Development of Plant Environmental DNA Analysis Method for Forest Soil

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Environmental DNA analysis is a useful method for identifying species that live in a place. In our laboratory, we are developing technology with the aim of applying this method to intraspecific diversity analysis. As a preliminary step of this study, we examined forest soil samples to confirm whether the plant DNA detected in the soil matches the composition of the surrounding plant species. Soil samples were collected from an artificial forest of *Cryptomeria japonica* and DNA was extracted using a commercially available soil DNA extraction kit. Multiple plant DNA barcode regions were amplified from the extracted DNA, and sequence data were obtained by next-generation DNA sequencing. In addition, plant samples growing in the vicinity of the sampling site were also collected, and sequence data were obtained by the same method after DNA extraction. As a result of analyzing the sequence data detected from the soil sample, the sequences were six species of woody plants including *C. japonica* and seven species of herbaceous plants growing in the vicinity. From these results, it was confirmed that plant environment DNA analysis of forest soil is possible, but it was also found that there is room for improvement in DNA extraction library construction methods. In the next step, we will conduct a similar analysis of forest soils with different vegetation to confirm the validity of the acquired data. And then we plan to develop it into an intraspecific level diversity analysis method after improving the basic technology by referring to the DNA extraction and library construction methods of ancient soil DNA conducted in previous studies.



Photo 1. Soil and plant samples were collected in an artificial forest of *Cryptomeria japonica*



Photo 2. Soil samples were collected from *ca*. 5 cm depth