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Community of Soil *Actinobacteria* in PTPN VI Oil Palm Plantation Jambi (Sumatra, Indonesia) Based on Amplicon Sequencing of 16S rRNA Gene

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ABSTRACT

In Sumatra, Indonesia, increased oil palm production encourages land expansion for oil palm plantations. And soil *Actinobacteria* have a potential role in agriculture and plantations ecosystems. The use of fertilizer and herbicide affects soil microbial diversity, including *Actinobacteria*. This research analyzed and investigated the community composition and diversity of *Actinobacteria* in soils of oil palm plantations in Jambi Sumatra. Amplicon-based analysis of the 16S rRNA gene (V3-V4 hypervariable region) was used to amplify actinobacterial full-length 16S sequences. The V3-V4 actinobacterial specific 16S rRNA gene sequencing was done using Next-Generation Sequencing. This study confirmed that actinobacterial specific 16S rRNA gene primer could amplify the actinobacterial 16S rRNA gene. Frankiales dominated the community composition of soilborne *Actinobacteria*. The diversity and community composition of soilborne *Actinobacteria* were not significantly affected by the interaction between fertilization and weed treatments. Furthermore, the use of NPK fertilizer significantly affected the abundance of *Kineosporiales*, whose abundance increased with the increasing concentration of NPK fertilizer. The interaction between fertilization and weeding treatments in the oil palm plantations has no impact on soil Actinobacteria's community composition and diversity.

1. Introduction

Palm oil is one agricultural commodity that significantly contributes to the Indonesian economy. Nearly 70% of oil palm plantations in Indonesia are located at Sumatra Island, and the remaining around 30% are at Kalimantan Island. Increased production of oil palm encourages land expansion for oil palm plantations. Most of the lowland forest on Sumatra Island has been converted to rubber (*Hevea brasiliensis*) and oil palm (*Elaeis guineensis*) plantations (monoculture of *Elaeis guineensis*) (Villamor *et al.* 2014). Deforestation and forest transformation impact bacterial diversity and community composition (Schneider *et al.* 2015).

Soil microbes are organisms that have essential roles in determining soil conditions. Soil microbes also contribute to the availability of nutrients in the soil, playing a significant role in plant growth (Nannipieri *et al.* 2003). Soil bacteria are reported as soil organic matter decomposers. They can also recycle the organic residues to make associations with plant roots that eventually help supply essential nutrients such as nitrogen, phosphorus, and potassium. The potential of soil bacteria such as *Pseudomonas corrugate*, *Azospirillum brasilense, Rhizobium* sp., *Bacillus subtilis*, and *Streptomyces nojiriensis* for increased plant growth as well as pest and disease control were reported (Bhattacharyya *et al.* 2016).

One of the microbial groups found in soil and known to have many vital roles is *Actinobacteria*. They belong to Gram-positive Bacteria and are widely distributed in nature, especially in soils (Ludwig *et al.* 2012; Castaneda and Barbosa 2017). *Actinobacteria* also play an essential role in agriculture and plantations ecosystems, among others, secreting secondary metabolites to counteract pathogenic fungi responsible for leaf spot, stem rot, and brown germ

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disease on oil palm (Pithakkit *et al.* 2015; Muzaimah *et al.* 2015).

Actinobacteria also have a potential role as plant growth-promoting rhizobacteria (PGPR). PGPR are free-living beneficial bacteria that important for agriculture. The PGPR mechanisms are the production of siderophores, indole acetic acid (IAA), nutrient solubilization, and antagonistic or beneficial synergistic effects (Franco-Correa *et al.* 2010). *Actinobacteria* is one of the most effective microbes in terms of siderophores production. Some genera of *Actinobacteria*, such as *Streptomyces* and *Thermobifida*, reported a grand synthesis of siderophores (Dimise *et al.* 2008).

Furthermore, IAA is a plant growth hormone and an active form of auxins. IAA plays a vital role in plant development through its life cycle. The production of IAA has been widely studied in *Actinobacteria*. Some genera of *Actinobacteria* such as *Streptomyces* sp., *Nocardia* sp., and *Kitasatospora* sp. have been reported as IAA producers (Dimkpa *et al.* 2008; Shrivastava *et al.* 2008; Sousa *et al.* 2008). Moreover, *Frankia* sp. has been known as nitrogen-fixing *Actinobacteria*. *Frankia* sp. play a role in symbiotic interaction with the plant roots called actinorhizal symbiosis (Khan *et al.* 2007).

Additionally, *Actinobacteria* also have a potential role as mycorrhiza helper bacteria. The majority of plants are symbiotic with mycorrhiza to increase the growth rate. *Streptomyces* and *Thermobifida improved* the mycelium growth of *Glomus* and *Gigaspora*. The existence of *Streptomyces* also has a beneficial effect and a good correlation with the germination of the mycorrhizal spores (Merzaeva and Shirokikh 2006).

