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1 **Impact of elevated CO₂ on root traits of a sapling community of three birches and an oak:**
2 **A free-air-CO₂ enrichment (FACE) in northern Japan**

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9 **ABSTRACT**

10 We evaluated the root response to elevated CO₂ fumigation of 3 birches (*Betula* sp.) and 1
11 deciduous oak (*Quercus* sp.) grown in immature volcanic ash soil (VA) or brown forest soil (BF).
12 VA is a nutrient-poor, phosphorus impoverished soil, broadly distributed in northern Japan. Each
13 species had been exposed to either ambient (375-395 μmol mol⁻¹) (aCO₂) or elevated (500 μmol
14 mol⁻¹) (eCO₂) CO₂ during the daytime (more than 70 μ mol m⁻²s⁻¹) over 4 growing seasons. The
15 results suggest that eCO₂ did not cause an increase in total root production when the community
16 had grown in fertile BF soil, however it did cause a large increase when the community was
17 grown in infertile VA soil. Yet, carbon allocation to plant roots was not affected by eCO₂ in
18 either the BF and VA soils. Rhizo-morphogenesis appeared to occur to a greater extent under
19 eCO₂. It seems that the saplings developed a massive amount of fine roots under the VA and
20 eCO₂ conditions. Unexpectedly, eCO₂ resulted in a larger total root mass when the community
21 was grown in VA soil than when grown in BF soil (eCO₂×VA vs. eCO₂×BF). These results may
22 hint to a site-specific potential of communities to sequester future atmospheric carbon. The

23 growing substance of plants is an important factor which root response to eCO₂ depends on,
24 however further studies are needed for a better understanding.

25 **Key words:** birch, elevated CO₂, FACE, oak, root, volcanic ash soil

26 **Key message:** The CO₂ effect on the root production of a broadleaved community was
27 insignificant when grown in brown forest soil, however it was positively large when grown in
28 volcanic ash soil.

29 **Author contribution statement:**

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

32 Makoto Watanabe: Data collection and article discussion

33 Tatsuro Nakaji: Guidance on the root research and article discussion

34 Xiaona Wang: Article discussion

35 Fuyuki Satoh: Management of the FACE system

36 Takayoshi Koike: Funds, excavation of roots, collection of data and management of all technical
37 procedures

38 **INTRODUCTION**

39 Roots are the hidden half of a plant and account for a high proportion of the biomass production
40 (Eshel and Beeckman 2013). They also act as an interface between soil and the plant body (e.g.
41 Fitter and Hay 2002). Most roots of woody plants have high plasticity to soil properties, such as
42 soil moisture content (e.g. Koike et al. 2003), nitrogen availability (Qu et al. 2003), temperature
43 of rhizosphere (Korotkii et al. 2002; Qu et al. 2009; Kajimoto et al. 2010) and other factors. The

44 growth dynamic of roots is strongly affected by atmospheric conditions (Wang et al. 2015a), as
45 in the case of the Aspen FACE (Pregitzer and Tihelm 2013). CO₂ levels have already hit a record
46 of ~400 μmol mol⁻¹ (NOAA 2014), and are projected to rise to 500 μmol mol⁻¹ with the
47 possibility to elevate even further until 2040 (IPCC 2007). It is known that elevated CO₂ (eCO₂)
48 alters above- and below-ground functions of plants (Koike et al. 1995; Beerling 1996; Karnosky
49 2003; Ozanne et al. 2003; Heath et al. 2005; Cheng et al. 2012; Slattery et al. 2013; Guo et al.
50 2015; Koike et al. 2015).

51 Soil fertility is one of the factors affecting the biomass production of the loblolly pine (Oren
52 et al. 2001) and several other tree species (e.g. Norby and Zak 2011; Pregitzer and Tihelm 2013)
53 under elevated CO₂ conditions. According to estimations, more than 50% of the global potential
54 arable lands are acidic (von Uexkull and Mutert 1995) and usually P-deficient (Zheng 2010;
55 Cordell and Neset 2014; Reijnders 2014; Ulrich and Frossard 2014). Specifically, the pH in
56 Japanese forests ranges from 4.8 to 5.2, except for red soil and podzolic soil (e.g. Hashimoto et al.
57 2012). Soils of Northern Japan are partly dominated by nutrient-poor immature volcanic ash or
58 pumice soils, where phosphorous (P) is usually impoverished (e.g. Eguchi et al. 2005; Kim et al.
59 2010; Hashimoto et al. 2012). Many plants grown under such nutrient limited conditions are
60 usually suffering from nutrient deficiency, and are consequently forced to develop fine roots
61 (Hermans et al. 2006) in order to establish symbioses with ectomycorrhizae (e.g. Smith and Read
62 2008; Qu et al. 2010).

