

# Understory Junipers, and Light Environment Effects on Biomass, Chemical Composition, and Nutrient Contents of Black Pine Seedlings

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In forest environments, the establishment, survival, and recruitment of seedlings of desired species can often be influenced by biotic interactions between the competing understory shrubs and the seedlings, as well as by the understory light environment. Previous studies regarding competing effects were mostly focused on the survival and growth of seedlings, but it is still largely unclear how competition with shrubs impacts the biomass of the seedlings and physiological traits connected to photosynthesis and nitrogen metabolism in various forest ecosystems. Moreover, there is limited knowledge on the influence of light conditions on the biomass of understory seedlings is limited for different species. The main objectives of this study were to examine the effects of understory junipers and light conditions on the above and belowground biomass of black pine seedlings (*Pinus nigra* Arnold). This study also examined the changes in chemical composition and plant nutrient contents in the organs of black pine seedlings (i.e., root, stem, and needle) with the presence of junipers in the understory. Seedling biomass was significantly affected by understory light conditions, while the presence of junipers negatively affected the root-biomass of black pine seedlings. Moreover, understory junipers influenced some photosynthetic pigments in black pine seedlings.

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## INTRODUCTION

Seedling growth and establishment in forests are significantly impacted by the complex interplay between environmental elements (Lhotka and Loewenstein 2008). In forest environments, the establishment, survival, and recruitment of seedlings of desired species can often be influenced by biotic interactions between competing understory shrubs and the seedlings (Huang *et al.* 2021). In previous studies, how understory seedlings react to competition with understory shrubs has been commonly observed in varying forest ecosystems (Tiscar and Linares 2011; Kara *et al.* 2017), but they documented inconsistent findings. Some studies have found that understory shrubs can aid seedling establishment by offering shade and shielding seedlings from extreme weather conditions (Gómez-Aparicio *et al.* 2004; Gómez-Aparicio *et al.* 2009). However, other studies have discovered that there may be a reduction in seedling survival and growth because understory shrubs may compete with the seedlings for nutrients (Götmark *et al.* 2011; Jensen and Löf 2017).

Although the effects of understory shrubs on seedling survival and growth have been widely examined, the knowledge of how understory plants affect the aboveground and belowground biomass of the seedlings is not well documented (Annighöfer *et al.* 2022).

Aboveground and belowground biomass of the seedlings is usually associated with the environmental adaptability of the species (Bachofen *et al.* 2019); therefore, quantifying the impacts of competing understory shrubs on the biomass of understory seedlings is crucial. Modeling seedling development and carbon sequestration heavily relies on such biomass equations. According to the light needs of understory seedlings, light transmittance onto the forest floor is another component that can either impede or encourage the survival, growth, and biomass of understory seedlings (Petritan *et al.* 2009; Kara and Topaçoğlu 2018). Our understanding of how light penetration into the forest floor affects seedling biomass in different forest ecosystems is currently limited. Ineffective regeneration efforts may result from a lack of understanding of the connections between understory competitive vegetation, understory light conditions, and seedling biomass.

Resources have a significant role in plant-plant interactions (*i.e.*, competition). When faced with competition, plants can display certain adaptive traits that may be morphological, anatomical, physiological, or biochemical in nature, which help them survive and thrive in stressful environments (Dawood *et al.* 2014). Plants may detect stress through their roots following competition and may send signals that cause changes in metabolism and the activation/synthesis of defense systems in various plant parts (Siopongco *et al.* 2008). In addition to the limited knowledge on the influence of understory shrubs on the biomass of understory seedlings, uncertainty also remains about how understory shrubs cause changes in chemical components and plant nutrients in the organs of the seedlings (*i.e.*, root, stem, and needle). Chemical substances known as pigments can show color by selective absorption of visible light wavelengths; they help in the process of photosynthesis, which is the basis of plant growth and a critical physiological mechanism for production (Bond *et al.* 2007; Vemmos *et al.* 2013). They can also enhance the ability of plants to adapt to changing environmental situations (Yer Çelik 2021). Therefore, studying the photosynthetic capability of seedlings that compete with understory shrubs would aid in understanding how well these organisms can grow, survive, and adapt to ever-changing environmental conditions (Guo *et al.* 2018).

The degree or direction of plant-plant interactions is substantially influenced by how well soil resources are used (Huang *et al.* 2021). Although the role that nutrients play in plant growth is known to be complex, certain nutrients are usually required for proper growth. Plant nutrient intake regulates and promotes growth, helps with photosynthesis, stimulates the growth of new roots, plays a role in energy-producing processes, increases the vigor and resistance of plants, and helps in the production of a plant hormone, *etc.* (Barker and Pilbeam 2015). The competition between understory seedlings and understory shrubs for resources including nutrients is regarded as a clear source of effect on the metabolic processes of plants (Gidman *et al.* 2003), which may eventually affect the growth and development of the seedlings (Kreslavski *et al.* 2012). Competition between seedlings and understory shrubs may impact their growth rates, net photosynthetic rates, and nutrient contents in leaves (Li *et al.* 2016). However, few studies have examined competitive interactions between different species in various ecosystems (Herb and Stefan 2006). Thus, a better understanding of the impact of understory shrubs on seedling nutrients is also needed.

