



Title	Photosynthetic traits of Siebold's beech seedlings in changing light conditions by removal of shading trees under elevated CO ₂
Author(s)	Watanabe, M.; Kitaoka, S.; Eguchi, N. et al.
Citation	Plant biology, 18, 56-62 https://doi.org/10.1111/plb.12382
Issue Date	2016-01
Doc URL	https://hdl.handle.net/2115/63957
Rights	This is the peer reviewed version of the following article: Plant Biology, Volume 18(S1), pages 56-62, January 2016, which has been published in final form at http://onlinelibrary.wiley.com/doi/10.1111/plb.12382/full . This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.
Type	journal article
File Information	PB18S1_56-62.pdf



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 Watanabe^{1, 2}, Satoshi Kitaoka³, Norikazu Eguchi², Yoko Watanabe^{2, 4},
Takami Satomura^{2, 5}, Kentaro Takagi⁴, Fuyuki Satoh⁴ and Takayoshi Koike^{1*}

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

1 **Abstract**

2 The purpose of this study was to obtain basic information on acclimation
3 capacity of photosynthesis of Siebold's beech seedlings to increasing light intensity
4 under future elevated CO₂ condition. We monitored leaf photosynthetic traits of
5 Siebold's beech seedlings in changing light conditions (before the removal of
6 shading trees, next year after the removal of shading trees and after acclimation to
7 open conditions) in a 10-year free air CO₂ enrichment experiment in northern Japan.
8 Elevated CO₂ did not affect the photosynthetic traits such as leaf mass per area,
9 nitrogen content and biochemical photosynthetic capacity of chloroplast (i.e.
10 maximum rate of carboxylation and maximum rate of electron transport) before the
11 removal of shading trees and after acclimation to open conditions; in fact, a higher
12 net photosynthetic rate was maintained under elevated CO₂. However, in next year
13 after the removal of the shading trees, there was no increase in photosynthetic rate
14 under elevated CO₂ conditions. This was not due to photoinhibition. In ambient
15 CO₂ conditions, leaf mass per area and nitrogen content were higher in next year
16 after the removal of shading trees than before, whereas there was no increase under
17 elevated CO₂ conditions. These results indicate that elevated CO₂ delays the
18 acclimation of photosynthetic traits of Siebold's beech seedlings to increasing light
19 intensity.

20

21 **Key words**

22 change of light condition, elevated CO₂, *Fagus crenata*, leaf nitrogen,
23 photoinhibition, photosynthesis

24

25

26 Introduction

27 Atmospheric CO₂ levels have increased dramatically since the industrial
28 revolution and have reached 400 μmol mol⁻¹ (Monastersky 2013). This increase is
29 predicted to continue throughout this century (Stocker *et al.* 2013). Increases in
30 CO₂ concentration generally enhance the leaf photosynthetic rate, as CO₂ is the
31 primary substrate for photosynthesis (Ainsworth & Rogers 2007). Higher net
32 photosynthetic rates were observed under elevated CO₂ in canopy pine and
33 sub-canopy deciduous trees in the Duke forest Free Air CO₂ Enrichment (FACE)
34 experiment (Ellsworth *et al.* 2012), and in five deciduous tree species in the Swiss
35 Canopy Crane project (Bader *et al.* 2010). Four understory tree species grown in
36 shaded conditions at the Duke Forest FACE site also showed a higher net
37 photosynthetic rate under elevated CO₂ conditions (Springer & Thomas 2007).
38 However, the extent of this enhancement is species-specific and depends on other
39 environmental factors such as nutrient conditions (e.g. Eguchi *et al.* 2004, 2008;
40 Ellsworth *et al.* 2004; Norby *et al.* 2010; Watanabe *et al.* 2011, 2013; Warren *et al.*
41 2015).

42 Siebold's beech (*Fagus crenata*) is a representative late-successional tree
43 species native to northern Japan (Koike 1988; Nakashizuka & Iida 1995; Peters
44 1997). These trees commonly experience dramatic changes in light conditions
45 over their life cycle. Seedlings generally grow under shaded conditions, and
46 become canopy trees following fall down and/or dieback of upper story trees
47 (Nakashizuka 1987). In Japan, gap formation in Siebold's beech forests is often
48 caused by typhoons (Nakashizuka 1987), which generally occur from late July to
49 September (Japan Meteorological Agency 2015).

50 Plants can acclimate to light conditions. For example, under high light
51 conditions, plants produce thick leaves with higher leaf mass per area (LMA) and
52 higher area-based nitrogen content (N_{area}) (Evans & Poorter 2001; Iio *et al.* 2005;
53 Kitaoka *et al.* 2009a, b; Poorter *et al.* 2009; Watanabe *et al.* 2014a; Nienemets *et*
54 *al.* 2015). As a result, photosynthetic parameters, such as maximum rate of
55 carboxylation (V_{cmax}) and the maximum rate of electron transport (J_{max}) are
56 generally higher in leaves acclimated to high-light conditions (Iio *et al.* 2005;
57 Lambers *et al.* 2008; Rodríguez-Calcerrada *et al.* 2008; Nienemets *et al.* 2015).
58 Nitrogen (N) allocation within the photosynthetic apparatus also acclimates to
59 light conditions. Nitrogen allocation to carboxylation-related enzymes (i.e.
60 Rubisco) increases under high-light conditions, while allocation to enzymes and
61 substances related to light harvesting, such as the light-harvesting complex and
62 the photosystems, decreases (Evans & Poorter 2001; Kitaoka & Koike 2004;
63 Rodríguez-Calcerrada *et al.* 2008).

64 Fully expanded leaves are constrained by anatomical limitations when it
65 comes to photosynthetic acclimation to increases in light intensity (Oguchi *et al.*
66 2005). Full acclimation to a new light environment, therefore, requires the
67 production of new leaves. Winter-deciduous tree species form leaf and shoot
68 primordia in winter buds during the previous year (Kozlowski & Clausen 1966,
69 Eschrich *et al.* 1989). Therefore, the leaf traits of these species depend on the light
70 conditions during the previous growing season, as well as during the bud burst.
71 This dependency is also species-specific. The layer of palisade cells in *Fagus*
72 *japonica* and *Acer tenuifolium* leaves is primarily determined by light conditions
73 in the current year, whereas leaf properties in *F. crenata* and *Fagus sylvatica* are

74 affected by light conditions in both the previous and current years (Eschrich *et al.*
75 1989; Koike *et al.* 1997; Tognetti *et al.* 1998; Uemura *et al.* 2000; Kitaoka *et al.*
76 2009b). Kitaoka *et al.* (2009b) reported that photosynthetic acclimation to gap
77 formation in seedlings of late-successional tree species (*Prunus ssiori* and
78 *Carpinus cordata*) was slower than that of mid- and mid-late-successional tree
79 species (*Magnolia hypoleuca* and *Quercus mongolica* var. *crispula*, respectively).

