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Effects of Pre-Hardening and Autumn Fertilization on Biomass Allocation and Root Morphology of *Pinus koraiensis* Seedlings

He Huang [1,†], Haibo Wu [1,2,†], Rosana López [3], Dongsheng Yin [4], Hailong Shen [1,2] and Peng Zhang [1,2,*]

1 Key Laboratory of Sustainable Forest Ecosystem Management-Ministry of Education, Northeast Forestry University, Harbin 150040, China
2 State Forestry and Grassland Administration Engineering Technology Research Center of Korean Pine, Harbin 150040, China
3 Department of Natural Systems and Resources, Universidad Politécnica de Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain
4 Forestry Research Institute of Heilongjiang Province, Harbin 150081, China
* Correspondence: zhangpeng@nefu.edu.cn
† These authors contributed equally to this work.

Abstract: The effects of pre-hardening fertilization and autumn fertilization on seedling growth have been studied separately, but studies on their combined effects are relatively scarce. We studied the effects of pre-hardening fertilization type and autumn fertilization level on biomass allocation and root morphology of container-grown seedlings of Korean pine (*Pinus koraiensis* Sieb. et Zucc.), a valuable evergreen conifer distributed from Changbai Mountain to the Xiaoxing'an Mountains in northeastern China. Three pre-hardening fertilization types (conventional fertilization, exponential fertilization, and controlled-release fertilizer) were all applied with 72 mg of nitrogen. We also applied four nitrogen levels of autumn fertilization: 0 mg/plant, 2 mg/plant, 4 mg/plant, and 6 mg/plant. We found that autumn fertilization increased Korean pine seedling biomass accumulation and root growth by 65.91%–92.15% and 108.86%–141.48%, respectively. There was significant interaction between pre-hardening fertilization type and autumn fertilization level on biomass allocation and root morphology. Seedlings with conventional fertilization during the growing season have the best response to autumn fertilization, particularly in the 2 mg/seedling and 4 mg/seedling treatments. Autumn fertilization can be applied to the cultivation of high-quality Korean pine seedlings.

Keywords: Korean pine; fertilization; biomass; root morphology

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1. Introduction

High quality seedlings are the basis of successful afforestation [1]. In sites with high environment pressure, outplanting performance of container seedlings is often better than that of bareroot seedlings [2]. Nutrient release in container seedling medium is slow; thus, fertilization is necessary to improve plant growth and promote resistance to biotic and abiotic stresses. After the dormancy of seedlings is released in spring, they grow rapidly before the hardening period in autumn. Fertilization during this period, pre-hardening fertilization, can effectively improve seedling quality. After the end of the growing season, the terminal bud is formed and the seedling height growth tends to stagnate, but secondary growth and radical growth may continue and thus plant biomass increases [3,4]. If fertilization stops after terminal bud formation, nutrient dilution caused by the decrease in nutrient concentration in seedlings affects seedling quality and afforestation performance [5]. Fertilization in this stage is normally referred to autumn fertilization. Because of its simple operation and remarkable effect, autumn fertilization has been widely applied to many conifer species, including *Picea abies* (L.) Karst. [6], *Picea mariana* [7], *Pinus taeda* L. [8], *Pinus resinosa* [9], and *Pinus tabulaeformis* Carr. [10].

Since the main purpose of autumn fertilization is avoiding nutrient dilution in seedlings [11], studies on autumn fertilization mostly focus on the nutrient concentration in seedlings [10,12,13], whereas less attention is paid to biomass accumulation and root morphology. Cuesta et al. [14] found that biomass had a higher effect on afforestation performance than nutrient concentration, and the higher the seedling biomass, the higher growth in the field. The effect of autumn fertilization on biomass may be related to the pre-hardening fertilization type. For example, the biomass of seedlings fertilized in autumn after conventional fertilization during the growing season did not increase significantly [5], but the biomass of seedlings fertilized in autumn after exponential fertilization during the growing season increased significantly [3]. Compared to the same amount of fertilizer applied each time with conventional fertilization, exponential fertilization provides nutrients at exponentially greater rates of application. Biomass allocation of seedlings also affected their performance in the field. For example, *Pinus pinea* L. seedlings with higher root biomass had greater height growth three years after planting [15]. The root is the most important organ for plants to absorb water and nutrients. Root length and area play a decisive role in the absorption of nutrients that reach the root surface mainly by diffusion. Therefore, it is of great significance to study the morphological characteristics of the root system. A well-developed root system is beneficial for plants to rapidly absorb water and nutrients, accumulate biomass [16], and enhance their resistance to environmental stress in unfavorable sites [17]. Fertilization can improve root morphology of plants, and the response of root morphology to different pre-hardening fertilization types was significantly different [18,19]. For example, root biomass, length, area, and volume of *Aquilaria malaccensis* and *Aquilaria sinensis* seedlings with exponential fertilization were significantly higher than those with conventional fertilization [19], whereas the effect of autumn fertilization on root morphology is still largely unknown.