63 In general, the allocation pattern of photosynthates to above- and below-ground differs
64 broadly among deciduous tree species (e.g. Schulze et al. 2005). This pattern strongly depends
65 on the successional traits of tree species; for example, early successional species (i.e. birch)
66 versus mid-late successional species (i.e. oak) (Sato 1995). Among birches, this tendency is

67 typically found in mountain birch (Koike et al. 2003). The majority of the early successional
68 species, such as birch, grow above- and belowground simultaneously. Yet, late successional
69 species, usually, first allocate photosynthates to their roots which store them, and then prepare
70 for quick growth in the succeeding year (e.g. Sato 1995). Consequently, these woody plants
71 flourish aboveground in the early part of the growing season and then gradually develop their
72 belowground structure. Under elevated CO₂, root growth is expected to increase, boosting the
73 absorption of mineral nutrients and water which support an increased aboveground body - if
74 increased.

75 A review of current literature revealed that several studies have dealt with responses of
76 individual species to elevated CO₂, while other studies tested the responses of communities to
77 elevated CO₂. However, there is a lack of evidence on the response of communities to elevated
78 CO₂ as a whole unit (e.g. Pregitzer et al. 2008).

79 We aimed to test if and how elevated CO₂ and soil infertility could impact the
80 belowground functioning of a community of trees in terms of carbon (C) allocation within plant
81 body and atmospheric C sequestration capacity. To this purpose, we established a free-air-CO₂
82 enrichment (FACE) system with 4 deciduous, broad-leaved species, which are known to be
83 physiologically (aboveground) affected by elevated CO₂ (Koike et al. 2015). We hypothesized
84 that the root impact of elevated CO₂ on the communities would depend on soil nutrient status due
85 to shifts in the photosynthetic function and the nutritive demands. We were expecting the soil
86 nutrient deficiency to induce a differential impact of elevated CO₂ on the belowground
87 functioning of the community.

88 In this study, we focused on a community of species – consisting of 2 ecological groups (3
89 early successional: birch, and 1 late successional: oak) - because these broad-leaved trees play an
90 integral role in the above-mentioned forest functions (Jensen and Anderson 1995).

91 MATERIALS & METHODS

92 **Experimental Design:** This study was conducted in the FACE system located in the Sapporo
93 Experimental Forest of Hokkaido University, Japan (43°06' N, 141°20' E, 60 m a.s.l.). The
94 main-plot treatment consisted of ambient (375–395 $\mu\text{mol mol}^{-1}$) and elevated (500 $\mu\text{mol mol}^{-1}$)
95 CO₂ treatments, with three site replications for each treatment (Filion et al. 2000). The sub-plot
96 treatments were brown forest (BF) soil, and BF mixed with immature volcanic ash plus pumice
97 (VA) soil. The latter is nutrient poor soil (Masyagina et al. 2006) which was excavated and
98 brought from Tomakomai Experimental Forest of Hokkaido University (42°40' N, 141°37' E, 30
99 m a.s.l.). It is widespread in Hokkaido and ideal for experiments mimicking soil-nutrient
100 deficiency scenarios.

101 Since BF soil is native to Sapporo Experimental Forest, half of each FACE ring was
102 excavated and refilled with a mixture of 65 % BF soil and 35 % VA soil. Chemical and physical
103 properties of BF and VA soils used in the present study are illustrated in Eguchi et al. (2008) and
104 Watanabe et al. (2013). The VA soil had about 17 % lower P₂O₅ content than the BF soil.

105 **Plant Materials:** The materials we used included saplings of 3 birch species (*Betula ermanii*
106 (Be: mountain birch), (*B. maximowicziana* (Bm: Monarch birch), *B. platyphylla* var. *japonica*
107 (Bp: white birch)), and a deciduous oak (*Quercus mongolica* var. *crispula* (Qm: oak). All the
108 saplings were obtained from Hokkaido Hort-green Co. Ltd. near Sapporo, and planted in the

109 FACE rings with a distance of 50 cm among them, in 2010 (after the snow had melted). Equal
110 numbers of individuals among species were planted in each research condition.

111 The mean values (\pm SD) of the height and trunk basal diameter (at 2 cm from the starting point
112 of root system) of the juveniles were measured for the 3 birches and the Oak; the variability
113 between the two gas treatments within each species was minimal (Table 1). The oak had been
114 suppressed by the birches (i.e. restricted relative light conditions) after 1.5 years with CO₂
115 fumigation (data not shown). Similarly, data from leaf area per ground area have shown that the
116 mountain birch was suppressed by the white and monarch birches (Hara et al. 2014). We
117 assumed that it was a phenomenon that could have occurred in pristine conditions and thus can
118 be considered as a realistic growth of a community. Nonetheless, the obtained growth data could
119 have been different if the trees had grown independently or in an uncompetitive environment.

