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Organic Acids Exudates and Enzyme Activities in the Rhizosphere based on Distance from the Trunk of Oil Palm in Peatland

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ABSTRACT

Enzyme activity in oil palm rhizosphere could be used as a quality indicator of peatland. Roots play an important role in producing exudates of organic acids that are deposited in the rhizosphere. This research aims to study root exudates and enzyme activities in oil palm rhizosphere based on the distance from the tree trunk. The research was done in an oil palm plantation at Koto Gasib, Siak District, Riau Province, Indonesia (0.74°–0.77° N and 101.77°–101.74° E) using the explorative method. The observation of oil palm rhizospheres was done by dismantling the root zone of the selected oil palm tree trunk. Oil palm root was collected at distances of 0–1, 1–2, and 3–4 m from the tree trunk while the adhered peat samples were taken at the surface layer of 0–25 cm depth within a quarter circle area of the canopy. The results showed that enzyme activities in oil palm rhizosphere decreased with increasing distance from the tree trunk. This decrease is attributed to the increase in organic acid root exudates and water content and a decrease in soil pH. Thickness of peat did not influence enzyme activity and organic acid content. Enzyme activities in the rhizosphere of severely degraded forest and shrubs were lower than those in oil palm rhizosphere. The organic acid exudates in the rhizosphere of oil palm, forest and shrubs consisted mainly of aliphatic compounds. Fertiliser application at 1–2 m from oil palm trees decreased organic acid content in the exudates. Results from this study also showed that the large N uptake by plants mainly originated from fertiliser application. Thus, nutrient supply for oil palm growth originated from fertiliser application rather than from peat decomposition.

Keywords: **Root exudates, rhizosphere, enzyme activities, oilpalm, peatland.**

INTRODUCTION

Peatlands have been used extensively for agriculture with approximately 1.7 million ha of the total of 14.9 million ha of Indonesian peatland under oil palm cultivation (Tropenbos International Indonesia 2012; Ritung *et al*. 2011). Currently there is increasing concern about the conversion of peatland into oil

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palm plantation due to carbon dioxide (CO_2) emissions as a result of land drainage and peat decomposition.

The changes in some chemical properties in oil palm plantations as a result of peat decomposition can be understood by studying rhizosphere enzyme activities. The changes in enzyme activity, especially the extracellular enzymes, are actually microbiota responses to environmental factors (Nannipieri *et al.* 2011). Enzyme activity is affected by plant root exudates in the form of organic acids (malic, acetic, succinic, citric and maleic acids) (Gianfreda 2015). Thus, the process of decomposition of organic matter caused by conversion of peatland into oil palm plantations can be investigated through the activity of enzymes affected by plant roots in the rhizosphere.

The enzyme often used as an indicator of organic carbon (C) mineralization in the soil is *β*-glucosidase (Stott *et al.* 2010). Some enzymes, such as urease, acid phosphatase, *β*-glucosidase and laccase serve as indicators of the decomposition process of organic matter in peatland. Sabiham *et al*. (2014) found that fertiliser application at the position nearest to the oil palm trees increased the nutrient levels in peat soil. According to Aon and Colaneri (2001), soil enzyme activities inhibited by N fertiliser are promoted by P and K fertilisers. Plant roots stimulate enzyme activity due to their positive effects on microbial activity and production of exudates (USDA 2010). Fertiliser application and the distribution and root mass of oil palm roots are influenced by enzyme activity. The aim of this study was to determine organic acid exudates and enzyme activities in the rhizosphere of peatland based on distance from the oil palm tree trunk.

METHODS

The research was done in an oil palm plantation in Pangkalan Pisang Village, Koto Gasib, Siak District, Riau Province, Indonesia (0.74°–0.77° N and 101.77°–101.74° E). The peat material analysis was conducted at the Laboratory of Chemistry and Soil Fertility, Department of Soil Science and Land Resources, Bogor Agricultural University (IPB), Bogor and at the Laboratory of Agrochemical Material Residue, Agricultural Environment Research Institute, Center for Agricultural Land Resource, Bogor Indonesia.

The research was an exploratory study in the form of field observation activities. Sites were selected based on peat thickness of <3 and >3 m and plant age of oil palm <6 years, 6–15 years and >15 years. The observations were done on transects perpendicular to the drainage channel (collection drain). In each transect two oil palm trees were dismantled 50 m and 100 m from the collection drain.

