Growth and Photosynthetic Responses of Seedlings of Japanese White Birch, a Fast-Growing Pioneer Species, to Free-Air Elevated O\textsubscript{3} and CO\textsubscript{2}

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Abstract: Plant growth is not solely determined by the net photosynthetic rate \(A\), but also influenced by the amount of leaves as a photosynthetic apparatus. To evaluate growth responses to CO\textsubscript{2} and O\textsubscript{3}, we investigated the effects of elevated CO\textsubscript{2} (550–560 \(\mu\text{mol mol}^{-1}\)) and O\textsubscript{3} (52 \(\text{nmol mol}^{-1}\) 1.7 \(\times\) ambient O\textsubscript{3}) on photosynthesis and biomass allocation in seedlings of Japanese white birch \((\textit{Betula platyphylla} \textit{var. japonica})\) grown in a free-air CO\textsubscript{2} and O\textsubscript{3} exposure system without any limitation of root growth. Total biomass was enhanced by elevated CO\textsubscript{2} but decreased by elevated O\textsubscript{3}. The ratio of root to shoot \((R:S\text{ ratio})\) showed no difference among the treatment combinations, suggesting that neither elevated CO\textsubscript{2} nor elevated O\textsubscript{3} affected biomass allocation in the leaf. Accordingly, photosynthetic responses to CO\textsubscript{2} and O\textsubscript{3} might be more important for the growth response of Japanese white birch. Based on \(A\) measured under respective growth CO\textsubscript{2} conditions, light-saturated \(A\) at a light intensity of 1500 \(\mu\text{mol m}^{-2} \text{s}^{-1}\) \(\text{(}\text{A}_{1500}\text{)}\) in young leaves \((\text{ca. 30 days old})\) exhibited no enhancement by elevated CO\textsubscript{2} in August, suggesting photosynthetic acclimation to elevated CO\textsubscript{2}. However, lower \(A_{1500}\) was observed in old leaves \((\text{ca. 60 days old})\) of plants grown under elevated O\textsubscript{3} (regulated to be twice ambient O\textsubscript{3}). Conversely, light-limited \(A\) measured under a light intensity of 200 \(\mu\text{mol m}^{-2} \text{s}^{-1}\) \(\text{(}\text{A}_{200}\text{)}\) was significantly enhanced by elevated CO\textsubscript{2} in young leaves, but suppressed by elevated O\textsubscript{3} in old leaves. Decreases in total biomass under elevated O\textsubscript{3} might be attributed to accelerated leaf senescence by O\textsubscript{3} indicated by the reduced \(A_{1500}\) and \(A_{200}\) in old leaves. Increases in total biomass under elevated CO\textsubscript{2} might be attributed to enhanced \(A\) under high light intensities, which possibly occurred before the photosynthetic acclimation observed in August, and/or enhanced \(A\) under limiting light intensities.

Keywords: photosynthetic acclimation; leaf senescence; light-saturated photosynthesis; light-limited photosynthesis; biomass allocation

1. Introduction

Atmospheric CO\textsubscript{2} concentration is increasing globally \([1,2]\), accompanied by an increase in tropospheric ozone \((\text{O}\textsubscript{3})\) concentration, particularly in East Asia \([3–6]\). O\textsubscript{3} pollution reduces plant growth and productivity through a reduction in photosynthesis, an increase in leaf respiration and acceleration of leaf senescence \([7–11]\). O\textsubscript{3} exposure reduces photosynthetic rate by a reduction in the maximum rate of Rubisco carboxylation \((V_{\text{c,max}})\) \([12–15]\) as well as a decrease in stomatal conductance \([14,16,17]\). Conversely, elevated CO\textsubscript{2} generally
increases plant growth with an enhancement of photosynthesis [18–20]. However, long-term elevated CO$_2$ leads to photosynthetic acclimation accompanied by a decrease in the Rubisco carboxylation capacity, indicated by a decrease in V$_{c,max}$ (so-called “photosynthetic downregulation”) frequently accompanied by leaf starch accumulation and leaf nitrogen reduction [19,21].

