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The Abundance of Nitrogen Fixing, Nitrifying, Denitrifying and Ammonifying Bacteria in the Soil of Tropical Rainforests and Oil Palm Plantations in Jambi

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Abstract

Alterations in the use of land for oil palm plantations can change the domination and activity of soil bacteria. More specifically, alteration in soil microbial communities can directly affect soil ecosystem functioning, particularly with respect to carbon and nitrogen cycles. Nitrogen can be a limiting nutrient, and the availability of nitrogen in the soil environment becomes a major factor in controlling the production of biomass. This research project aimed at studying the abundance of nitrogen-fixing, nitrogen-oxidizing, nitrogen-reducing, and ammonifying bacteria based on their functional genes in the tropical rain forests of Taman Nasional Bukit Duabelas (TNBD) and the oil palm plantations in Sarolangun Jambi. Samples were collected in November 2015. Soil sampling was performed randomly at three points representing each area of the tropical rainforests of TNBD and the seven- to eight-year-old oil palm plantations. Soil samples were collected using a soil sample core from 0–15 cm below the surface with depth strata of 0–5 cm, 5–10 cm, and 10–15 cm. Composite assessment was conducted on samples from each point corresponding to each respective depth strata. Soil samples were stored at -20°C prior to testing. Microbial abundance was measured using the most probable number (MPN) method. The abundance of microbes that play a role in nitrogen metabolism between strata of 5–10 cm and 10–15 cm does not appear to be different. The highest abundance of microbes in oil palm plantation land in Jambi was found in samples with nitrifying bacteria, later followed by denitrifying, nitrogen-fixing, and ammonifying bacteria. Ultimately, it was found that microbial abundance in oil palm plantations was higher than the corresponding rates in samples from tropical rainforests.

Abstrak

Kelimpahan Bakteri fiksasi nitrogen, Nitrifikasi, Denitrifikasi dan Amonifikasi pada Tanah Hutan Hujan Tropis dan Perkebunan Kelapa Sawit Jambi. Perubahan tata guna lahan menjadi perkebunan sawit dapat mengubah dominasi dan aktivitas bakteri tanah. Perubahan komunitas bakteri tanah secara langsung dapat mempengaruhi fungsi ekosistem tanah, terutama siklus karbon dan nitrogen. Nitrogen dapat menjadi pembatas nutrisi dan ketersediaan nitrogen di lingkungan tanah menjadi faktor utama dalam mengendalikan produksi biomassa. Penelitian ini bertujuan untuk mempelajari kelimpahan dari komunitas bakteri penambat N₂, pengoksidasi nitrogen, pereduksi nitrogen, dan amonifikasi berdasarkan gen fungsional di hutan hujan tropis Taman Nasional Bukit Duabelas (TNBD) dan lahan perkebunan sawit di Sarolangun Jambi. Pengambilan sampel tanah dilakukan secara random pada tiga titik yang mewakili setiap lahan hutan hujan tropis TNBD dan hutan transformasi perkebunan kelapa sawit yang telah berumur 7-8 tahun. Sampel tanah diambil menggunakan *soil sample core* pada ke dalaman 0-15 cm dengan ke dalaman 0-5 cm, 5-10 cm dan 10- 15 cm. Komposit dilakukan terhadap sampel dari masing-masing titik sesuai dengan tingkatan ke dalamnya. Sampel tanah disimpan pada suhu -20 °C sebelum pengujian. Kelimpahan bakteri tanah diukur dengan menggunakan metode *Most Probable Number* (MPN). Kelimpahan mikrob yang berperan dalam metabolisme nitrogen di lahan perkebunan sawit di Jambi tertinggi terbukti ditemukan pada bakteri nitrifikasi, kemudian diikuti oleh bakteri denitrifikasi, pemfiksasi nitrogen, dan amonifikasi. Keragaman mikrob pada tanah perkebunan sawit lebih tinggi dibandingkan dengan hutan hujan tropis.

