Root production of *Fagus crenata* Blume saplings grown in two soils and exposed to elevated CO_2 concentration: an 11-year free-air-CO_2 enrichment (FACE) experiment in northern Japan

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ABSTRACT

We examined the root production of a set of *Fagus crenata* (Siebold's beech) saplings grown in an infertile immature volcanic ash soil (VA) and another set in a fertile brown forest soil (BF) with both sets exposed to elevated CO_2. After the saplings had been exposed to ambient (370-390 μmol mol^{-1}) or elevated (500 μmol mol^{-1}) CO_2, during the daytime, for 11 growing seasons, the root systems were excavated. Elevated CO_2 boosted the total root production of saplings grown in VA and abolished the negative effect of VA under ambient CO_2, but there was no significant effect of elevated CO_2 on saplings grown in BF. These results indicate the projected elevated CO_2 concentrations may have a different impact in regions with different soil fertility while in regions with VA, a higher net primary production is expected. In addition, we observed large elevated-CO_2-induced

fine root production and extensive foraging strategy of saplings in both soils, a phenomenon that may partly a) adjust the biogeochemical cycles of ecosystems, b) form their response to global change and c) increase the size and/or biodiversity of soil fauna. We recommend that future researches consider testing a soil with a higher degree of infertility than the one we tested.

Key words: Air pollution, Atmospheric environment, Climate change, Ecophysiology, Greenhouse gas, NPP

Key message: Siebold's beech saplings had different root response to elevated CO_2 concentrations between fertile and infertile soils, and thus net primary productivity is likely to vary among regions

Author contribution statement:

Evgenios Agathokleous: Root measurements, data analysis and interpretation, and synthesis and production of the manuscript

Makoto Watanabe: Data collection and discussion on the study

Norikazu Eguchi: Discussion on the study based on the FACE from 2003

Tatsuro Nakaji: Guidance on root research and discussion on the study

Fuyuki Satoh: Management of the FACE system

Takayoshi Koike: Funds, excavation of the roots and collection of data, management of all the technical procedures and discussion

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1. INTRODUCTION

A hitherto weak point in knowledge on effects of future atmospheric carbon dioxide (CO_2) levels on trees is the lack of long-term studies; most studies have dealt with seedlings at a juvenile stage and for a short-term of exposure (e.g. Körner 2009; Norby and Zak 2011). Phytochemistry research on several aspen genotypes revealed that the effects of elevated CO_2 levels on forest trees are temporally dynamic over decadal time periods, and underlined the need for long-term research (Couture 2014). Such long-term studies should be conducted in different regions with different edaphoclimatological conditions in order to

66 shed light on CO_2 effects on trees after canopy closure and mature stage and to quantify the
67 effect (Leuzinger et al. 2011). The importance of studies conducted in different regions is
68 also highlighted by the latitude-dependency of the effect of atmospheric changes on forest
69 productivity (Silva and Anand 2013). Additionally, another weakness is the wide spacing
70 of plants which has been implemented in several experiments (Körner 2006). Wide spacing
71 leads to artifacts due to the influence of nutritional resources: If a first year effect is induced
72 by offering open space as a surrogate for ample nutrients, that signal will propagate into the
73 future (even if CO_2 exposure is terminated), which is even worse than short experimental
74 duration (Körner 2006). As such, most data do not truly represent the effects of future
75 elevated CO_2 levels on tree roots, the responses of which to elevated CO_2 levels are still not
76 well understood (Körner 2011; Wang et al. 2016).

77 Fine roots (production and turnover) partly adjust the biogeochemical cycles of ecosystems
78 and form their response to global change (Norby et al. 2004). Moreover, fine root data
79 along with models contribute to the understanding of the global belowground diversity and
80 biogeochemical processes in the terrestrial biosphere (McCormack 2015). Root dynamics
81 can explain elevated-CO_2-induced differences among ecosystems (Norby et al. 2004);
82 however, fine-root biomass can vary across years (Pregitzer et al. 2008; Wang et al. 2016),
83 as fine root production is also related to biotic factors, such as soil fauna (Lipson et al.
84 2014). In addition, future changes in allocation to belowground of trees in response to
85 elevated CO_2 levels are likely to alter the fungal community (Lipson et al. 2014; Wang et al.
86 2016).

87 Short-term experiments might not represent the actual responses of tree root system to CO_2
88 (Norby and Zak 2011; Kostiainen et al. 2014) due to physiological age and size dependency,
89 stand development, community composition, nitrogen deposition, ground-surface ozone,
90 etc. (Asshoff et al. 2006; Körner 2006; Pregitzer et al. 2008; Kostiainen et al. 2009, 2014;
91 Bader et al. 2013; Yan et al. 2014; Agathokleous et al. 2016a). Despite the importance of
92 roots, knowledge about their response to future elevated CO_2 concentrations remains
93 meager (e.g. Körner 2011; Wang et al. 2016). What is also surprising is the prevailing
94 unawareness in the role of soils in belowground responses of trees to atmospheric CO_2
95 despite that soils have greater influences on responses of plants to other applied treatments
96 (Spinnler et al. 2002, 2003; Körner 2011; Sigurdsson et al. 2013). To our knowledge, there
97 are no available studies on the decadal belowground response to CO_2 of trees grown in
98 different soils.

