Agr. Nat. Resour. 56 (2022) 511–524

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Changes in microbial community structure and diversity during decomposition of oil palm empty fruit bunches at different decomposition sites in humid tropical oil palm plantation

Pintaka Kusumaningtyas[a,*], Krishna Purnawan Candra[b], Abdul Sahid[c]

[a] *Chemistry Education Study Program, Department of Mathematic and Natural Science Education, Faculty of Teacher Training and Education, Mulawarman University, Samarinda 75119, Indonesia*

[b] *Department of Agricultural Product Technology, Faculty of Agriculture, Mulawarman University, Samarinda 75119, Indonesia*

[c] *Department of Agroecotechnology, Faculty of Agriculture, Mulawarman University, Samarinda 75119, Indonesia*

Article Info

Article history:
Received 8 September 2021
Revised 25 February 2022
Accepted 7 March 2022
Available online 17 June 2022

Keywords:
Decomposition,
Microbial community structure,
Microbial diversity,
Oil palm empty fruit bunch (OPEFB)

Abstract

Importance of the work: Environmental conditions during the decomposition of organic matter play an important role in the structure and diversity of microbial communities.

Objectives: To compare changes in the microbial community structure and diversity during decomposition of oil palm empty fruit bunch (OPEFB) under the oil palm canopy and on open land.

Materials & Methods: A polymerase chain reaction-denaturing gradient gel electrophoresis technique was used to compare microbial community structures at two different decomposition sites during 6 mth, 12 mth and 24 mth. The physico-chemical parameters—temperature, relative humidity, pH, total organic carbon (TOC) and total nitrogen—in OPEFB during decomposition were determined to analyze differences in the decomposition rates of OPEFB.

Results: Different stacking location sites (under the oil palm canopy and on open land) of OPEFB affected the microbial community structure and diversity. The bacterial and fungal diversity indices were significantly higher in OPEFB decomposed on open land ($H = 2.979$ and $D = 0.941$ for bacteria; $H = 1.969$ and $D = 0.828$ for fungi) than under the oil palm canopy ($H = 2.483$ and $D = 0.902$ for bacteria; $H = 1.866$ and $D = 0.776$ for fungi), but the richness indices for bacteria was significantly higher on open land (S = 6.026) than under the oil palm canopy (S = 4.201) and that for fungi was significantly lower on open land (S = 2.512) than under the oil palm canopy (S = 2.914).The significant decrease in TOC and texture of OPEFB decomposed under the canopy revealed that the decomposition rate of OPEFB for this site was faster than that on open land.

Main finding: Changes in the microbial community structure and diversity were closely correlated to changes in temperature, relative humidity, pH and TOC. These findings could contribute to the improvement of OPEFB waste management in oil palm plantations.

* Corresponding author.
E-mail address: pintaka@fkip.unmul.ac.id (P. Kusumaningtyas)

https://doi.org/10.34044/j.anres.2022.56.3.08

Introduction

Indonesia is the biggest palm oil producer in the world, where production reached 44.5 million t in 2021 (Indexmundi, 2020). Such rapid production growth is generally followed by an increase in the biomass residue produced in the palm oil industry. In palm oil extraction, oil palm empty fruit bunches (OPEFB) are produced as a large amount of the solid waste in huge (about 22–23% of the whole fruit bunch). Traditionally, OPEFB is burnt in palm oil mill incinerators as boiler fuel (Chang, 2014) and extensively dumped for composting and soil mulch (Siddiquee et al., 2017). However, due to environmental pollution, the direct burning of OPEFB has been avoided. Hence, the application of OPEFB by stacking it on land in the oil palm plantation has become the current trend for solid waste management by many oil palm mills in Indonesia as this is considered the most cost-effective and environmental-friendly system for disposal.

Stacking OPEFB in oil palm plantation areas needs to be managed properly to control environmental pollution. Improper stacking of OPEFB can result in a slow decomposition rate because the microbial communities decomposing this solid waste require optimal environmental conditions for their growth. OPEFB is a complex, highly fibrous, lignocellulosic material, consisting of cellulose (23.7–65.0%), hemicellulose (20.58–33.52%) and lignin (14.1–30.45%) (Chang, 2014). Lignocellulosic OPEFB is recalcitrant, so its decomposition takes a long time and becomes the major obstacle in its degradation. Despite many treatments reported to accelerate the composting process (Siddiquee et al., 2017; Hamzah et al., 2018), the treatments required additional labor and operational cost for field application. Therefore, most palm oil mills in Indonesia only stack the OPEFB on land in oil palm plantation areas without applying any treatment. Thus, the OPEFB is left to naturally decompose for months to years, depending on the environmental conditions at the disposal site.

Many microorganisms, including fungi and bacteria, are known to be involved in the decomposition of OPEFB, such as *Trichoderma* sp. (Hamzah et al., 2018; Rasyid et al., 2020; Siddiquee et al., 2017) and *Bacillus stratosphericus* (Marheni, 2019). Generally, the lignocellulose-degrading fungi and bacteria have genes encoding enzymes that degrade lignocellulose (Janusz et al., 2017). The decomposition of lignocellulose is greatly affected by the structure and function of bacterial and fungal communities (Zainudin et al., 2014; Grosso et al., 2018). Fungi are considered as the main microbial decomposers for organic matter with a more complex structure, such as lignin (Janusz et al., 2017; Andlar et al., 2018), while bacteria are considered as the major degraders for cellulose materials (López-Mondéjar et al., 2016; Buresova et al., 2019). Therefore, analyses of the structure of bacterial and fungal communities appear to be essential to monitor the environmental conditions influencing the OPEFB decomposition rate.

In East Kalimantan province, Indonesia most palm oil mills return the OPEFB to oil palm plantations and stack it directly on open land and under the oil palm canopy without applying any treatment (personal observation). Based on our personal communication with the operator in the field, no special reason was considered in selecting the location for the accumulation of OPEFB in the field. The determination of the OPEFB stacking location appeared to only based on the ease with which the transporting vehicle could reach the location, and the effect of stacking location on the decomposition rate of OPEFB has never been evaluated. The composition and dynamics of the bacterial and fungal populations involved in the decomposition process generally are sensitive to environmental changes, such as temperature, moisture, and others (Giacometti et al., 2013). These environmental changes will promote the succession of the microbial community involved in the decomposition process (H'ng et al., 2018) and directly influence the decomposition duration of organic material. Microclimatic conditions, such as the air temperature and relative humidity, between under the canopy and in the open land are very different. Van Westreenen et al. (2020) found that the air temperature under the canopy was up to 5 °C lower than the ambient temperature at noon and the relative humidity was about 25% higher in the canopy than in the ambient air.

The structure of microbial communities and their diversity in decomposing OPEFB have been reported by many researchers (Zainudin et al., 2014, 2017; Tahir et al., 2019). However, the structure and diversity of microbial communities in OPEFB decomposed under different microclimatic conditions have not been widely reported. Therefore, it is of great interest to investigate the change in the structure of microbial communities and their diversity during decomposition of OPEFB under the oil palm canopy and on open land. The findings from the present studies will help to provide a deeper understanding of OPEFB decomposition associated with the dump site environmental conditions that could lead to new insight into how the natural decomposition of OPEFB in oil palm plantation can be accelerated.

Materials and Methods

Sample collection

The OPEFB samples were collected from an oil palm plantation in the East Kalimantan region, Indonesia (01°26'S, 116°82'E). that has a humid tropical climate area with an average temperature of 26 °C and rainfall in the range 100–150 mm/mth. Samples were collected from three sampling points for 2 yr (April 2015–May 2017). Samples were obtained using a soil borer from OPEFB piled up on open land (OL) and under the canopy (UC) between oil palm plants that had been left for 6 mth, 12 mth and 24 mth (Supplementary data, Fig. S1). The collected OPEFB samples were transferred into sterile bags and transported to the laboratory in an ice box. For microbial analysis, the samples were placed in 50 mL sterile conical flasks and stored at -20 °C until further analysis.

