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Integrated Multidimensional Omics Analysis of Soil–Plant–Microbe Interactions in

Soybean Agroecosystems

（多次元オミクス統合解析によるダイズ農業生態系における

土壌–植物–微生物相互作用の解明）

Chapter 1 – General introduction

Under field conditions, crop performance can vary substantially due to complex interactions among diverse biotic and abiotic variables, including environmental conditions, management practices, regional differences, and interannual variability. Therefore, accurate evaluation of crop growth under field conditions requires an understanding not only of individual factors but also of the interactions among multiple drivers. Accordingly, plant responses to specific factors under field conditions reflect the net ecosystem response emerges from complex synergistic and antagonistic interactions among multiple components of agroecosystems—including plants, microbes, soils, climate, insects, and management practices—that operate across spatial and temporal scales. To better understand these responses, research approaches that systematically investigate interactions among multiple factors are required. Multi-omics analysis integrates multiple molecular layers, allowing the characterization of molecular interactions and cross-layer relationships, and have thus developed as effective approaches for elucidating the dynamic responses of biological systems. However, the application of multi-omics approaches to agricultural fields characterized by temporally and spatially heterogeneous multi-stress environments remains challenges. Thus, a “multidimensional omics analysis” that incorporates spatiotemporal hierarchy into multi-omics data can serve as an advanced framework for capturing

coordinated changes within agroecosystems. This study used soybean as a model crop and obtained multidimensional omics datasets across multiple fields, aiming to elucidate agroecosystem processes driven by the simultaneous interactions of multiple factors.

Soybean is one of the major crops in the world and an abundant source of proteins, oils, and minerals. Japanese domestic productivity is low, compared to the yield in U. S. and Brazil. Several research efforts and technological developments have been undertaken to enhance soybean productivity. However, the effectiveness and impacts observed with these studies has often been limited under commercial field conditions. While efforts to improve soybean productivity have long been a central focus of agricultural research, recent years have seen increasing demand not only for higher yields but also for sustainable and environmentally conservative soybean production systems. In this context, the use of organic fertilizers has increased recently. Among organic fertilizers, livestock manure compost represents one of the most widely used organic resources. The application of manure supplies carbon to soils and improves soil chemical, biological, and physical properties, thereby altering crop productivity and nutrient dynamics. However, these effects are known to vary depending on environmental conditions, management practices, and soil types. However, it remains unclear how changes in ecosystem interactions under organic fertilizer application regulate soybean productivity and nutrient dynamics across different field environments.

In soybean production, contamination of seeds with potentially toxic or non-essential elements also represents an important concern. In Japan, the hydrogen explosions at TEPCO's Fukushima Daiichi Nuclear Power Plant in 2011 resulted in the release and deposition of radioactive substances, including radiocesium (^{137}Cs), onto agricultural fields. After the accident, contaminated topsoil was physically removed. However, crops cultivated in decontaminated fields remain at risk of radioactive substances transfer to plant. Potassium (K) fertilization has been shown to effectively reduce cesium (Cs) uptake due to their chemical similarity. However, the effectiveness of K fertilization in reducing Cs transfer has been reported to vary depending on soil type and crop species. Moreover, due to the economic cost of fertilizers, continuous K application may not be sustainable to mitigate ^{137}Cs transfer from soil to plant. Therefore, alternative strategies beyond K fertilization should be explored for sustainable and effective risk management of Cs transfer.

In this doctoral thesis, I aim to elucidate multi-scale interactions within the soil–plant–microbe

system and uncover bioecological processes by integrating multidimensional omics datasets collected from environmentally diverse soybean fields. First, to address the variability in the effects of manure application across field conditions, I aimed to elucidate ecosystem responses to manure by analyzing multidimensional omics data across multiple soybean fields. Furthermore, manure has been reported to affect the Cs transfer, but underlying mechanisms remain unclear. Thus, I also investigated Cs dynamics under manure amendment using multidimensional omics data. Next, I focused on multi-element interactions involved in regulating spatiotemporal Cs dynamics. Finally, to investigate soybean-specific mechanisms of Cs transfer, I focused on effects of root nodule symbiosis on Cs dynamics.

