Increased soil temperature stimulates changes in carbon, nitrogen, and mass loss in the fine roots of *Pinus koraiensis* under experimental warming and drought

Seung Hyun HAN1*, Seongjun KIM1*, Hanna CHANG1*, Guanlin LI2*, Yowhan SON1*,

1Department of Environmental Science and Ecological Engineering, Graduate School, Korea University, Seoul, Republic of Korea
2Institute of Environment and Ecology, School of the Environment and Safety Engineering, Jiangsu University, Zhenjiang, P.R. China

**Abstract:** The effects of warming (+3 °C) and drought (−30% precipitation) on the fine root decomposition of *Pinus koraiensis* seedlings were examined using a litter bag method. The study site included a full factorial design with two temperature and two precipitation levels, with three replicates. Litter bags containing fine root litter of 2-year-old *P. koraiensis* seedlings were retrieved after 3, 6, and 12 months of decomposition. After 12 months, the mass loss of fine roots was significantly increased in response to warming (control = 31.1%, warming = 35.9%, drought = 29.2%, and warming plus drought = 35.5%); no change was observed until 6 months. Mass loss was not influenced by drought or by the interaction between warming and drought. Warming increased the nitrogen concentration of fine root litter but decreased the carbon concentration and carbon/nitrogen ratio after 6 and 12 months. This may be because warming stimulated nitrogen immobilization, which reduced the carbon/nitrogen ratio. Therefore, the carbon/nitrogen ratio may be affected by warming prior to changes in the mass loss of fine roots because roots with a low carbon/nitrogen ratio are generally characterized by high available nitrogen for decomposers. These results suggest that climate change (especially warming) may cause rapid decomposition of organic matter.

Key words: Carbon/nitrogen ratio, climate change, drought, fine root decomposition, soil temperature, warming

1. Introduction

Root decomposition represents a significant carbon flux in forest ecosystems because it controls the shift of carbon to the soil and acts as a significant source of CO₂ for the atmosphere (Solly et al., 2014). In particular, as fine roots are a key factor linking the water and nutrient cycles as well as carbon flux (Kim, 2012; Han et al., 2018), recent studies have addressed the fine root decomposition of various species in subtropical forests (Lin et al., 2011; Wang et al., 2013), tropical forests (Violita et al., 2016), and temperate forests (Solly et al., 2014; Sun et al., 2016; Liu et al., 2017). Moreover, there have been several review studies on fine root decomposition (Gill and Jackson, 2000; Silver and Miya, 2001; Zhang and Wang, 2015), but a limited number of studies have addressed the factors that regulate their decomposition dynamics (Sun et al., 2013).

Climate change may affect the release of carbon and nutrients from fine roots in the decomposition process and the litter quality of the decomposing root (Zhou et al., 2015). In particular, soil temperature and moisture act as key abiotic factors influencing root decomposition (Zhang and Wang, 2015). Changes in soil temperature and moisture in response to climate change directly alter litter quality and soil microbial activity (Liu et al., 2017). Higher temperature may stimulate soil microbial activities as well as the various metabolic processes of plants (Chung et al., 2013). In addition, previous studies have shown that reduced precipitation may inhibit soil microbial activities by drying the soil (Manzoni et al., 2012), while elevated precipitation may stimulate soil microbial activities (Li et al., 2018). In accordance with this reaction of soil microbial activities, warming may stimulate fine root decomposition (Chung et al., 2013), and drought may impede fine root decomposition (Wang et al., 2010).

Fine roots may decompose relatively slowly and release limited nitrogen concentrations during initial decomposition in general (Goebel et al., 2011). This phenomenon may cause nitrogen deficiency in plant growth because fine root nitrogen is largely retained during initial decomposition (Sun et al., 2013). However, in the decomposition process of fine root litter, nitrogen mineralization and nitrogen immobilization occur depending on soil environmental variables (Zhou et al., 2015). To understand the below-ground carbon and
nitrogen cycling with respect to climate change, it is necessary to examine the effects of climate change on fine root decomposition and to monitor nitrogen release in the decomposition process (Zhou et al., 2011).

Climate change could alter the soil environmental variables such as soil microclimate and soil microbial activity (Li et al., 2018), resulting in carbon and nitrogen cycling of the decomposition process. Despite that importance, only one study has monitored woody root decomposition and nutrient release from decomposing roots under experimental warming and precipitation manipulation (Liu et al., 2017). Since Liu et al. (2017) targeted a deciduous broadleaf tree (Quercus aliena) under climate change conditions (+1.8 °C soil temperature and ±50% precipitation manipulation), there was no information on the fine root decomposition of conifers under other climate change conditions. In a previous meta-analysis study, the fine root decomposition of conifers did not show a significant relationship with mean annual temperature and precipitation, while that of broadleaves was accelerated with increasing mean annual temperature and precipitation (Silver and Miya, 2001).