Korean pine (*Pinus koraiensis*) is the keystone species of the zonal climax vegetation of mixed broadleaved Korean pine forests in Northeast China. It has high ecological and economic value because of its high wood quality and rich seed nutrition. Korean pine is a member of the five-needle white pine subgroup of conifer species, which are unimodal and produce one whorl of branches annually. In the 1980s, due to unreasonable logging and utilization, the area of the original mixed broadleaved Korean pine forests decreased sharply. Renewal and restoration of the Korean pine has become the core issue of restoration [20]. Nowadays, in restoration practices in these forests, bareroot seedlings are commonly used despite some studies having shown that container seedlings have higher field survival rates [2]. Therefore, our study tested the effect of pre-hardening and autumn fertilization and the benefits of containers on biomass allocation and root morphological development of Korean pine. We transplanted 1-year-old seedlings into containers for a second growing season. During the growing season, we applied three fertilization schemes: conventional fertilization, exponential fertilization, and controlled-release fertilizer. Then, some seedlings were fertilized in autumn with three nitrogen levels, while the other seedlings were not fertilized in autumn, in order to: (1) explore whether autumn fertilization can improve the biomass and root morphology of Korean pine seedlings and (2) clarify whether the response of Korean pine container seedlings to autumn fertilization with different nitrogen levels is affected by pre-hardening fertilization types. In addition, we hypothesized that autumn fertilization could significantly improve the growth of seedlings. Likewise, we predicted the effect of autumn fertilization would be affected by the pre-hardening fertilization type.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Research was conducted at the Maoershan Forest Research Station (127° E, 45° N, 300 m above sea level) of Northeast Forestry University in Heilongjiang Province, China. The station is located in the Xiaoxing'an Mountains. The climate is temperate continental monsoon, with long winters and short summers. Average annual values for temperature, precipitation, evaporation, and relative humidity are 2.8 °C, 723 mm, 1094 mm, and 70%, respectively. The frost-free period is 120–140 d and total annual sunshine is 2471 h.

Before starting the experiment, we selected ten 1-year old and ten 2-year-old healthy bareroot Korean pine seedlings to determine the change in total nitrogen content to be applied during the container phase of seedling production. In order to determine N content, we followed the methods of Zhang et al. [21] for sample preparation. Samples were analyzed on an Elementar Vario EL cube (Elementar, Langenselbold, Germany). The resulting N content was 5.13 mg in 1-year-old seedlings and 27.99 mg in 2-year-old seedlings.

For the fertilization experiment, we selected 1-year-old healthy bareroot Korean pine seedlings with uniform (mean ± standard error) height (7.92 ± 0.10 cm) and root-collar diameter (2.61 ± 0.04 mm). Roots of tested seedlings were trimmed to ensure that the initial shape of the root systems was uniform. Seedlings were transplanted into pots of 15 cm height, 15 cm diameter, and 2.65 L volume which were filled with 500 g of a sterilized mixture of peat: perlite (1:1, *v:v*) on 25 April 2021. The medium had a bulk density of 0.25 g/cm^3 and a pH of 5.23. Total nitrogen (N), phosphorous (P), and potassium (K) values were 9.89, 67.12, and 9.59 g/kg, respectively. Available N, P, and K values were 698, 39, and 154 mg/kg, respectively. After transplanting, seedlings were placed in a greenhouse. Day/night temperature was set at 35/20 °C with relative humidity at 50/70%. Average photon flux density during daytime hours was ca. 500 µmol m^{-2} s^{-2}. Once expansion of the terminal buds was complete (28 May), pre-hardening fertilization (described below) commenced and continued 12 weeks until 24 August. About 1 week after, on 1 September, height growth stopped and all seedlings were moved from the greenhouse to an outdoor growing area to accelerate lignification and to receive autumn fertilization (described below).

