Interactive effects of warming and nitrogen addition on fine root dynamics of a young subtropical plantation

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ABSTRACT

Forest aboveground production and soil carbon (C) pools are closely linked to fine root dynamics. Uncertainty about the effects of warming and nitrogen (N) deposition on fine root dynamics limits our ability to predict how C will transfer between biological and atmospheric pools in tropical and subtropical forests. In order to examine the effects of warming and N deposition on fine roots in subtropical plantations, we used a randomized complete block design with factorial soil warming (ambient, ambient + 5 °C) and N deposition (ambient, ambient + 80 kg N ha^{-1} yr^{-1}) manipulation. Minirhizotrons were used to monitor fine root production, mortality and turnover rate of Chinese fir (*Cunninghamia lanceolata*) seedlings for two years, and seedling growth was measured. We found warming had positive effects on annual fine root production, mortality and turnover rate both under ambient and increased N addition. N addition had positive effects on annual fine root production, mortality and turnover rate in the warmed plots, but had no influence on annual fine root production, mortality and turnover rate in the unwarmed plots. Warming and N addition had an additive (not interactive) effect on fine root production, mortality and turnover rate. There was an interaction between warming and N addition on living fine root biomass in the second year. These changes can be largely attributed to belowground/aboveground C allocation. There was no evidence of root respiration acclimation to warming. In addition, increased fine root turnover rate after warming implies accelerated root C inputs to soils, which may affect soil C and nutrient dynamics. Nitrogen addition may exacerbate this. There was no acclimation of root respiration to warming, which may alter C balance and cause more CO_2 release to the atmosphere through autotrophic respiration.

1. Introduction

The impact of climate warming and atmospheric N deposition on patterns and processes of natural forests and plantations has been an important topic in global change studies. However, the majority of manipulative studies on the effects of warming and N deposition were conducted by using either warming or N addition as a treatment (Kern et al., 2004; Mo et al., 2008; Chen and Brassard, 2013; Noh et al., 2016). Because of the co-occurrence of warming and N deposition worldwide, the two major influential global change driver may potentially have interactive effects on forest ecosystem performance and function (Lupi et al., 2012). Therefore, single-factor experiments may be inadequate to fully understand the responses of natural forests or plantations to global warming and N deposition (Dermody et al., 2006). Experiments examining both independent and combined effects of warming and N deposition should be far more informative.

During the past two decades numerous manipulative experiments on the effects of warming or N deposition have been carried out globally (Melillo et al., 2002; Butler et al., 2012; Fan et al., 2015; Hasselquist et al., 2012). However, those studies are mainly concentrated in temperate and boreal regions, with few studies in the tropical and subtropical areas (Zhou et al., 2013; Cavaleri et al., 2015). Tropical and subtropical plant species may be more susceptible to warming than species in temperate or boreal regions as a consequence of millions of years of evolution under relatively narrow temperature variation in the tropics and subtropics (Wright et al., 2009; Krause

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et al., 2013). Compared with temperate and boreal forests, tropical and subtropical forest soils are highly weathered with relatively higher soil N availability (Zhang et al., 2011) and a faster N cycling rate. Thus, the effects of both warming and N deposition on forest ecosystems in the tropics and subtropics could be very different from temperate or boreal forests. Research in tropical and subtropical regions has largely lagged behind despite the fact that these regions are facing substantial increases in both temperature and N deposition (Field et al., 2014; Cavaleri et al., 2015; Kanakidou et al., 2016).

Previous studies have shown that warming and N deposition may directly or indirectly affect above- and belowground processes in forest ecosystems and the consequent responses to the climatic changes (Way and Oren, 2010; Burton et al., 2012; Lu, 2013). Both above- and belowground biomass allocation influences ecosystem function and associated soil carbon input as well as ecosystem carbon cycling (Kuzyakov and Domanski, 2000; Litton et al., 2007). However, the responses of belowground processes, such as fine-root production and turnover rate, have been much less studied than the aboveground processes (Pregitzer et al., 1995; Leppälammi-Kujansuu et al., 2014). Due to the logistical and technical difficulties of fine root extraction and accurate measurement in natural forests and plantations, studies on the effects of warming and N deposition on fine root production, mortality and turnover are extremely limited, especially in the tropics and subtropics (Zhou et al., 2013).

Rhizosphere temperature affects the growth, mortality and turnover of plant roots and consequently influences soil C sequestration and storage (Brunner and Godbold, 2007). To date, research results on the responses of fine roots to warming are inconsistent. For example, King et al. (1999) and Brassard et al. (2009) reported that warming increased the turnover of fine roots in a natural forest but Hollister and Flaherty (2010) and Chen and Brassard (2013) reported that fine root turnover rate was not affected by warming. Eissenstat et al. (2013) found a negative effect of warming on turnover rate of fine roots. The N deposition effects on fine root dynamics appears to have received more attention than warming effects during the past several decades. Some studies found that fine root turnover rate increased with N deposition (Pregitzer et al., 2000; Majdi and Andersson, 2005) but others found it decreased (e.g. Kern et al., 2004). Kang et al. (2016) reported that N deposition did not affect fine root turnover rate. The divergent findings from those studies indicate that the responses of fine root dynamics to N deposition is ecosystem-dependent and that a great deal of uncertainty exists regarding the effects N deposition on fine root dynamics. Previous studies on the effects of warming and N deposition on fine root have mostly focused on individual factors and little attention has been paid to the interaction of warming and N deposition due to cost constraints on this type of environmental research (Zuidema et al., 2013). However, with the increasing warming and N deposition in many regions of the globe, including southeast of Asia, studies on the potential interactive effects of warming and N deposition on root dynamics of natural forests or plantations cannot be neglected anymore (Majdi and Öhrvik, 2004).

Root tissue chemistry and physiology play a key role in controlling the metabolic and decomposition dynamics of roots (King et al., 2005; Zhou et al., 2011). Little information is available on how warming and N deposition will jointly affect the chemistry and physiological characteristics of fine roots, such as N, P, C:N ratio, N:P ratio, soluble sugars, starch and non-structural carbohydrate (NSC) concentration. Yang et al. (2013) reported that increased soil temperature is linked to increased N concentration and N:P ratio in root tissues due to enhanced root activity and N availability at higher temperature. And this may increase the root respiration rate mainly due to it is directly related to tissue N concentration (Pregitzer et al., 1998). It is also of note that roots in warmed soils have a greater respiration rate, which increases the C required for maintenance (Winkler et al., 1996) and may result in more rapid root mortality (Majdi and Öhrvik, 2004). Pregitzer et al. (2000) found that NSC accounted for 4%–23% dry weight of fine roots may act as a

marker of the functional response of fine roots for carbon consumption and storage. These compounds constantly provide C and energy for fine root activities and lack of such compounds could lead to dysfunction of fine roots (McDowell, 2011), and these carbohydrate reserves are allocated to compensatory growth when soil temperature increases. Thus, the information on fine root tissue chemistry should provide insight into the understanding the mechanisms of the effects of warming and N deposition on fine root dynamics.

