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Effects of Fertilization on Physiological Parameters in American Sycamore (*Platanus occidentalis*) during Ozone Stress and Recovery Phase

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ABSTRACT: American sycamore seedlings were grown in chambers with two different ozone concentrations (O_3-free air and air with additional O_3) for 45 days. Both the control and the O_3 chambers included non-fertilized and fertilized plants. After 18 days of O_3 fumigation, seedlings were placed in a clean chamber for 27 days. Seedlings under ozone fumigation showed a significant decrease in pigment contents and photosynthetic activity, and a significant increase in lipid peroxidation. Fertilization enhanced physiological damage such as the inhibition of photosynthetic activity and the increase of lipid peroxidation under ozone fumigation. During the recovery phase, the physiological damage level of seedlings increased with ozone fumigation. In addition, physiological damage was observed in the fertilized seedlings. Superoxide dismutase (SOD) and glutathione reductase (GR) activities of O_3-treated seedlings increased up to 33.8% and 16.3% in the fertilized plants. The increase of SOD activity was higher in the fertilized plants than in the non-fertilized plants. Negative effects of ozone treatment were observed in the biomass of the leaves and the total dry weight of the fertilized sycamore seedlings. The O_3-treated seedlings decreased in stem, root and total dry weight, and the loss of biomass was statistically significant in the fertilized plants. In conclusion, physiological disturbance under normal nutrient conditions has an effect on growth response. In contrast, in conditions of energy shortage, although stress represents a physiological inhibition, it does not seem to affect the growth response.

Key words: Biomass, Lipid peroxidation, Pigment content, Photosynthetic activity, Recovery phase

INTRODUCTION

Of all the air pollutants in a regional distribution, ozone (O_3) is considered to have the greatest impact on vegetation (Heck et al. 1988). Ozone is a photochemical oxidant, whose concentration has increased steadily since the industrial revolution and will continue to rise if current levels of anthropogenic activity are maintained. Chronic and acute exposures to the pollutant have been shown to stimulate leaf senescence and to decrease photosynthesis and growth in numerous plant species (Heath 1994, Farage 1996, Renaud et al. 1998). While it is generally assumed that ozone produces damage to leaf tissues by moving through stomata and reacting with cell components in the sub-stomatal cavities (Heath 1996), the primary attack site of ozone within leaves has not yet been definitively identified.

Most research has been conducted by adding ozone stress to plants and studying the plants' responses. Fluctuations in plants' ozone stress patterns may indicate a possibility that plants are repeatedly exposed to ozone stress. Thus, much research focuses on how a plant recovers from the ozone stress. For example, several experiments have been conducted on the recovery of the photosynthesis rate from drought stress (Boyer 1971, Heckathorn et al. 1997, Widodo et al. 2003). However, little is known about these recovery processes and the degree of recovery of physiological properties after ozone stress.

An increase in N fertilization may trigger short-term increases in plant photosynthesis and biomass production. According to Pell et al. (1995), an increased nitrogen supply can reduce *Populus tremuloides* seedlings' sensitivity to ozone with regard to biomass accumulation. This was also shown for *Betula pendula* seedlings, where no deleterious effects of O_3 were observed at high nitrogen levels (up to 150 kg N ha^{-1} yr^{-1}) (Pääkkönen and Holopainen 1995). In addition, Thomas et al. (2005) reported that nitrogen with fertilization rates of up to 80 kg N ha^{-1} yr^{-1} increased growth, biomass accumulation, and soluble carbohydrate concentrations, and slightly enhanced starch concentrations.

On the contrary, an excess of N can disturb normal plant metabolism, induce mineral imbalance, reduce frost hardness, and render plants more vulnerable to other environmental stresses (Utriainen and Holopainen 2001). Therefore, a possibility exists that crops may be adversely affected by elevated O_3 concentrations and by excess

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soil N. Leone et al. (1966) and Brewer et al. (1961) found that ozone-induced foliar injury of crop plants was enhanced when plants were grown in relatively high nitrogen treatments. In a recently published paper, Thomas et al. (2005) examined the simultaneous exposure of young spruce trees (*Picea abies*) to ozone and nitrogen fertilization. Ozone proved to have negative impacts on growth as well as starch concentrations in different plant parts. Thomas et al. (2006) also showed that a combined pollutant effect was found for leaf area and shoot elongation, and that ozone fumigation amplified the nitrogen effects.

Consequently, increasing nitrogen loads and ozone levels are likely to decrease tree health in forests by affecting growth, biomass accumulation, carbohydrate concentrations and nutrient ratios, and increasing susceptibility towards drought and insect infestations (Thomas et al. 2006).

