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Trench Construction in Peat Soil and the DGGE Analyses of Nif Gene and Activity of Dehydrogenase

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Basal stem rot (BSR) is a major disease in oil palm crops which also possible happen on peat soils. *Ganoderma boninense* is the pathogen that causes this disease and infects the plants through the root. Maintaining root health expects to reduce the intensity of the occurrence of BSR disease and its spread. In this research, trench construction and maintenance was carried out which aims to break the spread and activity of *Ganoderma.* Trench maintenance was carried out by giving empty bunches oil palm inoculated with decomposer, *Azotobacter* inoculants and humic acid. Four treatments tested were trenched (T1) and untrenched (T0) in combination with two level of *Ganoderma* attack, which are moderate (Mo) and severe (Se). The soil sample were retrieved from different point, which are in the trench area (TR), harvest path zone (H), and frond stack zone (M). The soil characteristics including microbe abundance, dehydrogenase activity, and PCR-DGGE of microbe community in each treatment were analyzed to reveal the effect of trench construction. The results showed that trench implementation reveals major results related to the abundance of microbes and fungi population, supported by the enhancement of dehydrogenase activity at the block with moderate *Ganoderma* attack. In addition, the DGGE study effectively separates the microbial population based on nif gene of four separate treatment into two clusters, splitting the groups depending on the *Ganoderma* level attack. This study shows enhancement of soil characteristics biologically and nutrients status of oil palm leaves especially P, as the results of trench construction in peatland.

Key words: DGGE, microbes, oil palm, peatland, trench

Busuk pangkal batang (BPB) adalah penyakit utama pada perkebunan kelapa sawit yang juga dapat terjadi pada tanah gambut. *Ganoderma* sp. adalah patogen penyebab penyakit BPB yang menginfeksi tanaman melalui akar. Dengan menjaga kesehatan akar diharapkan mampu mengurangi intensitas terjadinya penyakit BPB dan penyebarannya. Dalam penelitian ini, dilakukan pembuatan galian (*trench*) dan pemeliharaannya untuk menghentikan penyebaran dan aktivitas *Ganoderma.* Pemeliharaan galian dilakukan dengan memberikan tandan kosong kelapa sawit yang diinokulasi dengan dekomposer, *Azotobacter* dan asam humat. Empat perlakuan yang diuji adalah dengan galian (T1) dan tanpa galian (T0) di kombinasi dengan dua tingkat serangan *Ganoderma,* yaitu sedang (Mo) dan parah (Se). Sampel tanah diambil dari titik yang berbeda, yaitu di daerah galian (TR), jalur panen (H), dan jalur gawangan mati (M). Karakteristik tanah terdiri dari kelimpahan mikroba, aktivitas dehidrogenase, dan komunitas mikroba menggunakan PCR-DGGE di setiap perlakuan dan dianalisis untuk mengungkapkan efek konstruksi galian. Hasil penelitian menunjukkan efek positif dari implementasi galian terhadap kelimpahan mikroba dan populasi jamur, didukung oleh peningkatan aktivitas dehidrogenase di blok dengan intensitas serangan *Ganoderma* tingkat sedang. Selain itu, studi DGGE secara efektif memisahkan populasi mikroba berdasarkan gen nif dari empat perlakuan yang terpisah menjadi dua kelompok berdasarkan tingkat serangan *Ganoderma*. Studi ini menunjukkan peningkatan karakteristik tanah secara biologis dan status nutrisi daun kelapa sawit khususnya fosfor (P), sebagai hasil dari konstruksi galian di lahan gambut.

Kata kunci: DGGE, galian, kelapa sawit, lahan gambut, mikroba

Oil palm has been planted in peat soil since 1980 due to the decline in available suitable mineral soil. The productivity of oil palm in peatlands ranged from 12 to 27 ton/ha and yield up to 23% or 2% lower than mineral landfill. In 2010, the oil palm area reached 701.868 ha in Kalimantan and 500.000 ha in Riau (Wahyunto 2011).

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Peatlands are created by the deposition and burying of plant detritus-derived organic matter. It grows under conditions of near-continuous soil saturation, leading to anaerobic conditions that drastically slow down the decomposition. Peatland soils contain a wide variety of organic matter content and thickness while they frequently contain 80 to 100 percent organic matter that is multiple meters or thicker than others (Craft 2016). The main problem in peatland could be overcome by

drainage reparation, ameliorant application, and utilizing the biological ecosystem which plays role in the quality improvement of nutrients to support plant development (Rawat *et al.* 2018). Another problem that usually finds in the peatlands is the soil pathogen, such as *Ganoderma*. Lately, a report from Huan and Wahidin (2009) mentioned that *Ganoderma* sp. cases in peatlands increasing even in the first generation of oil palm. The trench construction method is one of the efforts to repair the peatland area. The field observation in the PT THIP area shows that trenches with 4 x 4 x 0.75 meters of size surrounding the oil palm infected by *Ganoderma* sp. successfully decreasing the infection rate (Huan and Wahidin 2009).