Chapter 2 – Changes in agroecosystem networks and causal structures under cattle manure application

Introduction

Organic fertilizers play an important role in building soil health by supplying slow-release nutrients, improving soil biological and physical properties, and enhancing carbon sequestration. However, their effects vary substantially depending on environmental conditions. Therefore, evaluating their agronomic and ecological impacts through multi-site, cross-disciplinary analysis is essential to identify robust response patterns. In this study, I aimed to clarify the robust effects of manure independent of cultivation conditions by employing a unified experimental design and collecting multi-omics datasets across multiple sites and years. Using this framework, I investigated how manure application reshapes interactions across data hierarchies within agroecosystems and identified the key drivers underlying these changes.

Materials and Methods

Soybean (*Glycine max* (L.) Merrill) was cultivated in six different fields across Japan in 2021 and 2022. In this field experiment, three treatments were prepared in each field: non-fertilizer, chemical fertilizer, and organic fertilizer (cattle manure) with three plot replicates. Sampling was conducted at three growth stages: pre-planting, flowering stage (R2), and harvesting stage (R8). Bulk soil was collected and stored for soil physical properties, ionome, metabolome, and microbiome analysis. Regarding microbiome, functional profiles were predicted using PICRUST2. Plant samples at R2 were dried, measured dry weight, and milled for ionome. Seed

samples at R8 were dried at room temperature, measured air-dried weight, and milled for ionome and metabolome. In each plot, soil sensors were installed at a depth of 10 cm to continuously monitor soil moisture and temperature throughout the growth period. Climate variables, including temperature and precipitation, were also recorded concurrently.

Multiple omics datasets measured consistently across both 2021 and 2022 were integrated. Data transformations were applied to each omics dataset according to its characteristics, for evaluation on the same scale.

Results and discussion

Principal component analysis and multivariate analysis of variance of the integrated multi-omics dataset showed that regional and annual factors explained the largest proportion of variation, followed by fertilization effects. To evaluate fertilization effects while accounting for differences among years and fields, mixed-effects models were constructed for each year with region as a random effect and fertilization treatment as a fixed effect. Variables that differed significantly under cattle manure and/or chemical fertilizer application compared with the non-fertilized control were identified, and their overlap was examined. Variables that consistently responded to cattle manure application in both years were significantly enriched in the soil ionome and soil metabolome, indicating that manure supplies nutrients across diverse environmental conditions. The largest number of variables belonged to those responding only to manure application in 2022, which were enriched in the soil microbiome. Within this group, soil bacterial α -diversity increased significantly. In addition, significant changes were observed in soil physical properties and plant growth. Together, these results indicate that manure not only supplies nutrients but also gradually improves soil physical and biological properties, thereby enhancing soybean growth across diverse environmental conditions. Bootstrap-based network topology analysis further revealed that bacterial functional networks shifted markedly under two consecutive years of manure application, indicating that even short-term manure amendment can drive structural differentiation of bacterial functions. These results suggest that short-term continuous manure application improves soil physical properties and promotes functional differentiation of soil bacteria, potentially contributing to enhanced plant growth.

Manure application has also been proposed as a strategy to mitigate Cs transfer because it supplies K. However, the underlying mechanisms remain unclear. Mixed-effects model analysis

confirmed that manure increased soil exchangeable K, whereas a significant reduction in plant Cs concentrations was observed only under consecutive manure application. This suggests that additional factors beyond K involve in suppression of Cs transfer. Further mixed-effects and network analysis indicated that manure application increases soil divalent cations and alters soil structure and microbial interactions, which may affect Cs transfer. In summary, these results suggest that manure promotes soil environments less conducive to Cs transfer not only by increasing K availability but also through changes in divalent cations and soil physical and biological processes.

