Title:
Photosynthetic traits of Siebold’s beech seedlings in changing light conditions by removal of shading trees under elevated CO₂

Names, affiliations and addresses of the authors:
Makoto Watanabe¹ 2, Satoshi Kitaoka³, Norikazu Eguchi², Yoko Watanabe² 4, Takami Satomura² 5, Kentaro Takagi⁴, Fuyuki Satoh¹ and Takayoshi Koike¹*

¹ Institute of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183–8509, Japan
² Silviculture and Forest Ecological Studies, Hokkaido University, Sapporo 060-8589, Japan
³ Forestry and Forest Products Research Institute, Tsukuba 305-8687, Japan
⁴ Field Science Center for Northern Biosphere, Hokkaido University, Sapporo 060–0809, Japan
⁵ Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-0795, Japan

Short title:
Beech photosynthesis in changing light conditions under elevated CO₂

Corresponding author:
Takayoshi Koike,
Tel: +81–11–706–3854, Fax: +81–11–706–2517
E-mail: tkoike@for.agr.hokudai.ac.jp
Faculty of Agriculture, Hokkaido University, Sapporo 060–8589, Japan
Abstract

The purpose of this study was to obtain basic information on acclimation capacity of photosynthesis of Siebold’s beech seedlings to increasing light intensity under future elevated CO$_2$ condition. We monitored leaf photosynthetic traits of Siebold’s beech seedlings in changing light conditions (before the removal of shading trees, next year after the removal of shading trees and after acclimation to open conditions) in a 10-year free air CO$_2$ enrichment experiment in northern Japan. Elevated CO$_2$ did not affect the photosynthetic traits such as leaf mass per area, nitrogen content and biochemical photosynthetic capacity of chloroplast (i.e. maximum rate of carboxylation and maximum rate of electron transport) before the removal of shading trees and after acclimation to open conditions; in fact, a higher net photosynthetic rate was maintained under elevated CO$_2$. However, in next year after the removal of the shading trees, there was no increase in photosynthetic rate under elevated CO$_2$ conditions. This was not due to photoinhibition. In ambient CO$_2$ conditions, leaf mass per area and nitrogen content were higher in next year after the removal of shading trees than before, whereas there was no increase under elevated CO$_2$ conditions. These results indicate that elevated CO$_2$ delays the acclimation of photosynthetic traits of Siebold’s beech seedlings to increasing light intensity.

Key words
change of light condition, elevated CO$_2$, Fagus crenata, leaf nitrogen, photoinhibition, photosynthesis
Introduction

Atmospheric CO$_2$ levels have increased dramatically since the industrial revolution and have reached 400 μmol mol$^{-1}$ (Monastersky 2013). This increase is predicted to continue throughout this century (Stocker et al. 2013). Increases in CO$_2$ concentration generally enhance the leaf photosynthetic rate, as CO$_2$ is the primary substrate for photosynthesis (Ainsworth & Rogers 2007). Higher net photosynthetic rates were observed under elevated CO$_2$ in canopy pine and sub-canopy deciduous trees in the Duke forest Free Air CO$_2$ Enrichment (FACE) experiment (Ellsworth et al. 2012), and in five deciduous tree species in the Swiss Canopy Crane project (Bader et al. 2010). Four understory tree species grown in shaded conditions at the Duke Forest FACE site also showed a higher net photosynthetic rate under elevated CO$_2$ conditions (Springer & Thomas 2007).

However, the extent of this enhancement is species-specific and depends on other environmental factors such as nutrient conditions (e.g. Eguchi et al. 2004, 2008; Ellsworth et al. 2004; Norby et al. 2010; Watanabe et al. 2011, 2013; Warren et al. 2015).