In rhizobia, the two major groups of nitrogen-fixing genes, the nif genes and fix genes, are present. The nif genes encode nitrogenase and mimic the nitrogen-fixing genes found in *Klebsiella pneumoniae* and other microbial groups structurally and functionally (Ruvkin and Ausubel 1980). While most of the nif genes are present on rhizobia plasmids, it has also been mentioned in *Bradyrhizobium chromosomes* (Sa'nchez *et al.* 2013). The nitrogen fixation mechanism is catalyzed by the enzyme nitrogenase, either in symbiotic or nonsymbiotic microorganisms, and this enzyme complex is encoded by the genes nifDK and nifH. There are two subunits of the enzyme nitrogenase, a molybdenum iron protein (Mo-Fe), subunit I and subunit II of an iron-containing protein (Fe). In different classes of microorganisms there is considerable difference in the organization and complexity of nif genes (Downie 1998). The nonsymbiotic K pneumoniae, according to Dean and Jacobson (1992), comprises at least 20 nif genes arranged in approximately eight operons. In most cases, the regulations of all nif genes are regulated by NifA (positive transcription activator) and NifL (negative regulator). Nif gene regulation is determined by the concentration of both oxygen and nitrogen in the system (Merrick and Edwards, 1995). When soil ammonia levels (NH3 or NH4) are high, NifL slows down the fixation of nitrogen to serve as a negative controller or gene expression by stopping NifA from functioning as an activator. If the concentration of O_2 is high, then nitrogenase synthesis decreases which leads to a decrease in BNF. The diversity of nitrogen-fixing bacteria in soil is commonly studied by DGGE (Rusmana *et al.* 2014).

The organic material application has proven to increase the carbon soil as well as the pathogen antagonism (Scotti *et al.* 2015). Besides, empty fruit bunches (EFB) applications also increasing the pH soil which responsible to increase the soil nutrition and diversity of microbes. The organic material also improving the physical, chemical, and biological soil characteristics (Haynes 2008). A report from Komariah *et al* (1993) and Nurani *et al* (2007) reported that the microbes' consortium and EFB application successfully increase the pH and the saturation of nutrients in peatlands, also decreasing the cation exchange capacity as well as the C/N ratio. Besides, Yusnaini (2009) revealing that organic compost application could increase the pH from 3.56 up to 5.47. Based on the previous results, we tried to make trench and maintain those trench with the addition of EFB compost, *Azotobacter*, humic acid, and analyze the soil characteristics, biological activity as well as the microbes community.

MATERIALS AND METHODS

This experiment was done in the oil palm field in Pasangkayu, Sulawesi. The soil type used was wet soil. The treatment tested was a combination of two different factor treatments, which are trenched (T1) and untrenched (T0); in combination with the intensity of *Ganoderma* attack i.e. severe (Se: >0.1) and moderate (Mo: <0.01) as shown in Table 1. The percentage of the *Ganoderma* attack was measured by counting the percentage of oil palm suffer from basal stem rot (BSR) disease caused by *Ganoderma*.

The trench size around 24 x 1 x 0.6 m was manually constructed, with 3 meters gap for every trench (Figure 1), followed by the maintenance through adding empty fruit bunches (EFB) oil palm, humic acid, and *Azotobacter* inoculum (Table 2). The trench was filled with one layer of EFB which contained 288 fruits which previously inoculated using decomposer and *Ganoderma* antagonist microbes directly using automatic applicator tools. The EFB in trench was then buried with the soil and frond piles. At the end of incubation time, around 4 weeks, the trench was opened and 1 kg of *Azotobacter* and 150 ml of humic acid were applied for each trench (Table 2). The trench was then layered with the frond piles and soil to optimized the decomposition process. In trenching plot, the inorganic fertilizer was applied in a lower dose i.e. 75% dose of recommended.

