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Title	Fine root turnover of Japanese white birch (<i>Betula platyphylla</i> var. <i>japonica</i>) grown under elevated CO ₂ in northern Japan
Author(s)	Wang, Xiaona; Fujita, Saki; Nakaji, Tatsuro et al.
Citation	Trees : structure and function, 30(2), 363-374 https://doi.org/10.1007/s00468-015-1282-4
Issue Date	2016-04
Doc URL	https://hdl.handle.net/2115/64929
Rights	The final publication is available at link.springer.com .
Type	journal article
File Information	Trees SF30-2_363-374.pdf



**Fine root turnover of Japanese white birch (*Betula platyphylla* var. *japonica*)
grown under elevated CO₂ in northern Japan**

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Authors' contribution statement:

Xiaona Wang: Original conception, in charge of experimental materials and
instrument, data collection and analysis, root tracing, and synthesis manuscript

Saki Fujita: Root tracing, English improvement

Makoto Watanabe: FACE site conduction, article discussion

Tatsuro Nakaji: Rhizotron development, article discussion

Fuyuki Satoh: Management of FACE system

Takayoshi Koike: Fund, management of all technical procedures, article discussion

Key message Elevated CO₂ reduced fine root dynamics (production and turnover) of
white birch seedlings, especially grown in volcanic ash soil compared with brown
forest soil.

Abstract

Increased atmospheric CO₂ usually enhances photosynthetic ability and growth of trees. In order to understand how increased CO₂ affect below-ground part of trees under varied soil condition, we investigated the responses of the fine root (diameter < 2mm) dynamics of Japanese white birch (*Betula platyphylla* var. *japonica*) which was planted in 2010. The three-year-old birch seedlings were grown in four experimental treatments comprising of two levels of CO₂, i.e. ambient: 380-390 and elevated: 500 $\mu\text{mol mol}^{-1}$, in combination with two kinds of soil: brown forest (BF) soil and volcanic ash (VA) soil which has few nutrients. The growth and turnover of fine roots were measured for three years (2011-2013) using the Mini-rhizotron. In the first observation year, live fine root length (standing crop) in BF soil was not affected by CO₂ treatment, but it was reduced by the elevated CO₂ from the second observation year. In VA soil, live fine root length was reduced by elevated CO₂ for all three years. Fine root turnover tended to decrease under elevated CO₂ compared with ambient in both soil types during the first and second observation year. Turnover of fine root production and mortality were also affected by the two factors, elevated CO₂ and different soil types. Median longevity of fine root increased under elevated CO₂ especially in VA soil at the beginning, and a shorter fine root lifespan appeared after two years of observation (2011-2012). These results suggest that elevated CO₂ do not consistently stimulate fine root turnover, particularly during the plant seedlings stage, as it may depend on the costs and benefits of constructing and retaining roots. Therefore, despite the other uncontrollable environment factors, carbon sequestration to the root system may be varied by CO₂ treatment period, soil type and plant age.

Key words: Elevated CO₂, Fine root longevity, Mini-rhizotron, Survival analysis, Volcanic ash soil

Introduction

The root is the hidden half of the plant and clearly regulates whole plant growth. Roots are roughly classified into coarse roots and fine roots with the latter being more physiologically active (Eshel and Beeckman 2013). What will be the effects of elevated carbon dioxide (CO₂) on root dynamics, especially on fine roots?

Since the beginning of this century, atmospheric CO₂ concentration has risen by approximately 30 % as a result from large increases in fossil fuel burning and deforestation (e.g. Meehl et al. 2007). The impacts of elevated CO₂ on forest trees and forest ecosystems are currently attracting great interest, including affects on exchange of energy and materials among soil, aboveground biomass, and the atmosphere (Lal 2005).

The average enhancement of photosynthesis for trees exposed to elevated CO₂ (300 $\mu\text{mol mol}^{-1}$) has been approximately 60 % (Norby et al. 1999). However, responses to exposure vary considerably by species (Naumburg et al. 2001; Koike et al. 2015), position in the crown (Takeuchi et al. 2001), nitrogen (N) fertility level (Watanabe et al. 2008), the season (Noormets et al. 2001b), and co-occurring pollutant concentrations (Noormets et al. 2001a; Koike et al. 2012). There is little certainty on tree growth and productivity under elevated CO₂ and even more is uncertain about effects on belowground parts (Scarascia-Mugnozza et al. 2001). In this study, we focused on fine root dynamics under elevated CO₂ with a Free Air CO₂ Enrichment (FACE) system.

Fine roots were defined as diameter ≤ 2 mm (Agathokleous et al. 2015). Although fine roots contribute to less than 2 % of tree biomass in forest ecosystems (e.g. Brunner and Godbold 2007), they comprise 33-67 % of the annual net primary productivity (NPP) in forest ecosystems (Gill and Jackson 2000). Moreover, despite the small biomass of fine roots relative to aboveground tissues in forest ecosystems, large amounts of carbon (C) and N cycle annually through fine roots, which grow, die,

83 and decompose very rapidly and have high N concentrations (Ruess et al. 2003).

84 Even though these below-ground processes, such as fine root production (FRP)
85 and mortality (FRM) are important. Little is understood by them (Norby and Jackson
86 2000; Aber and Melillo 2001; Fitter 2005). Since fine roots are increasingly
87 recognized crucial in balancing nutrient cycling in forest ecosystems, especially the C
88 sequestration to soil (Norby and Jackson 2000; Matamala et al. 2003; Norby et al.
89 2004), and so understanding the effect of CO₂ enrichment on root dynamics is pivotal.
90 In terms of fine root dynamics under elevated CO₂, however, the results of root
91 longevity and turnover are inconsistent among several CO₂ fumigation researches,
92 which result in great uncertainty about terrestrial C cycles (Pritchard et al. 2001;
93 Lichter et al. 2005; Hogberg and Read 2006).

