PEAT MICROBIAL FUNCTIONAL DIVERSITY IN PINEAPPLE PLANTATION

Nor Ayshah Alia, A.H.*, Khairatul, A., and Norziana, Z.Z.

Soil Science, Water, and Fertilizer Research Centre, Malaysian Agricultural Research and Development Institute, Serdang, Selangor, Malaysia

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Abstract: Quantification of peat microbial functional diversity in pineapple plantation area is needed to evaluate the response of long-term agricultural managed land. A total of three pineapple plantation in Pontian was chosen for this study. BiologEcoplate™ system was used to evaluate microbial functional diversity. The enumeration of aerobic culturable microorganism was performed using total plate count. Microbial activity, species richness, and aerobic culturable bacteria of PM1 were higher than PS. Microbial catabolic diversity was found not significant in the sampling location. Correlation analysis showed that microbial catabolic diversity was strongly associated with pH. The abundance of aerobic culturable fungi was also strongly associated with culturable actinomycetes. It is concluded that pH shaped the microbial functional diversity in peat by shifting the microbial community through selection and adaptation of microbes that can function in peat.

Keywords: Microbial activity, richness, catabolic diversity, culturable

Introduction

The contribution of microorganism to ecosystem function is widely recognized. Microbes are the main element of global biodiversity and they play a crucial role in the ecosystem functions such as nutrient cycling (Islam *et al.*, 2017). Microbial communities are the key element for understanding the influence of environmental disturbance on soil function. Microbial functional diversity is the characterization of physiology and metabolic trait in the microbial community. Pineapples have been cultivated in peat since 1940 in Malaysia. Determination of microbial functional diversity in the pineapple plantation is crucial as to evaluate the response of microbes in the long-term agricultural area where the pineapple yield reduction was observed. Hence, this study was performed with the objective to assess the peat microbial functional diversity in the selected pineapple plantation area based on pineapple plantation history.

Materials and Method

Soil sampling

The study took place in Pontian area, the oldest pineapple growing region in Malaysia. Soil type was classified as hemic peat soil with more than 65% organic matter. A total of three pineapple plantation was chosen which consisted of Kampung PuteriMenangis 1 (PM1), Kampung PuteriMenangis 2 (PM2), and Kampung ParitSikom (PS). Soil auger was used to collect soil at the depth of 20 cm from soil surface.

Soil properties

Soil pH was measured by mixing soil sample in water at 1: 2.5 soil : water ratio as according to standard method (Chapman and Pratt, 1978). Cation exchange capacity (CEC) and available phosphorus (P) was performed using flow injection analyzer (Lachat Instruments, USA). Total carbon and total nitrogen were measured according to the combustion method by using elemental analyzer (Flash 2000, Thermo Scientific, USA).

Microbial functional diversity analysis

Microbial functional diversity was characterized by assessing the microbial activity and community level physiological profile. BiologEcoplate™ system was used to evaluate these two criteria. Ecoplate™ has 96 well with three replicates whereby each one comprises 31 functional carbon sources relevant to ecosystem. Soil suspensions (soil 100 g and 1 litre of distilled water) were shaken for 30 min, and then filtered using 0.45 µm filter paper. Aliquots of 100 µl were inoculated in the Ecoplate™ and incubated at 27°C. The utilization of carbon source by microbes initiated the respiration in the cells and consequently reduces tetrazolium dye which produced the purple color. Colour intensity in each well was recorded as optical density (OD) at 590 nm wavelength. The OD was recorded at 48 hours to permit microbial utilization of any soluble organic carbon derived from the rhizosphere that could hinder in the sole carbon source-use response (Gomez et al., 2004).

Microbial activity

Average well colour development was determined from OD values recorded at 590 nm wavelength produced after 48 hours of Ecoplate™ incubation. Color development in each well was evaluated after the plate incubation. Average well color development was calculated as follows (Gomez et al. 2006):

$AWCD = \sum OD_i / 31$
where $OD_i$is the optical density value from each Ecoplate™ well.