120 **CO₂ treatment:** Fumigation of CO₂ was carried out in 4 consecutive Julian years (2010-2013).
121 Saplings were fumigated in each growing season during daytime, when the photosynthetic
122 photon flux (PPF) exceeded the 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (i.e. light compensation point of photosynthesis;
123 Koike 1988), from leaf emergence to leaf senescence (from May until late November). This
124 FACE system consists of 6 rings, 3 of which were fumigated by additional CO₂ in order to reach
125 the targeted concentration of 500 $\mu\text{mol mol}^{-1}$ (hereafter “eCO₂”). The diameter and height of
126 each FACE ring are 5 m. The 3 ring plots remained under ambient CO₂ (ca. 370-390 $\mu\text{mol mol}^{-1}$)
127 (hereafter “aCO₂”) as noted in researches by Eguchi et al. (2008) and Watanabe et al. (2013).

128 **Climatic & meteorological conditions:** The snow-free period lasts usually from early April to
129 early November. The monthly mean air temperature, wind speed, and relative humidity and the
130 monthly total sunshine duration and precipitation were averaged per year, and the means for the

131 entire experiment duration were calculated. The mean values for the years 2010-2013 were 9.40
132 (± 0.27 CI) °C, 3.45 (± 0.13 CI) m s⁻¹, 69.75 (± 0.94 CI) %, 1687 (± 126 CI) h, and 1301 (± 42
133 CI) mm, for each variable, respectively. The meteorological data were collected from a station
134 located in Sapporo (WMO, ID: 47412) at 43°03.6'N 141°19.7'E (Japan Meteorological Agency,
135 2015).

136 **Sampling and measurements:** At the end of the final growing season (2013), the trunk basal
137 diameter was measured for each grown sapling, and all the roots were excavated using a small
138 bulldozer. This mechanistic method was previously compared with the manual method (by hand)
139 and it was revealed that excavation by this method causes destruction of the roots during the
140 excavation process. Although the target species were different from the present 4 species, the
141 final excavated root system had less mass; approximately 70~90 % of the whole roots
142 (Matsunami 2008). Nevertheless, we assume there could be equal amounts of error across all the
143 subjects.

144 After the excavation, the following procedure was carried out: a) the roots of small classes were
145 sampled, and dried at 75 °C, for more than 5 days, to constant mass. The dry mass of
146 intermediate roots (\varnothing 2 - 4 mm) and fine roots (\varnothing < 2 mm) – including hyphae of
147 ectomycorrhizae – were determined; b) the whole root systems were left on the field to
148 physically dry; this was unavoidable because of the large size of the root systems. The next
149 summer, dry mass measurements were taken for the total root systems (TDM). Due to harsh
150 natural conditions, the identities of some root systems were lost, therefore these samples were
151 not measured. Nonetheless, the sample size was sufficient to perform parametric tests.

152 **Statistics:** The accepted threshold of significance was defined at $\alpha=.05$. Trunk basal diameter
153 data were first converted to trunk basal area (Area), and then the Trunk-basal-area:Total-root-
154 dry-mass rate (Area:TDM) was calculated. Area:TDM is a significant indicator of the plant
155 health, and is based on the Pipe Model theory of tree form (Shinozaki et al. 1964a, 1964b). Prior
156 to the analysis, the data of each variable were standardized (Saitanis et al. 2014, 2015). For the
157 standardization, T-scores ($T = z - score \times 10 + 50$) were used, with a mean equal to 50 and a
158 standard deviation equal to 10. Therefore, the mean T-score of each species in each soil and each
159 experimental unit, constituted the real-replicates in the overall analysis. As previously suggested
160 by Saitanis et al. (2014, 2015), this process also allows a fair intercomparison of measured
161 variables and removes any heterogeneity. In order to utilize the overall information and
162 generalize the results at a community level, two steps were followed: First, in relation to the
163 biomasses, data for each community (including all the species) in each treatment (i.e. soil and
164 CO₂) and block (FACE ring) were calculated by averaging the values of individuals of each
165 species in each treatment and then summing the returned values for all the species in each
166 treatment (this process treats any differences in ratios among species and gives better estimates).
167 In order to increase accuracy of the ratio and rates, the values for each individual were calculated
168 for each separately, and averaged per species in each of the research conditions for each block.
169 The values were averaged again for all the species (community) in each of the research
170 conditions for each block.

171 Second, the data from all the experimental units were pooled (resulting to n=3) and
172 analyzed. Prior to this analysis, the data were analyzed using individual species in order to test
173 for interactions with species; there were no significant ($p>0.05$) species-specific differences. All
174 the data were subjected to split-plot general linear model randomized by block (GLM), based on

175 Kuehl (1999), and, if there were significant effects (only for factors with more than two levels), a
 176 Tukey range post-hoc test was performed. For the presentation of the data, the non-standardized
 177 values were used.