Soil and Oil Palm Leaves Sample. The sample was taken two times i.e. before and after trench construction. We took the soil sample inside the trench especially for block L5 and L9 and in a similar area of

Table 1 The data of each treatment area

Block	Year of planting	Wide area (Ha)	Production (ton ha $^{-1}$)	Trenching	*Ganoderma* intensity attack (%)	Treatment code
F15	1999	44	23.84	T0	0.0022 (Mo)	T0-Mo
L5	2000	34.88	26.26	T1	0.0094 (Mo)	T1-Mo
L3	1998	31.46	23.62	T0	0.025 (Se)	T0-Se
L9	1995	31.22	21.23	T1	0.033 (Se)	T1-Se

T0: untrenched; T1: trenched; Mo: moderate; Se:severe

Table 2 The time of trenching construction and product application

Treatment code	Trenching end - date	Application of EFB compost +Humic acid+ *Azotobacter*	Application of EFB compost +Humic acid+ *Azotobacter*
		1st	2nd
T0-Mo	-	-	-
T0-Se	-	-	-
T1-Mo	October 2011	November 2011	January - April 2013
T1-Se	August 2011	September 2011	June -April 2013

T0: untrenched, T1: trenched, Mo: Moderate *Ganoderma* attack, Se: Severe *Ganoderma* attack, EFB: Empty fruit bunches oil palm

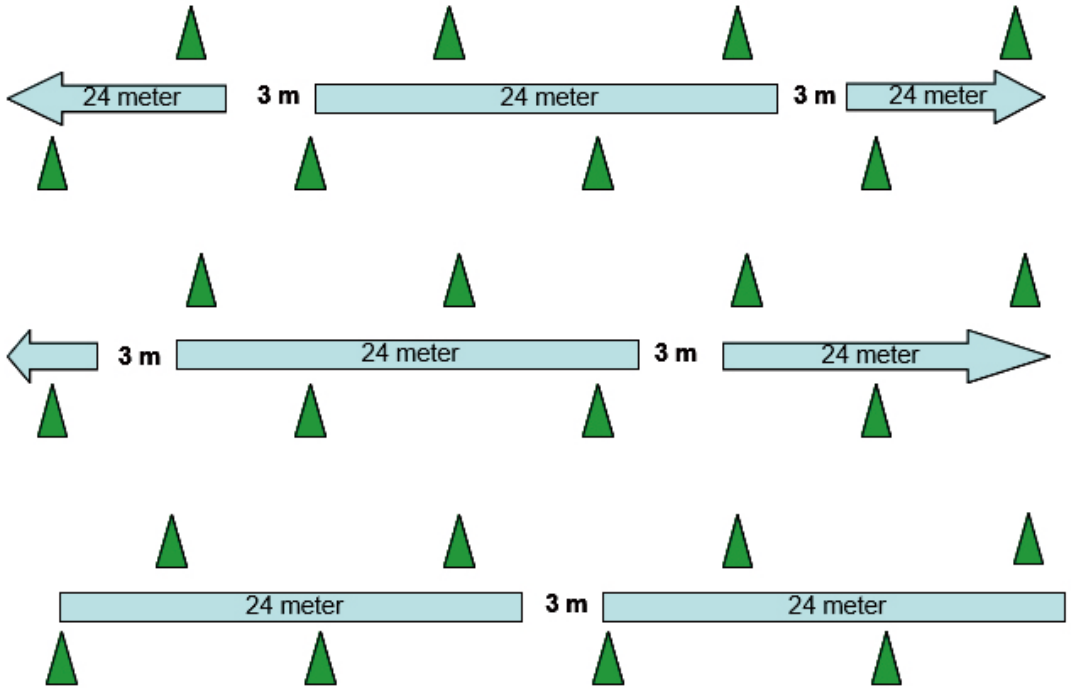

Fig 1 The trenching size and pattern applied in the oil palm crops. Green triangle indicated the oil palm tree.

trench for untrenched treatment (F15 and L3). A soil sample from trench was taken from the trench in the center of every 8 ha. The sample was composite of the 50 grams of soil from left, right, and the middle of the trench area. Then, the soil was labelled for further analysis. As a comparison, we also took the soil sample from the harvest path zone (H) and frond stack zone (M) following the same procedure. Leaves sample were taken from the same location as the soil in the form of 5 pieces of the leaves, the right and left side

Table 3 Empty fruit bunches (EFB) oil palm compost characteristics

Treatment code	Before			After			% tage of decreasing C/N ratio
	C (%)	N (%)	C/N ratio	C (%)	N (%)	C/N ratio	
T1-Mo	43.32	0.76	57	48.05	2.1	22.88	149
T1-Se	46.01	1.17	39.32	47.1	2.25	20.93	88

after the sweet spines. The parameters of leaf nutrients analyzed were N, P, and K content.

Dehydrogenase Activity Assay. To analyze the dehydrogenase activity, three soil samples were retrieved from a different point of each treatment: TR (trench area), M (frond stack zone), and H (harvest path zone). The dehydrogenase activity was measured in the darkroom. As much 5 g of soil sample was used. TTC (triphenyl tetrazolium chloride) 3% and Tris-HCL were added. The vial was then vigorously mixed for 24 h, in 37^{0}C. After incubation the methanol was added to each vial and shook for 2 hours, 125 rpm using a shaker. The suspension was filtered using Whatman paper number 5 and measured in 485 nm using a spectrophotometer.