Microbial DNA extraction

Total microbial DNA genome was extracted from decaying OPEFB samples using a PowerSoil® DNA Isolation Kit (MOBIO Laboratories, Inc., USA) based on available extraction procedure protocol No. 12888. The extracted DNA was detected using 0.8% agarose gel electrophoresis. The concentration and purity of the DNA were determined by measuring the absorbance at 260 nm, 280 nm and 320 nm using an ultraviolet-vis spectrophotometer (Genesys 10S, Thermo Scientific, USA). The extracted DNA samples were kept in clean 0.5 mL micro-centrifuge tubes and stored at -20 °C until used for polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE).

Polymerase chain reaction amplification of 16S-rDNA and 18S-rDNA

The total DNA of bacteria and fungi extracted from OPEFB were amplified by PCR according to the procedures described by Muyzer et al. (1993) for bacteria and May et al. (2001) for fungi using universal primers shown in Table 1. Bacterial diversity was assessed using 16S rDNA primers 341F and 534R (Invitrogen, Canada), while fungal diversity was measured using 18S rDNA primers NS1 and Fung (First Base, Malaysia). PCR amplifications were performed in a total volume of 25 µL reaction mixture containing 12.5 µL Kapa 3G Fast Ready Mix (KapaBiosystem, USA), 0.5 µL of each primer with a concentration of 20 pmol/ µL, 50 ng of template DNA extracted from OPEFB, and 16 µL of PCR-grade water. The PCR was performed using a PCR thermal cycler c1000 (BioRad, Germany).

The thermal cycling condition for amplification of bacterial 16S-rDNA was as follows: 1 cycle of denaturation at 95 °C for 5 min followed by 34 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min, and then continue at 72 °C for 10 min at the end of the cycles. The fragment size of this 16S-rDNA amplification product was about 200 bp. The thermal cycling condition for amplification of fungal 18S-rDNA was as follows: 1 cycle of denaturation at 95 °C for 30 sec followed by 36 cycles of denaturation at 95 °C for 30 sec, annealing at 55 °C for 30 sec, and extension at 72 °C for 1 min, and then continue at 72 °C for 10 min at the end of the cycles. The fragment size of this 18S-rDNA amplification product was about 370 bp. All PCR products were checked on 1.2 % agarose gel electrophoresis.

Polymerase chain reaction-denaturing gradient gel electrophoresis analysis

PCR products were run on 7% polyacrylamide gradient electrophoresis of 30–70% denatured gradient for 16S rDNA, and 20–55% for 18S rDNA from 100% denaturant containing 7 mol/L urea and 40% formamide. A sample of 25 µL was mixed with 5 µL loading dye (containing 0.5% bromophenol blue, 0.5% xylene cyanol, and 1x Tris-acetate-EDTA (TAE) buffer). DGGE was run using 1× TAE buffer at 120 V and 60 °C for 10 h using DCode™ Universal Mutation Detection System (Bio-rad, USA). Silver staining method was applied to stain the DGGE gel (Qiu et al., 2012). The consistency of DGGE banding pattern was confirmed by replication of the second DGGE of PCR products. Small pieces of representative DGGE bands were excised from the gel with a sterile blade and put in a 1.5 mL microtube containing 50 µL TE buffer.

Table 1 Polymerase chain reaction-denaturing gradient gel electrophoresis primers used for specific amplification of 16S-rDNA for bacteria and 18S-rDNA for fungi

Target	Primers	Sequence (5' → 3')	Reference
Bacteria	341F[a]	CCTACGGGAGGCAGCAG	Muyzer et al. (1993)
	534R	ATTACCGCGGCTGCTGG	
Fungi	NS1	GTAGTCATATGCTTGTCTC	May et al. (2001)
	Fung[b]	ATTCCCCGTTACCCGTTG	

F = forward primer; R = reverse primer;

[a] Primer with a 40-bp GC clamp (CGCCCGCCGCGCGCGGCGGG CGGGGCGGGGGCACGGGGGG)

[b] Primer with a 40-bp GC clamp. (CGCCCGCCGCGCCCCGCGCCCGG CCCGCCGCCCCCGCCCC)

The tube was incubated in boiling water for 10 min and then was incubated overnight at 4 °C to extract the DNA fragment from the gel, and then re-amplified with primers without GC clamp (Table 1). The purified PCR products were sent for sequencing to PT. Genetika Science, Indonesia. The sequence of PCR products was analysed for similarity with a sequence in The National Centre for Biotechnology Information (NCBI) database using the nucleotide-nucleotide Basic Local Alignment Search Online Tool (BLASTn, http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequence then aligned using the ClustalW program (MEGA X software version 10.2.6), and the phylogenetic tree was built based on the neighbor-joining algorithm with the same software.

Physicochemical analysis of decomposed OPEFB

Temperature and humidity of OPEFB pile were determined at the locus using temperature humidity meter (Mastec MS6505, China). The chemical characteristics for C-organic, N, and pH were determined by spectrophotometry, Kjeldahl and pH-H_2O method, respectively (Eviati and Sulaeman, 2009). The physical structure of composted OPEFB was determined by Scanning Electron Microscope (JSM-6510 JEOL Ltd., Japan). Before the OPEFB samples were subjected to the SEM analysis, samples were dried for 72 hours in an oven at 110–120 °C to remove moisture, and then coated with a layer of platinum.

Data analysis

The patterns of DGGE bands were analysed to determine the relatedness of microbial communities using diversity indices (Shannon-Wiener index, Simpson's index, and richness index) and Sorensen similarity coefficients based on the emergence of bands in each sample. The Shannon-Wiener index (H) was calculated using the equation $H = -\sum p_i(\ln p_i)$, in which p_i is the frequency of the i^{th} species. A higher H value indicates greater diversity. The Simpson's index (D) was calculated using the equation $D = 1 - \sum_{i=1}^{N}(n/N)^2$, in which n is the number of individuals of a single species and N is the number of individuals in a community. Species richness index was calculated using Margalef's diversity index (D_{mg}) by the equation $D_{mg} = \frac{(S-1)}{In\,(N)}$, in which S is the number of species in the sample and N is the total number of individual in the sample. The widely used Sorensen similarity coefficient was measured by the following equation $C_S = \frac{2\,ab}{a+b}$, in which Cs = similarity coefficient, a = the number of the band (species) in sample a, b = the number of bands (species) in sample b, and ab = the number band (species) shared by the two samples (Magurran, 2004). The DGGE profiles were subjected to cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA) program.

To support the UPGMA analysis, principal component analysis (PCA) was conducted using the SPSS 16.0 software (SPSS Inc.) to investigate shifts in the structure of bacterial and fungal communities. Diversity estimates (Shannon-Wiener, Simpson, and richness indices) and physicochemical properties were calculated for two-way ANOVA, followed by Duncan's test for significant differences among groups in SPSS version 16.0. The Pearson's correlation analysis was tested between bacterial and fungal diversity indices and physicochemical characteristics. Differences at $p < 0.05$ were regarded as statistically significant and at $p < 0.01$ as highly significant. Differences in microbial communities and physicochemical parameters among decomposition sites and time were determined using principal component (PC) analysis that was performed using the Minitab 21 software.

Results

Denaturing gradient gel electrophoresis analysis of structure of bacterial and fungal communities

In this study, DGGE was performed to demonstrate the structural changes in bacterial and fungal communities in OPEFB decomposed under oil palm canopy and on open land for 6 mth, 12 mth and 24 mth. The changes during decomposition were analyzed based on the number of bands, their migration and the intensity of the DGGE bands (Fig.1).

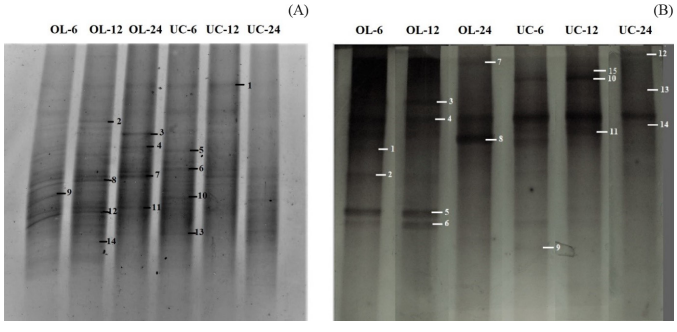

Fig. 1 Polymerase chain reaction-denaturing gradient gel electrophoresis analysis of communities of: (A) bacteria; (B) fungi in oil palm empty fruit bunches piled up on open land (OL) and under the canopy (UC) for different decomposition durations (6 mth, 12 mth and 24 mth) in palm oil plantation

There were clear differences in the DGGE patterns in the bacterial and fungal communities among OPEFB decomposed under the canopy and on open land. There were striking similarities between OPEFB decomposed for 6 mth and 12 mth on open land for both the bacterial and fungal communities, whereas the bacterial and fungal communities in OPEFB decomposed under the canopy varied with the different decomposition times.