Plant growth is not solely determined by photosynthetic rate, usually expressed as the rate of leaf-area-based CO$_2$ assimilation, but is also influenced by the amount of leaf area as a photosynthetic apparatus [22]. An increase in the shoot to root ratio (S:R ratio) was observed in some plants grown under elevated O$_3$ [23–25], which is considered a compensatory response against O$_3$-induced photosynthesis reduction by investing biomass into the photosynthetic apparatus [26].

Deciduous broadleaf forests are broadly distributed in Japan, including various tree species with different morphological and physiological traits along with forest succession [27,28]. Early successional species, such as birch species, have a succeeding-type shoot development, whereas mid- and late-successional species, such as Japanese oak (Quercus mongolica var. crispula) and Siebold’s beech (Fagus crenata), have a flush-type shoot development, generally flushing once a year in spring. Consequently, early successional pioneer species have a relatively higher S:R ratio than mid- and late-successional species (Shukla and Ramakrishnan, 1984; Kitao et al., 2005, 2015). Such species-specific differences in the shoot development pattern might influence the growth responses to elevated O$_3$ and CO$_2$.

A significant growth enhancement was previously observed in seedlings of two mid-successional species (Q. mongolica var. crispula and Q. serrata) and late successional species (F. crenata) grown under the combination of elevated O$_3$ and CO$_2$ [29,30]. These tree species exhibited an increased S:R ratio under O$_3$ exposure, with stimulation of new shoot development and enhanced photosynthetic rate under elevated CO$_2$.

As an early successional species, Japanese white birch has a preferable biomass allocation into shoots with successional leaf development [27,31]. We hypothesized that the intrinsically higher S:R ratio in Japanese white birch would restrict the compensatory response against O$_3$ exposure via a shift of biomass allocation into shoot. In this context, photosynthetic responses to O$_3$ and CO$_2$ exposure might directly affect the growth response of the pioneer Japanese white birch. To test this hypothesis, we investigated photosynthetic properties, growth, and biomass allocation in seedlings of Japanese white birch grown in a free-air, CO$_2$ and O$_3$ exposure system.

### 2. Materials and Methods

#### 2.1. Plant Materials

Bare-rooted, 1 year old seedlings of Japanese birch (Betula platyphylla var. japonica) obtained from a commercial nursery (Hokkaido Engei-Ryokka Center, Kitahiroshima, Hokkaido) were planted directly in soil inside the frames of a free-air CO$_2$ and O$_3$ exposure system that was established in the nursery of Forestry and Forest Products Research Institute in Tsukuba, Japan (36°00′ N, 140°08′ E, 20 m a.s.l.) in March 2011. Twelve frames (2 × 2 × 2 m), partly surrounded by transparent windscreens (15 to 65 cm, and 75 to 125 cm in height above the soil) to reduce wind while not interfering with air exchange inside–outside, were installed for free-air CO$_2$ and O$_3$ enrichment. The free-air, CO$_2$ and O$_3$ exposure system is described in detail in [29]. The height of the seedlings was ca. 15 cm. Six seedlings were planted within each frame (totally 72 plants in the 12 frames). The CO$_2$ and O$_3$ treatments were as follows: control (unchanged ambient air), elevated CO$_2$ (eCO$_2$, target: 550 µmol mol$^{-1}$), elevated O$_3$ (eO$_3$, target: 2.0 × ambient O$_3$), and elevated CO$_2$ + O$_3$ (eCO$_2$ + eO$_3$, 550 µmol mol$^{-1}$ CO$_2$ and 2.0 × ambient O$_3$). The twelve frames were divided into three replicates per gas treatment. The treatments lasted from the beginning of May to the middle of November 2011 (Table 1). During the experimental period, we had periodic precipitation (Figure 1), thus we only irrigated in the case of prolonged sunny days with no precipitation.
Table 1. CO$_2$ and O$_3$ concentrations in the free-air fumigation system. Values are means ± SD (n = 3) of daytime CO$_2$ and O$_3$ concentrations (6:00 to 18:00) during the growth season (May to November 2011).

