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Impact of elevated CO_2 on root traits of a sapling community of three birches and an oak: A free-air-CO_2 enrichment (FACE) in northern Japan

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ABSTRACT

We evaluated the root response to elevated CO_2 fumigation of 3 birches (*Betula* sp.) and 1 deciduous oak (*Quercus* sp.) grown in immature volcanic ash soil (VA) or brown forest soil (BF). VA is a nutrient-poor, phosphorus impoverished soil, broadly distributed in northern Japan. Each species had been exposed to either ambient (375-395 μmol mol^{-1}) (aCO_2) or elevated (500 μmol mol^{-1}) (eCO_2) CO_2 during the daytime (more than 70 μ mol $m^{-2}s^{-1}$) over 4 growing seasons. The results suggest that eCO_2 did not cause an increase in total root production when the community had grown in fertile BF soil, however it did cause a large increase when the community was grown in infertile VA soil. Yet, carbon allocation to plant roots was not affected by eCO_2 in either the BF and VA soils. Rhizo-morphogenesis appeared to occur to a greater extent under eCO_2. It seems that the saplings developed a massive amount of fine roots under the VA and eCO_2 conditions. Unexpectedly, eCO_2 resulted in a larger total root mass when the community was grown in VA soil than when grown in BF soil (eCO_2×VA vs. eCO_2×BF). These results may hint to a site-specific potential of communities to sequester future atmospheric carbon. The

growing substance of plants is an important factor which root response to eCO_2 depends on, however further studies are needed for a better understanding.

Key words: birch, elevated CO_2, FACE, oak, root, volcanic ash soil

Key message: The CO_2 effect on the root production of a broadleaved community was insignificant when grown in brown forest soil, however it was positively large when grown in volcanic ash soil.

Author contribution statement:

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

Makoto Watanabe: Data collection and article discussion

Tatsuro Nakaji: Guidance on the root research and article discussion

Xiaona Wang: Article discussion

Fuyuki Satoh: Management of the FACE system

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

INTRODUCTION

Roots are the hidden half of a plant and account for a high proportion of the biomass production (Eshel and Beeckman 2013). They also act as an interface between soil and the plant body (e.g. Fitter and Hay 2002). Most roots of woody plants have high plasticity to soil properties, such as soil moisture content (e.g. Koike et al. 2003), nitrogen availability (Qu et al. 2003), temperature 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 Tlhelm 2013). CO_2 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 CO_2 (eCO_2)
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 Tlhelm 2013)
53 under elevated CO_2 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 2014l 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

typically found in mountain birch (Koike et al. 2003). The majority of the early successional species, such as birch, grow above- and belowground simultaneously. Yet, late successional species, usually, first allocate photosynthates to their roots which store them, and then prepare for quick growth in the succeeding year (e.g. Sato 1995). Consequently, these woody plants flourish aboveground in the early part of the growing season and then gradually develop their belowground structure. Under elevated CO_2, root growth is expected to increase, boosting the absorption of mineral nutrients and water which support an increased aboveground body - if increased.

A review of current literature revealed that several studies have dealt with responses of individual species to elevated CO_2, while other studies tested the responses of communities to elevated CO_2. However, there is a lack of evidence on the response of communities to elevated CO_2 as a whole unit (e.g. Pregitzer et al. 2008).

We aimed to test if and how elevated CO_2 and soil infertility could impact the belowground functioning of a community of trees in terms of carbon (C) allocation within plant body and atmospheric C sequestration capacity. To this purpose, we established a free-air-CO_2 enrichment (FACE) system with 4 deciduous, broad-leaved species, which are known to be physiologically (aboveground) affected by elevated CO_2 (Koike et al. 2015). We hypothesized that the root impact of elevated CO_2 on the communities would depend on soil nutrient status due to shifts in the photosynthetic function and the nutritive demands. We were expecting the soil nutrient deficiency to induce a differential impact of elevated CO_2 on the belowground functioning of the community.

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

MATERIALS & METHODS

Experimental Design: This study was conducted in the FACE system located in the Sapporo Experimental Forest of Hokkaido University, Japan (43^{o}06' N, 141^{o}20' E, 60 m a.s.l.). The main-plot treatment consisted of ambient (375–395 μmol mol^{-1}) and elevated (500 μmol mol^{-1}) CO_2 treatments, with three site replications for each treatment (Filion et al. 2000). The sub-plot treatments were brown forest (BF) soil, and BF mixed with immature volcanic ash plus pumice (VA) soil. The latter is nutrient poor soil (Masyagina et al. 2006) which was excavated and brought from Tomakomai Experimental Forest of Hokkaido University (42^{o}40' N, 141^{o}37' E, 30 m a.s.l.). It is widespread in Hokkaido and ideal for experiments mimicking soil-nutrient deficiency scenarios.

