Chapter 6
Combined Effects of Ozone and Other Environmental Factors on Japanese Trees

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Abstract Plant responses to ozone (O_3) are highly dependent on other environmental factors. Elevated atmospheric CO_2 concentrations may reduce stomatal O_3 uptake in some tree species in certain environmental conditions. In addition, changes in the carbon availability and allocation pattern within a plant body under elevated CO_2 may confer compensative capacity against O_3 stress. The influence of the soil nitrogen load on growth and photosynthetic response to O_3 is highly species-specific. The soil nitrogen load enhanced the negative impact of O_3 on *Fagus crenata*, whereas lower O_3-induced growth reduction of *Larix kaempferi* was observed under a higher nitrogen load. It is predicted that soil-water stress would induce lower stomatal O_3 uptake due to stomatal closure, and therefore the negative impacts of O_3 on trees would decrease under water-stress conditions. In fact, some antagonistic effects of O_3 and soil water stress were observed in *F. crenata*, although these effects may depend on the severity of the water stress. The number of studies on the combined effects of O_3 and other environmental factors on Japanese forest tree species is limited and further research is crucial to accumulate the required datasets.

Keywords Ozone • Japanese forest tree species • Elevated CO_2 • Soil nitrogen load • Soil water stress

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6.1 Introduction

Forest ecosystems are suffering not only from a single stress factor but also from multiple stresses simultaneously. Since the industrial revolution, human activity has drastically changed our atmospheric environment. In particular, elevated atmospheric CO_2 concentrations, increasing nitrogen depositions in forests, and changes in water availability as a result of climate change are critical factors that affect forest health (Paoletti et al. 2010). Plant responses to O_3 are highly dependent on other environmental factors. The effects of O_3 on the eco-physiological traits of forest trees might be modified by these other factors. Here, we introduce experimental studies that deal with the combined effects of O_3 and elevated atmospheric CO_2, soil nitrogen load, and soil water stress on Japanese forest tree species.

6.2 Combined Effects of O_3 and Elevated Atmospheric CO_2

Atmospheric CO_2 levels have increased dramatically since the industrial revolution and have now reached 400 μmol mol^{-1} (Monastersky 2013). This increase is predicted to continue throughout this century (Stocker et al. 2013). In contrast to the effects of O_3, elevated CO_2 may enhance tree growth, if other factors such as nutrient and water availability do not constrain growth (e.g. Eguchi et al. 2004; Norby et al. 2010; Kitao et al. 2005). Whether recent increasing CO_2 concentrations ameliorate the negative impact of O_3 is a subject of continuous debate (Matyssek and Sandermann 2003). Generally, a higher atmospheric CO_2 concentration induces stomatal closure and therefore decreases O_3 uptake through stomata (Ainsworth and Rogers 2007). Greater amounts of carbohydrate due to a higher photosynthetic rate under elevated CO_2 may confer better detoxification and repair capacities against O_3 stress (Riikonen et al. 2004). Several studies of the combined effects of O_3 and elevated CO_2 on Japanese forest tree species have been carried out based on these hypotheses, mainly in this century.

Matsumura et al. (2005) conducted a study on the combined effects of elevated O_3 and CO_2 on the growth of three broad-leaved deciduous trees [*Betula platyphylla* var *japonica* (hereafter *B. platyphylla*), *Betula ermanii*, and *Fagus crenata*] and two evergreen conifers (*Pinus densiflora* and *Cryptomeria japonica*). The seedlings were exposed to ambient or elevated O_3 and CO_2 (1.5 times ambient concentrations of both gases) during two growing seasons, in open-top chambers. Significant interaction between elevated O_3 and CO_2 treatments was found for the whole-plant dry mass of *B. platyphylla* at the end of the experiment. While the exposure to O_3 significantly decreased the dry-matter growth of this species under ambient conditions, the negative effect of O_3 was not observed under elevated CO_2. However, there were no significant interactions of elevated O_3 and CO_2 treatments for the other four tree species.

