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## Invasion of Korean Pine (*Pinus koraiensis*) Seedlings into an Oak Forest in Korea: Biomass, Leaf Mass per Area, Chlorophyll and Nutrients

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### Abstract

The study aimed to estimate the standing biomass, nutrient distribution, leaf mass per area (LMA) and chlorophyll of Korean pine (*Pinus koraiensis* S. et Z.) seedlings after harvesting of overstory *Quercus mongolica* where Korean pine seedlings grow from seeds transported by red squirrels. Three years after harvesting, biomass, nutrient distribution, LMA and chlorophyll were measured, and allometric equations were applied to estimate the biomass of the foliage, branches, stems and roots of seedlings. Heights and diameters of Korean pine seedlings showed a reverse J-shaped distribution. Total biomass (kg ha<sup>-1</sup>) of Korean pine seedlings was 3,627 in the harvested stand and 1,432 in the control stand. The amounts of foliage were higher in both stands compared to other artificially planted Korean pine seedlings. The ratio of foliage biomass to total biomass was 32% in the harvested stand and 42% in the control stand. There were no significant differences in N and P concentration between the two stands among the different parts of the Korean pine seedlings. Total N and P contents were 22.50kg ha<sup>-1</sup> and 2.48kg ha<sup>-1</sup> for the harvested stand and 12.04kg ha<sup>-1</sup> and 1.26kg ha<sup>-1</sup> for the control stand, respectively. The ratio of N content of foliage to total N content was 58.9% in the harvested stand and 65.3% in the control stand. The ratio of P content of foliage to total P content was 53.0% in the harvested stand and 55.1% in the control stand. LMA was higher in the harvested stand than in the control stand. The LMA of current-year foliage was lower than that of older foliage in both stands. Total chlorophyll and chlorophyll b contents were higher in the control stand. There were no significant differences in chlorophyll a/b ratios between the two stands and between foliage ages. Korean pine seedlings originating from seeds transported by red squirrels showed increased LMA, decreased chlorophyll contents, and increased biomass resulting from improved light conditions following the harvesting of the overstory deciduous forest.

*Key words:* allometric equation, biomass, chlorophyll, invasion, LMA, Korean pine seedling

### Introduction

Korean pine (*Pinus koraiensis* S. et Z.) naturally grows together with deciduous broad-leaved trees and is a constituent of mixed forests in East Asia (Barnes *et al.* 1992, USDA Forest Service 2000, Wang 1994, Zyryanova *et al.* 2005). Korean pine is one of the important forest tree species as it has fruits that are edible and it produces good quality wood. Korean pine, which has been planted over several decades, accounts for 4% of the total forested area in Korea (Korea Forest Service 2005). Korean pine seedlings grow around planted forests as rodents such as red squirrels transport seeds in the forest (Miyaki 1987, Hayashida 1989, Kim *et al.* 1999, Jin *et al.* 2000). To produce Korean pine nuts and lumber, natural forest was replaced with artificial plantations in Korea. However, the costs and ecological disadvantages involved in pure artificial plantations have stirred debate among experts. Considering that Korean pine trees blend with broad-leaved trees in native forests, it seems to be a meaningful approach to convert existing oak forests, in

which Korean pine seedlings grow as a result of seeds transported by animals such as red squirrels, to mixed forests with Korean pine seedlings. There have been many studies of the invasion of broad-leaved trees into the needle-leaved forests in temperate regions (Namikawa and Wang 1996, Kitaoka and Koike 2004, Kitaoka and Koike 2005). But few studies have investigated the invasion of Korean pine seedlings into the forests in Korea (Lee 2002, Son *et al.* 2005). Korean pine seedlings naturally growing in oak forests show higher levels of density and lower growth rates than planted Korean pine trees (Kim *et al.* 1999, Jin *et al.* 2000). To promote the growth of Korean pine seedlings, it is necessary to increase the exposure to sunlight and the space between trees by removing overstory dominant trees. There will be changes in Korean pine seedlings if overstory dominant trees are harvested. Having more sunlight and space, Korean pine seedlings will show improved growth patterns physically and functionally. That is, the size and thickness of leaves will change along with functional

changes such as the amount of total chlorophyll and chlorophyll ratios (Kitaoka *et al.* 2003). This study investigated the biomass, LMA, chlorophyll, and nutrients of Korean pine seedlings after harvesting overstory dominant oak trees in *Quercus mongolica* forests.

