Fine roots branch orders of *Abies faxoniana* respond differentially to warming in a subalpine coniferous forest ecosystem

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**Abstract** Root is an important plant organ and has high heterogeneity. Global warming could change root and affect belowground ecological processes. There is little information on how fine roots branch orders responds to global change. This study examined the growth, morphological and physiological responses of fine roots of a subalpine coniferous species to warming. We investigated biomass, average diameter, specific root length (SRL), triphenyltetrazolium chloride (TTC) reducing capacity, carbon (C), total non-structural carbon (TNC) and fractions of the primal five branch order roots of *Abies faxoniana* in April, August, October and December. The decrease in total fine roots biomass after a growing season was significantly greater under warming treatment compared to control, suggesting that warming could accelerate the carbon input from root to soil, but the increment depended on tree species. Warming did not affect average diameter and SRL. Responses of biomass, TTC reducing capacity, C, TNC and fractions to warming significantly differed with root order and month. Significant warming effects were only observed in C and starch concentration of the first order and also TNC and soluble sugar concentration of the first three orders. The results indicated that the lower order roots (the first three orders) were more sensitive to warming, probably because they had more frequent, intense interactions with soil and low defense capability. Thus, global warming may dramatically alter root functions such as nutrients and water uptake as well as the cycle of C and nutrients at the whole subalpine coniferous forest ecosystem.

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Introduction

Global mean air temperature has been predicted to increase by 1.8–4.0 °C over this century (IPCC 2007), with a greater warming in the higher latitudinal and altitudinal ecosystems. This increase is associated with a stronger warming in nighttime temperature (daily minima) than daytime temperature (daily maxima) (Easterling et al. 1997). Warmer air temperatures would likely result in warmer soil temperatures, which could, in turn, affect the growth and function of root which is the hidden half of a plant under the ground. Root not only is responsible for plant growth and functional processes (Yin et al. 2014) but also plays an important role in regulating the response of terrestrial ecosystem to global climate warming (Schmidt et al. 2011; Phillips et al. 2012; Tefs and Gleixner 2012). Therefore, the ecological consequences on root arising from global warming have become an important issue of global change research (Bai et al. 2012). Most studies are conducted in the growing season or non-growing season, while little is known about the effect of global warming on root during the whole year.

Over the past few years, studies about terrestrial ecosystem structure and function have focused increasingly on the role of fine roots in carbon (C) cycling dynamics. Comprehensive reviews have showed that fine roots may account for nearly 30 % of total net primary production (Jackson et al. 1997). Fine roots of tree underwent constant replacement in root turnover and provided a large biomass input to the soil including both C and mineral nutrients (Trumbore and Gaudinski 2003). It was reported that tree fine roots in forest soil made up about 4 % of the total C in the soil (Brunner and Godbold 2007). Hence, fine roots are an important component of soil C. It has been widely recognized that soil temperature can exert a large influence on root biomass allocation and root turnover and that these in turn have considerable impact on soil C dynamic and functioning of terrestrial ecosystem (Yin et al. 2012). For example, Zhou et al. (2012) has proved that warming accelerate root death and turnover in tallgrass prairie. Previous studies on the effects of warming on soil C input were only investigated in a few individual species, such as Picea asperata (Yin et al. 2014). Few studies, however, had considered the other coniferous species. It is essential to incorporate the effects of warming on different coniferous species for better simulating and predicting the responses and feedbacks of C cycling in coniferous forest ecosystems to global warming.