178 Finally, in order to quantify the size of the difference between the community of plants grown
 179 under ambient CO₂ and elevated CO₂, under the different soils, and for each dependent variable
 180 (Table 2), the effect size *ESi* was calculated according to Cohen (1988). The formula was:

$$181 \quad ESi = \left(\frac{[C_{i(EA)} - C_{i(CTRL)}]}{\sqrt{[(n_{i(EA)} - 1) * (SD_{i(EA)})^2 + (n_{i(CTRL)} - 1) * (SD_{i(CTRL)})^2] / (n_{i(EA)} + n_{i(CTRL)} - 2)}} \right),$$

182 C_i , n_i , SD_i , stands for the mean score, the number of observations, and the standard deviation
 183 respectively, in the plants under CO₂ enriched air (*EA*) or ambient air (*CTRL*; control group), of
 184 the i case. The denominator stands for the pooled standard deviation. Absolute values of the
 185 effect size within the arbitrary segments 0.00-0.20, 0.20-0.50, 0.50-0.80, and 0.80+ indicate
 186 neutral, small, moderate, and large effects, respectively. *ES* was calculated only for the pairs with
 187 statistically significant difference in order to quantify the size of the difference. Data processing
 188 and statistical analysis were conducted using MS EXCEL 2010 (© Microsoft) and STATISTICA
 189 v.10 (© StatSoft Inc.) software.

190 RESULTS

191 CO₂ appeared to have a large main effect on all variables with the exception of Area:TDM.
 192 Besides, soil had only a large effect on Fine:Area and a medium effect on fine roots (Tables 2
 193 and 3). Concerning the CO₂ × soil interactions, only for Area:TDM and intermediate roots were
 194 not significant.

195 Regarding the Area, eCO₂ was responsible for a large effect, namely a 17 % smaller Area, cf.
196 aCO₂. There was a convergent interaction in Area due to a higher (ns) Area of the communities
197 under eCO₂×VA, compared to eCO₂×BF, and a 22 % significantly smaller (*ES* = 3.69) Area of
198 the community grown under aCO₂×VA, compared to aCO₂×BF (Table 3). Under eCO₂×VA,
199 there was not such a reduction. Instead, eCO₂×VA led to a 32 % larger (*ES* = 6.97) Area than
200 that of plants in aCO₂×VA. There was no significant effect of CO₂ on the communities grown in
201 BF soil.

202 Similarly, the large 20 % greater TDM induced by eCO₂ can be attributed to the communities
203 grown in VA soil and not in BF soil. Under VA soil and eCO₂, the TDM was 35 % higher (*ES* =
204 4.65) than under aCO₂×VA, and, unexpectedly, it was also 25 % higher than that under
205 eCO₂×BF (*ES* = 3.55).

206 Likely to TDM, the coarse root biomass was largely boosted (19 %) by eCO₂, regardless of the
207 soil treatment. This caused by a large increase of coarse root biomass in eCO₂×VA, which also
208 led to a divergent interaction. Under eCO₂×VA, coarse root biomass was largely higher than
209 under aCO₂×VA (35 %) and eCO₂×VA (25 %). It is revealed that there was no effect of eCO₂
210 when the communities were grown in BF soil. On the other hand, none of the factors had a
211 significant effect on Area:TDM (Table 2).

212 Fine root biomass was 27 % superior (*ES* = 2.90) in eCO₂ than in aCO₂, as a main effect.
213 Furthermore, it was 11 % suppressed (*ES* = 0.63) by VA soil, compared to BF soil. The
214 interaction of the two factors was significant and could be characterized as divergent with
215 differences in sizes as well. When the communities had grown in BF and VA soils, eCO₂ caused
216 large increases of fine root biomass by 14 and 39 % respectively. The moderate main effect of

217 the VA soil was produced by a 27 % lower ($ES = 5.09$) fine root biomass under $aCO_2 \times VA$,
218 compared to $aCO_2 \times BF$.

219 Intermediate root biomass experienced a large positive effect (+32 %) by eCO_2 , independent of
220 soil treatment, however it was not affected by the main effect of soil. There was also no
221 interaction between the 2 factors.

222 As a result, the Fine: Intermediate root dry mass ratio (Fine:Intermediate) was largely negatively
223 affected (-10 %) by eCO_2 as a main effect, and there was a significant interaction with soil that
224 tended to mimic a directional interaction. It seems that both the eCO_2 and VA soil alone or in
225 combination caused a higher increase of intermediate root than fine root biomass. Communities
226 under $aCO_2 \times BF$ had 22, 21, and 14 % higher Fine:Intermediate than under $eCO_2 \times BF$, $aCO_2 \times VA$,
227 and $eCO_2 \times VA$, respectively. Among the latter three treatments, there was no statistical
228 difference.

229 Finally, Fine:Area was largely increased by eCO_2 (27 %) and VA (42 %) factors, regardless of
230 each other factor. The factor of the interaction of these 2 variables was also significant. eCO_2 had
231 no significant effect on the Fine:Area of the community grown in BF, however it had a large
232 effect (+34 %) on the Fine:Area of the community grown in VA.