Chinese fir (*Cunninghamia lanceolata*) plantations are the most important commercial timber source in China, accounting for 6% of the world's plantations. They also play an important role in carbon sequestration in China (Piao et al., 2009). To our knowledge, there have been no field experiments for examining the effects of warming and N deposition on fine roots of Chinese fir plantations. In this experiment, using a two-factorial experimental block design combined with minirhizotron techniques and measurements of seedling growth, we examined the effects of soil warming and N addition on fine root production, mortality and turnover rate of Chinese fir. Our goal was to answer the following questions: (1) How will soil warming and N addition affect fine root production, mortality and turnover rate? (2) Are there synergistic effects between warming and N addition on fine roots? (3) How will soil warming and N addition affect the chemical and functional characteristics of fine roots? (4) How does altered belowground C allocation in response to N addition and/or warming effect aboveground productivity?

2. Materials and methods

2.1. Study site description

This study was conducted at Chenda Research Station of Fujian Normal University in Fujian province of China (26°19′N, 117°36′E), the elevation is 300 m above sea level. The study area has a subtropical monsoonal climate with a mean annual temperature of 19.1 °C and a mean annual frost-free period of approximately 300 days. The mean annual precipitation is 1750 mm with 75% occurring from March to August. The mean annual evapotranspiration and relative humidity are 1585 mm and 81% respectively. Evergreen broad-leaved species are the dominant vegetation in the study area. The parent material of the soils is granite and the soils are classified as red and yellow soils according to the China soil classification systems.

2.2. Experimental design

The warming and N addition experiment was established in October 2013. Randomized complete block factorial design was used in this experiment, with warming and N addition as fixed factors. A total of twenty 2 × 2 m plots were assigned to four treatments: control (C), warming (W, +5 °C), nitrogen addition (N, +80 kg N ha^{-1} yr^{-1}), and warming plus nitrogen addition (WN). The experiment was carried out on a flat area which is not significantly affected by hydrological conditions of neighbor forests (Fig. 1). PVC boards with a thickness of 0.8 cm were inserted into the soil at a depth of 70 cm to separate the plots, and the bottom of each plot has not been isolated. In the warming plots, heating cables (Nexans type TXLP, Oslo, Norway) with a heat output of 100 W m^{-2} and a diameter of 6.5 mm were installed at a soil depth of 10 cm with a distance of 20 cm between the cables. Temperatures in the warming plots were targeted at 5 °C higher than the non-warming plots. Cables were installed in the control plots to compensate for potential soil disturbance effects, but they were not turned on.

The temperature was controlled using individual proportional/integral/derivative (PID) controllers for each plot. Plot temperatures were measured with thermistor temperature probes and the plot temperature was set with reference to control-plot thermistors placed in unheated plots. This control method allowed the heated plots to respond quickly

Fig. 1. The study site a after a month and b after two years.

to changes in soil temperature due to changing soil moisture and daily fluctuations in soil temperature. One-year old *Cunninghamia lanceolata* seedlings with 25.7 ± 2.5 cm in height and 3.4 ± 0.4 mm in basal diameter were planted in November 2013. Each plot was divided into four 1 m × 1 m subplots and a Chinese fir seedling was planted in each subplot (Fig. 1). Temperature sensors (T109; Campbell Scientific Inc., Logan, UT, USA) were placed between cables in both the warming treatments and the controls. Three temperature sensors were used in each warming plot and two were used in each control plot. Two ECH2O-5 soil moisture probes (Decagon, Pullman, Washington, USA) were placed at a soil depth of 10 cm in each plot. Soil temperature and moisture were recorded at an interval of 30 min by a computer-based control system. A weather station near the research site was set up to record weather data. All the installations were completed in August 2013 and soil warming and N addition started in March 2014.

For the N addition treatment, N was applied monthly using a sprinkler filled with a solution of NH_4NO_3 (equivalent to 80 kg N ha^{-1} yr^{-1}). This is 220% of the local N deposition rate which averages 36.3 kg N ha^{-1} yr^{-1} (Zhang, 2013). The control and warming plots received deionized water at the same volume as the NH_4NO_3 solution in the N addition treatment.

2.3. Minirhizotron installation and root image collection

The minirhizotrons were made from clear plastic tubes of 5 cm in diameter to allow for repeated noninvasive measurements of root growth. They were installed at an angle of 45° from the soil surface under two of the four seedlings in each plot at a vertical depth of 50–55 cm. The total length of the minirhzotron was 90 cm. The aboveground part of the tube was covered with a polyethylene tape and sealed with a rubber cap to minimize heat exchange. The rubber cap was covered with another PVC cap to prevent from UV light damage. A total of 10 minirhizotrons (2 per plot) were installed into the plots of each treatment. To minimize installation effects, data were collected 6 months after minirhizotron installation. From March 2014 to February 2016, a digital camera (I-CAP version 4.01, Bartz Technology Corp, Carpinteria, USA) was used to acquire root images biweekly. Forty images (14 × 18 mm) were taken from each tube. Root diameter, new root production, length of live roots and root mortality were recorded (Huang et al., 2016). The roots with black color or shriveled appearance were classified as dead roots (Wells and Eissenstat, 2001). Root production and mortality were further pooled by season: spring (March to May), summer (June to August), autumn (September to November), and winter (December to February). Tree growth, including ground diameter, height and crown size, were measured at an interval of ten days.

2.4. Root production, mortality and turnover estimates

Fine root production between the sampling days was estimated by adding newly grown root lengths to all previous existing roots. Production and mortality were both expressed as root length per window area (m m^{-2}). Annual root turnover rate was calculated as annual root production divided by mean fine root standing crop.