Researchers have found that fine roots had a complex architecture and high heterogeneous function (Zadworny and Eissenstat 2011). Many root characteristics, such as root anatomy, morphology, physiology and so on, had striking differences among fine roots orders (Pregitzer et al. 2002; Valenzuela-Estrada et al. 2008; Espeleta et al. 2009; Rewald et al. 2012). Consequently, root branch order was considered as a fundamental characteristic that can be intimately linked to root functions (Fitter 2002). Furthermore, Xia et al. (2010) demonstrated that root systems of perennial plants may be organized like shoot systems, with rapidly cycling modular units devoted to energy-intensive resource-acquisition processes located at the distal ends of the branching perennial woody shoot or root systems. Due to their sensitivity to environment (Guo et al. 2004; Chen et al. 2013), large root length, strong physiological activity (Pregitzer et al. 1998) and large contribution to total net primary production (Jackson et al. 1997), fine roots are recognized all through as the keystone of plant root. Moreover, recent researches have highlighted the necessity for more information on how heterogeneous fine roots and associated ecological processes response to climate change (Guo et al. 2004; Kou et al. 2015). However, to date, relationships between fine roots branch orders and climate change are unclear. Especially, do the responses of distal lower order roots to climate change exhibit modularized character that distinctly different from those of higher-order woody roots?

The subalpine coniferous forest ecosystem in the Eastern Tibetan Plateau, located at the transition zone from the Qinghai-Tibet Plateau to the Sichuan Basin, is sensitive to global climatic change, and it is important consequences for global C (Wang et al. 2003). Moreover, the subalpine coniferous forest ecosystem has higher species diversity and complexity, including trees (i.e. Abies faxoniana, Betula albosinensis), shrubs (i.e. Lonicera spp., Rosa spp., Acanthopanax gracilithlus), and herbaceous plants (i.e. Smilax spp., Girardinia suborbiculata, Oxalis
griffiti, Anemone rivularis, Asarum himalaicum), and these plants are almost all perennials. Belowground interactions in such systems, both complementary and competitive, are a function of species rooting patterns (Jose et al. 2006). The growth, morphological, and physiological characteristics of lateral root in the contact zone of different root systems may be seen as an adaptive mechanism by which plants avoid excessive intra- and inter-specific competition (Zamora et al. 2007). In the subalpine coniferous forest ecosystem, A. faxoniana, (1) is widely distributed and important species; (2) is a dominant species in natural ecosystems; and (3) has shallow root system, and then is more susceptible to soil environment. Thus, understanding responses of A. faxoniana root to global warming is conducive to more preferentially maintain the stability of the whole ecosystems. It is hypothesized that (1) warming would stimulate root C input into soil for A. faxoniana; (2) warming effects on fine roots growth, morphology and physiology of A. faxoniana would be different among various branch orders.

Materials and methods

Experimental design and treatments

The experiment was conducted in a 15 × 6 m field located at the Maoxian Ecological Station of the Chinese Academy of Sciences, Sichuan Province, China (31°41′N, 103°53′E, 1820 m altitude), where mean annual temperature, precipitation and evaporation are 9.3 °C, 825.2 and 968.7 mm, respectively. There were eight 2 × 2 m plots (four warmed plots and four control plots). The warmed plot was heated by an infrared heater suspended 1.5 m above the middle of the plots according to previous studies (Yin et al. 2013). Consistent with the experiment design described by Yin et al. (2014) in the same experimental site, uniform 4-year-old A. faxoniana seedlings from a local nursery were selected in October 2010. The soil was classified as a mountain brown soil series (Chinese taxonomy). The soil properties determined in October 2010 were as follows: pH 6.8; total N, 4.0 g kg⁻¹; soil organic C, 61 g kg⁻¹; and bulk density, 0.91 g cm⁻³. Artificial night warming was conducted from 7:00 pm to 7:00 am (12 h day⁻¹) all year around. All of the plots were weeded as necessary and watered to maintain consistent soil water content.

Microclimate measurement

Air temperature (at a height of 20 cm above the ground) and relative humidity were measured using DS1923G temperature/humidity iButton data loggers. Soil temperatures (at a depth of 5 cm) were measured using DS1921G Thermochron iButton data loggers (DS1921G-F5, Maxim Integrated Products, Dallas Semiconductor, Inc., Sunnyvale, California, USA) in eight plots at an interval of 30 min during the experimental period. Daily air temperature (at 20 cm aboveground) and soil temperature (at 5 cm depth) in the warmed plots were on average increased by 2.15–3.17 °C, respectively, as compared with control plots (Fig. 1a, b). The mean air relative humidity was slightly lower in the warmed plots than the control plots, with humidity of 81.75 % in the warmed plots and 89.42 % in the control plots (Fig. 1c). Moreover, there was no obvious difference in soil water content between the control (23.41 %) and the warmed plots (23.53 %; Fig. 1d).

