Journal of Tropical Biodiversity and Biotechnology
Volume 07, Issue 02 (2022): jtbb68820
DOI: 10.22146/jtbb.68820

Research Article

Analysis of Soil Bacterial Diversity from Tropical Rainforest and Oil Palm Plantation In Jambi, Indonesia by 16S rRNA-DGGE Profiles

Risky Hadi Wibowo[1,2,3], Nisa Rachmania Mubarik[3]*, Iman Rusmana[3], Maggy Thenawidjaya Suhartono[4], Sipriyadi[1,2], Masrukhin[5]

1) Department of Biology, Faculty of Mathematics and Natural Sciences, University of Bengkulu, Kampus UNIB Kandang Limun, Bengkulu 38112, Indonesia
2) Graduate School of Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu, Kandang Limun, Bengkulu 38112, Indonesia.
3) Microbiology Study Program, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Dramaga-Bogor 16680, West java, Indonesia.
4) Department of Food Science and Technology, Faculty of Agricultural and Technology, IPB University, Dramaga- Bogor 16680, West java, Indonesia.
5) Research Center for Biosystematics and Evolution, National Research and Innovation Agency, Jln raya Jakarta-Bogor Km 46 Cibinong 16911, Indonesia
* Corresponding author, email: nrachmania@ipb.ac.id

Keywords:
16S rRNA
denaturing gradient gel electrophoresis (DGGE)
oil palm plantation
soil bacterial community
Submitted:
01 September 2021
Accepted:
15 June 2022
Published:
27 July 2022
Editor:
Miftahul Ilmi

ABSTRACT

Oil palm plantations are the most invasive land use changes in Southeast Asia. It must have affected unique natural biodiversity. This study aimed to investigate the diversity of soil bacteria based on 16S rRNA gene profiles from tropical forest and oil palm plantation in Jambi Province, Indonesia. Soil sample was taken from tropical forest and oil palm plantation from Jambi province, Indonesia. The forest site is in Bukit Duabelas National Park, and the nearby oil palm plantation is in Sarolangun District, Jambi Province, Indonesia. The diversity of bacterial communities from topsoil was studied using Denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene and common biodiversity indices. PCR amplification of 16S rRNA gene was successfully conducted primers-using 33F/ 518R primers. Phylogenetic approach was used for revealing the community shift of bacterial phyla and genera in both areas. Phylogenetic analysis showed there were 4 phyla of bacteria i.e., *Firmicutes*, *Alphaproteobacteria*, *Gammaproteobacteria*, and *Actinobacteria*, respectively. *Actinobacteria* was the most dominant group in both areas. The composition of soil bacterial community in the oil palm plantation, based on total number of bands 16S rRNA generated from DGGE was richer than that in the Bukit Dua Belas National Park. It was probably caused by plantation year circle more than 10 years and routine activities during the plantation management, such as applications of agricultural lime, herbicide and fertilizer.

INTRODUCTION

Tropical forests are unique ecosystems with a diverse range of endemic flora, animals, and microbes. The most abundant and diverse category of microorganisms in soil are prokaryotes (bacteria). These microorganisms are important for the majority of nutrient conversions in soil and are major

biogeochemical cycle drivers. On the top of the soil, the bacteria are vital in decomposition of the organic matters in soils. Due to the interdependence of the vegetation with the soil substrate, which relies on the maintenance of adequate water, canopy cover, organic litter inputs, and land use change, lowland forests are highly sensitive to the impacts of land use change (transformation) to agro-forest industry (such as oil palm plantation) (Ko et al. 2005; Yule 2010).

Indonesian forest had been damaged 3.5 million hectares per year since 1999 until 2006, 2 million hectares per year between 2006-2010, and 300,000 hectares per year between 2010-2012 (Wijayanti et al. 2014). Timber extraction is a major factor in the transition from primer to secondary forest. Migrants transform a portion of the forest into a temporary cropland. In Jambi, such land has the potential to evolve into permanent tree-based agricultural systems (agro-forests) (Palm et al. 2005). There were 420 thousand ha of production forest in Jambi heavily damaged by the high rate of conversion to industrial timber plantation, palm oil plantations, coal mines and the opening of other minerals, such as iron and gold. The extensive damage approximately 30% of the approximately 1.4 million ha remaining forest areas. The loss of environmental services provided by trees is a non-linear trend in Jambi Province, Sumatra, Indonesia. The oil palm plantation industry might be threatened a gradual simplification of complex agro-ecosystems in agro-forests with rising profitability (Mudiyarso et al. 2002).

Land-use change has more severe effects on the soil microbial community structure than agro forest age, and a change in microbial biomass is not necessarily accompanied by a change in microbial community diversity and activity. Several factors may be involved. The soil microbial community may have been impacted by land-use conversion that involved significant soil disruption, such as cultivation, compaction, and fertilizer application. Plant species have a significant selection influence on microbial communities in soil, including bacterial diversity, according to several studies such as by Xue et al. (2008).

Bacterial diversity can be measured using two approaches, cultivable and non-cultivable. Only a small proportion of soil microbial diversity is cultivated, and the majority of soil microorganisms are unable to be cultured. Molecular biology and protein engineering technologies, such as metagenomic analysis, are being used to solve this problem (Glogauer et al. 2011). Metagenomic research of whole microbial communities inhabiting a certain niche is a culture-independent genomic analysis (Armougom & Raoult 2009). Recently, using denaturing gradient gelelectrophoresis (DGGE) of PCR amplified DNA fragments to examine the structural diversity of microbial communities has become a new strategy to approach the challenges of cloning and sequencing DNA fragments. DGGE was created for the purpose of detecting point mutations in medical research (Fischer & Lerman 1983), Muyzer et al. (1993) on the other hand, was the one who introduced it into the microbial ecology.

Denaturing gradient gel electrophoresis (DGGE) provides for the qualitative and semiquantitative profiling of microbial populations by determining the richness and evenness of dominant microbial species using 16S rRNA gene amplicons. The number of 16SrRNA gene sequence similarity groupings can be used to estimate diversity (*i.e.* the number of DNA bands on the DGGE gel and the intensity of DGGE bands). For the sake of simplicity, each band is considered to represent a species, which is a functional taxonomic unit. As a result, the current study used the DGGE rRNA 16S gene profile to analyze and compare the diversity of soil bacteria in tropical rainforests before and after conversion to oil palm plantations in Jambi Province.

