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Title

Canopy carbon budget of Siebold's beech (*Fagus crenata*) sapling under free air ozone exposure

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Abstract

To determine the effects of ozone (O_3) on the canopy carbon budget, we investigated photosynthesis and respiration of leaves of Siebold's beech saplings under free air O_3 exposure (60 nmol mol^{-1} , during daytime) in relation to the within-canopy light gradient; we then calculated the canopy-level photosynthetic carbon gain (PCG) and respiratory carbon loss (RCL) using a canopy photosynthesis model. Susceptibilities of photosynthesis and respiration to O_3 were greater in leaves of upper canopy than in the lower canopy. The canopy net carbon gain (NCG) was reduced by O_3 by 12.4% during one growing season. The increased RCL was the main factor for the O_3 -induced reduction in NCG in late summer, while contributions of the reduced PCG and the increased RCL to the NCG were almost the same in autumn. These results indicate contributions of changes in PCG and RCL under O_3 to NCG were different between seasons.

Capsule:

Contributions of ozone-induced reduction in photosynthesis and increase in respiration to canopy net carbon gain of beech sapling were different between seasons.

Key words:

Free air ozone exposure; Canopy carbon budget; Light gradient; Siebold's beech; Sensitivity to ozone

1. Introduction

The increasing concentration of ozone (O₃) in the troposphere may lead to high risk of injury and productive decline in vegetation (Matyssek and Sandermann, 2003; Bytnerowicz et al., 2007; Sitch et al., 2007; Wittig et al., 2007, 2009). The concentration of O₃ has been increasing since the Industrial Revolution (Akimoto, 2003; Stevenson et al., 2006; Paoletti, 2007). A significant increase in O₃ concentrations in East Asia is predicted in the near future, because of rapid increases in emissions of the main O₃ precursors, nitrogen oxides and volatile organic compounds (Naja and Akimoto, 2004; Ohara et al., 2007; Yamaji et al., 2008). It is therefore important to assess the risk of increasing O₃ concentrations on forest trees in East Asia (Kohno et al., 2005; Watanabe et al., 2010; Watanabe et al., 2012).

A free-air O₃ exposure experiment using 10-year-old saplings of Siebold's beech (*Fagus crenata*) and oak (*Quercus mongolica* var. *crispula*) has been under way in Sapporo Experimental Forest, northern Japan, since 2011, to facilitate better risk assessment of O₃ (Hoshika et al., 2012a, b; Watanabe et al., 2013). Hoshika et al. (2012a) and Watanabe et al. (2013) reported an O₃-induced decline in the light saturated net photosynthetic rate and an enhanced dark respiration rate in the sun leaves of Siebold's beech.

Quantitative approach is highly important to assess the O₃ impact on carbon sink strength of forests. For this approach, the evaluation of canopy carbon budget of trees under elevated O₃ is needed, especially for bigger trees in view of the greater difficulty of harvesting than for seedlings. The lower photosynthetic activity and higher respiration observed in the upper canopy leaves of Siebold's beech (Hoshika et al., 2012a; Watanabe et al. 2013) may directly decrease whole-plant carbon gain.

However, the response of an individual leaf to O₃ may not directly contribute to the carbon budget of whole-canopy, because other factors such as light conditions within the canopy also affect the canopy-level carbon budget. Kitao et al. (2012) assessed O₃-induced changes in canopy level photosynthetic carbon gain and respiratory carbon loss in mature European beeches, based on the responses of leaf-level photosynthetic parameters to O₃ (Kitao et al. 2009); there was a 1.7% decline in gross carbon gain and an increase of 4.6% in respiratory carbon loss.

To determine the effect of O₃ on the carbon budget of the canopy, it is necessary to study the distinct responses of individual leaves to O₃ in relation to a gradient of light conditions within the canopy. Light limitations within the canopy may alter the susceptibility of leaves to O₃ injury; trees appear to differ in sensitivity according to their light demand (Matyssek and Sandermann, 2003). Contrasting results have been observed in light-demanding and shade-tolerant tree species. In shade-tolerant sugar maple (*Acer saccharum*) and European beech (*Fagus sylvatica*), shade leaves were sensitive to O₃ than sun leaves, whereas leaf photosynthesis and growth of light-demanding poplar (*Populus tristis* × *P. balsamifera*) seedlings were highly O₃-sensitive under high light conditions (Tjoelker et al., 1993, 1995; Kitao et al., 2009). Siebold's beech belongs to the genus *Fagus*, the same as the European beech, and its shaded leaves are therefore expected to have high O₃ susceptibility. According to a free air O₃ fumigation experiment at Sapporo Experimental Forest (Hoshika et al., 2012a), however, decreases in the light-saturated net photosynthetic rate in sun leaves of Siebold's beech sapling of 27%, 50% and 59% were observed as a result of daytime O₃ exposure (60 nmol mol⁻¹) for 3, 6 and 9 weeks, respectively. We accordingly consider the susceptibility of sun leaves of Siebold's beech to O₃ to

be relatively high. Are the shade leaves more sensitive to O₃?

To clarify the effects of O₃ on the carbon budget of the canopy, we investigated leaf gas exchange traits (i.e. photosynthesis and respiration) in leaves of Siebold's beech saplings under chronic free air O₃ exposure in relation to the within-canopy light gradient, and calculated the canopy-level photosynthetic carbon gain and respiratory carbon loss using a canopy photosynthesis model (Monsi und Saeki, 1953; Hirose, 2005) integrated with leaf-level gas exchange traits.

