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Author(s)	SUGAI, Tetsuto; YOKOYAMA, Satoko; TAMAI, Yutaka; MORI, Hirotaka; MARCHI, Enrico; WATANABE, Toshihiro; SATOH, Fuyuki; KOIKE, Takayoshi
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Effects of soil compaction on the seedlings growth and ectomycorrhizal fungal community in hybrid larch

SUGAI Tetsuto[1,2], YOKOYAMA Satoko [3], TAMAI Yutaka [4], MORI Hirotaka [4], MARCHI Enrico[5], WATANABE Toshihiro[2*], SATOH Fuyuki[6], KOIKE Takayoshi[2,3]

[1] Hokkaido Research Center, Forestry and Forest Products Research Institute, Sapporo 062-8516, Japan
[2] Plant Nutrition laboratory, Hokkaido University, Sapporo 060-8689, Japan
[3] Silviculture and Forest Ecological Studies, Hokkaido University, Sapporo 060-8589, Japan
[4] Forest Bioresource Technology laboratory, Hokkaido University, Sapporo 060-8589, Hokkaido, Japan
[5] Department of Agriculture, Food, environment and Forestry, University of Florence, Firenze 50145, Italy
[6] Field Science Center for Northern Biosphere, Hokkaido University, Sapporo 060-8589, Japan

Abstract

Soil physical composition is one of the fundamental factors regulating plant growth as well as soil microbes. For the rising expectation to optimize techniques of forestry machinery for sustainable forest managements in Japan, it is essential to assess the effects of soil compaction caused by machinery running on seedlings of afforestation tree species. Since most afforestation tree species interact with soil microbes, such as the symbiosis with ectomycorrhizal fungi, the effects of soil compaction would extend to the relationship between these microbes and planted seedlings. We evaluated the effects of soil compaction on growth and ectomycorrhizal fungi in hybrid larch seedlings. The experimental site was established with no compacted and compacted plots in the Sapporo experimental forest site of Hokkaido University, where two-year-old seedlings were planted. At compacted plots, the surface hardness was 25 kg cm^{-2} and the bulk density was 1.1 g cm^{-3}. Height growth of seedlings was significantly suppressed, and the dry weight was decreased 50% at compacted plots than that of seedlings grown at uncompacted plots. The dominant group of associated ectomycorrhizal fungi was changed by soil compaction. Our data showed that the effect of soil compaction can suppress growth of seedlings and shape the specific ectomycorrhizal fungal community.

Key words: Soil compaction, ectomycorrhizal fungi, hybrid larch, symbiosis.

Introduction

The physical properties of soil are mainly associated with the size and structure of soil particles. Mechanical force to soils disrupts its structure and anaerobic conditions. These changes in soil physical properties often occur with erosion, freezing, and compaction. Soil compaction is common in agricultural land, urban greening, and the planting area in artificial forests (Cambi *et al*. 2015, Correa *et al*. 2019). Soil compaction can be used for land management and harm the soil ecosystem due to excessive artificial activities. In managed forests, vehicle-based forestry machinery has significantly improved the safety and efficiency of the work. On the other hand, the use of heavy machines can suppress the productivity of the forest ecosystem because of soil compaction. In the case of Japan, where the use of forestry machinery is rapidly widespread even in forest floor (Matsuda et al 2002; Yamazaki and Yoshida 2020), there are high expectations for the development of technologies for optimal machinery utilization taking into consideration the negative impacts of soil compaction (Marchi *et al*. 2018).

While the effects of soil compaction on seedlings of afforestation tree species have been globally investigated, relatively few studies evaluated the relationship between seedlings and soil microbes under compacted soil (e.g., Bauman *et al*. 2013). Considering mycorrhizal fungi are associated with 80-90% of terrestrial plants, the question arises as to whether the symbiotic relationship would change specific for the physical conditions of soil.

This study revealed the effects of soil compaction on growth and community composition of ectomycorrhizal fungi in hybrid larch seedlings (Sugai *et al*. 2020). Hybrid larch F_1 (*Larix gmelinii* var. *japonica* × *L. kaempferi*) is expected to be a productive reforestation species because of its superior initial growth and resistance to red-back vole's damage (Ryu *et al*. 2009; Kita *et al*. 2009; 2018). This species is suitable for this study due to the significant body of knowledge on the symbiotic ectomycorrhizal fungi and their stress responses (e.g., Wang *et al*. 2018). To our knowledge, however, limited studies are available on evaluates the effect of soil compaction on seedling growth and symbiotic relationship with ectomycorrhizal fungi in hybrid larch (Kita *et al.* 2018). To obtain ecological insights for reforestation under forest management using forestry machinery as forest operation practice, this study evaluated the symbiotic relationship between infection of ectomycorrhizal fungi and a seedling planted in compacted soil.

Materials and Methods

We established the experiment at nursery in the Sapporo Experimental Forest of Hokkaido University,

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∗ Corresponding author: nabe@chem.agr.hokudai.ac.jp

Japan, where the soil is classified as brown forest soil (Dystric Cambisols). This experiment started in April 2018. After the tilling of the site, the treatment line was compacted by running heavy machinery, and the control line was made without soil compaction (Figure 1).

Figure 1. (A) The control plots, (B) the treatment plots of soil compaction in the experimental site, and (C) the heavy machinery weighing approximately 2.0 t used for the soil compaction.

Then, each track line was divided into five blocks, where two-year-old seedlings were planted in May, and weeds were carefully removed by hand. The surface soil's physical properties were evaluated with two parameters (Figure 2): (i) soil hardness measured by a soil hardness tester (Yamanaka's Soil Hardness tester, Fujiwara Scientific Co. Ltd, Tokyo) and (ii) soil bulk density measured by a soil extraction cylinder with 5 cm of diameter (Daiki Rika Kogyo Co. Ltd, Saitama). Both physical properties were evaluated four times during May to November in 2018.

