

Provenance causes variation on early growth and survival and sun protection on physiological responses of the vulnerable *Nothofagus glauca* (Phil.) Krasser in a common garden in Central Chile

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ABSTRACT

We assessed outplanting performance of *N. glauca* in two trials. In Trial 1, provenances from Pre- Andean (Linares, Longaví) and Coastal (Curanipe, Empedrado) origins were planted in a common garden under two sun protection treatments (i.e., black mesh shelter, and kaolin at 6% concentration), and a control treatment at full sun. In Trial 2, a local provenance was established under two site preparation treatments (i.e., old subsoiling from 4 years ago versus no-subsoiling). Growth, survival, gas exchange, and Chlorophyll fluorescence parameters were measured one year after outplanting. In Trial 1 survival was similar among treatments but larger seedlings were found under kaolin and in the local provenance Empedrado. The provenances varied by sun protection treatment in survival, and the Pre Andean provenance Linares tended to show the lowest survival at full sun. At the leaf-level physiology, provenances responded similarly in gas exchange and fluorescence parameters. In the sun protection treatments seedlings under kaolin increased stomatal conductance and electron transport, but decreased photosynthesis, water use efficiency, and efficiency of PSII. Provenances also varied by treatment in physiology. The Empedrado provenance exhibited the highest transpiration and stomatal conductance under kaolin whereas the Pre Andean provenance of Longaví showed the lowest photosynthesis and efficiency of PSII in the kaolin treatment. In Trial 2, root collar diameter, intercellular CO₂ and water use efficiency were higher in the old-subsoiling treatment. As a potential adaptation tool, seed sources from Pre Andean origins can be established in Coastal origins with no detriments on seedling survival.

1. Introduction

Nothofagus glauca (Phil.) Krasser (hualo) is an endemic and vulnerable species from Central Chile (Barstow et al., 2017). It has a narrow distribution along the country (34° to 37° S) and has been intensively degraded and fragmented by land use change (agriculture during last quarter of XIX century, wood and charcoal production, replacement by commercial forest plantations in the XX century), and fires (Le-Quesne and Sandoval, 2001; Wilson et al., 2005; Fajardo and Alaback, 2005; Echeverría et al., 2006; Balocchi et al., 2020; White et al., 2020), causing a decline in the natural populations of at least 30% (Baldwin et al., 2018). The species is classified as shade-intolerant and its natural

regeneration requires forest gaps with higher light availability (Donoso, 1981). However, excessive radiation may have harmful effects on the survival and growth of artificially established seedlings (i.e., active restoration), especially when combined with other stressing factors such as higher temperatures and lower soil moisture. Currently, there is concern about the restoration of the Mediterranean ecosystems in Central Chile, an area that has been affected by a mega-drought during the last decades (Garreaud et al., 2017 2020) compromising the success of the restoration programs and also the productivity of the natural populations.

Some management techniques are typically used to improve the rapid post-planting establishment such as site preparation and the use of

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shelters. Subsoiling is a widely used site preparation method in forest plantations. Subsoiling increases root penetration, soil aeration, nutrient availability, soil temperature, and reduces bulk density (Celma et al., 2019), improving seedling growth and survival. Whereas this method offers benefits such as maintained soil fertility and increased plant-available water (Lacclau et al., 2003; Mendham et al., 2003; Li et al., 2015), it can also produce soil degradation and compaction due to the harvesting of the previous plantation (Cambi et al., 2015; Andrade et al., 2017). *N. glauca* seedlings are typically planted in $30 \times 30 \times 40$ cm planting holes with infiltration trenches as the most common site preparation technique. Survival rates with this technique ranges from 33 to 55% for seedlings at full sun and under shelters (Peña-Rojas et al., 2013); however, no attempts have been made to assess the establishment of seedlings in areas previously subsoiled and used for pine silviculture, as a measure of a sustainable management.

The use of shelters improves plant performance by protecting plants from excessive sun radiation, increasing humidity, and buffering extreme temperatures (Padilla et al., 2011). It has been proved in other *Nothofagus* species (i.e., *N. alessandrii*) demonstrating positive effects on seedlings growth and survival (Quiroz et al., 2021), but at high costs in large scale projects. Kaolin clay coating applications are extensively used in fruit and grapevines production (Saour et al., 2003; Melgarejo et al., 2004; Wand et al., 2006) and may be a cheaper alternative for the establishment of *N. glauca* than the use of shelters. Kaolinite reduces leaf-to-air vapor pressure difference, protects photosystem II structure and function (Dinis et al., 2016), and increases stomatal conductance, net photosynthesis, and water use efficiency (Jifon and Syvertsen, 2003). Foliar applications of kaolinite have shown to reduce canopy temperature with no compromise of photosynthesis and productivity in apple trees (Glenn et al., 2001), but the effects might vary by species, plant architecture, environmental conditions, and scale of measurement (Boari et al., 2015). Similarly, kaolin has shown to increase stomatal conductance, photosynthesis, and transpiration rates in forest species such as *Pinus ponderosa*, *Pseudotsuga menziesii*, *Austrocedrus chilensis*, and *Eucalyptus urophylla* grown under controlled conditions (Varela et al., 2016; Santos et al., 2021). However, the effects of kaolin application on the outplanting performance of *N. glauca* seedlings are still poorly understood.