Chapter 3 – Effect of the mineral network on soybean Cs transfer mechanisms and their fluctuations through growth stages

Introduction

Cs is transported primarily via K transporters, and its uptake and translocation are therefore largely regulated by K. However, the effectiveness of K in reducing Cs transfer varies across field conditions, indicating that Cs dynamics cannot be explained by K alone. Cs also interacts with multiple elements, including alkali and alkaline earth metals, and these interactions may vary across plant growth stages and among plant organs. Therefore, evaluating Cs transfer requires a spatiotemporal understanding of elemental interactions in soils and plants and identification of the elemental groups involved in Cs transfer and their dynamics.

Materials and Methods

Soybean (*Glycine max* (L.) Merrill cv. Yukihomare) was cultivated in the long-term fertilizer experimental field at Hokkaido University in 2021 and 2024. To examine Cs transfer under different soil nutrient conditions, six fertilization treatments were established; complete fertilization, without nitrogen, without phosphorus, without K, without sulfur, and no fertilization. Plant (leaf, petiole, stem, pod, seed, and root), bulk soil, and rhizosphere soil were sampled at 5 growth stages. Plant samples were measured dry weight and milled for ionome. Soil samples were collected and the ionome was analyzed using three different extraction methods.

Results and discussion

Soybean seed and shoot Cs concentrations increased under K deficiency, and soil exchangeable

K showed a strong negative correlation with shoot Cs concentrations, indicating that Cs transfer is largely regulated by K dynamics. However, distinct Cs distribution patterns were also observed under deficiencies of elements other than K, suggesting that Cs transfer is influenced by multiple elements. Moreover, the negative relationship between soil exchangeable K and shoot Cs varied across growth stages, indicating that Cs transfer changes during plant development.

Network analysis based on soil mineral profiles further indicated that both monovalent and divalent soil cations are involved in Cs uptake. Soil Na showed a consistent positive correlation with shoot Cs, whereas K showed a negative correlation throughout plant development. These associations were particularly strengthened during early growth stages, suggesting that soil–plant elemental relationships shift during physiological transitions. In addition, correlations between these elements and shoot Cs differed among soil extraction fractions, highlighting the importance of considering elemental forms in soils.

Regarding Cs distribution within plants, Mg and Rb showed close associations with Cs as well as K. These relationships differed among plant organs, indicating spatiotemporal variation in elemental interactions. Further studies are needed to clarify how the internal transport pathways of these elements are linked to Cs transport.

Chapter 4 – Effect of nodule formation on the molecular mechanism of Cs transfer in legume plant

Introduction

Soybean exhibits a weaker suppression of Cs transfer by soil K compared with other crops, highlighting the need to elucidate soybean-specific mechanisms governing Cs transfer. In Chapter 2, an indirect association between soil bacteria and plant Cs concentrations was shown, implying that plant–microbe interactions may influence Cs transfer. Thus, one potential approach in soybean is to exploit symbiotic microbes such as rhizobia. However, the involvement of root nodule symbiosis in Cs transfer remains unclear. This study aimed to reveal how root nodules influence Cs translocation to the shoot. To further clarify the molecular mechanism of Cs transport in nodules, I examined the expression of candidate Cs-related transporters in nodule tissues of the model legume *Lotus japonicus*.

Materials and Methods

Three soybean (*Glycine max* (L.) Merrill) genotypes with different nodulation abilities [a normal nodulating genotype, a hyper-nodulating genotype, and a non-nodulating genotype, all derived from Enrei] were cultivated in a field at Hokkaido University in 2021 and 2024. The field experimental design was identical to that described in Chapter 3. Plant organs (leaf, petiole, stem, pod, seed, and root) and bulk soil (the 0-15 cm soil in the inter-row space) were sampled at the flowering and maturity stages for ionome analysis.

To study the distribution of ^{137}Cs in the nodules and roots, germinated soybean (*Glycine max* cv. Enrei) seeds were cultivated hydroponically and inoculated with rhizobia. After 18 days, ^{137}Cs was added and the plants were allowed to absorb it for 24 h. The root with attached nodules was frozen and cut for ^{137}Cs imaging.

