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Root production of *Fagus crenata* Blume saplings grown in two soils and exposed to elevated CO₂ concentration: an 11-year free-air-CO₂ 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 CO₂. After the saplings had been exposed to ambient (370-390 μmol mol⁻¹) or elevated (500 μmol mol⁻¹) CO₂, during the daytime, for 11 growing seasons, the root systems were excavated. Elevated CO₂ boosted the total root production of saplings grown in VA and abolished the negative effect of VA under ambient CO₂, but there was no significant effect of elevated CO₂ on saplings grown in BF. These results indicate the projected elevated CO₂ 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-CO₂-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 CO₂ 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 (CO₂) 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 CO₂ 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

shed light on CO₂ effects on trees after canopy closure and mature stage and to quantify the effect (Leuzinger et al. 2011). The importance of studies conducted in different regions is also highlighted by the latitude-dependency of the effect of atmospheric changes on forest productivity (Silva and Anand 2013). Additionally, another weakness is the wide spacing of plants which has been implemented in several experiments (Körner 2006). Wide spacing leads to artifacts due to the influence of nutritional resources: If a first year effect is induced by offering open space as a surrogate for ample nutrients, that signal will propagate into the future (even if CO₂ exposure is terminated), which is even worse than short experimental duration (Körner 2006). As such, most data do not truly represent the effects of future elevated CO₂ levels on tree roots, the responses of which to elevated CO₂ levels are still not well understood (Körner 2011; Wang et al. 2016).

Fine roots (production and turnover) partly adjust the biogeochemical cycles of ecosystems and form their response to global change (Norby et al. 2004). Moreover, fine root data along with models contribute to the understanding of the global belowground diversity and biogeochemical processes in the terrestrial biosphere (McCormack 2015). Root dynamics can explain elevated-CO₂-induced differences among ecosystems (Norby et al. 2004); however, fine-root biomass can vary across years (Pregitzer et al. 2008; Wang et al. 2016), as fine root production is also related to biotic factors, such as soil fauna (Lipson et al. 2014). In addition, future changes in allocation to belowground of trees in response to elevated CO₂ levels are likely to alter the fungal community (Lipson et al. 2014; Wang et al. 2016).

87 Short-term experiments might not represent the actual responses of tree root system to CO₂
88 (Norby and Zak 2011; Kostianen 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; Kostianen 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 CO₂ 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 CO₂
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 CO₂ 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° N) to southern Hokkaido (c. 42.8° 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 CO₂ levels and in fertile brown forest soil (BF) or infertile, immature volcanic ash plus pumice soil (VA) for 11 years, in a free-air CO₂ 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 CO₂ 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°06' N, 141°20' E, 60 m a.s.l), with a split-plot factorial design and employing the randomized block method (Filion et al. 2000). The CO₂ treatments were ambient and elevated CO₂, 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

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

2.2 Climatic & meteorological conditions: The snow-free period lasted from early May to mid. November. Meteorological data were collected from a station located in Sapporo (WMO, ID: 47412) at 43°03.6'N and 141°19.7'E (Japan Meteorological Agency, 2015). The monthly means of air temperature, wind speed, and relative humidity and the monthly totals of sunshine duration and precipitation were averaged per year (Table 1). The mean values 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) %, 1709.86 (± 27.37 se) h, and 1150.14 (± 43.55 se) mm, for each variable, respectively.

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

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

2.4 CO₂ treatment: Treatment with CO₂ was carried out in 11 consecutive Julian years (2003-2013). Seedlings were exposed to CO₂ in each growing season during daytime, when the photosynthetic photon flux (PPF) exceeded the $70 \mu\text{mol m}^{-2} \text{s}^{-1}$ (*i.e.* light compensation point of photosynthesis, Koike 1988), from leaf emergence to leaf senescence (from May until late November). This FACE system consisted of six rings from which the three were enriched with CO₂ to raise the atmospheric CO₂ concentration to a target of $500 \mu\text{mol mol}^{-1}$ (hereafter “eCO₂”); this concentration corresponds to the predicted CO₂ concentration for the Julian years 2040–2050 (Stocker et al. 2013). The three control plots remained under ambient CO₂ (about $370\text{-}390 \mu\text{mol mol}^{-1}$) (hereafter “aCO₂”) (see Eguchi et al. 2008; Watanabe et al. 2013). In order to control the CO₂ concentration, the Vaisala CARBOCAP® Carbon Dioxide Probe GMP343 (Vaisala ©), an accurate and rugged probe-type instrument for ecological measurements, was used. This FACE regime was in accordance with other 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 CO₂ concentrations, as measured in the center of each FACE site, during fumigation period 2003-2012 was 498 μmol mol⁻¹. The CO₂ 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 °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 ($Area = \pi \times \left(\frac{d}{2}\right)^2$ cm²). The level of significance was predefined at $\alpha=.05$. In order to treat the heterogeneity (Saitanis et al. 2015), the data of each variable were subjected to T-scoring standardization using the

