# Ecophysiological difference in co-existing beech and oak saplings grown in different soil types under a free-air ozone exposure system

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ORIGINAL ARTICLE

# Ecophysiological difference in co-existing beech and oak saplings grown in different soil types under a free-air ozone exposure system

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**ABSTRACT**

To know effects of elevated ozone (eO$_3$) response to beech (*Fagus crenata*) and oak (*Quercus mongolica* var. *crispula*) saplings, focusing on shoot elongation and leaf nutrient characteristics, beech and oak were grown in a free-air ozone-enrichment system (concentration: 35 ppb (aO$_3$) vs. 75 ppb (eO$_3$)) for two years under different soils (volcanic ash soil vs. brown forest soil). This treatment resulted in lower O$_3$ tolerance in beech than in oak. The ratio of 2$^{nd}$ shoot flush showed a characteristic response to eO$_3$ fumigation in beech and oak. For beech, the ratio was less than 0.5, while for oak, it ranged between 0.4 and 0.6. There was no difference in the photosynthetic rate of oak, although the photosynthetic rate of beech was slightly lower under elevated O$_3$. Principal component analysis provided a good summary of the traits of beech and oak in response to O$_3$ enrichment and soil combination, with O$_3$-enriched beech characterized by higher Ca and lower N, P, K and $A_{max}$ in the 1$^{st}$ component and lower Mg and Mn in the 2$^{nd}$ component. O$_3$ -treated oak in O$_3$-enriched brown forest soils was characterized by high N, P, K and $A_{max}$, while the influence of the 2$^{nd}$ component was small. Increased soil acidification will increase soil Mn and Al concentrations and may reduce the photosynthetic activity of beech saplings in eO$_3$.

## Introduction

Due to Asia' rapid economic growth, nitrogen dioxide (NO$_2$) as a precursor of ozone (O$_3$) exhibited levels that were 4 times higher than those of the European Union (EU) and the United States (U.S.A.) in 2020 (Feng et al. 2019; Kurokawa and Ohara 2020). Several reports indicate that current O$_3$ concentrations (approximately 60 ~ 80 ppb) could reduce final crop production by 30 ~ 60% (Koike et al. 2013; Feng et al. 2019).

Representative afforestation species of Sugi-cedar (*Cryptomeria japonica*) and Hinoki-cypress (*Chamaecyparis obtusa*) are more O$_3$-tolerant than beech (*Fagus crenata*) and oak (*Quercus serrata*, and *Q. mongolica* var. *crispula*), which represent the temperate zone, are less tolerant than these conifers (Yamaguchi et al. 2011). The gas-flux data revealed a decline in beech stands in northern Japan have declined but one of the dominant oak species stands remained unchanged (Kitao et al. 2016). In open-system O$_3$ experiments, Kitao et al. (2016) found that *Q. serrata* seedlings exhibited an increase secondary shoot elongation, while *Q. mongolica* seedlings displayed a slight increase in leaf mass. These results indicate differing interspecific responses to elevated O$_3$ (eO$_3$), which may be compensatory growth for growth inhibition. It is also possible that 2$^{nd}$ shoot elongation (flush) is inhibited by eO$_3$.

Based on a greenhouse-like open-top chamber (OTC) using regular glass (with no UV transmittance) on potted seedlings, it was observed that beech seedlings are more sensitive to eO$_3$ than oak species when it comes to biomass productivity (Matsumura 2001; Yamaguchi et al. 2011). Additionally, it was found that the sensitivity of beech to O$_3$ increases with an increase of nitrogen (N) deposition (Yamaguchi et al. 2007). These reports suggest that sensitivity to O$_3$ may be strongly influenced by soil types. Beech requires moderately fertile soils, whereas oak is relatively tolerant to drought (Matyssek et al. 2012; Leuschner and Ellenberg 2017). These edaphic responses of the two species suggest that they have different access to nutrient elements associated with shoot growth (Schulze et al. 2019). Nutrient conditions affect growth and photosynthesis, but $A_{max}$ at light and CO$_2$ saturation directly reflects the effect of nutrients in the plant body, excluding stomatal effects (Kitaoka et al. 2009). Q3

The additional deposition of N, as well as the eO$_3$, can reduce the allocation of photosynthates to the roots and their amount in many plant species (Agathokleous et al. 2015; Wang et al. 2016; Paoletti et al. 2020), including European beech (Haberer et al. 2008), Siebold's beech, and two oaks (*Quercus serrata* and *Q. mongolica*) native to Japan (e.g. Hoshika et al. 2015). These suggest significant changes in nutrient acquisition by the root system under eO$_3$. It is essential to understand the photosynthetic response of dominant tree species to eO$_3$ in relation to the shoot development patterns as well as soil types, especially nutrients and drought (Haberer et al. 2008; Hoshika et al. 2013; Agathokleous et al. 2015a; Agathokleous et al. 2015b). Q4

A stoichiometry study revealed the important role of re-translocation of foliar nutrients of three deciduous tree species (birch, beech, oak) grown in different soil types at eO$_3$ (Shi et al. 2017); however, the nutrient composition and the photosynthetic capacity of beech and oak tree saplings have not been examined (Watanabe and Yamaguchi 2011; Watanabe et al. 2013, 2018; Kitao et al. 2016). Plant sensitivity to O$_3$ is affected by soil types as well as specific nutrient balance, including Q5

symbiotic microbial activities (Watanabe et al. 2011; Matyssek et al. 2012; Wang et al. 2015). Furthermore, water-soluble elements are strongly influenced by soil moisture conditions, especially for beech species via stomatal regulation (Matyssek et al. 2012; Tobita et al. 2019). However, the growth functions of these deciduous broadleaved trees under e$O_3$ are influenced by nutrients (Shi et al. 2016).

This study aims to demonstrate the relationship between nutrient conditions, excluding N, such as representative micro-nutrients, and their effects on plant growth and $A_{max}$. According to previous research (Wang et al. 2015), even in the presence of e$O_3$, phosphorus (P) and manganese (Mn) are expected to have a positive correlation with the $A_{max}$ of beech and oak. This is because both elements are essential, in addition N, for photosynthesis under neutral soil pH (e.g. Kitao et al. 1999).

For this prediction, we measured seasonal changes in leaf mass per unit area (LMA) and leaf chlorophyll content (via SPAD) to determine the leaf maturity time of beech and oak saplings planted in two soil types. Subsequently, we observed 2$^{nd}$ or 3$^{rd}$ shoot growth and examined the $A_{max}$ and nutrients in the leaves. Based on the secondary shoot growth in summer and the correlation between nutrient concentrations and $A_{max}$, our data can enhance our understanding of how nutritional differences affect $O_3$ sensitivity in beech and oak.

## Materials and methods

### Plant materials

In mid-July 2014, 2-year-old seedlings of Siebold's beech (*Fagus crenata*; hereafter beech) and oak (*Quercus mongolica* var. *crispula*; hereafter oak), which were obtained from Hokkaido Holti-green Co. Ltd. near Sapporo, were planted at the nursery of the Sapporo Experimental Forest, Hokkaido University, northern Japan (43°04' N, 141°20' E, 15 m a.s.l.). The first tree is a typical late successional species characterized by flush growth of shoot development whereas the second tree is a mid-late successional tree species with a "two to three times flush growth of shoot. Intraspecific competition often occurs between the two species due to differences in their shoot structure (Kashimura 1978; Yagihashi et al. 2003; Kitao et al. 2019).

