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Research Article

Microbial proportion and heterotroph CO₂ flux from drainage peatland under oil palm plantation

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Abstract

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Keywords: greenhouse gases rhizosphere root exudates water management CO₂ fluxes from microbial decomposition in peatlands. Oil palm plants release root exudates transported to other places, i.e., shrub, by water movement, which can stimulate microbial activity. This study was conducted to learn the effects of differences of the soil layer and distance from the trunk in drainage peatland under oil palm plantation on total microbes, fungi, cellulolytic bacteria, ligninolytic fungi, and heterotroph fluxes CO₂, then compared to a shrub. Heterotroph respiration decreased with soil layer depth, where at the layer 0-20 cm released amount of CO₂ as much 6.0 ± 1.76 , at 20-40 cm is 5.18 ± 0.50 , and at 40-60 cm 5.27 ± 1.37 mg CO₂ 100g⁻¹ day⁻¹, and tended higher than in shrub where a layer of 0-20 cm released 5.51 \pm 1.69, then decrease at 20-40 cm to 4.83 \pm 1.37, and at 40-60 cm 4.30 + 1.09 mg CO₂ 100g⁻¹ day⁻¹. Total microbes (10⁷ CFU g⁻¹) and fungi (10⁵ CFU g⁻¹) were higher than total cellulolytic bacteria (10³ CFU g⁻¹) and ligninolytic fungi (10² CFU g⁻¹) in both under oil palm plantation and shrub. Organic acids affected the abundance of total microbes and fungi but did not affect cellulolytic bacteria and ligninolytic fungi on both sites, as shown by a lower population and cellulose and laccase enzymes. These findings showed that heterotroph CO₂ flux tended higher in oil palm plantations rather than in shrub.

The difference in soil layer can affect heterotroph respiration that means

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Introduction

Tropical peatlands are ecosystems that play an essential role in hydrological functions such as carbon storage and cycles. In Riau province, the peatland areas cover around 3.87 Mha (60% of the total peatland areas in Sumatera) (Page et al., 2011). At present, tropical peatland in Riau is widely cultivated for oil palm plantation, which that cultivation followed by drained and increased microbial activity (Jaenicke et al., 2010). Therefore, drained peatlands will change the potential C sink into a significant source of CO_2

release, causes a substantial increase in the decomposition rate of peat material (Hergoualc'h and Verchot, 2011; Itoh et al., 2017). Microbial activity is very related to CO_2 release from heterotroph respiration in peatlands, the crucial microbes related to peat decomposition are cellulolytic and ligninolytic. Widiastuti et al. (2020) reported these microbial groups to produce extracellular enzymes that can degrade cellulose or lignin. Those enzymes can be cellulase for cellulose, while laccase, Mn-peroxidase, and Fe-peroxidase for lignin. Batubara et al. (2018)

mentioned that the lower soil depth would decrease microbial activity and CO_2 release due to saturated conditions.

Microbial activity in drained peatlands not only affected by soil depth but also by the density of root vegetation. According to Sinuraya (2010), the root density of oil palm plants in peatlands is dominant at the closest distance from the trunk and decreases to the length of 4.5 m; oil palm roots are spread around 30-45 cm of soil depth. Root density can stimulate microbial activity because it is derived from root exudates and modified by microbial metabolism, a significant source of C release (Kuzyakov and Domanski, 2000; Kane et al., 2014). Organic acids derived from root exudates can increase microbial activity because they were low molecular compounds that quickly degraded and increased microbial activity in peat decomposition and release heterotroph CO₂ flux (Bengston and Bengtsson, 2007).

Heterotroph respiration in drained peatlands was reported as a major contributor to total emission from oil palm plantations. Previous research from Hoijer et al. (2010) said that heterotroph flux CO₂ from drained peatlands is 91.00 Mg CO₂ ha⁻¹ year⁻¹ which is estimated based on subsidence rate. Prananto et al. (2020) reported CO₂ flux from heterotroph respiration is around 39.60 Mg CO₂ ha⁻¹ year⁻¹, while Dariah et al. (2013) reported a heterotroph CO₂ flux around 38.20 Mg CO₂ ha⁻¹ year⁻¹ were both estimated by dark closed chamber method. Based on those previous research, it was uncertain emission rates, and there is no yet significant variation estimation in Indonesia compared to uncultivated drained peatland such as shrub.

