

Effects of nursery fertilization and shading on the seedling quality of the endemic *Abies cephalonica* Loudon

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Abstract

Forest restoration initiatives are accelerating worldwide, increasing the demand for high-quality nursery seedlings. Seedling performance after planting depends strongly on nursery practices that shape morphology, physiology, and stress tolerance. Despite this, species-specific protocols remain scarce, particularly for endemic Mediterranean conifers such as *Abies cephalonica*. This species faces high vulnerability due to climate-driven droughts and rising wildfire incidence, which severely limit natural regeneration. To support effective restoration, understanding how nursery techniques affect seedling quality is essential. We assessed, for the first time, the combined effects of shade, fertilization, and seed provenance on the growth and quality of Greek fir seedlings. The results showed that across treatments, seedlings remained relatively small at two years of age. Seed provenance primarily affected shoot morphology, with Parnitha seedlings showing greater height and higher H/D ratios than those from Vytina. Fertilization was the dominant factor enhancing seedling quality, significantly increasing RCD, biomass accumulation, and root system development. Shading effects were complex: 70% shade improved root collar diameter and reduced height/diameter ratio, while 50% shade maximized root and total biomass. Our study provides critical insights into how these widely used practices influence biomass allocation, root development, and overall seedling quality. The results offer practical recommendations for optimizing nursery protocols and improving restoration success for this threatened Mediterranean species.

Introduction

Recent policies at regional and international levels, such as the UN Decade of Ecosystem Restoration, the EU Biodiversity Strategy for 2030 and the EU Nature Restoration Regulation are promoting forest restoration worldwide. The plantation of seedlings grown in nurseries plays a key role in this restoration effort. Ensuring seedling survival and achieving effective forest restoration depend on a range of silvicultural practices. A key component of any successful restoration effort is the use of high-quality seedlings with strong potential for survival (Grossnickle 2012). Environmental conditions during nursery cultivation modify seedling morphological and physiological characteristics ultimately affecting outplanting performance (Villar-Salvador et al. 2026). Over the last decades, forest scientists and practitioners have focused on adjusting nursery cultivation protocols to species functional characteristics and the conditions in the planting sites following the Target Plant Concept (Dumroese et al. 2016). However, most nursery protocols aimed at cultivating high quality seedlings were developed based on common or economically important tree species (Clark et al. 2023). Given that the motivation for forest plantations is shifting from timber production to biodiversity conservation and restoration following disturbances (Martin et al. 2021), it is therefore urgent to develop specific protocols for a wide range of species, especially for endemics ones since they have received much less attention in scientific literature.

Morphological attributes are commonly used to differentiate seedling stocks according to their quality (Grossnickle 2012; Mataruga et al. 2023). Shoot height and root collar diameter are the most used morphological attributes, partly due to their ease of measurement, and many countries have established optimal ranges for these attributes in their regulations for plant material (Mataruga et al. 2023). Both shoot height and root collar diameter are proxies of seedling size and, thus, of a greater potential of outplanting performance (Andivia et al. 2021; Grossnickle 2005; Villar-Salvador et al. 2012). For plants of the same age, large seedlings usually show greater leaf and root mass, which is related to a higher growth and resource uptake capacity, key processes for seedling establishment and performance (Grossnickle 2005; Villar-Salvador et al. 2012). However, other studies have reported a lower outplanting performance of large seedlings, especially in drought prone ecosystems where higher transpiration and an imbalance in shoot and root biomass can increase vulnerability to drought stress (Oliet et al. 2019; Trubat et al. 2011). Thus, the evaluation of seedling quality in the nursery should also include parameters related to biomass allocation and especially to root growth capacity and architecture (Li et al. 2011; Tsakalidimi et al. 2005; 2013; Grossnickle and MacDonald 2018).

Fertilization is one of the most powerful tools to modify seedling morphology in the nursery (Landis 1985). Many studies have concluded that fertilization during the nursery phase promotes plant growth, nutrient storage and resistance to biotic and abiotic stresses (Andivia et al., 2012; 2014; Devetakovic et al. 2017; Tsakalidimi et al. 2021). In water limited

ecosystems, such as in the Mediterranean basin, seedling nutrient content is key to promote above- and below-ground growth after outplanting and thus resist summer drought and increase competitive ability (Villar-Salvador et al. 2012; Oliet et al. 2013). In these environments, seedlings are frequently artificially shaded in the nursery to avoid heat damage and reduce water stress (Puértolas et al. 2009). Shade can reduce plant growth, increase shoot to root biomass, slenderness and makes plants less water use efficient (Aranda et al. 2007; Mason et al. 2004), although these responses shift with species' tolerance to shade (Valladares and Niinemets 2008). Since fertilization and shade are among the most used techniques in Mediterranean nurseries and they can have opposite effects on seedling growth, biomass allocation and physiological responses (Luis et al., 2010), it becomes necessary to evaluate the iterative effect of both techniques on seedling quality.