94 Usually, a larger amount of C is allocated to roots under elevated CO₂. However,
95 experimental findings are inconsistent, with negative responses of elevated CO₂ also
96 being reported. This is due to the enhancement of plant growth under elevated CO₂,
97 which possibly vary with the timing of measurement and duration of CO₂ exposure
98 (Arnold et al. 2000; Higgins et al. 2002). With uncertain conclusions of root
99 production, the responses of root turnover and longevity under elevated CO₂ still
100 remain unclear. Several studies have found production and mortality of fine roots
101 being significantly increased under elevated CO₂ (Matamala and Schlesinger 2000;
102 Pregitzer et al. 2000; King et al. 2001; Pritchard et al. 2001). However, so far, the
103 stimulation of NPP by CO₂-enrichment at Duke FACE which has persisted for more
104 than 8 years amid speculation that nutrient limitations will eventually constrain to a
105 positive CO₂ response (Luo et al. 2004a, b; Finzi et al. 2006; Johnson 2006).

106 Moreover, as fine roots account for a large degree of NPP, which is strongly
107 affected by soil nutrient limitation (Oren et al. 2001), fine root dynamics is expected
108 to be dramatically affected by soil condition. Therefore, as reported, elevated CO₂
109 accelerated plant growth and increased plant nutrient demand as well as nutrient
110 uptake capacity (Bielenberg and Bassirirad 2005). Under infertile soil condition or

under nutrient limitation stress, fine roots adjust their dynamics to balance the costs and benefits of the whole plant. For instance, longer fine root length with lower turnover can reduce the root production cost, and relatively supply more benefits to the plant. However, these points are rarely addressed (e.g. Luo et al. 2004 a, b; Agathokleous et al. 2015).

White birch (*Betula platyphylla* var. *japonica*) is widely distributed and has well acclimated itself in several environmental conditions (Koike 1995). The distribution range of white birch includes a variety of regions, ranging from central Honshu to Far Eastern Asia (including Siberia) (Shi et al. 2010). Furthermore, this species exists under various conditions, and has a strong tendency to form a pure birch forest. White birch is densely planted in several regions of Hokkaido (Terazawa 2005) and in Russia (Zyryanova et al. 2010) due to the promising characteristics of species for green afforestation and its sap utilization.

To estimate the C cycling of boreal forest in East Asia under elevated CO₂, the root dynamics of birch plantation is emphasized since it dominates the forests. Specifically in northern Japan, the soil is widely covered by volcanic ash soil which usually is phosphorous (P) deficient and has relatively low N concentration (e.g. Kayama et al. 2009). Furthermore, P availability is regarded to be a limiting factor to tree growth due to several mechanisms, especially relating with N deposition (Vitousek et al. 2010). Therefore, assessment of future C sequestration should consider the limitations imposed by soil fertility.

In this study, we attempt to access the fine root dynamics of Japanese white birch under elevated CO₂ via the Mini-rhizotron system (Hendrick and Pregitzer 1996). This experiment involved two soil types, volcanic ash (VA) soil and brown forest (BF) soil. We hypothesize that 1) In BF soil, elevated CO₂ stimulates plant growth more than VA soil because of the nutrient limitation. Therefore, root length production is increased by elevated CO₂ in BF soil not in VA soil. 2) Over time, fine root turnover may be increased with elevated CO₂, with the turnover in BF soil being higher than

VA soil. 3) Fine roots will have a longer lifespan under elevated CO₂ and will also have a relatively longer root-length under VA soil condition than in BF soil, as a longer lifespan may lower the cost for root production in nutrient limited soil.

Materials and methods

Study site and FACE system

The experiment was conducted in a FACE system located in Sapporo Experimental Forest, Hokkaido University, Japan (43° 60' N, 141°20' E) (e.g. Eguchi et al. 2008; Watanabe et al. 2010) from 2011-2013. The FACE system was constructed in a size about 6.5 m width and 5.2 m height. The whole-plot treatment consisted of two levels of CO₂ [ambient (380-390 µmol mol⁻¹ CO₂) and elevated CO₂ (500 µmol mol⁻¹ CO₂)] with three site replications. The tanked CO₂ was supplied mainly in the daytime: above the light compensation point of photosynthesis of 70 µmol m⁻²s⁻¹ (Koike 1995), coving the whole photosynthesis period, and the CO₂ fumigation started from early June each year since 2010. We constructed six FACE rings in total, in order to account for the variance among sites for data analysis.

Plant materials and soil type

The present experiment had a split-plot factorial design and the randomized block method was employed. Three-year old seedlings of Japanese white birch (*Betula platyphylla* var. *japonica*) were planted randomly in each FACE site. There were two soil types: brown forest (BF) soil and pumice included volcanic ash (VA) soil (transferred from Tomakomai Experiment Forest) in each FACE site. The chemical and physical properties of these two soil types were described by Eguchi et al. (2008) using soil sampling of 5 cm depth in 2005. Importantly, they extracted exchangeable

phosphorus (P) in the soil with sodium bicarbonate solution, and found that P concentration greatly differed between soil types. P deficiency was more severe in VA soil ($0.58 \mu\text{g } 100 \text{ mg}^{-1}$) than BF soil ($4.48 \mu\text{g } 100 \text{ mg}^{-1}$).

Mini-rhizotron system

To investigate fine root dynamics, Mini-rhizotrons (MR-fine root observation tubes) and specialized camera or scanner equipment have been widely adopted for *in situ* observation (Heeraman and Juma 1993). This technique is a non-destructive method that can be used to monitor the same individual roots over selected time intervals, which can vary from days to years (Andersson and Majdi 2005). Compared to ingrowth core or sequential soil core method, MR has several advanced functions, such as identifying the same roots on successive dates (Hendrick and Pregitzer 1992; Majdi 1996), and quantifying the data on root length production, root length mortality, root longevity, root density and root diameter (Hendrick and Pregitzer 1996; Majdi and Andersson 2005).