The present study aimed to examine the initial response of fine root decomposition and carbon and nitrogen release from decomposing roots to experimental warming and drought in Pinus koraiensis seedlings, which are extensively used for plantations in the Republic of Korea (Korea Forest Service, 2017). Zhou et al. (2015) reported soil temperature is the predominant factor controlling fine root litter of P. koraiensis. Changes at the early stages of decomposition can trigger further changes in the subsequent process (Chen et al., 2001), because fine root decomposition occurs rapidly during the first year (Mun, 2004; Liu et al., 2017). We hypothesized that changes in soil temperature and moisture under a climate change experiment would alter the fine root decomposition rate and carbon and nitrogen release: warming would stimulate fine root decomposition, resulting in increased carbon and nitrogen release, and drought would inhibit fine root decomposition during the first year.

2. Materials and methods

2.1. Experimental site and design

The study site was located at the Environmental Ecology Arboretum of Korea University (37°35′35″N, 127°1′30″E), Seoul, Republic of Korea. An automatic weather station was deployed in the center of the study site, and daily air temperature and precipitation were measured continuously during the study period. Mean annual temperature was 13.4 °C, and annual precipitation was approximately 800 mm with 65% distributed in July and August during the study period. In May 2016, 20 experimental plots (1.0 × 1.5 m) were set up and 90 two-year-old P. koraiensis seedlings (mean height = 10.1 ± 0.2 cm) were planted in each plot. Soil of the study site had a loamy texture (sand:clay:silt = 54.8:26.8:18.4%), and soil pH, cation exchange capacity, and electrical conductivity of 7.2, 9.4 cmol C kg⁻¹, and 159.9 dS m⁻¹, respectively.

The experimental design consisted of four different treatments [control (C), warming (air temperature warming; TW), drought (precipitation decreased; PD), and warming plus drought (TW × PD)] with three replicates. To elevate the air temperature (+3 °C), an infrared heater (FTE-1000, Mor Electric Heating Association, Comstock Park, MI, USA) was set up in the TW and TW × PD plots. The two infrared heaters were installed 60 cm above the seedlings to give an even effect of warming to each plot and were hung on stainless steel structures. Drought (PD and TW × PD plots) was induced by covering 30% of the plot area with see-through acrylic panels. These warming and drought levels were designed based on those expected in South Korea over the next 50 years according to the RCP 8.5 climate change scenario (Climate information portal). Dummy heaters without infrared heaters and see-through acrylic panels that did not collect precipitation were used for the nonwarmed and the natural precipitation plots, respectively, to ensure the other environments remained unchanged. The warming and drought system worked in May after seedlings were planted.

During the study period, a multiparameter sensor (CS655, Campbell Scientific, Logan, UT, USA) was installed at 5- to 10-cm soil depth in each plot to monitor soil volumetric-water content and soil temperature, and the data were measured every 30 min. The experimental design is concretely described by Han et al. (2018), who used a similar experimental design.

2.2. Fine root decomposition

To ensure consistency in the initial conditions, we sampled fine roots (<2 mm diameter) by collecting the complete seedlings of 2-year-old P. koraiensis on 16 June 2016. The sampled seedlings were extra seedlings that had been planted outside the study plots. After removal of soil particles attached to the fine root samples with distilled water, the samples were air-dried at room temperature for 72 h to measure initial mass. In order to convert initial air-dried mass to dry mass, some initial root samples were weighed after drying at 65 °C. The effect of treatments on fine root decomposition was examined using the litter bag method. Thirty-six litter bags (four treatments × three treatment replicates × three collections times) were made of polyester net (5 cm × 5 cm) with a mesh size of 0.1 mm. On 23 June 2016, three litter bags with a label enclosing 0.30 g of fine root litter were installed at 10-cm depth in each plot. Three litter bags for three collection times were installed at the center of the plot. A litter bag was collected from each plot after 3 months (September 2016), 6 months...
(December 2016), and 12 months (June 2017). The retrieved samples were cleaned with distilled water and dried at 65 °C for 48 h prior to weighing. We calculated the fine root decay constant during the study period using the following decay model (Olson, 1963):

\[ X_t = X_0 \times e^{-kt}, \]

where \( X_t \) is the amount of remaining fine roots at time \( t \), \( X_0 \) is the initial fine root mass, \( e \) is the base of the natural logarithm, and \( k \) is the decay constant. Fine root samples from retrieved litter bags at each collection were analyzed for carbon and nitrogen concentrations using an elemental analyzer (varo MACRO, Elementar GmbH, Hanau, Germany).

