

RESEARCH ARTICLE

Shrub influences on seedling performance when restoring the slow-growing conifer *Pilgerodendron uviferum* in southern bog forests

Jan R. Bannister^{1,2} , Germán Travieso¹, Nicole Galindo¹, Manuel Acevedo³, Klaus Puettmann⁴, Christian Salas-Eljatib^{5,6}

Forest restoration is most efficient if it can take advantage of facilitative interactions between established vegetation and planted trees. However, positive and negative interactions have been identified in a number of plant communities. After centuries of anthropogenic fires, forest recovery has been extremely slow in southern bog forests previously dominated by the slow-growing and vulnerable conifer *Pilgerodendron uviferum* on Chiloé Island, Chile. Today, the landscape is dominated by secondary shrublands with scattered patches of *Sphagnum* moss and limited natural tree regeneration. We hypothesized that the retention of secondary shrubs facilitates the early performance of *P. uviferum* restoration plantings by providing better microsite conditions. To test this hypothesis, we compared the response of seedlings planted on sites prepared at two levels of intervention: after shrubs had been removed or where shrubs were retained. Shrub retention showed a nurse-plant effect on *P. uviferum* seedlings 4 years after planting, which resulted in reduced physiological stress (measured as Fv/Fm) for seedlings, as well as reduced browsing. Consequently, the seedlings growing in areas with shrub retention had larger height increment and higher vitality than those in areas where shrubs had been removed. Thus, the more open micro-site conditions created by shrub removal resulted in generally poorer seedling performance, although seedling mortality—which was low overall (approximately 2–4%)—showed no significant difference between the two levels of intervention. These findings have direct implications for the restoration of slow-growing conifers that can tolerate extreme wet conditions in highly degraded forests.

Key words: forest restoration, light availability, nurse-plant effect, plant physiology, seedling growth, waterlogged soils

Implications for Practice

- To improve tree seedling performance, initiatives to restore bog forests by establishing slow-growing conifers should include tree seedling planting between retained shrubs on natural mounds or small elevated areas in the landscape as a way to avoid seedling damage in waterlogged conditions.
- In *Sphagnum* bogs, safe sites for germination are not necessarily safe sites for tree seedling growth and survival. By facilitating height growth, however, seedlings have a better chance to outgrow *Sphagnum*.
- Field experiments along the entire distribution of *Pilgerodendron uviferum* are needed to clarify how restoration efforts with slow-growing conifers are best applied in the context of climate change, with warmer and drier summers.

benign microenvironments (Avendaño-Yáñez et al. 2014). In the case of ecological restoration, many studies have shown the benefits of facilitation by using shrubs as nurse plants for reforestation in Mediterranean ecosystems of Europe (Castro et al. 2002, 2004; Gómez-Aparicio et al. 2004; Gavinet et al. 2016); in tropical Africa (Duncan & Chapman 2003; Aerts et al. 2007); and tropical North and South America (Sánchez-Velásquez et al. 2004; Galindo et al. 2017). In these stressful environments, the facilitative interaction between established vegetation and planted tree species appears to override other negative interactions (Callaway & Walker 1997; Bustamante-Sanchez et al.

Author contributions: JRB designed the study and wrote the manuscript; JRB, GT, NG, CS-E analyzed data, performed research, and contributed with the creation of figures; MA, KP reviewed the manuscript and contributed to data interpretation.

¹Oficina Chiloé, Instituto Forestal, Castro, Chile

²Address correspondence to Jan R. Bannister, email jrbannister@infor.cl

³Sede Bio Bio, Instituto Forestal, Concepción, Chile

⁴Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331, U.S.A.

