Microbial Gene-Based Prediction of Methane Emission Potential in Tropical Peatlands for Climate Resilience

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Abstract. Tropical peatlands as carbon reservoirs play an important role in greenhouse gases regulation, which impact global climate change. Conversion of peatland into agricultural, plantation or managed production systems with drainage alters soil properties and influences methane emission. Microorganisms are key agents driving this process, but there is still limited understanding of how land-use change and soil moisture influence bacterial community and predicted methanogenesis gene marker. This study aims to predict methane emission potential in tropical peatlands by analysing soil bacterial community composition using next-generation sequencing across different land uses and soil moisture condition to predict methane emission potential. Soil samples were collected from eight land use types (flooded oil palm plantation, drained oil palm plantation, small holder oil palm plantation, rubber-pineapple mixed cropping, natural revegetation forest, revegetated jelutong forest, shrubland and ex-plot paludiculture) at two depths (0-15 cm and 15-30 cm), with three replications for each depth. Bacterial community composition was characterised using 16S rRNA amplicon sequencing, and the resulting taxonomic profiles were further analysed using PICRUSt to infer the potential abundance of functional genes associated with methanogenesis and correlation with soil moisture content. Soil moisture content was higher in the deeper soil layer. Increasing soil moisture significantly enhances the abundance of the meth-acetate pathway, which is predicted to result in higher methane emissions.

1 Introduction

Tropical peatlands are the most carbon-rich terrestrial ecosystems, storing around one-sixth of the global carbon pool [1]. In natural conditions, peatlands remain waterlogged; this saturated condition slows down organic matter decomposition, allowing plant residues to accumulate and form thick peat deposits [2]. Once the hydrological balance is disturbed, peatlands may shift from a carbon sink to becoming a source of greenhouse gas emissions [3]. In Indonesia, peatlands have been converted into agricultural fields, plantation areas, and other managed landscapes [4]. Land conversion is commonly accompanied by drainage. These changes alter oxygen availability, water-table level, substrate quality, and microbial activity [5]. As a consequence, the biogeochemical processes controlling carbon dioxide and methane emissions may also change.

Methane (CH_4) is produced mainly under anaerobic conditions through microbial degradation of organic substrates [6]. In peat soil, this process is closely linked to soil moisture, water saturation, and redox conditions. Wetter peat layers tend to create oxygen-limited microsites that support anaerobic decomposition and methanogenesis. In contrast, drained peat soil may suppress methane production but accelerate aerobic decomposition and carbon dioxide release. Peatland conversion alters peatland CH_4 dynamics [7]. Therefore, understanding the potential for methane emissionsin peatlands requires attention not only to land use types but also to soil moisture gradients and microbial community dynamics.

Microorganisms are central actors in peatland for producing CH_4 [8]. Bacteria are involved in the early and intermediate stages of organic matter decomposition, including hydrolysis, fermentation, and acetate production. Although methane production is ultimately carried out by methanogenic archaea, bacterial communities influence methanogenesis indirectly by supplying substrates such as acetate, hydrogen, and carbon dioxide [9]. Changes in bacterial community composition may provide important

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ecological signals regarding methane-related functional potential.

The development of next-generation sequencing has enabled the examination microbial communities in complex environmental samples with greater resolution [10]. The 16S rRNA amplicon sequencing approach is widely used to identify bacterial and archaeal taxa and compare microbial community composition across environmental gradients. In addition, functional prediction tools such as PICRUSt are used to infer potential metabolic pathways from taxonomic profiles [11]. While this method does not directly measure gene expression or methane flux, it offers a useful preliminary approach for predicting microbial functional capacity in ecological studies.

Despite the recognised role of tropical peatlands in greenhouse gas emissions, the ecological links among land-use change, soil moisture content, bacterial community structure, and methanogenesis-related functional potential remain poorly understood. In particular, limited information is available on the relationship between soil moisture content and the predicted abundance of METH_ACETATE_PWY, a pathway associated with acetate-dependent methanogenesis. Therefore, this study addresses this gap by analysing bacterial community composition under different land use types and soil depths, and by linking soil moisture content using bioinformatic tools, namely PICRUSt2. This approach provides an ecological indication of potential methane-related processes in tropical peat soils, although the predicted pathway abundance should be interpreted as functional potential rather than direct methane emission measurement.

2 Materials and Methods

2.1 Study Area and Soil Sampling

This study was conducted in the tropical peatland, Ogan Komering Ilir Regency, South Sumatra, Indonesia (104.9142°E, 3.4135°S). The sampling area representated different land-use management. Eight land uses were selected to capture variation between the plantation system, mixed agricultural system, revegetated area, and shrub-dominated area. Soil samples were taken from eight land uses, including oil palm plantation (OP), rubber-pineapple mix cropping (RPM), natural revegetation forest (NRF), revegetated Jelutong forest (JEL), shrubland dominated by *Hymenachnne acutigluma* (SH) and ex-paludiculture plot (PAL).