However, there are a lot of contradictory results concerning the ozone × nitrogen relationship (Maurer et al. 1997, Bielenberg et al. 2001). Although all plants demand N, they differ greatly in their requirements not only in terms of the concentration that promotes optimum growth but also in their capacity to use the nitrogen that is in soil. Despite the broad knowledge on ozone and nitrogen impacts alone, the combined effects of these two pollutants are poorly understood.

The combination of two factors will, therefore, have a significant impact on the physiological characteristics of trees. So far, few studies have been examined on the simultaneous effects of fertilization and ozone fumigation. We examined the combination of ozone fumigation and fertilization on the physiological parameters of American sycamore seedlings and the possible interactions of these two factors. In addition, we hypothesized mainly synergistic negative impacts on sensitive parameters. Finally, we examined the degree of recovery of those physiological characteristics involved in post-stress repair.

MATERIALS AND METHODS

Plant Material

The open-pollinated seedlings of American sycamore (*Platanus occidentalis*) were produced from seeds collected at the Department of Forest Genetic Resources, Korea Forest Research Institute. Seedlings were germinated in the spring and grown in a greenhouse in plastic containers with a medium of peat, vermiculite and perlite under ambient air temperatures for approximately six months. Six-month-old seedlings that reached a height of approximately 18 cm were transplanted into individual plastic pots (15 cm D × 20 cm H) containing artificial soil which consisted of 1:1:1 sand: peat moss: vermiculite (volume basis).

Fumigation System

Two 3 m × 3 m × 1.8 m (L × W × H, 16.2 m^3) exposure chambers were used in this study. Air was circulated through charcoal filters and O_3 was mixed into the air stream. The ozonated air entered from the bottom of each chamber and exited through the top of the chamber via two exhaust filters. Airflow, mean temperature and relative humidity were about 1 mL sec^{-1}, 25 ± 1℃ and 60 ± 5%, respectively. Air temperature and relative humidity were controlled at 22~24℃ and 60~80% RH. Light for plant growth was shone into the chambers through 1.6 mm thick glass. A photosynthetic photon flux was about 450 μmol m^{-2} s^{-1} and was provided for a 12-h photoperiod at plant height.

Ozone was generated using the Corona Discharge System (Model H450, Harim Engineering, Inc., Korea). Ozone gas was mixed with air filtered by the Zero Air System (Model 701, API, Inc., USA) and was delivered to each chamber with the Gas Exposing System (Model H1800, Harim Engineering, Inc., Korea). O_3 concentration at plant height was continuously monitored with a photometric O_3 analyzer (Model 400, API, Inc., USA), automatically controlled with a PWM (Pulse Width Modulated) system, and calibrated once a week. All measuring values were recorded every three seconds in the HARE 600 data logger (Harim Engineering, Inc., Korea). Control plants were exposed to charcoal-filtered air only.

During the experimental period, average ozone concentrations in the two chambers were 5 ± 1 ppb (control chamber) and 150 ± 10 ppb (fumigation chamber), respectively.

Ozone Exposure and Fertilization

At two weeks after planting, 12 seedlings were transferred into each of the two exposure chambers. Seedlings were exposed over an 18-day period from 31 July to 17 August 2006. Mean ozone concentration for seasonal 8-h daylight (9:00~17:00) was as follows: control, 5 ± 1 ppb; and elevated, 150 ± 10 ppb.

During the growing season, six seedlings of twelve seedlings in control and O_3 treatments were given 200 mL of fertilizer solution (1/5,000 of original solution) once a week. Liquid fertilizer was composed of 10% TN, 4% P, 5% K, 0.05% B, 0.05% Cu, and 0.15% Mn.

After the termination of O_3 fumigation, both the control plants and the O_3-treated plants were placed in a clean chamber to recover from ozone stress for 27 days. Pots were randomized in the greenhouse, watered every day, and moved every two or three weeks moved out the 45-day experimental period to minimize positional effects.

Photosynthetic Pigments

The leaves of both the control and the O_3-treated sycamores

were excised and soaked in DMSO in a glass vial. The vial was tightly capped and incubated in darkness at 70℃ for 2h. The concentration of the extracted pigments (total chlorophyll, chlorophyll a, chlorophyll b, and carotenoid) was calculated based on their absorbance values at 664, 645, and 470 according to Lichtenthaler (1987).