This research aimed to learn the effects of the differences in soil layer and distance from the trunk in drained peatland under oil palm plantation on microbial proportion (total microbes, fungi, cellulolytic bacteria, and ligninolytic fungi), heterotroph CO_2 flux, organic acids, and enzyme activity (cellulose and laccase activity), then compared to drained peat at shrub.

Materials and Methods

Study location

Soil sampling was conducted at tropical peatlands in Buatan Village, Siak Sri Indrapura Regency, Riau Province, Indonesia (0' 44' 44" N 101° 46' 22' B) with a thickness >550 cm, soil water content around 400-500%, maturity level around fibrist to hemist, and soil pH around 3.50 ± 0.42 . Sampling was carried out from two different locations, i.e., 1) under oil palm plantation cultivated 13 years and received fertilization according to the standard dose of NPK (15-6-24) and 2) shrub. Soil samples were taken from under oil palm trees at several distances, i.e., 1.5, 3.0, and 4.5 m from the trunk with three layers of soil depth, i.e., 0-20, 20-40, and 40-60 cm.

Fluxes of CO₂ measurement method

 CO_2 quantification was measured by root-free soil incubation method where 100 g of wet peat sample was placed in a 5000 g jar, then added two small jars containing 50 mL KOH 0.2 N and 100 mL of distilled water. The incubation period was 7 days, the determination of CO_2 fluxes was made by the acidbase titration method using HCl 0.1 N, indicators of phenolphthalein and methyl orange (Anas, 1989).

Quantification of total microbes, cellulolytic bacteria, and ligninolytic fungi

Total microbes (bacteria and fungi) was observed by extracting 10 g of peat soil into 90 mL of physiological solution (0.85% NaCl) and soak them for 30 minutes. Isolation for total microbes was carried out by pour plate method using serial dilution from 10⁻² to 10⁻⁷ and inoculated to Nutrient Agar medium (28 g L⁻¹); for the total fungi the solution was inoculated into Potatoes Dextrose Agar (39 g L⁻¹), for total cellulolytic bacteria, the solution was inoculated into 1% Carboxymethyl Cellulose medium (in 1 liter contain 1 g KH₂PO₄, 0.5 K₂SO₄, 0.5 g NaCl, 0.01 g FeSO₄, 0.01 g MnSO₄, 1 g NH₄NO₃, 10 g CMC, and 20 g agar), and for total ligninolytic fungi, the solution was inoculated into Potatoes Dextrose Agar medium enriched with 0.05% Guaiacol (in 1 liter contains 39 g PDA, 0.5 mL guaiacol, and 0.5 g antibiotic chloramphenicol). Quantification of total microbes, cellulolytic bacteria, and ligninolytic fungi was conducted by colony counter.

Cellulase and laccase activity assay

Cellulase activity assay was determined by extracting 5 g of peat soil added to 12.5 mL of citrate buffer containing 1.4% CMC, then mixed using vortex and incubated for 30 minutes. The mixture was then added with 5 mL DNS (3.5-dinitrosalicylic-acid) and heated for 10 minutes at 100 °C. Quantification of cellulase activity was made by spectrophotometer Shimadzu UV-VIS 1280 at a wavelength of 540 nm (Ghose, 1987).

Laccase activity was determined by extracting 5 g of peat soil added to 20 mL of 0.2 M phosphate buffer pH 4, then centrifuged at 5.000 rpm for 10 minutes until it was completely clear. Pipetting 1.2 mL of enzyme filtrate, then mixed with 0.3 mL of acetate buffer pH 5 and 1 mL of ABTS (2.2-azino-bis-3-ethlylbenzothiazoline-6-sulphonic acid) and incubated for 30 minutes. Quantification of cellulase activity was carried out by spectrophotometer Shimadzu UV-VIS 1280 at a wavelength of 420 nm (Eichlerova et al., 2012).

Organic acids, soil pH, water content analysis

Organic acids were measured by extracting soil samples with 0.1 N NaOH (Baziramakenga et al., 1995). Organic acids such as malic, lactic, citric, and

oxalic acids were quantified using HPLC (Shimadzu 20A Gradient LC System with U V-Vis Detector).