Keywords: *abundance, nitrogen-fixing, nitrifying, denitrifying, ammonifying, MPN*

Introduction

The conversion of rainforests into plantations and the expansion of agricultural industry have raised concerns regarding the changes of the microbial community involved in the nutrient cycle. The nitrogen cycle plays an important role in natural processes. Nitrogen is a macro element required by plants. However, most nitrogen molecules are in the form of N_2 in as much as 78% of the atmosphere (i.e., gasses). N_2 cannot be exploited directly by plants since they are only able to absorb the dissolved nitrogen in the soil using their roots. Still, nitrogen is needed by plants in large quantities, particularly for the sustenance of constituent proteins. Fulfillment of the supply of nitrogen in soil can be conducted by way of fertilizing or naturally with the help of microorganisms. Biological nitrogen fixation is an important natural process performed by the fixation of bacterial nitrogen as a result of the nitrogen gas atmosphere changing into ammonium. Ammonium is also a source of nitrogen in ecosystems. According to Arshad and Frankenberger [1], N_2 fixation in biological systems accounts for approximately 70% of all nitrogen fixed on Earth, and about 90% of a plant's nitrogen needs can be generated through a combination of these mechanisms. Nitrogen bacteria fixation may be conducted freely, and it can also coincide in symbiotes with plants. However, biological nitrogen fixation limited only to prokaryotes is not able to effectively perform nitrogen fixation due to the presence of eukaryotes [2].

The use of land for palm plantations can change the dominance and the activity of existing bacteria. The diversity of microbial activity associated with its function in the environment is an indication of changes in the global nitrogen cycle [3]. The transformative mechanisms of the nitrogen compounds by indigenous bacteria which

occur on plantations are important factors to look for in an effort to manage and anticipate nitrogen pollution. Furthermore, the intake of nitrogen compounds is dominated by anthropogenic activity comparable with population increases and the corresponding utilization of ecosystems [4]. Therefore, the determination of dominance and general activity of bacteria that play a role in the nitrogen cycle of land is very important.

Research needs to be conducted on the abundance of microbes that play a role in the nitrogen cycle, specifically with respect to conditions of abuse when forests are transformed into palm oil plantations in Jambi. This is because Jambi is an area in which land use changes are occurring very quickly. Therefore, this research examines the abundance of nitrogen-fixing bacteria, nitrogen oxidizers, reductions in nitrogen, and ammonification in palm plantations, forests, and land in Jambi. This research is expected to provide information on the abundance of the bacteria that play a role in the metabolism of nitrogen in rainforests and palm plantations in Jambi. In addition, the findings are expected to be a valuable reference to related research communities concerned with similar issues regarding bacteria.

Methods

Site description and sampling. Samples were taken from the tropical rainforest in Bukit Dua Belas National Park (with two location: TC, TD) and from an oil palm plantation (with two location: SA, SB) in the Sarolangun District, Jambi Province, Sumatera, Indonesia. All spatial geographical coordinates and altitudes were recorded via the use of GPS (eTrex Venture, Garmin, Lenexa, KS, USA). Detailed site information is listed in Table 1. Samples were collected in October 2015.

Table 1. Site Description: Location, Altitude, and Soil Analysis for Samples from the Tropical Rainforest and Oil Palm Plantation Sites in Jambi

Oil Palm Plantation				Tropical Rainforest			
Sample	Location	Altitude	T°C (soil thermo)	Sample	Location	Altitude	T°C (soil thermo)
A1 (SA)	01°56' 491'' LS 103° 15' 140'' BT	64 mdpl	29	C1 (TC)	01°56' 576'' LS 102° 34' 879'' BT	87 mdpl	27
A2 (SA)	01°56' 477'' LS 103° 15' 142'' BT	53 mdpl	29	C2 (TC)	01°56' 571'' LS 102° 34' 874'' BT	95 mdpl	28
A3 (SA)	01°56' 472'' LS 103° 15' 134'' BT	55 mdpl	29.5	C3 (TC)	01°56' 566'' LS 102° 34' 865'' BT	95 mdpl	28
B1 (SB)	01°56' 592'' LS 103° 15' 104'' BT	48 mdpl	29	D1 (TD)	01°56' 487'' LS 102° 34' 852'' BT	111 mdpl	27
B2 (SB)	01°56' 591'' LS 103° 15' 119'' BT	42 mdpl	28.5	D2 (TD)	01°56' 481'' LS 102° 34' 860'' BT	114 mdpl	27
B3 (SB)	01°56' 958'' LS 103° 15' 122'' BT	42 mdpl	28	D3 (TD)	01°56' 502'' LS 102° 34' 836'' BT	116 mdpl	27.5