Root sampling and branch order classification

Root samples were collected in the middle of April, August, October and December in 2013. We randomly selected three seedlings (including shoot and root) from each plot and sampled them by excavating the intact soil block of 50 × 50 × 40 cm (length × width × depth) around the seedling and then transported them to the laboratory by ice-boxes within 6 h. In the laboratory, the root in each soil block was sorted according to Guo et al. (2004). Five root samples per seedling were then selected, and each root sample contained at least eight intact distal root segments, including more than five root orders. The samples were dissected into different orders as described in a previous study (Pregitzer et al. 2002), in which the most distal root tips are classified as first-order root; and second-, third-, fourth-, and fifth-order root were dissected strictly according to the order of streams in geography.

Three order classes were separated as following in this study. The first order was be a unique unit of fine roots system, because they were the most distal root,
and exhibited primary anatomical structure, a high mycorrhizal colonization rate, most analogous to leaves (Withington et al. 2006) and most plastic responses to changes in the soil (Wang et al. 2006); second and third orders had exhibited highly synchronous changes in tissue nutrient flux and respiration, dominated root turnover, and hardly underwent secondary development, which was defined as a separate class (Guo et al. 2008; Xia et al. 2010); fourth and higher orders showed well-developed secondary anatomical structure, lacked mycorrhizal colonization, a much lower mortality rate than roots of the first three orders, and thus was defined as a separate class (Xia et al. 2010). The sorted root was separated
into two portions. The small portion was used to
determine root triphenyltetrazolium chloride (TTC)
reducing capacity. The other portion was used in root
biomass, morphological, and chemical analyses. All of
the root were then frozen (4 °C) until subsequent
processing.

Root measurements

According to Nan et al. (2013) and Pregitzer et al.
(2002), the diameter, length, and surface area of the
first five orders in the whole seedling were analyzed
using the WinRHIZO image analysis software (Régent
Instruments, Quebec, QC, Canada). The respective
volume of the first five orders per seedling was
calculated through the regression of the volume on
surface area. The respective biomass of the first five
orders per seedling was calculated using the formula
based on their volumetric percentage to those of the
fine roots (Cheng et al. 2008; Nan et al. 2013). Specific
root length (SRL) can be calculated as the ratio of the
total root length and biomass of each order. After plant
height and basal diameter were measured using tape
and Vernier caliper, the shoot biomass of each
seedling was measured after the sample was dried in
an oven at 70 °C for 48 h.

To reduce the error, we mixed all of the samples in
the same treatments according to the same root order.
Root TTC reducing capacity was determined with the
method described by Ruf and Brunner (2003). Total C
was determined with an elemental analyzer (Vario EL
Universal CHNOS Elemental Analyzer, Elementar,
Hanau, Germany) as Yin et al. (2014). Total non-
structural carbon (TNC) concentrations were analyzes
through a 2-step process in which sugar and sharch
concentrations were determined separately with mod-
ified phenol–sulfuric acid method (Buysse and Mer-
ckx 1993).

Statistical analysis

The values reported were the mean of four replicates.
Three-way analysis of variance (ANOVA) was per-
fomed to examine the effects of root orders, warming,
and month on all variables. In this study, the effect of
root order on the root traits was significant. So in each
branch order root, we examined the effect of month
and warming treatment by two-way ANOVA to reveal
the different responses of branch order to warming in
different months. The differences of root traits
between warming and control plots in the same root
order or month were analyzed using one-way
ANOVA. Tukey’s HSD test was also carried out for
multiple comparisons. The data were transformed
when necessary using ln-transformation to satisfy
normality and homogeneity of variances. The overall
data was statistically analyzed in the SPSS 13 program
(SPSS, Chicago).

Results

Plant height, basal diameter and biomass

Warming did not significantly affect plant height and
basal diameter among four sampling months, while
increased aboveground biomass, root biomass and total
biomass in October and December (Table 1). In addition,
the basal diameter, aboveground biomass, root biomass
and total biomass all varied significantly with month and
the interactions of warming and month (Table 1).

