学位論文審査の要旨

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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_2O)-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_2O）の産生および消去に関わる微生物の特徴に関する研究)

The thesis of 77 pages consists of 5 sections in the body of text with 166 literatures cited, 22 figures, 7 tables, and 1 diagram. One original article is attached as a reference paper. This study first developed an improved method for extraction of DNA from tropical peat soil that possessed extremely high contents of humic substances. Using soil DNA with this method, it became possible to determine bacterial and archaeal community structures in matured peat soils by using a 16S rRNA gene-targeted PCR-DGGE technique. Secondly, vertical gradient of N_2O emission potential from the top to 450-cm deep layers of soils was investigated at two oil palm plantations reclaimed from peat swamp forests. Also, potent N_2O quenchers were isolated and characterized. All the researches in this thesis contain important discoveries that suggest us new approaches for necessary for soil management and regulation of N_2O gas emission in the reclaimed peatlands.

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 showed 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 degrading and matured 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_2O Emission Potentials in Tropical Peatland

Using a culture-based N_2O emission assay, the N_2O 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_2O emission potential in deeper layers (200–400 cm), whereas the older plantation E10Y showed considerably more active N_2O emission potential in shallow soil (10–50 cm). N_2O emission potentials among the soil microbial communities at different soil depths of E10Y site showed positive correlations with NO_3^- and NH_4^+ contents, whereas soils of the E2Y site had N_2O emission potentials inversely proportional to the contents of NO_3^-. This contrasting vertical correlation between N_2O-emitting potentials and mineralized nitrogen contents suggests that active N_2O 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_2O Quenchers from Tropical Peatlands

Potent N_2O-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_2O-quenchable activity and was able to eliminate as much as 3000 ppmv (atmospheric level at 300 ppbv) of the supplemented N_2O in the headspace (22.57 mL) within 3 days. No inhibition of N_2O quenching was observed by addition of 10% acetylene, suggesting that the N_2O quenching may not be due to reduction of N_2O by N_2OR. The whole-genome sequencing for the *Chitinophaga* sp. using a pyrosequencer revealed that N_2O quenching activity of *Chitinophaga* sp. is without the *nosZ* gene, suggesting the possibility of other redox mechanisms.

Thus, the thesis study revealed the presence of a clear linkage between N_2O emission and soil properties including soil microbial community structures. Such linkages may offer a clue into future prospects for regulation of N_2O production in tropical peatlands.

Therefore, we acknowledge that the author is qualified to be granted the Degree of Doctor of Philosophy in Agriculture from Hokkaido University.