Siebold's beech (*Fagus crenata*) is a representative late-successional tree species native to northern Japan (Koike 1988; Nakashizuka & Iida 1995; Peters 1997). These trees commonly experience dramatic changes in light conditions over their life cycle. Seedlings generally grow under shaded conditions, and become canopy trees following fall down and/or dieback of upper story trees (Nakashizuka 1987). In Japan, gap formation in Siebold's beech forests is often caused by typhoons (Nakashizuka 1987), which generally occur from late July to September (Japan Meteorological Agency 2015).
Plants can acclimate to light conditions. For example, under high light conditions, plants produce thick leaves with higher leaf mass per area (LMA) and higher area-based nitrogen content \((N_{\text{area}})\) (Evans & Poorter 2001; Iio et al. 2005; Kitaoka et al. 2009a, b; Poorter et al. 2009; Watanabe et al. 2014a; Nienemets et al. 2015). As a result, photosynthetic parameters, such as maximum rate of carboxylation \((V_{\text{cmax}})\) and the maximum rate of electron transport \((J_{\text{max}})\) are generally higher in leaves acclimated to high-light conditions (Iio et al. 2005; Lambers et al. 2008; Rodríguez-Calcerrada et al. 2008; Nienemets et al. 2015). Nitrogen (N) allocation within the photosynthetic apparatus also acclimates to light conditions. Nitrogen allocation to carboxylation-related enzymes (i.e. Rubisco) increases under high-light conditions, while allocation to enzymes and substances related to light harvesting, such as the light-harvesting complex and the photosystems, decreases (Evans & Poorter 2001; Kitaoka & Koike 2004; Rodríguez-Calcerrada et al. 2008).

Fully expanded leaves are constrained by anatomical limitations when it comes to photosynthetic acclimation to increases in light intensity (Oguchi et al. 2005). Full acclimation to a new light environment, therefore, requires the production of new leaves. Winter-deciduous tree species form leaf and shoot primordia in winter buds during the previous year (Kozlowski & Clausen 1966, Eschrich et al. 1989). Therefore, the leaf traits of these species depend on the light conditions during the previous growing season, as well as during the bud burst. This dependency is also species-specific. The layer of palisade cells in *Fagus japonica* and *Acer tenuifolium* leaves is primarily determined by light conditions in the current year, whereas leaf properties in *F. crenata* and *Fagus sylvatica* are
affected by light conditions in both the previous and current years (Eschrich et al. 1989; Koike et al. 1997; Tognetti et al. 1998; Uemura et al. 2000; Kitaoka et al. 2009b). Kitaoka et al. (2009b) reported that photosynthetic acclimation to gap formation in seedlings of late-successional tree species (Prunus ssiori and Carpinus cordata) was slower than that of mid- and mid-late-successional tree species (Magnolia hypoleuca and Quercus mongolica var. crispula, respectively).

When a disturbance occurs during the growing season, leaves of Siebold’s beech that emerge the following spring may not be able to acclimate to the high light conditions. It is possible that leaves cannot enhance their photosynthetic rate, even under elevated CO₂ conditions. This may negatively affect growth of Siebold’s beech seedlings in future environmental conditions. Although several studies have focussed on the growth and photosynthetic responses of Siebold’s beech to elevated CO₂ conditions (Matsumura et al. 2005; Watanabe et al. 2010a; Hirano et al. 2012), no information is available on the effects of elevated CO₂ on photosynthetic responses to change in light conditions.

The aim of the present study is to obtain basic information on photosynthetic acclimation capacity of Siebold’s beech seedlings to gap formation under future elevated CO₂ conditions. We monitored photosynthetic traits of Siebold’s beech seedlings in changing light conditions (before the removal of shading trees, next year after the removal of shading trees and after acclimation to open conditions), in a 10-year FACE experiment in northern Japan.
Materials and methods

Experimental design and plant materials

We used the FACE system located in the Sapporo Experimental Forest, Hokkaido University, Japan (43°06´ N, 141°20´ E) (Eguchi et al. 2008; Watanabe et al. 2010b; Watanabe et al. 2013, 2014b). This site is in a transition zone between cool temperate and boreal forests, and is considered to be sensitive to global climate changes (Matsuda et al. 2002). We set two levels of CO₂ concentration, ambient (no addition of CO₂, 370-390 μmol mol⁻¹ CO₂) and elevated CO₂ (500 μmol mol⁻¹ CO₂), with three site replications for a total of six sites to analyze the data, including the variance among the six sites. The target CO₂ concentration in the elevated CO₂ treatment corresponded to the predicted CO₂ concentration in 2040-2050 (Stocker et al. 2013). Average values of temperature, daily maximum temperature, daily minimum temperature and precipitation during the growing season (May-October; 2003–2012) were 17.3 °C, 21.8 °C, 13.5 °C and 501.5 mm, respectively (Japan Meteorological Agency 2015).