MATERIALS AND METHODS

Soil Sampling and Laboratory Analysis

This study was carried out in the National Park of Bukit Dua Belas (NPBD) and the Humusindo Oil Palm Plantation in Jambi Province, Indonesia (Figure 1). Sampling sites of NPBD (latitude 01°56' 576" to 01-°56'502"S; longitude 102°34'879" to 102°34'836"E, altitude 87m to 116m asl) and oil palm plantation (latitude 01°56'491" to 01-°56'958"S; longitude 103°15'140" to 103°15'122"E, altitude 42m to 64m asl) were determined based on Purposive Random Sampling. The soil comprised relatively fertile, clay loam Acrisol soil in Bukit Duabelas and less fertile, loam Acrisol soil in Humusindo Oil Palm Plantation. Soil samples were collected from each two sampling sites in triplicate plots of 25 m². A total of 12 soil core samples were collected from each triplicate plots of the SW01 and SW02 (oil palm plantation aged 10-20 years) sites, TB03 and TB04 (tropical forest), and mixed to obtain a composite sample for each site. The soil samples were transported to the laboratory at room temperature and then stored at -20 °C until analysis. The

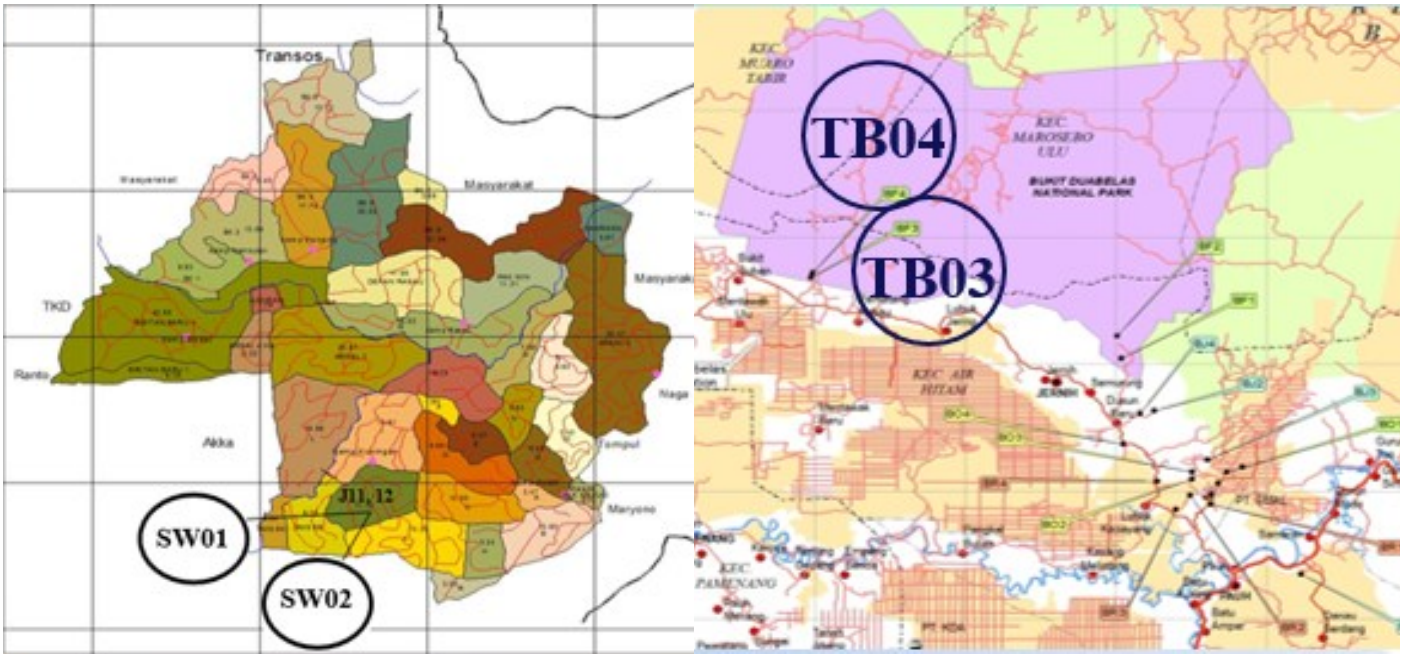


Figure 1. Map of Sampling location. Oil palm plantation of Humusindo (left) and National Park of Bukit Dua Belas (Right).

physico-chemical characteristics of soil were assessed using either field fresh soil or soil samples that had been air dried (Okalebo et al. 1993).

DNA Extraction and Quantification from Soil Samples

Extraction of community soil DNA was conducted using the Fast DNA® SPIN Kit for Soil (BIO 101 according to manufacturer's instruction). Approx. 0.5 g of fresh soil (stored at -20°C) was taken in a 2 mL E-tube containing lysing mixture. 978 µL SPB (sodium phosphate buffer) and 122 µL MT buffer were added in the tube and homogenized at maximum speed for 1 min. The suspension was centrifuged for 1 min at 14000 x g and the supernatant was transferred into a clear 2 ml tube and 250µL PPS (protein precipitation solution) was added followed by mixing the tube for 2 min. Then the tube was centrifuged again for 5 min at 14000 x g and the supernatant was transferred into a 15 mL tube. 1 mL of binding matrix suspension was added and the tube was turned upside down for at least 5 times to allow binding of DNA to the matrix. About 500 µL of the supernatant at the surface layer of the tube was discarded and resuspended the remaining supernatant in the binding matrix. The slurry was transferred into a Spin Tube in two aliquots of 500 µL each and centrifuged to discard the waste liquids. Finally, the DNA in the Spin filter was washed with 500 µL of SEWS-M (salt/ethanol wash solution, DNase-free) at 14000 x g for 1 min and the filtrate was discarded. The Spin was removed from the tube and air dried for 5 min at room temperature. The Spin was replaced into a fresh catch tube and 75-100 µL of DNA eluting solution (DES, Dnase /TE Buffer) was added while gently stirring the filter membrane with the pipette tip. Then, the tube was centrifuged at 14000 x g for 1 min to elute the DNA into the catch tube. The DNA content of the extract was checked at 1% agarose gel and then quantified using Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, USA) (Laurent et al. 2001).

PCR Amplification using 16S rRNA

T1 Thermocycler (BIOMETRA-Analytic Jena, Germany) was used to do PCR amplification of the 16S rRNA gene. P338F- GC (5' GCCCGC CGCGCGC GGCGGG CGG GGCGGGGGCACGGGGGGACTCC-TACGGGAGGCAGCAG-3') and P518R (5'-ATTA CCGCG GCTGCTGG -3') primers were used to amplify the 16S rRNA gene (Overeas et al. 1997) with PCR was set up for initial denaturation at 94°C for 5 minutes, then 35 cycles of 92°C denaturation for 30 seconds, 58°C annealing for 30 seconds, 72°C extension for 25 seconds, and 72°C extension for 3 minutes (Masrukhin et al. 2017). Electrophoresis in agarose gel (1% w/v) determined the PCR product, which was visualized in G:BOX gel documentation (Syngene, Frederick USA).

DGGE analysis of 16S rRNA gene

DGGE was performed by using *D Code Universal Mutation Detection System*

(Bio-Rad, Hercules, CA, US). As much as 25 µL sample (20 µL DNA + 55 µL *loading dye*) was loaded into 8% (w/v) polyacrilamide gel with 30–70% gradient denaturant. For 100% denaturant was 7M urea and 40% formamide. The electrophoresis process was performed at 150 V, 60 ºC for 5.5 h in 1 × TAE buffer (Tris- acetate- EDTA). The gel was stained by 0.1% (w/v) Ethidium Bromide (EtBr) for 15 min. The gel image documentation was performed using G:BOX *Gel Documentation* (Syngene, Frederick, USA). CLIQS 1D software (Total Lab) was used to estimate the DNA band volume and total band in the gel using DGGE bands. On the basis of the CLIQS 1D analysis results, clustering analysis was performed. Each DGGE band on the gel was excised and stored in a microtube with 100 liters of nuclease-free water. Overnight, the microtubes were stored in the refrigerator. For the re-PCR process, two microliters of the excised bands were used as the template. The re-PCR conditions were identical to the previous PCR (Chen et al. 2011).