Root biomass and TTC reducing capacity

Root biomass varied significantly with root order and
month, while there was no marked warming effect on
root biomass (Fig. 2a). Roots biomass increased with
root order. Irrespective of both warming and root order,
root biomass was larger in April than those in the other
three months, while there was no difference between the
later three months (Fig. 2a). There were not two-way
interactions of warming and month on root biomass
(Table 2). Root order and month had significant effects
on root TTC reducing capacity (Fig. 2b). The interac-
tions of warming and month on TTC reducing capacity
were detected in every root order (Table 2).

Average root diameter and SRL

Average root diameter and SRL were affected signif-
ically by root order and month, while there was no
obvious warming effect on them (Fig. 3). Average
diameter increased and SRL decreased with root order
(Fig. 3). ANOVA results indicated that average
diameter and SRL of all the first five orders were
different with month, and only the SRL of the first
order root had two-way interactions with warming and
month (Table 2).
Root C and total non-structural carbon (TNC) concentration

C concentration in root increased observably with root order (Fig. 4a), and for all the first five order roots, the warming effects on C concentration depended on month (Table 2). Warming significantly increased C concentration in August and October, but remarkably lowered it in April and December, except for the fourth and fifth orders (Fig. 4a). Root TNC concentration also increased with root order. TNC concentration significantly (especially the first three orders) reduced in April, August and October but significantly increased in December under warming treatment (Fig. 4b; Table 2). In addition, the interactive effects of warming and month were significant on TNC concentration of the first five orders (Table 2).

Table 1  Effects of warming and month on plant height, basal diameter, aboveground biomass, root biomass and total biomass in Abies faxoniana

| Month   | Treatments | Plant height (cm) | Basal diameter (mm) | Aboveground biomass (g) | Roots biomass (g) | Total biomass (g) |
|---------|------------|------------------|---------------------|------------------------|-------------------|-------------------|
| April   | Control    | 31.7 ± 3.0a      | 12.4 ± 1.3c         | 40.2 ± 6.4c            | 12.9 ± 1.9d       | 53.1 ± 8.2c       |
|         | Warming    | 39.3 ± 8.4a      | 12.8 ± 1.4c         | 42.6 ± 16.7c           | 17.3 ± 5.7c,d     | 59.9 ± 19.8c      |
| August  | Control    | 42.7 ± 5.5a      | 12.8 ± 2.3c         | 63.91 ± 3.3b,c        | 26.2 ± 2.4b,c     | 90.2 ± 2.7b       |
|         | Warming    | 23.3 ± 6.4a      | 12.7 ± 0.3c         | 78.7 ± 8.0b           | 36.1 ± 6.6b       | 113.8 ± 14.6b     |
| October | Control    | 31.7 ± 2.4a      | 13.9 ± 0.4c         | 46.3 ± 9.0c           | 17.9 ± 2.4d       | 64.2 ± 9.3c       |
|         | Warming    | 35.3 ± 1.2a      | 16.5 ± 2.0b         | 98.6 ± 21.0a          | 51.9 ± 10.3a      | 150.4 ± 31.3a     |
| December| Control    | 36.3 ± 3.2a      | 20.1 ± 1.1a         | 47.0 ± 15.1c          | 21.5 ± 5.2c       | 68.5 ± 19.5c      |
|         | Warming    | 26.3 ± 5.4a      | 16.7 ± 1.3b         | 90.9 ± 14.9a          | 39.9 ± 4.7b       | 130.8 ± 17.4a     |

P value  

Fo: P < 0.001  
Fw: P = 0.220  
Fm: P = 0.115

Values are mean ± SE. Values in rows with different letters denote significant differences among different month and treatments at P < 0.05 level for a given variable. P values smaller than 0.05 are bold.

Fig. 2  Effects of warming and month on root biomass (a) and TTC reducing capacity (b) in Abies faxoniana root order. Error bars represent one standard error of the mean. Fo, Fw and Fm respectively represent root order effect, warming effect, and month effect. ***, ** and * indicates significant difference between control and warming treatment in the same root order and month at P < 0.001, 0.01 and 0.05, respectively.