Two-year-old seedlings of ten deciduous tree species (three early-successional: alder, birches, four mid-successional: ash, elm, kalopanax, oak, and three late-successional species: Siebold’s beech, maple, basswood), from cool temperate forests in northern Japan (Koike 1988), were planted in brown forest soil in May 2003. The Siebold’s beech seedlings were shaded by the other tree species (shading trees) from the late 2004 growing season, because the other species were fast growing. The mean (maximum-minimum) tree height for all species except the Siebold’s beech in July 2007 was 273 (420–129) cm in the
ambient CO$_2$ treatment and 319 (502–198) cm in the elevated CO$_2$ treatment. The mean tree height of Siebold’s beech at the same time was 97 cm in the ambient and 103 cm in the elevated CO$_2$ treatment. Leaf area index (LAI) of the mixed-species canopy was approximately 5.6 $m^2$ $m^{-2}$ in both CO$_2$ treatments in 2007 (Eguchi 2008). Mean relative light intensity at the top level of the Siebold’s beech seedlings was 3.2% of that of the other species. There was no significant difference in light intensity between the CO$_2$ treatments. In late July 2007, after five growing seasons, above-ground of all species except for the Siebold’s beech were cut down at the base of the stem (5–8 cm above the ground). The Siebold’s beech seedlings were then grown until 2012 under open condition. After the removal of shading trees, the light intensity at the top level of the Siebold’s beech seedlings was the same as the above original mixed species canopy.

Fumigation with CO$_2$ was carried out in the daytime when the photosynthetic photon flux (PPF) exceeded 70 $\mu$mol $m^{-2} s^{-1}$, which is the light compensation point of photosynthesis. Fumigation took place every growing season, from leaf emergence to leaf senescence, from 2003 to 2012. This fumigation regime was analogous to those of other FACE experiments with woody plants (Karnosky et al. 2005; Liberloo et al. 2009; Norby et al. 2010; Ellsworth et al. 2012). The mean daytime CO$_2$ concentration in the centres of the FACE sites during fumigation periods was 498 $\mu$mol mol$^{-1}$. The CO$_2$ concentration remained within 500 ± 50 $\mu$mol mol$^{-1}$ for 64% of the fumigation period, and was within 500 ± 100 $\mu$mol mol$^{-1}$ for 86% of the fumigation period.
Measurement of leaf photosynthetic traits

The gas exchange rate of the fully mature leaves of the upper canopy was measured in 2006 and 2007 (before the removal of the shading trees), in 2008 (next year after the removal of the shading trees), and in 2011 and 2012 (after acclimation to open conditions) using an open gas-exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) with a light-emitting diode (LED) light source (LI-6400-02B in 2006, 2007, 2011 and 2012, LI-6400-40 in 2008, Li-Cor Inc., Lincoln, NE, USA). We assumed leaves could acclimate to a new light environment within four years, although whole-plant acclimation such as canopy structure and above ground to below ground biomass ratio may not be totally complete. Measurements were made from late July to early August in each year.

In 2007, we measured leaf photosynthetic traits before cutting the shading trees down. We randomly selected one or two seedlings from each site (three, five, and six seedlings per treatment, in 2006–2007, 2008, and 2011–2012, respectively). We measured photosynthetic traits in one leaf from each seedling. Leaf temperature and PPF were maintained during measurement at 25.0 ± 1.0 °C and 1500 μmol m⁻² s⁻¹, respectively. The leaf-to-air vapour pressure deficit was approximately 1.5 kPa. To obtain the intercellular CO₂ concentration (Cᵢ) response curve for the net photosynthetic rate (A), i.e. the A/Cᵢ curve, A was determined at 8–10 steps of external CO₂ concentration (Cₐ). We determined the value of A and the stomatal conductance of leaves for water vapour at Cₐ of growth CO₂ concentration (370 μmol mol⁻¹ for ambient and 500 μmol mol⁻¹ for elevated CO₂); these values were designated as A_growth and Gₛ. V_max and J_max were calculated from the A/Cᵢ curve (Farquhar et al. 1980; Long & Bernacchi 2003).
The Rubisco 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 the analysis of the A/C$_i$ curve were estimated from leaf temperature according to Bernacchi et al. (2001).