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

200 In order to find the effect size (*ES*) of the treatments and to compare the *ES* with that of a 4-
201 year experiment with a broadleaved community in the same plots (see Agathokleous et al.
202 2016b) the unbiased Cohen's δ (Cohen, 1988; Hedges and Olkin 1985) was calculated
203 (using T-scores) for each pair of treatments (Table 2). Values of δ reported by
204 Agathokleous et al. (2016b) were corrected (Hedges and Olkin 1985) and presented in
205 Table 2. Absolute *ES* values within the arbitrary segments 00.00-0.20, 0.20-0.50, 0.50-0.80
206 and 0.80+ indicate neutral, small, moderate and large effect, respectively. Finally, in order
207 to find the percentile gain in experimental conditions, the Cohen's U_3 index (Cohen 1977)
208 was calculated, along with the overlapping coefficient (OVL) (Reiser and Faraggi 1999).
209 Unbiased δ , U_3 and OVL were calculated only for the pairs with statistically significant
210 difference in order to quantify the size of the difference. Data processing and statistical
211 analyses were conducted using the MS EXCEL 2010 (Microsoft ©) and STATISTICA v.10
212 (StatSoft Inc. ©) software.

3. RESULTS

Elevated CO₂ had a significant impact to all the variables except trunk area (Table 2). Soil per se and its interaction with CO₂ 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, eCO₂ 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 eCO₂-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 aCO₂ and eCO₂ when the saplings had grown in BF was that of fine root biomass, where eCO₂ caused a large increase (41 %) (Tables 2 and 3). Furthermore, variant results were obtained when the saplings had grown in VA: eCO₂ 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 eCO₂, was apparently accounted for the significant reduction in intermediate root biomass by eCO₂ as a main factor (Table 2).

Under aCO₂, VA caused significant reductions of large size in the trunk area (36 %) and TDM (44 %). On the other hand, under eCO₂, VA caused a large reduction (71 %) in the intermediate root biomass and, consequently, a large increase (88 %) in the Area/TDM (Tables 2 and 3). Although, under eCO₂, saplings grown in VA had 19 % increased TDM and 14 % increased fine root biomass (compared to those grown under eCO₂ and BF), these differences were not significant ($p>0.05$) and, therefore, should be considered neither educationally nor practically/clinically significant (Wolf 1986).

Finally, the fine root biomass to trunk basal area (Fine:Area) rate was largely increased by eCO₂ (50 %), regardless of soil (Tables 2 and 3). Soil and the interaction between CO₂ and Soil were insignificant factors (Table 2).

4. DISCUSSION

To our knowledge, this is the first study evaluating the decadal independent or interactive effects between elevated levels of CO₂ and soil fertility on root production of a late-successional, deciduous, broadleaved species in a transition zone between cool temperate and boreal forests and at the Asian boreo-nemoral ecotone. Saplings were subjected to the treatments for the entire active growth period, and during the 11-year exposure, they were progressing towards the mature phase of wood production and canopy closure.

Interestingly, eCO₂ did not alter the trunk area while VA affected it. Particularly, saplings grown under aCO₂×VA had smaller trunk area, of a large *ES*, than those grown under aCO₂×BF. Similarly, although root biomass was significantly increased by eCO₂, as a

single factor, this was mainly due to the large negative effect of VA on saplings under ambient CO₂ environment (44 % lower, *cf.* BF). In fact, there were no statistically significant differences among the treatments a) aCO₂×BF, b) eCO₂×BF, and c) eCO₂×VA, however our data come from very wet years. The insignificant responses are in agreement with aboveground ecophysiological findings: The leaf area index (mean of the canopy of a community of ten species including beech) was higher in eCO₂ in 2nd growing season of CO₂ treatment, but not in the following growing seasons (Koike et al. 2015). In addition, leaf mass per area, area-based and mass-based N content of leaf, chlorophyll fluorescence and most photosynthetic traits of beech saplings in the BF were not affected by eCO₂ (Watanabe et al. 2016). A 10-year experiment with wet and dry years also revealed that there were no sustained increases in the biomass of a community of perennial plants (Newingham et al. 2013). It is nevertheless clinically noteworthy that eCO₂ led to a largely higher TDM of VA saplings, compared to aCO₂. In both variables (trunk area and TDM), eCO₂ mediated the negative impact of VA and as such the interaction of CO₂×soil was significant. Our findings (CO₂×VA), do not support the conclusion of Oren et al. (2001), based on light demanding pine stands, that “...fertility can restrain the response of wood carbon sequestration to increased atmospheric CO₂.”

