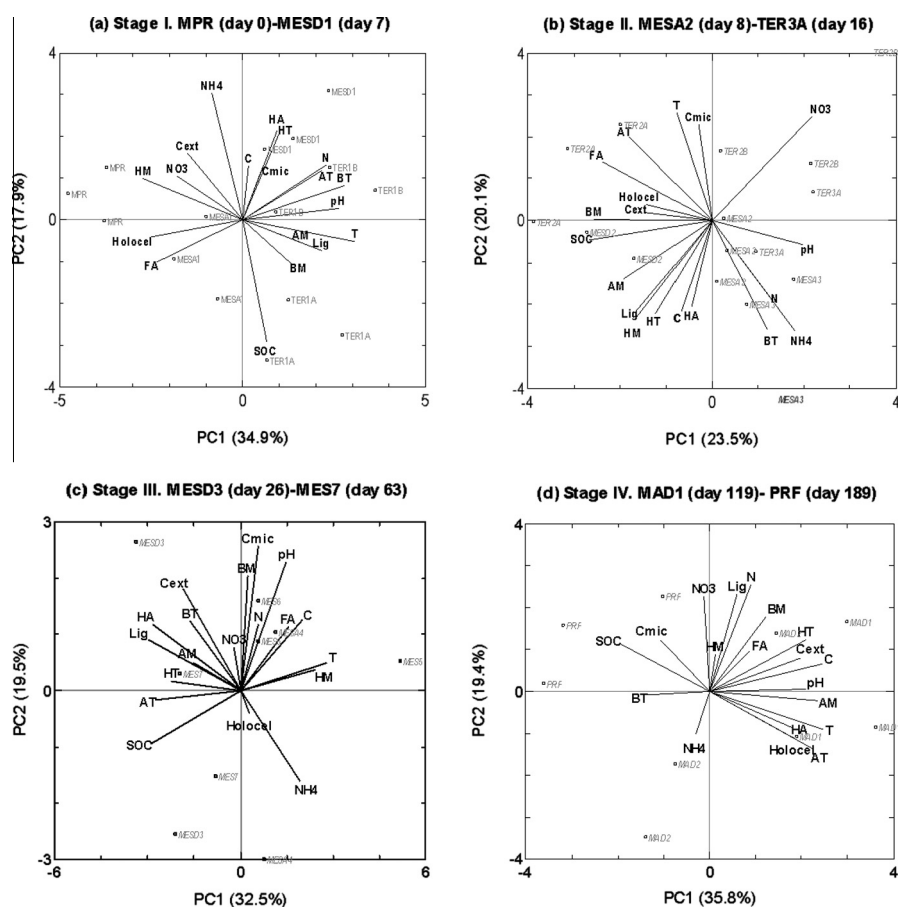
Fig. 6. Principal component analysis (PCA) of data showing variable loadings plots for each of the four periods identified by DA: (a) stage I (MPR-MESD1); (b) stage II (MESA2-TER3A); (c) stage III (MESD3-MES7); (d) stage IV (MAD1-PRF). Percent variability explained by each principal component (PC) is shown in parentheses. The variables used in the analyses are displayed: temperature (T), pH, mesophilic and thermophilic bacteria (BM, BT), actinobacteria (AM, AT), and fungi (FM, FT), microbial biomass C (C_{mic}), total carbon (C), total nitrogen (N), ammonium (NH_4), nitrate (NO_3), pyrophosphate alkaline-extractable carbon (Cext), fulvic acid-like (FA), humic acid-like (HA), holocellulose (Holocel), lignin (Lig), soluble organic carbon (SOC).

noteworthy to mention the high load score of soluble carbon fraction (SOC) at any stage of composting, even at the late ones (cooling and maturation). Thus, the importance of this fraction to determine carbon dynamics is not restricted to the first stages of composting as it was earlier described (Zmora-Nahum et al., 2005; Tejada et al., 2009). It is likely due to the crucial role of soluble carbon in the changes occurring in the bacterial and fungal community composition (Zhang et al. 2011). Finally, as the maturation was approached it was possible to observe a stronger association of the variables (Fig. 6a–d). This behavior could be related to the stabilization of organic matter at the end of the process.