The richness (Margalef index, *S*) and diversity indices (measured as Shannon's *H* and Simpson's *D*) of fungal and bacterial communities were calculated based on the number of DGGE bands per lane and their relative brightness (Table 2). The analysis of variance indicated that sites and time as well as their interaction significantly affected the bacterial and fungal communities in this study (Table 2). When each factor was considered, the values of *S, H* and *D* for bacteria were significantly higher on open land than those under the oil palm canopy and all diversity parameters declined as the incubation time was prolonged. However, when both factors (sites and time) were considered, the trend of the diversity was slightly changed, whereby, the bacterial diversity in OPEFB decomposed on open land increased until 12 mth of decomposition, and then dropped (see Table 2, Supplementary data, Figs. S2–S4). Conversely, under the oil palm canopy, bacterial richness and diversity (*S* and *D, respectively*) substantially decreased until 12 hr of decomposition, despite there being no significant difference in *S* between the 6 hr and 12 hr decomposition values. The diversity remained unchanged when decomposition continued to 24 hr except for *H* that significantly increased toward 24 hr.

For the fungal community, the values of *H* and *D* in OPEFB decomposed on open land were significantly higher than under the oil palm canopy, but *S* was significant higher under the oil palm canopy than on open land. There were similar tends in fungal diversity (Shannon and Simpson) between under the oil palm canopy and on open land, with no significant differences in fungal diversity for OPEFB decomposed for 6 mth and 12 mth. A substantial change in fungal diversity was only observed after decomposition for 24 mth. Interestingly, the richness of the fungal community under the oil palm canopy was significantly higher compared to on open land and the trend in fungal richness changes during decomposition greatly differed (see Supplementary data, Figs. S2–S4). On open land, there was no significant change in fungal richness over decomposition time; in contrast, under the oil palm canopy, the increase in richness occurred after decomposition for 12 mth, then substantially dropped after 24 mth.

Table 2 Bacterial and fungal diversity in decomposed oil palm empty fruit bunches on open land (OL) and under oil palm canopy (UC) for 6 mth, 12 mth and 24 mth

Treatment	Bacterial diversity			Fungal diversity		
	Shannon-Wiener index	Simpson diversity index	Species richness	Shannon-Wiener index	Simpson diversity index	Species richness
Site						
OL	2.979±0.123^{b}	0.941±0.008^{b}	6.026±0.453^{b}	1.969±0.207^{b}	0.828±0.053^{b}	2.512±0.151^{a}
UC	2.483±0.225^{a}	0.902±0.023^{a}	4.201±0.586^{a}	1.866±0.296^{a}	0.776±0.062^{a}	2.914±0.678^{b}
Time (mth)						
6	2.864±0.109^{c}	0.935±0.004^{c}	5.460±0.644^{b}	2.076±0.054^{b}	0.844±0.025^{b}	2.849±0.278^{b}
12	2.733±0.454^{b}	0.917±0.039^{b}	5.205±1.540^{b}	2.081±0.019^{b}	0.833±0.030^{b}	3.072±0.579^{c}
24	2.596±0.299^{a}	0.912±0.024^{a}	4.676±1.016^{a}	1.595±0.127^{a}	0.729±0.038^{a}	2.218±0.172^{a}
Site×Time						
OL×6	2.957±0.017^{b}	0.939±0.001^{a}	5.990±0.146^{b}	2.117±0.028^{b}	0.865±0.004^{ns}	2.613±0.086^{b}
OL×12	3.126±0.026^{c}	0.951±0.002^{b}	6.536±0.158^{c}	2.086±0.018^{b}	0.859±0.00^{ns}	2.579±0.095^{ab}
OL×24	2.854±0.001^{a}	0.933±0.002^{a}	5.554±0.101^{a}	1.704±0.023^{a}	0.761±0.01^{ns}	2.344±0.111^{a}
UC×6	2.772±0.033^{b}	0.932±0.001^{c}	4.931±0.321^{b}	2.036±0.037^{b}	0.824±0.013^{ns}	3.086±0.040^{b}
UC×12	2.341±0.026^{ab}	0.884±0.001^{a}	3.874±0.096^{a}	2.076±0.027^{b}	0.807±0.003^{ns}	3.566±0.150^{c}
UC×24	2.338±0.046^{a}	0.892±0.005^{b}	3.798±0.012^{a}	1.486±0.018^{a}	0.697±0.004^{ns}	2.092±0.112^{a}

Mean values (±SD) within a column of each factor or factor combination superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Multivariate analysis of denaturing gradient gel electrophoresis profile

UPGMA cluster analysis based on Sorensen's similarity coefficient (Cs) was used to compare the similarity of the bacterial and fungal communities during the decomposition of OPEFB for 6 mth, 12 mth and 24 mth on open land and under the oil palm canopy. The results of this analysis showed that there was a similar structure for bacterial and fungal communities in OPEFB decomposed on open land for 6 mth and 12 mth (Fig. 2), with the Cs values in the range 0.90–1.00 (Supplementary data, Table S1). Change in the structures of the bacterial and fungal communities on open land were observed after 24 mth decomposition (Cs values in the range 0.54–0.67). In contrast, on open land, the structures of the bacterial and fungal communities under the canopy changed with decomposition time, with the Cs values in the range 0.25–0.70, indicating that the succession of bacterial and fungal communities during the decomposing of OPEFB under the oil palm canopy took place more rapidly than on open land.

PC analysis provided supporting data to strengthen the clustering analysis using UPGMA. As shown in Fig.3, the results of PC analysis showed that the changes in the bacterial and fungal communities on open land and under the canopy were different during 6 mth, 12 mth and 24 mth of OPEFB decomposition. The PC analysis plots for the bacterial and fungal communities revealed two different clusters according to the first and the second components. For the bacterial community, the first principal component (PC1) and the second principal component (PC2) explained 41.38% and 20.78%, respectively, of the total variation, while for the fungal community, PC1 and PC2 explained 58.66% and 26.31%, respectively, of the total variation. The PC analysis for the bacterial and fungal communities in OPEFB decomposed on open land for 6 mth and 12 mth indicated clusters together with high similarity that subsequently separated after being decomposed for 24 mth. In contrast, the PC analysis for the bacterial and fungal communities in OPEFB decomposed under the canopy for 6 mth, 12 mth and 24 mth produced clusters that were more decentralized.

Phylogenetic analysis

To further understand the structure of the microbial communities in OPEFB decomposed at the two different decomposition sites, the 14 bands from 16S-rDNA DGGE and the 15 bands from 18S-rDNA DGGE were excised from the gel and sequenced (Fig. 1). The bands were selected based on their intensity in the samples. The sequencing results were aligned based on the NCBI-BLAST database and the similarity of most band sequences was >80–100% compared with those available in the GenBank database (see Supplementary data, Table S2). The phylogenetic trees were constructed using the neighbor-joining method with 1,000 bootstrap replicates (Fig. 4 and Fig. 5). Two bacterial phyla (*Proteobacteria* and *Actinobacteria*) and three fungal phyla (*Ascomycota, Basidiomycota* and *Mucoromycota*) were detected.