Phylogenetic and Cluster Analysis

The PCR products were sequenced in a company laboratory providing sequencing services. For the assembling and trimming process, the sequences of the 16S rDNA gene were analyzed using ChromasPro (Technelysium, AU). BLASTN (Basic Local Alignment Sequence Tools for Nucleotide) was used to compare the sequences to the GeneBank database (blast.ncbi.nlm.nih.gov). The phylogenetic analysis was carried out using MEGA 6.0 software, and the phylogenetic tree was constructed using the neighbor-joining method according to the Bayesian information criterion (BIC) score. MEGA 6.0 software was used to perform a hierarchical cluster analysis based on UPGMA correlation (Tamura et al. 2013).

Analysis of Shannon-Wienner Diversity Index (H') and Evenness Index (E')

Using the following equations, the Shannon-Wienner diversity index (H') and Pielou's equitability index (E') can be simply derived to represent hypothetical changes in the dominance among DGGE OTUs:

Bacterial diversity in the samples was estimated using Shannon-Wienner index of bacterial diversity (H'). The Shannon-Wienner diversity index was calculated as

$$H' = -\sum_{i=1}^{s} p_i Ln\, p_i$$

Based on Eichner's formula for relative band intensities (Eichner et al. 1999). Pi was defined as ni/N, where Ni is the area of a peak in intensity and N the sum of all peak areas in the lane profiles.

$$E_1 = \frac{H'}{H_{max}} = \frac{H'}{\ln(S)}$$

RESULTS AND DISCUSSION

Environmental Variables

Using the DGGE method to analyze the sequencing of the 16S rRNA gene, the composition of soil bacterial communities observed in two separate soil systems of tropical forest (TB) and oil palm plantations (SW) were tested and compared. The sequence of 16S rRNA gene was influenced by the chemical composition of two land types, such as total carbon (TC), C/N ratio, soil pH, total nitrogen (TN) and availability phosphate (AP) (Table 1). Organic matter content in the palm plantation tends to be lower, although not significant, compared to that in the Bukit Dua Belas National Park. This is most likely influenced by anthropogenic factors such as fertilizer use and land processing management.

Conversion of forestland into plantation can affect the soil chemical properties. This process changes the pH condition, carbon content, and modifies ratio of C/N and availability of phosphate in the soil. As a result of the conversion of forestland to plantation, one of the most important elements affecting the bacterial community is soil pH (Tripathi et al. 2012). Research conducted by Wan et al. (2014) showed that bacterial biomass increased with increasing pH and decreasing soil C/N ratio. pH is a major determinant of bacterial community diversity and composition, suggesting acidification has the potential to have a significant impact on bacterial-driven soil ecological processes (Wu et al. 2017). Soil pH in two oil palm plantation regions was higher than in NPBD, although the differences were not significant. This may be due to the soil acidic alleviation process during the land clearing for plantation. The tendency of ultisol forestland that is acidic, and poor of nutrients can be treated with ground limestone such as dolomite which contains Mg and Ca which are materials for soil acidic alleviation and have good effect toward soil properties such as increasing soil nutrients N and P, reducing the levels of Al and Mn, increasing soil pH and reducing the concentration of phytotoxins in highly acidic soil (Cristancho et al. 2014). Ca concentrations exhibited significant effects on active Rainforest Conversion Affects Active Bacteria communities and showed a positive correlation with increasing land use intensity from rainforest to oil palm plantation. This is most likely connected to liming practices and therefore to fertilizer application to counteract soil acidification (Tripathi et al. 2012). Previously observed changes of soil parameters after rainforest transformation to oil palm and rubber plantations indicated that the availability of N and other nutrients rely on continuous fertilization and liming (Allen et al. 2015). Thus, it is likely that the observed active bacterial community structure is highly dependent on ongoing treatment such as fertilizer application and liming.

According to Allen et al. (2015) that reduced carbon-to-nitrogen (C/N) ratio, which is known to be a significant additional driver of active bacterial communities, usually indicates a shift towards a more bacterial-dominated system. Ratio of C/N between oil palm plantation and tropical forests are not significantly different. Ratio of C/N ratio is an indicator that shows pro-

Table 1. Soil Variable Analysis.

Sample	pH (H_2O)	pH (KCl)	Total Carbon (%)	Total Nitrogen (%)	Phosphate Availability (mg/100mg)	Rasio of C/N
NPBD (TB01, TB02)	4.0± 0.70^{b}	3.7±1.20^{b}	1.33±0.23^{a}	0.11±0.48^{a}	18±0.70^{e}	12.1±0.77^{c}
Oil palm plantation (SW01, SW02)	4.6±0.42^{b}	4.0±0.70^{b}	0.98±0.33^{a}	0.09±0.01^{a}	15±0.70^{d}	10. ±0.98^{c}

Note: different letter notations indicate that the treatment given is significantly different, while the same letter notation indicates that the results obtained are not significantly different.

cess of mineralization and immobilization of N from organic material by microbial decomposers. The low C/N ratio indicates the soil containing more nitrogen, while the high C/N ratio indicates the low nitrogen content. It will make the competition between soil microbes and plants to obtain available nitrogen. The soil of palm plantations contains lower contents of nitrogen and carbon than that of NPBD, so its C/N ratio is also low. The soil with high C/N ratio needs the addition of nutrients to prevent it from nutrient deficiency, especially for nitrogen.

Generally, the change of forest land-use into plantations leads to decreasing level of organic C (Table 1). According to Allen et al. (2015) that tropical forest conversion causes biodiversity loss and promotes climate change, while also affecting the short- and long-term nutritional status of the converted land use systems. This condition was caused by some occurs much of the carbon been swept by erosion, runoff, lost in the form of gas, and also because of the absence of organic fertilization to supply of carbon in the soil. Organic carbon levels in soil cultivated with plantation crops range from low to moderate. Forestland has a higher carbon stock than plantation land, either in the body of vegetation, in the soil, or in the form of litter. It means that forestland is much more effective in absorbing and storing carbon than plantation (Monde 2009). The assessment criteria of physical and chemical properties of soil is based on Tropical Soil Quality Index (TSQI) (Arifin et al. 2012). Total carbon (0.98%) and total nitrogen (0.09 %) in oil palm plantation were classified as lowlevel category, and available phosphate (15 mg/100g) was classified as moderate level category, while total carbon (1.33%), nitrogen content (0.11%), were classified as moderate level category, and available phosphate (18 mg/100g) in forest land National Park of Bukit Dua Belas (NPBD) were classified as moderate level category. Phosphorus (P) is an essential element for all living organisms that makes up cell membranes, nucleic acids, proteins, and as the energy source such as GTP, ATP, and NADPH. This compound is also biological and environmental limiting factor and limiting nutrient for plants (Azziz et al. 2012). After nitrogen, phosphorus is the second most important critical element required by living organisms. Despite their abundance in nature, its availability is limited in the form insoluble phosphate. In nature, some bacteria species have the ability to dissolve phosphate. The abundance of phosphate in oil palm plantations decreases the amount of nutrients available to soil bacteria. The diversity and abundance of soil bacteria in oil palm plantations differs from that in the for-

est NPBD because of the availability of various nutrients. The low microbial biomass in oil palm plantations suggests that soil fertility and N availability may only temporarily decrease with the fertilization process and may not be as sustainable as in the original reference land use (Allen et al. 2015).