⁵Centro de Modelación y Monitoreo de Ecosistemas, Universidad Mayor, Santiago, Chile

⁶Laboratorio de Biometría, Universidad de La Frontera, Temuco, Chile

Introduction

The role of facilitation in plant communities has been well recognized (Brooker et al. 2008), especially in severe environments where the establishment of many species is restricted to a few

2011). In difficult growing conditions, shrubs appear to provide protection to planted seedlings by regulating microclimatic extremes, reducing solar radiation and soil temperature, conserving soil moisture, and/or enriching nutrient content (Castro et al. 2004; Padilla & Pugnaire 2006). Furthermore, in some cases shrubs may interfere with the vigorous growth of other competitive vegetation, e.g. grasses (Galindo et al. 2017), and provide protection against predation and browsing (Castro et al. 2004; Padilla & Pugnaire 2006; Aerts et al. 2007). However, complex combinations of negative and positive interactions have been identified in a number of plant communities. Several processes, including facilitation and competition, can occur simultaneously, and the specific outcome may vary due to a variety of factors, including different life stages, sizes, and densities of the interacting species (Callaway & Walker 1997). Several studies that explored shrubs as nurse plants for the restoration of forests in more mesic environments, such as temperate forest ecosystems, show that dense or tall shrubs reduce height and diameter growth of the planted seedlings (Blanco-García et al. 2011; Bustamante-Sanchez et al. 2011; Svriz et al. 2013), suggesting a dominance of competitive processes (Castro et al. 2004). Consequently, the dominance of the nurse-plant effect likely varies with the amount and traits of the species involved. For example, the nurse-tree effect is predicted to be more beneficial for tree species that can tolerate shade than for pioneer species (Blanco-García et al. 2011).

After centuries of deforestation by anthropogenic fires and logging (Holz & Veblen 2011; Bannister et al. 2014), Chiloé Island (North Patagonia, Chile) has seen dramatic changes from landscapes dominated by old-growth North Patagonian evergreen forests to extensive areas of land dominated by shrubs (Bannister & Donoso 2013; Bannister et al. 2017). Due to the poor drainage of the post-glacial soils in which North Patagonian forests develop, the removal and subsequent absence of trees has had dramatic and long-lasting effects on the water balance of these areas (Díaz et al. 2007). These hydrologic changes have led to waterlogging and associated arrested succession (*sensu* Putz & Canham 1992), with an alternative state characterized by scattered patches of *Sphagnum* moss, secondary *Bacharis* sp. shrublands, and limited or absent tree regeneration (Díaz et al. 2007; Bustamante-Sanchez et al. 2011). Prior to fire, these bog forests were mostly dominated by the slow-growing, long-lived, and vulnerable conifer *Pilgerodendron uviferum* in Chiloé Island. Thanks to its longevity (>880 years), extremely slow growth (<1 mm diameter per year), and tolerance to shade and physiological stress, this conifer has been shown to persist during millennia in extreme waterlogged sites in undisturbed conditions (Bannister et al. 2012a). However, in addition to the extreme growing conditions, limited seed sources after extensive fires resulted in areas in which only minor portions of stands were stocked with natural regeneration of *P. uviferum* after 80 years (e.g. 3% in Bannister et al. 2014). In this context, there is interest in a restoration approach that overcomes the seed limitations by planting seedlings and that also accounts for the impact of the existing shrub layer (Bannister 2015).

The chance for seedlings and saplings to dominate or persist in a stand is often determined by their performance in young stages (Bannister et al. 2012a; Soto et al. 2015). Several studies have highlighted the importance of monitoring the early performance of native species planted under different restoration techniques in degraded or burned forests (e.g. Bannister et al. 2013; Donoso et al. 2013; Soto et al. 2014, 2015). Many environmental variables can influence growth and survival in the early phases of tree development. For example, on Chiloé Island, microtopography in bog areas and the nurse canopy in upland areas have been found to influence the early performance of *P. uviferum* planted seedlings (Bannister et al. 2013).

In this study, we assess how growing conditions, as influenced by two different intervention levels of restoration (shrub removal or shrub retention), affect the performance of planted seedlings of *P. uviferum*. Specifically, we wanted to study the influences of these intervention levels on environmental conditions (e.g. light, water table) as they influence seedling growth and physiological stress. In this context, we hypothesized that in these burned bog forests, the retention of *Bacharis* sp. and *Gleichenia* sp. shrubs facilitated the early performance of *P. uviferum* restoration plantings (in terms of height and diameter growth and physiological stress) by promoting better microsite conditions. In contrast, we also hypothesized that the removal of the shrub layer created harsher microsite conditions with negative effects on seedlings' early performance. Our overall goal was to provide guidance for large-scale forest restoration efforts with slow-growing conifers in highly degraded forests, not only on Chiloé Island, but elsewhere under similar conditions, especially within the context of climate change.

