Trees (2016) 30:363–374
DOI 10.1007/s00468-015-1282-4

ORIGINAL PAPER

Fine root turnover of Japanese white birch (*Betula platyphylla* var. *japonica*) grown under elevated CO_2 in northern Japan

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Received: 17 April 2015 / Revised: 22 August 2015 / Accepted: 26 August 2015

Abstract

Key message **Elevated CO_2 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 CO_2 usually enhances photosynthetic ability and growth of trees. To understand how increased CO_2 affects below-ground part of trees under varied soil condition, we investigated the responses of the fine root (diameter <2 mm) 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 two levels of CO_2, i.e., ambient: 380–390 and elevated: 500 μmol 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 3 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 CO_2 treatment, but it was reduced by the elevated CO_2 from the second observation year. In VA soil, live fine root length was reduced by elevated CO_2 for all 3 years. Fine root turnover tended to decrease under elevated CO_2 compared with ambient in both soil types during the first and second observation years. Turnover of fine root production and mortality was also affected by the two factors, elevated CO_2 and different soil types. Median longevity of fine root increased under elevated CO_2, especially in VA soil at the beginning, and a shorter fine root lifespan appeared after 2 years of observation (2011–2012). These results suggest that elevated CO_2 does 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 CO_2 treatment period, soil type and plant age.

Keywords Elevated CO_2 · 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 (CO_2) on root dynamics, especially on fine roots?

Since the beginning of this century, atmospheric CO_2 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 CO_2 on forest trees and forest ecosystems are currently attracting great interest, including effects on exchange of energy and

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Communicated by T. Koike and K. Noguchi.

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materials among soil, aboveground biomass, and the atmosphere (Lal 2005).

The average enhancement of photosynthesis for trees exposed to elevated CO_2 (300 μmol 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 CO_2 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 CO_2 with a Free Air CO_2 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, fine roots grow, die, and decompose very rapidly, large amounts of carbon (C) and N cycle annually through them eventhough they have small biomass compared to aboveground tissues (Ruess et al. 2003).

Even though these below-ground processes, such as fine root production (FRP) and mortality (FRM), are important. Little is understood by them (Norby and Jackson 2000; Aber and Melillo 2001; Fitter 2005). Since fine roots are increasingly recognized crucial in balancing nutrient cycling in forest ecosystems, especially the C sequestration to soil (Norby and Jackson 2000; Matamala et al. 2003; Norby et al. 2004), and so understanding the effect of CO_2 enrichment on root dynamics is pivotal. In terms of fine root dynamics under elevated CO_2, however, the results of root longevity and turnover are inconsistent among several CO_2 fumigation researches, which result in great uncertainty about terrestrial C cycles (Pritchard et al. 2001a, b; Lichter et al. 2005; Hogberg and Read 2006).

Usually, a larger amount of C is allocated to roots under elevated CO_2. However, experimental findings are inconsistent, with negative responses of elevated CO_2 also being reported. This is due to the enhancement of plant growth under elevated CO_2, which possibly vary with the timing of measurement and duration of CO_2 exposure (Arnone et al. 2000; Higgins et al. 2002). With uncertain conclusions of root production, the responses of root turnover and longevity under elevated CO_2 still remain unclear. Several studies have found production and mortality of fine roots being significantly increased under elevated CO_2 (Matamala and Schlesinger 2000; Pregitzer et al. 2000; King et al. 2001; Pritchard et al. 2001a, b). However, so far, the stimulation of NPP by CO_2-enrichment at Duke FACE which has persisted for more than 8 years amid speculation that nutrient limitations will eventually constrain to a positive CO_2 response (Luo et al. 2004a, b; Finzi et al. 2006; Johnson 2006).

Moreover, as fine roots account for a large degree of NPP, which is strongly affected by soil nutrient limitation (Oren et al. 2001), fine root dynamics is expected to be dramatically affected by soil condition. Therefore, as reported, elevated CO_2 accelerated plant growth and increased plant nutrient demand as well as nutrient 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. 2004a, 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 CO_2, 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 CO_2 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 CO_2 stimulates plant growth more than VA soil because of the nutrient limitation. Therefore, root length production is increased by elevated CO_2 in BF soil not in VA soil. (2) Over time, fine root turnover may be increased with elevated CO_2, with the turnover in BF soil being higher than VA soil. (3) Fine roots will have a longer lifespan under elevated CO_2 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 to 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 CO_2 [ambient (380–390 μmol mol^{-1} CO_2) and elevated CO_2 (500 μmol mol^{-1} CO_2)] with three site replications. The tanked CO_2 was supplied mainly in the daytime: above the light compensation point of photosynthesis of 70 μmol m^{-2}s^{-1} (Koike 1995), coving the whole photosynthesis period, and the CO_2 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 μg 100 mg^{-1}) than BF soil (4.48 μg 100 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 and 15–30 cm using a scanner (CI-600 Root Scanner, CID Bio-Science, Inc., USA) 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 1 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 year^{-1}; this means that the root lifespan is longer than 1 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 3 years. We collected images in intervals of 3 weeks from April 2011 to October 2013, excluding snow periods (early November to next late April). The gathered images (21.59×19.56 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 unsuberized 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 were 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 (year^{-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).