Community-level physiological profile

Richness (R) and Shannon diversity Index (H) were used as the criteria for community-level physiological profile (Garland 1997). The values were calculated from the OD values of Ecoplate wells incubated at 48 h. Richness was determined as the total number of oxidized carbon substrates (BiologEcoPlate™ well with OD ≥ 0.25 as threshold for positive response of oxidized carbon substrates).

Shannon diversity Index was calculated as follows:

$H = \Sigma pi (\ln pi)$
where pi is the ratio of the activity on each substrate (ODi) to the sum of activities on all substrates (ΣODi) in BiologEcoPlate™ well whereas ln is the natural logarithm.

Enumeration of culturable microorganism

Culturable aerobic microorganism was enumerated using the common total plate count. Total plate count was done on three different selective media which includes nutrient agar (NA), potato dextrose agar (PDA), and starch casein agar (SCA). Nutrient agar, PDA, and SCA was used to quantify the abundance of bacteria, fungi, and actinomycetes, respectively. Nutrient agar and PDA were made according to the manufacturer (Merck, Germany) recommendation. SCA was made of 10 g soluble starch, 0.3 g casein, 2 g KNO3, 0.05 g MgSO4.7H2O, 2 g K2HPO4, 2 g NaCl, 0.02 g CaCO3, 0.01 g FeSO4.7H2O, 18 g of agar and 1 litre of distilled water. SCA agar was adjusted to 7±0.2 and autoclaved at 121°C for 15 minutes. Soil suspension was prepared by adding 10 g of soil into 90 ml of sterile distilled water and shake for 1 hour. A tenfold dilution series was performed on the soil suspension and aliquots of 1 ml were inoculated on selective media and incubated at 27°C for 48 hours for NA and PDA while SCA was incubated for 168 hours (7 days). Total plate count was measured using Colony Forming Unit (CFU) and expressed as $\log_{10}$ CFU/ml.

Statistical analysis

Pearson Coefficient Correlation was used to evaluate the relationship between microbial functional diversity, soil properties, and the population of aerobic culturable abundance. Average well colour development, R, H, and aerobic culturable microorganism were analyzed using ANOVA and multiple comparisons of means using Fisher's Least Significance Difference (LSD) test. All statistical analyses were performed with Minitab 17.

Results

Soil properties

Table 1 showed that pineapple plantation in PM1 and PM2 had similar range of pH, and phosphorus. It was noted that PS had low pH, carbon, nitrogen, and phosphorus concentration.

Table 1: Soil properties of selected pineapple plantation in Pontian

Location	pH	C (%)	N (%)	P (ug/g)	CEC (meg/100)
PM1	3.27	4.27	0.70	42.07	18.54
PM2	3.14	4.19	0.83	42.96	43.00
PS	2.96	4.17	0.63	32.42	32.28

C, Carbon; N, Nitrogen; P, Available Phosphorus; CEC, Cation Exchange Capacity

Microbial Activity

The ability of microbes to used specified substrate in BiologEcoplate was expressed as AWCD and can be applied as microbial activity. PM1 have higher microbial activity than PS (Figure 1). In contrary, PM2 did not have a significant microbial activity when compared with PM1 and PS.