In each FACE site, two birch seedlings were randomly selected as the observed target in each soil type, and the MR tube was installed matching each observed seedling. In total four birch seedlings were measured by four MR tubes buried beside the seedlings in one FACE site. All the seedlings were planted together with tubes in June 2010. We installed transparent acrylic tubes (0.5 m long with a 5.08 cm inside diameter) at an angle of 45° to the soil surface. We captured digital images at the soil depth of 0-15 cm and 15-30 cm using a scanner (CI-600 Root Scanner, CID Bio-Science, inc, U.S.A.) which was exactly matched to the tube size according to the schematic described by Maeght et al. (2013). Because it was difficult to distinguish birch roots from grass roots in the surface soil (0-15 cm), and enough number of roots were found in the subsurface soil (15-30 cm), therefore, we examined the roots in deep soil (15-30 cm) for an accurate analysis. A lag period of up to 12 months is

required to stabilize the density of fine roots, after installing MR tubes (Joslin and Wolfe 1999). Hence to avoid misrepresentation of root growth and death near the MR tube interface, we commenced image scanning one year after the planting. Root monitoring is a dynamic process, especially for turnover estimation. As in previous studies, in boreal forest, turnover value can be lower than 1 yr^{-1} , this means the root lifespan is longer than one year, see review by Yuan and Chen (2010). On the other hand, the effects of elevated CO_2 on tree growth showed a time dependent response. Therefore, the experiment with the root monitor was conducted for three years. We collected images in intervals of three weeks from April 2011 to October 2013, excluding snow periods (early November to next late April). The gathered images ($21.59 \times 19.56 \text{ cm}^2$) were used for detecting fine root dynamics.

Root image analysis

We used the program WinRHIZOTron (Regent Instruments, Quebec, Canada) to analyze the roots in the captured images. It was difficult to distinguish whether one root appeared from the time when we scanned the image. Therefore, roots that were unsubscriberized and white when observed for the first time were recorded as new, whereas those remaining white or changing to brownish in subsequent viewings were recorded as living. Roots were defined as dead (marked gone) when they turned black or wrinkled and later produced no new roots in subsequent viewings. For each tube, we traced the length and diameter of each individual root that appeared in the image area. The sum of the length of new roots and the increase in the length of existing roots during each observation interval was calculated as FRP. Likewise, FRM was evaluated from the length of root that was marked gone (turned black or disappeared) (Tingey et al. 2000; Satomura et al. 2007).

Fine root turnover (y^{-1}) can be estimated in two ways: (1) as the ratio of annual root length production to average live root length observed; (2) the inverse of median

root longevity (Majdi et al. 2005). We calculated the turnover of FRP and FRM following the first method, which follows the annual length-based method (Gill et al. 2002).

$$\text{Turnover of FRP (yr}^{-1}\text{)} = \text{ALRP}/\text{LRL}_{\text{max}} \text{ or } \text{ALRP}/\text{LRL}_{\text{mean}}$$

$$\text{Turnover of FRM (yr}^{-1}\text{)} = \text{ALRM}/\text{LRL}_{\text{max}} \text{ or } \text{ALRM}/\text{LRL}_{\text{mean}}$$

The ALRP is the annual length-based root production. It denotes the sum of the fine root length that is produced within one year. In parallel, ALRM is annual length-based root mortality. LRL is the live root length (standing crop) which denotes the fine root length of alive status. It represents the ability of fine root system. LRL_{max} and LRL_{mean} denote for the maximum and mean value of LRL during the corresponding year.

We define the fine root lifespan (median root longevity) obtained from MR, as the time during in which 50 % of the fine roots die (Andersson and Majdi 2005; Green et al. 2005). Additionally, fine root diameters (D) were classified into five orders: $D < 0.2$ mm, 0.2-0.3 mm, 0.3-0.4 mm, 0.4-0.5 mm and 0.5-2.0 mm. Roots of $D > 2.0$ mm were not estimated for all parameters in this study.

As the plant canopy was closed since 2012 (Hara 2014), we separated the first year (2011) data from next two years for calculating and plotting graphs of FRP and FRM.

Soil parameters

According to the report by Eguchi et al. (2008), nutrient concentration was relatively lower in VA soil than BF soil. We detected the C and N concentrations of the two soils in 2011 and 2012 with NC analyzers (NC-900, Sumica-Shimadzu, Kyoto, Japan).

Statistical analysis

All data were distributed normally, as verified by the Kolmogorove-Smirnov Test, the significant value was greater than 0.05. Then, the data were subjected to split-plot general linear model randomized. We performed general linear model-multivariate analysis of variance (ANOVA) to estimate the effects of different treatments (CO₂ and soil type) and their interaction on turnover of FRP and FRM over years. The fine root median and mean longevity were analyzed using nonparametric Kaplan-Meier survival function. Tukey-HSD was performed for the effect on fine root longevity under different treatment conditions, not for the effect on fine root longevity within diameter class. Statistical analysis unit is FACE site, all the data were undertaken by SPSS software (version 16.0).

Results

Soil C and N concentrations

Soil C and N concentration were measured (Table 1). C and N concentration in VA soil showed lower value than BF soil. Elevated CO₂ did not affect soil C and N concentrations.

Living fine root length

During the treatment period, LRL showed relatively higher values in the period of early growing season (June to Aug) from 2011 to 2013 (Fig. 1). In 2011, LRL did not differ significantly between ambient and elevated CO₂ treatment in BF soil, but it sharply increased in ambient treatment as opposed to the elevated CO₂ in 2012 and 2013. Contrastingly, in VA soil, LRL showed higher values in ambient than elevated CO₂ condition in all observation years (Fig. 1). Over these three years, LRL was extremely high under ambient conditions in VA soil compared to the other three

conditions. Elevated CO₂ markedly reduced LRL in VA soil during the three observed growing seasons (Fig. 1).

Fine root production and mortality

In BF soil, fine root production rate (root length based) was not affected by elevated CO₂ during 2011 except July and September when it was increased (Fig. 2 a). It was unaffected during 2012 but was reduced by elevated CO₂ in August of 2013 (Fig. 2 b). In VA soil, no significant differences were found between elevated CO₂ and ambient treatment in 2011 (Fig. 2 a), however it was reduced during the early growing season (June, July and August) in 2012 and 2013 (Fig. 2 b). No clear trend was found for the mortality rate in BF soil, and elevated CO₂ tended to reduce it in the late growing season: September and October (2012-2013) in VA soil (Fig. 3b).

Turnover of fine root production and mortality differed significantly among the treatments (Table 2). Elevated CO₂ did not significantly affect the turnover of production and mortality, but there was an interaction effect of year and CO₂ on mortality turnover. Over time, it was reduced by elevated CO₂ in two kinds of soil from 2011 to 2012. The soil type influenced production turnover significantly, showing lower values in VA soil than BF soil except in the final observation year. Turnover of production and mortality were significantly reduced with the time of the three observation years. The interaction effect of soil and year influenced the production turnover. There was no interaction effect of CO₂ and soil, nor CO₂, soil and year.