Soil sampling was performed randomly at three points representing each area of the tropical rainforest TNBD and the seven- to eight-year-old oil palm plantation. Soil samples were collected using a soil sample core from 0–15 cm below the surface with depth strata of 0–5 cm, 5–10 cm, and 10–15 cm. Composite assessment was conducted on the samples from each point corresponding to each respective depth strata. Soil samples were stored at -20 °C prior to testing.

Determination of the physicochemical parameters of the soil samples. The physicochemical parameters of the soil samples were determined according to the instructions provided by the International Soil Reference and Information Centre (ISRIC) standards [5]. The total organic carbon (TOC) equation was used to determine the TOC in the soil. The Kjeldahl method was used to determine the total nitrogen contents.

Determination of N₂ fixing bacteria An abundance of bacteria was calculated through the most probable number (MPN) method [6]. A total of 1 g of each soil sample was diluted with physiological saline solution (0.85%) triple repetitions for serial dilution. Further dilution of 1 ml for the third and final dilution was inoculated in 9 ml of an N-free liquid medium with the major components (L⁻¹) as follows: 1 g K₂HPO₄, 3 g KH₂PO₄, 0.065 g MgSO₄, 0.01 g FeCl₃·6H₂O, 0.07 g CaCl₂·2H₂O, and 5 g dextrose; the minor components included: 240 µg Na₂MoO₄·2H₂O, 3 µg H₃BO₄, 1,83 µg MnSO₄·H₂O, 290 µg ZnSO₄·7H₂O, 130 µg CuSO₄·5H₂O, and 120 µg CoCl₂·6H₂O (Phillips *et al.* 2000). Then, the N₂ gas was ejected into the medium using a sterile syringe for 3 minutes. Incubation was performed at room temperature for 7 days. A positive test indicating that a bacterial culture presenting N₂ fixation activity was declared with the formation of a blue color after the bacterial culture was given a phenol reagent of 10% alcohol, 0.5% nitroprusside, and a mixture of technical hypochlorite and 20% citric acid (1: 4) [7].

Determination of ammonifying bacteria. An abundance of bacteria was determined through the method of calculating the most probable number (MPN). A total of 1 g of each soil sample was diluted with physiological saline solution (0.85%) three times for serial dilution. Further dilution of 1 ml of the third final dilution was inoculated in 9 ml of an N-free liquid medium with the following major components (L⁻¹): 0.9 g Na₂HPO₄, 0.2 g KH₂PO₄, 0.1 g MgSO₄·7H₂O, 0.005 g FeCl₃·6H₂O, 0.0184 g CaCl₂·6H₂O, 0.25 g yeast extract, 5 g Na₂CO₃ for source C, and 5 g peptone. Incubation was performed at room temperature (27 °C) for 7 days. A positive test for ammonifying bacteria was determined by the formation of a blue color when the bacterial culture was given a phenol reagent of 10% alcohol, 0.5% nitroprusside, and a mixture of technical hypochlorite and 20% citric acid (1: 4) [7].

Determination of nitrifying bacteria. A total of 1 g of each soil sample was diluted with physiological saline solution (0.85%) three times for serial dilution. Further dilution of 1 ml of the third and final dilution was inoculated in 9 ml of an N-free liquid medium with the following major components (L⁻¹): 0.9 g Na₂HPO₄, 0.2 g KH₂PO₄, 0.1 g MgSO₄·7H₂O, 0.005 g FeCl₃·6H₂O, 0.0184 g CaCl₂·6H₂O, 0.25 g yeast extract, and 5 g Na₂CO₃ for source C. For NH₃ oxidizing bacteria, 1 g NH₄Cl was added, and for NO₂ oxidizing bacteria, 1 g NaNO₂ was added. Incubation was performed at room temperature (27 °C) for 7 days. Positive tests for nitrifying bacteria were indicated by the formation of a yellow color when the bacterial culture was given a brucine reagent and sulfuric acid [7].