Soil *Actinobacteria* have great potential as plant growth-promoting rhizobacteria. So this research was conducted to explore the diversity of soil *Actinobacteria* and analyze their relation with soil health. However, not all *Actinobacteria* can be cultured or grown in the laboratory. A solution to overcome the difficulties and limitations of the cultivation technique is metagenomic approaches. These approaches can be used to analyze the community and diversity of *Actinobacteria* without culturing process. A technique that can be used in metagenomic approaches is amplicon sequencing.

PT Perkebunan Nusantara (PTPN) VI is a state-owned enterprise in Jambi Province (Sumatra, Indonesia) established since 1996. The company is engaged in oil palm (90%), rubber, and tea processing agro-industry. The PTPN VI Jambi use fertilizers and herbicide on oil palm plantations. The fertilizers used in PTPN VI oil palm plantations are NPK (Nitrogen, Phosphorus, and Potassium) fertilizers, and glyphosate was used as an herbicide. The use of fertilizers and herbicides can affect soil microbial activities. The application of herbicides reduced the population of all the bacteria (Latha and Gopal 2010; Sathiyavani *et al.* 2015). Otherwise, the community of soil bacterial compositions was increased after the NPK fertilization treatments (Pan *et al.* 2014).

The community composition and diversity of soilborne *Actinobacteria* in soils of oil palm plantation in PTPN VI had not yet been described. So, this study aimed to analyze and investigate the community and diversity of *Actinobacteria* in the soil of PTPN VI plantation affected by the fertilizers and herbicides application in the soils.

2. Materials and Methods

2.1. Soil Sample Collection

The sampling sites were in PTPN VI oil palm plantation Jambi, Sumatra, Indonesia. Soil sampling was carried out at 4 locations of Oil Palm Management (OM) with four different treatments (core plots OM1, OM2, OM3, and OM4). The four treatments are a combination of different concentrations of NPK fertilizer and the weeding method used in the oil palm plantation system. There are two concentrations of fertilizer used, i.e., 260 N, 50 P, 220 K kg ha^{-1} $year^{-1}$ (conventional fertilization, designated “C”) and 136 N, 17 P, 187 K kg ha^{-1} $year^{-1}$ (reduced fertilization, designated "R"). There are two types of weeding treatments, i.e., using herbicides 500 cc ha^{-1} $year^{-1}$ (designated “H”) and mechanical weeding (designated “W”).

The four treatments combination of soil conditions, i.e., the soil was fertilized with 260 N, 50 P, 220 K kg ha^{-1} $year^{-1}$. It was weeded with glyphosate herbicide 500 cc ha^{-1} $year^{-1}$ (named “CH”), the soil was fertilized with 260 N, 50 P, 220 K kg ha^{-1} $year^{-1}$ and was weeded mechanically (named "CW"), the soil was fertilized with 136 N, 17 P, 187 K kg ha^{-1} $year^{-1}$. It was weeded with glyphosate herbicide 500 cc ha^{-1} $year^{-1}$ (named “RH”), and soil was fertilized with 136 N, 17 P, 187 K kg ha^{-1} $year^{-1}$ and was weeded mechanically (named “RW”). The NPK fertilizer was applied at about 1 m from the palm base, and the glyphosate herbicide was applied in a 2 m radius around palms. Mechanical weeding was conducted around each palm in a 2 m

radius from the palm base. Soil sampling was carried out at four core plots (OM1, OM2, OM3, and OM4) and four types of soil treatments (CH, CW, RH, and RW) in each core plot. Soil samples were taken from each soil treatment and were replicated five times for each soil treatment, resulting in a total of 80 soil samples (Figure 1).

Soil samples were taken from a depth of ~10 cm (topsoil) with a soil corer (~7 cm in diameter), and the sampling point was about 1 m from the oil palm tree. Three cores were taken per subplot, mixed, freed from roots and stones by hand, and supplemented with RNAprotect (Qiagen, Hilden, Germany). Subsequently, the soil samples were stored in cool boxes on ice packs and transported to the laboratory in Indonesia. The soil samples were frozen and stored at -20°C until shipment to the laboratory in Goettingen, Germany. The soil samples were transported in frozen using ice packs to Germany, and the soil samples were stored at -80°C until further use.

2.2. DNA Isolation and *Actinobacterial* Specific 16S rRNA Gene Amplification

To analyze the community and diversity of soil actinobacteria, DNA was isolated from all 80 soil samples by using the PowerSoil® DNA Isolation Kit (Qiagen, Hilden, Germany). DNA yields were measured using a Nanodrop Spectrophotometer ND-1000 (Thermo Fisher Scientific, Massachusetts, USA).