DNA Extraction from Soil Sample. Extraction was conducted by using the Power Soil DNA Isolation Kit (Mobio Laboratories, Carlsbad, CA, USA). Extraction was done according to the procedures of the kit from the company. The quality of DNA then checked using NanoDrop 2000 (Thermo Scientific, Wilmington, DE, USA).

Polymerase Chain Reaction Analyses of Nif Gene. DNA was amplified by using PolF / PolR primer sets. PCR was performed by using KAPA Hot Start Readymix (KAPA Biosystems, Wilmington, MA, USA). The primer used were F (PolF-GC): CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGC CCCCGCCCCTGCGAYCCSAARGCBGACTC and R (PolR): ATSGCCATCATYTCRCCGGA. Each PCR reaction contained 12.5 µL KAPA Hotstart Readymix, 1.25 µL forward primer (0.5 uM), 1.25 µL reverse primer (0.5 uM), 3 µL template (~100 ng) and 7 µL nuclease-free water. PCR was performed using T-Gradient Thermocycler (Biometra GmbH, Goettingen, Germany). The PCR thermal cycling conditions were performed as follows: initial denaturation (95 °C, 1 minutes), denaturation (95 °C, 15 s), annealing (55 °C, 15 s), extension (72 °C, 15 s), post-extension (72 °C, 5 minutes), for 30 cycles. Products were run at 1.5 % agarose gel for the checked correct size and stored at -20 °C until analyzed on DGGE.

Denaturing Gradient Gel Electrophoresis (DGGE) Analysis. DGGE analysis was performed using the D-Code Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA). PCR products (25ul) were separated in 1 mm thick in a 8% (W/V) polyacrylamide gel [acrylamide–bisacrylamide (37.5:1)] in 1× TAE buffer (40mM Tris, 20mM acetic acid, and 1mM EDTA) with a denaturing gradient from 35 to 60% (100% denaturant corresponds to 7 M urea and 40% (v/v) deionized formamide) in 130 V, 60 °C for 6 hours. The gel was stained in the SYBR safe solution (Invitrogen-Molecular Probes, Carlsbad, CA, USA) for 1 hour, and photographed by G:BOX (Syngene, Frederick, MD, USA) under UV transillumination. The DGGE result was then interpreted using NTSys software and Gel analyzer software. UPGMA method was applied to calculate the phylogenetic tree based on comparing DGGE band patterns.

RESULTS

Compost Characteristics. The results of the C analysis showed that the C content of compost was decrease in the range of 2.4% -12% while an increase in N ranged from 92-176% (Table 3). These results indicated the decomposition of microbes that emit CO_2 and form proteins along with the multiplication of microbial cells. These results were in line with the decrease in CN ratio in the range of 88-149%. The interesting point was the rate of decomposition of EFB in the plot with moderate *Ganoderma* attack was higher compared to that pathogen severe attack.

Microbiology Characteristics of Soil Before and After Trench Construction. The trench construction has been proven to increase the total of cellulolytic fungi, total bacteria, and nitrogen fixing bacteria especially in T1-Mo and T1-Se location. While the amount of phosphate solubilizing bacteria only increasing in the T1-Mo which has a moderate level of *Ganoderma* attack. In contrast, the total fungi in a location with trench construction showed reduction in volume (Table 4).

Dehydrogenase Activity Analysis. The graph

Table 4 The microbes population before and after trenching construction in each treatment

Treatment group	Before Trenching Application						After Trenching Application					
	Total of fungi (CFU)	Lignolytic fungi (CFU)	Cellulolytic fungi (CFU)	Total of bacteria (CFU)	Phosphate solubilizing bacteria (CFU)	Nitrogen fixing bacteria (CFU)	Total of fungi (CFU)	Lignolytic fungi (CFU)	Cellulolytic fungi (CFU)	Total of bacteria (CFU)	Phosphate solubilizing bacteria (CFU)	Nitrogen fixing bacteria CFU)
T0-Mo	15×10^5	0	6×10^3	75×10^6	0	0	4.0×10^5	0	3.3×10^5	1.3×10^{11}	0	0
T0-Se	24×10^5	0	2×10^3	6×10^6	0	0	1.2×10^4	0	3.3×10^4	1.5×10^{11}	1.6×10^9	0
T1-Mo	1×10^5	0	1×10^3	6×10^5	0	0	1.5×10^5	0	1.1×10^4	2.3×10^{11}	3.0×10^6	2.7×10^8
T1-Se	46×10^5	0	3×10^3	1×10^9	0	0	1.0×10^5	0	4.0×10^5	1.3×10^{11}	0	2.1×10^7