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

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

The ALRP is the annual length-based root production. It denotes the sum of the fine root length that is produced within 1 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: $D < 0.2$, 0.2–0.3, 0.3–0.4, 0.4–0.5 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 2 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 Kolmogorov–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 (CO_2 and soil type) and their interaction on turnover of FRP and FRM over yeas. 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 concentrations were measured (Table 1). C and N concentrations in VA soil showed lower value than BF soil. Elevated CO_2 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 CO_2 treatment in BF soil, but it sharply increased in ambient treatment as opposed to the elevated CO_2 in 2012 and 2013. Contrastingly, in VA soil, LRL showed higher values in ambient than elevated CO_2 condition in all observation years (Fig. 1). Over these 3 years, LRL was extremely high under ambient conditions in VA soil compared to the other three conditions. Elevated CO_2 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 CO_2 during 2011 except July and September when it was increased (Fig. 2a). It was unaffected during 2012, but was reduced by elevated CO_2 in August of 2013 (Fig. 2b). In VA soil, no significant differences were found between elevated CO_2 and ambient treatment in 2011 (Fig. 2a); however, it was reduced during the early growing season (June, July and August) in 2012 and 2013 (Fig. 2b). No clear trend was found for the mortality rate in BF soil, and elevated CO_2 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

Table 1 Soil C and N concentrations in ambient and elevated CO_2 during 2011 and 2012

Year	Nutrient	BF soil		VA soil		*p*		
		Ambient	Elevated	Ambient	Elevated	Soil	CO_2	Soil × CO_2
2011	C (mg 100 mg^{-1})	2.93 ± 0.18	3.24 ± 0.26	2.24 ± 0.16	2.24 ± 0.15	**	n.s.	n.s.
	N (mg 100 mg^{-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 100 mg^{-1})	2.78 ± 0.21	3.52 ± 0.29	1.75 ± 0.14	2.09 ± 0.15	**	n.s.	n.s.
	N (mg 100 mg^{-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.	•

Each value is the Mean ± SD of three replications, and statistical analysis unit is the FACE site

ANOVA: ** $P < 0.01$, *** $P < 0.001$, •$0.05 < P < 0.1$, *ns* not significant

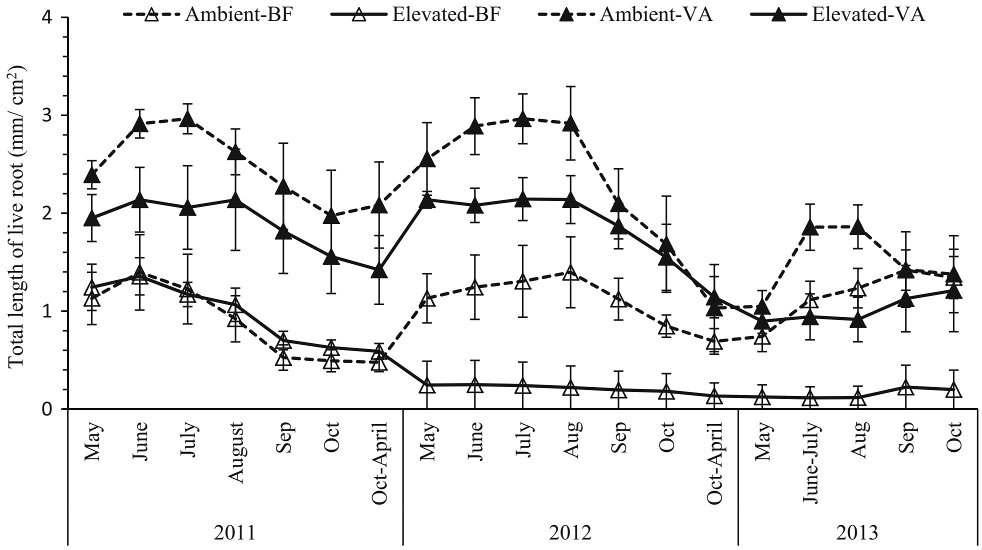

Fig. 1 Relative length of live fine root (standing crop) of white birch seedlings growing under elevated (500 µmol mol^{-1}) and control (370 µmol mol^{-1}) [CO_2] on volcanic ash (VA) and brown forest (BF) soil during 2011–2013. Each value is the mean of six replications; the *vertical bar* in the column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