Semi-nested amplification reactions were performed. The first amplification was done to amplify the *Actinobacterial* specific 16S rRNA full-length gene using a specific primer pair (27F 5'-AGAGTTTGATCCTGGCTCAG-3'16Sact1114R 5'-GAGTTGACCCCGGCRGT-3') as described in Martina *et al.* (2008). The first amplification was performed to amplify only the actinobacterial specific 16S rRNA gene. This process allows us to investigate only the community of *Actinobacteria* specifically. The second amplification was carried out to amplify the V3 to V4 region of the previously amplified 16S rRNA gene

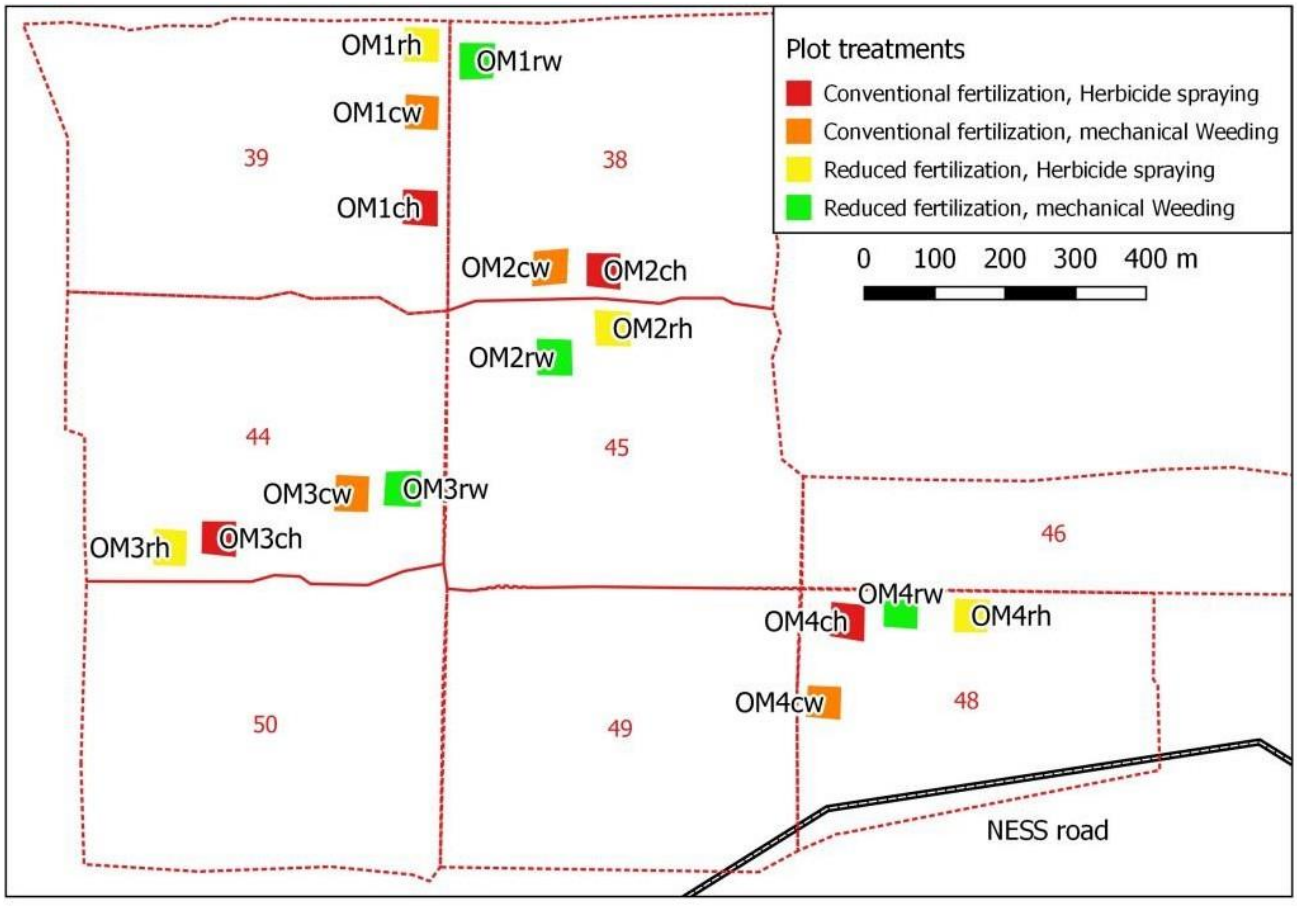

Figure 1. Map of soil sampling design in PTPN VI oil palm plantation at Jambi Sumatra, Indonesia

using the specific primer to identify *Actinobacteria* (341F 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGA CAGCCTACGGGNGGCWGCAG-3" and 805R 5'-GT CTCGTGGCTCGGAGATGTGTATAAGACAGGACTACH VGGTATCTACC-3"). The primers had been combined with specific adapters for the subsequent sequencing process (Klindworth *et al.* 2013).

