

**Study on the diversity and vertical distribution of soil
microorganisms in tropical peatlands of Sarawak, Malaysia,
and characterization of nitrous oxide (N₂O)-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壤微生物の多様性と
垂直分布、および熱帯泥炭土壌から分離した亜酸化窒素 (N₂O) の
産生および消去に関わる微生物の特徴に関する研究)

Ph.D. Dissertation
(The Special Postgraduate Program in Biosphere Sustainability Science)

Sharon Yu Ling LAU



Laboratory of Molecular and Ecological Chemistry
Division of Applied Bioscience
Graduate School of Agriculture
Hokkaido University
Sapporo, Japan

March 2015

SUMMARY

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 (N₂O) in this study. Poor understanding of the microbial mechanisms of these ecosystems may result in them becoming main key players in emission of N₂O 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 that is chemically intact. Several methods for 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). In addition, a humus-tolerant *Taq* polymerase compatible with DNA templates extracted from peat soil is identified and can be used for PCR-based evaluation of microbial community structure in the soil. 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, yielded the most comprehensive DNA fingerprinting (DGGE) 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 bacterial compositions including typically unculturable eubacteria such as those of class Deltaproteobacteria, phyla Actinobacteria, Bacteroidetes, and Acidobacteria, and archaea of phyla Thaumarchaeota and Crenarchaeota. Detection of these eubacteria and archaea has proven that the DNA extracted is highly suitable for use as template DNA in DGGE analysis of bacterial community structures in soil from adverse ecosystems.

2. Vertical Distribution of N₂O Emission Potentials in Tropical Peatland

Tropical peat swamp forests that have been reclaimed for agricultural use are generally active sources of nitrous oxide (N₂O) efflux; however the cause and mode for the emergence of N₂O emitters from the soil microbial communities of reclaimed tropical peat soil is unclear. This study aims to reveal the vertical distribution of N₂O emission potential and its correlation with mineral nitrogen contents in reclaimed soils. Using a culture-based N₂O emission assay, the N₂O emission potentials of soil at various depths (0–450 cm) were investigated in two oil

palm (*Elaeis guineensis*) plantations in Sarawak, Malaysia, which had been cultivated for a period of 2 years (E2Y) and 10 years (E10Y) after deforestation. The peat soil at E2Y showed a trend of high N₂O emission potential in deeper layers (200–400 cm), whereas the older plantation E10Y showed considerably more active N₂O emission potential in shallow soil (10–50 cm). N₂O emission potentials among the soil microbial communities at different soil depths of E10Y site showed positive correlations with NO₃⁻ and NH₄⁺ contents, whereas soils obtained from the E2Y site had N₂O emission potentials that were inversely proportional to the contents of NO₃⁻. This contrasting vertical correlation between N₂O-emitting potentials and mineralized nitrogen contents suggests that active N₂O 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, leading to dynamic changes in the nitrogen cycle in shallow soil.

3. Isolation and Characterization of N₂O Quenchers from Tropical Peatlands

Nitrous oxide reductase (N₂OR) encoded by *nos* gene is the enzyme responsible in reduction of nitrous oxide (N₂O) in the final step of denitrification and is reported to be the only conduit for N₂O degradation. The biochemistry of this process has been studied extensively in denitrifiers and current search is also extended to non-denitrifiers that possess the gene. Potent N₂O-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 N₂O quenching activity and was able to eliminate as much as 3000 ppmv (atmospheric level at 300 ppbv) of the supplemented N₂O in the headspace (22.57 mL) within 3 days. No acceleration of N₂O production was observed from the 10% acetylene inhibition assay. Additionally, the *nosZ* gene was not detected by PCR assay, using degenerate primer sets for both typical and atypical *nosZ* genes suggesting that its ability of N₂O quenching may not be through the exclusive N₂OR. Therefore, the whole-genome sequence of *Chitinophaga* sp. was determined using an Illumina HiSeq pyrosequencer and unassigned ORFs were annotated to conduct a BLAST search for N₂O-quenching genes. The pyrosequencing study revealed that N₂O quenching activity of *Chitinophaga* sp. is without the *nosZ* gene, suggesting the possibility of the presence of other redox mechanisms.