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Decoupling seedling establishment in a shade-intolerant species of a Mediterranean climate: Soil moisture determines survival but growth is promoted by irradiance

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ABSTRACT

In Mediterranean climates, drought is recognized as the main abiotic stress negatively affecting plant survival and growth after establishment. However, several factors besides water scarcity interact to affect plant performance, including stocktype, field establishment techniques, environmental variability, and the inherent ecological requirements of the target species. To fully comprehend the extent each of these factors influences plant performance, we analyzed plant survival, growth, and physiological performance of contrasting stocktypes of *Nothofagus glauca* seedlings, a shade-intolerant tree species from Mediterranean central Chile, established under different environmental conditions characterized by a shrubland versus a forest canopy cover. We hypothesize that nitrogen-loaded plants will have higher survival and growth during establishment, and that higher performance will be observed under the shrubland conditions, despite the presence of water constraints that are usually observed in more open canopies. Our experimental design induced contrasting environmental conditions in which the forest canopy was characterized by lower irradiance and water availability during summer versus the shrubland condition. As expected, bigger and nutrient-loaded *N. glauca* plants had better field performance in terms of survival and growth. After three years of assessment, we did not observe differences in survival between canopy conditions, but plants established under shrubland canopy presented significantly higher biomass accumulation and better water status. According to our relative importance analysis, we observed that soil water availability was the most relevant factor for plant survival, but plants showed more growth when established under a condition of increased irradiance. Considering that plant performance is explained by the combined response of survival and growth, we suggest that under Mediterranean conditions, each response could be independently affected by separate environmental factors as well as by target species biology (i.e., shade tolerant or shade-intolerant).

1. Introduction

Climate change caused by anthropogenic activities has promoted forest mortality due to heat waves and drought (Allen et al., 2015, 2010). Because forests are one of the main carbon stocks and have a direct contribution toward reductions of greenhouse gas emissions, several international initiatives have established worldwide goals to promote forest conservation, afforestation, and restoration to combat climate change (Bastin et al., 2019; Picard and Garavaglia, 2021). Areas with Mediterranean climate are particularly vulnerable to the effects of

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climate change, where the more frequent and prolonged droughts have driven Mediterranean forests to their limit of adaptability (Andrea, 2022; Giorgi, 2006; Peñuelas and Sardans, 2021). These extreme environmental conditions make forest restoration challenging and have adversely impacted reforestation initiatives in Mediterranean areas (Ramón Vallejo et al., 2012).

Several approaches can be considered to achieve forest restoration, with direct seeding and seedling planting being the most common active strategies (Acevedo et al., 2021a). While direct seeding, with its lower cost, is preferred, poor survival rates, performance, and seed use efficiency (seedling-to-seed ratio) have been reported in temperate and Mediterranean forests (Grossnickle and Ivetić, 2017; Palma and Laurance, 2015). On the contrary, establishment of nursery produced plants seems the most promising option for restoration of degraded lands impacted with severe abiotic stress. Research has delved into the morpho-physiological characteristics of nursery plants that are linked to higher survival rates and performance in arid, semi-arid, or Mediterranean climates. The general agreement indicates that larger and nitrogen-loaded plants have higher survival rates and performance (Cuesta et al., 2010; Oliet et al., 2013; Villar-Salvador et al., 2012). The correlation between high nitrogen (N) concentrations and high photosynthesis rates promotes root growth after planting, which allows plants to sustain higher water potential and positive carbon balance (Burdett et al., 1983; Cuesta et al., 2010; Uscola et al., 2015). However, survival rates and plant growth after establishment are not merely a function of plant morpho-physiological attributes, but an interplay between inherent plant traits and environmental variability caused by field conditions or anthropogenic disturbances. Plant performance and survival can also be promoted by site preparation techniques that aim to overcome the effects of drought, such as management of competitive vegetation and soil preparation to enable deep rooting. Thus, to successfully establish plants under environmentally challenging conditions, management based on empirical data regarding species' ecological requirements and biology and field environmental characteristics and history is prudent (Aleric and Kirkman, 2005).

Research into plant stocktypes for use in restoration of Mediterranean forests has focused on improving drought tolerance (Acevedo et al., 2020; Sloan et al., 2020; Uscola et al., 2015; Villar-Salvador et al., 2015). In addition to improving inherent stock quality, an array of greatly overlooked environmental variables that influence establishment success, such as light availability, can affect seedling performance (Cooper et al., 2014; Dey et al., 2012). On one hand, in deforested areas, high irradiance is predominant and can strongly reduce seedlings' performance even if water availability is optimal (González-Salvatierra et al., 2013), through damage to the photosynthetic apparatus that impairs carbon assimilation (Aleric and Kirkman, 2005). On the other hand, the presence of a pre-existent canopy cover can produce detrimental low irradiance levels inducing a competition effect, in which case management of stand density to promote photosynthesis and growth is recommended (Aleric and Kirkman, 2005; Dey et al., 2012). It has been recognized that N content plays a central role in plant acclimation to varying light levels (Lusk and Reich, 2000; Monnier et al., 2013; Niinemets, 1997), so besides the management of the pre-existent canopy cover, the use of N-loaded plants can also improve performance under detrimental irradiance levels. Beyond irradiance, a canopy also affects soil water balance (Kremer et al., 2022, 2021), leading to contrasting levels of water availability. Although a canopy can attenuate the impacts of water deficit by reducing temperature and evaporative demand (Black et al., 2005), a canopy can contribute to water scarcity (Prévosto et al., 2011; Rodríguez-Calcerrada et al., 2010, 2006; Walters et al., 2006). For example, a *Pinus halepensis* canopy can prevent 15–35 % of autumnal rainfall from reaching the soil (Maestre et al., 2003; Maestre and Cortina, 2004), decreasing soil moisture and water availability with increasing stand density. In agreement, Walters et al. (2023) indicated that the facilitative effects of an overstory on plant survival and growth can be further influenced by water availability and species morphological traits.

Thus, with the objective of inducing a facilitative effect of the overstory on seedlings survival and growth, the use of nurse plants has proved useful in the establishment of late-successional trees (Fagundes et al., 2018; Gómez et al., 2004; Gómez-Aparicio et al., 2005; Siles et al., 2008). The facilitative effects of nurse plants have been explained through an increase in soil water availability (Gómez et al., 2004; Lozano et al., 2020; Lu et al., 2018), and/or conditions more conducive to growth (i.e., lower radiation, air temperature, and higher relative humidity) (Giday et al., 2019; Liu et al., 2021). This assumption agrees with research indicating that survival and growth is enhanced in partially shaded versus full sun environments (Montgomery et al., 2010; Walters et al., 2016; Willis et al., 2015). In this context, it is worth to assess different establishment techniques that aim to increase resource availability and accelerate root growth. For example, increasing planting depth can improve plant survival and growth after establishment by allowing high root growth and access to deeper water sources (Bocio et al., 2004; Fonseca et al., 2011; Oliet et al., 2015). Also with the objective of modifing the above-mentioned environmental conditions, weed control has been shown to improve survival, growth, and water status of *Quercus* species (Rey Benayas, 2005; Rey Benayas et al., 2007) by increasing soil moisture and light availability.

Thus, it is clear that water and light availability are interacting factors in Mediterranean climates that can affect plant performance during establishment, but are usually assessed independently, or the relative importance of each factor remains unclear. Consequently, species tolerance and performance under simultaneous stresses are poorly understood despite their ubiquitous coexistence in nature (Battaglia et al., 2000; Niinemets and Valladares, 2004). A general hypothesis suggests that shade and drought tolerance are negatively associated (Kubiske et al., 1996; Niinemets and Valladares, 2004), because plant traits linked to drought (i.e., increased root-to-shoot ratio) and shade tolerance (i.e., increased leaf area and shoot biomass) are opposing. This opposition suggests the existence of a trade-off, such that shade-tolerant species are more vulnerable to drought than more light-demanding ones (Sack, 2004). Thus, the effect of light and water availability and its interaction on plant performance will also depend on light requeriments (i.e., shade tolerance) of the target species and its acclimation capacity. The assessment of the plant morpho-physiological responses to different environmental factors can reveal information regarding seedlings functional responses and trade-offs (Sánchez-Gómez et al., 2006), and how plant attributes developed during nursery will impact plants acclimation and performance.

Consequently, to achieve successful plant establishment, we propose a holistic approach where stocktype morpho-physiological attributes, environmental variability, establishment techniques, and species' inherent ecological traits are examined as interacting factors that determine plant survival and growth. To explore this approach, we used contrasting stocktypes of *Nothofagus glauca* (Phil.) Krasser, an endemic tree species from Mediterranean central Chile, established under environmentally different sites characterized by the presence of a shrubland and a forest canopy. In support of the accumulated evidence, we hypothesize (1) that N-loaded nursery plants will have higher survival and growth because of improved water status and photosynthetic capacity. Because *N. glauca* has been characterized as a pioneer and shade-intolerant tree species (Fajardo and Alaback, 2005), we also hypothesize (2) that shrubland conditions will yield higher seedling survival and growth despite the presence of water constraints that are usually observed in more open canopies (3) that water availability will be the most relevant factor influencing *N. glauca* survival and growth.

2. Materials and methods

2.1. Nursery phase

2.1.1. Seedling production

We collected *N. glauca* seeds during autumn (March) of 2015 in the

Curepto commune, Maule region, Chile (lat −35.08°, long −72.03°), and promptly stored them at 6 °C. To obtain plants with contrasting morphological traits, we grew two stocktypes, one for a single growing season (1Y; sown 2017) and another for two seasons (2Y; sown 2016). Seeds were soaked 48 h in 200 mg L^{-1} of gibberellic acid (Giberplus 2.0; Anasac, Chile) and immediately sown into expanded polystyrene trays (84 cavities, 14 cm deep, 130 mL $cavity^{-1}$, 336 cavities m^{-2}; Aislapol, Santiago, Chile) filled with composted *Pinus radiata* bark with a nutritional composition as described by (Acevedo et al., 2021b). We used 30 trays in total (15 trays per stocktype) to produce 2520 seedlings. Plants were grown outdoors (ambient conditions) at the nursery of the Centro Tecnológico de la Planta Forestal of Instituto Forestal (INFOR) in Concepción, Central Chile (Meditarranean climate, lat −36.84°, long −73.13°).