The seedlings were exposed to an experimentally enhanced $O_3$ regime with a free-air $O_3$ enrichment system ($O_3$-FACE) (Shi et al. 2017) for two growing seasons. The details of $O_3$-FACE are elaborated in the following subsection. Monthly sampling of 4 saplings per species for each treatment (8 treatments in total) was performed with 3 replications during the $O_3$-FACE operated snow-free period. The height of the trees varied between 2.1 m for beech and 2.3 m for oak under ambient $O_3$ (a$O_3$) and 1.7 m for beech, 2.5 m for oak under e$O_3$, respectively. The $O_3$ exposure took place between early August and mid-November of 2014, from mid-June to early December of 2015, and from mid-June to the end of November of 2016, excluding the snow season. Sapporo's annual average temperatures and total precipitation for 2014, 2015, and 2016 were 8.2°C and 1129 mm, 10.3°C and 1331 mm, and 9.3°C and 1331 mm, respectively.

Table 1. Soil element (nutrient) conditions at free-air ozone exposure site with two types of soil. Brown forest soil (BF); volcanic ash soil (VA). Ambient (Amb); ozone ($O_3$).

| element | mean (SE) | | | | p-value | | |
|---|---|---|---|---|---|---|---|
| condition | Amb-BF | Amb-VA | $O_3$-BF | $O_3$-VA | $O_3$ | soil | $O_3 \times$ soil |
| pH | 5.24 (0.03) | 4.26 (0.04) | 5.32 (0.02) | 4.57 (0.04) | - | - | - |
| N | 2.98 (0.02) | 2.47 (0.29) | 2.64 (0.13) | 2.34 (0.05) | 0.162 | 0.044* | 0.527 |
| P | 2.79 (0.29) | 2.01 (0.03) | 2.65 (0.25) | 2.04 (0.14) | 0.632 | 0.022* | 0.682 |
| K | 21.65 0.92) | 22.2 (1.05) | 23.33 (1.45) | 23.18 (1.51) | 0.363 | 0.763 | 0.784 |
| Ca | 247.97 (42.98) | 209.87 (34.40) | 295.5 (12.90) | 273.18 (22.08) | 0.290 | 0.392 | 0.799 |
| Mg | 20.23 (1.63) | 15.46 (1.02) | 24.51 (1.08) | 20.33 (0.70) | 0.023* | 0.013* | 0.805 |

Unit of element: N (mg g$^{-1}$) and the rest elements (mg 100 g$^{-1}$)

### Treatments

The experimental site consisted of 6 circular plots, of which 3 were used for (a$O_3$) and 3 for e$O_3$. Each circular plot was 6.5 m in diameter with 5.2 m high dispersal pipes surrounding the circle (Kim et al. 2011). The distance between a$O_3$ plots and e$O_3$ plots was about 40 m. The method of $O_3$ exposure applied in this study was based on the system used in the Kranzberg Forest, Germany (Nunn et al. 2005).

We produced $O_3$ in the field with an $O_3$ generator (Fuji, Tokyo, Japan). Watanabe et al. (2013) described the free-air $O_3$ fumigation ($O_3$-FACE) field station in detail. The $O_3$ was supplied from the upper part of FACE with Teflon-coated tubes at 80 cm intervals with 0.3 mm holes at 50 cm intervals. In order to simulate the high $O_3$ concentration in the near future under natural conditions, the $O_3$ concentration of the e$O_3$ plots was regulated as 75 ± 8 nmol mol$^{-1}$ during daylight hours (about 8 h d$^{-1}$), while the a$O_3$ plots were subjected to daytime $O_3$ concentration of 30 ± 4 nmol mol$^{-1}$ as the simulation of the current $O_3$ condition in urban centers. These were monitored by the $O_3$ monitor (Type 408, 2B Technologies, Boulder, CO, U.S.A.)

Each plot in the experimental system was divided into two sections and set with 2 different soil types: brown forest soil (BF; Dystric Cambisol), which acted as control in this study and was original soil of the nursery, and volcanic ash soil (VA; Vitric Andosol), which was a mixture collected from the Tomakomai Experimental Forest of Hokkaido University, located 60 km south of Sapporo, at about a 25 cm surface depth supported the nutrient absorption by the fine roots of two tree species (Shi et al. 2017). BF soil is a fertile soil type containing sufficient nutrients necessary for plant growth (Kim et al. 2011; Kayama et al. 2011). The VA soil was mixed with immature volcanic ash soil, which is considered highly acidic with a pH of around 4.0; it contains high levels of aluminum (Al) and iron (Fe), but low levels of primary and secondary nutrients (Watanabe et al. 2010; Kayama et al. 2011). The pH of the soil mixture was about 4.38. More details on soil nutrients and pH are given in Table 1. pH was measured with a portable sensor (LAQUA D-220P-S, Horiba, Kyoto, Japan) in each soil type where beech and oak saplings were grown.

### Measurements

Shoots of beech and oak trees can elongate multiple times during the summer season (Kashimura 1978). To assess the impact of $O_3$ and soil types on this process, we monitored 100–120 cm shoots located from stem base of individuals in each treatment, recorded the secondary elongation of shoots as a ratio of total individuals, and evaluated the results.

The leaf greenness was detected using a SPAD chlorophyll meter (Minolta, Tokyo, Japan) (Eguchi et al. 2006), and leaf mass per unit area (LMA) was used for leaf maturity (Watanabe et al. 2018). For oak, the mean SPAD value was obtained from three leaves from the base of the shoot in the 2nd flush. We also observed the shoot development (leaf flush, new shoot development, leaf yellowing) of beech and oak. Fresh leaves were collected from the second or the third leaf counted from the top of a shoot of beech or oak exposed to sunlight. All collected leaf samples were dried at 70°C for 5–7 days after rinsing the surface with distilled water and ground to a powder in a mill. For both beech and oak, three individuals were selected for each treatment, and one leaf per individual was tested.

The gas exchange rate of mature sun leaves of beech and the first flush of sun leaves of oak was measured during July and August 2016 using with an open gas exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, U.S.A.) to adjust leaf age of around 70 ~ 80 days at peak (Koike 1990). Four saplings were randomly selected in each gas plot ($aO_3$ vs. $eO_3$) under two soil types (BF soil vs. VA soil) of each species, and one leaf of each selected tree was used for gas exchange measurement. The gas exchange rates were measured between 08:00 and 15:00 hours (except 13:00 ~ 14:00) to avoid mid-day depression. The leaf temperature and photosynthetic photon flux density during the measurement were maintained at $25.0 \pm 0.5°C$ and $1500 \, \mu mol \, m^{-2} \, s^{-1}$, respectively. The leaf-to-air vapor pressure deficit (VPD) was $1.5 \pm 0.2$ kPa. To obtain the intercellular $CO_2$ concentration ($C_i$) response curve of the net photosynthetic rate ($A$) ($A/C_i$ curve), $A$ was determined at 10 $CO_2$ concentration levels in the chamber ($C_a$, 60–1700 $\mu mol \, mol^{-1}$). Basic measurement procedure of $A/C_i$ curve was following to Long and Bernacchi (2003).

