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Laccase activity as an indicator of peat degrading microbes in several types of peatlands use in West Sumatra, Indonesia

Aktivitas laccase sebagai indikator mikroba pendegradasi gambut pada beberapa tipe penggunaan lahan gambut Sumatera Barat, Indonesia

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ABSTRACT

Land clearing and drainage activities on peatlands had boosted the decomposition rate of organic matter, caused by an increase in soil temperature and soil microbial activity. Enzyme activity is a sensitive indicator to measure changes in the decomposition process especially for organic matter is Laccase. *This research aimed to study* Laccase *as an indicator of lignin degradation in several types of peatland use in Pesisir Selatan, West Sumatra, namely forests, shrubs, oil palm plantations, agricultural land, and bare land. The Laccase activity was measured by using the* ABTS *method. The results of our study show that* Laccase *activity in several types of peatland use from the highest to the lowest follows the order: agricultural land (LP) 3.20 μg.g^{-1}.hour^{-1} is higher than bare land (LT) 1.46 μg.g^{-1}.hour^{-1}, and consecutively is higher than shrub (SB) 1.17 μg.g^{-1}.hour^{-1}, smallholder oil palm plantation (PSr) 0.66 μg.g^{-1}.hour^{-1}, private company oil palm plantation (PSs) 0.70 μg.g^{-1}.hour^{-1}, and forest (H) 0.06 μg.g^{-1}.hour^{-1}. Soil characteristics that affect the enzyme activity are water content, which is negatively correlated with* the Laccase *enzyme. The higher the water content in the peat material, the lower the Laccase activity. Therefore, it is necessary to control the soil water content high to prevent the rate of peat decomposition.*

***Keywords**:* Laccase, *enzyme, oil palm plantation, land use, peatland.*

ABSTRAK

Aktivitas pembukaan lahan dan drainase pada lahan gambut menyebabkan peningkatan laju dekomposisi bahan organik sehingga suhu tanah dan aktivitas mikroba tanah meningkat. Aktivitas enzim menjadi indikator yang sensitif untuk mengukur perubahan proses dekomposisi terutama dekomposisi bahan organik adalah Laccase. Penelitian ini bertujuan mengkaji aktivitas Laccase sebagai indikator degradasi lignin pada beberapa tipe penggunaan lahan gambut di Pesisir Selatan, Sumatera Barat**,** seperti: hutan, semak belukar, perkebunan sawit, lahan pertanian dan lahan terbuka. Aktivitas Laccase dianalisis menggunakan metode ABTS. Hasil penelitian ini menunjukkan bahwa aktivitas Laccase pada beberapa tipe penggunaan lahan dari yang paling tinggi ke yang paling rendah mengikuti urutan sebagai berikut: lahan pertanian (LP) 3,20 μg.g^{-1}.jam^{-1} lebih tinggi dari lahan terbuka (LT) 1,46 μg.g^{-1}.jam^{-1} dan secara berturut-turut lebih tinggi dari semak belukar (SB) 1,17 μg.g^{-1}.jam^{-1}, perkebunan sawit rakyat (PSr) 0,66 μg.g^{-1}.jam^{-1}, perkebunan sawit swasta (PSs) 0,70 μg.g^{-1}.jam^{-1}dan hutan (H) 0,06 μg.g^{-1}.jam^{-1}. Karakteristik tanah yang mempengaruhi tinggi rendahnya aktivitas enzim tersebut adalah kadar air dimana kadar air berkorelasi negatif dengan aktivitas Laccase. Semakin tinggi kadar air dalam bahan gambut maka aktivitas laccase menurun. Oleh sebab itu perlu menjaga kadar air tanah tetap tinggi untuk mencegah laju dekomposisi gambut

Kata kunci: *Laccase*, enzim, perkebunan sawit, penggunaan lahan, lahan gambut.

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INTRODUCTION

Land clearing and drainage activities on peatlands have driven an increase in the rate of decomposition of organic matter that has the potential to emit CO2 into the atmosphere. The construction of drainage channels in oil palm plantations results in a decrease in the groundwater level, causing soil temperature and soil microbial activity to increase. Changes in these conditions generate carbon residues in peatlands to be emitted into the atmosphere. One of the carbon dioxide emissions from peatlands result from the root respiration process, which is known as autotrophic respiration. In contrast, soil organic matter decomposition by microbes is known as heterotrophic respiration (Luo & Zhou, 2006).