Irrigation needs were monitored with soil moisture sensors (3 sensors per stocktype; ECH_2O EC-5; METER, Pullman, WA, USA). Sensor values for volumetric water content ($m^3\ m^{-3}$) were calibrated with gravimetric mass to estimate the percentage of available water according to Dumroese et al. (2015). During nursery production, irrigation and fertigation (fertilizer mixed with water) were applied the same way. Irrigation was performed when substrate lost 50 % of available water, and fertigation was alternated with irrigation events. For fertigation, we applied N in a proportion of $1NO_3^-$:$1NH_4^+$ and in a 300 mg N L^{-1} concentration. We also added constant rates of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) at concentrations of 100, 127, 80, 40, and 84 mg L^{-1}, respectively. For micronutrients we added constant rates of iron (Fe), zinc (Zn), manganese (Mn), Boron (B), and copper (Cu) of 8, 8, 6, 3, and 1 mg L^{-1}, respectively.

2.1.2. Morphology and nutrient status

Immediately prior to outplanting, we assessed both stocktypes (1Y and 2Y) for stem length (cm; measured from the base of the stem), root collar diameter (RCD) (mm; measured at the root collar), and biomass. For biomass, plants were separated into components (leaves, stem, and roots) and dried at 65 °C in a forced-ventilation oven for 48 h to achieve constant weight. Biomass partitioning was calculated by dividing each plant component by total biomass to obtain ratios of leaf mass, stem mass, and root mass. For each tray, we composited samples from whole plants and ground and analyzed them for N, P, K, and Ca concentrations using colorimetric (N and P) or atomic absorption (K and Ca) methods according to Temminghoff and Houba (2004).

2.1.3. Root growth potential

Root growth potential (RGP) was assessed in June 2018. We randomly selected 15 plants of each stocktype (1Y and 2Y) and removed all white root tips corresponding to new and non-suberized roots (Villar-Salvador et al., 2004). Each seedling was planted into a 10-L pot filled with sand. Pots were installed in ambient conditions and irrigated twice weekly at full container capacity. After 120 days, we assessed RGP through the average length of the three longest roots to the nearest millimeter and the number of all new white and non-suberized roots (Burdett, 1979).

2.2. Field Phase

2.2.1. Site description and experimental design

Our field sites were near the seed collection site near Curepto (lat 35.10°, long 72.02°) in a site owned and managed by Arauco, a forestry company. This area is characterized by a Mediterranean climate with wet winters and dry summers (Acevedo et al., 2020). In July of 2018, we established 2352 seedlings, distributed equally in two contrasting vegetative conditions: forest (lat −35.15°, long −72.05°) and shrubland (lat −35.18°, long −72.09°) (Figs. 1B and 1C, respectively). Soil in the shrubland is predominantly clay with a pH of 6.2, an organic matter content of 1.4 %, and an available N, P, and K content of 5.6 ± 0.8, 3.2 ± 1.5, and 51.9 ± 14.8 mg kg^{-1}, respectively. Soil in the forest is a sandy loam with a pH of 5.6 and an organic matter content of 5.5 %, available N, P, and K content was 9.2 ± 8.5, 3.1 ± 3.0, and 130.6 ± 78.1 mg kg^{-1}, respectively. Within each vegetative condition (hereafter "canopy condition"), we established 24 plots (2 stocktypes *x* 2 planting hole sizes *x* 2 levels of vegetation management *x* 3 replicates), and each plot (13 m width *x* 13 m length, 169 m^2 of plot area) was hand planted with 49 *N. glauca* seedlings on a 2-m *x* 2-m spacing and arranged in 7 rows *x* 7 columns disposition (Fig. 1A). We included two planting hole sizes (20 cm width *x* 20 cm length *x* 20 cm depth and 20 cm width *x* 20 cm length *x* 40 cm depth) to further assess variation in micro-environmental conditions. Also, we assessed the effect of different vegetation management levels with two treatments: with vegetation management (WVM) and without vegetation management (WoVM). For the WVM treatment, existing vegetation within 1 m of the planting hole was sprayed with a systemic herbicide (Roundup®, Bayer) two weeks before and five months after seedlings were outplanted (December 2018). We assessed the light environment of each canopy condition using three randomly selected plots within each condition. Within each plot, we collected 20, randomly distributed, pre-dawn hemispheric photographs in November 2018 from three plots per canopy condition. A fisheye lens (DX Fisheye-NIKKIR, 10.5 mm, f/2.8 G ED; Nikon) mounted on a digital camera (D5100; Nikon) was used to photograph canopies. The tripod-mounted camera was 60 cm above ground, horizontally leveled, and north-oriented. Photographs were analyzed with a plant canopy analysis system (HemiView 2.1SR5; Delta-T Devises Ltd., Burwell, UK) to obtain leaf area index (LAI) and groundcover index.

2.2.2. Environment

We installed 16 sensors for volumetric soil water content ($m^3\ m^{-3}$) (1 sensor *x* 2 canopy conditions *x* 2 stocktypes *x* 2 planting hole sizes *x* 2 levels of vegetation management) (ECH_2O EC-5; METER Group, Pullman, WA, USA). Also, we installed eight (1 replicate *x* 2 canopy condition *x* 2 stocktypes *x* 2 levels of vegetation management) environmental monitoring units, each having three sensors: photosynthetically active radiation (QSO-S; METER Group), and relative humidity and air temperature (VP-4; METER Group). Sensors were connected to data loggers (Em50 and Em5b; METER Group) that recorded and stored measurements every 60 minutes during the duration of the experiment (see Fig. 1 for sensors and data logger distribution).

To estimate gravimetric soil water content (%) from soil humidity sensors, we measured gravimetric soil water content on 11 occasions during the field phase between 2018 and 2021 (2018: September; 2019: January, April, June, October; 2020: January, July, November; 2021: January, May, September). On each of the 48 plots (24 plots *x* 2 canopy conditions), we randomly obtained five samples from the top 20 cm of soil. Samples were weighed, dried in a forced ventilation oven at 105 °C for 48 h, and reweighed. We calculated gravimetric soil water content using Eq. 1:

$$\%soil\ water = \frac{weight\ of\ wet\ soil(g) - weight\ of\ dry\ soil(g)}{weight\ of\ dry\ soil(g)} \quad x \quad 100 \tag{1}$$

2.2.3. Survival and biomass

Survival of the original 49 plants per plot (2352 plants total) was censused 11 times on the dates described in Section 2.2.2. We determined organ (leaf, stem, and root) and total biomass of surviving seedlings in August 2018, April 2019, and September 2021 as described in Section 2.1.2. To obtain root biomass we established a 1.5 m radius around each plant and carefully excavated to obtain the whole root system (up to 1.8 m in depth). For each sample date, biomass was obtained for 48 plants (1 plant *x* 2 canopy conditions *x* 2 stocktypes *x* 2 planting hole sizes *x* 2 levels of vegetation management *x* 3 replicates).