The observation of oil palm rhizosphere was done by dismantling the root zone of the selected oil palm tree trunk on each transect on the *frond windrow*, that is, space between two rows of plants. Oil palm roots were collected at distances of 0–1, 1–2, and 3–4 m from the tree trunk within a quarter circle area of the canopy (Fig. 1). Adhered peat samples were taken at the surface layer of 0–25 cm depth within 1-2 mm from the roots. Differences in root distribution and fertiliser application in the oil palm circle served as benchmark changes in the rhizosphere of oil palm on peatland (Fig. 1). Peat samples were collected 3-4 months after the last regular fertiliser application (for nutrients N, P, K) by plantation management. The amount of N and P fertilisers applied by plantation management was as much as 19 (N), 15 (P) kg/tree (for oil palm <6 years); 6 (N), 1(P) kg/tree (for oil palm 6-15 years) and 7 (N), 3(P) kg/tree (for oil palm >15 years) before soil sampling was done. For comparison purposes, samples were also taken from peat soil profiles (0-25 cm) of severely degraded forest and shrub vegetation in peatland.

Water content in the peat was determined by gravimetric method. The pH value measurement(1:2) of peat material (10 g of material: 20 ml of deionised water) was performed using a pH meter (2700 Autech Instrument). Total N and P detemination by digestion method with 98% H_2SO_4 solution. Total P was determined by 60 % $HClO_4$ solution and blue colour intensity was measured by Spectrofotometer 490 nm Shimadzu UV 1280 (Page *et al*. 1982). Organic acid exudates were extracted with deionised water (5 g peat matter: 10 ml deionised water); the mixture was ???? for about 30 min and centrifuged at 4000 rpm. Organic acids in the supernatant were measured by high-performance liquid chromatography (HPLC) Shimadzu 20A.

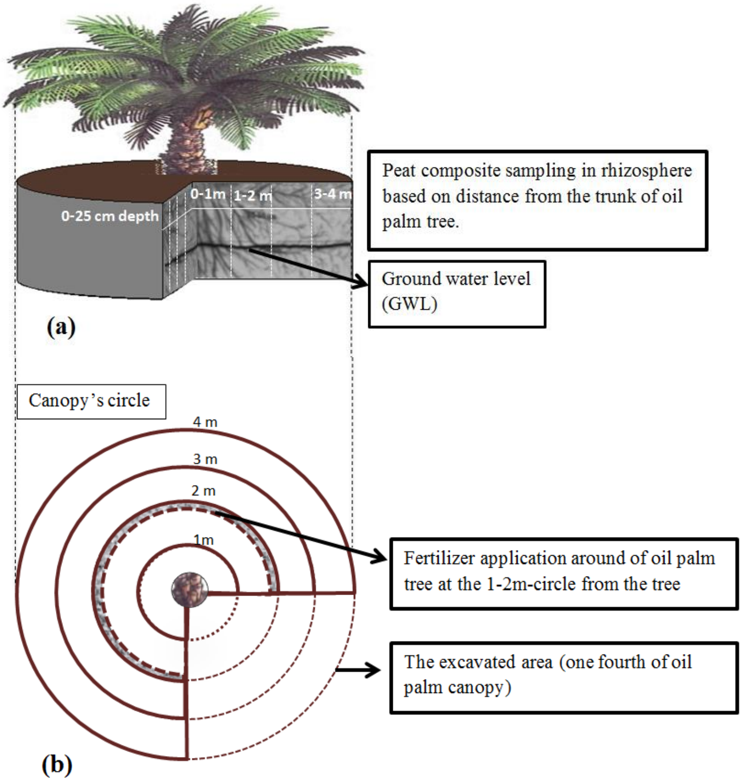

Fig. 1: Peat composite sampling in rhizosphere based on (a) distance from the trunk of oil palm and (b) fertiliser application around of oil palm tree

Activities of enzymes urease, phosphatase, β-glucosidase, and laccase activities were measured In the case of urease, the release of ammonium by the non-buffered method was measured (Schinner *et al.* 1996). Phosphatase activity was determined by measuring the release of phosphorus (P) from organic P by the p-nitrophenyl phosphate buffer method (Schinner et al. 1996). *β*-glucosidase activity was determined to measure the breakdown of cellulose into glucose using the *β*-glucosido-saligenin (salicin) method (Schinner *et al.* 1996). Finally, laccase activity was determined by measuring lignin degradation activity using the 2, 2-azinobis (3-ethyl-benzothiazoline-6-sulphonate) method (Eichlerová *et al.* 2012).

