Effects of Ammonium Sulfate and/or Ozone on the growth and photosynthesis of Japanese larch and hybrid larch F_1

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Introduction

A huge amount of air pollutants emitted with industrial developing is one of the most serious problems in Asia. The amount of nitrogen (N) deposition has increased in northern hemisphere because of rapid development of industry as well as agriculture. In northeast Asia, ammonium sulfate $(NH_4)_2SO_4$ is also a composer of N deposition. Although the amount of gaseous sulfate emission has decreased with use of the desulfurization, N deposition as a form of ammonium sulfate is monitored in Japan because of transboundary air pollution emitted by windward countries. N deposited to soil is considered to become an inorganic nutrient to some extent. If the amount of N deposition will continuously increase, pH of some kinds of soil would decrease. As a result, several inorganic nutrients become imbalance and ions of heavy metals are leaching (Ca^{2+}, Mg^{2+}, etc.). Finally, tree growth is inhibited by soil acidification caused by N deposition. Recently, ground-level Ozone (O_3) has again been increasing in Japan. Relatively higher O_3 concentration has been monitored in Japan although the concentration of precursor substances has decreased. One of the main reasons why O_3 has again been increasing is attributed to transboundary pollution in northeastern part of Asia. O_3 absorbed via stomata has oxidation stress on chloroplasts and also decreases dry mass production and changes of photosynthate allocation.

As edaphic condition in northern Japan is composed of immature volcanic ash, vigor and health of forests are declining because of soil acidification. In Hokkaido, Japanese larch (*Larix kaempferi*) is mainly planted and is ready for harvested. On the other hand, the hybrid larch F_1 is expected to a strong candidate of afforestation species. This new species is produced crossing between Dahurian x Japanese larch (*Larix gmelinii* var. *japonica* × *Larix kaempferi*) to improve weak-points of Japanese larch. F1 has high tolerance against vole feeding damage and high initial growth rate. Therefore, it is important to evaluate the physiological and growth responses to atmospheric environment change for future reforestation. However, most of advanced researches had been evaluating of the effect of ammonium nitrate (NH_4NO_3) on forest trees because of main source of N deposition. According to Watanabe et al. (2006), application of ammonium nitrate to soil decreases the O_3 sensitivity of Japanese larch. However in this research, soil pH condition did not decrease because ammonium nitrate is easily leached from soil. As denitrification characteristics of this ammonium nitrate, N deposition may not be simulated, especially soil acidification. Therefore, it is necessary to re-consider that effects of N deposition on larch seedlings in combination with elevated O_3. In this research, we hypothesized that application of ammonium sulfate to larch seedlings may increase their O_3 sensitivity. For accessing this

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hypothesis, we use open top cambers for evaluating O_3 sensitivity of Japanese larch and hybrid larch planted in immature volcanic ash with/without ammonium sulfate.

Materials and Methods

We used 2-year-old seedlings of Japanese larch (*Larix kaempferi*) and hybrid larch F_1 (hereafter F_1, *Larix gmelinii* var. *japonica* × *Larix kaempferi*). These seedlings were planted in 7 liter pots filled with 1:1 (v/v) mixture of Kanuma pumice soil and Akadama volcanic ash soil. We ran an open-top chamber (OTC) experiment from June to September with O_3 (60 ppb: during daytime). The whole-plot treatment was comprised with 2 levels of O_3 and 2 levels of nitrogen addition (Control, O_3, N, O_3+N) with 4 replications, resulting in a total of 16 OTCs. In each OTC, 4 seedlings of each tree species were assigned, resulting in a total of 128 seedlings.

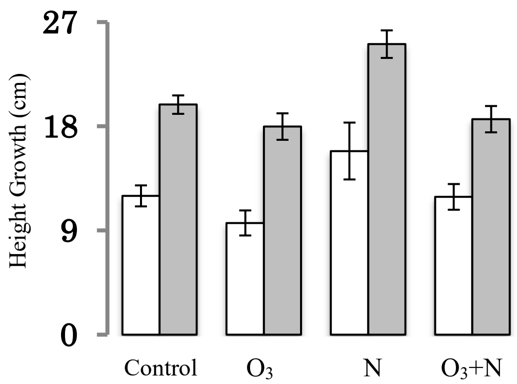
We set the O_3 concentration to 60 ppb in O_3 and O_3+N treatment and 30 ppb in control and N treatment. The target O_3 concentration was the environmental standard of photochemical oxidants in Japan. From June to September in 2015, we added 500 ml of $(NH_4)_2SO_4$ solution to the surface of each pot soil at approximately 2 weeks interval. The total amount of N added to the potted soil was 50 kg $ha^{-1}year^{-1}$. Soil of pots of control and O_3 was supplied with tap water.