Determination of Denitrification Bacteria. A total of 1 g of each soil sample was diluted with physiological saline solution (0.85%) with some serial dilution. Further dilution of 1 ml of the third and final dilution was inoculated in 9 ml of an N-free liquid medium with the following major components (L⁻¹): 0.9 g Na₂HPO₄, 0.2 g KH₂PO₄, 0.1 g MgSO₄·7H₂O, 0.005 g FeCl₃·6H₂O, 0.0184 g CaCl₂·6H₂O, 0.25 g yeast extract, and 5 g Na₂CO₃ for source C. For the reduction of bacteria, 1 g NaNO₃ and DNRA 5 g glucose was added. N₂ gas was ejected into the medium using a sterile syringe for 3 minutes. Incubation was performed at room temperature for 7 days. A positive test for NO₃⁻denitrifikasi bacteria was indicated by the formation of a pink color after the administration of 1% sulfanilamide and 0.1% Naftalena Etilena Diamina (NED) reagent. A positive test of DNRA-denitrifying bacteria was indicated by the formation of a blue color when the bacterial culture was given a phenol reagent of 10% alcohol, 0.5% nitroprusside, and a mixture of technical hypochlorite and 20% citric acid (1: 4) [7].

Results and Discussion

Soil characteristics. The results of the analysis of the soil characteristics and physical chemistry indicate that the soil in the oil palm plantation contained 39% sand, 30% dust, and 31% clay. Tropical rainforest soils contained 74% sand, 5% dust, and 21% clay. The total contents of organic carbon in the soil of the oil palm plantation and the tropical rainforest were 0.98%, and 1.33%, respectively. The total contents of organic nitrogen in the soil of the oil palm plantation and the tropical rainforest were 0.09% and 0.11%, respectively.

The abundance of N₂-fixing bacteria. Analysis of the results of the N₂-fixing microbe population in the tropical rainforest and oil palm plantation was determined not to be different. The tropical rainforest had the lowest microbial abundance value at 3.04 Log cells g⁻¹ (TC) in the strata 0–5 cm. Meanwhile the highest was in oil palm plantation in strata 10–15 cm (6.08 Log cells g⁻¹) (SA). The abundance profile of N₂-

fixing microbes after increasing the depth was shown to have increased (Figure 1). Furthermore, the microbial abundance in the oil palm plantation was higher than that of the tropical rainforest. In addition, the microbial abundance in the observed location seemed consistent with measurable organic levels (Table 1). It is therefore presumed that the organic materials contained in the site can be used or overhauled by microbes as a carbon source for metabolic energy and growth.

The abundance of ammonifying bacteria. The analysis of the ammonifying microbe population in the tropical rainforest and oil palm plantation was determined not to be different. The oil palm plantation had the lowest abundance of microbes at 4.18 Log cells g⁻¹ (SB) in the strata of 0–5 cm; the highest value was in the oil palm plantation at the strata of 5–10 cm (5.56 Log cells g⁻¹). The abundance of ammonifying microbes increased in the strata of 5–10 cm (Figure 2). The microbial abundance in the oil palm plantation was therefore higher than that in the tropical rainforest.

The abundance of nitrifying bacteria. Oxidizing bacteria NH₃ with the highest abundance value was found in the strata of 0–5 cm (7.56 Log cells g⁻¹). The abundance

of these bacteria was shown to progressively decrease with increasing depth (Figure 3). The lowest levels were present in the strata of 10–15 cm (3.18 Log cells g⁻¹)

The highest abundance of oxidizing bacteria NO₂⁻ was found in the strata of 0–5 cm (7.18 Log cells g⁻¹). However, the abundance of these bacteria decreased in the strata of 10–15 cm (Figure 4). The lowest value was present in the strata of 10–15 cm (3.18 Log cells g⁻¹).