2.5. Fine root respiration, biomass and mycorrhizal colonization measurement

For the fine root sampling, six soil cores were randomly taken with a 3.5-cm soil corer in each plot in January 2015 and 2016. The soil cores were divided into four layers: 0–10, 10–20, 20–40 and 40–60 cm. Soil samples were immediately stored in an icebox and then transferred to the laboratory for analysis. Large roots were carefully picked out from the soil samples with forceps and then were wet-sieved with a 0.5 mm-mesh sieve. The sieved soils were placed in deionized water at a temperature of 1 °C and stirred continuously to float the fine roots to the water surface for collection. Fine roots were sorted into the categories of living and dead roots (diameter < 1 mm and up to 1–2 mm). The living or dead fine roots were determined based on color and appearance. Fine root respiration was measured with Clark-style liquid-phase oxygen electrode systems (Hansatech Instruments Ltd., UK) (Clark et al., 2010). Excised roots were placed in an aqueous physiological buffer (10 mM MES buffer and 1 mM $CaSO_4$), submerged in a thermostatic bath (18 °C) for 10–15 min, and then put into an oxygen electrode for determination of root respiration at a temperature of 18 °C. Root respiration was calculated in n mol O_2 g^{-1} s^{-1}. All fine root samples were oven-dried at 100 °C for 1 h to deteriorate enzymes (Guo et al., 2004) and then at 65 °C for 48 h to determine the total fine root biomass. Fine root mycorrhizal colonization was measured with acid fuchsin staining method (Kormanik et al., 1980), and calculating the extent of the infection as the percentage of root tips.

2.6. Fine root chemical analyses

The dried fine roots were ground and the total C and N concentrations were measured using a C-N analyzer (Elementar Vario, MAX, Germany). Sulfuric acid and perchloric acid were applied to digest subsamples which were used to determine phosphorus (P) in fine roots. P concentrations were determined using a continuous flow analyzer (Skalar San + +,Netherlands). Starch and sugar contents were determined using the phenol–sulfuric acid method (Buysse and Merckx, 1993). Non-structural carbohydrates (NSC) concentrations were calculated from the sugar and starch concentrations. Soluble sugar was extracted by placing 40 mg of dry ground fine roots in 10 ml of ethanol (80%) overnight and then centrifuged (Thermo Scientific, USA) for 15 min (4000 rpm). The extraction process was repeated three times and the three extractants were mixed for sugar analysis. Starch concentration was determined from the residue after drying at 100 °C for 3 h, hydrolyzing with 3 ml of 3% chlorine hydride in boiling water for 0.5 h and then filtered. Glucose and starch concentrations were determined from the supernatants and filtrates using phenol-sulfuric acid and a UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) at

490 nm.

2.7. Soil sampling and chemical analyses

In April 2015 and April 2016, soil samples were randomly collected at 6 locations in each plot by using a 3.5-cm soil sampler. The soil cores were divided into three layers: 0–10, 10–20 and 20–40 cm. Soils were dried to a constant weight in oven at a temperature of 105 °C to measure soil water content. Roots and all organic debris were removed from the soil samples that were subsequently air-dried for analyses of soil pH, total C, total N, NO_3^--N, NH_4^+-N and available P. Soil samples for determining pH, total C and total N were ground and passed through a 2-mm sieve. Soil pH was determined using a pH meter with a soil: water ratio of 1:2.5. Total C and N were determined using an elemental analyzer (Elementar Vario MAX). The inorganic N in the supernatant was measured using a continuous flow analyzer (Skalar san + +, Netherlands). A soil sample of 3 g was mixed with 30 ml of Mehlich-3 extracting solution and then shaken and centrifuged for 5 min. The supernatant was used to determine the available P using a continuous flow analyzer (Skalar San + +, Netherlands).

2.8. Statistical analysis

The differences of tree height, tree height increment, fine root production, fine root mortality, fine root turnover rate, living fine root biomass, fine root N and P concentration, C:N ratio, N:P ratio, soluble sugars, starch, NSC concentration, respiration rate, mycorrhizal colonization, total soil C, total soil N, NH_4^+-N, NO_3^--N, inorganic N, available P and pH between the treatments were determined using one-way ANOVA. Least significant difference (LSD) test was used to identify the significance. The significance between the two years was tested by using a paired T test. Repeated measures analysis of variance was applied to determine the effects of season, warming and N addition on tree height, fine root production and mortality, season was the within group factor and warming and N were between group factors. Two-way ANOVA was used to test the effects of warming, N addition and interactive effects on tree growth, cumulative fine root production and mortality, fine root turnover rate, living fine root biomass. Three-way ANOVA was used to test the effects of warming, N addition and diameter class on fine root N and P concentration, C:N ratio, N:P ratio, soluble sugars, starch, NSC concentration and respiration rate. All statistical analyses were conducted with SPSS 20.0 statistical software (SPSS Inc., Chicago, IL, USA). Diagrams were drawn using Origin 9.0 software (Origin Lab, Massachusetts, USA). The statistical tests were considered significant among the treatments at $P < 0.05$.

3. Results

3.1. Soil micrometeorology

Mean annual air temperatures in the during the study period, ~18.5 °C (Fig. 2a), was lower than the long-term mean annual temperature, 19.1 °C. Annual precipitation was 1994.2 mm in the first year and 2160.4 mm in the second year, which was higher than the long-term mean annual precipitation of 1749 mm (Fig. 2a). The 5 °C warming treatment, which stably kept the temperature of the treated plots 5 °C higher than that of the control plots (Fig. 2b), reduced soil moisture (0–10 cm) by 19.5% and 19.8% for the first and second years, respectively (Fig. 2c).

3.2. Tree height growth

Warming and interaction of warming and N addition all significantly affect the tree height but the effects varied between the two years (Fig. 3a). In the first year, the interaction of warming and N addition significantly suppressed seedling growth (Fig. 3b; Table 1), and
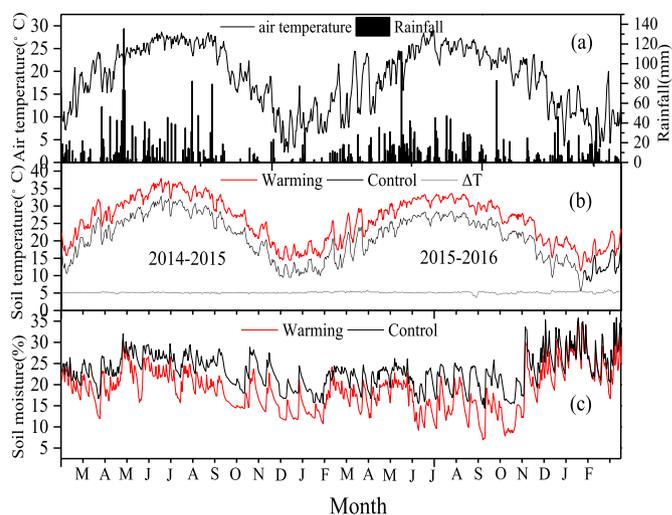