Figure 2. (A) A view of the site where the experimental site was set up, and (B) Survey of soil profile at the soil compaction treatment.

Height growth of seedlings was measured from planting to the end of October using a measuring tape with 0.1 cm resolution. Then, seedlings were dug up, dismantled by organ, and oven-dry weights (after 72 hours at 70°C) were measured. When digging out the seedlings, root tips were collected (Figure 3), and evaluated for the association of ectomycorrhizal fungi via the BLAST analysis (ITS region analysis of DNA) based on the protocol described in Wang *et al*. (2018).

Figure 3. (A) A condition of the excavated bare roots associated with ectomycorrhizal fungi, (B) a view of its root within soil, and (C) the microscopic observation of root tips.

Results and Discussion

The average of surface hardness was approximately 25 kg cm^{-2} and the bulk density was 1.1 g cm^{-3} at compacted plots. Compared to the non-compacted plots, the height growth of seedlings was suppressed approximately 25%, and the dry weight was up to 50% lower at the compacted plots (Figure 4).

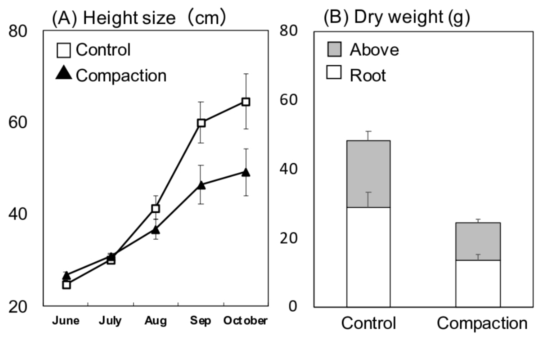

Figure 4. (A) Seasonal changes of height size and (B) dry mass of hybrid larch seedling in October under soil compaction (Mean value ± SE, n=7-8).

Although the soil tillage condition could not occur within forests except for a managed nursery, the negative effects of soil compaction were obvious in this study. Based on the relative abundance of ectomycorrhizal fungi, the dominant group shifted from Ascomycetes (47.9%) at non-compacted plots to Basidiomycetes (55.8%) at compacted plots (Figure 5). *Suillus* sp. showed stable abundance regardless of treatments, which may be attributed to the fungal species-specific for larch as a host (e.g., Wang *et al.* 2015)

Our data showed that soil compaction could suppress the growth of seedlings and shape the specific ectomycorrhizal fungal community. On the other hand, the effect of compaction could also affect vegetation plants (Bockstette *et al.* 2017) as well as a planted seedling although we did not directly evaluate this due to the regular removal of weed plants. For the ecological understanding of the competitive relationship between competitive plant species and the seedling, the interspecific difference in responses to soil compaction needs to be evaluated.

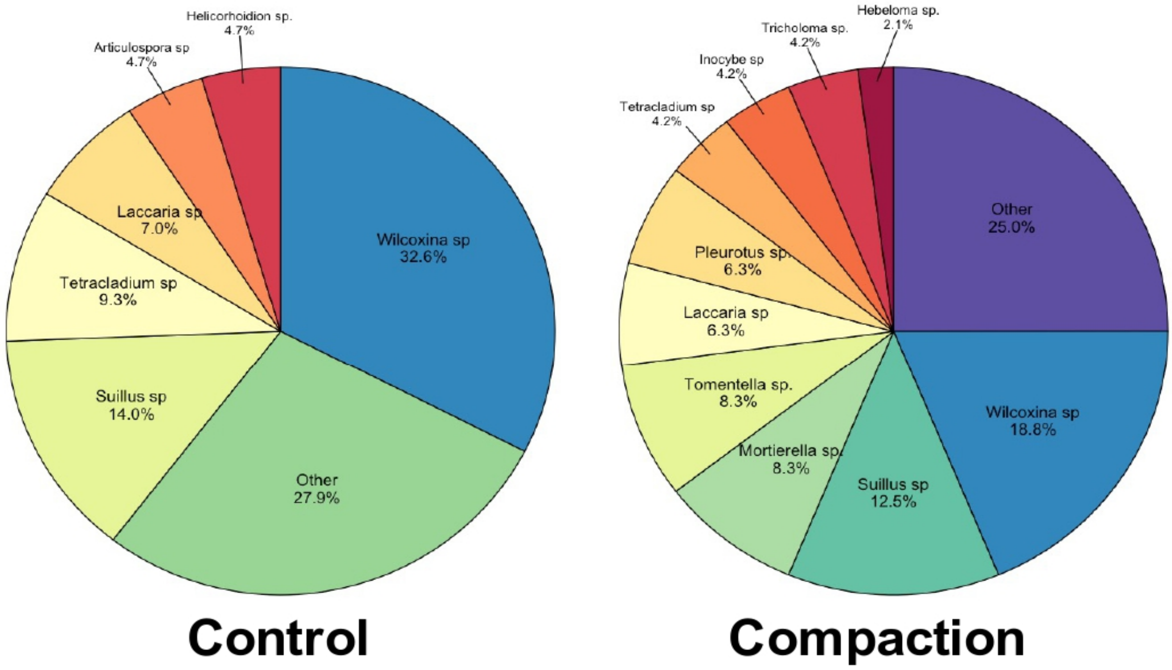

Figure 5. Pie chart of relative abundance for ectomycorrhizal fungi associated with roots of hybrid larch seedlings under soil compaction (n = 5). The classification of the ectomycorrhizal fungi identified in this study was described at the genus level.

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