According to Agus et al. (2010) and Hergoualc'h and Verchot (2013) that CO_2 emissions resulting from the respiration process of roots are neutralized through photosynthesis by plants, while respiration resulting from peat decomposition by microbes contributes to greenhouse gases. The main component in the heterotroph respiration process is a microbial activity in decomposing litter and peat material. The groundwater level is a determining factor for the boundary between anaerobic and aerobic conditions of peatland, which affects the activity of microorganisms. The change from anaerobic to aerobic conditions could increase available O_2 affecting microbial activity. Nugroho et al. *(*2013) stated that the drained peatlands would change the conditions from anaerobic to aerobic. These results increased the microbial activity of organic matter decomposers.

Enzyme activity is a sensitive indicator to measure changes in the decomposition process. The enzyme associated with the decomposition of organic matter is Laccase. Laccase is one of the enzymes produced by lignin-degrading microbes (such as white root fungi), where the primary carbon source is lignocellulose, which results from the breakdown of lignin. These microbes secrete many enzymes that can break down the lignin structure (Farragher, 2013). The Laccase is an enzyme oxidizing a wide variety of aromatic and non-aromatic compounds used as a hydrogen donor (Farragher, 2013).

Lignin becomes a substrate for microorganisms in the decomposition process of organic matter. Also, lignin is the main compound of peat material, where peatland degradation is indicated by lignin oxidation mediated by lignin-degrading microbes, which is catalyzed by the Laccase enzyme (Theurl & Buscot, 2010). Based on the background, this research was conducted to study Laccase as an indicator of lignin-degrading microbes in several types of peatland use in Pesisir Selatan Regency, West Sumatra, and to identify the correlation of Laccase activity with peat surface characteristics

METHODOLOGY

The study began by collecting secondary data to identify peatlands distribution and land use as a determiner of soil sampling; they are the map of Degraded Peatlands of West Sumatra (BBSDLP, 2013), and satellite imagery data in 2020 obtained from Landsat 8 OLI level 1 (https://earthexplorer.usgs.gov/).

Land use data in 2020 is overlaid with peatland maps and administrative map of Pesisir Selatan, so that we know the distribution of peatlands and the type of land use in the area. The results show that the research location covers areas in the Districts of Pancung soal, Tapan, and Lunang Silaut. Soil sampling points were carried out for each land use, namely forest (H), shrubs (SB), agricultural land (LP), bare land (LT), and oil palm plantation. In the technical field, the soil sampling in oil palm plantations is divided into smallholder oil palm plantations (PSr) and private company oil palm plantations (PSs). Soil samples were taken for each land use with two replications. The distribution sampling points are presented in Figure 1.

Soil samples used for soil properties analysis and enzyme activity were disturbed soil samples and undisturbed soil samples taken at a depth of 0-20 cm from the soil

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surface. Analysis of soil properties includes water content (based on dry mass), bulk density (BD), pH, organic carbon, and ash content.

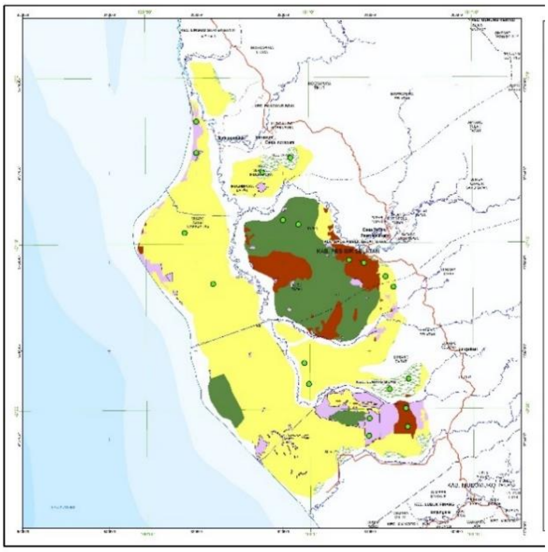

Figure 1. Soil sampling points in several peatland uses in Pesisir Selatan, West Sumatra

Before analyzing enzyme activity, approximately 300 grams of soil sample were taken in the field and then stored in polyethylene plastic bags to maintain soil moisture. The sample was then put into a cooling box to prevent soil temperature fluctuation that could damage the sample. After that, the soil was stored in a refrigerator. Prior to analysis in the laboratory, the peat sample was left at room temperature for 1-2 days; then, the temperature was maintained until the analysis was performed in the laboratory.