| Treatment       | Control | eCO$_2$ | eO$_3$  | eCO$_2$ + eO$_3$ |
|-----------------|---------|---------|---------|------------------|
| CO$_2$ (µmol mol$^{-1}$) | 377 ± 1.0 | 563 ± 9.0 | 381 ± 1.2 | 546 ± 2.3       |
| O$_3$ (nmol mol$^{-1}$)  | 30.2 ± 0.4 | 30.2 ± 0.7 | 52.2 ± 2.1 | 51.6 ± 3.1       |

2.2. Measurements of Gas Exchange

Gas exchange measurements were conducted in the beginning of August 2011, for randomly selected young (∼30 day old) and old (∼60 day old) leaves of a representative seedling per frame, i.e., three replications for each treatment combination (CO$_2$ × O$_3$), using a portable photosynthesis system (Model LI-6400; LI-COR, Lincoln, NV, USA). A total of 12 seedlings were selected, and one young leaf (∼13th leaf position) and one old leaf (∼7th leaf position from the bottom leaf of main shoot) in the same seedling were used (a total of 24 leaves). Japanese birch has heterophyllous leaves (i.e., early leaves flushed in spring and late leaves succeedingly developed during summer) [15,31]. We used late leaves of different ages (young and old), where young leaves were flushed in July and old leaves flushed in June. Because of the succeeding-type leaf development, both young and old leaves were developed under a sunlit condition—considered sun leaves.

Measurements were conducted between 8:00 and 16:00. As the sunrise was around 4:30, and the sunset was around 19:00 in the beginning of August, we considered that the leaves had been photosynthetically activated under sunlight at the onset of the gas exchange measurements. The CO$_2$ response evaluation was conducted as follows: at a saturating photon flux density (PFD) of 1500 µmol m$^{-2}$ s$^{-1}$, we first measured the net photosynthetic rate (A) at a CO$_2$ concentration of 100 µmol mol$^{-1}$ after 15 min acclimation, then measured A at CO$_2$ concentrations of 200, 380, 550, and 1000 µmol mol$^{-1}$ in sequence after ∼5 min acclimation for each CO$_2$ concentration. Measurements were conducted under a block temperature of 27 ºC (leaf temperature, 31.5 ± 0.3 ºC, mean ± SE, n = 24) and a relative humidity of ∼80%. We monitored A, stomatal conductance (g$_s$), and intercellular CO$_2$ concentration (C$_i$) throughout the measurements and recorded their values after they reached the steady state. We then investigated light-acclimated photosynthesis as follows: after the measurement of A at a CO$_2$ concentration of 1000 µmol mol$^{-1}$, CO$_2$ concentration was set to the respective growth CO$_2$ concentration. At the respective growth CO$_2$ concentration, A was measured at a PFD of 2000 µmol m$^{-2}$ s$^{-1}$ after a 10 min acclimation. Then, A was measured at the PFDs of 1500, 1000, 600, 300, 200, and 100 µmol m$^{-2}$ s$^{-1}$ in sequence after a 4 min acclimation for each PFD. We considered A measured at a PFD of 1500 under the respective growth CO$_2$ concentrations (i.e., CO$_2$ concentration of 380 µmol mol$^{-1}$ for
control and eO$_3$ treatments, and 550 µmol mol$^{-1}$ for eCO$_2$ and eCO$_2$ + eO$_3$ treatments) as a measure of the light-saturated, net photosynthetic rate (A$_{1500}$). As the quadratic equation for the light-response curve [32] failed to fit in some cases, especially for old leaves, we used A measured at a PFD of 200 µmol m$^{-2}$ s$^{-1}$ under the respective growth CO$_2$ concentration as a direct measure of the light-limited photosynthetic rate (A$_{100}$).

We used a traditional, steady-state A/C$_i$ response technique [33] but with fewer CO$_2$ points. The maximum rates of Rubisco carboxylase (V$_{c,max}$) and RuBP regeneration (J$_{max}$) were estimated based on the protocol using linear regression in [33]. In the case that A is limited by Rubisco carboxylation, A is expressed as $A = V_{c,max} f^\prime - R_d$, where $f^\prime = \frac{C_i - C_o}{C_i + K_c (1 + \frac{C_i}{C_o})}$ and $R_d$ is mitochondrial respiration under light. $\Gamma^\ast$ is the CO$_2$ compensation point, $K_c$ Rubisco Michaelis constant for CO$_2$, $K_o$ Rubisco Michaelis constant for O$_2$, and $O_2$ oxygen concentration in air. $\Gamma^\ast$, $K_c$, and $K_o$ at the leaf temperature during gas exchange measurements were estimated based on the temperature responses of them [34].