Black pine (*Pinus nigra* Arnold) is one of the most widespread and economically important tree species across Europe and Asia Minor (Köseoğlu and Kara 2019). Although

silvicultural disturbances are thought to promote the regeneration of black pine, it is yet unclear how other factors, such as understory shrubs, drive this process. Our knowledge of how competing understory vegetation affects the biomass of black pine seedlings in particular is relatively poor. *Juniperus oxycedrus* L. subsp. *oxycedrus* (hereafter, juniper), which is a thermophile shrub, may be commonly found in a variety of locations across the Mediterranean and near the Black Sea region (Orhan *et al.* 2011; Vilar *et al.* 2016). The species is also known as an indicator species and a common understory shrub in black pine forests (Güner *et al.* 2011). To expose mineral soil and reduce their competition with the seedlings, junipers are frequently removed when natural regeneration in black pine stands is aimed (Odabaş *et al.* 2004). Few studies have monitored the competitiveness of seedlings of desired species against given understory shrubs (Schnitzer 2005; Guo *et al.* 2018). However, there is still ambiguity over how black pine seedlings react to junipers in the understory throughout the range of species.

Previous studies regarding competition effects have mostly focused on survival and growth, but it is still largely unclear how competition impacts biomass, and physiological traits connected to photosynthesis and nitrogen metabolism in various forest ecosystems. Understory juniper-seedling relationships regarding chemical components and plant nutrients remain insufficiently explored in black pine forests as well. Moreover, the influence of light conditions on the biomass of black pine seedlings is not well documented. The main objectives of this study were to examine the effects of understory junipers and light conditions on the aboveground and belowground biomass of black pine seedlings, and the changes in chemical components and plant nutrients in the organs of black pine seedlings (*i.e.*, root, stem, and needle) based on the presence of junipers. The quantitative knowledge about juniper-seedling relationships in the understory would help forest managers understand how understory seedlings may respond to junipers when aiming to regenerate and conduct site preparation in black pine stands. Knowledge of the interspecific variations in plant features linked to physiology or morphology can be used as a basis to select species when establishing plantations or modifying or controlling understory species richness.

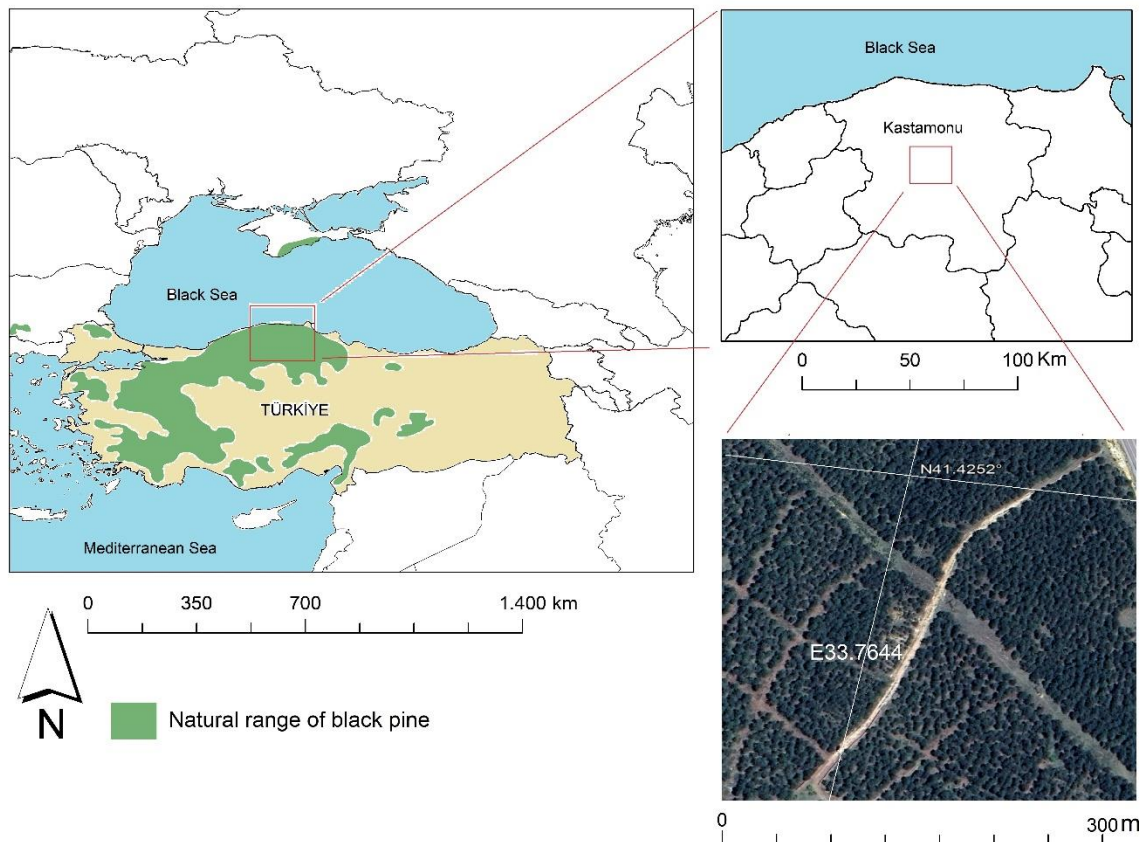
## EXPERIMENTAL

### Study Site

This study was conducted in a black pine forest in the northern Turkish city of Kastamonu (Fig. 1). This area is within the native black pine distribution and is a component of the Euro-Siberian phyto-geographic region. The research area is illustrative of the usual features of a continental climate, with cold winters and rainy summers. The average monthly temperature and the mean total yearly precipitation are 9.7 °C and 480 mm, respectively. The terrain varies from virtually level to gradually sloping. Loamy clay dominates the soil, which is often moderately deep (between 40 cm and 80 cm). The elevation of the research site varies from 700 m to 800 m above sea level. A forest stand dominated by black pine was selected for the research. The other dominant tree species of the research site is Scots pine (*Pinus sylvestris* L.).

