Chapter 5
Effects of Ozone on Japanese Trees

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Abstract The effects of ozone (O_3) on tree species in Japan have been studied since the 1970s. Based on the results from O_3 fumigation studies, current ambient levels of O_3 have negative impacts on the growth and physiological functions of Japanese forest tree species, although there is a big variation of O_3 sensitivity between species. Stomatal O_3 uptake is one of the key factors that can explain the differences in O_3 sensitivity between species, and modeling of this factor has been intensively studied during the past decade. Although O_3 generally induces stomatal closure, less efficient stomatal control, so-called stomatal sluggishness, is also induced by chronic exposure to O_3. These opposite phenomena result in complex responses of stomata to O_3. Detailed gas exchange analysis has revealed that O_3-induced reductions in the photosynthetic rate of Japanese forest tree species were mainly due to a biochemical limitation in chloroplasts, but not due to stomatal closure. Risk assessments of the O_3 impact on Japanese forest tree species, based on the results of experimental studies, national monitoring data of air pollutant concentrations, and vegetation surveys, indicate that the areas with high O_3-induced reduction in growth do not necessarily correspond to the areas with relatively high O_3 exposure. Free-air O_3 fumigation systems in Japan were developed in 2011. Studies with this novel technology have clarified differences in leaf O_3 sensitivities between canopy positions, and have estimated the effects of O_3 on whole-canopy carbon budgets. As future perspectives, not only we need clarification of the

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physiological mechanisms of O_3 impact, but we also need clarification of the effects of interactions between trees and other biotic factors such as diseases, herbivores, and symbiotic microbes.

Keywords Ozone • Japanese forest tree species • Growth • Physiological functions • Stomatal function

5.1 Introduction

The Japanese archipelago is located on the eastern edge of the Eurasian continent and extends from southwest to northeast in an arcuate shape. The length of the island chain is approximately 3,000 km, from the subtropics to the subarctic, covering a broad range of functional types of trees. Forests cover 67 % of Japan's total land area. In general, the number of tree species in the East Asian temperate biome is higher than that in the European and North American temperate biomes (Fine and Ree 2006).

Japan was the first country in East Asia to be recognized as suffering from serious air pollution, from the 1960s to the early 1970s (e.g., Izuta 2003, 2006; Nouchi 2001). As mentioned in Chap. 1, although the concentration of O_3 in Japan fell in the late 1970s, it has increased again from the 1980s. Furthermore, a continuous increase of O_3 concentration is predicted in this century. Therefore, the effects of O_3 on forest tree species will be serious in the near future. The understanding of tree responses to elevated O_3 is important to predict future forest contributions to our life through timber production, carbon sequestration, disaster prevention, and biodiversity conservation.

In Japan, studies on the effects of O_3 on plants have been published since the 1970s (Nouchi et al. 1973; Furukawa et al. 1983). These studies revealed the acute effects of O_3 on trees in roadsides and parks, such as *Prunus yedoensis*, *Ginkgo biloba*, and *Zelkova serrata* (Fujinuma et al. 1987; Furukawa 1991; Izuta 2006). Experimental studies on the effects of chronic O_3 exposure on the growth and physiology of forest trees commenced in the 1990s. These studies indicated that exposure to ambient levels of O_3 below 100 ppb for several months was sufficient to inhibit growth and physiological functions in sensitive Japanese forest tree species, such as *Fagus crenata*, *Z. serrata*, and *Pinus densiflora* (Izuta et al. 1996, 1998; Matsumura et al. 1996, 1998; Shan et al. 1997). In this chapter, we summarize the effects of O_3 on the growth and physiological characteristics of Japanese forest tree species, based on the experimental studies conducted mainly since the 1990s. In addition, advanced free-air O_3 fumigation studies of representative Japanese deciduous tree species, showing the effects of O_3 on defense capacity against biotic stresses, and symbiosis with ectomycorrhizal fungi are described. Nomenclature follows that described by Ohwi (1983).

5.2 Effects of O_3 on Growth and Physiological Characteristics of Japanese Trees

5.2.1 Effects of O_3 on Growth

5.2.1.1 Dry-Matter Growth

When plants are exposed to elevated O_3, their dry-matter growth is generally decreased. There are many studies concerning the effects of O_3 on the growth of Japanese forest tree species, using O_3 fumigation chambers (Fig. 5.1; e.g. Kohno et al. 2005; Yamaguchi et al. 2011). For example, Matsumura et al. (1996) reported that the whole-plant dry mass of *Cryptomeria japonica*, *Chamaecyparis obtusa*, and *Z. serrata* seedlings was reduced by increasing the exposure to O_3 from 0.4 to 3.0 times ambient O_3 concentration for 24 weeks (Fig. 5.2). Ozone-induced growth reductions have been reported for all functional types of tall tree species, such as deciduous broad-leaved (Matsumura 2001; Matsumura et al. 1996, 1998, 2005: Yonekura et al. 2001a, b; Watanabe et al. 2007; Yamaguchi et al. 2007), deciduous conifer (Matsumura 2001; Watanabe et al. 2006; Koike et al. 2012;

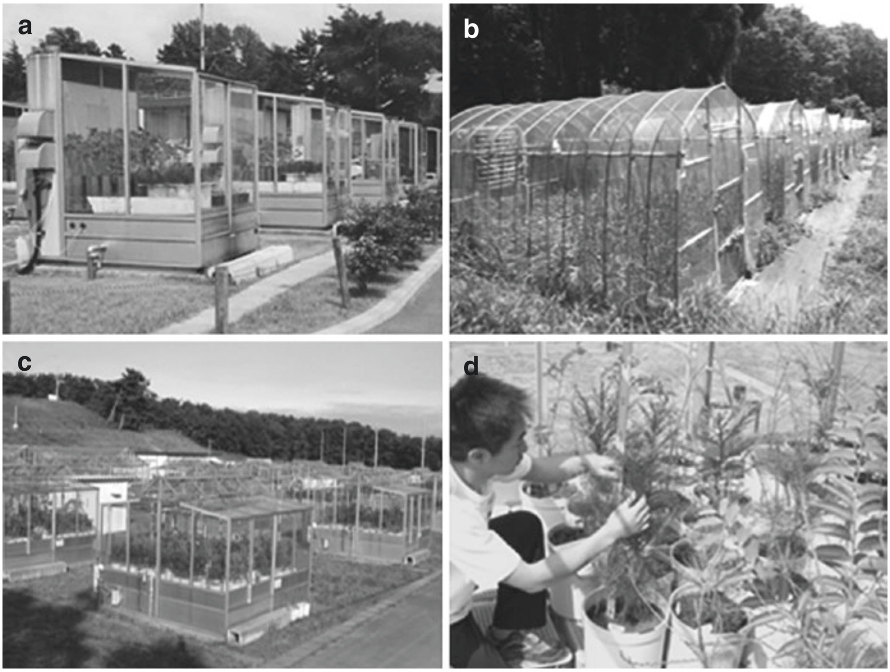

Fig. 5.1 (**a**) Naturally lit environmental control chambers, (**b**) greenhouse-type chambers, (**c**) open-top chambers, and (**d**) growth measurement of the seedlings in the open-top chamber

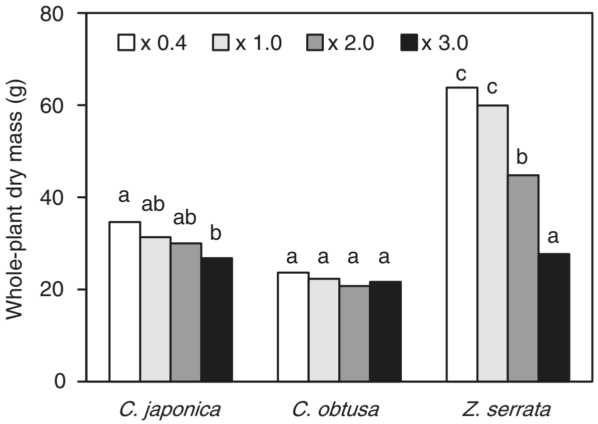

Fig. 5.2 Whole-plant dry mass of *Cryptomeria japonica*, *Chamaecyparis obtusa*, and *Zelkova serrata* seedlings exposed to four levels of ozone for 24 weeks. The seedlings were exposed to four simulated profiles with diurnal fluctuations of ozone: 0.4 (×0.4), 1.0 (×1.0), 2.0 (×2.0), and 3.0 (×3.0) times the ambient ozone concentrations. The average concentrations of ozone during the daytime (12 h) at ×0.4, ×1.0, ×2.0 and ×3.0 treatments were 16, 39, 74, and 114 nmol mol^{-1}, respectively. Values with different letters are significantly different at $P<0.05$ (Data source: Matsumura et al. 1996)

Wang et al. 2015), evergreen broad-leaved (Matsumura and Kohno 2003; Watanabe et al. 2008), and evergreen conifer (Kohno and Matsumura 1999; Matsumura 2001; Matsumura et al. 1996, 1998, 2005; Nakaji and Izuta 2001; Nakaji et al. 2004; Watanabe et al. 2006).