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Root soluble sugar and starch concentration

As the components of TNC, soluble sugar and starch concentration increased with root order in all the four months (Fig. 5). Soluble sugar concentration of the first three order roots was reduced in April, August and October while that of the later two order roots hardly affected by warming (Fig. 5a; Table 2). There was not warming effect on soluble sugar concentration in December. ANOVA results showed that only the starch concentration of the first order root was influenced by warming (Table 2), with decrease in

Table 2 Results of two-way ANOVA showing the \( P \) values for responses of root character in *Abies faxoniana* root order to warming and month

| Root order | Effect | Biomass (g) | Diameter (mm) | SRL (cm g\(^{-1}\)) | TTC reducing capacity (mg g\(^{-1}\) FW h\(^{-1}\)) | Carbon concentration (g kg\(^{-1}\)) | TNC concentration (g kg\(^{-1}\)) | Soluble sugar concentration (g kg\(^{-1}\)) | Starch concentration (g kg\(^{-1}\)) |
|------------|--------|-------------|--------------|--------------------|----------------------------------|-------------------------------|-----------------|-------------------|------------------|
| 1st root   | Fw     | 0.588       | 0.376        | 0.932              | 0.146                            | \(<0.001\)                    | \(<0.001\)      | \(<0.001\)        | 0.013            |
|            | Fm     | \(<0.001\)  | \(<0.001\)   | \(<0.001\)         | \(<0.001\)                      | \(<0.001\)                   | \(<0.001\)      | \(<0.001\)        | 0.001            |
|            | Fw x m | 0.201       | 0.433        | \(<0.001\)         | \(<0.001\)                      | \(<0.001\)                   | \(<0.001\)      | \(<0.001\)        | \(<0.001\)       |
| 2nd–3rd root| Fw     | 0.053       | 0.990        | 0.971              | 0.977                            | 0.887                        | 0.041           | \(<0.001\)        | 0.389            |
|            | Fm     | 0.008       | 0.032        | \(<0.001\)         | \(<0.001\)                      | \(<0.001\)                   | \(<0.001\)      | \(<0.001\)        | \(<0.001\)       |
|            | Fw x m | 0.655       | 0.242        | \(<0.001\)         | \(<0.001\)                      | \(<0.001\)                   | \(<0.001\)      | \(<0.001\)        | 0.003            |
| 4th–5th root| Fw     | \(<0.001\)  | 0.061        | 0.575              | 0.267                            | \(<0.001\)                   | \(<0.001\)      | \(<0.001\)        | \(<0.001\)       |
|            | Fm     | 0.003       | 0.061        | 0.373              | \(<0.001\)                      | \(<0.001\)                   | \(<0.001\)      | \(<0.001\)        | \(<0.001\)       |
|            | Fw x m | 0.510       | 0.322        | 0.872              | \(<0.001\)                      | \(<0.001\)                   | \(<0.001\)      | \(<0.001\)        | \(<0.001\)       |

Fw, Fm and Fw x m respectively represent warming effect, month effect, and interaction effect of warming and month. \( P \) values smaller than 0.05 are bold

SRL specific root length, TNC total non-structural carbon

Fig. 3 Effects of warming and month on average root diameter (a) and specific root length (SRL, b) in *Abies faxoniana* root order. Error bars represent one standard error of the mean. Fo, Fw and Fm respectively represent root order effect, warming effect, and month effect. \(* * *\), \(* *\) and \(*\) indicates significant difference between control and warming treatment in the same root order and month at \( P < 0.001, 0.01 \) and 0.05, respectively

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Discussion

In the present study, warming increased the whole plant biomass of the *A. faxoniana* plots in October and December, but did not have a significant effect on those in April and August. It indicated that the effect of warming on plant biomass depended on month.

April, August and October and increase in December (Fig. 5; Table 2).