99 Siebold's beech (*Fagus crenata* Blume; Fagaceae) is a late-successional, deciduous,
100 broadleaf tree native to Japan (Koike et al. 1998). It has a distribution from Kyushu (c.
101 30.5^{o} N) to southern Hokkaido (c. 42.8^{o} N) and a climatic threshold close to the cool-
102 temperate zone (Horikawa 1972; Fang and Lechowicz 2006). Thus, it is a dominant species
103 in the cool temperate zone of Japan (Asuka et al. 2004a). Among the beech species,
104 Siebold's beech is adapted to and occurs in the most humid conditions of cold areas (Fang
105 and Lechowicz 2006). Phenological events (*e.g.* flowering and autumnal surcease of
106 growth) of late successional taxa, such as Siebold's beech, are primarily controlled by
107 photoperiod and not temperature (Körner and Basler 2010). This species is vital to

ecosystem functioning and biodiversity conservation (Asuka et al. 2004b; Hara 2010), and northern Siebold's beech forests are included in the World Heritage (UNESCO 2002).

The objective of the present study was to quantify the belowground net primary production (NPP), in terms of root production, and carbon (C) allocation balance between aboveground and belowground part of Siebold's beech saplings grown under ambient or elevated CO_2 levels and in fertile brown forest soil (BF) or infertile, immature volcanic ash plus pumice soil (VA) for 11 years, in a free-air CO_2 enrichment (FACE) system. This system was established in northern Japan, in a transition zone between cool temperate and boreal forests, a part of the Asian boreo-nemoral ecotone and sensitive to global climate changes (Uemura 1992; Matsuda et al. 2002). Thus, this research will provide a new piece of information which can be used to determine the future C abundance in trees and NPP (Körner 2003, 2006; Leuzinger et al. 2011; Norby and Zak 2011). We hypothesized that the response of saplings to elevated CO_2 levels would be affected by soil fertility.

2. MATERIALS & METHODS

2.1 Experimental Design: The present research was conducted in the FACE system located in the Sapporo Experimental Forest of Hokkaido University, Japan ($43^{o}06'$ N, $141^{o}20'$ E, 60 m a.s.l), with a split-plot factorial design and employing the randomized block method (Filion et al. 2000). The CO_2 treatments were ambient and elevated CO_2, with three site replicates for each treatment. The design of these FACE facilities was based on the system used at the Stillberg, Davos, in the Swiss Alps (Hättenschwiler et al. 2002). The soil treatments were BF (Matsui 2001) and VA (Kato 1983), both at each site with a

129 distance of 1.5 m between them. VA is a nutrient-poor soil that was excavated and brought
130 from Tomakomai Experimental Forest of Hokkaido University (42°40’ N, 141°37’ E, 30 m
131 a.s.l.); this soil is widespread in Hokkaido island. Since BF is native to the Sapporo
132 Experimental Forest, half of each FACE rings was excavated to a depth of about 15 cm,
133 and it was refilled with VA. For the purpose of soil physical properties uniformity, the
134 same process was followed for BF, *i.e.* the excavated soil was back-filled. Although roots
135 can go much deeper, usually most of the nutrients accessed by plants are those found by
136 exploring fine roots at top soil; in this case, most of the lateral and fine roots were
137 distributed between the soil surface and a depth of 10–15 cm. Chemical and physical
138 properties of BF and VA used in the present study can be found in Watanabe et al. (2013).
139 The sites were completed in autumn 2002.

140 **2.2 Climatic & meteorological conditions**: The snow-free period lasted from early May to
141 mid. November. Meteorological data were collected from a station located in Sapporo
142 (WMO, ID: 47412) at 43°03.6'N and 141°19.7'E (Japan Meteorological Agency, 2015). The
143 monthly means of air temperature, wind speed, and relative humidity and the monthly totals
144 of sunshine duration and precipitation were averaged per year (Table 1). The mean values
145 for the years 2003-2013 were 9.31 (± 0.08 se) °C, 3.54 (± 0.04 se) m s^{-1}, 68.5 (± 0.39 se) %,
146 1709.86 (± 27.37 se) h, and 1150.14 (± 43.55 se) mm, for each variable, respectively.