### 2.3. Statistical analysis

Repeated-measures ANOVA and two-way ANOVA were used to determine the effects of warming and drought on fine root decomposition throughout the study period and at each collection time, respectively. Tukey’s multiple range test was performed to analyze the pairwise differences between the treatments. Linear regression was performed to determine the correlations between treatment effect on the decay constant and carbon/nitrogen ratio during 1 year and treatment-induced differences in mean annual soil temperature and moisture. Treatment effect on decay constant and carbon/nitrogen ratio was calculated as the percentage difference in values from PD, TW, and TW × PD plots relative to C plots, and treatment-induced differences in soil temperature and moisture were based on the difference in measurements from PD, TW, and TW × PD plots relative to C plots. All statistical analyses were performed by SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

### 3. Results

#### 3.1. Treatment effects on soil temperature and moisture

Warming, drought, and time (month) had significant effects on soil temperature and moisture content (Table 1). During the study period (July 2016 to June 2017), compared with C plots, warming elevated mean soil temperature by 4.12, 1.28, and 4.10 °C in TW, PD, and TW × PD plots, respectively (Figure 1a). Warming and drought decreased mean soil moisture content by 1.41, 0.97, and 1.48 vol % in TW, PD, and TW × PD plots, respectively, compared with C plots (Figure 1b).

#### 3.1. Treatment effects on fine root decomposition

The mass loss of fine roots was significantly affected by warming but not by drought or the interaction between warming and drought (Table 1). After 12 months, the mass loss of fine roots was significantly increased by warming, while no significant differences were detected until 6 months (Figure 2a). Warming increased the decay constant by 19.79% and 27.21% in TW and TW × PD plots, respectively (C = 0.37 ± 0.00, PD: 0.35 ± 0.01, TW: 0.45 ± 0.03, and TW × PD: 0.44 ± 0.01; Figure 2b) compared with the C and PD plots. Additionally, collection time significantly affected the mass loss of fine roots (Table 1). Mass loss of fine roots increased markedly up to 3 months, after which it decreased. The mass loss of fine roots progressed by 26.15% after 3 months, 31.94% after 6 months, and 32.94% after 12 months.

#### 3.2. Carbon and nitrogen concentrations of fine root litter

Warming and collection times significantly affected the carbon and nitrogen concentrations and the carbon/nitrogen ratio during the study period (Table 1).
nitrogen ratio of decomposing roots; however, these were not affected by drought or the interaction between warming and drought (Table 1). The fine root carbon concentration was influenced by warming only after 12 months, whereas fine root nitrogen concentration and the carbon/nitrogen ratio were influenced by warming after both 6 and 12 months. The initial fine root carbon concentration was 45.91 ± 0.15%, and gradually decreased over time, with the highest values observed in C plots (44.37 ± 0.06%) and the lowest values observed in TW plots (41.59 ± 0.56%) after 12 months (Figure 3a). The initial fine root nitrogen concentration was 0.86 ± 0.01%, and this increased over time, with the highest values observed in TW × PD plots (1.34 ± 0.05%) and the lowest values in C plots (1.12 ± 0.05%) after 12 months (Figure 3b). The initial carbon/nitrogen ratio of fine roots was 53.64 ± 0.46. The carbon/nitrogen ratio of decomposing roots increased over time, with the highest values observed in C plots (39.83 ± 1.48) and the lowest values in TW × PD plots (31.13 ± 1.50) after 12 months (Figure 3c).

3.3. Correlations between decay constant and soil temperature and moisture
During the study period (Figures 4a–4d), there were positive relationships of treatment effect on the decay constant and carbon/nitrogen ratio with treatment-induced difference in soil temperature (P < 0.001 and P < 0.05, respectively; Figures 4a and 4c). However, there were no significant relationships of treatment effect on decay constant and carbon/nitrogen ratio with treatment-induced difference in soil moisture (P > 0.05; Figures 4b and 4d).
4. Discussion

Our findings support the hypothesis that warming stimulates fine root decomposition (Table 1; Figure 2). These results are similar to those of a previous meta-analysis study that showed fine root decomposition increased linearly with elevating mean air temperature in forest ecosystems (Silver and Miya, 2001). In general, fine root decomposition is controlled by various factors, including soil environment, litter quality, and decomposer activities (Zhang et al., 2008). Our findings suggest that warming stimulates soil microbes, including microbial biomass and enzyme activities, leading to increased mass loss and decay constant of fine roots (Zhou et al., 2013; Zhao et al., 2016). Notably, in a previous study at the same site with similar systems, warming enhanced soil microbial biomass and activity (Li et al., 2017).