Provenance (seed source) also influences the establishment of planted seedlings. Local seed sources are better adapted to local conditions, improving survival, and growth (Broadhurst et al., 2008; Van der Mijnsbrugge et al., 2010; Broadhurst and Boshier, 2014). Several common-garden experiments using provenances in *Nothofagus* species have demonstrated the genetic determinism of phenological traits, survival, and growth (Premoli et al., 2007; Torres-Ruiz et al., 2019; Ignazi et al., 2020; Gutiérrez and Navarrete, 2021). In a common garden experiment with *N. pumilio*, Ignazi et al. (2020) found that marginal populations of the species presented reduced performance in plant architecture, whereas with *N. obliqua* Gutiérrez and Navarrete (2021) found an outstanding performance in growth and survival for local provenances. In *N. glauca*, Vergara et al. (2014) found differences in adaptive properties among provenances, but there is still little knowledge on how these differences are expressed in a common environment and to what extent they reflect adaptation to local conditions.

As seedlings are typically less able to resist stress than adult trees, it is important to understand the factors influencing the early establishment of a species. In this context, we established two trials with *N. glauca* in a coastal site. In Trial 1 we established a common garden with four provenances (from Coastal and Pre Andean geographical origins) with three levels of sun protection (black shelter that provides 60% shade, kaolin at 6%, and a control treatment at full sun). In Trial 2 we established a local provenance under two levels of site preparation (4-year-old subsoiling versus no-subsoiling). The working hypotheses of this study were that the use of sun protection treatments and subsoiling may ameliorate the post-planting growth stresses of *N. glauca* seedlings, and that those responses may vary by provenance. The aim of this study was

to assess the effect of provenance (seed source), sun protection, and site preparation on the growth, survival, leaf-level physiology, and Chlorophyll fluorescence of *N. glauca* one year after outplanting.

2. Material and methods

2.1. Plant material

Seeds from four provenances (seed sources) of *N. glauca* from coastal and Pre Andean geographical origins were selected for this study (Table 1). Seeds were soaked for 24 h in a solution with aqueous gibberellic acid at a concentration of 400 mg L^{-1} . Seeds were sown in October 2019 on 130 mL pots filled with composted bark of radiata pine, fumigated with sodium hypochlorite at 10% and cultured until early July 2020.

2.2. Site description and trials establishment

Two field trials were established in July 2020 in the Las Cañas locality (Constitución, Chile, $35^{\circ} 30' \text{ S}$, $72^{\circ} 22' \text{ W}$, 445 m.a.s.l) with 1–0 seedlings. According to Köppen climatic classes, the climate is classified as Cfb (temperate oceanic climate with no dry season and warm summer). The area has an annual rainfall of 885 mm, a mean annual temperature of 11.6° C and a maximum average temperature of 18.7° C in the hottest summer months of January and February. The soil at the experimental site is classified as a Constitución series (Alfisol, in the Typic Rhodoxeralfs family) with a silty clay loam texture (20% sand, 48% lime and 32% clay). It rests on a substratum made up of highly weathered metamorphic rocks with a clayey matrix, and it preferably occupies the highest sector and western slopes of the Coastal Mountain Range. The main chemical properties for the common garden and the provenances origin are shown in Supplementary Material (Table S1). By using SPAWN Hydrology (a free online hydrologic budget model) we estimated that field capacity, wilting point, and available water content were 0.36 , 0.20 , and $0.16 \text{ m}^3 \text{ m}^{-3}$, respectively. The site was previously occupied by a *Pinus radiata* D. Don plantation, which was felled in 2014. The trials were planted on sites of Forestal Mininco S.A. company, and the site preparation consisted of chemical weed control. Granular NPK (12:32:5) fertilizer was applied by hand at the planting hole (Multicote™ 4M at 25 g plant^{-1}). The site was fenced to avoid herbivore damage and seedlings were hand planted in $30 \times 30 \times 40$ cm planting holes at a spacing of $2 \times 2 \text{ m}$ (i.e., tree stocking of $2500 \text{ stems ha}^{-1}$). Seedlings were protected from animal damage with a sturdy plastic fence (40 cm tall $\times$ 20 cm wide) secured with two 5 mm iron bars.