2.2. Pre-Hardening and Autumn Fertilization

Pre-hardening fertilization treatments consisted of conventional fertilization (CF; equal amount of fertilizer is applied weekly during the growing period), exponential fertilization (EF), and controlled-release fertilizer (CRF). As total seedling N content increased about 22.86 mg during the container phase of nursery production, and we assumed a N utilization rate of ca. 33% [22], our target N application amount was rounded up slightly to 72 mg.

For CRF, we used APEX controlled-release fertilizer (N:P:K = 18:6:12). Therefore, we applied 400 mg (72 mg N target/0.18 N) of CRF evenly to the surface of the growing medium once at the onset of pre-hardening fertilization.

For CF and EF, N was supplied by urea (46% N), which is commonly used in Chinese nurseries [23]. The amount of N applied weekly for CF was 6 mg (72 mg N target/12 weeks). For EF, we determined the weekly amount of N to apply by following the methods of Dumroese et al. [24]:

$$N_{\mathrm{T}} = N_{\mathrm{S}}\ (\mathrm{e}^{rt} - 1) \quad (1)$$

where N_{T} is the total amount of nitrogen applied; N_{S}, is the amount of nitrogen in seedlings before the first fertilization; t is the times of pre-hardening fertilization; r is an undetermined coefficient indicating the relative addition rate of nitrogen; and e^{rt} is the growth rate of N content in seedlings after t-times exponential nitrogen application. As mentioned above, N_{T}, N_{S}, and t were 72, 5.13, and 12, respectively. Using Equation (1), the relative addition rate (r) was calculated to be 0.2259. The formula for calculating the specific N amount each time was:

$$N_{\mathrm{t}} = N_{\mathrm{S}}\ (\mathrm{e}^{rt} - 1) - N_{t-1} \quad (2)$$

where N_{t-1} is the total amount of N accumulated before the current fertilization and the rest of the parameters are the same as those in Equation (1).

For CF and EF, once a week, the target amount of urea was dissolved in 20 mL N-free nutrient solution that supplied macro- and micronutrients (Table 1; Sinopharm Chemical Reagent Co., Ltd., Beijing, China) and applied evenly to the surface of the growing medium. Seedlings were irrigated 3–5 times per week according to the weather conditions. To minimize leaching losses of fertilizer, irrigation was not applied the day before or the day after fertilization. Container seedlings were randomly rearranged every 2 weeks to minimize edge effects.

Table 1. The concentration of nutrient elements in nitrogen-free nutrient solution and the configuration of fertilizers.

Macronutrients	Target Concentration (mol/L)	Fertilizer (mol/L)	Micronutrients	Target Concentration (mol/L)	Fertilizer (mol/L)
P	3.87×10^{-3}	85%H_3PO_4 (4.57×10^{-3})	Fe	1.43×10^{-4}	$FeSO_4 \cdot 7H_2O$ (1.43×10^{-4})
K	7.69×10^{-3}	K_2SO_4 (3.85×10^{-3})	Mn	2.91×10^{-5}	$MnCl_2 \cdot 4H_2O$ (2.91×10^{-5})
Ca	4.00×10^{-3}	$CaCl_2 \cdot 2H_2O$ (3.56×10^{-3})	Zn	9.85×10^{-6}	$ZnSO_4 \cdot 7H_2O$ (9.62×10^{-6})
Mg	3.33×10^{-3}	$MgSO_4 \cdot 7H_2O$ (3.33×10^{-3})	Cu	4.69×10^{-6}	$CuSO_4 \cdot 5H_2O$ (7.31×10^{-6})
S	3.75×10^{-3}	$MgSO_4 \cdot 7H_2O$ (2.88×10^{-2})	Mo	4.17×10^{-7}	$Na_2MoO_4 \cdot 2H_2O$ (3.89×10^{-7})
—	—	—	B	9.09×10^{-5}	H_3BO_3 (9.10×10^{-5})

Autumn fertilization was applied 2 weeks after pre-hardening fertilization finished. Autumn fertilization treatments consisted of control (AF0; lack of N in the nutrient solution), low level of N addition (AF2; 2 mg/seedling), medium level of N addition (AF4; 4 mg/seedling), and high level of N addition (AF6; 6 mg/seedling). All four levels had the same base N-free nutrient solution (Sinopharm Chemical Reagent Co., Ltd., Beijing, China) that supplied macro- and micronutrients, except for N (Table 1). N was supplied as urea and applied in the N-free nutrient solution as described for pre-hardening fertilization CF. N fertilization was applied once per week during 3 consecutive weeks, and thus the total amounts of N added in the autumn fertilization were 0, 6, 12, and 18 mg/seedling in the 4 treatments, respectively. Seedlings were irrigated 3–5 times per week according to the weather conditions. The experiment included 144 seedlings (3 pre-hardening fertilization × 4 autumn fertilization × 12 seedlings).