Contrary to our hypothesis, drought did not significantly inhibit the rate of fine root decomposition (Table 1). Similar effects of reduced precipitation on the decomposition of fine roots have been previously reported. The effect of mean annual precipitation (MAP) on fine root decomposition was not significant, while a negative relationship was observed between MAP and coarse root decomposition (Schuur, 2001; Zhang and Wang, 2015). Moreover, the effect of MAP on conifer fine root decomposition was not significant in terrestrial ecosystems (Silver and Miya, 2001). The reduced level of precipitation used in the present study may have been insufficient to induce substantial changes in fine root decomposition (Zhang and Wang, 2015). It seemed that the prevention of rainfall by seedling canopy and abnormally low annual precipitation during the study period (only 55% of the previous 30-year average) may weaken the drought effect on soil moisture (Yun et al., 2016; Li et al., 2018). Furthermore, the warming effect could be attributed to decreased soil moisture by the drying effect (Li et al., 2018), leading to similar effects of drought and warming on soil moisture. However, regardless of warming and drought effects, decreased soil moisture by the two treatments did not affect fine root decomposition (Figure 4b).

Changes in the carbon and nitrogen concentrations of decomposing roots seem to correspond with changes in fine root mass loss. Carbon and nitrogen concentrations changed dramatically in the first 3 months of decomposition, during which fine roots lost the largest amount of mass. Throughout the study period, the carbon concentration of fine roots decreased, while the nitrogen concentration increased, which resulted in a simultaneous reduction in the carbon/nitrogen ratio. The increase in nitrogen concentration might be related to nitrogen immobilization. In general, microbes prefer to immobilize soil nitrogen when the carbon/nitrogen ratio exceeds 30 in forest ecosystems (Huang et al., 2004). In the present
In this study, the carbon/nitrogen ratio of all plots was between 30 and 55 during the decomposition process (Figure 3c). Previous studies have shown that nitrogen immobilization occurs at high carbon/nitrogen ratios (Mun, 2004; Wang et al., 2010). In particular, the increase in soil microbial biomass and activity caused by warming may accelerate the loss of carbon from the root litter, lowering the carbon/nitrogen ratio of TW and TW × PD plots after 6 and 12 months (Li et al., 2017). The decreased carbon/nitrogen ratio of decomposing roots might contribute to the fast decomposition observed in TW and TW × PD plots because the carbon/nitrogen ratio is an important factor regulating microbial activity (Gholz et al., 2000; Silver and Miya, 2001; Sariyildiz, 2015). In general, root litter with low carbon/nitrogen ratio is characterized by lower amounts of lignin as well as high available nitrogen for decomposers (Heal et al., 1997). The process of nitrogen immobilization would then be followed by net nitrogen release, as reported in previous studies investigating fine root decomposition (Chen et al., 2001; Mun, 2004). Meanwhile, contrary to the warming effect, drought did not significantly affect the carbon and nitrogen concentrations or the carbon/nitrogen ratio of decomposing roots.

The carbon concentration, nitrogen concentration, and carbon/nitrogen ratio of decomposing roots, and the decomposition rate of fine roots were subject to warming rather than drought effects. Fine root decomposition was dependent only on the increased soil temperature under warming. These results suggest that climate change (especially warming) may induce the rapid decomposition of organic matter, increasing soil nutrient availability (Chung et al., 2013). However, the present study used living roots from seedlings that might have higher initial nitrogen concentration than that of mature P. koraiensis.
or naturally dead roots (Kim, 2002). Therefore, in order to accurately predict decomposition dynamics in the *P. koraiensis* forest under climate change, further studies should be conducted on fine roots of mature *P. koraiensis*.

In summary, warming altered the fine root decay constant and carbon and nitrogen release; however, these parameters were not influenced by drought or the interaction between warming and drought. It is possible that warming-induced changes in soil temperature stimulated soil microbial activity, leading to increased fine root decomposition. However, the warming- and drought-induced reduction in soil moisture had no effect on root decomposition. Meanwhile, warming stimulated carbon release and nitrogen immobilization. Because of these changes, the carbon/nitrogen ratio in warming plots remained low. The carbon/nitrogen ratio was first changed by warming, which led to changes in the fine root decomposition. These results indicate that warming-induced changes in the soil microclimate may increase the release of carbon from fine roots in the early stage. Because this study dealt with the short-term effects on fine root decomposition, additional long-term studies with more replicates are needed to examine nitrogen mineralization (nitrogen release). Nevertheless, these results will contribute to our understanding of below-ground root decomposition in response to climate change.

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**References**


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