Since BF soil is native to Sapporo Experimental Forest, half of each FACE ring was excavated and refilled with a mixture of 65 % BF soil and 35 % VA soil. Chemical and physical properties of BF and VA soils used in the present study are illustrated in Eguchi et al. (2008) and Watanabe et al. (2013). The VA soil had about 17 % lower P_2O_5 content than the BF soil.

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

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

The mean values (± SD) of the height and trunk basal diameter (at 2 cm from the starting point of root system) of the juveniles were measured for the 3 birches and the Oak; the variability between the two gas treatments within each species was minimal (Table 1). The oak had been suppressed by the birches (i.e. restricted relative light conditions) after 1.5 years with CO_2 fumigation (data not shown). Similarly, data from leaf area per ground area have shown that the mountain birch was suppressed by the white and monarch birches (Hara et al. 2014). We assumed that it was a phenomenon that could have occurred in pristine conditions and thus can be considered as a realistic growth of a community. Nonetheless, the obtained growth data could have been different if the trees had grown independently or in an uncompetitive environment.

CO_2 treatment: Fumigation of CO_2 was carried out in 4 consecutive Julian years (2010-2013). Saplings were fumigated 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 consists of 6 rings, 3 of which were fumigated by additional CO_2 in order to reach the targeted concentration of 500 μmol mol^{-1} (hereafter "eCO_2"). The diameter and height of each FACE ring are 5 m. The 3 ring plots remained under ambient CO_2 (ca. 370-390 μmol mol^{-1}) (hereafter "aCO_2") as noted in researches by Eguchi et al. (2008) and Watanabe et al. (2013).

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

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

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

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

Statistics: The accepted threshold of significance was defined at α=.05. Trunk basal diameter data were first converted to trunk basal area (Area), and then the Trunk-basal-area:Total-root-dry-mass rate (Area:TDM) was calculated. Area:TDM is a significant indicator of the plant health, and is based on the Pipe Model theory of tree form (Shinozaki et al. 1964a, 1964b). Prior to the analysis, the data of each variable were standardized (Saitanis et al. 2014, 2015). For the standardization, T-scores ($T = z - score \times 10 + 50$) were used, with a mean equal to 50 and a standard deviation equal to 10. Therefore, the mean T-score of each species in each soil and each experimental unit, constituted the real-replicates in the overall analysis. As previously suggested by Saitanis et al. (2014, 2015), this process also allows a fair intercomparison of measured variables and removes any heterogeneity. In order to utilize the overall information and generalize the results at a community level, two steps were followed: First, in relation to the biomasses, data for each community (including all the species) in each treatment (i.e. soil and CO_2) and block (FACE ring) were calculated by averaging the values of individuals of each species in each treatment and then summing the returned values for all the species in each treatment (this process treats any differences in ratios among species and gives better estimates). In order to increase accuracy of the ratio and rates, the values for each individual were calculated for each separately, and averaged per species in each of the research conditions for each block. The values were averaged again for all the species (community) in each of the research conditions for each block.

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

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

Finally, in order to quantify the size of the difference between the community of plants grown under ambient CO_2 and elevated CO_2, under the different soils, and for each dependent variable (Table 2), the effect size *ESi* was calculated according to Cohen (1988). The formula was:

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

C_i, n_i, SD_i, stands for the mean score, the number of observations, and the standard deviation respectively, in the plants under CO_2 enriched air (*EA*) or ambient air (*CTRL*; control group), of the *i* case. The denominator stands for the pooled standard deviation. Absolute values of the effect size within the arbitrary segments 0.00-0.20, 0.20-0.50, 0.50-0.80, and 0.80+ indicate neutral, small, moderate, and large effects, respectively. *ES* was calculated only for the pairs with statistically significant difference in order to quantify the size of the difference. Data processing and statistical analysis were conducted using MS EXCEL 2010 (© Microsoft) and STATISTICA v.10 (© StatSoft Inc.) software.

RESULTS

CO_2 appeared to have a large main effect on all variables with the exception of Area:TDM. Besides, soil had only a large effect on Fine:Area and a medium effect on fine roots (Tables 2 and 3). Concerning the CO_2 × soil interactions, only for Area:TDM and intermediate roots were not significant.