Soil samples were taken using a soil auger at two depth intervals: 0-15 cm and 15-30 cm. For each land use and depth, three replications were taken. Each samples units was a composite sample from eight cores and was homogenised. The collected soil samples were placed into sterile plastic bags. Samples intended for molecular analysis were kept in a cool box during field transportation and stored at low temperature before DNA extraction. Samples for soil moisture analyses were separated and transported to the laboratory for gravimetric measurement.

2.2 Soil moisture measurement

Soil moisture content was determined using the gravimetric method. Fresh soil samples were collected from each land-use type and soil depth. Each fresh sample was weighed immediately to obtain the wet weight, then oven-dried at 105ºC for 24 hours. After drying, the samples were collected in a desiccator and reweighed to obtain the dry weight. Soil moisture content was calculated based on the equation below.

$$SMC\ (\%) = \frac{w_w - w_d}{w_d}\ x\ 100 \qquad (1)$$

Where SMC is the percentage of soil moisture content (%), Ww is the wet weight of the soil sample (g), and Wd is the oven-dry weight of the soil sample (g).

2.3 Functional Prediction of Methanogenesis form the Acetate Pathway Using PICRUSt2

Functional prediction of methanogenesis from the acetate pathway was conducted using PICRUSt2 based on 16S rRNA amplicon sequencing data from soil samples collected across six land use types and two soil depths. The microbial community data were processed into amplicon sequence variants (ASVs), consisting of an ASV abundance table and representative sequences. These files served as the main input for the PICRUSt2 pipeline. First, the representative sequences were placed into a reference phylogenetic tree to estimate their evolutionary relationship with known microbial genomes. The predicted gene abundances were then normalised by 16S rRNA gene copy number to reduce bias caused by differences in copy number among microbial taxa. After normalisation, PICRUSt2 inferred gene family abundances and mapped them to metabolic pathways using the MetaCyc database.

This study specifically focused on the methanogenesis from the acetate pathway (METH_ACETATE_PWY), which was selected as the target pathway to represent the potential capacity of the microbial community to produce methane through acetate utilisation. The predicted abundance of this pathway was converted into relative abundance and compared among land use types and soil depths. PICRUSt2 predicts function from 16S rRNA marker-gene data; the results were interpreted as the potential functional capacity of the microbial community, not as direct evidence of actual in situ methanogenic activity.

2.4 Statistical Analyses

Statistical analyses were performed using RStudio. The effect of land use, soil depth and their interaction on soil moisture content and METH_ACETATE_PWY abundance were assessed using two-way ANOVA. The data were checked for normality and homogeneity of variance to ensure that the assumptions of parametric analysis were adequately met. When significant main or interaction effects were detected, mean separation was conducted using the Least Significant Difference (LSD)

test at the 5% significance level. The LSD test was used to compare soil depths within the same land use types and to compare land use types within the same depth. The correlation between soil moisture content and METH_ACETATE_PWY abundance was further examined using Pearson's correlation coefficient to evaluate the strength and direction of their linear relationship.

3 Result and Discussion

3.1 Soil moisture content across land uses and depth

Soil moisture content varied significantly across land use types and soil depths. Overall, the 15-30 cm soil layer showed higher soil moisture than the upper layer. Significant depth-related increases were observed in all land uses, except SH, because SH has the highest water table level (-10 cm). At the 0-15 cm depth, PAL and SH had the highest soil moisture content, while NRF showed the lowest moisture content. At the 15-30 cm depth, PAL maintained the highest soil moisture, while NRF remained the lowest. This pattern is consistent with the hydrological behaviour of peat soils, in which the surface layer is more exposed to evaporation, drainage effects, and water table level. Whereas deeper peat layers tend to remain more hydraulically connected to groundwater and retainwater [12].

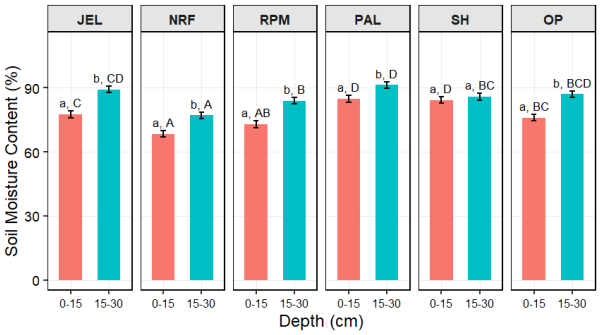

Fig. 1. Soil moisture content across land uses and depths

3.2 PredictedMETH_ACETATE_PWY abundance across land uses and depth

METH_ACETATE_PWY (MEP) represents the methanogenesis from the acetate pathway, also known as the acetoclastic methanogenesis. In this pathway, acetate is converted into methane and carbon dioxide under anaerobic conditions. Therefore, the predicted abundance of MEP may indicate the potential capacity of the microbial community to support acetate-based methane production in peat soils [13].