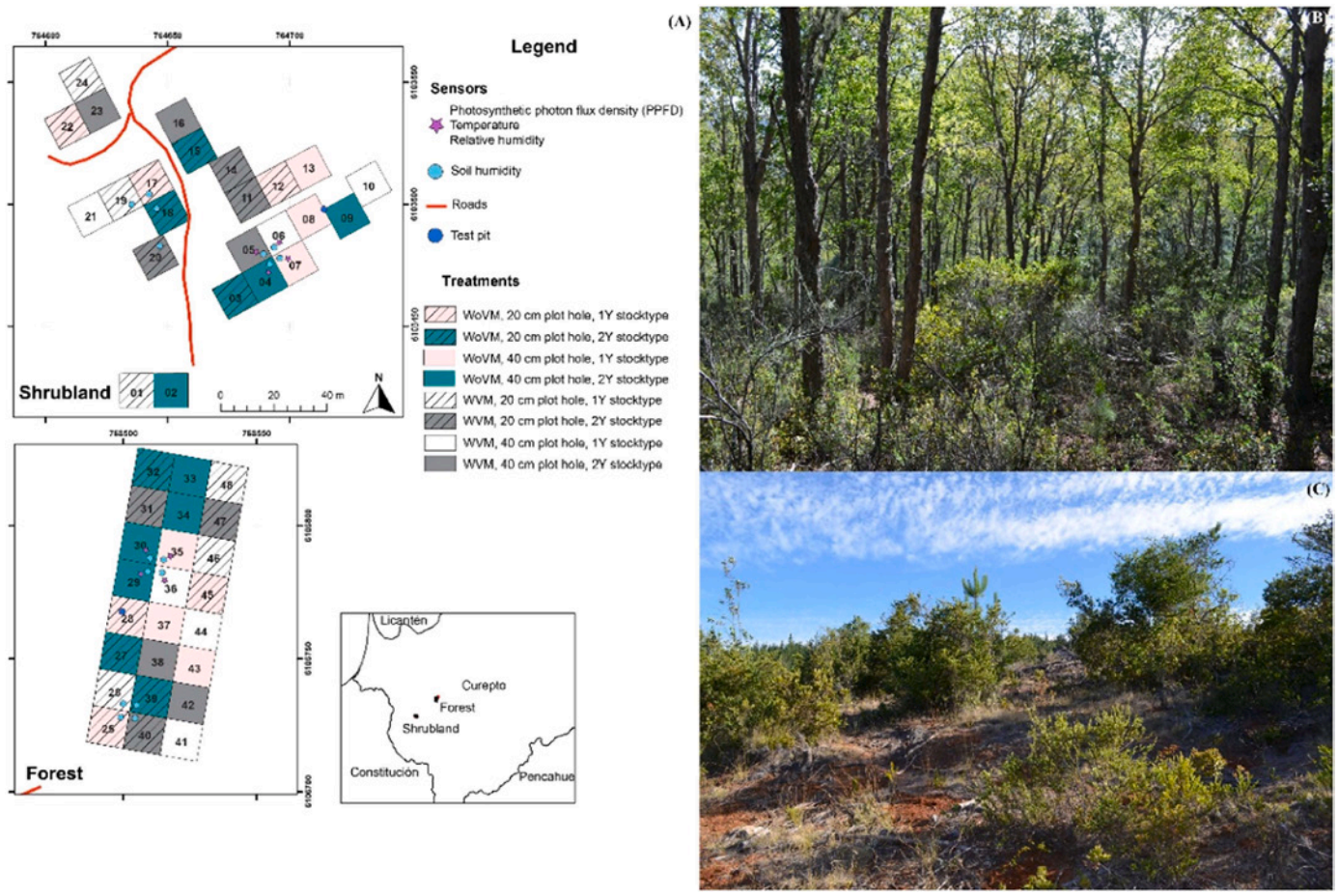

Fig. 1. Distribution of plots, sensors, and test pits within forest and shrubland canopy conditions (A). WVM: with vegetation management; WoVM: without vegetation control; 1Y: one-year-old stocktype; 2Y: two-year-old stocktype. General view of forest (B) and shrubland (C) canopy conditions.

2.2.4. Pre-dawn water potential

We measured pre-dawn water potential (Ψ_{pd}) nine times (2018: September; 2019: January, April, June, October; 2020: January, July, November; 2021: January, May, September). Measurements were made on fully developed leaves from the upper third of 72 randomly selected plants (3 plants *x* 2 stocktypes *x* 2 planting hole sizes *x* 2 levels of vegetation management *x* 3 replicates), according to Alvarez et al. (2019). During the evening before each measurement, leaves were wrapped in aluminum foil. At pre-dawn, leaves were collected and kept on ice until the determination of Ψ_{pd} (within the same morning) using a pressure chamber (Scholander; PMS Instruments, Albany, OR, USA).

2.2.5. Leaf gas exchange and chlorophyll fluorescence

At the end of the field phase (October 2021), we measured photosynthetic light response curves on a leaf area basis (*A*-*Q* curves) on *N. glauca* plants with contrasting stocktypes (1Y and 2Y) and canopy conditions (2 canopy conditions *x* 2 stocktypes *x* 6 replicates = 24 total). Gas exchange and fluorescence were measured simultaneously with a portable photosynthesis system and a fluorescence module (Ciras-3 and CFM-3; PP Systems, Amesbury, MA, USA). Measurements were performed on a fully expanded leaf from the upper third of each plant between 09:00 and 15:00. Leaves were dark acclimated in the cuvette for 30 min, after which a saturating light pulse (8000 μmol photons m^{-2} s^{-1}) was applied to obtain maximum quantum efficiency of photosystem II (PSII) (F_v/F_m) (Maxwell and Johnson, 2000). Then, plants were acclimated for at least 5 min to cuvette conditions set to 400 μmol mol^{-1} CO_2, 1000 μmol photons m^{-2} s^{-1} of photosynthetically active photon flux density (PPFD), 25 °C leaf temperature, and 60 % relative humidity. *A*-*Q* curves were measured under steady-state conditions by recording CO_2 assimilation rate at PPFD of 1440, 1200, 1000, 800, 600, 500, 400, 200, 100, 50, and 0 μmol photons m^{-2} s^{-1}. After each measurement, a pulse of actinic light was applied to measure steady-state fluorescence yield (F_s) and the maximum light-adapted fluorescence (F_m). Once actinic light was removed, a pulse of far-red light was applied to oxidize quinone Q_A and estimate minimum fluorescence in the light (F_0). We calculated the actual quantum efficiency of PSII (ΦPSII) according to (Genty et al., 1989), while the electron transport rate (ETR) and the photochemical (*q*) and non-photochemical quenching (NPQ) of chlorophyll a were calculated according to (Brooks and Niyogi, 2011). We fitted light response curves using the nonrectangular hyperbola model (Prioul and Chartier, 1977), and we extracted the parameters using the a spreadsheet calculator developed by Lobo et al. (2013). Net photosynthetic rate (A_N) was calculated using Eq. 2:

$$A_N = \frac{\phi_{(I_o)} \; x \; I + \; A_{gmax} - \sqrt{(\phi_{(I_o)} \; x \; I + \; A_{gmax})^2 - 4\theta x \; \phi_{(I_o)} \; x \; I \; x \; A_{gmax}}}{2\theta} - \; R_D \qquad (2)$$

where, I is the photosynthetic photon flux density (μmol photons m^{-2} s^{-1}), $\phi_{(Io)}$ is the quantum yield at $I = 0$ μmol photons m^{-2} s^{-1} (μmol CO_2 μmol $photon^{-1}$), A_{gmax} is the maximum gross photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), θ is the convexity factor (dimensionless), and R_D is the dark respiration rate (μmol CO_2 m^{-2} s^{-1}).

2.2.6. Photosynthetic pigments

We quantified photosynthetic pigments from 24 leaf samples, selecting fully developed leaves from the upper third of the stem (2 canopy conditions *x* 2 stocktypes *x* 6 replicates). Harvested leaves were immediately placed into liquid nitrogen and subsequently stored in a freezer at −80 °C until needed. Chlorophylls and carotenoids were extracted from 100 mg of leaves with 2 mL acetone. The extract was filtered in a 0.22 μm PTFE filter and disposed in a glass vial for the autosampler. Using a spectrophotometer, absorbance was measured at 750, 662, 645, and 474 nm using a series of dilutions at 1/20 to obtain a linear range. Pigment quantification was determined according to Lichtenthaler and Buschmann (2001). Individual carotenoids were quantified by high-performance liquid chromatography and a reverse phase column (RP-18; Purospher STAR Supelco), using an acetonitrile: methanol:isopropanol (85:10:5 v/v/v) mix as mobile phase with a 1.5 mL min^{-1} flow rate at 30 °C. We identified carotenoids (e.g., α-carotene, β-carotene, lutein) according to their absorption spectra and retention time (Shimadzu; model LC-20AT).

2.2.7. Whole plant nitrogen (N)

In August 2018 and September 2021, we assessed whole plant N content following the methodology described in Section 2.1.2. For this purpose, we sampled 48 plants in total (1 plant *x* 2 canopy condition *x* 2 stocktypes *x* 2 planting hole sizes *x* 2 levels of vegetation management *x* 3 replicates).

2.3. Data analysis

For the nursery phase, variables of morphology, nutrient status, and root growth potential were assessed with a one-way variance analysis (1Y vs. 2Y) using generalized linear mixed models (PROC GLIMMIX; SAS Institute, Inc., Cary, NC, USA). The Akaike Information Criteria (AIC) was used for the selection of distributions.

The field phase was established using a split-plot design with two canopy conditions (forest vs. shrubland) that corresponded to the whole plots. Within each plot we randomized our fixed effects of stocktype (1Y vs. 2Y), planting hole sizes (20 cm vs. 40 cm), and levels of vegetation management (with and without) that corresponded to the split plots. Our three replicates corresponded to the random effects. The variables of survival, root-to-total and stem-to-total biomass partitioning, total biomass, soil water content (SWC), Ψ_{pd} and whole plant N were analyzed with a split-plot model with repeated measures (Kuehl, 2001) (GLMM and PROC GLIMMIX; SAS Institute, Inc.), with selection of distributions considering the AIC. The photosynthetic parameters of $A_{N(Imax)}$, R_D, I_{comp}, $\Phi_{(Icomp)}$, and F_v/F_m, and photosynthetic pigments were assessed using a two-way analysis of variance (stocktype and canopy condition) (PROC GLIMMIX; SAS Institute, Inc.) for the GLMM models, also selecting the lowest AIC for selection of distributions. We determined differences among means with a Tukey (HSD) test for multiple comparisons with a 95 % level of confidence. Chlorophyll fluorescence curves to different PPFD were modeled (PROC NLIN; SAS Institute, Inc.) using the Gauss-Newton method through a derivative-free algorithm. The variable ΦPSII was modeled with the first order decay kinetic model according to Eq. 3:

$$y = \beta_1 \quad x \quad e^{(-\beta_2 \quad xPPFD)} \quad + \quad \beta_3 \tag{3}$$

The variable NPQ in response to increasing PPFD was modeled with the Michaelis-Menten model described in Eq. 4:

$$y = (\beta_1 \quad xPPFD)/ \quad (\beta_2 + PPFD) \tag{4}$$

The extra sums of squares principle was used to assess the effect of canopy condition (Bergerud, 1996). We performed a relative importance analysis for the survival variable independently for forest and shrubland canopy conditions. Additionally, individual survival evaluation occasions underwent separate analyses. The Random Forest algorithm served as the predictive model, assessing variables with the highest relative weights in explaining survival, measured in terms of mean square error. Predictor and survival variable information underwent normalization by standardizing values between zero and one. The calculations were executed using the randomForest library (Liaw and Wiener, 2002) and VIP library (Greenwell and Boehmke, 2020) (R software; R Core Team, 2023). Visualizations were made with graphing software (SigmaPlot 14; Systat Software Inc., San José, CA, USA).