Photosynthetic Parameters

All gas exchange of fully expanded leaves was measured using an infrared gas analyzer (Li-6400, LI-COR, USA). To calculate carboxylation efficiency (*CE*) and the photorespiration rate, the net photosynthetic vs. internal CO_2 partial pressure (*A-Ci*) response curve was measured on fully expanded leaves in each treatment at 0, 50, 100, 200, 300, 360, 400, 500 μmol CO_2 mol^{-1} air (Farquhar et al. 1980, Kim and Lee 2001). Leaf chamber was maintained at 1,100 μmol m^{-2} s^{-1} photon flux density (PFD), 25℃ leaf chamber temperature, and 60% relative humidity. The carboxylation efficiency was determined from the slope of linear regression using the linear portion of *A-Ci* curve (0~200 μmol CO_2 mol^{-1} air); the photorespiration rate was estimated based on the y-intercept of the linear regression (Ro et al. 2001).

The light response curve was measured at 0, 20, 50, 100, 200, 500, 1000, 1500 and 2000 μmol m^{-2} s^{-1} PFD). Leaf chamber was maintained at 25℃ leaf chamber temperature, 60% relative humidity, and 400 μmol CO_2 mol^{-1} air. Apparent quantum yield (*AQY*) was used to calculate photochemical efficiency (*PE*) (Sharp et al. 1984, Evans 1987, Kim and Lee 2001). The apparent quantum yield was determined from a linear regression using the linear portion of 0 to 100 μmol m^{-2} s^{-1} PFD. The dark respiration rate and the light compensation point were estimated based on the y-intercept and x-intercept of the linear regression, respectively.

Lipid Peroxidation

Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) produced by the thiobarbituric acid reaction as described by Heath and Packer (1968). Crude extract was mixed with the same volume of 0.5% (w/v) thiobarbituric acid solution containing 20% (w/v) trichloroacetic acid. The mixture was heated at 95℃ for 30 min and then quickly cooled in an ice-bath. The mixture was centrifuged at 3,000×g for 10 min and the absorbance of supernatant was monitored at 532 and 600 nm. After subtracting the non-specific absorbance (600 nm), MDA concentration was determined by its molar extinction coefficient (155 mM^{-1} cm^{-1}). The results were expressed as μmol MDA g^{-1} FW.

Antioxidant Enzyme Activities

Fresh leaves (0.1 g) were homogenized under ice-cold conditions with 5 mL of 50 mM phosphate buffer (pH 7.0), 10mM ascorbic acid (AsA), and 1.0% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 20,000×g for 30 min and the supernatant was collected for enzyme assays.

Superoxide dismutase (SOD) was assayed on the basis of the inhibition of reduction of nitro-blue tetrazolium in the presence of xanthine at 530 nm according of Beauchamp and Fridovich's method (1971). Activity of glutathione reductase (GR) was assayed as described in Carlberg and Mannervik (1985). The assay was carried out in a reaction mixture containing 50 mM phosphate buffer (pH 7.8), 0.1 mM NADPH, 0.5 mM GSSH, and 0.1 mL enzyme extract. The change in A_{340} was recorded for 5 min after the addition of the enzyme extract. Catalase (CAT) activity was determined by following a two-step procedure (Fossati et al. 1980): The rate of dismutation of H_2O_2 to water and molecular oxygen is proportional to the concentration of catalase. Therefore the sample containing catalase was incubated in the presence of a known concentration of H_2O_2. After incubation for exactly one minute the reaction was quenched with sodium azide. The amount of H_2O_2 racaining in the reaction mixture was then determined by the oxidative coupling reaction of 4-aminophenazone (4-aminoantipyrene) and 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) in the presence of H_2O_2 and catalyzed by horseradish peroxidase (HRP). The resulting quinoneimine dye was measured at 520 nm. The activities of all enzymes were measured using UV-120 (SHIMADZU, Japan).

Biomass

At harvest, shoots and roots were carefully removed, and then thoroughly rinsed twice with distilled water. After drying the tissues at 70℃, shoot and root dry weights were recorded.

Statistical Analysis

The data were statistically analyzed using the SAS System for Windows, Version 8.01 (SAS Institute, USA). Mean values per treatment were compared by GLM. When significant differences ($p < 0.05$) were indicated, Duncan's multiple range tests were performed.

RESULTS

Photosynthetic Pigments

The photosynthetic pigment contents of sycamore seedlings were analyzed at the end of ozone treatment and recovery phase and are given in Table 1.