Soil DNA extraction and amplification of 4 Locations

The purity ratio of DNA obtained from four locations is low, around 1.35 to 1.50 (Table 2). The A260/280 ratio of good-purity DNA should be between 1.8 and 2.0, and the A260/230 ratio should be above 2.0. Total DNA with low purity, pure DNA and RNA with an A260/280 ratio of 1.8-2.0 and an A260/230 ratio higher than 2.0 were obtained using the FastDNA SPIN KIT for soil (MP Biomedicals, USA) (Yeates et al. 1998; Sambrook & Russell 2001). A280 is the wavelength for protein, A260 is for DNA and A230 is for phenol, and also humic acid. Humic acid contamination in the soil can interfere in the DNA quantification process because humic acid absorbed well A230 and A260 nm (Yeates et al. 1998). In addition the A260/A30 ratio is very low at 0.07- 0.28, this value indicates the presence of humic acid or humic acid-like molecules detected in the A230 wavelength absorption. Using nested PCR and primers p338F and p518R, the amplification of the 16S rRNA gene of bacteria in soil from tropical forests and oil palm plantations was analyzed. This technique used two-time PCR, the first and second phase PCR produced 187 bp products.

Table 2. Quantity and Quality of DNA Extraction from 4 Soil Samples.

No.	Sample	Nucleid acid Concentration (ng/µl)	260/280	260/230
1.	SW01	85.0	1.37	0.14
2.	SW02	38.0	1.50	0.07
3.	TB03	236.3	1.35	0.28
4.	TB04	144.8	1.40	0.20

The diversity of bacterial 16S rRNA Based on Method Non-Culture DGGE

This study used a non-culture approach, which not only can obtain bacteria that has been known for dominant and easily cultured, but also uncultured bacteria by genes of 16S rRNA obtained directly from environmental samples. The uncultured bacteria can be used to determine the breadth of bacterial distribution in an environment, as well as providing metabolic and biochemical information that has not before been discovered (Harris et al. 2004).

Based on the results of the DGGE, namely DNA band profile, diversity index of quantification of the BNA band intensity, dendogram analysis, OTU identity of DNA, and phylogenetic relationships among OTU(s). The results of 16S rRNA gene PCR product separation showed that the pattern of the bacterial communities varied in each sample and result of DGGE interpretation (Figure 2A). Band distribution pattern on polyacrylamide gel showed that the bacterial community in the soil samples of oil palm planta-

tion (16-24 bands) is more diverse than soil samples of NPBD (6-8 bands). Based on the results obtained, each band represents 1 species of bacteria which means that the diversity of bacteria in the oil palm plantation soil sample is more diverse than in the NPBD.

The results of cluster analysis, which compare similarities in all samples based on DGGE band location (Figure 2B), divided the samples into two groups. The soil bacterial community in the soil samples of palm plantations SW01 and SW02 have ± 99% similarity, according to an analysis of similarity patterns with binary data. Meanwhile, a community sample of soil patterns in tropical forests TB03 and TB04 also have similarities of ± 99% but it has its clusters differ with soil samples in oil palm plantations. This indicated the presence of different patterns of community with soil samples in oil palm plantations.

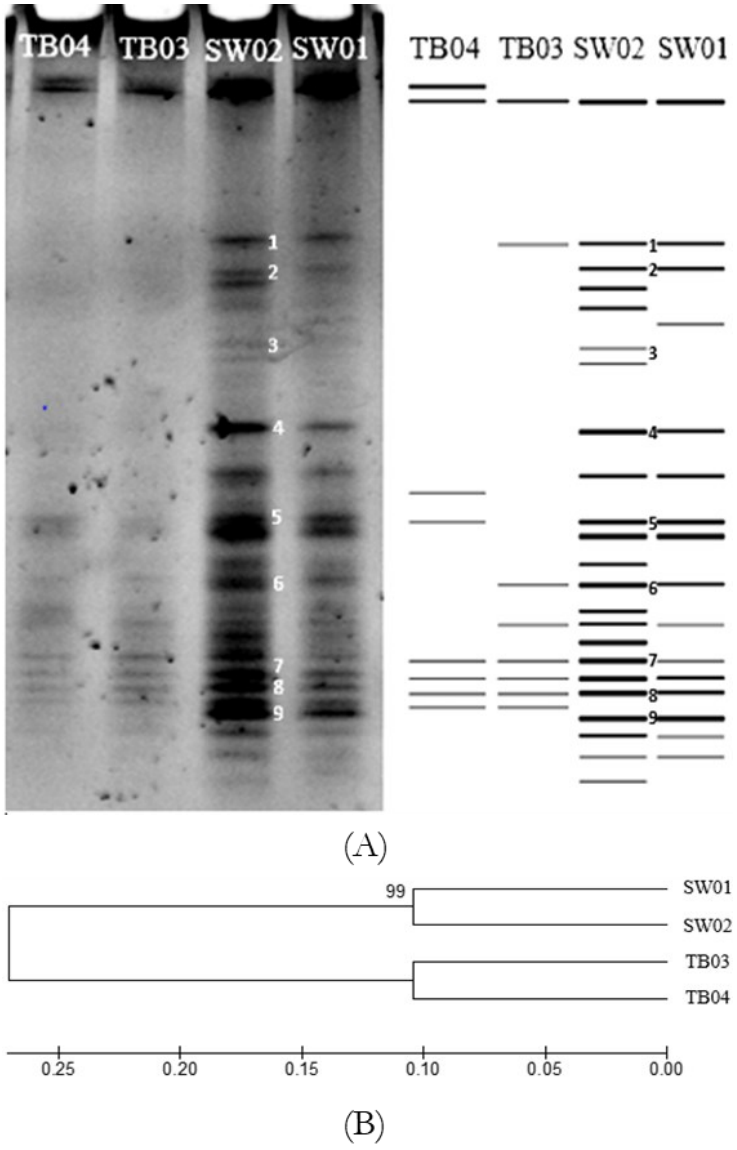


Figure 2. (A) DGGE profile of 16S rRNA from soil samples (left). DGGE illustration by CLIQS 1D software (right). Nine bands were excised for further analysis. (B) Clustering analysis of soil bacterial diversity, based on 16S rRNA gene showed different cluster between oil palm plantation of Humusindo (SW) and National Park of Bukit Dua Belas (TB) area.

Phylogenetic Analysis and BLAST

From four soil samples, DGGE analysis of the whole bacterial community identified 9 different bands. Purified DNA of DGGE bands were amplified with the same primer without GC clamps with a size of ± 180 bp product (Figure 3). Sequence alignment were conducted using Mega 6 software. The alligned sequence is a 16S rRNA sequence with a base length of about 173bp using 338f and 518r primers which amplified the third region of 16S rRNA genes (Overeas et al. 1997). The nine bands that have been successfully sequenced are then compared among the OTU sequences to see the same sequence sequence and the different base pairs sequences of the nine DGGE OTU sequences (Figure 4). The results obtained are that the nine OTU sequences have sustainable areas in some sequences marked with black areas. The gray area shows sequences between OTUs that have the same region (semi conserved region) whereas the white area is a variable region showing the difference of the basic sequences of each OTU from DGGE.