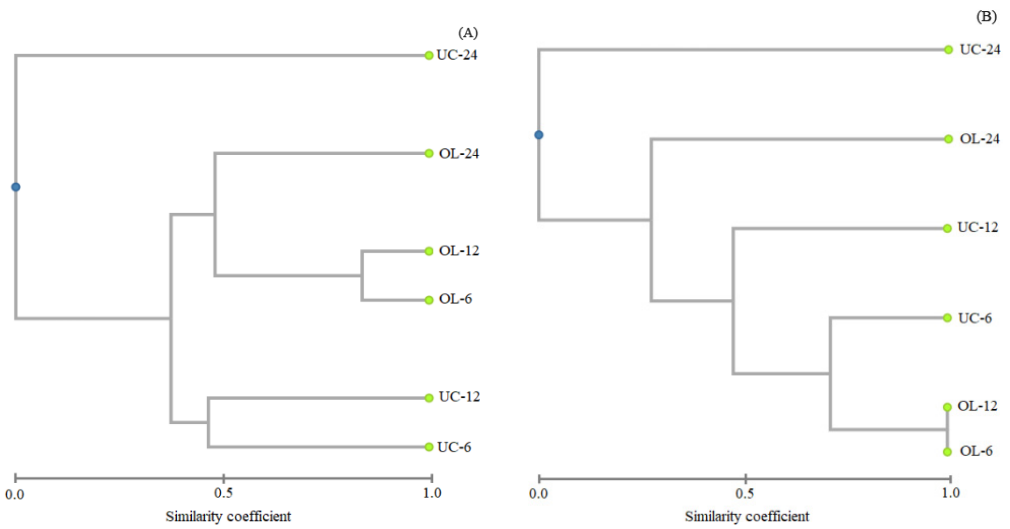

Fig. 2 Unweighted pair group method with arithmetic mean clustering analysis of the denaturing gradient gel electrophoresis pattern of communities of: (A) bacteria; (B) fungi on different oil palm empty fruit bunch decomposition sites of open land (OL) and under canopy (UC), during decomposition periods of 6 mth, 12 mth and 24 months

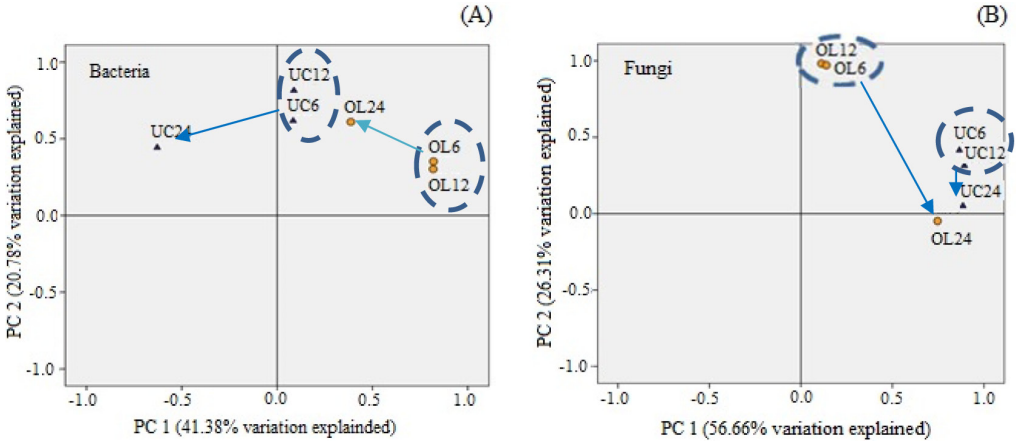

Fig. 3 Principal component (PC) analysis loading plot of communities of: (A) bacteria; (B) fungi from oil palm empty fruit bunches decomposed under oil palm canopy (UC) and on open land (OL) for 6 mth, 12 mth and 24 mth based on denaturing gradient gel electrophoresis pattern, where arrows indicate progression of decomposition stages as a function of time and dashed circles indicate grouping based on similarity of microbial composition

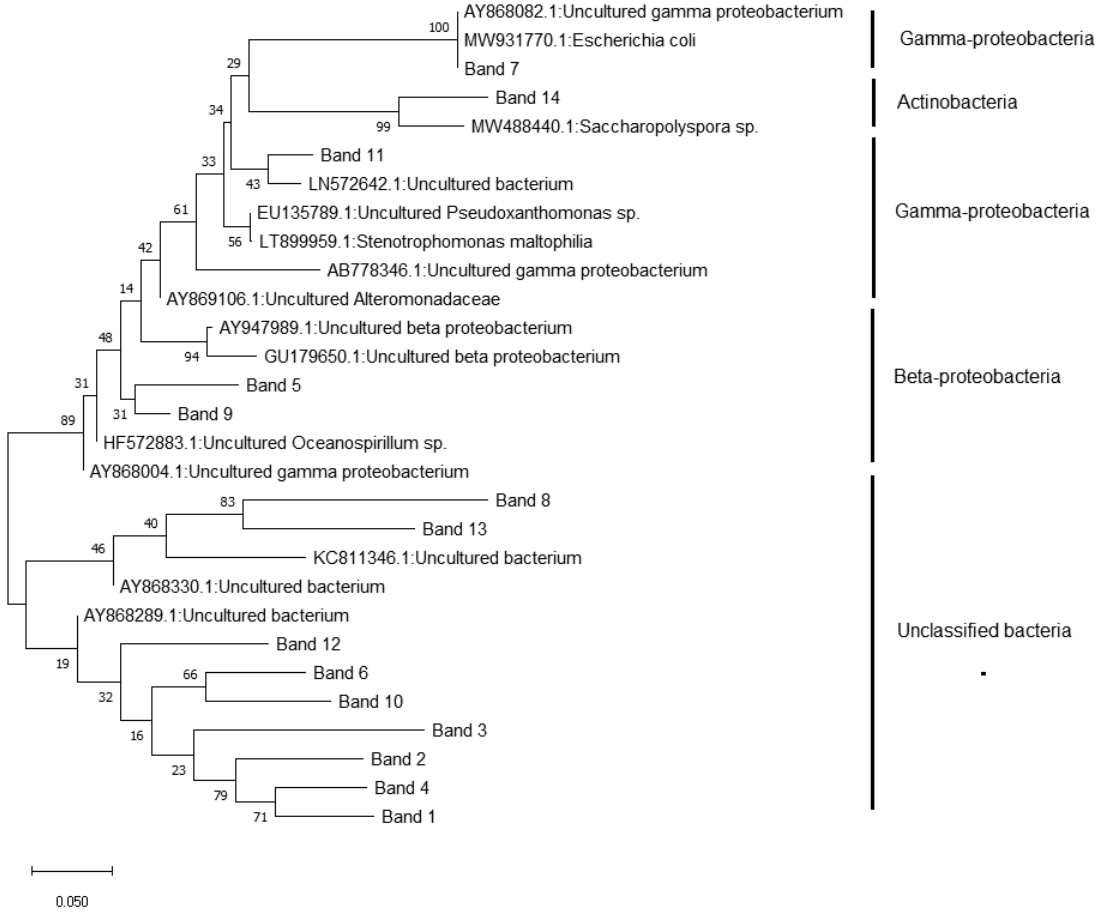

Fig. 4 Phylogenetic relationship analysis between 16S rDNA of polymerase chain reaction-denaturing gradient gel electrophoresis products based on NCBI database using neighbor-joining method

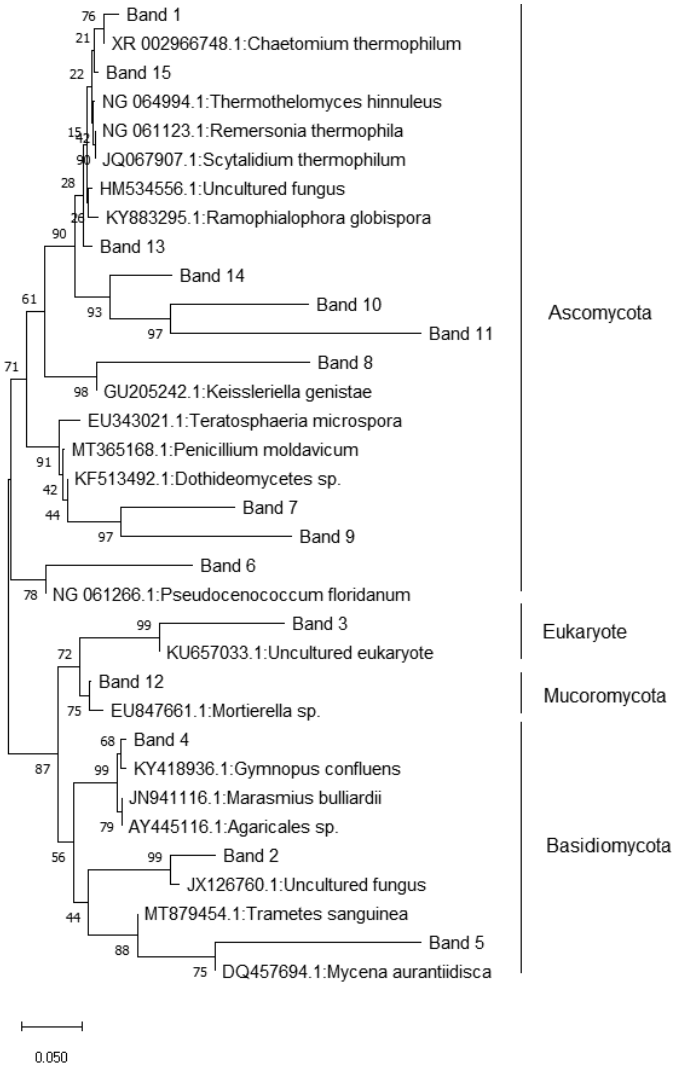

Fig. 5 Phylogenetic relationship analysis between 18S rDNA of polymerase chain reaction-denaturing gradient gel electrophoresis products based on NCBI database using neighbor-joining method