3. Results

3.1. Nursery phase

3.1.1. Morphology and nutrient status

Our production plan achieved morphologically contrasting stocktypes (Table 1). Not surprising, the two-year-old plants (2Y) had more height and root, stem, shoot, and total biomass (all $p < 0.005$) than one-year-old plants (1Y), although root collar diameter (RCD) was similar (Table 1). Stocktypes also displayed differences in nutrient concentration; 2Y plants had significantly more N and K than 1Y plants, and 1Y plants had more P than 2Y plants (Table 1).

3.1.2. Root growth potential

Stocktype was significant for root growth potential; 2Y plants had more new roots than 1Y plants (63.7 ± 4.4 vs. 45.6 ± 4.3; $p = 0.0430$). In contrast, the average length of new roots was lower in 2Y plants compared to 1Y plants (21.5 ± 0.3 cm vs. 27.6 ± 0.7 cm; $p = 0.0012$).

3.2. Field phase

3.2.1. Environment

During the field experiment (September of 2018 and September of 2021) and according to the Climate Explorer (http://explorador.cr2.cl), mean monthly minimum temperatures were observed in July (2.7, 2.4, 4.4, and 1.7 °C for each year, respectively). Mean monthly maximum temperatures were recorded in December (26.5, 28.7, 29.2, and 26.6 °C for each year, respectively). Annual accumulated precipitation reached 385 (September 2018 through December 2019), 216 (2020), and 773 (January 2021 through September 2021) mm. The percentage of annual precipitation that fell during May and June in 2019, 2020, and 2021 was 80, 75, and 77 %, respectively.

Forest and shrubland canopy conditions were characterized by different vegetation cover levels, which in turn produced differences in environmental variables underneath the canopies. The forest was characterized by greater LAI compared to the shrubland (1.64 ± 0.24 m^2 m^{-2} vs. 0.40 ± 0.16 m^2 m^{-2}). The same trend was observed in the groundcover index, which was significantly higher in the forest (63.6 ± 13.3 %) than in the shrubland (3.1 ± 8.6 %). These differences in LAI did not, however, affect maximum temperature nor minimum relative humidity dynamics during the duration of the experiment (September 2018 to September 2021; Supplementary Fig. 1 A and 1B). In contrast, we observed differences in forest and shrubland for PPFD of planted seedlings and gravimetric soil water content. We measured a nearly 4-fold difference in maximum PPFD values during summer (December to January) between shrubland (~2000 μmol photons m^{-2} s^{-1}) and forest (~512 μmol photons m^{-2} s^{-1}). Winter values (June to September) had a similar pattern, with PPFD in the shrubland (~1178 μmol photons m^{-2} s^{-1}) nearly 3-fold greater than in the forest (~414 μmol photons m^{-2} s^{-1}) (Fig. 2).

Measurement time and canopy condition interacted to affect gravimetric soil water content ($p < 0.0001$, see supplementary table 1).

Table 1
Mean (± standard error) values for morphological traits and macronutrient concentration (%) for two *Nothofagus glauca* nursery stocktypes (1Y, one-year-old; and 2Y, two-year-old). Values in bold indicate significant differences at $p < 0.05$ according to Tukey HSD.

Stocktype	Stem length (cm)	RCD (mm)	Biomass (g)						Macronutrients (%)		
			Root	Stem	Leaf	Shoot	Total	Shoot/root (g g^{-1})	N	P	K
1Y	26.3 ± 2.0	5.20 ± 0.2	1.08±0.12	1.09 ± 0.12	0.12 ± 0.04	1.16 ± 0.13	2.24 ± 0.24	1.09 ± 0.07	1.84 ± 0.02	0.53 ± 0.01	0.49 ± 0.01
2Y	49.3 ± 2.3	5.82 ± 0.37	1.77 ± 0.15	3.18 ± 0.30	0.10 ± 0.03	3.23 ± 0.30	5.00 ± 0.43	1.85 ± 0.11	2.34 ± 0.17	0.44 ± 0.01	0.70 ± 0.01
p-value	**<0.0001**	0.2247	**0.003**	**<0.0001**	0.7811	**<0.0001**	**<0.0001**	**<0.0001**	**0.0004**	**0.0041**	**0.0004**
df	1	1	1	1	1	1	1	1	1	1	1

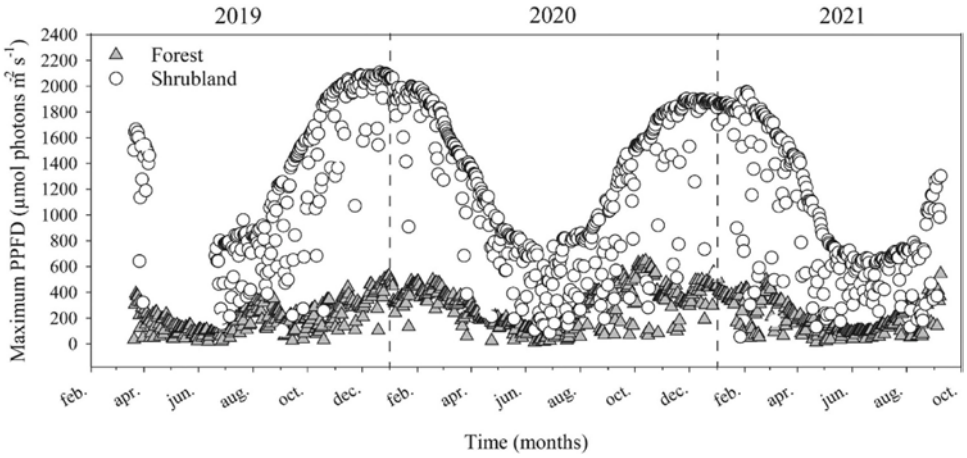

Fig. 2. Dynamic of daily maximum photosynthetically active photon flux density (PPFD, µmol photons m^{-2} s^{-1}) for forest (grey triangles) and shrubland (white circles) canopy conditions between March 2019 until September 2021.

During the wet cycle of the Mediterranean climate cycle (winter through spring: June through November), gravimetric soil water content was higher in the forest than in the shrubland (Fig. 3). Through the dry cycle (summer into autumn: November through May), however, gravimetric soil water content was higher in the shrubland than in the forest ($p < 0.0001$), generating differences in soil water content of 2–5 % between conditions.

3.2.2. *Survival and biomass*

The three-way interaction of canopy condition (forest or shrubland), stocktype (1Y or 2Y), and level of vegetation management (with or without) was significant ($p = 0.0075$) for survival, as were the two-way interactions of measurement time *x* canopy condition ($p < 0.0001$), measurement time *x* stocktype ($p = 0.0493$), and measurement time *x* level of vegetation management ($p = 0.0079$) (for *p*-values of all assessed interactions, see supplementary table 1). Regardless of canopy

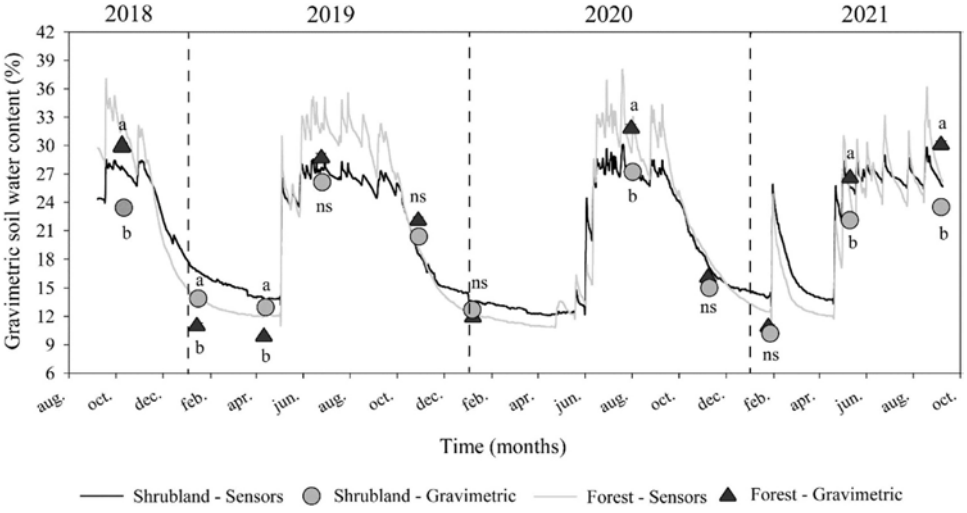

Fig. 3. Dynamic of gravimetric soil water content (%) estimated from volumetric soil water content (m^3 m^{-3}) for shrubland (circles) and forest (triangles) conditions. Sensor-measured soil water content during the field experiment is shown in black (shrubland) and gray (forest) lines. Different letters indicate significant differences between canopy conditions at the same measurement time according to Tukey HSD at $p < 0.05$. Ns: non-significant.

condition, stocktype, or level of vegetation management, survival decreased rapidly during the first season of establishment (September 2018 to October 2019) to values between 58 % and 41 % (Fig. 4). During the second growing season, survival decreased between 25 % and 8 % depending on treatment, reaching values ranging between 33 % and 46 % before the beginning of the third growing season (November 2019). Survival reached steady state values during the third growing season independently of canopy condition, stocktype, and level of vegetation management. Canopy condition was significant for survival; values were higher in the forest (76.7 ± 3.5 %) than in shrubland (54.0 ± 3.0 %) until April 2019, after which no differences were observed