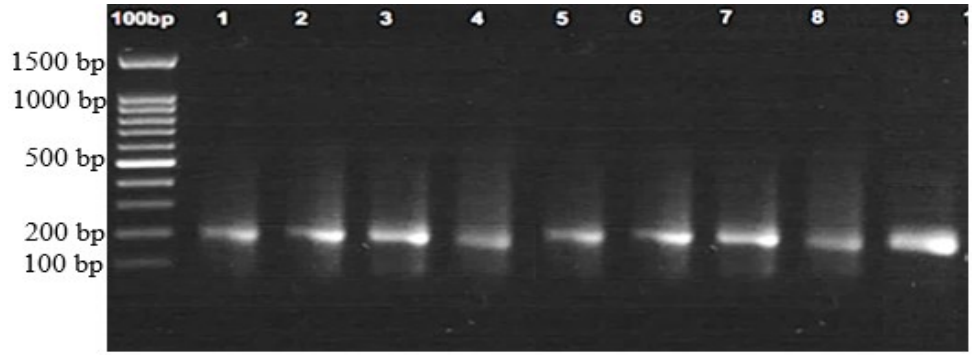


Figure 3. Amplification of 16S rRNA gene soil bacteria from four sample locations with product size ±180 bp using primer p338F (Non GC Clamps) and p518R. Lane 100 bp as a marker, lane 1 to line 9 : clone band of DGGE.

Based on sequence analysis and database in Genebank, the obtained results showed 6 bands of DGGE were identified as uncultured bacterium. However, there were 3 DGGE band identified as *Enterococcus canis* (band 1) with the similarity percentage of 94%, *Pseudomonas aeruginosa* (band 2) by 100% and the percentage of similarity *Weisselia confusa* (band 3) was about 93% (Table 3). Generally, the percentage of sequence similarity of DGGE results with Genebank database were about 89-100%.

Neighbor joining tree with bootstrap 1000x was used to construct the phylogenetic tree. The sequence analysis of 16S rDNA DGGE bands showed 3 different phylla of bacteria which were found in both of location i.e Actinobacteria, Proteobacteria (Bethaproteobacteria, and Gammaproteobacteria), Firmicutes (Figure 5). Betaproteobacteria**,** Gammaproteobacteria, and Firmicutes are 3 subphylum appearing on DGGE results from soil samples of oil palm plantations. While Actinobacteria are a group that always appear in both samples and the most dominating in both soil samples both TNBD forest and oil palm plantations.

DGGE results showed that group of species that have been identified

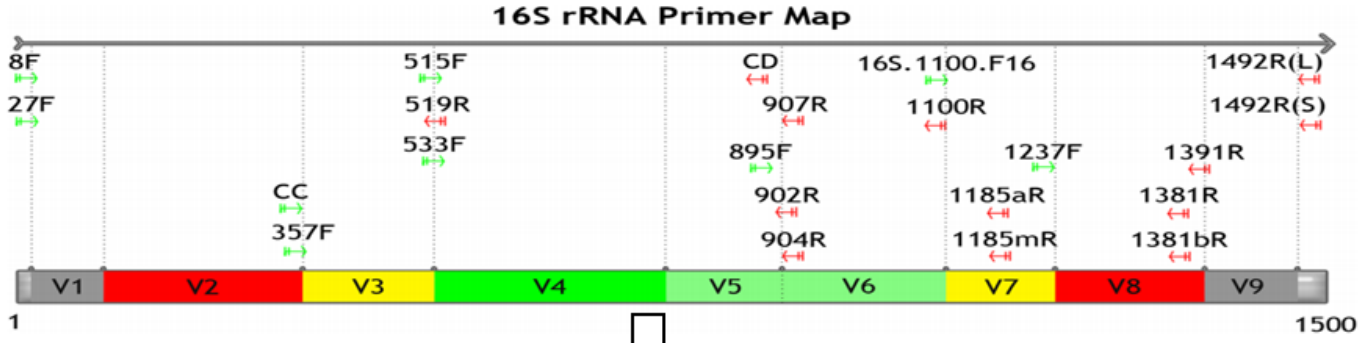
Table 3. Blast N result of DGGE bands base on 16S rRNA gene.

No.	Closely relative description	Quary Cover	E-Value	Identity	Accesion number	Family
Clone band 1	*Enterococcus canis* strain NBRC 100695	99%	3e-62	94%	NR_113931.1	Bacili/Firmicutes
Clone band 2	*Pseudomonas aeruginosa* P-hrb07	99%	2e-98	100%	GU214819.1	Gamma-Proteobacteria
Clone band 3	*Weisselia confusa*	99%	4e-76	93%	AM117158.1	Bacili/Firmicutes
Clone band 4	Uncultured Bacteria QM-73-158	92%	6e-84	100%	Ku928795.1	Beta-proteobacteria
Clone band 5	Uncultured bacterium clone A15C08	95%	6e-38	91%	JQ379115.2	Actinobacteria
Clone band 6	Uncultured bacterium clone VHA39_	99%	2e-69	95%	KX753094.1	Actinobateria
Clone band 7	Uncultured bacteria clone BButL 8P5CO2	98%	2e-83	99%	LK024724.2	Actinobacteria
Clone band 8	Uncultured bacteria clone MA-537	99%	2e-83	99%	KM 207493.1	Actinobacteria
Clone band 9	Uncultured bacteria clone Out 26067	54%	3e-16	89%	KX878538.1	Actinobacteria

were almost belong to the uncultured species in abundance. In oil palm plantations, the Actinobacteria group was the most dominant, with a higher relative abundance than forest soils. According to Lee-Cruz et al. (2013), concluded the group Actinobacteria was more abundant in palm oil than on forest land. Actinobacteria Anthropogenic activities have impacted the existence of oil palm plantations through the fertilization process. Actinobacterial shift communities on oil palm plantations, as well as ectomycorrhizal fungal communities, have been characterized as a result of anthropogenic intervention and forest conversion (Kerfahi et al. 2014). The increasing the number of Actinobateria especially actinomycetales group on deforested land explain why the increased availability of nutrients in supporting the growth of the Actinobacteria as copiotropic bacteria (Wagner & Horn 2006).

DGGE results showed that group of species that have been identified were almost belong to the uncultured species in abundance. In oil palm plantations, the Actinobacteria group was the most dominant, with a higher relative abundance than forest soils. According to Lee-Cruz et al. (2013), concluded the group Actinobacteria was more abundant in palm oil than on forest land. Actinobacteria Anthropogenic activities have impacted the existence of oil palm plantations through the fertilization process. Actinobacterial shift communities on oil palm plantations, as well as ectomycorrhizal fungal communities, have been characterized as a result of anthropogenic intervention and forest conversion (Kerfahi et al. 2014). The increasing the number of Actinobateria especially actinomycetales group on deforested land explain why the increased availability of nutrients in supporting the growth of the Actinobacteria as copiotropic bacteria (Wagner & Horn 2006).