Laccase activity was measured by the ABTS method modified by Eichlerová et al. (2012).

The extracellular enzyme activity of Laccase was determined by spectrophotometer using a substrate of 0.5 mM ABTS (*2.2 – Azino - bis (3-ethylbenzothiazoline-6-sulphonic acid)*. A spectrophotometer monitored the oxidation of ABTS at a wavelength of 420 nm. In this study, we adopted a modified method carried out by Harianti (2017), where 7.5 mm of 100 mM buffer acetate solution was added to 5 g of peat material. Then 7.5 ml of 0.5 mM ABTS solution was added and incubated in a water bath with slowly shaking at 25 ºC for 30 minutes. The suspension was then filtered, the filtrate was measured using a spectrophotometer at a wavelength of 420 nm. One unit of enzyme activity was described as the amount of enzyme that oxidizes 1 M ABTS per minute at 25˚C.

$$\text{Laccase activity} = \frac{(S-C).5.100.\%dm}{5} = \ldots\ \mu g.g^{-1}.hour^{-1}$$

where,

S = absorbance of peat samples
C = absorbance without peat samples (blanks)
5 = mass of peat samples
100 = calibration factor
%dm = percentage of dry mass of peat samples

RESULTS AND DISCUSSION

Types of peatlands use

Based on the analysis of satellite imagery and field observations, peatland use of Pesisir Selatan can be grouped into six land use classes: Forest, Shrub, Private Company Oil Palm Plantation, Smallholder oil Palm Plantation, Seasonal Agricultural Land, and Bare Land. The description of each land use is presented in Table 1.

Table 1. Peatland use classes in Pesisir Selatan

No.	Land use	Description
1.	Forest (H)	Primary forest and secondary forest. The flooded wetland secondary forest showed remains of logging activity, and there are traces of burning trees at the border of the forest, so it is classified as a secondary forest. Whereas in the primary forest, the condition of the forest is still natural without any disturbance from logging and land

		clearing activities.
2.	Shrub (SB)	Land that has been cleared but not cultivated, so it is grown by various types of small and low woody shrubs.
3.	Private Company Oil Palm Plantation (PSs)	All oil palm plantations owned by private companies.
4.	Smallholder Oil Palm Plantation (PSr)	All oil palm plantations owned by farmers.
5.	Seasonal Agricultural Land (LP)	Rice fields, corn, and other food crops. This land also includes other annual crops cultivated with semi-intensive to intensive tillage.
6.	Bare Land (LT)	Cleared or uncultivated area. The land is covered with grass <20% and might be previously used for agricultural activities.

Characteristics of Surface Peatlands

Water content and pH

Water content and pH of peatland would affect nutrient supply and biochemical reactions. Based on Table 2, it can be seen the water content of peat in several peatland uses. The soil water content between land uses shows the water content following the order of H>PSs>PSr>SB>LT>LP with the values sequentially of 421.06% > 276.6% > 275.44% > 240.48% > 213 ,38% > 123.1%. This sequence shows that the highest water content is found in the forest. The highest water content in the forest is due to the high capacity of the forest to hold water. It is caused by the canopy being larger and denser than other land uses. In addition, the forest does not have drainage channels, so this condition makes the peatlands always in an anaerobic condition.

The water content of peatland on smallholder oil palm plantations and private company oil palm plantations was 275.44% and 276.6%, respectively (Table 2). This value is higher than that of shrubs by 240.48%. The high water content in oil palm plantations is caused by the canopy being wider than shrubs. It is supported by field observations that the age of oil palm plants is about 15-20 years, so the canopy is broader and denser than shrubs. It influences the soil to be moist/wet instead of the shrubs.

The water content in seasonal agricultural land is the lowest (123.1%) compared to other land uses. It is due to intensive land management, as evidenced by the value of soil bulk density (BD) 0.6-0.9 $g.cm^{-3}$ (Table 3) and water management during cultivation. The land is frequently exposed in under aerobic conditions. In addition, the plant canopy on agricultural land (rice) is smaller than other land uses such as forests, oil palm, and shrubs. In contrast to bare land, the water content is slightly higher (213.38%) than agricultural land. It is because the bare land is not treated by intensive tillage, so the water content of peat can still be maintained.