compared dehydrogenase activity in each soil sample from each treatment from three locations, which are the frond stack zone, harvest path zone area, and trench area of oil palm crops. According to the data, the highest dehydrogenase activity showed in the trench (TR) sample in each location, range from 9.23 to 10.51 in the location without trench (T0) and 2.56 to 20.11 in the location with trench (T1). Interestingly, the trench (T1) showed higher dehydrogenase activity than non-trench location (T0), specifically in blocks F15 and L5. These two blocks have a history of *Ganoderma*'s moderate attack. In addition, the sample in the frond stack zone (M) has lower dehydrogenase activity than the harvest path zone (H). While in the L3 and L9 block, which have a severe history of *Ganoderma*, showed no constant pattern of dehydrogenase activity. Unlike in the F15 and L5 locations, the harvest path zone (H) location in the L3 and L9 block has lower dehydrogenase activity than the frond stack zone (M) sample (Figure 2).

DGGE Analysis of Nif Gene. The DNA extraction given the results in varied yield range from 31 to 101 ng/µl, and standard purity OD 260/280 range from 1.8 to 1.9 (Table 5). The phylogenetic tree of DGGE analysis revealing the closeness of microbes population based on the nif gene detected in each location (Figure 4). There are two clusters which built in the tree. The first cluster consists of TR-T0-Mo, H-T1-Mo, M-T0-Mo, TR-T0-Se, H-T0-Mo, M-T1-Mo, and TR-T1-Mo. The phylogenetic evolution showed that the microbes population in the first cluster closer to the reference sample, which is compost, in particular the TR-T0-Mo sample. The majority of cluster I member is the location with a moderate level of *Ganoderma* attack. Whilst, the second cluster consists of TR-T1-Se, M-T0-Se, M-T1-Se, H-T1-Se, and H-T1-Mo. The tree revealed that the location in cluster II mostly has a severe *Ganoderma* attack.

In general, from both clusters, the microbes population from the frond stack zone (M) soil sample was closer to the trench sample (TR). In addition, the microbe population from a harvest path zone (H) location usually formed the new tree branch. The phylogenetic data also supported with the distance analysis data to strengthen the analysis (Figure 5).

Nutrient Status of Leaves Before and After Trench Construction. The results of the nutrient analysis at moderate *Ganoderma* attacks showed increasing of N levels in the treatment without trench approximately 293% and 91% each for T0 and T1 (Table 6) whereas in plots with severe *Ganoderma* attack, the increase in leaf N levels was 338% and 103 % for T0 and T1, respectively. These results indicated that by making trench the rate of increase in N of the leaves reduced compared to that without trench. Interestingly, an increase in leaf N content was higher at severe *Ganoderma* attack rates compared to those at moderate attack rates.

For leaf P levels, at moderate *Ganoderma* attack, the increase of P was 147% (T0) and 7 times (T1) while at severe was 19% and 11 times each for T0 and T1 respectively. These data showed that trench yield the higher P leaf content compared to those untrenched. For K leaves an increase of 10 times and 6 times respectively for T0 and T1 at moderate *Ganoderma* attack while at severe *Ganoderma* attack was 19 and 13 times for T0 and T1 respectively. The increase in nutrient status that occured in trenched block seems to be lower compared to untrenched block. The K nutrient status of severe *Ganoderma* attack was higher compared to that moderate *Ganoderma* attack. It seems that the tendency of K leaf nutrient was similar with N levels.

DISCUSSION

This study finds that the construction of trench able to increase the existence of bacteria nitrogen fixation. This caused by the trench development contains *Azotobacter*, humic acid, and EFB compost for plant development. A study reported that obligatory aerobes such as *Azotobacter vinelandii* able to protect nitrogenase from oxygen and remove nitrogen by using cytochrome oxidases (Poole and Hill, 1997). Nitrogen in soil parent material is not present, given the fact that nitrogen content in the atmosphere is highest of all atmospheric gasses (Hedin *et al.* 2009). Soil nitrogen inputs for plant nutrition and crop productivity are thus largely dependent on organic matter degradation, inorganic fertilizer applications, and biological nitrogen fixation (BNF) through nitrogenase enzyme activity (Vitousek *et al.* 2013).