Although fine root (diameter < 2 mm) constitutes only a small fraction of root system, it is an important source of soil organic C and plays a key role in the regulation of ecosystem C cycle, and may account for nearly 30% of total net primary production (Jackson...
et al. 1997). Temperature is a key factor that dominates root dynamic and C turnover, and a lot of studies have demonstrated that fine roots biomass increased with soil warming (Leppälammi-Kujansuu et al. 2013; Jaana et al. 2014). Nevertheless, our results demonstrated that effects of warming on fine roots biomass varied with root order and month (Fig. 2a; Table 2), and that only biomass of the later two orders in August was enhanced by warming, similarly with previous study where root was classified by their diameter (Pilon et al. 2013).

In this study, the decrease in total fine roots biomass between April and December under warming treatment was significantly larger compared with control (2.94 ± 0.63 and 2.04 ± 0.47 g plant\(^{-1}\) for warming and control treatment, respectively). Bai et al. (2012) observed that warming had no effect on root lifespan, survival rate and longevity of the root initiated in summer. According to Yin et al. (2014), the root lifespan of the experimental seedlings is about or more than 100 days. Our results estimated that warming can accelerate the carbon input from root to soil with an increase of about 44 %, i.e. 0.90 g fine roots plant\(^{-1}\), and equal to about 0.38 g C plant\(^{-1}\) (C concentration was 422 g kg\(^{-1}\), the mean value in this study). Similar results have also been reported in previous studies. For example, Jaana et al. (2014) has proved that warming increased fine roots C input to the soil in Picea abies, and the increased amplitude was about 184 %. Likewise, Yin et al. (2014) also showed that warming accelerated the carbon input from root to soil in P. asperata, and the increased amplitude was about 85 %. The results showed clearly that warming accelerated fine roots C input to soil, and the increased impact varied with tree species. It has been reported that tree species can have a remarkable effect on root production and root turnover rate (Luke et al. 2014), root decomposition (Fan and Guo 2010), C released from the root (i.e., root exudates; Jones et al. 2009) as well as soil environment (Hobbie 1992) and soil microflora (Zhao et al. 2014), which may result in the establishment of different C exchange of root-soil interface and thus brought differences in C input from root.

Root growth is closely dependent on soil temperature (Pregitzer et al. 2000), since soil temperature affected growth of root system components, initiation and branch, orientation and direction of growth (Kaspar and Bland 1992). In our study, root diameter and SRL of A. faxoniana were not sensitive to warming irrespective of root order and month. The lack of a remarkable warming effect on root morphology was consistent with the finding of the studies conducted in dry meadow and subalpine forest ( Björk et al. 2007; Duan et al. 2013). The effects of warming on root diameter and SRL, however, varied among different studies. For example, Leppälammi-Kujansuu et al. (2013) reported that warming significantly increased SRL in fine roots of Norway spruce. The variability of these results may result from the diversity of soils and plant species.

Root physiology is one of the crucial research priorities of improving predictions of tree response to environment changes (Smithwick et al. 2013). Changes in direct physiological activity of root under environmental change would lead to alteration in plant growth and root lifespan. TTC reducing capacity is directly linked to respiration rate and is a reliable indicator of respiration activity (Ruf and Brunner 2003; Yin et al. 2014). A lot of studies have demonstrated that fine roots TTC reducing capacity increases with warming (Jarvi et al. 2013). Nevertheless, our results clearly demonstrated that warming effects on TTC reducing capacity of A. faxoniana varied with month (Table 2; Fig. 2b). Moreover, individual branch of fine roots also exhibited different responses to warming in the same month. Similarly, warming effects on root C, TNC and fractions were different with order, which also depended greatly on month in this study. Warming effects on C concentration of A. faxoniana were similar to that of P. asperata reported by Yin et al. (2014), while the effects of warming on TNC and fractions were different between two species, especially in April and December (Yin et al. 2014), which may be explained by inherent differences in growth patterns and phenology between two species. The results are consistent with some previous studies which also have demonstrated significant seasonal difference (Turnbull et al. 2004) and highly species-specific (Shi et al. 2015) between temperature and root TNC.