Additionally, the annual length-based root production (ALRP) and annual length-based root mortality (ALRM) of each tube in all treatments were positively correlated (Fig. 4).

Fine root longevity

307

308 The median fine root longevity was estimated by the different treatments and root
309 diameters. Overall, median root longevity differed with different treatments, and it
310 was increased under elevated CO₂ in 2011 for BF soil and VA soil (Table 3). From
311 2012 to 2013, compared to the ambient treatment, the relative longer median fine root
312 longevity under elevated CO₂ was gradually reduced in BF soil. The increase of
313 median fine root longevity compared to ambient treatment, was weakened by elevated
314 CO₂ in VA soil.

315 Median root longevity of different diameter classes showed significant responses
316 to different treatments. The thinnest fine root ($D < 0.2$ mm) was not affected by
317 elevated CO₂ in all conditions in 2011 and 2012, but it was increased by elevated CO₂
318 in BF soil and reduced in VA soil in 2013 (Table 4). Root longevity of roots with
319 diameters between 0.2 mm and 0.3 mm were markedly increased by elevated CO₂
320 except in 2013 in BF soil, where longevity was, in contrast, reduced by elevated CO₂.
321 The root longevity of roots with diameters between 0.3-0.4 mm was increased by
322 elevated CO₂. But there were no effects on the median longevity of the roots with
323 diameters between 0.4-0.5 mm by elevated CO₂ in 2012, the same results were
324 attained for roots with a diameter larger than 0.4 mm in 2013.

325

326 **Discussion**

327

328 Fine root length standing crop

329

330 We found the LRL (live root length or length-based standing crop) was significantly
331 influenced by elevated CO₂ treatment. In BF soil, LRL was not affected by elevated
332 CO₂ in first year, but it was reduced by elevated CO₂ from the second year onwards.
333 In contrast, under VA soil, elevated CO₂ reduced the LRL for all three observed years
334 (Fig. 1). Generally, elevated CO₂ stimulates plant growth (Norby and Zak 2011) and

more carbon is allocated to the roots (Lukac et al. 2003), therefore, that is why elevated CO₂ was assumed to increase the root/shoot ratio in earlier studies.

However, other studies have revealed less pronounced effects (Bielenberg and Bassirirad 2005) or even negative responses of elevated CO₂ (Arnone et al. 2000; Higgins et al. 2002). These present results have proved this point and suggest that the stimulated effects of elevated CO₂ diminished over time. On the other hand, the down-regulation of photosynthesis was frequently observed in the seedling and sapling stage of various tree species (Tissue and Lewis 2010). This was the reason why a higher relative LRL was found under the ambient condition and not at elevated CO₂ condition from 2012 in the two kinds of soil.

The different results between BF soil and VA soil, suggested that the LRL is strongly related with C and N condition of soil. Our results found that the soil N concentration in VA soil was relatively lower than BF soil as reported before (Eguchi et al. 2008). This perhaps led to the total length of live root in VA soil to be higher than BF soil in all treatments during the three years, because wide roots system were efficient for limited nutrient uptake (Ryser 2006). Moreover, the down-regulation of photosynthesis can be clearly found under immature VA soil conditions (Mao 2013), this is because it limits photosynthate allocation to belowground. For this reason, LRL in VA soil was consistently lower under elevated CO₂ than ambient treatment.

As we mentioned, the canopy was closed since 2012 in our experimental site according to Hara (2014), thus, the effects of elevated CO₂ to root growth was minified. In our case, elevated CO₂ even reduced root growth, such as the fine root production rate of VA soil in the early growing season (June, July and August), and distinctly decreased the LRL in BF soil since 2012. As elevated CO₂ accelerates plant growth, plant nutrient demand and its uptake capacity may also be accelerated (Bielenberg and Bassirirad, 2005). As a result, higher nutrient demand under limited nutrient environments, particularly infertile soil, may have reduced or even restricted

plant growth, especially belowground growth. Thus, a reduced LRL of white birch was found for three years in VA soil.

Additionally, the negative effect of elevated CO₂ on LRL from the second year in BF soil and throughout the three years in VA soil can be hypothesized that it potentially derived from the changes of root production and mortality. For instance, changes of higher mortality or lower production under elevated CO₂ can lead to a reduced LRL. Overall, we did not find any consistent trend for root production rate and mortality rate (Fig. 2, 3). Also a strong correlation of ALRP and ALRM was found in all treatment conditions suggest the root production and mortality were equal within one year (Fig. 4). Therefore, we deduced that the different LRL patterns may depend on the fine root turnover and lifespan, which we will further discuss below.

Turnover of root production and mortality

Elevated CO₂ did not affect the turnover of FRP and FRM (Table 2). Our results are consistent with the results of Pritchard et al. (2008), whereby elevated CO₂ did not significantly alter turnover of loblolly pine, despite an increased root length, production and mortality. One possible reason is the treatment period of elevated CO₂. Our case is still shorter than six years monitoring as Pritchard et al. (2008) did. Another possibility is the soil depth; the roots in 15-30 cm soil depth may have been inactive. Moreover, an interactive effect with year was found, and that elevated CO₂ reduced turnover of FRM over time. It could contribute to a longer lifespan with CO₂ enrichment as we detected (Table 3). Additionally, Eissenstat et al. (2000) concluded that elevated CO₂ may be associated with longer root lifespan, by decreasing the root N concentration and reducing the root maintenance respiration. There was also a similar report by Arnone et al. (2000) that the longer lifespan was also found under elevated CO₂. Turnover of FRM was increased by elevated CO₂ in 2013, therefore there was lower LRL in the third year compared to 2011 and 2012 during our

observation (Fig. 1), and this could lead to a reduction of root lifespan under elevated CO₂ (Table 4).

Soil only significantly affected turnover of FRP, but not turnover of FRM. VA soil had low turnover capacity of FRP in 2011 and 2012, and this may explain why lifespan in VA soil was relatively higher than BF soil with the same CO₂ treatment (Table 3). Another possibility is symbiotic effect of ectomycorrhiza (ECM), as roots with ECM symbiosis can live much longer or with lower production than non-colonized roots (King et al. 2002). This result was demonstrated by Bidartondo et al. (2001). He found that roots (D = 0.3~0.6 mm) of *Pinus muricata* had longer root longevity when they were colonized with ECM. Therefore, present results found the lower turnover and longer lifespan of fine root with limited nutrient in VA soil in the first-two observed years. Thus, there is a slower root dynamics in VA soil than in the BF soil during the early period of CO₂ treatment.