V$_{c,max}$ is estimated as the slope of the A and $f^\prime$ relationship, and $R_d$ is estimated as the $y$-intercept. We used A at the CO$_2$ concentrations of 100, 200, and 380 µmol mol$^{-1}$ for V$_{c,max}$ and J$_{max}$ estimation, as A is generally limited by Rubisco carboxylation under ambient CO$_2$ and saturated light [35,36]. Furthermore, we confirmed the linearity of the three points of A as a function of $f^\prime$ (Supplemental Figure S1), supporting that A at 380 µmol mol$^{-1}$ CO$_2$ was limited by Rubisco carboxylation.

Similarly, when $A$ is limited by RuBP regeneration under saturating light, $J_{max}$ is determined as the slope of the following equation: $A = J_{max} g^\prime - R_d$, where $g^\prime = \frac{C_i - C_o}{43 C_i + 105 \Gamma^\ast}$. In this case, $R_d$ was determined as explained above [33]. A measured at the CO$_2$ concentrations of 550 and 1000 µmol mol$^{-1}$ was used for the $J_{max}$ determination since linearity was observed in the relationship between A+$R_d$ at these CO$_2$ concentrations and $g^\prime$ through the origin (A + $R_d$ = $J_{max}$ $g^\prime$) (Supplemental Figure S1). This supports that A at 550 and 1000 µmol mol$^{-1}$ CO$_2$ was limited by RuBP regeneration.

Notably, $g_s$ stayed above 0.08 mol m$^{-2}$ s$^{-1}$ during the A/C$_i$ measurements, which is the threshold of accurate A/C$_i$ response measurement, regarding stomatal patchiness [37]. V$_{c,max}$ and $J_{max}$, estimated at the leaf temperature of $\approx$32 °C, were normalized to those at 25 °C by using their temperature responses according to [34].

2.3. Growth and Biomass Allocation

At the end of the experiments (November 2011), all seedlings were harvested. The soils were carefully removed by hand, using small, stainless-steel rakes (Bonsai rake, Kikuwa, Sanjo, Niigata, Japan). The biomasses of the leaves, stems, and roots were measured after oven-drying at 70 °C to a constant weight. We also collected all leaves that were shed before harvest. Senescent leaves with an abscission layer, which were easily detached by hand, were collected every day from 1 September 2011 to the end of the experiments; we considered them shed leaves. We calculated the ratio of shoot to root (S:R ratio), defined as [leaf + shed leaf + stem dry mass]/[root dry mass], and leaf weight ratio (LWR), calculated as [leaf + shed leaf dry mass]/[total dry mass]. As some seedlings died during the growth season irrespective of the treatment, due to some unidentified reason, 7 to 14 plants per treatment combination were sampled, where initially 18 plants were planted per treatment combination (totally 72 plants).

2.4. Leaf Nitrogen Content

The area-based leaf nitrogen content (N$_{area}$) was determined for the leaves used for the gas exchange measurements by the combustion method, using an analysis system composed of an N/C determination unit (SUMIGRAPH, NC 800, Sumika Chem. Anal. Service, Osaka, Japan), a gas chromatograph (GC 8A, Shimadzu, Kyoto, Japan), and a data processor (Chromatopac, C R6A, Shimadzu). The leaf area of the sampled whole leaves was determined, using a scanner (LiDE210, Canon, Tokyo, Japan) and an image analysis software (LIA32 ver 0.3781, http://www.agr.nagoya-u.ac.jp/~shinkan/LIA32/, accessed on 24 May 2021). The leaf mass per area (LMA = leaf dry mass/leaf area) was
calculated from the leaf area and leaf dry mass measured after oven-drying at 70 °C. Using LMA, dry-mass-based leaf N was converted to \( N_{\text{area}} \).