After 18 days of ozone exposure, pigment content in the leaves of seedlings showed significant ($p < 0.05$) reduction under ozone fumigation, and the reduction rates of pigments were higher in the fertilized plants than in non-fertilized plants (Table 1). In addition,

Table 1. Effects of ozone fumigation and fertilization on pigment concentrations of *Platanus occidentalis*

Treatment	The end of ozone fumigation (mg g^{-1})			Recovery phase (mg g^{-1})		
	Chl a	Chl b	Chl a+b	Chl a	Chl b	Chl a+b
Non-fertilization						
Control	1.38 ± 0.01^{c}	0.37 ± 0.01^{c}	1.75 ± 0.02^{c}	1.49 ± 0.01^{d}	0.37 ± 0.01^{d}	1.86 ± 0.01^{d}
150 ppb	1.20 ± 0.11^{c}	0.33 ± 0.03^{d}	1.53 ± 0.15^{c}	1.62 ± 0.01^{c}	0.45 ± 0.01^{c}	2.07 ± 0.01^{c}
Fertilization						
Control	2.47 ± 0.13^{a}	0.64 ± 0.01^{a}	3.11 ± 0.15^{a}	2.03 ± 0.01^{b}	0.54 ± 0.01^{b}	2.57 ± 0.01^{b}
150 ppb	1.77 ± 0.11^{b}	0.44 ± 0.02^{b}	2.22 ± 0.13^{b}	2.89 ± 0.01^{a}	0.82 ± 0.01^{a}	3.70 ± 0.01^{a}
Ozone (O_3)	***	***	***	***	***	***
Fertilization (F)	***	***	***	***	***	***
$O_3 \times F$	**	***	**	***	***	***

All the values are means of three replicates ± SD; Values in each row followed by the same letter indicate no significant differences ($p < 0.05$) according to Duncan's test; mixed effects linear model: $^{**}p < 0.01$, $^{***}p < 0.001$.

the ozone × fertilization interaction was observed for the content of photosynthetic pigment. At the end of ozone treatment, chlorophyll b content in the leaves of O_3-treated plants without fertilization was significantly lower than that in the control plants (13%), but chlorophyll a and total chlorophyll content of O_3-treated plants was not significantly lower than those of the control plants. In the fertilized plants, the reduction rates of chlorophyll a, b, and total chlorophyll content were 28.3%, 31.3%, and 28.6%, respectively.

In contrast, at day 27 of the recovery phase, chlorophyll a, b, and total chlorophyll content in O_3-treated plants was significantly ($p < 0.05$) higher in both the non-fertilized and fertilized plants than in the control plants. In non-fertilized plants, the chlorophyll a, b, and total chlorophyll content in O_3-treated plants were 8.7%, 21.6% and 11.3% higher than the control plants, respectively, and their increasing rates were higher the fertilized plants than non-fertilized plants. In the fertilized plants, chlorophyll a, b, and total chlorophyll content were 42.4%, 51.9% and 44.0% higher than the control plants, respectively.

Concentrations of chlorophyll a, b, and total chlorophyll in the control and O_3-treated plants were higher at recovery phase than at the end of ozone treatment, except for the control plants with fertilization; the effect of ozone treatment on pigments was more sensitive under fertilization.

Photosynthetic Parameters

At the end of ozone fumigation, photosynthetic parameters showed significant ($p < 0.05$) differences under ozone treatment and fertilization (Table 2). The carboxylation efficiency of O_3-treated plants decreased significantly in comparison with control plants, and the reduction rate was higher in the fertilized plants (42.4%) than in the non-fertilized plants (24.3%). The photorespiration rate was significantly different (28.7% of control) between the control and O_3-treated plants in the fertilized plants. The apparent quantum yields for non-fertilized and fertilized O_3-treated plants were 25.6% and 18.9% of control, respectively.

Under ozone fumigation, both the dark respiration rate and the light compensation point increased significantly relative to control plants in both non-fertilized and fertilized plants. The dark respiration rate increased more in the fertilized plants (76.9% of control) than in the non-fertilized plants (63.9% of control), whereas the light compensation point was higher in the non-fertilized plants (117.1% of control) than in the fertilized plants (96.9% of control). The ozone × fertilization interaction for carboxylation efficiency was observed, but the ozone × fertilization interactions for photorespiration rate, apparent quantum yield, dark respiration rate, and light compensation point were not.

There were no significant differences between treatments during the recovery phase (Table 2), except that the carboxylation efficiency and photorespiration rate showed significant difference between non-fertilized and fertilized plants. The ozone × fertilization interaction for photosynthetic parameters was not observed.