Fine root lifespan of white birch under elevated CO₂

The median longevity was initially increased by elevated CO₂ in both BF soil and VA soil, but this did not continue from the second year in BF soil, and appeared to be a convergent effect of elevated CO₂ in VA soil (Table 3). As it has been reported, plants under elevated CO₂, generally increase water use efficiency and dramatically stimulate aboveground growth (Qu et al. 2004; Koike et al. 2010). Furthermore, under elevated CO₂, root uptake provides nutrient resources primarily for CO₂-stimulated growth in aboveground biomass, with more modest production in fine roots or longer lifespan roots (Housman et al. 2006). In VA soil, the root median longevity was consistently increased under elevated CO₂. One possibility is that nutrient limitation resulted in a lower turnover of FRP and FRM under elevated CO₂ over the years, because the root longevity was inversely related to the duration of the resource supply (Pregitzer et al. 1993). Therefore, there was a longer root lifespan due to limited

nutrient availability. Another reason is that plants preferentially enhance the growth of aboveground as we discussed above, and this may readily occur in nutrient limited condition as root lifespan would be increased if construction costs relative to maintenance costs are high, or if the nutrient availability is low (Eissenstat et al. 2000).

Root diameter is known to change by multi-year studies with elevated CO₂ treatment (Pritchard et al. 2008). Therefore, root responses of different diameter classes to the treatments were predicted (see Table 4). The median lifespan of fine root ($D < 2$ mm) was significantly affected by different treatments. The thinnest roots ($D < 0.2$ mm) were affected under elevated CO₂ since 2013 after two growing seasons. The fine root lifespan of the other diameter orders increased initially and were unaffected under elevated CO₂ ($D > 0.4$ mm) in 2013. As reported, plants usually increase mycorrhizal colonization and decrease root N concentration under elevated CO₂ (Pritchard and Rogers 2000; Tingey et al. 2000). Given that root longevity is negatively correlated with tissue N concentration (Pregitzer et al. 1998), we concluded that the mycorrhiza symbiosis was strongly stimulated under elevated CO₂ in the beginning (e.g. Wang et al. 2015). As a result, fine root performed a longer lifespan with no distinct effect by CO₂ enrichment in this study. Moreover, mycorrhizal colonization under elevated CO₂ has been found to stabilize over time. Shinano et al. (2007) found that with elevated CO₂, ECM colonized with the Japanese larch (*Larix kaempferi*) at an increasing rate during the first year treatment, and later equilibrated to a stable lower rate. In our case, the shorter longevity of fine root during the third year was likely to be derived from the decreased ECM colonization. It is possible that ECM assisted birch seedlings to survive in new soil conditions, and the shorter root lifespan revealed a completed aboveground growth. After this establishment, the plants started to develop root systems (Eissenstat et al. 2000).

Additionally, regardless of the mycorrhizal symbiosis in the rhizosphere (McNear 2013), the different responses of roots in different diameter classes indicate

root heterogeneity, such as specific root area, specific root length and root tissue density. The location of a root and its branching system of a root, potentially influences the root lifespan (Guo et al. 2004). Further understandings of these characteristics are required to deeply clarify the root dynamics under changing environments.

Conclusions

Elevated CO₂ reduced standing crop of fine root length of white birch in VA soil, with a lower turnover of production and mortality compared with BF soil. This may indicate a slow root dynamics of white birch in VA soil during the early period of CO₂ enrichment. Elevated CO₂ increased root longevity, especially in VA soil over the three observed growing seasons, suggesting that soil nitrogen or nutrient status strongly affects root longevity. The shorter turnover of fine root production under elevated CO₂ compared with ambient CO₂ in VA soil during the third growing season, indicates birth and death of the fine root increased, therefore possibly leading to a rise in C sequestration to soil. This result may be further due to the elevated CO₂ causing changes of mycorrhizal colonization, root specific characteristics, and/or the position of a root in the branching root system. These factors cannot be ignored, thus more efforts are required to expand our knowledge of root research and thoroughly understanding the responses of root dynamics under a changing environments.

Acknowledgments

We thank Mr. Ito Hirotaka for his contribution of the installation of the Mini-rhizotron system. We also thank Prof. Heljä-Sisko Helmisaari and Dr. Jaana Leppälammii-Kujansuu for their guidance on data analysis. Thanks are also given to Dr. Anthony Garrett of SCITEXT of Cambridge, U.K. and Ms. Amelie Vanderstock

474 of Biological Institute of The University of Sydney, Australia for English
475 improvement. This study was supported by the Japan Society for the Promotion of
476 Science New field and Type B program (to T. Koike, 21114008 & 26660119).

