Observation of species richness of Ectomycorrhiza in hybrid larch F_1 under elevated CO_2 and O_3

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Introduction

The larch (*Larix* species) is a dominant afforestation tree in the northeastern part of Eurasia (*1*, *2*). It is superior to other coniferous species in its symbiotic relationship with mycorrhizae (*2*, *3*, *4*). We have recently developed a new hybrid larch F_1 (*Larix gmelinii* var. *japonica* × *L. kaempferi*, hereafter F_1) to overcome various environmental difficulties (*5*). Qu *et al.* (*2*, *4)* found that ectomycorrhiza (ECM) infection increases the growth of F_1 by 1.5-2.0 times compared to the non ECM-infected ones.

Concentrations of atmospheric CO_2 and ground-surface Ozone (O_3) are sharply increasing (*6*). Some studies found that elevated CO_2 increase the ECM mass and mycorrhizal infection, colonization and the amount of extramatrical hyphae (*7*, *8*). In contrary, elevated CO_2 did not enhance carbon allocation to root growth or mycorrhiza formation, or even a decreased trend in the mycorrhiza formation (*9*).

The role of ground-surface O_3 in altering plant growth and development has been the subject of thousands of publications over the last several decades. Still, there is limited understanding regarding the possible effects of O_3 on belowground processes. These negative effects on above ground link to belowground response, such as reduced the specific rate of inorganic N-uptake by roots (*10*), decreased standing fine root mass and fungi sporocarp production (*11*, *12)*.

Comprehensively, it is hard to predict how particular ECM community attributes will respond to CO_2 enrichment and O_3 fumigation. The reports about species and composition of ECM with larch affected by CO_2 and O_3 are limited and still are not solved clearly. The goal of this study is to investigate the type of ECM and changes of ECM composition in response to elevated CO_2 and O_3 in F_1.

Materials and methods

1. Plant materials and treatments

We set the Open Top Chamber (OTC) system in experimental forest site of Hokkaido University, carried out four treatments: control (ambient free air as no gas treatment), elevated CO_2, O_3 and the combination CO_2+O_3. The fumigated concentration of CO_2 and O_3 were 600 μmol mol^{-1} and daytime 60 nmol mol^{-1}, respectively. Totally 16 chambers (volume=1.2×1.2×1.2m) were constructed with four replications, as well as four replications of larch seedlings were planted in each chamber (see framework in Fig. 1).

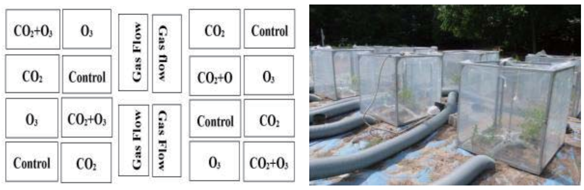

Fig.1 The layout of open top chamber system in experimental site (air into chambers were filtered to avoid O_3 then added O_3 and CO_2)

Two-year-old seedlings of hybrid larch F_1 were planted in brown forest soil in May 2011. Fertilization and water were supplied at the beginning to keep based nutrition and appropriate soil moisture content.

2. Morphology and Molecular analysis

The whole root of seedlings were dug out in October 2012 after two growing seasons and put into big plastic with soil sticking in it, then took back in the laboratory immediately, stored at 4 °C in the refrigerator for further analysis (Fig. 2). All root samples were carefully washed with tap water until no soil particle stick on the fine root, then ECM colonization rate were checked by microscope. 500 root tips were counted for each replication and calculated the colonization rate (CR) as follow formula:

$$CRi = \frac{Ni}{500n} \times 100\%$$

Ni: the number of infected root tip of 500 tips; *i*: an ECM type label; n: replication, n=4.

The different ECM types initially identified by the microscope from morphology were finally identified with molecular methods. First we extracted the ribosomal DNA

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高 CO_2，O_3 条件下のグイマツ F_1 における外生菌根の種の豊富さと観察

(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 (*13*).