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

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

95

96

97

98 **Materials and methods**

99 **Experimental design and plant materials**

100 We used the FACE system located in the Sapporo Experimental Forest,
101 Hokkaido University, Japan (43°06' N, 141°20' E) (Eguchi *et al.* 2008; Watanabe
102 *et al.* 2010b; Watanabe *et al.* 2013, 2014b). This site is in a transition zone
103 between cool temperate and boreal forests, and is considered to be sensitive to
104 global climate changes (Matsuda *et al.* 2002). We set two levels of CO₂
105 concentration, ambient (no addition of CO₂, 370-390 μmol mol⁻¹ CO₂) and
106 elevated CO₂ (500 μmol mol⁻¹ CO₂), with three site replications for a total of six
107 sites to analyze the data, including the variance among the six sites. The target
108 CO₂ concentration in the elevated CO₂ treatment corresponded to the predicted
109 CO₂ concentration in 2040-2050 (Stocker *et al.* 2013). Average values of
110 temperature, daily maximum temperature, daily minimum temperature and
111 precipitation during the growing season (May-October; 2003–2012) were 17.3 °C,
112 21.8 °C, 13.5 °C and 501.5 mm, respectively (Japan Meteorological Agency
113 2015).

114 Two-year-old seedlings of ten deciduous tree species (three
115 early-successional: alder, birches, four mid-successional: ash, elm, kalopanax, oak,
116 and three late-successional species: Siebold's beech, maple, basswood), from cool
117 temperate forests in northern Japan (Koike 1988), were planted in brown forest
118 soil in May 2003. The Siebold's beech seedlings were shaded by the other tree
119 species (shading trees) from the late 2004 growing season, because the other
120 species were fast growing. The mean (maximum-minimum) tree height for all
121 species except the Siebold's beech in July 2007 was 273 (420–129) cm in the

122 ambient CO₂ treatment and 319 (502–198) cm in the elevated CO₂ treatment. The
123 mean tree height of Siebold's beech at the same time was 97 cm in the ambient
124 and 103 cm in the elevated CO₂ treatment. Leaf area index (LAI) of the
125 mixed-species canopy was approximately 5.6 m² m⁻² in both CO₂ treatments in
126 2007 (Eguchi 2008). Mean relative light intensity at the top level of the Siebold's
127 beech seedlings was 3.2% of that of the other species. There was no significant
128 difference in light intensity between the CO₂ treatments. In late July 2007, after
129 five growing seasons, above-ground of all species except for the Siebold's beech
130 were cut down at the base of the stem (5–8 cm above the ground). The Siebold's
131 beech seedlings were then grown until 2012 under open condition. After the
132 removal of shading trees, the light intensity at the top level of the Siebold's beech
133 seedlings was the same as the above original mixed species canopy.

134 Fumigation with CO₂ was carried out in the daytime when the
135 photosynthetic photon flux (PPF) exceeded 70 μmol m⁻² s⁻¹, which is the light
136 compensation point of photosynthesis. Fumigation took place every growing
137 season, from leaf emergence to leaf senescence, from 2003 to 2012. This
138 fumigation regime was analogous to those of other FACE experiments with
139 woody plants (Karnosky *et al.* 2005; Liberloo *et al.* 2009; Norby *et al.* 2010;
140 Ellsworth *et al.* 2012). The mean daytime CO₂ concentration in the centres of the
141 FACE sites during fumigation periods was 498 μmol mol⁻¹. The CO₂
142 concentration remained within 500 ± 50 μmol mol⁻¹ for 64% of the fumigation
143 period, and was within 500 ± 100 μmol mol⁻¹ for 86% of the fumigation period.

144

145

146 **Measurement of leaf photosynthetic traits**

147 The gas exchange rate of the fully mature leaves of the upper canopy was
148 measured in 2006 and 2007 (before the removal of the shading trees), in 2008
149 (next year after the removal of the shading trees), and in 2011 and 2012 (after
150 acclimation to open conditions) using an open gas-exchange system (LI-6400,
151 Li-Cor Inc., Lincoln, NE, USA) with a light-emitting diode (LED) light source
152 (LI-6400-02B in 2006, 2007, 2011 and 2012, LI-6400-40 in 2008, Li-Cor Inc.,
153 Lincoln, NE, USA). We assumed leaves could acclimate to a new light
154 environment within four years, although whole-plant acclimation such as canopy
155 structure and above ground to below ground biomass ratio may not be totally
156 complete. Measurements were made from late July to early August in each year.
157 In 2007, we measured leaf photosynthetic traits before cutting the shading trees
158 down. We randomly selected one or two seedlings from each site (three, five, and
159 six seedlings per treatment, in 2006–2007, 2008, and 2011–2012, respectively).
160 We measured photosynthetic traits in one leaf from each seedling. Leaf
161 temperature and PPF were maintained during measurement at 25.0 ± 1.0 °C and
162 $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. The leaf-to-air vapour pressure deficit was
163 approximately 1.5 kPa. To obtain the intercellular CO_2 concentration
164 (C_i)-response curve for the net photosynthetic rate (A), i.e. the A/C_i curve, A was
165 determined at 8–10 steps of external CO_2 concentration (C_a). We determined the
166 value of A and the stomatal conductance of leaves for water vapour at C_a of
167 growth CO_2 concentration ($370 \mu\text{mol mol}^{-1}$ for ambient and $500 \mu\text{mol mol}^{-1}$ for
168 elevated CO_2); these values were designated as A_{growth} and G_s . V_{cmax} and J_{max} were
169 calculated from the A/C_i curve (Farquhar *et al.* 1980; Long & Bernacchi 2003).

170 The Rubisco Michaelis constants for CO₂ (K_c) and O₂ (K_o) and the CO₂
171 compensation point in the absence of dark respiration (Γ^*) for the analysis of the
172 A/C_i curve were estimated from leaf temperature according to Bernacchi *et al.*
173 (2001).