Table 2. Water content and pH in several peatland uses in Pesisir Selatan

Land use types	LP	LT	SB	PSr	PSs	H
Water content (%)	123,10	213,38	240,48	275,44	276,60	421,06
pH	5,13	4,31	4,67	4,18	4,27	5,08

Note: LP = seasonal agricultural land, LT = bare land, SB = shrubs, PSr = Smallholder Oil Palm Plantation, PSs = Private Company Oil Palm Plantation, PSr = Smallholder Oil Palm Plantation, H = Forest

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Table 3**.** Bulk Density for several peatlands uses.

Land use types	LP	LT	SB	PSr	PSs	H
BD (g.cm-^{3})	0,6	0,32	0,32	0,27	0,25	0,2

Note: LP = seasonal agricultural land, LT = bare land, SB = shrubs, PSr = Smallholder Oil Palm Plantation, PSs = Private Company Oil Palm Plantation, PSr = Smallholder Oil Palm Plantation, H = Forest

The pH of peatland ranged from 4 to 5 (Table 2). The pH value in agricultural land tends to be higher (5,13) compared to pH in other land uses. The high pH on agricultural land is caused by adding lime by farmers before growing plants, such as corn and secondary crops. In addition, the high pH of agricultural land is also caused by the practice of farmers burning the remnant harvested biomass and burying it back into the soil. Like bare land, shrubs and forests also have a relatively high pH of 4.31-5.08. It was caused by both lands having been burned, leaving ashes containing high cations on the soil surface. This ash affects increased soil pH.

Based on Mardiana's (2006) research, the pH of burned peatlands has a pH of 6.40, and the pH of unburned peat (natural forest) is 4.20. It is also supported by Yudasworo (2001) in his research stating that burnt land increases the soil pH due to the addition of minerals left above the soil surface, including carbonates, alkaline cations such as sodium, potassium, calcium, and magnesium. These salts donate OH^- more than H^+ in the soil. The mechanism is as follows: If peatlands are added with ash (oxide, such as CaO, MgO, Na_2O, K_2O, CuO), OH- would increase in the soil solution, and then the pH will rise. The reaction is as follows: $CaO + H_2O\ Ca^{2+} + 2OH^-$

The pH values in private company oil palm plantations and smallholder oil palm plantations tend to be similar, ranging from 4.18-4.27. It is due to the peat in this location being classified as transitional peat. Like the case with transitional peat in Air Sugihan Kiri, South Sumatra has a higher pH range of H_2O between 4.1 to 4.3 (Hartatik *et al.* 2004). The acidity of tropical peatland is generally about 3-5, caused by poor drainage and hydrolysis of organic acids, dominated by fulvic and humic acids (Widjaja-Adhi, 1988). According to Salampak (1999), peat reaction is closely related to the content of organic acids, namely humic and fulvic acids.

Bulk density

Bulk density (BD) between land uses can be seen in Table 3. The BD at depth of 0-20 cm in several land uses follow the order of LP>LT>SB>PSr>PSs>H with values consecutively is 0.6 g.cm^{-3} > 0.32 g.cm^{-3} = 0.32 g.cm^{-3} >. 0.27 g.cm^{-3} > 0.25 g.cm^{-3} > 0.2 g.cm^{-3}. This sequence shows that the highest BD is found in agricultural land. The high BD in agricultural land is predicted by the incorporation of peat material with mineral soil during land preparation prior to planting.

In the forest, soil BD at depth of 0-20 cm is the lowest (0.2%) compared to other land uses, indicating most of the soil volume is dominated by pore space. The pores could be filled with water or air. While on peatland forest, the groundwater table (0 cm) is shallow so that all soil pores are filled with water. The pores of peat soil could store large amounts of water. The ability of peat soil to store water can be seen from the water content in field conditions which reaches 421.06%. BD of mixed swamp forest in the surface layer ranged from 0.10 g.cm^{-3} – 0.15 g.cm^{-3}, while the subsurface layer ranged from 0.05 g.cm^{-3} – 0.10 g.cm^{-3} (Driessen and Sudjadi, 1984).

Table 4. Ash and organic carbon content in several types of peatlands use

Land use types	LP	LT	SB	PSr	PSs	H
Ash content (%)	92,13	85,79	45,89	63,87	64,01	68,17
Organic carbon (%)	7,87	14,21	40,04	36,18	35,99	31,83

Note: LP = seasonal agricultural land, LT = bare land, SB = shrubs, PSr = Smallholder Oil Palm Plantation, PSs = Private Company Oil Palm Plantation, PSr = Smallholder Oil Palm Plantation, H = Forest.