The study stand is a natural forest. It represented an even-aged stand structure and had an area of around five hectares. The average height of trees was 10.2 m, while the dominant tree ages ranged from 30 to 50. Early in 2020, the stand was thinned from below, and exhibited a partial canopy afterwards. The typical understory cover, juniper, is sporadic

within the study stand. Over the course of the study site, no other understory vegetation predominated.



**Fig. 1.** Location of Kastamonu city, and study stand

### Study Design

A total of 30 well-developed junipers were chosen at random from the understory of the study stand. Overstory black pine trees were more than 2 m distant from the chosen junipers. Although factors that affect the distribution of junipers were not examined in this study, given the personal observations, the presence of junipers seemed to depend on light availability within the stand. Across the stand, junipers had an average root collar diameter (RCD) of 25 mm, ranging from 11 mm to 43 mm. The average height of junipers was 60 cm, ranging between 33 cm and 90 cm. Three black pine seedlings were planted next to each chosen juniper (a total of 90 seedlings) within a distance of 20 cm (Fig. 2) to monitor the competitive impacts of junipers on the planted seedlings (Lhotka and Lowenstein 2015). The research stand is in close proximity to the Daday Nursery Directorate, where the seedlings for the planting were attained. Bare-root one-year-old black pine seedlings were used. The planted seedlings had a mean RCD of 4.43 mm, ranging from 2.22 to 6.84, and an average height of 26.9 cm, varying from 16.5 cm to 42.8 cm. For control purposes, the same number of seedlings (90 total) were planted at least 2 m away from the selected junipers (Fig. 2) to reduce their intraspecific competition with the junipers (Kara *et al.* 2017). All black pine seedlings were hand-planted on the day they were shipped from the nursery in March 2020. To avoid the intraspecific competition with mature trees, the researchers made sure that the seedlings were not planted near the trees. The seedlings were not irrigated after planting because planting was done immediately after a rainy period.





**Fig. 2.** Black pine seedlings planted near to juniper, and away from juniper

At the end of the third growing season after planting (*i.e.*, October 2022), 60 planted seedlings (*i.e.*, 30 planted near junipers, and 30 planted away from junipers) were uprooted by hand and using small shovels. Afterwards, they were delivered to the laboratory for sampling. The needles of the seedlings were removed, and seedlings were properly cleansed of dirt and other debris in the laboratory. They were then separated into the main stem and root. All components (*i.e.*, roots, needles, and stems) were oven-dried for 72 h at 70 °C (Yamashita *et al.* 2016), in a Nüve FN 400 oven (Nüve Laboratory & Sterilization Technology). After that, dried samples were weighed with a precision scale to determine the root biomass (RB), stem biomass (SB), needle biomass (NB), and total aboveground (TAG) biomass of the seedlings.

Because light transmittance through forest canopy (TPAR) may influence seedlings biomass, light measurements were also taken and included in analyses. First, photosynthetically active radiation (PAR) was measured above each planted seedling understory. The PAR measurements were taken using a CI-110 Plant Canopy Imager (CID Bio-Science Inc., Camas, WA, USA), on a nearly cloudless day, between 11:00 and 13:00 h time as suggested (Kara and Topaçoğlu 2018). During the PAR measurements, the device was leveled, and extra care was taken to prevent the operator's shadow from blocking the sensors. In an open area, another PAR measurement was taken concurrently. Then, the TPAR (%) for each seedling was attained using the formula below:

$$\text{TPAR (\%)} = \frac{\text{PAR understory}}{\text{PAR in open}} \times 100 \quad (1)$$

### Biochemical and Mineral Analyses

Chlorophylls, carotenoids, and xanthophylls were extracted with ethanol according to the methods described by Kukric *et al.* (2012) and Chang *et al.* (2013) with some modifications. For extraction, a representative portion of sample ( $0.1 \pm 0.001$  g) (mass) was accurately weighted, ground, and quantitatively transmitted in a glass test tube. Then,

ethanol was added to make it up to 5 mL, the test tubes were kept in the dark for 15 min with occasional shaking at room temperature, and finally centrifuged. Chlorophyll, carotenoid, and xanthophyll content were analyzed spectrophotometrically by absorption measurements at 350 to 700 nm with 1.0 nm interval and calculated according to the following equations:

$$\text{Chlorophyll a (mg/g)} = (13.7 \times 665) - (5.76 \times A_{649})/\text{mass} \times 200 \quad (2)$$

$$\text{Chlorophyll b (mg/g)} = (25.8 \times A_{649}) - (7.6 \times A_{665})/\text{mass} \times 200 \quad (3)$$

$$\text{Carotenoid (mg/g)} = (4.7 \times A_{440}) - (0.263 \times \text{CHLA} + \text{CHLB})/\text{mass} \times 200 \quad (4)$$

$$\text{Xanthophylls (lutein) (mg/g)} = (11.51 \times A_{480}) - (20.61 \times A_{495})/\text{mass} \times 200 \quad (5)$$

The proline content, which protects plants from numerous stresses and aids in their quicker recovery after stress, was examined in needle, stem, and root samples. The proline content was examined using the method outlined by Bates *et al.* (1973). First, dry needle, stem, and root samples of seedlings were grounded. Using acidic ninhydrin reagent, 500 mg of samples of each part (*i.e.*, needle, stem, and root) were crushed and homogenized in 3% aqueous sulfosalicylic acid. At 520 nm, absorbance of the homogenate was measured. The calibration curve was used to calculate the proline content, which was expressed as  $\mu\text{mol/g}$  of fresh weight.