In addition, the trench system construction in the location where *Ganoderma* attacks at a moderate level positively affect the dehydrogenase activity. Additional of EFB in the trench location expected to enhance the pH of soil. B. Trisakti *et al* (2017) reported that the EFB with piece size <1 cm to 12-15 cm have high pH range 9.15 ± 0.25 to 9.25 ± 0.18. This basic condition leads to inhibition of pathogen growth, which only survive in acidic environment (Saidi *et al.* 2008). However, in the location with severe *Ganoderma* attack level, trench implementation shows

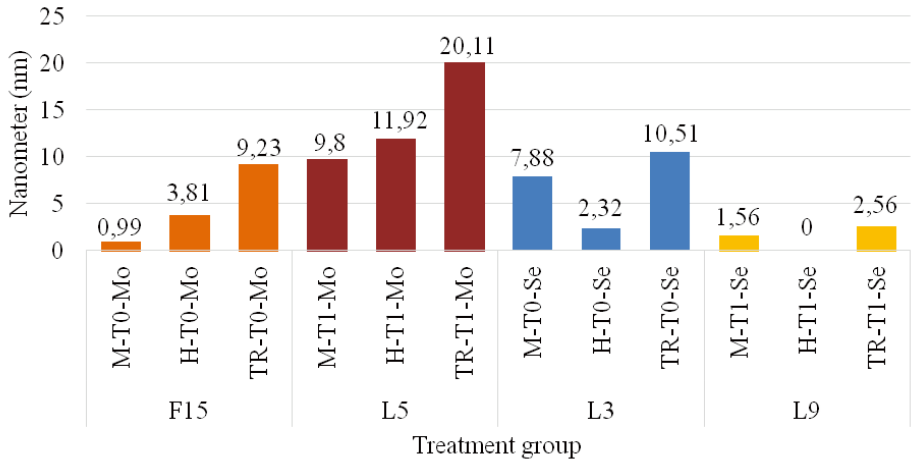

Fig 2 The dehydrogenase activity of each treatment tested.

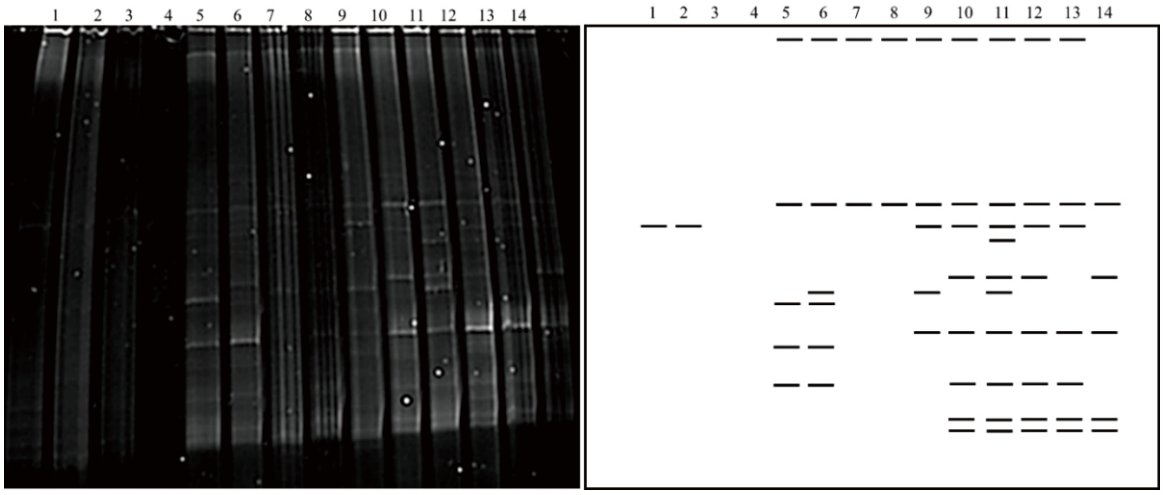

Fig 3 The PCR-DGGE result. Lane 1: compost; 2: TR-T0-Mo; 3: H-T0-Se; 4: empty; 5: M-T1-Mo; 6: TR-T1-Mo; 7: M-T0-Mo; 8: TR-T0-Se; 9: H-T0-Mo; 10: TR-T1-Se; 11: H-T1-Se; 12: M-T0-Se; 13: M-T1-Se; 14: H-T1-Mo.