2. Materials and methods

2.1 Study site and experimental design

The study site was Sapporo Experimental Forest of Hokkaido University, in northern Japan (43°04' N, 141°20' E, 15 m a.s.l.). The mean temperature and precipitation during the growing season from May to October in 2012 were 17.5°C and 644 mm (Japan Meteorological Agency, 2012 for precipitation). The snow-free period is usually from mid-April to late-December. The soil type was brown forest soil (i.e. Cambisol). We prepared two plots, one for 'ambient' plot and another for elevated O₃. These two plots were about 20 m apart. Each plot was a rectangle of sides 5.5 m × 7.2 m, and the height was 5.5 m. Ten seedlings of two-year-old beech and oak were planted in May 2003 in each plot, and were grown under ambient conditions. The area for Siebold's beech in the experimental site was approximately 10 m², indicating 10,000 tree ha⁻¹ of planting density. Canopy of each trees are overlapped to an extent in 2012.

We commenced our O₃ exposure experiment in 2011, when the trees were 10 years old. The mean tree height and mean stem diameter at breast height of beech

at the start were 3.3 ± 0.4 m and 26.7 ± 5.9 mm. There was no significant difference between the plots in heights and stem diameters.

The method of O₃ exposure in our system employs the system used at Kranzberg Forest in Germany (Nunn et al., 2002; Werner and Fabian, 2002). Details of the fumigation system have been described in Watanabe et al. (2013). The target O₃ concentration above canopy was 60 nmol mol^{-1} during daylight hours. The O₃ concentration above the canopy was monitored continuously by an O₃ monitor (Mod. 202, 2B Technologies, Boulder CO, USA), and the observed value was used as feedback to the three-way valve so as to regulate the O₃ concentration, using the PID algorithm. The duration of O₃ exposure was 98 days from 6 August to 11 November in 2011, and 178 days from 17 May to 10 November in 2012. When ambient O₃ concentration was above 60 nmol mol^{-1} , O₃ fumigation was terminated. We observed such termination for 16 hour during the second growing season in 2012. During the second growing season, we also monitored the O₃ concentration below the canopy using another same-type O₃ monitor. The monitoring interval of O₃ concentration was two minutes and those values were averaged for every hour. The mean \pm standard deviations of one-hour average O₃ concentration during daylight hours in the O₃ fumigated plot in 2012 was $61.5 \pm 13.0 \text{ nmol mol}^{-1}$ (above canopy) and $51.2 \pm 12.2 \text{ nmol mol}^{-1}$ (below canopy); that in the ambient plot was $27.5 \pm 11.6 \text{ nmol mol}^{-1}$.

2.2 Measurement and modeling of leaf photosynthesis and respiration

The gas exchange rates of the 1st flush leaf were measured during 10-16 June, 26-31 August and 9-16 October 2012 using an open gas exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA). Six saplings were selected randomly in

each plot (i.e. ambient and elevated O₃). We measured the gas exchange rate for two leaves from each tree selected; one leaf was from the upper canopy (above 2.4 m) and the other from the lower canopy (below 1.2 m). We also measured gas exchange in the middle canopy (around 1.8 m) of three saplings in each plot. In total, 15 leaves per plot were analyzed. We used only first flush leaves in these measurements, because the number of second flush leaves was very small (0.16% of the total leaf number taken over all saplings). The leaf temperature and leaf-to-air vapor pressure deficit (VPD) during measurement were $25.0 \pm 0.5^\circ\text{C}$ and 1.5 ± 0.2 kPa. To obtain the light-response curve of the net photosynthetic rate (A), i.e. the A -light curve, A was determined at ten photosynthetic photon flux (PPF, 1500, 1100, 800, 500, 400, 300, 200, 100, 75, 50 and $0 \mu\text{mol m}^{-2} \text{s}^{-1}$). The gas exchange rates were measured between 0800 and 1500 hours. In the preliminary examination, we observed little difference between A -light curve measured in morning and that in afternoon within a same leaf. After the gas exchange rate had been measured, we collected leaf samples in order to determine the leaf mass per area (LMA). These leaf samples were dried in an oven for 5 days at 70°C and then weighed. The LMA was calculated from the area and dry mass of the leaves.

A nonrectangular hyperbola was used to fit the A -light curve (Hirose and Werger, 1987), as follows:

$$A = \frac{[\varphi I_L + A_{\text{sat}} - \{(\varphi I_L + A_{\text{sat}})^2 - 4\varphi\theta I_L A_{\text{sat}}\}^{0.5}]}{2\theta} - R \quad (1),$$

where I_L is the PPF incident on a leaf, A_{sat} is the light-saturated gross photosynthetic

rate per unit leaf area, ϕ is the initial slope of the curve, θ is the curvature factor, and R is the respiration rate per unit leaf area. In the present study, the R above light compensation point of photosynthesis was considered to be day respiration (R_{day}), and was calculated as 50% of dark respiration (R_{d}), which was determined from gas exchange rate at $0 \mu\text{mol m}^{-2} \text{s}^{-1}$ of PPF in A -light curve. We considered the range from light compensation point to $0 \mu\text{mol m}^{-2} \text{s}^{-1}$ of PPF as transient zone from R_{day} to R_{d} and the R within this light range was calculated according to relative PPF to light compensation point. In the curve fitting of A -light curve, the step of PPF at $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ in all measurements was over light compensation point. The value of A_{sat} calculated from this curve fitting is the theoretical values corresponding to infinite PPF, and is therefore higher than the measured value of A at $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF.