2.3. Seedling Measurements

Three seedlings were randomly selected from each pre-hardening fertilization × autumn fertilization combination on 19 October. Seedlings were harvested and separated into roots, stems, and needles. Stems and needles were immediately placed in a forced-air oven at 70 °C until constant weight was reached to measure the dry mass.

All roots were initially rinsed in water to remove coarse growing media from the root system but not damage fine roots. Roots were then gently washed 3–4 times, and any fine roots that separated from the system were collected. Roots of each seedling were then carefully spread onto a tray ensuring no overlap and scanned using an Expression 10000XL 1.0 scanner (dpi = 400; Epson Telford, Ltd., Telford, UK). Roots were then analyzed with WinRHIZO (Pro2004b) software (Instruments Regent Co., Ville de Québec, QC, Canada) to obtain total root length (TRL), total root area (TRA), and total root volume (TRV). For each root system, roots were further divided into 2 diameter classes ($\leq$1 mm and >1 mm) and length, area, and volume were determined for each class. Roots were then dried in a forced-air oven at 70 °C until constant weight was reached to determine dry mass.

Total seedling biomass was estimated as the sum of the root, stem, and needle biomass. Shoot-to-root ratio (S:R) was estimated as the ratio between shoot (stem and needle) dry mass and root dry mass. Specific root length (SRL) was calculated as the ratio between total root length and root biomass.

2.4. Data Analysis

Our experiment used a split-plot design, with pre-hardening fertilization as the whole plot and autumn fertilization as the split plot. A two-way analysis of variance (ANOVA) was used to test the main effects of pre-hardening and autumn fertilization and their interaction on biomass (root, stem, needle, total), S:R, TRL, TRA, TRV, and SRL. When significant effects were found at $\alpha = 0.05$ level, post hoc Duncan's tests were applied for multiple comparisons. Statistical analyses were performed using SPSS 26.0 for Windows (SPSS, Chicago, IL, USA) and figures were plotted with SigmaPlot 10.0 (SYSTAT, San Jose, CA, USA).

3. Results

3.1. Effect of Fertilization on Biomass Production and Allocation

In the absence of autumn fertilization, root, needle and total biomass of CRF were significantly higher than CF and EF (Figure 1). However, autumn fertilization had a significant effect on the biomass of all organs in CF and EF with not much difference between levels except for a slightly significant decrease in needle biomass under AF6 in EF. (Figure 1; Table 2). We also found a significant effect of the interaction between pre-hardening fertilization × autumn fertilization for all biomass traits except for stem biomass (Figure 1; Table 2) due mainly to the limited response to autumn fertilization of plants in the CRF treatment, which only showed higher root biomass in AF6 but did not show any other significant increase in biomass (Figure 1).

