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Year 2023	**Summary of Thesis**

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(Title)

Investigation of sampling methods and quantification of root exudates from *Abies sachalinensis* and *Quercus crispula*

【Introduction】The carbon cycle in forest ecosystems is greatly affected by the net primary production in the aboveground part of trees, as well as the dynamics of soil respiration, root growth, and mortality in the belowground part. Root exudates, such as sugars, increase the activity of rhizosphere microorganisms and promote soil respiration. Especially in boreal forests, the decomposition rate of soil organic matter is slow, and may be affected by the amount of root exudates. The amount of root exudates released is said to be dependent on tree species and root morphology. However, it remains unclear regarding differences in the amount of root exudates among boreal forest species and the effect of tree age on the amount of root exudates.

There are two major methods for collecting root exudates in situ; immersing the target roots in a syringe filled with glass beads and culture solution for at least one day and then collecting the eluted root exudates (syringe method), and clipping the target roots onto a glass fiber filter moistened with ultrapure water for several hours and then collecting the filter that absorbed root exudates (filter method). The amount of root exudates collected may differ between the syringe and filter methods because of differences in the target root sizes and collection conditions, such as installation time.

The objective of this study was to clarify the effects of different sampling methods on root exudates of boreal forest tree species. To achieve this, we 1) compared root exudates between two major boreal forest tree species, i.e., Abies sachalinensis (fir) and Quercus crispula (oak), and between their growth stages, and then 2) compared root exudates among the sampling methods using the species with the certain growth stage selected in 1), in which variability of the exudates among samples was little.

【Methods】In the first experiment (Experiment 1), root exudates were collected from mature (approximately 60 years old) and sapling (8 years old) stage samples of fir, and mature (approximately 65 years old) and sapling (8 years old) stage samples of oak, using the filter method. The target roots were dug out, cleaned, and seven glass fiber filters were placed in the ground. In the second experiment (Experiment 2), the tree roots selected in Experiment 1 were used for the filter method with ultrapure water, the filter method with culture solution, and the syringe method with culture solution. Fifteen samples were collected for each method. Root morphometry in the filters and syringes and carbon (C) and nitrogen (N) contents of the soil, in which the target root

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were growing, were also measured.

【Results and Discussion】In Experiment 1, the mean root exudate per root weight in the filter was not significantly different between tree species or growth stages (Fig. 1; Tukey-Kramer method, $p < 0.05$). Conversely, the variability of root exudates per root weight was smaller in the mature fir samples. The N content of the soil was significantly higher in the mature fir trees than in the other trees. Root exudates from mature fir tended to be higher at the lower soil N content. These results suggest that the site of the mature fir trees was relatively fertile and had high microbial activity, which in turn caused little variability of root exudates among the samples in the mature fir trees.

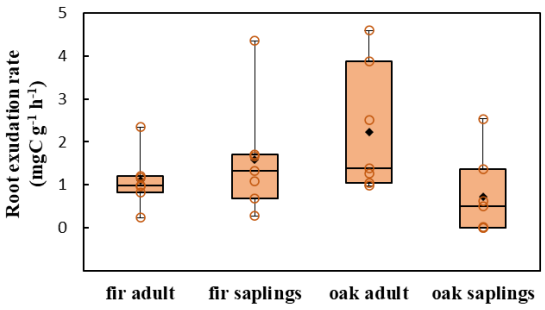

Fig.1 Amount of root exudates [mgC g^{-1} h^{-1}] from each tree.

Based on the results of Experiment 1, mature fir trees were selected as the target trees for Experiment 2. The results showed that root exudate was greater in the filter method than in the syringe method (Fig. 2; $p < 0.05$), and that the variability of the root exudates among the samples was also greater in the syringe method. This is attributable to the fact that the filter method required 2 hours of incubation time, while the syringe method required 24 hours. In other words, the amount of root exudate per unit time varied over the course of a day, but the syringe method was thought to provide an averaged amount of root exudate over a 24-hour period. While the filter method using culture solution resulted in the high variability of root exudate volume among samples, root exudate volume correlated with specific root surface area, tissue density, moisture content [%], and C-to-N ratio. Taken together, the syringe method could be useful to obtain root exudate volume averaged over a 24-hour period. On the other hand, the filter method could grasp the relationship between root exudates and root traits.

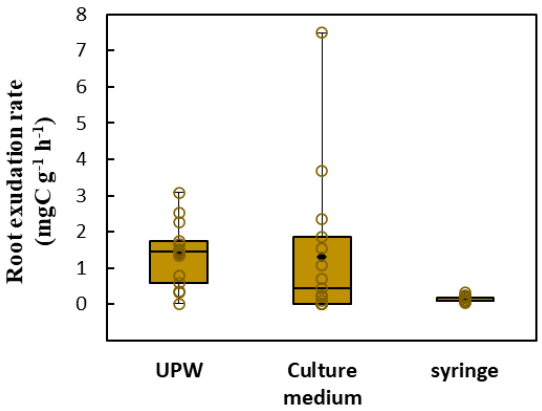

Fig.2 Amount of root exudates [mgC m^{-1} h^{-1}] for each sampling method. UPW represents the filter method using ultrapure water, culture solution represents the filter method using culture solution, and syringe represents the syringe method using culture solution.

【Summary】While the filter method yielded a greater amount of root exudate than the syringe method, it was found that there was a greater variability of the root exudates among the samples. The syringe method could provide root exudates averaged over the long installation time. On the other hand, the filter method, especially the one using culture solution, could provide the amount of root exudate reflecting root traits of each sample.

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