The relationships between O_3 exposure and growth responses of tree species have been studied since the late 1990s. These studies have revealed big differences of sensitivity to O_3 between forest tree species in Japan (Table 5.1). As a general trend of differences between functional types, the O_3 sensitivity of deciduous tree species is higher than that of evergreen tree species. As a general trend between functional types, O_3 sensitivity of deciduous tree species is higher than that of evergreen tree species. The foliage of evergreen tree species is exposed various biotic and abiotic stresses such as strong wind, low temperature, herbivore and diseases as compared to that of deciduous tree species due to their longer leaf longevity. Therefore, the foliage of evergreen trees should have higher physical and chemical tolerance to these stress factors, and this might confer higher tolerance to O_3 exposure. This tendency is also found in trees in other world regions such as Europe and China (Büker et al. 2015; Zhang et al. 2012). However, there is a big variation in O_3 susceptibility within functional types. For example, *Castanopsis sieboldii*, a representative evergreen tree species in warm temperate forests in Japan, has high susceptibility to O_3 exposure, which is comparable to that of *F. crenata* (Watanabe et al. 2008).

O_3, which enters the leaf through stomata, causes damage to plants. Accordingly, the effects of O_3 are described in the following formula,

Table 5.1 Classification of ozone sensitivity for Japanese forest tree species

Sensitivity class	Functional type		Species	AOT40 corresponding to 10 % reduction of growth
High	Broad-leaved	Deciduous	*Populus maximowiczii* *Populus nigra* *Fagus crenata* *Zelkova serrata*	8–15 ppm h
		Evergreen	*Castanopsis sieboldii*	
	Coniferous	Deciduous	*Larix kaempferi*[a]	
		Evergreen	*Pinus densiflora*	
Moderate	Broad-leaved	Deciduous	*Quercus serrata* *Betula platyphylla* var. *japonica*	16–30 ppm h
		Evergreen	*Quercus myrsinaefolia* *Cinnamomum camphora*	
	Coniferous	Evergreen	*Abies homolepis*	
Low	Broad-leaved	Deciduous	*Quercus mongolica* var. *crispula*[b]	31 ppm h<
		Evergreen	*Lithocarpus edulis* *Machilus thunbergii*	
	Coniferous	Evergreen	*Pinus thunbergii* *Cryptomeria japonica* *Chamaecyparis obtuse*	

After Kohno et al. (2005), Yamaguchi et al. (2011)

High sensitivity: dry mass growth was significantly reduced by the ambient level of ozone

Moderate sensitivity: dry mass growth was significantly reduced by 1.5 or 2.0 times the ambient level of ozone

Low sensitivity: dry mass growth was significantly reduced by 1.5 or 2.0 times the ambient level of ozone

AOT40: accumulated exposure over a threshold of 40 nmol mol^{-1} O_3 during daylight hours for 6 months (April–September)

[a] *Larix leptolepis*

[b] *Quercus mongolica* var. *grosseserata* or *Q. crispula*

$$O_3 \text{ effect} = \text{stomatal } O_3 \text{ uptake} \times \text{damage caused by a unit of } O_3 \text{ uptake}$$

It should be noted that both factors explaining the O_3 effect are regulated by complex physiological mechanisms. In Europe, analyses of accumulated stomatal O_3 uptake and growth reduction have been carried out since the first decade of the 2000s (Karlsson et al. 2004, 2007; Büker et al. 2015). On the other hand, uptake-based analysis of O_3 effects on Japanese forest tree species is an on-going study (see Sect. 5.2.2.1). The latter factor in the formula may be related to the detoxification of O_3 and reactive oxygen species derived from O_3, and processes of repair and compensation of O_3 damage (Fuhrer and Booker 2003); however, the worldwide knowledge is not yet complete enough to explain this latter factor in the formula.

5.2.1.2 Leaf Turnover

Ozone affects leaf turnover in trees, which can continuously produce new leaves during a growing season. In the experiment of Pell et al. (1994), O_3-induced acceleration of abscission of old leaves, and simultaneous increase of new leaf production, were observed in *Populus tremuloides* seedlings. These phenomena are considered as a kind of compensation response to elevated O_3 stress. A similar tendency was observed in *C. sieboldii* seedlings in the experiment of Watanabe et al. (2008) (Fig. 5.3). In their experiment, *C. sieboldii* seedlings were grown under 12 different experimental conditions, comprised of four gas treatments (charcoal-filtered air and three levels of O_3 at 1.0, 1.5, and 2.0 times the ambient concentration), in open-top chambers during the two growing seasons from April 2004 to November 2005. The exposure to O_3 significantly increased the abscission of the old leaves of *C. sieboldii* seedlings. But new leaf emergence was stimulated with increasing levels of O_3 exposure. In addition, the net photosynthetic rate of second-flush leaves was higher than that of first-flush leaves. Ozone-induced stimulation of new leaf production was also observed in the seedlings of *F. crenata* (Watanabe et al. 2010b), *Betula platyphylla* var. *japonica* (hereafter *B. platyphylla*; Hoshika et al. 2013b), *Quercus serrata,* and *Quercus mongolica* var. *crispula* (hereafter *Q. mongolica*; Kitao et al. 2015). However, the efficiency of the compensation response

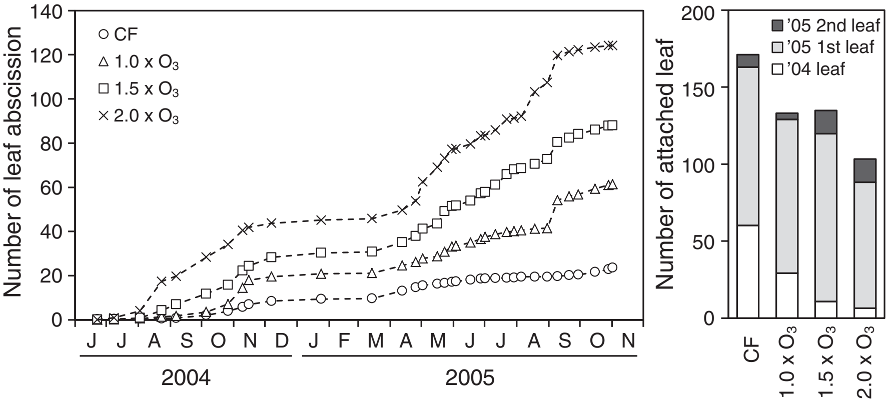

Fig. 5.3 Time course of leaf abscission (*left*) and number of attached leaves in each age class, on 1 November 2005, of *Castanopsis sieboldii* seedlings grown under charcoal-filtered air (CF) or 1.0, 1.5, and 2.0 times ambient concentration of ozone: '04 leaf, emerged in 2004; '05 first leaf, emerged in May 2005; '05 second leaf, emerged from July to October 2005 (Data source for the right figure is Watanabe et al. (2008), while data for the left figure is unpublished data from Watanabe et al. (2008))

to O_3 stress is not clear, because with old leaf falling there is loss of carbon and new leaf production requires another carbon cost. Actually, the total number of *C. sieboldii* leaves was decreased by the exposure to O_3, even with the increase of second-flush leaves (Fig. 5.3, right).