174 In 2008, we also measured chlorophyll fluorescence at C_a of growth
175 conditions (370 $\mu\text{mol mol}^{-1}$ for ambient and 500 $\mu\text{mol mol}^{-1}$ for elevated CO₂)
176 using an open gas-exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA)
177 with a LED light source and fluorometer (LI-6400-40, Li-Cor Inc., Lincoln, NE,
178 USA). The minimum (F_o) and maximum fluorescence (F_m) were determined after
179 overnight dark adaptation with a saturating light of 7000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF for 1 s.
180 The F_v/F_m was then calculated as $(F_m - F_o)/F_m$. The quantum yield of PSII electron
181 transport (Φ_{PSII}), photochemical quenching (qP), and photochemical efficiency of
182 the open PSII (F_v'/F_m') were determined after acclimation to a 25.0 ± 1.0 °C leaf
183 temperature and 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF. From the fluorescence yield, F_s and the
184 maximum fluorescence, F_m' , (determined at the saturating light of 7000 $\mu\text{mol m}^{-2}$
185 s^{-1} PPF for 1 s) were used to calculate Φ_{PSII} ($= (F_m' - F_s)/F_m'$) (Genty *et al.* 1989).
186 F_o' was determined by turning off the actinic light and applying far-red light
187 immediately after a saturating light pulse. This parameter was used to derive qP
188 and F_v'/F_m' ($= (F_m' - F_o')/F_m'$), calculated according to Schreiber *et al.* (1994). The
189 fraction of excess energy was also calculated, using the formula $(1 - qP)F_v'/F_m'$
190 (Demmig-Adams *et al.* 1996).

191 After the gas exchange rate and chlorophyll fluorescence measurements,
192 we collected 2-3 leaf discs (12 mm diameter) in order to determine the leaf mass
193 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 acetanilide (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).

218 Higher values of A_{growth} were observed in elevated CO_2 than in ambient CO_2
 219 before the removal of shading trees (2006 and 2007) and after acclimation to open
 220 conditions (2011 and 2012), although the increase before the removal of the
 221 shading trees was marginal (Fig. 1a). In contrast, A_{growth} in the elevated CO_2
 222 treatment tended to be lower than that in ambient CO_2 in the next year after the
 223 removal of shading trees (2008). Although there were no significant interactions
 224 between elevated CO_2 and year-to-year variation for the other parameters (Table
 225 1), there was a significant effect of elevated CO_2 in the next year after the removal
 226 of the shading trees. In this period, the elevated CO_2 treatment induced significant
 227 reductions in G_s , V_{cmax} , LMA, and N_{area} , and marginal reductions in J_{max} (Fig. 1b, c,
 228 d, Fig. 2a, c). From a time course perspective, all photosynthetic parameters
 229 except for N_{mass} increased from 2007 to 2008 in the ambient CO_2 treatment,
 230 whereas there was no increase in the elevated CO_2 treatment.

231 There was a clear correlation between V_{cmax} and J_{max} throughout the
 232 experimental period (Fig. 3). No significant effect of elevated CO_2 was found on
 233 the slope and intercept of regression line. No significant difference in regression
 234 lines between CO_2 treatments was found when we analysed the regression lines in
 235 each period (before the removal of shading trees, next year after the removal of
 236 shading trees and after acclimation to open conditions) separately.

237 Elevated CO_2 did not significantly change the slope of the regression line
 238 between A_{growth} and N_{area} ; however, the intercept of the regression line for the
 239 elevated CO_2 treatment was significantly higher than that for the ambient CO_2
 240 treatment (Fig. 4a) when a common slope was applied to both regression lines. No
 241 significant differences in slope and intercept of the regression lines were found

242 between V_{cmax} and N_{area} , or J_{max} and N_{area} (Fig. 4b, c).

243 The Φ_{PSII} tended to decrease under elevated CO_2 (Table 2). Although there
244 was no significant difference between ambient CO_2 and elevated CO_2 for the other
245 parameters, there was a relatively large reduction of qP (35%) in the elevated CO_2
246 treatment.

247

248 Discussion

249 In this study, we did not observe any negative effects of CO_2 on
250 gas-exchange parameters and N content in leaves before the removal of shading
251 trees and after acclimation to open conditions (Table 1, Figs. 1 and 2). As a result,
252 A_{growth} increased by 54% before the removal of shading trees and 31% after
253 acclimation to open conditions. Increases in photosynthesis rate with increased
254 CO_2 have also been found in other FACE experiments (e.g. Springer & Thomas
255 2007; Bader *et al.* 2010; Ellsworth *et al.* 2012). However, this does not mean that
256 elevated CO_2 does not permanently induce photosynthetic down-regulation in
257 Siebold's beech. Nitrogen availability is one of the most important factors for
258 photosynthetic down-regulation under elevated CO_2 (Saxe *et al.* 1998; Norby &
259 Zak 2011). In general, elevated- CO_2 -induced declines in photosynthetic capacity
260 are prominent in low-N soil conditions (e.g. Curtis *et al.* 2000; Kubiske *et al.*
261 2002). Norby *et al.* (2010) and Warren *et al.* (2015), found that although
262 photosynthetic production of sweetgum (*Liquidambar styraciflua*) was increased
263 in elevated CO_2 conditions during the first few years of the experiment, it
264 ultimately declined, due to a reduction in N availability during the latter period of
265 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 F_v/F_m and $(1-qP)F_v'/F_m'$, 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, Φ_{PSII} decreased under elevated CO₂. The contribution of qP reduction to the decline of Φ_{PSII} under elevated CO₂ was larger than that of F_v'/F_m' reduction. F_v'/F_m' 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, V_{cmax} 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 Φ_{PSII} under elevated CO₂.

Nitrogen is a nutrient that strongly relates to photosynthetic capacity (Lambers *et al.* 2008). In the present study, A_{growth} , V_{cmax} and J_{max} were linearly correlated to N_{area} and the relationship between V_{cmax} and N_{area} , and 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 next year after the removal of shading trees (Fig. 2c) led to lower photosynthetic activity (indicated by V_{cmax} and J_{max}), and therefore, lower A_{growth} . N_{area} is calculated as a product of LMA and N_{mass} ,