RESULTS AND DISCUSSION

Total Weight of Oil Palm Roots

Higher root biomass was consistent with palm age and increased soil CO_2 flux, suggesting that root respiration and microbial activity are associated with root exudates as a major component of soil respiration in tropical peatland under oil palm (Melling *et al*. 2014). The older the oil palm, the higher the weight of the main roots (root diameter (Ø) >0.5 mm) and feeding roots (Ø <0.5 mm) at a depth of 0–25 cm (Table 1). The total weight of roots and feeding roots decreased with increasing distance from the tree. The total weight of roots in peat (thickness <3 m) was higher compared to the peat (thickness >3 m) (Table 1). Sabiham et al. (2014) found that in the 0–15 cm peat layer, the quaternary roots had the highest density compared to primary, secondary, and tertiary roots.

pH Value and Water Content

The water content of the peat in the rhizosphere of oil palm ranged from 150%–450%, and this was found to increase with increasing distance from the tree trunk for all treatments (Fig. 2). This might be due to the rapid water absorption in the root zone, which consisted of more than 50 % of the total weight of roots. The water content was negatively correlated with enzyme activity, indicating that increasing soil water content might lead to decreasing enzyme activity (Table 2). Water content of peat soils from Bibai marsh, Hokkaido in Japan was found to vary from about 200% to more than 2000% of dry weight (Hamamoto *et al.* 2010).

The pH of the rhizosphere was in the range of 3.5–4.0. The pH value of peat decreased with increasing distance from the palm tree, which might be due to the increase in water content, root activities and the release of organic acids. In the rhizosphere of severely degraded forests, peat moisture content was higher compared to shrub vegetation because the stand in the severely degraded forest maintained a higher water content than that of the shrub vegetation (dominated by ferns). The pH values in the rhizosphere of severely degraded forest and shrub vegetation tended to be similar (within the range of 3–3.5), but lower than the pH of peat in oil palm rhizosphere. This is because the peat in oil palm rhizosphere held

TABLE 1

Weights of oil palm main-roots (Ø >0.5mm) and feeding-roots (Ø < 0.5mm) in relation to oil palm ages, peat thickness and distance from oil palm tree trunk.

Oil palm age (years old)		Total weight of roots (kg/m^3)					Feeding root weight (kg/m^3)				
		Distance from tree trunk (m)					Distance from tree trunk (m)				
		0-1	1-2	2-3	3-4	Total	0-1	1-2	2-3	3-4	Total
	n	**Peat thickness <3 m**					**Peat thickness <3 m**				
<6	4	1.96 ± 0.59	2.14 ± 0.28	0.82 ± 0.081	0.15 ± 0.05	5.06 ± 0.99	1.33 ± 0.43	1.27 ± 0.17	0.58 ± 0.057	0.11 ± 0.03	3.39 ± 0.687
6-15	4	4.36 ± 0.78	2.18 ± 0.26	3.11 ± 1.55	1.50 ± 1.01	11.13 ± 3.59	2.99 ± 0.53	1.74 ± 0.21	1.12 ±0.55	1.40 ± 0.94	7.25 ± 2.23
>15	4	8.28 ±5.53	4.04 ± 3.47	2.26 ± 0.27	1.23 ± 0.67	15.82 ± 9.94	3.00 ± 2.01	2.38 ± 2.04	1.3 ± 0.15	1.08 ± 0.59	7.76 ± 4.79
		Peat thickness >3 m					**Peat thickness >3 m**				
<6	4	2.55 ± 0.33	2.70 ± 0.84	1.40 ± 0.24	0.57 ± 0.67	7.24 ± 2.10	0.96 ± 0.12	2.33 ± 0.73	0.92± 0.16	0.55± 0.65	4.76 ± 1.66
6-15	4	5.00 ± 4.74	1.82 ± 0.20	0.89 ± 0.24	0.69 ± 0.73	8.42 ± 5.92	3.58 ± 3.39	1.47 ± 0.97	0.65 ± 0.17	0.58 ± 1.15	6.28 ± 5.68
>15	4	5.32 ± 3.56	2.82 ± 0.44	2.02 ± 1.12	0.89 ± 0.41	7.23 ± 5.55	2.41 ± 1.61	2.12 ± 0.33	0.96 ± 0.53	0.68 ± 0.31	6.17 ± 3.09