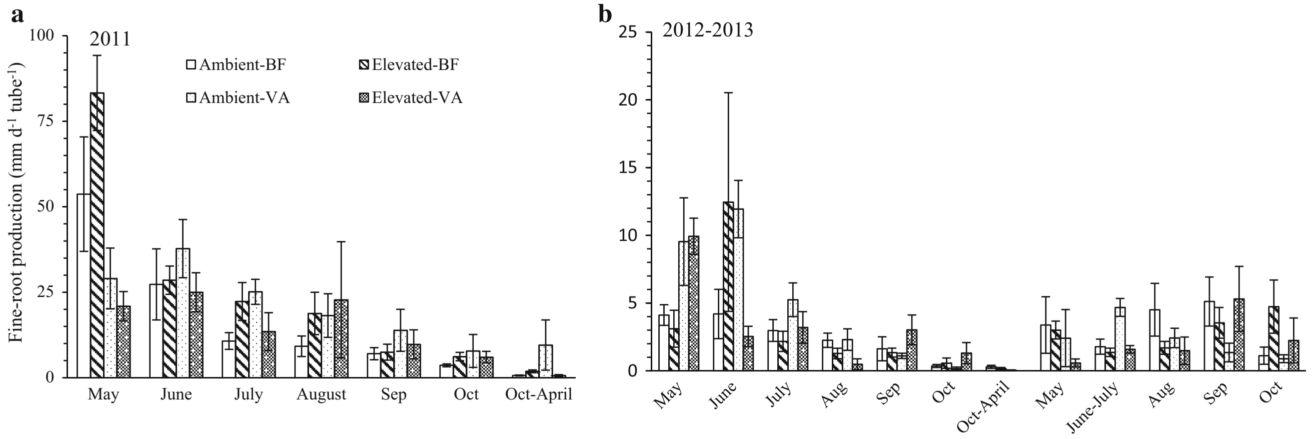

Fig. 2 Fine root length production rate of birch seedlings growing under elevated and ambient [CO_2] on volcanic ash (VA) and brown forest (BF) soil. Each value is the mean of six replications; the *vertical bar* in the column denotes SE. The letters **a** and **b** denote the graph of different year

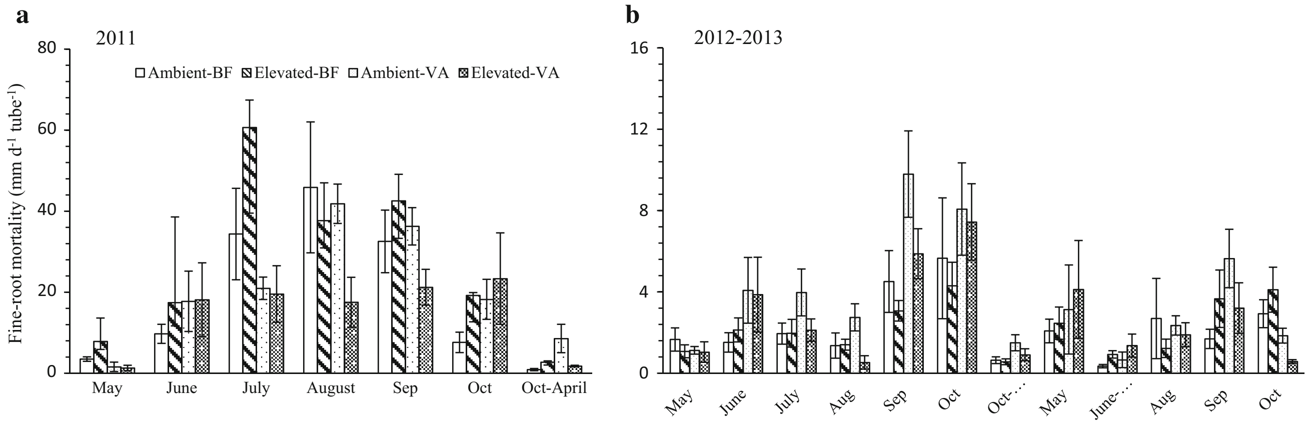

Fig. 3 Fine root length mortality rate of birch seedlings growing under elevated and ambient [CO_2] on volcanic ash (VA) and brown forest (BF) soil. Each value is the mean of six replications; the *vertical bar* in the column denotes SE. The letters **a** and **b** denote the graph of different year

