博士（農学）　程　　郅

学 位 論 文 題 名

Impact of 90-year-application of chemical fertilizer on the community structure and function of arbuscular mycorrhizal fungi

（90年の化学肥料連用がアーバスキュラー菌根菌の群集構造および機能に及ぼす影響）

学位論文内容の要旨

Arbuscular mycorrhizal (AM) fungi are ubiquitous in terrestrial ecosystems and form symbiotic associations with most land plants. The primary benefit of the associations is facilitated uptake of immobile nutrients, especially phosphorus (P), through hyphal networks constructed in the soil. Therefore, AM fungi play a significant role in plant P-nutrition not only in natural ecosystems but also in agroecosystems. A drastic increase in chemical fertilizer application occurred in 1990s in the world, which has greatly improved crop productivity. Massive application of chemical fertilizer, however, has caused negative effects on environments. It has been well documented that increases in soil P level generally reduce AM fungal colonization in the roots, but little is known about how P-fertilizer application affects the community structure and function of AM fungi. In the long-term experimental field in Hokkaido University, the same fertilizer management has been implemented since 1914. The present study investigated the impact of long-term application of chemical fertilizer on the community structure and function of AM fungi by using the resource in the experimental field.

1. Impact on the diversity and community structure

In this chapter, two hypotheses were addressed: firstly, the long-term application of chemical fertilizer has reduced AM fungal diversity and altered the community compositions, and secondly, the effect of single application of P-fertilizer on the communities is the same as, or at least similar to that of the 90-year-application of P. To answer the first hypothesis both greenhouse (trap culture) and field experiments were conducted using the molecular ecological approach. For the second hypothesis, P-enrichment (trap culture) experiment was conducted. Soils were collected from four

plots with different fertilizer management in the long-term experimental field. *Lotus japonicus* was grown on the soils in a greenhouse, while *Glycine max* was grown in the plots in the field. DNA was extracted from their roots, and the diversity and community compositions of the fungi were determined based on the large subunit ribosomal RNA gene sequences. The 90-year-application of nitrogen (N) and potassium (K) in the absence of P consistently increased AM fungal diversity and resulted in formation of a distinctive fungal community compared with those in the other treatments. In addition, this effect could not be cancelled by single application of P to the soil. On the other hand, only a limited impact of balanced application of N, P, and K on the AM fungal communities was observed. These observations suggested that the presence/absence of P-fertilizer acted as a selection pressure against AM fungal communities, but the action may appear only on a long time scale.

2. Impact on the function of the communities and individual isolates with respect to soil phosphorus-fertility

In this chapter, a hypothesis that the long-term application of P-fertilizer resulted in predominance of inefficient AM fungi in terms of plant growth promotion was addressed. To answer the hypothesis two experiments in which the effectiveness of the fungi was assessed both at community- and individual isolate-levels were conducted. Soils were collected from P-fertilized and -unfertilized plots and used as AM fungal inocula to examine the effectiveness at a community level. *G. max* was grown with or without the inocula in the greenhouse and assessed the growth and AM fungal community compositions. Twenty single-spore cultures of the fungi were raised from each of the P-fertilized and -unfertilized soils to examine the effectiveness at an isolate-level. *Tagetes patula* was inoculated with the isolates and grown in the greenhouse. The effectiveness of the AM fungal communities from the P-fertilized and -unifertilized plots was not significantly different, although distinctive difference in the composition was observed between the two inocula. The effectiveness of the individual isolates varied extensively, but no significant difference in the frequency of efficient/inefficient isolates was observed between the P-fertilized and -unfertilized soils from which the isolates were raised. These observations suggest that 90-year-application of P-fertilizer did not necessarily lead to the predominance of inefficient AM fungi.

The present study shed new light on the high-input modern agriculture by demonstrating that the impact of heavy application of chemical fertilizer on the plant symbiotic fungi that play a significant role in crop productivity.

学位論文審査の要旨

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This thesis consists of 21 figures, 3 tables, 61 references, Introduction, two chapters, and General discussion in a total of 85 pages with two accompanying publications.

Arbuscular mycorrhizal (AM) fungi are ubiquitous in terrestrial ecosystems and form symbiotic associations with most land plants. The primary benefit of the associations is facilitated uptake of immobile nutrients, especially phosphorus (P), through hyphal networks constructed in the soil. Therefore, AM fungi play a significant role in plant P-nutrition not only in natural ecosystems but also in agroecosystems. A drastic increase in chemical fertilizer application occurred in 1990s in the world, which has greatly improved crop productivity. Massive application of chemical fertilizer, however, has caused negative effects on environments. It has been well documented that increases in soil P level generally reduce AM fungal colonization in the roots, but little is known about how P-fertilizer application affects the community structure and function of AM fungi. In the long-term experimental field in Hokkaido University, the same fertilizer management has been implemented since 1914. The present study investigated the impact of long-term application of chemical fertilizer on the community structure and function of AM fungi by using the resource in the experimental field.

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