290 and the lower N_{area} in the elevated- CO_2 treatment in the next year after the removal
 291 of shading trees was primarily due to lower LMA, since there was no significant
 292 difference in N_{mass} between the ambient- and elevated- CO_2 treatments (Fig. 2).
 293 LMA increased from 2007 to 2008 in the ambient CO_2 treatment, whereas there was
 294 no difference between 2007 and 2008 in the elevated CO_2 treatment. The
 295 anatomical properties of Siebold's beech leaves are affected by light conditions in
 296 the previous growing season (Uemura *et al.* 2000). Eschrich *et al.* (1989) observed
 297 that differentiation into sun-leaf and shade-leaf primordia of the European beech
 298 (*Fagus sylvatica*) occurs in early August. Although there are many differences
 299 (climate of the study site, species and age of tree) between that study and ours, the
 300 timing of removal of shading trees in the present study (late July) was similar to the
 301 timing of the differentiation in that study (Eschrich *et al.* 1989). Sigurdsson (2001)
 302 reported earlier bud formation under elevated- CO_2 conditions in young black
 303 cottonwood (*Populus trichocarpa*). It is possible that the differentiation of leaf
 304 primordia occurred prior to the removal of shading trees under elevated- CO_2
 305 conditions and, as a result, leaf acclimation to increasing light intensity in the
 306 following year was delayed. It should be noted that the increase in LMA throughout
 307 the experiment was not only caused by the change in light conditions, but also by
 308 increasing tree age. We observed an increase in LMA during steady light conditions,
 309 i.e. from 2006 to 2007 and from 2011 to 2012 (Fig. 2a), and LMA generally
 310 increases with increasing tree age (e.g. Niinemets 2006; Poorter *et al.* 2009). The
 311 LMA in the final year of the experiment (2012) reached approximately 90 g m^{-2}
 312 (Fig. 2a), which is comparable to that of the upper canopy leaves of mature
 313 Siebold's beech trees (Iio *et al.* 2005; Uemura *et al.* 2006).

314 Although the reduction in V_{cmax} in the next year after the removal of
315 shading trees was severe, the extent of the reduction in A_{growth} was rather small.
316 This is mainly due to a higher volume of substrate for photosynthesis (i.e. high
317 CO_2 concentration). This reduction was not observed in 2011; therefore, even
318 when negative effects of elevated- CO_2 conditions on photosynthetic functions are
319 induced, the effect is marginal. Thus, photosynthetic production may not decrease
320 severely in future CO_2 conditions.

321 A meta-analysis conducted by Ainsworth & Long (2005) predicted that the
322 ratio of J_{max} to V_{cmax} ($J_{\text{max}}/V_{\text{cmax}}$) would increase under elevated- CO_2 conditions.
323 However, we found the relationship between J_{max} and V_{cmax} in elevated CO_2
324 conditions was not differed from that in ambient CO_2 conditions (Fig. 3). This
325 indicates that there was no shift in N allocation between carboxylation and electron
326 transport capacities (Evans & Poorter 2001; Kitaoka & Koike 2004;
327 Rodríguez-Calcerrada *et al.* 2008). The relatively low enhancement of CO_2
328 concentration ($500 \mu\text{mol mol}^{-1}$) in the elevated- CO_2 treatment in the present study
329 could explain the lack of shift of N allocation. Similarly, the change in light
330 conditions did not significantly affect the relationship between J_{max} and V_{cmax} (data
331 not shown). Onoda *et al.* (2005) reported no seasonal variation of $J_{\text{max}}/V_{\text{cmax}}$ in the
332 Siebold's beech, indicating little temperature dependency. The shift of $J_{\text{max}}/V_{\text{cmax}}$
333 across environmental conditions is species-specific (Niinemets *et al.* 1998;
334 Hikosaka *et al.* 1999; Medlyn *et al.* 2002; Ainsworth & Long 2005), and therefore it
335 is possible that in the Siebold's beech, $J_{\text{max}}/V_{\text{cmax}}$ remains constant across
336 environmental conditions.

337 Our results indicate that elevated- CO_2 conditions ($500 \mu\text{mol mol}^{-1}$; the

338 predicted concentration for 2040–2050), enhance the net photosynthetic rate of
339 the Siebold's beech in both shaded and open conditions. We did not observe
340 stomatal closure, or reduction of N content and photosynthetic N use efficiency
341 under elevated CO₂ conditions, indicating there was no down-regulation of
342 photosynthesis in leaves. In contrast, the acclimation of photosynthesis of Siebold's
343 beech seedlings to increasing light intensity was delayed under elevated-CO₂
344 conditions. Photosynthetic increases under elevated CO₂ conditions did not occur
345 in the next year after the removal of shading trees. This was not due to
346 photoinhibition, but to lower area-based N content. Although the delay of
347 photosynthetic acclimation is marginal, further studies could investigate the
348 impacts on regeneration capacity, such as growth performance, of Siebold's beech
349 seedlings, in order to predict dynamics of forests of this tree species under future
350 elevated CO₂ conditions.

351

352 **Acknowledgments**

353 This study was partly supported by Japan Society for the Promotion of Science
354 through the programs of a Grant-in-Aid for Young Scientists (B) (24710027 and
355 15K16136, to M. Watanabe), a Grant-in-Aid for Scientific Research on Innovative
356 Areas (21114008, to T. Koike), Challenging Exploratory Research (26660119, to
357 T. Koike) and Grant-in-Aid for Scientific Research (A) (23255009, to F. Satoh).

358

359 **References**

360 Ainsworth E.A., Long S.P. (2005) What have we learned from 15 years of free-air
361 CO₂ enrichment (FACE)? A meta-analytic review of the responses of

362 photosynthesis, canopy properties and plant production to rising CO₂. *New*
363 *Phytologist* **165**, 351–372.

364 Ainsworth E.A., Rogers A. (2007) The response of photosynthesis and stomatal
365 conductance to rising [CO₂]: mechanisms and environmental interactions.
366 *Plant, Cell and Environment* **30**, 258–270.

367 Bader M.K.F., Siegwolf R., Körner C.H. (2010) Sustained enhancement of
368 photosynthesis in mature deciduous forest trees after 8 years of free air CO₂
369 enrichment. *Planta* **232**, 1115–1125.

370 Baker N.R. (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo.
371 *Annual Review of Plant Biology* **59**, 89–113.

372 Bernacchi C.J., Singsaas E.L., Pimentel C., Portis A.R., Long S.P. (2001)
373 Improved temperature response functions for models of Rubisco-limited
374 photosynthesis. *Plant, Cell and Environment* **24**, 253–259.

375 Curtis P.S., Vogel C.S., Wang X., Pregitzer K.S., Zak D.R., Lussenhop J., Kubiske
376 M., Teeri J.A. (2000) Gas exchange, leaf nitrogen, and growth efficiency of
377 *Populus tremuloides* in a CO₂-enriched atmosphere. *Ecological*
378 *Applications* **10**, 3–17.

379 Demmig-Adams B., Adams W.W. III., Barker D.H., Logan B.A., Bowling D.R.,
380 Verhoeven A.S. (1996) Using chlorophyll fluorescence to assess the fraction
381 of absorbed light allocated to thermal dissipation of excess excitation.
382 *Physiologia Plantarum* **98**, 253–264

383 Eguchi N. (2008) Study on the changes in CO₂ fixation and storage capacity of
384 deciduous tree species native to cool temperate zone with increasing
385 ambient CO₂ concentration. Doctoral dissertation of The Graduate School

386 of Agriculture of Hokkaido University, Sapporo, Japan.

