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Coffee husk composting: An investigation of the process using molecular and non-molecular tools

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ABSTRACT

Various parameters were measured during a 90-day composting process of coffee husk with cow dung (Pile 1), with fruit/vegetable wastes (Pile 2) and coffee husk alone (Pile 3). Samples were collected on days 0, 32 and 90 for chemical and microbiological analyses. C/N ratios of Piles 1 and 2 decreased significantly over the 90 days. The highest bacterial counts at the start of the process and highest actinobacterial counts at the end of the process (Piles 1 and 2) indicated microbial succession with concomitant production of compost relevant enzymes. Denaturing gradient gel electrophoresis of rDNA and COMPOCHIP microarray analysis indicated distinctive community shifts during the composting process, with day 0 samples clustering separately from the 32 and 90-day samples. This study, using a multi-parameter approach, has revealed differences in quality and species diversity of the three composts.

1. Introduction

Coffee production and processing constitutes an important sector of the agro-industry in Ethiopia, accounting for up to 65% of the total exports from the country. In addition, approximately 15 million people (20% of the population) directly make a living from the coffee industry (Wiersum et al., 2008). Industrial coffee production uses either a dry or a wet processing method for the removal of the shell and mucilaginous parts from the cherries (Pandey et al., 2000; Murthy and Naidu, 2012), resulting in the production of coffee husk and pulp, respectively. According to these authors, around two tons of such lignocellulosic residues are obtained after the de-hulling of 2–6 tons coffee cherries. In Ethiopia, coffee cherries are mostly processed using the dry method, thereby leading to an annual release of more than 240,000 tons of coffee husk into the environment (CTA, 1999). This represents a serious environmental problem mainly due to the high content of tannins and phenolic compounds contained in this product (Fan et al., 2003). In fact, these authors reported that coffee husks contains more than 9% phenolic compounds, and as such, their direct release into the environment could inhibit plant root growth and lead to an increase in greenhouse gas emissions through anaerobic decomposition. Therefore, it is of great importance to counterpart the high coffee production projected in future years with the proper use and disposal of the resulting residues in order to contribute towards more sustainable production and consumption systems (Murthy and Naidu, 2012). In Ethiopia, this has also been shown for other types of agricultural residues like sorghum and mustard meal (Lulu and Insam, 2000).

The treatment of coffee by-products through oxygen-driven biological methods, such as composting, would serve a dual purpose, i.e. fertiliser production and environmental protection (Murthy and Naidu, 2012). Indeed, composting has become one of the most widely-known and accepted technologies for the recycling of agricultural waste materials under aerobic conditions. It transforms waste materials into a high quality amendment/fertiliser, rich in organic matter and nutrients (Insam and de Bertoldi, 2007). The transformation process involves the succession of specialised microbial communities that express a wide array of enzymes responsible for the changes in the physico-chemical properties of the substrate (Mondini et al., 2004). Monitoring the presence and activities of specific intracellular and/or extracellular enzymes during composting, therefore, provide great insights into the development of the waste biodegradation processes (Vargas-Garcia et al., 2010).

On the other hand, molecular methods based on polymerase chain reaction (PCR) amplification of rRNA genes allow the profiling of complex microbial communities, thereby avoiding the biases associated with culturing-based methods. Denaturing gradient gel

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electrophoresis (DGGE) of PCR-amplified 16S and 18S rRNA gene fragments has been reported as a reliable and fast fingerprinting technique for determining the bacterial and fungal communities inhabiting complex environmental samples (Muyzer and Smalla, 1998). In addition, specific compost-targeted microarrays have also been used to investigate bacterial and fungal communities present in composts (Franke-Whittle et al., 2005, 2009; Hultman et al., 2010). The COMPOCHIP includes several oligonucleotide probes for the detection of some major human, animal and plant pathogenic bacteria, as well as probes targeting plant growth promoting organisms and composting degrading bacteria (Franke-Whittle et al., 2005, 2009). The combined use of both DGGE and the COMPOCHIP microarray offers a deeper insight into the microbial communities during organic waste composting (Danon et al., 2008; Fernández-Gómez et al., 2012).

In this study, cow dung and fruit/vegetable wastes were used as co-substrates in the composting of coffee husks. As coffee husks have a high C/N ratio, amendment with cow dung and fruit/vegetable wastes which are rich in easily biodegradable nitrogen compounds, was conducted, in order to reduce the C/N ratio and to increase the rate of degradation. There were two main aims of the present study; firstly to monitor the physico-chemical changes during the composting of coffee husks, with a special emphasis on extracellular enzymatic activities. The other main aim was to investigate the microbial community dynamics during the major phases of the composting process, using both culture-dependent and culture-independent approaches. Increased knowledge about the changes occurring during the process from a microbial viewpoint should contribute to the further development of efficient strategies for the management and valorisation of waste products generated by the coffee industry.

2. Methods

2.1. Composting process and sample collection

Two different composts were prepared by mixing coffee husks with cow dung and with fruit/vegetable wastes in proportions of 4:1 and 2:1 (on fresh weight basis), respectively, taking into account their C to N ratios. Coffee husks were collected from coffee processing areas located in Bebeka and Mizan Teferi towns (Southwestern, Ethiopia). The fruit/vegetable wastes and the cow dung were collected from the market center and dairy enterprise located in Addis Ababa, respectively. The physico-chemical properties of the materials are shown in Table 1.

Composting was conducted at the Gerji compost demonstration site owned by Addis Ababa Environmental Protection Authority (EPA). About 2000 kg of each mixture was composted in roughly trapezoidal piles with a volume of 1.5 m^3: coffee husk + cow dung (CHCD, Pile 1); coffee husk + fruit/vegetable wastes (CHFVW, Pile 2). A pile containing only coffee husk (CH, Pile 3) was also included in the experimental set-up. All three piles were composted following the aerobic windrow method (Diaz et al., 2002) and subjected to manual turning every day during the first week and once per week until the end of the process. Moisture in the piles was adjusted by adding water to keep the level at approximately 60%. In addition, all piles were covered with a perforated plastic material in order to prevent excessive moisture loss. The temperature was measured in triplicate on days 0, 32 and 90 of composting using a digital thermometer. Three composite samples representing each pile (around 500 g each) were collected separately on days 0, 32 and 90, resulting in a total of twenty-seven samples. The samples were immediately stored at −20 °C upon arrival at the laboratory for molecular analyses and at 4 °C for the remaining analyses. All analyses were carried out in triplicate.

2.2. Physico-chemical analysis

The moisture content of composts was determined after oven-drying (105 °C) of samples (10 g, fresh weight) for 24 h. Total C was determined by dry combustion at 550 °C and total N content (TN) by the Kjeldahl digestion analysis. pH and electrical conductivity (EC) were measured in aqueous extracts (1:10, w/v) using a pH meter (HD8602, Italy) and a conductivity meter (CC401 ELMEIRON, Poland), respectively. Samples were analysed in triplicate.

2.3. Enumeration of total aerobic heterotrophs, actinobacteria and fungi

Estimations of total aerobic heterotrophs, actinobacteria and fungi were determined by direct plating on appropriate media. For the enumeration of total aerobic heterotrophs, plate count agar (PCA, Oxoid, England) was used, whereas actinobacteria and fungi were counted on starch casein agar (SCA) and potato dextrose agar (PDA) media, respectively. Compost samples were ground to 0.25 mm, serially diluted in sterile water and inoculated onto the agar using the plate frequency technique. Plates were incubated for three days at 30 ± 2 °C for the growth of bacteria and fungi, and between five and seven days for actinobacteria. After incubation, any observed visible growth was scored positive and the populations of the different groups in the compost samples were

Table 1
Physico-chemical properties of the raw materials and compost from piles during the composting process on days 0, 32 and 90.

Parameters	Raw Materials			Pile 1			Pile 2			Pile 3		
	CH	FVW	CD	0	32	90	0	32	90	0	32	90
Moisture (%)	13.0 (0.6)	74.3 (1.0)	64.3 (0.12)	54.3 (4.8)^{a}	64.3 (0.7)^{a}	57.7 (1.2)^{a}	56.3 (6.7)^{a}	65.3 (0.7)^{a}	58.3 (0.9)^{a}	58.7 (0.9)^{b}	61.0 (2.1)^{ab}	65.3 (0.9)^{a}
pH (1:10)	5.32 (0.05)	6.21 (0.18)	7.8 (0.01)	6.37 (0.09)^{c}	8.16 (0.03)^{a}	7.89 (0.05)^{b}	6.07 (0.09)^{c}	8.16 (0.03)^{a}	7.79 (0.06)^{b}	5.23 (0.09)^{c}	8.21 (0.06)^{a}	7.46 (0.02)^{b}
EC (dS m^{-1})	2.24 (0.15)	2.25 (0.15)	0.85 (0.01)	1.23 (0.01)^{b}	1.84 (0.09)^{a}	1.77 (0.01)^{a}	1.40 (0.05)^{b}	1.99 (0.09)^{a}	1.75 (0.02)^{a}	1.25 (0.03)^{b}	1.93 (0.16)^{a}	1.57 0.04)^{ab}
Organic C (%)	54.5 (0.4)	40.1 (0.1)	23.0 (0.1)	48.1 (0.3)^{a}	41.5 (0.9)^{b}	35.4 (0.7)^{c}	57.5 (0.9)^{a}	43.0 (1.01)^{b}	36.2 (0.4)^{c}	54.5 (0.1)^{a}	43.2 (1.2)^{b}	41.7 (0.3)^{b}
Total N (%)	1.83 (0.03)	1.32 (0.01)	1.82 (0.03)	2.76 (0.07)^{b}	2.92 (0.03)^{b}	3.19 (0.03)^{a}	2.37 (0.02)^{b}	2.80 (0.13)^{a}	2.84 (0.03)^{a}	1.84 (0.01)^{c}	1.99 (0.01)^{b}	2.31 (0.06)^{a}
C to N ratio	29.8 (0.7)	30.5 (0.2)	12.6 (0.1)	17.5 (0.3)^{a}	14.2 (0.3)^{b}	11.1 (0.2)^{c}	24.9 (0.2)^{a}	15.4 (0.8)^{b}	12.7 (0.1)^{c}	29.5 (0.2)^{a}	22.7 (0.5)^{b}	18.1 (0.3)^{c}

Values are means (n = 3) with standard error in brackets. CH = coffee husk. FVW = fruit/vegetable wastes. CD = cow dung. Pile 1: coffee husk + cow dung; Pile 2: coffee husk + vegetable/fruit waste; Pile 3: coffee husks. EC: electrical conductivity. For each pile different letters indicate significant differences between samples (Tukey HSD test; α = 0.05).

estimated using the Most Probable Number (MPN) method (Tiquia et al., 2002).

