

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.

Keywords Air pollution · Atmospheric environment · Climate change · Ecophysiology · Greenhouse gas · NPP

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 et al. 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 (Körner et al. 2005; Leuzinger et al.

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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 et al. 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).

Short-term experiments might not represent the actual responses of tree root system to CO₂ (Norby and Zak 2011; Kostianen et al. 2014) due to physiological age and size dependency, stand development, community composition, nitrogen deposition, ground-surface ozone, etc. (Asshoff et al. 2006; Körner 2006; Pregitzer et al. 2008; Kostianen et al. 2009, 2014; Bader et al. 2013; Yan et al. 2014; Agathokleous et al. 2016a). Despite the importance of roots, knowledge about their response to future elevated CO₂ concentrations remains meager (e.g., Körner 2011; Wang et al. 2016). What is also surprising is the prevailing unawareness in the role of soils in belowground responses of trees to atmospheric CO₂ despite that soils have greater influences on responses of plants to other applied treatments (Spinnler et al. 2002, 2003; Körner 2011;

Sigurdsson et al. 2013). To our knowledge, there are no available studies on the decadal belowground response to CO₂ of trees grown in different soils.

Siebold's beech (*Fagus crenata* Blume; Fagaceae) is a late-successional, deciduous, broadleaf tree native to Japan (Koike et al. 1998). It has a distribution from Kyushu (c. 30.5° N) to southern Hokkaido (c. 42.8° N) and a climatic threshold close to the cool-temperate zone (Horikawa 1972; Fang and Lechowicz 2006). Thus, it is a dominant species in the cool temperate zone of Japan (Asuka et al. 2004a). Among the beech species, Siebold's beech is adapted to and occurs in the most humid conditions of cold areas (Fang and Lechowicz 2006). Phenological events (e.g., flowering and autumnal surcease of growth) of late successional taxa, such as Siebold's beech, are primarily controlled by 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 and 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 and 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 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 eight 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 μmol m⁻² 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 μmol 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 370–390 μmol 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.

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

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

2.5 Sampling and 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 2009); 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 5 days. The dry masses of intermediate ($d=2\text{--}4$ 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 ($\text{Area} = \pi \times \left(\frac{d}{2}\right)^2 \text{ cm}^2$). 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 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 (GLM) randomized by block, based on Kuehl (1999), and, if needed, Tukey range, post hoc 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 ,

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 four 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 (370–390 $\mu\text{mol mol}^{-1}$) 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

	GLM results		Means (\pm se) and Tukey's range test results ($\text{CO}_2 \times \text{soil}$)			
	CO_2	Soil	$\text{CO}_2 \times \text{Soil}$	$\text{eCO}_2 \times \text{BF}$	$\text{aCO}_2 \times \text{VA}$	$\text{eCO}_2 \times \text{VA}$
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)	0.227^{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)	0.908^{b} (0.090)	1.748^{a} (0.060)
Area/TDM ($\text{cm}^2\text{-kg} \times 10$)	$F = 10.86, p < 0.01$	$F = 01.16, p = 0.31$	$F = 00.07, p = 0.81$	0.233^{a} (0.014)	0.273^{a} (0.008)	0.209^{a} (0.028)
Fine root biomass (g)	$F = 42.48, p < 0.01$	$F = 00.01, p = 0.93$	$F = 03.39, p = 0.10$	0.207^{b} (0.036)	0.090^{a} (0.013)	0.242^{b} (0.031)
Intermediate root biomass (g)	$F = 23.82, p < 0.01$	$F = 15.26, p < 0.01$	$F = 20.72, p < 0.01$	0.730^{a} (0.051)	0.790^{a} (0.064)	0.209^{b} (0.033)
Fine/intermediate (g/g)	$F = 20.90, p < 0.01$	$F = 20.27, p < 0.01$	$F = 19.58, p < 0.01$	0.285^{a} (0.053)	0.268^{a} (0.059)	2.463^{b} (0.480)
Fine/area ($\text{g}/\text{cm}^2 \times 10$)	$F = 42.20, p < 0.001$	$F = 01.44, p = 0.26$	$F = 00.14, p = 0.72$	0.691^{b} (0.065)	0.394^{a} (0.028)	0.780^{b} (0.086)