477

478 **Conflict of interest:**

479 We declare that our research has no conflict of interest.

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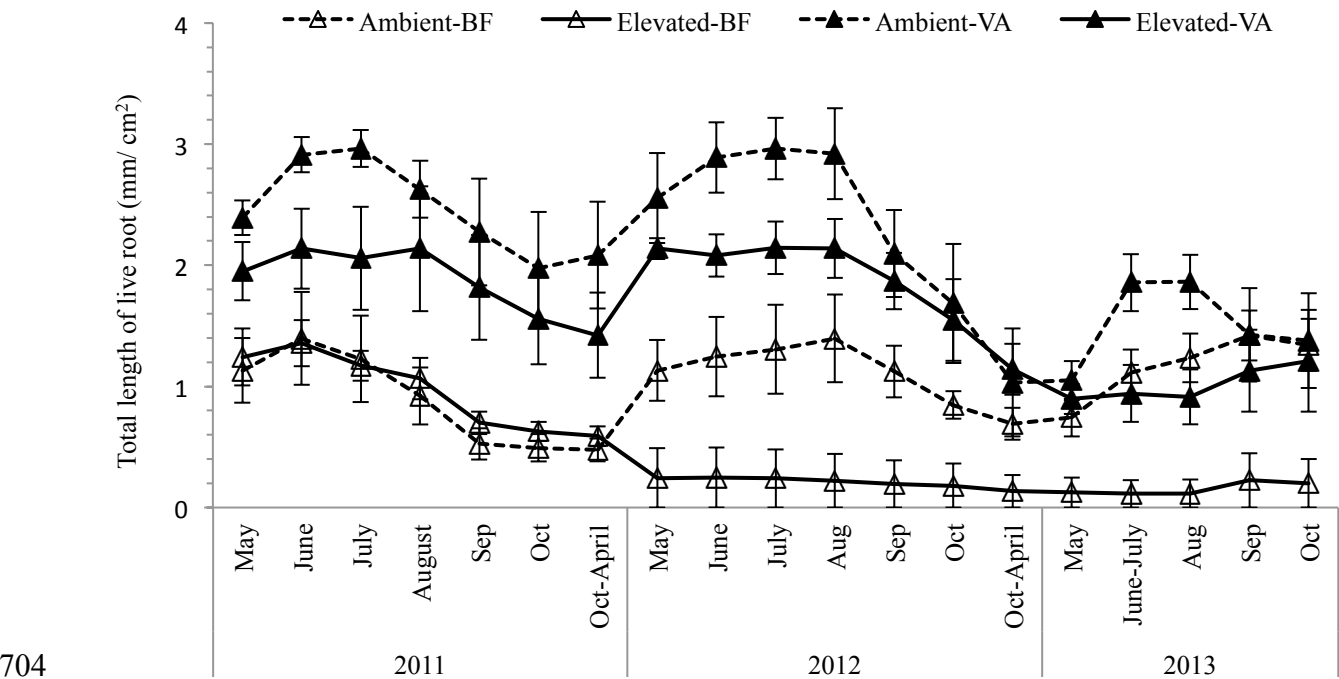
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 701



704
705 **Fig. 1** Relative length of live fine root (standing crop) of white birch seedlings growing under elevated (500 $\mu\text{mol mol}^{-1}$) and control (370 μmol
706 mol^{-1}) [CO_2] on volcanic ash (VA) and brown forest (BF) soil during 2011 to 2013. Each value is the mean of six replications, the vertical bar in
707 the column denotes SE.

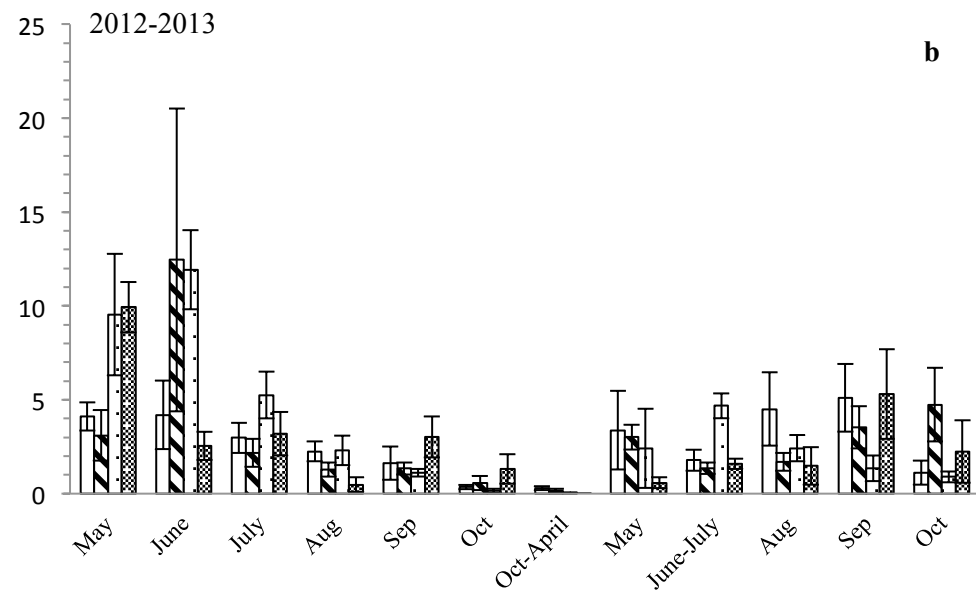
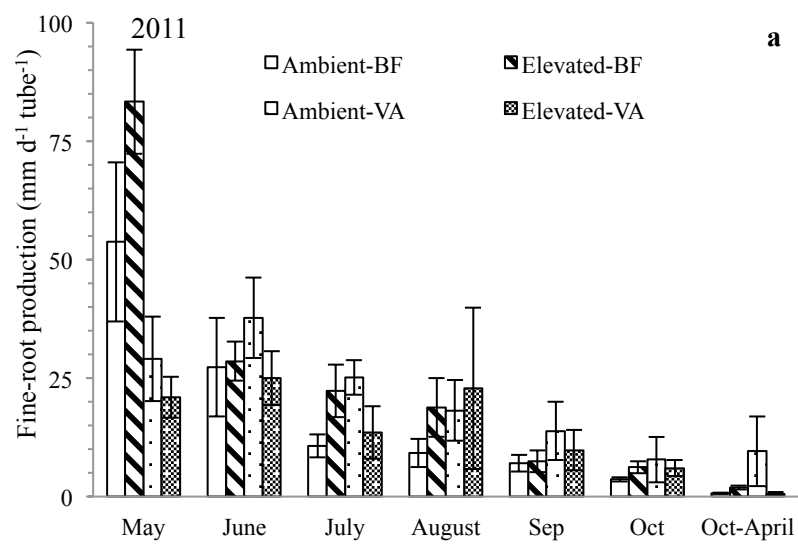


Fig. 2 Fine root length production rate of birch seedlings growing under elevated and ambient [CO₂] on volcanic ash (VA) and brown forest (BF) soil. Each value is the mean of six replications, the vertical bar in the column denotes SE. The letter **a** and **b** denote the graph of different year.