Regarding the Area, eCO_2 was responsible for a large effect, namely a 17 % smaller Area, cf. aCO_2. There was a convergent interaction in Area due to a higher (ns) Area of the communities under eCO_2×VA, compared to eCO_2×BF, and a 22 % significantly smaller (*ES* = 3.69) Area of the community grown under aCO_2×VA, compared to aCO_2×BF (Table 3). Under eCO_2×VA, there was not such a reduction. Instead, eCO_2×VA led to a 32 % larger (*ES* = 6.97) Area than that of plants in aCO_2×VA. There was no significant effect of CO_2 on the communities grown in BF soil.

Similarly, the large 20 % greater TDM induced by eCO_2 can be attributed to the communities grown in VA soil and not in BF soil. Under VA soil and eCO_2, the TDM was 35 % higher (*ES* = 4.65) than under aCO_2×VA, and, unexpectedly, it was also 25 % higher than that under eCO_2×BF (*ES* = 3.55).

Likely to TDM, the coarse root biomass was largely boosted (19 %) by eCO_2, regardless of the soil treatment. This caused by a large increase of coarse root biomass in eCO_2×VA, which also led to a divergent interaction. Under eCO_2×VA, coarse root biomass was largely higher than under aCO_2×VA (35 %) and eCO_2×VA (25 %). It is revealed that there was no effect of eCO_2 when the communities were grown in BF soil. On the other hand, none of the factors had a significant effect on Area:TDM (Table 2).

Fine root biomass was 27 % superior (*ES* = 2.90) in eCO_2 than in aCO_2, as a main effect. Furthermore, it was 11 % suppressed (*ES* = 0.63) by VA soil, compared to BF soil. The interaction of the two factors was significant and could be characterized as divergent with differences in sizes as well. When the communities had grown in BF and VA soils, eCO_2 caused 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×VA,
218 compared to aCO_2×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×BF had 22, 21, and 14 % higher Fine:Intermediate than under eCO_2×BF, aCO_2×VA,
227 and eCO_2×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. eCO_2 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 eCO_2. 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 aCO_2. Meanwhile, it appeared that eCO_2 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 eCO_2 nor VA soil affected the Area:TDM, VA soil in combination with eCO_2 led to a
250 marginally ($p>0.05$) higher belowground allocation than aboveground (i.e., trunk area) compared
251 to aCO_2×BF (-14%), aCO_2×VA (-9%) and eCO_2×VA (-11%). Despite this, under ambient CO_2,
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 aCO_2.

254 Our results agree with the findings of quantitative reviews which state that, on average,
255 eCO_2 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 eCO_2, while the sequestration of atmospheric C, in eCO_2,

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

The usual response of trees to elevated CO_2 is an increased root growth and root turnover of fine roots (e.g. Karnosky 2003; Pregitzer et al. 2008; de Oliveira et al. 2012; King et al. 2013; Cao et al. 2008). Our results support this response with the large effect of eCO_2 on TDM when the community was grown in VA soil. On the other hand, there was no effect of eCO_2 when the community was grown in BF. Insignificant increases of root biomass due to 2-growing-season fumigation with eCO_2 were also found in *Larix gmelinii* var. *japonica* × *L. kaempferi* seedlings grown in well-homogenized BF soil in FACE (Watanabe et al. 2013) or OTCs (Wang et al. 2015a), in the same region. Furthermore, short-term fumigation with elevated CO_2 had no significant effects on the root biomass of neither the *Betula papyrifera* seedlings grown in slight fertilization (Zhang et al. 2006) nor the *Fagus crenata* seedlings (Hirano et al. 2012). In general, root longevity is positively correlated with poor soil-nutrient status and well aeration of soils (Fitter and Hay 2002), which in our case may be attributed to high porosity of VA soil with pumice (e.g. Eguchi et al. 2005; Masyagina et al. 2006).

The eCO_2 increased fine roots, of which the majority naturally colonizes A horizon (e.g. Jaloviar et al. 2009) in both the BF and VA soils. It seems fine roots are ecologically important components as they account for some 13 % of net primary production (NPP) (Ostonen et al. 2005) and a high proportion of total litter (e.g. Leppalammi-Kujansuu et al. 2014). Thus, increased production of fine roots may have an ecologically important benefit in nutrient cycling in a forest. As observed in the Area, although VA soil unexpectedly suppressed fine roots under aCO_2, with the interaction of VA×eCO_2, the fine roots still reached levels similar to that of BF×eCO_2. We expected that under the influence of either eCO_2 or VA soil, the saplings would

undergo a higher need to seek nutrients thereby increasing the fine root area/biomass in order to access more nutrients. Instead, this was the case only for eCO_2 since VA soil caused a reduction of fine root biomass, until the canopy closed (Hara et al. 2014). At present, however, we cannot give a plausible reason for this phenomenon.