The simple linear regression method was used to estimate the parameters of the A -light curve from the LMA:

$$\begin{aligned}
 A_{\text{sat}} &= a_{\text{s}} \text{LMA} + b_{\text{s}} \\
 \phi &= a_{\text{p}} \text{LMA} + b_{\text{p}} \\
 R_{\text{d}} &= a_{\text{r}} \text{LMA} + b_{\text{r}}
 \end{aligned}
 \tag{2},$$

where a and b are regression coefficients. We tested the effect of O_3 on the slope and intercept of the regression lines using statistical software (SMATR, Version 2.0, <http://bio.mq.edu.au/ecology/SMATR/index.html>). When we found no significant effect of O_3 on slope or intercept, we applied a single regression line throughout both gas treatments. No significance for the regression line between θ and LMA and for the effect of O_3 on θ (T -test), the average θ throughout the plots in each measurement

period was used in the following calculation of canopy carbon budget.

In addition to this regression analysis, we determine averages of net photosynthetic rate, stomatal conductance and ratio of intercellular CO₂ concentration to ambient CO₂ concentration under PPF at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ ($A_{n_{1500}}$, G_s and C_i/C_a , respectively) in the leaves of upper and lower canopies. The effects of O₃ were analyzed by *T*-test in each leaf position ($n = 6$).

2.3 Calculation of canopy carbon budget

Model vegetation for calculating the canopy carbon budget was established based on the saplings of Siebold's beech grown in experimental site. We counted number of all leaves in all saplings. Fifteen leaves per tree were collected from various heights of the canopy and mean individual leaf area was measured in each tree. The whole-plant leaf area in each tree was determined as leaf number multiplied by individual leaf area.

The vertical distribution of leaf area was estimated from the stem cross-sectional area at various heights (Waring et al. 1982). We assumed that the stem cross-sectional area at a given height is proportional to the leaf area above that height. This assumption is based on "pipe-model theory" which indicates proportionality between the amount of non-assimilating organs (i.e. stem and branch) and assimilating organs (i.e. leaf) (Shinozaki et al. 1964a, b; Waring et al. 1982). We determined the diameter of the main stem from ground to the top with a 30 cm interval, and then calculated the relative diameter to diameter just below lowest branch. The relative diameter was multiplied by whole-canopy leaf area. This value was considered as sum of the leaf area above the measurement height h (LA_h). The

leaf area between height h and next height ($h+1$) is calculated as LA_{h+1} minus LA_h .

The distribution of PPF within the canopy was described by Beer's law (Monsi und Saeki, 1953; Hirose, 2005):

$$I = I_0 \exp(-KF_h) \quad (3),$$

where I is the PPF within the canopy on a horizontal plane at height h , I_0 is PPF above the canopy, K is the light extinction coefficient, and F_h is the cumulative leaf area from the top of the canopy down to height h . The value of I_L is calculated from the relation:

$$I_L = K I \quad (4),$$

To estimate K , we monitored the light intensity above and below the canopy using a HOBO Pendant temperature/light data logger (UA-002-64, Onset Computer, Co., MA, USA) for a whole month, August 2012. The value of K was determined to be 0.30 from the whole-canopy leaf area and light intensities above and below canopy; these parameters are denoted in equation (3) by F_h , I_0 and I respectively. Figure 1 shows the estimated vertical distribution of leaf area and relative light intensity on a horizontal plane of model vegetation, averaged over 20 trees. Because the planting density was $10,000 \text{ tree ha}^{-1}$, the averaged leaf area was used as leaf area index (LAI, $\text{m}^2 \text{ m}^{-2}$).

We analyzed the relation between light intensity on a leaf and LMA at various heights in the canopy. Color acetate films (Y-1W, Taisei Environment &

Landscape Co., Katsushika, Japan) were set on leaves at various heights for 14 days (1-14 August 2013) and the relative light intensity on a leaf (I_R) was determined. We collected the leaves that were used for light intensity measurement and determined LMA according to the method stated above. Because there was no significant effect of O_3 on slope and intercept of regression lines between I_R and LMA (Fig. 2), we calculated a single regression line for both plots,

$$\text{LMA} = 75.7 I_R + 23.5 \quad (5).$$

From this equation we calculated LMA at each height in the model vegetation. In addition, we analyzed correlation between the I_R and relative light intensity calculated in model vegetation (Fig. 1), and the correlation coefficient was 0.931.

Photosynthetic carbon gain and respiratory carbon loss of the canopy were calculated every hour in our model vegetation and the results accumulated for early summer (17 May – 21 July, 66 days), late summer (22 July - 19 September, 60 days) and autumn (20 September – 10 November, 52 days) in 2012, with corresponding measurement of gas exchange. The distribution of PPF within the canopy was estimated from equation (3), with I_0 monitored continuously by a quantum sensor (LI-190, Li-Cor Inc., Lincoln, NE, USA) in the open plot of the experimental forest. We calculated the LMA at a given height from equation (5), and then obtained the parameters of the A-light curve from equation (2). The photosynthetic rate and respiration rate per unit area was calculated using the parameters of the A-light curve and I_L from equation (4). The area-based photosynthetic rate and respiration rate were multiplied by the leaf area at each height.