2.2.1. Trial 1

The trial followed a randomized complete block design with four provenances (Longaví, Linares, Curanipe, Empedrado), two sun protection treatments (black mesh shelters that provided 60% shade, kaolin at 3% concentration, plus a control treatment at full sun). The kaolin used in this experiment was processed kaolin (Sorround WP, BASF Company) applied as a water suspension at 3% concentration on December 15, 2020 (first application), and 6% concentration on December 16, 2020 (second application) to ensure kaolin adhesion uniformity. We established a total of 1680 seedlings (4 provenances $\times$ 3 sun protection treatments $\times$ 5 blocks $\times$ 28 seedlings per plot). On August 18, 2020, we recorded the phenological stages resting buds, swollen buds, and expanding leaves as in Rusch (1993). Each seedling was recorded as belonging to a particular phenological stage when at least one bud and/or leaf had attained a given phenophase.

2.2.2. Trial 2

The trial followed a randomized complete block design with two site preparation treatments (old subsoiling at up to 80-cm depth versus no subsoiling). The subsoiling was made in 2018 four years after the *P. radiata* plantation was harvested. We established a total of 450 seedlings

Table 1Location and climatic parameters for the four provenances of *N. glauca* represented in the common garden experiment.

Provenance (seed sources)	Geographical origin	Latitude (°S)	Longitude (°W)	Altitude (m.a. s.l.)	MAP (mm)	MAT (°C)	T Jan (°C)	T July (°C)	De Martone aridity index
Longaví	Coastal	36° 08'	71° 30'	500	1262	12.6	27.5	2.5	55.8
Linares		35° 51'	71° 17'	560	1041	11.9	26.7	1.6	47.5
Curanipe		35° 51'	72° 36'	550	820	13.0	24.0	5.7	35.6
Empedrado		35° 35'	72° 22'	450	861	11.6	23.7	3.4	39.9
Common garden		35° 30'	72° 22'	445	855	11.6	23.5	3.4	39.6

MAP = Mean Annual Precipitation, MAT = Mean Annual Temperature. The De Martonne aridity index was estimated as $MAP/(MAT + 10)$, T Jan = Average temperature during January, T Jul = Average temperature during July.

of the local provenance Empedrado (2 site preparation treatments $\times$ 3 blocks $\times$ 9 replicate plots of 25 seedlings each). The phenological stages were not recorded in this trial. We measured three common indicators of soil compaction: bulk density (BD, $g\ cm^{-3}$), porosity (POR, %), and soil resistance to penetration (PR, kPa) (Cambi et al., 2015). BD was measured at 0–20 cm and 20–30 cm depth in both site preparation treatments with a cylindrical metal sampler (height 5 cm, diameter 5.9 cm). Six samples were randomly taken per depth and treatment. BD cores were dried for 24 h at 105 °C before weighing. The particle density (PD) was measured by a pycnometer (Multipycnometer, Quantachrome, Boynton Beach, FL, USA) on subsample of soil samples used to determine BD. POR was determined by the following equation: $POR = ((PD-BD)/PD) \times 100$. PR was measured with the cone penetrometer testing (CPT) (Beckett et al., 2018). Briefly, using a penetrometer (Hand Penetrometer Eijelkamp) with a 2 cm² tip, 12 measurements were taken per block and treatment at 5, 15, 30, 70, and 100 cm depth and then averaged to obtain the soil penetration resistance.

2.3. Survival and morphological assessments

In both trials, seedling height (H, cm) and root collar diameter at the ground line (DAC, mm) were measured on all seedlings at the planting date and one year after outplanting. The increments in H (ICNh, cm) and D (ICNdac, mm) were derived as the difference between both measurement dates. H and D were collected in June 2021 because height growth ceases there during this month. H and D were recorded using graded height poles and digital calipers, respectively. Survival (SUR) was measured one year after outplanting and was considered a categorical trait (i.e., live seedling = 1, dead seedling = 0).