2.5. Statistical Analysis

As one representative plant per FACE frame (experimental unit; \( n = 3 \) for each treatment combination) was used for the gas exchange measurements and leaf N analysis, a two-factorial ANOVA was used to test the effects of \( \text{CO}_2 \), \( \text{O}_3 \) and their interaction on the photosynthetic properties and leaf N content of young and old leaves (R Development Core Team 2014). A linear mixed model was applied to analyze the total biomass and S:R ratio, with \( \text{CO}_2 \) and \( \text{O}_3 \) treatments as fixed factors and the frame as a random effect. We used the \text{lmer} function of the R package \text{lme4} for the model fitting [38], and the ANOVA function of the R package car for the analysis of the deviance table [39]. The level of significance was 0.05.

3. Results

3.1. Growth Responses of Japanese white Birch Seedlings to Elevated \( \text{CO}_2 \) and \( \text{O}_3 \)

The total biomass was increased by the elevated \( \text{CO}_2 \) but decreased by the elevated \( \text{O}_3 \) (Figure 2a). Conversely, the S:R ratio showed no significant difference among the treatment combinations (Figure 2b). Similar to the S:R ratio, LWR showed no significant difference among the treatment combinations (Figure 2c). The shed leaf to total leaf dry mass, defined as \([\text{shed leaf dry mass}] / [\text{leaf + shed leaf dry mass}]\), was significantly increased by the elevated \( \text{O}_3 \) (Figure 2d).

![Figure 2](image)

Figure 2. Dry mass of plant organs (a), shoot to root ratio (b), leaf weight ratio (c), and shed to total leaf dry mass (d) in seedlings of Japanese white birch grown under combinations of \( \text{CO}_2 \) and \( \text{O}_3 \) treatments. Seedlings were planted to the ground in the frames in March 2011, and harvested in November 2011. Enrichment of \( \text{CO}_2 \) and \( \text{O}_3 \) concentrations was conducted from May to November 2011. Shoot to root ratio is defined as \([\text{leaf + shed leaf + stem dry mass}] / [\text{root dry mass}]\). Leaf weight ratio = \([\text{leaf + shed leaf dry mass}] / [\text{total dry mass}]\). Shed to total leaf dry mass = \([\text{shed leaf dry mass}] / [\text{leaf + shed leaf dry mass}]\). Control—ambient air, \( \text{eCO}_2 \)—elevated \( \text{CO}_2 \), \( \text{eO}_3 \)—elevated \( \text{O}_3 \), and \( \text{eCO}_2 + \text{eO}_3 \)—elevated \( \text{CO}_2 \) and \( \text{O}_3 \). Chi-square (\( \chi^2 \)) and probability (\( P \)) of the effects of \( \text{CO}_2 \), \( \text{O}_3 \) and their interaction are indicated in the panel. Values are mean ± SE (\( n = 7–14 \)).
3.2. Net Photosynthetic Rate and Stomatal Conductance in Young and Old Leaves of Japanese Birch Seedlings Grown under the Combination of CO₂ and O₃

The light-saturated net photosynthetic rate under a light intensity of 1500 μmol m⁻² s⁻¹ \((A_{1500})\) showed no significant difference in young leaves (≈ 30 day old) among the treatment combinations, whereas decreases in \(A_{1500}\) were observed in old leaves (≈ 60 day old) of plants grown under elevated O₃ (Figure 3, upper panel). Conversely, the light-limited net photosynthetic rate, measured under a light intensity of 200 μmol m⁻² s⁻¹ \((A_{200})\), showed higher values in young leaves of plants grown under elevated CO₂, whereas elevated O₃ decreased \(A_{200}\) in old leaves (Figure 3, lower panel). In all cases, no significant effect of the CO₂ × O₃ interaction was observed. Stomatal conductance under a saturating light intensity of 1500 μmol m⁻² s⁻¹ \((g_{s1500})\) showed no significant difference among the treatment combinations both in young and old leaves (Figure 4, upper panel). Although stomatal conductance under a limiting light intensity of 200 μmol m⁻² s⁻¹ \((g_{s200})\) showed no significant difference in young leaves, \(g_{s200}\) was significantly decreased by elevated O₃ (Figure 4, lower panel).