The Mediterranean Basin is a critical biodiversity hotspot, hosting a variety of endemic species, including eight taxa of the *Abies* genus, making it a significant center of distribution for these conifers. Among these, *Abies cephalonica* Loudon, commonly known as Greek fir, has an extremely restricted distribution in Southern and Central Greece with highly isolated populations (Fig. 1) (Politi et al. 2011; Daskalakou et al. 2019; Ioannidis et al., 2021). This species thrives in mountainous regions of the Mediterranean, typically at elevations between 800 and 2000 m (Jagodzinski et al., 2011). It is shade-tolerant and relatively soil moisture-demanding species (Schutt 1994). The species can regenerate on very low light levels and seedlings may live under deep shade for 60 years or more (Daskalakou et al. 2019). The regeneration of Greek fir, similar to that of many other fir species, occurs within forest management systems (e.g. shelterwood cuttings) that utilize the protective environment created by the canopy cover of mature trees (Holgen and Hanell 2000; Mason 2013; Vacek et al. 2015).

Extreme summer droughts have increased in the relatively more humid and colder regions of Greece due to climate change (Dimitrakopoulos et al. 2011). Consequently, a shift of wildfires toward higher altitudinal ecosystems, including fir ecosystems, has been observed (Arianoutsou et al. 2010; Politi et al. 2011; Lehtonen et al. 2016). That increases the displacement risk of these mountainous fir ecosystems, since Greek fir has not developed fire-adapted mechanisms, such as serotinous cones and nor does it maintain a seed bank to secure its regeneration after a wildfire (Politi et al. 2011). Additionally, the species cannot regenerate asexually, which raises the risk of the forest failing to re-establish itself, leading to severe consequences such as biodiversity loss, soil erosion, and landscape degradation (Ganatsas et al. 2012a). Previous studies showed null post-fire regeneration of *A. cephalonica* in Mt. Parnitha, three years after the wildfire despite the existence of nearby unburned fir stands (Ganatsas et al. 2012).

As the fire events in these ecosystems is expected to increase due to climate change, Greek fir forests are becoming highly vulnerable, and therefore there is an urgent need to restore burnt Greek fir forests within their native range (Ioannidis et al. 2021) by development effective reforestation projects (Spanos et al. 2010; Ganatsas et al. 2012b). As a result, there has been an increasing need to produce vigorous, high-quality nursery seedlings of this species. Unfortunately, we lack specific studies evaluating how common nursery techniques, such as fertilization and shade, or seed provenances influence seedling quality of Greek fir, hampering the implementation of effective nursery protocols to produce high quality seedlings, which constitutes an obstacle to its ecosystem successful restoration.

In the present study, we evaluated the influence of shade, fertilization and seed provenance on the growth and quality of nursery-produced Greek fir seedlings. We hypothesized: (1) to find differences in seedling growth between seed provenances, (2) that artificial shade will favor above- and below-ground growth and quality of fir seedlings, as the species is considered as shade-tolerant, and (3) that fertilization will increase seedlings growth and quality.

Materials and Methods

Seed collection

Seeds of Greek fir originating from two provenances in southern Greece, Parnitha and Vytina (Fig. 1), with different geographical and environmental conditions (Table 1) were collected in autumn 2017. Undamaged seeds were collected

from mature forest stands and from at least 30 mature trees (approx. 100–150 years old). Seeds from each location were pooled, so the genetic variability was only considered at the provenance level. Seeds were stored in partially sealed Kraft paper bags (permeable to carbon dioxide and oxygen yet largely impermeable to moisture) at 2°C until the experiment began the following March.

Table 1
Geographical location and environmental factors for the two seed provenances of *A. cephalonica* in Greece.

Seed Provenance	Longitude, Latitude	Altitude (m)	Mean annual Temperature (°C)	Min Temperature of coldest month (°C)	Max Temperature of warmest month (°C)	Annual Rainfall (mm)
Parnitha	38°10'25.2"N 23°43'39.5"E	1280	10.7	1.6	19.8	720
Vytina	37°40'01.0"N 22°12'12.2"E	1250	10.9	3.0	19.3	1050

Nursery experiment

The experiment was conducted in an open-air nursery of the Laboratory of Silviculture of the Aristotle University of Thessaloniki, northern Greece (40,537970 N, 22,995253 E). Greek fir seeds were sown (one seed per pot) in March (2018) in plastic trays (Quick pots with 24 cavities each, cavity volume 330 cm³, depth 16 cm). The pots were filled with peat and perlite (2:1, v/v).