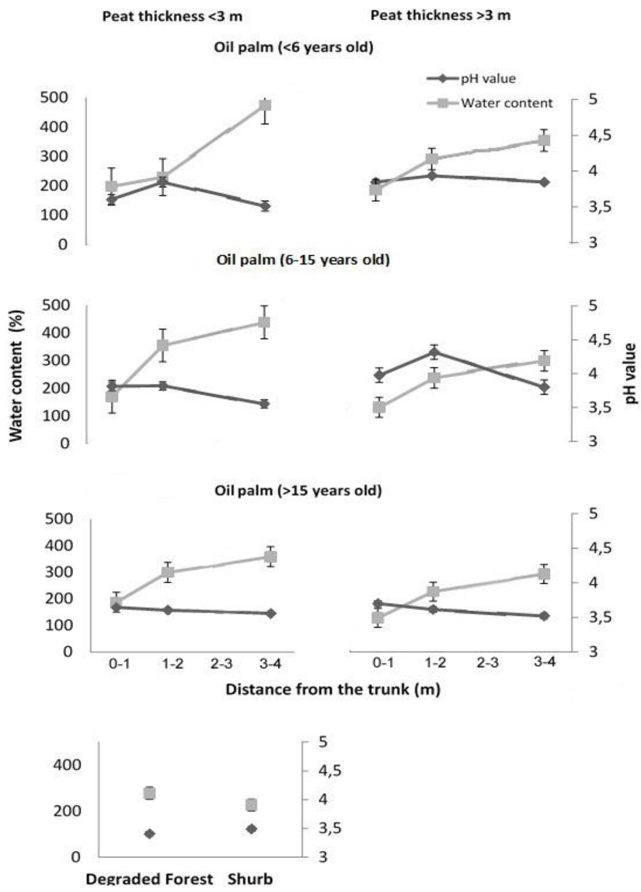

Fig. 2: Water content and pH in rhizospheres of oil palm based on distance from oil palm tree trunk, oil palm age and peat thickness and rhizosphere of degraded forest and shrub in peatland.

almost twice the water content compared to that of the severely degraded forest and shrub vegetation. The pH of the peat was very low (2.68–3.74) but was relatively high in all of the peat layers sampled at the agricultural site in central Kalimantan, Indonesia (Könönen *et al.* 2015).

The increase in organic acid content decreased the pH of peat due to the donation of protons from the organic acid group. This is associated with the concentration of soil nitrogen (N) and phosphorous (P), where low availability of P triggers the release of citric and oxalic acids into the rhizosphere to solubilise P and promote NH_4^+ uptake by plants, thus decreasing the pH. pH is positively correlated with urease and phosphatase in which the decrease in pH suppresses enzyme activities. Blonska (2010) observed the same results for urease and dehydrogenase activities which were found to increase with increasing pH in peatland.

Total N and P Content

Total N content in the oil palm rhizosphere (0.5%–1.5%) was lower than in the rhizosphere of severely degraded forest and shrub vegetation (1%–2%). This might be due to the high application of Nitrogen fertiliser by oil palm plantations (average of 2.3% to 2.7% N). The large N uptake by plants is largely due to fertiliser application.

The total N content of peat in young (lesss than 6 year old) and old (more than 15 year old) oil palm rhizosphere for peat thicknesses of <3 and >3 m tended to decrease with increasing distance from the tree trunk (Fig. 3). In contrast, total N content significantly decreased, at a distance of 1–2 m from the tree trunk and increased at a distance of 3–4 m from the tree trunk in 6–15- year-old oil palm. This is because of the large N uptake by roots of oil palm at the 1–2 m distance, although fertiliser was applied further away from the tree trunk, and the high N requirement of 6–15-year-old oil palm (Fig.3). The total N content of peat correlated positively with citric and butyric acids (Table 2). The increase in total N correlated with increased organic acid content, especially at a distance of 3–4 m from the oil palm tree (Fig. 3). The high root activity at a distance of 3–4 m, due to high root exudates, leads to high organic acid content, triggering an increase in microbial abundance and microbial activities, thereby increasing the total N content.

Total P content of peat in oil palm rhizosphere ranged from 100–300 ppm, whereas P contents in the rhizosphere of severely degraded forest and shrub vegetation were only around 100 ppm. The high P content in oil palm rhizosphere was due to the application of large amounts of P fertiliser. The P contents in <6- and >15-year-old oil palm rhizosphere tended to decrease with decreasing distance from the tree trunk. For 6–15-year-old oil palm with peat thickness of >3 m, the total P content increased at a distance of 3-4 m from the tree trunk because the organic acid exudates increased P solubility. The release of high organic acids content from carboxylic groups, especially citric acid (Fig. 4) shows the low solubility of P in the rhizosphere, while oil palm needs very high available P. According to Neumann *et al*. (2000), the plant roots secrete a number of organic acids, especially citric acid (in mature plants), to acidify the rhizosphere.