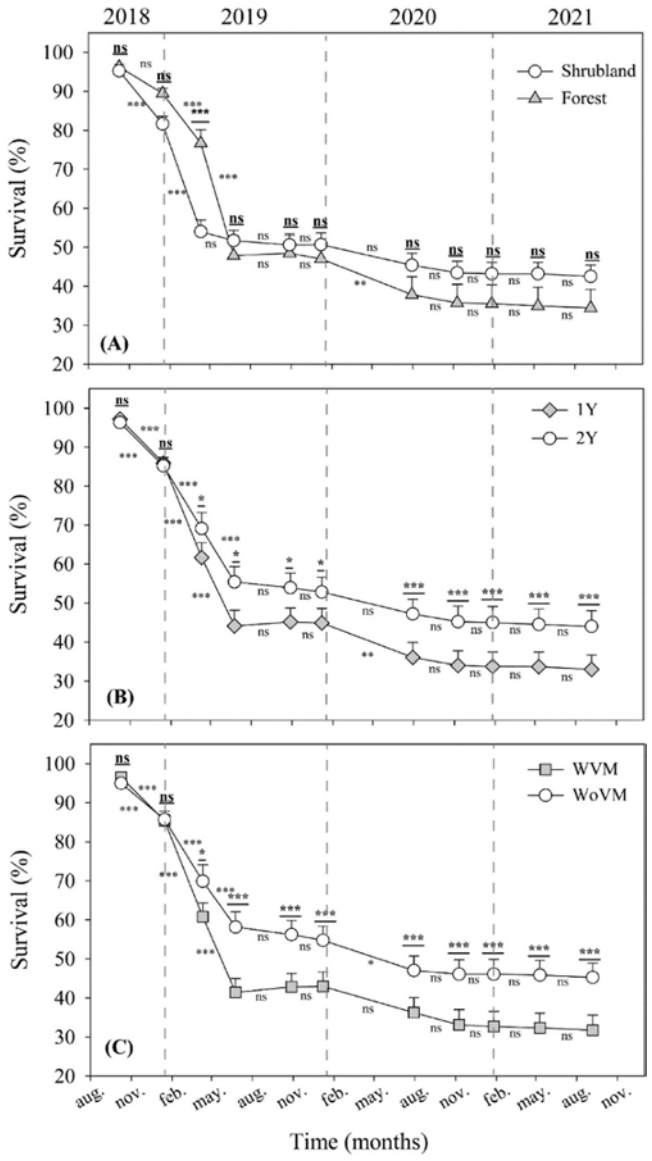

Fig. 4. Dynamics of *Nothofagus glauca* survival during the field experiment (September 2018 to September 2021) in relation to (A) canopy conditions of shrubland (white circles) and forest (grey triangles), (B) seedling stocktype 1Y (one-year-old; grey diamonds) and 2Y (two-year-old; white circles), and (C) seedling establishment with vegetation management (WVM) and without vegetation management (WoVM). Symbols indicate mean survival + standard deviation. Signs (ns or asterisks) indicate level of significance: ns: non-significant; *, **, and *** = significant at $p < 0.05$, $p < 0.01$, and $p < 0.0001$, respectively, according to Tukey HSD. Underlined signs on top of symbols indicate differences between treatments at the same measurement time. Non underlined signs between symbols indicate significant differences between measurement times under the same treatment.

(Fig. 4A). Stocktype was significant for survival; 2Y plants survived more (44.0 ± 4.1 %) than 1Y plants (32.9 ± 3.7 %) (Fig. 4B). Level of vegetation management was also significant for survival and followed a similar trend observed in the canopy and stocktype treatments, where regardless of level of vegetation management, survival decreased during the first growing season after which it reached steady state values. During the following seasons, plant survival was higher without vegetation management (45.3 ± 3.7 %) than with vegetation management (31.7 ± 3.9 %) (Fig. 4C). The three-way interaction of canopy condition *x* stocktype *x* level of vegetation management revealed that survival was highest for 2Y seedlings established under forest canopy and without vegetation control (71.3 ± 20.5 %), while 1Y plants established under forest canopy and with vegetation management yielded the lowest survival (33.3 ± 31.9 %).

Briefly, the three-way interactions of planting hole sizes *x* stocktypes *x* levels of vegetation management and planting hole sizes *x* canopy condition *x* levels of vegetation management were both significant ($p < 0.0001$) for survival. Although we did not observed a general trend, the deep (40 cm) planting hole displayed slightly higher survival compared to its shallow (20 cm) counterpart (supplementary Fig. 2A and 2B).

The interactions of measurement time *x* canopy condition ($p < 0.0001$) and measurement time *x* planting hole sizes ($p = 0.0128$) were significant for whole plant biomass accumulation. Plants established in the shrubland accumulated more total biomass than plants in the forest (Table 2). Biomass increased significantly every year in plants in the shrubland; these plants gained almost 5-fold their initial biomass by the end of the experiment. In contrast, plants established under forest canopy only presented an increase in total biomass during the first growing season, which then remained unchanged until the end of the experiment (Table 2).

Stocktype ($p < 0.0001$) and planting hole sizes ($p < 0.0001$) were significant for whole plant biomass accumulation. Two season stocktype plants (2Y) had, independent of canopy condition, gained more biomass compared to 1Y plants (16.31 ± 2.08 g vs. 8.39 ± 1.64 g). Plants established in deep planting holes had more biomass at the end of the first growing season than plants in shallow holes (15.71 ± 3.0 g vs. 9.62 ± 1.37 g), although this significant effect was absent at the end of the experiment (26.5 ± 5.8 g in 20 cm vs. 23.0 ± 5.0 g in 40 cm). Total root biomass was affected by the interaction between planting hole size *x* measurement time ($p = 0.0182$) and planting hole size *x* stocktype ($p = 0.0438$). After the first season, plants established in the deeper plot holes had higher root biomass than the shallow plot holes (7.3 ± 1.34 g vs. 4.96 ± 0.76 g, respectively), no differences were observed at the end of the experiment. In the 1Y stocktype, plants established in deeper planting holes had higher root biomass (6.06 ± 1.58 g) compared to the shallow planting hole (4.75 ± 1.57 g), no differences were observed between the 2Y stocktypes established in both planting holes.

When assessing biomass partitioning, we observed that the measurement time *x* canopy condition *x* stocktype interactions were significant for root-to-total biomass ratio ($p = 0.0276$) and stem-to-total biomass ratio ($p = 0.004$) (Fig. 5). At outplanting, the 2Y stocktype had a lower root-to-total biomass ratio compared to the 1Y stocktype (Figs. 5A, 5B). During the first season in the field, all treatments

Table 2
Mean (± standard error) total biomass (g) values of *Nothofagus glauca* seedlings established under shrubland and forest canopy conditions and measured throughout the field experiment. Within a canopy condition, different letters indicate significant differences among years at $p < 0.05$ according to Tukey HSD. Asterisks (*) indicate significant differences between canopy conditions within the same year at $p < 0.05$ according to Tukey HSD.

Canopy condition	Total Biomass (g)		
	2018	2019	2021
Shrubland	3.62 ± 0.29 **c**	16.97 ± 3.08 **b** *	41.45 ± 5.48 **a** *
Forest	3.62 ± 0.29 **b**	8.30 ± 0.93 **a**	7.43 ± 0.82 **a**

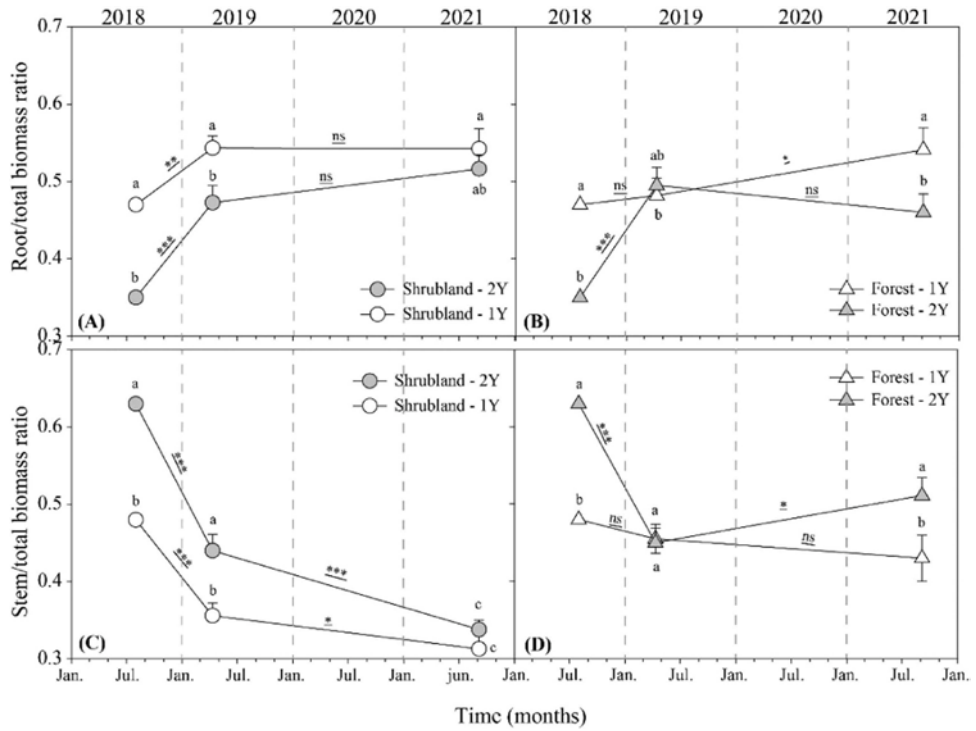

Fig. 5. Dynamics of root-to-total biomass partitioning (A, B) and stem-to-total biomass partitioning (C, D) of *Nothofagus glauca* 1Y (white symbols) and 2Y (grey symbols) stocktypes established in shrubland (circles) (A, C) and forest (triangles) (B, D) canopies. Symbols indicate means + standard deviation. Different letters indicate significant differences between different canopy condition at the same measurement time according to Tukey HSD at $p < 0.05$. Ns: non-significant; *, **, and *** = significant at $p < 0.05$, $p < 0.01$, and $p < 0.0001$, respectively.

increased biomass distribution towards roots except for the 1Y stocktype established under the forest canopy. At the end of the experiment, 2Y plants under forest canopy (Fig. 5B) had lower biomass distribution towards roots compared to all other treatment combinations. Stem-to-total biomass ratio followed an opposite pattern (Figs. 5C, 5D). In the final measurement time, 2Y plants under the forest canopy (Fig. 5D) had higher distribution towards stems than their 1Y cohorts, but both stocktypes had higher values than their counterparts under shrubland canopy (Fig. 5C).