We determined $A$, stomatal conductance, and the ratio of $C_i$ to $C_a$ at $C_a = 390 \, \mu mol \, mol^{-1} \, CO_2$ ($A_{sat}$, $G_s$, and $C_i/C_a$, respectively). The maximum rates of carboxylation ($Vc_{max}$) and electron transport ($J_{max}$) were calculated from the A/Ci curve (Farquhar et al. 1980; Long and Bernacchi 2003). Values of the Rubisco and Michaelis constants for $CO_2$ ($K_c$) and $O_2$ ($K_o$), and the $CO_2$ compensation point in the absence of dark respiration ($\Gamma^*$) for analysis of the $A/C_i$ curve were according to Long and Bernacchi (2003). All A/Ci fitting parameters were calculated by the Plant-Ecophys package of R language (Duursma and Struik 2015). Since $Vc_{max}/J_{max}$ is almost constant in young trees under $eO_3$ (Watanabe et al. 2018), $A_{max}$ is regarded as an adequate parameter for estimating the photosynthetic capacity of both tree species. In addition, we can evaluate the potential capacity of photosynthesis without any stomatal regulation under $CO_2$ and light saturation like slach1 mutant (Saji et al. 2017).

To measure the concentrations of Magnesium (Mg), potassium (K), Calcium (Ca), Mn, 50 mg leaf samples were digested with nitric acid and hydrogen peroxide. P was determined according to the Bray II method (Wuenscher et al. 2015). Analysis of leaf concentrations was then performed using an inductively coupled plasma mass spectrometer (ICP-MS; IRIS/IRIS Advantage ICAP, Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.). The combustion method with an NC analyzer (NC-900, Sumica, Osaka, Japan) was used to measure the N concentration in the leaf samples.

### Statistics

All statistical analyses were performed using by R language (R Core Team 2016). Two-way analysis of variance (Two-way ANOVA) was used to test the effects of $O_3$ fumigation and the soil types as well as their combinations. The relationship between photosynthetic traits and leaf nutrient elements was tested by principal component analysis (PCA). Before applying ANOVA, homoscedasticity, normality and outliers were checked. The significance levels were set at $p < 0.05$, although we suggested $p$-values from 0.05 to 0.10 to indicate marginal significance.

## Results

### Soil types

The pH of soils at the study site, specifically BF and VA, were 5.25 and 4.38, respectively (see Table 1). The concentrations of nitrogen (N) and phosphorus (P) in leaf tissue were significantly greater in BF soil compared to VA soil ($p < 0.05$). Additionally, Magnesium (Mg) concentration was higher in BF soil than in VA soil ($p < 0.05$), particularly under $eO_3$ treatment. No interaction effects between soil and $O_3$ were detected concerning N, P, and Mg. There were no significant differences in K, Mn, and Al concentration in leaf tissue between $O_3$ levels and soil types. Moreover, measurements of phenological observations, SPAD, LMA, and photosynthetic traits were taken. Beech species flush their leaves uniformly in early May, and the emergence of new leaves doesn't occur until the leaves begin to senesce around mid-to-late September. The SPAD value sharply increased in mid-June, gradually continued to increase through mid-July, and remained unchanged at the beginning of September. Based on Figure 1, the SPAD value of beech in $aO_3$ was slightly higher than that in e $O_3$, regardless of soil type, during July and early August.

### Phenological observation, SPAD, LMA and photosynthetic traits

Beech flushed leaf in early May, followed by a senescence period from mid-to-late September, except very few under $aO_3$ in July. The SPAD of beech showed a sharp increase in mid-June, followed by a gradual increase in mid-July, and remained constant at the start of September. During July and early August, the SPAD in $aO_3$ was marginally higher than that in $eO_3$, regardless of the soil type (see Figure 1).

In mid-May, oak partially displayed 3–5 leaves as the 1st flush, and by early June, it successfully developed 3–4 leaves as the 2nd flush. No difference in the SPAD value was observed between the 1st and 2nd flushes of oak. Furthermore, in August, the LMA value of beech tended to be larger independent of soil type at e $O_3$ ($p = 0.11$).
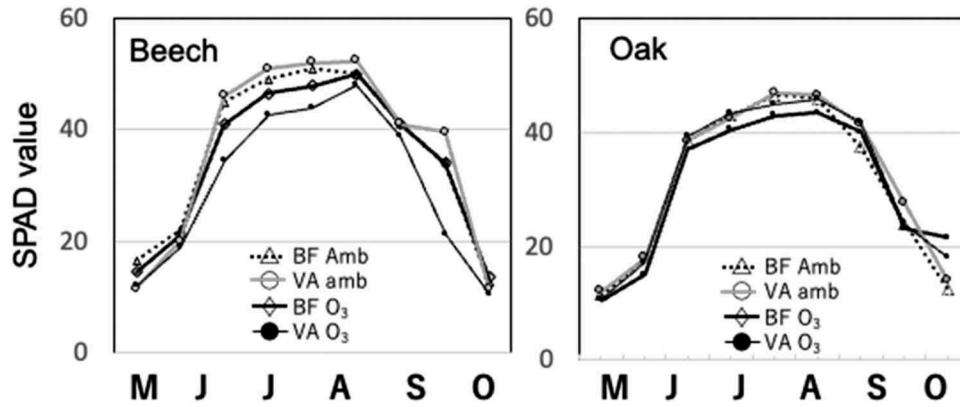
**Figure 1.** Seasonal changes in SPAD (chlorophyll) of beech and oak leaves exposed to ambient vs. elevated $O_3$ grown in brown forest soil mixed with volcanic ash soil. Different line colors indicate different treatments, ambient with brown forest soil (BF Amb, blue), ambient with volcanic ash soil (VA Amb, gray), ozone fumigation with brown forest soil (BF $O_3$, orange), and ozone fumigation with volcanic ash soil (VA $O_3$, sunlight yellow).

Regardless of soil type, an increase in LMA was seen in oak under $eO_3$ conditions during the early growing season. However, there were no significant differences in LMA during leaf senescence or after early frost past mid-September. Leaf senescence of oak in the 1st and 2nd flush began in early October and mid-October, respectively.

The ratio of 2nd flush demonstrated a distinctive response to $O_3$ exposure in beech and oak, as shown in Figure 2. Regardless of soil, one to two individuals of beech exhibited 2nd flush in mid-July in $aO_3$. No 2nd flush was observed in August. In contrast, 3 to 4 oak individuals per ring showed 2nd flush in July in $aO_3$, 1 to 2 trees in $eO_3$ showed fall growth in July regardless of soil, and 1 to 2 trees in $eO_3$ showed 2nd flush in August regardless of soil and $O_3$ levels.

During the leaf senescence period, LMA gradually decreased, ranging from 62 to 63 g m$^{-2}$ around August to 56–58 g m$^{-2}$ towards mid-October ($P < 0.05$). The decline of LMA in both beech and oak trees was higher in $aO_3$ surroundings as compared to $eO_3$, regardless of soil types, towards October. In 2nd flush of oak growth, the mean LMA of three leaves from the bottom of the shoot, grown in VA, was approximately 5 g m$^{-2}$ larger and maintained the highest value of 63–66 g m$^{-2}$ in early October when the leaves were destroyed by early frost.

In beech trees, $A_{max}$ tended to decrease with exposure to $eO_3$ (Table 2). Additionally, both $Vc_{max}$ and $J_{max}$, which are biochemical characteristics of photosynthesis, were significantly reduced by $eO_3$. However, there were no significant effects observed within the BF and VA soil. In contrast, oak did not show a significant decrease in $A_{max}$ due to $eO_3$, and no significant effects of $eO_3$ were observed in $Vc_{max}$ and $J_{max}$, as shown in $A_{max}$. Additionally, there were no significant differences between BF and VA soils.

### Principal component analysis (PCA)

The PCA indicated that the 1st principal component (pc) had a contribution ratio of 0.51, whereas the 2nd pc contributed 0.22. The 1st pc comprised of $A_{max}$ and N, P, K with Ca as the 2nd pc. When N, P, K, and $A_{max}$ were high, the score was low, and the value of Ca was greater when the score was high.

