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SHORT COMMUNICATION

Contrasting effects of warming on pioneer and fibrous roots growth in *Abies faxoniana* seedlings at low and high planting density

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Abstract

Key message **Distinct differences in pioneer and fibrous roots acclimation to climate warming.**

Abstract This study was conducted to determine whether belowground parts of plants at different planting density differ in their responses to elevated temperature (ET). We investigated plant growth, pioneer and fibrous roots growth, root nonstructural carbohydrates, and root colonization of *Abies faxoniana* seedlings grown in environment-controlled chambers with two different planting densities. Warming has more pronounced positive effects at low density. Although ET did not affect total root biomass, fibrous roots biomass increased under ET at low planting density while pioneer roots biomass was unaffected by ET, indicating that this species may maintain the main framework of the root system with a high capability for water and N absorption under ET. ET increased root nonstructural carbohydrates concentration and ectomycorrhiza colonization in fibrous roots. Increased root nonstructural carbohydrates in response to ET might be associated with the increased roots ectomycorrhizal infection under ET. The present study provided experimental evidence of distinct differences in pioneer and fibrous roots acclimation to climate change.

Keywords Colonization · Competition · Pioneer and fibrous roots · Root partitioning · Nonstructural carbohydrates

Introduction

Considerable attention has been paid to assessing the effects of climate change on aboveground plant parts in the past decades (Körner 2000; van Wijk et al. 2004; Day et al. 2008; Way and Oren 2010; Dawes et al. 2011). However, relatively little attention has been given to the belowground parts (Olsrud et al. 2010), particularly under competition conditions (Zhang et al. 2008; Hagiwara et al. 2010; Toillon et al. 2013). On the other hand, in woody plants, roots are normally classified into two distinct types: fibrous and pioneer roots (Polverigiani et al. 2011; Zadworny and Eissenstat 2011). Pioneer roots are mainly exploratory roots whereas fibrous roots are for water and nutrient absorption (Xia et al. 2010; Polverigiani et al. 2011; Zadworny and Eissenstat 2011). Despite the different functions of these two root types, there are very limited studies that analyze the ecophysiological responses of pioneer and fibrous roots to climate change.

Root nonstructural carbohydrates could play the role as root exudates that provide energy to the fungus (Lewis et al. 1994; Kobe et al. 2010). It has been suggested that carbon allocation to roots might result in enhanced mycorrhizal colonization under elevated CO_2 (Staddon et al. 2002; Kobe et al. 2010; Keel et al. 2012). However, studies

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of the effects of climate change on mycorrhizae are often short term, which do not reflect long-term trends (Staddon et al. 2003; Clemmensen et al. 2006; Ferguson and Nowak 2011). Furthermore, less is known about the effects of density on plant growth under climate warming.

Abies faxoniana (Rehd. et Wils.), an evergreen alpine and subalpine endemic woody plant species, is widely distributed in the Sichuan and Gansu provinces, Southwest China. It plays a very important role in preventing soil erosion, and in retaining ecological stability. Although *A. faxoniana* is an important forest tree species in China, its belowground responses to elevated temperature over longer time remain largely unknown. Moreover, little is known how warming influences this species at different planting densities. In our study, we examined the effects of long-term exposure to elevated temperature on belowground response in terms of plant growth, pioneer and fibrous roots growth, root nonstructural carbohydrates, and ectomycorrhizal infection in *A. faxoniana* at different planting densities. The objective of this study was to investigate the effects of warming and planting densities on belowground responses of *A. faxoniana* seedlings. We expect that there would be differences in these two types of root responses to elevated temperature. We hypothesized that positive temperature effects on plant growth would result in greater demand for N, thus eventually resulting in increased nonstructural carbohydrates accumulation and root colonization for N uptake and assimilation.