The amelioration of O_3 effects under elevated CO_2 was also observed in the study reported by Koike et al. (2012). They studied the effects of elevated O_3 (60 nmol mol^{-1} for 7 h per day) and CO_2 concentrations (600 μmol mol^{-1} for daylight hours)

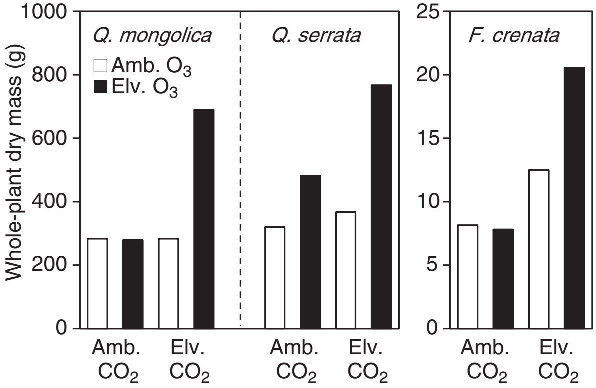

Fig. 6.1 Whole-plant dry mass of *Quercus mongolica* var. *crispula* and *Quercus serrata* and *Fagus crenata* seedlings grown under elevated ozone (O_3) and CO_2 concentrations. See detail for the setup of the experiments in the main text (Data sources: Kitao et al. 2015 for *Q. mongolica* and *Q. serrata* and Watanabe et al. 2010 for *F. crenata*)

on the growth and photosynthesis of the hybrid larch F_1 (*Larix gmelinii* var. *japonica* × *Larix kaempferi*) and its parental (i.e., *L. gmelinii* var. *japonica* and *L. kaempferi*) seedlings, using open-top chambers located at Sapporo, northern Japan. Ozone reduced the height and diameter increments of the main stem, and the needle dry mass of the hybrid larch F_1, while there was almost no effect of O_3 on *L. gmelinii* var. *japonica* and *L. kaempferi*. The decrease in diameter increment of the hybrid larch F_1 was only observed under ambient CO_2 conditions. Under elevated CO_2 conditions, no difference in diameter increments between ambient and elevated O_3 treatments was observed. Reports from another experiment at the same facility in Sapporo indicated no elevated CO_2-induced amelioration of O_3 impact in *B. ermanii*, *B. platyphylla*, *Betula maximowicziana*, and hybrid larch F_1 seedlings (Hoshika et al. 2012; Wang et al. 2015).

In the study of Kitao et al. (2015), two mid-successional oak species, *Quercus mongolica* var. *crispula* (hereafter *Q. mongolica*) and *Quercus serrata* seedlings were grown and fumigated in conditions of elevated O_3 (twice-ambient concentration) in combination with elevated CO_2 (550 μmol mol^{-1}) in an octagonal free-air fumigation system 2 m in height for two growing seasons. The seedlings were directly planted on the ground to avoid any limitation of root growth. Both the oak species showed a significant enhancement of whole-plant dry mass under the combination of elevated O_3 and CO_2 treatments (Fig. 6.1), which was considered to be a result of a preferable biomass partitioning into leaves induced by O_3 (see Sect. 5.2.1.3) and a predominant enhancement of photosynthesis under elevated CO_2. This over-compensative response in the two Japanese oak species resulted in greater plant growth under the combination of elevated O_3 and CO_2 than under elevated CO_2 alone. A similar tendency was observed in *F. crenata* seedlings (Fig. 6.1; Watanabe et al. 2010). In this experiment, 2-year-old seedlings of *F. crenata* were grown in four experimental treatments, comprising two O_3 treatments (charcoal-filtered air and 100 nmol mol^{-1} O_3; 6 h/day, 3 days/week) in combination with two CO_2 treatments (350 and 700 µmol mol^{-1}) for 18 weeks in environmental controlled-growth chambers with an artificial light system. The dry-matter growth of the seedlings was greater under the combination of elevated O_3 and CO_2 than under the elevated CO_2 alone. A marked increase of second-flush leaves was observed under the combination of elevated O_3 and CO_2 concentrations. Watanabe et al. (2010) considered that the greater investment of carbohydrate due to the higher photosynthetic rate in first-flush leaves contributed to the marked increase in second-flush leaf emergence under the combination of elevated O_3 and CO_2.

The results of the above-mentioned studies did not completely support the hypothesis: "elevated CO_2 ameliorates the negative impacts of O_3". Interestingly, the amelioration phenomenon was not consistent even in the same species (e.g. Matsumura et al. 2005 vs Hoshika et al. 2012 for *B. platyphylla*, Koike et al. 2012 vs Wang et al. 2015 for hybrid larch F_1). On the other hand, when the amelioration or compensation of the O_3 impact under elevated CO_2 was observed, an elevated CO_2-induced decrease in stomatal conductance was also found (Matsumura et al. 2005; Watanabe et al. 2010; Kitao et al. 2015), except for the hybrid larch F_1 (Koike et al. 2012), and vice versa. Therefore, modification of stomatal O_3 uptake may be one of the critical functions that determines the amelioration effect produced by elevated CO_2. However, it is hard to explain the over-compensation phenomenon (Watanabe et al. 2010; Kitao et al. 2015) in terms of lower stomatal O_3 uptake. Changes in the availability of carbohydrate within a plant body and changes in the carbon allocation pattern between plant organs under elevated CO_2 may be key functions for understanding the combined effects of O_3 and elevated CO_2.