233 **DISCUSSION**

234 We predicted that root growth and root architecture of the saplings would be affected by eCO_2
235 and soil infertility; thus, the response of the community to eCO_2 would depend on the soil
236 fertility and species seral traits. Based on the results, and in contrast to our expectations, this 4-
237 year fumigation with eCO_2 resulted in neither a greater Area nor an increased TDM (or coarse

238 root biomass) when the community had grown in the fertile BF soil. eCO₂ also drastically
239 increased Area, coarse root biomass and TDM when the community had grown in the infertile
240 VA soil. Moreover, no specific seral traits were found in final production of root biomass
241 (p>0.05; data not shown), which may be due to only one final assessment of the production of
242 root biomass. In addition, root growth may be restricted after canopy closure (Sato 1995; Hara et
243 al. 2014; Wang et al. 2015b).

244 Area:TDM and TDM were assessed in order to evaluate the root responses to eCO₂. The
245 former is involved in the tree functional traits, i.e. C allocation, and the latter is important for C
246 sequestration capacity of the ground. Although VA suppressed the Area, it did not suppress the
247 TDM under aCO₂. Meanwhile, it appeared that eCO₂ had a surprisingly large hostile effect on
248 the VA soil, and thus the negative effects of the VA soil on Area were compensated. Although
249 neither eCO₂ nor VA soil affected the Area:TDM, VA soil in combination with eCO₂ led to a
250 marginally (p>0.05) higher belowground allocation than aboveground (i.e., trunk area) compared
251 to aCO₂×BF (-14%), aCO₂×VA (-9%) and eCO₂×VA (-11%). Despite this, under ambient CO₂,
252 soil infertile conditions are usually expected to alter the C partitioning to favor root growth
253 (Hermans et al. 2006), yet this was not the case under aCO₂.

254 Our results agree with the findings of quantitative reviews which state that, on average,
255 eCO₂ does not alter the allometry of plants (Poorter and Nagel 2000; Poorter et al. 2012).
256 However, the results of our statistical analyses differ to the general finding which notes that soil
257 infertility does alter the allometry (Poorter and Nagel 2000; Poorter et al. 2012) as explained by
258 the *functional equilibrium* theory (Brouwer 1962; Poorter and Nagel 2000). Our findings pointed
259 out that C allocation within a plant body was not altered in this community of trees by a long-
260 term experimental fumigation with eCO₂, while the sequestration of atmospheric C, in eCO₂,

261 increased only in the community grown in VA soil but not BF, which may be due to physical
262 structure of BF (e.g. Eguchi et al. 2005).

263 The usual response of trees to elevated CO₂ is an increased root growth and root turnover of
264 fine roots (e.g. Karnosky 2003; Pregitzer et al. 2008; de Oliveira et al. 2012; King et al. 2013;
265 Cao et al. 2008). Our results support this response with the large effect of eCO₂ on TDM when
266 the community was grown in VA soil. On the other hand, there was no effect of eCO₂ when the
267 community was grown in BF. Insignificant increases of root biomass due to 2-growing-season
268 fumigation with eCO₂ were also found in *Larix gmelinii* var. *japonica* × *L. kaempferi* seedlings
269 grown in well-homogenized BF soil in FACE (Watanabe et al. 2013) or OTCs (Wang et al.
270 2015a), in the same region. Furthermore, short-term fumigation with elevated CO₂ had no
271 significant effects on the root biomass of neither the *Betula papyrifera* seedlings grown in slight
272 fertilization (Zhang et al. 2006) nor the *Fagus crenata* seedlings (Hirano et al. 2012). In general,
273 root longevity is positively correlated with poor soil-nutrient status and well aeration of soils
274 (Fitter and Hay 2002), which in our case may be attributed to high porosity of VA soil with
275 pumice (e.g. Eguchi et al. 2005; Masyagina et al. 2006).

276 The eCO₂ increased fine roots, of which the majority naturally colonizes A horizon (e.g.
277 Jaloviar et al. 2009) in both the BF and VA soils. It seems fine roots are ecologically important
278 components as they account for some 13 % of net primary production (NPP) (Ostonen et al.
279 2005) and a high proportion of total litter (e.g. Leppalammi-Kujansuu et al. 2014). Thus,
280 increased production of fine roots may have an ecologically important benefit in nutrient cycling
281 in a forest. As observed in the Area, although VA soil unexpectedly suppressed fine roots under
282 aCO₂, with the interaction of VA×eCO₂, the fine roots still reached levels similar to that of
283 BF×eCO₂. We expected that under the influence of either eCO₂ or VA soil, the saplings would

284 undergo a higher need to seek nutrients thereby increasing the fine root area/biomass in order to
285 access more nutrients. Instead, this was the case only for eCO₂ since VA soil caused a reduction
286 of fine root biomass, until the canopy closed (Hara et al. 2014). At present, however, we cannot
287 give a plausible reason for this phenomenon.