Fig. 2. Air temperature, rainfall (a), soil temperature (b) and soil water content (c) at 10 cm (n = 5) above ground for the control and the warming treatment.
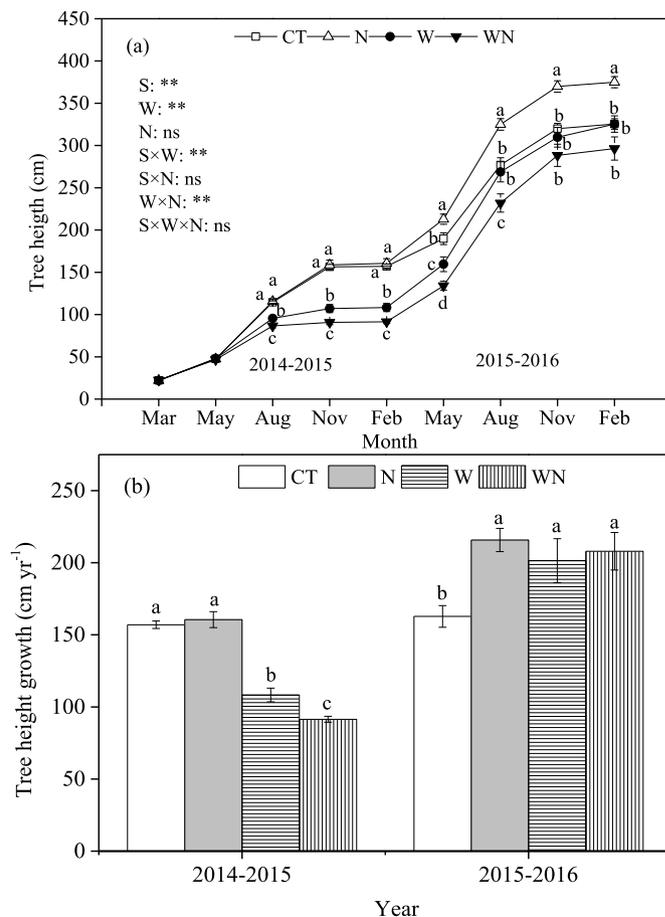

Fig. 3. The seasonal change of tree height (a) and the tree height increment (b) for different treatments (mean ± SE, n = 5). Different lowercase letters indicate significant differences among the treatments ($P < 0.05$). S, season effect; W, experimental warming effect; N, nitrogen addition effect; S × W, the interactive effect of season and warming; S × N, the interactive effect of season and nitrogen addition; W × N, the interactive effect of warming and nitrogen addition; S × W × N, the interactive effect among season, warming and nitrogen addition; ns, not significant at the level of $P = 0.05$; * significant at the level of $P = 0.05$; ** significant at the level of $P = 0.01$.

Table 1
The effect of warming (W), and nitrogen addition (N) on tree height growth, fine root (length) cumulative yearly production, cumulative yearly mortality, turnover rate and living fine root biomass index (n = 5), the values in table are P values.

Year	Source	Tree height growth	Cumulative production	Cumulative mortality	Turnover rate	Living fine root biomass
2014–2015	W	0.0001	0.02	0.0001	0.01	0.0001
	N	0.14	0.38	0.93	0.16	0.005
	W × N	0.03	0.95	0.28	0.69	0.86
2015–2016	W	0.001	0.003	0.0001	0.0001	0.06
	N	0.10	0.02	0.008	0.002	0.82
	W × N	0.001	0.83	0.36	0.93	0.04

the growth of tree height was obviously inhibited in summer. The tree height was 31.0% and 41.8% lower in W and WN respectively compared with CT (Fig. 3a). In the second year, the growth of tree height was accelerated in all the treatments (Fig. 3a). In contrast to the first year the tree height increment of all the treatments was greater than the CT (Fig. 3b).

3.3. Seasonal patterns of fine root production and mortality

Season and warming had significant effects on fine root production and mortality, and the interaction of season and warming had significant effects only on fine root production (Fig. 4). There was a clear seasonal variation in fine root production and mortality throughout the experimental period. In the first year, the warming effect was most prominent in the spring while in the second year the significant effect occurred in the autumn and winter. In the first year, compared with the CT, fine root production was greater in W and WN in the spring (Fig. 4a) and fine root mortality was greater in W in the spring (Fig. 4b). In the second year, compared with the CT, fine root production was greater in W and WN in the autumn and winter (Fig. 4a) and fine root mortality was greater in W in the summer and winter and it was greater in WN in the summer and autumn (Fig. 4b).
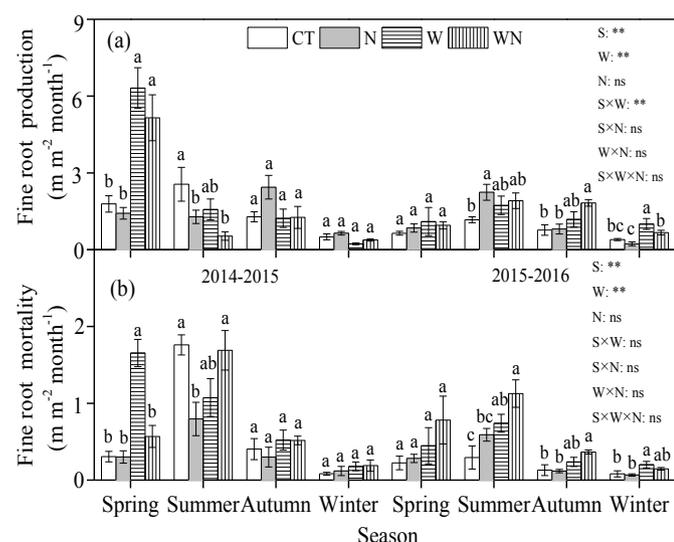
3.4. Annual cumulative fine root length production, mortality, biomass and turnover rate

There was a difference between the two years in annual fine root production, mortality and living fine root biomass, but not in fine root turnover rate. In the first year, warming had significant positive effect on annual cumulative fine root production, mortality and turnover rate, and had significant negative effect on living fine root biomass; nitrogen addition had negative effects on living fine root biomass (Table 1). Compared with the CT, warming increased annual cumulative fine root production, mortality, turnover rate by 42.6%, 278.2%, 41.8%, respectively (Fig. 5a, Fig. 6a); warming, N addition and WN decreased living fine root biomass by 47.9%, 31.9% and 54.9%, respectively (Fig. 6b); WN significantly increased fine root turnover rate by 72.2% (Fig. 6a).