2.4. Gas-exchange and Chlorophyll fluorescence

At mid February 2021, saturated photosynthesis rate (A_{sat} , $\mu mol\ CO_2\ m^{-2}\ s^{-1}$), transpiration rate (E , $mmol\ H_2O\ m^{-2}\ s^{-1}$), intercellular CO_2 concentration ($\mu mol\ mol^{-1}$), stomatal conductance (g_s , $mmol\ H_2O\ m^{-2}\ s^{-1}$) and intrinsic water use efficiency ($WUE_i = A_{sat}/g_s$) were measured on a random subsample of 108 seedlings in Trial 1 (i.e., 3 blocks $\times$ 4 provenances $\times$ 3 sun protection treatments $\times$ 3 seedlings), and in 18 seedlings in Trial 2 (i.e., 3 blocks $\times$ 2 site preparation treatments $\times$ 3 seedlings). In the same samples, under light-adapted conditions, and upon the application of a saturating flash, we also measured the fraction of light absorbed in the PSII antennae that is dissipated thermally (D), the PSII operating efficiency in light conditions ($\phi PSII$), the maximum efficiency of PSII in light conditions (Fv'/Fm'), the electron transport rate through PSII ($ETR\ \mu mol\ m^{-2}\ s^{-1}$), the photochemical quenching (qP), and the fraction of PSII centers that are 'open' (qL). These measurements were taken between 09:00 and 12:00 (local time) using a LI-6800 photosynthesis system (LI-COR Inc., Lincoln, NE, USA). Temperature, air CO_2 concentration, and light source were set at ambient conditions to 25 °C, 400 ppm, and 1800 $mmol\ m^{-2}\ s^{-1}$, respectively.

2.5. Data analyses

To evaluate the differences in growth, increments, and survival

among treatment factors all traits were analyzed with analysis of variance. For growth, increments, and survival, average responses were recorded in the 28 and 25-seedlings plot of Trial 1 and Trial 2, respectively. For level physiology and Chlorophyll fluorescence, average responses were recorded in the 3-seedlings plot of Trial 1 and Trial 2. The mean values showing significant differences were compared with the Tukey test at $P \leq 0.05$. Prior to the analyses, the data were examined and conformed to the normality and homogeneity of variance assumptions required for the analysis of variance. To meet the assumption of normality and constant variances, the data were transformed according to the Box-Cox transformation when appropriate. In the case of survival (i.e., 1, 0), we used a GLMM with a binomial distribution and the logit link function. The model terms were as follows:

$$Y = \mu + B + P + T + P \times T + \epsilon \quad (1)$$

$$Y = \mu + B + SP + \epsilon \quad (2)$$

where, Y is the observed phenotypic value, μ is the overall mean, B is the random effect of block, P is the fixed effect of provenance (Longaví, Linares, Curanipe, Empedrado), T is the fixed effect sun protection treatment (black shelter, kaolin, full sun), SP is the fixed effect of site preparation treatment (old subsoiling, no-subsoiling), ϵ is the error term. Differences in soil physical parameters measured in Trial 2 were assessed with a two-way ANOVA considering the effects of site preparation treatment and the depth of sampling. All the statistical analyses were performed with SPSS version 18.0 software (SPSS Inc, Chicago, Illinois, USA).

3. Results

3.1. Effects of provenance and sun protection. Trial 1

The phenological observations carried out one month after the trial establishment indicate that the Coastal provenances had on average 58, 33, and 8% of resting buds, swollen buds, and expanding leaves, whereas in the Pre Andean provenances, those values were 75, 22, and

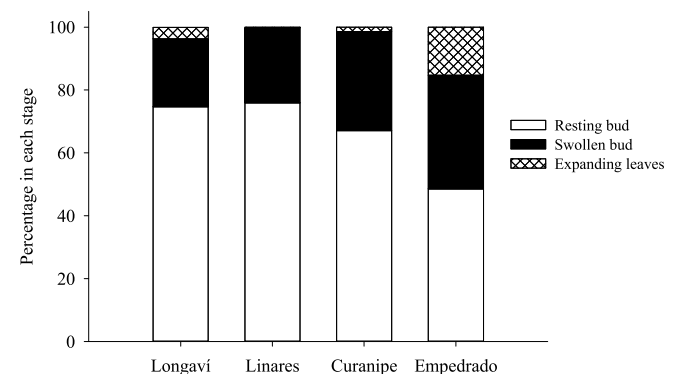


Fig. 1. Phenological stages of *N. glauca* provenances in Trial 1 registered in August 2020.

2% (Fig. 1).