147 **2.3 Plant Materials**: Seedlings of Siebold’s beech, obtained from Hokkaido Hort-green
148 Company Co. Ltd. (located near Sapporo city), were used as experimental subjects. These
149 seedlings originated from Kuromatsunai town (42°40.14'N 140°18.26'E), the northern

150 boundary of beech stands in Japan (Koike et al. 1998). In order to limit tree growth and
151 avoid compound interest effects (Körner 1995, 2006), 2-year-old seedlings (h = 15.3 cm ±
152 1.5 cm SD, d = 0.42 mm ± 0.08 mm SD) were planted in the FACE rings at a distance of 30
153 cm among them, after the snow had melted in 2003, with an equal number of 8 individuals
154 in each research condition. In surrounding areas within the same plots (not between beech
155 saplings to avoid interspecific competition), plants of different species or families, such as
156 alder, birches, larch and oak, were also planted at different times throughout the 11
157 experimental years (Eguchi et al. 2008; Watanabe et al. 2013; Agathokleous et al. 2016b,
158 see Koike et al. 2015 for a complete list of references).

159 **2.4 CO_2 treatment**: Treatment with CO_2 was carried out in 11 consecutive Julian years
160 (2003-2013). Seedlings were exposed to CO_2 in each growing season during daytime, when
161 the photosynthetic photon flux (PPF) exceeded the 70 μmol m^{-2} s^{-1} (*i.e.* light compensation
162 point of photosynthesis, Koike 1988), from leaf emergence to leaf senescence (from May
163 until late November). This FACE system consisted of six rings from which the three were
164 enriched with CO_2 to raise the atmospheric CO_2 concentration to a target of 500 μmol mol^{-1}
165 (hereafter "eCO_2"); this concentration corresponds to the predicted CO_2 concentration for
166 the Julian years 2040–2050 (Stocker et al. 2013). The three control plots remained under
167 ambient CO_2 (about 370-390 μmol mol^{-1}) (hereafter "aCO_2") (see Eguchi et al. 2008;
168 Watanabe et al. 2013). In order to control the CO_2 concentration, the Vaisala CARBOCAP®
169 Carbon Dioxide Probe GMP343 (Vaisala ©), an accurate and rugged probe-type instrument
170 for ecological measurements, was used. This FACE regime was in accordance with other
171 FACE regimes for studying trees (Karnosky et al. 2005; Liberloo et al. 2009; Norby et al.

2010; Ellsworth et al. 2012). More information can be found in previous publications (see Koike et al. 2015 for a complete list of references). The mean daytime CO_2 concentrations, as measured in the center of each FACE site, during fumigation period 2003-2012 was 498 μmol mol^{-1}. The CO_2 concentration was 500 ± 50 or 500 ± 100 μmol mol^{-1} for 64 or 89 % of the fumigation period, respectively.

2.5 Sampling & measurements: At the end of the last growing season (2013), the trunk basal diameter was measured for each beech sapling and all the roots were excavated using a small bulldozer. This mechanistic method was previously compared with the manual method (by hand), in different species, and it was revealed that excavation by this method results in 10-30 % less root biomass (Matsunami 2008); this assumes equal error across all the subjects. After the excavation, the following procedures were followed. First, the root tips in horizon A (10-15 cm) were immediately sampled and dried at 75 ^{o}C for more than five days. The dry masses of intermediate (d = 2 - 4 mm) and fine (d = < 2 mm) roots – including ectomycorrhizae – were determined. Second, the whole root systems were left on the field to physically dry; this was unavoidable because of the huge root systems. The next summer (2014), measurements were taken for the total root system dry mass (TDM). Forty one beech saplings were sampled and measured, with an average number of 3 (± 1 CI) randomly selected saplings from each soil in each experimental unit.

2.6 Statistics: Trunk diameter data were transformed to area ($Area = \pi \times \left(\frac{d}{2}\right)^2\ cm^2$). The level of significance was predefined at α=.05. In order to treat the heterogeneity (Saitanis et al. 2015), the data of each variable were subjected to T-scoring standardization using the

formula $T = \left(\frac{X-\mu}{\sigma}\right) \times 10 + 50$, where X is the raw score, μ the mean, and σ the standard deviation. Consequently, the mean became equal to 50 and the standard deviation equal to 10. The average T-score of each treatment in each experimental unit constituted the real replicate in the overall analysis (n = 3). All the data were subjected to split-plot general linear model randomized by block (GLM), based on Kuehl (1999), and, if needed, Tukey range, posthoc test was followed. For data presentation purpose, the untransformed, instead of transformed, values are presented.

In order to find the effect size (*ES*) of the treatments and to compare the *ES* with that of a 4-year experiment with a broadleaved community in the same plots (see Agathokleous et al. 2016b) the unbiased Cohen's δ (Cohen, 1988; Hedges and Olkin 1985) was calculated (using T-scores) for each pair of treatments (Table 2). Values of δ reported by Agathokleous et al. (2016b) were corrected (Hedges and Olkin 1985) and presented in Table 2. Absolute *ES* values within the arbitrary segments 00.00-0.20, 0.20-0.50, 0.50-0.80 and 0.80+ indicate neutral, small, moderate and large effect, respectively. Finally, in order to find the percentile gain in experimental conditions, the Cohen's U_3 index (Cohen 1977) was calculated, along with the overlapping coefficient (OVL) (Reiser and Faraggi 1999). Unbiased δ, U_3 and OVL were calculated only for the pairs with statistically significant difference in order to quantify the size of the difference. Data processing and statistical analyses were conducted using the MS EXCEL 2010 (Microsoft ©) and STATISTICA v.10 (StatSoft Inc. ©) software.