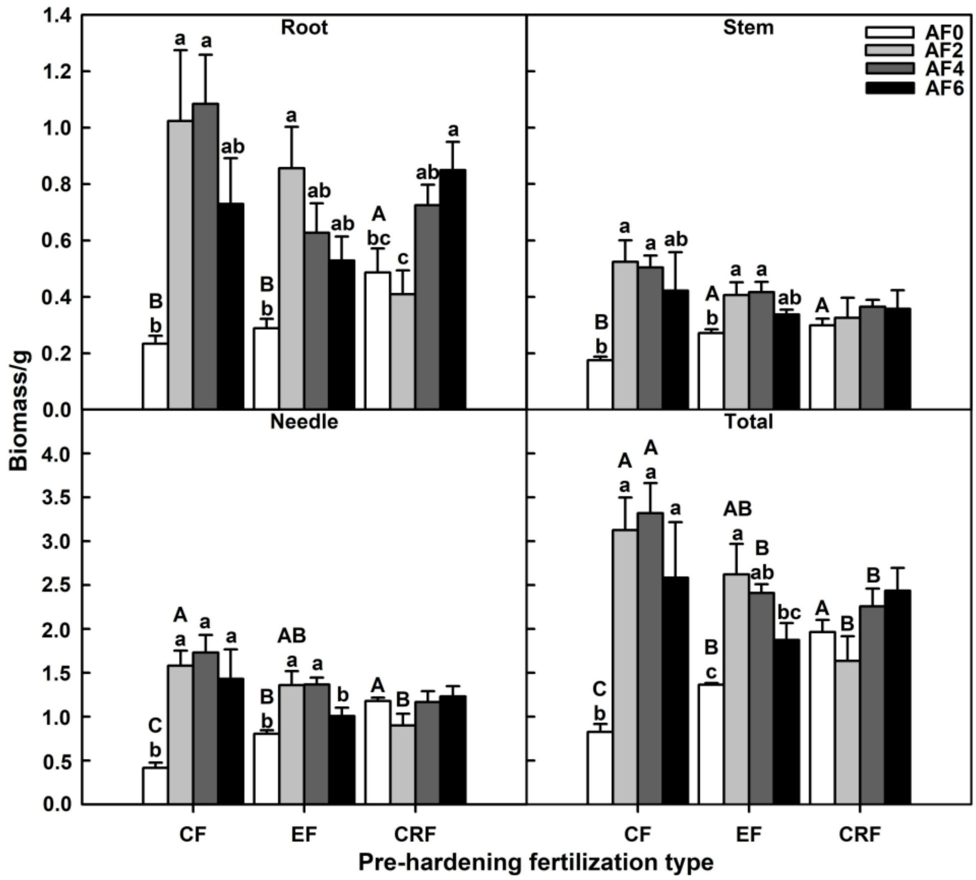

Figure 1. Root, stem, needle, and total biomass in seedlings of *Pinus koraiensis* in response to pre-hardening fertilization type (CF, EF, and CRF) and autumn fertilization level (AF0, AF2, AF4, and AF6) treatments. Bars and arrows represent the mean and corresponding standard error, respectively. Different capital letters indicate a significant effect of biomass among pre-hardening fertilization types ($p < 0.05$). Different lowercase letters indicate significant differences in biomass among autumn fertilization levels ($p < 0.05$). Means were compared using Duncan's tests.

Table 2. Statistics (df, degrees of freedom; *p*-values; and significance levels) of two-way ANOVAs testing the main and interaction effects of pre-hardening fertilization type and autumn fertilization level on biomass allocation of *Pinus koraiensis* seedlings.

Source	df	*p*-Values				
		Needle	Stem	Root	Total	Shoot-to-Root Ratio
PF	2	0.231 ns	0.248 ns	0.097 ns	0.114 ns	0.138 ns
AF	3	<0.001 **	0.003 **	<0.001 **	<0.001 **	0.010 **
PF × AF	6	0.002 **	0.201 ns	0.015 *	0.004 **	0.026 *

Note: ns denotes a non-significant effect; * and ** indicate significant effects at 0.05 and 0.01 significance levels, respectively. Abbreviations: PF: pre-hardening fertilization type; AF: autumn fertilization level.

Regarding biomass allocation, S:R after pre-hardening fertilization of CRF was significantly lower than CF and EF (Table 3). The high-nitrogen level of autumn fertilization (AF6) significantly decreased S:R of EF and CRF ($p < 0.05$), but S:R of CF was fairly constant regardless of the autumn fertilization level.

Table 3. Effects of pre-hardening fertilization type and autumn fertilization level on shoot-to-root ratio (S:R) (mean ± SE) of *Pinus koraiensis* seedlings at the end of experiment. Different capital letters indicate a significant effect of S:R among pre-hardening fertilization types ($p < 0.05$). Different lowercase letters indicate significant differences in S:R among autumn fertilization levels ($p < 0.05$). Means were compared using Duncan's tests.

Pre-Hardening Fertilization Type	Autumn Fertilization Level	S:R
CF	AF0	3.04 B ± 0.02
	AF2	2.34 ± 0.69
	AF4	2.14 ± 0.30
	AF6	2.51 A ± 0.07
EF	AF0	3.60 Aa ± 0.10
	AF2	2.11 b ± 0.14
	AF4	2.99 ab ± 0.45
	AF6	2.60 Ab ± 0.21
CRF	AF0	2.68 Ca ± 0.04
	AF2	3.07 a ± 0.19
	AF4	2.13 b ± 0.18
	AF6	1.88 Bb ± 0.14

3.2. Effect of Fertilization on Root Traits

Pre-hardening fertilization type, autumn fertilization level, and their interaction had extremely significant effects on TRL, TRA, and morphology of fine roots (diameter ≤ 1 mm; $p < 0.01$; Table 4). In the absence of autumn fertilization, morphology of fine roots (diameter ≤ 1 mm) in CF was significantly higher than EF and CRF ($p < 0.05$), whereas the other root traits were similar among the three pre-hardening fertilization types.