Ash content and organic carbon

The ash content among land uses followed the order of LP>LT>H>PSs>PSr>SB with the value sequentially was 92 .13% > 85.79% > 68.17% > 64.01% > 63.87% > 45.89% (Table 4). This sequence shows that the highest ash content is found in agricultural land use. The high ash content in agricultural land in all soil layers is affected by agricultural activities, such as burning biomass after harvesting. Ash content in bare land is about 85.79%. This value is higher than other land uses (SB, aPSr, PSs, and H). It is because the bare land previously has been burned so that could enrich soil ash content.

Ash content in shrubs is the lowest (45.89%), where the shrubs are overgrown with grasses and ferns. This plant species is grouped as short-lived plants, so the biomass could return quickly to the soil, enriching more organic carbon into the soil. The ash content indicates the level of decomposition of peat and the mineral content incorporated in it. The higher the ash content, the further the decomposition rate of peat material (Sukarman et al. 2012).

Organic carbon content between land uses can be seen in Table 4. Carbon organic content between land uses follows the order SB>PSr>PSs>H>LT>LP with the value sequentially is 40.04% > 36.18 % > 35.99% > 31.83% > 14.21% > 7.87%. This sequence shows that the highest organic carbon is found in the shrub. It was due to the shrubs being overgrown with grasses and ferns. This plant species is grouped as short-lived plants, so the biomass could return quickly to the soil, enriching more organic carbon into the soil.

Organic carbon content in agricultural land (LP) is the lowest (7.87%). Low organic carbon in agricultural land use is caused by intensive agricultural practices used for cultivation, such as: rice, corn, and secondary crops. Hence, the soil is frequently in an oxidizing condition. Likewise, soil tillage will be able to accelerate the rate of peat decomposition so that organic carbon levels become low. In addition, farmers also burn remaining harvested biomass, causing low soil organic carbon.

Laccase activity on several types of peatlands use

Activity Laccase on several peatland-uses in Pesisir Selatan is displayed in Figure 2. The highest Laccase is found in agricultural land (LP) 3.20 $\mu g.g^{-1}.hour^{-1}$, while the lowest Laccase is in forest vegetation (H) 0.06 $\mu g.g^{-1}.hour^{-1}$. If we compare to several land uses, Laccase from the highest to the lowest follows the order: agricultural land (LP) 3.20 $\mu g.g^{-1}.hour^{-1}$ > bare land (LT) 1.46 $\mu g.g^{-1}.hour^{-1}$ > shrubs (SB) $\mu g.g^{-1}.hour^{-1}$ > smallholder oil palm plantations (PSr) 0.66 $\mu g.g^{-1}.hour^{-1}$ > private company oil palm plantations (PSs) 0.70 $\mu g.g^{-1}.hour^{-1}$ > forest (H) $\mu g.g^{-1}.hour^{-1}$.

The high Laccase in agricultural land is due to the low water content of 123.10%, as shown in Table 2. Low water content could drive the peat environment more oxidative and allow changes in the peat physical properties, as the BD value becomes high (0.6 $g.cm^{-3}$), thereby supporting increased enzyme activity. Similarly, in bare land, shrubs, and oil palm plantations, the water content of peatlands is <300%, which follows the sequence: bare land (LT) 213.38% > shrubs (SB) 240.48% > smallholder oil palm plantations (PSr) 275.44 % > private company oil palm plantations (PSs) 276.60%. It causes the laccase enzyme activity becomes decrease

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with increasing water content. It was proven that the water content in the forest (H) was much higher by 421.06%, while the enzyme activity was low by 0.06 µg.g^{-1}.hour^{-1} (Table 2), meaning that the higher the water content, the lower the enzyme activity. It is due to the anaerobic condition of the soil environment, which can suppress microbial activity in decomposing of peat material (Hijri *et al*., 2022). It is supported by Harianti (2017), stating that the value of water content has a significant negative correlation with Laccase. The decreasing of Laccase enzyme activity was significantly influenced by the increasing water content. Thevenot et al. (2010) also found that the high water content and soil moisture could affect enzyme activity.