3. RESULTS

Elevated CO_2 had a significant impact to all the variables except trunk area (Table 2). Soil per se and its interaction with CO_2 had also significant impact on trunk area, TDM, intermediate root biomass, and ratio of fine root biomass to intermediate root biomass (Fine/Intermediate); the impact on trunk area to TDM rate (Area/TDM) and fine root biomass was insignificant. Particularly, eCO_2 led to a large increase in TDM, fine root biomass, and Fine/Intermediate of 20, 53, and 81 %, respectively, and a large decrease in Area/TDM and intermediate root biomass of 21 % and 39 % (Tables 2 and 3). The eCO_2-induced increase of fine root biomass was visible even to the naked eye (Fig 1). VA induced a large increase (80 %) in the Fine/Intermediate and a decrease in trunk area (18 %), TDM (13 %), and intermediate root biomass (33 %), of large, medium, and large *ES*, respectively (Table 3).

According to Tukey range tests (n = 3), the only statistically significant difference between the aCO_2 and eCO_2 when the saplings had grown in BF was that of fine root biomass, where eCO_2 caused a large increase (41 %) (Tables 2 and 3). Furthermore, variant results were obtained when the saplings had grown in VA: eCO_2 did not significantly alter the trunk area and the Area/TDM, but it did induce a large increase in TDM, fine root biomass, and Fine/Intermediate, of 48, 63, and 90 %, respectively, and a large decrease in intermediate root biomass (73 %) (Tables 2 and 3). The largely reduced mass of intermediate root biomass (Table 3), when saplings had grown in VA and exposed to eCO_2, was apparently accounted for the significant reduction in intermediate root biomass by eCO_2 as a main factor (Table 2).

235 Under aCO_2, VA caused significant reductions of large size in the trunk area (36 %) and
236 TDM (44 %). On the other hand, under eCO_2, VA caused a large reduction (71 %) in the
237 intermediate root biomass and, consequently, a large increase (88 %) in the Area/TDM
238 (Tables 2 and 3). Although, under eCO_2, saplings grown in VA had 19 % increased TDM
239 and 14 % increased fine root biomass (compared to those grown under eCO_2 and BF), these
240 differences were not significant ($p>0.05$) and, therefore, should be considered neither
241 educationally nor practically/clinically significant (Wolf 1986).

242 Finally, the fine root biomass to trunk basal area (Fine:Area) rate was largely increased by
243 eCO_2 (50 %), regardless of soil (Tables 2 and 3). Soil and the interaction between CO_2 and
244 Soil were insignificant factors (Table 2).

245 ## 4. DISCUSSION

246 To our knowledge, this is the first study evaluating the decadal independent or interactive
247 effects between elevated levels of CO_2 and soil fertility on root production of a late-
248 successional, deciduous, broadleaved species in a transition zone between cool temperate
249 and boreal forests and at the Asian boreo-nemoral ecotone. Saplings were subjected to the
250 treatments for the entire active growth period, and during the 11-year exposure, they were
251 progressing towards the mature phase of wood production and canopy closure.

252 Interestingly, eCO_2 did not alter the trunk area while VA affected it. Particularly, saplings
253 grown under aCO_2×VA had smaller trunk area, of a large *ES*, than those grown under
254 aCO_2×BF. Similarly, although root biomass was significantly increased by eCO_2, as a

255 single factor, this was mainly due to the large negative effect of VA on saplings under
256 ambient CO_2 environment (44 % lower, *cf.* BF). In fact, there were no statistically
257 significant differences among the treatments a) aCO_2×BF, b) eCO_2×BF, and c) eCO_2×VA,
258 however our data come from very wet years. The insignificant responses are in agreement
259 with aboveground ecophysiological findings: The leaf area index (mean of the canopy of a
260 community of ten species including beech) was higher in eCO_2 in 2nd growing season of
261 CO_2 treatment, but not in the following growing seasons (Koike et al. 2015). In addition,
262 leaf mass per area, area-based and mass-based N content of leaf, chlorophyll fluorescence
263 and most photosynthetic traits of beech saplings in the BF were not affected by eCO_2
264 (Watanabe et al. 2016). A 10-year experiment with wet and dry years also revealed that
265 there were no sustained increases in the biomass of a community of perennial plants
266 (Newingham et al. 2013). It is nevertheless clinically noteworthy that eCO_2 led to a largely
267 higher TDM of VA saplings, compared to aCO_2. In both variables (trunk area and TDM),
268 eCO_2 mediated the negative impact of VA and as such the interaction of CO_2×soil was
269 significant. Our findings (CO_2×VA), do not support the conclusion of Oren et al. (2001),
270 based on light demanding pine stands, that "…fertility can restrain the response of wood
271 carbon sequestration to increased atmospheric CO_2."