Temperature dependency of photosynthetic parameters (i.e. A_{sat} , ϕ and R_d) were incorporated for the estimation of carbon budget. The respiration rate was corrected according to the relation between R_d and temperature in genus *Fagus* (Iio et al., 2004; Rodríguez-Calcerrada et al., 2010). The temperature dependency of A_{sat} was also analyzed based on the reports for genus *Fagus* (Iio et al., 2004; Jurik et al., 1988). Iio et al. (2004) reported the temperature dependency for both light-saturated net photosynthetic rate and R_d . The A_{sat} (light-saturated gross photosynthetic rate) was calculated with an assumption that R_{day} equals to 50% of R_d . To estimate A_{sat} for Jurik et al. (1988), we assumed the ratio of R_{day} to light-saturated net photosynthetic rate at 25°C in Iio et al. (2004) and that in Jurik et al. (1988) was the same. Then, the relation between leaf temperature and A_{sat} in Iio et al. (2004) and Jurik et al. (1988) were connected. Because we could not confirm a regulation of air humidity for the experiment in Jurik et al. (1988), the plot with leaf temperature at 34°C was omitted from the calculation. The temperature dependency of ϕ was according to Ehleringer et al. (1997). The temperature of the leaves was assumed to be the same as the air temperature.

Because we predicted decline in photosynthesis owing to high VPD in the field condition as reported by Iio et al. (2004), we evaluate the response of net photosynthetic rate to VPD in mid-June. We measured net photosynthetic rate of the the upper canopy leaves of eight trees (four trees in each gas treatment) using an open gas exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) with standard leaf chamber without artificial light source. The chamber angle was changed every one hour to avoid the shading by chamber edge. Air temperature in the chamber was adjusted to ambient temperature. We observed decline in light-saturated net

photosynthetic rate at PPF over $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ with increasing VPD (Fig. 3). Regression line throughout the gas treatments was obtained and was used to carbon budgeted estimation because there was no significant difference in regression lines between ambient and elevated O_3 conditions.

3. Results

3.1 Response of photosynthesis and respiration to ozone with gradient of leaf mass per area

Figure 4 shows relations between the photosynthetic parameters from A-light curve analysis and LMA. The slopes of the regression line for A_{sat} under elevated O_3 in August and October were significantly lower than those at ambient O_3 levels. In contrast, the slope of the regression line for R_d rose with elevated O_3 . We found no significant difference in slope or intercept of the regression line between ambient and elevated O_3 conditions for ϕ in all the measurement periods, or for A_{sat} and R in June.

3.2 Photosynthetic traits of upper and lower canopy leaves under elevated ozone

The $A_{n_{1500}}$ in upper canopy leaves was significantly reduced by elevated O_3 in August and October, while there was no significant effect of O_3 in lower canopy (Table 1). The exposure to O_3 marginally reduced G_s in both height ($P < 0.1$) in June and August. We observed significant increase of C_i/C_a in the leaves of the upper canopy under elevated O_3 in October. There was no significant response of C_i/C_a to elevated O_3 in other period for upper canopy and all period for lower canopy.

3.3 Canopy carbon budget due to photosynthesis and respiration

Canopy carbon budget due to photosynthesis and respiration in model vegetation of Siebold's beech is shown in Table 2. We apply the same equation for both ambient and elevated O₃ condition to estimate carbon budget in early summer because there was no significant effect of O₃ on photosynthetic parameter (Fig. 4).

Ozone-induced reduction in photosynthetic carbon gain of whole-canopy of Siebold's beech sapling for late summer was 1.9%. This was mainly contributed by reduction in photosynthetic carbon gain in the relatively upper part (above 2.4 m) of the canopy (Fig. 5). An O₃-induced increase in respiratory carbon loss was found throughout the canopy. The increase of respiratory carbon loss by O₃ in late summer was 64.7%, and was prominence than reduction in photosynthetic carbon gain. The O₃-induced reduction in net carbon gain was 28.5%, due mainly to the large reduction in respiratory carbon loss.

Although the photosynthetic carbon gain and respiratory carbon loss in both treatments in autumn were both lower than those in late summer, the reduction in respiratory carbon loss was greater. The O₃-induced increase in respiratory carbon loss in autumn was also larger (56.3%) than the reduction in photosynthetic carbon gain (7.3%). However, contributions to the O₃-induced reduction in net carbon gain were almost the same between photosynthetic carbon gain (1.4 mol reduction) and respiratory carbon loss (1.4 mol increase).

Net carbon gain for whole growth period was decreased by 12.4%. The 79% of reduction in net carbon gain under elevated O₃ was caused by increase in respiratory carbon loss.

4. Discussion

Photosynthesis and respiration were more susceptible to O_3 in the leaves of the upper canopy than the lower canopy. The same has been found in poplars but the reverse in the sugar maple and European beech (Tjoelker et al., 1993, 1995; Kitao et al., 2009). It is not easy to explain the contrasting results within the single genus *Fagus*, although experimental conditions (climate, O_3 exposure regime, tree ages and size etc.) differed between the experiments (Nunn et al., 2007; Kitao et al., 2009). Susceptibility to O_3 in relation to light gradient might be influenced by other factors as well as ecological traits for light demand.

The large decline in A_{sat} and increase in R with exposure to O_3 in the upper canopy having high LMA in the present study is typical of the contrariness to European beech in Kitao et al. (2009). The negative effect of O_3 on photosynthetic activity reported by Kitao et al. (2009) was relatively small throughout the light gradient in the canopy. According to Table 1, O_3 induced reduction in G_s was found in the leaves of both upper and lower canopies in June and August. However, the reduction in G_s is not considered as a main factor of reduction in net photosynthetic rate under elevated O_3 in August because there was no decrease of C_i/C_a . Measurement in the previous growing season also indicated the O_3 -induced reduction in photosynthetic activity in the leaves of upper canopy was due not to stomatal closure, but to biochemical limitation (Watanabe et al., 2013).