Data analysis

Data obtained from this research were analyzed by variance with the F test at the confidence interval of 95%. Enzyme activity and organic acids were tabulated with Microsoft Excel 2016 and presented descriptively.

Results and Discussion

Proportion of microbes, fungi, cellulolytic bacteria, and ligninolytic fungi

The results showed that different layers of soil depths significantly affected the abundance of total microbes and cellulolytic bacteria, but they did not affect the abundance of total fungi and ligninolytic fungi, under oil palm plantation, while the several distances from the trunk did not significantly affect the abundance of all four total microbes (Table 1). At shrub, the more depth soil layer did not significantly affect the abundance of all total microbes (Table 2). The abundance of total cellulolytic bacteria and ligninolytic fungi decreased with the more depth of soil layer due to the saturated conditions. According to Brouns et al. (2016), the microbial population on drained agricultural peatlands will decrease due to waterlogged layers. The proportion of total cellulolytic bacteria (10³ CFU g⁻¹) and ligninolytic fungi (10² CFU g⁻¹) were lower than total microbes (10⁷ CFU g⁻¹) and fungi (10⁵ CFU g⁻¹) in both sites. Despite of the population of lignocellulolytic microbes was lower, it is also possible to increase if drainage is carried out excessively.

Table 1. Total microbes, fungi, cellulolytic bacteria, and ligninolytic fungi at several soil layers and distances from trunk under oil palm plantations.

Treatment Factor	Microbial Proportion (CFU g ⁻¹)				
	Total Fungi	Total Microbes	Cellulolytic bacteria	Ligninolytic Fungi	
Soil depth (cm)	P=0.767	P=0.087	P=0.000*	P=0.149	
0-20	3.690° x 105	1.564 ^{ab} x 10 ⁷	$3.150^{\circ} \times 10^{3}$	1.444b x 10 ²	
20-40	3.708 ^a x 10 ⁵	1.774° x 10 ⁷	$1.083^{a} \ge 10^{3}$	0.888 ^{ab} x 10 ²	
40-60	3.294° x 105	1.286 ^a x 10 ⁷	2.372 ^b x 10 ³	0.222a x 10 ²	
Distance from trunk (m)	P=0.625	P=0.145	P=0.053	P=0.650	
1.5	3.451° x 105	1.751ª x 10 ⁷	1.722 ^a x 10 ³	1.111 ^a x 10 ²	
3.0	3.323° x 105	1.322 ^a x 10 ⁷	2.672 ^b x 10 ³	$0.555^{a} \ge 10^{2}$	
4.5	3.918 ^a x 10 ⁵	1.552 ^a x 10 ⁷	2.211 ^{ab} x 10 ³	$0.888^{a} \ge 10^{2}$	

Notes: Numbers followed by the same letter in the same column show no significant difference based on DMRT test at the level of 5% (p<0.05).

Table 2. Total microbes, total fungi, cellulolytic bacteria, and ligninolytic fungi at several soil layers in peat shrub.

Soil depth (cm)	Microbial proportion (CFU g ⁻¹)				
	Total Fungi	Total Microbes	Cellulolytic bacteria	Lignolytic Fungi	
	P=0.570	P=0.785	P=0.512	P=0.259	
0-20	3.440 ^a x 10 ⁵	1.536 ^a x 10 ⁷	$1.766^{a} \ge 10^{3}$	$1.666^{a} \ge 10^{2}$	
20-40	2.826 ^a x 10 ⁵	1.356 ^a x 10 ⁷	2.850 ^a x 10 ³	$0.333^{a} \ge 10^{2}$	
40-60	2.006 ^a x 10 ⁵	1.206 ^a x 10 ⁷	$1.366^{a} \ge 10^{3}$	$0.000^{a} \ge 10^{2}$	

Notes: Numbers followed by the same letter in the same column show no significant difference based on DMRT test at the level of =5% (p<0.05).

This proportion can indicate that the release of CO_2 in this study was from ligninolytic activities and other microbial activities. This is because the accumulation of resistant organic materials dominates tropical peatlands such as in study sites. Resistant material can inhibit lignocellulolytic microbes and being slowgrowing microbes. According to Keuskamp et al. (2013), the microbial communities capable of oxidizing recalcitrant were under stress and became slow-growing on peatlands. The strain of some bacteria and fungi that can decompose recalcitrant compounds such as lignin and carbon complex such as cellulose are very limited and specific compared to the total microbes that respire through other metabolisms that were not observed in this study. One of the microbes known to degrade recalcitrant organic matter is white-rot fungi (Paul and Clark, 1996). Girkin et al. (2018) mentioned that microbes could use energy efficiently and prefer to consume low molecular compounds such as glucose, amino acids, organic acids derived from root exudates.