As to the Area/TDM, the only significant difference was that of aCO₂ vs. eCO₂; apparently, eCO₂ led to a higher TDM per trunk area, decreasing thus the Area/TDM. Area/TDM derives from the *Pipe Model* theory of tree form and can be used as an index for foliage mass against stem mass (Shinozaki et al. 1964a, b), indicating C allocation within plant body. According to meta-analyses, on average, eCO₂ does not change plant allometry

(Poorter and Nagel 2000; Poorter et al. 2012). However, our results indicate changed plant allometry, and this is in contrast to the previous findings (Agathokleous et al. 2016b) from a sapling community of three birches and an oak exposed to eCO₂ for 4 years in the same facilities. In the latter case, the saplings were more widely spaced (50 cm vs. 30 cm in the present study). The present and previous results (Agathokleous et al. 2016b), at a wet region, are in agreement and differ to the general conclusion that soil infertility affects plant allometry (Poorter and Nagel 2000; Poorter et al. 2012), according to the *functional equilibrium* theory (Brouwer 1962; Poorter and Nagel 2000). It can only be postulated that the degree of soil infertility was not adequate to change the plant allometry during 11 growing seasons. In long-term experiments, in contrast to short-term experiments, there is an input of nutrients, through litterfall and decomposition, which may increase the soil fertility.

Regarding the results of the root classes, the large eCO₂-induced fine root biomass was certainly very high and clinically significant (Wolf 1986). This is in agreement with our previous results (Table 3, Agathokleous et al. 2016b), but does not coincide with the findings of Bader et al. (2009) where unchanged or reduced fine root biomass of trees occurred at a mature deciduous forest exposed to 7 years FACE. We optically observed an increased length of the small class of roots (Fig 1). Essentially, there was no individual or interactive soil effect, even though we were expecting VA to be a critical factor altering fine roots production through a force to seek nutrients. This is another indication that future research should consider more nutrient-starving soil. The significant independent and interactive effects of CO₂ and soil are again attributed to an effect of VA under eCO₂,

which caused a reduction of the intermediate root biomass and thus an increase of the Fine/Intermediate. We cannot confidently explain why the intermediate root biomass was reduced by VA only under eCO₂, but we could say that it may be explained by the large increase of fine root biomass – even higher than in eCO₂×BF. Through this morphogenesis of expanding fine roots at the expense of intermediate roots, saplings under eCO₂ succeed to compensate the negative effects of VA. It is also possible that root turnover was faster under eCO₂ (Wang et al. 2016), and fewer roots grew older which might decreased intermediate roots.

Saplings underwent extensive foraging strategy of fine roots, as indicated by higher Fine:Area, under eCO₂ (Ostonen et al. 2011; Leppalammi-Kujansuu et al. 2014) so as to increase fine root mass and length in order to achieve greater absorbing area (Ostonen et al. 2011). This effect was as large as it was in our previous study (Agathokleous et al. 2016b), however VA had no significant effects which is inconsistent with our previous findings (Agathokleous et al. 2016b) where VA had a large effect. In the latter case, an initial effect might be caused by wider spacing and as such propagated in following years.

Short-term exposure of very young or small seedlings to CO₂ and artifacts usually reveal high responses of tree species to elevated CO₂ (Pregitzer et al. 1995; Tissue et al. 1997; Kgope et al. 2009; Lavola et al. 1995; Duan et al. 2014), which can be even higher than those of herbaceous species (Körner 2006). Artifacts are caused by inappropriate growth conditions such as wide spacing and fertile artificial substrates. Our results do not correspond with some of those short-term experiments where a high, and likely

overestimated, total root biomass response to elevated CO₂ 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 CO₂ 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 $\mu\text{mol mol}^{-1}$ of CO₂ 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 CO₂ treatment, *cf.* ambient. Kostianinen et al. (2014) exposed 4 clones of *Populus tremuloides* and *Betula papyrifera* saplings to 560 $\mu\text{mol mol}^{-1}$ of CO₂ 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 $\mu\text{mol mol}^{-1}$) 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 4th to 6th growing season after exposure to 550 $\mu\text{mol mol}^{-1}$ of CO₂ 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 $\mu\text{mol mol}^{-1}$ of CO₂ for 10 years and found that elevated CO₂ led to $\approx$ 20 % greater fine and total root mass.