The 2nd pc score was defined by the secondary elements Mg, as well as the trace elements of Mn and Al. Higher scores indicated higher values for Mn and Al, while larger values for Mg indicated lower values for the second primary component score.

Beech was more easily distinguished by $eO_3$ than oak. $O_3$ exposure with BF resulted in lower levels of N, P, K, and $A_{max}$, as well as higher Ca content, as characterized by the 1st pc having little influence on the 2nd pc. Similar trends were observed in $eO_3$ with VA. Additionally, the N, P, K, and $A_{max}$ were high in $aO_3$ with BF.

Oak in $aO_3$ with both BF and VA exhibited comparable responses, with the small influence of the 1st pc and the amount of Mg in the 2nd pc being the defining characteristics. Oak exhibited lower variation within treatments in the 1st pc, while the differences in treatment were
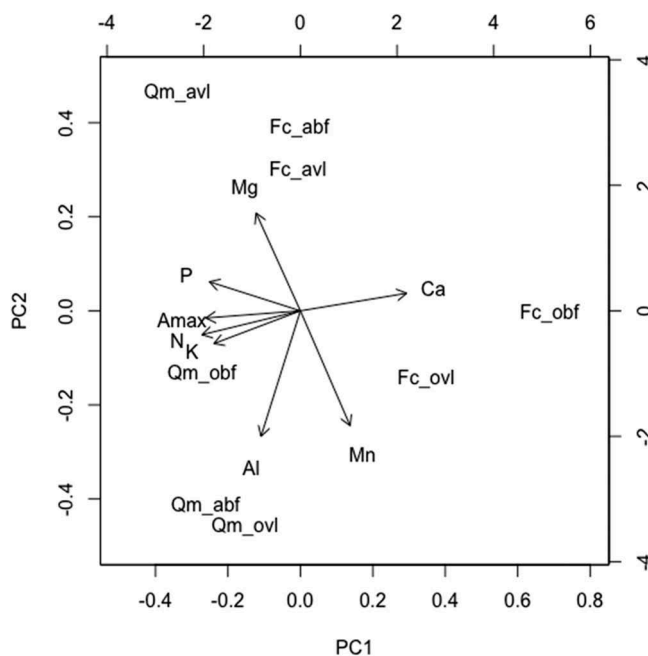


**Figure 2.** Results of principal component analysis of beech and oak grown under both ambient and $O_3$ fumigation treatments. Abbreviations: al: leaf aluminum concentration, $A_{max}$: photosynthetic rates at light and $CO_2$ saturation, Ca: leaf calcium c., Fc_abf: beech grown under $aO_3$ with brown forest (BF), Fc_avl : beech grown under $aO_3$ with volcanic ash (VA) soil, Fc_obf: beech grown under $eO_3$ with BF, Fc_ovl: beech grown under $eO_3$ with VA, K: leaf potassium c., Mg: leaf magnesium c., Mn: leaf manganese c., N: leaf nitrogen c., P: leaf phosphorous c., Qm_abf: oak grown under $aO_3$ with BF soil, Qm_avl: oak grown under $aO_3$ with VA, Qm_obf: oak grown under $eO_3$ with BF, Qm_ovl: oak grown under $eO_3$ with VA.

**Table 2.** Leaf nutrients and $A_{max}$ of beech and oak trees are grown under factorial combinations of two $O_3$ levels and two soil types.

| Sp. | | Mean (SE) | | | | F-value of two-way ANOVA | | |
|---|---|---|---|---|---|---|---|---|
| | | Amb-BF | Amb-VA | $O_3$-BF | $O_3$-VA | $O_3$ | Soil | $O_3 \times$ soil |
| Beech | $A_{max}$ | 332.83 (26.92) | 353.33 (43.62) | 214.55 (17.37) | 291.61 (42.56) | 4.23† | 1.56 | 0.52 |
| | $J_{max}$ | 125.89 (7.32) | 130.97 (2.52) | 96.37 (6.04) | 101.52 (7.55) | 22.66** | 0.68 | <0.01 |
| | $Vc_{max}$ | 48.95 (4.17) | 54.66 (2.12) | 37.63 (3.58) | 41.99 (5.25) | 9.25* | 1.63 | 0.03 |
| | N | 43.65 (2.48) | 44.77 (1.62) | 33.4 (1.45) | 38.00 (2.53) | 10.66** | 1.12 | 0.45 |
| | P | 3.76 (0.19) | 3.35 (0.14) | 2.56 (0.16) | 3.19 (0.28) | 8.54* | 0.11 | 4.55 |
| | K | 5.38 (0.73) | 5.83 (0.86) | 4.87 (0.43) | 4.93 (0.27) | 0.96 | 0.14 | 0.08 |
| | Mg | 3.8 (0.18) | 4.13 (0.32) | 3.38 (0.38) | 2.90 (0.27) | 6.92* | 0.04 | 1.53 |
| | Al | 0.19 (0.01) | 0.22 (0.02) | 0.24 (0.01) | 0.26 (0.01) | 16.53** | 4.03† | 0.34 |
| | Mn | 0.43 (0.02) | 0.49 (0.04) | 0.63 (0.03) | 0.60 (0.04) | 15.66** | 0.19 | 1.41 |
| | Ca | 19.15 (1.20) | 19.4 (1.18) | 25.53 (0.15) | 20.95 (1.85) | 5.57* | 1.91 | 2.38 |
| Oak | $A_{max}$ | 392.09 (55.49) | 342.51 (24.72) | 332.40 (34.64) | 339.06 (42.05) | 0.38 | 0.36 | 0.40 |
| | $J_{max}$ | 135.10 (8.10) | 134.47 (3.22) | 127.71 (7.92) | 136.85 (10.74) | 0.10 | 0.29 | 0.38 |
| | $Vc_{max}$ | 46.88 (2.90) | 47.97 (1.49) | 48.77 (2.26) | 50.23 (3.47) | 0.62 | 0.23 | <0.01 |
| | N | 45.63 (4.51) | 42.8 (0.78) | 46.03 (1.28) | 47.2 (2.43) | 0.41 | 0.05 | 0.30 |
| | P | 3.46 (0.42) | 4.25 (0.20) | 3.46 (0.48) | 3.97 (0.34) | 0.01 | 1.95 | 0.1 |
| | K | 10.68 (1.24) | 12.1 (0.25) | 14.57 (1.39) | 9.90 (0.99) | 0.22 | 1.50 | 5.31* |
| | Mg | 3.79 (0.18) | 4.11 (0.25) | 3.40 (0.38) | 2.96 (0.23) | 5.82* | 0.04 | 1.36 |
| | Al | 0.45 (0.14) | 0.21 (0.01) | 0.34 (0.14) | 0.36 (0.07) | 0.001 | 0.71 | 0.96 |
| | Mn | 0.64 (0.03) | 0.34 (0.02) | 0.43 (0.05) | 0.66 (0.03) | 2.13 | 0.69 | 49.3** |
| | Ca | 15.78 (0.29) | 14.58 (1.22) | 14.00 (0.75) | 15.98 (1.06) | 0.002 | 0.05 | 1.69 |

Q14   The mean values and results of the two-way ANOVA of $A_{max}$ between nutrient elements of beech and oak leaves grown by combination of $O_3$ fumigation and different soils as shown in table 1. SE, standard error; $A_{max}$, maximum photosynthetic rate (nmol $g^{-1}s^{-1}$; under light and $CO_2$ saturation). N measured in mg $g^{-1}$, other nutrients (P, K, Mg, al, Mn, and Ca) in mg 100 $g^{-1}$. Symbols on the values indicates significant differences, †$p < 0.1$; *$p < 0.05$; **$p < 0.01$.

distinguished by the variation in the 2$^{nd}$ pc. No discernible pattern was observed for e$O_3$. Oak trees in a$O_3$ with BF and e$O_3$ with VA soils were less impacted by the 1$^{st}$ pc. Moreover, in e$O_3$ with forest soils, oaks were identified by their Al content.