272 As to the Area/TDM, the only significant difference was that of aCO_2 *vs.* eCO_2; apparently,
273 eCO_2 led to a higher TDM per trunk area, decreasing thus the Area/TDM. Area/TDM
274 derives from the *Pipe Model* theory of tree form and can be used as an index for foliage
275 mass against stem mass (Shinozaki et al. 1964a, b), indicating C allocation within plant
276 body. According to meta-analyses, on average, eCO_2 does not change plant allometry

277 (Poorter and Nagel 2000; Poorter et al. 2012). However, our results indicate changed plant
278 allometry, and this is in contrast to the previous findings (Agathokleous et al. 2016b) from
279 a sapling community of three birches and an oak exposed to eCO_2 for 4 years in the same
280 facilities. In the latter case, the saplings were more widely spaced (50 cm *vs*. 30 cm in the
281 present study). The present and previous results (Agathokleous et al. 2016b), at a wet
282 region, are in agreement and differ to the general conclusion that soil infertility affects plant
283 allometry (Poorter and Nagel 2000; Poorter et al. 2012), according to the *functional*
284 *equilibrium* theory (Brouwer 1962; Poorter and Nagel 2000). It can only be postulated that
285 the degree of soil infertility was not adequate to change the plant allometry during 11
286 growing seasons. In long-term experiments, in contrast to short-term experiments, there is
287 an input of nutrients, through litterfall and decomposition, which may increase the soil
288 fertility.

289 Regarding the results of the root classes, the large eCO_2-induced fine root biomass was
290 certainly very high and clinically significant (Wolf 1986). This is in agreement with our
291 previous results (Table 3, Agathokleous et al. 2016b), but does not coincide with the
292 findings of Bader et al. (2009) where unchanged or reduced fine root biomass of trees
293 occurred at a mature deciduous forest exposed to 7 years FACE. We optically observed an
294 increased length of the small class of roots (Fig 1). Essentially, there was no individual or
295 interactive soil effect, even though we were expecting VA to be a critical factor altering
296 fine roots production through a force to seek nutrients. This is another indication that
297 future research should consider more nutrient-starving soil. The significant independent and
298 interactive effects of CO_2 and soil are again attributed to an effect of VA under eCO_2,

299 which caused a reduction of the intermediate root biomass and thus an increase of the
300 Fine/Intermediate. We cannot confidently explain why the intermediate root biomass was
301 reduced by VA only under eCO_2, but we could say that it may be explained by the large
302 increase of fine root biomass – even higher than in eCO_2×BF. Through this morphogenesis
303 of expanding fine roots at the expense of intermediate roots, saplings under eCO_2 succeed
304 to compensate the negative effects of VA. It is also possible that root turnover was faster
305 under eCO_2 (Wang et al. 2016), and fewer roots grew older which might decreased
306 intermediate roots.

307 Saplings underwent extensive foraging strategy of fine roots, as indicated by higher
308 Fine:Area, under eCO_2 (Ostonen et al. 2011; Leppalammi-Kujansuu et al. 2014) so as to
309 increase fine root mass and length in order to achieve greater absorbing area (Ostonen et al.
310 2011). This effect was as large as it was in our previous study (Agathokleous et al. 2016b),
311 however VA had no significant effects which is inconsistent with our previous findings
312 (Agathokleous et al. 2016b) where VA had a large effect. In the latter case, an initial effect
313 might be caused by wider spacing and as such propagated in following years.

314 Short-term exposure of very young or small seedlings to CO_2 and artifacts usually reveal
315 high responses of tree species to elevated CO_2 (Pregitzer et al. 1995; Tissue et al. 1997;
316 Kgope et al. 2009; Lavola et al. 1995; Duan et al. 2014), which can be even higher than
317 those of herbaceous species (Körner 2006). Artifacts are caused by inappropriate growth
318 conditions such as wide spacing and fertile artificial substrates. Our results do not
319 correspond with some of those short-term experiments where a high, and likely

overestimated, total root biomass response to elevated CO_2 was found: There was an increase in biomass when saplings had grown in VA, however, there was an insignificant response when grown in BF. On the other hand, the fine root biomass was not only high, but often even higher than in some short-term experiments, for saplings grown in both soils.