The provenance by sun protection treatment interaction was significant only for survival, explained by the provenance differences found at the full sun treatment (Table S2). The Pre Andean provenance of Linares showed the lowest survival (71%) at full sun compared with the other provenances (c.a. 80%) (Fig. 2a). At the provenance and sun protection levels, the largest seedlings were those from the local provenance Empedrado and those established under the kaolin treatment (Table S2). At the leaf-level physiology, there was a significant interaction between provenances and sun protection treatments in E , A_{sat} , and g_s (Table S3). The coastal provenance of Empedrado exhibited the highest E and g_s under the kaolin treatment, whereas the Pre Andean provenance of Longaví showed the lowest A_{sat} in the same treatment but the highest performance for E and g_s under shelters (Fig. 2b c d). At the sun protection level, seedlings in the kaolin treatment exhibited higher E , C_i , and g_s , but the highest A_{sat} and WUE_i were found in seedlings established under shelters and at full sun (Table S3). For fluorescence parameters, there was a significant interaction between provenances and sun protection treatments for D and F_v'/F_m' (Table S4). The Pre Andean provenance of Longaví showed the highest D and lowest F_v'/F_m' under

the kaolin treatment (Fig. 2e f). At full sun, provenances Longaví and Empedrado exhibited the highest and lowest F_v'/F_m' respectively. The main effect of sun protection level indicates that, except for D and F_v'/F_m' , the rest of parameters were found to be high in seedlings under the kaolin treatment.

3.2. Effects of old subsoiling. Trial 2

We found no differences between treatments for BD. PR was higher and POR was lower in the no-subsoiling treatment. As expected, PR and BD were higher at deep depths, whereas the opposite was true for POR (Table 2). At the seedling level, we found that only DAC , C_i , and WUE_i were higher in the old-subsoiling treatment (Table 3).

4. Discussion

4.1. Seedling performance under sun protection treatments

In this study, we expected that sun protection would exert positive effects on the early establishment of *N. glauca* because the species