Heterotroph CO₂ flux

The observation results showed that soil depth and distance from trunk did not significantly release the different heterotroph CO_2 fluxes under oil palm

plantation (Table 3), while at shrub soil depth also did not significantly release the difference of CO2 flux (Table 4). However, the results showed a tendency that the more depth soil layer decreased the amount of CO₂ released. Soil respiration is affected by various edaphic factors, in which soil depth is one of the main factors controlling CO₂ fluxes in peatlands. The more depth of soil layer can increase soil saturation and result in the decrease of heterotroph CO₂ released. This result was similar to previous research from Batubara et al. (2018) that the more depth soil layer would decrease the net of heterotroph CO₂ flux because the microbial activity is very limited in saturated conditions. In comparison, the different distances from oil palm trunk were not affected to heterotroph CO2 flux because microbial activity around oil palm plants is influenced by roots exudates. Thus can increase the CO₂ release, root exudates such as organic acids in this study were released and moved to other places by water movement. It can be seen from the spatial distribution of organic acids in several distances from the trunk and in shrub that was also not different (Figure 2). Results of the t-test analysis showed that the heterotroph CO₂ flux from peatlands under oil palm plantations and shrubs was not different (p>0.05) (Figure 1). However, the heterotroph CO₂ fluxes from drained peatlands under oil palm plantations tended to be higher than a shrub. The Heterotroph CO₂ fluxes in peatland under oil palm plantations tended to be higher because fertilization stimulates microbial activity to produce CO₂.

Table 3. Fluxes CO₂ (mg 100g⁻¹ day⁻¹) at several soil layers and distances from trunk under oil palm plantations.

Treatment Factor	Fluxes CO ₂ (mg 100g ⁻¹ day ⁻¹)
Soil depth (cm)	P=0.297
0-20	6.00^{a}
20-40	5.18 ^a
40-60	5.27 ^a
Distance from trunk (m)	P=0.288
1.5	6.00 ^a
3.0	5.47 ^a
4.5	5.00 ^a

Notes: Numbers followed by the same letter in the same column show no significant difference based on DMRT test at the level of =5% (p<0.05).

Table 4. Fluxes CO₂ (mg 100g⁻¹ day⁻¹) at several soil layers at peat shrub,

Soil depth (cm)	Fluxes CO ₂ (mg 100g ⁻¹ day ⁻¹)
	<i>P</i> =0.595
0-20	5.51ª
20-40	4.83 ^a
40-60	4.30 ^a

Notes: Numbers followed by the same letter in the same column show no significant difference based on DMRT test at the level of =5% (p<0.05).

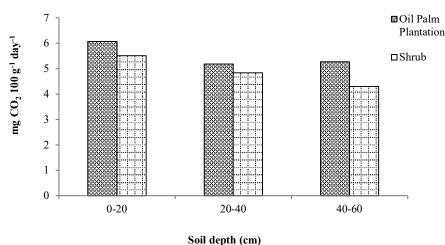


Figure 1. Heterotroph CO₂ fluxes from two different land use at oil palm plantation and shrub.

Previous research from Fenner and Freeman (2011) and Brouns et al. (2016) indicated that respiration rates in agricultural peatlands were higher than natural peatlands because nutrient addition can increase enzyme activity and respiration rates. Previous research from Sabiham et al. (2014) also found fertilizer application under oil palm plants increased the nutrient levels and respiration rate in peat soils.

Organic acids under oil palm plantation and shrub

Organic acid components observed in this study were acetic, lactic, citric, malic, and oxalic acids which are dominant in cultivated peatlands (Nurzakiah et al., 2020). The result showed that there was no different concentration of organic acids spatially from peatland under oil palm plantation with several distances from

trunk and shrub (Figure 2). The higher concentration of five types of organic acids vertically was in the soil layer of 20-40 cm. Concentrations of organic acids in peatlands under oil palm plantation and shrub were not different because spatially affected by radial diffusion rate, water movement, sorption to the solid phase, and microbes exudates consumption (Proctor and He, 2021).