### Materials and Methods

The study was conducted in a *Quercus mongolica* forest located in Research Forest of the Kangwon National University (37°49'N, 127°51'E) in central Korea where the average annual precipitation is 1,295 mm and precipitation amounts during the period from July to August account for 70% of the annual precipitation. The average annual temperature was 10.9°C over the last 30 years with a maximum temperature of 36.6°C and a minimum temperature of -9.6°C. In 2002, a total of eight 10m × 10m plots were established in the forest for harvesting and control. In four plots overstory *Q. mongolica* were harvested. Diameter at breast height (DBH) for overstory *Q. mongolica* and diameter at root collar (RCD) for Korean pine seedlings were measured.

In late September 2005, thirty Korean pine seedlings in the control stand were selected based on diameter distribution for biomass estimation. Seedlings were cut at the ground level and divided into foliage, branches, and stems. Roots were manually excavated to a depth that allowed the removal of practically all the roots. The fresh weight of each component was measured in the field. Subsamples of each component were weighed, oven-dried at 65°C to a constant weight and weighed again to determine moisture content. Total fresh weights were converted to dry weights using the calculated moisture content. Relationships between diameter at root collar and biomass were analyzed. An allometric model of the form  $Y=aRCD^b$  ( $Y$ , oven-dry weight of the biomass component of a seedling; RCD, root collar diameter;  $a$  and  $b$ , parameters) was used to develop equations for all biomass components of the seedlings. The equations were then solved for all surveyed seedlings within the plots, giving estimates of plot biomass for each seedling component. Biomass for Korean pine seedlings in the harvested stand was estimated from the allometric equations developed by Ji (2004).

Seedling sub-samples of each component were collected for nitrogen (N) and phosphorus (P) analyses. Nitrogen and P concentrations were analyzed colorimetrically with a Lachat continuous flow ion analyzer (Lachat QuikChem AE, Wisconsin, USA) after digestion with a sulfuric acid digest (Son and Gower 1992). To estimate LMA, 100 needles were collected from different foliage age groups for both stands. Leaf area and dry weight were measured using WinNeedle (Regent Instruments Inc., Canada) to calculate the ratio of leaf weight to leaf area ( $g\ cm^{-2}$ ). Foliage sub-samples were extracted using 80% acetone to measure chlorophyll content at different foliage ages. Light intensity was measured with a spectrophotometer (U-1100, Hitachi, Japan) at 645nm and 633nm. Total chlorophyll content, and chlorophyll  $a$  and  $b$  were

determined by the methods of Arnon (1949).

## Results and Discussion

### Stand structure

Heights and diameters of overstory oak trees and understory Korean pine seedlings showed different distribution patterns (Figure 1). Oak trees showed no distinct distribution patterns in diameter and height while Korean pine seedlings had reverse J-shaped distribution patterns in diameter and height. The average diameter at the root collar was 1.2cm in Korean pine seedlings and the majority of Korean pine seedlings had the value < 2.0cm for diameter at the root collar. The study stand had two distinct stratum with *Q. mongolica* in the dominant canopy layer at an average top height of 21.5m and *P. koraiensis* in the understory stratum with an average height of 0.7m. *Q. mongolica* had an average DBH of 21.5cm. Species densities were also markedly different with *Q. mongolica* at 750 and *P. koraiensis* at 4300 stems per hectare.