Autumn fertilization with three nitrogen levels improved the root morphology of seedlings, but the N doses affected plants of the pre-hardening fertilization types differently. In general, root length, area, and volume of CF and EF increased with low and medium levels of autumn fertilization but decreased with the highest level, AF6, whereas in CRF we did not observe this decline and values in AF4 and AF6 were not significantly different (Figure 2a–c). The CF treatment seemed to be more responsive to autumn fertilization and showed higher TRL, TRA, TRV, and root length, area, and diameter of roots ≤ 1 mm than EF and CRF for AF4 (Figure 2a–c).

Table 4. Statistics (df, degrees of freedom; *p*-values; and significance levels) of two-way ANOVAs testing the main and interaction effects of pre-hardening fertilization type and autumn fertilization level on root morphology of *Pinus koraiensis* seedlings.

Source	df	*p*-Values									
		TRL	TRA	TRV	SRL	Root with Diameter ≤ 1 mm			Root with Diameter > 1 mm		
						Length	Area	Volume	Length	Area	Volume
PF	2	0.002 **	0.002 **	0.050 *	0.094 ^{ns}	0.007 **	<0.001 **	<0.001 **	0.035 *	0.051 ^{ns}	0.135 ^{ns}
AF	3	<0.001 **	<0.001 **	0.006 **	0.091 ^{ns}	<0.001 **	<0.001 **	<0.001 **	0.004 **	0.003 **	0.031 *
PF × AF	6	0.002 **	0.006 **	0.177 ^{ns}	0.232 ^{ns}	0.006 **	0.002 **	0.001 **	0.010 **	0.048 *	0.313 ^{ns}

Note: ns denotes a non-significant effect; * and ** indicate significant effects at 0.05 and 0.01 significance levels, respectively. Abbreviations: PF: pre-hardening fertilization type; AF: autumn fertilization level.