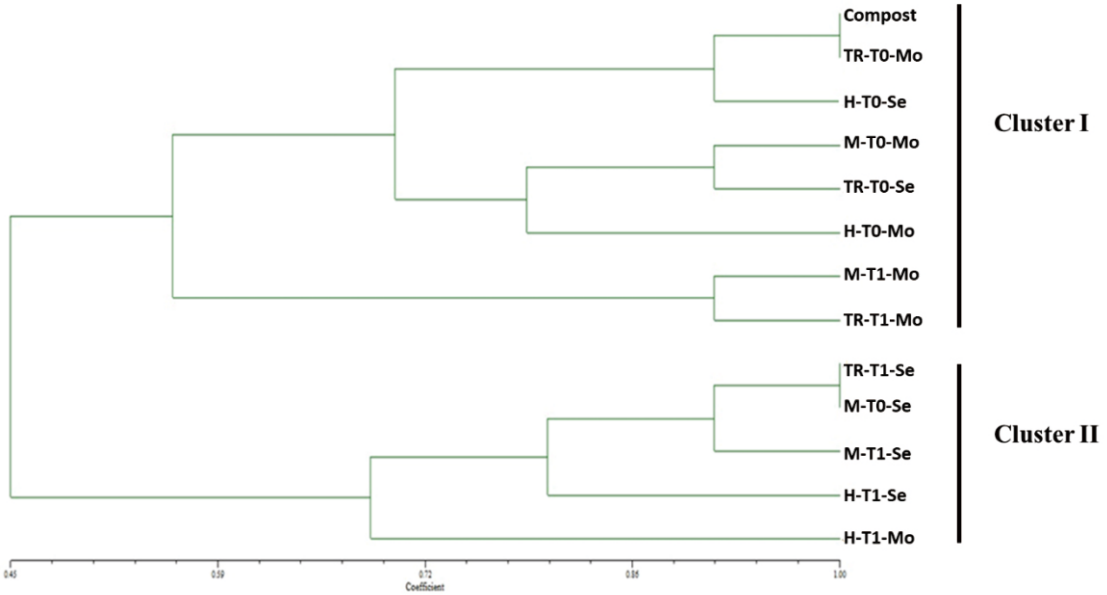

Fig 4 Phylogenetic tree construct of microbes community from each sample based on nif gene.

Table 5 Yield and purity of DNA extracted from soil sample

Block	Sample	Concentration (ng/µl)	OD (260/280)
	Compost	42.4	1.92
F15	TR-T0-Mo	60.3	1.86
	M-T0-Mo	101.1	1.88
	H-T0-Mo	90.7	1.86
L3	TR-T0-Se	43	1.81
	M-T0-Se	32	1.9
	H-T0-Se	48.1	1.84
L5	TR-T1-Mo	42.9	1.88
	M-T1-Mo	46.8	1.84
	H-T1-Mo	32.5	1.89
L9	TR-T1-Se	48.4	1.87
	M-T1-Se	47.1	1.88
	H-T1-Se	31.4	1.92

Table 6 The nutrient status of leaves before and after trenching construction in each treatment

Treatment code	Before			After			% increase		
	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)	N	P	K
T0-Mo	0.74	0.083	0.067	2.91	0.205	0.74	293	147	1004
T0-Se	0.65	0.162	0.038	2.86	0.19	0.75	338	19	1874
T1-Mo	1.68	0.023	0.202	3.21	0.182	1.503	91	691	644
T1-Se	1.61	0.017	0.100	3.27	0.2	1.443	103	1080	1326

	Compost	TR-T0-Mo	H-T0-Se	M-T1-Mo	TR-T1-Mo	M-T0-Mo	TR-T0-Se	H-T0-Mo	TR-T1-Se	H-T1-Se	M-T0-Se	M-T1-Se	H-T1-Mo
Compost	1.00E+14												
TR-T0-Mo	1.00E+14	1.00E+14											
H-T0-Se	9.17E+13	9.17E+13	1.00E+14										
M-T1-Mo	5.00E+13	5.00E+13	5.83E+13	1.00E+14									
TR-T1-Mo	4.17E+13	4.17E+13	5.00E+13	9.17E+13	1.00E+14								
M-T0-Mo	7.50E+13	7.50E+13	8.33E+13	7.50E+13	6.67E+13	1.00E+14							
TR-T0-Se	6.67E+13	6.67E+13	7.50E+13	6.67E+13	5.83E+13	9.17E+13	1.00E+14						
H-T0-Mo	6.67E+13	6.67E+13	5.83E+13	5.00E+13	5.83E+13	7.50E+13	8.33E+13	1.00E+14					
TR-T1-Se	4.17E+13	4.17E+13	3.33E+13	4.17E+13	3.33E+13	5.00E+13	5.83E+13	5.83E+13	1.00E+14				
H-T1-Se	2.50E+13	2.50E+13	1.67E+13	2.50E+13	3.33E+13	3.33E+13	4.17E+13	5.83E+13	8.33E+13	1.00E+14			
M-T0-Se	4.17E+13	4.17E+13	3.33E+13	4.17E+13	3.33E+13	5.00E+13	5.83E+13	5.83E+13	1.00E+14	8.33E+13	1.00E+14		
M-T1-Se	5.00E+13	5.00E+13	4.17E+13	5.00E+13	4.17E+13	5.83E+13	6.67E+13	6.67E+13	9.17E+13	7.50E+13	9.17E+13	1.00E+14	
H-T1-Mo	5.00E+13	5.00E+13	5.83E+13	3.33E+13	2.50E+13	5.83E+13	6.67E+13	5.00E+13	7.50E+13	5.83E+13	7.50E+13	6.67E+13	1.00E+14

Fig 5 Genetic distance of microbes species from each sample based on nif gene.

no consistent effect of dehydrogenase activity. It may take longer time on this location with severe level of *Ganoderma* attack to restore microbial activity.

