学位論文内容の要旨

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学位論文題名

The use of digestate from animal wastes and its impacts on the soil microbiome and nutrient dynamics in agricultural soils

（家畜糞尿由来消化液の利用が農地土壌微生物叢と栄養素ダイナミクスに及ぼす影響の評価）

Digestate is the semi liquid byproduct of the biogas production process. Due to its basic pH and high nutrient content (i.e inorganic nitrogen, phosphorous, organic carbon), digestate use as a fertilizer has been increasing. Additionally, to improve its efficiency as a fertilizer, digestate can be separated into solid and liquid fractions using a flocculant. However, the effect of digestate and its separated fractions on the soil microbiome and nutrient dynamics in agricultural soils is not clear. Therefore, this study, involving fieldwork and incubation experiments was conducted to address this research need. The field work involved sampling soils from farmers' fields in Kamishihoro town in Hokkaido to determine the effect of land use, seasons and fertilizer application on the soil microbiome and related functions. The second experiment was an incubation to determine the effect of soil pH on microbial stability against the application of digestate from dairy wastes. The third experiment was an incubation to analyze the effect of solid-liquid separation of digestate on soil nutrient dynamics and Japanese mustard spinach yields in different soils.

1.Land use and season drive changes in soil microbial communities and related functions in agricultural soils

The field study was conducted in an Andosol (volcanic soil) dominated agricultural region in cool temperate climate to determine the effect of land use (cropland, grassland), season (spring, summer) and fertilizer (digestate) on soil microorganisms and related functions. Soils were sampled from farmers' fields, DNA extracted and sequenced targeting 16S rRNA region. In result, land use had a significant effect on beta diversity and evenness with higher values recorded in cropland than grassland. However, grassland had a higher number of unique operational taxonomic units (OTUs) (10303) compared to cropland (5112). In cropland, season had a significant effect on beta diversity, evenness, OTU numbers and Shannon index with higher values recorded in summer compared to spring. Based on predicted soil functions, nitrogenase (*nifH*) had significantly higher values in cropland-summer while nitrite reductase (*nirK*) and ammonia monooxygenase (*amoA*) were significantly higher in cropland-spring. In grassland, season had a significant effect on beta diversity only. These results indicate that grassland microorganisms were stable and more resistant to seasonal changes than cropland, suggesting that conventional tillage practices have a negative effect on soil microbial stability. Additionally, grassland-spring (7059) had a higher number of unique OTUs than grassland-summer (2597). Based on predicted soil functions, *nifH* was significantly higher in grassland-spring while *nirK* and *amoA* were significantly higher in grassland-summer. These results

indicate that the impact of seasons on soil microorganisms' distribution and abundance in cropland and grassland may directly affect soil functions.

2.Liming improves the stability of soil microbial community structures against the application of digestate made from dairy wastes

The incubation experiment was performed to understand the effect of lime application (pH = 6.5 and 5.5 for the soils with and without lime, respectively) and fertilizer (digestate, urea and control) on the soil microbial community structures, stability and gene functions. Soils were sampled weekly after the application of fertilizers for a month. For microbial community analysis, DNA was extracted and sequenced targeting 16S rRNA region. For gene abundances (i.e 16S rRNA, ammonia oxidizing archaea (AOA), ammonia oxidizing bacteria (AOB), nitrous oxide reductase (*nosZ*) and nitrite reductase (*nirS*), quantitative PCR was conducted. In result, the relative abundance of *Actinobacteria* was influenced more strongly by digestate in lime soils, while *Alphaproteobacteria* was influenced more strongly by digestate in the no lime soil. In no lime treatments, digestate had a significant effect on more operational taxonomic units (146) compared to lime (127), indicating that lime application increased soil microbial community's stability. Liming and fertilizer had a significant effect on 16S rRNA gene copy numbers with the highest values observed in lime plus digestate treatments. Soil pH had a significant on AOA, *nosZ* and *nirS* gene copy numbers with the highest values observed in lime treatments. In the lime treatments digestate application had a positive impact on AOB gene copy numbers but this was not the case for soils without liming treatments. These results indicate that soil pH and fertilizer type should be taken into consideration for the management of functional gene abundance in agricultural soils.

3.The effect of solid-liquid separation of digestate on soil nutrient dynamics and Japanese mustard spinach yields in different soils

The incubation experiment was setup to analyze the effect of soil types (Kamishihoro and Arakida) and fertilizers (digestate, solid digestate, liquid digestate, chemical and control) on soil and plant (Japanese mustard spinach) nutrients, gene abundance and nitrous oxide (N_2O) emissions. Based on the results, soil type significantly influenced the above ground plant biomass and Nitrogen (N) content with higher values observed in Kamishihoro compared to Arakida. Kamishihoro had significantly higher soil inorganic N (NO_3^- and NH_4^+) content compared to Arakida soil. Fertilizer had a significant effect on soil NO_3^- (solid-Kamishihoro, digestate-Arakida) and NH_4^+ (liquid-Kamishihoro, chemical-Arakida) content. The results indicate that digestate and its derived fertilizers (solid digestate, liquid digestate) can be used to influence soil inorganic N content in agricultural soils. Digestate derived fertilizers influenced soil pH with highest values observed in liquid in both Kamishihoro and Arakida soils. Arakida soil had significantly higher 16S rRNA and ITS gene copy numbers compared to Kamishihoro. Regarding soil N_2O emissions, Kamishihoro soil had significantly higher emissions compared to Arakida. Additionally, digestate and solid fertilizer had significantly higher N_2O emissions compared to liquid in both Kamishihoro and Arakida soils. The results indicate the importance of soil type and digestate derived fertilizers in modulating soil gene abundance and N_2O emissions in agricultural soils. Furthermore, the separation of digestate into solid and liquid fractions can be considered a reliable method to regulate aspects of soil nutrient dynamics in agricultural soils.