Species were detected in DGGE results that belong to a group Firmicutes / bacilli were *Enterococcus canis* and *Weisselia confuse*. Most of the species that belong to Firmicutes phylum have a high and good resistance varied to dryness and extreme environmental factors. Because these microorganisms




```
Band_1  CAATGG-ACG AAAGCCTGAT GGAGCACCGC CGCGTGAGTG ATGAAGGTTT [ 50]
Band_2  CAATGG-GCG AAAGCCTGAT CCAGCCATGC CGCGTGTGTG AAGAAGGTCT [ 50]
Band_3  TTCTGGTAAG ATACCGTCAA ACAGTCAACA GTTACTCTCA CTGTCGTTCT [ 50]
Band_4  CAATGG-GGG AAACCCTGAC GCAGCAACGC CGCGTGGAGG ATGAAGTCCC [ 50]
Band_5  CAATGG-GCG AAAGCCTGAC GCAGCGACGC CGCGTGGGGG ATGAAGGCCT [ 50]
Band_6  ---------- TGAGAATGAC GCAGCTACGC TGCGTGG-TA ATGAAGGCCT [ 50]
Band_7  CAATGG-GGG AAACCCTGAC GCAGCAACGC CGCGTGGAGG ATGAAGTCCC [ 50]
Band_8  ---------- --AGCCTGAC GCAGCGACGC CGCGTGG-GG ATGAAGGCCT [ 50]
Band_9  CAATGG-GCG AAAGCCTGAC GCAGCGACGC CGCGTGGGGG ATGAAGGCCT [ 50]

Band_1  TC--GGATCG TAAAACTCTT GTTGTTAGAGA -AGAACGACA GTGAGAGTAA [100]
Band_2  TC--GGATTG TAAAGCACTT TTAAGTTGGGA -GGAAGGGCA GTAAGTTAAT [100]
Band_3  TCTCTAACAA CAGAGTTTTT ACGAGCCG--- -AAGACCTTC ATCACTCACG [100]
Band_4  TT--GGGATG TAAACTCCTT TTCGATC---- -GGGACGATA ATGAC----- [100]
Band_5  TC--GGGTTG TAAACCCCTT TTCAGCAGGGA CGAAGCGAAA GTGAC----- [100]
Band_6  TC--GGATTG TAAACCTCTT TTCGGCA---- -GGGACGAAG ATGAG----- [100]
Band_7  CTTAGGGGTG TAAACTCCTT TTCGATCC--- -GGGACGATT ATGACC---- [100]
Band_8  TC--GGGTTG TAAACCCCTT TTCGGCA---- -GGGACGAAG CTGAA----- [100]
Band_9  TC--GGGTTG TAAACCCCTT TTCAGCAGGGA CGAAGCGAAA GTGAC----- [100]

Band_1  CTGTTCACTG TTTGACGGT ATCTAACCAGA AAGCCACGGC -TAA---ATA [150]
Band_2  ACCTTGCTGT TTTGACGTT ACCAACAGAAT AAGCACCGGC -TAA---CTT [150]
Band_3  CGGCGTTGCT CCATCAGGC TTTCGCCCATT GGATGGAAGA TTCC---CTA [150]
Band_4  ---------- ------GGT ACCGGAAGAAG AAGCACCGGC -TAA---CTC [150]
Band_5  ---------- ------GGT ACCTGCAGAAG AAGCGCCGGC -TAA---CTA [150]
Band_6  ---------- ------GTG ACCGTTCCTGG AAACGAAGGA -TAA---CTT [150]
Band_7  ---------- ------GGT ACCGGATGAAG AAGCACCGGC GTAAACCCCC [150]
Band_8  ---------- ------GGG ACCGTACCTGC AAACGACGGG -CCA---GTT [150]
Band_9  ---------- ------GGT ACCTGCAGAAG AAGCGCCGGC -TAA---CTA [150]

Band_1  CGTGCCAGCA GCCGCGGTAATAA [173]
Band_2  CGTGCCAGCA GCCGCGGTAATAA [173]
Band_3  C-TGCTGCCT CCCGTAGGAGTAG [173]
Band_4  TGTGCCAGCA GCCGCGGTAATAT [173]
Band_5  CGTGCCAGCA GCCGCGATAATAA [173]
Band_6  CACTACAACC CACGCGCCAATAC [173]
Band_7  TGTGCCAGCA GCCGCGGTA---- [173]
Band_8  AACTACGTGC CCCCAGCCGCGAA [173]
Band_9  CGTGCCAGCA GCCGCGATAATA- [173]
```

Figure 4. Sequence Alignment of 9 16S rRNA gene sequences in the third region (V3) of soil bacteria obtained from DGGE analysis.