2.4. Enzyme Activity using API ZYM™ kit

The relative enzymatic activity in the compost samples was determined by using the API ZYM™ kit according to the manufacturer's protocol. Each strip contained 20 microcupules containing dehydrated chromogenic substrates for different enzymes and a control. For this study, compost samples (5 g, fresh weight) were mixed with 50 ml of sterile water, homogenized in a horizontal shaker for 10 min, allowed to settle for another 10 min and the supernatant was collected for further analysis of the enzymatic activities. An aliquot (65 µl) of the supernatant was dispensed into each of the twenty microcupules. The API ZYM™ strips were covered and incubated at 37 °C for 4.5 h. Then, 30 µl of each reagent (ZYM A and ZYM B) was added to each microcupule. After 5 min, color development was evaluated and a numerical value ranging from 1 to 5 (1 = 5 nM; 2 = 10 nM; 3 = 20 nM; 4 = 30 nM; 5 = 40 nM) was assigned according to the color chart provided by the manufacturer. The results were reported as reactions of low intensity [1], moderate intensity [2–3], and high intensity [4–5], as described by Tiquia et al. (2002).

2.5. DNA extraction and PCR-DGGE analysis of microbial community

Total DNA was extracted from 0.25 g of each triplicate compost sample using the PowerSoil DNA Isolation Kit (MO Bio Laboratories Inc., Carlsbad, USA) according to the manufacturer's protocol, with the following modifications in order to improve the DNA yield. After an abbreviated bead beating step (10 min), samples were frozen at −80 °C for 30 min and then heated for 10 min at 60 °C. This freeze–thaw step was repeated once, before continuing with the standard protocol. The PCR amplification of bacterial and fungal communities was performed as described by Fernández-Gómez et al. (2010). Proper sizes of amplification products were verified by electrophoresis in 1% agarose gels, and PCR product concentration was determined with PicoGreen dsDNA quantification reagent (Invitrogen, Carlsbad, USA). Fluorescence was measured using an Anthos Zenyth 3100 multimode reader (Anthos Labtec, Austria) and the Software for Anthos Multimode Detectors (Version 2.0.0.13).

The denaturing gradient gel electrophoresis (DGGE) of bacterial and fungal communities was performed by loading 60 ng of PCR products in a 7% (w/v) polyacrylamide gel in 1 X TAE (20 mM Tris–HCl, 10 mM acetate, 0.5 mM Na_2EDTA) containing a denaturing gradient of 40–70% and 30–60%, respectively (100% denaturants consisting of 7 mol/L urea and 40% formamide). A 100 bp DNA ladder (Genecraft®, Germany) served as marker. Gels were run in an INGENYphorU System (Ingeny International BV, The Netherlands) at 60 °C for 16 h at 100 V. Gels were stained with silver nitrate using the Hoefer Automated Gel Stainer (Amersham Pharmacia Biotech, Germany), air dried and scanned for subsequent image analysis.

2.6. Microarray analysis

The COMPOCHIP microarray, spotted with 414 probes targeting compost-relevant microorganisms, including plant, animal and human pathogens, and bacteria related to plant disease suppression was used. All the probes included on the COMPOCHIP microarray were designed so to have similar melting temperatures, and probe sequences ranged in length from 17 to 25 nucleotides. Fluorescence labelling of target DNA by PCR using the 8F and 1492R primers, hybridization, scanning of arrays and image analysis were conducted as described by Franke-Whittle et al. (2009).

2.7. Data analysis

Chemical and microbiological data were analysed by repeated measures analysis of variance (ANOVAR) in which piles represented the subjects. In addition, the composition of parent material (CHCD, CHFVW and CH) was fixed as the between-subject factor, and the composting time (0, 32 and 90 days) was fixed as the within-subject factor. All the variables met the sphericity condition (Mauchlýs test), except for pH, EC and organic C. In these cases the sphericity violation was corrected with the Geisser-Greenhouse (G-G) procedure (Potvin et al., 1990). Significant differences in the main effects were further analysed by paired comparisons with the Tukey HSD test. Pearson product moment correlation (r^2) was used to calculate general correlation between enzyme activities and microbial MPN counts. The statistical analyses were performed using SPSS v16.0. A principal component analysis (PCA) from the enzyme activities was also performed using Statistica v9 software.

A comparison of bacterial and fungal DGGE patterns was made with the GelCompar II software package (Applied Math, Belgium). DGGE bands were normalised using the reference position defined by the molecular-weight marker in order to align the bands for proper comparison. A cluster analysis was performed using Dice correlation coefficients and the unweighted-pair-group method with arithmetic averages (UPGMA) clustering algorithm. The program settings were at 1.0% optimisation and 1.0% position tolerance.

Principal component analysis (PCA) of the total signal-to-noise ratio (SNR) from microarray data of the study was conducted, using CANOCO for windows 4.5 (Ter Braak and Smilauer, 2002).

3. Results and discussion

3.1. Changes in physico-chemical parameters during composting

In this study, four temperature phases were seen for the different compost piles, as shown in Fig. 1. As is typical with composting processes, four different temperature phases were observed: (i) an initial mesophilic phase (18–42 °C), which lasted for 2 days in Piles 1 and 2 but for 24 days in Pile 3; (ii) a thermophilic phase (45–70 °C) which lasted for 45 days in the co-composted piles and for 16 days in the control; (iii) a second mesophilic phase for 22 and 38 days in the co-composted and the control piles, respectively; and (iv) a maturity (curing) phase for 12 days. However, significant differences were evident in the temperature changes among the three piles in function of the composting time (compost pile × time ANOVAR $F_{27,72} = 27.78$, $P = 0.00001$). Piles 1 and 2 exhibited a short initial mesophilic phase and self-heated to 60 °C within three days (Fig. 1). In contrast, Pile 3 (composed of coffee husk alone) showed a much longer initial mesophilic phase which lasted for almost three weeks, prior to the start of the thermophilic stage which peaked on day 32, and lasted for only one week. The second mesophilic phase followed and continued until day 78 when the cooling phase started. These results suggest that co-composting of coffee husk with either cow dung or fruit/vegetable wastes accelerated the composting process (Díaz et al., 2002). These findings are in agreement with those of Boulter-Bitzer et al. (2006), who produced four composts from mixtures of different feedstocks (horse, chicken and paunch manure, bone meal ash, bark mix, soybean meal, and milogranite), and reported increases in temperature within the first 10 days from ambient to 55–70 °C, and a second increase in temperature after 31 days, prior to a cooling phase. The sharp decrease in temperature in Piles 1 and 2 during days 10 and 24 could be a result of a lower level of microbial activity due to a lower availability of easily degradable organic materials.

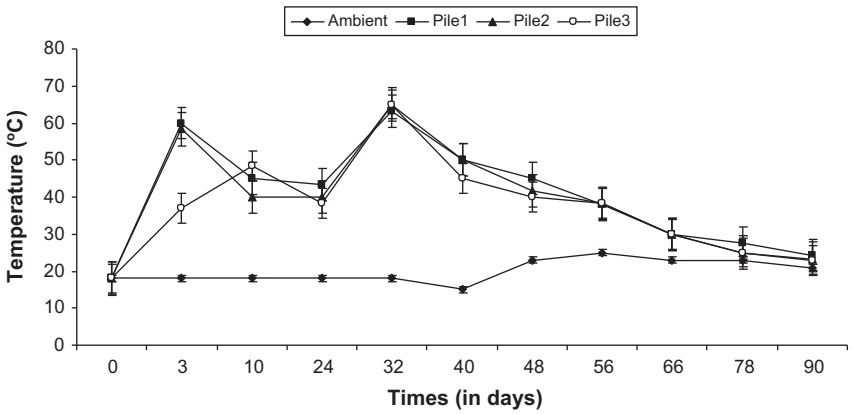

Fig. 1. Temperature changes during composting of three different piles. Values are expressed as means and standard error of triplicates of ambient T in Pile1 (coffee husk + cow dung), Pile2 (coffee husk + fruit/vegetable waste), and Pile3 (coffee husk).

The key physico-chemical properties of the composts at the start, after 32 and after 90 days of composting are shown in Table 1. Overall, the moisture content was in the range 54–65% (mass/mass) for the three piles, although significant differences were found over time (ANOVAR $F_{2,12} = 4.27$, $P = 0.04$). In the two co-composted piles, highest moisture levels were seen in the active phase, with approximately 10% moisture losses occurring afterwards. The reduction in moisture in Piles 1 and 2 can be explained by microbial heat generation causing enhanced desiccation (Rebollido et al., 2008). Moisture loss during the composting process can be considered an indicator of the decomposition rate because the heat generation that accompanies decomposition drives evaporation. In contrast, moisture levels increased during the process in Pile 3. This finding can most likely be attributed to the short thermophilic phase, which resulted in reduced evaporation of moisture into the environment.