Methods

Study Area

The study area was located near Lake Chaiguata (Tantauco Park, approximately 43°10'S and 74°05'W) in the south of Chiloé Island, North Patagonia (Fig. 1). This region contains extensive disturbed areas of previously *Pilgerodendron uviferum*-dominated bog forests, most of which had burned in an extensive fire in 1942–1943 (Holz & Veblen 2011). Southern Chiloé is in the south temperate biogeographic region of Chile (Bannister et al. 2012b). It has a cool-temperate climate with a strong oceanic influence, a mean annual temperature of 10°C, and high annual precipitation that can reach up to 6,000 mm (Di Castri & Hajek 1976; Pérez et al. 2008). The study area has been shaped by the last glaciation (approximately 13,000 years BP), which created a mix of hills (glacial till deposits) and shallow valleys, over a basis of Pre-Cambrian and Tertiary Metamorphic rock, with extremely acidic, poorly drained soils with Gley horizons (Villagrán 1988). Altitudes range between 150 and 280 meters above sea level.

The study area was mostly covered by *Sphagnum* bogs, cushion plants, and dense secondary shrublands dominated by *Bacharis* and *Gleichenia* species, with standing fire-killed trees of *P. uviferum* and *Tepualia stipularis*. In areas with better

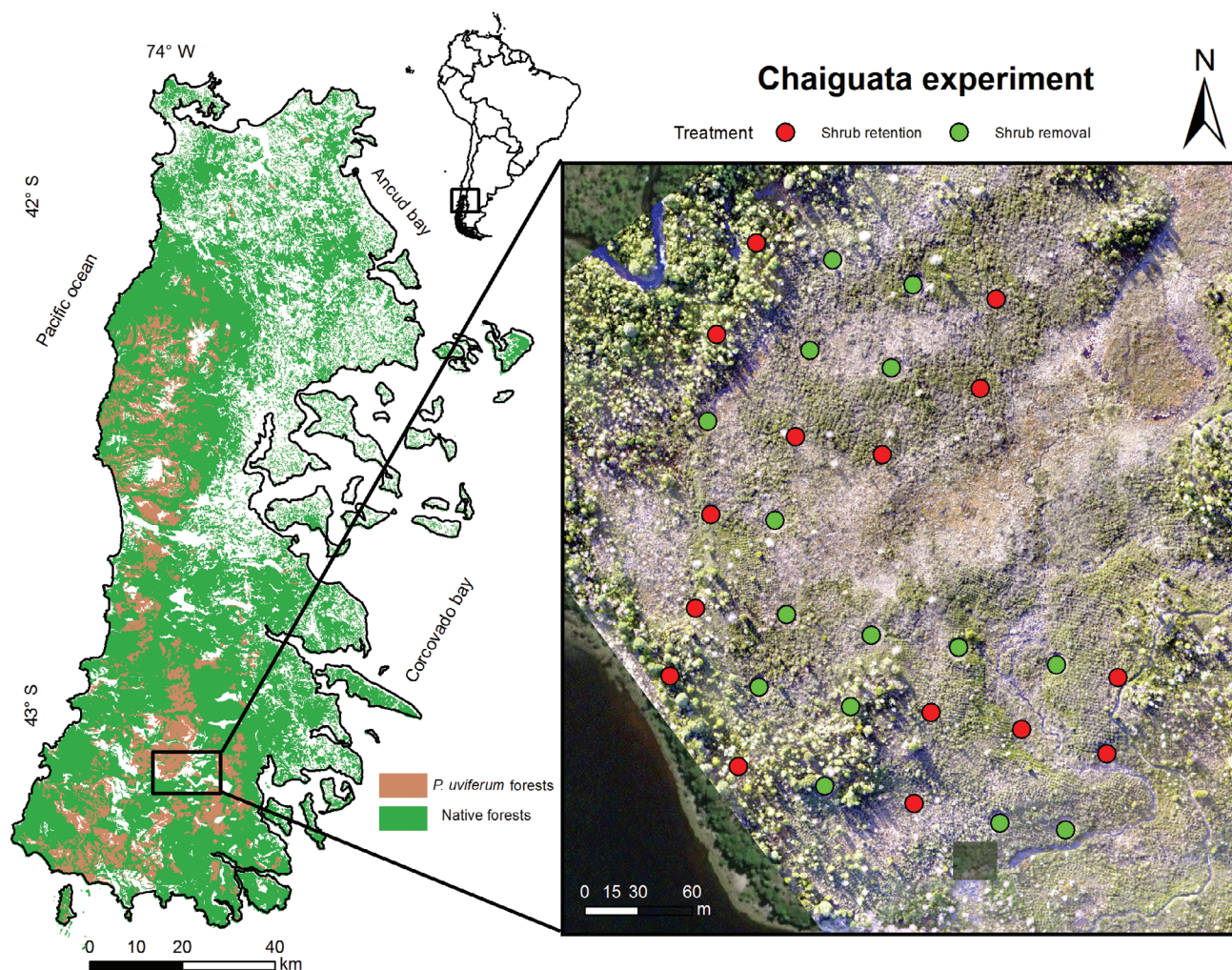


Figure 1. Study area in Chiloé Island, North Patagonia (left), and location of the 30 experimental units near Lake Chaiguata (right).

drainage (slightly higher elevation), rare natural regeneration of broadleaf species like *T. stipularis*, *Nothofagus nitida*, *Drimys winteri*, and *Weinmannia trichosperma* (Fig. 2A & 2B) was also present.

Experiment Design