## Discussion

Previous studies have reported that various tree species with distinct shoot development patterns – flush type, succeeding growth type, and intermediate type – display unique resource retranslation characteristics in temperate regions (Larcher 2003; Watanabe et al. 2013; Shi et al. 2017). According to data from SPAD and LMA (Figure 1), beech leaves reach leaf maturation under e$O_3$ within 1.5 months after emergence, while oak leaves complete maturation in August (Figure 2). Although research indicates that oak has a higher tolerance for $O_3$ than beech (Yamaguchi et al. 2011), the delayed maturation of oak leaves suggests that unmatured oak leaves may have been exposed to $O_3$ for longer periods than beech. Leaf Mass per Area (LMA) demonstrates a strong correlation with $A_{max}$ and is influenced by light conditions. Additionally, it characterizes distinct traits of beech and oak saplings following exposure to e $O_3$, even during free-air exposure in July and August. The second flush for oak occurred in mid-July and was unaffected by soil type or $O_3$ levels, as seen in the results of two oak species at the $O_3$-FACE near Tokyo, documented by Kitao et al. (2015). Similar findings were also present in beech and two oak species grown in Andisol soil originating from volcanic ash under e$O_3$, as Tobita et al. (2019).

Many plant species absorb P, water, and N through the functional activities of symbiotic ectomycorrhizae (Schulze et al. 2019), which are affected by soil pH and fertility (Marschner 2011). The $A_{max}$ for beech and oak species in VA under e$O_3$ levels was slightly higher than that of the BF species. This increase can be attributed to the production of antioxidants in both species under nutrient-limited

conditions. According to further research, the nutrient utilization traits of beech and oak differ. Beech commonly requires fertile soil, as noted by Kitao et al. (2016). Additionally, beech utilizes K in conjunction with P (Supple-Table 1). Synergy and antagonism effects among elements must be taken into account (Marschner 2011). Larcher (2003) found that despite Mg serving as the skeleton material of chlorophyll, the positive correlation between Mg and $A_{max}$ was weaker in oak than in beech. The nutrient balances found between Mg and Ca (Shi et al. 2016, 2017) may create a synergy effect while playing no role in the contrast of Mn between beech and oak. Additionally, oak had a higher $A_{max}$ than beech, due to the influence of soil nutrient conditions (Table 1). In truth, tree growth is affected not only by the chemical or nutritional properties of the soil but also by physical factors (Schulze et al. 2019).

Recently, Watanabe et al. (2020) suggested that the photosynthetic nitrogen use efficiency (PNUE) in beech does not change with nutrient application, but PNUE may increase photosynthesis per N and residence time in leaves. This research may add another explanation for the increase in N, which will decrease the net photosynthetic activity at a$CO_2$ levels under e$O_3$ (Yamaguchi et al. 2007).

The correlation between $A_{max}$ and Mn, Al varied between beech and oak (Supple-Table 1). Mn and Al exhibited negative correlation with $A_{max}$ in beech, indicating that soil acidification poses a greater threat to beech than $O_3$ stress in suburban regions (Gregg et al. 2003; Kohno 2017; Moser-Reischl et al. 2019). On the other hand, Supple.-Table 1 revealed that while oak did not exhibit a clear correlation between Mn and $A_{max}$, a weak positive correlation was uncovered between Al and $A_{max}$ in oak leaves. This trend appears uncommon. Notably, Watanabe and Azuma's (Paoletti et al. 2021) screening experiments on 819 plant species indigenous to Japan recently uncovered Al's unique characteristics. According to them (2021), the coefficient of variation (CV) for Al was approximately 5.5 among species, while the CV for macro-elements was less than 1.0, and that

for micro-elements ranged from 1 to 6, excluding Ni. It is worth noting that some species do require Ni as a micro-element. The Al tendency in oak trees, as suggested by the study, is backed by the concept that even though there are substantial dissimilarities in the absorption of nutrients containing Al between various plant species (Watanabe and Azuma 2021), oak can be categorized as a species that has a need for Al, which is depicted in the advancement of root growth (Tomioka et al. 2007; Tomioka and Takenaka 2007).

The PCA results demonstrated distinct features between beech and oak in BF soil with exposure to $eO_3$. Oak exposed to $eO_3$ in brown forest soil retained larger K, N, and P concentrations than in $aO_3$, with no suppression in $A_{max}$. Although, there was an indication of a decreasing trend in Ca accumulation in leaves. This trend suggests that the deposition on leaf cell walls is delayed due to Ca absorption by the transpiration stream. This may occur because of transpiration suppression, which avoids $O_3$ absorption (Hoshika et al. 2013). In contrast, beech did not show notable suppression in K, N, P, and $A_{max}$ compared to the control. Despite that, high Ca accumulation indicates that the leaf cell walls may have matured early and turned tough, which may inhibit future photosynthesis. In populated areas, individuals with excessive Ca supply exhibit lower NPK concentrations than those with optimal Ca supply (Weng et al. 2022). Additionally, Ca has been found to function as a signaling response to pollutants, particularly $O_3$, in stomata (McAinsh et al. 2002). These discoveries suggest that beech is affected by $eO_3$ levels since it absorbs $O_3$ through its stomata. In contrast, oak is highly tolerant to $O_3$ (Yamaguchi et al. 2011) and achieves higher $A_{max}$ than beech.

Ca is a reliable indicator of soil fertility (Marschner 2011; Foth and Ellis 2020). According to Kitao et al. (2016), beech prefer to grow in fertile and moist soil. Ca serves to strengthen the cell walls of leaves, and once deposited in the cell wall, it remains largely immobile (Marschner 2011; Watanabe and Azuma 2021). Whilst oak underwent a 2nd flush even by September, beech did not, even in $aO_3$ conditions. During the process of leaf aging or shedding, crucial elements are re-translocated from aging leaves to new or non-photosynthetic organs (Marschner 2011; Shi et al. 2016). A weak correlation between Ca and $A_{max}$ of oak observed in this study can be attributed to the pattern of shoot development, specifically the presence of a 2nd flush. It is important to consider the carryover effects of $O_3$ on the growth of perennial plants, particularly fixed-growth tree types such as beech (Yonekura et al. 2001, 2004). However, future changes in beech and oak under rapidly changing environmental conditions such as N deposition and soil acidification remain unclear.

The decline in vegetation function in East Asia is partly due to changes in $NO_2$ emissions (Kurokawa and Ohara 2020; Qu et al. 2022). $NO_2$ is the precursor of $O_3$ under ultraviolet radiation, which suppresses photosynthetic function through oxidation (e.g. Gregg et al. 2003). In particular, the agricultural system in Northeast China has been changing, especially soybean cultivation; farmers use commercial chemical fertilizers instead of *Rhizobium* sp. ($N_2$-fixing microbe) (Qiu 2009; Ju et al. 2009; Lu et al. 2015; Li et al. 2017; Hou et al. 2018). As a result, additional N leads to N deposition, causing an imbalance of N cycling in this region (Izuta 2017; Takahashi et al. 2020; Qu et al. 2022).