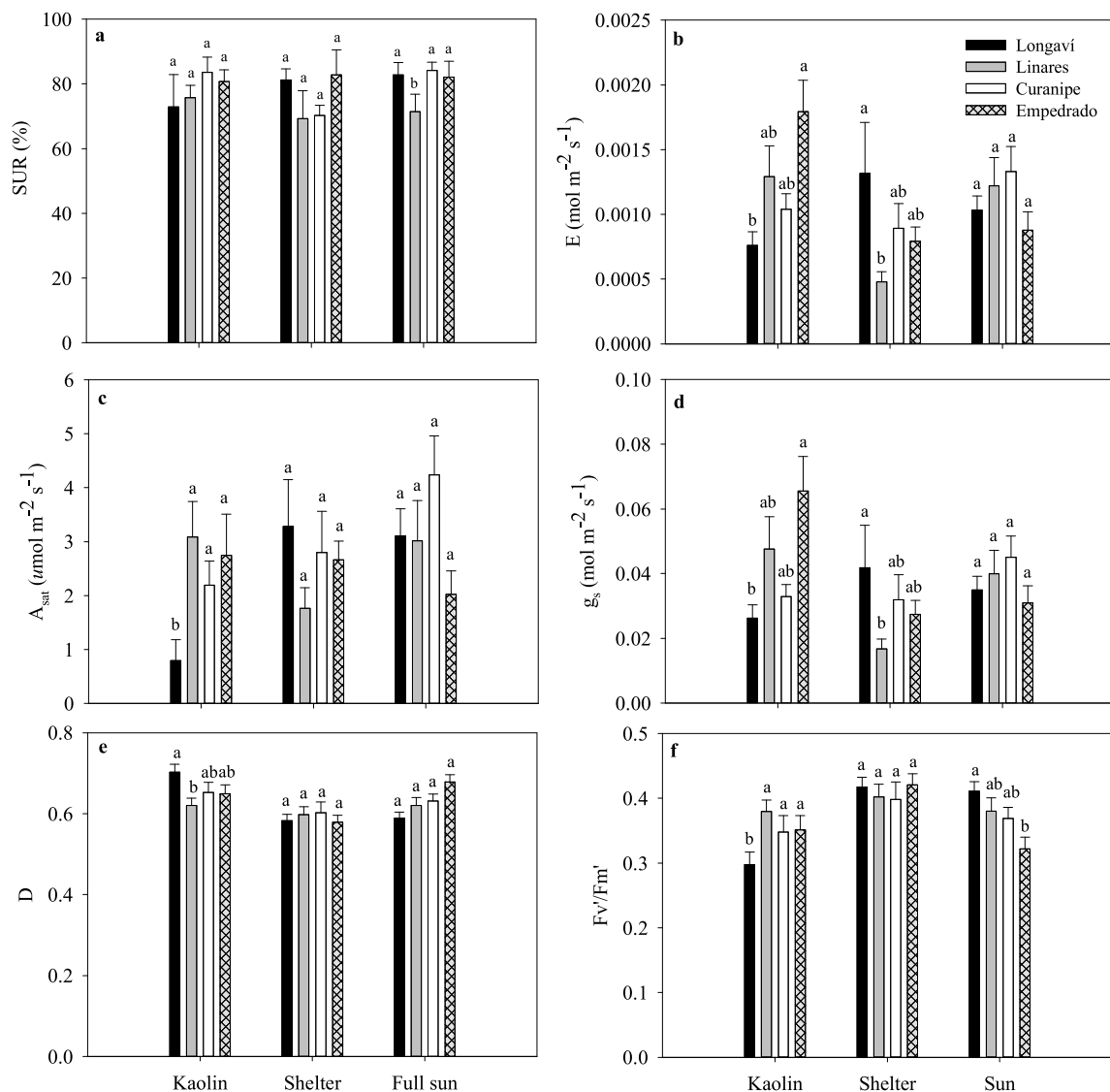


Fig. 2. Provenance by sun protection treatment interaction for outplanting survival (SUR), gas exchange parameters, F_v'/F_m' , and D in *N. glauca*. Lowercase letters indicate differences for provenances within a treatment ($p \leq 0.05$).

Table 2

Penetration resistance (PR), soil bulk density (BD), and porosity (POR) (mean $\pm$ standard deviation) for two soil preparation levels at different depths.

	PR (kPa)	BD (g cm ⁻³)	POR (%)
Treatment			
Old- subsoiling	1115 $\pm$ 66 b	1.22 $\pm$ 0.04 a	56.5 $\pm$ 2.8 a
No- subsoiling	2307 $\pm$ 86 a	1.12 $\pm$ 0.04 a	52.5 $\pm$ 2.1 b
Depth (cm)			
5	999 $\pm$ 70 D	–	–
15	1436 $\pm$ 97 C	–	–
30	1668 $\pm$ 118 BC	–	–
70	1915 $\pm$ 139 B	–	–
100	2538 $\pm$ 181 A	–	–
0–20	–	1.07 $\pm$ 0.03 B	58.2 $\pm$ 1.4 A
20–30	–	1.27 $\pm$ 0.04 A	50.5 $\pm$ 1.8 B
Significance level			
Treatment (T)	0.000	0.099	0.076
Depth (D)	0.000	0.002	0.012
T $\times$ D	0.121	0.401	0.382

Different lower and uppercase letters indicate differences between treatments and among depths, respectively ($p \leq 0.05$). Significant values are in bold type.

Table 3

Mean values $\pm$ SE for growth, increment, survival, leaf-level physiology, and Chlorophyll fluorescence in *N. glauca* seedlings established in Trial 2. Different lowercase letters indicate significant differences between site preparation treatments ($p < 0.05$).

	Site preparation treatment	
	Old- subsoiling	No- subsoiling
Morphological responses		
DAC (mm)	6.5 $\pm$ 0.37 a	5.6 $\pm$ 0.11 b
H (cm)	42.6 $\pm$ 2.41 a	41.3 $\pm$ 1.19 a
INC _{dac} (mm)	2.3 $\pm$ 0.43 a	1.4 $\pm$ 0.09 a
INC _h (cm)	6.0 $\pm$ 2.20 a	2.3 $\pm$ 0.71 a
SUR (%)	83.6 $\pm$ 3.4 a	79.1 $\pm$ 2.8 a
Leaf-level physiology		
E (mol m ⁻² s ⁻¹)	0.0012 $\pm$ 0.0001 a	0.0013 $\pm$ 0.0002 a
A _{sat} (μmol m ⁻² s ⁻¹)	2.45 $\pm$ 0.74 a	1.04 $\pm$ 0.47 a
C _i (μmol mol ⁻¹)	288 $\pm$ 19 b	345 $\pm$ 16 a
g _s (mol m ⁻² s ⁻¹)	0.034 $\pm$ 0.005 a	0.037 $\pm$ 0.006 a
WUE _i (A _{sat} /g _s)	58.5 $\pm$ 12.4 a	22.6 $\pm$ 10.1 b
Chlorophyll fluorescence		
D	0.68 $\pm$ 0.01 a	0.69 $\pm$ 0.01 a
φPSII	0.06 $\pm$ 0.00 a	0.05 $\pm$ 0.00 a
Fv'/Fm'	0.31 $\pm$ 0.01 a	0.30 $\pm$ 0.01 a
ETR	51.0 $\pm$ 6.6 a	42.8 $\pm$ 6.4 a
qP	0.21 $\pm$ 0.02 a	0.18 $\pm$ 0.02 a
qL	0.15 $\pm$ 0.01 a	0.14 $\pm$ 0.01 a

DAC = Root collar diameter at the ground line one year after establishment; H = Height; INC_{dac} = Increment in DAC; INC_h = increment in H; SUR = survival; E = Transpiration one year after establishment rate; A_{sat} = Assimilation rate; C_i = Interacellular CO₂; g_s = Stomatal conductance to water vapor; WUE_i = Intrinsic water use efficiency; D = fraction of light absorbed in the PSII antennae that is dissipated thermally; φPSII = PSII operating efficiency in light conditions; Fv'/Fm' = maximum efficiency of PSII in light conditions; ETR = electron transport rate through PSII; qP = photochemical quenching; qL = fraction of PSII centers that are 'open'.