3.2.3. *Pre-dawn water potential*

The three-way interaction of measurement time *x* canopy condition *x* levels of vegetation management was significant ($p = 0.0109$) for pre-dawn water potential (Ψ_{pd}) (Fig. 6). The initial Ψ_{pd} at establishment was -0.35 ± 0.03 MPa in all treatments. At the end of the first growing season (April 2019) and independent of level of vegetation management, Ψ_{pd} remained unchanged in plants under the shrubland canopy. In contrast, Ψ_{pd} of plants under forest canopy decreased, with lower Ψ_{pd} values observed in plants with vegetation control (-4.12 ± 0.1 MPa) compared to plants without vegetation control (-3.76 ± 0.09 MPa) (Fig. 6). During the second growing season, Ψ_{pd} decreased between October 2019 until January 2020 in both canopy conditions, although water status of plants established under shrubland canopy was higher than under forest canopy. This behavior was repeated during the third

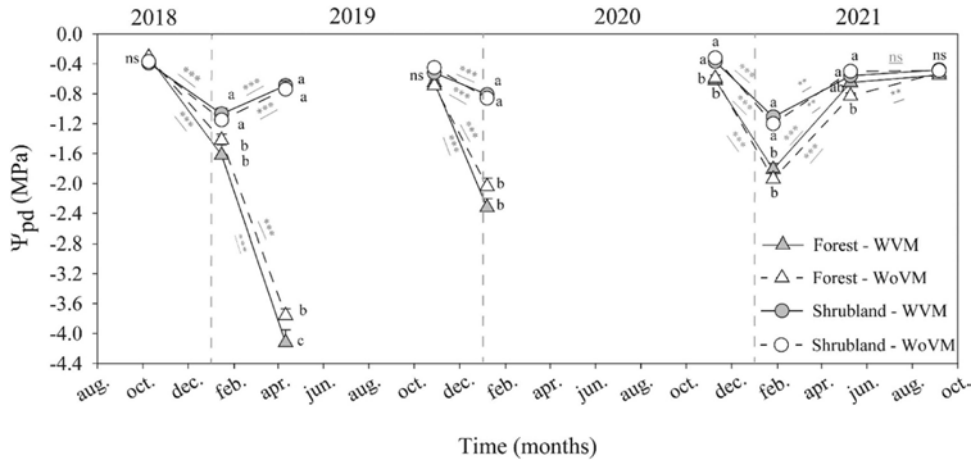

Fig. 6. Mean values for pre-dawn water potential (Ψ_{pd}) (+ standard deviation) in *Nothofagus glauca* seedlings established under forest (triangles) and shrubland (circles) canopy conditions and established with (WVM) and without (WoVM) vegetation management. Different letters indicate significant differences between treatments at the same measurement time. Underlined symbols indicate significant differences between measurement time within the same treatment. Ns: non-significant, **, and *** = significant at $p < 0.01$, and $p < 0.0001$, respectively.

growing season (October 2020 – January 2021) and no influence of level of vegetation management was observed. At the end of the experiment (October 2021), no significant differences were observed between canopy condition or level of vegetation management.

3.2.4. Leaf gas exchange, chlorophyll fluorescence and photosynthetic pigments

Canopy condition was significant for the three parameters extracted from the *A*-*Q* curves (Table 3): maximum photosynthetic rate obtained at $I{=}I_{max}$ ($A_{N(Imax)}$), the dark respiration rate (R_D), and the light compensation point (I_{comp}). Stocktype was significant for $A_{N(Imax)}$ (Table 3). Specifically, plants in the shrubland showed an increase in I_{comp} and R_D, while we observed a decrease in $A_{N(Imax)}$ in plants established under forest canopy. Then, 2Y plants increased $A_{N(Imax)}$ compared to 1Y plants at the end of the experiment. The interaction of canopy condition and stocktype was significant for quantum yield at $I{=}I_{comp}$ ($\phi_{(Icomp)}$) (Table 3); higher values were observed in the forest canopy independent of stocktype, followed by 2Y plants and finally by 1Y plants under the shrubland canopy (Table 3). The interaction of canopy condition and stocktype was significant for F_v/F_m; compared to all the other treatment combinations, the lowest values were measured in 1Y plants established under the shrubland canopy (Table 3).

When analyzing chlorophyll fluorescence curves, we observed that canopy condition and stocktype were significant for photochemistry. At the end of the experiment, plants established under the forest canopy had higher quantum efficiency of the PSII (ΦPSII) (Fig. 7A) than shrubland plants; this effect was observed mainly at lower flux densities in the curve (between 0 and 600 μmol photons m^{-2} s^{-1}). In contrast, plants established under shrubland canopy increased thermal dissipation of excess energy, observed through higher non-photochemical quenching (NPQ), compared to plants established under forest canopy (Fig. 7B). The 2Y stocktype showed an increased ΦPSII compared to the 1Y stocktype (data not shown). For the photosynthetic pigments chlorophyll a, chlorophyll *b*, α and β-carotene, and lutein, neither canopy condition, stocktype, nor their interaction were significant (all $p > 0.05$).

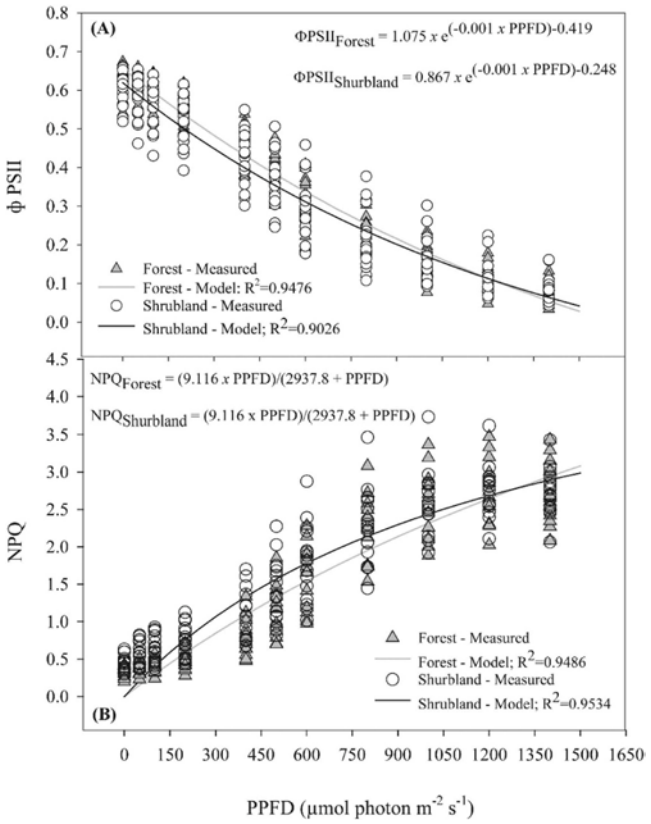

Fig. 7. Dynamics of photochemical efficiency of the PSII (ΦPSII) (A), and non-photochemical quenching (NPQ) (B) to increasing photosynthetic photon flux density (PPFD) of *Nothofagus glauca* plants established under forest and shrubland canopy conditions. Circles and triangles indicate measured data (forest in triangles, shrubland in circles) and lines indicate modelled data (forest in grey, shrubland in black). Adjusted model for forest and shrubland field conditions for ΦPSII and NPQ are shown on each panel.

3.2.5. Whole plant nitrogen

The three-way interaction of measurement time *x* canopy condition *x* stocktypes was significant ($p = 0.0030$) for whole plant N content. At the beginning of the field experiment, 2Y plants had more N than the 1Y plants (117.0 ± 0.0 vs. 41.3 ± 0.0 mg plant^{-1}). Plants of both stocktypes established under shrubland canopy increased their N content throughout the experiment, whereas plants under the forest canopy decreased their N content. Thus, at the end of the field experiment, shrubland plants from both stocktypes had higher N content (224.6 ± 54.0 mg plant^{-1} in 1Y stocktype and 189.8 ± 24.2 mg plant^{-1} in 2Y stocktype) than stocktypes established under forest canopy (43.4 ± 5.0 mg plant^{-1} for 2Y stocktype vs. 18.1 ± 2.2 mg plant^{-1} for 1Y stocktype). The two-way interaction of measurement time *x* canopy condition was significant ($p = 0.0226$) for N concentration, which decreased in plants under both shrubland and forest canopies during the experiment. At the end of the experiment, plants in the shrubland had more N than plants established under forest canopy (5.20 ± 0.35 mg g^{-1} vs. 4.37 ± 0.26 mg g^{-1}).

3.3. Analysis of relative importance

In our analysis of relative importance of measured environmental variables for survival (Fig. 8), measurements of soil water content

Table 3
Mean (± standard deviation) values of maximum net photosynthetic rate obtained at $I{=}I_{max}$ ($A_{N(Imax)}$), dark respiration rate (R_D), light compensation point (I_{comp}), and the quantum yield at $I{=}I_{comp}$ ($\phi_{(Icomp)}$), and maximum efficiency of the PSII (F_v/F_m) of *Nothofagus glauca* plants at the end of the field experiment, and *p*-values of different stocktypes (S) and canopy conditions (CC). Different letters within columns indicate significant differences among means. ns: non-significant.