In addition, water stress is often caused by changes in precipitation pattern (Yonekura et al. 2001, 2004). As a result, photosynthetic activity is certainly affected by plant nutrient load; in addition, plant physiological functions are strongly influenced by soil pH and the status of water-soluble elements. Although Mn is an essential microelement and is released below pH 5.0, additional Mn reduces photosynthetic activity, while Al released below pH 4.5 has a negative effect on root meristem development (Marschner 2011; Schulze et al. 2019; Watanabe and Azuma 2021). Regardless of the $O_3$ level, the pH of the studied soils was lower than 5.2 (Table 1); consequently, in VA soils, the pH values independent of $O_3$ levels (about 4.6 and 4.3, respectively) imply that Mn was already released (pH < 5.0) and Al was gradually released (pH < 4.5).

The amount of $SO_2$ deposition has decreased with the rapid installation of desulfurization equipment in China, and consequently, $SO_4$ deposition toward Japan has also decreased (Takahashi et al. 2020). As mentioned above, N deposition is increasing in China (Qu et al. 2022). The increase in $NH_4^+$ and $SO_4^{2-}$ is due to the conversion of some of the $(NH_4)HSO_4$ to $(NH_4)_2SO_4$, which is already found on the west coast of the U.S.A., where pollution has been worsening (Butler et al. 2016). Compared to oak, beech growth activity is expected to decrease with increasing soil Al content accompanied by decreasing soil pH under elevated ground-level $O_3$ (Table 2).

These nutrient responses of beech and oak species are strongly influenced by the direct effects of $eO_3$ combined with drought (Haberer et al. 2008) and other environmental stresses (Agathokleous et al. 2015; Paoletti et al. 2021). Although several studies have been conducted on mature European beech exposed to $eO_3$ or N deposition, an essential role of phytohormones in fine root formation was found (Matyssek et al. 2012). Further studies are needed to analyze the roots of beech and oak to better understand the role of fine roots.

Finally, based on the flux tower experiments, beech stands have already shown $O_3$ induced symptoms of decline, but there were no clear symptoms of decline in oak stands. In general, beech prefers nutrient-rich and moist sites where water-soluble nutrients play a crucial role for its growth under $eO_3$ compared to oak stands. From our findings on different soil conditions in the $O_3$-FACE, the reduced activity of beech under $eO_3$ may be due to the imbalance of nutrients compared to oak.

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## Disclosure statement

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## Author contributions

formal analyses and writing, S.K.; main investigation, C.S.; draft preparation, S.K., T.K.; writing – review and editing, T.W.; conducted plant nutrition research, T.K.; project administration, All authors have read and agreed to the published version of the manuscript."

## References

Agathokleous E, Saitanis CJ, Wang X, Watanabe M, Koike T. 2015. A review study on past 40 years of research on effects of tropospheric $O_3$ on belowground structure, functioning, and processes of trees: a linkage with potential ecological implications. Water Air Soil Pollut. 227(1):33. doi: 10.1007/s11270-015-2715-9.

Braun S, Schindler C, Rihm B. 2020. Foliar nutrient concentrations of European beech in Switzerland: relations with nitrogen deposition, ozone, climate and soil chemistry. Front For Glob Change. 3:33. doi: 10.3389/ffgc.2020.00033.

Q8

Butler T, Vermeylen F, Lehmann CM, Likens GE, Puchalski M. 2016. Increasing ammonia concentration trends in large regions of the USA derived from the NADP/AMoN network. Acid Rain Its Environ Recent Sci Adv. 146:132–140. doi: 10.1016/j.atmosenv.2016.06.033.

Duursma RA, Struik PC. 2015. Plantecophys - an R package for analysing and modelling leaf gas exchange data. PLoS ONE. 10(11): e0143346. doi: 10.1371/journal.pone.0143346.

Eguchi N, Ichie T, Ji D, Karatsu K, Koike T. 2006. Accurate estimation of nitrogen concentration in deciduous tree leaves in a field study using a portable non-destructive nitrogen detector. J Plant Physiol. 163(6):680–683. doi: 10.1016/j.jplph.2005.09.004.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic $CO_2$ assimilation in leaves of $C_3$ species. Planta. 149(1):78–90. doi: 10.1007/BF00386231.

Feng Z, Shang B, Li Z, Calatayud V, Agathokleous E, Stevens C. 2019. Ozone will remain a threat for plants independently of nitrogen load. Funct Ecol. 33(10):1854–1870. doi: 10.1111/1365-2435.13422.

Foth HD, Ellis BG. 2020. Soil fertility. 2nd. Boca Raton: CRC Press. doi:10.1201/9780203739341.

Gregg JW, Jones CG, Dawson TE. 2003. Urbanization effects on tree growth in the vicinity of New York City. Nature. 424(6945):183–187. doi:10.1038/nature01728. Internet.

Haberer K, Herbinger K, Alexou M, Rennenberg H, Tausz M. 2008. Effects of drought and canopy ozone exposure on antioxidants in fine roots of mature European beech (*Fagus sylvatica*). Tree Physiol. 28(5):713–719. doi: 10.1093/treephys/28.5.713.

Hoshika Y, Tatsuda S, Watanabe M, Wang X, Watanabe Y, Saito H, Koike T, Koike T. 2013. Effect of ambient ozone at the somma of Lake Mashu on ▢ growth and leaf gas exchange in *Betula ermanii* and *Betula platyphylla* var.*japonica*. Environ Exp Bot. 90:12–16. doi: 10.1016/j.envexpbot.2012.11.003.

Hoshika Y, Watanabe M, Kitao M, Häberle K-H, Grams TEE, Koike T, Matyssek R. 2015. Ozone induces stomatal narrowing in European and Siebold's beeches: a comparison between two experiments of free-air ozone exposure. Environ Pollut. 196:527–533. doi: 10.1016/j.envpol.2014.07.034.

Hou X, Zhan X, Zhou F, Yan X, Gu B, Reis S, Wu Y, Liu H, Piao S, Tang Y. 2018. Detection and attribution of nitrogen runoff trend in China's croplands. Environ Pollut. 234:270–278. doi: 10.1016/j.envpol.2017.11.052.

Izuta T. 2017. Effects of soil acidification on Asian trees. In: Izuta T, editor. Air pollut impacts plants east asia. Tokyo: Springer Japan; pp. 257–270. doi:431-56438-6_17",1,0,0>10.1007/978-4-431-56438-6_17.

Ju X-T, Xing G-X, Chen X-P, Zhang S-L, Zhang L-J, Liu X-J, Cui Z-L, Yin B, Christie P, Zhu Z-L, et al. 2009. Reducing environmental risk by improving N management in intensive Chinese agricultural systems. Proc Natl Acad Sci. 106(9):3041. doi: 10.1073/pnas.0813417106.

Kashimura T. 1978. Decrease of frost tolerance in *Fagus crenata*, *Quercus mongolica* var. *grosseserrata*, and *Q. serrata* during spring. In: mem late Prof K Yoshioka. Colloquium Of Tohoku Plant Ecology. 450–465.

Q9

Kayama M, Satoh F, Koike T. 2011. Photosynthetic rate, needle longevity, and nutrient contents in *Picea glehnii* growing on strongly acidic volcanic ash soil in northern Japan. Photosynt. 49(2):239–245. doi: 10.1007/s11099-011-0030-2.

Kim Y, Watanabe M, Imori M, Sasa K, Takagi K, Hatano R, Koike T. 2011. Reduced atmospheric $CH_4$ consumption by two forest soils under elevated $CO_2$ concentration in a FACE system in northern Japan. J Jpn Soc Atmospheric Environ. 46(1):30–36. doi: 10.1016/j.atmosenv.2011.10.034.