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

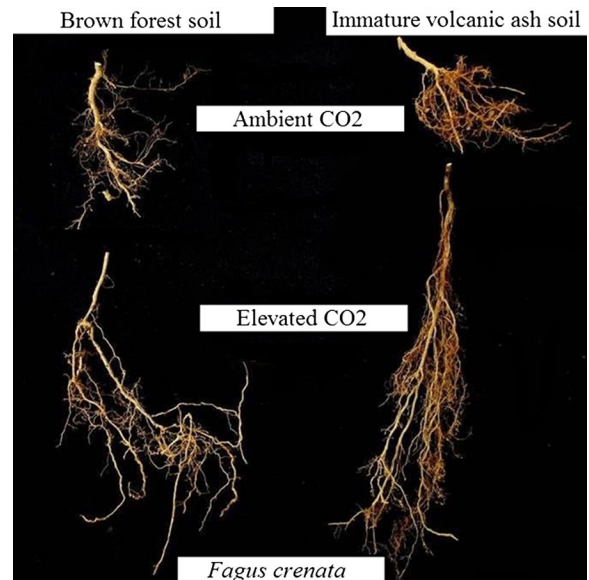


Fig. 1 Typical difference of 10 cm root tips. Samples were obtained from Siebold's beech (*Fagus crenata*) saplings which were grown in two different types of soil (BF brown forest soil or VA volcanic ash soil including pumice) and exposed either to ambient CO_2 (370–390 $\mu\text{mol mol}^{-1}$) or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

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 $a\text{CO}_2$ and $e\text{CO}_2$ when the saplings had grown in BF was that of fine root biomass, where $e\text{CO}_2$ caused a large increase (41 %) (Tables 2 and 3). Furthermore, variant results were obtained when the saplings had grown in VA; $e\text{CO}_2$ 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 $e\text{CO}_2$, was apparently accounted for the significant reduction in intermediate root biomass by $e\text{CO}_2$ as a main factor (Table 2).

Under $a\text{CO}_2$, VA caused significant reductions of large size in the trunk area (36 %) and TDM (44 %). On the other hand, under $e\text{CO}_2$, 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 $e\text{CO}_2$, saplings grown in VA had 19 % increased TDM and 14 % increased fine root biomass (compared to those grown under $e\text{CO}_2$ 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 $e\text{CO}_2$ (50 %), regardless of soil (Tables 2 and 3). Soil and the interaction between CO_2 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 CO_2 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, $e\text{CO}_2$ did not alter the trunk area while VA affected it. Particularly, saplings grown under $a\text{CO}_2 \times \text{VA}$ had smaller trunk area, of a large *ES*, than those grown under $a\text{CO}_2 \times \text{BF}$. Similarly, although root biomass was significantly increased by $e\text{CO}_2$, as a single factor, this was mainly due to the large negative effect of VA on saplings under ambient CO_2 environment (44 % lower, *cf.* BF). In fact, there were no statistically significant differences among the treatments (a) $a\text{CO}_2 \times \text{BF}$, (b) $e\text{CO}_2 \times \text{BF}$, and (c) $e\text{CO}_2 \times \text{VA}$; 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 $e\text{CO}_2$ in 2nd growing season of CO_2 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 $e\text{CO}_2$ (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 $e\text{CO}_2$ led to a largely higher TDM of VA saplings, compared to $a\text{CO}_2$. In both variables (trunk area and TDM), $e\text{CO}_2$ mediated the negative impact of VA and as such the interaction of $\text{CO}_2 \times \text{soil}$ was significant. Our findings ($\text{CO}_2 \times \text{VA}$) 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 CO_2 ."