With reference to short-term experiments, long-term CO_2 experiments with saplings usually provide contradictory evidence (e.g. Bader and Körner 2010; Norby et al. 2010; Bader et al. 2013; Li et al. 2014; Warren et al. 2015). For instance, Li et al. (2014) found that 11 years of FACE treatment with 475 μmol mol^{-1} of CO_2 led to widely-ranged (-6 to +28%) annual plant production of grazing pasture and as little as 3 % higher (or even lower) final pasture production for the elevated CO_2 treatment, *cf.* ambient. Kostiainen et al. (2014) exposed 4 clones of *Populus tremuloides* and *Betula papyrifera* saplings to 560 μmol mol^{-1} of CO_2 for 11 years and found that most saplings responses to treatments were observed in the early phase of the experiment. Similarly, Dawes et al. (2015) reported that 9 years of FACE treatment (+200 μmol mol^{-1}) did not significantly change the coarse root biomass or total biomass of either *Larix decidua* or *Pinus uncinata*, approximately 40-year-old trees. Interestingly, Norby et al. (2004, 2010) found 24 % higher NPP –the prime contributor being a more than doubled annual fine root production- in plants of a more widely spaced, deciduous community during the 4^{th} to 6^{th} growing season after exposure to 550 μmol $mol^{-1}$of CO_2 began. However, the NPP enhancement declined to just 9 % after 11 growing seasons. In contrast, Pregitzer et al. (2008) exposed a community of *Populus tremuloides* to 560 μmol mol^{-1} of CO_2 for 10 years and found that elevated CO_2 led to ≈ 20 % greater fine and total root mass.

342 It is obvious that there is a wide range of responses in short-term and long-term research.
343 Nonetheless, in most of the long-term cases the elevated CO_2-induced differences were
344 insignificant (e.g. Norby et al. 2010; Newingham et al. 2013; Kostiainen et al. 2014; Li et al.
345 2014; Dawes et al. 2015) and this is consistent with our findings. The total root growth
346 simulation in mature Siebold's beech stands with closed canopies was much smaller or
347 neutral for the present two common types of soil when compared to the control treatment of
348 aCO_2×BF. However, if we take into account the large simulation caused by eCO_2 under VA,
349 compared to aCO_2×VA, it will be very important for relevant regions.

350 The key issue is that CO_2 enrichment must be applied to closed canopy stands to avoid the
351 compound interest effect artifact (including wide spacing, fertile artificial substrates, etc.).
352 What is needed is intact undisturbed natural soil *in situ* such as Sigurdsson at al. (2013)
353 used for experiments with mature Norway spruce (*Picea abies* (L.) Karst) trees exposed to
354 elevated CO_2 for 3 years, where limited nutrient availability in soil restricted the tree
355 response to elevated CO_2 (Sigurdsson at al. 2013). Spinnler et al. (2002, 2003) found that
356 beech (*Fagus sylvatica* L.) responded negatively to 4-year CO_2 enrichment when grown in
357 acidic soil, but responded positively when grown in calcareous soil, where growth
358 stimulation was observed –due to compound interest effect- during the first 2-3 growing
359 seasons. In agreement with the findings of Spinnler et al. (2002, 2003), our findings show
360 that we would draw false conclusions, if we had chosen to experiment with only one soil
361 type (brown forest soil).

362 Increased belowground allocation in poor soil caused by elevated CO_2 levels, as observed
363 in our study, may have wider consequences in the long term. Saxe et al. (2001) noted
364 "climatic adaptation seems to be the most important component in the evolutionary process
365 of temperate and boreal tree species." The root response of trees may affect the
366 performance of the whole trees and the interactions and distributions of populations and
367 species (de Kroon 2007). Fine roots contribute significantly to NPP (DeLucia et al. 1999),
368 however, the NPP impact by CO_2 is species specific and depends on other factors such as
369 nitrogen deposition (Norby et al. 2010; Yan et al. 2014). The variant NPP responses may
370 affect species' composition of forests under future climate change (Yan et al. 2014).

371 Overall, Siebold's beech saplings may not experience significant belowground effects in
372 regions with fertile soils, but may experience significant positive effects in regions with
373 infertile soils. The former case can be translated to insignificant effects on NPP, while in
374 the latter case to a higher NPP and quicker reach of high storage age, so called *buying time*,
375 (Körner 2006) in such regions. Regarding the large increase in biomass production caused
376 by eCO_2 under VA, there is no benefit if we compare it with $aCO_2 \times BF$, but the net (real)
377 surplus will be large to areas with VA. Natural forests at mountainous and remote regions
378 often have infertile soils and are not easily accessible to humans, while urban forests and
379 trees at plains usually have fertile soils. At remote infertile areas eCO_2 impacts could be
380 higher than at nearby areas and this should be taken into account when planning relevant
381 experiments.

5. CONCLUSIONS

The response to eCO_2 and soil fertility of saplings of the late-successional, deciduous, broadleaved Siebold's beech (*Fagus crenata*) was studied after 11 years of exposure, in a transition zone between cool temperate and boreal forests and at the Asian boreo-nemoral ecotone.

Elevated CO_2 led to a large enhancement of the total root production of saplings grown in VA, compensating the negative effect of VA under aCO_2, however, there was no significant effect of eCO_2 on saplings grown in BF. Since the effects of eCO_2×VA on total root production were not significantly different from aCO_2×BF, the eCO_2-enhancement is not quantitatively noteworthy compared to other soils, but this enhancement will be practically significant for regions with VA (or similar soil). In such regions, a higher NPP may be noticed, meaning that the projected elevated CO_2 concentrations may have a different impact in regions with different soil fertility.