Seedlings from both provenances were assigned to four treatments combining two fertilization doses (5 g and 10 g per liter of substrate) of a water-soluble complete fertilizer (N:P:K 30:10:10 + micronutrients) (Tsakalimi et al. 2021) with two shading levels (50% and 70% of total sunlight). These fertilization doses corresponded to 486 mg of fertilizer seedling⁻¹ and 972 mg of fertilizer seedling⁻¹, respectively. Half of the fertilizer was incorporated into the substrate before sowing, while the remaining half was applied via irrigation during the period of rapid seedling growth (April–May). For each fertilization treatment, half of the seedlings were grown under 70% shade and the other half under 50% shade. Shading treatments were implemented from early June to late September. The treatments were arranged in a completely randomized block design, and there were three trays (24 seedlings each) per treatment combination, for each seed provenance (totally 288 seedlings, i.e. 24×3×2×2). The seedlings were irrigated by an overhead irrigation system as needed.

Survival, stem height and root collar diameter of all seedlings were measured after two growing seasons and twice: before summer (at the end of May 2020) and after summer at the end of the growing season (November 2020). For biomass and root morphology measurements, five seedlings were randomly selected from each treatment combination (fertilization × shade) and from the two provenances on late November. Seedlings were harvested and separated into roots and shoots. All roots were initially rinsed in water to remove coarse growing media from the root system but not to damage fine roots. Roots were then gently washed 3–4 times, and any fine roots that separated from the system were collected. All roots were kept fresh, and each separated and spread out in aluminum foil until they were processed (Tsakalimi et al. 2005, 2009). For root analysis, the roots of each seedling were carefully spread onto a tray ensuring no overlapping and then scanned using an Expression 12000XL flatbed scanner. The images were digitized at 600 DPI as 16-bit greyscale images. Roots were then analyzed with RhizoVision Explorer (2.0.3), an open-source software for root image analysis (Seethepalli et al. 2021), to obtain total root length (TRL), total root surface area (TRA), total root volume (TRV), and root length in three diameter classes $d \leq 1$ mm, $1 < d \leq 2$ mm and $2 < d \leq 3$ mm. Roots and shoots were then dried in a 70 °C forced-air oven until constant weight was reached and then weighed to obtain the dry mass.

Statistical Analysis

All statistical tests were performed using the SPSS 29 statistical software. Assumptions of residual normality and variance homogeneity were tested using the tests via GLM and UNIVARIATE procedure. A factorial ANOVA (Univariate) was performed to evaluate the fixed effects of seed provenance, fertilization and shade, as well as their interaction on Greek fir seedlings morphological characteristics. In all cases, a Waller Duncan post hoc test was performed to determine the significance of differences ($p < 0.05$) between the studied effect levels. Levene's Test for Equality of Variances (for equal variances assumed) was also performed. To test the differences between the two seed provenances, the two fertilization dosages and the two shade levels on Greek fir seedlings' growth, the Independent Samples T-test analysis was performed. For the destructive samplings (biomass and root morphology measurements), because the number of samples was not large and sufficient for full statistical analyses among seed provenances, the effect of fertilization and shading was examined overall for the two seed provenances.

Results

Above-ground morphological traits

In May 2020, as well as at the end of the second cultivation period in the nursery (November 2020), seedlings originating from Parnitha exhibited significantly greater height and height-to-diameter ratio (H/D) compared with those originating from Vytina (Table 2). In contrast, seed provenance did not have a statistically significant effect on root collar diameter (RCD).

Across both assessment periods, and irrespective of seed provenance, RCD and H/D ratio were significantly influenced by fertilization dose and shading level, whereas shoot height remained unaffected (Tables 3 and 4). By the end of the second cultivation period, seedlings that received 10 g of fertilizer developed a larger diameter (2.71 ± 0.07 mm) and a lower H/D ratio (3.29 ± 0.09) than those that received 5 g (2.48 ± 0.07 mm in RCD and 3.60 ± 0.09 in H/D). A similar pattern was observed regarding shading treatment: seedlings grown under 70% shade exhibited a greater diameter (2.73 ± 0.07 mm) and a lower H/D ratio (3.12 ± 0.09) compared with those grown under 50% shade (2.49 ± 0.07 mm in RCD and 3.71 ± 0.08 in H/D). These differences associated with fertilization and shading levels were also evident in the measurements taken before the summer period (Tables 3 and 4).

Examining the interactions among the applied treatments, the only interaction that significantly affected seedling above-ground growth was that of seed provenance $\times$ fertilization ($P \times F$) (Table 5). This interaction was significant for shoot height and H/D.

Table 2

Effects of seed provenance (Parnitha vs. Vytina) on Greek fir seedlings' growth after two growing seasons in nursery, as calculated with the Independent Samples T-test.

Parameter	Before summer (May)			After summer (Nov.)		
	<i>Sig.</i> <i>Two-Sided</i> <i>p</i>	Parnitha	Vytina	<i>Sig.</i> <i>Two-Sided</i> <i>p</i>	Parnitha	Vytina
Shoot Height (H) (cm)	< 0.001	8.312 (0.20)	7.064 (0.22)	< 0.001	8.987 (0.17)	7.532 (0.29)
Root collar diameter (RCD) (mm)	0.433	2.097 (0.05)	2.038 (0.05)	0.315	2.576 (0.06)	2.703 (0.10)
H/D	< 0.01	4.092 (0.11)	3.578 (0.12)	< 0.001	3.568 (0.07)	2.948 (0.13)

Values in each provenance are Means and the standard error of mean is in parenthesis.

