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# 学 位 論 文 審 査 の 要 旨

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## 学 位 論 文 題 名

**Impacts of anthropogenic disturbances on the community structures and functions of soil microorganisms**
（人為的攪乱が土壌微生物群集の組成や機能に及ぼす影響）

The thesis consists of 123 pages in English, 28 figures, 9 tables, 6 chapters, and 1 reference paper. On a global scale, anthropogenic disturbances such as land-use changes, pollution, and introductions of exotic species have impacted the original natural ecosystems, causing changes in the balance of ecosystem functions. This study focused mainly on soil microbial communities, which have various life-supporting ecosystem functions because such disturbances have also influenced them. Despite the importance of soil microbes, little was known about whether the disturbed microbial communities further accelerate the loss of functionality. Also, nitrogen (N) cycle-related functions are essential within the variable microbial functions due to their impact on the environment and plant growth. Thus, the objective of this study was valid. In addition, previous microbiome studies primarily focused on the Global North (i.e., North America, Europe, and East Asia), while knowledge of the microbiome in other regions was lacking, especially in Africa. Although the loss of soil carbon (C) and nitrogen (N) due to excess farming management in Africa is prominent, how much soil degradation affects soil microbial diversity and nutrient cycling functions, which could contribute to further soil degradation, was unclear. Thus, this study focused on filling the research gap in soil microbiome research.

Additionally, this study focused on the recent globalization leading to a rapid movement of exotic animals or plants to other local areas with human activities. In the former glacier northern hardwood forests in North America, native earthworms were thought to be cleared out during the last ice age. However, European people introduced non-native earthworms to that area, causing an alteration of ecosystem services such as nutrient availability. The study used this problem as a model case of the human impact of globalization. It evaluated the effect of the problem concerning soil microbial functions using novel approaches such as stable isotope probing.

1) The changes in nitrification potential and related microbial communities by farming management in sub-Saharan Africa

The first experiment measured the changes in nitrification potential and its relationship with soil nitrifying microbial communities altered by farming management, targeting three sites where all had neighboring natural lands and farmlands. The study revealed that the nitrification potential increased in all farmlands than in the natural lands. The abundance of ammonia-oxidizing bacteria (AOB) also tends to increase in farmlands, but it did not correlate with the nitrification potential. On the contrary, the abundance of ammonia-oxidizing

archaea (AOA) was lower in the farmlands than in the natural lands. Previously, it was thought AOB was more strongly correlated to the nitrification potential, but this was not the case in the sites in sub-Saharan Africa, where this study focused. Species in AOB clusters 3a.2 and 3b relatively increased in the farmlands, most likely due to increased soil pH and ammonium addition by farming. This result suggests that the total abundance of AOB and the internal changes in their community structure can upregulate nitrification potential, possibly leading to further N loss.

2) Changes in soil microbial diversity by forest-to-farmland conversion in various scales in sub-Saharan Africa

The second experiment performed a microbial ecological investigation on the changes in microbial diversity through forest-to-farmland conversion at the sample scale, the location-scale (e.g., within one farmland in Kenya), and the metacommunity scale (e.g., among multiple farmlands across Kenya and Malawi). This study found that the heterogeneity of soil prokaryotic and fungal communities was lower in the farmlands than in the natural lands, indicating a community homogenization caused by farming management. The microbial taxonomic heterogeneity was also correlated with the heterogeneities of C cycle- and N cycle-related functions and fungal lifestyles at the location-scale. Moreover, the homogenization of fungal communities at both location and metacommunity scale was correlated with the relative abundance of pathogenic fungi. Farming management could reduce the local niche of some rare microbiomes on a small scale. Overall, this study suggested that farming management in sub-Saharan Africa leads to a homogenization of microbial taxa, possibly leading to a loss of heterogeneous functions and a rise of pathogenic fungi.

3) The evaluation of the impact of invasive earthworm species on soil microbiome using stable isotope probing

Finally, the third experiment conducted a field investigation of earthworms, gas, and soil biochemical properties and incubation in a laboratory to conduct a stable isotope DNA probing, targeting a northern hardwood forest in Minnesota, where a gradation in invasion level of non-native earthworms is observed (Minimally invaded site, Site M; Highly invaded site, Site H). Previous studies suggested the changes in nitrification potentials due to the earthworm invasion in this area, but the mechanisms were unknown. This study identified that the abundance of AOA was more significant in Site M than in Site H. In addition, the shallow soil in Site H exhibited a slower nitrification activity than in Site M, most likely due to a decrease in the AOA abundance. However, not AOA, but relatively larger AOB and nitrite-oxidizing bacteria (NOB) were detected as they actively facilitate nitrification in all the soils except for the shallow soil in Site H. The increases in AOB and NOB may be because the ammonium concentration used in the incubation was too high for AOA, which prefers low ammonium environments. These results suggest that the nitrification activity of the shallow soil in Site H is slow regardless of the original field nitrifiers' abundances. This study concluded that the earthworm could negatively affect some soil functions, such as nitrification, and introducing foreign species to soil ecosystems must be performed carefully.

The series of studies in this thesis mainly evaluated the effects of the anthropogenic activities on soil functions by analyzing the soil microbiome. The results of these studies accumulated knowledge on the soil microbiome, particularly in sub-Saharan Africa. The knowledge can be used to establish sustainable soil management techniques, critically needed in the area. Overall, these results are highly evaluated as a significant contribution to the development of sustainable agriculture.

Therefore, we acknowledge that the author is qualified to be granted the degree of Doctor of Philosophy in the field of Food Resources from Hokkaido University.