Materials and methods

Growth chambers and seedlings

The experiments were carried out in fumigation chambers at the Maoxian Ecological Station (31°41′07″N, 103°53′58″E, and altitude 1820 m) in Southwest China. Individuals of *A. faxoniana*, preselected for uniform height, were planted into 12 boxes (100 × 100 × 50 cm) filled with sieved surface sandy soil taken from a 30-year-old natural *A. faxoniana*: forest and moved to growth chambers and grown under a controlled environment. Half of the boxes had a density of two individuals per box (low density) and the other half had four individuals per box (high density). The seedlings were irrigated to keep the soil water content at 30–35%, as logged by a computer, which was an approximation of the optimal water content. The computer-controlled temperature system enabled the temperature to be adjusted automatically inside the chambers to ensure the ambient condition, or to achieve a rise in temperature. The treatments were started on 23 March 2011, and ended in September 2014. The raised air temperature was within 1.5–2.5 °C for 80% (ET chambers) of the exposure time. The mesocosms were then placed into six fumigation chambers. Each chamber contained two mesocosms in total. The two different temperature treatments were assigned at random to the different chambers (three chambers for each temperature). The experiment thus consisted of a factorial design of two growth patterns and two temperate levels, resulting in four treatments, replicated thrice.

Growth analysis

For each treatment, plants were randomly selected and harvested at the end of the experiment. Fibrous roots were distinguished qualitatively from pioneer roots based on their relative diameters (pioneer roots, range 0.7–1 mm, fibrous roots range 0.4–0.6 mm). All roots analyzed in the experiment were first or second order (Polverigiani et al. 2011; Zadworny and Eissenstat 2011). Biomass samples were dried (70 °C, 48 h) to constant weight and weighed. Scanned individual roots were then dried and weighed. Images of individual roots were analyzed for length and diameter using WINRHIZO software (Regent Instruments Inc., Quebec, Canada).

Carbon, nitrogen analysis

Pioneer roots fibrous roots were analyzed for carbon (C) and nitrogen (N). Dried samples were ground to a fine powder and passed through a mesh (pore diameter ca. 275 μm). The C and N concentrations in each tissue were determined by flash combustion using a Carlo-Erba EA 1108 analyzer.

Total soluble sugars and starch determination

Fine roots were heated at 70 °C for 48 h to constant weight. The dry tissues were incubated in 80% (w/v) ethanol at 70 °C for 30 min and centrifuged at 5000×*g* for 10 min. Total soluble sugar and starch were estimated by the sulfuric acid assay using 0.2% anthrone in concentrated H_2SO_4 as reagent. The tubes were then placed in boiling water for 15 min, cooled, and the absorbance read at 625 nm according to Yemm and Willis (1954). Both soluble sugar and starch were calibrated using glucose as standard.

Ectomycorrhizal infection

The ectomycorrhizal colonization was estimated by counting the total number of ectomycorrhizal tips per seedling and by calculating percentage of ectomycorrhizal root tips per seedling (Dehlin et al. 2004).

Statistical analyses

All measurements were tested by a two-way ANOVA for the effects of temperature and planting density. Before ANOVA, data were checked for normality and the homogeneity of variances, and log-transformed to correct deviations from these assumptions, when needed. The analyses were performed with the general linear ANOVA model (GLM) procedure of SPSS 11.0 (SPSS Inc., Chicago, IL, USA). Post hoc comparisons were tested using the Tukey's test at a significance level of $P < 0.05$.

Results

Total biomass, root biomass, pioneer and fibrous roots biomass

Elevated temperature (ET) increased total biomass and shoot biomass at both planting densities, but this increase was less pronounced at high planting density (Fig. 1a, c). The ET treatment did not affect total root biomass (Fig. 1b). The ET treatment decreased root/shoot ratio at both planting densities (Fig. 1d). The ET treatments did not affect pioneer roots biomass but increased fibrous roots biomass at low planting density (Fig. 1e, f). Compared to fibrous roots, pioneer roots had higher biomass across all treatments (Fig. 1e, f).