In 2008, we also measured chlorophyll fluorescence at $C_a$ of growth conditions (370 μmol mol$^{-1}$ for ambient and 500 μmol mol$^{-1}$ for elevated CO$_2$) using an open gas-exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) with a LED light source and fluorometer (LI-6400-40, Li-Cor Inc., Lincoln, NE, USA). The minimum ($F_o$) and maximum fluorescence ($F_m$) were determined after overnight dark adaptation with a saturating light of 7000 μmol m$^{-2}$ s$^{-1}$ PPF for 1 s. The $F_{v}/F_{m}$ was then calculated as $(F_m-F_o)/F_m$. The quantum yield of PSII electron transport ($\Phi_{PSII}$), photochemical quenching (qP), and photochemical efficiency of the open PSII ($F_{v}'/F_{m}'$) were determined after acclimation to a 25.0 ± 1.0 °C leaf temperature and 1500 μmol m$^{-2}$ s$^{-1}$ PPF. From the fluorescence yield, $F_s$ and the maximum fluorescence, $F_{m}'$, (determined at the saturating light of 7000 μmol m$^{-2}$ s$^{-1}$ PPF for 1 s) were used to calculate $\Phi_{PSII}$ (= $(F_{m}'-F_o')/F_{m}'$) (Genty et al. 1989).

$F_o'$ was determined by turning off the actinic light and applying far-red light immediately after a saturating light pulse. This parameter was used to derive qP and $F_{v}'/F_{m}'$ (= $(F_{m}'-F_o')/F_{m}'$), calculated according to Schreiber et al. (1994). The fraction of excess energy was also calculated, using the formula $(1-qP)F_{v}'/F_{m}'$ (Demmig-Adams et al. 1996).

After the gas exchange rate and chlorophyll fluorescence measurements, we collected 2-3 leaf discs (12 mm diameter) in order to determine the leaf mass per area (LMA) and nitrogen content. The LMA was calculated from the area and
dry mass of leaves. The N content of the leaves was determined using a gas chromatography (GC-8A, Shimadzu, Kyoto, Japan) after combustion with circulating O₂ using a NC analyser (Sumigraph NC-900, Sumika Chemical Analysis Service, Osaka, Japan). A calibration curve was made by using acetonilide (N = 10.36%, C = 71.09, Wako, Osaka, Japan).

Statistical analysis

Statistical analyses were run using R software (version 2.15.0; R Development Core Team 2012). We used a repeated measures analysis of variance (repeated measures ANOVA) to analyse the effects of elevated CO₂, year-to-year variation and their interaction on all leaf parameters except for chlorophyll fluorescence. We used a T-test to discern the effects of elevated CO₂ on leaf parameters within each year. Mean values from each site were used in statistical analysis, resulting in three replications for the analysis. We tested the effect of elevated CO₂ on the slope and intercept of the regression lines between leaf traits parameters using (S) MATR (Version 2; see http://www.bio.mq.edu.au/ecology/SMATR/). When there was no significant difference between slopes of the regression lines for treatments, we applied a common slope and analysed the difference of the intercepts of the regression lines.

Results

Overall, leaf photosynthetic traits (except for N_mass) increased throughout the experimental period (Table 1, Figs. 1 and 2). There was a significant interaction between elevated CO₂ and year-to-year variation for A_growth (Table 1).
Higher values of $A_{\text{growth}}$ were observed in elevated CO$_2$ than in ambient CO$_2$ before the removal of shading trees (2006 and 2007) and after acclimation to open conditions (2011 and 2012), although the increase before the removal of the shading trees was marginal (Fig. 1a). In contrast, $A_{\text{growth}}$ in the elevated CO$_2$ treatment tended to be lower than that in ambient CO$_2$ in the next year after the removal of shading trees (2008). Although there were no significant interactions between elevated CO$_2$ and year-to-year variation for the other parameters (Table 1), there was a significant effect of elevated CO$_2$ in the next year after the removal of the shading trees. In this period, the elevated CO$_2$ treatment induced significant reductions in $G_s$, $V_{\text{cmax}}$, LMA, and $N_{\text{area}}$, and marginal reductions in $J_{\text{max}}$ (Fig. 1b, c, d, Fig. 2a, c). From a time course perspective, all photosynthetic parameters except for $N_{\text{mass}}$ increased from 2007 to 2008 in the ambient CO$_2$ treatment, whereas there was no increase in the elevated CO$_2$ treatment.

There was a clear correlation between $V_{\text{cmax}}$ and $J_{\text{max}}$ throughout the experimental period (Fig. 3). No significant effect of elevated CO$_2$ was found on the slope and intercept of regression line. No significant difference in regression lines between CO$_2$ treatments was found when we analysed the regression lines in each period (before the removal of shading trees, next year after the removal of shading trees and after acclimation to open conditions) separately.

