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| Title               | Study on the diversity and vertical distribution of soil microorganisms in tropical peatlands of Sarawak, Malaysia, and characterization of nitrous oxide (N <sub>2</sub> O)-emitters and quenchers from the tropical peat soils [an abstract of entire text] |
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## 学位論文の要約

博士の専攻分野名称：博士（農学）

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## 学位論文題名

Study on the diversity and vertical distribution of soil microorganisms in tropical peatlands of Sarawak, Malaysia, and characterization of nitrous oxide (N<sub>2</sub>O)-emitters and quenchers from the tropical peat soils

(サラワク・マレーシア熱帯泥炭地における土壌微生物の多様性と垂直分布、および熱帯泥炭土壌から分離した亜酸化窒素 (N<sub>2</sub>O) の産生および消去に関わる微生物の特徴に関する研究)

Tropical peat swamp forests are globally important ecosystems that lack proper study on their ecology and biodiversity. Peat swamp forests are thought to show low rates of nutrient cycling and organic matter decomposition due to the accumulation of woody peat and the adverse environmental conditions. However, growing numbers of publications are proving this otherwise and with the improvement of scientific methods, new findings are gradually being discovered. One of the largest gaps in knowledge regarding these ecosystems is how these microorganisms are related to greenhouse gas production and consumption, specifically focusing on nitrous oxide (N<sub>2</sub>O) in this study. Poor understanding of the microbial mechanisms of these ecosystems may result in them becoming main key players in emission of N<sub>2</sub>O to the atmosphere. To expound further into better understanding of this subject matter, this study is conducted and summarized into three major parts as follows:

### **1. Improved DNA Extraction Method to Access Microbial Diversity of Tropical Peatlands**

Woody tropical peat soil is a histosol with an intermediate-to-strong acidic nature, consisting of >75% organic matter mainly with degraded wood materials extraordinarily rich in humic substances. Due to its chemical and physical properties, tropical peat soil is one of the most difficult sources for obtaining pure soil DNA. Several methods for effective and reproducible DNA extraction from woody peat soil were tested to obtain high-quality DNA suitable for use as template DNA for 16S rRNA gene-targeted denaturing gradient gel electrophoresis (DGGE), along with an appropriate choice of a humus-tolerant *Taq* polymerase. Results show that DNA extraction using a modified conventional method, followed by removal of humic substances using 1.5% agarose gel electrophoresis in Tris/Borate/EDTA (TBE) buffer as an important step, yielded the

most comprehensive DNA fingerprinting profile for soil eubacteria and archaea. The DGGE profiles of the DNA samples from both top (0–50 cm) and deep (350–400 cm) layers of tropical peat soils exhibited microbial compositions including unculturable eubacteria of class *Deltaproteobacteria*, phyla *Actinobacteria*, *Bacteroidetes*, and *Acidobacteria*, and archaea of phyla *Thaumarchaeota* and *Crenarchaeota*.

## **2. Vertical Distribution of N<sub>2</sub>O Emission Potentials in Tropical Peatland**

Tropical peat swamp forests that have been reclaimed for agricultural use are generally active sources of nitrous oxide (N<sub>2</sub>O) efflux; but the cause and mode for the emergence of N<sub>2</sub>O emitters from the soil microbial communities of reclaimed tropical peat soil are unclear. Using a culture-based N<sub>2</sub>O emission assay, the N<sub>2</sub>O emission potentials of soil at various depths (0–450 cm) were investigated in two oil palm plantations with a period of 2 years (E2Y) and 10 years (E10Y) after deforestation in Sarawak, Malaysia. The peat soil at E2Y showed a trend of high N<sub>2</sub>O emission potential in deeper layers (200–400 cm), whereas the older plantation E10Y showed considerably more active N<sub>2</sub>O emission potential in shallow soil (10–50 cm). N<sub>2</sub>O emission potentials among the soil microbial communities at different soil depths of E10Y site showed positive correlations with NO<sub>3</sub><sup>−</sup> and NH<sub>4</sub><sup>+</sup> contents, whereas soils of the E2Y site had N<sub>2</sub>O emission potentials inversely proportional to the contents of NO<sub>3</sub><sup>−</sup>. This contrasting vertical correlation between N<sub>2</sub>O-emitting potentials and mineralized nitrogen contents suggests that active N<sub>2</sub>O emission in deep soil of E2Y has maintained the original C/N ratio of the peat soil, whereas at E10Y, such a regulatory system has been lost due to advanced soil degradation.

## **3. Isolation and Characterization of N<sub>2</sub>O Quenchers from Tropical Peatlands**

Potent N<sub>2</sub>O-quenchers were isolated and identified as *Burkholderia* sp. and *Chitinophaga* sp. from soils collected at various depths from an oil palm plantation on peat. *Chitinophaga* sp. showed an extraordinary N<sub>2</sub>O-quenchable activity and was able to eliminate as much as 3000 ppmv (atmospheric level at 300 ppbv) of the supplemented N<sub>2</sub>O in the headspace (22.57 mL) within 3 days. No inhibition of N<sub>2</sub>O quenching was observed by addition of 10% acetylene, suggesting that the N<sub>2</sub>O quenching may not be due to reduction of N<sub>2</sub>O by N<sub>2</sub>OR. The whole-genome sequencing for the *Chitinophaga* sp. using a pyrosequencer revealed that N<sub>2</sub>O quenching activity of *Chitinophaga* sp. is without the *nosZ* gene, suggesting the possibility of other redox mechanisms.