High light intensity is found in the upper canopy and generally leads to the generation of reactive oxygen species (ROS) from the water-water cycle in chloroplasts, inducing oxidative stress (Asada, 1996; Apel and Hirt, 2004). In addition, higher air temperature results in greater generation of ROS (Apel and Hirt,

2004). There is a possibility that O₃-induced lower G_s in June and August in the present study increased leaf temperature owing to the reduction in transpiration and thereby stimulated generation of ROS. Therefore, the large decline in photosynthetic activity under elevated O₃ in the upper canopy might be due to relatively high ROS at increased leaf temperature caused by O₃-induced stomatal closure in combination with higher light intensity. To test this hypothesis it would be necessary to make a comparative analysis of temperature and oxidative stresses (e.g. measurement of malondialdehyde, an indicator of lipid oxidation: see The Japanese Society of Photosynthesis Research, 2003) between leaves in the upper- and lower-canopy.

We observed no decrease in G_s under elevated O₃ in upper canopy in October even lower $A_{n_{1500}}$ in O₃ treatment (Table 1). Although the G_s generally decrease by the exposure to O₃, (Wittig et al. 2007), no change or increase of G_s has also been reported (Yamaguchi et al. 2007, Mills et al. 2009). Ozone-induced ethylene synthesis might relate the no decrease of G_s in autumn in the present study because ethylene is known to inhibit abscisic acid-induced stomatal closure in *Arabidopsis* (Tanaka et al., 2005).

Although a clear reduction in A_{sat} was observed with elevated O₃ in leaves of the upper canopy (Fig. 4), the decline in canopy photosynthetic carbon gain was fairly small, especially in summer (Table 2). We did not find any significant effect of O₃ on the relation between ϕ and LMA, implying that there was almost no difference in the gross photosynthetic rate between ambient and elevated O₃ at low light intensity. We estimated the value of K to be 0.30. This means that the leaves received only 30% of I_0 even at the top of the canopy, so that for most leaves the photosynthetic rate was not saturated by light. In fact, the O₃-induced reduction in

the photosynthetic carbon gain was found only in the upper part of the canopy (Fig. 5). The effect of O_3 on the photosynthetic carbon gain was therefore fairly small.

We also found no significant effect of O_3 on ϕ in the autumn of previous growing season (data not shown). A similar result in Siebold's beech has been reported by Yonekura et al. (2001). They suggested that O_3 -induced degradation of carboxylation capacity with decreasing ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) was more sensitive to O_3 than ϕ , and that this is the main factor in O_3 -induced reduction in the light-saturated net photosynthetic rate. We compared photosynthetic carbon gains for various ϕ and A_{sat} using model simulation (Fig. 6). Photosynthetic carbon gain responded to changes in ϕ over the whole range of I_R . We found some contribution to photosynthetic carbon gain of changes in A_{sat} for relatively high I_R only. At lower I_R , the contribution of A_{sat} to photosynthetic carbon gain was very small. Therefore, maintaining ϕ under elevated O_3 might be a way to minimize the negative effect of O_3 on photosynthetic carbon gain in Siebold's beech. From the viewpoint of metabolism, on the other hand, Hörtensteiner and Feller (2002) reported different degradation processes for stromal proteins and thylakoid-binding proteins during leaf senescence. A_{sat} is mainly regulated by activity of Rubisco (stromal proteins), while ϕ is regulated by light-harvesting and electron transport functions on thylakoid membrane. Although senescence-like symptoms are frequently observed in the leaves of woody plants exposed to O_3 (e.g. Yonekura et al., 2004; Watanabe et al. 2013), there are several differences as compared to normal senescence (Matyssek and Sandermann, 2003). The O_3 stress may stimulate degradation process only for stromal proteins in the leaves of Siebold's beech.

There was a clear increase in respiratory carbon loss under elevated O₃ (Table 2). Several reports have found an O₃-induced increase in dark respiration, due presumably to enhancement of O₃-related detoxification involving O₃-derived ROS, and/or repair of damaged tissues (Landolt et al., 1997; Matyssek and Sandermann, 2003; Kitao et al., 2009; Watanabe et al. 2013). Our calculation of the canopy carbon budget revealed a significant contribution of respiratory carbon loss enhanced by O₃ to the reduction in net carbon gain. In late summer in particular, the increase in respiratory carbon loss was the main factor in the reduction of net carbon gain induced by elevated O₃ even for whole growth period. Although the O₃-induced changes of respiration in autumn were similar in pattern to those in summer, the absolute value of the respiration was lower in autumn because of the lower temperature. This is the main reason for the larger reduction in net carbon gain induced by O₃ in summer than in autumn.

In the present study, we estimated canopy carbon budget with a model integrated photosynthetic traits of individual leaf and canopy structure. We revealed that contributions of reduction in photosynthetic carbon gain and increase of respiratory carbon loss under elevated O₃ to canopy net carbon budget. Because this estimation is relatively extrapolative, we consider main uncertainties in the estimation and perspectives for future work should be discussed.

The evaluation of K (light extinction coefficient) was based on two point measurements (i.e. top and bottom of the canopy) and vertical distribution of leaf area was estimated from diameter of main stem based on “pipe model theory”. Although those methods are convenient, they might have relatively large uncertainty. Because our main purpose was to estimate potential impact of O₃ on canopy carbon

budget from photosynthetic and respiratory traits with a gradient of LMA, we estimated “model vegetation” by the relatively simple methods. However, long-term chronic O₃ exposure may change the canopy structure, because of differing sensitivities in the physiology of leaves to O₃ between the upper and lower canopy, as we found. Hence, the effect of O₃ on canopy structure is another important factor for the adequate estimation of O₃ risk on carbon budget of forest tree species, and the validation of estimation especially for canopy structure and light condition within canopy are needed.

