Biodiversity of ectomycorrhiza of 3 larch species grown under different phosphorous and nitrogen levels in weathered volcanic ash soil

Graduate School of Agriculture, Hokkaido University
Xiaona WANG
Qiaozhi MAO

Research Center for Eco-Environment Sciences, Chinese Academy Sciences, China
Laiye QU

Research Faculty of Agriculture, Hokkaido University, Japan
Yutaka TAMAI
Makoto WATANABE

Natural Resource Ecology Laboratory, Colorado State University, US
Akihiro Koyama

Research Faculty of Agriculture, Hokkaido University, Japan
Takayoshi KOIKE

Introduction

Larch is one of the promising tree species for forestation and timber production in Hokkaido (*1*) where most soil are originated from volcanic ash (*2*). Therefore, soil is usually suffering from phosphorous (P) deficiency. Recently we develop new hybrid larch F_1 to overcome several environmental difficulties (*3*). It is superior to other coniferous species for symbiotic relationship with mycorrhiza (*3, 4*). Qu et al., (*3*) found ectomycorrhiza (ECM) infection increased growth of larch and its hybrid larch F_1 with 1.5 to 2.0 times under nutrient poor soil in northern Japan. This symbiotic interaction may play an essential role in the success of forest rehabilitation in infertile soil conditions and is often a reason why ECM communities are very diverse in established forests (*5, 6, 7*). Is this relation stable under changing environment, such as high nitrogen (N) loading?

N deposition is increasing in East Asia due to the rapid development of industries and overuse of N fertilizer (*8*). The evidence showed continued deposition from atmospheric N perhaps causes P becoming more limiting nutrient in Hokkaido simultaneously (*9*), therefore considerable focus on soil available P uptake in our forest soil region is imperative for P deficiency (*10*).

Given these effective factors we conducted this experiment addresses the following questions: 1) how about increasing N deposition interacted effect on ECM infection rate with/ without P? 2) Could it change the symbiotic relationship between ECM and host larches under the over loading?

Materials and methods

1. Plant materials and treatments

Single seedling of Dahurian larch (*Larix gmelinii* var. *japonica*) originated from The Kuril Islands, Japanese larch (*L. kaempferi*) native to the central Japan and their hybrid larch F_1 (*L. gmelinii* var. *japonica* × *L. kaempferi*): 3-year-old planting stock was planted in 15 L pot filled with simulated immature volcanic ash soil. The potted plant with matched tray to keep nutrient was placed under full sunlight at Sapporo Experimental Forest of Hokkaido University, Japan (N43°.07’, E141°.38’, 15 m a.s.l.). The potted plants were well watered, and the fertilization was applied by a factorial combination of P and N with the concentration of 0, 50 kg P $ha^{-1}.yr^{-1}$ and 0, 100 kg N $ha^{-1}.yr^{-1}$, respectively. The 4 treatments were as control (P0N0), high P (P50N0), high N (P0N100) and high N×P (P50N100) with each 6 replication. P and N were supplied every two weeks from June 2010 until October 2011, except from October to April.

2. Morphology and Molecular analysis

The ECM types were identified by morphology and molecular analysis. First, harvested roots were washed clean with tap water carefully and then used microscope (Olympus szx-ILLK100, Japan) to observe the infection rate. One hundred root tips of each 6 plant with the PN treatment were selected randomly and counted under the microscope for identifying the ECM type and infection rate. We recorded the number of different types based on their morphology. And then get the infection rate (IR):

$$IRi = \frac{Ni}{100n} \times 100\%$$

Ni: Infected root tip number of 100 tips; i: Infected type of ECM (i.e. tentatively named as A-G); n: replication, n=6.

The different ECM types initially identified by the microscope from morphology were finally identified with molecular methods. First we extracted the ribosomal DNA (rDNA) from the root tips use DNeasyTM Plant Mini Kit (QIAGEN), then identified by polymerase chain reaction with primer 1F/4 RFLP (restriction fragment length polymorphism) analysis of the ITS (Internal Transcribed Spacer)-region of rDNA, finally compared the base sequence with data library(*14*).

王暁娜·毛巧芝（北海道大学大学農学院、札幌 060-8589）・曲來葉（中国科学院·生態·環境研究センター、北京）、
玉井裕·渡辺誠·小池孝良（北海道大学大学院農学研究院、札幌 060-8589）、KOYAMA, Aki(コロラド州立大学)
未成熟火山灰土を模したどじょうで育成したカラマツ属３種の外生菌根の種多様性

3. Statistical analysis

The infection rate data was analyzed on the factor of species and fertilization respectively. Statistical analysis was undertaken using R software, version 2.15.0 (*11*). We ran distance-based redundancy analyses for ECM diversity and got the results of ANOVA of species and fertilization effects on ECM diversity.

Results and Discussion

1. The ECM types and infection rate in three larch species:

Seven types of ECM infected with 3 larch species were identified (Fig. 1). According to taxonomy, all the seven types were Hymenomycetes of Basidiomycotina and divided into two orders: Agaricales and Aphyllophrales. For the family taxa, Agaricales contained three families: Boletaceae (Type A,G), Cortinariaceae (Type B, E), Russulaceae (Type C); Aphyllophrales involved one family Thelephora (Type D,F).