One soil sample from each soil treatment (CH, CW, RH, RW) was chosen randomly as a control. Only one amplification reaction was performed on control samples. The amplification was performed to directly amplify the hypervariable V3 to V4 region of the 16 rRNA gene. The specificity of the primer used was investigated to increase the possibility of actinobacterial specific 16S rRNA gene amplification. The V3-V4 hypervariable regions can analyze the soil *Actinobact* (Schuwirth *et al.* 2005; Bukin *et al.* 2019).

The reaction mixture for the first amplification (25 µl total volume) contained 5 µl 5x GC buffer, 1 µl primer 27F, 1 µl primer 16Sact1114R, 0.2 µl $MgCl_2$, 2.5 µl DMSO, 1 µl dNTPs, 0.5 µl polymerase, 11.8 µl DEPC water, and 2 µl template DNA. The thermal cycling for amplification of actinobacterial specific 16S rRNA genes was set up for 30 cycles with an initial denaturation at 98° C for 1 minute, denaturation at 98° C for 45 seconds, annealing at 55°C for 45 seconds, elongation at 72°C for 30 seconds, and final elongation at 72°C for 5 minutes. Amplicons were purified using MagSi® NGSPREPplus (Magna Medics, Wiesenbach, Germany). Purified DNA was estimated by using a Nanodrop Spectrophotometer ND-1000 (Thermo Fisher Scientific, Massachusetts, USA).

The second amplification was performed similarly to the first amplification except the volume used for $MgCl_2$ was 0.75 µl, 1.25 µl DMSO, 0.5 µl dNTPs, 0.25 µl polymerase, 14.75 µl DEPC water, and 0.5 µl template DNA.

The amplicons were purified using MagSi® NGSPREPplus (Magna Medics, Wiesenbach, Germany) before proceeding to the next sequencing stage. The Goettingen Genomics Laboratory used the Nextera DNA library kits (Ilumina Inc., San Diego, USA) for library preparation and determined the hypervariable V3 to V4 region sequences actinobacterial specific 16s rRNA gene amplicons by using Ilumina Miseq (Ilumina Inc., San Diego, USA).

2.3. Analysis of 16S rRNA Gene Sequences

The resulting hypervariable V3 to V4 region of actinobacterial specific 16s rRNA gene sequences were processed and analyzed using QIIME 1.9 (Caporaso *et al.* 2010). Quality filtering was performed using fastp (Chen *et al.* 2018). Paired-end sequences were joined with PEAR 0.9.11 (Zhang *et al.* 2014). The remaining primer sequences were clipped with cutadapt 1.16. Additionally, size filtering, dereplication, denoising, and removal of the chimera sequences were performed with VSEARCH 2.8.0 (Rognes *et al.* 2016). All reads were mapped against the SILVA database 132 with the BLAST tool. Taxonomic classification was performed with QIIME script parallel_assign_taxonomy_blast.py. Determination of operational taxonomic units (OTUs) was performed at a genetic divergence of 3% (similarity 97%) with pick_open_reference_otus.py based on SILVA 132 NR SSU database as reference (Quast *et al.* 2013). OTU tables were created using make_otu_table.py. Singletons, chloroplasts, unclassified OTUs, and extrinsic domain OTUs were removed from the table by employing filter_otu_table.py. Biodiversity and the rarefaction curve were calculated using QIIME script alpha_rarefaction.py. Non-metric multidimensional scaling (NMDS) was performed in R (R Development Core Team 2017) based on a weighted UniFrac distance matrix (Lozupone *et al.* 2011).

Significant differences between samples and controls were determined calculated with t-test analysis and ANOVA using R (R Development Core Team 2017). The same analysis was performed to determine the treatments (fertilization and weeding) that significantly influenced the diversity and community of soil *Actinobacteria*. The results were interpreted with a significance level of $p < 0.05$ (significance level 5%).

3. Results

3.1. Diversity and Community Composition of Soilborne *Actinobacteria*

Eighty soil samples were successfully analyzed based on the V3 to V4 hypervariable region of the actinobacterial specific 16S rRNA gene. Sequencing the 16S rRNA gene from all sampling plots resulted in 7,948,649 reads and 6,807,533 high-quality reads, comprised of 1847 OTUs with 97% sequence identity (3% dissimilarity).