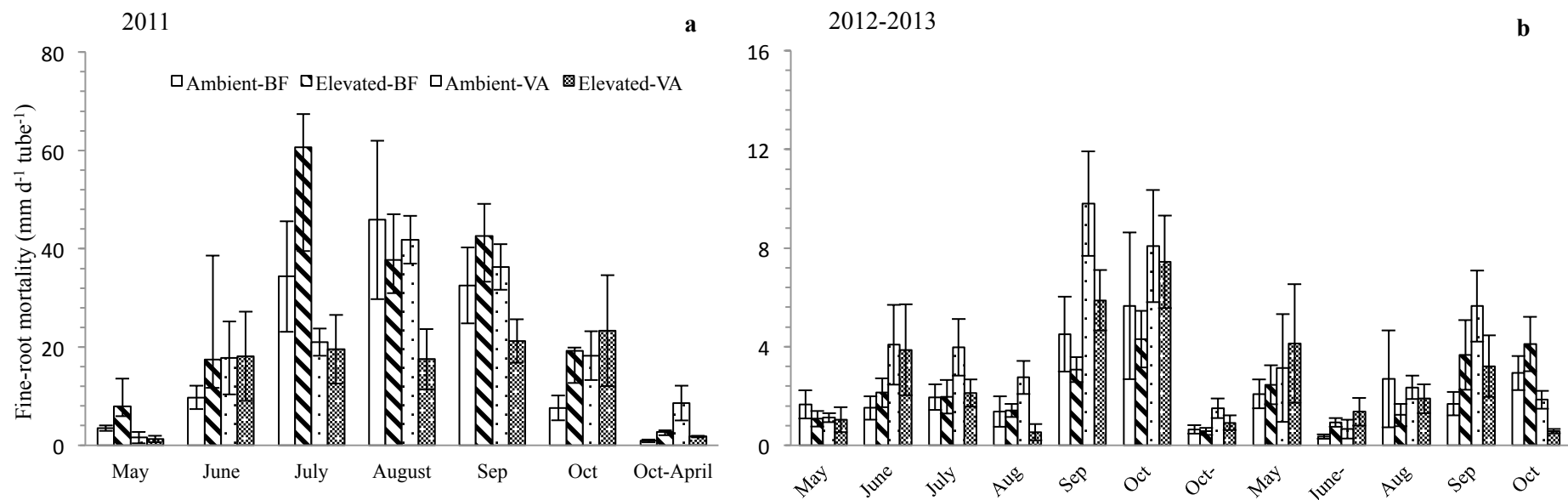


Fig. 3 Fine root length mortality rate of birch seedlings growing under elevated and ambient [CO₂] on volcanic ash (VA) and brown forest (BF) soil. Each value is the mean of six replications, the vertical bar in the column denotes SE. The letter **a** and **b** denote the graph of different year.

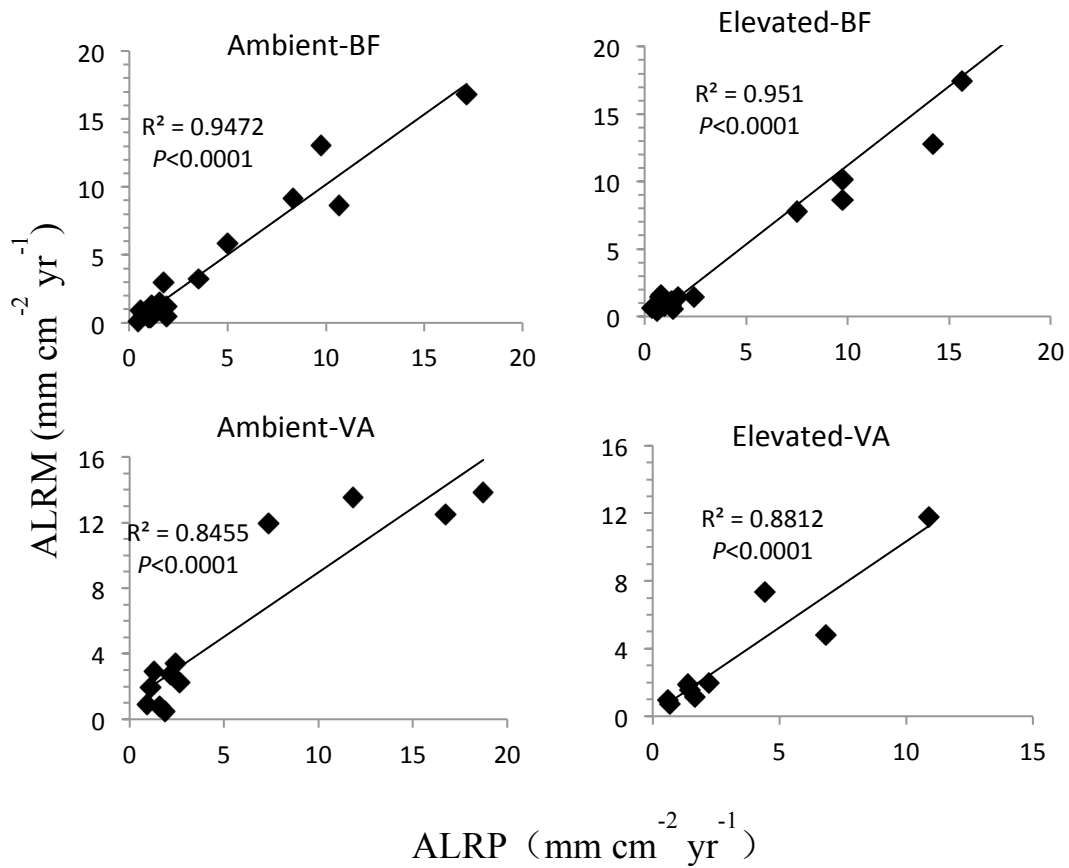


Fig. 4 Relationship between annual length-based root production (ALRP) and annual length-based root mortality (ALRM) in each tube and treatment. Pearson correlation test showed $P < 0.0001$.