The pH values of the different compost piles increased from acidic values at the start of the experiment to alkaline values on day 32. Specifically, this pH rise from day 0 to 32 was more pronounced for Pile 3 than for the two co-composted piles (compost pile x time ANOVAR $F_{4,12} = 27.32$, $P = 0.00001$), probably due to the production of more ammonia during ammonification and mineralisation of organic nitrogen in coffee husks. Between days 32 and 90, pH values were found to decrease minimally (Table 1). Indeed, such increase in pH over time can be attributed to a release of ammonia associated with protein degradation, and its subsequent decrease to a volatilisation of ammoniacal nitrogen and H^+ release resulting from a microbial nitrification process (Gómez-Brandón et al., 2008). The presence of volatile fatty acids may also influence the pH development in the compost piles, as shown in previous studies (Beck-Friis et al., 2001). These latter authors underscored that the dynamics of fatty acid formation and decomposition seem to be important in controlling biological activity and in turn, the gaseous emissions during the composting process. They observed that, when the thermophilic phase began in the self-heated household waste compost, fatty acids were decomposed followed by a rapid increase in pH and NH_3 emissions.

Electrical conductivity increased significantly in all piles from the start of the experiment (Table 1; ANOVAR $F_{2,12} = 60.36$, $P = 0.00001$), probably due to the release of ions, such as phosphate, ammonium and potassium throughout the composting process (Bernal et al., 2009). EC decreased after 32 days (Table 1), and after 90 days, composts had EC values suitable for soil application (<threshold value of 3 mS cm^{-1}; Gómez-Brandón et al., 2008).

Composting time had a significant effect on the total organic carbon (TOC) content in all composts, but this effect varied depending on the starting material (ANOVAR $F_{4,12} = 7.01$, $P = 0.002$). The highest TOC reductions were found in Pile 2 (about 37%), followed by Pile 1 (26%), and Pile 3 (23%), reflecting a notable mineralisation of organic matter over time. In our study, the control pile (coffee husk compost) had the lowest CO_2 loss, probably because of the higher concentration of recalcitrant compounds. This pile contained no additional organic matter that could fuel the kinetic process; hence, its decomposition occurred more slowly.

Total nitrogen (TN) percentage showed an increasing trend with composting duration (Table 1; ANOVAR $F_{2,12} = 46.60$, $P = 0.00001$) which is due to the concentration effect caused by carbon loss associated with mineralisation of the organic matter. The C/N ratio decreased significantly over time (Table 1), especially in the co-composted piles (compost pile x time ANOVAR $F_{4,12} = 27.16$, $P = 0.00001$), as C was lost in the form of CO_2 through microbial respiration and N was recycled (Ryckeboer et al., 2003). A higher C/N ratio at the end of the composting process for the coffee husk compost (Pile 3) was found, indicating a slow kinetic process in this compost. The addition of cow dung and fruit/vegetable wastes to coffee husk, however, significantly contributed to the decomposition of the ligno-cellulosic compounds of the husk, resulting in higher N losses in these mixed composts corroborating the data of Sánchez-Monedero et al. (2001).

3.2. Changes in microbial numbers during composting

Total mesophilic counts of bacteria, actinobacteria and fungi in the three different compost piles indicated significant variations in relation to temperature changes during the composting process (Table 2). At the start of the process, high numbers of bacteria were obtained in Pile 1 (9.58 log MPN g^{-1} dw) and Pile 2 (9.36 log MPN g^{-1} dw). Yet, both piles were found to contain significantly lower numbers of bacteria (6.37 log MPN g^{-1} dw, Pile 1; 6.79 log MPN g^{-1} dw, Pile 2) during the active phase of composting as the temperatures peaked. Bacterial numbers increased again in the maturation phase (Table 2). These findings were expected, based on the findings of others (Hassen et al., 2001). In contrast, bacterial numbers in Pile 3 did not differ significantly for the three sampling times (Table 2), thereby resulting in a significant interaction between compost pile and time (ANOVAR $F_{4,12} = 29.03$, $P < 0.001$).

As occurred with mesophilic bacteria, actinobacterial numbers varied significantly during composting only for Piles 1 and 2 (Table 2; ANOVAR $F_{4,12} = 9.39$, $P = 0.001$). Specifically, the highest number of actinobacteria was found after 90 days of composting in both co-composted piles. Indeed, this group of organisms is able to utilise big polymers and accounts for a majority of the microorganisms present in mature composts (Steger et al., 2007).

Table 2
Enumeration of total aerobic heterotrophic bacteria, actinobacteria and fungi in compost samples collected from the three different piles on days 0, 32 and 90. Data are expressed as log MPN g^{-1} dw.

Microbial groups	Pile 1			Pile 2			Pile 3		
	0	32	90	0	32	90	0	32	90
Total aerobic bacteria	9.58 (0.10)[a]	6.37 (0.08)[c]	8.61 (0.29)[b]	9.36 (0.08)[a]	6.79 (0.13)[c]	8.21 (0.08)[b]	7.89 (0.03)[a]	7.85 (0.20)[a]	7.59 (0.26)[a]
Actinobacteria	8.28 (0.05)[b]	7.93 (0.34)[b]	9.71 (0.09)[a]	8.57 (0.15)[b]	7.57 (0.19)[c]	9.48 (0.14)[a]	8.41 (0.02)[a]	8.20 (0.13)[a]	7.94 (0.45)[a]
Fungi	7.46 (0.05)[b]	5.20 (0.08)[c]	9.22 (0.03)[a]	8.51 (0.20)[a]	5.34 (0.14)[b]	8.21 (0.08)[a]	7.89 (0.18)[a]	5.35 (0.14)[c]	6.25 (0.09)[b]

Values are means ($n = 3$) with standard error in brackets. Pile 1: coffee husk + cow dung; Pile 2: coffee husk + fruit/vegetable waste; Pile 3: coffee husk.
For each pile, different letters indicate significant differences between samples (Tukey HSD test; $\alpha = 0.05$).

Actinobacteria are also considered important for compost hygienisation.

In all piles, fungal numbers were significantly reduced at temperatures above 50 °C and increased later when temperatures declined (Table 2; ANOVAR $F_{2,12} = 499{,}31$, $P = 0.000001$). This is because most fungi are unable to thrive at temperatures above 50 °C (Ryckeboer et al., 2003). Although the exact role of fungi may not be clear, the return of fungal communities at the end of composting probably can be explained as a result not only of the reduced temperatures, but also of the less aggressive environmental factors prevailing and the availability of cellulose and lignin in the remaining piles (Hassen et al., 2001).

3.3. Evolution of extracellular enzymes during composting

Analysis of API ZYM™ testing of the three different compost piles showed varying diversity and relative abundance of enzymes as the composting process proceeded (Table 3). In most cases, the activity of enzymes was highest at day 32, during the active phase of composting, and decreased significantly to a negligible level at the end of the process, indicating the stabilisation of the pile (Mondini et al., 2004). In Pile1, phosphatases (alkaline, acid and naphthol phosphohydrolase), esterases (esterase and esterase–lipase), β-galactosidase and β-glucosidase were the highest in the active phase of composting. In Pile 2, phosphatase and esterase groups showed increasing activity from low to high and moderate level of concentration as composting process advanced, respectively. On the other hand, the protease enzymes decreased in activity level with time of composting. Synthesis of phosphatases which are induced by phosphohydrolate compounds, are considered to be indicators of microbial presence; and protease which converts polypeptides into amino groups can be considered as good indicator of organic matter decomposition. Increased activities of amino peptidase and protease enzymes in Piles 1 and 2 are a sign of high concentration of protein macromolecules at the beginning and thermophilic phases (Vargas-Garcia et al., 2010).

Table 3
Relative activity of extracellular enzymes extracted from different compost piles on days 0, 32 and 90.

Enzymes	Pile1			Pile2			Pile3		
	0	32	90	0	32	90	0	32	90
I-Phosphatase									
Alkaline phosphatase	1	5	3	1	4	3	0	3	1
Acid phosphatase	1	5	3	2	4	3	0	4	0
Naphthol phosphohydrolase	1	5	3	1	4	3	0	5	0
II-Esterases									
Esterase	1	5	3	1	2	2	0	4	1
Esterase lipase	2	5	1	1	3	2	0	1	1
Lipase	2	1	1	1	2	3	0	1	1
III-Amino-peptidase									
Leucine-arylamidase	4	5	1	3	4	1	0	3	1
Valine-arylamidase	4	3	1	3	3	1	1	2	1
Cysteine-arylamidase	4	2	1	4	3	1	1	2	1
IV-Proteases									
Trypsin	4	2	1	4	1	2	1	1	0
α Chymotrypsin	4	2	1	4	1	2	1	1	1
V-Glycosyl-hydrolases									
α-galactosidase	1	1	0	0	0	1	0	1	0
β-galactosidase	1	5	0	0	3	1	0	3	0
β-glucuronidase	1	2	0	0	2	0	0	2	0
α-glucosidase	1	4	0	0	2	0	0	0	1
β-glucosidase	1	5	0	0	2	0	0	4	0
N-acetyl-β-glucosaminidase	3	4	0	0	2	0	0	4	1
α mannosidase	1	1	0	0	0	0	0	0	0
α fucosidase	0	0	0	0	0	0	0	0	0

Averages of triplicate values are expressed as intensity of enzymes (1 – poor, 2–4 – moderate, 5 – excellent).

Piles 1 and 2 expressed a maximum and moderate activity of the enzymes β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase and N-acetyl β-glucosaminidase at day 32, respectively. Maximum activity of β-glucosidase can be associated with higher presence of readily metabolisable substrates, important in the hydrolysis of β-glucosides, such as cellobiose, (Vargas-Garcia et al., 2010). Accordingly, maximum activity of this enzyme was observed in Pile 1 at the bio-oxidative stage.