5.2.1.3 Carbon Allocation

Ozone changes the carbon allocation in tree species between above- and below-ground organs. In many tree species, O_3 induces greater inhibition of root growth than above-ground growth, resulting in an increase in the ratio of shoot biomass to root biomass (S/R) under elevated O_3 (Wittig et al. 2009). Matsumura et al. (1996) demonstrated increases of S/R in *Z. serrata* seedlings with increasing accumulated O_3 exposure (concentration × time) in a 24-week O_3 fumigation study. Also, an increase of S/R has been reported in other tree species in Japan, such as *C. japonica* (Miwa et al. 1993; Matsumura et al. 1998; Kohno and Matsumura 1999), *C. obtusa* (Kohno and Matsumura 1999), *Chamaecyparis pisifera* (Kohno and Matsumura 1999), *B. platyphylla* (Matsumura et al. 1998), *Abies homolepis* (Matsumura et al. 1998), *F. crenata* (Yamaguchi et al. 2007), *Q. serrata*, and *Q. mongolica* (Kitao et al. 2015). This change in carbon allocation might be a result of a compensation response to maintain growth under elevated O_3 conditions (Kitao et al. 2015). However, the mechanism of the change in carbon allocation of tree species under elevated O_3 conditions is not understood yet. If the carbon allocation to below-ground organs (i.e., roots) is decreased, carbon input from the root system to soil brought about by root turnover and exudate may be decreased. On the other hand, relative above-ground carbon input from leaf and branch abscissions increased. The decomposition processes, including animal and microbial decomposers, are quite different between carbon from above ground and that from below ground. Therefore, the changes in carbon allocation may be a critical matter from the viewpoint of the carbon cycle in a forest.

5.2.1.4 Phenology

The acceleration of foliar senescence is one of the common responses of trees to elevated O_3 conditions (e.g. Pell et al. 1999), although the phenomena induced by O_3 are somewhat different to those of natural senescence (Matyssek and Sandermann 2003). Although the acceleration of foliar senescence and the above-mentioned old leaf abscission for producing new leaves, as a kind of compensation response under elevated O_3 conditions, might occur via the same mechanism, any difference has not been clarified. Yonekura et al. (2004) examined the effects of O_3 on the phenological characteristics of *F. crenata* seedlings. The seedlings were exposed to 60 nmol mol^{-1} O_3 for 7 h per day during one growing season (May to October) in naturally lit growth chambers. All the seedlings were removed from the growth chambers after the growing season, and grown under field conditions until the next spring. The exposure to O_3 during the growing season induced early leaf fall. In the next spring, bud break of the seedlings was delayed, although these authors did not conducted O_3 exposure during the bud-break observation. This phenomenon is called the "carry-over effect". A similar phenomenon was observed in mature *Fagus sylvatica* in the Kranzberg Forest in Germany (Nunn et al. 2005). The carry-over effect, not only in relation to phenology but also in relation to growth, morphology, and physiology, was reported in *B. pendula* seedlings in northern Europe (Oksanen and Saleen 1999).

From the ecological viewpoint, the impact of O_3 on phenology is highly important for forest dynamics. The light environment of the deciduous forest floor undergoes seasonal changes. And seedlings in the forest floor respond to the changes in light condition (e.g. Kitaoka and Koike 2004). Because the responses to changes in light condition are species-specific, shortening of the leafy period in canopy trees induced by elevated O_3 will affect seedlings in the forest floor in various ways, and may cause a change in species composition, indicating change in forest dynamics. On the other hand, the phenological changes of seedlings in the forest floor under elevated O_3 conditions also have the potential to affect forest dynamics. For example, a phenological gap is important for the seedlings of *F. crenata* (Terazawa and Koyama 2008). The leaf emergence of *F. crenata* is earlier than that of other tree species such as *Q. mongolica* and *Magnolia obovate* (= *M. hypoleuca*). Even though the seedlings of *F. crenata* in the forest floor are covered by upper-storey trees of other species, they can use light resources before the leaf emergence of upper-storey trees. However, if the leaf emergence of *F. crenata* seedlings is delayed under elevated O_3 conditions, the seedlings may not use the phenological gap efficiently and the growth of the seedlings may be inhibited.

5.2.2 O_3 Effects on Physiology

5.2.2.1 Stomatal Functions

Stomata are small pores on leaves whose aperture is actively regulated by the plant in response to multiple abiotic and biotic factors, and stomatal conductance, which is an index of stomatal apertures, is a major determinant of CO_2 uptake (photosynthesis) and water loss (transpiration). Ozone enters leaves through the stomata and causes damage to physiological processes such as photosynthesis (Reich 1987). Stomatal O_3 flux or uptake is considered to be closely related to O_3 impacts on forest trees (Mills et al. 2010). Stomatal conductance is one of the most important factors for the estimation of stomatal O_3 flux. In the 2000s, modeling studies have been developed to estimate stomatal O_3 flux for assessing the impact of O_3 in Japanese forest trees. A multiplicative numerical approach (Jarvis-type model; Jarvis 1976) has been commonly used to estimate stomatal conductance (Mills et al. 2010). This approach assumes that stomatal conductance is a multiplicative function of leaf age and environmental factors (e.g., light intensity, temperature, and humidity deficits and soil-moisture stress). Several efforts to estimate stomatal O_3 flux have thus been made in forest tree species in Japan (e.g. Hoshika et al. 2012c; Azuchi et al. 2014; Kinose et al. 2014). Kinose et al. (2014) reported the importance of a species-specific stomatal response for determining stomatal O_3 flux. In their study, the cumulative stomatal uptake of O_3 (CUO) of *F. crenata*, *Q. serrata*, and *Q. mongolica* seedlings in spring, from April to May, was relatively low, whereas the O_3 concentration was relatively high. On the other hand, the CUO of *B. platyphylla* seedlings was relatively high, mainly because of rapid leaf maturation and a lower optimal temperature for stomatal opening. In addition to the Jarvis-type model, a model based on the empirical relationship between the photosynthetic rate and stomatal conductance (i.e., the Ball-Woodrow-Berry model; Ball et al. 1987) was recently applied to estimate stomatal O_3 flux (Hoshika et al. 2015a). These modeling studies also found that O_3 affected stomatal conductance. Hoshika et al. (2012c) reported that O_3 decreased the maximum stomatal conductance of *F. crenata* leaves. Although simple functions are used for estimating O_3 effects on stomatal conductance (Hoshika et al. 2012c; Azuchi et al. 2014; Kinose et al. 2014), the actual stomatal responses to O_3 are more complex than those used in the current models (Hoshika et al. 2015c). Also, the mechanisms of O_3 effects on stomatal conductance are still under investigation (Kangasjärvi et al. 2005; Mills et al. 2009). Stomatal behavior under elevated O_3 is thus a major concern.

According to the meta-analysis of O_3 effects on tree species done by Wittig et al. (2007), O_3 was found to reduce stomatal conductance. There are several reports on lower stomatal conductance in the leaves of Japanese forest tree species under elevated O_3 (e.g. Hoshika et al. 2012c, 2013b). The reduction of stomatal conductance may limit stomatal O_3 flux into leaves. This raises the question of whether O_3-induced stomatal closure could act as an avoidance response to O_3 stress. Hoshika et al. (2013a) examined this hypothesis for *F. crenata* using an optimal stomatal model, which was based on the optimization theory of stomatal conductance for maximizing carbon gain while minimizing accompanying water loss and O_3 flux. Eleven-year-old saplings of *F. crenata* were exposed to 60 nmol mol^{-1} O_3 by a free-air fumigation technique (see Sect. 5.4) for daylight hours during the growing season. Analysis based on the optimal stomatal model suggested that O_3 flux was efficiently limited by stomatal closure in early summer (June). Their results indicate that O_3-induced stomatal closure in early summer could be considered as an avoidance response to O_3 stress, to allow maximum photosynthetic capacity to be reached. It should be noted, however, that it is not likely that sufficient evolutionary time has elapsed to develop plant adaptation to elevated O_3, which is a "new" stress with a short history as compared with times for plant evolution (Jacob 1999). Therefore, such stomatal closure may be just a reaction to O_3 and fortunately it avoids O_3 stress for trees. In late summer (August) and autumn (early October), on the other hand, the decrease of stomatal conductance by O_3 exposure became small. Previous studies suggest that O_3 also causes less efficient stomatal control, especially a weaker capacity to close stomata, so-called stomatal sluggishness (Paoletti and Grulke 2005). Yamaguchi et al. (2007) found that O_3 increased the stomatal conductance of *F. crenata* seedlings under light-saturated conditions in autumn (Fig. 5.4). Watanabe et al. (2014b) pointed out an inconsistent stomatal response to O_3 (60 nmol mol^{-1} during daylight hours), due to