As to the area/TDM, the only significant difference was that of $a\text{CO}_2$ vs. $e\text{CO}_2$; apparently, $e\text{CO}_2$ 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, $e\text{CO}_2$ 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 $e\text{CO}_2$ for 4 years in the same facilities. In the latter case, the saplings were more widely spaced (50 vs. 30 cm in the present study).

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. (2016b) but with corrected δ estimate (Hedges and Olkin 1985)

	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	n/a	n/a 5.573 (L)	n/a	n/a
TDM (kg)	<u>1.001 (L)</u> 1.430 (L)	0.842	0.617	<u>0.544 (M)</u> n/a	0.707	0.786	n/a n/a	n/a	n/a	<u>7.782 (L)</u> 3.720 (L)	1.000	0.000
Area/TDM (cm ² kg × 10)	<u>2.003 (L)</u> n/a	0.977	0.317	n/a n/a	n/a	n/a	n/a n/a	n/a	n/a	n/a n/a	n/a	n/a
Fine root biomass (g)	<u>2.630 (L)</u> 2.680 (L)	0.996	0.189	n/a 0.585 (M)	n/a	n/a	<u>6.395 (L)</u> 2.800(L)	1.000	0.001	<u>4.490 (L)</u> 7.004 (L)	1.000	0.025
Intermediate root biomass (g)	<u>1.359 (L)</u> 4.306 (L)	0.913	0.497	<u>0.995 (L)</u> n/a	0.840	0.619	n/a 3.999 (L)	n/a	n/a	<u>8.013(L)</u> 6.919 (L)	1.000	0.000
Fine/intermediate (g/g)	<u>1.220 (L)</u> 0.817 (L)	0.889	0.542	<u>1.194 (L)</u> n/a	0.884	0.551	n/a 3.203 (L)	n/a	n/a	<u>4.537 (L)</u> n/a	1.000	0.023
Fine/area (g/cm ² × 100)	<u>3.875 (L)</u> 0.942 (L)	0.100	0.053	n/a 2.350 (L)	n/a	n/a	<u>5.223 (L)</u> n/a	1.000	0.009	<u>4.238(L)</u> 4.201 (L)	1.000	0.034

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 are 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. 2010; Lavola et al. 2013; 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 μmol 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. Kostianen et al. (2014) exposed four clones of *Populus tremuloides* and *Betula papyrifera* saplings to 560 μmol 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 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 4th to 6th growing season after exposure to 550 μmol 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 *P. tremuloides* to $560 \mu\text{mol mol}^{-1}$ of CO_2 for 10 years and found that elevated CO_2 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 CO_2 -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 $\text{aCO}_2 \times \text{BF}$. However, if we take into account the large simulation caused by eCO_2 under VA, compared to $\text{aCO}_2 \times \text{VA}$, it will be very important for relevant regions.

The key issue is that CO_2 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 CO_2 for 3 years, where limited nutrient availability in soil restricted the tree response to elevated CO_2 (Sigurdsson et al. 2013). Spinnler et al. (2002, 2003) found that beech (*Fagus sylvatica* L.) responded negatively to 4-year CO_2 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 CO_2 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 (De Lucia et al. 1999); however, the NPP impact by CO_2 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 eCO_2 under VA, there is no benefit if we compare it with $\text{aCO}_2 \times \text{BF}$, 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 eCO_2 impacts could be higher than at nearby areas and this should be taken into account when planning relevant experiments.

5 Conclusions

The response to eCO_2 and soil fertility of saplings of the late-successional, deciduous, broadleaved Siebold's beech (*F. 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 $\text{eCO}_2 \times \text{VA}$ on total root production were not significantly different from $\text{aCO}_2 \times \text{BF}$, 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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Compliance with Ethical Standards

Conflict of Interest Authors declare no conflict of interest. The funding source (JSPS) is a non-profit organization. JSPS had not involvement in study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the article for publication.

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