ABSTRACT

The purpose of this study was to obtain basic information on acclimation capacity of photosynthesis in Siebold’s beech seedlings to increasing light intensity under future elevated CO₂ conditions. We monitored leaf photosynthetic traits of these seedlings in changing light conditions (before removal of shade trees, the year after removal of shade trees and after acclimation to open conditions) in a 10-year free air CO₂ enrichment experiment in northern Japan. Elevated CO₂ did not affect photosynthetic traits such as leaf mass per area, nitrogen content and biochemical photosynthetic capacity of chloroplasts (i.e. maximum rate of carboxylation and maximum rate of electron transport) before removal of the shade trees and after acclimation to open conditions; in fact, a higher net photosynthetic rate was maintained under elevated CO₂. However, in the year after removal of the shade trees, there was no increase in photosynthesis rate under elevated CO₂ conditions. This was not due to photoinhibition. In ambient CO₂ conditions, leaf mass per area and nitrogen content were higher in the year after removal of shade trees than before, whereas there was no increase under elevated CO₂ conditions. These results indicate that elevated CO₂ delays the acclimation of photosynthetic traits of Siebold’s beech seedlings to increasing light intensity.

INTRODUCTION

Atmospheric CO₂ levels have increased dramatically since the industrial revolution and have now reached 400 μmol mol⁻¹ (Monastersky 2013). This increase is predicted to continue throughout this century (Stocker et al. 2013). Increases in CO₂ concentration generally enhance leaf photosynthetic rates, as CO₂ is the primary substrate for photosynthesis (Ainsworth & Rogers 2007). Higher net photosynthesis rates were observed under elevated CO₂ in canopy pine and sub-canopy deciduous trees in the Duke forest Free Air CO₂ 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 higher net photosynthesis rate under elevated CO₂ 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 shade conditions, and become canopy trees following the fall and/or dieback of upper storey 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, plants produce thicker leaves with a higher leaf mass per area (LMA) and higher area-based nitrogen content (N/area) (Evans & Poorter 2001; Iio et al. 2005; Kitaoka et al. 2009a,b; Poorter et al. 2009; Watanabe et al. 2014a; Niinemets et al. 2015). As a result, photosynthetic parameters, such as maximum rate of carboxylation (Vₘₐₓ) and maximum rate of electron transport (Jₘₐₓ), are generally higher in leaves acclimated to high light conditions (Iio et al. 2005; Lambers et al. 2008; Rodríguez-Calcerrada et al. 2008; Niinemets 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 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 light conditions during the previous growing season, as well as during bud burst. This dependency is also species-specific. The layers of palisade cells in Fagus japonica and Acer tenuifolium leaves are 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 year (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 sissori and Carpinus cordata) was slower than that of mid- and mid-late successional tree species (Magnolia hypoleuca and Quercus mongolica var. cris-pula, respectively).

When a disturbance occurs during the growing season, leaves of Siebold’s beech that emerge in the following spring may not be able to acclimate to high light conditions. It is possible that leaves cannot enhance their photosynthesis 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 (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 changes in light conditions.

The aim of the present study was 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 these seedlings in changing light conditions (before removal of shade trees, the year after the removal of shade trees and after acclimation to open conditions) in a 10-year FACE experiment in northern Japan.

MATERIAL 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, 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 replicates for a total of six sites, to analyse data including variance among the six sites. The target CO₂ concentration in the elevated CO₂ treatment corresponded to the predicted CO₂ concentration for 2040–2050 (Stockler 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 (shade trees) from the late 2004 growing season, because the other species were fast-growing. The mean (maximum–minimum) tree height for all species except Siebold’s beech in July 2007 was 273 cm (range 129–420 cm) in the ambient CO₂ treatment and 319 cm (range: 198–502 cm) in the elevated CO₂ treatment. The mean tree height of Siebold’s beech at the same time was 97 cm in ambient and 103 cm in elevated CO₂ treatment. Leaf area index (LAI) of the mixed species canopy was approximately 5.6 m²·m⁻² in both CO₂ treatments in 2007 (Eguchi 2008). Mean relative light intensity at the top 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 CO₂ treatments. In late July 2007, after five growing seasons, aboveground growth of all species except Siebold’s beech was cut down to the stem base (5–8 cm above the ground). The Siebold’s beech seedlings were then grown until 2012 under open conditions. After removal of these shade trees, the light intensity at the top of the Siebold’s beech seedlings was the same as that above the original mixed species canopy.