Lipid Peroxidation

The extent of lipid peroxidation was measured as malondialdehyde (MDA) concentration. At the end of ozone treatment, MDA content in the leaves of both non-fertilized and fertilized plants increased significantly ($p < 0.05$) in comparison with control plants (Fig. 1). The increase rate of MDA was higher in the fertilized plants (73.9% of control) than in the non-fertilized plants (33.7% of control).

During the recovery phase, there was no significant difference between control and O_3-treated plants without fertilization, whereas

Table 2. Effects of ozone fumigation and fertilization on photosynthetic parameters of *Platanus occidentalis*

A) The end of ozone fumigation

Treatment	Carboxylation efficiency (mmol CO_2 mol^{-1})	Photo respiration rate (μmol CO_2 $m^{-2}s^{-1}$)	Apparent quantum yield (mmol CO_2 mol^{-1})	Dark respiration rate (μmol CO_2 $m^{-2}s^{-1}$)	Light compensation point (μmol $m^{-2}s^{-1}$)
Non-fertilization					
Control	0.037 ± 0.005^{b}	1.98 ± 0.24^{b}	0.043 ± 0.004^{b}	0.36 ± 0.21^{c}	8.2 ± 4.3^{c}
150 ppb	0.028 ± 0.009^{c}	1.79 ± 0.50^{b}	0.032 ± 0.005^{c}	0.59 ± 0.22^{bc}	17.8 ± 5.1^{b}
Fertilization					
Control	0.059 ± 0.008^{a}	3.03 ± 0.34^{a}	0.053 ± 0.005^{a}	0.78 ± 0.10^{b}	16.3 ± 2.5^{b}
150 ppb	0.034 ± 0.004^{bc}	2.16 ± 0.12^{b}	0.043 ± 0.003^{b}	1.38 ± 0.25^{a}	32.1 ± 5.4^{a}
Ozone (O_3)	***	***	***	***	***
Fertilization (F)	***	***	***	***	***
$O_3 \times F$	**	n.s.	n.s.	n.s.	n.s.

B) Recovery phase

Treatment	Carboxylation efficiency (mmol CO_2 mol^{-1})	Photo respiration rate (μmol CO_2 $m^{-2}s^{-1}$)	Apparent quantum yield (mmol CO_2 mol^{-1})	Dark respiration rate (μmol CO_2 $m^{-2}s^{-1}$)	Light compensation point (μmol $m^{-2}s^{-1}$)
Non-fertilization					
Control	0.031 ± 0.007^{a}	1.81 ± 0.44^{ab}	0.034 ± 0.007^{a}	0.31 ± 0.14^{a}	9.0 ± 2.1^{a}
150 ppb	0.021 ± 0.007^{b}	1.39 ± 0.31^{b}	0.038 ± 0.005^{a}	0.26 ± 0.16^{a}	6.3 ± 3.5^{a}
Fertilization					
Control	0.038 ± 0.009^{a}	2.21 ± 0.58^{a}	0.033 ± 0.007^{a}	0.21 ± 0.16^{a}	5.9 ± 2.6^{a}
150 ppb	0.036 ± 0.010^{a}	2.12 ± 0.62^{a}	0.032 ± 0.014^{a}	0.27 ± 0.25^{a}	5.7 ± 3.1^{a}
Ozone (O_3)	n.s.	n.s.	n.s.	n.s.	n.s.
Fertilization (F)	***	***	n.s.	n.s.	n.s.
$O_3 \times F$	n.s.	n.s.	n.s.	n.s.	n.s.

All the values are means of three replicates ± SD; Values in each row followed by the same letter indicate no significant differences ($p < 0.05$) according to Duncan's test; mixed effects linear model: $^{**}p < 0.01$, $^{***}p < 0.001$.

ozone treatment significantly increased the MDA content of O_3-treated plants with fertilization (56.3% of control).

Antioxidative Enzyme Activities

At the end of ozone treatment, the activities of antioxidative enzymes in the leaves of the seedlings were not significantly different between control and O_3-treated plants, whereas SOD and GR activities showed significant ($p < 0.05$) differences between non-fertilized and fertilized plants (Table 3). SOD and GR activities of fertilized O_3-treated seedlings increased up to 33.8% and 16.3% of control plants.

During the recovery phase, SOD activity of O_3-treated plants increased significantly in comparison with control plants. In addition, the increase rate of SOD activity was higher in the fertilized plants (57.3% of control) than in the non-fertilized plants (22.6% of control). However, GR and CAT activities showed no significant difference.

