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**Study on the effects of elevated CO₂, O₃ and high nitrogen loading on the
rhizosphere dynamics of deciduous trees**

(落葉樹の根圏動態に対する高CO₂とO₃及び高窒素負荷の影響に関する研究)

Recently, rapid economic growth, industrialization and urbanization have caused a series of environmental pollutions mainly due to tremendous energy consumption. Subsequent increase in atmospheric carbon dioxide (CO₂) concentration, nitrogen oxide deposition and tropospheric ozone (O₃) are considered to incite environmental change, threatening forest ecosystems. In northeast Eurasia and Asia, birch and larch represent essential components as well as being promising species for afforestation. Since northern Japan is mostly covered with volcanic ash and pumice soil, ectomycorrhizal (ECM) symbiosis is fundamental to sustain the growth of these trees. This symbiotic relation directly affects rhizosphere activities, such as fine root development. In this study, I tried to elucidate the response of fine root dynamics, species richness of ECM fungi under elevated CO₂ and O₃ as well as high nitrogen loading, aiming to obtain basic information for future afforestation with birch and larch species under changing environments.

At elevated CO₂ concentration, plants usually enhance the growth of aboveground parts and allocate more photosynthates to belowground. This allocation increases the respiration of both coarse and fine roots. Fine-root dynamics play an important role in carbon (C) cycling of belowground and influence C sequestration to the soil. In chapter 2, to investigate the effect of elevated CO₂ on fine-root dynamic of Japanese white birch (*Betula platyphylla* var. *japonica*), I monitored their dynamics using the Free Air CO₂ Enrichment (FACE) facility of Hokkaido University for three years (2011-2013). Elevated CO₂ was maintained at 500 μmol/mol which simulating the situation around 2040 according to IPCC prediction, and currently the ambient air contains 380-395 μmol/mol of CO₂. Mini-rhizotron (MR) instrument was used: all MR tubes were set up with planting seedlings in 2010. The image scanning in the field started in 2011, one year later, to avoid the gap between tube and soil. Except for the CO₂ treatment, I also applied two soil types, i.e., brown forest (BF) soil and volcanic ash (VA) soil, which is widely distributed in northern Japan. Live fine-root length (LRL), fine-root production (FRP), mortality (FRM) and root lifespan were analyzed after tracing images by the Win-Rhizotron software. LRL was estimated as the total length in each area unit. FRP (FRM) was calculated according to the annual length-based method. It equals to the annual length-based root production (mortality) to live root length. Fine root lifespan was determined by fine root longevity using the Kaplan-Meier survival function. LRL increased under elevated CO₂ in the first year but showed no significant increase thereafter in BF soil. However, in VA soil, it decreased with CO₂ enrichment for all three growing seasons. Independent of treatments and soils, turnover of FRP and FRM ranged from 0.25 to 1.69 yr⁻¹. The turnover of both FRP and FRM was relatively lower under elevated CO₂ in the first two years, and increased from the third growing season by elevated CO₂ in both soils. Elevated CO₂ increased fine-root lifespan in BF soil during the first year and in VA soil during the three years. But the response of root longevity to elevated CO₂ differed according to root diameter classes.

CO₂ is the basic C source for photosynthesis related to plant growth, while O₃ is a phytotoxic air pollutant of major concern for forest decline. In general, elevated CO₂ decreases stomatal conductance,

which may reduce harmful effect of O₃ via stomatal function. How does this combination of CO₂ and O₃ effect growth of the underground parts of larch? For tree growth and fine root dynamics, ECM symbiosis is a vital issue. In chapter 3, to clarify the combined effects of elevated CO₂ and O₃ on tree growth and ECM symbiosis, I used the Open Top Chamber (OTC) system to estimate the response of hybrid larch (F₁) (*Larix. gmelinii* var. *japonica* × *L. kaempferi*) for two years (2011 and 2012). Treatments consisted of i) charcoal-filtered ambient CO₂ (almost no O₃, 385 μmol/mol), ii) 60 nmol/mol O₃, iii) high CO₂ (600 μmol/mol), and iv) their combination. Elevated CO₂ increased the ECM colonization rate but not the diversity of ECM types. Higher net photosynthetic rates at the growth CO₂ level increased the biomass of underground parts and stems, which, in turn, increased the ECM colonization rate. Elevated O₃ negatively affected the ECM colonization rate and more strongly, species abundance. The growth of F₁ was restricted, and the biomass was reduced by O₃. However, specific ECM species, such as *Suillus grevillei* was selected as the one of the capable species that flourishes under enhanced O₃. ECM efficiently absorbed Phosphorous (P) and other elements.

Moreover, the ECM symbioses with host plants greatly depend on C gain and allocation. Although N is an essential element for plant growth, the recent increase of N deposition surely brings an imbalance and is another critical factor of changing environment. N deposition usually increases tree growth, as it is an essential nutrient. How does N deposition affect the ECM symbiosis with host plants, especially in relation to another important nutrient, such as P? In chapter 4, to estimate the ECM symbiosis under different levels of N deposition with P efficiency, I planted the seedlings of three larch species in pots and placed them outdoors in open air, i.e., Japanese larch (JL: *Larix. kaempferi*), Dahurian larch (DL: *L. gmelinii* var. *japonica*) and F₁. Four nutrient levels were applied, using two levels of N (0 and 100 kg ha⁻¹yr⁻¹) and two levels of P (0 and 50 kg ha⁻¹yr⁻¹). After two years of nutrient application, seven types of ECM were identified to colonize the three larch species. The ECM colonization rate was reduced by 19.8 % for DL, and increased by 39.4 % for JL, 63.7 % for F₁ in high N condition, respectively. P application positively affected the ECM colonization for the three larches. ECM diversity was not significantly affected by N or P treatment except DL. ECM community structure of JL significantly differed among the nutrient regimes, but this was not the case with DL or F₁. Increasing N load obviously reduced P concentration in needles of the parents, but F₁ was not affected.

In summary, elevated CO₂ did not accelerate root turnover in infertile soil condition, especially at the beginning of CO₂ enrichment. Root dynamics of birch seedlings indicated great activity in the third year, most possibly due to mycorrhizal symbiosis. Under elevated CO₂ and O₃, I selected F₁ as the best ECM-colonized species, and found the ECM symbiosis extremely assisting seedling growth under external stress. Uptake of essential elements such as P via ECM symbiosis remained the same or accelerated under elevated O₃ in F₁. ECM community structure greatly changed, and ECM species belonging to genus *Suillus* predominated. Comparing the ECM symbiosis of F₁ seedlings with its parents in terms of N and P treatment, F₁ was considered to retain ECM diversity even when exposed to changes in the levels of N and P. In particular, under high N loading, P in the needle was reduced for DL and JL, but not affecting F₁. This might be attributed to the specific ECM symbiosis between *S. grevillei* and F₁, where the symbiotic relationship remained before and after the nutrient treatments.

From the view of larch afforestation, coping with P deficiency and/or infertile soil conditions is important for seedlings under changing environment, such as elevated CO₂, O₃ and N loading. The birch and F₁ are promising species and is a good candidate for reforestation under such conditions. However, it requires the vital partner, i.e., ECM, especially at the seedling stage. In conclusion, for survival under changing environment in future, it is necessary to develop the ECM inoculation method for seedlings.