Fumigation with CO₂ was carried out in the daytime when the photosynthetic photon flux (PPF) exceeded 70 µmol 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 containing woody plants (Karnosky et al. 2005; Liberloo et al. 2009; Norby et al. 2010; Ellsworth et al. 2012). The mean daytime CO₂ concentration in the centres of the FACE sites during fumigation periods was 498 µmol·mol⁻¹. The CO₂ concentration remained within 500 ± 50 µmol·mol⁻¹ for 64% of the fumigation period, and was within 500 ± 100 µmol·mol⁻¹ for 86% of the fumigation period.

Measurement of leaf photosynthetic traits

The gas exchange rate of fully mature leaves of the upper canopy was measured in 2006 and 2007 (before removal of shade trees), in 2008 (the year after removal of shade trees) and in 2011 and 2012 (after acclimation to open conditions) using an open gas exchange system (LI-6400; Li-Cor, 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). We assumed leaves could acclimate to a new light environment within 4 years, although whole-plant acclimation such as canopy structure and aboveground to belowground 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 shade 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 ca. 1.5 kPa. To obtain A/Ci curves, the curves for intercellular CO₂ concentration (Ci) to
Beech photosynthesis in changing light conditions and elevated CO₂

Statistical analysis

Statistical analyses were run using R software (version 2.15.0; R Development Core Team 2012). We used repeated measures ANOVA to analyse the effects of elevated CO₂, year-to-year variation and their interactions on all leaf parameters except chlorophyll fluorescence. We used a t-test to discern 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 analyses. We tested the effect of elevated CO₂ on the slope and intercept of the regression lines between leaf traits using (S) MATR (version 2; Falster et al. 2006). When there was no significant difference between slopes of regression lines for treatments, we applied a common slope and analysed the difference of intercepts of the regression lines.

RESULTS

Overall, leaf photosynthetic traits (except Nmass) increased throughout the experimental period (Table 1, Figs 1 and 2). There was a significant interaction between elevated CO₂ and year-to-year variation in Agrowth (Table 1). Higher values of Agrowth were observed in elevated CO₂ than in ambient CO₂ before removal of shade trees (2006 and 2007) and after acclimation to open conditions (2011 and 2012), although the increase before removal of shade trees was marginal (Fig. 1a). In contrast, Agrowth in elevated CO₂ treatment tended to be lower than that in ambient CO₂ in the year after removal of shade trees (2008). Although there were no significant interactions between elevated CO₂ and year-to-year variation for the other parameters (Table 1), there was a significant effect of elevated CO₂ in the year after removal of shade trees. In this period, the elevated CO₂ treatment induced significant reductions in Gs, Vcmax, LMA and Narea and marginal reductions in Jmax (Figs 1b, c, d and 2a, c). From a time course perspective, all photosynthetic parameters except Nmass increased from 2007 to 2008 in the ambient CO₂ treatment, whereas there was no increase in the elevated CO₂ treatment.

There was a clear correlation between Vcmax and Jmax throughout the experimental period (Fig. 3). No significant effect of elevated CO₂ was found on the slope and intercept of the regression line. No significant difference in regression lines between CO₂ treatments was found when we analysed the regression lines in each period (before removal of shade trees, year after removal of shade trees and after acclimation to open conditions) separately (data not shown).

Elevated CO₂ did not significantly change the slope of the regression line between Agrowth and Narea; however, the intercept of the regression line for the elevated CO₂ treatment was significantly higher than that for the ambient CO₂ 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 Vcmax and Narea or Jmax and Narea (Fig. 4b, c).

The FriPSII tended to decrease under elevated CO₂ (Table 2). Although there was no significant difference between ambient CO₂ and elevated CO₂ for the other parameters, there was a relatively large reduction of qP (35%) in the elevated CO₂ treatment.