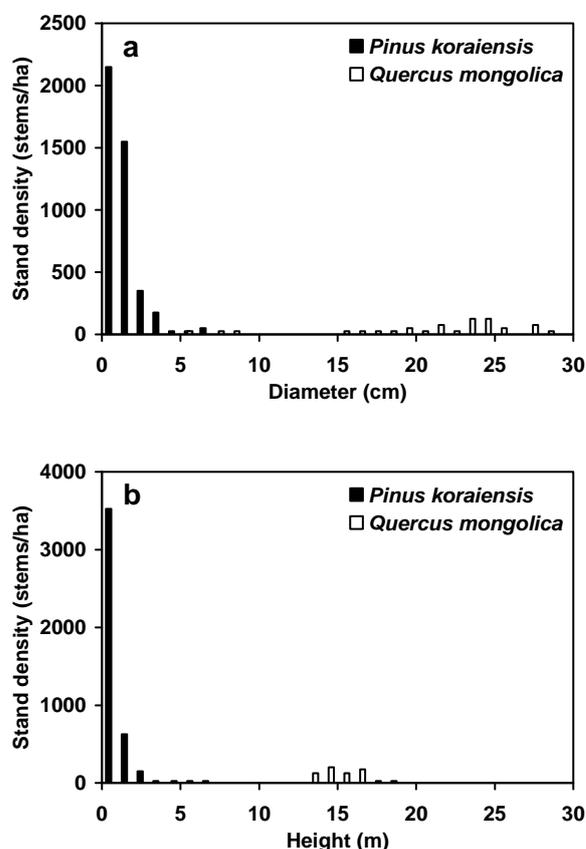


Fig. 1. Frequency distribution of trees in diameter (a) and height (b) classes for *Pinus koraiensis* and *Quercus mongolica* in a mixed-species stand in central Korea.

### Allometric equations and biomass

All allometric models expressing dry weights (g) of the seedling components as a function of RCD (mm) showed high  $R^2$  values and low standard errors of estimates (Figure 2). Based on regression equations