typically regenerates in shady microsites. Seedlings sprayed with kaolin exhibited superior height, but increments in diameter and height were low and not statistically different. This low increments can be partially attributed to the low carbon fixation in all seedlings (i.e., A_{sat} from 2.2 to 3.2 μmol m⁻² s⁻¹, Table S3) which were very low compared to the A_{sat} performance in 4-year-old saplings of other *Nothofagus* species which can reach up to 18 μmol m⁻² s⁻¹ (van Gardingen, 1987; Varela et al., 2012). Maximum values for A_{sat}, E, C_i, and g_s were higher under kaolin, but; contrarily to our expectations, A_{sat} and WUE_i were lower. The reduction in A_{sat} caused by kaolin has been observed in apple and tomato (Le Grange et al., 2004; Cantore et al., 2009) and was attributed to reduction in the light available to the leaf for photosynthesis (Wünsche

et al., 2004; Rosati, 2007) or to heat stress (Glenn et al., 2003). Photo-synthetically active radiation (PAR) is reduced from 20 to 40% in plants with kaolin applications (Rosati, 2007). In our study, plants under kaolin had reduced WUE_i because of a decreased A_{sat} whereas g_s and C_i increased (Medrano et al., 2002), but also due to a decrease in non-stomatal limitations (i.e., Fv'/Fm'). We found a significant effect of provenance by sun protection treatment on E, A_{sat}, and g_s. Consistently, all provenances did not differ when established at full sun, but the opposite was found in the sun protection treatments. Within the kaolin treatment, the local provenance Empedrado (Coastal origin), exhibited higher E and g_s, whereas the provenance Longaví, from Pre Andean origin, exhibited the lowest A_{sat}. Under shelters however, both provenances from the Pre Andean origins exhibited the highest and lowest E and g_s.

Despite seedlings sprayed with kaolin increased the total electron flow through PSII (i.e., higher φPSII, ETR, qP, and qL), they have a decrease in Fv'/Fm' which could be associated to the lower A_{sat} under kaolin that promoted an imbalance between photochemical activity at PSII level and electron requirement for photosynthesis. Thus, it might be possible that the photoprotective capacity of *N. glauca* leaves sprayed with kaolin was exceeded and photoinhibitory damage in the PSII have occurred (Valladares and Pearcy, 1997). Dinis et al. (2018) found that kaolin treated grapevines increased Fv'/Fm' in the morning hours but no differences were reported for measurements at midday. Thus, in our experiment the decrease in Fv'/Fm' could have been completely recovered at the end of the afternoon. Diurnal course of dark-adapted leaves could provide some arguments to solve this question, but this is beyond of our study. Contrarily, sheltered seedlings showed increased Fv'/Fm' but a limited regulation for electron transport that was coupled with a reduction in the opening of reaction centres (reduced qL). It thus seems that kaolin treatment whereas increases height, it reduces photosynthesis and increases transient photoinhibition in young *N. glauca* seedlings. Small seedlings of *N. glauca* with heights lower than 40 cm (Table S2) are growing close to the soil surface and may not be well shielded from re-irradiated high soil surface temperatures. Hence, despite the fact that kaolin may be a cheaper alternative for the establishment of *N. glauca* than the use of shelters, its prescription at an operational scale does not seem appropriate at least at this stage of the plantation development.

Overall, survival was high and similar among sun protection treatments suggesting that water could have driven survival more than light. The available water content of c.a., 147 mm in the 70 cm of soil profile (average of 1.32 g cm⁻³ of bulk density $\times$ 700 mm of soil depth $\times$ 16% of available water), provided water and allowed *N. glauca* to sustain similar survival the first year after outplanting, minimizing the effects of sun protection. Tullus et al. (2010) pointed out that available water content over 150 mm in the first 75 cm of the soil profile is adequate to sustain height growth in 7-years-old aspen. Additionally, we registered a total of c.a., 919 mm of precipitation from winter 2019 to winter 2020 which might have been enough to sustain homogenous survival between site preparation treatments the first dry season in summer 2020.