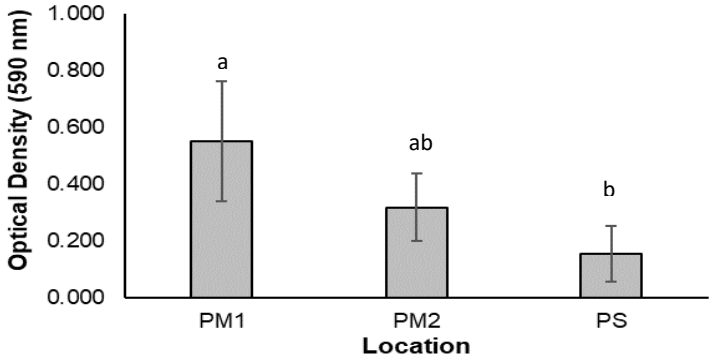
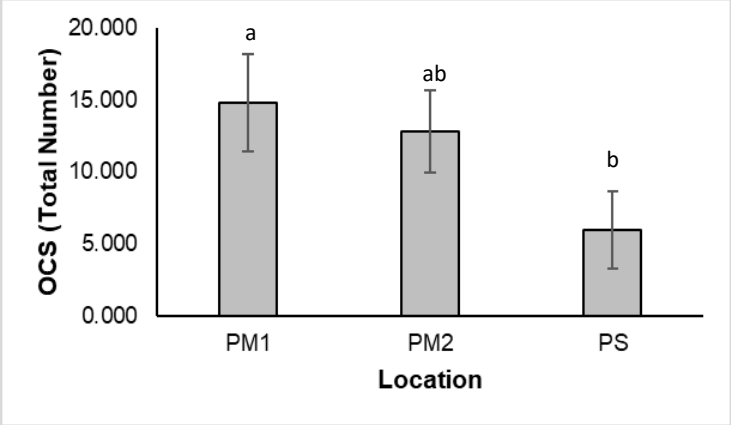


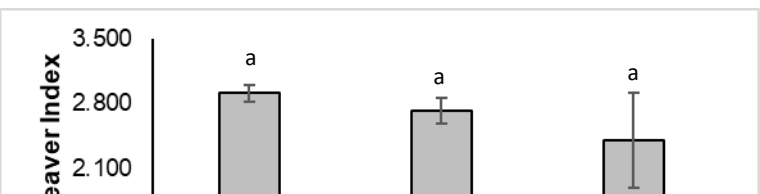
Figure 1: Mean separation of microbial activityperformed using Fisher's Least Significance Difference. PM1, Kampung PuteriMenangis 1; PM2, Kampung PuteriMenangis 2; PS, Kampung ParitSikom

Community-level physiological profile

Figure 2a showed that species richness in PM1 was higher than PS. The species richness in PM2 followed the same pattern as in microbial activity.



a



b

Figure 2: Mean separation of community-level physiological profile performed using Fisher's Least Significance Difference. PM1, Kampung PuteriMenangis 1; PM2, Kampung PuteriMenangis 2; PS, Kampung ParitSikom

Species richness was not influenced by location. In addition, microbial catabolic diversity did not affect by the location of pineapple plantation (Figure 2b).

Microbial culturable abundance

Table 2 showed that the abundance of aerobic culturable bacteria in PS was slightly higher than PM1. The location of pineapple plantation did not have an effect on the abundance of aerobic culturable fungi and actinomycetes.

Table 2: The abundance of culturable aerobic microorganism

Location	Bacteria (Log10 CFU/ml)	Fungi (Log10 CFU/ml)	Actinomycetes (Log10 CFU/ml)
PuteriMenangis 1	5.45 ± 0.16a	2.56 ± 0.32a	1.68 ± 0.53a
PuteriMenangis 2	6.14 ± 0.25ab	2.29 ± 0.74a	0.82 ± 0.33a
ParitSikom	5.61 ± 0.15b	2.31 ± 0.41a	0.83 ± 0.56a

Mean Log_{10} CFU/ml measured based on different selective medium and data are expressed in means ± SE. Mean comparison was performed using Fisher's Least Significance Difference. Mean with the same letter is not significant ($p \leq 0.05$)

Correlation between soil and microbiological properties

Correlation analysis was performed between the soil properties, microbial functional diversity and the abundance of aerobic culturable abundance (Table 3). It was shown that pH has a positive association with Shannon Weaver Index. The abundance of aerobic culturable fungi also positively correlated with the population of actinomycetes.