Table 3

Effects of fertilization regime (two doses of water-soluble fertilizer N:P:K 30:10:10) on Greek fir seedlings' growth after two growing seasons in the nursery, as calculated with the Independent Samples T-test.

Before summer (May)				After summer (Nov.)		
Parameter	<i>Sig.</i> <i>Two-Sided</i> <i>p</i>	5g	10 g	<i>Sig.</i> <i>Two-Sided</i> <i>p</i>	5g	10 g
Shoot Height (H) (cm)	0.394	7.627 (0.21)	7.897 (0.23)	0.099	8.262 (0.22)	8.802 (0.23)
Root collar diameter (D) (mm)	< 0.001	1.920 (0.04)	2.210 (0.05)	< 0.05	2.479 (0.07)	2.714 (0.07)
H/D	< 0.05	4.127 (0.14)	3.633 (0.09)	< 0.05	3.597 (0.09)	3.289 (0.09)

Values in each provenance are Means and the standard error of mean is in parenthesis.

Table 4

Effects of shading level (50% vs. 70%) on Greek fir seedlings' growth after two growing seasons in nursery, as calculated with the Independent Samples T-test.

Before summer (May)				After summer (Nov.)		
Parameter	<i>Sig.</i> <i>Two-Sided</i> <i>p</i>	50%	70%	<i>Sig.</i> <i>Two-Sided</i> <i>p</i>	50%	70%
Shoot Height (H) (cm)	0.516	7.865 (0.21)	7.659 (0.22)	0.823	8.547 (0.22)	8.472 (0.24)
Root collar diameter (D) (mm)	< 0.05	1.982 (0.05)	2.172 (0.05)	< 0.05	2.490 (0.07)	2.733 (0.07)
H/D	< 0.01	4.074 (0.12)	3.636 (0.11)	< 0.001	3.709 (0.08)	3.121 (0.09)

Values in each provenance are Means and the St error of mean in parenthesis.

Table 5

Main effects of provenance (Greek fir seeds originated from Partitha vs. Vytina), fertilization dose, shading level (50% vs. 70%) and their interactions on seedlings growth after two growing seasons in nursery; before summer (May) and after summer (November), as calculated by a factorial ANOVA.

Before summer (May)							
Parameter	Seed Provenance (P)	Fertilization regime (F)	Shading level (S)	P x F	P x S	F x S	P x F x S
Shoot height (H) (cm)	***	n.s.	n.s.	**	n.s.	n.s.	n.s.
Root collar diameter (D) (mm)	n.s.	***	*	n.s.	n.s.	n.s.	n.s.
H/D	**	*	**	**	n.s.	n.s.	n.s.
After summer (Nov.)							
Shoot height (H) (cm)	***	n.s.	n.s.	*	n.s.	n.s.	n.s.
Root collar diameter (D) (mm)	n.s.	**	*	n.s.	n.s.	n.s.	n.s.
H/D	***	*	***	**	n.s.	n.s.	n.s.

Levels of significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s.: not significant.

Root traits

Fertilization significantly affected the root morphology of two-year-old Greek fir container seedlings, while shading and the interaction between fertilization and shading did not show a significant effect (Table 6). At the end of the second cultivation period in the nursery, those seedlings that received the higher dose of fertilizer had significantly longer roots (total root length increased by 132%), greater root volume (by 114%), root surface area (by 125%) and longer fine roots with diameter $d \leq 1$ mm (by 136%) than those fertilized with 5 g (Fig. 2). Neither fertilization or shading had a significant effect on the length of roots with diameter higher than 1 mm.

Table 6

The effect of two different fertilization regimes (two doses of water-soluble complete fertilizer N:P:K 30:10:10) and two shading levels (50% vs. 70%) on root traits and biomass of Greek fir two-year-old container seedlings, at the end of the second cultivation period in nursery as calculated by a factorial ANOVA.