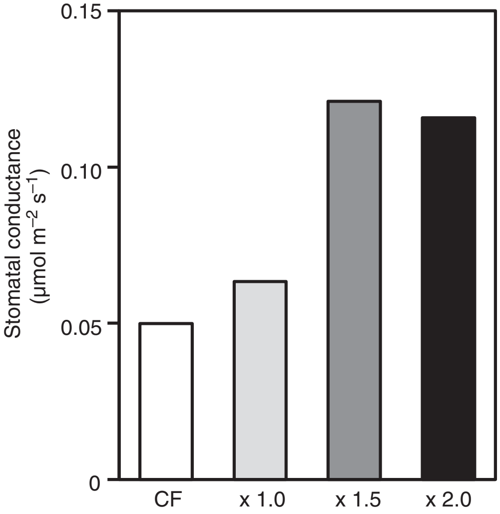

Fig. 5.4 Stomatal conductance of *Fagus crenata* seedlings in September 2005. The seedlings were grown under charcoal-filtered air (CF) or 1.0, 1.5, and 2.0 times ambient concentration of ozone for two growing seasons, from April 2004 to September 2005 (Data source: Yamaguchi et al. 2007)

both stomatal closure and stomatal sluggishness, in *Betula maximowicziana* seedlings. Hoshika et al. (2015b) reported that O_3-induced stomatal closure decreased with increasing leaf age in *F. crenata*. Also, O_3 may increase night-time stomatal conductance, as reportedly occurs in *F. crenata* in October (Hoshika et al. 2013a).

The impairment of stomatal control may lead to a slower stomatal response to environmental stimuli. Hoshika et al. (2012b) found a significantly slower stomatal response to light variation (alternative light exposure of photosynthetic photon flux density between 100 and 1,500 µmol m^{-2} s^{-1}) for *F. crenata* saplings. Although the mechanisms of stomatal sluggishness are still not known, Omasa (1990) suggested that such a stomatal response was caused by a slight increase in the permeability of epidermal cell membranes and alterations of the osmotic pressure modulating a balance in turgor between guard and subsidiary cells. Mills et al. (2009) found that O_3 reduced stomatal sensitivity to abscisic acid (ABA). Ozone-induced ethylene synthesis might also be related to stomatal sluggishness, because ethylene is known to inhibit the action of ABA in stomatal closure (Tanaka et al. 2005; Wilkinson and Davies 2010). Moreover, it was recently demonstrated that gene expression under O_3 impact inhibited the CO_2 signaling involved in stomatal closure (Dumont et al. 2014). The effect of elevated O_3 on stomatal reaction to changes in environmental conditions should be considered in dynamic models for the estimation of stomatal O_3 flux.

In this section, we have explained two kinds of stomatal sluggishness, one where stomata do not close to a sufficient level (steady-state evidence) and the other in which there is a slower stomatal response (kinetic evidence). Whether or not these two types of stomatal sluggishness are supported by the same mechanism is a future topic for study.

5.2.2.2 Photosynthesis

Approximately 40 % of a plant's dry mass consists of carbon, fixed during photosynthesis. This process of CO_2 assimilation with water and light energy is vital for the growth and survival of substantially all plants (Lambers et al. 2008). Many studies have reported decreased leaf photosynthetic rates (usually in light-saturated conditions) under elevated O_3. A decreased photosynthetic rate is considered to be one of the most important factors that induces growth reduction in trees. According to Watanabe et al. (2006), reductions in net photosynthetic rates by exposure to O_3 were observed in *Larix kaempferi* and *P. densiflora* seedlings, which showed relatively large growth reduction. On the other hand, in *C. japonica* seedlings, the extent of growth reduction was relatively small and there was no significant reduction in the net photosynthetic rate under elevated O_3. Similar tendencies were reported in *F. crenata* (sensitive to O_3) and *Q. mongolica* (tolerant to O_3) saplings (Watanabe et al. 2013).

The rate of photosynthetic carbon assimilation is determined by both the supply and the demand for CO_2. The supply of CO_2 to the chloroplasts from ambient air is governed by diffusion in the gas and liquid phases. Stomatal conductance is one of the main parameters controlling CO_2 diffusion in the gas phase and therefore this process controls the supply of CO_2 to the chloroplasts. The demand for CO_2 is determined by the rate of CO_2 assimilation in the chloroplast, which is governed by the component structures of the chloroplast and by biochemical reactions, such as carboxylation in the stroma and electron transport and adenosine triphosphate (ATP) synthesis in thylakoid membranes. The question is: which factor (i.e., supply or demand) mainly limits the rate of photosynthesis under elevated O_3?

As mentioned above, O_3 generally reduces stomatal conductance (Wittig et al. 2007). On the other hand, there is a high correlation between the net photosynthetic rate and stomatal conductance (Ball et al. 1987) under normal ambient conditions (i.e., no O_3 effect). The ratio of the intercellular CO_2 concentration (C_i) to the ambient CO_2 concentration (C_a), i.e., C_i/C_a, at a given vapor pressure deficit is constant under a certain range of atmospheric CO_2 concentrations (e.g. Sage 1994). If the biochemical assimilation capacity decreases, stomatal conductance also decreases for constant C_i/C_a. Therefore, it should be noted that the co-reduction of the net photosynthetic rate and stomatal conductance may not indicate stomatal limitation of photosynthesis.

The analysis of the C_i-response curve of the net photosynthetic rate (A/C_i curve) is one of the strongest methods with which to quantify the supply and demand for CO_2 in photosynthesis (Fig. 5.5; Farquhar et al. 1980). The maximum rate of carboxylation (V_{cmax}) and the maximum rate of electron transport (J_{max}) are representative parameters of CO_2 demand, and stomatal limitation is the representative parameter of CO_2 supply. Detailed information on photosynthesis and the general environmental responses (light, humidity and CO_2 responses) of forest tree species are described in specialized books (e.g. Lambers et al. 2008). Limitation by the rate of triose phosphate utilization (TPU) is also considered as a factor that regulates the photosynthetic rate (Sharkey 1985). But there is no report on the effect of O_3 on the rate of TPU utilization for Japanese forest tree species.

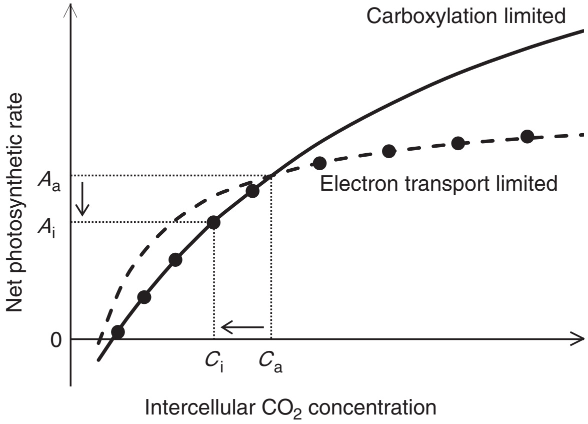

Fig. 5.5 Idealized intercellular CO_2 concentration-response curve of the net photosynthetic rate (A/C_i curve). C_a ambient CO_2 concentration, C_i intercellular CO_2 concentration, A_a theoretical net photosynthetic rate at $C_i = C_a$ (absence of limitation in diffusion of CO_2 from ambient air to intercellular air space), A_i net photosynthetic rate at C_i. The net photosynthetic rate at given C_i is plotted on the *lower line* of carboxylation-limited or electron transport-limited photosynthesis as *filled circles*. Stomatal limitation (L_s, %) is calculated as $L_s = (A_a - A_i) / A_a \times 100$

Although many simulations of the carbon cycle in vegetation use Farquhar's biochemical model for the calculation of photosynthesis, to date only a limited number of studies have determined the parameters of this model. Watanabe et al. (2013) analyzed the A/C_i curve in the leaves of *F. crenata* saplings exposed to free air O_3 enrichment (see Sect. 5.4) (Fig. 5.6). Elevated O_3 (60 nmol mol^{-1} during daytime) significantly reduced the light-saturated net photosynthetic rate (A_{sat}), V_{cmax}, and J_{max}, while there was no significant effect of O_3 on stomatal conductance and stomatal limitation. These results indicate the O_3-induced reduction of photosynthetic activity in *F. crenata* was due not to stomatal closure, but to biochemical limitations. In addition, the C_i/C_a was increased under elevated O_3, indicating that the stomata did not close enough to keep the C_i/C_a constant (i.e., there was stomatal sluggishness, see subsection "*Stomatal functions*" above). Ozone-induced decreases of V_{cmax} or carboxylation efficiency, which is calculated as the initial slope of the A/C_i curve and is closely correlated with V_{cmax}, have also been reported in the leaves of other Japanese forest tree species: *F. crenata* (Izuta et al. 1996; Yonekura et al. 2001b; Yamaguchi et al. 2007), *B. platyphylla* (Matsumura et al. 1998), *B. maximowicziana* (Watanabe et al. 2014b), *Z. serrata* (Matsumura et al. 1998), and *P. densiflora* (Nakaji and Izuta 2001).