Among the 14 selected 16S-rDNA DGGE bands, most of the band sequences affiliated with unculturable bacteria were related to the classes *β-Proteobacteria* (2 bands), *γ-Proteobacteria* (2 bands) and unclassified bacteria (9 bands). Only one of the 16S-rDNA DGGE band sequences (band no.11) was affiliated with the phylum *Actinobacteria*. The fungal classes identified in most of the selected 18S-rDNA DGGE bands were affiliated with culturable fungus (see Supplementary data, Table S3) related to the *Sordariomycetes* (6 bands), *Agaricomycetes* (2 bands) and *Mortierellaceae* (1 bands). Only two sequences of 18S-rDNA DGGE bands were affiliated with uncultured fungus relating to *Basidiomycota* and *Ascomycota*, while one other sequence was affiliated with an uncultured eukaryote. Among these groups, one fungal species (*Gymnopus confluens*) belonging to phylum *Basidiomycota* and the class *Agaricomycetes* was detected in all OPEFB samples with a high intensity signal and became the most dominant band in OPEFB decomposed under the oil palm canopy. *Chaetomium thermophilum* (phylum *Ascomycota*, class *Sordariomycetes*) was only detected in OPEFB decomposed for 6 mth on open land, whereas other species belonging to the same class, such as *Remersonia thermophila* and *Thermothelomyces hinnuleus*, were only detected in OPEFB decomposed under the oil palm canopy. Interestingly, *Mortierella* sp. (phylum *Mucoromycota*, class *Mortierellaceae*) was also only found in OPEFB decomposed for 24 mth under the oil palm canopy.

Physicochemical changes in oil palm empty fruit bunches during the decomposition process

Changes in the physicochemical parameters of OPEFB decomposed under the oil palm canopy and on open land for 6 mth, 12 mth and 24 mth are shown in Table 3. No significant differences in temperature and humidity changes were observed during OPEFB decomposition at both sites; however, there were significant changes in the pH and C/N ratio during decomposition (Table 3). The difference in the C/N ratio between both sites was due to the difference in the changes of TOC and TN during decomposition (see Supplementary data, Table S5).

To understand the influence of differences in the structure and diversity of the microbial communities on the OPEFB decomposition rate, SEM analysis was conducted for OPEFB decomposed for 24 mth. The micrographs for decomposed OPEFB at the two different stacking sites are shown in Fig. 7. The micrographs revealed that the surface condition of the OPEFB piled on open land was still rich in fiber and contained silica bodies (SB). In contrast, there were no fibers or SBs in the OPEFB stored under the oil palm canopy, indicating that the OPEFB at this site had been entirely turned into soil.

Relationship between diversity of microbial communities and physicochemical properties

PC analysis was used to assess the relationships between the sample plots and physico-chemical profiles (Fig. 6). The two principal axes, explaining the first and second components, accounted together for 85.9% of the physico-chemical data variability. The first axis (PC1) explained 52.4% of the variation and was positively correlated with temperature, pH and TN, but negatively correlated with

humidity. The second axis (PC 2) explained 32.5% of the variation and was positively correlated with TOC, but negatively correlated with the temperature and humidity. Ordination biplot in the plane showed that the sample plots, based on physico-chemical changes, were divided into four quadrants, namely OL-0 and UC-0 in the first quadrant, OL-6 and OL-12 in the second quadrant, UC-6, UC-12 and OL-24 in the third quadrant and UC-24 further away in the fourth one. These indicated that the microbial community in the OPEFB decomposed under the oil palm canopy was more active than for the open land site.

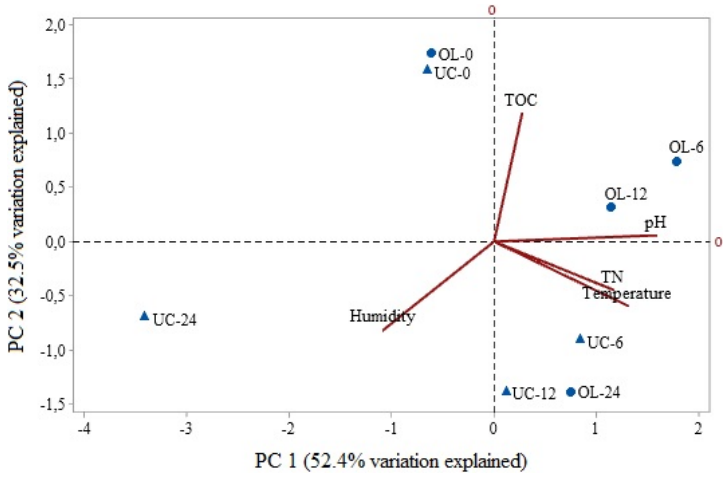

Fig. 6 Principal component (PC) analysis of physicochemical properties of oil palm empty fruit bunch (OPEFB) samples decomposed under oil palm canopy (UC, triangles) and on open land (OL, circles) for 0 mth, 6 mth, 12 mth and 24 mth, where red lines indicate progression of changes in physicochemical parameters (TOC = total organic carbon and TN = total nitrogen) as a function of time during OPEFB decomposition for 6 mth, 12 mth and 24 mth

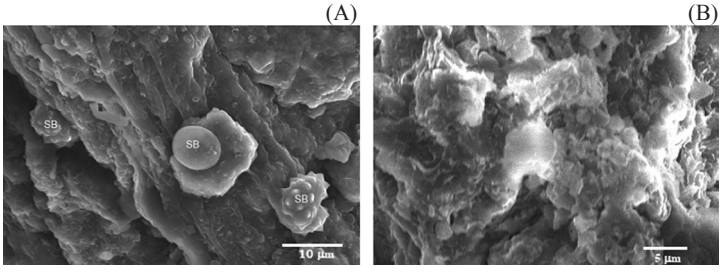

Fig. 7 Microstructure from scanning electron microscopy of oil palm empty fruit bunches decomposed for 24 mth: (A) piled on open land; (B) piled under canopy, where SB = silica body

Table 3 Physico-chemical properties of decomposed oil palm empty fruit bunches on open land (OL) and under oil palm canopy (UC) during the decomposition process