Fertilization regime (F)		Total Root Length (cm)	Total Root Volume (cm ³)	Total Root Surface Area (cm ²)	Root Length (cm) with $d \leq 1$ mm	Root Length (cm) with $1 < d \leq 2$ mm	Root Length (cm) with $2 < d \leq 3$ mm	Shoot biomass (g)	Root biomass (g)	R/S	Total biomass (g)
5g	Mean	782.7	10.4	246.5	715.8	61.3	5.6	0.71	0.63	0.87	1.35
	Std. error	± 184	± 2.7	± 58.8	± 167	± 20.4	± 2.0	± 0.04	± 0.08	± 0.08	± 0.17
10g	Mean	1815	22.3	555.5	1689	111.6	14.66	1.31	1.10	0.84	2.42
	Std. error	± 315.2	± 5.03	± 101.9	± 302.7	± 31.2	± 5.4	± 0.09	± 0.09	± 0.04	± 0.18
<i>p</i> value		*	*	*	*	ns	ns	**	**	ns	**
Shading level (S)											
50%	Mean	1189	17.9	396	1076.8	99.5	12.5	1.17	1.01	0.93	2.13
	Std. error	± 317.5	± 4.1	± 95.4	± 301.9	± 28.3	± 4.1	± 0.13	± 0.11	± 0.07	± 0.23
70%	Mean	1236.8	12.9	354.5	1165.7	64.9	6.1	0.91	0.72	0.77	1.63
	Std. error	± 341.5	± 4.6	± 108.5	± 317	± 23.5	± 3.5	± 0.11	± 0.12	± 0.04	± 0.23
<i>p</i> value		ns	ns	ns	ns	ns	ns	ns	*	ns	*
F x S	<i>p</i> value	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Levels of significance: * $p < 0.05$; * $p < 0.01$; ns: not significant.

Seedling biomass

Fertilization had a significant effect ($p < 0.01$) on both aboveground and belowground biomass of the two-year-old Greek fir container seedlings (Table 6). The results show a clear increasing trend in root, shoot, and total seedling biomass with higher fertilizer doses. Shading also exerted a statistically significant influence on root and total biomass, with greater biomass under the low shading intensity. However, the interaction between fertilization and shading was non-significant (Table 6). Seedlings received higher fertilization dose, regardless of the shading level, presented higher root (0.99–1.24 g), shoot (1.16–1.46 g) and total biomass (2.13–2.70 g) than those fertilized with the lower dose (Fig. 3).

Discussion

This study demonstrates that the growth and morphological traits of Greek fir (*Abies cephalonica*) container seedlings is mainly influenced by independent effects of seed provenance, fertilization, and shading. Aboveground growth patterns were

partially dependent on provenance, whereas fertilization emerged as the dominant factor affecting both shoot and root traits. Shading intensity reduced above- and below-ground biomass.

Two-year-old container-grown fir seedlings, regardless of treatments applied, exhibited relatively small sizes, with mean height of 8.5 cm and root collar diameter (RCD) of 2.6 mm, consistent with previous reports suggesting that seedlings of this species require four to five years to reach 20 cm in height (Panetsos 1975). Similar dimensions were found in two-year-old container seedlings of other forest species with slow-growing pattern as *Pinus nigra* and *Picea abies* (Tsakalimi et al. 2026; Ivetic and Nicolescu 2026). Overall, these results highlight the challenges of producing high-quality nursery seedlings of slow-growing species and the difficulty of maintaining sufficient nursery stocks to meet restoration needs, given that these species must be cultivated for at least two to three years.

Seed provenance effects were more pronounced in shoot morphology than in root traits. Two-year-old fir seedlings originated from Parnitha consistently exhibited greater shoot height and H/D ratios than those from Vytina, reflecting intrinsic population-level differences in aboveground growth. Similar patterns were observed in field plantings of the same fir provenances, where Parnitha seedlings - originating from a drier site than those from Vytina - also showed superior height growth (Ioannidis et al. 2021). These differences in climatic conditions likely contribute to the species adaptation to each specific site conditions. Comparable patterns have been also reported in oak species, where seedlings from drier provenances tend to be larger (Ramírez-Valiente et al. 2009, 2011). Larger seedlings may better withstand water stress due to enhanced carbon gain, which promotes root growth and their competitive ability (Cuesta et al. 2010; Villar-Salvador et al. 2012). Divergent selective pressures may thus drive local adaptation and shape genetic variance within populations (Ramírez-Valiente et al. 2011). Nonetheless, common garden studies encompassing more Greek fir populations across multiple years are needed to confirm these trends.

Fertilization emerged as the primary determinant of seedling quality. Seedlings receiving higher dose ($972 \text{ mg seedling}^{-1}$) of water-soluble complete fertilizer exhibited significantly greater RCD and lower H/D ratios compared to seedlings receiving the lower dose ($486 \text{ mg seedling}^{-1}$), which are key indicators of seedling quality and field performance under Mediterranean climatic conditions (Tsakalimi et al. 2013; Ivetić et al. 2016; Guimaraes et al. 2024). However, seedlings' shoot height remained unaffected by fertilization dose. That may be related to the intrinsic low growth rate of this species during the juvenile phase or may reflect species-specific resource allocation patterns. Nutrients are critical for physiological processes underpinning planting success, particularly by promoting traits such as early root growth that enhance drought avoidance (Oliet et al. 2013). Fir seedlings received a greater dose of fertilization markedly increased their root, shoot and total biomass and enhanced their root system development, including total root length, volume, surface area, and fine-root length with $d \leq 1$ up to $d \leq 3$ mm. According to Spanos et al. (2008), during early development, the Greek fir root system initially consists of a taproot and first-order lateral roots, with second- and a few third-order laterals forming progressively with age. These findings reflect robust stimulation of both structural and absorptive components of the root system, suggesting improved capacity for nutrient and water acquisition and thus reducing seedling water stress in the field conditions since seedlings' ability to take up water is highly affected by its root system size and distribution (Grossnickle 2005; 2012). Comparable effects have been reported in larch seedlings (Agathokleous et al. 2023) where higher fertilization also contributed to the increase of seedlings root collar diameter, aboveground and root biomass, and in *Pistacia chinensis* container seedlings (Song et al. 2019) where the increase of the dosage of controlled-release fertilizer (400 and 500 mg seedling^{-1}) significantly affected root morphology and architecture. Similar findings have been reported for other forest species, where nitrogen fertilization enhances growth and nutritional status, representing a beneficial nursery practice (Oliet et al. 2009; Grossnickle and MacDonald 2018b). Given the importance of root development in reducing transplanting shock and the low intrinsic growth rate of this species, these results might have key implications for enhancing outplanting performance.