In Pile 3, the relative abundance of many of these enzymes showed significantly varying levels of activities at day 32, but negligible at the beginning and end of the process probably because of the occurrence of non-aggressive environmental factors such as medium temperature and moisture and slight alkalinisation (pH 8.16–8.21; Hassen et al., 2001).

In general, key enzymes of agronomic value and relevant for the characterization of the composting processes showed similar trends regarding presence and compost age, especially for the two co-composted piles. Similar findings were also reported and by Vargas-Garcia et al. (2010). The difference in the activities of various extracellular hydrolytic enzymes can be attributed to the substrates used for composting. Indeed, the degradation of larger polymers such as cellulose, hemicelluloses and lignin in compost is the result of the combined and successive actions of many microorganisms (Tiquia et al., 2002), and as such, the differing activity levels of the various enzymes during the composting process is not unexpected.

In the present investigation, correlation analysis was performed using microbial count and enzyme activity for the three sampling times (0, 32 and 90). Results indicated that total aerobic heterotrophs, actinobacteria and fungi in the coffee husk plus cow dung and coffee husk with fruit/vegetable waste composts were positively correlated with a number of enzymes (Table 4). In Pile 1, a positive correlation was seen for actinobacteria with three phosphatase enzymes that are involved in the release of phosphate from organic compound. Fungal and actinobacterial numbers in

Table 4
Pearson correlation (r^2) between enzymatic activities and microbial populations in the different compost piles.

	Pile 1			Pile 2			Pile 3		
	Total aerobic bacteria	Actinobacteria	Fungi	Total aerobic bacteria	Actinobacteria	Fungi	Total aerobic bacteria	Actinobacteria	Fungi
Phosphatases									
Acid phosphatase	−0.29	0.71*	0.43	−0.39	0.35	0.11	−0.03	0.11	0.74*
Alkaline phosphatase	−0.28	0.70*	0.43	−0.49	0.21	−0.48	−0.54	−0.48	0.74*
Phosphohydrolase	−0.29	0.71*	0.43	−0.59	0.15	−0.14	−0.45	−0.37	0.88**
Esterases									
Esterase	−0.37	0.61	0.32	−0.82**	−0.25	−0.52	−0.23	−0.04	0.79*
Esterase–lipase	0.44	0.93**	0.93**	−0.24	0.51	0.31	−0.43	−0.66	0.79*
Lipase	0.94**	0.61	0.90**	−0.51	0.36	0.00	−0.52	−0.44	0.38
Amino-peptidases									
Leucine-acrylamidase	0.77*	0.80*	0.98**	−0.25	−0.82**	−0.60	−0.36	−0.52	0.66
Valine-arylamidase	0.88**	0.23	0.60	−0.09	−0.78**	−0.42	0.38	0.51	0.39
Cysteine-arylamidase	0.87**	0.04	0.43	0.13	−0.71*	−0.42	0.34	0.60	0.39
Proteases									
Trypsine	0.87**	0.04	0.43	0.82**	0.14	0.46	0.31	0.49	−0.24
Chymotrypsine	0.86**	0.04	0.43	0.82**	0.14	0.46	0.22	0.52	0.14
Glycosyl-hydrosylase									
α-galactosidase	0.94**	0.61	0.90**	0.06	0.82**	0.59	0.46	0.39	0.39
β-galactosidase	0.40	0.93**	0.92**	0.06	0.79*	0.59	−0.59	−0.70*	0.80*
β-glucuronidase	0.67*	0.88**	0.99**	0.06	0.82**	0.58	−0.30	−0.63	0.46
α-glucosidase	0.44	0.93**	0.92**	0.06	0.77*	0.58	−0.58	−0.38	0.40
β-glucosidase	0.40	0.93**	0.92**	0.06	0.77*	0.58	−0.31	−0.34	0.91**
N-acetyl β-glucosaminidase	0.77*	0.80*	0.98**	0.04	0.83**	0.59	−0.40	−0.22	0.79*
α-Mannosidase	−0.22	−0.92**	0.83**	0.00	0.00	0.00	0.00	0.00	0.00
α-Fructosidase	−0.72*	−0.31	0.07	0.00	0.00	0.00	0.00	0.00	0.00

* Correlation is significant at 0.05 level.
** Correlation is significant at 0.01 level. Pile 1: Coffee husk + cow dung; Pile 2: Coffee husk + fruit/vegetable waste; Pile 3: coffee husk alone.

Pile1 were also significantly positively correlated with β-galactosidase ($r = 0.92$ and $r = 0.93$, respectively), β-glucuronidase ($r = 0.99$ and $r = 0.88$), α-glucosidase and β-glucosidase ($r = 0.92$ and $r = 0.93$). These enzymes are very important in the hydrolysis of lactose and cellobiose. In this study, fungal and actinobacterial abundance were positively correlated with esterase–lipase ($r = 0.93$). However, total aerobic heterotrophs were significantly correlated with lipase ($r = 0.94$), valine arylamidase ($r = 0.88$), cystein arylamidase ($r = 0.87$), trypsine ($r = 0.87$), chymotrypsine ($r = 0.86$) and α galactosidase ($r = 0.94$).

In Pile 2, total aerobic heterotrophs showed significantly positive correlation with trypsin and chymotrypsine ($r = 0.82$) whereas actinobacteria had significant correlations with a number of glycosyl-hydrolase enzymes such as α-galactosidase ($r = 0.82$), β-galactosidase ($r = 0.79$), β-glucuronidase ($r = 0.82$), α-glucosidase and β-glucosidase ($r = 0.77$). In Pile 3, however, only fungi were highly significantly correlated with phosphohydrolase ($r = 0.88$) and β-glucosidase ($r = 0.91$). Unlike the reports of Tiquia et al. (2002), the population of fungi was positively correlated with phosphohydrolase in the present study. Tiquia et al. (2002) also reported that β-galactosidase, which is involved in the hydrolysis of cellobiose, showed significant positive correlation with fungi ($r = 0.98$).

Fig. 2 shows the loading plot of PCA for the three composts collected on days 0, 32 and 90. Overall, PCA grouped all samples collected on day 0 differently from samples collected on days 32 and 90. Also, the younger composts (Piles 1 and 2) grouped distant from those of Pile 3 (higher PC2 scores). Therefore, we can conclude that a certain degree of decomposition had occurred in the dung and fruit/vegetable wastes before they were used in co-composting, as a considerable number of the enzymes investigated were active on day 0. The day 90 composts from Piles 1 and 2 clustered close together indicating that the enzyme profiles in the mature composts were similar. Furthermore, the composts collected in the active phase were grouped loosely along the PC1. This is consistent with the correlation analysis (Table 4) that indicated that the important enzymes in composting included phosphatases, esterase–lipases, proteases (trypsin and chymotrypsin), β-glucosidases and β-galactosidases.

3.4. PCR-DGGE

The bacterial and fungal DGGE profiles were generated from a total of 27 samples including three replicate compost samples taken on days 0, 32 and 90 from Piles 1, 2 and 3 (Figs. 3 and 4). Overall, DGGE profiles of triplicate samples had a degree of similarity higher than 95%.

DGGE patterns showed that the bacterial community profiles clustered into two main groups with 46% similarity (Fig. 3). Samples from day 0 grouped together and in distance from the 32 and 90 days samples, which were very similar (clustered with 84% similarity). This indicates a change in the bacterial community composition concurrent with the dynamics of the composting process. The beginning of the process was probably characterised by a bacterial community involved in the degradation of labile organic substrates, while the bacterial DGGE profiles of DNA after 90 days of composting presumably reflects a community involved in the stabilisation of organic matter. However, towards the end of the process, the microbial community structure tended to stabilise, as suggested by the high similarity of DGGE profiles. Similar results were found by Takaku et al. (2006) and Novinscak et al. (2009).

In contrast, the three composts collected on day 0 were found to group separately (Fig. 3), as the two different co-substrates used introduced different and diverse microbial communities into the composting process. Interestingly, Pile 2 (coffee husks and vegetable and fruit wastes) and Pile 3 (coffee husks only) were found to group together within this cluster (68% similarity). Also, compost samples from Piles 2 and 3 clustered together and distinctly from

Pile 1 after 32 days of composting. Nevertheless, all 90 day compost samples were found to group together (Fig. 3). This suggests that the resulting composts were homogenous materials with a well-defined bacterial community.

UPGMA analysis also clustered fungal profiles into two groups, with 63% similarity (Fig. 4): one group comprised samples from day 0, while the other comprised composts from days 32 and 90. Within this cluster, these two composts were found to group separately with a degree of similarity of 72% (Fig. 4). Composting time had a significant effect on fungal communities, as was shown by fungal counts (Table 2). Cumulatively, these findings suggest there was a succession of fungal communities during the composting process related to the different phases involved in the process. Moreover, as for bacteria, fungal DGGE profiles of the three composts collected on days 0 and 32 clustered separately (Fig. 4). These groupings were not observed in 90 days samples (Fig. 4), indicating that a lower diversity was achieved at the end of the composting period.

3.5. Microarray

The SNRs obtained after hybridisation of samples on the COMPOCHIP microarray were determined and are shown in Table 5. The changes in bacterial communities in the three different piles, and over the course of composting are clearly shown in this Table. Fig. 5 shows a principal component analysis (PCA) loading plot, whereby the two first axes explain 65.2% of the variance, the first axis representing 44.3% of the variance, the second axis representing 20.9%. The lengths of the arrows indicate the significance for compost sample differentiation, and arrows point in the direction of samples with above average signal. Probes with similar arrow directions have high covariance, meaning they tend to occur jointly on the microarrays. Certain probes (indicated by the arrows) can be seen to be more influential in discriminating the samples, namely, the probes KO 443 and 444 (*Stenotrophomonas maltophilia*), KO609, KO 610 and KO 614 (*Brevundimonas/Caulobacter*), KO 500 (*Derxia gummosa*) KO 612, 615,616 and 617 (*Flavobacterium/Flexibacter*), KO 541 (*Pseudomonas putida*), KO 252 (*Acinetobacter*) and KO 342 (*Actinomyces* sp.). Canonical analysis clearly shows the different grouping of the compost samples according to the age of the compost. The composts collected on day 0 were found to cluster separately on the positive side of the first principal component axis, while the 90 days composts all clustered quite closely together on the negative side of the first principal component axis. These groupings occurred because of the greater diversity of microorganisms in the composts at the start of the experiment, while at the end of the composting period, the diversity and bacterial numbers had decreased, and the various composts were more similar. The result supports the findings of the DGGE study.