The total polyphenol contents were determined according to the method of Folin and Denis (1915). After being homogenized in 15 mL of acetone (80%), 500 mg of dry powdered bark was filtered through a Buckner's funnel. The final volume was adjusted to 50 mL with 80% acetone after the residue was rinsed multiple times with the solvent. The Folin-Denis reagent was made by combining 100 g of sodium tungstate with 20 g of phosphomolybdic acid in approximately 800 mL distilled water and 200 mL of 25% phosphoric acid. The mixture was then refluxed for 2 to 3 h to room temperature before the final volume was adjusted to 1000 mL with distilled water. With distilled water, the reaction mixture was diluted to a final volume of 50 mL. On a double beam UV-visible spectrophotometer (SPECTRO Analytical Instruments, Kleve, Germany), the absorbance of the blue color that appeared after 20 min was determined at 660 nm. With the use of a standard curve containing 0.1 mg/mL tannic acid, the total amount of polyphenols was determined and represented as 100 g of dry weight.

To determine the phenylalanine ammonia lyase (PAL) activities, 0.1 g of fresh needle was homogenized with 50 mM sodium phosphate, which contains 1% polyvinylpyrrolidone (PVP), 1 mM phenylmethylsulfonyl fluoride (PMSF), with pH 6.5. Next, the homogenate was centrifuged at 4 °C for 25 min in 10,000 g. The supernatant attained from this homogenate was used for the PAL. Spectrophotometric method was used to determine PAL (Pascholati *et al.* 1986; Goldson *et al.* 2008). A 100  $\mu\text{L}$  enzyme extract and 1000  $\mu\text{L}$ , 0.2% phenylalanine solution were mixed and allowed to react for 1 h at 37 °C, and transformation of L-phenylalanine to trans cinnamic acid was read following absorption measurements at 290 (A 290) nm. The PAL activity was determined by preparing the cinnamic acid standards and was defined in terms of  $\mu\text{mol}$  cinnamic acid per hour.

Soluble sugars in needle, stem, and root samples were also determined. At both the cellular and organismal levels, the soluble sugars—particularly sucrose and glucose—play a crucial role in the structure and metabolism of plants. Soluble sugars were determined by utilizing the anthrone method (Pearson *et al.* 1976). The leaf, stem, and root samples were

also analyzed for concentrations of nutrient elements (Na, K, Ca, Cl, Fe, Mg, Cr, Co, Cu, Mn, Ni, P, S, and Zn) using SPECTRO brand (SPECTRO Analytical Instruments, Kleve, Germany) XEPOS model X-ray fluorescence (XRF) instrument at Central Research Laboratory at Kastamonu University.

### Statistical Analyses

The effects of junipers and light on above and belowground biomasses of black pine seedlings were examined using a mixed-effect multiple regression model. The TPAR (%) and seedling groups (*i.e.*, planted near junipers [hereafter, PSN<sub>J</sub>] and planted away junipers [hereafter, PSA<sub>J</sub>], were utilized as fixed-effects variables (*i.e.*, independent variables), while RB, SB, NB, and TAG were used as the response variables (*i.e.*, dependent variables). Junipers were treated as random effects in the models. It should be noted that an individual juniper was considered as the experimental unit (*i.e.*, replication). The models were defined by the equation below,

$$R_v = \beta_0 + R_e + X^T + \varepsilon \quad (6)$$

where  $R_v$  is the dependent variable,  $\beta_0$  is the intercept,  $R_e$  is the random effect,  $X^T$  is the transposed matrix of the independent variables, and  $\varepsilon$  is the error term.

Comparison of seedling groups (*i.e.*, PSN<sub>J</sub> and PSA<sub>J</sub>) in terms of photosynthetic pigments, proline, and soluble sugar contents were also tested using a mixed-effect regression model ( $\alpha$ -level = 0.05). With the use of residual analysis, the normality and homogeneity of data were examined, and no deviations from these model assumptions were observed. During statistical analyses, “lme”, “aov” and “TukeyHSD” functions of R-Statistical software (R Development Core Team 2021) were utilized.

## RESULTS

The RB values of seedlings ranged from 0.85 to 2.24 g across all seedlings, while SB varied between 1.49 and 5.04 g. The PSA<sub>J</sub> seedlings had significantly higher RB compared to PSN<sub>J</sub> seedlings (Table 1). Even though SB and NB were slightly greater in PSN<sub>J</sub> seedlings, the differences were not statistically significant. Moreover, a similar amount of TAG biomass was examined with the two seedling groups (Table 1).

**Table 1.** Descriptive Statistics for the Variables of Studied Seedling Groups

Variables	Seedling Groups		p-value
	PSN <sub>J</sub>	PSA <sub>J</sub>	
RB	1.15 (0.86 to 1.78)	1.48 (0.85 to 2.24)	P < 0.05
SB	3.32 (1.49 to 4.55)	3.06 (1.87 to 5.04)	P > 0.05
NB	2.79 (1.44 to 3.99)	2.60 (0.88 to 5.38)	P > 0.05
TAG	6.11 (2.93 to 12.01)	5.66 (2.75 to 10.42)	P > 0.05

RP, SB, NB, and TAG refer to dry root biomass, dry stem biomass, dry needle biomass, and total aboveground biomass, respectively. PSN<sub>J</sub> and PSA<sub>J</sub> donate to seedlings planted near junipers, and seedlings planted away from junipers, respectively. Numbers in parenthesis give the range.