288 There was an unexplained decrease in fine root biomass in VA soil under aCO₂. Yet the
289 Fine:Area, which is an appropriate index to assess the belowground responses in relation to
290 nutrient cycling, showed something different. Namely, VA soil induced a large (*ES* = 3.54)
291 increase when the community had been exposed to aCO₂ (cf. aCO₂×BF). Among the interaction
292 levels, the largest Fine:Area was observed in eCO₂×VA: it was 34 % higher than aCO₂×VA and
293 49 % (*ES* = 8.13) higher than eCO₂×BF. A different foraging strategy of fine roots among the
294 research conditions is clear, in accordance with the findings of Ostonen et al. (2011) and
295 Leppalammi-Kujansuu et al. (2014). Particularly, fine roots underwent extensive foraging
296 strategy (Ostonen et al. 2011), i.e. higher Fine:Area, in VA soil, in both CO₂ conditions.
297 Moreover, eCO₂ had a synergistic effect with VA soil leading to the highest Fine:Area. These
298 results coincide with the results of Area:TDM, albeit insignificant, and indicating greater
299 responses to eCO₂ in relation to nutrient cycling when the community was grown in VA soil.

300 Differences of intermediate root biomass were attributed only to eCO₂ which increased the
301 biomasses of the communities grown in both the BF and VA soils, compared to the pairwise
302 aCO₂. Consequently, there was a higher amount of intermediate roots than fine roots both in VA
303 soil and eCO₂. The ratio of fine roots to intermediate roots indicates the balance of the two root
304 classes, and its modification is dependent on the needs of the plants. For instance, plants in the
305 need of increasing nutrient uptake are expected to modify a higher proportion of fine roots
306 compared to intermediate roots. However, this could be misleading in such cases which take into

307 account single observations of only standing biomasses. A single observation, as in our case, of
308 the living fine roots, does not stand for the total production of fine roots, but only a partial
309 production of that given time. In our study, this ratio should be taken with a degree of caution as
310 fine roots were increased by eCO₂ as well. One explanation for the higher amount of
311 intermediate roots than fine roots could be the mortality and regeneration of fine roots that occur
312 (Pritchard et al. 2008) more frequently throughout the growing season which deposit a
313 significant amount of C. As such, we can only speculate that there was an accelerated turnover of
314 fine roots in all research conditions except aCO₂×BF.

315 Despite our expectations that soil infertility might lead to a maximum fine root production
316 independent of the CO₂ treatment, CO₂ treatment did appear to have a combination effect with
317 soil infertility, resulting in higher production of fine roots. Could this phenomenon be an
318 ecological growth method of saplings in order to mitigate the negative effects via plasticity? In
319 other terms, this phenomenon could be a kind of “*rhizo-morphogenesis*” as the root architecture
320 is changing under these external stressors. This might be induced by a better communication
321 between above- and below-ground under eCO₂, thus resulting in mitigation of the VA soil
322 separated effects.

323 In general, eCO₂ increases root biomass and the demand for N, P, and K (Norby et al. 1986;
324 Li et al. 2013), however, even if the concentrations of elements in the soil are adequate, a
325 proportionally smaller root system accompanied by less microbial activity is quite hard to
326 support the dissimilation organs. On the other hand, eCO₂ acts as an external force for plants in
327 order to extract more nutrients from the soil: eCO₂ increased NaHCO₃- and NaOH-extractable
328 organic P in the rhizosphere of wheat by 160 and 53 %, respectively (Jin et al. 2014).

329 It seems that the growing substance of plants is an important factor to which the plant
330 response to CO₂ usually depends on (e.g. Conroy et al. 1990), and makes it difficult to draw
331 general conclusions. It is also critical that nutrient dynamics between different elements may
332 change under elevated CO₂ (Cong et al. 2015; Li et al. 2015; Wang et al. 2015a). However, the
333 responses are still not well understood since many factors such as soil temperature, light
334 condition, nitrogen deposition, O₃, etc. (Cao et al. 2008; Danyagri et al. 2014; Cong et al. 2015;
335 Wang et al. 2015a) may confound the nutrient allocation under elevated CO₂.

336 We should mention that increased microbial activity contributes to P and root-derived
337 materials immobilization in rhizosphere under elevated CO₂ (Jin et al. 2014; Wang et al. 2015a).
338 Considering fine root dynamics, we should include the interaction of soil microbial activity (e.g.
339 ectomycorrhizae) that could be a critical and determinant factor in the modulation of nutritional
340 starvation and elevated CO₂ interaction. Unfortunately, we did not find any significant species-
341 specific difference in root biomasses and structure of fine roots. In order to understand this, we
342 should trace the seasonal trends of above- and belowground (Sato 1995). Moreover, future
343 studies should consider further investigations on soil microbial activity under the interaction of
344 nutrient-poor soils and elevated CO₂.