Biomass

At the end of ozone fumigation, the biomass of the seedlings was not significantly affected by ozone treatment, but fertilization significantly increased the leaf dry weight and the ratio of shoot to root. In addition, the negative effects for ozone treatment were observed at leaf and total dry weight of the fertilized sycamore

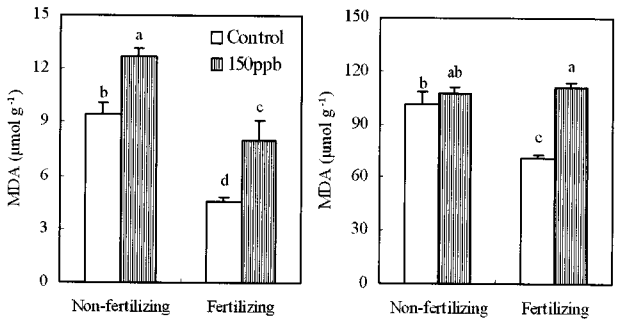

Fig. 1. Effect of ozone fumigation and fertilization on MDA content in the leaves of *Platanus occidentalis*. All the values are means of three replicates ± SD; The same letter indicate no significant differences ($p < 0.05$) according to Duncan's test.

seedlings (Table 4).

During the recovery phase, ozone treatment significantly affected the reduction of the stem, root, and total dry weight of seedlings, and the reduction of biomass was statistically significant ($p < 0.05$) in the fertilized plants. The reduction rates of leaf, stem, and root of the fertilized seedlings were 26.5%, 31.9%, and 34.0% of control plants.

DISCUSSION

Photosynthetic Pigments

The reduction of chlorophyll content is observed generally under ozone treatment, and many studies have reported the decrease of chlorophyll content in the leaves of plants exposed to ozone (Anderson et al. 2003, Calatayud and Barreno 2004, Lee et al. 2006). In our results, the foliar content of chlorophyll decreased under ozone fumigation; specifically, the reduction of chlorophyll a, b, and total chlorophyll content of O_3-treated plants was greater at fertilization than at non-fertilization, because chlorophyll content in control plants increased greatly through fertilization (Table 1). In O_3-treated plants, however, the increase of chlorophyll content was not so large as in the control plants, because the availability of fertilizer in O_3-treated plants might be depressed. Thus, the difference of chlorophyll content between control and O_3-treated plants was obviously greater under fertilization.

During the recovery phase, the chlorophyll content in O_3-treated plants was higher than in the control plants, and was more remarkable in the fertilized plants. This may be a result of the O_3-treated plants showing a compensation response with the disappearance of ozone stress. That is, O_3-treated plants might increase the synthesis of chlorophyll in order to recover the growth reduction, and fertilization might contribute to this recovery process.

Photosynthetic Parameters

Ozone-related decreases in chlorophyll content strongly correlate with reduction in photosynthetic rates. Prolonged exposure to high ozone concentrations resulted in both decreased chlorophyll content and decreased photosynthetic rates (Anderson et al. 2003, Han et al. 2007). However, substantial decreases in photosynthetic rates occurred earlier than did substantial decreases in chlorophyll concentration. This suggests that either the photosynthetic process is highly sensi-

Table 3. Effect of ozone fumigation and fertilization on antioxidative enzyme activities of *Platanus occidentalis*

Treatment	The end of ozone fumigation			Recovery phase		
	SOD (unit g^{-1})	GR (nmol g^{-1})	CAT (unit g^{-1})	SOD (unit g^{-1})	GR (nmol g^{-1})	CAT (unit g^{-1})
Non-fertilization						
Control	554 ± 23^{a}	137 ± 16^{c}	$1{,}786 \pm 80^{ab}$	$1{,}181 \pm 23^{b}$	107 ± 34^{a}	$1{,}783 \pm 31^{a}$
150 ppb	471 ± 34^{ab}	120 ± 21^{c}	$1{,}763 \pm 80^{ab}$	$1{,}448 \pm 52^{a}$	77 ± 21^{a}	$1{,}694 \pm 88^{a}$
Fertilization						
Control	296 ± 80^{c}	196 ± 13^{b}	$1{,}864 \pm 64^{a}$	815 ± 27^{c}	100 ± 27^{a}	$1{,}700 \pm 41^{a}$
150 ppb	396 ± 16^{b}	228 ± 14^{a}	$1{,}662 \pm 27^{b}$	$1{,}282 \pm 152^{b}$	109 ± 27^{a}	$1{,}705 \pm 92^{a}$
Ozone (O_3)	n.s.	n.s.	n.s.	***	n.s.	n.s.
Fertilization (F)	***	***	n.s.	***	n.s.	n.s.
$O_3 \times F$	**	n.s.	n.s.	n.s.	n.s.	n.s.