Table 1. A summary of repeated measures ANOVA for effects of elevated CO₂ and year-to-year variation on net photosynthesis rate (A), annual growth parameter (Agrowth), stomatal conductance to water vapour (Gs), maximum rate of carboxylation (Vcmax), maximum rate of electron transport (Jmax), leaf mass per area (LMA), mass-based N content (Nmass), and area-based N content (Narea) in leaves of Siebold’s beech seedlings.

<table>
<thead>
<tr>
<th>Trait</th>
<th>CO₂ × year</th>
<th>CO₂ × year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agrowth</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>Gs</td>
<td>n.s.</td>
<td>***</td>
</tr>
<tr>
<td>Vcmax</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Jmax</td>
<td>n.s.</td>
<td>***</td>
</tr>
<tr>
<td>LMA</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Nmass</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Narea</td>
<td>n.s.</td>
<td>***</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01; ***P < 0.001; n.s. not significant.
DISCUSSION

In this study, we did not observe any negative effects of CO2 on gas exchange parameters and N content in leaves before removal of shade trees and after acclimation to open conditions (Table 1, Figs 1 and 2). As a result, A\text{growth} increased 54% before removal of shade trees and 31% after acclimation to open conditions (2011 and 2012). Increases in photosynthesis rate with increased CO2 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 imply that elevated CO2 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 CO2 (Saxe et al. 1998; Norby & Zak 2011). In general, elevated CO2-induced declines in photosynthetic capacity are well known 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) increased in elevated CO2 conditions during the first few years of the experiment, it ultimately declined due to a reduction in N availability in the later period of the experiment. In the present study,
Beech photosynthesis in changing light conditions and elevated CO₂

Adams et al. (2016) indicated that elevated CO₂ did not stimulate photoinhibition (Table 2). In addition, qP reduction to the capacity of PSII, while qP is affected by the status of components downstream of PSII, such as PSI and carboxylation (Baker 2008). In fact, V_{cmax} in elevated CO₂ was lower than in ambient CO₂ (Fig. 1a). Therefore, the lower carboxylation capacity is likely one of the main reasons for the decrease in Φ_{PSII} under elevated CO₂.

Nitrogen is a nutrient strongly related to photosynthetic capacity (Lambers et al. 2008). In the present study, A_{growth}, V_{cmax} and J_{max} were linearly correlated with N_{area} and the relationship between V_{cmax} and N_{area} and between J_{max} and N_{area} was the same in both CO₂ treatments (Fig. 4b, c). Therefore, it is likely that the lower N_{area} in the elevated CO₂ treatment in the year after removal of shade trees was primarily due to lower LMA and N_{mass} and the lower N_{area} in the elevated CO₂ treatment in the year after removal of shade trees was primarily due to lower LMA, since there was no significant difference in N_{mass} between the ambient and elevated CO₂ treatments (Fig. 2). LMA increased from 2007 to 2008 in the ambient CO₂ treatment, whereas there was no difference between 2007 and 2008 in the elevated CO₂ 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 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 shade trees in the present study (late July) was similar to the timing of differentiation in the study of Eschrich et al. (1989). Sigurdsson (2001) reported earlier bud formation under elevated CO₂ conditions in young black cottonwood (Populus trichocarpa). It is possible that the differentiation of leaf primordia occurred prior to removal of shade trees under elevated CO₂ 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

Table 2. Parameters of chlorophyll fluorescence in leaves of Siebold’s beech seedlings grown under ambient or elevated CO₂ (500 μmol mol⁻¹ CO₂) conditions in the year after removal of shade trees (2008)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ambient CO₂</th>
<th>Elevated CO₂</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₀/F_m</td>
<td>0.814 (0.005)</td>
<td>0.810 (0.002)</td>
<td>n.s.</td>
</tr>
<tr>
<td>F₉/F_m</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>Φ_{PSII}</td>
<td>0.101 (0.014)</td>
<td>0.061 (0.011)</td>
<td>0.088</td>
</tr>
<tr>
<td>(1-qP)F₀'/F₀</td>
<td>0.303 (0.006)</td>
<td>0.319 (0.023)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

SE is shown in parentheses (n = 3).

