

# マレーシア熱帯泥炭地における微生物群集構造とそれらの生物地球化学的循環への寄与

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(Summary)

Microbial Community Structure and Their Contribution  
to Biogeochemical Cycles in Malaysian Tropical  
Peatland

Agriculture

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## SUMMARY

Southeast Asian tropical peatland forests are a one-of-a-kind dual ecosystem that combines rainforest and peatland. Its topography is heavily influenced by hydrological conditions, which in turn determine the vegetation structure and peat types. Tropical peatland forests in Malaysia with approximately 2.7 Mha (approx. 1.7 Mha are in Borneo Malaysia and approx 1.0 Mha are in Peninsular Malaysia) are considered as extreme ecosystems because of their low pH (generally below pH 4), anaerobic and oligotrophic conditions. This tropical peatland plays a vital role in nutrient cycling, serving as a source of groundwater, reducing excess nutrient flows from surface waters, sequestering and storing atmospheric carbon (C), hence, regulating the global C balance and concentrations of greenhouse gases (GHG), e.g., methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) in the atmosphere, making them critical in terms of climate change. Also, high C storage in tropical peatland resulting from the photosynthetic accumulation and low decomposition due to anaerobic conditions have been shown to support unique groups of microorganisms.

In recent years, southeast Asian tropical peatlands have been rapidly deforested due to agricultural expansion fuelled by the increasing demand for food and renewable energy sources. Changes in the tropical peatland functions due to agricultural drained-based, e.g., oil palm plantation, may change this ecosystem services. The oil palm plantation needed drainage canal networks for controlling the water table level and making the soil suitable for planting. Yet, their presence has the negative impact of drying out peat soils near the ground surface. The exposed surface peat enhances aerobic decomposition, presumably resulting in higher CO<sub>2</sub> emissions, which reverses the role of peatlands as C sinks to C sources. Furthermore, peat soil considered to be poor soil due to acidic conditions and limited nutrient availability. The addition of fertilizer in the oil palm plantation to promote palm productivity is likely to generate peat mineralization, thus, potentially stimulating soil nitrous oxide (N<sub>2</sub>O) emission. Furthermore, persistent hydrological changes can alter the soil properties and C substrate quality, as well as microbial communities below-ground. Therefore, the conversion of peatlands to agricultural landscapes could affect soil conditions that would in turn affect microbial communities, and that all these changes together can affect GHG emissions. The research presented in this thesis aimed to understand better how tropical peatlands respond to disturbance by investigating GHG emissions, physicochemical variables, peat characteristics, peat microbial communities, and the link between these components in Malaysian peatland.

Two field assessments were conducted separately at North Selangor peat swamp forest, Malaysia. The first field study was conducted to investigate the temporal GHG emissions corresponding to the environmental variables and peat characteristics of three land-use in Malaysian peatland systems, (i) natural peat swamp forest (NF), (ii) recovery peat swamp forest (RF), and (iii) oil palm plantation (OP). Biweekly temporal measurements of CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O fluxes were conducted using a closed-chamber method from July 2017 to December 2018, along with the continuous measurement of environmental variables and a one-time measurement of the soil physicochemical properties. Cumulative emissions were calculated by plotting emissions against time, interpolating linearly between emission samplings, and integrating the area under the curve. Pearson correlation was used to determine correlations between GHG emissions and hydrology parameter variables. Then, one-way ANOVA with post-hoc test was used to compare GHG emissions and environmental parameters at the different sites. Calculated cumulative gases flux values were used to compare the GHG emissions between sites, while mean values were used for environmental parameters. In addition, to compare the overall Global Warming Potential (GWP) load of the three GHGs, annualized emissions of CH<sub>4</sub> and N<sub>2</sub>O were converted to CO<sub>2</sub> equivalents (CO<sub>2</sub>-eq) over a 20-year horizon because this time period was considered to be the most appropriate for evaluating the impacts of land-use change that typically occur over 20-30 year time periods in tropical regions. Annualized emissions were calculated using the cumulative emissions multiplied by a constant of 365/total days of cumulative emission. The GWP value of 86 and 268 were used for CH<sub>4</sub> and N<sub>2</sub>O, respectively. Our results indicate that the significant differences in environmental conditions of the drained OP, historically drained rewetting-restored RF and natural peatland NF systems affected CH<sub>4</sub> and N<sub>2</sub>O most, with little effect on CO<sub>2</sub> emissions. The cumulative CH<sub>4</sub> emission was significantly higher in the forested sites (RF and NF), which was linked to a significantly higher water table level ( $p < 0.05$ ). The similar high cumulative CO<sub>2</sub> emission trends at the RF and OP sites indicated that the RF rewetting-restored peatland system continued to have high decomposition process of the available labile C and higher proportion of root respiration despite having a significantly higher water table level than the OP site ( $p < 0.05$ ). The highest cumulative N<sub>2</sub>O emission at the drained-fertilized OP and rewetting-restored RF sites was linked to the available substrates for high decomposition (low C/N ratio) together with soil organic matter mineralization that provided inorganic nitrogen (N), enabling ideal conditions for microbial mediated N<sub>2</sub>O emissions. Furthermore, The CO<sub>2</sub> emissions contributed 78% of the total 20-year GWP at the OP. However, the much lower of total 20-year GWP at OP than at the forested sites, despite the high CO<sub>2</sub> emissions at OP, was

due to the higher CH<sub>4</sub> emissions at the NF and RF sites. Overall, the measured peat properties did not vary significantly between the three land uses. However, the lower C/N ratio at the OP and the RF sites indicated higher decomposition rates in the drained and historically drained peat than in the undrained natural peat (NF), which was associated with high cumulative CO<sub>2</sub> and N<sub>2</sub>O emissions.