Table 2 Turnover of fine root production and mortality of each observed year (yr^{-1})

Year	Soil	CO_2	Production		Mortality	
			$ALRP/LRL_{max}$	$ALRP/LRL_{mean}$	$ALRM/LRL_{max}$	$ALRM/LRL_{mean}$
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

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* not significant

CO_2 did not significantly affect the turnover of production and mortality, but there was an interaction effect of year and CO_2 on mortality turnover. Over time, it was reduced by elevated CO_2 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 was 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 CO_2 and soil, or CO_2, 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).

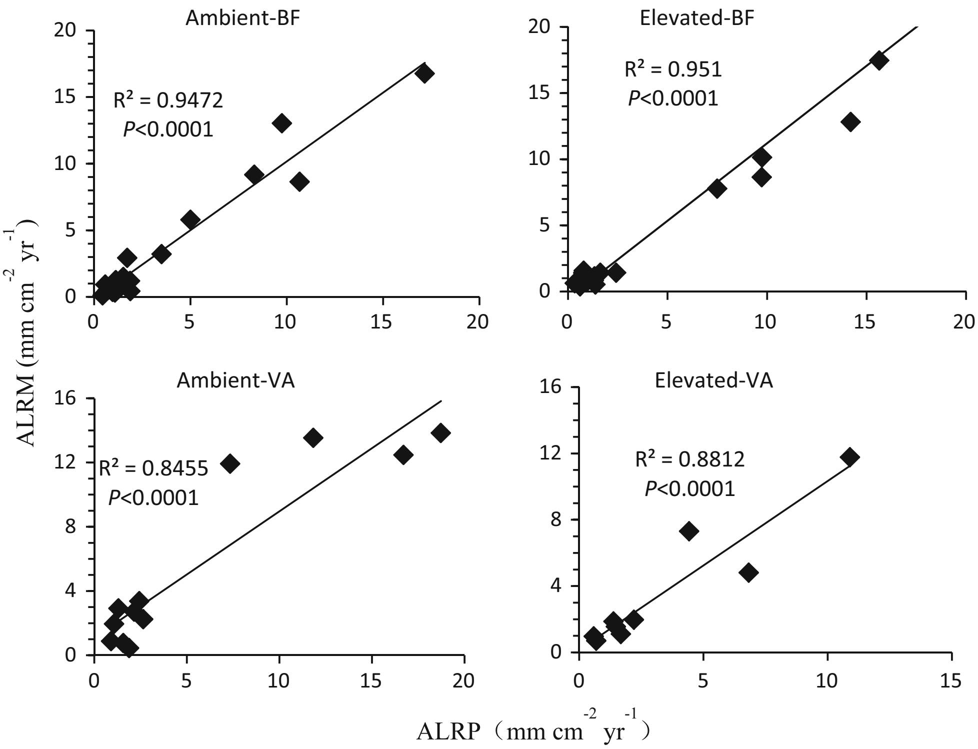

Fig. 4 Relationship between annual length-based root production (ALRP) and annual length-based root mortality (ALRM) in each tube and treatment. Pearson correlation test showed $P < 0.0001$

Fine root longevity

The median fine root longevity was estimated by the different treatments and root diameters. Overall, the median root longevity differed with different treatments, and it was increased under elevated CO_2 in 2011 for BF soil and VA soil (Table 3). From 2012 to 2013, compared to the ambient treatment, the relatively longer median fine root longevity under elevated CO_2 was gradually reduced in BF soil. The increase of median fine root longevity compared to ambient treatment was weakened by elevated CO_2 in VA soil.

Median root longevity of different diameter classes showed significant responses to different treatments. The thinnest fine root ($D < 0.2$ mm) was not affected by elevated CO_2 in all conditions in 2011 and 2012, but it was increased by elevated CO_2 in BF soil and reduced in VA soil in 2013 (Table 4). Root longevity of roots with diameters between 0.2 and 0.3 mm was markedly increased by elevated CO_2 except in 2013 in BF soil, where longevity was, in contrast, reduced by elevated CO_2. The root longevity of roots with diameters between 0.3 and 0.4 mm was increased by elevated CO_2. But there were no effects on the median longevity of the roots with diameters

Table 3 Fine root lifespan (weeks) of birch seedlings growing under elevated and ambient [CO_2] on volcanic ash (VA) soil and brown forest (BF) soil