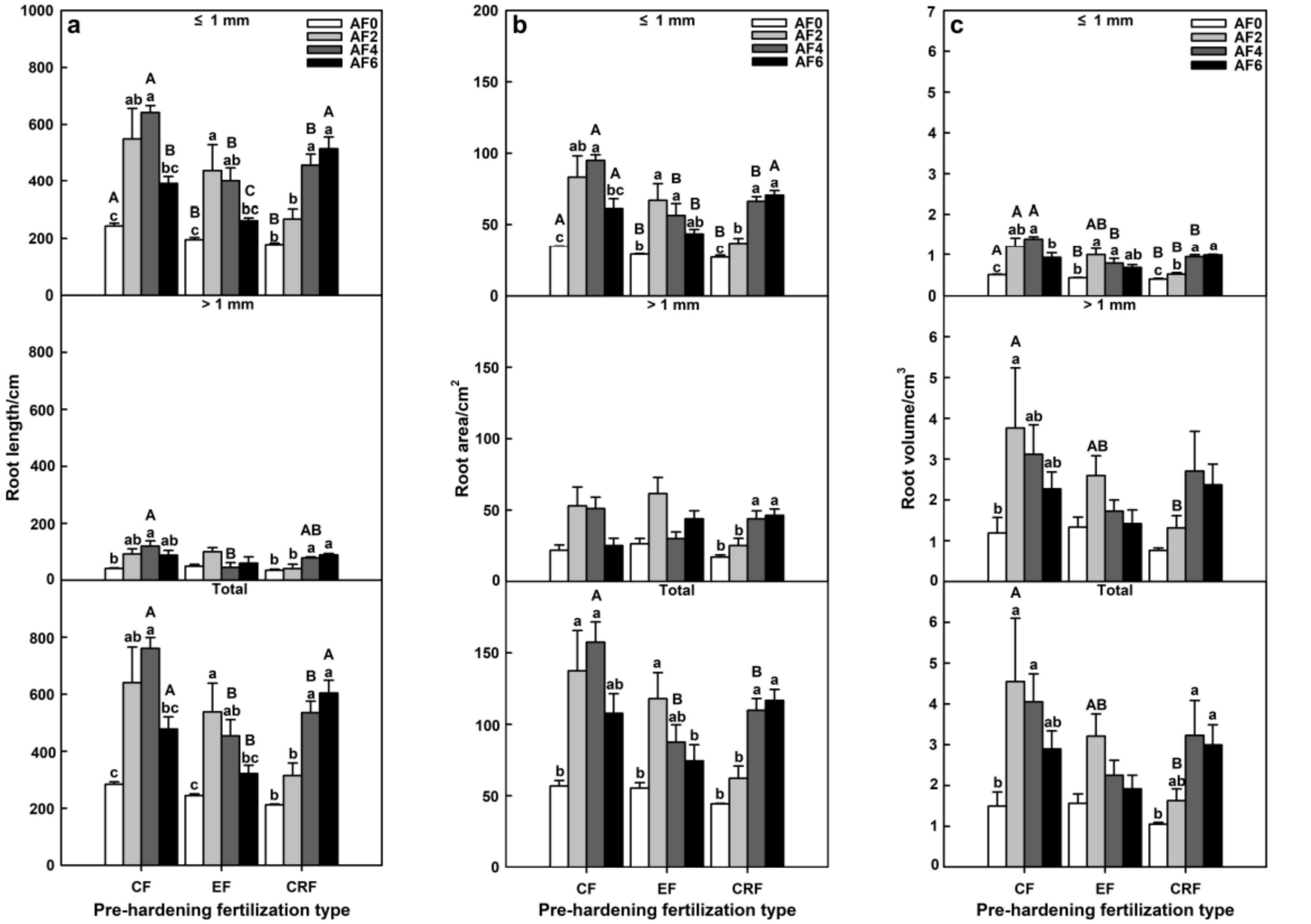

Figure 2. Root length (**a**), area (**b**), and volume (**c**) in seedlings of *Pinus koraiensis* in response to pre-hardening fertilization type (CF, EF, and CRF) and autumn fertilization level (AF0, AF2, AF4, and AF6) treatments. Bars and arrows represent the mean and corresponding standard error, respectively. Different capital letters indicate a significant effect of root traits among pre-hardening fertilization types ($p < 0.05$). Different lowercase letters indicate significant differences in root traits among autumn fertilization levels ($p < 0.05$). Means were compared using Duncan's tests.

In the absence of autumn fertilization, SRL of CF was significantly higher than EF and CRF ($p < 0.05$; Table 5). Autumn fertilization had no significant effect on SRL.

Table 5. Effects of pre-hardening fertilization type and autumn fertilization level on specific root length (SRL) (mean ± SE) of *Pinus koraiensis* seedlings at the end of experiment. Different capital letters indicate a significant effect of S:R among pre-hardening fertilization types ($p < 0.05$). Means were compared using Duncan's tests.

Pre-Hardening Fertilization Type	Autumn Fertilization Level	SRL (cm/g)
CF	AF0	738.72 A ± 16.14
	AF2	650.30 ± 64.29
	AF4	733.15 ± 104.06
	AF6	691.25 ± 83.92
EF	AF0	505.57 C ± 3.16
	AF2	626.03 ± 50.85
	AF4	738.68 ± 77.17
	AF6	622.42 ± 52.23
CRF	AF0	580.10 B ± 1.91
	AF2	797.89 ± 71.13
	AF4	745.25 ± 51.02
	AF6	721.02 ± 47.72

4. Discussion

Height growth of seedlings tends to stagnate at the end of the growing season, but stem cambium and the root system continue growing [3]. As our first hypothesis, autumn fertilization significantly increased biomass accumulation and modified the root morphology of Korean pine seedlings growing in containers, as has been observed in previous studies with *Quercus ilex* [25,26]. We also found that, except for stem biomass, there was a significant interaction of pre-hardening fertilization type and autumn fertilization in biomass allocation, which is similar to the results of biomass and nitrogen concentration in roots obtained in seedlings of Chinese cork oak (*Quercus variabilis* Bl.) [27]. These results validated our second hypothesis that the response of Korean pine container seedlings to autumn fertilization is affected by the type of pre-hardening fertilization.