In our study, significant warming effects were only observed on C and starch concentration of the first order, TNC and soluble sugar concentration of the first three orders in A. faxoniana. These evidences indicated that the lower order roots (the first three orders) were more sensitive to warming. Pregitzer et al. (2002) reported increases in root N concentration after
fertilization were found in only the first three root orders in three North American trees. Guo et al. (2004) suggested only the first four orders exhibited a trend of increasing biomass after N fertilization in *Pinus palustris*. Also, Yin et al. (2014) reported that significant warming effect was observed in TNC and its fraction concentration of only the first three or four order roots in *P. asperata*. Thus, lower order roots are more sensitive to environmental alterations such as changes in soil temperature and fertility. Other studies were unable to recognize this phenomenon, probably because roots were sampled only once or during the same season (Eissenstat et al. 2000).

Why do the lower order roots have more sensitive to soil environment? One of the reasons may be that low order roots have more frequent, intense interaction with soil. Lower order roots are generally absorptive root (Guo et al. 2008). They have high metabolic and respiration rates (Xia et al. 2010), mycorrhizal colonization (Guo et al. 2008), and root exudates (Brown and Gange 1990) and thus release more chemical cues such as carbon dioxide, amino acids and sugars into the rhizosphere soil. What’s more, the changes in the microbial community composition and their activity resulting from the changes of soil environment (i.e. temperature, moisture, nutrient availability; Zhao et al. 2014) may more easily influence low order roots, because low order roots have high N concentration (Guo et al. 2004) and thus supply greater protein and amino acids to feed soil microorganisms (Sun et al. 2011). The other one possible reason was that higher order roots comprised mainly of defensive component (Guo et al. 2008), generally with higher cellulose (Guo et al. 2004) and higher TNC concentration, which may enhance defensive functions (Norisada et al. 2005; Hunter 2008). The phenomenon that lower order roots are more strongly responsive to environmental alterations may has important implication to understanding how global warming affects root growth and functions, as well as C budget and nutrient cycles in forest ecosystems. Firstly, there was good evidence that the lower order roots were the main body of absorptive root. They died rapidly and dominated root turnover (Valenzuela-Estrada et al. 2008; Xia et al. 2010). Global warming primarily influence the lower order roots, including infected mycorrhizal fungi (Guo et al. 2008), which may greatly alter root functions such as nutrients and water uptake as well as the cycle of C and nutrients at the whole ecological system (Hunter 2008). Secondly, the lower order roots having more sensitive to soil environmental alterations in this study supports the view of “root modules” in woody plants (Pregitzer et al. 2002; Xia et al. 2010), i.e. roots of the lower orders may function as a module with the similar longevities in their life cycles as well as the similar anatomical structures (non-woody, primary structure) and physiological functions (absorptions) (Guo et al. 2008). Thirdly, roots of tree and herbaceous species are interconnected and complex systems at the subalpine coniferous forest ecosystem. Root systems of the component species and their associated rhizosphere played major roles in belowground resource competition at the mixed-species forest ecosystem (Jose et al. 2004, 2006). The changes of tree lower order roots under global warming may affect the growth and function of herbaceous species roots by rhizosphere effect (i.e. root exudates, Xiong et al. 2013), then alter belowground interactions and even the whole ecosystem stability. Thus, this information is critical in developing and refining comprehensive interactions among the component species at the mixed-species forest ecosystem under global climate change.

**Conclusions**

Results showed warming accelerate the carbon input from root into soil in *A. faxoniana*, with the increased amplitude of about 44 %. Effects of warming on TTC reducing capacity, C, TNC and fractions varied with root order, and the lower order roots (the first three orders) were more sensitive to warming because they have more frequent, intense interaction with soil and low defense capability. It meant that global warming may greatly alter root functions as well as the cycle of C and nutrients at the whole ecological system because the lower order roots were the main body of absorptive root and dominated root turnover. As a result, the response differences of fine roots branch orders to warming could have important implication for subalpine coniferous forest dynamics. It should be kept in mind, however, that the results offered here must be viewed with caution since the date come from seedlings and the experiment period was only lasted two years. Future studies about adult trees and/or longer-term studies should be conduct to greatly advance our understanding of how tree root–soil
interactions influence belowground ecological processes of forest ecosystem under global climate change.

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