In the "samples soil" (nested-PCR) dataset, most of the classified sequences (93.4%) belong to phylum *Actinobacteria*. Like *Planctomycetes, Proteobacteria*, and others, the other phyla were smaller than 2% (Figure 2). Conversely in the "control soil" (single-PCR) dataset, *Acidobacteria* (25.5%), *Proteobacteria* (18.5%),

and *Chloroflexi* (14.7%) were the most abundant phyla. In the "control soil," the relative abundance of *Actinobacteria* is no more than 9.5%. The relative abundance of phylum *Actinobacteria* in "samples soil" is significantly different from the controls ($p<0.05$), with *Actinobacteria* as the dominant phylum.

The number of actinobacterial OTUs in soil samples was weeded by glyphosate was higher than the soil samples was weeded mechanically. Moreover, the alpha diversity indices Shannon, Simpson, and Chao1 were also higher in soil samples weeded by glyphosate. However, the statistical test confirmed that the number of OTUs, the alpha diversity indices Shannon, Simpson, and Chao1 in the four soil treatments did not show significant ($p<0.05$) differences (Table 1).

The similarity of the soilborne *Actinobacteria* diversity in the four different soil treatments was confirmed with non-metric multidimensional scaling (NMDS, Figure 3). The NMDS plot also explained that the fertilization and weeding treatments did not affect the diversity of soilborne *Actinobacteria* in the oil palm plantations.

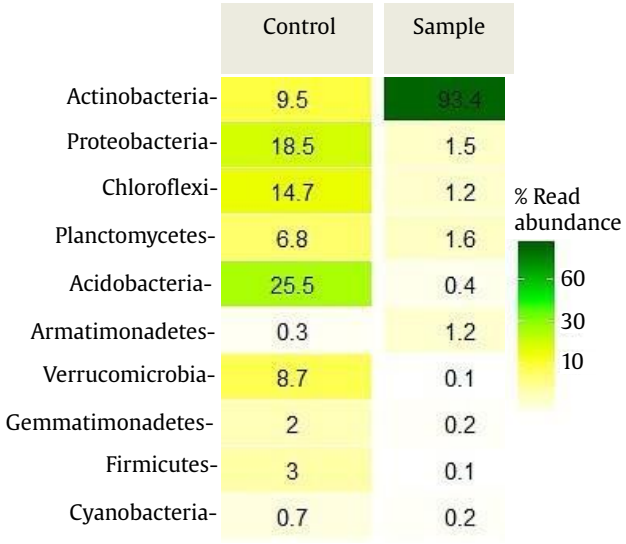

Figure 2. Communities of soil bacteria in PTPN VI oil palm plantation Jambi Sumatra. Community compositions are displayed as relative abundance at the phylum level based on the sequences of the 16S rRNA gene (control: single-PCR, sample: nested-PCR)

3.2. Effects of Fertilizer and Weeding Treatments on *Actinobacteria* Community

Around 50% of *Frankiales* consistently dominated in the four different soil conditions. The community composition of soilborne *Actinobacteria* in the four different soil treatments showed similar trends (Figure 4). The abundance of order *Micromonosporales*, IMCC26256, *Microtrichales*, *Micrococcales*, *Corynebacteriales*, OTU-97-2, *Streptomycetales*, *Propionibacteriales*, and *Kineosporiales* in the four

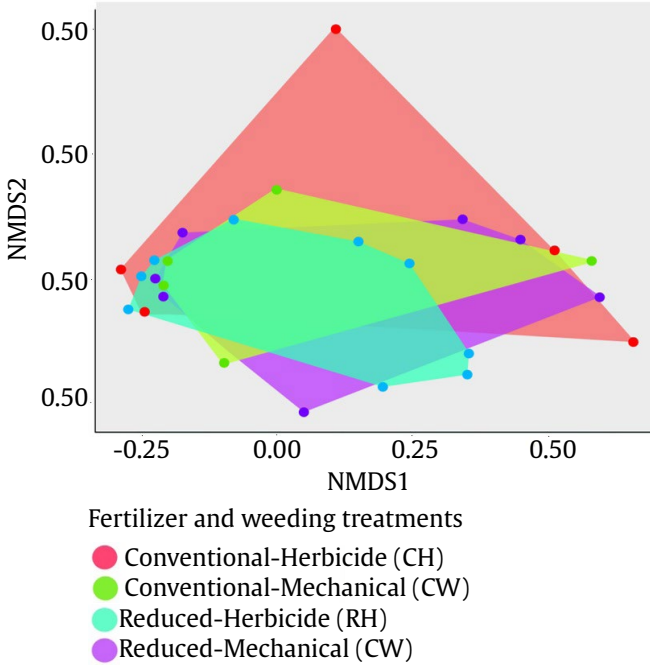

Figure 3. Non-metric multidimensional scaling (NMDS) of soilborne *Actinobacteria* in four different soil treatments in the oil palm plantations Jambi, Sumatra based on weighted Unifrac distance matrics