345 Summarizing, in agreement with similar studies (e.g. de Oliveira et al. 2012), our results
346 show that soil type has the potential to modify the response of trees to eCO₂. However, in
347 contrast to our expectations, the data support the hypothesis that the root growth of the
348 community in BF soil was not altered by eCO₂ while in the infertile VA soil it was positively
349 altered. Elevated CO₂ led to largely higher coarse root biomass (*ES* = 3.55), TDM (*ES* = 4.08)
350 and Fine:Area (*ES* = 8.13) when the community was grown in VA soil than when the community
351 was grown in BF soil. This indicates the magnitude of response varies considerably between

352 communities growing in different regions and that models dealing with the estimation of C
353 allocation and storage under elevated CO₂ scenarios may overestimate the potential in some
354 regions and underestimate it in others.

355 CONCLUSIONS

356 Elevated CO₂ did not cause an increase in trunk basal area or total root mass when the
357 community had grown in fertile BF soil after canopy closure, but it did cause a large increase in
358 both characteristics when the community had grown in infertile VA soil. Furthermore, eCO₂
359 caused an evident rhizo-morphogenesis through a large increase in fine root production in both
360 soils. On the other hand, eCO₂ had no effect on C allocation to roots even in the case where
361 eCO₂ increased Area and TDM in VA soil. Similarly, VA soil did not cause any significant
362 disturbance to C allocation under ambient or elevated CO₂, albeit there was an insignificant trend
363 for higher allocation to belowground when combined with eCO₂. Nevertheless, our results do not
364 support the drawing of general conclusions because the adaptability of plants to VA soil as well
365 as to eCO₂ may differ among species of different growth traits and soil physicochemical
366 structure.

367 We conclude that eCO₂ fumigation of 4 growing seasons has no potential to alter the total
368 root production of a community of the 4 deciduous broad-leaved trees when it is growing in
369 brown forest soil. Tree species growing in less fertile soils, such as VA with high porosity, may
370 respond more vigorously to eCO₂ if they are well adapted to the single effects of such soils. New
371 directions should also deal with more infertile soil than the one used in our study. Moreover,
372 ectomycorrhizae research under similar conditions would be essential as their symbiosis may
373 directly or indirectly help plants to mediate the VA- or CO₂-induced suppressing stressors.

374

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575 Captions

576 **Table 1** The mean (\pm SD) values of the initial height and diameter (at 2 cm) of the juveniles

577 **Table 2** Summary of the GLM results for the measured variables trunk basal area (Area), total
578 belowground dry mass (TDM), coarse root dry mass, Area:TDM rate, fine and intermediate roots
579 dry masses, Fine:Intermediate dry mass ratio and Fine:Area rate. Text in bold indicates statistical
580 significance. Data obtained from communities (*Betula ermanii*, *B. maximowicziana*, *B.*
581 *platyphylla* var. *japonica*, and *Quercus mongolica* var. *crispula*) of saplings exposed to (370–390
582 $\mu\text{mol mol}^{-1}$) or elevated (500 $\mu\text{mol mol}^{-1}$) CO_2 and grown either in brown forest soil or immature
583 volcanic ash plus pumice soil for 4 consecutive years. Three real replications were used for each
584 treatment

585 **Table 3** Mean real values (\pm SD) of the measured variables trunk basal area (Area), total
586 belowground dry mass (TDM), coarse root dry mass, Area:TDM rate, fine and intermediate roots
587 dry masses, Fine:Intermediate dry mass ratio and Fine:Area rate. The lowercase letters above the
588 mean values indicate the significant differences among the 4 combination treatments while the
589 uppercase indicate the differences within the 2 main treatments. The results of each variable
590 obtained by one GLM analysis or a Tukey range, post-hoc test, after significant results of the
591 GLM analysis, based on standardized data. Means within each variable marked with different
592 letter differ statistically significantly at a level of significance $\alpha=0.05$. Uppercase letters stand for
593 the differences within each main factor and lowercase letters stand for the differences within the
594 interactive effects of the two main factors. In case of no significant effects post-hoc test was not
595 followed, rather means marked with the same letter. ES_v indicates the Cohen effect size of the v
596 case for each pair with statistically significant difference and *n/a* means not applicable. Data
597 obtained from communities (*Betula ermanii*, *B. maximowicziana*, *B. platyphylla* var. *japonica*,

598 and *Quercus mongolica* var. *crispula*) of saplings exposed to (370–390 $\mu\text{mol mol}^{-1}$) or elevated
599 (500 $\mu\text{mol mol}^{-1}$) CO_2 and grown either in brown forest soil or immature volcanic ash soil for 4
600 consecutive years. Each value is the product of three replications