Nitrogen is a nutrient that is strongly related to biochemical assimilation capacity (e.g. Lambers et al. 2008). A large fraction of the nitrogen in leaves is incorporated into proteins associated with the photosynthetic process (Evans 1989; Evans and Seemann 1989), and there is generally a positive correlation between the photosynthetic rate and the nitrogen content of leaves (e.g. Kitaoka and Koike 2004). The net photosynthetic rate (A) is described as follows:

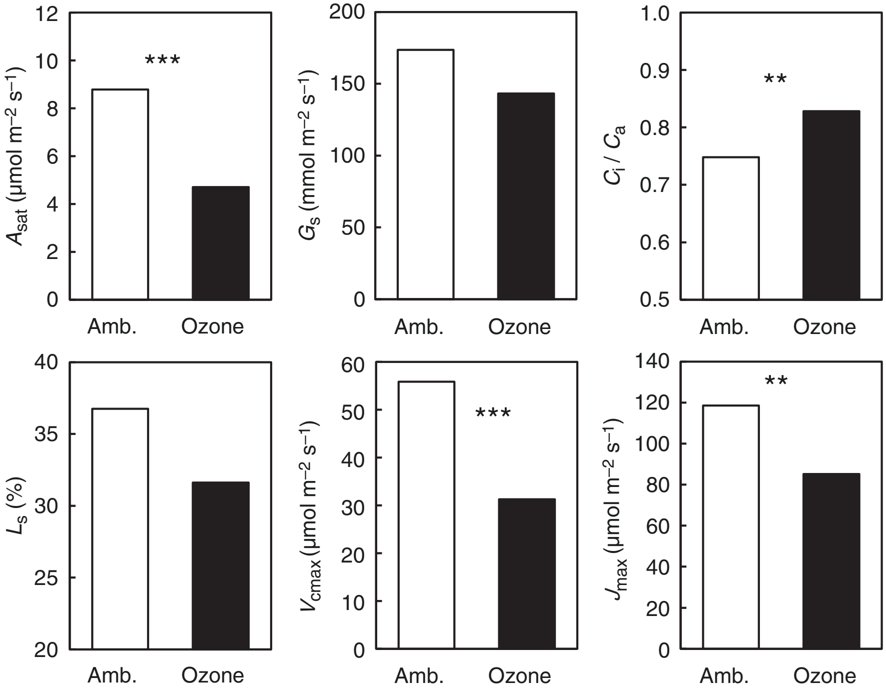

Fig. 5.6 Leaf photosynthetic traits of *F. crenata* saplings grown under ambient and elevated concentrations of O_3. A_{sat} light-saturated net photosynthetic rate at 380 μmol mol^{-1} CO_2, G_s stomatal conductance to water vapor, C_i/C_a ratio of intercellular CO_2 concentration to ambient CO_2 concentration, L_s stomatal limitation of photosynthesis, V_{cmax} maximum rate of carboxylation, J_{max} maximum rate of electron transport. **, $P<0.01$, ***, $P<0.001$ (Data source: Watanabe et al. 2013)

$$A\left(\mu\mathrm{mol\,m^{-2}s^{-1}}\right) = \mathrm{PNUE}\left(\mu\mathrm{mol\,mol^{-1}s^{-1}}\right) \times \text{leaf nitrogen content}\left(\mathrm{mol\,m^{-2}}\right),$$

where PNUE is photosynthetic nitrogen use efficiency (net photosynthetic rate per unit leaf nitrogen content). Generally, exposure to O_3 has little effect on leaf nitrogen content, except in autumn. In autumn, nitrogen resorption, and therefore decreases in leaf nitrogen content, may start earlier in leaves exposed to O_3 due to the O_3-induced acceleration of foliar senescence (see Sect. 5.2.1.4). On the other hand, several studies have reported decreased PNUE in woody species native to Japan under elevated O_3 (Watanabe et al. 2007, 2015; Yamaguchi et al. 2007; Hoshika et al. 2013b). These findings indicate that O_3 reduced leaf nitrogen allocation to the photosynthetic apparatus. As shown in Fig. 5.7, a decrease of the nitrogen fraction in the photosynthetic apparatus under elevated O_3 was demonstrated in the leaves of *F. crenata* saplings (Watanabe et al. 2013).

Yonekura et al. (2001b) monitored the time course of gas exchange traits in the leaves of *F. crenata* seedlings (A/C_i curve and light response curve of A) under elevated O_3. The negative effect of O_3 was first observed in regard to carboxylation

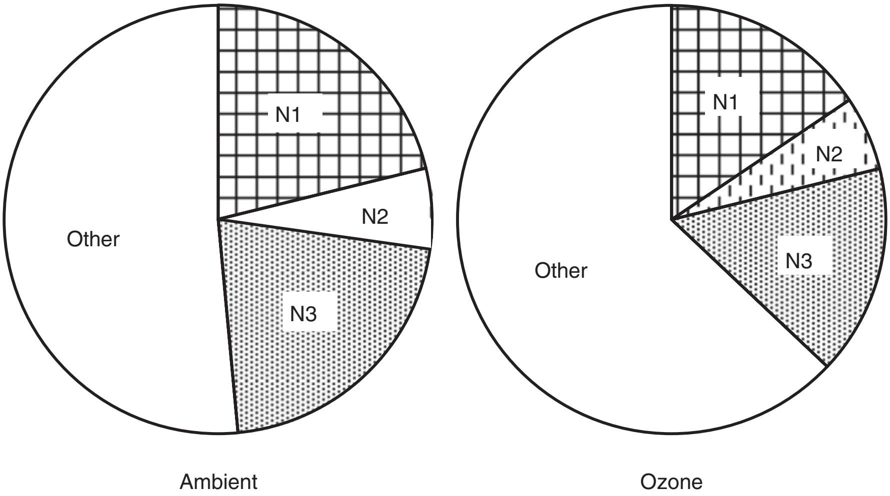

Fig. 5.7 Nitrogen fraction in leaves of *Fagus crenata* saplings. N1: Rubisco, N2: bioenergetics (electron carriers except for photosystems, coupling factor, and Calvin cycle enzymes except for Rubisco), N3: light-harvesting complex and photosystems (After Watanabe et al. 2013)

efficiency (initial slope of A/C_i curve) from mid-June, and in A_{sat} and light and CO_2 saturated A (which has a high correlation with J_{max}), which was decreased under elevated O_3 from mid-July. These results suggest that O_3 first causes a reduction of carboxylation capacity (i.e., a reduction in ribulose-1,5-bisphosphate carboxylase/oxygenase [Rubisco] content and/or its specific activity), and then a reduction in the capacity of other photosynthetic functions is induced as a result of the feedback regulation of the photosynthetic rate to match photosynthetic capacities with carboxylation capacity. According to Yamaguchi et al. (2007, 2010a), O_3 decreased the ratio of soluble protein content (mainly Rubisco) to nitrogen content in the leaves of *F. crenata* seedlings. Furthermore, they reported that the decrease in Rubisco in the leaves of *F. crenata* seedlings under elevated O_3 was accompanied by an increase in the acidic amino acid content, indicating the degradation of Rubisco protein. Brendley and Pell (1998) also found O_3-induced acceleration of proteolysis in a hybrid poplar (*Populus maximowiczii* A. Henry × *trichocarpa* Torr. & A. Gray). Reduction in Rubisco content was also observed in other Japanese forest tree species (*Q. serrata*; Watanabe et al. 2007, *P. densiflora*; Nakaji and Izuta 2001, *C. sieboldii*; Watanabe et al. unpublished data).