Organic Acid Content of Root Exudates

Organic acids are part of the root exudates released into the rhizosphere; they are the source of energy for microbes in enzyme synthesis, which affect physical and chemical properties of peat (Carvalhais *et al.* 2010). The carboxylic acid-containing organic acid of peat material in oil palm rhizosphere aged <6 years at peat depths of <3 m and >3 m tended to decrease with increasing distance from the tree trunk, while carboxylic acid containing organic acid content for oil palm aged 6–15 years and >15 years decreased at a distance of 2 m and increased at a distance of 3–4 m from the tree trunk (Fig. 3). This could be due to oil palm roots not releasing root exudates at a distance of 1–2 m from the tree trunk as an effect

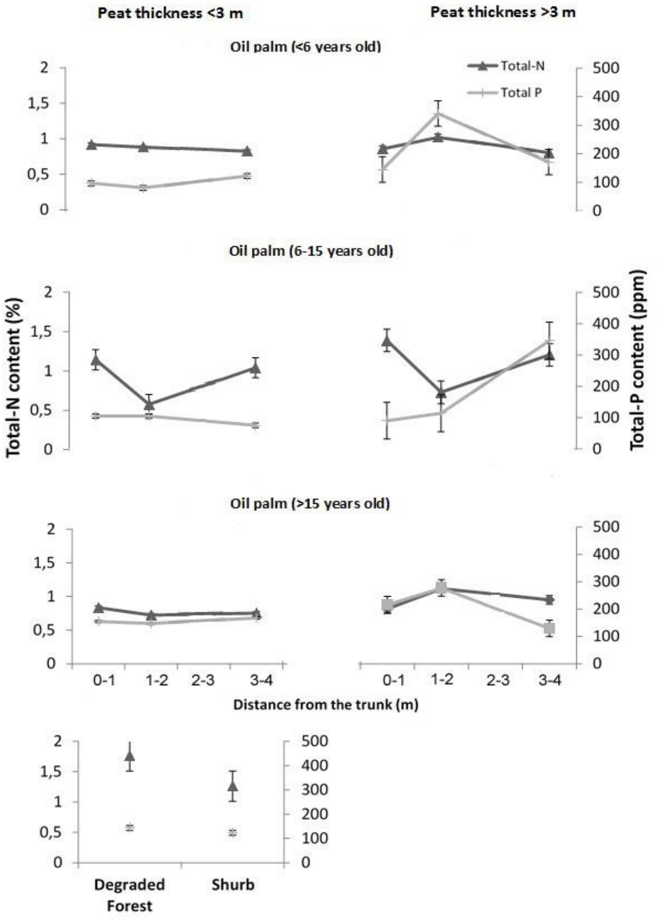

Fig. 3: Total Nitrogen and Phosphorous contents in rhizosphere of oil palm based on distance from oil palm tree trunk, oil palm age and peat thickness and rhizosphere of degraded forest and shrub in peatland.

of intensive fertiliser application in the oil palm tree circle at a distance of 1–2 m (Fig. 1). The increase in carboxylic acid content at a distance of 3–4 m from the tree might be due to an increase in root activity because of increasing plant age (i.e., in 6–15-and >15-year-old plants), root hairs (feeding roots) and decreased pH (Fig. 2). The expansion of the root total weight by root hairs reached >50% of the total weight of the roots (Table 1). According to Badri and Vivanco (2009), root hair cells are involved in root secretion of organic compounds. Garcia et al. (2001) showed that root exudation is positively correlated with root growth; actively growing root systems secrete more exudates.

pH value was significantly negatively correlated with oxalic and citric acid contents and positively correlated with enzyme activities (urease and phosphatase; Table 2). The organic acid content in the oil palm rhizosphere showed a positive correlation with each group of organic acids (malic, acetic, citric, oxalic and

TABLE 2
Pearson's correlation coefficient test between rhizospheres pH, water content for total N and P, root exudate organic acids, and enzyme activities in peatland.