The separate grouping of the 3 different composts collected on day 0 is to be expected, with the different co-substrates used to make the piles. Both cow dung and vegetable and fruit wastes offer a different and diverse input of microbial communities into the composting process, which allowed the three compost piles to group distinctly after a canonical analysis of the microbial communities detected by the microarray. Of interest was the finding of *Brevundimonas* and *Caulobacter* in the starting composts, especially in Piles 2 and 3. Members of the genus *Brevundimonas* were also found to be dominant at the start of a compost process treating sewage sludge and yard waste (Danon et al., 2008). In their study, *Caulobacter* was found in composts after a curing time of 41 days. Pedro et al. (2001) also found *Brevundimonas* present in the mesophilic phase of industrial and agricultural waste composting. These bacteria may play an important role in the composting process.*Chryseobacterium* was also found in higher numbers in the starting composts, in particular, in Piles 2 and 3 (Pile 1 had fewer and lower signals). Lower levels of *Chryseobacterium* were found at the end of the composting process in these same composts. In a study by Franke-Whittle et al. (2009), *Chryseobacterium* levels were found to be higher in fresher composts than in more mature composts. *Chryseobacterium* species are known for their importance in the degradation of complex biopolymers in composting situations (Al Khadi et al., 2004).*Sphingobacterium* was detected in the compost Piles 2 and 3 at the start of the experiment, but not at the end of the experiment. *Sphingobacterium* is an aerobic bacterium, known to have broad degradation activity, and frequently occur in compost. It was also reported after 3 and 9 days (but not after 21 and 92 days) in composts in a study by Cayuela

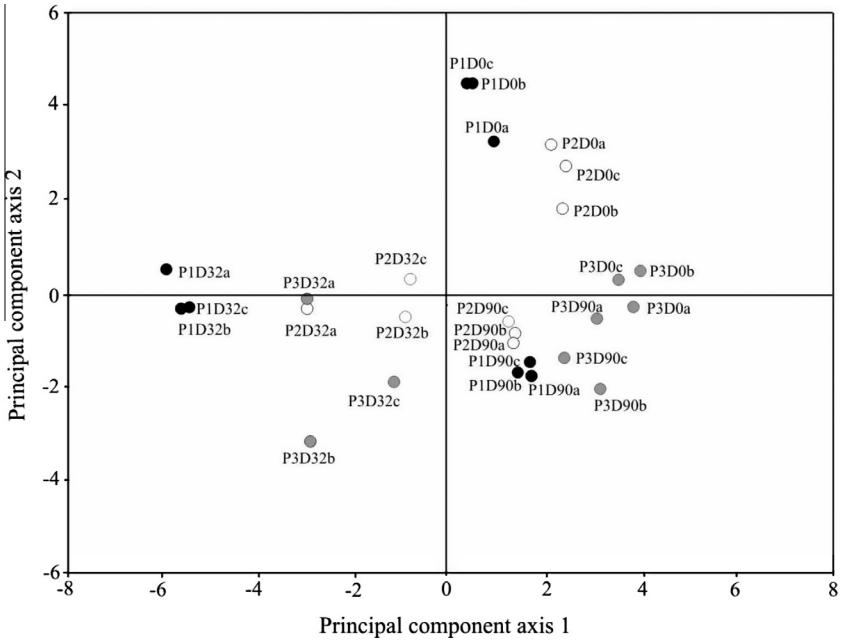

Fig. 2. Principal Component Analyses (PCA) of compost enzymes on days 0 (D0), 32 (D32) and 90 (D90) from the three compost piles. P1: Pile 1 – coffee husk + cow dung; P2: Pile 2 – coffee husk + fruit/vegetable waste; P3: Pile 3 – coffee husk.

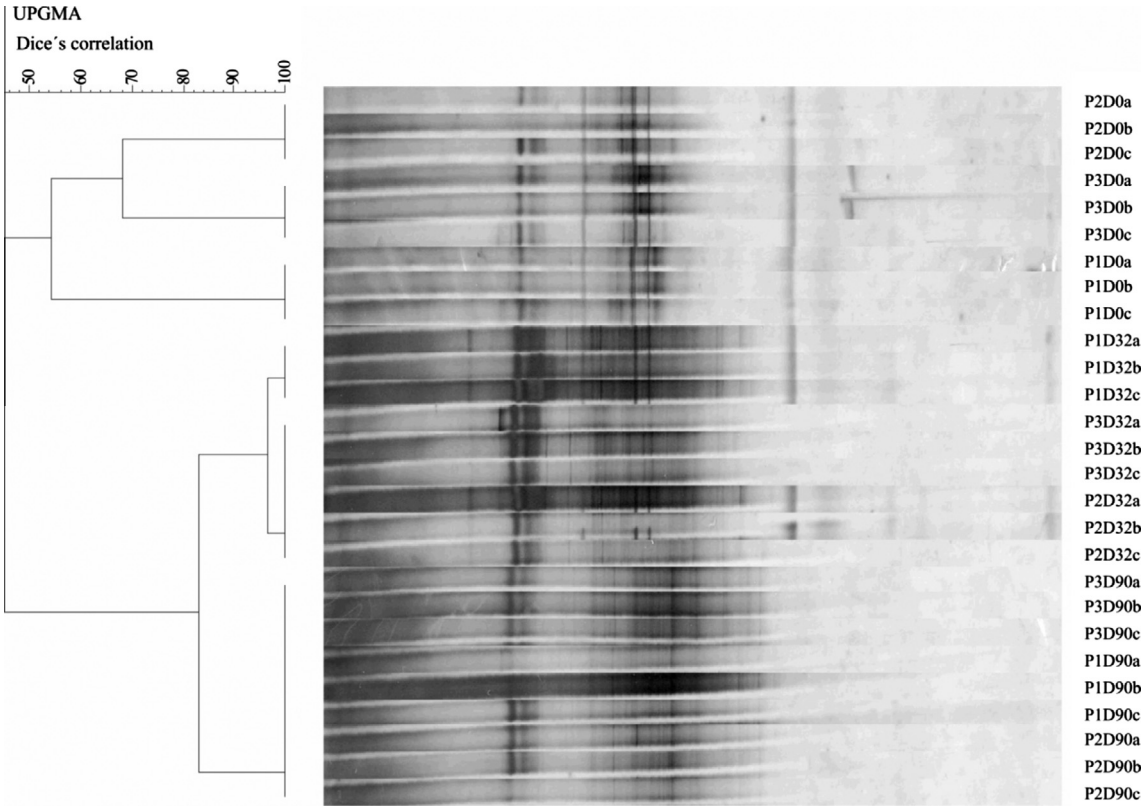

Fig. 3. Cluster analysis of bacterial DGGE fingerprints based on 16S rDNA of compost samples collected on days 0 (D0), 32 (D32) and 90 (D90). Values indicate the percentage of similarity, based on the Dice correlation coefficient. P1: Pile 1 – coffee husk + cow dung; P2: Pile 2 – coffee husk + fruit/vegetable waste; P3: Pile 3 – coffee husk.

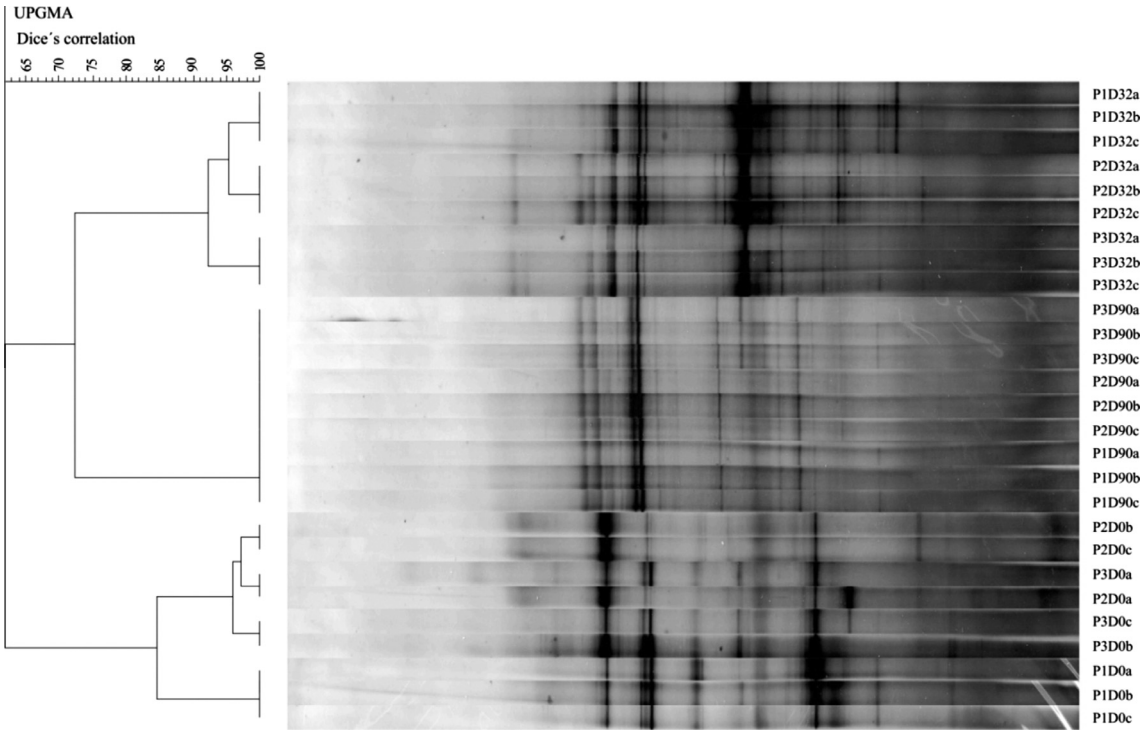

Fig. 4. Cluster analysis of fungal DGGE fingerprints based on 18S rDNA of compost samples collected on days 0 (D0), 32 (D32) and 90 (D90). Values indicate the percentage of similarity, based on the Dice correlation coefficient. P1: Pile 1 – coffee husk + cow dung; P2: Pile 2 – coffee husk + fruit/vegetable waste; P3: Pile 3 – coffee husk.