The abundance of denitrifying bacteria. The abundance of reducing bacteria NO₃⁻ in rainforest and palm plantations in Jambi tend to increase with the increasing depth (Figure 5.). The highest abundance found in the strata of 10–15 cm (7.66 Log cells g⁻¹) and lowest abundance found in the of strata 0–5 cm (4.43 Log cells g⁻¹).

The abundance of DNRA bacteria. Dissimilatory nitrate reduction to ammonium (DNRA) could be found with abundance levels that tended to increase in line with increasing depth (Figure 6). The highest abundance levels were found in the strata of 10–15 cm (7.66 Log cells g⁻¹) and the lowest in the strata of 0–5 cm (6.04 Log cells g⁻¹).

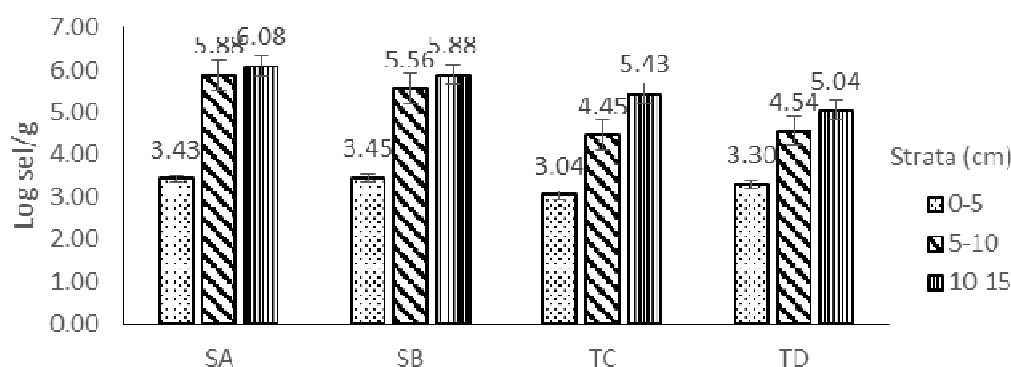


Figure 1. Profile of Microbe Abundance in N₂ Fixation from the Oil Palm Plantation (SA, SB) and Tropical Rainforest (TC, TD)

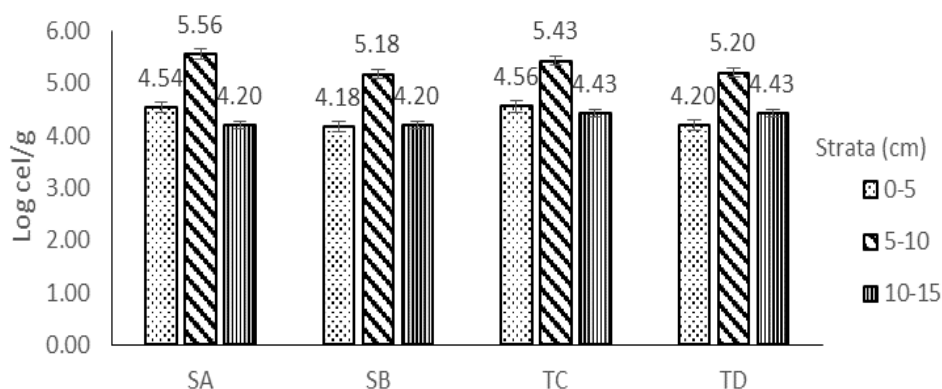


Figure 2. Microbial Abundance Profile of Ammonifying Bacteria in the Soil of the Oil Palm Plantation (SA, SB) and the Tropical Rainforest (TC, TD)

The highest abundance of nitrogen-fixing bacteria was found in the palm oil plantation in the strata of 10–15 cm (6.08 Log cells g^{-1}), and the lowest was found in the tropical rainforest in the strata of 0–5 cm (3.04 Log cells

g^{-1}). The highest abundance of NH_3^- oxidizing bacteria was found in the tropical rainforest in the strata of 0–5 cm (7.56 Log cells g^{-1}). The lowest abundance was found in the tropical rainforest in the strata of 10–15 cm