Treatment	Temperature (°C)	Relative humidity (%)	pH	Total organic carbon (%)	Total nitrogen (%)	Carbon-to-nitrogen ratio
Site						
OL	32.98±3.64^{b}	81.21±7.80^{a}	8.19±0.83^{b}	19.99±4.00^{b}	0.57±0.11^{b}	37.34±12.85^{b}
UC	31.44±5.03^{a}	87.38±5.52^{b}	6.81±2.13^{a}	14.68±6.35^{a}	0.46±0.07^{a}	32.60±14.48^{a}
Time (mth)						
0	27.33±0.58^{a}	82.15±5.04^{ab}	7.13±0.29^{b}	25.42±0.99^{c}	0.46±0.03^{a}	55.59±2.25^{c}
6	35.53±0.29^{e}	78.25±6.82^{a}	9.01±0.30^{d}	15.58±4.49^{b}	0.54±0.03^{b}	28.86±8.06^{b}
12	36.43±0.64^{d}	83.79±5.57^{b}	8.04±0.47^{c}	15.32±4.40^{b}	0.51±0.04^{b}	29.86±7.91^{b}
24	29.55±3.91^{b}	93.00±1.80^{c}	5.81±2.58^{a}	13.02±2.84^{a}	0.54±0.20^{b}	25.55±5.09^{a}
Site×Time						
OL×0	27.27±0.67^{a}	81.37±5.92	7.00±0.20^{a}	25.69±1.10^{c}	0.46±0.03^{a}	55.53±1.98^{c}
OL×6	35.65±0.05^{c}	73.10±5.20	9.13±0.40^{e}	19.64±0.32^{b}	0.55±0.03^{b}	35.80±2.54^{b}
OL×12	35.90±0.10^{c}	78.78±1.48	8.45±0.15^{b}	19.33±0.35^{b}	0.53±0.04^{ab}	36.81±2.28^{b}
OL×24	33.10±0.30^{b}	91.60±1.10	8.17±0.04^{b}	15.31±2.06^{a}	0.72±0.06^{c}	21.20±1.10^{a}
UC×0	27.40±0.62^{b}	82.93±5.15	7.27±0.35^{b}	25.15±1.01^{b}	0.45±0.04^{b}	55.56±2.95^{c}
UC×6	35.40±0.40^{e}	83.40±3.10	8.89±0.14^{c}	11.53±0.92^{a}	0.53±0.04^{c}	21.92±3.39^{a}
UC×12	36.95±0.45^{d}	88.80±0.20	7.63±0.13^{b}	11.31±0.24^{a}	0.50±0.05^{bc}	22.92±2.59^{a}
UC×24	26.00±0.60^{a}	94.40±1.00	3.45±0.02^{a}	10.73±0.35^{a}	0.36±0.02^{a}	29.89±2.62^{b}

Mean values (±SD) within a column of each factor or factor combination superscripted with different lowercase letters are significant ($p < 0.05$) different

Pearson's correlation (r) analysis was applied to better understand the relationship between the changes in the physicochemical properties of OPEFB and richness and diversity of the microbial communities (see Supplementary data, Table S4). The diversity indices for the fungal community showed highly significant positive correlations with the changes in temperature ($r = 0.864$ for the H value and $r = 0.805$ for the D value, $p < 0.01$) and pH ($r = 0.819$ for the H value and $r = 0.861$ for the D value, $p < 0.01$). In addition, relative humidity had a highly significant negative correlation with the fungal diversity ($r = -0.768$ for the H value and $r = -0.888$ for the D value, $p < 0.01$), confirming that as the relative humidity in the OPEFB piles increased, the fungal diversity decreased. However, the TOC had a significant positive correlation with the bacterial diversity ($r = 0.829$ for the H value and $r = 0.762$ for the D value, $p < 0.05$) and a highly significant positive correlation richness ($r = 0.872$, $p < 0.01$). Nevertheless, the TN of OPEFB was not correlated with either bacterial or the fungal diversity.

Discussion

DGGE band patterns are useful in the assessment of microbial communities in environmental samples (Orlewska et al., 2018). Although DGGE is not a quantitative method due to the bias introduced by PCR, the increases or decreases in band intensities in different samples can be used to indicate a relative increase or decrease in the relative abundance of the microbes in the community (Stamper et al., 2003). Therefore, in the current study, the relative band intensities were used to investigate the changes in the structures of the microbial communities during OPEFB decomposition. The number of DGGE bands was used to estimate the number of species present in each sample, even though one band could possibly represent many species and certain microbes produce multiple bands on DGGE gel (Muyzer et al., 1993). Based on the analysis of the DGGE profile, the structures and the numbers of bacteria and fungi distinctly differed between the OPEFB decomposed under the oil palm canopy and on open land. Based on the UPGMA and PCA analyses, major changes in the structures of the bacterial and fungal communities during decomposition for 6 mth, 12 mth and 24 mth were more evident in the OPEFB decomposed under the canopy. However, on open land, there were no significant changes in the structures of the bacterial and fungal communities between the OPEFB decomposed for 6 mth and 12 mth, although a significant change was observed after decomposition for 24 mth.

The differences in the structures of the microbial communities between the OPEFB decomposed under the oil palm canopy and on open land were also evident in the differences in the richness and diversity indices (see Table 2), which were significantly higher on open land than under the oil palm canopy. These might have been caused by differences in the temperature and relative humidity in the different OPEFB piles (see Table 3). Temperature and relative humidity are two major factors affecting microbial activity in soil (Gomez et al., 2020). East Kalimantan province has a humid tropical climate with high precipitation and abundant sunshine, so the temperature tends to produce warm to hot conditions, with the relative humidity being high in the range 70–90% due to the high precipitation in this region. On open land, the surface of the OPEFB was exposed to most of the sunlight and so became warmer, resulting in increased saturated water vapor pressure and a subsequent decrease in the relative humidity in the OPEFB pile. Conversely, OPEFB under the oil palm canopy was not exposed to direct sunshine, so the relative humidity tended to be higher (82–95%). Relative humidity exceeding 80% can cause the death of aerobic microbes because of a lack of oxygen (Stanaszek-Tomal, 2020). Consequently, the diversity indices for the bacteria and fungi under the oil palm canopy were lower than those on open land. This was consistent with the measured data in the current study, showing that the relative humidity was negatively correlated with bacterial and fungal diversity. Nevertheless, the majority of fungi require a higher relative humidity for their optimal growth, so this resulted in the fungal richness in the OPEFB decomposed under the oil palm canopy being significantly higher than on open land (Talley et al., 2002).

The decomposition process of organic materials is dynamic, with very intense biological activity that causes changes in environmental conditions, including the temperature, pH and C/N ratio, supporting microbial succession (Meng et al., 2019). Most notable is the increasing temperature, since during decomposition, heat is released through the oxygen-consuming microbial metabolism, resulting in increased temperature, with a rapid increase during the decomposition process and a slower decrease until the degradation process was completed (Hock et al., 2009; Shen et al., 2011). In the current study, an increase in the pile temperature was observed at both decomposition sites from 27 °C initially to 35 °C after decomposition for 6 mth, with a different trend in the change in pile temperature under the oil palm canopy compared to on open land. Under the oil palm canopy, the temperature continued to increase until it reached 37 °C after decomposition for

12 mth and then it decreased until it had returned to the initial temperature (26 °C) after decomposition for 24 mth, indicating that the degradation process had finished (Trisakti et al., 2018). In contrast on open land, there was no noticeable change in the temperature in the OPEFB piles after decomposition for 12 mth, with the temperature after decomposition after 24 mth being above 30 °C, demonstrating that the decomposition process was still ongoing at a slow rate with the late stages of decomposition not yet reached.

The final decomposition stage was supported by observation of the OPEFB texture and microstructural analysis using SEM after 24 mth of decomposition. On open land, the texture of the decomposed OPEFB had almost turned to that of soil, although it still contained fiber. The texture of the decomposed OPEFB under the oil palm canopy had entirely decomposed into soil and contained no fiber. The photomicrographs of the OPEFB (Fig. 7) decomposed on open land showed the presence of SBs, attached to circular pores along the OPEFB strands. In addition, the microfibrillar structure was observed in the decomposed OPEFB on open land, indicating that the decomposition process of the OPEFB was not yet complete. In contrast, no SBs or microfibril structures were found in the decomposed OPEFB under the oil palm canopy, indicating that the OPEFB has been completely degraded. The differences in the OPEFB decomposition rates between under the oil palm canopy and on open land were also confirmed by the changes in other physicochemical parameters, such as the pH, TOC and C/N ratio. There was a significant change in the pH value in the OPEFB decomposed under the oil palm canopy from alkaline to acidic, indicating microbial activity during decomposition. Additionally, a significant decrease in the TOC content reflected microbial-induced degradation of the OPEFB. Under the oil palm canopy, the TOC content in the OPEFB substantially decreased after decomposition for 6 mth and then became stable, indicating that the decomposition process was complete. On open land, the TOC decreased slowly, suggesting that the decomposition of OPEFB on open land took place at a slower rate than under the oil palm canopy. These results suggested that changes in the structure of the fungal and bacterial communities were likely due to the changes in substrate availability, in particular the carbon source. There was a highly significant positive correlation between TOC with bacterial richness but not for the fungal community, suggesting that this parameter was likely to influence or be influenced by bacterial species.

