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Effects of simulated nitrogen deposition on ectomycorrhizae community structure in hybrid larch and its parents grown in volcanic ash soil: The role of phosphorous

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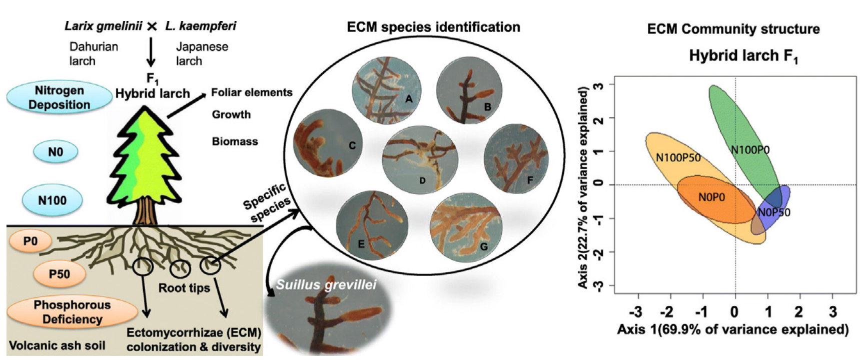
HIGHLIGHTS

- Effects of atmospheric N deposition on ectomycorrhizae remain underexplored.
- We tested single and mixed effects of N and P loading to three larches.
- N and P loadings affected the ectomycorrhizae colonization and diversity.
- Hybrid larch F_1 displayed heterosis over its parents in all the treatments.
- The ectomycorrhizae community structure varies upon host genetic base.

GRAPHICAL ABSTRACT



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ABSTRACT

With the rapid industrial development and modern agricultural practices, increasing nitrogen (N) deposition can cause nutrient imbalance in immature volcanic ash soil commonly found in Japan. Larch species, widely distributed in northeast Eurasia, are associated with ectomycorrhizal (ECM) fungi which play a critical role in nutrient acquisition for their hosts. In this study, we investigated species richness and diversity of ECM fungi associated with a hybrid larch (F_1) and its parents, Dahurian larch (*Larix gmelinii* var. *japonica*) and Japanese larch (*L. kaempferi*), under simulated N deposition (0 and 100 kg ha^{-1} yr^{-1}) with/without phosphorous (P) (0 and 50 kg ha^{-1} yr^{-1}). Seedlings planted in immature volcanic ash with low nutrient availability were subjected to the N and P treatments for fifteen months. We found that response of ECM community structure to the increased nutrient availability depended on host genotypes. Nutrient addition significantly affected ECM structure in Japanese larch, but no such significant effect was found for Dahurian larch. Effects of the nutrient addition to ECM fungal community in F_1 were intermediate. F_1 was tolerant to high N loading, which was due to consistent, relatively high association with *Suillus* sp. and *Hebeloma* sp. F_1 showed heterosis in relative biomass, which was most apparent under high N treatments. This co-variation of ECM fungal community structure and F_1 biomass in response to N loading suggest that ECM community structure might play an important role in host growth. The

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present findings indicate effects of N deposition on ECM fungal community structure can depend on larch species, thus it is challenging to predict general trends.

1. Introduction

Nitrogen (N) deposition has been increasing since pre-industrial era as a result of industrial development and overuse of N fertilizer (Park et al., 2002; Galloway et al., 2008; Morino et al., 2011). Although N is often known as a limiting nutrient in terrestrial ecosystems (LeBauer and Treseder, 2008), chronic deposition of atmospheric N leads to accumulation of N in the soil. Once N accumulates above a specific threshold, it can suppress root growth and induce imbalances in plant nutrients (Qu et al., 2003; Crowley et al., 2012). Furthermore, it may induce phosphorus (P) limitation in forest ecosystems (Gradowski and Thomas, 2006; Du et al., 2016). Long-term N deposition can negatively impact ecological properties and processes (Liu et al., 2011), such as P and N cycling (Du et al., 2016), soil chemistry (Vitousek et al., 1997), biological diversity (Clark and Tilman, 2008), flux of greenhouse gases (Song et al., 2013), and forest declines (e.g. Aber et al., 1989; Smith et al., 2009).

Japan is a part of the Pacific 'Ring of Fire', therefore, forest soil is often derived from volcanic ash (Schmincke, 2004). A broad area in northern Japan is covered by immature volcanic ash, which has relatively low nutrients (Kayama et al., 2015). In volcanic ash soil, N deposition may lead to a nutrient imbalance, causing P deficiency (Frey et al., 2004; Wang et al., 2016). In such nutrient-poor soils, ectomycorrhizal (ECM) fungi establish symbioses with plant roots and play a critical role in nutrient acquisition (Smith and Read, 1997, 2008; Qu et al., 2010; Agerer, 2012). The ECM colonization of seedlings can occur from existing mycelial networks (Jonsson et al., 1999) or from resistant ECM propagules present in the soil, mainly in the form of spores (Buscardo et al., 2010). For example, the ECM propagule availability was limited in volcanic desert of Mount Fuji, where mycelial networks dominated dwarf willow roots (Nara, 2006a), whereas ECM propagules were the dominant vectors for the initial colonization of Douglas-fir seedlings in a harvested forest (Teste et al., 2009). As the major root symbionts in the forest ecosystems, ECM can improve the survival ability of trees by absorbing and providing water and essential plant nutrients from soils (Smith and Read, 1997). In general, under infertile conditions, roots associated with ECM fungi can take up significantly higher amounts of water, P, and N (Aquino and Plassard, 2004; Alves et al., 2010; Wang et al., 2016) compared to roots with no ECM (Buscot et al., 2000; Wallander, 2000; Alves et al., 2010; Qu et al., 2010; Wang et al., 2016).