In 2014, we established an experiment near Lake Chaiguata in an area that was burned in an extensive anthropogenic fire between 1942 and 1944 (Holz 2010) and where *P. uviferum* bog forests were dominant before the fire. We established 30 experimental plots (113 m²), with 41 *P. uviferum* seedlings planted in a regular grid in each plot. To assess the effects of shrub removal on seedlings, we randomly assigned one of two levels of intervention (i.e. either shrub removal or shrub retention) to the plots, with 15 plots containing each intervention level. The shrub removal intervention completely removed the shrub layer (dominated by *Bacharis* sp. and *Gleichenia* sp.) before seedlings were planted (Fig. 2C). The shrub retention intervention retained all shrubs, with the exception of a 50-cm

radius circle (Fig. 2D), which was needed to allow tree planting. The experiment was established in a nature conservation area where cattle are excluded; therefore, no protection from cattle was needed.

The seed for the 1,230 *P. uviferum* seedlings used in this study had been collected previously in the study area, and the seedlings were then grown in a local nursery for 4 years. During the first year, plants were grown as container seedlings inside a greenhouse; seedlings were then grown in a nursery bed for 3 years. The 4-year-old bare-root seedlings, with a mean height of 22.5 cm, were then planted in the experimental plots during the winter (June–July) of 2014.

Data Collection

We measured the following variables for all of the planted *P. uviferum* seedlings every winter after planting: root collar diameter (mm), total height (cm), and estimated vitality (1: <10%, 2: 10–25%, 3: 26–50%, 4: >50% of dead foliage, sensu García-Garduño 2016). We computed the periodic annual