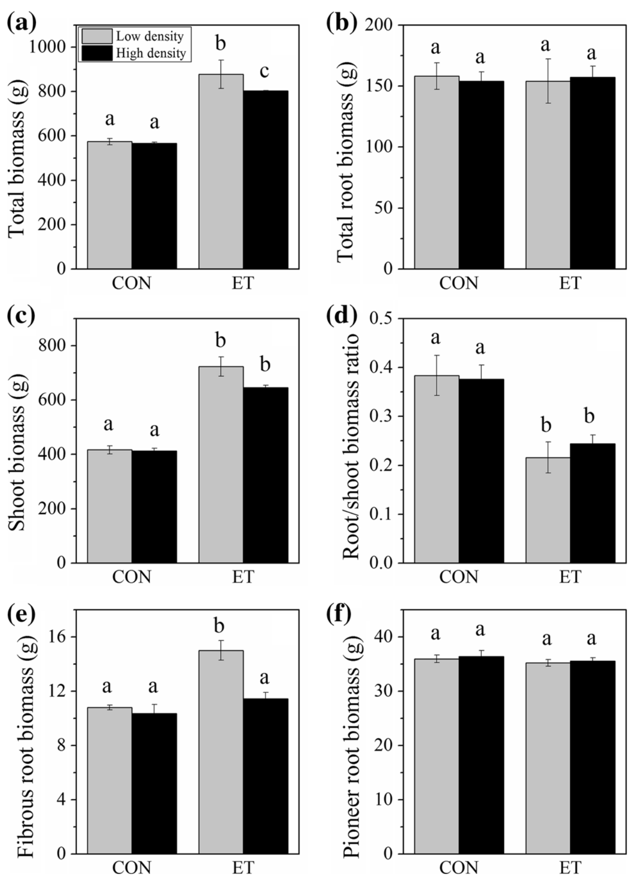

Fig. 1 The effects of elevated air temperature on plant growth of *A. faxoniana* seedlings under low- and high-density treatments. The values not sharing the same letters are significantly different ($P < 0.05$) according to Tukey's test. The values are mean ± SE, $n = 5$

Pioneer and fibrous root N and C/N ratio

The ET treatments did not significantly change pioneer and fibrous roots N at both planting densities (Fig. 2a, c). Fibrous root C/N ratio decreased under ET at both planting densities while pioneer root C/N ratio was unaffected by ET (Fig. 2b, d).

Root sugars and ectomycorrhizal colonization

The ET treatment significantly increased fibrous soluble sugars at high planting density (Fig. 3a). By contrast, the ET treatment had no significant effect on these parameters in pioneer when compared with control seedlings (Fig. 3b). Compared to fibrous roots, pioneer roots had higher roots sugars across all treatments (Fig. 3a, b). No responses of ectomycorrhizal colonization to ET were observed in pioneer roots (Fig. 4b). Ectomycorrhizal colonization in fibrous roots was significantly increased under ET at high planting density (Fig. 4a). Compared to fibrous roots,

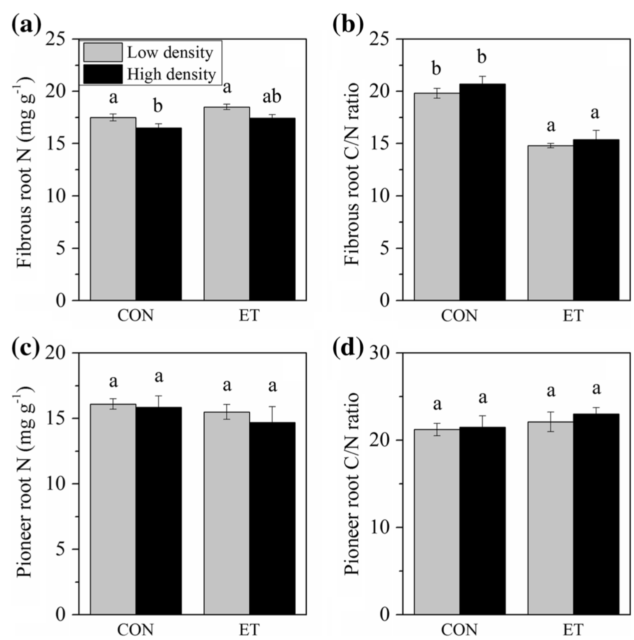

Fig. 2 The effects of elevated air temperature on pioneer and fibrous roots C, N and C/N ratio of *A. faxoniana* seedlings under low- and high-density treatments. The *values not sharing the same letters* are significantly different ($P < 0.05$) according to Tukey's test. The values are mean ± SE, $n = 4$