We found that autumn fertilization promoted biomass accumulation of Korean pine seedlings. Several studies have found that seedlings with higher biomass and more internal nutrient reserves are more resistant to environmental stress after afforestation and can compete better with understory vegetation during establishment [5,28–30]. This has been related to a greater ability to allocate more resources to roots after planting and better overcome stress [14]. We also found that biomass of seedlings fertilized with EF during the growing season was higher with low levels of autumn fertilization but lower with high level of autumn fertilization, and a similar pattern was showed by seedlings in CF. However, in the CRF treatment we did not observe a decline in biomass production at high levels of N. Fertilizers used for CF and EF were urea, which is a traditional water-soluble fertilizer. Fertilization with different N sources can produce diverse growth responses in some species [31], as in *P. taeda* seedlings fertilized with urea which reduced growth compared with seedlings fertilize with ammonium [32]. In Korean pine, controlled-release fertilizer can improve nitrogen utilization efficiency of seedlings compared with urea, which may be the reason why CRF seedlings can maintain high biomass production under high autumn fertilization levels [33]. Another explanation could be related to luxury consumption, and thus pines in CF and EF and high doses of N would increase nutrient concentration in some tissues at the expense of growth [34]. This result suggests that pre-hardening fertilization type should be considered when evaluating the response of seedlings to autumn fertilization and the importance of appropriate autumn fertilization levels [23,35].

Biomass allocation patterns also determine the afforestation performance of seedlings. If the proportion of aboveground biomass is too large, the transpiration rate of the above-ground part may exceed the rate of water absorption by the roots, resulting in wilting and even the death of seedlings. Thus, S:R lower than 2.5 is considered ideal since seedlings are less susceptible to water stress after planting [36], although some studies have reported the opposite, and plants with high S:R showed lower mortality after planting in semi-arid areas apparently due to higher availability of photosynthates to resume root growth [37]. We found that autumn fertilization reduced the S:R of Korean pine seedlings, which indicates that autumn fertilization can increase the proportion of underground biomass by promoting root growth. We also found that, except for CF-AF6, EF-AF4, EF-AF6, and CRF-AF2, S:R in the other autumn fertilization treatments was less than 2.5.

The root system is an important organ for plants to absorb and utilize nutrients and anchor aboveground parts [38]. Root morphology is also an important standard to reflect seedling quality. Root morphological indexes such as TRL and TRA are closely related to the establishment, growth, and survival of seedlings after afforestation. Seedlings with well-developed root structures have a high ability to absorb water and nutrients and tolerate drought and barren sites [39]. We found that autumn fertilization increased the TRL and TRA of Korean pine container seedlings. Additionally, an appropriate autumn fertilization level can effectively improve not only the root morphology of seedlings but also their hydraulic capacity [40]. The increment of photosynthesis capacity following fertilization rapidly increases the distribution of photosynthate in roots, with the subsequent increases in TRL and TRA. The higher absorption capacity of roots and better absorption efficiency of nutrients and water in seedlings prevent nutrient deficiencies and negative water potentials during periods of water scarcity [19,41]. After analyzing the root morphological index after dividing the diameter class, we found that pre-hardening fertilization type and autumn fertilization level have an extremely significant effect on root morphology with diameter $\leq$ 1 mm ($p < 0.01$). Fine roots ($D \leq 1$ mm) are susceptible to soil nutrient availability and are the main part in the root system for nutrient absorption [42], so the improvement of the root system by fertilization is mainly reflected in fine roots. In nutrient-dense areas, the root system is dominated by fine roots because of its large absorption area and high absorption efficiency [43].

In our study, compared with seedlings fertilized by EF and CRF in the absence of autumn fertilization, seedlings fertilized by CF had higher SRL. The reason may be that pre-hardening fertilization types of EF and CRF can better satisfy the nitrogen needs of seedlings, so EF and CRF seedlings can obtain sufficient nutrients without improving their own resource utilization efficiency, and photosynthates are preferentially allocated to the growth of aboveground organs. However, CF seedlings, in an attempt to improve resource utilization efficiency, preferentially allocate photosynthates to roots and expand root extension to absorb the sufficient nutrients.

5. Conclusions

Autumn fertilization can effectively promote the growth of Korean pine container seedlings. There was significant interaction between pre-hardening fertilization type and autumn fertilization level on biomass allocation and root morphology. Interestingly, and as occurred with biomass, a medium autumn fertilization level resulted in longer roots and higher volume than in the treatment with high autumn fertilization level in seedlings with conventional and exponential pre-hardening fertilization. Considering biomass accumulation and root morphology, autumn fertilization with a medium nitrogen level, 4 mg/seedling per week for 3 weeks, after conventional fertilization during the growing season (CF-AF4) is the most appropriate fertilization treatment for 1-year-old Korean pine container seedlings.

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