Table 5
Microarray results showing SNR values of the different composts. The green represents hybridisation signals with SNR values above the threshold of 2.

		P1D0			P2D0			P3D0			P1D90			P2D90			P3D90		
		a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Acinetobacter	KO 252	10,28	6,45	23,63	2,01	1,28	2,31	2,54	2,63	1,40	1,86	2,60	2,32	2,36	3,94	3,74	2,87	2,02	2,86
Acinetobacter calcoaceticus	KO 254	2,54	1,38	1,71	2,68	1,38	3,14	1,85	2,50	1,62	2,07	2,17	1,98	2,33	2,48	1,37	1,75	2,16	1,70
Acinetobacter lwofii	KO 233	5,01	1,06	2,92	1,44	0,95	1,52	1,07	1,08	1,03	1,09	1,04	0,99	1,04	1,09	1,07	1,08	1,07	1,02
Actinomyces sp.	KO 342	3,62	1,18	1,55	4,61	2,08	8,74	5,19	5,01	2,03	27,50	5,57	21,91	10,30	14,23	10,95	26,79	21,54	14,31
Agrobacterium tumefaciens	KO 23	2,28	1,56	2,27	2,01	1,13	1,49	1,71	1,71	1,67	1,43	2,09	1,56	1,89	2,09	2,63	2,98	1,75	3,16
Alcaligenes faecalis	KO 350	8,26	1,68	2,38	2,81	1,17	1,98	1,81	2,78	1,59	1,13	1,27	1,29	1,46	1,19	1,25	1,50	1,19	1,10
Alpha proteobacteria	KO 240	27,22	13,46	9,37	25,36	5,97	19,27	28,25	26,16	10,08	55,44	67,77	43,96	27,25	75,22	78,60	79,85	22,73	33,07
Azotobacter beijerinckii	KO 277	6,21	4,94	5,18	4,70	2,88	5,02	6,46	7,27	8,24	4,37	8,86	4,61	6,82	9,01	6,34	3,73	2,46	3,51
Brevundimonas	KO 614	1,57	1,02	1,19	6,80	19,12	31,84	23,07	2,02	7,84	1,18	1,13	1,58	1,22	1,21	1,88	2,57	1,49	2,54
Brevundimonas	KO 612	7,06	0,98	1,00	14,87	16,17	15,76	1,46	3,00	1,08	0,99	1,03	1,02	1,02	1,14	1,47	1,13	1,16	1,16
Brevundimonas/Caulobacter	KO 610	12,84	1,00	1,48	26,79	15,77	16,22	5,59	12,93	6,41	1,55	1,40	1,41	1,06	2,04	2,44	2,47	1,95	1,46
Brevundimonas/Caulobacter	KO 609	7,34	0,97	1,22	15,78	13,98	14,92	4,50	5,54	6,37	1,31	1,17	1,26	1,01	1,41	1,60	1,29	1,51	1,27
Brevundimonas/Caulobacter	KO 611	1,65	1,02	1,06	7,10	2,88	4,21	1,49	1,82	1,38	1,08	0,99	1,04	1,01	1,03	1,08	1,05	1,09	1,08
Chryseobacterium	KO 495	12,50	2,84	3,80	9,31	4,99	4,30	30,83	34,93	24,69	2,14	1,57	2,32	1,46	2,65	21,53	7,90	6,99	19,41
Chryseobacterium	KO 494	1,94	1,20	1,51	4,69	2,26	3,72	5,30	14,01	10,84	1,49	1,31	1,51	1,47	1,35	14,13	2,44	1,46	3,06
Chryseobacterium	KO 496	1,24	1,19	1,28	2,55	2,08	1,73	2,77	3,51	2,25	1,05	1,01	1,16	1,11	1,29	3,52	1,22	1,17	1,96
Clostridium A	KO 255	1,20	1,29	5,17	1,47	1,16	1,32	1,59	2,14	1,67	1,06	1,07	1,13	1,26	1,35	0,97	1,03	1,05	1,18
Comomonas/Acidovorax	KO 274	1,16	1,08	8,41	1,95	1,23	1,51	1,16	1,31	1,13	1,16	1,26	1,30	1,12	1,44	1,13	1,08	1,08	1,05
DELTA495a	KO 639	14,98	2,24	4,00	7,58	2,26	4,85	3,82	5,69	6,53	12,54	7,96	8,36	11,03	16,80	12,17	13,26	16,33	7,92
Derxia gummosa	KO 500	25,80	3,51	14,70	7,30	5,20	6,28	2,46	5,39	9,72	0,97	1,01	1,05	1,23	1,07	1,13	1,04	1,08	1,12
Derxia gummosa	KO 502	1,59	1,27	1,43	7,20	2,22	3,24	11,55	6,31	4,30	1,04	0,98	1,12	1,31	1,21	6,92	1,72	1,46	2,38
EnterobacteriaceaeD	KO 246	5,64	1,91	5,15	1,23	1,06	1,38	13,60	5,13	7,26	1,77	1,70	1,32	1,22	5,46	1,70	1,58	1,11	1,54
Flavobacteria	KO 615	2,44	1,06	4,00	27,08	5,55	16,59	5,35	9,68	14,32	4,53	5,09	4,30	1,86	4,28	29,58	14,04	11,33	9,97
Flavobacterium/Flexibacter	KO 617	1,12	1,01	1,40	25,40	11,61	28,75	1,34	2,13	1,71	3,99	2,56	2,14	1,58	3,52	5,48	2,65	4,16	3,65
Flavobacterium/Flexibacter	KO 616	0,99	1,00	1,01	8,74	6,98	8,60	1,20	1,23	1,13	1,25	1,35	1,10	1,35	1,23	1,48	3,78	2,71	3,10
Flavobacterium/Flexibacter	KO 618	1,01	1,03	1,00	3,60	1,35	1,22	1,01	0,98	1,01	1,02	1,02	1,04	1,04	1,10	1,15	1,59	1,52	1,37
Lactobacillus panis	KO 505	6,66	5,15	4,72	2,93	1,53	2,25	2,65	3,38	2,64	1,28	1,10	1,23	1,52	1,17	1,29	1,33	1,26	1,14
Low G+C	KO 319	5,63	1,91	11,61	4,41	2,36	10,06	1,86	2,35	1,83	22,66	17,03	14,94	9,84	14,41	29,88	51,30	34,77	49,28
Methylobacterium	KO 513	6,93	5,39	4,67	2,45	1,66	3,33	2,27	2,51	1,84	1,54	1,34	1,28	1,54	1,47	1,36	1,51	1,41	1,18
Methylobacterium	KO 514	5,08	1,08	1,05	1,06	1,06	1,15	1,74	1,13	1,10	1,00	0,96	1,14	1,02	0,96	1,00	1,02	1,03	1,00
Methylobacterium	KO 512	3,47	1,35	1,04	1,03	1,03	1,22	1,60	1,22	1,23	1,04	1,02	0,97	1,03	1,04	1,01	1,04	1,07	1,02
Microbacterium	KO 515	2,31	1,29	1,15	1,59	1,25	1,55	1,41	1,43	1,23	2,05	2,57	1,52	2,27	2,31	3,12	3,26	3,66	3,72
Microbacterium	KO 516	1,72	1,27	1,04	1,32	1,15	1,38	1,32	1,28	1,17	1,86	2,23	1,54	2,13	1,71	2,83	2,44	3,11	2,90
Nitrobacter	KO 293	2,63	2,95	7,25	3,29	2,07	3,22	4,73	5,22	2,19	4,07	9,06	4,28	3,92	5,82	3,91	5,89	3,87	4,86
Nitrosomonas communis	KO 301	1,54	2,13	2,99	2,07	1,75	2,27	2,20	3,22	1,64	1,58	2,14	1,91	2,03	3,35	1,20	1,67	1,86	1,43
Nitrosomonas oligotropha	KO 419	7,65	2,12	3,63	1,57	1,17	1,49	1,11	1,71	2,06	0,94	1,06	1,00	1,10	1,07	1,13	1,07	0,99	1,01
Nitrospira/Nitrosovibrio/Nitrosomonas	KO 295	9,25	3,46	6,00	1,99	2,50	1,35	1,51	3,29	1,52	1,34	1,30	1,41	1,19	1,46	1,25	1,28	1,10	1,32
Pantoea	KO 528	9,35	1,13	4,12	1,06	1,27	1,48	9,96	3,09	13,87	1,14	0,99	1,13	1,30	3,83	1,22	1,22	1,17	1,00
Pantoea	KO 529	1,00	1,07	1,01	1,03	1,03	1,01	2,53	2,85	5,02	0,97	0,94	0,97	0,99	1,46	1,02	1,03	0,99	0,99
Propionibacterium	KO 433	4,35	1,19	2,35	1,44	1,23	1,32	1,12	1,15	1,66	0,97	0,95	1,00	1,06	0,98	1,06	1,06	1,00	1,01
Pseudomonas	KO 536	24,31	13,05	20,07	11,79	8,38	9,23	13,80	16,90	17,85	5,21	2,71	3,61	2,12	3,06	7,43	5,39	6,79	2,69
Pseudomonas	KO 537	8,40	11,32	3,29	0,92	1,33	3,66	1,04	1,09	1,07	1,72	1,46	1,56	1,17	1,53	2,08	1,37	1,51	1,21
Pseudomonas aeruginosa	KO 244	4,78	1,59	2,57	1,60	1,22	1,83	1,19	1,62	1,64	1,55	1,73	1,62	1,57	1,84	1,54	1,43	1,23	1,23
Pseudomonas aeruginosa	KO 12	1,51	1,38	1,58	1,51	1,32	1,77	1,51	2,64	2,42	1,58	2,04	1,61	1,43	2,01	3,18	1,69	1,46	1,96
Pseudomonas aeruginosa	KO 435	2,92	1,93	4,16	1,07	1,13	1,19	2,75	1,63	2,42	1,04	1,04	1,06	1,12	1,03	0,99	1,06	0,98	1,09
Pseudomonas putida	KO 541	31,19	4,63	16,56	4,61	1,89	3,27	9,71	9,77	7,08	1,79	1,56	1,50	1,09	2,39	1,58	1,31	1,75	1,11
Rhodococcus	KO 544	6,25	1,02	1,05	1,03	1,04	1,02	1,00	1,04	1,03	1,01	1,03	0,99	1,06	1,07	1,02	1,05	0,99	1,03
Salmonella	KO 245	16,68	1,34	3,03	2,03	1,14	1,98	4,49	5,05	7,03	2,20	2,74	1,73	1,68	4,58	2,49	2,11	1,65	1,83
Sphingobacterium	KO 548	2,93	0,97	1,08	1,80	1,40	2,51	4,73	22,17	3,39	1,03	1,02	1,00	1,13	1,07	1,06	1,18	1,44	1,24
Sphingobacterium	KO 549	7,23	1,06	1,41	6,03	4,14	4,65	8,00	21,33	4,48	2,22	1,76	1,67	1,16	1,63	2,20	2,90	2,63	1,80
Sphingobacterium	KO 550	1,54	1,10	1,54	2,88	2,37	2,73	3,66	5,37	2,22	1,24	1,41	1,47	1,63	1,31	1,38	1,44	1,31	1,53
Stenotrophomonas maltophilia	KO 443	95,55	3,59	42,55	21,29	8,52	16,10	7,36	7,16	33,36	1,17	1,08	1,09	1,21	1,30	1,97	1,57	1,52	1,38
Stenotrophomonas maltophilia	KO 444	52,33	1,99	15,82	1,74	1,53	2,82	1,32	1,81	3,44	0,97	1,01	1,09	1,14	1,06	1,01	1,03	1,01	1,05
Stenotrophomonas maltophilia	KO 243	32,79	9,09	26,62	9,28	2,54	5,84	8,19	6,37	6,57	4,06	6,23	4,05	3,30	6,35	4,96	8,50	5,51	6,39
Streptomyces	KO 630	1,43	1,02	1,38	1,34	1,32	1,08	1,43	1,43	1,40	3,60	3,32	1,95	1,82	3,59	4,26	6,26	6,41	2,24
Streptomyces	KO 629	1,66	1,02	1,02	1,14	1,00	1,04	1,01	1,17	1,02	2,71	2,26	1,50	1,17	2,01	2,44	2,72	3,99	1,81
Thermoactinomyces	KO 555	3,78	2,22	3,66	2,41	1,48	1,47	2,31	3,10	2,19	1,32	1,40	1,29	1,26	1,54	1,28	1,31	1,13	1,09
Thermus thermophilus	KO 1	1,80	1,60	2,15	2,44	2,01	1,90	2,05	2,30	2,16	1,82	2,26	3,00	2,60	2,14	2,14	2,36	1,85	2,41
Vibrio cholerae	KO 453	1,05	0,99	4,77	1,05	0,97	0,98	1,05	1,00	1,15	0,98	1,03	1,07	1,12	1,06	1,07	1,10	1,03	1,05
Xanthomonas	KO 561	8,11	1,32	2,80	4,44	1,56	4,58	2,55	3,53	2,50	1,06	1,07	1,12	1,06	1,10	1,07	1,08	1,03	1,05
Xanthomonas	KO 455	7,60	2,42	5,39	2,62	1,84	2,73	1,78	2,06	3,77	1,04	1,06	1,04	1,13	0,97	1,06	1,04	0,99	0,98
Xylella/Xanthomonas/Stenotrophomonas	KO 241	22,23	9,86	30,02	9,69	3,39	9,49	28,57	13,59	10,34	17,24	16,95	11,96	8,03	17,09	17,16	17,11	4,27	8,60
Xylella/Xanthomonas/Stenotrophomonas	KO 242	3,36	3,57	13,86	3,95	2,24	2,92	3,90	5,07	3,27	4,61	10,19	4,59	3,62	4,98	2,67	9,04	2,85	6,07
Yersinia	KO 458	4,75	1,52	2,45	1,08	1,32	2,13	1,22	2,71	3,05	0,99	0,95	1,18	1,00	1,02	1,04	0,99	1,00	0,99