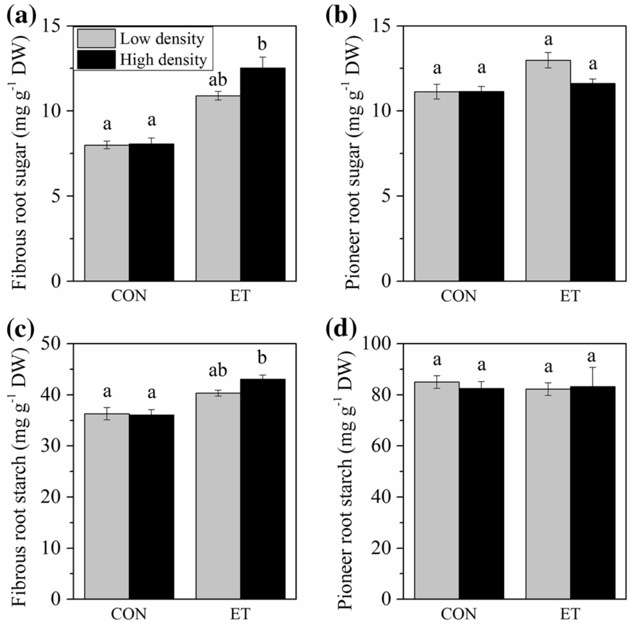

Fig. 3 The effects of elevated air temperature on pioneer and fibrous roots starch and soluble sugars of *A. faxoniana* seedlings at low- and high-density treatments. The *values not sharing the same letters* are significantly different ($P < 0.05$) according to Tukey's test. The values are mean ± SE, $n = 4$

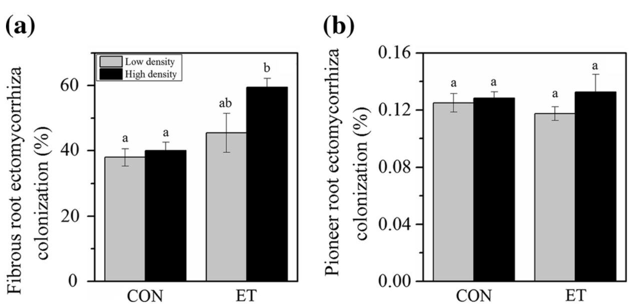

Fig. 4 The effects of elevated air temperature on pioneer and fibrous roots ectomycorrhiza colonization of *A. faxoniana* seedlings at low- and high-density treatments. The *values not sharing the same letters* are significantly different ($P < 0.05$) according to Tukey's test. The values are mean ± SE, $n = 4$

pioneer roots had little root ectomycorrhizal colonization across all treatments (Fig. 4a, b).

Discussion

We found that plant biomass responses to elevated temperature (ET) depended on whether plants were grown at low planting density or high planting density. Total biomass and shoot biomass increased with increasing temperatures at both planting densities. However, the increases were less pronounced for *A. faxoniana* at high planting density, indicating competitive repressive effects on the growth (Benomar et al. 2012). Our study demonstrates that high planting density decreased the temperature-induced total biomass enhancement of seedlings. Nevertheless, there were no differences in aboveground parts (shoot biomass) when subjected to ET at low and high density, indicating high planting density effects being stronger on belowground parts than on aboveground parts. At the same time, *A. faxoniana* increased biomass allocated to shoot growth but decreased biomass allocated to root growth when exposed to ET, which feeds back to faster growth. Although warming increased fibrous roots biomass at low planting density, warming decreased root/shoot ratio at both planting densities, predisposing seedlings to water deficit as less soil water can be taken up. This effect would be more severe in warmer climate with increased evapotranspiration.