Figure 3. Net photosynthetic rate under saturating (upper panel) or limiting light intensity (lower panel) in old (closed bar) and young leaves (open bar) of Japanese white birch seedlings grown under the CO₂ and O₃ treatment combinations. Control—ambient air, eCO₂—elevated CO₂, eO₃—elevated O₃, and eCO₂ + eO₃—elevated CO₂ and O₃. Young leaves were ≈ 30 days old, and old leaves were ≈ 60 days old. Measurements were conducted under the respective growth CO₂ concentrations, where a CO₂ concentration of 380 μmol mol⁻¹ was applied for control and eO₃ plants, and that of 550 μmol mol⁻¹ was applied for eCO₂ and eCO₂ + eO₃ plants. F-value and probability (P) of the effects of CO₂, O₃ and their interaction are indicated in the panel. Values are mean ± SE (n = 3).

3.3. Maximum Rates of Rubisco Carboxylation \((V_{c,max})\) and RuBP Regeneration \((J_{max})\) in Young and Old Leaves of Japanese Birch Seedlings Grown under the Combination of CO₂ and O₃

The maximum rate of Rubisco carboxylation \((V_{c,max})\) was decreased by the elevated CO₂ in young leaves, whereas \(V_{c,max}\) was decreased by both the elevated CO₂ and O₃ in older leaves (Figure 5, upper panel). The maximum rate of RuBP regeneration \((J_{max})\) was also decreased by the elevated CO₂ in young leaves and decreased by the elevated CO₂ and O₃ in old leaves (Figure 5, lower panel). No significant effect of the CO₂ × O₃ interaction on \(V_{c,max}\) and \(J_{max}\) was observed.
Figure 4. Stomatal conductance ($g_s$) under saturating (upper panel) or limiting light intensity (lower panel) in old (closed bar) and young leaves (open bar) of Japanese white birch seedlings grown under the CO$_2$ and O$_3$ treatment combinations. Control—ambient air, eCO$_2$—elevated CO$_2$, eO$_3$—elevated O$_3$, and eCO$_2$+eO$_3$—elevated CO$_2$ and O$_3$. Young leaves were ≈30 days old, and old leaves were ≈60 days old. Measurements were conducted under the respective growth CO$_2$ concentrations, where a CO$_2$ concentration of 380 µmol mol$^{-1}$ was applied for control and eO$_3$ plants, and that of 550 µmol mol$^{-1}$ was applied for eCO$_2$ and eCO$_2$+eO$_3$ plants. $F$-value and probability ($P$) of the effects of CO$_2$, O$_3$ and their interaction are indicated in the panel. Values are mean + SE ($n=3$).

Figure 5. Maximum rates of Rubisco carboxylation ($V_{c,max}$, upper panel) and RuBP regeneration ($J_{max}$, lower panel) in old (closed bars) and young leaves (open bars) of Japanese white birch seedlings grown under CO$_2$ and O$_3$ treatment combinations. Control—ambient air, eCO$_2$—elevated CO$_2$, eO$_3$—elevated O$_3$, and eCO$_2$+eO$_3$—elevated CO$_2$ and O$_3$. $V_{c,max}$ and $J_{max}$ were normalized to those at 25 ºC. Young leaves were ≈30 days old, and old leaves were ≈60 days old. $F$-value and probability ($P$) of the effects of CO$_2$, O$_3$ and their interaction are indicated in the panel. Values are mean + SE ($n=3$).
3.4. Leaf Nitrogen Content

The area-based leaf nitrogen content ($N_{\text{area}}$) generally decreased in older leaves, while leaves of plants grown under elevated CO$_2$ showed significantly lower $N_{\text{area}}$ for both young and old leaves of Japanese white birch seedlings (Figure 6). Conversely, no significant effect of the CO$_2$ $\times$ O$_3$ interaction was observed.