4. Conclusions

This work concerns the in depth study of transformation of main forms of organic matter during composting of lignocellulosic waste and its relationships with biological and physical factors. The first thermophilic stage of composting is crucial in determining the further evolution of soluble and polymeric carbon fractions but dynamics of carbon is still important at the maturation stage. Multivariable analysis reveals that evaluation of soluble organic carbon (SOC), microbial biomass C (C_{mic}), pH and inorganic nitrogen (N–NH_4 and N–NO_3) can be of great use in identifying the composting stage.

References

Böhm, K., Smidt, E., Tintner, J., 2013. Application of Multivariate Data Analyses in Waste Management. In: Freitas, L.V., Barbosa, A.P. (Eds.), Multivariate Analysis in Management, Engineering and the Sciences. InTech, http://dx.doi.org/10.5772/53975.

Bustamante, M.A., Paredes, C., Marhuenda-Egea, F.C., Pérez-Espinosa, A., Bernal, M.P., Moral, R., 2008. Co-composting of distillery wastes with animal manures: carbon and nitrogen transformations in the evaluation of compost stability. Chemosphere 72, 551–557.

Bustamante, M.A., Paredes, C., Morales, J., Mayoral, A.M., Moral, R., 2009. Study of the composting process of winery and distillery wastes using multivariate techniques. Bioresour. Technol. 100, 4766–4772.

Campitelli, P., Ceppi, S., 2008. Chemical, physical and biological compost and vermicompost characterization: a chemometric study. Chemom. Intell. Lab. Syst. 90, 64–71.

Cayuela, M.L., Mondini, C., Insam, H., Sinicco, T., Franke-Whittle, I., 2009. Plant and animal wastes composting: effects of the N source on process performance. Bioresour. Technol. 100, 3097–3106.

Ciavatta, C., Govi, M., Vittori, L., Antisari, L., Sequi, P., 1991. Characterization of humified compounds by extraction and fractionation on solid polyvinylpyrrolidone. J. Chromatogr. A 509, 141–146.

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.

Guardia, A.D., Mallard, P., Teglia, C., Marin, A., Le Pape, C., Launay, M., Benoist, J.C., Petiot, C., 2010. Comparison of five organic wastes regarding their behaviour

during composting: part 2, nitrogen dynamic. Waste Manage. (Oxford) 30, 415–425.

Hassen, A., Belguith, K., Jedidi, N., Cherif, A., Cherif, M., Boudabous, A., 2001. Microbial characterization during composting of municipal solid waste. Bioresour. Technol. 80, 217–225.

Herber, D., Phipps, P., Stronoe, R., 1971. Chemical Analysis of Microbial Cells. In: Norris, J., Ribbons, D. (Eds.), Methods in Microbiology. Academy Press, New York.

Hofman, J., Dusek, L., 2003. Biochemical analysis of soil organic matter and microbial biomass composition – a pilot study. Eur. J. Soil Biol. 39, 217–224.

Huang, D.L., Zeng, G.M., Feng, C.L., Hu, S., Lai, C., Zhao, M.H., Su, F.F., Tang, L., Liu, H.L., 2010. Changes of microbial population structure related to lignin degradation during lignocellulosic waste composting. Bioresour. Technol. 101, 4062–4067.

Iglesias, E., Pérez, V., 1992. Determination of maturity indices for city refuse composts. Agric. Ecosyst. Environ. 38, 331–343.

Kuok, F., Mimoto, H., Nakasaki, K., 2012. Effects of turning on the microbial consortia and the in situ temperature preferences of microorganisms in a laboratory-scale swine manure composting. Bioresour. Technol. 116, 421–427.