It is obvious that there is a wide range of responses in short-term and long-term research. Nonetheless, in most of the long-term cases the elevated CO₂-induced differences were insignificant (e.g. Norby et al. 2010; Newingham et al. 2013; Kostianen et al. 2014; Li et al. 2014; Dawes et al. 2015) and this is consistent with our findings. The total root growth simulation in mature Siebold's beech stands with closed canopies was much smaller or neutral for the present two common types of soil when compared to the control treatment of aCO₂×BF. However, if we take into account the large simulation caused by eCO₂ under VA, compared to aCO₂×VA, it will be very important for relevant regions.

The key issue is that CO₂ enrichment must be applied to closed canopy stands to avoid the compound interest effect artifact (including wide spacing, fertile artificial substrates, etc.). What is needed is intact undisturbed natural soil *in situ* such as Sigurdsson et al. (2013) used for experiments with mature Norway spruce (*Picea abies* (L.) Karst) trees exposed to elevated CO₂ for 3 years, where limited nutrient availability in soil restricted the tree response to elevated CO₂ (Sigurdsson et al. 2013). Spinnler et al. (2002, 2003) found that beech (*Fagus sylvatica* L.) responded negatively to 4-year CO₂ enrichment when grown in acidic soil, but responded positively when grown in calcareous soil, where growth stimulation was observed –due to compound interest effect- during the first 2-3 growing seasons. In agreement with the findings of Spinnler et al. (2002, 2003), our findings show that we would draw false conclusions, if we had chosen to experiment with only one soil type (brown forest soil).

Increased belowground allocation in poor soil caused by elevated CO₂ levels, as observed in our study, may have wider consequences in the long term. Saxe et al. (2001) noted “climatic adaptation seems to be the most important component in the evolutionary process of temperate and boreal tree species.” The root response of trees may affect the performance of the whole trees and the interactions and distributions of populations and species (de Kroon 2007). Fine roots contribute significantly to NPP (DeLucia et al. 1999), however, the NPP impact by CO₂ is species specific and depends on other factors such as nitrogen deposition (Norby et al. 2010; Yan et al. 2014). The variant NPP responses may affect species’ composition of forests under future climate change (Yan et al. 2014).

Overall, Siebold’s beech saplings may not experience significant belowground effects in regions with fertile soils, but may experience significant positive effects in regions with infertile soils. The former case can be translated to insignificant effects on NPP, while in the latter case to a higher NPP and quicker reach of high storage age, so called *buying time*, (Körner 2006) in such regions. Regarding the large increase in biomass production caused by eCO₂ under VA, there is no benefit if we compare it with aCO₂×BF, but the net (real) surplus will be large to areas with VA. Natural forests at mountainous and remote regions often have infertile soils and are not easily accessible to humans, while urban forests and trees at plains usually have fertile soils. At remote infertile areas eCO₂ impacts could be higher than at nearby areas and this should be taken into account when planning relevant experiments.

5. CONCLUSIONS

The response to eCO₂ 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 CO₂ led to a large enhancement of the total root production of saplings grown in VA, compensating the negative effect of VA under aCO₂, however, there was no significant effect of eCO₂ on saplings grown in BF. Since the effects of eCO₂×VA on total root production were not significantly different from aCO₂×BF, the eCO₂-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 CO₂ concentrations may have a different impact in regions with different soil fertility.

A large eCO₂-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 eCO₂ 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 ($\pm$ 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 $\alpha=0.05$. Data obtained from Siebold's beech (*Fagus crenata*) saplings exposed to ambient ($370\text{--}390\ \mu\text{mol mol}^{-1}$) or elevated ($500\ \mu\text{mol mol}^{-1}$) 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 CO₂ (370–390 $\mu\text{mol mol}^{-1}$) or to elevated CO₂ (500 $\mu\text{mol mol}^{-1}$) for 11 growing seasons (2003-2013)