387 Eguchi N., Fukatsu E., Funada R., Tobita H., Kitao M., Maruyama Y., Koike T.

388 (2004) Changes in morphology, anatomy, and photosynthetic capacity of

389 needles of Japanese larch (*Larix kaempferi*) seedlings grown in high CO₂

390 concentration. *Photosynthetica* **42**, 173–178.

391 Eguchi N., Karatsu K., Ueda T., Funada R., Takagi K., Hiura T., Sasa K., Koike T.

392 (2008) Photosynthetic responses of birch and alder saplings grown in a free

393 air CO₂ enrichment system in northern Japan. *Trees* **22**, 437–447.

394 Ellsworth D.S., Reich P.B., Naumburg E.S., Koch G.W., Kubiske M.E., Smith

395 S.D. (2004) Photosynthesis, carboxylation and leaf nitrogen responses of 16

396 species to elevated pCO₂ across four free-air CO₂ enrichment experiments

397 in forest, grassland and desert. *Global Change Biology* **10**, 2121–2138.

398 Ellsworth D.S., Thomas R., Crous K.Y., Palmroth S., Ward E., Maier C., DeLucia

399 E., Oren R. (2012) Elevated CO₂ affects photosynthetic responses in

400 canopy pine and subcanopy deciduous trees over 10 years: a synthesis

401 from Duke FACE. *Global Change Biology* **18**, 223–242.

402 Eschrich W., Burchardt R., Essiamah S. (1989) The induction of sun and shade

403 leaves of the European beech (*Fagus sylvatica* L.): anatomical studies.

404 *Trees* **3**, 1–10.

405 Evans J.R., Poorter H. (2001) Photosynthetic acclimation of plants to growth

406 irradiance: the relative importance of specific leaf area and nitrogen

407 partitioning in maximizing carbon gain. *Plant, Cell and Environment* **24**,

408 755–767.

409 Farquhar G.D., von Caemmerer S., Berry J.A. (1980) A biochemical model of
 410 photosynthetic CO₂ assimilation in leaves of C3 species. *Planta* 149, 78–90.

411 Genty B., Briantais J.-M., Baker N.R. (1989) The relationship between the
 412 quantum yield of photosynthetic electron transport and quenching of
 413 chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990**, 87–92.

414 Hikosaka K., Murakami A., Hirose T. (1999) Balancing carboxylation and
 415 regeneration of ribulose-1,5-bisphosphate in leaf photosynthesis:
 416 temperature acclimation of an evergreen tree, *Quercus myrsinaefolia*. *Plant,*
 417 *Cell and Environment* **22**, 841–849.

418 Hirano A., Hongo I., Koike T. (2012) Morphological and physiological responses
 419 of Siebold's beech (*Fagus crenata*) seedlings grown under CO₂
 420 concentrations ranging from pre-industrial to expected future levels.
 421 *Landscape and Ecological Engineering* **8**, 59–67.

422 Iio A., Fukasawa H., Nose Y., Kato S., Kakubari Y. (2005) Vertical, horizontal and
 423 azimuthal variations in leaf photosynthetic characteristics within a *Fagus*
 424 *crenata* crown in relation to light acclimation. *Tree Physiology* **25**, 533–544

425 Japan Meteorological Agency (2015) Statistical Information of Meteorology.
 426 Available via DIALOG. <http://www.jma.go.jp/jma/menu/report.html> (in
 427 Japanese) Accessed 25 Feb 2015.

428 Lambers H., Chapin III. F.S., Pons T.L. (2008) *Plant physiological ecology,*
 429 *second edition*. Springer, New York, USA: 605 pp.

430 Liberloo M., Lukac M., Calfapietra C., Hoosbeek M.R., Gielen B., Miglietta F.,
 431 Scarascia-Mugnozza G.E., Ceulemans R. (2009) Coppicing shifts CO₂
 432 stimulation of poplar productivity to above-ground pools: a synthesis of

433 leaf to stand level results from the POP/EUROFACE experiment. *New*
 434 *Phytologist* **182**, 331–346.

435 Long S.P., Bernacchi C.J. (2003) Gas exchange measurements, what can they tell
 436 us about the underlying limitations to photosynthesis? Procedures and
 437 sources of error. *Journal of Experimental Botany* **54**, 2393–2401.

438 Karnosky D.F., Pregitzer K.S., Zak D.R., Kubiske M.E., Hendrey G.R., Weinstein
 439 D., Nosal M., Percy K.E. (2005) Scaling ozone responses of forest trees to
 440 the ecosystem level in a changing climate. *Plant, Cell and Environment* **28**,
 441 965–981.

442 Kitaoka S., Koike T. (2004) Invasion of broad-leaf tree species into a larch
 443 plantation: seasonal light environment, photosynthesis and nitrogen
 444 allocation. *Physiologia Plantarum* **121**, 604–611.

445 Kitaoka S., Watanabe M., Watanabe Y., Kayama M., Nomura M., Sasa K. (2009a)
 446 Growth of regenerated tree seedlings associated with microclimatic change
 447 in a mature larch plantation after harvesting. *Landscape and Ecological*
 448 *Engineering* **5**, 137–145.

449 Kitaoka S., Watanabe Y., Koike T. (2009b) The effects of cleared larch canopy and
 450 nitrogen supply on gas exchange and leaf traits in deciduous broad-leaved
 451 tree seedlings. *Tree physiology* **29**, 1503–1511.

452 Koike T. (1988) Leaf structure and photosynthetic performance as related to the
 453 forest succession of deciduous broad-leaved trees. *Plant Species Biology* **3**,
 454 77–87.