Table 1. Number of sequences and OTUs (97% similarity) and alpha diversity indices of soil *Actinobacteria* in the four different soil treatments

Soil samples	Number of reads		Number of OTUs	Alpha diversity indices		
	Total	High-quality		Shannon	Simpson	Chao1
Conventional –Herbicide (CH)	1,858,239	1,622,538	484	4.60	0.97	511
Conventional-Mechanical (CW)	2,292,847	1,967,018	460	4.48	0.96	482
Reduce-Herbicide (RH)	1,941,323	1,755,690	477	4.59	0.97	493
Reduce-Mechanical (RW)	1,856,240	1,462,287	467	4.52	0.97	486

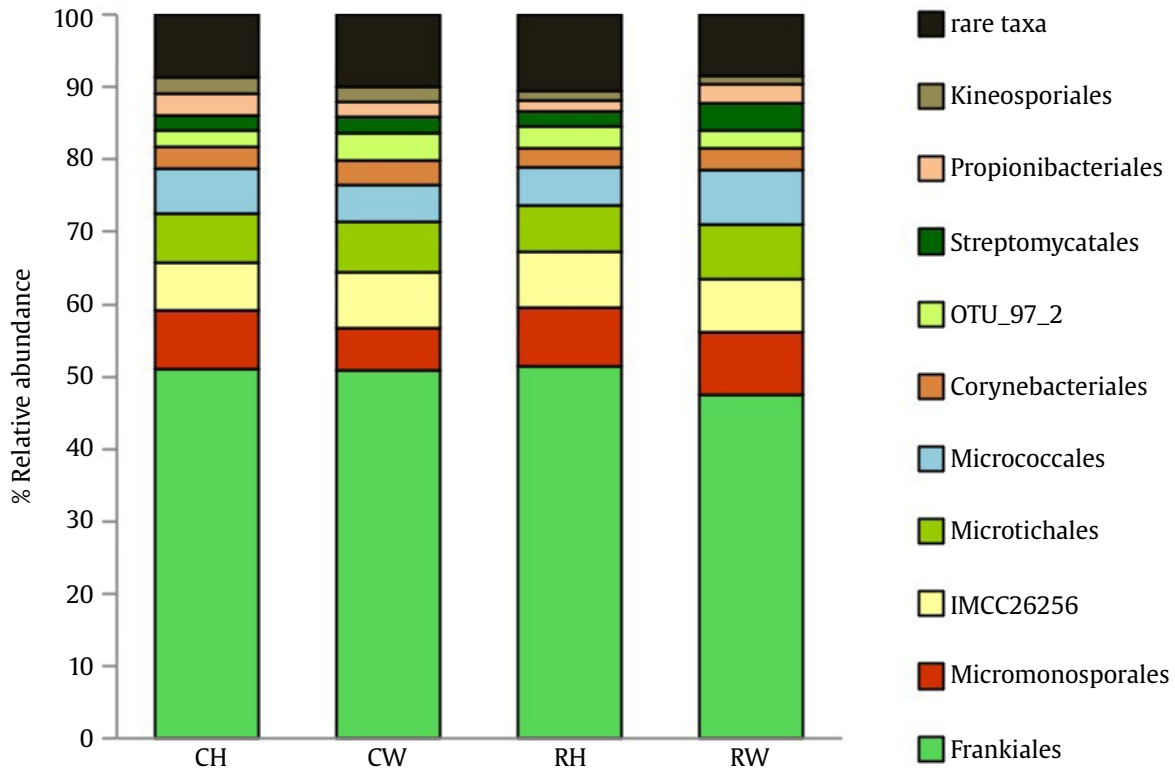

Figure 4. Community composition of soilborne *Actinobacteria* in four different soil treatments in oil palm plantations. Community compositions are displayed in relative abundances at order level based on 16S rRNA sequences. Actinobacterial taxa with an abundance below 2% were summarized as 'rare taxa'

different soil treatments also resulted in similar patterns.

Statistical tests ANOVA confirmed the interaction between fertilization and weeding treatments had no significant effect ($p<0.05$) on the community composition of soilborne *Actinobacteria* in the oil palm plantation. Furthermore, the abundance of *Kineosporiales* was significantly ($p<0.05$) affected by NPK fertilizer. The higher concentration of NPK fertilizer used, the greater abundance of *Kineosporiales* (1.25% in reduced fertilization increased to 2.2% in conventional fertilization).