All the values are means of three replicates ± SD; Values in each row followed by the same letter indicate no significant differences ($p < 0.05$) according to Duncan's test; mixed effects linear model: $^{**}p < 0.01$, $^{***}p < 0.001$.

Table 4. Effect of ozone fumigation and fertilization on biomass and the ratio of shoot to root of *Platanus occidentalis*

(A) The end of ozone treatment

Treatment	Biomass (g)				Shoot : root
	Leaf	Stem	Root	Total	
Non-fertilization					
Control	0.88 ± 0.23^{b}	0.55 ± 0.19^{b}	0.77 ± 0.23^{a}	2.20 ± 0.61^{b}	1.90 ± 0.31^{b}
150 ppb	0.94 ± 0.43^{b}	0.57 ± 0.17^{b}	0.71 ± 0.22^{a}	2.21 ± 0.79^{b}	2.09 ± 0.24^{b}
Fertilization					
Control	1.76 ± 0.59^{a}	0.86 ± 0.23^{a}	1.03 ± 0.36^{a}	3.64 ± 1.16^{a}	2.58 ± 0.30^{a}
150 ppb	1.18 ± 0.30^{b}	0.65 ± 0.18^{ab}	0.67 ± 0.21^{a}	2.50 ± 0.63^{b}	2.81 ± 0.48^{a}
Ozone (O_3)	n.s.	n.s.	n.s.	n.s.	n.s.
Fertilization (F)	**	n.s.	n.s.	n.s.	***
$O_3 \times F$	n.s.	n.s.	n.s.	n.s.	n.s.

(B) Recovery phase

Treatment	Biomass (g)				Shoot : root
	Leaf	Stem	Root	Total	
Non-fertilization					
Control	1.06 ± 0.12^{c}	0.78 ± 0.12^{c}	1.48 ± 0.51^{b}	3.32 ± 0.71^{bc}	1.32 ± 0.31^{b}
150 ppb	0.84 ± 0.18^{c}	0.62 ± 0.14^{c}	1.24 ± 0.27^{b}	2.49 ± 0.51^{c}	1.47 ± 0.35^{b}
Fertilization					
Control	2.49 ± 0.64^{a}	1.44 ± 0.17^{a}	2.00 ± 0.35^{a}	5.93 ± 1.04^{a}	1.97 ± 0.25^{a}
150 ppb	1.83 ± 0.51^{b}	0.98 ± 0.11^{b}	1.32 ± 0.78^{b}	4.13 ± 0.73^{b}	2.17 ± 0.39^{a}
Ozone (O_3)	n.s.	***	**	**	n.s.
Fertilization (F)	***	***	n.s.	***	***
$O_3 \times F$	n.s.	n.s.	n.s.	n.s.	n.s.

All the values are means of three replicates ± SD; Values in each row followed by the same letter indicate no significant differences ($p < 0.05$) according to Duncan's test; mixed effects linear model: $^{**}p < 0.01$, $^{***}p < 0.001$.

tive to changed chlorophyll content or that changes in photosynthetic physiology, other than those directly related to chlorophyll concentration, were expressed in seedlings exposed to the elevated ozone regimes (Anderson et al. 2003).

The initial slope of the *ACi* curve, indicative of carboxylation activity, decreased upon ozone exposure, suggesting a decrease in the activity of the CO_2-fixing enzyme of C_3 plants, ribulose 1, 5-bisphosphate carboxylase/oxygenase (Rubisco). A loss of rubisco activity was also found in experiments carried out with realistic ozone fumigation of various deciduous and evergreen species beech (Lütz et al. 2000), birch (Matyssek et al. 1991), Norway spruce (Wallin et al. 1990) and Scots pine (Skärby et al. 1987).

In our results, ozone fumigation decreased carboxylation efficiency. In particular, the reduction of carboxylation efficiency in the fertilized plants (42.4%) was larger than the reduction of non-fertilized plants (24.3%). This result was consistent with the photorespiration rates. During the recovery phase, however, carboxylation efficiency and the photorespiration rate of the fertilized O_3-treated plants recovered up to the level of the control plants. This phenomenon demonstrates that carboxylation efficiency and photorespiration rate respond sensitively to stress under fertilization.