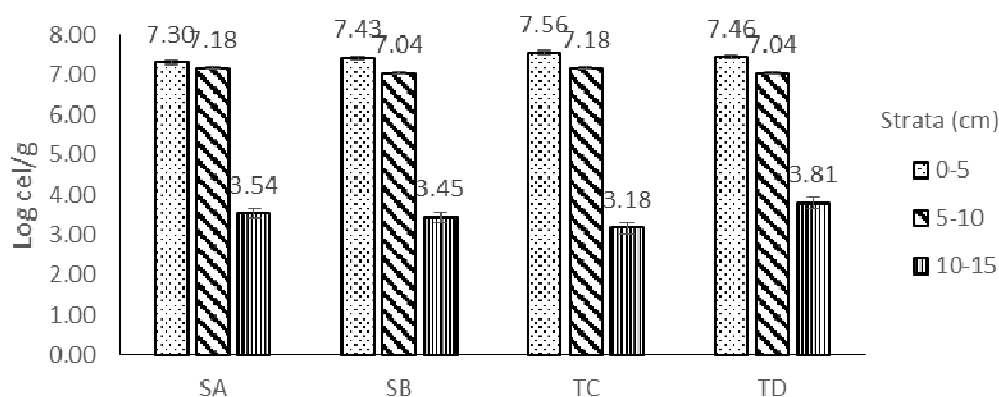


Figure 3. Microbial Abundance profile of NH_3^- Nitrifying Bacteria in the Soil of the Oil Palm Plantation (SA, SB) and the Tropical Rainforest (TC, TD)

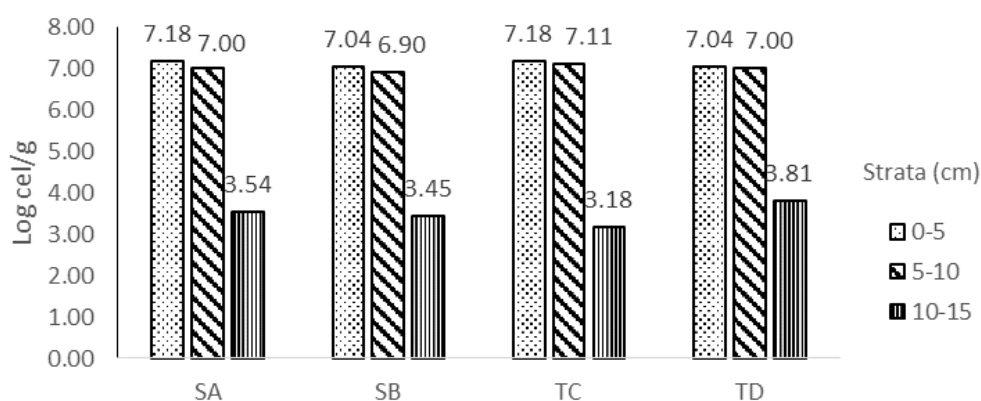


Figure 4. Microbial Abundance Profile of NO_2^- Nitrification Bacteria in the Soil of the Oil Palm Plantation (SA, SB) and the Tropical Rainforest (TC, TD)