669 **Table 1**

Julian Year	Air Temperature (°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 ($\pm$ se) & Tukey's range test results ($\text{CO}_2 \times \text{Soil}$)			
	CO_2	Soil	$\text{CO}_2 \times \text{Soil}$	a $\text{CO}_2 \times \text{BF}$	e $\text{CO}_2 \times \text{BF}$	a $\text{CO}_2 \times \text{VA}$	e $\text{CO}_2 \times \text{VA}$
Trunk Area ($\text{cm}^2/100$)	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 ($\text{cm}^2/\text{kg} \times 10$)	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/Intermedia te (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 ($\text{g}/\text{cm}^2 \times 100$)	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 (cm²)	n/a <u>1.560(L)</u>	n/a	n/a	1.159(L) <u>n/a</u>	0.877	0.562	n/a <u>n/a</u>			n/a <u>5.573(L)</u>		
TDM (kg)	1.001(L) <u>1.430(L)</u>	0.842	0.617	0.544(M) <u>n/a</u>	0.707	0.786	n/a <u>n/a</u>			7.782(L) <u>3.720(L)</u>	1.000	0.000
Area/TDM (cm²/kg ×10)	2.003(L) <u>n/a</u>	0.977	0.317	n/a <u>n/a</u>			n/a <u>n/a</u>			n/a <u>n/a</u>		
Fine root biomass (g)	2.630(L) <u>2.680(L)</u>	0.996	0.189	n/a <u>0.585(M)</u>			6.395 (L) <u>2.800(L)</u>	1.000	0.001	4.490(L) <u>7.004(L)</u>	1.000	0.025
Intermediate root biomass (g)	1.359(L) <u>4.306(L)</u>	0.913	0.497	0.995(L) <u>n/a</u>	0.840	0.619	n/a <u>3.999(L)</u>			8.013(L) <u>6.919(L)</u>	1.000	0.000
Fine/Intermediate (g/g)	1.220(L) <u>0.817(L)</u>	0.889	0.542	1.194(L) <u>n/a</u>	0.884	0.551	n/a <u>3.203(L)</u>			4.537(L) <u>n/a</u>	1.000	0.023
Fine/Area (g/cm² × 100)	3.875(L) <u>0.942(L)</u>	0.100	0.053	n/a <u>2.350(L)</u>			5.223 (L) <u>n/a</u>	1.000	0.009	4.238(L) <u>4.201(L)</u>	1.000	0.034

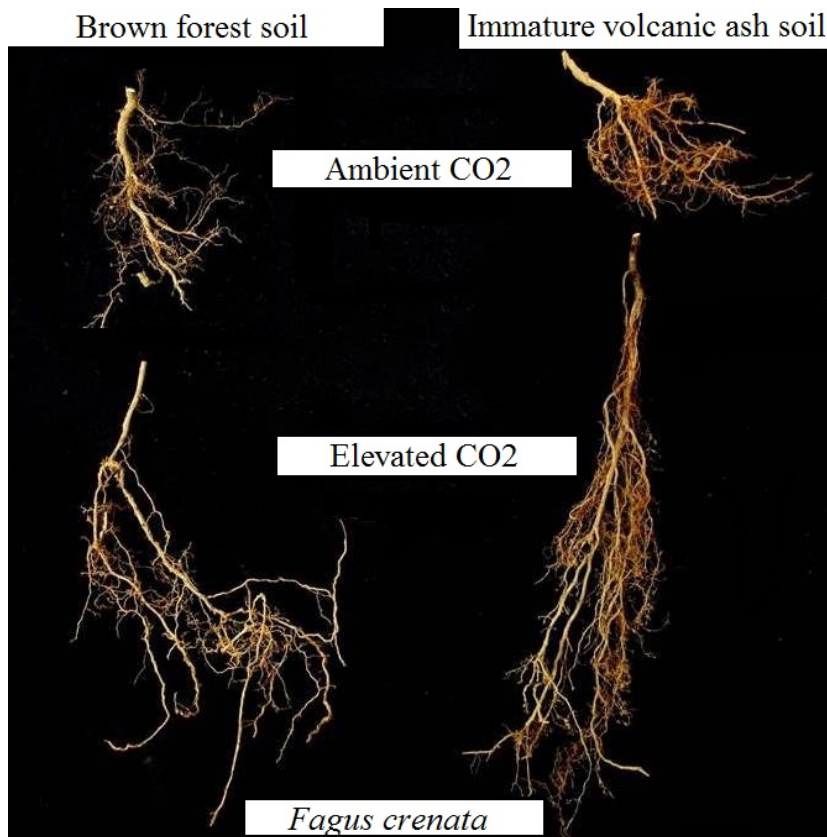
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693 **Fig 1**



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