Elevated CO$_2$ did not significantly change the slope of the regression line between $A_{\text{growth}}$ and $N_{\text{area}}$; however, the intercept of the regression line for the elevated CO$_2$ treatment was significantly higher than that for the ambient CO$_2$ treatment (Fig. 4a) when a common slope was applied to both regression lines. No significant differences in slope and intercept of the regression lines were found
between $V_{\text{cmax}}$ and $N_{\text{area}}$, or $J_{\text{max}}$ and $N_{\text{area}}$ (Fig. 4b, c).

The $\Phi_{\text{PSII}}$ tended to decrease under elevated CO$_2$ (Table 2). Although there was no significant difference between ambient CO$_2$ and elevated CO$_2$ for the other parameters, there was a relatively large reduction of $qP$ (35%) in the elevated CO$_2$ treatment.

**Discussion**

In this study, we did not observe any negative effects of CO$_2$ on gas-exchange parameters and N content in leaves before the removal of shading trees and after acclimation to open conditions (Table 1, Figs. 1 and 2). As a result, $A_{\text{growth}}$ increased by 54% before the removal of shading trees and 31% after acclimation to open conditions. Increases in photosynthesis rate with increased CO$_2$ have also been found in other FACE experiments (e.g. Springer & Thomas 2007; Bader *et al.* 2010; Ellsworth *et al.* 2012). However, this does not mean that elevated CO$_2$ does not permanently induce photosynthetic down-regulation in Siebold’s beech. Nitrogen availability is one of the most important factors for photosynthetic down-regulation under elevated CO$_2$ (Saxe *et al.* 1998; Norby & Zak 2011). In general, elevated-CO$_2$-induced declines in photosynthetic capacity are prominent in low-N soil conditions (e.g. Curtis *et al.* 2000; Kubiske *et al.* 2002). Norby *et al.* (2010) and Warren *et al.* (2015), found that although photosynthetic production of sweetgum (*Liquidambar styraciflua*) was increased in elevated CO$_2$ conditions during the first few years of the experiment, it ultimately declined, due to a reduction in N availability during the latter period of the experiment. In the present study, seedlings (approximately 3.3 m height in
both CO₂ treatments in 2012) were not as large as those used in other FACE experiments. The availability of soil N may have been sufficient for growth during this experiment. If N availability decreases with increasing plant size, down regulation of photosynthesis may occur in the future.

An increase in the photosynthetic rate was not observed in the elevated-CO₂ treatment in the next year after the removal of shading trees. This may have been due to photoinhibition; however, there was no difference in Fᵥ/Fₘ and (1--qP)Fᵥ/Fₘ′, which are indicators of photoinhibition in leaves (Genty et al. 1989; Demmig-Adams et al. 1996), between the ambient CO₂ and elevated CO₂ treatments. This indicates elevated CO₂ did not stimulate photoinhibition (Table 2).

In addition, Φₚₛᵢᵢ decreased under elevated CO₂. The contribution of qP reduction to the decline of Φₚₛᵢᵢ under elevated CO₂ was larger than that of Fᵥ/Fₘ′ reduction. Fᵥ/Fₘ′ represents the capacity of photosystem II, while qP is affected by the status of components downstream of photosystem II, such as photosystem I and carboxylation (Baker 2008). In fact, Vₖₘₐₓ in elevated CO₂ was lower than in ambient CO₂ (Fig. 1c). Therefore, the lower carboxylation capacity is likely one of the main reasons for the decrease in Φₚₛᵢᵢ under elevated CO₂.