The predicted abundance of METH_ACETATE_PWY (MEP) varied across soil depth. The deeper peat layer generally showed higher MEP than the surface layer, indicating a higher predicted acetate-related methanogenesis potential. In the 15-30 cm layer, all land use types exhibit higher MEP than the 0-15 cm layer, due to higher soil moisture. Significant depth-related increases were particularly evident in revegetated Jelutong forest (JEL), Ex-Paludiculture plot (PAL) and Shrub-dominated with *Hymenachne acutigluma* (SH). Land-use types also influenced the predicted abundance of MEP. The SH site showed the highest abundance at both depths, reaching the greatest value at 15-30 cm, followed by PAL. RPM consistently exhibited the lowest abundance.

Across land-use types, variation in both composition and concentration of root exudate inputs can regulate gas emission [14]. In this pathway, acetate is initially activated to acetyl-CoA by phosphorylation, before being further transformed by the acetyl-CoA decarbonylase [13]. This indicates that differences in plant communities may affect emissions by modifying the type and amounts of organic compounds released through root activity.

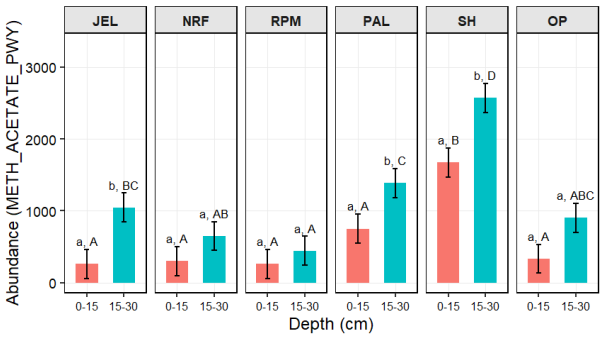

Fig. 2. Predicted abundance of METH_ACETATE_PWY across land uses and depths

3.3 Correlation between soil moisture and the methane gene pathway

The correlation analysis showed a positive and statistically significant relationship between soil moisture content (SMC) and the predicted abundance of METH_ACETATE_PWY. The Pearson correlation coefficient indicated a moderate positive association ($r=0.53$), while the regression model explained approximately 28.1% of the variation in MEP abundance ($R^2=0.281$). This relationship was statistically significant ($p=0.000877$), indicating that higher soil moisture was associated with increased predicted MEP.

This finding is ecologically reasonable because higher soil moisture can reduce oxygen diffusion within the peat matrix, promote anaerobic microsites, and may provide a more favourable environment for anaerobic microbial processes linked to acetate-based methane production. In anaerobic peatland soil, acetate is a major precursors for CH_4 formation, and acetoclastic methanogenesis represents an important pathway by which acetate is converted into methane and CO_2. Under anaerobic conditions, where electron acceptors other than bicarbonate are largely unavailable, methane becomes the terminal product [15].

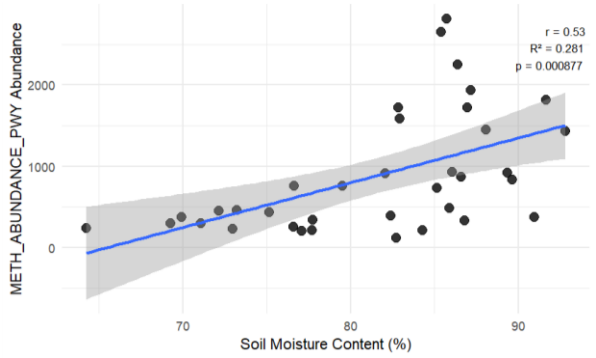

Fig. 3. Correlation between soil moisture content and predicted METH_ACETATE_PWY abundance

4 Conclusion

This study showed that land use and soil depth influence soil moisture content and the predicted abundance of METH_ACETATE_PWY (MEP) in tropical peatland. The deeper layer shows higher soil moisture content and higher predicted MEP. A significant positive correlation between soil moisture content and MEP abundance indicated that wetter peat conditions may favour methane formation. This finding implied that maintaining peatland hydrological conditions is important for managing methane-related microbial potential in tropical peatlands. However, because the pathway abundance was derived from PICRUSt2 prediction, further validation is needed using direct methane flux measurement.

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