The soil microbial communities depend on organic matter for their nutrients and C supply and become critical drivers in biogeochemical processes such as the C-N cycles. Hence, it is vital to comprehend how microbial communities are affected to fully comprehend the impact of environmental changes on tropical peatland functions. The second field study was conducted to explore the variation of tropical peat microbial communities between undisturbed-peat natural peat swamp forest (NF) and disturbed-peat oil palm plantation (OP) at different peat depths and how it related to biogeochemical cycling using the high-throughput Next Generation Sequencing (NGS). A total of 18 peat samples at three different depths, 0 – 5cm (surface layer), 20 – 25cm (sub-soil layer), and 40 – 50cm (bottom layer), were collected in September 2019 (dry season) from both sites. DNA was extracted from three replicate soil samples at each depth using the ISOIL beat beating soil extraction kit, and were sent to GENEWIZ for Next Generation Sequencing (NGS). Sequences were rarefied prior to the calculation of alpha and beta diversity statistics. The alpha diversity indices, such as Chao1 and Shannon indices were generated for each soil sample (represent depth) based on the OTU table. Beta diversity was performed by Principal coordinate analysis (PCoA) using unweighted UniFrac. A one-way analysis of similarity (ANOSIM) was performed to determine the differences between groups. The Bray–Curtis similarity index was used as a metric of similarity between the microbial communities based on the abundance of OTUs between samples. This study shows that the peat microbial community structure in tropical peatlands was dominated by bacteria and responded to depth and land-use change. The dominant microbial taxa observed at the NF in this study are selectively similar to those in other studies on tropical peatland forests, yet their proportion of abundances are different. The dominant phyla that accounted for more than 3% of the mean relative abundance of the overall microbial communities of both NF and OP sites were *Acidobacteria*, followed by *Proteobacteria*, *Thermoplasmatota*, and *Actinobacteria*. Furthermore, high relative abundance of *Acidobacteria* and *Proteobacteria* at 0 – 5cm depth in both sites and constantly decreased with depth down, indicated that high degradation of biomass in the surface layer compared to the deeper layers of peat soil. Indices of Chao1 and Shannon values showed that the richness and diversity of microbial communities were not significantly different between NF and OP sites. However, PCoA analysis

demonstrated dissimilarities in microbial community composition between peat depths and the study sites. Metastats gap analysis presented five bacterial genera that were significantly different between NF and OP sites ( $p < 0.05$ ). *Bryobacter* spp., *Acidibacter* spp. and *WPS-2* spp. were significantly higher in OP than NF, whilst *Syntrophotobacter* spp. and *Subgroup – 13* spp. were vice versa. Furthermore, Linear discriminant analysis effect size (LEfSe) analysis identified one archaeal genus of *Bathyarchaeia* spp. was significantly higher in OP than NF ( $p < 0.05$ ). However, approximately 60.5% of the sequences were not classified to any known genera.

Based on the findings, this study can assert that the rewetting-restored efforts of tropical peatland may not immediately reduce GHGs, because it depends on the length of time since the peatland started recovering. The higher total GHG emissions at the RF site in this study (rewetting restoration started 6-7 years previously), mainly due to increased CH<sub>4</sub> emissions, suggest that emissions may not be fall during the initial stage of peatland recovery. Therefore, further research is needed to clarify the timeline for changes in emissions after the start of peatland restoration. In addition, research on restored peatlands should be widened to include different types of restored sites and different histories of the previous land-used before the implementation of rewetting-restoration efforts. Also, this study has demonstrated the differences in the structure of microbial communities between a natural peat swamp forest (NF) and a drained and cultivated peatland oil palm plantation (OP). The peat microbial community structure in both tropical peatlands was dominated by bacteria, but varied with depth and land-use. The microbial communities in the oil palm plantation were closely related to the soil surface communities of the natural forest system. The dominant microbial taxa observed in the peatland forest system in this study are similar to those in other studies on tropical peatland forests in Malaysia, yet the proportions of their abundances were different, which might reflect the distinct degrading process of plant residues and the formation of peat under different environmental conditions. In this study, approximately 60.5% of the sequences were not classified to any known genera, suggesting that there could be new, unknown microbial biota present in this extremely acid ecosystem. To connect taxonomic composition and function is a challenge, and to connect microbial community structure and ecosystem functioning is even more of a dilemma. Therefore, future studies should focus on specific taxa that have demonstrated a significant response to land-use change in order to gain a more detailed understanding of the implications of microbial turnover for peatland ecosystem functioning.