LMA is a representative parameter for estimating acclimation of leaf to given light condition. Therefore, we used LMA for estimating photosynthetic activity throughout canopy and obtained clear regression lines between photosynthetic parameters and LMA. Kitao et al. (2009, 2012) also analyzed the relation between LMA and photosynthetic parameters and estimated canopy-level carbon budget in a mature beech stand. Further studies are possibly to employ the canopy nitrogen distribution for an estimation of canopy carbon budget, (e.g. Hirose and Werger, 1987) for mechanistic understanding of photosynthetic response to O₃

Our model of canopy photosynthesis assumed diffuse radiation when estimating the relative light intensity at each height in the canopy, although the actual light conditions were a mixture of diffuse and direct radiation. According to our model calculation and measurement, canopy photosynthesis is generally enhanced with increasing fraction of diffuse radiation (Hirose, 2005). Our estimates of canopy photosynthesis with only diffuse radiation may therefore be overestimates of the photosynthetic carbon gain. However, the contribution of photosynthetic carbon gain to net carbon gain was relatively low. We therefore believe that our assumption of

diffuse radiation did not significantly affect our estimates of the O₃-induced reduction in net carbon gain.

5. Conclusion

In the present study, leaf-level photosynthetic and respiratory traits of Siebold's beech saplings in relation to the within-canopy light gradient and canopy carbon budget under elevated O₃ were investigated. The new findings are (1) higher O₃ sensitivity in upper canopy leaves as compared to lower canopy leaves of Siebold's beech saplings, and (2) seasonal difference in contributions of the reduction in photosynthetic carbon gain and the increase in respiratory carbon loss under elevated O₃ to net canopy carbon gain. Those information are highly important for assessing risk of O₃ impacts on Siebold's beech forests in Japan. As future studies, clarification of the physiological mechanism responsible for the difference in O₃ sensitivities between upper and lower canopy leaves within a canopy, and variation among the tree species, and also differing sensitivity within photosynthetic parameters (i.e. A_{sat} and ϕ) are all important for understanding the effect of O₃ on the canopy carbon budget in various forest types, as well as the ecological strategy of trees in response to O₃ stress.

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Table 1 Net photosynthetic rate, stomatal conductance and ratio of intercellular CO₂ concentration to ambient CO₂ concentration under photosynthetic photon flux density at 1500 μmol m⁻² s⁻¹ ($A_{n,1500}$, G_s and C_i/C_a , respectively) in the upper and lower canopy leaves of Siebold's beech grown under ambient and elevated concentrations of ozone.

		$A_{n,1500}$	G_s	C_i/C_a
June				
Upper	Ambient	10.5	0.20	0.74
	Ozone	10.0	0.17	0.72
	T-test	ns	0.081	ns
Lower	Ambient	4.9	0.12	0.79
	Ozone	4.5	0.08	0.74
	T-test	ns	0.078	ns
August				
Upper	Ambient	12.1	0.30	0.77
	Ozone	8.6	0.22	0.80
	T-test	**	0.076	ns
Lower	Ambient	4.9	0.17	0.81
	Ozone	4.3	0.13	0.81
	T-test	ns	0.095	ns
October				
Upper	Ambient	8.1	0.22	0.79
	Ozone	4.4	0.21	0.87
	T-test	**	ns	**
Lower	Ambient	3.2	0.07	0.76
	Ozone	3.6	0.08	0.75
	T-test	ns	ns	ns

T-test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. not significant ($n = 6$). The actual P value is shown if $0.05 < P < 0.10$.

Table 2 Carbon budget per unit ground area (mol m^{-2}) due to photosynthesis and respiration of canopy in model vegetation (Fig. 1) of Siebold's beech grown under ambient and elevated concentrations of ozone.

	Ambient	Ozone	Difference	Relative change (%)
Early summer (17 May - 21 July)				
Photosynthetic carbon gain	49.1		-	-
Respiratory carbon loss	12.6		-	-
Net carbon gain	36.5		-	-
Late summer (22 July - 19 September)				
Photosynthetic carbon gain	32.9	32.3	-0.6	-1.9
Respiratory carbon loss	9.4	15.5	6.1	64.7
Net carbon gain	23.5	16.8	-6.7	-28.5
Autumn (20 September - 10 November)				
Photosynthetic carbon gain	18.5	17.2	-1.4	-7.3
Respiratory carbon loss	2.4	3.8	1.4	56.3
Net carbon gain	16.1	13.4	-2.7	-16.9
Whole growth period (17 May - 10 November)				
Photosynthetic carbon gain	100.6	98.6	-2.0	-2.0
Respiratory carbon loss	24.4	31.9	7.4	30.4
Net carbon gain	76.1	66.7	-9.4	-12.4

Difference = Ozone - Ambient

Because there was no significant effect of O_3 on photosynthetic parameter (Fig. 4), we apply the same equation for both ambient and elevated O_3 condition to estimate carbon budget in early summer.