Year	Longevity	Ambient BF	Elevated BF	Ambient VA	Elevated VA	*P* 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): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 4 Fine root lifespan (weeks) in different root diameter classes of birch seedlings growing under elevated and ambient [CO_2] on volcanic ash (VA) soil and brown forest (BF) soil

Year	D (mm)	Ambient BF	Elevated BF	Ambient VA	Elevated VA	P value
2011	$D < 0.2$	12 (2.76)	12 (0.69)	15 (1.08)	15 (3.93)	ns
	D 0.2–0.03	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 0.2-0.03	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 0.2–0.03	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

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

Significance was tested by Log Rank (Mantel-Cox): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, *ns* not significant

between 0.4 and 0.5 mm by elevated CO_2 in 2012; the same results were attained for roots with a diameter larger than 0.4 mm in 2013.

Discussion

Fine root length standing crop

We found that the LRL (live root length or length-based standing crop) was significantly influenced by elevated CO_2 treatment. In BF soil, LRL was not affected by elevated CO_2 in first year, but it was reduced by elevated CO_2 from the second year onwards. In contrast, under VA soil, elevated CO_2 reduced the LRL for all three observed years (Fig. 1). Generally, elevated CO_2 stimulates plant growth (Norby and Zak 2011) and more carbon is allocated to the roots (Lukac et al. 2003), therefore, that is why elevated CO_2 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 CO_2 (Arnone et al. 2000; Higgins et al. 2002). These present results have proved this point and suggest that the stimulated effects of elevated CO_2 diminished over time. On the other hand, the downregulation of photosynthesis was frequently observed in the seedling and sapling stages 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 CO_2 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 conditions 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 3 years, because wide roots system were efficient for limited nutrient uptake (Ryser 2006). Moreover, the downregulation 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 CO_2 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 CO_2 to root growth was minified. In our case, elevated CO_2 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 CO_2 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 3 years in VA soil.

Additionally, the negative effect of elevated CO_2 on LRL from the second year in BF soil and throughout the 3 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 CO_2 can lead to a reduced LRL. Overall, we did not find any consistent trend for root production rate and mortality rate (Figs. 2, 3). Also a strong correlation of ALRP and ALRM was found in all treatment conditions suggesting that the root production and mortality were equal within 1 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 CO_2 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 CO_2 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 CO_2. Our case is still shorter than 6 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 CO_2 reduced turnover of FRM over time. It could contribute to a longer lifespan with CO_2 enrichment as we detected (Table 3). Additionally, Eissenstat et al. (2000) concluded that elevated CO_2 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 CO_2. Turnover of FRM was increased by elevated CO_2 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 CO_2 (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 CO_2 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, the 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 CO_2 treatment.

Fine root lifespan of white birch under elevated CO_2

The median longevity was initially increased by elevated CO_2 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 CO_2 in VA soil (Table 3). As it has been reported, plants under elevated CO_2, generally increase water use efficiency and dramatically stimulate aboveground growth (Qu et al. 2004; Koike et al. 2010). Furthermore, under elevated CO_2, root uptake provides nutrient resources primarily for CO_2-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 CO_2. One possibility is that nutrient limitation resulted in a lower turnover of FRP and FRM under elevated CO_2 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 the 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 CO_2 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 CO_2 since 2013 after two growing seasons. The fine root lifespan of the other diameter orders increased initially and was unaffected under elevated CO_2 ($D > 0.4$ mm) in 2013. As reported, plants usually increase mycorrhizal colonization and decrease root N concentration under elevated CO_2 (Pritchard and Rogers 2000; Tingey et al. 2000). Given that the root longevity is negatively correlated with tissue N concentration (Pregitzer et al. 1998), we concluded that the mycorrhiza symbiosis was strongly stimulated under elevated CO_2 in the beginning (e.g., Wang et al. 2015). As a result, fine root performed a longer lifespan with no distinct effect by CO_2 enrichment in this study. Moreover, mycorrhizal colonization under elevated CO_2 has been found to stabilize over time. Shinano et al. (2007) found that with elevated CO_2, 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 influence 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 CO_2 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 CO_2 enrichment. Elevated CO_2 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 CO_2 compared with ambient CO_2 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 CO_2 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 changing environments.

Author 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.

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 Leppälammi-Kujansuu for their guidance on data analysis. Thanks are also given to Dr. Anthony Garrett of SCITEXT of Cambridge, UK and Ms. Amelie Vanderstock of Biological Institute of The University of Sydney, Australia for English improvement. This study was supported by the Japan Society for the Promotion of Science New field and Type B program (to T. Koike, 21114008 and 26660119).

Compliance with ethical standards

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

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