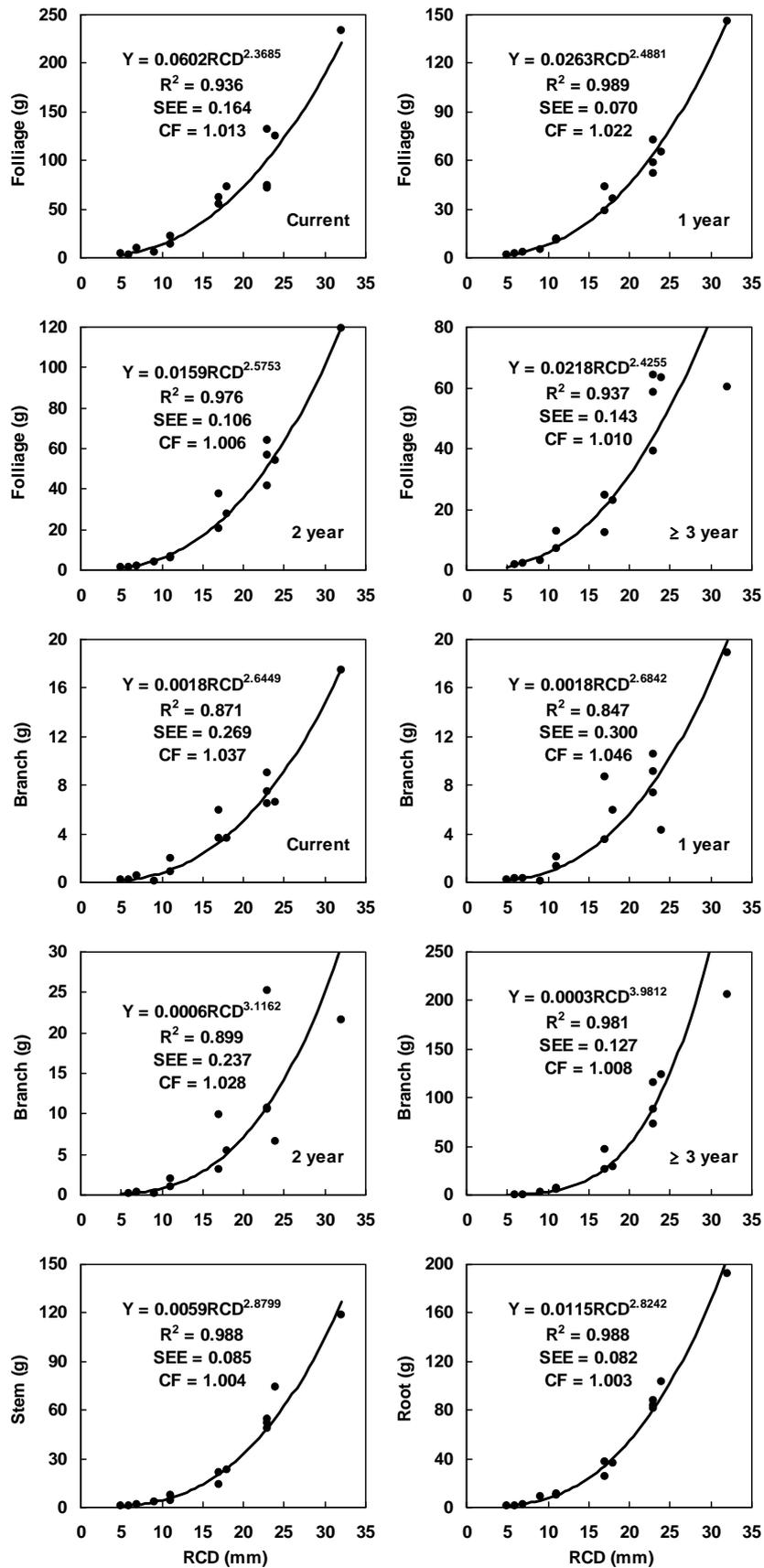


Fig. 2. Relationships between dry weight of tree components and diameter at root collar (RCD) for *Pinus koraiensis* seedlings. The curves fit are exponential functions,  $Y=aRCD^b$  (Y, component drymass; a and b, parameters). SEE, the standard error of estimate. CF, the correction factor.

given in Fig. 2 and the results of Ji (2004), aboveground and underground biomass of Korean pine seedlings in the control and harvested stands was estimated. Total biomass was 3,627 kg ha<sup>-1</sup> in the harvested stand, which was 2.5 times higher than the 1,432 kg ha<sup>-1</sup> value for the control stand (Table 1). Total biomass of the control stand was lower than the 2.7 t ha<sup>-1</sup> value reported in a previous study (Son *et al.* 2005) in which the biomass was estimated for Korean pine seedlings located in an area not far from the forest used in this study. In general, the aboveground biomass is large in the stems among planted Korean pine trees, and is followed by the branches and foliage (Kwon 1982, Lee 1983, Lee and Park 1987, Son *et al.* 2001, Yi 1998). However, this study found a foliage > branch > stem order in the harvested stand in which the amount of biomass was higher in foliage and a foliage = branch > stem order in the control stand in which the amounts of foliage and branch biomass were almost the same with both being larger than the amount of stem biomass. The ratio of foliage biomass to total biomass was 42% in the control stand, which was larger than the 32% in the harvested stand. The branch biomass was larger than that of the stem in the control stand but smaller than that of the stem in the harvested stand. It is therefore assumed that a larger amount of biomass is allocated to foliage because of low light intensity stemming from shade conditions in the understory where the Korean pine seedlings are located (Son *et al.* 2005).

### LMA and chlorophyll

There were differences in the LMA (g cm<sup>-2</sup>) of foliage between the harvested and control stands (Figure 3). LMA was significantly lower in the control stand due to high shielding rates ( $p < 0.05$ ). LMA levels were positively correlated to foliage age. Significantly lower LMA was observed in current-year foliage than in 1-year- and 2-year-old foliage. These results were consistent with the findings of Son *et al.* (2005). LMA (g cm<sup>-2</sup>) was 0.013 in current-year foliage, 0.017 in 1-year-old foliage and 0.018 in 2-year-old foliage in the control stand. And these values were almost same as those reported in an earlier study (Son *et al.* 2005), but significantly lower than the range (0.023-0.028 g cm<sup>-2</sup>) reported by Son *et al.* (2001) for planted Korean pine stands. Specific leaf area (SLA) and LMA are used as important growth parameters to measure responses to shading (Larcher 1995, Kitaoka and Koike 2005, Qu *et al.* 2005). A reduction in LMA in response to changes in shading indicates that thinner leaves with lower mass developed under shaded conditions (Qu *et al.* 2005, Kitaoka *et al.* 2003). When foliage becomes thinner, it can utilize limited sunlight more effectively and consume as much carbon dioxide as is possible in shaded conditions. The lower LMA levels of the control stand, compared with the LMA levels of planted Korean pine seedlings and those of the harvested stand, are attributed to physical conditions (sun leaf and shade leaf) under different light intensities (Bartelink 1997, Son *et al.* 2001).

Table 1. Biomass (kg ha<sup>-1</sup>) and standard error (parentheses,  $n=4$ ) of *Pinus koraiensis* seedling components in harvested and control stands.

Component		Harvested stand	Control stand
Foliage	current	435.39 (31.62)†a	228.14 (17.67)b
	1yr	266.47 (19.06)a	150.21 (10.70)b
	2yr	264.15 (17.52)a	122.87 (8.24)b
	≥3yr	193.75 (13.45)a	100.39 (7.47)b
Branch	current	48.01 (3.70)a	17.74 (1.14)b
	1yr	71.03 (5.40)a	20.37 (1.27)b
	2yr	89.08 (6.41)a	31.94 (1.94)b
	≥3yr	667.96 (39.20)a	407.30 (50.90)b
	dead	28.25 (1.73)a	6.35 (0.47)b
Stem		1139.90 (69.20)a	133.72 (7.76)b
Root		423.26 (28.52)a	213.61 (12.56)b
Total		3627.24 (228.54)a	1432.64 (84.16)b

† Means with different letters within a row are statistically different at  $p < 0.05$ .