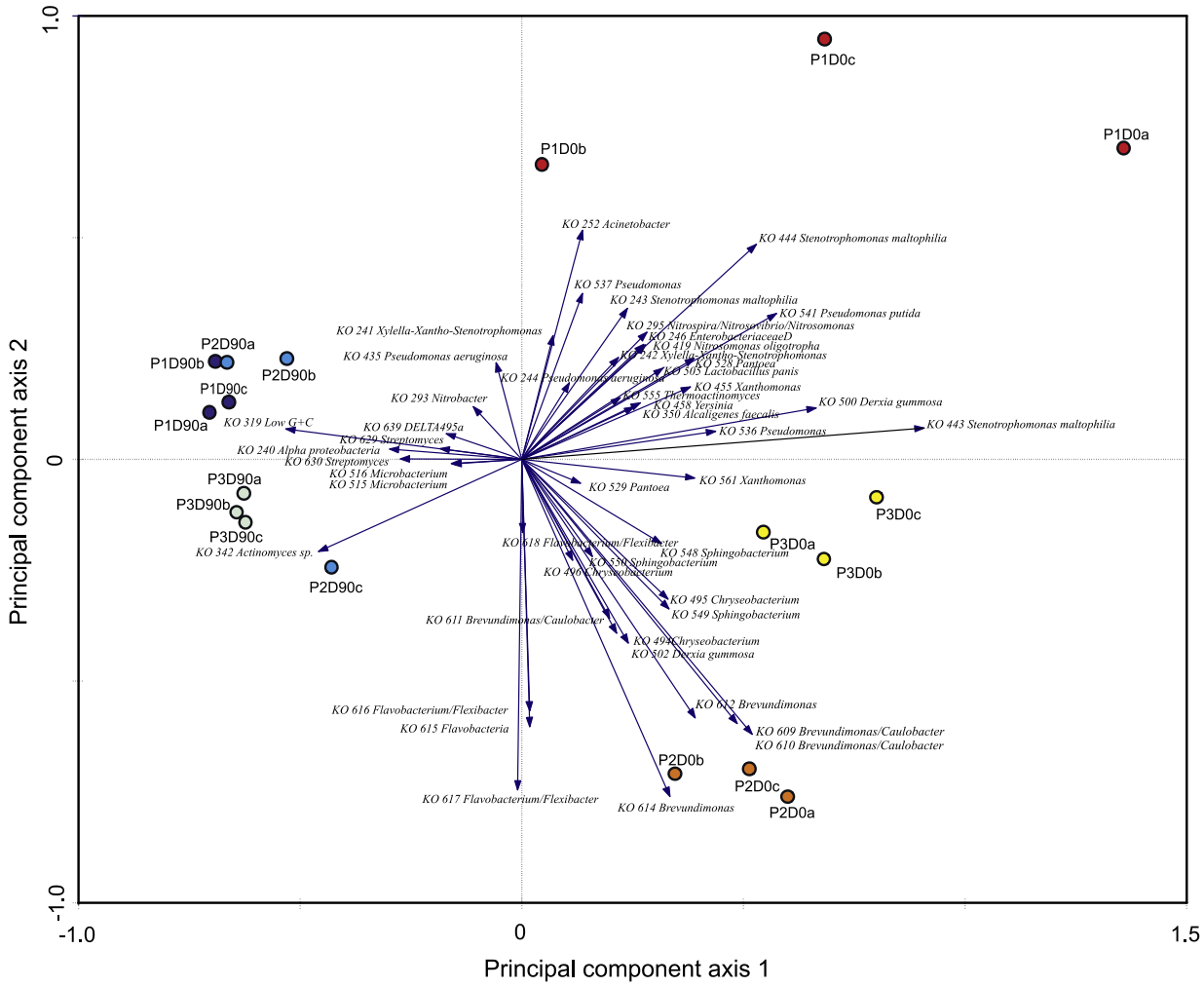

Fig. 5. Principal component analysis (PCA) of compost communities from day 0 (D0) and day 90 (D90) composts analysed by the COMPOCHIP microarray. The two axes represent 65.2% of the explained variance. The vectors show the covariance structure of the probe signals. P1: Pile 1 – coffee husk + cow dung; P2: Pile 2 – coffee husk + fruit/vegetable waste; P3: Pile 3 – coffee husk.

et al. (2009), where the impact of different nitrogen rich animal wastes on the composting of straw and cotton wastes was investigated. A study by Franke-Whittle et al. (2009) similarly revealed the presence of *Sphingobacterium* predominantly in 2 week old composts of various types (green compost, manure mix compost, and anaerobic digestate compost), although *Sphingobacterium* was also detected after 8 weeks in the green compost.