	pH	**Water Content**	**Total N content**	**Total P content**	**Malic acids**	**Acetic acids**	**Oxalic acids**	**Citric acids**	**Butyric acids**	**Urease**	**Acid Phosphatase**	**β-glucosidase**	**Laccase**
pH	1	-0.342	-0.206	0.210	-0.042	-0.319	**-0,536***	**-0.524***	-0.217	**0.780****	**0.490***	0.139	0.179
Water content		1	-0.098	-0.049	-0.057	-0.049	0.130	-0.095	-0.245	-0.324	**-0.532***	**-0.690****	**-0.559***
Total N content			1	0.234	0.111	0.257	0.244	**0.560***	**0.490***	0.147	0.071	0.106	-0.053
Total P content				1	-0.312	-0.037	-0.067	-0.016	-0.130	0.436	0.259	0.094	-0.032
Malic acids					1	**0.551***	0.458	0.313	**0.734****	0.052	0.137	-0.019	0.100
Acetic acids						1	**0.780****	**0.641****	**0.661****	-0.148	-0.329	-0.098	0.261
Oxalic acids							1	**0.617****	0.414	-0.260	0.416	0.041	0.062
Citric acids								1	**0.759****	0.155	-0.115	0.258	0.373
Butyric acids									1	-0.019	0.122	0.191	0.327
Urease										1	**0.571***	0.158	0.145
Acid Phosphatase											1	0.339	0.224
B-glucosidase												1	**0.842****
Laccase													1

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level

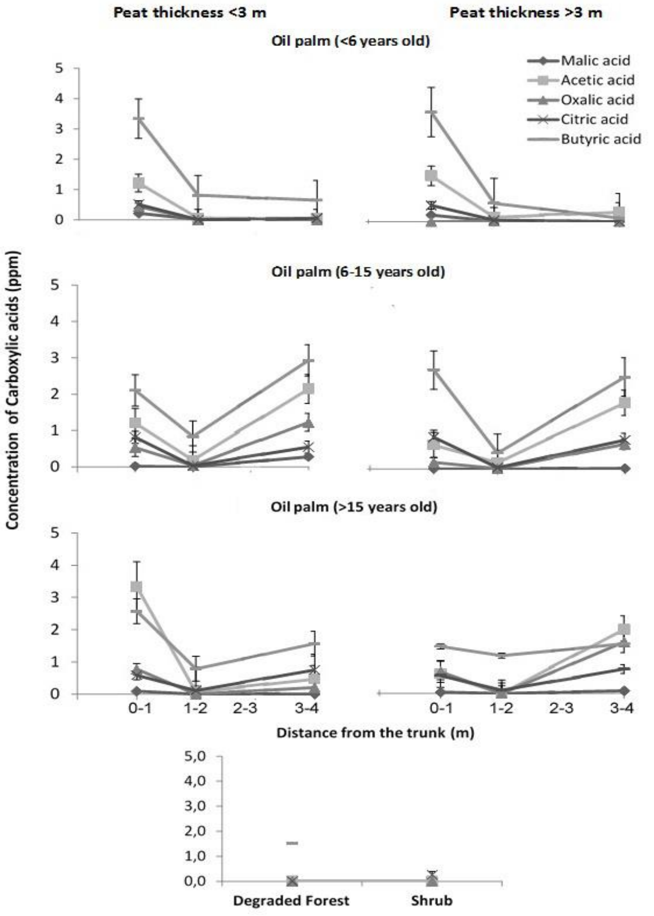

Fig. 4: Aliphatic acids (carboxylic acids) concentrations (ppm) in rhizosphere of oil palm based on distance from oil palm tree trunk, oil palm age and peat thickness and rhizosphere of degraded forest and shrub in peatland.

butyric acids; Table 2). The carboxylic acid containing organic acid in the rhizosphere of severely degraded forest and shrub vegetation was lower than in the oil palm rhizosphere at various ages, which indicates that the root of oil palm actively secretes organic acid exudates that might affect peat conditions. The active secretion of root exudates by plant roots indicates the low solubility of nutrients needed by plants (Carvalhais *et al.* 2010). The high amounts of organic acids (malic and citric acid) released into the rhizosphere is a response to low P uptake by plants so that these acids can solubilise the phosphate, making it more available for plant use (Neumann and Martinoia 2002). A similar trend was observed with, the content of aromatic organic acid (phenolic acid) (Fig. 3). The synthesis of carboxylic anions and their ultimate exudation in the rhizosphere also depend upon the cation-anion balance (Hinsinger *et al.* 2003)(Not in ref list???).