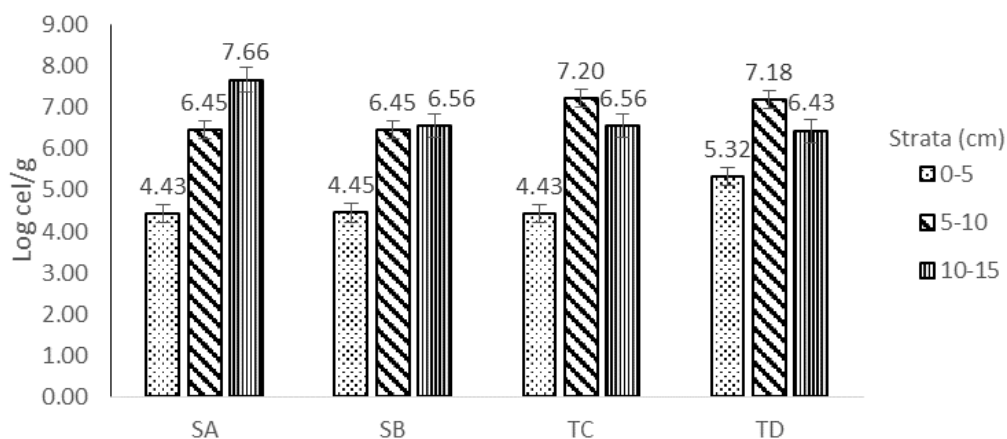


Figure 5. Microbial Abundance Profile of Denitrifying Bacteria in the Soil of Oil Palm Plantation (SA, SB) and Tropical Rainforest (TC, TD)

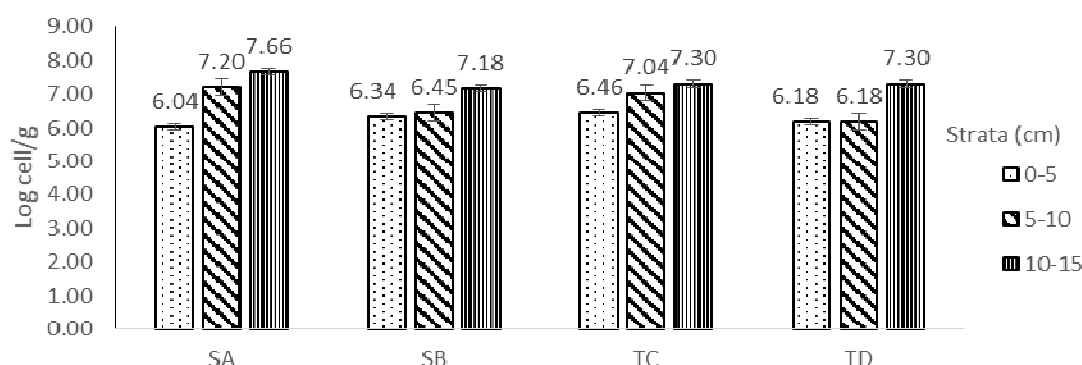


Figure 6. Microbial Abundance Profile of Reducing Bacteria to DNRA in the Soil of the Oil Palm Plantation (SA, SB) and the Tropical Rainforest (TC, TD)

(3.18 Log cells g^{-1}). The abundance of NO_3^- reducing bacteria increased with the increasing soil depth. The highest abundance was found in the oil palm plantation in the strata of 10–15 cm (7.66 Log cells g^{-1}) and the lowest was in the strata of 0–5 cm (4.43 Log cells g^{-1}). NO_3^- reducing bacteria into NH_4^+ (DNRA) could be found as the abundance increased with the increase of depth. The highest abundance was found in the oil palm plantation in the strata of 10–15 cm (7.66 Log cells g^{-1}) and the lowest abundance in the strata of 0–5 cm (6.04 Log cells g^{-1}). The highest abundance of ammonifying bacteria was found in the oil palm plantation in the strata of 5–10 cm (5.56 Log cells g^{-1}) and the lowest in the strata of 0–5 cm (4.18 Log cells g^{-1}). The highest abundance of microbes which have a role in the metabolism of nitrogen in oil palm plantations in Jambi was found in nitrogen-reducing bacteria, followed by nitrogen-oxidizing, nitrogen-fixing, and ammonifying bacteria.

The highest abundance of N_2 fixation bacteria was found in the oil palm plantation in the strata of 10–15 cm, where the availability of dissolved O_2 is lower than that of the above strata. The clay soils had higher N_2 fixation bacteria abundance levels than those of sandy soil. The type of soil texture can therefore lead to different numbers of N_2 fixation bacteria. The soil texture of the oil palm plantation was clay, and that of the tropical rain forest was sandy. Clay fractions in the soils are important for imparting specific physical properties, forming micro- and macroaggregates [8], and providing microaerophilic or anaerobic conditions that are favorable to N_2 fixation. The concentrations of clay in soil can be correlated with nitrogenase activity [9], and this is because the enzyme nitrogenase is very sensitive to O_2 [10]. Nitrogenase inactivation will occur when O_2 is available in the environment [11,12]. As for the accumulation of the compound NH_4^+ and NO_3^- , it is thought not to inhibit N_2 fixation bacterial activity. This contrasts with what was found in the strata of 10–15 cm, where the coating had the lowest O_2 availability and accumulation of compounds higher than NH_4^+ were thought to have been able to inhibit the growth of N_2 fixation bacteria. These