455 Koike T., Miyashita N., Toda H. (1997) Effects of shading leaf structural
 456 characteristics in successional deciduous broadleaved tree seedlings and

457 their silvicultural meaning. *Forest Resources and Environment* **35**, 9–25.
 458 Kozłowski T.T., Clausen J.J. (1966) Shoot growth characteristics of
 459 heterophyllous woody plants. *Canadian Journal of Botany* **44**, 827–843.
 460 Kubiske M.E., Zak D.R., Pregitzer K.S., Takeuchi Y. (2002) Photosynthetic
 461 acclimation of overstory *Populus tremuloides* and understory *Acer*
 462 *saccharum* to elevated atmospheric CO₂ concentration: interactions with
 463 shade and soil nitrogen. *Tree Physiology* **22**, 321–329.
 464 Matsuda K., Shibuya M., Koike T. (2002) Maintenance and rehabilitation of the
 465 mixed conifer-broadleaf forests in Hokkaido, northern Japan. *Eurasian*
 466 *Journal of Forest Research* **5**, 119–130.
 467 Matsumura H., Mikami C., Sakai Y., Izuta T., Yonekura T., Miwa M., Kohno Y.
 468 (2005) Impacts of elevated O₃ and/or CO₂ on growth of *Betula platyphylla*,
 469 *Betula ermanii*, *Fagus crenata*, *Pinus densiflora* and *Cryptomeria japonica*
 470 seedlings. *Journal of Agricultural Meteorology* **60**, 1121–1124.
 471 Medlyn B.E., Dreyer E., Ellsworth D., Forstreuter M., Harley P.C., Kirschbaum
 472 M.U.F., Le Roux X., Montpied P., Strassmeyer J., Walcroft A., Wang K.,
 473 Loustau D. (2002) Temperature response of parameters of a biochemically
 474 based model of photosynthesis. I. Seasonal changes in mature maritime pine
 475 (*Pinus pinaster* Ait.). *Plant, Cell and Environment* **25**, 1155–1165.
 476 Monastersky R. (2013) Global carbon dioxide levels near worrisome milestone.
 477 *Nature* **497**, 13–14.
 478 Nakashizuka T. (1987) Regeneration dynamics of beech forests in Japan.
 479 *Vegetatio* **69**, 169–175.
 480 Nakashizuka T., Iida S. (1995) Composition, dynamics and disturbance regime of

481 temperate deciduous forests in Monsoon Asia. *Vegetatio* **121**, 23–30.

482 Niinemets Ü. (2006) The controversy over traits conferring shade-tolerance in
483 trees: ontogenetic changes revisited. *Journal of Ecology* **94**, 464–470.

484 Niinemets U., Keenan T.F., Hallik L. (2015) A worldwide analysis of
485 within-canopy variations in leaf structural, chemical and physiological traits
486 across plant functional types. *New Phytologist* **205**, 973–993.

487 Niinemets Ü., Kull O., Tenhunen J.D. (1998) An analysis of light effects on foliar
488 morphology, physiology, and light interception in temperate deciduous
489 woody species of contrasting shade tolerance. *Tree Physiology* **18**, 681–
490 696.

491 Norby R.J., Warren J.M., Iversen C.M., Medlyn B.E., McMurtrie R.E. (2010)
492 CO₂ enhancement of forest productivity constrained by limited nitrogen
493 availability. *Proceedings of the National Academy of Sciences* **107**, 19368–
494 19373.

495 Norby R.J., Zak D.R. (2011) Ecological lessons from free-air CO₂ enrichment
496 (FACE) experiments. *Annual Review of Ecology, Evolution, and*
497 *Systematics* **42**, 181–203.

498 Oguchi R., Hikosaka K., Hirose T. (2005) Leaf anatomy as a constraint for
499 photosynthetic light acclimation: differential response in leaf anatomy to
500 increasing growth irradiance among three deciduous trees. *Plant, Cell and*
501 *Environment* **28**, 916–927.

502 Onoda Y., Hikosaka K., Hirose T. (2005). The balance between RuBP
503 carboxylation and RuBP regeneration: a mechanism underlying the
504 interspecific variation in acclimation of photosynthesis to seasonal change

505 in temperature. *Functional Plant Biology* **32**, 903–910.

506 Peters R. (1997) *Beech forests*. Kluwer, Dordrecht, Nederland: 169 pp.

507 Poorter H., Niinemets Ü., Poorter L., Wright I.J., Villar R. (2009) Causes and
508 consequences of variation in leaf mass per area (LMA): a meta-analysis.
509 *New Phytologist* **182**, 565–588.

510 R Development Core Team (2012) R: A language and environment for statistical
511 computing. *R Foundation for Statistical Computing*. Vienna. ISBN
512 3-900051-07-0. Available at <http://www.R-project.org>. (Accessed 28 April
513 2015). Saxe H., Ellsworth D.S., Heath J. (1998) Tree and forest functioning
514 in an enriched CO₂ atmosphere. *New Phytologist* **139**, 395–436

515 Rodríguez-Calcerrada J., Reich P.B., Rosenqvist E., Pardos J.A., Cano F.J.,
516 Aranda I. (2008) Leaf physiological versus morphological acclimation to
517 high-light exposure at different stages of foliar development in oak. *Tree*
518 *Physiology* **28**, 761–771.

519 Saxe H., Ellsworth D.S., Heath J. (1998) Tansley Review No. 98 Tree and forest
520 functioning in an enriched CO₂ atmosphere. *New Phytologist* **139**, 395–436

521 Schreiber U., Bilger W., Neubauer C. (1994) Chlorophyll fluorescence as a
522 nonintrusive indicator for rapid assessment of in vivo photosynthesis. In:
523 Schulze E.-D., Caldwell M.M. (Eds), *Ecophysiology of Photosynthesis*.
524 Springer-Verlag, Berlin, Germany: 49–70.

525 Sigurdsson B.D. (2001) Elevated [CO₂] and nutrient status modified leaf phenology
526 and growth rhythm of young *Populus trichocarpa* trees in a 3-year field study.
527 *Trees* **15**, 403–413.

528 Springer C.J., Thomas R.B. (2007) Photosynthetic responses of forest understory

529 tree species to long-term exposure to elevated carbon dioxide concentration
 530 at the Duke Forest FACE experiment. *Tree Physiology* **27**, 25–32.

531 Stocker T.F., Qin D., Plattner G.-K., Tignor M., Allen S.K., Boschung J., Nauels
 532 A., Xia Y., Bex V., Midgley P.M. (2013) *IPCC, 2013: Climate Change*
 533 *2013: The Physical Science Basis. Contribution of Working Group I to the*
 534 *Fifth Assessment Report of the Intergovernmental Panel on Climate Change.*
 535 Cambridge University Press, Cambridge, UK and New York, USA: 1535
 536 pp.

537 Tognetti R., Minotta G., Pinzauti S., Michelozzi M., Borghetti M. (1998)
 538 Acclimation to changing light conditions of long-term shade-grown beech
 539 (*Fagus sylvatica* L.) seedlings of different geographic origins. *Trees* **12**,
 540 326–333.

541 Uemura A., Harayama H., Koike N., Ishida A. (2006) Coordination of crown
 542 structure, leaf plasticity and carbon gain within the crowns of three
 543 winter-deciduous mature trees. *Tree Physiology* **26**, 633–641.