In the second year, warming and N addition had significant positive effects on annual cumulative fine root production, mortality and turnover rate, and the interaction of warming and N addition had significant effect on living fine root biomass (Table 1). Compared with the CT, warming and WN increased annual cumulative fine root production by 70.7% and 130.4%, respectively; and warming and WN increased annual cumulative fine root mortality by 205.3% and 402.0%, respectively (Fig. 5); warming, N addition and WN increased fine root turnover rate by 30.4%, 15.7% and 45.2%, respectively (Fig. 6a).
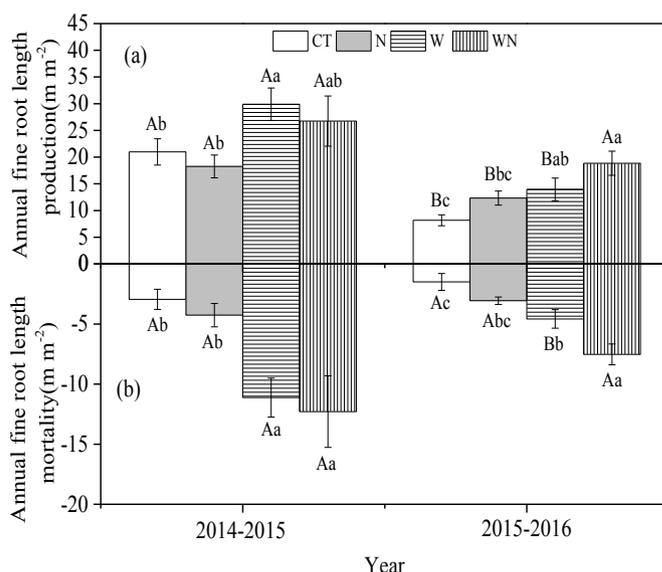

Fig. 4. Seasonal patterns of fine root production (a), fine root mortality (b) for different treatments at a soil depth of 0–40 cm (mean ± SE, n = 5). Different lowercase letters indicate significant differences in the same season (P < 0.05). S, season effect; W, experimental warming effect; N, nitrogen addition effect; S × W, the interactive effect of season and warming; S × N, the interactive effect of season and nitrogen addition; W × N, the interactive effect of warming and nitrogen addition; S × W × N, the interactive effect among season, warming and nitrogen addition; ns, not significant at the level of P = 0.05; * significant at the level of P = 0.05; ** significant at the level of P = 0.01.

Fig. 5. Total annual fine root length production (a) and mortality (b) for different treatments (mean ± SE, n = 5). Different lowercase letters indicate significant differences among the treatments in the same year, and different uppercase letters indicate significant differences between the first year and the second year (P < 0.05).
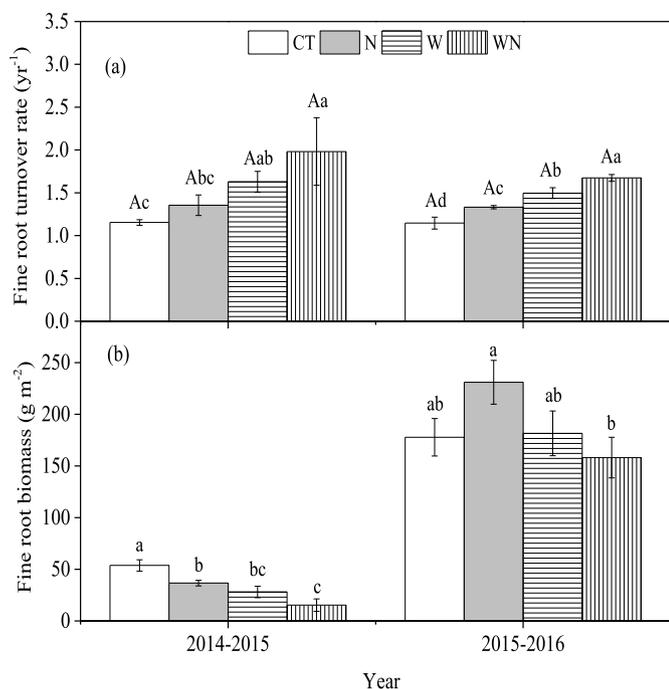
Fig. 6. Annual fine root turnover rate (a) and living fine root biomass (b) for different treatments (mean ± SE, n = 5). Different lowercase letters indicate significant differences among the treatments in the same year, and different uppercase letters indicate significant differences between the first and the second year (*P* < 0.05).

3.5. Fine root chemistry and physiology

In the first year, warming and N addition had significant positive effects on fine root N concentration and N: P ratio but significant negative effects on fine root C: N ratio and soluble sugar. The interaction of warming and N addition had significant effects on fine root N concentration, N: P ratio, C: N ratio, soluble sugar and NSC (Table 2). Compared with the CT, fine root N concentration increased and C:N ratio decreased in all the treatments; warming and WN increased fine root N:P ratio (Fig. 7); warming and N addition decreased fine root starch and NSC concentrations (Fig. 8).

In the second year, warming had significant positive effects on fine root N concentration, N: P ratio and respiration rate, but negative
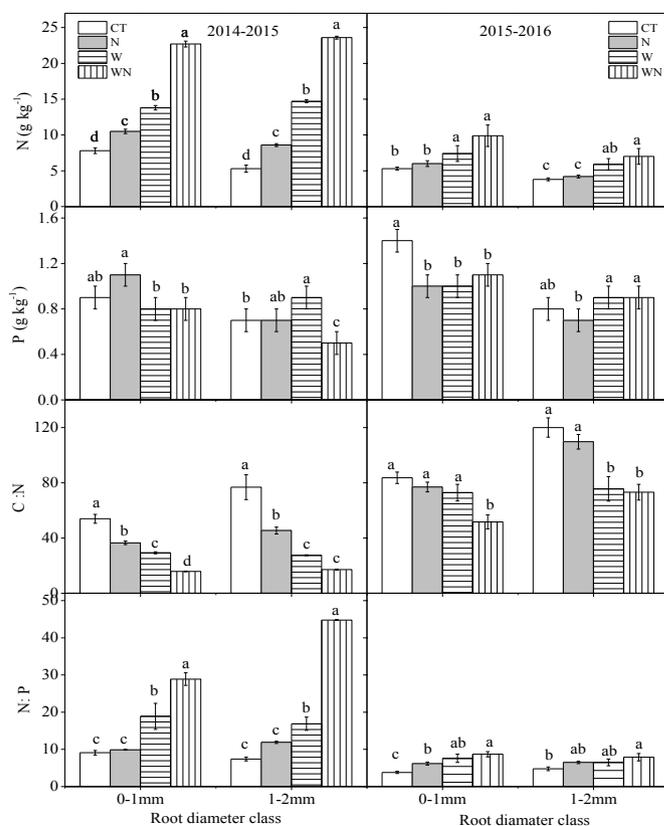

Fig. 7. Fine root N and P concentration, C:N ratio, N:P ratio for different treatments (mean ± SE, n = 5). Different lowercase letters indicate significant differences among treatments within the same diameter class (*P* < 0.05).

effects on fine root C: N ratio, soluble sugar, starch and NSC. N addition had significant positive effects on fine root N concentration, N: P ratio, but significant negative effects on fine root C: N ratio and P concentration. The interaction of warming and N addition had significant effect on fine root P concentration (Table 2). Compared with the CT, W and WN increased fine root N concentration; fine root P concentration reduced in all the treatments and WN increased N:P ratio (Fig. 7). Compared with the CT, warming increased the fine root respiration for the 1–2 mm roots and WN increased fine root respiration for the 0–1 mm roots (Fig. 8).