5.2.2.3 Respiration

The response of the dark respiration rate (R_d) in the leaves of Japanese forest tree species to elevated O_3 varied between experiments. Izuta et al. (1996) reported over 40 % reduction in R_d of *F. crenata* seedlings exposed to 75 and 150 nmol mol^{-1} O_3

(6 h per day for 18 weeks), although the reduction was not statistically significant. According to Wittig et al. (2007), R_d generally decreases under elevated O_3. In contrast, however, increased R_d was observed in *F. crenata* and *Q. mongolica* saplings under elevated O_3 (Watanabe et al. 2013). No significant effects of O_3 on R_d were reported in *C. japonica*, *C. obtusa*, and *Z. serrata* seedlings (Matsumura et al. 1996) and *F. crenata* seedlings (Yonekura et al. 2001b). Although a conclusive mechanism of these differences has not been clarified, the extent of damage caused by O_3 may be one of the factors responsible for this discordance. If the O_3 damage is severe, all metabolic processes, including R_d, would be decreased. On the other hand, minor damage by O_3 may induce an increase in R_d to enhance the detoxification capacity of O_3-related reactive oxygen species, and/or the repair of damaged tissues (Landolt et al. 1997; Matyssek and Sandermann 2003). There is no study about the effects of O_3 on photorespiration in the leaves of Japanese forest tree species.

5.3 Risk Assessment of Ozone Impact

Risk assessments of the impact of O_3 on tree growth in Japan have been conducted, based on experimental studies, since 2003 (Takagi and Ohara 2003). Kohno et al. (2005) estimated the critical level of O_3 for forest trees, based on experimental studies of 18 tree species, and they mapped the area in which the level of O_3 corresponds to at least a 10 % growth reduction of O_3-sensitive tree species throughout Japan.

Based on an experimental study on the combined effects of O_3 and soil nitrogen loading (see Sect. 6.3 and Yamaguchi et al. 2007 for details), the risk of a negative O_3 impact on the growth of *F. crenata,* with consideration of changes in O_3 sensitivity caused by nitrogen deposition, was determined (Watanabe et al. 2012). The values for the average and maximum O_3-induced relative growth reduction in *F. crenata* across Japan were estimated to be 3.2 % and 9.7 %, respectively. On the other hand, when the value for nitrogen deposition was assumed to be zero, the estimated values for average and maximum relative growth reduction were 2.3 % and 5.7 %, respectively. The inclusion of atmospheric nitrogen deposition data thus increased the estimated values for average and maximum relative growth reduction (by 38 % and 71 %, respectively). These authors emphasize that a change in the sensitivity to O_3 associated with atmospheric nitrogen deposition is an important consideration in the risk assessment of the impact of O_3 on the growth of *F. crenata* in Japan.

Watanabe et al. (2010a) indicated that not only the accumulated O_3 exposure but also the variety of tree habitat, differences in sensitivity to O_3, and differences in annual carbon absorption among the tree species must be taken into account to assess the risk of O_3 impact on annual carbon absorption in Japanese conifers. They integrated a forest growth model from a national forest monitoring data set and the results of an experimental study, and estimated the O_3 impact on annual forest carbon absorption in each prefecture throughout Japan for three representative Japanese conifers: *L. kaempferi*, *P. densiflora*, and *C. japonica*. The areas with high O_3-induced reduction in annual carbon absorption did not necessarily correspond to the areas with relatively high O_3 exposure. Widespread distribution of O_3-sensitive tree species, such as *P. densiflora* and *L. kaempferi*, and high annual carbon absorption were important factors that induced a high risk of O_3 impact on annual carbon absorption. The O_3-induced reduction in the total annual forest carbon absorption of the above three representative tree species in Japan was estimated to be 0.8 %.

Risk assessments of the effects of O_3, based on stomatal O_3 flux, which could account for the influence of climatic and ontogenetic factors, are now being developed in Japan (Izuta 2012). Hoshika et al. (2012a) compared the maps of accumulated O_3 exposure (e.g. the accumulated exposure over a threshold of 40 nmol mol^{-1}, AOT40) and cumulative O_3 uptake for *Z. serrata* in Japan and suggested that stomatal closure induced by a high vapor pressure deficit caused a decoupling of stomatal O_3 uptake from high O_3 exposure. This indicates that a stomatal flux-based approach may provide higher precision in the assessment of O_3 impacts on a regional scale (Watanabe and Yamaguchi 2011). Hoshika et al. (2015a) focused on O_3-induced steady-state stomatal sluggishness (see Sect. 5.2.2.1), and examined the effects of O_3-induced stomatal sluggishness on carbon assimilation and transpiration in temperate deciduous forests in the Northern Hemisphere. They conducted a simulation by combining a detailed multi-layer land surface model and a global atmospheric chemistry model. They reported that O_3-induced stomatal sluggishness may change the balance of the carbon and water cycle in temperate deciduous forests. According to their simulation, water use efficiency, i.e., the ratio of net CO_2 assimilation to transpiration, in temperate deciduous forests would decrease by up to 20 % when considering stomatal sluggishness data (i.e., the relationship between minimum conductance and CUO) obtained from the experimental study on *F. crenata* at the Sapporo experimental forest (see Sect. 5.4).

The risk assessment of O_3 impact requires relatively extensive studies. Data from experimental studies, field monitoring, and vegetation surveys are combined. Therefore, there are uncertainties at each level. Here, we discuss some uncertainties in the risk assessment of O_3 impact on Japanese forest tree species. According to previous studies, the extent of responses in plant traits (e.g., reduction of biomass, and leaf-level photosynthesis) to O_3 is species-specific (e.g. Yamaguchi et al. 2011), and even genotype-specific (Paludan-Müller et al. 1999). There are also differences in sensitivities between seedlings and mature trees (Karnosky et al. 2007; Matyssek et al. 2007). Pretzsch et al. (2010) reported comparable growth sensitivity to O_3 in mature *F. sylvatica* trees and juvenile seedlings, although the mechanism of the O_3-induced growth reduction may differ. Other environmental factors may affect the O_3 sensitivity of trees (see Chap. 6). To overcome these uncertainties, we need to develop a mechanism for understanding the effects of O_3 on trees in terms of whole-tree physiology and vegetation production; better modeling of the processes of plant growth and physiology is also needed.

Monitoring stations for O_3 in Japan have been mainly located in urban areas, because the aim of monitoring is the protection of human health. There are limited numbers of monitoring stations in the mountains and rural areas. However, several phenomena indicate that the O_3 concentration in mountainous and rural areas is higher than that in urban regions (e.g. Yamaguchi et al. 2010b). Furthermore, the atmospheric concentration of O_3 in mountainous areas sometimes shows diurnal variation that is different from that in urban areas (mainly flatland); in particular, a typical phenomenon in mountainous areas is that there is little change in O_3 concentration under the inversion layer. Reconsideration of the location of monitoring stations for O_3 is needed for the accurate assessment of O_3 impact on forest trees in Japan.

5.4 Free-Air Ozone Fumigation Experiment

Previous studies have applied chamber experiments to study the effects of O_3. Although chamber experiments offer an advantage as a study mechanism owing to their controllability of O_3 concentration, artifacts may arise in the environmental conditions as a result of differences in micro-meteorological conditions and the absence of biotic stresses such as herbivores and diseases. Most studies in Japan, as well as those around the world, have used pot-grown seedlings due to the limitations of the study facility. Accurate simulation of forest conditions is therefore questionable (Kolb and Matyssek 2001).