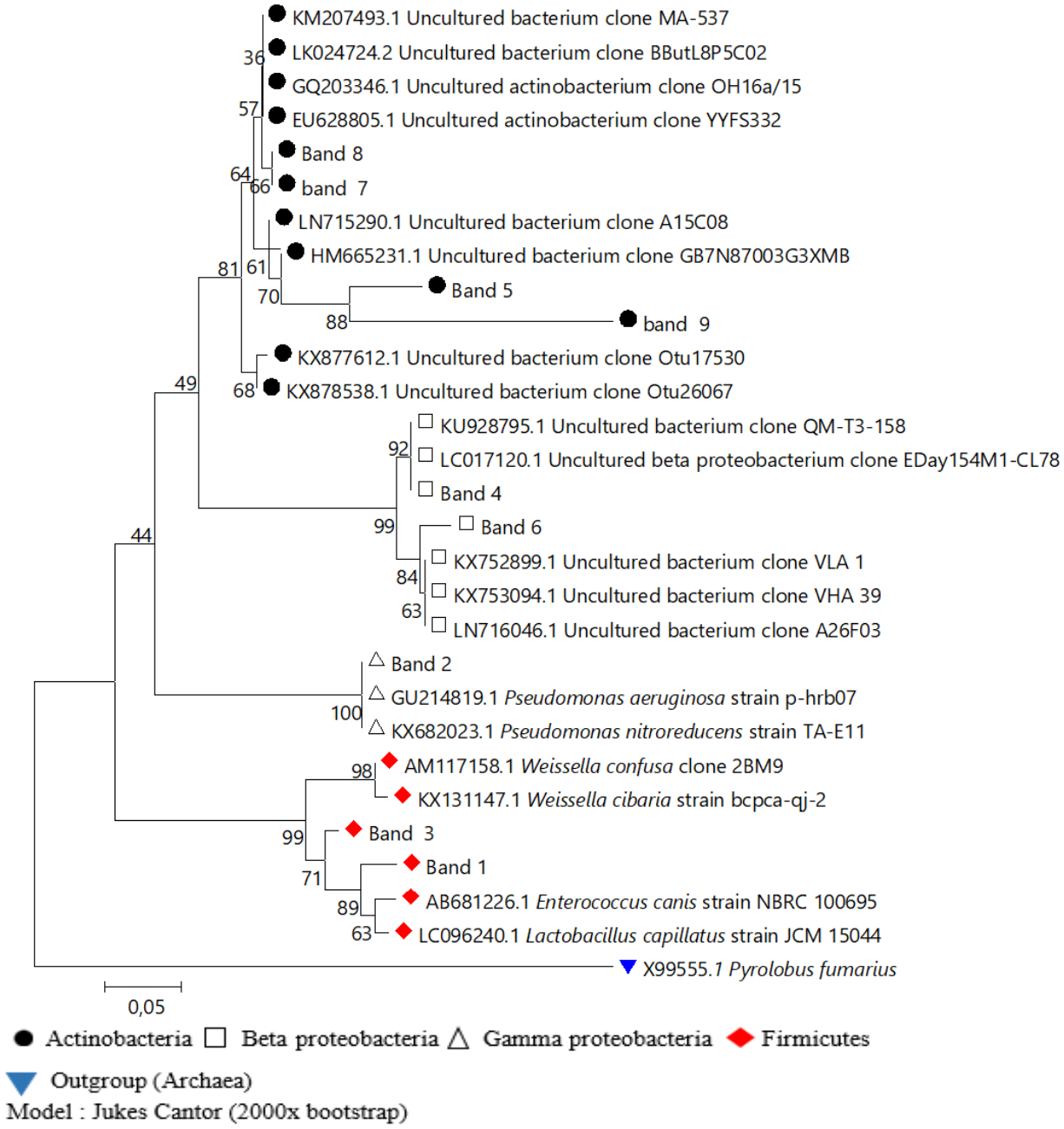


Figure 5. Phylogenetic tree of 9 DGGE bands of 16S rRNA. The tree was constructed using neighbour joining method through 2000x bootstrap.

are known to thrive in carbon-rich environments and land surface temperatures vary throughout the day, a significant rise in the relative abundance of Firmicutes sequences observed following land conversion is detected.

Species detected in DGGE results were classified in Gammaproteobacteria ie *Pseudomonas aeruginosa*, from the group Betaproteobacteria included into uncultured bacteria. This study found that there is no species belong to the group Alfaproteobacteria, Deltaproteobacteria and Epsilonproteobacteria. These results are consistent with the study by Janssen et al. (2002) which stated that the bacterial phylum Proteobacteria was the highest abundance. Alphaproteobacteria was the most abundant class followed by Gammaproteobacteria, Betaproteobacteria, Deltaproteobacteria, and Epsilonproteobabteria. The higher comparison of proteobateria in oil palm plantations compared to primary forest land because of the large influx of fertilization and

organic matter which showed the soil fertility and could decrease of competition of the microbs. The increase of bioavailable nitrogen through fertilization in intensively managed soils provides other bacterial taxa with improved growth conditions. This observation is confirmed by studies on N availability in the investigated land use systems, which decreased along the described land use gradient (Allen et al. 2015). The C to N ratio, a predictor for nitrogen availability, was lower in managed soils due to fertilizer usage (Schneider et al. 2015). Betaproteobacteria were copiotrophic, and their relative abundances were highest in soils with high C availability, either as a nutrient or as an essential ingredient of the soil. The relative abundances of Betaproteobacteria and Bacteroidetes increased while the relative abundances of Acidobacteria decreased (Fierer et al. 2007). Total carbon in oil SW sites was found to be lower than in TB sites in this study.

Shannon-Wienner (H') Diversity and Equitability (e') Index

Shannon-Wienner (H') diversity index was used to estimate the diversity of microbes in each sampling site. The results showed that the bacterial soil community diversity index (H') based on 16S rRNA gene at four locations ranged at 1.90 – 2.93. Meanwhile the equitability index (E') at four locations ranged at 0.914 – 0.939 (Figure 6).

Figure 6. Shannon-Wienner Diversity Index and Equitability Index of Bacterial community based on 16S rRNA gene.

The composition of soil bacterial community revealed in the DGGE profile showed SW01 and SW02 have relatively high abundance of bacteria compared with TB03 and TB04. This result was also confirmed by the diversity index of Shannon-Wienner. The differences of the bacterial diversity among the samples can be influenced by some factors both abiotic and biotic (Hallman et al. 1997). The soil results of cultivation produce a diversity of microbial higher than the forest soil, this can be happen due to the activity of cultivation that do like tilling the soil and run off from fertilizers produced will decrease competition nutrients that will produce more diverse of microbes (Lee-Cruz et al. 2013).

CONCLUSION

Conversion of forestland into oil palm plantation increased constantly over the last decades, which led to massive deforestation, especially on Jambi Province. It also can affect the chemical properties of the soil so it changes pH conditions may affect the soil bacterial diversity in two locations of oil palm plantations and tropical forests. Based on the number of bands 16S rRNA obtained through DGGE, the soil bacterial population in oil palm plantation seems to be more diverse than that in Bukit Dua Belas National Park. The identification of 16S rRNA and phylogenetic analysis of 16S rRNA found out four different phyla in the oil palm plantation, i.e. *Proteobacteria* (*Betaproteobacteria* and *Gammaproteobacteria*), *Firmicutes*, and *Actinobacteria*. Diversity analysis showed that Shannon-Wienner diversity index (H') of soil bacterial community in four locations ranged between 1.90 and 2.93, while the equitability index (E') ranged between 0.914 and 0.934. We stated there is an increase of soil bacteria diversity from rainforest to oil palm plantations. Some specific bacterial and archaeal taxa are present in rainforest soils and absent in other land use systems. Thus, although there is an increase in diversity in managed systems, but the conversion of rainforests to managed systems leads to a loss of bacterial biodiversity, which is coupled with a loss of traits. The long-term effect of this condition is not known and has to be determined in long-term studies, e.g., analysis of the recovery potential of soil bacterial communities after reforestation or in the absence of management treatments like fertilization.

AUTHORS CONTRIBUTION

All authors have reviewed the final version of the manuscript and approved it for publication. R.H.W., I.R. and N.R.M. designed the research, N.R.M., I.R. and M.T.S. supervised all the process, S. and M. collected and analyzed the molecular data and R.H.W. wrote the manuscript.

ACKNOWLEDGMENTS

This research was funded by DIPA BIOTROP 2016 (SEAMEO BIOTROP) PhD Grant with contract number: 0044.26/PSRP/SC/SPK-PNLT/III/2016 to Risky Hadi Wibowo and Directorate General of Higher Education Republic of Indonesia through a fellowship PhD Program (BPPDN) 2013 and to all people that help our research.

CONFLICT OF INTEREST

The authors state no conflict of interest from this manuscript.

REFERENCES

Allen et al., 2015. Soil Nitrogen-Cycling Responses to Conversion of Lowland Forests to Oil Palm and Rubber Plantations In Sumatra, Indonesia. *PLoS One*, 10(7), e0133325. doi: 10.1371/journal.pone.0133325

Arifin et al., 2012. Proposing A Suitable Soil Quality Index for Natural, Secondary and Rehabilitated Tropical Forests in Malaysia. *African Journal of Biotechnology*, 11(14). doi: 10.5897/AJB11.2903.