544 Uemura A., Ishida A., Nakano T., Terashima I., Tanabe H., Matsumoto Y. (2000)
 545 Acclimation of leaf characteristics of *Fagus species* to previous-year and
 546 current year solar irradiances. *Tree Physiology* **20**, 945–951.

547 Warren J.M., Jensen A.M., Medlyn B.E., Norby R.J., Tissue D.T. (2015) Carbon
 548 dioxide stimulation of photosynthesis in *Liquidambar styraciflua* is not
 549 sustained during a 12-year field experiment. *AoB PLANTS* **7**, 1–13.

550 Watanabe M., Hoshika Y., Inada N., Koike, T. (2014a) Canopy carbon budget of
 551 Siebold's beech (*Fagus crenata*) sapling under free air ozone exposure.
 552 *Environmental Pollution* **184**, 682–689.

553 Watanabe M., Kitaoka S., Eguchi N., Watanabe Y., Satomura T., Takagi K., Satoh
 554 F., Koike T. (2014b) Photosynthetic traits and growth of *Quercus mongolica*
 555 var. *crispula* sprouts attacked by powdery mildew under free air CO₂
 556 enrichment. *European Journal of Forest Research* **133**, 725–733.

557 Watanabe M., Mao Q., Novriyanti E., Kita K., Takagi K., Satoh F., Koike T.
 558 (2013) Elevated CO₂ enhances the growth of hybrid larch F₁ (*Larix gmelinii*
 559 var. *japonica* × *L. kaempferi*) seedlings and changes its biomass allocation.
 560 *Trees* **27**, 1647–1655.

561 Watanabe M., Umemoto-Yamaguchi M., Koike T., Izuta T. (2010a) Growth and
 562 photosynthetic response of *Fagus crenata* seedlings to ozone and/or
 563 elevated carbon dioxide. *Landscape and Ecological Engineering* **6**, 181–
 564 190.

565 Watanabe M., Watanabe Y., Kitaoka S., Utsugi H., Kita K., Koike T. (2011)
 566 Growth and photosynthetic traits of hybrid larch F₁ under elevated CO₂
 567 concentration with low nutrient availability. *Tree Physiology* **31**, 965–975.

568 Watanabe Y., Satomura T., Sasa K., Funada R., Koike T. (2010b) Differential
 569 anatomical responses to elevated CO₂ in saplings of four hard wood species.
 570 *Plant, Cell and Environment* **33**, 1101–1111.

571

572 Table 1 A summary of Repeated Measures ANOVA for effects of elevated CO₂
573 and year-to-year variation on net photosynthetic rate at growth CO₂ concentration
574 (A_{growth}), stomatal conductance to water vapor (G_s), maximum rate of
575 carboxylation (V_{cmax}) and maximum rate of electron transport (J_{max}), leaf mass per
576 area (LMA), mass-based nitrogen content (N_{mass}), and area-based nitrogen content
577 (N_{area}) in the leaves of Siebold's beech seedlings

	CO ₂	Year	CO ₂ × Year
A_{growth}	*	***	**
G_s	n.s.	***	n.s.
V_{cmax}	n.s.	***	n.s.
J_{max}	n.s.	***	n.s.
LMA	n.s.	***	n.s.
N_{mass}	n.s.	n.s.	n.s.
N_{area}	n.s.	***	n.s.

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

579

580 Table 2 The parameters of chlorophyll fluorescence in leaves of Siebold's
 581 beech seedlings grown under ambient and elevated CO₂ (500 µmol mol⁻¹
 582 CO₂) conditions in the year after the removal of shading trees (2008).

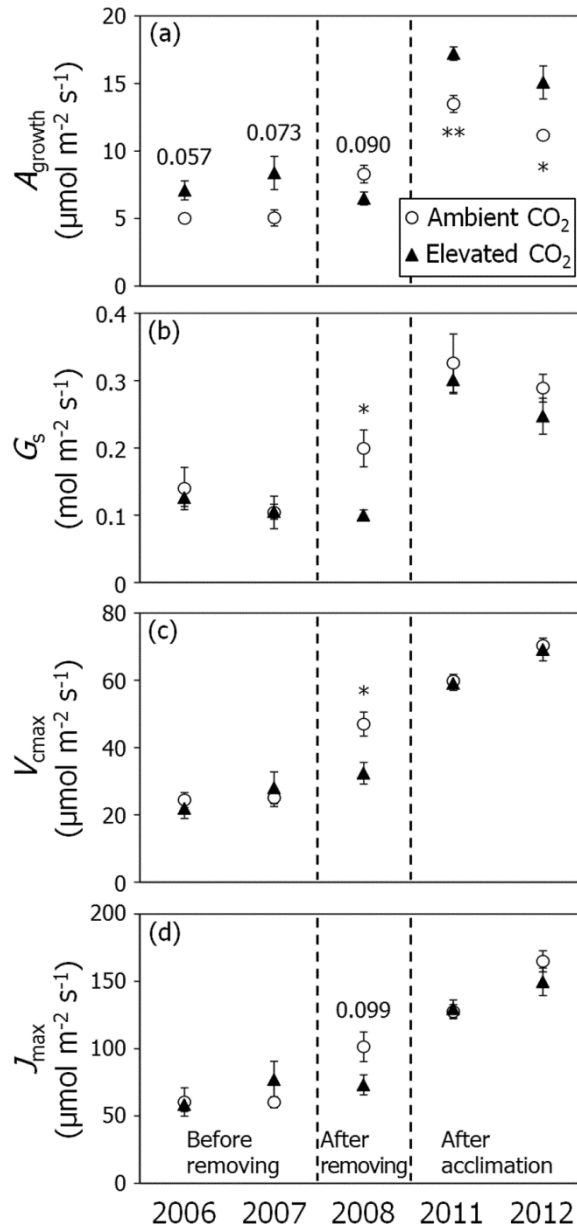
	Ambient CO ₂	Elevated CO ₂	T-test
F_v / F_m	0.814 (0.005)	0.810 (0.002)	n.s.
F_v' / F_m'	0.404 (0.009)	0.380 (0.012)	n.s.
qP	0.251 (0.031)	0.163 (0.032)	n.s.
Φ_{PSII}	0.101 (0.014)	0.061 (0.011)	0.088
$(1 - qP) F_v' / F_m'$	0.303 (0.006)	0.319 (0.023)	n.s.