In the oil palm aged <6 years, the decrease in phenolic organic acid content was consistent with the distance from the tree trunk (Fig. 5), while in the oil palm of 6–15 years and >15 years, phenolic acid content increased with increasing distance from the tree trunk because of the large contribution of hair root exudates in the release of organic acids. Phenolic acids content was very low in the rhizosphere of severely degraded forest and shrub vegetation compared with oil palm rhizosphere. Aliphatic organic acid (carboxylic acid) content was higher in the rhizosphere of peat compared to that of aromatic organic acids (phenolic acids), where carboxylic acids were at concentrations of <20 μlL^{-1} and phenolic acids at <2 μlL^{-1} . This finding is similar to the observation by Tuason and Arocena (2008) that the concentrations of low molecular weight aliphatic organic acids in soil samples and root exudates in the rhizosphere are higher than those of

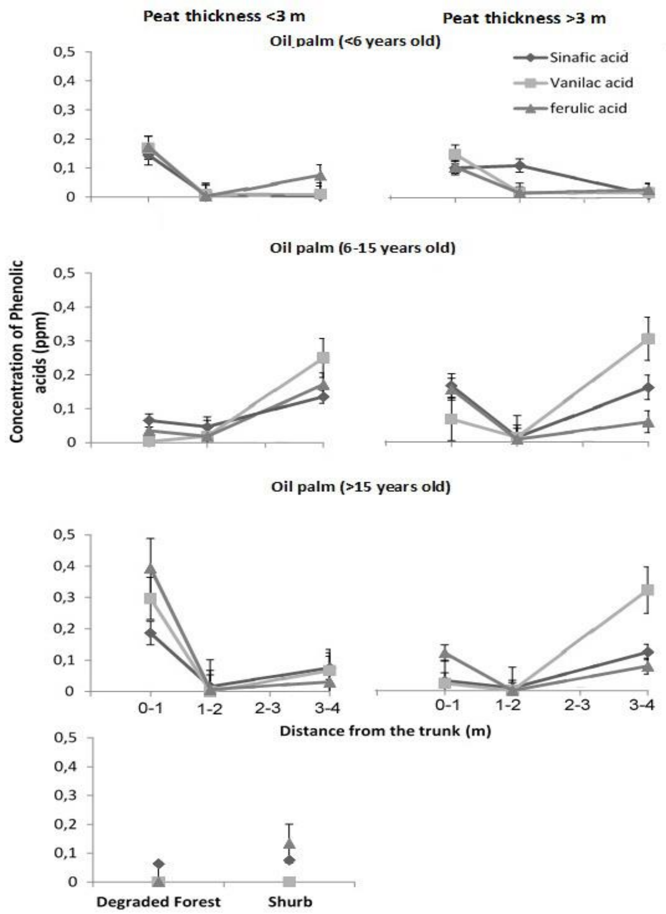

Fig. 5: Aromatic acids (phenolic acids) concentration (ppm) in rhizosphere of oil palm based on distance from oil palm tree trunk, oil palm age and peat thickness and rhizosphere of degraded forest and shrub in peatland.

aromatic acids. In contrast to the tropical peatland in Kalimantan and Jambi, the concentrations of phenolic acid (ferulyc and synapic acids) in our study were higher than those of carboxylic acids (Sabiham 1997(not in ref list???); Mario and Sabiham 2002).

Enzyme Activity

Enzyme and microbial activities are related to and associated with decomposition of organic matter. Peat thickness did not significantly influence enzyme activity. Urease activity in the rhizosphere of the peatland was very low (<0.5 μg g^{-1} h^{-1}) because of the low pH of peat (Fig. 2); there was a significant negative correlation between peat pH and urease activity. The nitrogen supply to meet the plant requirement was largely derived from the fertiliser applied. This indicated that the availability of N and P were not associated with peat decomposition but from the high N fertiliser application in the circle of the tree. According to Aon and Colaneri. (2001) a decrease in urease activity could be explained by the activation of nitrification and denitrification which causes the suppression of urease production.

Phosphatase activity ranged from 2–6 μg g^{-1} h^{-1} and was higher than urease activity, indicating that phosphate requirement by plants is not met by the fertiliser addition. Phosphatase activity in peat of <3 m thickness was lower than that in peat of >3 m thickness and tended to decrease with increasing age of the plant. This is because the P fertiliser applied (which was sufficient at age <6 years) was insufficient due to low solubility. Plants can survive under low P conditions by secreting phosphohydrolase (phosphatase) to solubilise organic phosphate into soluble inorganic phosphate in the rhizosphere (Lefebvre *et al*.1990; Duff *et al.* 1994). The increase in phosphatase activity in the rhizosphere of oil palm seedlings suppresses the production of oxalic acid (Widiastuti *et al.* 2015)(2003 in ref list).