Table 3: Coefficient Correlation using Pearson between selected soil properties, microbial functional diversity, and the abundance of aerobic culturable microorganism

Variable	1	2	3	4	5	6	7	8	9	10	11
1. pH											
2. C	0.910 0.271										

3. N	0.430 0.717	0.019 0.988									
4. P	0.874 0.323	0.595 0.594	0.815 0.394								
5. CEC	-0.481 0.680	-0.801 0.409	0.584 0.603	0.005 0.997							
6. AWCD	0.982 0.123	0.973 0.149	0.250 0.839	0.765 0.445	-0.640 0.558	0.589 0.599					
7. R	0.977 0.136	0.802 0.408	0.612 0.580	0.957 0.186	-0.284 0.817	0.861 0.340	0.918 0.259				
8. H	**0.999** **0.034**	0.887 0.305	0.478 0.683	0.899 0.289	-0.434 0.714	0.768 0.442	0.970 0.156	0.987 0.103			
9. B	-0.130 0.917	-0.529 0.646	0.839 0.366	0.368 0.760	0.932 0.236	0.579 0.607	-0.318 0.794	0.083 0.947	-0.078 0.951		
10. F	0.776 0.435	0.967 0.163	-0.236 0.848	0.372 0.758	-0.926 0.246	0.140 0.911	0.882 0.312	0.624 0.571	0.741 0.468	-0.727 0.482	
11. A	0.810 0.399	0.980 0.127	-0.181 0.884	0.423 0.722	-0.904 0.282	0.195 0.875	0.907 0.276	0.667 0.535	0.778 0.433	-0.687 0.518	**0.998** **0.036**

C, Carbon; N, Nitrogen, P, Available P; CEC, Cation Exchange Capacity; AWCD, Average Well Colour Development; R, Species Richness; H, Shannon Weaver Index; B, Bacteria abundance; F, Fungi abundance; A, Actinomycetes abundance

Upper cell content is Pearson correlation while the lower cell content is P-value (≤0.05). Cell with bold font indicates significant correlation

Discussion

Microbial functional diversity in peat ecosystem was studied particularly in pineapple plantation area. The microbial activity and species richness were affected in 2 different sites studied. Having the same soil profiles, perhaps microbial activity and species richness in the sites studied were affected by location and land management. It was noted that the 2 sites studied (PM1 and PS) have a different soil chemical property such as, nitrogen, and phosphorus. Nitrogen and phosphorus had been reported to increase microbial activity in peat (Mandic-Mulec et al., 2014 and Nusantara et al., 2019).

Microbial catabolic diversity was not affected by the different location in the pineapple plantation area. This indicates that microbial communities have a species that could utilize the carbon sources provided in the peat. Results from correlation study showed that microbial catabolic diversity had strong positive relationship with pH. Peat is an acidic soil and only microbe that can tolerate low pH able to survive and thrive in this ecosystem. Prior research has shown that fungi and actinomycetes can adapt with low pH (Jefrey, et al., 2011 and Zhang et al., 2017). This might explain on the positive relationship between the abundance of fungi and actinomycetes in this study. It is suggested that these fungi and actinomycetes contributed to the function of peat hence clarified the microbial catabolic diversity in this study.

Albeit this study had observed that there was no correlation between microbial functional diversity and culturable microbial abundance, it was noted that the pattern of culturable bacterial abundance in PM1 and PS was the same as their microbial activity and species richness. This could indicate on the contribution of aerobic culturable bacteria in microbial activity and species richness. It is known that less than 2% of the bacteria are culturable (Wade, 2002) and efforts on culturing bacteria are continuously done such as an improvement on the cultivation technique (Martiny, 2019). However, this study used nutrient agar to quantify bacterial abundance and might not able to capture other culturable bacteria that needs different or specific substrate for growth.

Conclusion

This study was conducted to determine the peat microbial functional diversity in pineapple plantation in Pontian. It is suggested that microbial activity and species richness were affected by location and agricultural management in pineapple plantation area. From this study, it is concluded that pH shaped the microbial functional diversity in peat by shifting the microbial community through selection and adaptation of microbes that can function in peat. Comprehensive understanding on the microbial community including microbial taxonomic diversity and also the characterization of unculturable microbes can be performed by using metagenomic approach such as next generation sequencing analysis.

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