![Figure 6. Area-based leaf nitrogen content in old (closed bars) and young leaves (open bars) of Japanese white birch seedlings grown under CO$_2$ and O$_3$ treatment combinations. Control—ambient air, eCO$_2$—elevated CO$_2$, eO$_3$—elevated O$_3$, and eCO$_2$ $\times$ eO$_3$—elevated CO$_2$ and O$_3$. Young leaves were $\approx$ 30 days old, and old leaves were $\approx$ 60 days old. $F$-value and probability ($P$) of the effects of CO$_2$, O$_3$ and their interaction are indicated in the panel. Values are mean $\pm$ SE ($n$ = 3).](image)

4. Discussion

In the present study, we observed growth enhancement by elevated CO$_2$ but suppression by elevated O$_3$ in Japanese white birch seedlings grown in a free-air CO$_2$ and O$_3$ exposure system without any limitation of root growth as it has been reported for the pioneer tree species Populus tremuloides [40]. However, the growth responses in Japanese white birch were quite different from those observed in mid- and late-successional tree species. Mid-successional tree species (Japanese oak (Quercus mongolica var. crispula) and Konara oak (Q. serrata)), and late-successional tree species (Sebold’s beech (Fagus crenata)), showed an enhancement of shoot growth relative to root growth by O$_3$ exposure, which fully compensated the reduction in the photosynthetic rate by O$_3$ regarding plant growth, and even led to significant increases in total dry mass under the combination of elevated CO$_2$ and O$_3$ [29,30,41]. Thus, growth responses to elevated CO$_2$ and O$_3$ might depend on both photosynthetic responses and biomass allocation into plant organs, especially into leaves.

Japanese white birch seedlings showed little change in biomass allocation as is indicated by the almost constant value of the S:R ratio (around 2) and leaf weight ratio ($\approx$0.23) (Figure 2). As a pioneer tree species, Japanese white birch produces new leaves continuously throughout the growth season, while mid- and late-successional species generally flush several leaves at once in spring [27,42]. Such a difference in the shoot development pattern caused a different biomass allocation pattern, indicated by the S:R ratio; Japanese white birch showed a value of 2 but Japanese oak and Konara oak showed a value of 1 in control plants grown in ambient air [29]. Japanese white birch could not change the ratio under elevated O$_3$, whereas Japanese oak and Konara oak, grown in the same FACE system, changed the ratio from 1 to 2 in the case of elevated O$_3$ exposure [29], maybe because O$_3$-induced hormonal changes might stimulate the intrinsically conservative shoot growth in the two oak species [43–45]. Thus, without any changes in biomass allocation, the growth of Japanese white birch might be influenced more directly by photosynthetic responses to elevated CO$_2$ and O$_3$, in comparison with mid- or late-successional tree species.
Growth responses of Japanese white birch among the treatment combinations could not be fully explained by the differences in $A_{1500}$ measured in mid-summer, where a higher total dry mass was observed under elevated CO$_2$ despite no significant enhancement in $A_{1500}$ (Figures 2 and 3). Conversely, decreases in total biomass in plants grown under O$_3$ exposure could be attributed to the lower $A_{1500}$ in old leaves. Although the light-saturated photosynthetic rate is often used as a measure of plant responses to environmental stresses, diurnal changes in environmental conditions, and the leaf-age-dependent photosynthetic capacity should be taken into consideration for assessing plant growth as an integration of photosynthetic performance.

Regarding the photosynthetic capacity indicated by $V_{c,max}$ and $J_{max}$, photosynthetic downregulation apparently occurred even in young leaves in August 2011, accompanied by a reduction in N$_{area}$ [18,46,47]. Conversely, old leaves decreased photosynthetic capacity ($V_{c,max}$ and $J_{max}$) under both elevated CO$_2$ and elevated O$_3$, suggesting that O$_3$-accelerated leaf senescence may be due to oxidative damage additionally occurred upon photosynthetic downregulation by the elevated CO$_2$ [12,13,15,48]. Elevated CO$_2$ can affect the photosynthetic capacity in a short term (significantly, even for young leaves), whereas O$_3$ only affects it over a longer term. Furthermore, although elevated O$_3$ did not affect leaf production (Figure 2c), O$_3$-accelerated leaf senescence induced earlier leaf shedding in autumn (Figure 2d), which might also cause an adverse effect on plant growth. Conversely, photosynthetic performance (indicated by $A_{1500}$, Figure 3) of young leaves under the respective growth CO$_2$ concentrations showed no significant differences among the treatment combinations, suggesting that the photosynthetic downregulation (reduction in $V_{c,max}$ or $J_{max}$; Figure 5) could be compensated by the elevated CO$_2$ by enhancing photosynthesis and suppressing photorespiration via higher intercellular CO$_2$ concentrations [35,48]. Such a compensative effect of the elevated CO$_2$ seemed still effective in old leaves, which showed lower $A_{1500}$ only under elevated O$_3$.