Lashermes, G., Barriuso, E., Villio-Poitrenaud, M.L., Houot, S., 2012. Composting in small laboratory pilots: performance and reproducibility. Waste Manage. (Oxford) 32, 271–277.

López, M.J., Elorrieta, M.A., Vargas-García, M.C., Suárez-Estrella, F., Moreno, J., 2002. The effect of aeration on the biotransformation of lignocellulosic wastes by white-rot fungi. Bioresour. Technol. 81, 123–129.

Maeda, K., Toyoda, S., Shimojima, R., Osada, T., Hanajima, D., Morioka, R., Yoshida, N., 2010. Source of nitrous oxide emissions during the cow manure composting process as revealed by isotopomer analysis of and amoA abundance in betaproteobacterial ammonia-oxidizing bacteria. Appl. Environ. Microbiol. 76, 1555–1562.

Marambe, B., Ando, T., 1990. Phenolic acids as potential seed germination-inhibitors in animal-waste composts. Soil Sci. Plant Nutr. 38, 727–733.

Morita, H., 1980. Total phenolic content in the pyrophosphate extracts of two peat soil profiles. Can. J. Soil Sci. 60, 291–297.

Paradelo, R., Moldes, A.B., Barral, M.T., 2013. Evolution of organic matter during the mesophilic composting of lignocellulosic winery wastes. J. Environ. Manage. 116, 18–26.

Šafařík, I., Šantrůčková, H., 1992. Direct determination of total soil carbohydrate content. Plant Soil 143, 109–114.

Said-Pullicino, D., Erriquens, F.G., Gigliotti, G., 2007. Changes in the chemical characteristics of water-extractable organic matter during composting and their influence on compost stability and maturity. Bioresour. Technol. 98, 1822–1831.

Sánchez-Monedero, M.A., Roig, A., Cegarra, J., Bernal, M.P., 1999. Relationships between water-soluble carbohydrate and phenol fractions and the humification indices of different organic wastes during composting. Bioresour. Technol. 70, 193–201.

Somogyi, M., 1951. Notes on sugar determination. J. Biol. Chem. 73, 599–612.

Stevenson, F.J., 1994. Humus Chemistry: Genesis, Composition, Reactions, second ed. John Wiley and Sons, London.

Tang, J.C., Kanamori, T., Inoue, Y., Yasuta, T., Yoshida, S., Katayama, A., 2004. Changes in the microbial community structure during thermophilic composting of manure as detected by the quinone profile method. Process Biochem. 39, 1999–2006.

Tejada, M., García-Martínez, A.M., Parrado, J., 2009. Relationships between biological and chemical parameters on the composting of a municipal solid waste. Bioresour. Technol. 100, 4062–4065.

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.

Vargas-García, M.C., Suárez-Estrella, F., López, M.J., Moreno, J., 2010. Microbial population dynamics and enzyme activities in composting processes with different starting materials. Waste Manage. (Oxford) 30, 771–778.

Yousefi, J., Younesi, H., Ghasempoury, S.M., 2013. Co-composting of municipal solid waste with sawdust: improving compost quality. Clean-Soil Air Water 41, 185–194.

Zeng, Y., Guardia, A.D., Ziebal, C., Macedo, J.D., Dabert, P., 2013. Impact of biodegradation of organic matters on ammonia oxidation in compost. Bioresour. Technol. 136, 49–57.

Zhang, J., Zeng, G., Chen, Y., Yu, M., Yu, Z., Li, H., Yu, Y., Huang, H., 2011. Effects of physico-chemical parameters on the bacterial and fungal communities during agricultural waste composting. Bioresour. Technol. 102, 2950–2956.

Zmora-Nahum, S., Markovitch, O., Tarchitzky, J., Chena, Y., 2005. Dissolved organic carbon (DOC) as a parameter of compost maturity. Soil Biol. Biochem. 37, 2109–2116.