A large eCO_2-induced fine root biomass (with higher biomass per basal trunk area) was observed, and it was certainly high, for both soils. Unexpectedly, there were no individual or interactive soil effects, something pointing out that future research should consider more nutrient-starving soil. A morphogenesis of roots was evident in saplings exposed to eCO_2 and VA, through which saplings succeed to compensate the negative effects of VA by expanding fine roots at the expense of intermediate roots.

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Captions

Table 1 The yearly averages of the monthly average air temperature, average wind speed, average relative humidity, total sunshine duration, and total precipitation, for the experimental Julian years 2003-2013

Table 2 Summary of the GLM results and mean untransformed values (± se) of the measured variables trunk basal area (Trunk Area), total belowground dry mass (TDM), Area/TDM rate, fine and intermediate roots dry masses, ratio of fine root biomass to intermediate root biomass (Fine/Intermediate) and fine root to trunk basal area rate (Fine/Area). The lowercase letters above the mean values indicate the significant differences among the 4 combination treatments. The results of each variable obtained by a GLM analysis or a Tukey range, post-hoc test, after significant results of the GLM analysis, based on standardized data. Means within each variable marked with different lowercase letters differ statistically significantly at a level of significance α=0.05. Data obtained from Siebold's beech (*Fagus crenata*) saplings exposed to ambient (370–390 μmol mol^{-1}) or elevated (500 μmol mol^{-1}) CO_2 and grown either in brown forest soil or immature volcanic ash plus pumice soil for 11 consecutive years. Three real replicates were used for each experimental condition

Table 3 The unbiased Cohen δ, Cohen U_3 index and overlapping coefficient (OVL) of the measured variables trunk basal area (Trunk Area), total belowground dry mass (TDM), Area/TDM rate, fine and intermediate roots dry masses, ratio of fine root biomass to intermediate root biomass (Fine/Intermediate) and fine root to trunk basal area rate (Fine/Area). The effect size (*ES*), for each pair with statistically significant difference, is

indicated by the letters M and L for Moderate and Large effect, respectively. “n/a” shows that there was no statistically significant effect and therefore the values are not available. The small-size and underlined values in the columns “δ” are the values found by Agathokleous et al. (2015b) but corrected for estimate bias.

Fig. 1 Typical difference of 10 cm root tips. Samples were obtained from Siebold’s Beech (*Fagus crenata*) saplings which were grown in two different types of soil (BF: brown forest soil or VA: volcanic ash soil including pumice) and exposed either to ambient CO_2 (370–390 μmol mol^{-1}) or to elevated CO_2 (500 μmol mol^{-1}) for 11 growing seasons (2003-2013)

669 **Table 1**

Julian Year	**Air Temperature (^{o}C)**	**Wind Speed (m s^{-1})**	**Relative Humidity (%)**	**Sunshine Duration (h)**	**Precipitation (mm)**
2003	8.8	3.6	68	1787.1	0916.0
2004	9.7	3.6	66	1668.4	1130.5
2005	8.9	3.6	68	1700.5	1236.5
2006	9.1	3.8	68	1725.4	1145.5
2007	9.4	3.4	68	1730.1	1028.5
2008	9.5	3.5	68	1844.5	0843.0
2009	9.4	3.6	68	1604.4	1147.0
2010	9.8	3.4	69	1526.9	1325.0
2011	9.3	3.3	69	1753.6	1253.5
2012	9.3	3.5	70	1819.6	1279.0
2013	9.2	3.6	71	1647.9	1347.0
Average	**9.3**	**3.5**	**69**	**1709.9**	**1150.1**

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686 **Table 2**

	GLM results			Means (± se) & Tukey's range test results (CO_2 × Soil)			
	CO_2	**Soil**	**CO_2 × Soil**	**aCO_2×BF**	**eCO_2×BF**	**aCO_2×VA**	**eCO_2×VA**
Trunk Area ($cm^2/100$)	F = 00.55, *p* = 0.48	**F = 06.63, *p* < 0.05**	**F = 08.26, *p* < 0.05**	0.357^{a} (0.038)	0.306^{ab} (0.024)	0.227^{b} (0.003)	0.313^{ab} (0.015)
TDM (kg)	**F = 16.92, *p* < 0.01**	**F = 05.95, *p* < 0.05**	**F = 43.62, *p* < 0.01**	1.617^{a} (0.046)	1.421^{a} (0.104)	0.908^{b} (0.090)	1.748^{a} (0.060)
Area/TDM ($cm^2/kg \times 10$)	**F = 10.86, *p* < 0.01**	F = 01.16, *p* = 0.31	F = 00.07, *p* = 0.81	0.288^{a} (0.016)	0.233^{a} (0.014)	0.273^{a} (0.008)	0.209^{a} (0.028)
Fine root biomass (g)	**F = 42.48, *p* < 0.01**	F = 00.01, *p* = 0.93	F = 03.39, *p* = 0.10	0.122^{a} (0.021)	0.207^{b} (0.036)	0.090^{a} (0.013)	0.242^{b} (0.031)
Intermediate root biomass (g)	**F = 23.82, *p* < 0.01**	**F = 15.26, *p* < 0.01**	**F = 20.72, *p* < 0.01**	0.750^{a} (0.085)	0.730^{a} (0.051)	0.790^{a} (0.064)	0.209^{b} (0.033)
Fine/Intermediate (g/g)	**F = 20.90, *p* < 0.01**	**F = 20.27, *p* < 0.01**	**F = 19.58, *p* < 0.01**	0.249^{a} (0.036)	0.285^{a} (0.053)	0.268^{a} (0.059)	2.463^{b} (0.480)
Fine/Area ($g/cm^2 \times 100$)	**F = 42.20, *p* < 0.001**	F = 01.44, *p* = 0.26	F = 00.14, *p* = 0.72	0.347^{a} (0.012)	0.691^{b} (0.065)	0.394^{a} (0.028)	0.780^{b} (0.086)