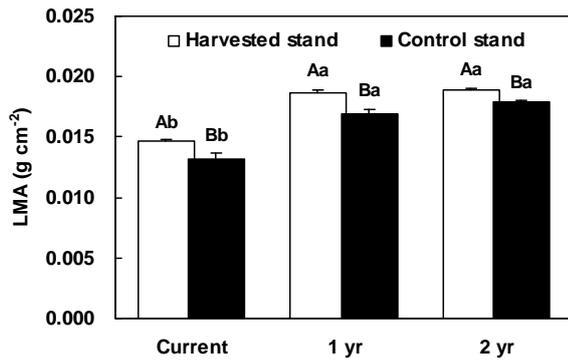


Fig. 3. Leaf mass per area (LMA, g cm<sup>-2</sup>) of *Pinus koraiensis* seedlings in harvested and control *Quercus mongolica* stands. Vertical bars represent standard error of the means. Different letters are significantly different at  $p=0.05$  level. Capital letters indicate differences between stands within same foliage age, and small letters indicate differences between foliage ages within a stand.

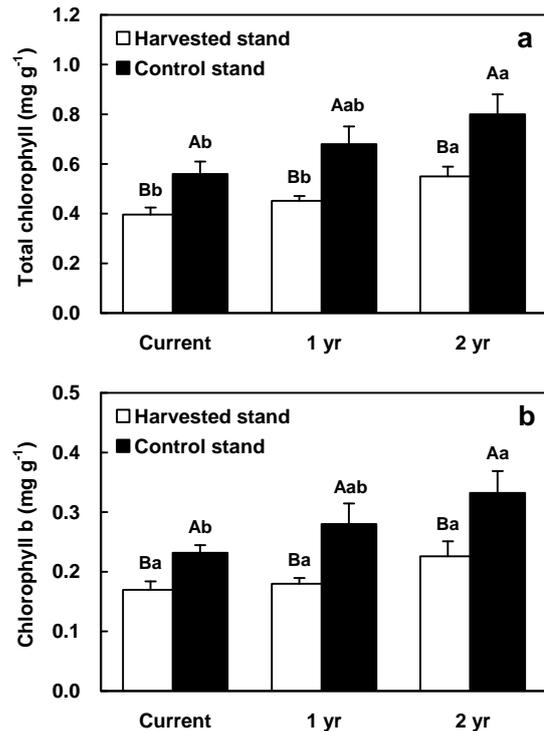


Fig. 4. Total chlorophyll (a), chlorophyll b (b) of *Pinus koraiensis* seedlings in harvested and control *Quercus mongolica* stands. Vertical bars represent standard error of the means. Different letters are significantly different at  $p=0.05$  level. Capital letters indicate differences between stands within same foliage age, and small letters indicate differences between foliage ages within a stand.

Total chlorophyll content and chlorophyll b content of foliage (current-year, 1-year-old and 2-year-old) were higher in the control stand than in the harvested stand (Figure 4). Wang *et al.* (2001) reported that sunlight blockage had the same effects in pine trees. Total chlorophyll content and chlorophyll b content of foliage increased with foliage age. Chlorophyll a and b contents in the control stand were higher than the previously reported values that ranged from 0.24 to 0.30 mg g<sup>-1</sup> for chlorophyll a and from 0.16 to 0.19 mg g<sup>-1</sup> for chlorophyll b for similar foliage ages (Son *et al.* 2005). Korean pine seedlings in the harvested stand showed higher LMA and total chlorophyll, and chlorophyll a and b contents than those in the control stand. These results were consistent with Wang *et al.* (2001) and Kitaoka *et al.* (2003), and mean that the increased photosynthetic capacity of Korean pine seedlings in the harvested stand resulted in increased biomass.