Belowground, ET did not affect total root biomass at either planting density. Our results are different from those reported for coniferous species (Tingey et al. 2000). On the other hand, it has been reported that pioneer roots are mainly for nutrient acquisition, while fibrous roots serve a role in water and nutrient absorption (Polverigiani et al. 2011). Meanwhile, our results showed that pioneer roots were unaffected but fibrous roots increased under ET at low plant density, indicating that pioneer roots showed less growth plasticity in response to warming. This may enable plants to maintain the main framework of the root system with a high capability for water and N absorption (Xia et al. 2010; Polverigiani et al. 2011; Zadworny and Eissenstat 2011). In our study, we found that pioneer roots and fibrous roots N concentrations were maintained in response to ET. The maintenance of root N could have resulted from the increase in soil N mineralization and availability under warming conditions (Lukac et al. 2010) through increased ectomycorrhizal colonization in fibrous roots. The corresponding decrease in fibrous root C/N ratio under ET may indicate that the turnover rate of fibrous roots increases under ET (Withington et al. 2006; Terzaghi et al. 2013) and, consequently, the fibrous roots life-span decreases. By contrast, ET did not change C/N ratios in pioneer roots, which means that turnover rate of pioneer roots was unaffected by ET. The high turnover rate under ET conditions probably reflects the higher physiological activity in fibrous roots compared with pioneer roots.

In our study, pioneer roots had higher nonstructural carbohydrate than those in fibrous roots, suggesting that the pioneer root system has an important role in photosynthate storage (Norby et al. 1999; Zadworny and Eissenstat 2011). The increased nonstructural carbohydrate could also be mobilized to support mycorrhizae (Kobe et al. 2010). In our study, increased ectomycorrhizal colonization in fibrous roots under ET at high planting density is consistent with this mechanism. The increased requirements for nutrients at high planting density may stimulate belowground C partitioning towards ectomycorrhizal growth, to compensate for the leaf N requirements for growth in *A. faxoniana* under ET. Moreover, we found that ectomycorrhizal colonization is less in pioneer roots than in fibrous roots as previously proposed (Polverigiani et al. 2011).

Root traits may change with age (Wells and Eissenstat 2003; Zadworny and Eissenstat 2011). This suggests that studies in contrasting fibrous with pioneer roots can be influenced by root age. We assumed that similarity age among fibrous and pioneer roots as fibrous and pioneer roots samples evaluated arose only from first or second order. In our study, the effects of soil depth on roots have not been quantified. However, all roots analyzed in the experiment were first or second order to minimize the influence due to root position within the experiment boxes. Further, whole root systems in our study were exposed to spatially uniform soil root temperature. We found that fibrous roots responded more strongly than pioneer roots as indicated by more enhancements in biomass. This may enable plants under elevated temperature conditions to have high capability for exploration nutrient resources. Moreover, visual inspections of all root systems at harvest time showed that the plants enhanced lateral root formation under warming conditions, which is especially important for phosphate acquisition (Zhu et al. 2006). It has been reported that soil temperature affects phytohormone production (Veselova et al. 2005), which in turn affects plants growth (Koevoets et al. 2016). Further research on this topic is required to provide more insight into how changes in hormone metabolism are involved in the different growth plasticity between pioneer roots and fibrous roots in response to warming.

In conclusion, the long-term exposure of *A. faxoniana* seedlings to warming experiment showed that the total plant biomass was increased under ET, and this increase was less pronounced at high planting density, demonstrating that plant–plant competition may mitigate the effects of climate warming. Belowground, ET did not affect total root biomass, regardless of planting density. Fibrous roots biomass was increased by ET at low planting density, while pioneer root biomass was unaffected by ET under both planting densities. ET also resulted in significantly higher nonstructural carbohydrate in fibrous roots. Increased fibrous roots nonstructural carbohydrate in response to ET might be associated with the increased fibrous roots ectomycorrhizal infection under ET. The present study provided experimental evidence of distinct differences in pioneer and fibrous roots acclimation to climate warming.

Author contribution statement YL contributed to all the experimental process, conducting the experiment, dealing with the data and writing the paper. YZ, SL and SP mainly contributed to the experimental process. BD supervised the research work and revised the manuscript.

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Compliance with ethical standards

Conflict of interest All authors have read and approved the final manuscript and have no conflicts of interest with regard to this research or its funding.

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