Nitrogen is a nutrient that strongly relates to photosynthetic capacity (Lambers et al. 2008). In the present study, A₉ᵣₒₜ₉, Vₖₘₐₓ and Jₘₜₚₐₓ were linearly correlated to Nₐₑᵣₐₑ and the relationship between Vₖₘₐₓ and Nₐₑᵣₐₑ, and Jₘₜₚₐₓ and Nₐₑᵣₐₑ, was the same in both CO₂ treatments (Fig. 4b, c). Therefore, it is likely that the lower Nₐₑᵣₐₑ in the elevated-CO₂ treatment in the next year after the removal of shading trees (Fig. 2c) led to lower photosynthetic activity (indicated by Vₖₘₐₓ and Jₘₜₚₐₓ), and therefore, lower A₉ᵣₒₜ₉. Nₐₑᵣₐₑ is calculated as a product of LMA and Nₘₐₜₜₐₑ.
and the lower $N_{\text{area}}$ in the elevated-CO$_2$ treatment in the next year after the removal of shading trees was primarily due to lower LMA, since there was no significant difference in $N_{\text{mass}}$ between the ambient- and elevated-CO$_2$ treatments (Fig. 2). LMA increased from 2007 to 2008 in the ambient CO$_2$ treatment, whereas there was no difference between 2007 and 2008 in the elevated CO$_2$ treatment. The anatomical properties of Siebold’s beech leaves are affected by light conditions in the previous growing season (Uemura et al. 2000). Eschrich et al. (1989) observed that differentiation into sun-leaf and shade-leaf primordia of the European beech ($Fagus$ $sylvatica$) occurs in early August. Although there are many differences (climate of the study site, species and age of tree) between that study and ours, the timing of removal of shading trees in the present study (late July) was similar to the timing of the differentiation in that study (Eschrich et al. 1989). Sigurdsson (2001) reported earlier bud formation under elevated-CO$_2$ conditions in young black cottonwood ($Populus$ $trichocarpa$). It is possible that the differentiation of leaf primordia occurred prior to the removal of shading trees under elevated-CO$_2$ conditions and, as a result, leaf acclimation to increasing light intensity in the following year was delayed. It should be noted that the increase in LMA throughout the experiment was not only caused by the change in light conditions, but also by increasing tree age. We observed an increase in LMA during steady light conditions, i.e. from 2006 to 2007 and from 2011 to 2012 (Fig. 2a), and LMA generally increases with increasing tree age (e.g. Niinemets 2006; Poorter et al. 2009). The LMA in the final year of the experiment (2012) reached approximately 90 g m$^{-2}$ (Fig. 2a), which is comparable to that of the upper canopy leaves of mature Siebold’s beech trees (Iio et al. 2005; Uemura et al. 2006).
Although the reduction in $V_{cmax}$ in the next year after the removal of shading trees was severe, the extent of the reduction in $A_{growth}$ was rather small. This is mainly due to a higher volume of substrate for photosynthesis (i.e. high CO$_2$ concentration). This reduction was not observed in 2011; therefore, even when negative effects of elevated-CO$_2$ conditions on photosynthetic functions are induced, the effect is marginal. Thus, photosynthetic production may not decrease severely in future CO$_2$ conditions.

A meta-analysis conducted by Ainsworth & Long (2005) predicted that the ratio of $J_{max}$ to $V_{cmax}$ ($J_{max}/V_{cmax}$) would increase under elevated-CO$_2$ conditions. However, we found the relationship between $J_{max}$ and $V_{cmax}$ in elevated CO$_2$ conditions was not differed from that in ambient CO$_2$ conditions (Fig. 3). This indicates that there was no shift in N allocation between carboxylation and electron transport capacities (Evans & Poorter 2001; Kitaoka & Koike 2004; Rodríguez-Calcerrada et al. 2008). The relatively low enhancement of CO$_2$ concentration (500 μmol mol$^{-1}$) in the elevated-CO$_2$ treatment in the present study could explain the lack of shift of N allocation. Similarly, the change in light conditions did not significantly affect the relationship between $J_{max}$ and $V_{cmax}$ (data not shown). Onoda et al. (2005) reported no seasonal variation of $J_{max}/V_{cmax}$ in the Siebold’s beech, indicating little temperature dependency. The shift of $J_{max}/V_{cmax}$ across environmental conditions is species-specific (Niinemets et al. 1998; Hikosaka et al. 1999; Medlyn et al. 2002; Ainsworth & Long 2005), and therefore it is possible that in the Siebold’s beech, $J_{max}/V_{cmax}$ remains constant across environmental conditions.

Our results indicate that elevated-CO$_2$ conditions (500 μmol mol$^{-1}$; the
predicted concentration for 2040–2050), enhance the net photosynthetic rate of
the Siebold’s beech in both shaded and open conditions. We did not observe
stomatal closure, or reduction of N content and photosynthetic N use efficiency
under elevated CO\textsubscript{2} conditions, indicating there was no down-regulation of
photosynthesis in leaves. In contrast, the acclimation of photosynthesis of Siebold’s
beech seedlings to increasing light intensity was delayed under elevated-CO\textsubscript{2}
conditions. Photosynthetic increases under elevated CO\textsubscript{2} conditions did not occur
in the next year after the removal of shading trees. This was not due to
photoinhibition, but to lower area-based N content. Although the delay of
photosynthetic acclimation is marginal, further studies could investigate the
impacts on regeneration capacity, such as growth performance, of Siebold’s beech
seedlings, in order to predict dynamics of forests of this tree species under future
elevated CO\textsubscript{2} conditions.