Armougom et al., 2009. Monitoring bacterial community of human gut microbiota reveals an increase in *Lactobacillus* in obese patients and methanogens in anorexic patients. *PLoS ONE*, 4(9), e7125. doi: 10.1371/journal.pone.0007125

Azziz et al., 2012. Abundance, diversity and prospecting of culturable phosphate solubilizing bacteria on soils under crop-pasture rotations in a no -tillage regime in Uruguay. *Apply of Soil Ecology*, 61, pp.320-326. doi:10.1016/j.apsoil.2011.10.004

Chen et al., 2011. Using DGGE and 16S rRNA Gene Sequence Analysis to Evaluate Changes in Oral Bacterial Composition. *Chinese Journal of Dental Research*, 14(2). pp.95-103. PMID: 22319750; PMCID: PMC4417478.

Cristancho et al., 2014. Aluminum speciation of amended acid tropical soil and its effects on plant root growth. *Journal Plant Nutrition*, 37(6), pp.811-827. doi: 10.1080/01904167.2014.881856

Eichner et al., 1999. Thermal gradient gel electrophoresis analysis of bioprotection from pollutant shocks in the activated sludge microbial community. *Applied and Environmental Microbiology*, 65(1), pp.102-109. doi: 10.1128/aem.65.1.102-109.1999

Fierer, N., Bradford, M.A. & Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. *Ecology*, 88, pp.1354-1364. doi: 10.1890/05-1839

Fischer S.G. & Lerman, L.S., 1983. DNA fragments differing by single base-pair substitutions separated in denaturing gradient gels: correspondence with melting theory. *Proceedings of National Academic of Sciences*, USA. 80 (6), pp.1579-1583. doi: 10.1073/pnas.80.6.1579

Glogauer et al., 2011. Identification and charachterization of new true lipase isolated through metagenomic approach. *Microbiology Cell factories*, 10, pp.10-54. doi: 10.1186/1475-2859-10-54

Hallman et al., 1997. Bacterial endophytes in agricultural crops. *Canadian Jurnal Microbiology*, 43, pp.895-914. doi: https://doi.org/10.1139/m97-131

Harris, J.K., Kelley, S.T. & Pace, N.R., 2004. New perspective on uncultured bacterial phylogenetic division OP11. *Apply Environmental of Microbiology*, 70(2), pp.845-849. doi: 10.1128/AEM.70.2.845-849.2004

Janssen et al., 2002. Improved culturabililty of soil bacteria and isolation in pure culture of novel members of the divisions acidobacteria, actonobacteria, proteobacteria, and verrucomicrobia. *Applied and Environmental of Microbiology*, 68(5), pp.2391-2396. doi: 10.1128/AEM.68.5.2391-2396.2002

Kerfahi et al., 2014. The impact of selective-logging and forest clearance for oil palm on fungal communities in Borneo. *PLoS ONE*, 9(11), e111525. doi: 10.1371/journal.pone.0111525

Ko, W.H., Wang, I.T. & Ann, P.J., 2005. A simple method for detection of lipolytic microorganisms in soils. *Journal of Soil Biology and Biochemistry*, 37 (3), pp.597-599. doi: 10.1016/j.soilbio.2004.09.006

Laurent et al., 2001. Fitness and competitive growth advantage of new gentamicin-susceptible MRSA Clones spreading in French hospitals. *Journal of Antimicrobial Chemotherapy*, 47, pp.277-283

Lee-Cruz et al., 2013. The impact of logging and forest conversion to oil palm on soil bacterial communities in Borneo. *Applied and Environmental Microbiology*, 79(23), pp.7290-7297. doi: 10.1128/AEM.02541-13

Masrukhin., Rusmana, I. & Mubarik, N.R., 2017. Effect of biofertilizer on the diversity of nitrogen - fixing bacteria and total bacterial community in lowland paddy fields in Sukabumi West Java, Indonesia. *Malaysian Journal of Microbiology*, 13(2), pp.139-146. doi: 10.21161/MJM.92916

Monde, A., 2009. Degradation of Carbon Stock (C) Due to the Use of Forest Land to Become Cocoa Land in Nopu River Basin, Central Sulawesi. Agriculture faculty. University of Tadulako. *Journal of Agroland*, 16(2), pp.110 – 117.

Murdiyarso et al., 2002. Environmental benefits and sustainable land-use options in the Jambi transect, Sumatra, Indonesia. *Journal of Vegetation Science*, 13, pp.429–438.

Muyzer, G., Waal, E.C. & Uitierlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16s rRNA. *Applied and Environmental Microbiology*, 59(3), pp.695-700. doi: 10.1128/aem.59.3.695-700.1993

Okalebo, J.R., Gathua, K.W. & Woomer, P.L., 1993. *Laboratory methods of soil and plant analysis: A working manual*, Nairobi: UNESCO.

Overeas, L., Fomey, L. & Daae, F.L., 1997. Distribution of bacterioplankton in meromictic lake saelevannet, as determined by denaturing gradient gel electrophoresis of PCR amplified gene fragments coding for 16S rRNA. *Applied and Environmental Microbiology,* 63, pp.3367-3373. doi: 10.1128/aem.63.9.3367-3373.1997

Palm et al., 2005. *Slash and Burn The Search for Alternative*, New York: Columbia University Press.

Sambrook, J. & Russell., 2001. *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Pr.

Schneider et al., 2015. Impact of LowlandRainforest Transformation on Diversity and Composition of Soil Prokaryotic communities in Sumatra (Indonesia*). Frontiers in Microbiology*, 6, 1339. doi: 10.3389/fmicb.2015.01339

Tamura et al., 2013. Mega 6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30(12), pp.2725-2729. doi: 10.1093/molbev/mst197

Tripathi et al., 2012. Tropical soil bacterial communities in Malaysia: pH dominates in the equatorial tropics too. *Microbial Ecology*, 64, pp.474-484. doi: 10.1007/s00248-012-0028-8

Wagner, M. & Horn, M., 2006. The Plantomycetes, Verrucomicrobia, Chlamydiae and Sister Phyla Comprise a Superphylum with Biotechnological and Medical Relevance. *Current Opinion in Biotechnology*, 17, pp.241–249. doi: 10.1016/j.copbio.2006.05.005

Wan et al., 2014. Soil C:N ratio is the major determinant of soil microbial community structure in subtropical coniferous and broadleaf forest plantations. *Plant and Soil*, 381(1-2). doi:10.1007/s11104-014-2277-4

Wijayanti et al., 2014. Diversity and the Composition of Fatty Acids of Lipolytic Bacteria Isolated from Soil and Aquatic Sediment in a Forest and on an Oil Palm Plantation. *Makara Journal of Science*, 18(3), pp.71-78. doi: 10.7454/mss.v18i3. 3718

Wu et al., 2017. pH Is The Primary Determinant of The Bacterial Community Structure In Agricultural Soils Impacted By Polycyclic Aromatic Hydrocarbon Pollution. *Scientific Report*, 7, 40093. doi: 10.1038/srep40093

Xue et al., 2008. Soil microbial community structure in diverse land use systems : A comparative study using biolog, DGGE, and PFLA analyses. *Pedosphere*, 18(5), pp.653-663.

Yeates et al., 1998. Methods for microbial DNA extraction from soil for PCR amplification. *Biological Procedures Online*, 1(1), pp.40-47. doi: 10.1251/bpo6

Yule, C.M., 2010. Loss of biodiversity and ecosystem functioning in Indo-Malayan peat swamp forests. *Biodiversity Conservation*, 19, pp.393–409. doi: 10.1007/s10531-008-9510-5