findings are consistent with the fact that the negative effect of ammonia on N_2 fixation has been known for a long time, as expression of nitrogenase is very often inhibited by the presence of NH_4^+ [13,14].

The abundance of bacterial nitrification at strata 0–5 cm and 5–10 was not found to be different. It is alleged to be due to the concentrations of dissolved O_2 in the strata. NH_3^- oxidizing bacteria require O_2 to perform their activities [15,16], so an environment rich in O_2 will strongly support such bacteria. NH_3^- oxidizing bacteria also utilize the NH_3^- oxidation process to obtain energy for their growth [15,17].

The activity of denitrifying bacteria reduction of NO_2^- , specifically by utilizing alternative electron acceptors for NO_2^- , functions as a substitute for oxygen. Reducing the bacterial abundance of NO_3^- increases with increasing depth, and this is allegedly closely related to the concentrations of dissolved O_2 and organic compounds. The changes of dissolved O_2 concentrations with increasing depth are thought to increase the activity of NO_3^- reducing bacteria. With the decrease in NO_3^- compound accumulation, which affects the increasing abundance of NO_3^- , the presence of reducing bacteria may indicate that NO_3^- is used as a substitute of electron acceptors for O_2 in the process of oxidizing the organic compounds. In this case, the carbon organic compounds act as electron donors. The electron is obtained from the oxidation of the carbon compounds stored in the molecules of NADH and $FADH_2$, which will act as electron donors between respiration chains. Furthermore, the type of source C hardness is known to affect the activity reduction of NO_3^- . The concentration of dissolved O_2 is too low which is allowing the higher NO_3^- reduction activity to strongly support the growth of NO_3^- bacteria and thus reducing anaerobic activity in the environmental conditions.

The process of NO_3^- reduction is directly related to the process of electron transfer, where the organic compound acts as an electron donor and an electron acceptor as NO_3^- . Denitrifying bacteria can also benefit from NO_2^- ,

NO₃⁻ or N₂O as electron acceptors in a last replacement of O₂ in a chain of respiration [18,19]. DNRA is an NO₃⁻ reducing bacteria with a fermentative nature such that the presence of organic compounds can also affect its activities. The availability of sulfides is also become influential in reducing bacterial activity (i.e., NO₃⁻ may inhibit a two-stage reaction and denitrification end so that the trigger for the onset of NO₃⁻ reduction activity trails through to the DNRA [20]). Bacteria are anaerobic, so DNRA activities are strongly influenced by the availability of O₂ [21].

The process of ammonification is very closely related to the process of overhauling the organic material. According to Perez *et al.* [22] the high concentrations of protein, polypeptide, and peptide can be a major factor affecting the intensive growth of bacteria, and the significant growth of bacteria is suspected to increase the activity of the ammonification [23].

The abundances of soil bacterial communities varied between the samples taken from the tropical rainforest and the oil palm plantation. Despite the higher abundances in the managed land-use systems, fertilization temporarily increased bacterial abundances. An increase of soil bacteria abundance in the oil palm plantation was also recorded. This finding is in contrast to the idea that animals, fungi, and plants negatively impact bacteria abundance levels in the conversion of rainforests lands to agricultural lands.

Conclusion

The abundance of microbes that play a role in nitrogen metabolism between the strata of 5–10 cm and 10–15 cm does not differ. The highest abundance of microbes in the oil palm plantation in Jambi were found to be nitrifying bacteria, later followed by denitrifying, nitrogen-fixing, and ammonifying bacteria. Ultimately, the microbial abundance in the oil palm plantation were higher than that of the tropical rainforest.

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