Shading exerted nuanced effects on seedling quality. It significantly influenced root collar diameter (RCD), height-to-diameter (H/D) ratio, and both root and total biomass, whereas shoot height and root morphology remained unaffected. Seedlings grown under 70% shading exhibited greater RCD and lower H/D ratios than those under 50% shading, indicating

enhanced stem sturdiness under low irradiance. Conversely, root and total biomass peaked under 50% shading, highlighting the complex and species-specific responses of Greek fir seedlings to light availability. Mason et al. (2004) investigated the effect of a wide range of full light conditions (0% – 100%) on semi-shade-tolerant species concluded that species-specific growth responses exhibited minimal variation under high light conditions, whereas performance under low light generally aligned with the shade tolerance rankings reported in the literature. Overall, the increase of shading may reduce total seedling growth, but responses vary among species- specific light requirements at an early developmental stage. For the semi-shade-tolerant *Pinus nigra*, Tsakalimi et al. (2021) found that 50% shading during the nursery phase markedly enhanced seedling shoot height and RCD over time. By contrast, for the light-demanding *Pinus canariensis*, Luis et al. (2010) observed only minor shading effects on growth, mainly slight changes in root:shoot ratio, while Puertolas et al. (2009) reported weak or negligible morphological responses to shade in *Quercus ilex*. Since outplanting performance, especially in drought prone ecosystems, seems to be better predicted by plant biomass, especially root biomass (Andivia et al. 2021; Grossnickle 2005; Villar-Salvador et al. 2012), moderate shading during summer should be considered in the cultivation of this species in the nursery. Interestingly, we did not find a significant interaction of fertilization and shading on the morphological traits analyzed. Both, shading and fertilization can induce similar responses in seedlings by increasing mass allocation to the shoot (Canham et al. 1996). In our study, fertilization increased both shoot and root biomass, but shading only increased root biomass. This lack of interactive effect of both factors has been also reported for other species (e.g. Dehlin et al. 2004; Villar-Salvador et al. 2004), suggesting that the independent effects of light and nutrients are more important than how they jointly influence growth for the analyzed species.

This study demonstrates the importance of a species-specific nursery protocol for producing high-quality *A. cephalonica* seedlings, which is essential for successful restoration and reforestation. By considering the species' ecophysiological traits, high fertilization dose and moderate shade levels in the summer can enhance outplanting establishment, addressing current knowledge gaps in the nursery production of seedlings of this species. Given the limited post-fire regeneration of *A. cephalonica* (Ganatsas et al., 2012; Ioannidis et al., 2021), these findings provide critical insights for improving seedling quality, which might then contribute to increasing survival and growth following field outplanting and thus facilitating effective ecosystem restoration. Future studies should assess the impact of varying fertilization regimes and extended nursery cultivation periods on attaining target seedling sizes, and subsequently evaluate their field performance following outplanting.

Declarations

Declarations

Conflict of interest

The authors declare no competing interests.

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Author Contribution

Author Contributions: M.T.: Conceptualization; Investigation; Methodology; Formal analysis; Data curation; Writing—original draft, E.A.: Writing—review & editing, Validation; M.B.: Investigation, Data curation ; N.O.: Investigation; P.G.: Conceptualization; Project administration; Data curation; Writing—review & editing; Supervision; Resources. All authors reviewed the manuscript.

Data Availability

Data is provided within the manuscript.