4.2. How did coastal vs. Pre Andean seed sources perform after outplanting?

Except for survival and growth, we detected no differences in leaf-level physiology, and Chlorophyll fluorescence among provenances from the Coastal and Pre Andean origins. The local provenance Empedrado had superior growth whereas the Pre Andean provenances tended to grow less (Longaví) and survive less (Linares). This might be explained because Pre Andean provenances are adapted to sites with cold winters and high levels of precipitation (Table 1). As Pre Andean soils were originated from volcanic ashes, it is expected that the Pre Andean provenances might be affected in growth by the lower pH and Ca found at the common garden. The growth potential of a species is strongly influenced by the temperature and precipitation at seed origin

(Thomson et al., 2009; Callaham, 2013). In our study, the average annual temperature of both provenances and that of the planting site are similar but precipitations are lower at the planting site (Table 1) and might have influenced seedling growth and development. It is known that coastal origins of *N. glauca* are adapted to a thermal amplitude and precipitation of 8 °C and 800–1000 mm year⁻¹, whereas in the Pre Andean origins those values are 14 °C and 2000 mm year⁻¹, respectively (Amigo et al., 2000). Our preliminary analysis of phenological stages indicates that, one month after planting, seedlings from Pre Andean provenances were mainly in the stage of resting buds whereas the Coastal provenances had more swollen buds and some expanding leaves (Fig. 1). Thus, the slightly lower growth and survival of Pre Andean provenances might be explained by delayed budburst; which comes at the cost of sub-optimal acquisition of available resources in spring (Soliani and Aparicio, 2020), and the low precipitation in the common garden; compared to that of the site origin. However, this hypothesis needs further research.

In our study, there were negligible differences in all leaf-level physiological traits among the provenances. This lack of provenance differentiation could be explained by the narrow range of distribution of *N. glauca*, which spans over a range of just 400 km in central Chile, and the genetic similarity between the Coastal and Pre Andean origins (Vergara et al., 2014). These low differences in genetic configuration between sources could explain the low differences in adaptive capacity and physiological responses between provenances. If we consider WUE_i as a proxy for adaptation to the drought conditions of the planting site (Hatfeld and Dold, 2019), we can say that all the provenances responded similarly while maintaining a positive but similar and low increments in height and diameter.

4.3. Did the old subsoiling exert a positive effect on early performance of *N. glauca*?

Contrarily to our expectations, the old-subsoiling did not exert a significant effect on the early performance of *N. glauca* at the study site. Despite that penetration resistance was significantly lower in the treatment with old-subsoiling, mean survival for the two site preparation treatments varied from 79 to 83% suggesting that values of penetration resistance reported at the root zone presence (less than 70 cm of soil depth) are not restrictive for the root growth of *N. glauca* during the first year after planting. Although species such as *P. radiata* have a small number of roots that still penetrate soil with strengths up to 7000 kPa, root growth of many trees is limited at soil strengths greater than 3000 kPa (Sands et al., 1979; Greacen and Sands, 1980; Whalley et al., 1995; Ampoorter et al., 2011; Bécel et al., 2012). Olarieta et al. (2012) found that *Quercus ilex* age 10 years experiences a 40% decrease in growth at resistance values over 7000 kPa. In the case of seedlings, Alameda and Villar (2009), in a potted experiment, reported that resistance values over 600 kPa reduces root growth of 17 woody species. In our study, the higher values close to 2500 kPa were only found at depths close to 70 cm in the no-subsoiled treatment. Before planting, average root length in *N. glauca* seedlings was less than 10 cm (data not shown). We thus assume that *N. glauca* had no roots yet at 70 cm depth and the majority of roots are concentrated in upper soil layers. Palfner et al. (2008) reported a mean root length of 20 cm in 2-year-old *Nothofagus alpina* seedlings growing in a volcanic soil and that at 25–45 cm of depth, roots were almost completely absent. Our results suggest that the positive effects of subsoiling on soil properties is short-lived (Hamza and Anderson, 2005); therefore, it will need to be practiced at regular intervals. McBeath et al. (2010) found that the effects of site preparation on soil properties was minimal after 4 growing seasons.

5. Conclusions

This study investigated the variations in growth, survival, and leaf-level physiology of Coastal and Pre Andean provenances of *N. glauca*

growing in a common-garden in a coastal site. In general, differences in seedling performance in all traits under study were not large among provenances, suggesting good adaptation of the species to different site conditions. As *N. glauca* has a narrow geographic distribution, the introduction of provenances from colder Pre Andean origins; with appropriate sun protection, to more temperate Coastal origins, might be one potential adaptation tool to dampen the adverse effects of future climatic environments. We found no substantial evidence that kaolin and old-subsoiling significantly improves *N. glauca* establishment at least at this stage of the plantation development. However, given the relatively small number of provenances in our trial, this hypothesis needs further research and the consideration of long-term data with a greater number of Coastal and Pre Andean provenances, and the analysis of soil variability at the experimental site.

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.indic.2022.100192>.

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