The linear mixed effect models indicated that biomasses of all components (*i.e.*, root, stem, needle, and total aboveground of black pine seedlings were significantly affected by TPAR (%). Biomass values increased with increasing TPAR (%) across all seedlings ( $p < 0.05$ ) (Table 2). Moreover, being away from junipers positively affected RB

of black pine seedlings, but influence of junipers on SB, NB, and TAGB was not statistically significant ( $p > 0.05$ ) (Table 2).

**Table 2.** Influences of Light Transmittance (TPAR (%)), and Junipers (*i.e.*, Away Versus Near Junipers) on Biomass of Black Pine Seedlings

Variables	RB	SB	NB	TAG
Intercept	74.26 (0.21)**	2.7669 (0.67)**	1.9818 (0.81)*	4.7487 (1.39)*
TPAR	0.0114 (0.01)***	0.0248 (0.01)*	0.0321 (0.01)**	0.0569 (0.02)*
Juniper	0.2085 (0.12)*	ns	ns	ns

ns, \*, \*\*, \*\*\* are not significant,  $p > 0.05$ ,  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively. The DRP, DSB, DNB, and TAGB refer to dry root biomass, dry stem biomass, dry needle biomass, and total aboveground biomass, respectively. Numbers in brackets within the results refer to the standard error (SE).

Table 3 displays the average levels of photosynthetic pigments (*i.e.*, Chlorophyll *a*, Chlorophyll *b*, total chlorophyll, and carotenoids) in needle samples of the seedling groups (*i.e.*, PSN<sub>J</sub> and PSA<sub>J</sub>). Some concentrations varied significantly among the seedling groups. While the differences of Chlorophyll *a* were not notable among the seedling groups, PSN<sub>J</sub> seedlings had greater amount of Chlorophyll *b* and total chlorophyll than PSA<sub>J</sub> seedlings (Table 3). A similar amount of total carotenoid was examined in the seedling groups ( $p > 0.05$ ). In addition, Chlorophyll *a/b* (Ch-*a*/Ch-*b*) ratio of PSA<sub>J</sub> seedlings was relatively higher than PSN<sub>J</sub> seedlings. Moreover, anthocyanin and xanthophyll were significantly higher in PSA<sub>J</sub> seedlings than PSN<sub>J</sub> seedlings (Table 3).

**Table 3.** Mean Concentrations of Photosynthetic Pigments (mg/g) in Needles of Black Pine Seedlings in Different Seedling Groups

Components	PSN <sub>J</sub>	PSA <sub>J</sub>	p-Value
Chlorophyll <i>a</i>	0.647 ± 0.0007	0.609 ± 0.001	0.103
Chlorophyll <i>b</i>	1.055 ± 0.002	0.775 ± 0.001	< 0.0001
Total Chlorophyll	1.702 ± 0.003	1.385 ± 0.001	< 0.0001
Total Carotenoid	3.961 ± 0.011	3.671 ± 0.014	0.1511
Chlorophyll <i>a/b</i> ratio	0.613 ± 0.0007	0.786 ± 0.002	< 0.0001
Xanthophyll	0.406 ± 0.001	0.687 ± 0.001	< 0.0001
Anthocyanin	0.5612 ± 0.001	0.7579 ± 0.002	< 0.0001

PSN<sub>J</sub> and PSA<sub>J</sub> denote to seedlings planted near junipers and seedlings planted away junipers, respectively.

Mean concentrations of proline ( $\mu\text{mol/g}$ ), total polyphenol (TP) (mg/g), phenylalanine ammonia lyase (PAL, mg/g), glucose (mg/g), and sucrose (mg/g) in different parts of seedlings (*i.e.*, needle, stem, and root) for the seedling groups are given in Table 4. Some concentrations varied significantly among the seedling groups ( $p < 0.05$ ). In needles, proline, glucose, and sucrose were significantly greater in PSA<sub>J</sub> seedlings, while TP was higher in needles of PSN<sub>J</sub> seedlings (Table 4). In stems, the concentrations of all components except TP were statistically insignificant between PSN<sub>J</sub> and PSA<sub>J</sub> seedlings ( $p > 0.05$ ). As for roots, TP, glucose, and sucrose were significantly greater in PSA<sub>J</sub> seedlings compared to the PSN<sub>J</sub> seedlings ( $p < 0.005$ ) (Table 4). The change of PAL activity, which plays a role in the synthesis of phenolic compounds in organs had no statistical significance ( $p > 0.05$ ).