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688 **Table 3**

	aCO_2 vs. eCO_2			BF vs. VA			aCO_2×BF vs. eCO_2×BF			aCO_2×VA vs. eCO_2×VA		
	δ	**U_3**	**OVL**	***δ***	**U_3**	**OVL**	***δ***	**U_3**	**OVL**	***δ***	**U_3**	**OVL**
Trunk Area (cm^2)	n/a 1.560(L)	n/a	n/a	1.159(L) n/a	0.877	0.562	n/a n/a			n/a 5.573(L)		
TDM (kg)	1.001(L) 1.430(L)	0.842	0.617	0.544(M) n/a	0.707	0.786	n/a n/a			7.782(L) 3.720(L)	1.000	0.000
Area/TDM (cm^2/kg ×10)	2.003(L) n/a	0.977	0.317	n/a n/a			n/a n/a			n/a n/a		
Fine root biomass (g)	2.630(L) 2.680(L)	0.996	0.189	n/a 0.585(M)			6.395 (L) 2.800(L)	1.000	0.001	4.490(L) 7.004(L)	1.000	0.025
Intermediate root biomass (g)	1.359(L) 4.306(L)	0.913	0.497	0.995(L) n/a	0.840	0.619	n/a 3.999(L)			8.013(L) 6.919(L)	1.000	0.000
Fine/Intermediate (g/g)	1.220(L) 0.817(L)	0.889	0.542	1.194(L) n/a	0.884	0.551	n/a 3.203(L)			4.537(L) n/a	1.000	0.023
Fine/Area (g/cm^2 × 100)	3.875(L) 0.942(L)	0.100	0.053	n/a 2.350(L)			5.223 (L) n/a	1.000	0.009	4.238(L) 4.201(L)	1.000	0.034

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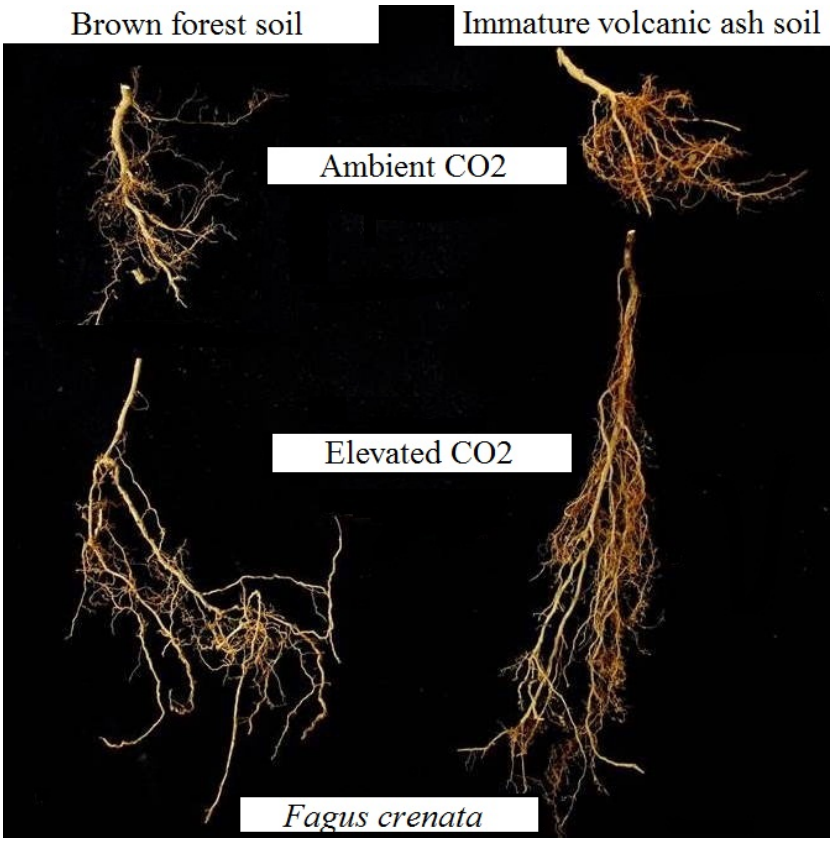
693 **Fig 1**



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