In fact, the data of biology and chemical characteristic before trench construction shows that the soil sample which has a severe level of *Ganoderma* attack already have higher nitrogen content, total fungi, and bacteria compare to the location with moderate *Ganoderma* attack. This might lead to the hypothesis that over-adding nutrition, in this case, trench development, to the soil that already has high nutrition could reduce the soil performance or might be reaching the saturated level of nutrition in the soil. The dehydrogenase is one of soil enzyme that becomes the indicator of ecosystem status. Soil enzyme acts as mediators and catalysts of important soil function, involving organic inputs decomposition, the transformation of soil organic native, releasing the inorganic nutrient, N_2 fixation, nitrification, genitrification, and xenobiotics detoxification (Dick 1997). Dehydrogenase is an enzyme that transfers protons and electrons from substrates to acceptors to oxidize organic soil matter. This enzyme is known as an essential part of intact cells but does not remain in the soil (Das and Varma 2011). It is known that the dehydrogenase enzyme will occur if only the soil bacteria exist in the sample. *Pseudomonas* genus observed abundantly in the soil where dehydrogenase activity is high (Walls-Thumma 2000). The correlation between dehydrogenase activity and *Ganoderma* sp. level in soil could be explained by the fact that the abundance of *Pseudomonas* sp. in the soil has inhibition activity to the *Ganoderma* sp. (Bivi *et al.* 2010).

By coupling PCR amplification of taxonomic targets with sequence dissimilarities examined by denaturing gradient gel electrophoresis (DGGE), molecular fingerprints of variety can be obtained by splitting DNA fragments of equal length but of separate sequences (Muyzer *et al.* 1993; Muyzer *et al.* 1996), resulting in a DGGE fingerprint that is unique to the sample location. A PCR-DGGE is able to detect up to 95-99% of the bacterial population, and many candidate sequences can be used as genetic biomarkers, including variable regions within the 16S ribosomal DNA gene (Tsen *et al.* 1998), the 23S ribosomal DNA gene, and the rpoB gene (encoding the RNA polymerase β subunit) (Dahllof *et al.* 2000). The DGGE analysis divides the microbial community based on the nif gene existence into two clusters. We hypothesize that if the nif gene playing role in the nitrogen fixation in the soil, then the bacteria community will be different from one another, especially our sampling area comprising various treatment group. The cluster I consist of microbial communities which found in the moderate level of *Ganoderma* location. In contrast, cluster II involving microbial found in the severe effect of *Ganoderma* sp. The data shows that the microbial community in the soil with moderate and severe *Ganoderma* attack have its microbial type. Hypothetically, specific microbial communities have the ability to fight against the *Ganoderma* with different intensity levels.

Interestingly, the microbial in the trenching location has a similar characteristic to the frond stack zone. This might correlate with sun exposure in the frond stack zone that could enrich the microbial population. Previous studies found that the nif genes encoding active nitrogenase can be passed to non-nitrogen-fixing prokaryotes to allow the removal of ambient nitrogen gas in ammonia as a supply of nitrogen (Temme *et al.* 2012; Setten *et al. 2*013; Wang *et al.* 2013; Han *et al.* 2015). The nif gene detected by the DGGE might contain culture and non-cultured bacteria. This leads to the hypothesis that the specific bacteria in each sampling location might not be cultured as reported in Hadianta *et al.* (2014). However, we still unsure about the uncultured bacteria nif that we have identified. Further study needed to be done to confirm this statement.

The development of trenching in four different locations shows a significant effect related to the abundance of microbes and fungi population, supported by the enhancing of dehydrogenase activity in the location with a moderate level of *Ganoderma* attack. Also, the DGGE analysis successfully clustering the microbial community by two clusters from four different locations, dividing the classification based on the *Ganoderma* level attack.

The leaf nutrient status showed that trench could induce enhancement of leaf nutrient especially for P, in contrast to N and K leaf content. The increasing of *Ganoderma* sp attacks followed by an increase in leaf N and K nutrient status.

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