There was an unexplained decrease in fine root biomass in VA soil under aCO_2. Yet the Fine:Area, which is an appropriate index to assess the belowground responses in relation to nutrient cycling, showed something different. Namely, VA soil induced a large (*ES* = 3.54) increase when the community had been exposed to aCO_2 (cf. aCO_2×BF). Among the interaction levels, the largest Fine:Area was observed in eCO_2×VA: it was 34 % higher than aCO2×VA and 49 % (*ES* = 8.13) higher than eCO_2×BF. A different foraging strategy of fine roots among the research conditions is clear, in accordance with the findings of Ostonen et al. (2011) and Leppalammi-Kujansuu et al. (2014). Particularly, fine roots underwent extensive foraging strategy (Ostonen et al. 2011), i.e. higher Fine:Area, in VA soil, in both CO_2 conditions. Moreover, eCO_2 had a synergistic effect with VA soil leading to the highest Fine:Area. These results coincide with the results of Area:TDM, albeit insignificant, and indicating greater responses to eCO_2 in relation to nutrient cycling when the community was grown in VA soil.

Differences of intermediate root biomass were attributed only to eCO_2 which increased the biomasses of the communities grown in both the BF and VA soils, compared to the pairwise aCO_2. Consequently, there was a higher amount of intermediate roots than fine roots both in VA soil and eCO_2. The ratio of fine roots to intermediate roots indicates the balance of the two root classes, and its modification is dependent on the needs of the plants. For instance, plants in the need of increasing nutrient uptake are expected to modify a higher proportion of fine roots compared to intermediate roots. However, this could be misleading in such cases which take into

account single observations of only standing biomasses. A single observation, as in our case, of the living fine roots, does not stand for the total production of fine roots, but only a partial production of that given time. In our study, this ratio should be taken with a degree of caution as fine roots were increased by eCO_2 as well. One explanation for the higher amount of intermediate roots than fine roots could be the mortality and regeneration of fine roots that occur (Pritchard et al. 2008) more frequently throughout the growing season which deposit a significant amount of C. As such, we can only speculate that there was an accelerated turnover of fine roots in all research conditions except aCO_2×BF.

Despite our expectations that soil infertility might lead to a maximum fine root production independent of the CO_2 treatment, CO_2 treatment did appear to have a combination effect with soil infertility, resulting in higher production of fine roots. Could this phenomenon be an ecological growth method of saplings in order to mitigate the negative effects via plasticity? In other terms, this phenomenon could be a kind of "*rhizo-morphogenesis*" as the root architecture is changing under these external stressors. This might be induced by a better communication between above- and below-ground under eCO_2, thus resulting in mitigation of the VA soil separated effects.

In general, eCO_2 increases root biomass and the demand for N, P, and K (Norby et al. 1986; Li et al. 2013), however, even if the concentrations of elements in the soil are adequate, a proportionally smaller root system accompanied by less microbial activity is quite hard to support the dissimilation organs. On the other hand, eCO_2 acts as an external force for plants in order to extract more nutrients from the soil: eCO_2 increased $NaHCO_3$- and NaOH-extractable 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 CO_2 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 CO_2 (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, O_3, 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 CO_2.

336 We should mention that increased microbial activity contributes to P and root-derived
337 materials immobilization in rhizosphere under elevated CO_2 (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 CO_2 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 CO_2.

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 eCO_2. 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 eCO_2 while in the infertile VA soil it was positively
349 altered. Elevated CO_2 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

communities growing in different regions and that models dealing with the estimation of C allocation and storage under elevated CO_2 scenarios may overestimate the potential in some regions and underestimate it in others.