Although both O_3 and elevated CO_2 may affect secondary metabolism, and therefore may change plant defense capacity against biotic stresses such as pathogens and herbivores (Peltonen et al. 2005), there has been only one report on the combined effects of O_3 and elevated CO_2 on secondary metabolite concentrations in the leaves of Japanese tree species. Karonen et al. (2006) investigated the quantitative response of proanthocyanidins (PAs) to elevated CO_2 and O_3 in the seedlings of *B. platyphylla*, *B. ermanii*, and *F. crenata*. PAs are a major group of phenolic compounds, which are some of the main secondary metabolites in the leaves of many woody plants. The seedlings in the study were the same as those investigated by Matsumura et al. (2005). Total PA concentrations in the leaves of all species examined were similarly affected by the different treatments. PA concentrations were significantly higher in seedlings treated with the combination of O_3 and elevated CO_2 as compared with the other treatments. Significant species × treatment interaction was observed in results for polymeric PA concentrations in *B. platyphylla* and *B. ermanii*. In *B. platyphylla*, leaves treated with the combination of O_3 and elevated CO_2 differed significantly from leaves subjected to all other treatments. The authors concluded that the strongest effect of the O_3 and elevated CO_2 combination on leaf PA content resulted from the additive effect of these environmental factors on phenolic biosynthesis.

6.3 Combined Effects of O_3 and Soil Nitrogen Load

Nitrogen is the element required in the largest amounts by plants after carbon. The availability of nitrogen is therefore a decisive factor for forest production (Magnani et al. 2007), although excess nitrogen availability may have negative impacts on plants (See Chap. 18). Nitrogen deposition from the atmosphere to forest ecosystems has been increasing since the industrial revolution. Generally, there is a spatial correlation between high exposure to O_3 and high nitrogen deposition (Watanabe et al. 2012). In addition, changes in nitrogen availability as a result of increasing nitrogen deposition may affect plant sensitivity to O_3, because nitrogen is a component of enzymes that catalyze many metabolic processes, including the defense against O_3 (Dizengremel et al. 2013). Therefore, it is important to clarify the interaction between elevated O_3 and nitrogen availability for Japanese forest tree species.

Nakaji and Izuta (2001) presented the first report on the combined effects of O_3 and nitrogen load on forest tree species in Japan. They observed additive effects (i.e., no interaction) of O_3 (60 nmol mol^{-1} for 7 h) and excess soil nitrogen load (28–340 kg N ha^{-1} $year^{-1}$) on growth and photosynthetic traits in *Pinus densiflora* seedlings. Similar trends were observed in the same species by Nakaji et al. (2004). The influence of soil nitrogen load on the growth and physiological traits (such as photosynthesis) of six representative forest tree species in Japan (*Q. serrata*, *F. crenata*, *Castanopsis sieboldii*, *L. kaempferi*, *P. densiflora*, and *C. japonica*) were studied (Watanabe et al. 2006, 2007, 2008; Yamaguchi et al. 2007a, b, 2010). The seedlings were assigned to 12 experimental treatments, comprised of the combination of four levels of gas exposure (charcoal-filtered air and three levels of O_3 at 1.0, 1.5, and 2.0 times ambient concentration) and three levels of nitrogen load (0, 20, and 50 kg ha^{-1} $year^{-1}$; N0, N20, and N50, respectively), and grown for two growing seasons. At the end of the experiment, it was found that the nitrogen load did not change the sensitivities of growth to elevated O_3 in *Q. serrata*, *C. sieboldii*, *P. densiflora*, and *C. japonica* seedlings. On the other hand, significant interactions for