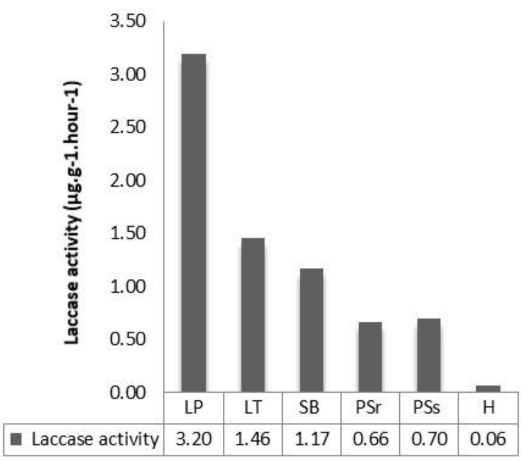

Figure 2. Laccase activity in several land-uses on peatlands. LP = seasonal agricultural land, LT = bare land, SB = shrubs, PSr = Smallholder Oil Palm Plantation, PSs = Private Company Oil Palm Plantation, PSr = Smallholder Oil Palm Plantation, H = Forest

Correlation of Laccase with peat surface characteristics

Peatland use changes generate transformations in enzyme activity in the soil surface layer. Enzyme activity implies the peat decomposition process. Soil and plant ecology on the surface affect enzyme activity as a footprint for the interaction of peat and vegetation (Harianti 2017). The relationship between Laccase activity and peat characteristics can be seen in Table 5.

The soil water content significantly correlated with Laccase (r^2 = -0.914, Table 5). Decreasing Laccase activity is significantly influenced by the increase of soil water content, implying that the higher the water content, the lower the Laccase activity in peatlands. It is due to the anaerobic conditions, which can suppress microbial activity in decomposing peat material. This reason is supported by Brzezinâska *et al*. (1998), stating that the low amount of oxygen in the soil influence the activity of soil microorganisms, biochemical and enzyme reactions.

Retaining a sufficiently high water content > 300% w/w is a critical requirement in controlling GHG emissions on peatlands. Related to this research, one of the GHG emissions comes from peat decomposition, so increasing water content is the main factor in reducing peat decomposition. Increasing the water content of the peat is carried out by maintaining the water table at >40-60 cm from the peat surface. The peat environment is characterized by high hydration and could suppress enzyme activity in the soil through changes in the number of microbes (Freeman & Kang 1999). Water content is an essential factor in several catalytic processes, especially those related to hydrolytic reactions such as synthesis, production, and activity of extra and intracellular enzymes (Gianfreda & Bollag, 1996).

Soil pH was positively correlated with Laccase (Table 5), meaning that decreasing soil pH could suppress enzyme activity. The optimum pH for a particular enzyme could be different because it arrives from diverse microbes. Several studies have shown that Laccase has an optimum pH of 3.5-6 (Irshad et al. 2011; Madhavi & Lele, 2009). The ash content was positively correlated with Laccase, meaning that the enzyme activity increased in the matured peatlands, which had a higher ash content. It could increase GHG emissions. Organic carbon content was negatively correlated with Laccase (Table 5), meaning that if peatlands have high organic carbon, it could support slowing down peat material decomposition (Almeida et al. 2015).

Table 5. Matrix of Pearson Correlation between Laccase and peat characteristics

Parameter	BD	pH	KA (%)	Ash (%)	Org-C (%)	MAT (cm)	Laccase ($\mu g.g^{-1}.hour^{-1}$)
BD	1	0.453	-0.862*	0.604	-0.604	0.406	0.987**
pH		1	0.025	0.260	-0.260	0.967**	0.362
KA (%)			1	-0.463	0.463	0.019	-0.914*
Ash (%)				1	-1.000**	0.260	0.632
Org-C (%)					1	-0.260	-0.632
MAT (cm)						1	0.348
Laccase ($\mu g.g^{-1}.hour^{-1}$)							1

Note: BD = Bulk density, KA = water content, Org-C = organic carbon, MAT = water table from soil surface
* Correlation is significant at the 95%
** Correlation is significant at the 99%

CONCLUSION

Based on the results of this study, it can be concluded that the Laccase activity on several land-uses from the highest to the lowest follows the order: agricultural land (LP) > bare land (LT) > shrubs (SB) > smallholder oil palm plantations (PSr) > private company oil palm plantations (PSs) > forest (H). The enzyme activity is influenced by water content, in which the water content is negatively correlated with the Laccase. It means that the higher the water content in the peat material, the lower the laccase activity. Therefore, it is necessary to control the soil water content high to prevent the rate of peat decomposition.

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