Interestingly, there was an increased TN content in the OPEFB decomposed on open land for 24 mth that may have been caused by the results of microbial degradation products on the availability of substrate releasing ammonia nitrogen (Lim et al., 2015), which was consistent with the high pH value in the OPEFB decomposed on open land. Conversely, the TN content in the OPEFB decomposed under the oil palm canopy was reduced, perhaps due to the presence of fungi consuming more N sources to produce fleshy stems and fibrous caps and causing the decrease in the TN content in decomposed OPEFB. These findings revealed that the activity of microbial communities in OPEFB decomposed under the oil palm canopy was higher than that on open land. It seems fungi are the key decomposer responsible for OPEFB decomposition, consistent with Ko et al. (2017) who reported that fungi were considered to play key roles in decomposing recalcitrant compounds, such as lignin. Therefore, the abundance of fungi in the OPEFB decomposed under the oil palm canopy facilitated the faster decomposition of the OPEFB.

The C/N ratio can be used as an indicator of the decomposition process (Brito et al., 2008). Several studies reported the C/N ratio of final OPEFB compost was less than 20 (Giacometti et al., 2013; Lim et al., 2015). Nevertheless, in the current results, the C/N ratios of the OPEFB decomposed for 24 mth at both sites were still higher than the value accepted as indicating complete decomposition, indicating that some OPEFB organic matter was still difficult to decompose during the later stages. However, the C/N ratio in the OPEFB decomposed for 24 mth under the oil palm canopy became stable and even increased slightly, revealing that the OPEFB decomposition had reached the late stages of decomposition. In terms of the bacterial community structure, most of the selected 16S-rDNA PCR-DGGE bands had low similarity (<97%) with the sequences of the Genbank database (see Supplementary data, Table S2). Most of the selected bacterial DGGE bands sequences were unidentified bacteria. Only two bacterial phyla were identified in this study: *Proteobacteria* and *Actinobacteria*. Two classes of *Proteobacteria* (*β*- and *γ*-*Proteobacteria*) have been reported as lignin-degrading bacteria (Janusz et al., 2017), and have been reported as the most abundant phylum in decaying OPEFB (Tahir et al., 2019). In addition, the dominance of *Proteobacteria* has been reported in soil (Ko et al., 2017), decaying wood (Kielak et al., 2016) and paddy straw (Zhan et al., 2018). This phylum was relatively more abundant in the OPEFB decomposed on open land. Baharuddin et al. (2009) and Ahmad et al. (2011) showed that *Proteobacteria* dominated at the beginning of OPEFB decomposition and they were found throughout the decomposition process. In the current study, only one band was identified as *Actinobacteria*,

indicating that this phylum was less abundant. *Actinobacteria* has been reported as the major lignocellulose degrader, with the highest abundance usually observed during thermophilic stages (Zainudin et al., 2017). This phylum was detected as one of the predominant bands in the OPEFB decomposed on open land and the band intensity related to this phylum decreased in the OPEFB decomposed under the canopy until toward the end of decomposition process.

Similar to the bacteria, the fungal community also underwent a succession of taxa that were able to decompose the OPEFB. *Ascomycota* and *Basidiomycota* were the two predominant fungal phyla detected in this study, while *Mucoromycota* was only detected in the OPEFB decomposed under the canopy for 24 mth. *Ascomycota* is one of the most diverse and ubiquitous phyla of the eukaryotes and had the highest percentage of all known phyla in the current study. *Ascomycetes* are mostly able to degrade cellulose and hemicellulose, although they have limited ability to convert lignin (Janusz et al., 2017). A species of the *Ascomycota* related to *Chaetomium thermphilum* (97% similarity) was only detected in the early stage of the OPEFB decomposition on open land. Fungal species belonging to this genus are important species in the decomposition of plants and other cellulose-rich materials due to the ability to produce cellulolytic enzymes (Coronado-Ruiz et al., 2018; El-Dawy et al., 2020). Another species related to *Ascomycota*, that were dominant under the canopy and on open land, was observed to decrease gradually throughout the decomposition process. A species of *Basidiomycota* related to *Gymnopus confluens* (98% similarity) was the most dominant throughout the decomposition process at the two different decomposition sites. The populations of this species changed throughout the decomposition process. Janusz et al. (2017) reported that ligninolytic enzymes have been detected in several species related to the *Gymnopus* genus. Consistent with that report, investigation of fungal succession during the OPEFB decomposition showed that *Basidiomycota* dominated in the earlier stages of decomposition, while *Ascomycota* dominated in the later stages. The current study detected *Mucoromycotina*, related to *Mortierella*, only in the OPEFB decomposed under the canopy for 24 mth. This indicated that lignocellulosic hydrolysate, consisting of glucose and xylose, had been available as a carbon source for *Mortierella* growth (Zeng et al., 2013). These data suggested that the differences between the decomposition sites under the oil palm canopy and on open land resulted in differences in the structural changes that occurred in the bacterial and fungal communities and that these played an important role in determining the OPEFB decomposition rates in and around oil palm plantations in the humid tropical zone.

The current findings should be useful for identifying stacking sites for OPEFB in and around oil palm plantations that can produce faster decomposition, as these different decomposition sites greatly influence the microclimate around the OPEFB piles. Based on the current results, it can be concluded the biggest impact on the decomposition of OPEFB was under the oil palm canopy. Further investigation is needed to obtain more detailed information on the contributions of bacterial and fungal communities on the complete decomposition of OPEFB until it turns into topsoil for further use as mulch in oil palm plantations.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

Mrs Ariana, M.Si provided technical assistance. PT. Perkebunan Nusantara VI East Kalimantan Region collaborated in collecting samples and PT. Genetika Science Indonesia carried out the molecular genetic analysis.

References

Ahmad, M.N., Mokhtar, M.N., Baharuddin, A.S., Hock, L.S., Ali, S.R.A., Abd-Aziz, S., Rahman, N.A.A., Hasan, M.A. 2011. Changes physicochemical and microbial community during co-composting of oil palm frond. BioResource 6: 4762–4780.

Andlar, M., Rezić, T., Marđetko, N., Kracher, D., Ludwig, R., Šantek, B. 2018. Lignocellulose degradation: An overview of fungi and fungal enzymes involved in lignocellulose degradation. Eng. Life Sci. 18: 768–778. doi.org/10.1002/elsc.201800039

Baharuddin, A., Kazunori, N., Abd-Aziz, S., et al. 2009. Characteristics and microbial succession in co-composting of oil palm empty fruit bunch and partially treated palm oil mill effluent. Open Biotechnol. J. 3: 87–95.

Brito, L.M., Coutinho, J., Smith, S.R. 2008. Methods to improve the composting process of the solid fraction of dairy cattle slurry. Bioresource Technol. 99: 8955–8960. doi.org/10.1016/J.BIORTECH.2008.05.005

Buresova, A., Kopecky, J., Hrdinkova, V., Kamenik, Z., Omelka, M., Sagova-Mareckova, M. 2019. Succession of microbial decomposers is determined by litter type, but site conditions drive decomposition rates. Appl. Environ. Microb. 85: e01760-19. doi.org/10.1128/AEM.01760-19

Chang, S. 2014. An overview of empty fruit bunch from oil palm as feedstock for bio-oil production. Biomass Bioenerg. 62: 174–181. doi.org/https://doi.org/10.1016/j.biombioe.2014.01.002

Coronado-Ruiz, C., Avendaño, R., Escudero-Leyva, E., Conejo-Barboza, G., Chaverri, P., Chavarría, M. 2018. Two new cellulolytic fungal species isolated from a 19th-century art collection. Sci. Rep. 8: 7492. doi.org/10.1038/s41598-018-24934-7

El-Dawy, E.G., Shebany, Y.M., Gherbawy, Y.A. 2020. Molecular characterization and cellulolytic capacity of *Chaetomium globosum* isolates. ARRB 35: 14–22. doi.org/10.9734/arrb/2020/v35i930267

Eviati, Sulaeman. 2009. Chemical Analysis for Soil, Plant, Water and Fertilizer, 2nd ed. Soil Research Institute. Bogor, Indonesia.