	$A_{N(Imax)}$ (μmol CO_2 m^{-2} s^{-1})	R_D (μmol CO_2 m^{-2} s^{-1})	I_{comp} (μmol photon m^{-2} s^{-1})	$\phi_{(Icomp)}$ (μmol CO_2 μmol photon^{-1})		F_v/F_m	
Canopy condition (CC)				**Stocktype**		**Stocktype**	
				1Y	**2Y**	**1Y**	**2Y**
Shrubland	5.35 ± 0.79 **b**	0.81 ± 0.09 **a**	42.10 ± 3.30 **a**	0.017 ± 0.004 **c**	0.022 ± 0.004 **b**	0.723 ± 0.007 **b**	0.749 ± 0.007 **a**
Forest	6.87 ± 0.24 **a**	0.31 ± 0.05 **b**	12.73 ± 1.91 **b**	0.024 ± 0.005 **a**	0.025 ± 0.007 **a**	0.765 ± 0.007 **a**	0.754 ± 0.009 **a**
Stocktype (S)							
1Y	5.30 ± 0.73 **b**	0.49 ± 0.35 ns	35.14 ± 27.84 ns	–		–	
2Y	6.92 ± 0.33 **a**	0.83 ± 0.79 ns	26.98 ± 20.69 ns	–		–	
Source of variation	*p-values*						
CC	**0.0345**	**0.0011**	**0.0003**	**<0.0001**		**0.0033**	
S	**0.0315**	0.1433	0.2207	**0.0015**		0.2855	
CC *x* S	0.0794	0.8181	0.3936	**0.0042**		**0.0157**	

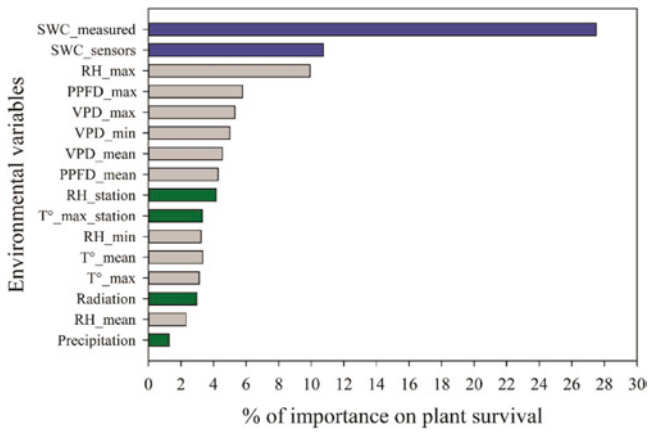

Fig. 8. Analysis of relative importance (%) of environmental and meteorological variables on survival on *Nothofagus glauca* plants. Blue bars represent gravimetric soil water content. Grey bars represent micro-environmental variables measured on each canopy condition. Green bars represent meteorological variables measured at the nearest meteorological station. SWC: soil water content, RH: relative humidity; PPFD: photosynthetic photon flux density; VPD: vapor pressure deficit; and T°: temperature.

(SWC), measured through soil weight and by sensors, were the main variables that explained plant survival (28 % and 11 %, respectively). Maximum environmental variables of relative humidity (RH), PPFD, and vapor pressure deficit (VPD) further explained survival by 9.9, 5.8, and 5.3 %, respectively. Radiation and precipitation measured in a meteorological station, and mean RH were the variables that least explained survival. By analyzing the evolution of the relative importance of the five most relevant environmental factors, we observed that throughout the field experiment, soil water content was the most relevant factor for *N. glauca* survival, especially during the first and second season after establishment (Fig. 9). The importance of this factor decreased to 28.1 % during the summer of the third season (February 2021), but then showed a sustained increase until the end of the experiment, reaching 65.8 %.

4. Discussion

4.1. Effect of stocktype on N. glauca field performance

In agreement to our first hypothesis, larger and N-loaded *N. glauca* plants cultivated during two seasons (2Y) in the nursery had better performance during field establishment, observed through higher survival rates that were sustained during three seasons in the field. This result agrees with the ecophysiological model proposed by Villar-Salvador et al. (2012), where larger plants with nutrient loading have higher survival rates in Mediterranean-climate plantations. At the beginning of the field experiment, 2Y plants displayed higher total N content, which could have promoted increased biomass accumulation due to higher N remobilization to sustain new growth (Millard and Grelet, 2010; Uscola et al., 2015). Also, higher photosynthetic capacity of 2Y plants support the existence of a positive feedback cycle, where initial N supports photosynthesis rates that in turn promote survival and growth (Cuesta et al., 2010). Considering that *N. glauca* is described as a shade-intolerant species (Fajardo and Alaback, 2005), the improved performance of these two traits in 2Y plants could also improve their competitive advantage during early establishment (Morrissey et al., 2010), especially by increasing light interception.

The higher shoot-to-root ratio of the 2Y stocktypes compared to smaller 1Y plants could have created a morphological imbalance between water absorption through roots and transpirational losses (Cortina et al., 2013; Trubat et al., 2008). Our results did not show, however, that 2Y plants were subjected to higher water stress than their 1Y counterparts, as stocktype was not significant for Ψ_{pd}, further rejecting the premise that smaller and N-deprived plants with lower transpiration rates could perform better in water-limited environments (Cortina et al., 2013; Trubat et al., 2008). According to Andivia et al. (2021), the higher transpiration rates of taller plants would be compensated by increased water absorption capacity promoted by increased root growth rates. Indeed, the 2Y stocktype allocated significantly more biomass towards root during the first growing season in the field, agreeing with the increased root grow potential observed. This behavior was probably sustained by higher N content of 2Y plants, which could have improved root growth and water absorption, thereby avoiding water stress in the field, thus compensating its increased height.

4.2. N. glauca stocktypes response to irradiance and water availability in the field

Our experimental setup of forest vs. shrubland canopies created differences in environmental conditions of irradiance and soil water content. First, in the forest canopy, the presence of an overstory reduced PPFD four-fold compared to the shrubland. Second, the presence of a forest canopy influenced the dynamics of SWC along the year; in the wet season (June to September), SWC was greater in the forest, probably due to increased organic matter content; whereas in the dry season (December to April), SWC was higher in the shrubland. Here, the lack of differences in relative humidity and air temperature between canopy conditions, supports the idea that the increased amount of overstory in

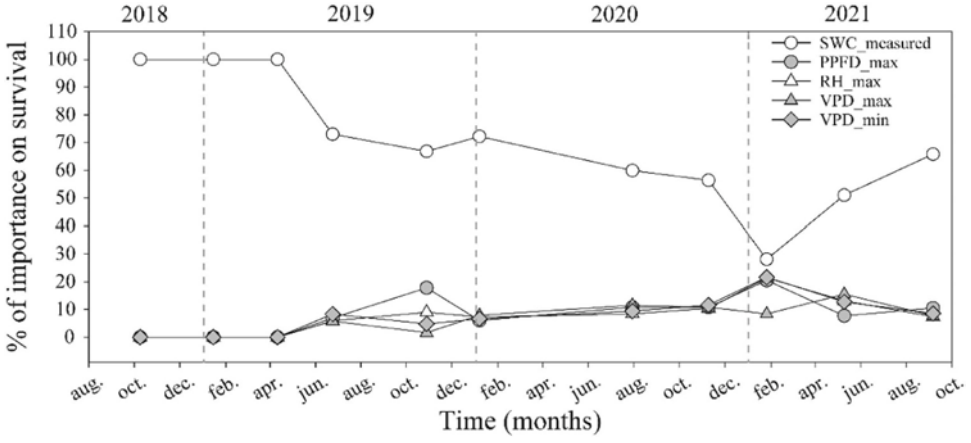

Fig. 9. Dynamic of importance (%) of environmental variables on *Nothofagus glauca* survival during three seasons after outplanting. SWC: soil water content; PPFD: photosynthetic photon flux density; RH: relative humidity; and VPD: vapor pressure deficit.

the forest led to additional transpiration during the dry season, which is the main driver of lower SWC in the forest vs. shrubland.

In water-limited environments such as in Mediterranean climates, the presence of an overstory can benefit the performance of plants established below by ameliorating evaporative demand (Black et al., 2005). However, the forest canopy clearly led to a stressful environment with a combination of water scarcity and low irradiance that negatively affected *N. glauca* growth after establishment. Although survival did not differ between canopy conditions, plants established in the shrubland showed higher biomass accumulation three years after planting. The lower SWC in the forest vs. shrubland during summer induced lower Ψ_{pd} that reached −4.12 MPa during the first season, indicating that these plants were subjected to severe water stress. During the following growing seasons (2020 and 2021), forest Ψ_{pd} increased and approached the values observed in the shrubland (-1.10 MPa approximately). These sustained Ψ_{pd} values in plants from the shrubland seem to establish a threshold of minimum leaf water potential for plant survival and growth. This observed value is close to the water potential at 50 % loss of leaf hydraulic conductivity (P_{50}) reported for *N. glauca* (-0.94 MPa) (Bucci et al., 2012) and higher (less negative) than the reported value for leaf water potential at turgor loss point (Ψ_{TLP}) of −1.95 MPa. On the contrary, Ψ_{pd} of plants under the forest canopy long surpassed the Ψ_{TLP}, especially during the first growing season. The maintenance of leaf water potentials above the Ψ_{TLP} under the shrubland canopy may have sustained cell turgor and allowed expansive growth (Bartlett et al., 2012), which agrees with the significantly greater biomass accumulation.