4. Discussion

4.1. Diversity and Community Composition of Soilborne *Actinobacteria*

The presence of specific bacterial phyla in the plantation's soil was reported to have many important ecological functions. *Proteobacteria* and *Acidobacteria* were dominant phyla in the oil palm plantation Jambi, Sumatra. Phylum *Proteobacteria* and *Acidobacteria* were previously reported as generally high abundant in soils, and they have an important role in the decomposition of soil carbon (Hansel *et al.* 2008; Leff *et al.* 2012; Schneider *et al.* 2015; Wijayanti *et al.* 2019). Furthermore, *Chloroflexi* was also found in the oil palm plantation soil. The *Chloroflexi* was mainly associated with oil palm and crop management in the agricultural system (Bouskill *et al.* 2013; Orr *et al.* 2015; Wijayanti *et al.* 2019). The abundance of *Actinobacteria* in the oil palm plantations soil was less than *Proteobacteria, Acidobacteria*, and *Chloroflexi*. According to Yun *et al.* (2016), *Actinobacteria* was more abundant in soils with a neutral or alkaline pH. However, previous studies confirmed that the oil palm plantations soil in Jambi, Sumatra has a low pH of about 4.5 (acidic soil) (Wijayanti *et al.* 2019).

The actinobacterial specific 16S rRNA gene primer has been successfully amplified only the actinobacterial 16S rRNA gene. The results were indicated by the significant differences in the abundance phylum *Actinobacteria* between "soil samples" (nested-PCR) and control soil" (single-PCR). In the "samples soil" dataset, most of the classified sequences belong to phylum *Actinobacteria*. According to the previous studies, Martina *et al.* (2008) reported that the primers 16Sact1114R and

27F are specific primers to amplify actinobacterial specific 16S rRNA genes. The primers were able to identify *Actinobacteria* to the genus level.

The other actinobacterial-specific primers are Com2xf/Ac1186r (Schafer *et al.* 2010) and S-C-Act-0235-a-S-20/S-CAct-0878-a-A-19 (Stach *et al.* 2003). The *Actinobacteria* specific primer detected around 87% sequences were assigned to actinobacterial genera. Tang *et al.* (2016) confirmed that 75 of 425 clones belonged to *Actinobacteria* by employing the primers. Compared with the two actinobacterial-specific primers, the primer used in this study (27F/16Sact1114R) was expected to have a higher level of specificity because by using the primers, the number of *Actinobacteria* detected was higher. However, further research is still needed to compare the level of specificity of all the actinobacterial-specific primers systems. Indeed, the use of actinobacterial-specific primers increased the amplification of actinobacterial 16S rRNA genes, although some 16S rRNA genes from other bacterial phyla were still amplified in smaller amounts.

The diversity and species richness of soilborne *Actinobacteria* in the four different soil treatments in the oil palm plantation was similar. It revealed that the utilization of NPK fertilizers and glyphosate herbicide in the oil palm management system did not affect the diversity of soil *Actinobacteria.* This result corresponds to the studies that reported that the diversity of *Actinobacteria* was not affected by crop management. However, the diversity and abundance of soil *Actinobacteria* in the long-term fertilization field were primarily affected by the sampling time and seasonal variation (Chhabra *et al.* 2013; Orr *et al.* 2015). In this study, we collected the samples in the same year. Therefore the diversity of soil *Actinobacteria* did not differ significantly.

We expected the *Actinobacteria* was indigenous microbes in the oil palm plantations soil. The *Actinobacteria* already existed in the soil before the NPK fertilizer and herbicide were applied to the oil palm plantations system. Furthermore, the previous study reported that ancient actinobacterial DNA showed no damage (Zaremba-Niedzwiedzka and Andersson 2013). *Actinobacteria* are microbes that can survive and adapt well in various environments. However, the ecological function of actinobacteria is mostly unknown. Therefore, the diversity of soil *Actinobacteria* based on 16S rRNA gene sequences in the four different soil treatments was similar. Tang *et al.* (2016) suggested that each ecologically similar study site shows similar *Actinobacteria* diversity due to identical environmental conditions. We collected soil samples from one study site in this study, although there were four different treatments. It might be that the causes of soil Actinobacteria's diversity did not differ significantly.

4.2. Effects of Fertilizer and Weeding Treatments on *Actinobacteria* Community

The community composition of soilborne *Actinobacteria* in the four different soil treatments consistently was dominated by *Frankiales*. The order *Frankiales* was soil *Actinobacteria* found abundantly in Sumatra's forest and oil palm plantations. Berkelmann *et al.* (2018) reported that *Frankiales* dominated the soil bacterial community in rubber forest and oil palm plantations in Jambi, Sumatra. Moreover, *Frankiales* was also reported to be the most abundant *Actinobacteria* in the rainforest soil in Sumatra (Schenider *et al.* 2015). In general, *Frankiales* are related to *Actinobacteria* that are symbiotic with plant roots and play a role in forming the nodules for nitrogen fixation and related to the nitrogen cycle in the environment (Franco-Correa *et al.* 2010). The *Frankiales'* existence in the soil of palm plantations was also related to the application of NPK fertilizers.