**Table 4.** Influence of Junipers on Mean Concentrations of Proline ( $\mu\text{mol/g}$ ), TP ( $\text{mg/g}$ ), PAL ( $\text{mg/g}$ ), Glucose ( $\text{mg/g}$ ), and Sucrose ( $\text{mg/g}$ ) in Needles, Stem, and Root of Black Pine Seedlings that Grown Near or Away Junipers

Parts	Components	Seedling Groups		p-Value
		PSN <sub>J</sub>	PSA <sub>J</sub>	
Needle	Proline	24.98 $\pm$ 0.02	36.39 $\pm$ 0.05	0.033
	TP	100.67 $\pm$ 0.12	64.23 $\pm$ 0.14	< 0.001
	PAL	1.41 $\pm$ 0.51	0.90 $\pm$ 0.41	0.065
	Glucose	53.45 $\pm$ 0.11	62.76 $\pm$ 0.14	0.022
	Sucrose	166.5 $\pm$ 0.39	171.2 $\pm$ 0.32	< 0.001
Stem	Proline	20.42 $\pm$ 0.02	22.58 $\pm$ 0.08	0.293
	TP	82.69 $\pm$ 0.12	96.12 $\pm$ 0.06	< 0.001
	PAL	1.16 $\pm$ 0.01	1.24 $\pm$ 0.01	0.126
	Glucose	51.38 $\pm$ 0.12	52.23 $\pm$ 0.15	0.138
	Sucrose	146.5 $\pm$ 0.67	151.3 $\pm$ 0.38	0.315
Root	Proline	24.55 $\pm$ 0.03	22.17 $\pm$ 0.08	0.325
	TP	87.88 $\pm$ 0.58	66.36 $\pm$ 0.04	< 0.001
	PAL	1.23 $\pm$ 0.01	1.16 $\pm$ 0.01	0.412
	Glucose	70.60 $\pm$ 0.09	80.73 $\pm$ 0.15	< 0.001
	Sucrose	169.4 $\pm$ 0.24	203.4 $\pm$ 0.13	< 0.001

Table 5 exhibits the changes in elements (ppm) in different parts of black pine seedlings by seedling groups. In general, concentrations of Na, Mg, P, S, K, and Ca were mostly higher than other elements. While P, S, Cl, and K concentrations were higher in needles, Na was greater in stems, and Mg and Ca concentrations were higher in roots (Table 5). In needles of PSN<sub>J</sub>, accumulations of Na, Mg, Cl, and Ca concentrations were greater. In stem tissues of PSN<sub>J</sub>, Na, Mg, S, P, K, and Ca concentrations were higher than other elements.

**Table 5.** Changes in Elements (ppm) in Different Parts of Black Pine Seedlings by Seedling Groups

Elements	Leaf			Stem			Root		
	PSN <sub>J</sub>	PSA <sub>J</sub>	p-Value	PSN <sub>J</sub>	PSA <sub>J</sub>	p-Value	PSN <sub>J</sub>	PSA <sub>J</sub>	p-Value
Na	3290	2630	p < 0.01	3470	3430	p > 0.05	3520	3050	p < 0.01
Mg	1767	1304	p < 0.01	1493	1199	p < 0.01	3396	2520	p < 0.01
P	1555	1739	p < 0.01	1086	1238	p < 0.01	718.5	957.3	p < 0.01
S	1992	2089	p > 0.05	984.1	970.5	p > 0.05	1051	1197	p < 0.01
Cl	487.9	305.2	p < 0.01	219.6	304.5	p < 0.01	249.7	231.1	p > 0.05
K	12840	12870	p > 0.05	8945	7871	p < 0.01	7543	7671	p > 0.05
Ca	12790	11610	p < 0.01	15710	10150	p < 0.01	23820	22830	p > 0.05
Cr	10	5.5	p < 0.01	17.4	11.2	p < 0.01	86.2	71.3	p < 0.01
Mn	44.6	41.4	p > 0.05	41.5	34.3	p < 0.01	111.4	79.3	p < 0.01
Fe	576.1	299.4	p < 0.01	937.2	460.5	p < 0.01	5696	4510	p < 0.01
Co	15.9	14	p > 0.05	18	23.8	p < 0.01	20.8	19.2	p > 0.05
Ni	43.9	31.1	p < 0.01	40.8	34.6	p < 0.01	52.1	45.6	p < 0.01
Cu	31.9	10.7	p < 0.01	37.1	14.4	p < 0.01	40.1	14.3	p < 0.01
Zn	79.1	45.7	p < 0.01	77.1	48.8	p < 0.01	77.7	40.2	p < 0.01

## DISCUSSION

Understory light availability is one of the most important drivers that promotes or inhibits the growth of seedlings (Daryaei *et al.* 2019), which can be mainly provided through silvicultural implications including thinning. The understory light environment has a significant impact on early growth of black pine because it is a semi-tolerant species to shade (Kara *et al.* 2021). The lack of light in the understory may have a detrimental impact on further growth of black pine seedlings (Tiscar and Linares 2011). These claims are supported by this study, which shows how light availability influences the biomass black pine seedlings. Light usually plays a more crucial role in growth and biomass of black pine seedlings especially after age of three (Genç 2020).

The competition of understory seedlings with understory vegetation for resources may also limit seedling growth (Kuusipalo 1983). Even though some studies revealed that understory vegetation has a positive or no effect on the growth of pine seedlings (Hyppönen *et al.* 2013), the consensus is that understory vegetation has a negative effect on seedling growth by competing for soil moisture and other resources (Smidt and Puettmann 1998; Yılmaz *et al.* 2018). Black pine requires a partial canopy because of its moderate shade tolerance in early stages, and this canopy structure allows establishment of other understory vegetation including junipers (Odabaşı *et al.* 2004; Genç 2020). A study that examined black pine recruitment in central-eastern Spain concluded that control of competing vegetation improved seedling emergence in pure stands (Lucas-Borja *et al.* 2016). In a similar study, Tardós *et al.* (2019) monitored the impacts of understory removal on black pine seedling establishment and found that the removal enhanced seedling recruitment. The reason why junipers only affected root biomass is likely that black pine seedlings primarily develop their root system rather than aboveground parts (Köseolu and Kara 2019). For this reason, the influence of junipers on stem and needle biomass might have been insignificant. Moreover, the allelopathic potential of junipers on the growth of neighboring plants (Young and Bush 2009; Semerdjieva *et al.* 2022) can also be associated with the negative impacts of junipers on root biomass of black pine seedlings examined in the present study.