Giacometti, C., Demyan, M.S., Cavani, L., Marzadori, C., Ciavatta, C., Kandeler, E. 2013. Chemical and microbiological soil quality indicators and their potential to differentiate fertilization in temperate agroecosystems. Appl. Soil Ecol. 64: 32–48. doi.org/10.1016/j.apsoil.2012.10.002

Gomez, E.J., Delgado, J.A., Gonzalez, J.M. 2020. Environmental factors affect the response of microbial extracellular enzyme activity in soils when determined as a function of water availability and temperature. Ecol. Evol. 10: 10105–10115. doi.org/10.1002/ece3.6672

Grosso, F., Iovieno, P., Alfani, A., De Nicola, F. 2018. Structure and activity of soil microbial communities in three Mediterranean forests. Appl. Soil Ecol. 130: 280–287. doi.org/10.1016/j.apsoil.2018.07.007

H'ng, P.S., Chai, E.W., Chin, K.L., et al. 2018. Evolution of organic matter within sixty days of composting of lignocellulosic food industry waste in Malaysia. Compost Sci. Util. 26: 16–26. doi.org/10.1080/1065657X.2017.1342105

Hamzah, N.H.C., Yahya, A., Man, H.C., Baharuddin, A.S. 2018. Effect of pretreatments on compost production from shredded oil palm empty fruit bunch with palm oil mill effluent anaerobic sludge and chicken manure. BioResources 13: 4998–5012.

Hock, L., Baharuddin, A.S., Ahmad, M.N., Shah, U.K.M., Rahman, N.A.A., Abd-Aziz, S., Hassan, M.A., Shirai, Y. 2009. Physicochemical changes in windrow co-composting process of oil palm mesocarp fiber and palm oil mill effluent anaerobic sludge. Aust. J. Basic Appl. Sci. 3: 2809–2816.

Indexmundi. 2022. Indonesia palm oil production by year. United States Department of Agriculture. Boston, MA, USA. https://www.indexmundi.com/agriculture/?country=id&commodity=palm-oil, 20 February 2022.

Janusz, G., Pawlik, A., Sulej, J., Świderska-Burek, U., Jarosz-Wilkolazka, A., Paszczyński, A. 2017. Lignin degradation: Microorganisms, enzymes involved, genomes analysis and evolution. FEMS Microbiol. Rev. 41: 941–962. doi.org/10.1093/femsre/fux049

Kielak, A.M., Scheublin, T.R., Mendes, L.W., van Veen, J.A., Kuramae, E.E. 2016. Bacterial community succession in pine-wood decomposition. Front. Microbiol. 7: 231. doi.org/10.3389/fmicb.2016.00231

Ko, D., Yoo, G., Yun, S.T., Jun, S.C., Chung, H. 2017. Bacterial and fungal community composition across the soil depth profiles in a fallow field. J. Ecology Environ. 41: 34. doi.org/10.1186/s41610-017-0053-0

Lim, L.Y., Chua, L.S., Lee, C.T. 2015. Effects of microbial additive on the physiochemical and biological properties of oil palm empty fruit bunches compost. J. Eng. Sci. Technol. 10: 10–18.

López-Mondéjar, R., Zühlke, D., Becher, D., Riedel, K., Baldrian, P. 2016. Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems. Sci. Rep. 6: 25279. doi.org/10.1038/srep25279

Magurran, A.E. 2004. Measuring Biological Diversity. Blackwell Publishing. Oxford, UK.

Marheni. 2019. *Bacillus stratosphericus* from *Oryctes rhinoceros* larvae as decomposer of oil palm empty fruit bunches. IOP Conf. Ser.: Earth Environ. Sci. 260: 012133. doi.org/10.1088/1755-1315/260/1/012133

May, L., Smiley, B., Schmidt, M. 2001. Comparative denaturing gradient gel electrophoresis analysis of fungal communities associated with whole plant corn silage. Can. J. Microbiol. 47: 829–841. doi.org/10.1139/w01-086

Meng, Q., Yang, W., Men, M., et al. 2019. Microbial community succession and response to environmental variables during cow manure and corn straw composting. Front. Microbiol. 10: 529. doi.org/10.3389/fmicb.2019.00529.

Muyzer, G., de Waal, E., Uitterlinden, A. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59: 695–700.

Orlewska, K., Piotrowska-Seget, Z., Cycon, M. 2018. Use of the PCR-DGGE method for the analysis of the bacterial community structure in soil treated with the cephalosporin antibiotic cefuroxime and/or inoculated with a multidrug-resistant *Pseudomonas putida* strain MC1. Front. Microbiol. 9: 1387. doi.org/10.3389/fmicb.2018.01387

Qiu, S., Chen, J., Lin, S., Lin, X. 2012. A comparison of silver staining protocols for detecting DNA in polyester-backed polyacrylamide gel. Braz. J. Microbiol. 43: 649–652. doi.org/10.1590/S1517-83822012000200029

Rasyid, B., Ala, A., Kuswinanti, T., Sapareng, S. 2020. Exploring functional fungi on organic matter decomposition of oil palm empty bunches as bio-resource in land remediation. Biodiversitas 21: 4247–4252. doi.org/10.13057/biodiv/d210941

Shen, Y., Ren, L., Li, G., Chen, T., Guo, R. 2011. Influence of aeration on CH_4, N_2O and NH_3 emissions during aerobic composting of a chicken manure and high C/N waste mixture. Waste Manage. 31: 33–38. doi.org/10.1016/j.wasman.2010.08.019

Siddiquee, S., Shafawati, S.N., Naher, L. 2017. Effective composting of empty fruit bunches using potential *Trichoderma* strains. Biotechnol. Rep. 13: 1–7. doi.org/10.1016/j.btre.2016.11.001

Stamper, D., Walch, M., Jacobs, R.N. 2003. Bacterial population changes in a membrane bioreactor for graywater treatment monitored by denaturing gradient gel electrophoretic analysis of 16S rRNA gene fragments. Appl. Environ. Microbiol. 69: 852–860. doi.org/10.1128/AEM.69.2.852-860.2003

Stanaszek-Tomal, E. 2020. Environmental factors causing the development of microorganisms on the surfaces of national cultural monuments made of mineral building materials—Review. Coatings 10: 1203. doi.org/10.3390/coatings10121203

Tahir, A., Barnoh, N.F.M, Yusof, N., et al. 2019. Microbial diversity in decaying oil palm empty fruit bunches (OPEFB) and isolation of lignin-degrading bacteria from a tropical environment. Microbes Environ. 34: 161-168. doi.org/10.1264/jsme2.ME18117

Talley, S.M., Coley, P.D., Kursar, T.A. 2002. The effects of weather on fungal abundance and richness among 25 communities in the Intermountain West. BMC Ecol. 2: 7. doi.org/10.1186/1472-6785-2-7

Trisakti, B., Mhardela, P., Husaini, T., Irvan, Daimon, H. 2018. Production of oil palm empty fruit bunch compost for ornamental plant cultivation. IOP Conf. Ser.: Mater. Sci. Eng. 309: 012094. doi.org/10.1088/1757-899X/309/1/012094

Van Westreenen, A., Zhang, N., Douma, J.C., Evers, J.B., Anten, N.P.R., Marcelis, L.F.M. 2020. Substantial differences occur between canopy and ambient climate: Quantification of interactions in a greenhouse-canopy system. PLoS One 15: e0233210. doi.org/10.1371/journal.pone.0233210

Zainudin, M.H.M., Hassan, M.A., Md Shah, U.K., et al. 2014. Bacterial community structure and biochemical changes associated with composting of lignocellulosic oil palm empty fruit bunch. BioResources 9: 316–335. doi.org/10.15376/biores.9.1.316-335

Zainudin, M.H.M., Ramli, N., Hassan, M.A., Shirai, Y., Tashiro, K., Sakai, K., Tashiro, Y. 2017. Bacterial community shift for monitoring the co-composting of oil palm empty fruit bunch and palm oil mill effluent anaerobic sludge. J. Ind. Microbiol. Biotechnol. 44: 869–877. doi.org/10.1007/s10295-017-1916-1

Zeng, J., Zheng, Y., Yu, X., Yu, L., Gao, D., Chen, S. 2013. Lignocellulosic biomass as a carbohydrate source for lipid production by *Mortierella isabellina*. Bioresource Technol. 128: 385–391. doi.org/10.1016/j.biortech.2012.10.079

Zhan, Y., Liu, W., Bao, Y., Zhang, J., Petropoulos, E., Li, Z., Lin, X., Feng, Y. 2018. Fertilization shapes a well-organized community of bacterial decomposers for accelerated paddy straw degradation. Sci. Rep. 8: 7981. https://doi.org/10.1038/s41598-018-26375-8