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Figures

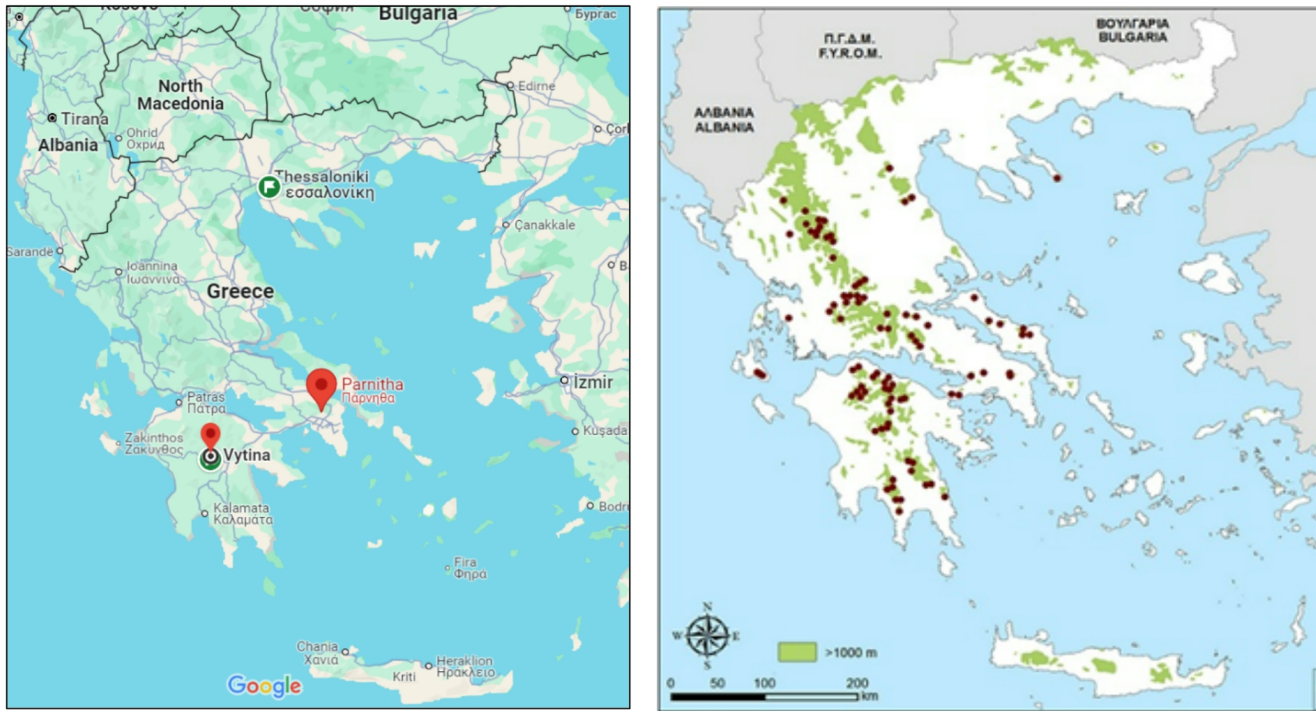


Figure 1

Location of the two seed provenances of Greek fir (Parnitha, and Vytina) (left map). Natural distribution of Greek fir (*A. cephalonica*) in Greece (brown circles) (right map).

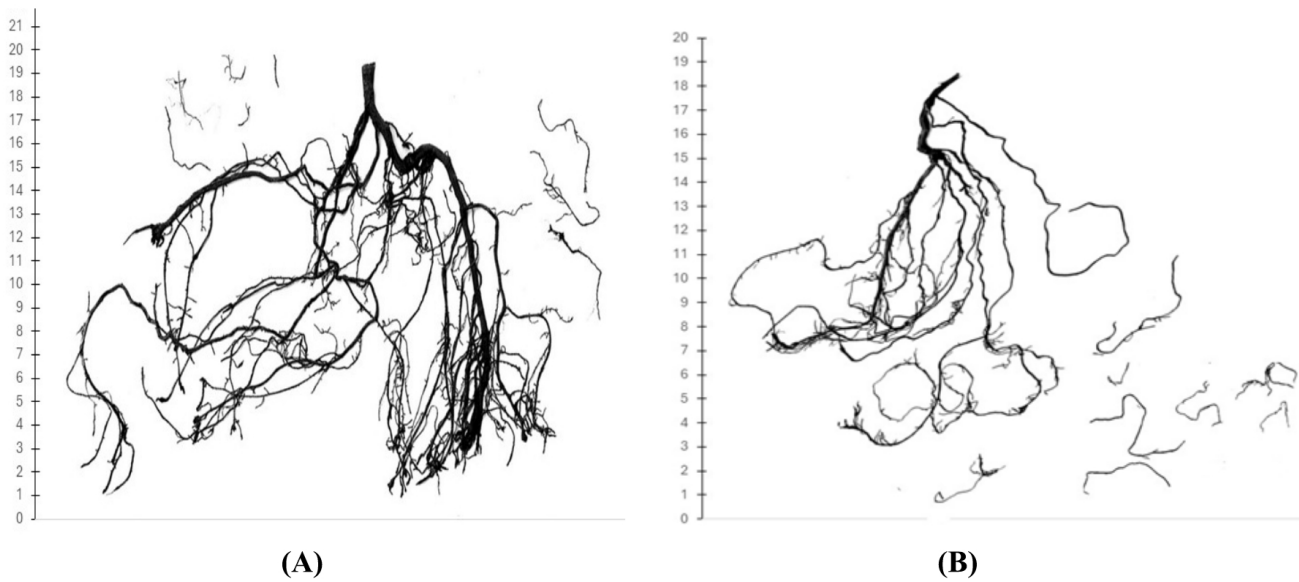


Figure 2

Schematic model of two random sample root systems of Greek fir (*Abies cephalonica*) two-year old seedlings, representing: (A) a root system from seedlings raised in higher fertilization dose (10 g) and 50% shading level, (B) a root system from seedlings raised in lower fertilization dose (5 g) and 50% shading level.