In addition, at the end of ozone treatment, the apparent quantum yield, dark respiration rate, and light compensation point estimated, based on the light response curve of control and O_3-treated plants, increased remarkably with fertilization. During the recovery phase, all values of O_3-treated plants recovered to control levels.

It is widely recognized that respiratory processes increase in response to ozone (Darrall 1989, Dizengremel and Petrini 1994). Calatayud et al. (2006) suggested that the inhibitory effect of O_3 on the photosynthesis induction reactions depends on N concentration. All results on chlorophyll a fluorescence parameters were in accordance with a greater sensitivity to O_3 among those plants grown with a higher N supply rate (Calatayud et al. 2006). In addition, the rapid recovery of all photosynthetic parameters of the fertilized plants in

our experiment is related to this.

Lipid Peroxidation

MDA content, which represents the state of membrane lipid peroxidation, has been correlated with plants' exposure to O_3 (Ranieri et al. 1996). An increase in lipid peroxidation indicated that damage was occurring at the membrane levels.

In our study, ozone treatment increased MDA content, which manifested as physiological damage, among O_3-treated plants, but fertilization decreased this physiological damage. Calatayud et al. (2006) reported that MDA content was higher in O_3-treated plants and it was in this group that maximum effects occurred with the higher N concentration, confirming oxidative stress. Our result, which showed higher MDA content in the O_3-treated plants with fertilization, was in accordance with the above result.

An extraordinary result was also observed in our experiment, where physiological damage increased abruptly at recovery phase when compared with the result at the end of ozone fumigation. This may be indicative of the potential damage accumulated during ozone treatment represented during the recovery phase.

Enzyme Activities

The changes of antioxidative enzyme activities that scavenge the active oxygen species generated by ozone toxicity were obviously observed under fertilization. This means that fertilization plays an important role in these species. This effect was particularly evident in SOD and GR activity; SOD activity effectded sensitively during ozone treatment and recovery phase, while GR activity changed only during ozone treatment. CAT did not show any effect for treatment.

The detoxifying system, known to be present in plant cell compartments including chloroplasts, is activated upon ozone exposure (Kangasjärvi et al. 1994, Noctor and Foyer 1998, Willekens et al. 1994). Total SOD activity is thus increased in ozone treated birch (Tuomainen et al. 1996), Norway spruce trees (Schubert et al. 1997), and maple trees (Han et al. 2007). It is, however, to be noted that conflicting results about increased SOD activity in ozone- exposed trees exist.

Biomass

During ozone treatment and recovery, the growth of non-fertilized plants remained unaffected, but the fertilized plants responded to ozone fumigation because the shortage of metabolic energy source among non-fertilized plants precluded physiological damages generated by ozone fumigation. However, fertilized control plants grew normally, while O_3-treated plants showed significant difference due to growth inhibition by physiological damage.

Ozone-induced depression in biomass was observed in O_3-treated plants grown with higher nitrogen supply but not in those grown with limited nitrogen. This observation could reflect compensation for lower levels of nitrogen or the inability to detect changes in biomass due to the reduced weight of plants grown at the lowest nitrogen supply (Pell et al. 1990). Others have also reported that both foliar injury and plants' biomass response to O_3 were more pronounced when plants were grown with an adequate nitrogen supply (Leone et al. 1966, Ormrod et al. 1973).

Nitrogen had a significant positive impact on the shoot growth of spruce saplings. These findings are consistent with various other publications, e.g. Kainulainen et al. (2000) or Utriainen and Holopainen (2001a), where additional nitrogen also enhanced shoot growth in *Picea abies*. Similar findings have been published for other conifer species, e,g, *Pinus sylvestris* (Manninen et al. 2002, Utriainen et al. 2001, Utriainen and Holopainen 2001b). Our findings of the negative growth effect caused by ozone are also in accordance with the findings of other groups (Karlsson et al. 1996, Ottoson et al. 2003, Wallin et al. 2002).

In our study, nitrogen fertilization and ozone fumigation did not interact, which means that the observed effects can be seen as additive, where nitrogen fertilization might level off the negative impact of ozone on shoot growth. This is in accordance with Utriainen and Holopainen (2001a) or Kainulainen et al. (2000), who also found no interaction of ozone and nitrogen on growth of *Picea abies*, or *Pinus sylvestris*.

Accordingly, the optimum energy condition for normal metabolism is needed to understand the exact physiological responses of plants under stress. Physiological disturbance under normal conditions has an effect on growth response. In contrast, in conditions of energy shortage, although stress represents a physiological inhibition, it does not seem to affect the growth response.

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