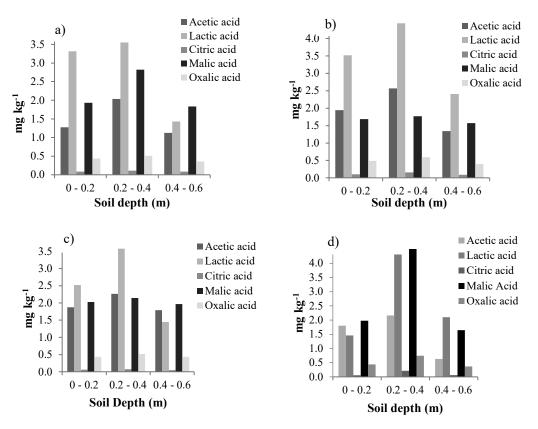


Figure 2. a) Organic acids at the 1.5 m, b) organic acids at the 3 m, c) organic acids at the 4.5 m from oil palm trunk, and d) organic acids from shrub.

In vertical gradients of soil depth, there was no consistent increase or decrease in organic acids concentration due to the considerable vertical heterogeneity of peatlands. Girkin et al. (2018) reported that the addition of organic acid concentration rapidly increased CO₂ fluxes by microbial metabolism. CO₂ release with OH⁻ while microbes consume H⁺ by degrading organic acids (Gramss et al., 2003). Organic acids in peatlands also can stimulate microbial activity, such as degrading soil organic matter (Kuzyakov, 2002). However, in this study, the role of organic acids in increasing microbial activity in peat decomposition remains unclear. This was shown by the abundance of total cellulolytic bacteria (10³ CFU g⁻¹) and ligninolytic fungi (10² CFU g⁻¹) that were lower than total microbes in peatlands (Tables 1 and 2).

Cellulose and laccase enzyme activity

Cellulose and laccase enzyme activity from peat soil from oil palm plantations was not significantly affected by several distances from the trunk and tended to be higher than that of shrubs. However, it is still

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classified as low activity (Figure 3). Enzymes activities are very related and associated with the decomposition process of peat material. The hydrolysis of cellulose into glucose and the oxidation of phenolic compounds into simple chains are complex reactions that were influenced by environmental factors. Cellulose and laccase enzyme activity in peat soil is affected by soil water content; this is because both are the type of enzymes that use O_2 as electron acceptors in the degradation process (Dashtban et al., 2009). Mulyawan et al. (2019) reported that differences in soil water content showed different cellulose and laccase activities. In this study, enzyme activity in both sites was not significantly different; it can be caused by soil water content in both sites also was not significantly different (p=0.128>0.05). These results were in line with Harianti et al. (2017), where the enzyme activities from rhizosphere with several distances from oil palm trunk and shrub tended to be similar because soil water content was not different. Soil water content in peatland could affect chemical and biological processes, such as decomposition activity (Nurzakiah et al., 2014).

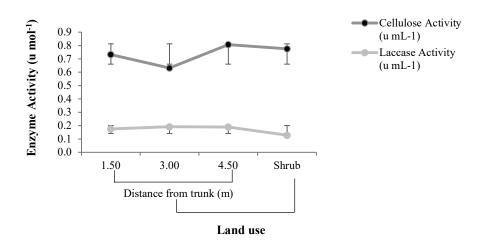


Figure 3. Activity of Cellulose and laccase enzyme (u mol⁻¹) from peat soil at some distances of trunk under oil palm plantations and shrub.

Conclusion

Soil depth tended to affect the heterotroph CO₂ flux, which the more depth of soil layer decreased flux of CO₂ in peat soils from tropical drainage peatlands. Heterotroph CO₂ fluxes were tended higher under oil palm plantation, although the total microbes in both sites were not different. The availability of organic acids did not significantly stimulate the abundance of cellulolytic bacteria and ligninolytic fungi, as shown by the population of cellulolytic bacteria and ligninolytic fungi lower than total microbes and fungi, also from the activity of cellulose and laccase enzyme. It concluded that the source of heterotroph CO₂ fluxes are not only from lignocellulolytic microbes in this study. Water management such as rewetting and maintaining groundwater levels are important to reduce heterotroph CO₂ release in peatland for sustainable oil palm production.

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