**Acknowledgments**

This study was partly supported by Japan Society for the Promotion of Science
through the programs of a Grant-in-Aid for Young Scientists (B) (24710027 and
15K16136, to M. Watanabe), a Grant-in-Aid for Scientific Research on Innovative
Areas (21114008, to T. Koike), Challenging Exploratory Research (26660119, to
T. Koike) and Grant-in-Aid for Scientific Research (A) (23255009, to F. Satoh).

**References**

Ainsworth E.A., Long S.P. (2005) What have we learned from 15 years of free-air
CO\textsubscript{2} enrichment (FACE)? A meta-analytic review of the responses of


Eguchi N. (2008) Study on the changes in CO₂ fixation and storage capacity of deciduous tree species native to cool temperate zone with increasing ambient CO₂ concentration. Doctoral dissertation of The Graduate School
of Agriculture of Hokkaido University, Sapporo, Japan.


Koike T., Miyashita N., Toda H. (1997) Effects of shading leaf structural characteristics in successional deciduous broadleaved tree seedlings and
their silvicultural meaning. *Forest Resources and Environment* 35, 9–25.


Matsumura H., Mikami C., Sakai Y., Izuta T., Yonekura T., Miwa M., Kohno Y. (2005) Impacts of elevated O₃ and/or CO₂ on growth of *Betula platyphylla*, *Betula ermanii*, *Fagus crenata*, *Pinus densiflora* and *Cryptomeria japonica* seedlings. *Journal of Agricultural Meteorology* 60, 1121–1124.


Nakashizuka T., Iida S. (1995) Composition, dynamics and disturbance regime of


Table 1 A summary of Repeated Measures ANOVA for effects of elevated CO\(_2\) and year-to-year variation on net photosynthetic rate at growth CO\(_2\) concentration \((A_{growth})\), stomatal conductance to water vapor \((G_s)\), maximum rate of carboxylation \((V_{cmax})\) and maximum rate of electron transport \((J_{max})\), leaf mass per area \((LMA)\), mass-based nitrogen content \((N_{mass})\), and area-based nitrogen content \((N_{area})\) in the leaves of Siebold’s beech seedlings

<table>
<thead>
<tr>
<th></th>
<th>CO(_2)</th>
<th>Year</th>
<th>CO(_2) × Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A_{growth})</td>
<td>*</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>(G_s)</td>
<td>n.s.</td>
<td>***</td>
<td>n.s.</td>
</tr>
<tr>
<td>(V_{cmax})</td>
<td>n.s.</td>
<td>***</td>
<td>n.s.</td>
</tr>
<tr>
<td>(J_{max})</td>
<td>n.s.</td>
<td>***</td>
<td>n.s.</td>
</tr>
<tr>
<td>LMA</td>
<td>n.s.</td>
<td></td>
<td>n.s.</td>
</tr>
<tr>
<td>(N_{mass})</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>(N_{area})</td>
<td>n.s.</td>
<td>***</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* \(P < 0.05\); ** \(P < 0.01\); *** \(P < 0.001\); n.s. not significant
Table 2 The parameters of chlorophyll fluorescence in leaves of Siebold’s beech seedlings grown under ambient and elevated CO₂ (500 μmol mol⁻¹ CO₂) conditions in the year after the removal of shading trees (2008).

<table>
<thead>
<tr>
<th></th>
<th>Ambient CO₂</th>
<th>Elevated CO₂</th>
<th>T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fᵥ/Fₘ</td>
<td>0.814 (0.005)</td>
<td>0.810 (0.002)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Fᵥ'/Fₘ'</td>
<td>0.404 (0.009)</td>
<td>0.380 (0.012)</td>
<td>n.s.</td>
</tr>
<tr>
<td>qP</td>
<td>0.251 (0.031)</td>
<td>0.163 (0.032)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Φₜₚₜ</td>
<td>0.101 (0.014)</td>
<td>0.061 (0.011)</td>
<td>0.088</td>
</tr>
<tr>
<td>(1 - qP) Fᵥ'/Fₘ'</td>
<td>0.303 (0.006)</td>
<td>0.319 (0.023)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Standard error is shown in parentheses (n = 3).