In addition to the photosynthetic pigments that play a direct role in photosynthesis in plant tissues, there are also other types of pigments (*i.e.*, polyphenols) that create color, odor, and aroma (Tanaka *et al.* 2008; Tran 2018). Photosynthetic pigments are effective in absorbing light energy and retaining this energy in the chemical bonds of ATP and NADPH molecules in biomass synthesis reactions (Li *et al.* 2016). Polyphenolic compounds are bioactive molecules that play a role in cleaning toxic molecules with their high antioxidant properties, preventing pathogen attacks, stimulating tolerance to stresses, such as UV stress and extreme temperatures, and increasing mechanical resistance by strengthening the cell wall structure (Tanaka *et al.* 2008; Zhu *et al.* 2016). In this study, changes in Chlorophyll-*a*, Chlorophyll-*b*, and total chlorophyll were lower in PSA<sub>J</sub> seedlings.

The similar Chlorophyll-*a* content of the seedlings in both groups can be associated with the adaptation of the seedlings to balance the ratio of the above and belowground parts of the seedlings (Schall *et al.* 2012). It should be recalled that PSN<sub>J</sub> seedlings were planted near junipers; thus, those seedlings were likely in competition for light with junipers, because most of them were shaded to varying degrees by the crowns of the junipers. In contrast, PSA<sub>J</sub> seedlings were not in competition with junipers. It has been revealed in previous studies that the change of chlorophyll pigments in coniferous species, such as black pine, Scots pine, spruce, and fir, varies according to plant age, leaf age, and light (or shade) conditions (Niinemets 2012; Turfan *et al.* 2021; Deligöz *et al.* 2018; Hernandez

Velasco and Mattsson 2019; Pakharkova *et al.* 2020). The lower Ch-*a*/Ch-*b* ratio and xanthophyll content of PSN<sub>J</sub> seedlings were likely due to their competition with junipers for light (Deligöz *et al.* 2018; Tran 2018). In a similar study, Tran (2018) reported that exposure of *Fraxinus latifolia* seedlings to low light conditions can cause a decrease in the Chl-*a*/Chl-*b* ratio.

Previous studies found that high xanthophyll concentration is an indicator of light absorption capacity and the amount of carbonaceous compounds (Niinemets and Valladares 2004; Snyder *et al.* 2004). Previous research also pointed out that the xanthophyll ratio in leaf tissue is usually associated with anthocyanin accumulation (Manetas *et al.* 2002; Tanaka *et al.* 2008). In general, the higher the xanthophyll content, the higher the amount of anthocyanin in leaves (Snyder *et al.* 2004), which is consistent with the current study. Anthocyanin and xanthophyll pigments are molecules that increase the protection of plants in leaf tissue against abiotic and biotic stress conditions (Tanaka *et al.* 2008). Zhu *et al.* (2016) examined the effects of different light regimes on the leaf tissues of seedlings and found more accumulation of anthocyanins in the leaves of the seedlings that were exposed to full light, which is consistent with this study where higher anthocyanins were found in PSA<sub>J</sub> seedlings. They further stated that the accumulated anthocyanin played a role in the reduction of photo-oxidative damage to the leaves. Under light competition conditions, young and fresh leaves especially complete the development of their photosynthetic apparatus more slowly, and therefore they accumulate less anthocyanin (Yu *et al.* 2021).

The total polyphenol (TP) content of the seedlings significantly differed between the seedling groups (*i.e.*, PSN<sub>J</sub> and PSA<sub>J</sub>) in the leaf, stem, and root ( $p < 0.001$ ). The amount of TP was higher in the leaves and roots of the PSN<sub>J</sub> seedlings, while it was higher in the stem tissues of the PSA<sub>J</sub> seedlings. The higher amount of TP in leaves and roots of PSN<sub>J</sub> seedlings can be associated with the change of secondary metabolic pathways according to organs (Niinemets and Valladares 2004; Deligöz *et al.* 2018). The higher phenols in the stem tissue of PSA<sub>J</sub> seedlings were considered to result from the cell wall activity related to stem development. The stem balances the below and above-ground volume of the plant and is also important in the transmission of matter from the soil to the leaf and from the leaf to the roots (Poorter *et al.* 2012). Because PSA<sub>J</sub> seedlings did not experience a major competition with junipers for light and other resources, their metabolism likely accelerated and caused an increase in stem growth rate (Anterola *et al.* 2002; Dixon *et al.* 2002). In PSN<sub>J</sub> seedlings, these reactions might have progressed more slowly, and secondary metabolite accumulation decreased due to slowing metabolism (Franceschini and Schneider 2014).

Osmolytes or osmoprotectants are amino acids with low molecular weight, reduced sugars, polyols, proteins, and polyamines (Szabados and Saviouré 2010; Gago *et al.* 2022). These compounds play major roles in physiological processes, such as the control of osmosis/turgor events, detoxification of toxic molecules, and protection of cellular membrane integrity in plants. The PSA<sub>J</sub> seedlings had significantly higher proline content in their needles. It has been reported in the literature that proline is a stress indicator and its amount in plant tissues usually increases under water stress (Szabados and Saviouré 2010). Therefore, it is likely that increasing light availability resulted in a decrease in moisture content for PSA<sub>J</sub> seedlings, and consequently an increase in proline content in leaves of PSA<sub>J</sub> seedlings. Moreover, low proline content in both seedling groups can be associated with seedling size and age. Many metabolic reactions, including water transport, usually decelerate during the early stages of seedling development in autumn and winter

seasons (Poorter *et al.* 2012; Goudiaby *et al.* 2022), which can cause low amount of proline contents.