Kitao M, Agathokleous E, Yazaki K, Komatsu M, Kitaoka S, Tobita H. 2021. Growth and photosynthetic responses of seedlings of Japanese white birch, a fast-growing pioneer species, to free-air elevated $O_3$ and $CO_2$. Forests. 12(6):675. doi: 10.3390/f12060675.

Q10

Kitao M, Komatsu M, Yazaki K, Kitaoka S, Tobita H. 2015. Growth overcompensation against $O_3$ exposure in two Japanese oak species, *quercus mongolica* var. *crispula* and *quercus serrata*, grown under elevated $CO_2$. Environ Pollut. 206:133–141. doi: 10.1016/j.envpol.2015.06.034.

Kitao M, Lei TT, Koike T. 1999. Effects of manganese in solution culture on the growth of five deciduous broad-leaved tree species with different successional characters from northern Japan. Photosynt. 36(1):31–40. doi: 10.1023/A:1007058418199.

Kitao M, Tobita H, Kitaoka S, Harayama H, Yazaki K, Komatsu M, Agathokleous E, Koike T. 2019. Light energy partitioning under various environmental stresses combined with elevated $CO_2$ in three deciduous broadleaf tree species in Japan. Climate. 7(6):79. doi: 10.3390/cli7060079.

Kitao M, Yasuda Y, Kominami Y, Yamanoi K, Komatsu M, Miyama T, Mizoguchi Y, Kitaoka S, Yazaki K, Tobita H, et al. 2016. Increased phytotoxic $O_3$ dose accelerates autumn senescence in an $O_3$-sensitive beech forest even under the present-level $O_3$. Science Reports. 6(1). 10.1038/srep32549.

Kobno Y. 2017. Decline of *Fagus crenata* in the Tanzawa Mountains, Japan. In: Izuta T, editor. Air pollut impacts plants east asia. Tokyo: Springer Japan; pp. 151–162. doi:431-56438-6_10",1,0,0>10.1007/978-4-431-56438-6_10.

Koike T. 1990. Autumn coloring, photosynthetic performance and leaf development of deciduous broad-leaved trees in relation to forest succession. Tree Physiol. 7(1–2–3–4):21–32. doi: 2-3-4.21",1,0,0>10.1093/treephys/7.1-2-3-4.21.

Koike T, Watanabe M, Hoshika Y, Kitao M, Matsumura H, Funada R, Izuta T. 2013. Effects of ozone on forest Ecosystems in east and Southeast Asia. In: Matyssek R, Clarke N, Cudlin P, Mikkelsen T, Tuovinen J-P, Wieser G Paoletti E, editors. Clim change air pollut glob chall, 13. Elsevier; pp. 371–390. doi: 08-098349-3.00017-7",1,0,0>10.1016/B978-0-08-098349-3.00017-7.

Kurokawa J, Ohara T. 2020. Long-term historical trends in air pollutant emissions in Asia: regional emission inventory in ASia (REAS) version 3. Atmospheric Chem Phys. 20(21):12761–12793. doi: 10.5194/acp-20-12761-2020.

Larcher W. 2003. Physiological plant ecology: ecophysiology and stress physiology of functional groups [internet]. 4th ed. Verlin, Heidelberg, NewYork: Springer.

Leuschner C, Ellenberg H. 2017. Life forms and growth types of Central European plant species. In: Leuschner C Ellenberg H, editors. Ecol Cent Eur for Veg Ecol Cent Eur I. Cham: Springer International Publishing; pp. 23–28. doi:319-43042-3_2",1,0,0>10.1007/978-3-319-43042-3_2.

Li H, Feng W, He X, Zhu P, Gao H, Sun N, Xu M. 2017. Chemical fertilizers could be completely replaced by manure to maintain high maize yield and soil organic carbon (SOC) when SOC reaches a threshold in the northeast China plain. J Integr Agric. 16 (4):937–946. doi: (16)61559-9",1,0,0>10.1016/S2095-3119(16) 61559-9.

Long SP, Bernacchi CJ. 2003. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis?

Procedures and sources of error. J Exp Bot. 54(392):2393–2401. doi: 10.1093/jxb/erg262.

Lu Y, Chadwick D, Norse D, Powlson D, Shi W. 2015. Sustainable intensification of China's agriculture: the key role of nutrient management and climate change mitigation and adaptation. Agric Ecosyst Environ. 209:1–4. doi: 10.1016/j.agee.2015.05.012.

Marschner H. 2011. Marschner mineral nutrition of higher plants - 3rd. Edition Academic Press.

Matsumura H 2001. Impacts of ambient ozone and/or acid mist on the growth of 14 tree species: an open-top chamber study conducted in Japan. Water Air Soil Pollut. 130(1/4):959–964. doi: 10.1023/A:1013919221030.

Matyssek R, Schnyder H, Oßwald W, Ernst D, Munch JC, Pretzsch H. eds. 2012. Growth and defence in plants: resource allocation at multiple scales. Berlin Heidelberg: Springer-Verlag. doi:642-30645-7",1,0,0>10.1007/978-3-642-30645-7.

McAinsh MR, Evans NH, Montgomery LT, North KA. 2002. Calcium signaling in stomatal responses to pollutants. New Phytol. 153(3):441–447. doi: 10.1046/j.0028-646X.2001.00336.x.

Moser-Reischl A, Rötzer T, Biber P, Ulbricht M, Uhl E, Qu L, Koike T, Pretzsch H. 2019. Growth of *abies sachalinensis* along an urban gradient affected by environmental pollution in Sapporo, Japan. Forests. 10(8):707. doi: 10.3390/f10080707.

Nunn AJ, Reiter IM, Häberle K-H, Langebartels C, Bahnweg G, Pretzsch H, Sandermann H, Matyssek R. 2005. Response patterns in adult forest trees to chronic ozone stress: identification of variations and consistencies. Environ Pollut. 136(3):365–369. doi: 10.1016/j.envpol.2005.01.024.

Paoletti E, Feng Z, De Marco A, Hoshika Y, Harmens H, Agathokleous E, Domingos M, Mills G, Sicard P, Zhang L, et al. 2020. Challenges, gaps and opportunities in investigating the interactions of ozone pollution and plant ecosystems. Sci Total Environ. 709:136188. doi: 10.1016/j.scitotenv.2019.136188.

Paoletti E, Hoshika Y, Arab L, Martini S, Cotrozzi L, Weber D, Ache P, Neri L, Baraldi R, Pellegrini E, et al. 2021. Date palm responses to a chronic, realistic ozone exposure in a FACE experiment. Environ Res. 195:110868. doi: 10.1016/j.envres.2021.110868.

Qiu J. 2009. Nitrogen fertilizer warning for China. Nature. doi: 10.1038/news.2009.105.

Qu L, Wang Y, Shi C, Wang X, Masui N, Rötzer T, Watanabe T, Koike T. 2022. Vigor and Health of urban Green Resources under elevated O₃ in far east Asia. In: Hufnagel D El-Esawi D, editors. Veg Dyn Chang Ecosyst Hum Responsib. Rijeka: IntechOpen. doi:10.5772/intechopen.106957.

R Core Team. 2016. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Saji S, Bathula S, Kubo A, Tamaoki M, Aono M, Sano T, Tobe K, Timm S, Bauwe H, Nakajima N, et al. 2017. Ozone-sensitive arabidopsis mutants with deficiencies in photorespiratory enzymes. Plant Cell Physiol. 58(5):914–924. doi:10.1093/pcp/pcx027.