720 **Table 1** Soil C and N concentration in ambient and elevated CO₂ during 2011 and 2012.
 721

Year	Nutrient	BF soil				VA soil				<i>p</i>		
		Ambient		Elevated		Ambient		Elevated		Soil	CO ₂	Soil×CO ₂
2011	C (mg 100mg ⁻¹)	2.93	± 0.18	3.24	± 0.26	2.24	± 0.16	2.24	± 0.15	**	n.s.	n.s.
	N (mg 100mg ⁻¹)	0.25	± 0.01	0.27	± 0.01	0.18	± 0.01	0.19	± 0.01	***	n.s.	n.s.
	C / N	11.69	± 0.23	11.96	± 0.41	12.23	± 0.59	12.17	± 0.35	n.s.	n.s.	n.s.
2012	C (mg 100mg ⁻¹)	2.78	± 0.21	3.52	± 0.29	1.75	± 0.14	2.09	± 0.15	**	n.s.	n.s.
	N (mg 100mg ⁻¹)	0.24	± 0.02	0.28	± 0.02	0.15	± 0.01	0.17	± 0.01	***	n.s.	n.s.
	C / N	11.50	± 0.19	12.43	± 0.48	11.92	± 0.18	12.20	± 0.28	n.s.	n.s.	•

722 Each value is the Mean ± SD of three replications, and statistical analysis unit is the FACE site. ANOVA: ** *P*<0.01,*** *P*<0.001, •
 723 0.05<*P*<0.1, ns denotes not significant.
 724

725 **Table 2** Turnover of fine root production and mortality of each observed year (yr⁻¹).

			Production	Mortality		
Year	Soil	CO ₂	ALRP/LRLmax	ALRP/LRLmean	ALRM/LRLmax	ALRM/LRLmean
2011	BF	Ambient	1.04(0.15)	1.54(0.19)	1.14(0.17)	1.68(0.22)
		Elevated	0.93(0.06)	1.35(0.12)	0.98(0.07)	1.43(0.14)
	VA	Ambient	1.00(0.21)	1.23(0.22)	1.07(0.08)	1.33(0.11)
		Elevated	0.84(0.14)	1.06(0.12)	0.95(0.23)	1.22(0.30)
2012	BF	Ambient	1.17(0.15)	1.68(0.25)	1.19(0.11)	1.69(0.12)
		Elevated	1.07(0.03)	1.43(0.09)	0.93(0.08)	1.23(0.09)
	VA	Ambient	0.70(0.08)	0.96(0.12)	0.96(0.14)	1.33(0.20)
		Elevated	0.71(0.04)	0.92(0.06)	0.78(0.06)	1.01(0.12)
2013	BF	Ambient	0.64(0.09)	0.88(0.17)	0.25(0.05)	0.33(0.06)
		Elevated	0.88(0.13)	1.04(0.14)	0.76(0.19)	0.90(0.22)
	VA	Ambient	0.71(0.06)	0.93(0.08)	0.56(0.17)	0.74(0.23)
		Elevated	0.78(0.16)	1.00(0.25)	0.78(0.10)	0.97(0.08)
CO ₂			ns	ns	ns	ns
Soil			*	**	ns	ns
Year			*	**	***	***
CO ₂ ×Year			ns	ns	**	**
Soil×Year			**	**	ns	ns
CO ₂ ×Soil			ns	ns	ns	ns
CO ₂ ×Soil×Year			ns	ns	ns	ns

726 Each value is the Mean (SE) of six replications, and statistical analysis unit is FACE site. ANOVA: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns
727 denotes not significant.

Table 3 Fine root lifespan (weeks) of birch seedlings growing under elevated and ambient [CO₂] on volcanic ash (VA) soil and brown forest (BF) soil.

Year	Longevity	Ambient-BF	Elevated-BF	Ambient-VA	Elevated-VA	<i>P</i> value
2011	Median	15(0.29)c	18(0.44)b	18(0.32)b	44(2.87)a	***
	Mean	23(0.42)	26(0.35)	25(0.38)	32(0.53)	***
	N	1364	2419	1643	680	
2012	Median	18 (1.35)c	18 (2.31)b	18 (1.08)c	24 (3.74)a	**
	Mean	24 (1.12)	27(0.97)	25(0.78)	29(1.09)	**
	N	284	414	574	314	
2013	Median	21(2.92)a	18(2.44)a	12(0.72)c	15(1.62)b	***
	Mean	32(1.27)	33(1.04)	22(0.82)	25(1.33)	***
	N	672	842	886	399	

Each value is the median and mean longevity (SE) calculated by survival function with six replications; N denotes the number of available roots. Tukey-HSD post-hoc test was shown with small letters, and statistical analysis unit is FACE site. Significant was tested by Log Rank (Mantel-Cox): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

735 **Table 4** Fine root lifespan (weeks) in different root diameter classes of birch seedlings growing under elevated and ambient [CO₂] on volcanic
736 ash (VA) soil and brown forest (BF) soil.
737

Year	D (mm)	Ambient-BF	Elevated-BF	Ambient-VA	Elevated-VA	<i>P</i> value
2011	D<0.2	12(2.76)	12(0.69)	15(1.08)	15(3.93)	ns
	D 0.2-.03	15(0.29)	18(0.45)	18(0.34)	44(2.91)	***
	D 0.3-0.4	18(1.14)	29(3.61)	15(0.70)	18(1.95)	***
	D 0.4-0.5	15(0.80)	24(2.88)	15(0.43)	50(4.24)	***
	D>0.5	18(1.33)	21(3.49)	15(0.80)	18(2.88)	***
2012	D<0.2	12(3.97)	9(1.54)	12(6.00)	39(9.80)	ns
	D 0.2-.03	18(1.16)	21(4.14)	18(1.11)	24(3.51)	***
	D 0.3-0.4	15(4.06)	36(3.81)	18(4.90)	48(2.49)	***
	D 0.4-0.5	21(2.41)	42(2.60)	15(4.32)	27(10.51)	ns
	D>0.5	27(20.17)	----	42(13.35)	----	***
2013	D<0.2	9(1.49)	12(0.75)	9(1.97)	3(1.96)	**
	D 0.2-.03	30(4.86)	21(3.71)	12(0.73)	18(1.43)	**
	D 0.3-0.4	18(3.62)	48(5.55)	15(4.15)	48(7.76)	**
	D 0.4-0.5	24(10.64)	51(4.51)	24(10.22)	33(9.26)	ns
	D>0.5	27(13.98)	57(7.90)	45(5.23)	63(3.16)	ns

738 Each value is median longevity (SE) calculated by survival function with corresponding roots belonging to different diameter classes, “----”
739 denotes the unavailable result due to the insufficient roots, and statistical analysis unit is FACE site. Significance was tested by Log Rank
740 (Mantel-Cox): * $P<0.05$, ** $P<0.01$, *** $P<0.001$, ns denotes not significant.