The reduced sugars (*i.e.*, glucose and sucrose) were higher in PSA<sub>J</sub> seedlings. Both compounds reached the highest value in root tissues, followed by needle and stem tissues. The low glucose and sucrose contents in PSNJ seedlings can be associated with their competition with junipers (Bai *et al.* 2012). Because these compounds were higher in root and leaf tissues of PSA<sub>J</sub> seedlings also substantiates this assertion. Because the lack of competition in these seedlings stimulates root development, it may be the reason for high glucose and sucrose source-pool balance (Poorter *et al.* 2012; Gago *et al.* 2022). The increase in root volume under the soil causes more water requirements. High glucose and sucrose may have balanced the water content and turgor/osmosis events in leaves and roots (Chin and Sillett 2016; Rezai *et al.* 2018). Accordingly, Stephenson *et al.* (2014) found that carbon accumulation in plants increased as the size and volume of the plant increased.

Minerals that have important functions on optimal growth conditions of plants are generally classified as major elements and trace (heavy metals) elements. Major elements are found in high amounts in plant tissues, while trace elements are found in much lower concentrations (Bolat and Kara 2017). The concentration of P, S, Cl, and K macro nutrients was greater in leaves, while Na concentration accumulated more in stem, and Mg and Ca concentrations were higher in the roots. In general, the order of the major elements depending on the amount was Ca > K > Na > Mg > S > P > Cl. The element concentrations observed in the leaves, stems, and roots of the seedlings are in line with the literature studies, which examined *Pinus* (Pietrzykowski *et al.* 2013); *Cupressus* (Chin and Sillett 2016; Turfan 2022), and *Picea* (Krüger *et al.* 2021; Major and Mosseler 2021) species. Major elements are essential in plant development because they regulate physiological events such as osmotic regulation (K, Cl), stomatal movements (K, Cl), carbohydrate and protein metabolism (K, P, S, Ca), cell walls strengthening (Ca), enzyme activations (K, Mg, Ca), and chlorophyll biosynthesis (Mg). The high amount of Cl in leaves has been considered as an adaptation to stomatal behavior because stomatal movements in leaves are closely related to K/Cl ions (Zenda *et al.* 2017; Major and Mosseler 2021). High Cl content in the stems of PSA<sub>J</sub> group seedlings has been associated with the regulation of water movement in the stems (Zenda *et al.* 2017).

In general, Cr, Mn, Fe, Ni, Cu, and Zn concentrations were relatively higher in PSNJ seedlings, while Co concentration was found higher in PSA<sub>J</sub> seedlings. The results regarding trace elements were similar to the findings of Alaimo and Varrica (2020), Eltner and Sivacioğlu (2021), and Turfan (2022). Researchers determined that the most abundant trace elements in leaves, stem bark, and roots are Fe, Mn, Zn, Ni, Cu, and Cr, respectively. In addition, Fe, Mn, Zn, and Cu are functional in the regulation of electron transport and redox in chloroplasts and mitochondria, as well as acting as cofactors of many enzymes in plants (Alaimo and Varrica 2020; Major and Mosseler 2021). It is likely that the accumulator properties of juniper roots cause more trace element accumulation around the root of black pine seedlings (Petrova 2020); thus, the advantage of these elements for black pine roots may be effective for the PSNJ seedlings in long-term (An *et al.* 2014). The fact that trace elements are generally high in the roots has been associated with the mobility of the elements. The elements Fe and Cu are considered immobile, whereas Mn and Zn are mobile elements (Bolat and Kara 2017; Rietra *et al.* 2017).

Although understory junipers appeared to compete with black pine seedlings in the study stand, it should be noted that junipers, which is a native species in the area, could play an indispensable role in maintaining and functioning of the ecosystem (Kalapos and

Mázsa 2001). Thus, complete removal of the junipers should be avoided while treating understory vegetation during natural regeneration of these forests. It should also be emphasized that invading species may dominate the area if the native species like junipers are completely eradicated (Bakacsy and Szepesi 2023). Further research can be recommended to examine the complete and partial removal of junipers on the ecosystem functioning in black pine forests.

## CONCLUSIONS

1. Dry root biomass, dry stem biomass, dry needle biomass, and total aboveground biomass of black pine seedlings were influenced by the light transmittance through the overstory canopy, while understory junipers negatively affected root-biomass of the black pine seedlings.
2. The presence of understory junipers near black pine seedlings influenced the Chlorophyll *b*, total chlorophyll, anthocyanin, and xanthophyll of the pine seedlings.
3. Greater proline, glucose, and sucrose were determined in seedlings planted away from junipers, while total polyphenol was higher in seedlings planted near junipers
4. Initial findings would enable forest managers to use silvicultural practices that modify the understory light availability and regulate understory competing species. However, due to its potential roles in functioning of black pine ecosystems, complete removal of junipers should be avoided in these forests.
5. Knowledge about how juniper effects on seedling development vary over time is still limited. Therefore, long-term monitoring of the study area may be recommended.
6. Further research can also be recommended to examine the influence of complete and partial removal of junipers on the ecosystem functioning in black pine forests.

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