L. japonicus was cultivated in sterilized pots with rhizobial inoculation. Three different Cs treatments were applied. Harvested plants were separated into shoots, roots, and nodules for ionome and transcriptome analysis.

Results and discussion

Through field experiments conducted using soybean genotypes with different nodulation abilities, comparison of elemental concentrations in each organ revealed that Cs was highly accumulated in nodules. Similarly, spatial mapping of ^{137}Cs in the root system revealed that ^{137}Cs was predominantly distributed in nodules. These results highlight the importance of nodules as key sites for Cs accumulation. Moreover, Cs accumulation in nodules was particularly pronounced under low-K conditions. This finding implies that K is preferentially translocated to the shoot rather than Cs, thereby limiting Cs mobility and promoting its accumulation in nodules. In addition, shoot Cs concentration was negatively correlated with nodulation, particularly number of nodules. These results suggest that nodules can accumulate Cs and thereby suppress its translocation to the shoot. Similarly, in *L. japonicus*, root nodule symbiosis suppressed Cs translocation to shoot, and candidate K transporters potentially involved in Cs transport within nodules were identified. These findings provide fundamental insights into the potential use of root nodule symbiosis as a strategy for mitigating Cs transfer to shoots.

Chapter 5 – General discussion

In this study, I aimed to systematically characterize agroecosystem responses to agricultural

practices by integrating multidimensional omics datasets collected from soybean fields across diverse environmental conditions. This thesis indicates the potential of multidimensional omics approaches in agroecosystem research and provides a conceptual and analytical foundation for advancing such approaches. Multidimensional omics analysis enabled the detection of organ-specific elemental distributions, exemplified not only by Cs accumulation in nodules but also by K accumulation in petioles. Manure application was also suggested as a potential strategy to suppress Cs transfer. Because previous studies have shown that manure can promote nodule formation, manure application may enhance the suppressive effect of rhizobial symbiosis on Cs transfer. However, further investigation is required to verify this possibility.

Conclusion

In this doctoral research, I applied an integrative multidimensional omics framework to datasets collected from soybean fields across diverse environmental conditions to elucidate the net ecological effects arising from the simultaneous interactions of multiple factors. Using this framework, I examined two case studies: the robust cross-field impacts of manure application and the effects of multiple factors on Cs transfer.

Manure application consistently improved soil chemical properties across fields. When applied over consecutive years, it also enhanced soil physical structure and bacterial functional differentiation, contributing to synergistic improvements in plant growth. In addition to increasing soil exchangeable K and thereby reducing Cs transfer, manure altered soil divalent cation levels, soil organic carbon, physical structure, and microbial communities. These multivariate responses imply that manure promotes soil environments less conducive to Cs transfer through not only chemical effects but also physical and biological pathways.

Multidimensional ionomic datasets incorporating spatiotemporal dimensions showed that Cs transfer is not governed solely by K availability. Instead, the analysis revealed multi-element interactions involved in Cs transfer and their spatiotemporal variability. Cs uptake was positively associated with Na throughout all growing period, and mineral interaction networks expanded during early growth. Moreover, Cs distribution within plants was closely linked to organ-specific interactions with Rb and Mg.

Although soil microbes were initially implicated only indirectly, Chapter 4 indicated that

nodules accumulate Cs and thereby suppress its translocation to the shoot. Ionomics and transcriptomic analysis further identified candidate K transporters potentially involved in Cs transport within nodules.

These findings indicate that manure application can reliably improve soil conditions across diverse field environments, representing a structural shift in agroecosystems. They also highlight the potential for sustainable Cs mitigation strategies that rely not only on K fertilization but also on broader interactions among soil, plants, and microbes. Furthermore, the analytical framework developed in this study advances integrated multi-omics approaches for understanding complex agroecosystem processes. Future work should include physiological and ecological validation of the hypotheses generated by multidimensional omics analysis.