The height and diameter of seedlings were measured at beginning and end of the experiment to determine growth. At mid-October, lower part of each seedling was wrapped with 1.5 mm mesh for collecting needle litter. We used this litter for the measurements of leaf nitrogen contents. In early September, the gas exchange rates of mature needle of 4 samples were measured using an open gas exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA). We determined the net photosynthetic rate (A_{growth}) for the vapor at the chosen values of CO_2, i.e., 380ppm for the ambient treatment. Net photosynthetic rate at 1700µmol mol^{-1} (A_{max}, indicating maximum rate of ribulise-1,5-bisphosphate regeneration) was determined by the intercellular CO_2 concentration-response (A/Ci) curve using Farquhar et al. (1980) model. The leaf temperature was maintained at 25°C and the relative humidity was maintained approximately 70 %. After measurements, the leaf area was measured using a scanner (Canon, LIDE-80) and image analysis system (USA, Image J). All collected needles were dried at 70 °C. Then their nitrogen concentration was measured by a NC analyzer (Elemntar, VarioEL III). From the difference in N contents of needles of early September and of leaf litter, we estimated the re-translocation rate of nitrogen in needles (hereafter RN).

Statistical analyses were performed using R software, version 3.2.2. Two-way analysis of variance (ANOVA) was used to test the effects of O_3 and nitrogen addition. In each analysis, a chamber was tested within each treatment and added as a random effect to the model. When a significant interaction of O_3 and nitrogen addition was detected, Tukey's HSD test was performed to identity significant differences among the 4 treatments.

Results

In N treatment, the diameter growth of both species increased and the height growth and leaf mass of F_1 increased. The height growth of both species decreased in O_3 treatment but the diameter growth did not change significant (Fig.1). Although a significant interaction of nitrogen and O_3 was detected in height growth of F_1, there was no significant difference in control, O_3 and O_3+N treatments except N treatment. A_{max} of F_1 decreased with O_3 treatment. A_{growth} and A_{max} of Japanese larch increased with N treatment (Table 1). However, there was no significant change in A_{max} and A_{growth} of both species with O_3+N treatment. Nitrogen content per leaf area (N_{area}) and per mass (N_{mass}) of Japanese larch decreased with O_3 treatment. N_{area} and N_{mass} of Japanese larch increased in N treatment. Additionally, there were significant interactions of O_3 and N application to N_{mass} and RN of Japanese larch.



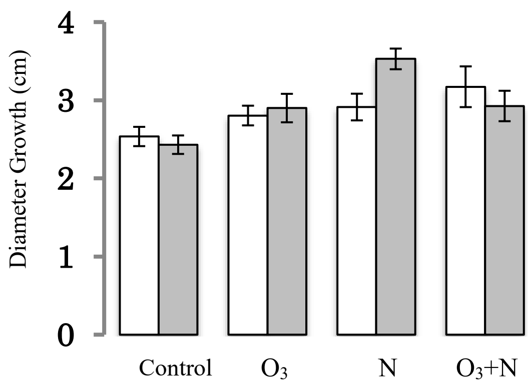


Figure 1. Effects of O_3 and $(NH_4)_2SO_4$ on the height and diameter growth of Japanese larch and F_1. White bar: Japanese larch, Gray bar: F_1. All values are average±SE (n=4).

Table 1. Effects of O_3 and $(NH_4)_2SO_4$ on the photosynthesis and leaf nitrogen content of Japanese larch and F_1. All values are average (n=4). A_{max}, A_{growth} (µmol m^{-2} s^{-1}), N_{area} (mgN mm^{-2}), N_{mass} (100mgN mg^{-1}), RN (%)

	Japanese larch					Hybrid larch F_1				
	A_{max}	A_{growth}	N_{area}	N_{mass}	RN	A_{max}	A_{growth}	N_{area}	N_{mass}	RN
Control	19.05	4.71	0.83	1.18	52.9	26.05	5.71	1.13	1.28	53.9
O_3	19.24	4.45	0.55	0.65	26.1	20.85	5.58	1.06	1.08	46.7
N	33.77	11.16	1.27	1.37	36.1	32.32	10.61	1.15	1.31	51.9
O_3+N	25.75	6.68	0.94	1.18	42.4	21.67	6.36	1.06	1.26	50.7

Conclusion

In one growing season, effect of $(NH_4)_2SO_4$ application did not change significantly the O_3 sensitivity of photosynthesis at both species. However, there was significant interaction of O_3 and N application on height growth and needle nitrogen condition. So, it is necessary to evaluate not only photosynthesis parameter, for example, A_{max} and A_{growth}, but also nitrogen use efficiency to growth and the pattern change of photosynthate and several nutrient allocations in individual seedlings treated with different conditions.

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