Photosynthetic downregulation occurs with enhanced plant growth, which results from nitrogen dilution due to increased biomass over the nitrogen acquisition capacity by the root system [19,49]. In this context, the leaves of plants grown under elevated CO$_2$ were downregulated as of the time point of measuring photosynthesis in August 2011 as a consequence of growth enhancement, which was supported by the lower N$_{area}$ in the young leaves of plants grown under elevated CO$_2$. Soil N availability might have been limited as of that time because of the relatively poor root system in seedlings of Japanese white birch, indicated by the high S:$R$ ratio.

It is also noteworthy that a significantly higher light-limited photosynthetic rate ($A_{200}$) was observed in young leaves of plants grown under elevated CO$_2$ (Figure 3). Elevated CO$_2$-induced photosynthetic downregulation accompanied by decreased N$_{area}$ might not influence the total electron flow under limiting light (Supplemental Figure S2). Conversely, a reduction in electron partitioning into photorespiration under elevated CO$_2$ resulted in a higher photosynthetic rate under limiting light ($A_{200}$) [50]. As Japanese white birch develops new leaves continuously during the growing season [27,42], most leaves are partially shaded except for the topmost ones. Accordingly, all leaves could not necessarily receive saturating light even around noon. Therefore, the higher A under limiting light in the elevated-CO$_2$-grown plants could also contribute to the greater growth under elevated CO$_2$. Such an increase in light-limited photosynthesis would be of relevance for shade tolerance among tree species with different successional traits [51].

Regarding $g_s$, elevated O$_3$ decreased $g_s$ only in old leaves of Japanese white birch under limiting light. This suggests that O$_3$-induced stomatal closure, which contributes to preventing O$_3$ influx [17,52], might not function well in Japanese white birch. Relatively higher $g_s$ ($g_{s1500}$, up to 0.4 mol m$^{-2}$ s$^{-1}$) was observed in the control plants of Japanese white birch, compared with those in Q. mongolica (up to 0.3 mol m$^{-2}$ s$^{-1}$) and Q. serrata (up to 0.2 mol m$^{-2}$ s$^{-1}$) (cf. [29]), which suggests that photosynthesis of Japanese white birch might be more sensitive to elevated O$_3$ exposure as a consequence of the higher phytotoxic O$_3$ dose through the stomata [53].
In conclusion, a pioneer species, Japanese white birch, had no compensative biomass allocation into shoot growth under elevated O₃, in contrast to mid- and late-successional species. This may be attributed to its intrinsically higher S:R ratio not allowing further changes. Accordingly, photosynthetic responses, including earlier leaf senescence, might be associated with the growth responses to elevated CO₂ and O₃. Nevertheless, age-dependent changes in photosynthetic capacity, and light-limited photosynthesis should be taken into consideration to predict growth response of Japanese white birch in future-coming atmospheric conditions.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f12060675/s1, Figure S1: Light-saturated net photosynthetic rate (A) as a function of \( f' \), and \( A + R_d \) as a function of \( g' \) in a leaf of Japanese white birch. Figure S2: Electron transport rate under saturating or limiting light intensity in old and young leaves of Japanese white birch seedlings grown under CO₂ and O₃ treatment combinations.

Author Contributions: M.K. (Mitsutoshi Kitao) and H.T. designed the study. M.K. (Mitsutoshi Kitao), H.T., S.K., K.Y. and M.K. (Masabumi Komatsu) collected the photosynthetic data, performed the analysis, and hence equally contributed to this study. M.K. (Mitsutoshi Kitao) led the writing with input from E.A. All authors also discussed the results and commented on the manuscript. All authors have read and agreed to the published version of the manuscript.

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