Soil N availability can affect ECM fungal communities. For example, N availability was a major determinant of ECM fungal communities across north-western Europe (Cox et al., 2010). Root tip abundance and mycelial production of ECM fungi decreased with increasing N deposition (Ostonen et al., 2011; Kjøller et al., 2012). A meta-analysis revealed that mycorrhizal abundance decreased 32% and 15% with P and N addition, respectively (Treseder, 2004). There is also a great deal of studies regarding effects of changing environment, such as warming, CO_2 and O_3, on mycorrhizal communities (e.g. Treseder, 2004; Treseder et al., 2016; Wang et al., 2016). However, we still have limited ecological understanding about how ECM fungal community respond to chronic N deposition in nutrient-poor soils (Veresoglou et al., 2015; Weemstra et al., 2016).

Larches (*Larix* sp.) are ectomycorrhizal deciduous conifers which occur in a vast area of the Eastern Eurasian continent (Qu et al., 2004, 2010; Abaimov, 2010; Wang et al., 2016). Dahurian larch (*Larix gmelinii* (Rupr.) Rupr.) is a representative dominant tree species in the Eurasian area (Abaimov et al., 2000; Kayama et al., 2009; Mao et al., 2010), and has also been intensively planted for afforestation in northeastern China (Zhang et al., 2000; Zhao et al., 2011). Similarly, Japanese larch (*Larix kaempferi* (Lamb.) Carr.), which is a native species to northern Japan, has been planted widely in Japanese forests. However, it suffers from abiotic and biotic stresses, such as cold weather, wild animal grazing and shoot blight disease (Ryu et al., 2009). To overcome these stresses, a new hybrid larch F_1 (hereafter, F_1) has been developed by hybridizing Dahurian and Japanese larch species (Ryu et al., 2009). It has been shown that ECM fungal colonization increased growth of Japanese and F_1 larches by 1.5–2.0 fold in nutrient-poor soil (Qu et al., 2010). However, it is hitherto unknown which ECM species contributes to this observation, and how characteristics of ECM fungal community in F_1 differ from those of its parents. Baxter and Dighton (2005) reported that host trees with a diverse ECM species are regarded as equally efficient in mediating abundant P acquisition as trees with fewer ECM species. On the other hand, growth and photosynthetic function of larch seedlings colonized by multiple ECM species were superior to those of seedlings colonized by only one ECM species (Qu et al., 2004; Choi, 2008). It is therefore important to investigate the ECM diversity under N deposition scenarios and provide new insights that could be applied in management practices.

Despite the importance of larch as plantation species in Eurasia (e.g. Abaimov, 2010), only a few studies have investigated the symbiotic relationship between ECM fungi and larch species regarding N deposition (Qu et al., 2003; Choi et al., 2005; Shinano et al., 2007), and even less have focused on ECM symbiosis in immature volcanic ash soil (Leski and Rudawska, 2012). Wang et al. (2016) found that ECM composition of F_1 was altered when the atmosphere was enriched with ozone and carbon dioxide. Notably, specific ECM species assisted F_1 and compensated the ozone-induced injury. Furthermore, earlier studies have shown that F_1 shows a better growth performance than its parents (Ryu et al., 2009; Agathokleous et al., 2017), however, ECM colonization and its role concerning these three larch species under N deposition still remains underexplored.

In this study, we investigated how F_1 larch and its parents responded to N addition under different P loadings. We specifically attempted to answer the following questions: (1) Are there any effects of N and P application on colonization and species richness of ECM fungi? (2) Do ECM communities respond to N and P differently among the three larches? (3) Does ECM community structure vary with the host larch species? In order to answer these questions we conducted an experiment using F_1 and its parent species planted in immature volcanic ash soil under two levels of N in combination with or without P loading. We expected that response of ECM fungi of F_1 to increased nutrient levels would be similar to that of Dahurian larch due to paternal inheritance of chloroplast and growth characteristics (Szmidt et al., 1987; Abaimov, 2010).

2. Materials and methods

2.1. Plants and soil materials

The experiment was conducted in Sapporo Experimental Forest of Hokkaido University, Japan (N43.07, E141.38, 15 m above sea level.) from 2009 to 2011. The snow-free period lasted from early May to early November. The average temperature and relative humidity at the experimental site were 20.2 °C and 74.3%, respectively during the growing season from May to October.

Three-year-old seedlings of the three larches species were planted in 15-L pots. Japanese and Dahurian larches were provided from a nursery near Bibai, Hokkaido. Hybrid larch F_1 was provided by Forestry Research Institute, Hokkaido Research Organization located in Bibai. All seedlings were kept in low temperature cabinets in a storeroom until planting. The seedlings were examined for initial ECM colonization, diameter

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