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

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

585 F_v / F_m was determined after an overnight dark adaptation, while F_v' / F_m' , qP,
 586 Φ_{PSII} and $(1 - qP) F_v' / F_m'$ were determined after acclimation to $25.0 \pm$
 587 1.0°C of leaf temperature and $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetic photon
 588 flux. The intensity of saturation pulse was $7000 \mu\text{mol m}^{-2} \text{s}^{-1}$ of PPF for 1 s.
 589 During the measurement, CO₂ concentration within a chamber was maintain
 590 at 370 and 500 µmol mol⁻¹ for ambient and elevated CO₂ treatment,
 591 respectively

592



593

594 **Fig. 1** (a) Net photosynthetic rate at growth CO_2 concentration (A_{growth}), (b)
595 stomatal conductance to water vapour (G_s), (c) maximum rate of carboxylation
596 (V_{cmax}) and (d) maximum rate of electron transport (J_{max}) of Siebold's beech
597 seedlings grown under ambient and elevated CO_2 ($500 \mu\text{mol mol}^{-1}$) conditions,
598 before the removal of shading trees (2006 and 2007), next year after the removal
599 of shading trees (2008) and after acclimation to open conditions (2011 and 2012).
600 A *T*-test was performed in each year: * $P < 0.05$; ** $P < 0.01$; actual P values
601 were shown when $0.05 < P < 0.1$.

602

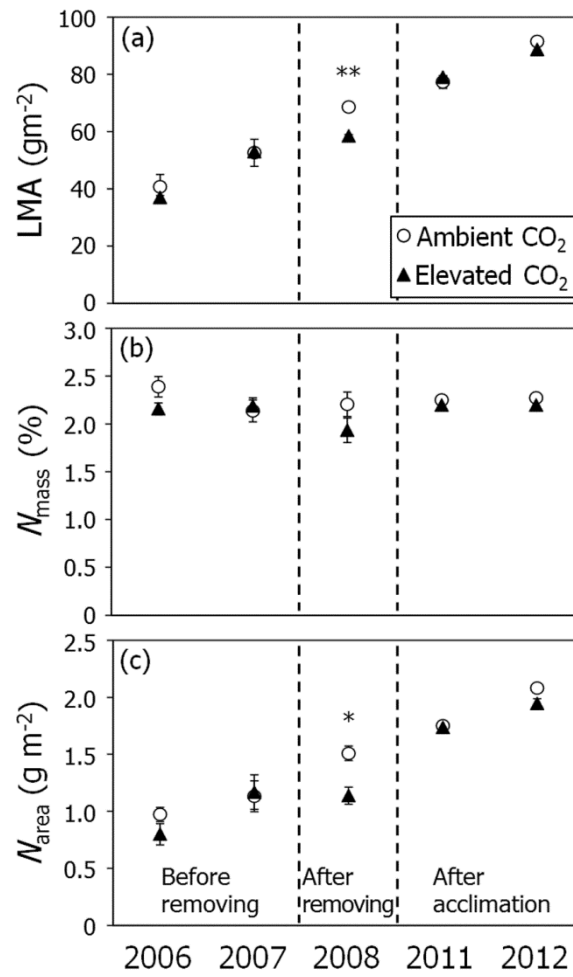
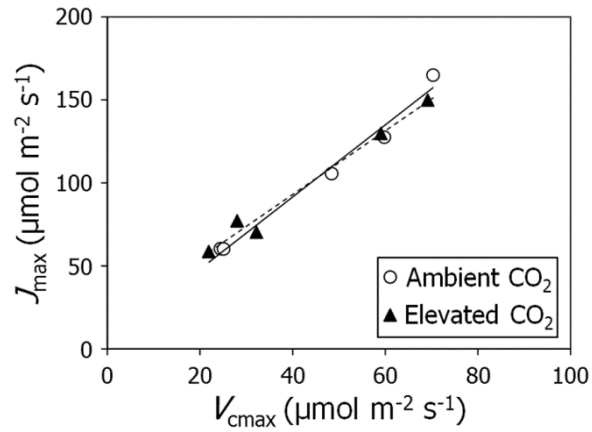


Fig. 2 (a) Leaf mass per area (LMA), (b) mass-based nitrogen content (N_{mass}) and (c) area-based nitrogen content (N_{area}) in the leaves of Siebold's beech seedlings grown under ambient and elevated CO₂ (500 μmol mol⁻¹) conditions, before the removal of shading trees (2006 and 2007), next year after the removal of shading trees (2008) and after acclimation to open conditions (2011 and 2012). A *T*-test was performed in each year: * $P < 0.05$; ** $P < 0.01$.



613

614

615 **Fig. 3** Maximum rate of electron transport (J_{max}) as a function of maximum rate of
 616 carboxylation (V_{cmax}) of Siebold's beech seedlings grown under ambient and
 617 elevated CO₂ (500 μmol mol⁻¹) conditions. Each data point indicates the mean
 618 value in each year. Regression line: $y = 4.8 + 2.2x$, $R^2 = 0.98^{***}$ for ambient CO₂
 619 (solid line) and $y = 16.3 + 1.9x$, $R^2 = 0.98^{***}$ for elevated CO₂ (dashed line). There
 620 was no significant difference of the slope and intercept of regression lines
 621 between ambient and elevated CO₂ treatments.

622

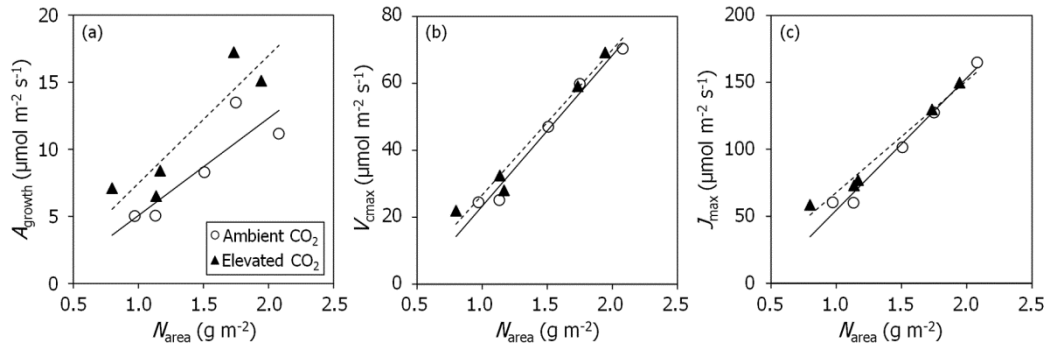


Fig. 4 (a) Net photosynthetic rate at growth CO_2 concentration (A_{growth}), (b) maximum rate of carboxylation (V_{cmax}) and (c) maximum rate of electron transport (J_{max}) as a function of area-based nitrogen content (N_{area}) in leaves of Siebold's beech seedlings grown under ambient and elevated CO_2 ($500 \mu\text{mol mol}^{-1}$) 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).