The community of soilborne *Actinobacteria* in the oil palm plantations was also composed of *Micromonosporales*, IMCC26256, *Microtrichales, Micrococcales, Corynebacteriales*, OTU-97-2, *Streptomycetales, Propionibacteriales*, and *Kineosporiales*. The existence of order *Micrococcales* and *Streptomycetales* was related to the fertilization system. *Micrococcal* and *Streptomycetales* were identified as a bioindicator for fertilizer treatments in agricultural soil (Harkes *et al.* 2019), although this study revealed that the fertilizer treatments did not influence the abundance of *Micrococcales* and *Streptomycetales*. Additionally, *Propionibacterales* were reported predominately also found in agricultural soil. Unfortunately, the important role of these taxa is still not known. *Micromonosporales* could dissolve phosphate in the environment (phosphate solubilizers) (Wang *et al.* 2016; Yeager *et al.* 2017). Therefore, the abundance of *Micromonosporales* in the plantation soil is supposedly related to NPK fertilizer application.

The interaction between fertilization and weeding treatments did not significantly affect the community composition of soilborne *Actinobacteria* in the oil palm plantation. Furthermore, the abundance of

Kineosporiales was significantly affected by NPK fertilizer.

Kineosporiales was reported as a bioindicator for fertilization treatments in agricultural soil (Harkes *et al.* 2019). Previous studies reported that the diversity of soil *Actinobacteria* overrepresented under NPK fertilizer treatments (Pan *et al.* 2014). Specifically, the diversity of *Actinobacteria* was increased by nitrogen (N) fertilizer (Wang *et al.* 2018). However, N addition decreases the decomposition of recalcitrant carbon (C), reducing the members of the phylum *Actinobacteria*, as they play an important role in the carbon cycle (Craine *et al.* 2007; Ventura *et al.* 2007). Additionally, another study confirmed that the utilization of NPK fertilizers has various effects on microbial community composition that depend on the duration of the experiment (Ramirez *et al.* 2010).

Besides NPK fertilizer, herbicide spraying in the oil palm plantations may affect the soil bacterial composition (Lane *et al.* 2012). Glyphosate (N-(phosphonomethyl)-glycine) is one of the most commonly used herbicides in agricultural systems in developed countries. Glyphosate is a herbicide with a broad spectrum, nonselective, and post-emergence herbicide. It is generally applied to control weeds on agricultural and plantation fields (Baylis *et al.* 2000; Duke and Powles 2008). Sebiomo *et al.* (2011) reported that the soils treated with glyphosate had decreased the population of microbes. Otherwise, Araujo *et al.* (2003) and Haney *et al.* (2002) explained the diversity of *Actinobacteria* increases in soils treated with glyphosate compared to untreated soil. However, early studies confirmed that soil *Actinobacteria* are unaffected by glyphosate (Wardle and Parkinson 1990). Allegrini *et al.* (2015) also confirmed no significant differences in diversity and abundance of soil microbial profiles after glyphosate application.

The half-life time of glyphosate in the soil also contributes to microbes' resistance or susceptibility to the herbicide. The abiotic environmental factors and the biodegradation could attenuate glyphosate toxicity, which has been well described (Toretta *et al.* 2018). Hence, the microbial community tolerance to glyphosate is not consistent, depending on the history exposure, the concentration of herbicide used, and the particular taxa of soil microbes themselves. Additionally, Duke and Powles (2008) confirmed the range of sensitivity to glyphosate within the soil microbial community is not fully known.

This study confirmed that fertilization and weeding treatments in the oil palm plantations do not affect soilborne Actinobacteria's community composition and diversity. Torsvik *et al.* (2008) reported that *Actinobacteria* are a group of bacteria with a high physiological and ecological plasticity level. These allow *Actinobacteria* to survive and adapt well in various environments. Moreover, the diversity and community composition of soilborne *Actinobacteria* analyzed in this study were DNA. According to Carini *et al.* (2016), DNA-based studies lead to around 55% increase in the observed soil prokaryotes. This result could lead to a significant misinterpretation of the taxon relative abundances. After the death of the cells, the relic DNA remained in the soil, which may obscure the treatment effects and relationship between bacterial taxa and environmental conditions. Lupatini *et al.* (2013) also reported that the community composition and diversity of soil bacteria do not reflect the active bacteria or activity of the bacterial community. The similar diversity and community structure pattern of soil bacteria did not show a similar function. Those phenomena could contribute to the insignificance of differences in soilborne Actinobacteria's diversity and community composition in the four different treatments in oil palm plantations. Active bacterial studies (based on RNA) are preferable to investigate the diversity and community composition of *Actinobacteria*.

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