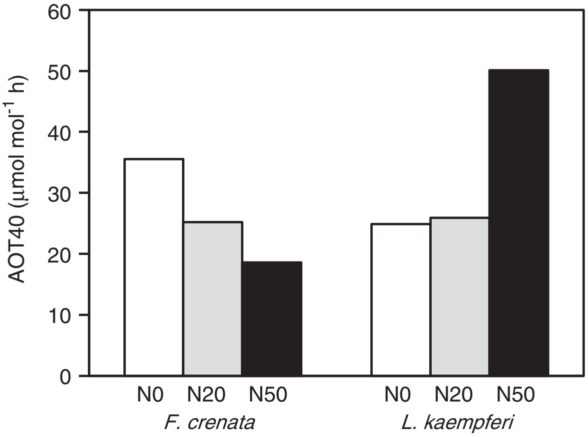

Fig. 6.2 Accumulated exposure over a threshold of 40 nmol mol^{-1} (AOT40) of ozone that induced 5 % reduction in the whole-plant dry mass increment of *Fagus crenata* and *Larix kaempferi* seedlings. The seedlings were grown under three levels of soil nitrogen load (0, 20, and 50 kg ha^{-1} $year^{-1}$; N0, N20, and N50, respectively) (Data source: Watanabe et al. 2008)

dry-matter growth between O_3 and nitrogen load were found in *F. crenata* and *L. kaempferi* seedlings (Fig. 6.2, Watanabe et al. 2006; Yamaguchi et al. 2007a).

There was an increase in the sensitivity of the growth of *F. crenata* to O_3 under a relatively high nitrogen load (Yamaguchi et al. 2007a). To clarify the reason for the enhancement of *F. crenata* O_3 sensitivity with increasing nitrogen load, Yamaguchi et al. (2007a, 2010) studied photosynthetic traits and nitrogen metabolism in the leaves during the second growing season. An ozone-induced reduction in the light-saturated net photosynthetic rate (A_{sat}) was observed with all nitrogen treatments in September. On the other hand, there was a significant interactive effect of O_3 and nitrogen load on A_{sat} in July. The exposure to O_3 significantly reduced the A_{sat} in seedlings with N20 and N50 treatments, but not in those with the N0 treatment. A similar tendency was observed in the concentrations of total soluble protein and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), while there was no significant interaction of O_3 and nitrogen load for stomatal conductance and area-based nitrogen content. These results indicate that the higher O_3 sensitivity of photosynthesis to elevated O_3 under a relatively high nitrogen load in *F. crenata* seedlings was not due to stomatal closure, but was due to the greater reduction of nitrogen allocation to soluble proteins, including Rubisco, and therefore indicated reduced biochemical assimilation capacity in chloroplasts.

In contrast to findings in *F. crenata*, the O_3-induced reduction in the growth of *L. kaempferi* seedlings was ameliorated by the nitrogen load (Fig. 6.2; Watanabe et al. 2006). Pell et al. (1995) reported that the largest reduction in growth of *Populus tremuloides* seedlings by exposure to O_3 was observed under the condition of nitrogen supply that induced the highest tree growth rate. In the case of *L. kaempferi* seedlings, there was no growth enhancement by the nitrogen loading. There is a possibility that the excessive nitrogen was used for detoxification and repair capacities against O_3 stress. Kam et al. (2015) also studied the combined effects of O_3 (60 nmol mol^{-1} during daytime) and nitrogen load, at 50 kg ha^{-1}, for one growing season on hybrid larch F_1 (*L. gmelinii* var. *japonica* × *L. kaempferi*) and *L. kaempferi* seedlings using a free-air O_3 fumigation system (See Chap. 5). They reported an additive effect of O_3 and nitrogen load on growth in both species, indicating that other environmental conditions affect the influence of nitrogen load on O_3 sensitivity in Japanese forest tree species.

6.4 Combined Effects of O_3 and Soil Water Stress

Water is the most abundant molecule on the Earth's surface. The availability of water is the factor that most strongly restricts plant production in terrestrial ecosystems on a global scale. Low water availability limits the productivity of many natural ecosystems. The progression of climate change at global, regional, and landscape levels modifies the water status of plants. Because periods with relatively high concentrations of atmospheric O_3 often occur in combination with low soil moisture in dry summers, there is the possibility that many tree species are simultaneously affected by O_3 and water stress.