T-test: n.s. not significant; actual P values were shown when 0.05 < P < 0.1.

Fᵥ/Fₘ was determined after an overnight dark adaptation, while Fᵥ'/Fₘ', qP, Φₜₚₜ and (1 - qP) Fᵥ'/Fₘ' were determined after acclimation to 25.0 ± 1.0°C of leaf temperature and 1500 μmol m⁻² s⁻¹ of photosynthetic photon flux. The intensity of saturation pulse was 7000 μmol m⁻² s⁻¹ of PPF for 1 s.

During the measurement, CO₂ concentration within a chamber was maintained at 370 and 500 μmol mol⁻¹ for ambient and elevated CO₂ treatment, respectively.
Fig. 1 (a) Net photosynthetic rate at growth CO₂ concentration ($A_{growth}$), (b) stomatal conductance to water vapour ($G_s$), (c) maximum rate of carboxylation ($V_{cmax}$) and (d) maximum rate of electron transport ($J_{max}$) of Siebold’s beech seedlings grown under ambient and elevated CO₂ (500 μmol mol⁻¹) conditions, before the removal of shading trees (2006 and 2007), next year after the removal of shading trees (2008) and after acclimation to open conditions (2011 and 2012). A $T$-test was performed in each year: * $P < 0.05$; ** $P < 0.01$; actual $P$ values were shown when $0.05 < P < 0.1$. 

$A_{growth}$, $G_s$, $V_{cmax}$, $J_{max}$.
Fig. 2 (a) Leaf mass per area (LMA), (b) mass-based nitrogen content ($N_{\text{mass}}$) and (c) area-based nitrogen content ($N_{\text{area}}$) in the leaves of Siebold’s beech seedlings grown under ambient and elevated CO$_2$ (500 μmol mol$^{-1}$) conditions, before the removal of shading trees (2006 and 2007), next year after the removal of shading trees (2008) and after acclimation to open conditions (2011 and 2012). A T-test was performed in each year: * $P < 0.05$; ** $P < 0.01$. 
Fig. 3 Maximum rate of electron transport ($J_{\text{max}}$) as a function of maximum rate of carboxylation ($V_{\text{cmax}}$) of Siebold’s beech seedlings grown under ambient and elevated CO$_2$ (500 μmol mol$^{-1}$) conditions. Each data point indicates the mean value in each year. Regression line: $y = 4.8 + 2.2x$, $R^2 = 0.98^{***}$ for ambient CO$_2$ (solid line) and $y = 16.3 + 1.9x$, $R^2 = 0.98^{***}$ for elevated CO$_2$ (dashed line). There was no significant difference of the slope and intercept of regression lines between ambient and elevated CO$_2$ treatments.
Fig. 4 (a) Net photosynthetic rate at growth CO$_2$ concentration ($A_{\text{growth}}$), (b) maximum rate of carboxylation ($V_{\text{cmax}}$) and (c) maximum rate of electron transport ($J_{\text{max}}$) as a function of area-based nitrogen content ($N_{\text{area}}$) in leaves of Siebold’s beech seedlings grown under ambient and elevated CO$_2$ (500 μmol mol$^{-1}$) conditions. Each data point indicates the mean value in each year. The solid lines indicate the regression lines for ambient CO$_2$ conditions, and the dashed lines for elevated CO$_2$ conditions. Regression equations: (a) $y = -2.2 + 7.3x$, $R^2 = 0.77$ ($P = 0.052$) for ambient CO$_2$ and $y = -2.1 + 9.5x$, $R^2 = 0.82^*$ for elevated CO$_2$, (b) $y = -21.8 + 45.1x$, $R^2 = 0.98^{***}$ for ambient CO$_2$ and $y = -16.8 + 43.4x$, $R^2 = 0.97^{**}$ for elevated CO$_2$ and (c) $y = -44.2 + 98.8x$, $R^2 = 0.98^{***}$ for ambient CO$_2$ and $y = -15.8 + 83.6x$, $R^2 = 0.98^{***}$ for elevated CO$_2$.

There was no significant difference in the slope of regression lines between ambient and elevated CO$_2$ for all relationships. When a common slope was applied to both regression lines, there was a significant difference in the intercept between ambient and elevated CO$_2$ for (a), while no significant difference was found for (b) and (c).