学 位 論 文 審 査 の 要 旨

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学 位 論 文 題 名

The impact of lead pollution and *Azospirillum brasilense* Sp7 inoculation in lemongrass cultivation on soil nitrogen cycling and microbial communities

（鉛汚染及び *Azospirillum brasilense* Sp7 株の接種を伴う
レモングラスの植栽が土壌中の窒素循環と微生物群集に及ぼす影響）

The thesis comprises 165 pages in English, with 15 figures, 72 tables, 4 chapters, and 1 reference paper.

Soil heavy metal contamination is a global environmental issue, stemming from sources such as mining, industrial waste incineration, exhaust emissions, and excessive use of chemical fertilizers and pesticides. Heavy metals like lead (Pb) are non-biodegradable, biologically inactive, and highly toxic. Contaminated soil degrades ecosystem functions, causes vegetation loss, and poses health risks due to the dispersal of polluted topsoil. Thus, effective remediation requires both removal of heavy metals and restoration of soil fertility.

The nitrogen cycle, closely linked to soil fertility, is sensitive to heavy metal pollution and often serves as an indicator of environmental impact. However, few studies assess both inorganic nitrogen levels and functional gene abundance under heavy metal stress. Most focus on specific processes or genes, leaving unclear how the entire nitrogen cycle is affected.

In recent years, combining plant growth-promoting microorganisms (PGPMs) with heavy metal-tolerant plants has emerged as a cost-effective and eco-friendly remediation approach. However, microbial strains and inoculation methods vary widely, highlighting the need to identify effective strategies tailored to specific plant species. While many studies focus on plant growth, their effects on soil functions remain underexplored. Lemongrass has recently gained attention for its tolerance to heavy metals and economic value, yet studies involving microbial inoculation under heavy metal stress are limited.

This study addresses these gaps by: (1) evaluating the nitrogen cycle under Pb contamination through measurements of inorganic nitrogen and functional genes; (2) assessing the effects of microbial inoculation and lemongrass cultivation on nitrogen cycling under Pb stress; and (3) comparing different inoculation methods on both nitrogen cycling and plant growth.

1) Effects of Pb on denitrification with nitrate addition:

A soil incubation study examined changes in denitrification and microbial communities under Pb exposure at four levels (0, 200, 400, 1600 mg Pb/kg soil), with or without nitrate. At 1600 mg/kg, Pb inhibited NO_3^- and NO_2^- reduction and increased N_2O emissions. The *narG* gene decreased significantly, while *nirS* showed no consistent trend. 16S rRNA abundance declined, though Shannon diversity increased. Proteobacteria declined rapidly at 1600 mg/kg, and Azotobacter dropped to less than 50% of the control at ≥400 mg/kg Pb.

2) Inoculation of lemongrass with *Azospirillum brasilense* Sp7 in Pb-contaminated soil:

An eight-week pot experiment was conducted using three inoculation methods: seed, root, and combined. Combined inoculation yielded the highest leaf and root growth, while root-only inoculation appeared suppressive. By week four, the combined method led to the highest abundance of nitrogen-related genes (*nxrB*, *amoA*, *nirK*, *nirS*, *norB*, *nosZ*) and 16S rRNA, with some significantly higher than controls.

Overall, the two experiments revealed that Pb contamination at 400–1600 mg/kg impairs denitrification, reduces functional gene abundance and microbial biomass, and alters microbial community structure. In contrast, inoculation with *A. brasilense* Sp7—especially via seed and root—enhanced lemongrass growth, increased functional gene abundance, and improved nitrogen cycling in Pb-contaminated soil.

These results suggest that microbial inoculation and phytoremediation using lemongrass can positively affect nutrient cycling and soil recovery under heavy metal stress. The findings also offer insights into how altered denitrification may influence plant nitrogen use efficiency. Nevertheless, limitations remain, including the lack of transcriptomic data, Pb quantification in soil and plants, and long-term field validation. Future research should address these gaps through RNA-level analyses, Pb measurement, and field trials to assess long-term inoculation effects.

The thesis evaluated the impacts of Pb contamination on nitrogen cycling, microbial communities, and microbial inoculation using inorganic nitrogen analysis, gene quantification, community structure assessment, and plant growth measurement. These studies contribute to the understanding of nitrogen cycling in contaminated soils and offer valuable knowledge applicable to soil fertility management and bioremediation. The findings are considered a significant contribution to both foundational science and applied strategies for sustainable land use.

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.