Table 2
The effect of diameter class (D), warming (W), and nitrogen deposition (N) on fine root N and P concentration, C: N, N: P, soluble sugar, starch, NSC and root respiration index (n = 5), the values in table are P values.

|	Source	N	P	C:N	N:P	Soluble sugar	Starch	NSC	Respiration
2014–2015	D	0.02	0.001	0.005	0.002	0.01	0.18	0.03	0.0001
	W	0.0001	0.01	0.0001	0.0001	0.0001	0.29	0.12	0.43
	N	0.0001	0.58	0.0001	0.0001	0.003	0.42	0.30	0.32
	D × W	0.0001	0.04	0.004	0.003	0.06	0.04	0.33	0.16
	D × N	0.57	0.001	0.28	0.0001	0.21	0.10	0.09	0.86
	W × N	0.0001	0.002	0.02	0.0001	0.14	0.0001	0.0001	0.98
	D × W × N	0.61	0.17	0.10	0.002	0.39	0.28	0.44	0.64
2015–2016	D	0.003	0.0001	0.0001	0.75	0.12	0.80	0.47	0.29
	W	0.0001	0.85	0.0001	0.0001	0.001	0.01	0.001	0.04
	N	0.05	0.02	0.03	0.003	0.40	0.23	0.11	0.35
	D × W	0.68	0.004	0.01	0.11	0.48	0.94	0.98	0.72
	D × N	0.49	0.76	0.38	0.92	0.63	0.78	0.45	0.10
	W × N	0.32	0.001	0.69	0.40	0.14	0.40	0.28	0.79
	D × W × N	0.63	0.03	0.21	0.63	0.68	0.85	0.96	0.11
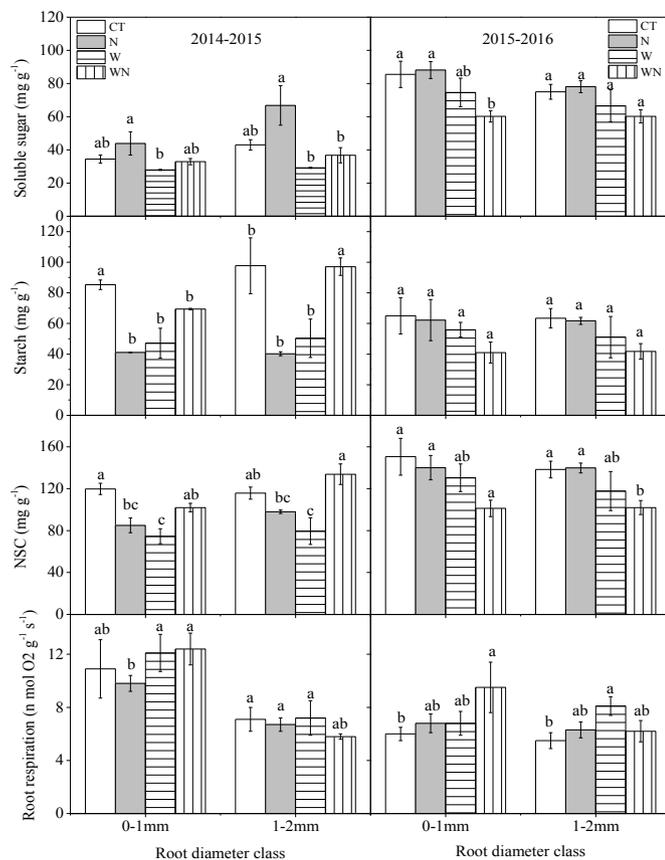
Fig. 8. Fine root soluble sugars, starch, non-structural carbohydrate (NSC) concentration and respiration rate for different treatments (mean ± SE, n = 5). Different lowercase letters indicate significant differences among treatments within the same diameter class (*P* < 0.05).

3.6. Soil chemistry and mycorrhizal colonization

In the first year, compared with the CT, N addition and WN increased the NH_4^+-N concentration; warming and WN increased the NO_3^--N and inorganic N concentration; warming increased available P concentration but WN and N addition decreased available P concentration; WN reduced soil pH (Table 3). In the second year, compared with the CT, W decreased NH_4^+-N concentration; the NO_3^--N concentration reduced in all the treatments; warming and WN reduced inorganic N concentration; WN increased available P concentration (Table 3). Mycorrhizal colonization increased in all the treatments

during the two years (Table 3). The inorganic N concentration and mycorrhizal colonization in the second year was lower than in the first year (Table 3).

4. Discussion

4.1. Effects of experimental warming

In this study, the annual cumulative fine root production and mortality were stimulated after warming (Fig. 5), which is in accordance with some previous studies in the temperate zones (Majdi and Öhrvik, 2004; Wan et al., 2004). Increased fine root production might be due to shifted C allocation to root growth in order to uptake more water resources as a response to water stress associated with soil warming (Fig. 2c). The increased root mortality might be due to warming-induced drought, especially during summer season. Warming also increases the risk of embolism, especially for smaller diameter roots (Sperry et al., 2002). Extended duration of soil drying after warming could increase root mortality, particularly when trees were not adapted to drought conditions (Eissenstat and Yanai, 1997). In our study, the interaction of season and warming had significant effects on fine root production, indicating that warming effect varied seasonally (Fig. 4). Some studies showed that warming effects on fine roots depended on seasonal variation in air temperature (Prieto et al., 2009) and soil moisture (Sardans et al., 2006). Our result showed that warming enhanced fine root production and mortality during the spring of the first year (Fig. 4), which was likely due to elevated soil temperatures and non-limiting soil moisture in the spring (Fig. 2). Warming may increase environmental temperature to an optimal range and thus increase photosynthetic rates (Sage and Kubien, 2007), which in turn results in extra belowground carbon allocation (Pregitzer and King, 2005). Pregitzer et al. (2000) hypothesized that in temperate and boreal environments, the initiation time and rate of new root growth in the spring was directly related to the accumulated heat in soils. In our study warming increased fine root mortality in spring of the first year (Fig. 4b) and the aboveground growth of the plants was also inhibited in this period (Fig. 3a). During the second year, the seasonal pattern of fine root production was less altered after warming compared with the first year. Fine root production showed no significant difference between the warming treatment and the control in spring but significantly increased in autumn and winter in the warming treatment in the second year (Fig. 4a). These effects may also be attributed to abundant precipitation in autumn and winter during this year (Fig. 2a), which significantly improved soil water availability.