Nothofagus glauca has been described as a shade-intolerant pioneer species, and a gap-strategist in mature forests (Fajardo and Alaback, 2005), and the significantly higher biomass accumulation of plants developed in the shrubland canopy condition supports this statement. Despite this improved growth performance compared to plants from the forest canopy, shrubland plants were subjected to high levels of irradiance that indeed caused stress in the photosynthetic apparatus, observed through a decrease in photosynthetic capacity and efficiency and higher R_D rates. Although reductions in F_v/F_m are indicative of photo-damage, small decreases in this parameter (bellow 0.84) can also be interpreted as a photo-protection mechanism (Adams III et al., 2006), which is in agreement with the higher NPQ of plants established in the shrubland condition, indicating that these plants acclimated to higher irradiances through thermal dissipation of excess energy. As no differences in qP and ETR were observed between conditions (data not shown), we suspect that higher NPQ in shrubland plants avoided an over-reduction of PSII centers, thus preventing chronic photoinhibition. Similar results in regard to the activation of photo-protective mechanisms were reported in two co-occurring deciduous oak species established under a high-light environment (Rodríguez-Calcerrada et al., 2006). It is important to note, however, that these photosynthetic responses were observed at the end of the field experiment in surviving plants, and that plants sustained similar mortality rates during the first growing season regardless of canopy condition. In plants established under the shrubland canopy, the combination of higher Ψ_{pd} and summer SWC, activation of photo-protective mechanisms, and a decrease in photosynthetic capacity could indicate that, higher irradiance was the main driver of mortality in these plants instead of water stress. Further, although plants in the shrubland condition received significantly higher PPFD than their forest counterparts, the presence of shrub-like vegetation has previously been shown to induce large micro-environmental variability between and within individuals (Gómez-Aparicio et al., 2005; Raffaele and Veblen, 1998). Thus, considering the existence of high heterogeneity in the light environment surrounding individual plants, it is possible that plants that died during the first growing season under the shrubland canopy received higher levels of radiation that did not allowed an acclimation response, while surviving plants could have been facilitated by neighboring pre-existent plants. This is in agreement with a meta-analysis reported by Holmgren et al. (2012), where shade-intolerant species established in drought-prone environments showed adverse effects in both extremes of the light gradient, hence explaining similar mortality rates of *N. glauca* established under deep shade (forest) or high light (shrubland). In light of these results, we believe that the shrubland canopy condition induced a nursing effect on surviving *N. glauca* plants established under its canopy, through increased SWC in summer and an irradiance level that allowed acclimation and promoted growth, as observed in other species from Mediterranean climates (Gómez et al., 2004; Padilla and Pugnaire, 2006; Siles et al., 2008)

Considering plant field performance as an interplay between survival and growth (Grossnickle, 2012), it is important to understand biomass allocation patterns that allow plants to cope with environmental stress in the field. In this regard, contrasting hypotheses in the literature state that (1) an intraspecific trade-off occurs in which water stress will have a higher impact on plants growing in shade because of a greater allocation of biomass towards shoots vs. roots (Kubiske et al., 1996; Kupers et al., 2019; Smith and Huston, 1989); and that (2) shade will ameliorate drought impact due to greater protection from stressful factors, such as higher irradiances, temperature, and VPD (Holmgren et al., 2000; Sack, 2004). Our results support the first statement because plants established under the forest canopy showed that biomass was mainly allocated towards shoots for light interception, rather than promoting root growth for water stress avoidance. On the contrary, plants established under the shrubland canopy allocated higher biomass towards roots vs. shoots during the first growing season, possibly due to increased PPFD. We suspect that higher biomass allocation towards roots in plants established under the shrubland canopy allowed a greater N accumulation at the end of the field experiment, this in turn would have promoted photosynthesis and the growth of new organs, leading to a positive feedback cycle (Villar-Salvador et al., 2012). Similarly, establishment of plants on deeper planting holes also increased root biomass and led to increased survival under specific conditions (see suppl. Fig. 2). Although we did not observed an effect of planting hole size on plant water status, it could have improved nutrient acquisition further supporting our previous statement. Following *N. glauca* survival and growth responses to shrubland and forest conditions, our second hypothesis was partially confirmed, as only growth was significantly higher in the shrubland condition.

4.3. Environmental determinants of N. glauca field survival

Contrary to controlled experiments, on field studies, factors such as irradiance and water supply are patchy and interact at different levels to affect plant responses, complicating the assessment of the key environmental variables that drive plant field performance. Consequently, we performed a relative importance analysis that showed that the main driver of *N. glauca* field survival was soil water content, followed by air relative humidity. As expected, the available SWC was a determinant for survival during the first two growing seasons, thus confirming our third hypothesis. Likewise, the vegetation management treatment induced a decrease in *N. glauca* water status, leading to decrease survival in the following seasons. Afterwards, we observed a decrease in its relevance in the third growing season, concurring with an increase in Ψ_{pd}, above the Ψ_{TLP} reported for *N. glauca*, possibly indicating that once surviving plants are not water-limited, other environmental factors such as irradiance gain relevance to determine its performance.

Although research in Mediterranean climates reports that water availability is the main driver of plant survival and performance (Osem et al., 2012; Rodríguez-Calcerrada et al., 2010), irradiance also plays a role, especially in shade-intolerant species (Cooper et al., 2014; Holmgren et al., 2012). Considering that maximum irradiance had the fourth higher relative importance, we suggest that survival of *N. glauca* established under the shrubland canopy was greatly influenced by higher irradiance, which is evidenced in the impact on mechanisms of excess energy dissipation and photosynthesis.

The accomplishment of forest restoration programs based on

planting depends on seedling establishment success. To meet this goal, extensive research links plant morpho-physiological and behavioral traits to field establishment success. In this regard, the Target Plant Concept (Dumroese et al., 2016) indicates that these plant traits should be determined based on environmental limiting factors present on the site. In agreement, Grossnickle (2012) and Grossnickle and MacDonald (2018) reviewed which seedling traits best correlated with high survival and growth. This perspective assumes, however, that survival and growth are evenly affected by the same environmental stressor, and although our results agree with the impact of nursery plant traits on field performance, we also observed that survival and growth are each independently affected by different environmental factors.

Although research has aimed to understand the effects of light and water availability on plants survival and growth (Black et al., 2005; Sánchez-Gómez et al., 2006; Walters et al., 2023), research regarding plant traits to comply with the TPC for restoration too often assumes that established plants will need to overcome a single limiting factor for survival and growth. However, under our experimental conditions, we observed that water availability is key for survival, while irradiance is needed to promote growth. Although survival or growth have been used interchangeably to explain plant performance, Grossnickle and MacDonald (2018) explain that high performance is achieved once seedlings are coupled to their environments, overcome environmental stress, and initiated growth leading to survival. Thus, considering our results, specific levels of water availability and irradiance are required to achieve high performance in *N. glauca*. For forest restoration plantings, it is important to consider that survival and growth could be affected by different environmental factors, which could further depend on target species biology (i.e., shade tolerant or shade-intolerant).

4.4. Management implications for establishment of a shade-intolerant species under Mediterranean conditions

Results from this research are useful to determine best practices for establishment of a shade-intolerant species under Mediterranean conditions according to resource availability. Regarding stocktype selection, we found that larger plants loaded with nitrogen had the best field survival and growth. In this particular research, larger and N-loaded plants were achieve through growth of seedlings for two season in the nursery, but N loading can also be attaind by exponential fertilization or late-season fertilization in the nursery (Birge et al., 2006; Boivin et al., 2004; Dumroese et al., 2023, 2005).

In regard to establishment techniques, we tested different sizes of the plot hole and competitive vegetation management, but we did not find a clear trend in which a specific combination of treatments significantly improved *N. glauca* survival or growth. We also observed that the application of herbicide had detrimental effects on survival and plant water status, which is in opposition to most literature regarding this subject (Dinger and Rose, 2010, 2009). Although it was not specifically tested, it is possible that the removal of competing vegetation allowed the pre-existing vegetation to produce more fine roots to advantage of the improving growing conditions. Thus, considering that this is costly procedure, we advice against its use in environments similar to the ones described above. However, this should be further evaluated under different environmental conditions as other reports have shown positive effects of vegetation management (Dinger and Rose, 2010, 2009). Regarding the use of deeper planting holes, the literature has shown an improvement in plant water status and root development (Fonseca et al., 2011; Oliet et al., 2015), leading to higher survival rates. Despite that we observed increased survival in plants established in deeper planting holes, these differences were subtle and does not seem to be related to increased plants water status. However, this treatment induced higher root biomass production, which could promote nutrient acquisition leading to a positive feedback cycle as disbribed by Villar-Salvador et al. (2012).

According to our relative importance analysis, water availability was the most relevant factor for *N. glauca* survival, and severely imptacted survival in forest conditions during the first summer after establishment. Under this scenario, it would be advisible to decrease stand density to decrease rainfall interception and allow higher soil water content. Also considering that *N. glauca* is a shade-intolerant species, a decrease in stand density will also allow an increase in light which in turn promotes growth. Although we were unable to characterize the light environment of individual plants in the shrubland condition, we believe that surviving plants at the end of the experiments were facilitated by neighbouring shrub in this condition. This agrees with Holmgren et al. (2012) whose work indicates that higher performance of shade-intolerant plants is achieved at intermediate irradiance levels instead of fully open canopies. Facilitation could also be attained by planting adjacent to pre-existing vegetation, or in canopy gaps.

CRediT authorship contribution statement

Andree Álvarez: Resources, Methodology, Formal analysis. **Claudia Stange:** Resources, Methodology. **Simón Sandoval:** Software, Methodology, Formal analysis. **Eduardo Cartes:** Project administration, Funding acquisition, Data curation. **Jan R Bannister:** Writing – review & editing, Validation, Conceptualization. **R Kasten Dumroese:** Writing – review & editing, Validation. **Marta González:** Resources, Funding acquisition, Conceptualization. **Manuel Alejandro Acevedo:** Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Carolina Estela Alvarez-Maldini:** Writing – review & editing, Writing – original draft, Visualization, Resources, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foreco.2024.122190.

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