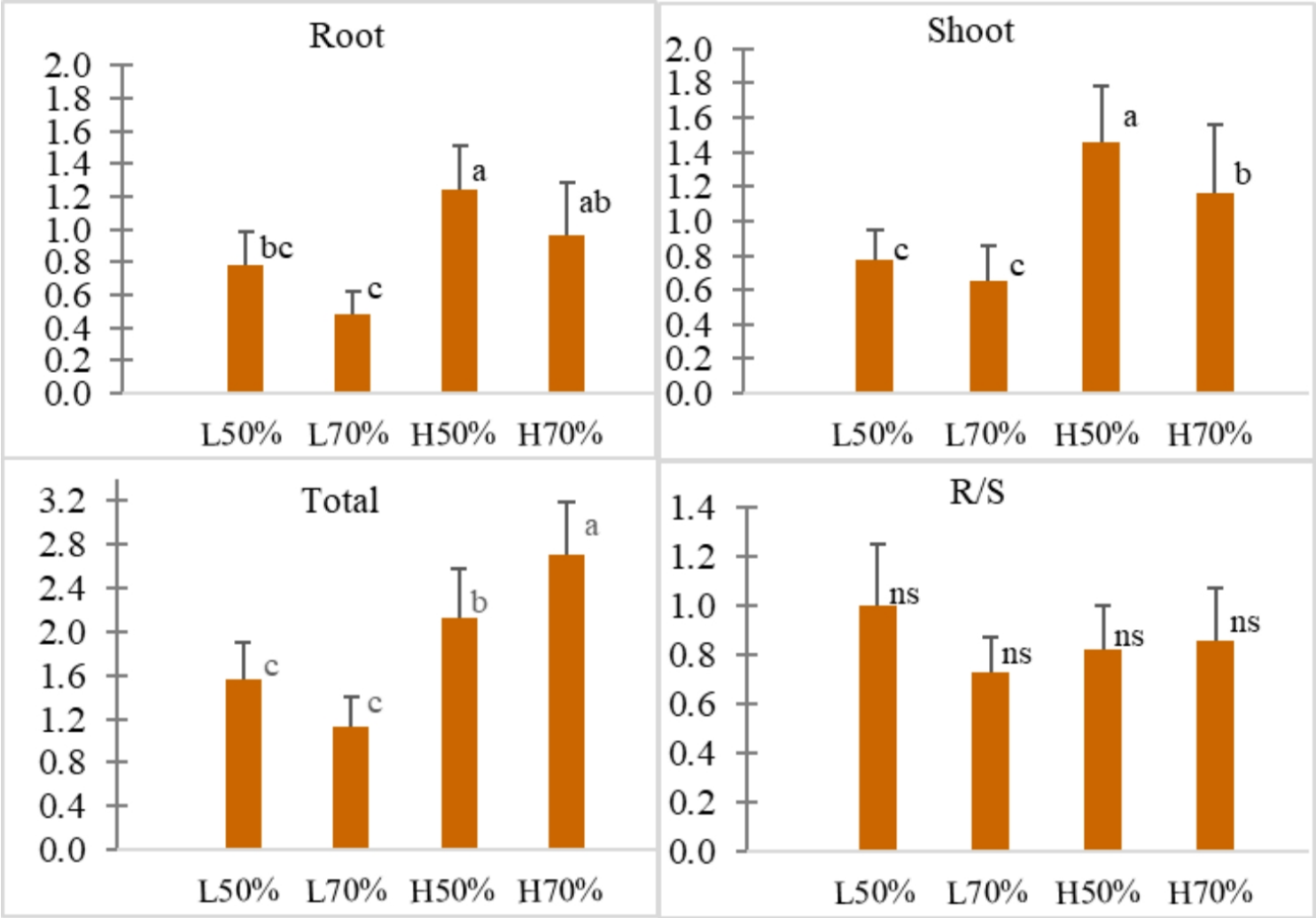


Figure 3

Root, shoot, total biomass and R/S in two-year-old container seedlings of Greek fir, at the end of the growing season in nursery, in response to different treatments applied with combination of two fertilization doses (L- low), H- high) and shading levels (50%, 70%). Bars and arrows represent the mean and corresponding standard error, respectively. Different low case letters indicate a significant difference between means (one-way ANOVA, $p < 0.01$; ns: non-significant). Means were compared using Waller Duncan's test.