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

F₀/F_m was determined after overnight dark adaptation, while F₉/F_m, qP, Φ_{PSII} and (1-qP)F₀'/F₀ were determined after acclimation to 25 ± 1 °C leaf temperature and 1500 μmol m⁻² s⁻¹ PPF. Saturating light pulse was 7000 μmol m⁻² s⁻¹ for 1 s. During measurement, CO₂ concentration within a chamber was maintained at 370 or 500 μmol mol⁻¹ for ambient or elevated CO₂ treatment, respectively.

Fig. 4. (a) Net photosynthesis rate at growth CO₂ concentration (A_{growth}), (b) maximum rate of carboxylation (V_{cmax}) and (c) maximum rate of electron transport (J_{max}) as a function of ambient N content (N_{area}) in leaves of Siebold’s beech seedlings grown under ambient or elevated CO₂ (500 μmol mol⁻¹ CO₂) conditions. Each data point indicates mean value in each year. The solid lines are regression lines for ambient CO₂ conditions, and dashed lines for elevated CO₂ conditions. Regression equations: (a) y = -2.2 + 7.3x, R² = 0.77 (P = 0.052) for ambient CO₂ and y = -2.1 + 9.5x, R² = 0.82 * for elevated CO₂; (b) y = -21.8 + 45.1x, R² = 0.98 ** for ambient CO₂ and y = -16.8 + 43.4x, R² = 0.97 * for elevated CO₂ and (c) y = -44.2 + 98.8x, R² = 0.98 ** for ambient CO₂ and y = -15.8 + 83.6x, R² = 0.98 ** for elevated CO₂. There was no significant difference in slope of regression lines between ambient and elevated CO₂ 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 CO₂ for (a), but no significant difference was found for (b) and (c).
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 ca. 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 year after removal of shade 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 CO₂ concentration). This reduction was not observed in 2011; therefore, even when negative effects of elevated CO₂ conditions on photosynthetic functions are induced, the effect is marginal. Thus, photosynthetic production may not decrease severely in future CO₂ conditions.

A meta-analysis by Ainsworth & Long (2005) predicted that the ratio of J_max to V_cmax (J_max/V_cmax) would increase under elevated CO₂ conditions. However, we found the relationship between J_max and V_cmax in elevated CO₂ conditions did not differ from that in ambient CO₂ conditions (Fig. 3). This indicates that there was no shift in N allocation between carboxylation and electron transport capacity (Evans & Poorter 2001; Kitaoka & Koike 2004; Rodríguez-Calcerrada et al. 2008).

The relatively low increase of CO₂ concentration (500 μmol·mol⁻¹) in the elevated CO₂ treatment in the present study could explain the lack of a shift in 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 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 Siebold’s beech, J_max/V_cmax remains constant across environmental conditions.

Our results indicate that elevated CO₂ conditions (500 μmol·mol⁻¹; predicted concentration for 2040–2050) enhance net photosynthesis rate of 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 CO₂ conditions, indicating there was no down-regulation of photosynthesis in the leaves. In contrast, acclimation of photosynthesis in Siebold’s beech seedlings to increasing light intensity was delayed under elevated CO₂ conditions. No increase in photosynthesis under elevated CO₂ conditions occurred in the year after removal of shade trees. This was not due to photoinhibition, but to lower area-based N content. Although the delay in photosynthetic acclimation was marginal, further studies should 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 under future elevated CO₂ conditions.

ACKNOWLEDGEMENTS

This study was partly supported by Japan Society for the Promotion of Science through the programmes 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


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


Hirano A., Hongo I., Koike T. (2012) Morphological and physiological responses of Siebold’s beech (Fagus crenata) seedlings grown under CO₂ concentrations ranging from pre-industrial to expected future levels. Landscape and Ecological Engineering, 8, 59–67.
Beech photosynthesis in changing light conditions and elevated CO₂

Watanabe, Kitaoka, Eguchi, Watanabe, Satomura, Takagi, Satô & Koike