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616 **Table 1**

	Ambient CO ₂		Elevated CO ₂	
	Height (cm)	Diameter (mm)	Height (cm)	Diameter (mm)
<i>Betula ermanii</i>	30.6 (0.8)	4.2 (0.1)	29.4 (1.2)	4.1 (0.2)
<i>B. maximowicziana</i>	64.4 (1.1)	6.1 (0.2)	61.3 (1.4)	6.3 (0.3)
<i>B. platyphylla</i> var. <i>japonica</i>	45.1 (0.9)	4.1 (0.2)	44.1 (1.0)	3.9 (0.3)
<i>Quercus mongolica</i> var. <i>crispula</i>	55.1 (0.9)	9.3 (0.4)	54.2 (1.1)	9.8 (0.5)

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631 **Table 2**

	CO₂	Soil	CO₂ × Soil
Area	F = 22.56, p < 0.010	F = 01.83, p = 0.213	F = 21.75, p < 0.010
TDM	F = 14.67, p < 0.010	F = 01.76, p = 0.221	F = 14.68, p < 0.010
Coarse root	F = 09.72, p < 0.050	F = 01.64, p = 0.236	F = 11.27, p < 0.010
Area:TDM	F = 00.18, p = 0.681	F = 00.75, p = 0.413	F = 04.42, p = 0.069
Fine roots	F = 79.15, p < 0.001	F = 10.65, p < 0.050	F = 18.91, p < 0.010
Intermediate roots	F = 73.00, p < 0.001	F = 04.19, p = 0.075	F = 01.23, p = 0.300
Fine:Intermediate	F = 05.75, p < 0.050	F = 04.62, p = 0.064	F = 16.75, p < 0.010
Fine:Area	F = 22.04, p < 0.010	F = 66.02, p < 0.001	F = 10.71, p < 0.050

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646 **Table 3**

	CO ₂		Soil				CO ₂ x Soil					
	aCO ₂	eCO ₂	ES _a	BF	VA	ES _b	aCO ₂ xBF	eCO ₂ xBF	ES _c	aCO ₂ xVA	eCO ₂ xVA	ES _d
Area (cm²)	5.438 ^A (0.864)	6.545 ^B (0.533)	1.69	6.149 ^A (0.377)	5.834 ^A (1.251)	<i>n/a</i>	6.064 ^a (0.501)	6.159 ^a (0.323)	<i>n/a</i>	4.736 ^b (0.371)	6.931 ^a (0.400)	6.97
TDM (kg)	0.737 ^A (0.096)	0.918 ^B (0.154)	1.55	0.796 ^A (0.059)	0.859 ^A (0.216)	<i>n/a</i>	0.774 ^a (0.085)	0.796 ^a (0.039)	<i>n/a</i>	0.678 ^a (0.074)	1.040 ^b (0.113)	4.65
Coarse root (kg)	0.702 ^A (0.097)	0.867 ^B (0.160)	1.36	0.751 ^A (0.071)	0.818 ^A (0.209)	<i>n/a</i>	0.740 ^{ab} (0.089)	0.744 ^a (0.067)	<i>n/a</i>	0.647 ^a (0.080)	0.989 ^b (0.121)	4.08
Area:TDM (cm² g⁻¹ x10)	0.344 ^A (0.021)	0.338 ^A (0.031)	<i>n/a</i>	0.347 ^A (0.022)	0.335 ^A (0.029)	<i>n/a</i>	0.337 ^a (0.020)	0.358 ^a (0.021)	<i>n/a</i>	0.352 ^a (0.021)	0.319 ^a (0.029)	<i>n/a</i>
Fine roots (g)	12.824 ^A (2.368)	17.529 ^B (0.835)	2.90	16.040 ^A (1.520)	14.314 ^B (3.935)	0.63	14.822 ^a (0.807)	17.242 ^b (0.885)	3.50	10.812 ^c (1.099)	17.816 ^b (0.844)	8.75
Intermediate roots (g)	23.892 ^A (3.196)	35.384 ^B (2.087)	4.66	31.015 ^A (5.999)	28.261 ^A (7.299)	<i>n/a</i>	24.855 ^a (3.217)	36.015 ^b (2.146)	5.00	21.769 ^a (1.291)	34.754 ^b (2.257)	8.65
Fine:Intermediate	0.557 ^A (0.086)	0.502 ^B (0.045)	0.88	0.554 ^A (0.090)	0.505 ^A (0.041)	<i>n/a</i>	0.613 ^a (0.045)	0.479 ^b (0.036)	-	0.485 ^b (0.029)	0.524 ^b (0.047)	<i>n/a</i>
Fine:Area (g cm⁻² x10)	0.285 ^A (0.068)	0.388 ^B (0.141)	1.02	0.247 ^A (0.036)	0.425 ^B (0.103)	2.55	0.227 ^a (0.038)	0.263 ^{ab} (0.032)	<i>n/a</i>	0.338 ^b (0.039)	0.513 ^c (0.043)	5.25

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