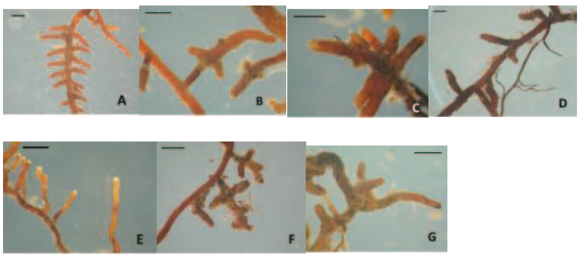

Fig.1 Different types of ECM identified by microscope and molecular skill comprehensively. (black bar=1mm)

Note: A *Suillus laricinus* B *Inocybe* sp. C *Russula* sp. D *Tomentella* sp. E *Hebeloma* sp. F *Thelephora terrestris* G *Suillus grevillei*

The high ECM infection rate for 3 larch species with different PN were in the order of Dahurian larch P50×N0, Japanese larch P0×N100 and hybrid larch F_1 P0×N100. In contrast, the low infection rate were ordered from Dahurian larch P0×N100, Japanese larch P50×N100, hybrid larch F_1 P0×N0, respectively.

Table 1. Dominated ECM types of three larch species in four different fertilizations.

	DL				JL				HL			
	CK	HP	HN	HPN	CK	HP	HN	HPN	CK	HP	HN	HPN
A	1	5	3	6	3	3	1		1	1	2	1
B	2	4		5								
C		1	1	3			2	1	2		2	2
D	3	2	2	2	4	2	3	5		3		5
E		3		4	2			3	3		1	3
F	4		4	1	1	1		2			4	
G							4		4	2		4

Note: DL: Dahurian larch, JL: Japanese larch, HL: Hybrid larch F_1, A~G show types of ECM, CK=Control, HP=P50N0, HN=P0N100, PN=P50N100; 1-7 means the ECM types infection rate by descending order. The 2 in HN of HL means type A and C showed same infection rate.

Different ECM types showed difference symbiosis in N and P adaptability between 3 larch species: 5 ECM types appeared in three tree species at same time (Type A, C, D, E, and F). Type B was found that only infected with Dahurian larch, and type G was found both in Japanese larch and hybrid larch F_1, but not the Dahurian larch. Seven ECM types infected with 3 larch trees were classified by taxa, it indicates the fundamental biological classification, all the infected ECM belonged to Basidiomycotina and a majority of them belonged to Agaricales. Type A and G (=*Suillus* sp.) were the most common fungi species infected with larch especially *Suillus grevillei* was highly ecologically specific for *Larix* spp. (*12*) (Table.1).

2. Biodiversity of ECM of 3 larch species with 4 fertilizations

We could get the initial basic information that Japanese larch showed significant difference of ECM diversity but there was no significant difference between Dahurian larch and hybrid larch F_1 among the 4-fertilization-effect. Based on distance-based redundancy analyses, we could extract 50.5 % information of variance explained of Japanese larch showed significant difference (P=0.015). Fertilization as the factor we found the parent Dahurian larch showed no significant difference on fertilization loading (p=0.560). The hybrid larch F_1 represented some traits between their parents, 4 fertilization effects were not significant (P=0.420).

Under the 4 fertilization loadings, Japanese larch showed highest species richness of ECM than Dahurian larch and hybrid larch F_1, probably because Japanese larch possess higher growth rate compared with Dahurian larch or its hybrid larch F_1 (*7, 13*). Mycorrhiza infection usually increases plant growth among many species (*3*). Abundant nutrient were intensively needed absorbing from the soil for the seedlings to support the aboveground growth, therefore Japanese larch presented intensive symbiotic relationship with ECM, especially *Suillus* spp. (*15, 16*).

3. Biodiversity of ECM of 4 fertilizations among 3 larches

In order to understand the ECM symbiosis difference and diversity among 3 larches, we analyzed the infection rate data using tree species as the factor. The results of 4 fertilization levels among 3 larches showed different significance. For the control (P0N0) and high P treatment (P50N0), ECM infection rates showed significant among 3 species respectively. In control treatment (P0N0), ECM diversity between Dahurian and Japanese larch was significant while in high P (P50N0) the significant difference appeared between Dahurian and hybrid larch F_1, however, infection rate showed no significance of ECM diversity during 3 larches under high N (P0N100) and high P×N (P50N100).

From the control to high N (P0N100) showed the same results of high P50N0 to high P×N (P50N100) treatment which species richness of ECM among 3 larches were disappeared. It indicated that low N condition could remain the ECM diversity because in infertile soil (P0N0) the seedlings demand considerable N and P at the beginning of the growth period because the photosynthates were small in our case (17). Therefore these ECM could make easily symbiosis with host plants. When the soil nutrient leveled up to P50N100, the high P×N condition was a super saturated nutrient habitat, ECM assisting nutrient absorption was not necessary requirement because the plant could absorb nutrient through the own root.

4. ECM types affected by N and P

In general, 3 ECM species (type A, B, G) appeared in low N conditions, replaced by type C, E, F under high N level. We considered that type C, E, F may be the dominated symbiotic ECM types under high N deposition in the volcanic ash soil. As type B only infected with Dahurian larch, this type B maybe more closely related to establish symbiotic relationship with Dahurian than other two.

Conclusion

The complex specific biodiversity in ECM and symbiosis was found in volcanic ash condition. ECM infection and biodiversity were altered by different PN treatment. Different ECM types dominated in different nutrient condition, the biodiversity at species level decreased with increasing the N loading, N may be a subordinate nutrient for maintaining ECM diversity as compared with P. Three larch species were performed different properties. Dahurian larch was less sensitive to what than Japanese larch and hybrid larch F_1 with different P and N level, the ECM symbiosis performed similarity between Japanese larch and hybrid F_1. Hybrid larch F_1 showed more sensitive with P level presented high adaptability under high N but P deficient condition.

Acknowledgments

We thank Miss Rina Yamakawa for her kind support in the experiments. This study was partly supported by Japanese Society of Promotion of Science (Innovation research: 211140 and Type B 23380078) to T.K.

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