3. Statistical analysis

All the result was calculated by software of SPSS.

Results and Discussion

1. The ECM observation

Six types of ECM were identified from hybrid larch species after morphology and molecular analysis (Fig. 2 & Table 1).

Fig.2 Field investigation and microscope observation of ECM colonized with fine roots (bar in right photo is 5 mm).

According to the mycorrhiza taxon, all the six ECM types belong to the class of Basidiomycetes (Type A, C, D, F) and Ascomycetes (Type B, E) (see table 1). Type C and D are specialist species for larch trees and other types are generalist. (*14*)

Table 1 Taxonomic rank of all ECM species (adopted from Wang *et al*., *19*)

ID	ECM Type	Taxon
A	*Tomentella* sp.	Basidiomycetes (generalist)
B	*Peziza* sp.	Ascomycetes (generalist)
C	*Suillus laricinus*	Basidiomycetes (specialist)
D	*Suillus grevillei*	Basidiomycetes (specialist)
E	*Cadophora finlandica*	Ascomycetes (generalist)
F	*Laccaria cf.laccata*	Basidiomycetes (generalist)

2. Colonization rate of ECM

The total colonization rate of ECM was influenced by high CO_2 and O_3. ECM colonization rate increased by elevated CO_2 comparing with control site rising from 59% to 70% ($P \leq 0.05$) significantly, however reduced by high O_3 level sharply down to 29% ($P \leq 0.01$). In the CO_2+O_3 mixed fumigation, the ECM colonization rate was slightly enhanced to 37% comparing with O_3 treatment.

Elevated CO_2 usually increase the mycorrhizal infection level because larger amount of photosynthates will be allocated to belowground stimulating the symbiosis with ECM. The previous studies also support this point (*11*, *15*). Elevated CO_2 showed the highest ECM colonization rate comparing with other treatment. With O_3 fumigation, the colonization rate of ECM also increased (*11*, *16*), however, some genotypes of ECM fungi were reduced or no significant effect with O_3 exposure (*17*, *18*).

The reason for this probably because O_3 led to the stomata closure, and reduce photo-assimilation (*20*). Another possibility is the whole plant biomass was reduced, and moreover according to T/R ratio relatively fewer biomass were allocated to belowground for root, in hence there is not sufficient nutrient to sustain root growth and lower the potential of ECM symbiosis with root system (*11*, *15*).

3. ECM composition in different fumigations

The six types of colonized ECM took different composition among four treatments. Overall the composition types A, C, D and F showed the majority colonizing type of ECM with F_1. Under elevated CO_2, the ECM composition was similar with control treatment. Whereas at high O_3 and mixed fumigation, the ECM composition was significantly altered from control and elevated CO_2 ($P \leq 0.01$), as well between high O_3 and mixed condition, ECM composition presented slightly difference.

From the control to elevated CO_2, the infected species of ECM was same only the amount of species proportion was changed, type D was increased from 26% to 35% and type C decreased by 11%. Under O_3 exposure, type B could not colonized with F_1, type D was significantly increased to 46% and type A was sharply decreased to 2% comparing with control. In the mixed fumigation, type C increased to 60% performed the dominated species as control treatment.

This proved that larch specialists (*Suillus* sp.) were dominant ECM even at high O_3, *Tomentella* sp. and *Laccaria cf. laccata* were co-dominant ECM which increase hosts' activities with enough CO_2 and vice versa. Meanwhile it predicted *Suillus* sp. has high efficiency of the symbionts than other generalist species.

Conclusion

Elevated O_3 plays negative effect on the growth and ECM symbiosis of F_1. Even though elevated CO_2 compensates the harmful impact of O_3 via enhancing the biomass and ECM symbiosis, the functional diversity might be changed depending on the composition of ECM. As it is essential for afforestation in future, especially in Hokkaido, we should focus more on *Suillus* sp. and Type A, C, D and F during the period of seedlings in field or nursery

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