Free-air O_3 fumigation with field-grown trees is a novel technique for assessing the effects of O_3 in field conditions without volume restrictions on root growth. Studies employing this technology have been conducted in Europe and the United States since the 1990s (e.g. Karnosky et al. 2007; Matyssek et al. 2007; Oksanen et al. 2007; Díaz-de-Quijano et al. 2012). Although the concerns regarding O_3 in the Asian region are acute and important, no free-air fumigation study of forest tree species had been reported in this region until recent years. Three free-air O_3 fumigation experiments are running at present in Japan; two systems are located in the Sapporo experimental forest, Hokkaido University, Sapporo (43°04′ N, 141°20′ E, 15 m above sea level) (e.g. Watanabe et al. 2013; Koike et al. 2013) and the other one is located in the nursery of the Forestry and Forest Products Research Institute in Tsukuba (36°00′ N, 140°80′ E, 20 m a.s.l.) (Kitao et al. 2015). Some of the results of the free-air O_3 fumigation experiments have already been described (e.g. Hoshika et al. 2012b, c; Watanabe et al. 2013). Here, we introduce the details of the fumigation system and the results of canopy-scale study in the Sapporo experimental forest (Watanabe et al. 2014a, 2015). The results from the Tsukuba experiment are shown in Chapter 6.

The size of the system in the Sapporo experimental forest is a 5.5 × 7.2 m rectangle, and the height is 5.5 m (Figs. 5.8 and 5.9). In this system, 10-year-old saplings (as of 2011) of *F. crenata* and *Q. mongolica*, and 3-year seedlings of *B. platyphylla*, which are representative species in northern Japan, were grown. *F. crenata* and *Q. mongolica* have similar distribution and growth traits, which are late successional with shade tolerance, although *Q. mongolica* prefers a relatively higher light condition than *F. crenata* (Hokkaido Forest Tree Breeding Association 2008).

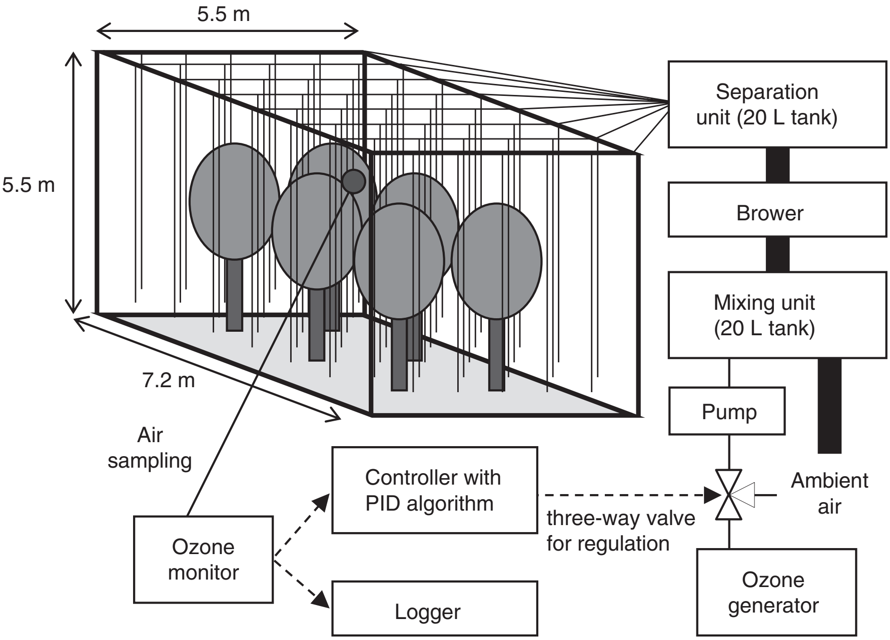

Fig. 5.8 Diagram of free-air ozone fumigation system located in the Sapporo experimental forest, northern Japan (After Watanabe et al. 2013)

Fig. 5.9 Overview of free-air ozone fumigation system at Sapporo experimental forest (*left*), inside of the system (*upper right*), and gas exchange measurement in leaves of *Fagus crenata* saplings (*lower right*)

On the other hand, *B. platyphylla* is a typical pioneer species in Northern Japan. The target O_3 concentration was 60 nmol mol^{-1} during the daylight hours of the growing season (i.e., the leafy period). The O_3 generated from an O_3 generator was mixed with ambient air, using a three-way valve to control the concentration. The air containing O_3 was then diluted with ambient air in a mixing unit and passed into the canopies through 48 fluorine resin tubes hanging from a fixed grid above the trees down to a height of 50 cm above the ground. Each tube has ten holes (2-mm diameter) at 50-cm intervals. The signal of O_3 monitoring at canopy height was used as feedback to the three-way valve so as to regulate the O_3 concentration, using the proportional-integral-differential (PID) algorithm.

When we consider the effects of O_3 on whole-canopy carbon uptake, it is important to understand the differences in O_3 susceptibility between canopy positions. There are considerable variations of leaf traits within a developed canopy. One of the most important factors that determine leaf traits is light intensity. For example, thick leaves with a higher leaf mass per area (LMA) and higher area-based nitrogen content (N_{area}) are produced under high light conditions (e.g. Evans and Poorter 2001; Kitaoka et al. 2009a, b; Poorter et al. 2009; Niinemets et al. 2015). As a result, photosynthetic parameters, such as V_{cmax} and J_{max}, are generally higher in leaves exposed to higher irradiance (Iio et al. 2005; Rodríguez-Calcerrada et al. 2008; Niinemets et al. 2015). Watanabe et al. (2014a, 2015) investigated the response of A_{sat} of upper and lower canopy leaves of *F. crenata* and *Q. mongolica* saplings in a free-air O_3 exposure experiment (Fig. 5.10). There were considerable differences between species. Ozone-induced significant reduction in A_{sat} of *F. crenata* was

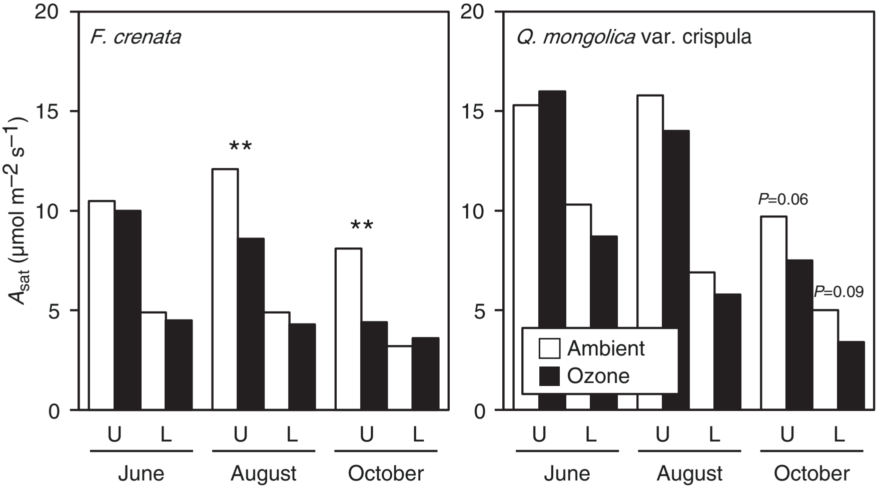

Fig. 5.10 Light-saturated net photosynthetic rate (A_{sat}) in upper (U) and lower (L) canopy leaves of *Fagus crenata* and *Quercus mongolica* var. *crispula* saplings grown under ambient and elevated concentrations of ozone (60 nmol mol^{-1} during daylight hours). Significant reduction in A_{sat} of *F. crenata* under elevated O_3 was observed only in upper canopy leaves (**, $P<0.01$), while A_{sat} in both the upper and lower canopy leaves of *Q. mongolica* showed similar marginal reduction by exposure to O_3 in October (Data source: Watanabe et al. 2014a, 2015)

observed only in upper canopy leaves in August and October, indicating higher O_3 susceptibility in upper canopy leaves. On the other hand, a marginal decrease of A_{sat} under elevated O_3 was found in both the upper and lower canopy leaves of *Q. mongolica* in October. Species-specific differences in O_3 susceptibility between canopy positions have already been indicated in studies in Europe and the United States (Tjoelker et al. 1993, 1995; Kitao et al. 2009). However, there were big variations in environmental conditions among the previous studies that evaluated O_3 sensitivity, and therefore uncertainties were raised regarding species specific comparisons. On the other hand, the findings of Watanabe et al. (2014a, 2015) were obtained from the same experimental site during the same growing season. In addition, relative light intensities in the lower canopy were similar in the two species examined, at around 10–15 %. Therefore, the results strongly confirm the species specificity in the difference of O_3 sensitivity between upper and lower canopy leaves even in the same environmental conditions.