In our study, warming significantly increased fine root turnover rate throughout the two years (Fig. 6a). Field observations across sites and through time had also indicated that fine root turnover rates likely

Table 3
Soil chemical and mycorrhizal infection properties at the 0–10 cm depth for different treatments (mean ± SE, n = 5).

Year	Treatment	Total soil C (g kg^{-1})	Total soil N (g kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Inorganic N (mg kg^{-1})	Available P (mg kg^{-1})	pH	Mycorrhizal infection (%)
2014–2015	CT	14.3 ± 1.0Aa	1.7 ± 0.1Aa	5.3 ± 0.8Ac	3.1 ± 0.7Ab	8.4 ± 1.5Ac	1.6 ± 0.1Aab	4.7 ± 0.1Aa	29.0 ± 0.6Ad
	N	13.6 ± 2.5Aa	1.4 ± 0.4Aa	7.4 ± 1.0Ab	3.7 ± 1.4Ab	11.1 ± 2.4Abc	1.1 ± 0.1Ac	4.6 ± 0.2Aab	37.3 ± 0.6Ac
	W	12.7 ± 2.2Aa	1.2 ± 0.3Aa	5.6 ± 0.8Abc	7.3 ± 2.3Aa	12.9 ± 3.1Ab	1.9 ± 0.1Aa	4.6 ± 0.3Aab	44.9 ± 2.1Ab
	WN	13.9 ± 0.9Aa	1.3 ± 0.1Aa	9.2 ± 2.2Aa	9.6 ± 2.1Aa	18.8 ± 4.3Aa	1.5 ± 0.1Ab	4.4 ± 0.2Ab	50.4 ± 2.7Aa
2015–2016	CT	12.8 ± 0.7Aa	1.1 ± 0.1Ba	5.1 ± 0.4Aa	1.5 ± 0.1Ba	6.6 ± 0.5Ba	1.3 ± 0.1Bb	4.3 ± 0.1Ba	8.2 ± 0.9Bc
	N	11.6 ± 0.4Aa	1.0 ± 0.1Aa	5.1 ± 0.3Ba	0.9 ± 0.1Bb	6.0 ± 0.4Bab	1.5 ± 0.1Bb	4.3 ± 0.1Ba	15.5 ± 3.5Bb
	W	12.3 ± 0.7Aa	1.0 ± 0.1Aa	3.8 ± 0.4Bb	1.0 ± 0.2Bb	4.8 ± 0.6Bc	1.5 ± 0.1Ab	4.3 ± 0.1Ba	24.3 ± 5.2Ba
	WN	12.0 ± 0.4Ba	1.0 ± 0.1Ba	4.1 ± 0.2Bab	1.1 ± 0.1Bb	5.2 ± 0.3Bbc	1.8 ± 0.1Aa	4.3 ± 0.1Ba	31.0 ± 5.6Ba

Different lowercase letters indicate significant differences among the treatments in the same year, and different uppercase letters indicate significant differences between the first and the second year.

increased with increasing temperature (Tierney et al., 2003; Kitajima et al., 2010). Nitrogen concentration of the fine roots significantly increased and C:N ratio significantly decreased in the warming treatment (Fig. 7), indicating an increase in root mortality (Withington et al., 2006). Some studies have suggested that root respiration under warming conditions might increase in order to obtain the necessary C for maintenance of root functions (e.g., Winkler et al., 1996), which potentially resulted in more rapid root mortality (Eissenstat et al., 2000). However, studies also showed that the effects of warming on root respiration might be short-lived as plants could acclimate to temperature changes within a certain range (Atkin et al., 2000). Some researches have shown that root respiration acclimated to warming (Bradford et al., 2008; Jarvi and Burton, 2013), especially early in the warming study. Some studies have shown that root respiration rate at a reference temperature had been down-regulated if acclimation to a higher growth temperature had occurred (Atkin et al., 2005a, 2005b). In our study, there was no difference in root respiration rate between the warming and the control plots (Fig. 8), which was measured at a common temperature (18 °C). This suggests that there was no evidence of acclimation during the relatively short period in our study. Since soil temperature are 5 °C warmer, root respiration rate at field temperature should be much greater in the warmed treatments than in the control, and this may increase carbon use of roots, decrease fine root NSC (Fig. 8), and result in greater mortality and turnover rate. This may alter plant C balance and cause more CO_2 release to the atmosphere through autotrophic respiration (Woodwell and Mackenzie, 1995).

Living fine root biomass was lower in the warming treatment than in the control treatment during the first year (Fig. 6b), which could be due to greater root mortality than root production (Fig. 6a), leading to a reduced standing biomass. Some studies have shown that during the dry season, interaction between high temperature and water stresses might occur, which could negatively affect plant photosynthesis and growth, leading to reduced above- and below-ground biomass (Norby et al., 2004; Sanaullah et al., 2012). In our study, especially during the first summer, when high soil temperatures and water stresses interacted to negatively affect photosynthesis and growth, causing the reduced above- and below-ground biomass. However, by the end of the second year, fine root biomass in warming plots was no longer significantly different from that in the control plots. This was primarily attributed to the higher annual rainfall in the second year (Fig. 2a), which significantly improved soil water availability and ameliorated water limitations. Furthermore, roots grew deeper in the soil (unpublished data) as it was essential for Chinese fir seedling to use deep soil water to alleviate water stress, and the roots had begun to fully exploit the soil during the second year. Thus, both the above- and below-ground biomass increased.

4.2. Effects of nitrogen addition

In our study, annual fine root production showed a decreasing trend in response to N addition during the first year (Fig. 5a). Some studies suggested that increasing nutrient availability in soils plants tended to invest less in root nutrient foraging and thus more resources were allocated to above-ground biomass (Vogel et al., 2008). In the second year, fine root production showed an increasing trend (Fig. 5a) which supported the hypothesis that additional new roots grew to absorb more nutrients for supporting the plant growth. Various studies have found that trees at different growth stages exhibited different characteristics due to its ecological plasticity. For example, with increased soil N availability fine root production and turnover rates of young trees usually increased (Børja et al., 2008; Jagodzinski and Katuckd, 2011).