Figure captions

- Fig. 1.** Vertical distribution of leaf area and relative light intensity of model vegetation of Siebold's beech used in calculating canopy carbon budget.
- Fig. 2.** Leaf mass per area (LMA) of Siebold's beech grown under ambient (open) and elevated ozone (filled) conditions as a function of relative light intensity.
- Fig. 3.** Light saturated net photosynthetic rate at PPF over $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ in leaves of Siebold's beech saplings grown under ambient (open) and elevated ozone (filled) conditions as a function of leaf-to-air vapor pressure deficit (VPD). Regression line throughout the gas treatments was obtained because there was no significant difference in regression lines between ambient and elevated ozone condition.
- Fig. 4.** Photosynthetic parameters obtained from the light-response curve of the photosynthesis as a function of leaf mass per area (LMA) in leaves of Siebold's beech saplings grown under ambient (open) and elevated ozone (filled) conditions. A_{sat} : light-saturated gross photosynthetic rate, ϕ : initial slope of the curve, R : respiration rate. When we detected no significant difference due to ozone of the slope and intercept, we used a single regression line in all cases.
- Fig. 5.** Gross photosynthetic carbon gain (circle) and respiratory carbon loss (triangle) per unit ground area of model vegetation of Siebold's beech grown under ambient (open) and elevated ozone (filled) conditions in late summer (left) and autumn (right). Values are calculated from the vertical distribution of leaf area and the light intensity (Fig. 1), the leaf mass per area (Fig. 2), and leaf-level photosynthesis and respiration (Fig. 4).

Fig. 6. Relations between photosynthetic carbon gain per unit leaf area and initial gradient (ϕ , left) and light saturated gross photosynthetic rate (A_{sat} , right), calculated from the light-response curve of the net photosynthetic rate, for differing relative light intensity (I_{R}). See materials and methods for details of ϕ , A_{sat} and I_{R} . The simulation was conducted for one month with the climatic conditions corresponding to August 2012 at the experimental site in our present study. The values of A_{sat} in the left figure and ϕ in the right figure were fixed as $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $0.04 \text{ mol mol}^{-1}$ respectively. Leaf mass per unit area was fixed as 80 g m^{-2} .