The combined effects of O_3 and soil water stress on the growth and leaf characteristics of *F. crenata* seedlings were investigated for two growing seasons (Yonekura et al. 2001a, b, 2004; Watanabe et al. 2005). Three-year-old seedlings of *F. crenata* were exposed to charcoal-filtered air or 60 nmol mol^{-1} O_3, for 7 h a day, from May to October 1999 in naturally lit growth chambers. Half of the seedlings in each gas treatment group received 250 ml of water at 3-day intervals (well-watered treatment), while the rest received 175 ml of water at the same

Table 6.1 Effects of ozone (O_3) and soil water stress (WS) on growth and leaf physiological traits of *Fagus crenata* seedlings in first and second growing seasons

	O_3		WS		$O_3 \times$ WS	
	1st	2nd	1st	2nd	1st	2nd
Growth						
Leaf dry mass	↓	↓	–	↓	–	–
Bud dry mass	–	–	↓	↓	–	–
Stem dry mass	↓	↓	↓	↓	–	–
Root dry mass	↓	↓	–	↓	–	–
Whole-plant dry mass	↓	↓	↓	↓	–	–
Leaf area	–	↓	–	↓	–	–
Leaf number	–	↓	–	↓	–	–
Bud number	–	↓	↓	↓	–	–
Leaf number per bud	×	↓	×	–	×	–
S/R	–	↑	–	–	–	–
Leaf traits						
A_{sat}	↓	↓	↓	↓	–	Antag.
G_s	–	–	↓	↓	–	–
E	–	–	↓	↓	–	–
A_{max}	↓	↓	–	↓	–	Antag.
CE	↓	↓	–	–	–	Antag.
QY	–	–	–	↓	–	–
F_v/F_m	↓	↓	–	–	–	–
R_d	–	–	–	–	–	–
Water potential	–	–	↓	↓	–	–
Chl concentration	–	↓	–	–	–	–
Rubisco concentration	↓	↓	–	↓	–	–
TSP concentration	–	–	–	–	–	–
Carb. concentration	↓	↓	–	↑	–	–
Starch concentration	↓	↓	–	↑	–	–

After Yonekura (2006)

See text for the details of the gas and soil water treatments

↓: significant decrease, ↑: significant increase, –: not significant, ×: not determined, antag.: antagonistic effects of O_3 and WS

S/R shoot-to-root biomass ratio, A_{sat} light-saturated net photosynthetic rate, G_s stomatal conductance, E transpiration rate, A_{max} light and CO_2 saturated net photosynthetic rate, CE carboxylation efficiency, QY quantum yield, F_v/F_m maximum quantum yield, R_d dark respiration, Chl chlorophyll, Rubisco ribulose-1,5-bisphosphate carboxylase/oxygenase TSP total soluble protein, Carb. water-soluble carbohydrates intervals (water-stressed treatment). There were group differences in the combined effects of O_3 and water stress (Table 6.1). Additive effects (no significant interaction) of O_3 and water stress were detected in the growth and leaf traits in the first growing season. In the second growing season, on the other hand, there were significant effects of interactions between O_3 and water stress on some photosynthetic parameters, indicating water stress-induced amelioration of the negative effects of O_3. Lower leaf water potential and stomatal conductance may induce lower stomatal O_3 uptake. Furthermore, as reported by Watanabe et al. (2005), soil water stress in *F. crenata* seedlings increased the concentration of glutathione, a representative antioxidant, in the leaves, and these authors raised the possibility that the increased glutathione under soil water stress may moderate the negative impacts of O_3 on photosynthetic functions in *F. crenata* seedlings. It should be noted that, although antagonistic effects of O_3 and water stress were found in leaf traits, there was no significant effect of water stress on the growth response to O_3. Additive effects of O_3 and water stress on *F. crenata* and *B. ermanii* were also reported by Shimizu and Ito (2013) and Shimizu and Feng (2007), respectively. The extent of O_3 and water stress may modulate the interactive effects of the two stress factors.

6.5 Future Perspectives

The results obtained from the experimental studies described above indicate that most of the environmental factors examined have the potential to change O_3 sensitivities in Japanese forest tree species. The physiological mechanisms of changes in O_3 sensitivity seem to differ between environmental factors, although some of the mechanisms are the same (e.g. both elevated CO_2 and soil water stress decrease stomatal conductance and therefore stomatal O_3 uptake). To predict the effects of O_3 on Japanese forests under changing environments, deeper clarification of the modification effects of other environmental factors on O_3 sensitivity is crucial. However, the number of these studies has not yet been sufficient to achieve comprehensive understanding. Although studies with mature trees under natural conditions, such as free-air enrichment studies, are, of course, important, we consider that the accumulation of data on the combined effects of O_3 and other environmental factors on Japanese forest tree species, even with studies of seedlings, is important at the present time.

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