β-glucosidase and laccase activities are the activities of enzymes involved in decomposing peat derived from cellulose–hemicellulose and lignin. β-glucosidase and laccase activities in the oil palm rhizosphere tended to decrease with increasing distance from the tree trunk (Fig. 6). The enzyme activities of β-glucosidase and laccase were higher than those of phosphatase and urease, which indicates that the nutrient supply from the peat decomposition process (lignocellulolytic) is in the rhizosphere. This is due to the high root activity involved in releasing organic acid exudates to dissolve organic matter and absorb nutrients and the low water content of peat.

The high enzyme activity at adistance of 0–1 m from the tree trunk was caused by the high activity of roots and low water content of peat, which led to an oxidative condition that increases the microbial activities in the rhizosphere. However, application of fertilisers a ta distance of 1–2 m from the tree trunk led to reduced enzyme activities and decreased organic acids content (Fig. 4 and 5), whereas a decrease in enzyme activities at a distance of 3–4 m from the trunk was caused by an increase in water content and low pH of peat. β-glucosidase and

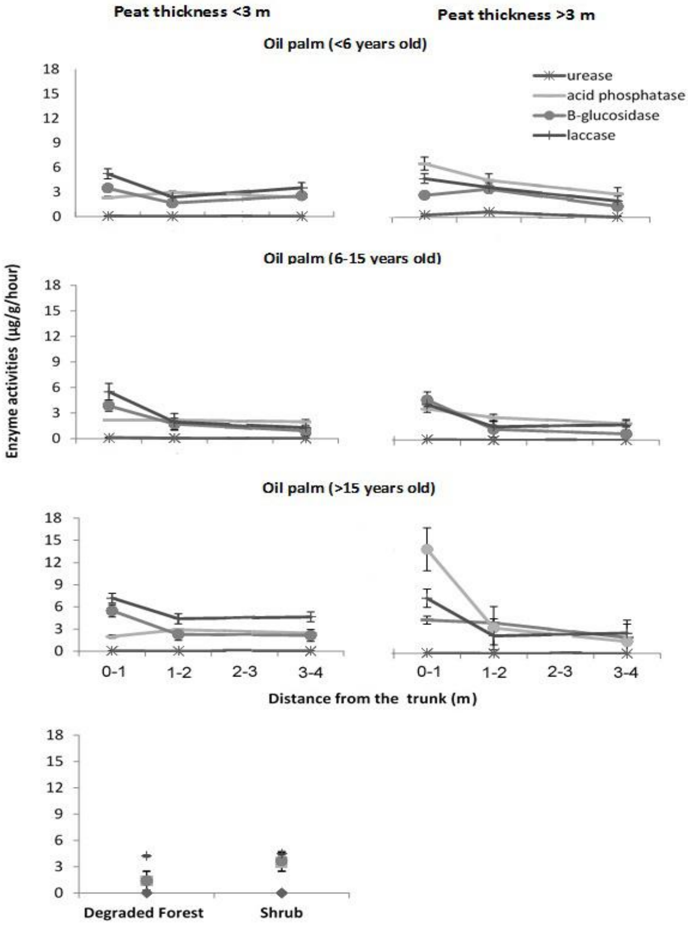

Fig. 6: Enzyme activities in rhizosphere of oil palm based on distance from oil palm tree trunk, oil palm age and peat thickness and rhizosphere of degraded forest and shrub in peatland.

laccase activities were significantly negatively correlated with the water content (Table 2). The enzyme activities in the rhizosphere of severely degraded forest and shrub vegetation tended to be similar as in the rhizosphere of oil palm trees in peat of different thickness, indicating that peat decomposition also occurs in unproductive peatland of different thickness.

CONCLUSION

The enzyme activity in the oil palm rhizosphere decreased with increasing distance from the tree trunk due to an increase in water content, adecrease in pH, and an increase in organic acid root exudates, especially carboxylic organic acids. Aliphatic organic acids (carboxylic group) released into the rhizosphere were higher than aromatic organic acids. The thickness of peat did not influence enzyme activity and organic acid content. Fertiliser application in the circle of the oil palm tree trunk decreased the content of organic acid exudate and enzyme activity at a distance of 1–2 m from the tree trunk, while the nutrient supply for plants was derived from fertiliser and not from peat decomposition.

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