Fig. 1

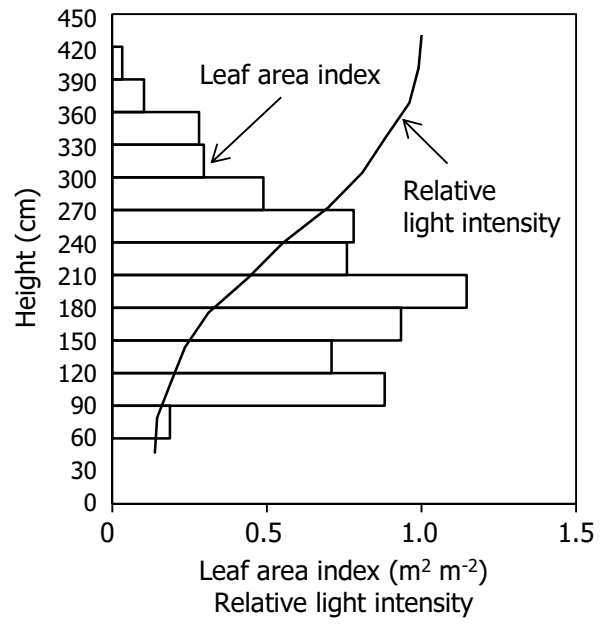


Fig. 2

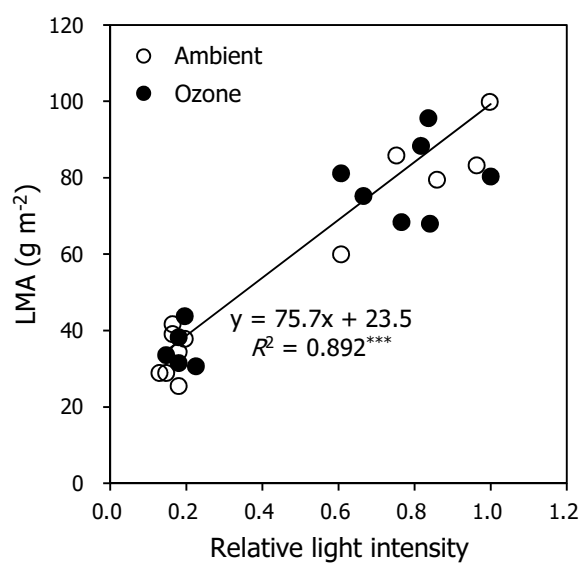


Fig. 3

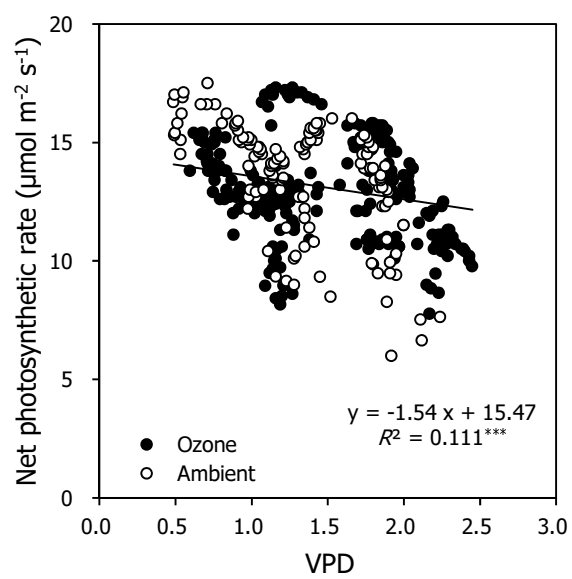


Fig. 4

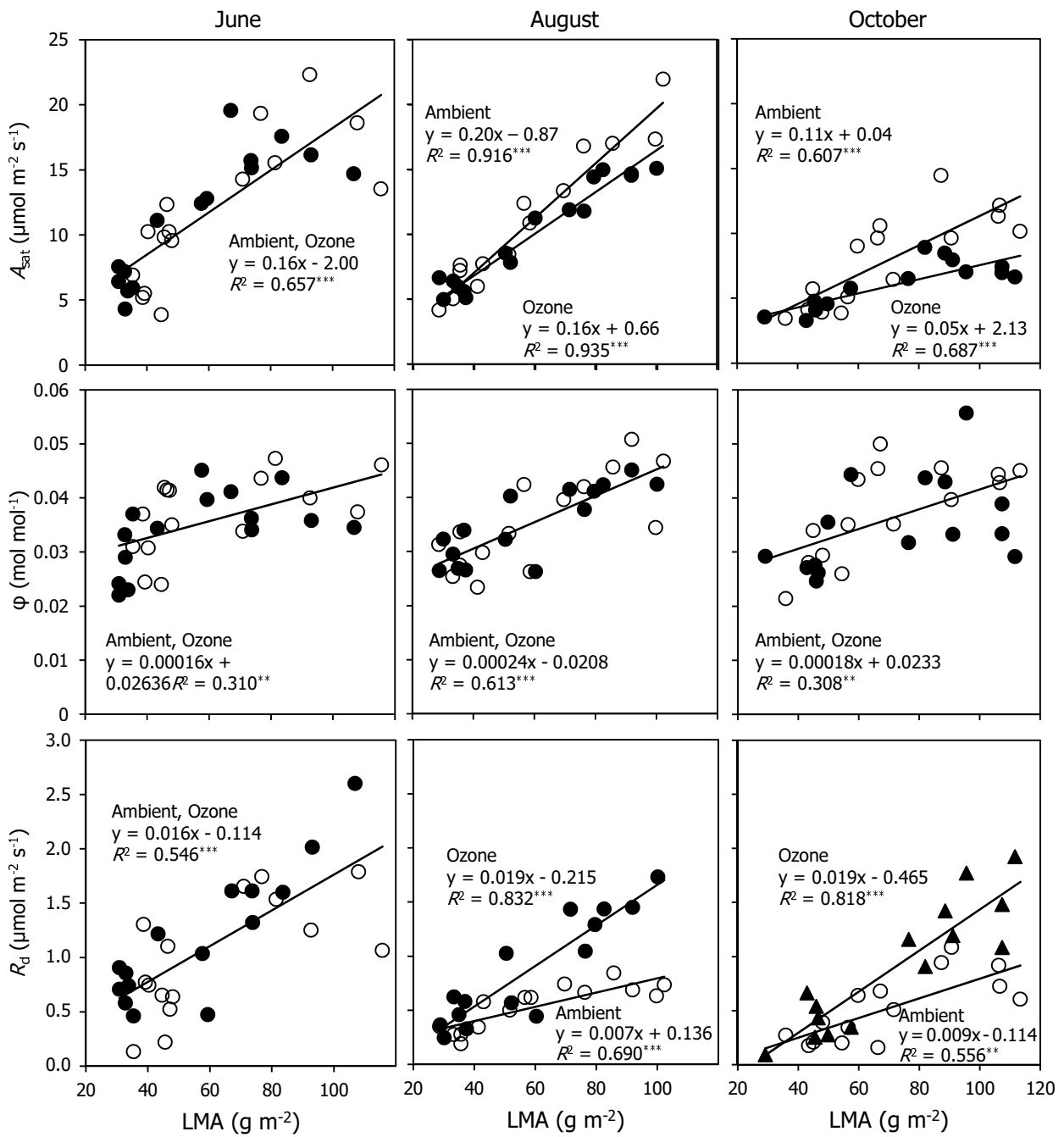


Fig. 5

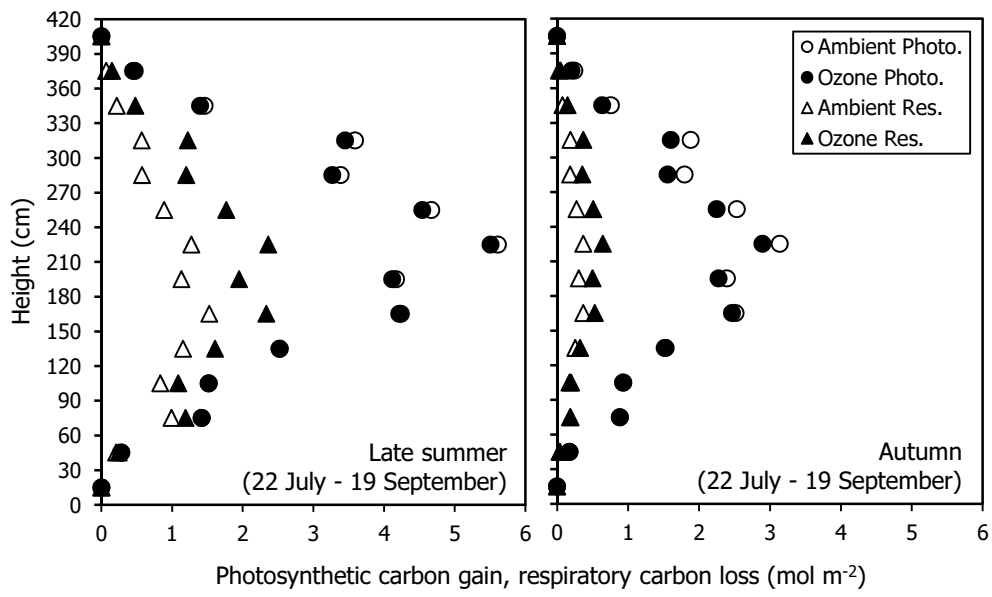


Fig. 6