SNR values above the detection limit were also found in most compost piles at the start and end of the experiment for the *Flavobacteria/Flexibacter* probes KO 615,616, 617 and 618. *Flavobacteria* are common in environmental samples and include opportunistic pathogens. Its presence in composts has been reported by others (Danon et al., 2008).

The genus *Stenotrophomonas* includes species which are plant and human pathogens, and the presence of these bacteria could be of concern in a mature compost product. The *S. maltophilia* probes KO 243, 443 and 444 yielded positive signals in the day 0 compost piles, while only the probe KO 243 yielded a positive signal in the 90 days composts. This indicates that the KO 243 probe works more efficiently than the other *S. maltophilia* probes, and that this organism has survived the composting process.

In the mature composts, microbial diversity and numbers were lower. The group probes DELTA 495a, Alpha proteobacteria and Low G + C yielded hybridisation signals in all three compost piles. Hybridisation was also seen in all day 90 composts with the KO 241 *Xylella/Xanthomonas/Stenotrophomonas*, *Azotobacter beijerinckii* KO 277 and the KO 342 *Actinomyces* probes. The proportion of *Actinomycetes* relative to other bacteria has been suggested as an indicator of compost maturity (Palmisano and Barlaz, 1996). Thus, it is not surprising that microarray analysis revealed high levels of *Actinomycetes* in the composts from longer curing times.

Low SNR values were also obtained for the *Streptomyces* probes KO 629 and KO 630 as well as the *Microbacterium* probes KO 515 and KO 516 for all 90 days composts. Interestingly, these two organisms were not detected at all in the immature composts. This finding supports the results of Danon et al. (2008), whereby *Microbacterium* was also found in composts collected 41 and 131 days after the start of the experiment. Silva et al. (2000) also reported the finding of *Microbacterium* on coffee cherries.

4. Conclusions

The use of manure and fruit/vegetable wastes as co-composting materials of coffee husks was shown to result in higher losses in carbon. The mesophilic phase of the composting process was longer when coffee husks were composted alone. While the microbial communities in the three compost piles at the start of the process and after 32 days of composting were found to be different, the communities were more similar at the end of the composting process, as shown by DGGE fingerprints and microarray analysis. The microbial communities in the three compost piles at the start of

the experiment also differed from each other, according to DGGE fingerprints and microarray analysis. Overall, the use of a multi-parameter approach has broadened our knowledge on the microbiology of the composting of coffee husks.

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References

Al Khadi, S.F., Myers, K.M., Rasooly, A., Chizhikov, V., 2004. Genotyping of *Clostridium perfringens* toxins using multiple oligonucleotide microarray hybridization. Mol. Cell. Probes 18, 359–367.

Beck-Friis, B., Smårs, S., Jönsson, H., Kirchmann, H., 2001. Emissions of carbon dioxide, ammonia and nitrous oxide from organic household waste in a compost reactor under different temperature regimes. J. Agric. Eng. Res. 78, 423–430.

Bernal, M.P., Alburquerque, J.A., Moral, R., 2009. Composting of animal manures and chemical criteria for compost maturity assessment. A review. Bioresour. Technol. 100, 5444–5453.

Boulter-Bitzer, I.J., Trevors, J.T., Boland, G.J., 2006. A polyphasic approach for assessing maturity and stability in compost intended for suppression of plant pathogen. Appl. Soil Ecol. 34, 65–81.

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

CTA (Coffee and Tea Development Authority), 1999. Ethiopia Cradle of the Wonderbean Coffee Arabica (Abissinica). Addis Ababa, Ethiopia.

Danon, M., Franke-Whittle, I.H., Insam, H., Chen, Y., Hadar, Y., 2008. Molecular analysis of bacterial community succession during prolonged compost curing. FEMS Microbiol. Ecol. 65, 133–144.

Díaz, M.J., Madejón, E., Ariza, J., López, R., Cabrera, F., 2002. Co-composting of beet vinasse and grape marc in win-drows and static pile systems. Compost Sci. Util. 10, 258–269.

Fan, L., Soccol, A.T., Pandey, A., Soccol, C.R., 2003. Cultivation of Pleurotus mushrooms on Brazilian coffee husk and effects of caffeine and tannic acid. Micología Aplicada Int. 15, 15–21.

Fernández-Gómez, M., Nogales, R., Insam, H., Romero, E., Goberna, M., 2010. Continuous-feeding vermicomposting as a recycling management method to revalue tomato-fruit wastes from greenhouse crops. Waste Manage. 30, 2461–2468.

Fernández-Gómez, M., Nogales, R., Insam, H., Romero, E., Goberna, M., 2012. Use of DGGE and COMPOCHIP for investigating bacterial communities of various vermicomposts produced from different wastes under dissimilar conditions. Sci. Total Environ. 414, 664–671.

Franke-Whittle, I.H., Klammer, S.H., Insam, H., 2005. Design and application of an oligonucleotide microarray for the investigation of compost microbial communities. J. Microbiol. Meth. 62, 37–56.

Franke-Whittle, I.H., Knapp, B.A., Fuchs, J., Kaufmann, R., Insam, H., 2009. Application of COMPOCHIP microarray to investigate the bacterial communities of different composts. Microbiol. Ecol. 57, 510–521.

Gómez-Brandón, M., Lazcano, C., Domínguez, J., 2008. The evaluation of stability and maturity during the composting of cattle manure. Chemosphere 70, 436–444.

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.

Hultman, J., Kurola, J., Raininsalo, A., Kontro, M., Romantschuk, M., 2010. Utility of molecular tools for optimization of large scale composting. In: Insam, H., Franke-Whittle, I.H., Goberna, M. (Eds.), Microbes at Work. From Wastes to Resources. Springer, Berlin, Heidelberg, pp. 135–152.

Insam, H., de Bertoldi, M., 2007. Microbiology of the composting process. In: Golueke, C., Bidlingmaier, W., de Bertoldi, M., Diaz, L. (Eds.), Compost Science and Technology. Elsevier, pp. 25–48.

Lulu, B., Insam, H., 2000. Medium-term effects of a single application of mustard residues on soil microbiota and C contents of vertisols. Biol. Fertil. Soils 31, 108–113.

Mondini, C., Fornasier, F., Sinicco, T., 2004. Enzymatic activity as a parameter for the characterization of the composting process. Soil Biol. Biochem. 36, 1587–1594.

Murthy, P.S., Naidu, M., 2012. Sustainable management of coffee industry by-products and value addition – a review. Resour. Conserv. Recycl. 66, 45–58.

Muyzer, G., Smalla, K., 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie van Leeuwenhoek 73, 127–141.

Novinscak, A., DeCoste, N.J., Surette, C., Filion, M., 2009. Characterization of bacterial and fungal communities in composted biosolids over a 2 year period using denaturing gradient gel electrophoresis. Can. J. Microbiol. 55, 375–387.

Palmisano, A.C., Barlaz, M.A., 1996. Microbiology of Solid Waste. CRC Press Inc., p. 224.

Pandey, A., Soccol, C.R., Nigan, P., Brand, D., Mohan, F., Rovossos, S., 2000. Biotechnological potential of coffee pulp and husk for bio-process. J. Biochem. Eng. 6, 153–162.

Pedro, M.S., Haruta, S., Hazaka, M., Shimada, R., Yoshida, C., Hiura, K., Ishii, M., Igarashi, Y., 2001. Denaturing gradient gel electrophoresis analyses of microbial community from field-scale composter. J. Biosci. Bioeng. 91 (2), 159–165.

Potvin, C., Lechowicz, M.J., Tardif, S., 1990. The statistical analyses of ecological response curves obtained from experiments involving repeated measures. Ecology 71, 1389–1400.

Rebollido, R., Martinez, J., Aguilera, Y., Melchor, K., Koerner, I., Stegmann, R., 2008. Microbial populations during composting process of organic fraction of municipal solid waste. Appl. Ecol. Environ. Res. 6 (3), 61–67.

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

Sánchez-Monedero, M.A., Roig, A., Paredes, C., Bernal, M.P., 2001. Nitrogen transformation during organic waste composting by the Rutgers system and its effects on pH, EC and maturity of the composting mixtures. Bioresour. Technol. 78, 301–308.

Silva, C.F., Schwan, R.F., Dias, E.S., Wheals, A.E., 2000. Microbial diversity during maturation and natural processing of coffee cherries of *Coffea arabica* in Brazil. Int. J. Food Microbiol. 60, 251–260.

Steger, K., Sjögren, Å.M., Jarvis, Å., Jansson, J.K., Sundh, I., 2007. Development of compost maturity and *Actinobacteria* populations during full-scale composting of organic household waste. J. Appl. Microbiol. 103, 487–498.

Takaku, H., Kodaira, S., Kimoto, A., Nashimoto, M., Takagi, M., 2006. Microbial communities in the garbage composting with rice hull as an amendment revealed by culture-dependent and -independent approaches. J. Biosci. Bioeng. 101, 42–50.

Ter Braak, C.J.F., Smilauer, P., 2002. CANOCO Reference Manual and Canodraw for Windows User's Guide: Software for Canonical Community Ordination (Version 4.5). Microcomputer power, Ithaca, NY, USA.

Tiquia, S.M., Wan, J.H.C., Tam, N.F.Y., 2002. Microbial population dynamics and evolution of enzyme activities during composting. Compos. Sci. Util. 10, 150–161.

Vargas-Garcia, M.C., Suarez-Estrella, F., Lopez, M.J., Moreno, J., 2010. Microbial population dynamics and enzyme activities in composting processes with different starting materials. Waste Manage. 30, 771–778.

Wiersum, K.F., Gole, T.W., Gatzweiler, F., Volkmann, J., Bognetteau, E., Wirtu, O., 2008. Certification of wild coffee in Ethiopia: experiences and challenges. Forest, Trees Livelihoods 18, 9–21.