CONCLUSIONS

Elevated CO_2 did not cause an increase in trunk basal area or total root mass when the community had grown in fertile BF soil after canopy closure, but it did cause a large increase in both characteristics when the community had grown in infertile VA soil. Furthermore, eCO_2 caused an evident rhizo-morphogenesis through a large increase in fine root production in both soils. On the other hand, eCO_2 had no effect on C allocation to roots even in the case where eCO_2 increased Area and TDM in VA soil. Similarly, VA soil did not cause any significant disturbance to C allocation under ambient or elevated CO_2, albeit there was an insignificant trend for higher allocation to belowground when combined with eCO_2. Nevertheless, our results do not support the drawing of general conclusions because the adaptability of plants to VA soil as well as to eCO_2 may differ among species of different growth traits and soil physicochemical structure.

We conclude that eCO_2 fumigation of 4 growing seasons has no potential to alter the total root production of a community of the 4 deciduous broad-leaved trees when it is growing in brown forest soil. Tree species growing in less fertile soils, such as VA with high porosity, may respond more vigorously to eCO_2 if they are well adapted to the single effects of such soils. New directions should also deal with more infertile soil than the one used in our study. Moreover, ectomycorrhizae research under similar conditions would be essential as their symbiosis may directly or indirectly help plants to mediate the VA- or CO_2-induced suppressing stressors.

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Captions

Table 1 The mean (± SD) values of the initial height and diameter (at 2 cm) of the juveniles

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

Table 3 Mean real values (± SD) of the measured variables trunk basal area (Area), total belowground dry mass (TDM), coarse root dry mass, Area:TDM rate, fine and intermediate roots dry masses, Fine:Intermediate dry mass ratio and Fine:Area rate. The lowercase letters above the mean values indicate the significant differences among the 4 combination treatments while the uppercase indicate the differences within the 2 main treatments. The results of each variable obtained by one 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 letter differ statistically significantly at a level of significance $\alpha=0.05$. Uppercase letters stand for the differences within each main factor and lowercase letters stand for the differences within the interactive effects of the two main factors. In case of no significant effects post-hoc test was not followed, rather means marked with the same letter. ES_v indicates the Cohen effect size of the ν case for each pair with statistically significant difference and *n/a* means not applicable. Data obtained from communities (*Betula ermanii*, *B. maximowicziana*, *B. platyphylla* var. *japonica*,

598 and *Quercus mongolica* var. *crispula*) of saplings exposed to (370–390 μmol mol^{-1}) or elevated
599 (500 μmol 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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Table 1

	Ambient CO_2		Elevated CO_2	
	Height (cm)	Diameter (mm)	Height (cm)	Diameter (mm)
Betula ermanii	30.6 (0.8)	4.2 (0.1)	29.4 (1.2)	4.1 (0.2)
B. maximowicziana	64.4 (1.1)	6.1 (0.2)	61.3 (1.4)	6.3 (0.3)
B. platyphylla* var. *japonica	45.1 (0.9)	4.1 (0.2)	44.1 (1.0)	3.9 (0.3)
Quercus mongolica* var. *crispula	55.1 (0.9)	9.3 (0.4)	54.2 (1.1)	9.8 (0.5)

631 **Table 2**

	CO_2	Soil	CO_2 × 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 × Soil					
	aCO_2	**eCO_2**	***ES_a***	**BF**	**VA**	***ES_b***	**aCO_2×BF**	**eCO_2×BF**	***ES_c***	**aCO_2×VA**	**eCO_2×VA**	***ES_d***
Area (cm^2)	5.438^{A} (0.864)	6.545^{B} (0.533)	1.69	6.149^{A} (0.377)	5.834^{A} (1.251)	*n/a*	6.064^{a} (0.501)	6.159^{a} (0.323)	*n/a*	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)	*n/a*	0.774^{a} (0.085)	0.796^{a} (0.039)	*n/a*	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)	*n/a*	0.740^{ab} (0.089)	0.744^{a} (0.067)	*n/a*	0.647^{a} (0.080)	0.989^{b} (0.121)	4.08
Area:TDM (cm^2 g^{-1} ×10)	0.344^{A} (0.021)	0.338^{A} (0.031)	*n/a*	0.347^{A} (0.022)	0.335^{A} (0.029)	*n/a*	0.337^{a} (0.020)	0.358^{a} (0.021)	*n/a*	0.352^{a} (0.021)	0.319^{a} (0.029)	*n/a*
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)	*n/a*	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)	*n/a*	0.613^{a} (0.045)	0.479^{b} (0.036)	- 4.00	0.485^{b} (0.029)	0.524^{b} (0.047)	*n/a*
Fine:Area (g cm^{-2} ×10)	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)	*n/a*	0.338^{b} (0.039)	0.513^{c} (0.043)	5.25

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