Watanabe et al. (2014a) estimated the canopy carbon budget for one growing season of *F. crenata* saplings under elevated O_3 (Fig. 5.11). They determined the light-response curve of the photosynthetic rate and respiration rate of leaves in various light conditions. Then the canopy-level photosynthetic carbon gain (PCG) and respiratory carbon loss (RCL) were calculated using a canopy photosynthesis model (Monsi and Saeki 1953; Hirose 2005). The canopy net carbon gain (NCG) was reduced by 12.4 % by the exposure to O_3 (60 nmol mol^{-1} during daytime) during one growing season. The main factor in the O_3-induced reduction in NCG in late summer was the increased RCL. However, the contributions of the reduced PCG and the increased RCL to the NCG were almost the same in autumn. Watanabe et al. (2014a) concluded that contributions of changes in PCG and RCL to NCG under elevated O_3 were different between seasons.

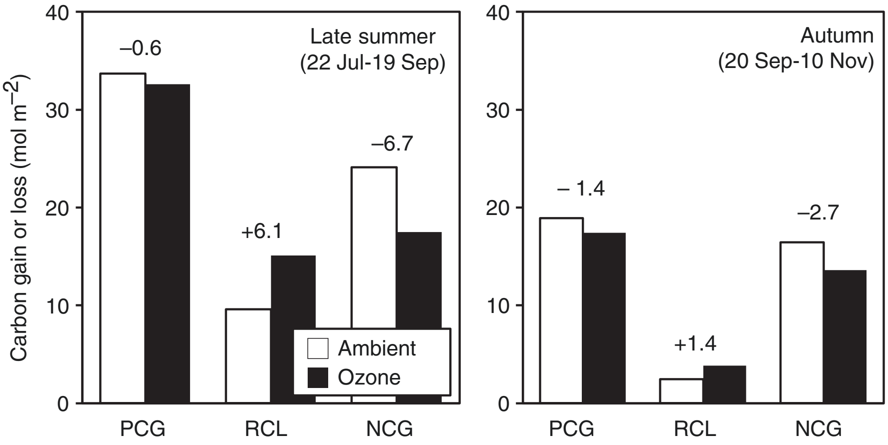

Fig. 5.11 Carbon budget per unit ground area (mol m^{-2}) due to photosynthesis and respiration in canopy of *Fagus crenata* grown under ambient and elevated concentrations of ozone. *PCG* photosynthetic carbon gain, *RCL* respiratory carbon loss, *NCG* net carbon gain. The value on the bar is the difference between ambient and elevated ozone (Data source: Watanabe et al. 2014a)

5.5 Biotic Relations

5.5.1 Defense Capacity Against Biotic Stresses

The defense capacity of plants against disease and insect attack is modified by environmental conditions (e.g. Percy et al. 2002; Oßwald et al. 2012; Watanabe et al. 2014c). There are several lines of evidence showing changes in the concentrations of defense chemicals in leaves under elevated O_3 (Percy et al. 2002; Peltonen et al. 2005). Sakikawa et al. (2016) reported that damage of leaves of *B. platyphylla* saplings by an insect herbivore (mainly by the leaf beetle, *Agelastica coerulea*) was decreased under free-air O_3 exposure. Similar trends were observed in seedlings of the same species by Vanderstock et al. (2016). At the present time, however, information on the effects of O_3 on plant-insect/disease interaction in Japanese forest tree species is very limited. Further research is needed.

5.5.2 Symbiosis with Ectomycorrhizal Fungi

The majority of below-ground root systems in woody plants are symbiotically colonized by ectomycorrhizal (ECM) fungi (Marschner 2012). Up to 30% of the total carbohydrate assimilated through the photosynthetic process can be used in the growth and maintenance of ECM (Hampp and Nehls 2001). In turn, ECM fungi usually act as efficient patronage for the root system of the host plant by absorbing water and essential nutrients, such as phosphorus and sometimes nitrogen (e.g., Quoreshi et al. 2003; Cairney 2011). Ozone usually has negative effects on photosynthetic assimilation. As a result, the amount of carbon allocated to below-ground systems is reduced (Grantz and Farrar 2000; King et al. 2005). These effects are expected to decrease ECM colonization and to affect species-host compatibility. In fact, there is evidence of a reduction in ECM colonization caused by exposure to O_3. In the O_3 fumigation study reported by Yamaguchi et al. (2007, 2010a), the ECM colonization rates (the ratio of ECM fine root tip to all fine root tip) in *F. crenata* seedlings were also determined, and a trend of significant decrease in the ECM colonization rate was found (Fig. 5.12). Wang et al. (2015) also reported a reduction in ECM colonization under elevated O_3 (60 nmol mol^{-1} during daylight hours) in hybrid larch F_1 (*Larix gmelinii* var. *japonica* × *L. kaempferi*) seedlings. Furthermore, they revealed changes in the composition of the ECM community under elevated O_3 – O_3 decreased the fraction of *Tomentella* sp., while the fraction of *Cadophora finlandia* was increased.

5.6 Future Perspectives

A relatively large data set is available for the effects of O_3 on forest tree species in Japan, compared with data for Europe and North America. However, a large part of the data was obtained from juvenile seedlings under controlled chamber conditions

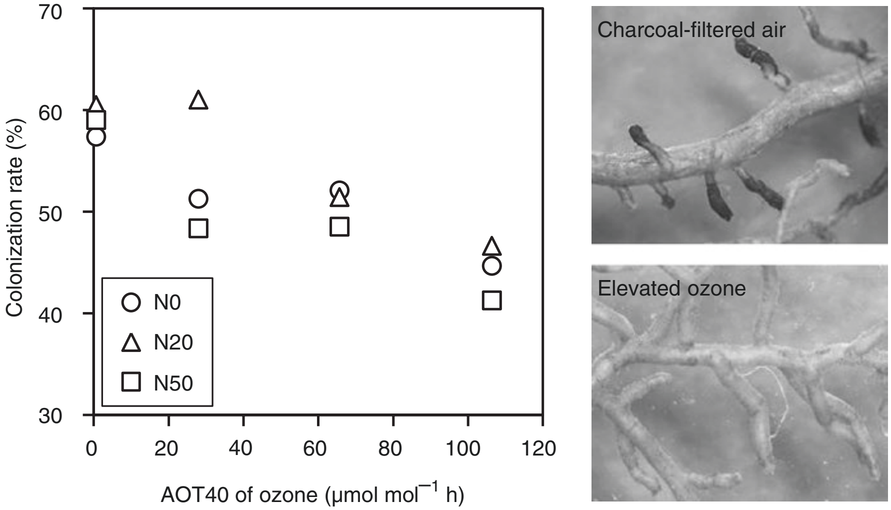

Fig. 5.12 The relationship between accumulated exposure over a threshold of 40 nmol mol^{-1} (AOT40) of ozone and the rate of ectomycorrhizal colonization in the fine root tips of *Fagus crenata* seedlings (*left*). Seedlings were exposed to four levels of ozone in combination with three levels of nitrogen loading: 0, 20, and 50 kg ha^{-1} year^{-1} (N0, N20, and N50, respectively). Fine root tips of *F. crenata* seedlings grown under charcoal-filtered air (*upper* photo) and elevated ozone (*lower* photo). Little ectomycorrhizal colonization was observed in the root tips of *F. crenata* seedlings grown under elevated ozone (Yamaguchi et al. (unpublished data))

with short-term exposure (1–3 years). It is clear that long-term (>10 years) free-air O_3 fumigation study with mature trees at the vegetation scale will be the direction of study in the near future. On the other hand, an epidemiological approach would also be useful for understanding the impact of O_3 on large trees in a forest (e.g. Karlsson et al. 2006). The free-air fumigation method requires expensive experimental facilities for realistic O_3 simulation and only a few individual trees can usually be investigated. Studies for understanding the physiological mechanisms of O_3 effects in an environmental control chamber are, of course, still important.

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