Schulze ED, Beck E, Buchmann N, Clemens S, Müller-Hohenstein K, Scherer-Lorenzen M. 2019. Plant ecology. 2nd. Berlin Heidelberg: Springer-Verlag. doi:662-56233-8",1,0,0>10.1007/978-3-662-56233-8.

Shi C, Eguchi N, Meng F, Watanabe T, Satoh F, Koike T. 2016. Retranslocation of foliar nutrients of deciduous tree seedlings in different soil condition under free-air O₃ enrichment. iForest. 9(5):835. doi: 10.3832/ifor1889-009.

Shi C, Watanabe T, Koike T. 2017. Leaf stoichiometry of deciduous tree species in different soils exposed to free-air O₃ enrichment over two growing seasons. Environ Exp Bot. 138:148–163. doi: 10.1016/j.envexpbot.2017.03.012.

Takahashi M, Feng Z, Mikhailova TA, Kalugina OV, Shergina OV, Afanasieva LV, Heng RKJ, Majid NMA, Sase H. 2020. Air pollution monitoring and tree and forest decline in East Asia: a review. Sci Total Environ. 742:140288. doi: 10.1016/j.scitotenv.2020.140288.

Tobita H, Komatsu M, Harayama H, Yazaki K, Kitaoka S, Kitao M. 2019. Effects of combined CO₂ and O₃ exposures on net CO₂ assimilation and biomass allocation in seedlings of the late-successional *Fagus crenata*. Climate. 7(10):117. doi: 10.3390/cli7100117.

Tomioka R, Takenaka C. 2007. Enhancement of root respiration and photosynthesis in *Quercus serrata* thunb. seedlings by long-term aluminum treatment. Environ Sci Int J Environ Physiol Toxicol. 14(3):141–148. https://pubmed.ncbi.nlm.nih.gov/17622218/.

Tomioka R, Uchida A, Takenaka C, Tezuka T. 2007. Effect of aluminum on nitrate reductase and photosynthetic activities in *Quercus serrata* seedlings. Environ Sci Int J Environ Physiol Toxicol. 14(3):157–165. https://pubmed.ncbi.nlm.nih.gov/17622220/.

Wang X, Agathokleous E, Qu L, Watanabe M, Koike T. 2016. Effects of CO₂ and O₃ on the interaction between root of woody plants and ectomycorrhizae. J Agric Meteorol. 72(2):95–105. doi: 14-00045",1,0,0>10.2480/agrmet.D-14-00045.

Wang X, Qu L, Mao Q, Watanabe M, Hoshika Y, Koyama A, Kawaguchi K, Tamai Y, Koike T. 2015. Ectomycorrhizal colonization and growth of the hybrid larch F₁ under elevated CO₂ and O₃. Environ Pollut. 197:116–126. doi: 10.1016/j.envpol.2014.11.031.

Watanabe M, Hiroshima H, Kinose Y, Okabe S, Izuta T. 2020. Nitrogen use efficiency for growth of *Fagus crenata* seedlings under elevated ozone and different soil nutrient conditions Seedlings Under Elevated Ozone And Different Soil Nutrient Conditions. Forests. 11(4):371. doi: 10.3390/f11040371.

Watanabe M, Hoshika Y, Inada N, Koike T. 2018. Photosynthetic activity in relation to a gradient of leaf nitrogen content within a canopy of Siebold's beech and Japanese oak saplings under elevated ozone. Sci Total Environ. 636:1455–1462. doi: 10.1016/j.scitotenv.2018.04.423.

Watanabe M, Hoshika Y, Inada N, Wang X, Mao Q, Koike T. 2013. Photosynthetic traits of Siebold's beech and oak saplings grown under free air ozone exposure in northern Japan. Environ Pollut. 174:50–56. doi: 10.1016/j.envpol.2012.11.006.

~~Watanabe M, Kinose Y, Izuta T. 2018. Photosynthesis of three evergreen broad-leaved tree species, *castanopsis sieboldii*, Quercus glauca, and Q. myrsinaefolia, under elevated ozone, *Quercus glauca*, and *Q. myrsinaefolia*, under elevated ozone. iForest. 11(3):360. doi: 10.3832/ifor2493-011.~~

Watanabe M, Matsuo N, Yamaguchi M, Matsumura H, Kohno Y, Izuta T. 2010. Risk assessment of ozone impact on the carbon absorption of Japanese representative conifers. European J For Res. 129(3):421–430. doi: 10.1007/s10342-009-0316-0.

Watanabe M, Watanabe Y, Kitaoka S, Utsugi H, Kita K, Koike T. 2011. Growth and photosynthetic traits of hybrid larch F₁ (*larix gmelinii* var. *japonica × L. kaempferi*) under elevated CO₂ concentration with low nutrient availability. Tree Physiol. 31(9):965–975. doi: 10.1093/treephys/tpr059.

Watanabe M, Yamaguchi M. 2011. Risk assessment of ozone impact on 6 Japanese forest tree species with consideration of nitrogen deposition. Jpn J Ecol. 61:89–96. doi: 10.18960/seitai.61.1_89.

Watanabe T, Azuma T. 2021. Ionomic variation in leaves of 819 plant species growing in the Botanical Garden of Hokkaido University, Japan. J Plant Res. 134(2):291–304. doi: 10.1007/s10265-021-01254-y.

Weng X, Li H, Ren C, Zhou Y, Zhu W, Zhang S, Liu L. 2022. Calcium regulates growth and nutrient absorption in poplar seedlings. Front Plant Sci. 13:887098. doi: 10.3389/fpls.2022.887098.

Wuenscher R, Unterfrauner H, Peticzka R, Zehetner F. 2015. A comparison of 14 soil phosphorus extraction methods applied to 50 agricultural soils from Central Europe. Plant Soil Env. 61(2):86–96. doi: PSE",1,0,0>10.17221/932/2014-PSE.

Yagihashi T, Matsui T, Nakaya T, Taoda H, Tanaka N. 2003. Classification of *Fagus crenata* forests and *Quercus mongolica* var. *grosseserrata* forests with regard to climatic conditions. Jpn J Ecol. 53(2):85–94. doi: 10.18960/seitai.53.2_85.

Yamaguchi M, Watanabe M, Iwasaki M, Tabe C, Matsumura H, Kohno Y, Izuta T. 2007. Growth and photosynthetic responses of *Fagus crenata* seedlings to O₃ under different nitrogen loads. Trees. 21(6):707–718. doi: 10.1007/s00468-007-0163-x.

Yamaguchi M, Watanabe M, Matsumura H, Kohno Y, Izuta T. 2011. Experimental studies on the effects of ozone on growth and photosynthetic activity of Japanese forest tree species. Asian J Atmospheric Environ. 5(2):65–78. doi: 10.5572/ajae.2011.5.2.065.

Yonekura T, Honda Y, Oksanen E, Yoshidome M, Watanabe M, Funada R, Koike T, Izuta T. 2001. The influences of ozone and soil water stress, singly and in combination, on leaf gas exchange rates, leaf ultrastructural characteristics and annual ring width of *Fagus crenata* seedlings. J Jpn Soc Atmospheric Environ. 36(6):333–351. doi: 10.11298/taiki1995.36.6_333.

Yonekura T, Yoshidome M, Watanabe M, Honda Y, Ogiwara I, Izuta T. 2004. Carry-over effects of ozone and water stress on leaf phenological characteristics and bud frost hardiness of *Fagus crenata* seedlings. Trees. 18(5):581–588. doi: 10.1007/s00468-004-0345-8.