### Nutrients

Nitrogen (N) and P concentrations for Korean pine seedlings were higher in the control stand than in the harvested stand although there was no significant difference (Table 2). It appears that seedling component N and P concentrations tended to be higher for the control stand than the harvested stand because of excess N and P absorption under low light conditions (Waring and Schlesinger 1985). Nitrogen and P concentrations were highest in foliage followed by the branches, roots, and stems. Nitrogen and P contents of different parts of the seedlings were about 2 times higher in the harvested stand than in the control stand (Table 3). Total N content in the control stand was lower than the value of 17.6 kg ha<sup>-1</sup> for similar age seedlings (Son *et al.* 2005). The ratio of foliage biomass to total biomass was 32% in the harvested stand and 42% in the control stand

(Table 1) while the ratio of N content of foliage to total N content was 58.9% in the harvested stand and 65.3% in the control stand (Figure 5). The ratio of P content of foliage to total P content was 53% in the harvested stand and 55.1% in the control stand. These values were similar to those found in Korean pine seedlings naturally growing in an oak forest (Son *et al.* 2005).

It appears that harvesting resulted in minor changes in nutrient conditions compared to biomass and their allocation among components of Korean pine seedlings. In general, the current results indicated sharp differences in morphology and slight changes in the physiological characteristics of Korean pine seedlings after harvesting. However, our data were obtained only 3 years after harvesting. It is possible that these characteristics will change with an increase in years after treatment. Therefore, longer-term and more detailed research would be necessary to identify the influence of harvesting on the changes in microenvironments and growth of Korean pine seedlings naturally regenerated near plantations.

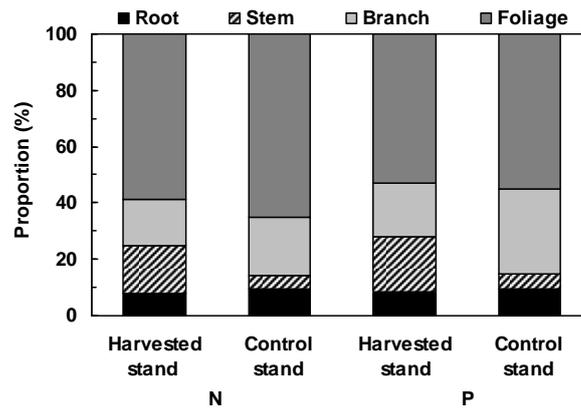


Fig. 5. Allocation of nitrogen (N) and phosphorus (P) to tree components for *Pinus koraiensis* seedlings in harvested and control *Quercus mongolica* stands.

Table 2. Average nitrogen (N) and phosphorus (P) concentrations (% of dry weight) and standard error (parentheses,  $n=3$ ) of *Pinus koraiensis* seedling components in harvested and control stands.

Component	Nitrogen (N)		Phosphorus (P)		
	Harvested stand	Control stand	Harvested stand	Control stand	
Foliage	current	1.307(0.032)†a	1.357(0.047)a	0.167(0.003)a	0.147(0.024)a
	1yr	1.283(0.074)a	1.287(0.047)a	0.090(0.006)b	0.107(0.018)a
	≥2yr	1.290(0.023)a	1.270(0.112)a	0.077(0.009)b	0.090(0.015)a
Branch	current	0.833(0.054)a	0.860(0.143)a	0.110(0.006)a	0.120(0.010)a
	1yr	0.553(0.071)b	0.643(0.058)ab	0.083(0.012)a	0.090(0.010)ab
	≥2yr	0.437(0.071)b	0.500(0.035)b	0.047(0.012)b	0.077(0.015)b
Stem	0.383(0.020)	0.410(0.040)	0.043(0.003)	0.050(0.006)	
Root	0.477(0.041)	0.540(0.026)	0.050(0.006)	0.057(0.007)	

† Means with different letters within a column of each component are statistically different at  $p<0.05$ .

Table 3. Nitrogen (N) and phosphorus (P) contents ( $\text{kg ha}^{-1}$ ) of *Pinus koraiensis* seedlings in harvested and control stands.

Component	Nitrogen (N)		Phosphorus (P)	
	Harvested stand	Control stand	Harvested stand	Control stand
Foliage	current	5.691	0.727	0.335
	1yr	3.419	0.240	0.161
	≥2yr	5.907	0.353	0.201
Branch	current	0.400	0.053	0.021
	1yr	0.393	0.059	0.018
	≥2yr	3.308	0.356	0.338
Stem	4.366	0.548	0.490	0.067
Roots	2.019	1.153	0.212	0.122
Total	25.502	12.046	2.489	1.263

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