In the present study, fine root turnover rate significantly increased in N addition treatment compared with the control in the second year (Fig. 6a). Wang et al. (2012) found that root turnover rate increased one year after N deposition. However, Burton et al. (2000) found that root turnover rate decreased with increasing soil N availability. Previous

studies showed that the effects of N addition on plant growth varied with N load, site characteristics, duration of N input, and plant species (e.g. Mayor et al., 2014). In our study, the experiment utilized the first two years of data from young trees that began the experiment as seedlings. Thus, as the experiment progressed, the root systems were expanding the portion of soil exploited as tree size increased. The results from our study might not be the same as what might have been observed for the same species in a more mature plantation, where the soil is fully occupied by roots.

Living fine root biomass significantly decreased in response to N addition during the first year (Fig. 6b). From a synthesis of 54 studies, Li et al. (2015) showed that N addition decreased living fine root biomass. Wang et al. (2012) showed that living fine root biomass decreased at a soil depth of 0–20 cm after one-year N addition. Leppälammi-Kujansuu et al. (2013) suggested that long-term N addition (22a) increased living fine root biomass, especially in surface soil. These studies showed that the change of living fine root biomass was probably related to the duration of N addition. In this study, the living fine root biomass in the N addition had an increasing trend in the second year compared with the first year (Fig. 6b), possibly due to the accelerated aboveground growth of the plants (Fig. 3), which increased the demand for N.

4.3. Interactive effects of warming and nitrogen addition

In our study, the interaction of warming and N addition had no significant impact on annual fine root production and mortality in both years (Table 1). The interactive effects of warming and N addition on fine root dynamics have seldom been reported, especially for subtropical forests. Leppälammi-Kujansuu et al. (2014) suggested that the interaction of warming and N fertilization had synergistic effects, accelerating the death of fine roots. Majdi and Öhrvik (2004) suggested that warming combined with fertilization may increase root production and reduce root mortality in boreal forests at low fertility sites where water is not a limiting factor. In our study, coupling between seasonal drought and warming plus N addition treatment significantly increased the annual fine root mortality (Fig. 5b).

In the present study, the interaction of warming and N addition had no significant impact on fine root turnover rate in both years (Table 1). Leppälammi-Kujansuu et al. (2014) found that soil warming and fertilization with ample water availability increased the fine root turnover rate of Norway spruce. They suggested that fertilization and warming have an additive (not interactive) effect, with increased fine root N concentration further enhancing fine root mortality and increased turnover rate. In our study, increased N availability in the soil resulting from warming plus N addition may lead to increased N concentration and N:P ratio and also decreased C:N ratio in fine roots (Fig. 7). Such changes may cause nutrient imbalance and root N toxicity and increase fine root turnover rate. This phenomenon was most obvious in the first year but was much milder in the second year, which might mainly result from the diluting effects caused by enhancement of the growth of Chinese fir. NSC concentration of fine roots did not decrease in the warming plus N addition treatments (Fig. 8). This may be primarily due to enhance photosynthesis in the WN and may have also led to increased supply of photosynthate to the roots, offsetting increased root respiration. Research has shown that chronic N addition can lead to base cation leaching and associated soil acidification. Soil acidification and increased mobility of Al could impact root tissue health and absorption of essential micronutrients (Marschner, 2011). Smithwick et al. (2013) found that decreased soil pH can also cause a series of physical and chemical changes in soils which may accelerate the death of fine roots. In our study, soil pH values decreased in warming plus N addition in the first year (Table 3), which may be one of the reasons for the observed increases in fine root turnover rate. In addition, another mechanistic explanation of greater root death with the WN treatment could be more negative soil water potential due to the combined effects

of greater nutrient mineralization and added nutrients. This could cause a situation where the water potential in the soil is more negative than in the roots, thus decreasing water uptake and increasing plant water stress.

Our finding that living fine root biomass in the warming plus N addition treatment was the lowest during the two years of the experiment (Fig. 6b) maybe explained by the highest fine root turnover rate (Fig. 6a). The interaction of warming and N addition had no significant impact on living fine root biomass in the first year but did have significant effects on living fine root biomass in the second year. In the first year, the plants were at the seedling stage with less N demand and the stress of warming and extra N addition could inhibit aboveground growth (Fig. 3). In the same warming experiment, we found that some plants died in warming and N addition plots in the summer of the first year and the mortality rate reached 10% (unpublished data). In the second year, with more N demand by the plants, the stress of warming and N addition decreased and therefore both above- and below-ground biomass increased. In addition, warming, N addition and WN increased the mycorrhizal colonization during the two years (Table 3). Meanwhile, the fine root P concentration had different level of decline in the two years, especially, the P concentration significantly decreased for 0–1 mm roots in the second year (Fig. 7). Therefore, the growth of Chinese fir may be more limited by P, and N addition may aggravate the limitation of P. Corkidi et al. (2002) also showed that N addition to the P-deficient soil aggravated the P deficiency of plants. Therefore, plants may increase the mycorrhizal colonization to obtain limited nutrients in this study.

The underlying mechanisms of the responses fine roots in subtropical plantations to the interaction of climate warming and N deposition are uncertain and deserve to be explored in future studies. In addition, ontogeny could be just as likely to be the underlying cause of differences in any of these variables between years, the difference in growth rate and physiology of a 2-year old vs a 3-year old tree can be quite dramatic. This may swamp any effect of seasonal or inter-annual differences in meteorological variables.

5. Conclusions

In a subtropical Chinese fir plantation in south China, experimental warming alone increased fine root production, mortality, and turnover rate, and N addition alone showed no effect on fine root production and mortality. We found warming and N addition had an additive (not interactive) effect on fine root production, mortality and turnover rate. We also found that there were significant interactive effects of warming and N addition on tree height, and on living fine root biomass in the second year. Warming, N addition and their interaction changed fine root stoichiometry and physiology, which may regulate fine root performances to a certain extent. This study provided new information on fine root response of subtropical plantations to global change. Increased fine root turnover in the warming treatment implies that warming could accelerate root C inputs to soils, which may affect soil C and nutrient dynamics. Chinese fir seedling growth was sustained by increasing fine root production by shifted C allocation belowground in warming and N addition treatment to compensate for the accelerated root mortality and sustain the fine root biomass. These findings might be valuable to understanding climate controls over the productivity and C balance of subtropical and tropical forests under future climate.

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