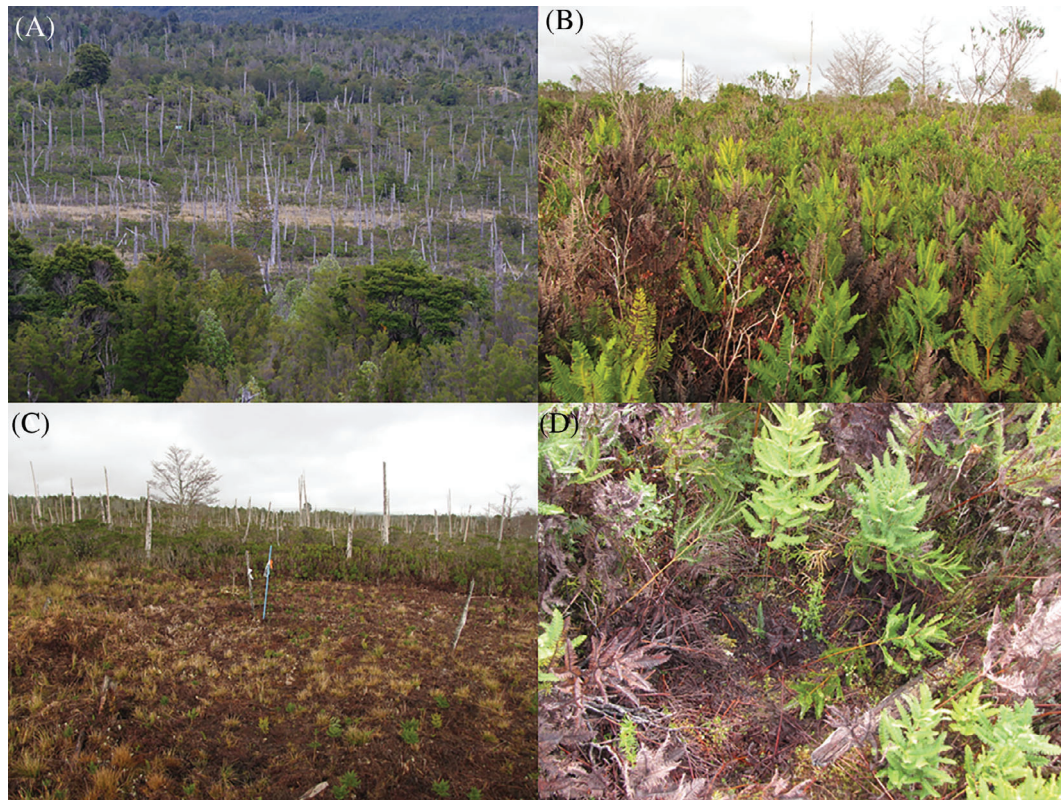


Figure 2. (A) Landscape previously dominated by forests of *Pilgerodendron uviferum* on Chiloé Island that had burned previously, in 1942–1943; (B) secondary native shrublands of *Bacharis* sp. and *Gleichenia* sp. that have grown since the fires and hamper tree seedling planting; and (C) *P. uviferum* seedlings planted under two different intervention levels: shrub removal (C) and shrub retention (D).

increment (pai) for root collar diameter (pai_d) and height (pai_h) of each seedling for growth analysis. Neither browsed seedlings (most likely by *Pudu puda*, a native small deer) nor dead seedlings were included in the growth analysis. Mortality, survival, and incidence of browsing were also recorded and averaged per plot. Light availability for seedlings was determined based on the one-point overcast sky condition method (sensu Parent & Messier 1996). This method considers the percentage of photosynthetic photon flux density (PPFD: $\mu\text{mol m}^{-2} \text{second}^{-1}$) reaching each seedling in relation to a simultaneous PPFD measured in an open area (%PPFD). This was carried out with a LICOR 1400 Data logger and LI250 point quantum sensors.

The potential photochemical quantum yield of photosystem II (Fv/Fm) was measured for a randomly chosen subsample of five living seedlings per plot. Every 2 months for 1 year (April 2017–April 2018), the same five seedlings were measured using an OS30p + chlorophyll fluorometer. Decreases in Fv/Fm values indicate the occurrence of photoinhibitory damage in response to environmental conditions, and this damage will often be interpreted as the first manifestation of physiological stress (Maxwell & Johnson 2000). Additionally, needles were collected from the top of at least 10 living seedlings in plots with the different intervention levels for nutrient analysis in winter 2018 (one composite sample per plot).

We also measured the water table level every 2 months for 1 year (April 2017–April 2018) with a piezometer at the center of each plot. During the same year, air relative humidity and temperature, and soil moisture, were measured in two randomly selected plots (one for each intervention level) with Em50 Decagon data loggers to provide more detailed information about the study area. The soil measurements were taken every hour for 1 year and were calibrated by gravimetric analyses. Finally, using a soil auger, the soil depth and pH were measured at the center of each plot. Furthermore, soil samples were extracted from each plot (at 10 cm depth) for nutrient analysis using small test pits. The nutrient levels of the soil samples were measured in the Laboratory of Forest Nutrition and Soils at the Universidad Austral de Chile in Valdivia, following the methods of Sadzawka et al. (2006).

Statistical Analyses

We studied the effects of the two intervention levels (shrub removal and shrub retention) on plot-level response variables. These response variables represented soil and leaf properties (i.e. nutrient concentrations of needles and soil), micro-environment features (i.e. air temperature and relative humidity, PPFD, mean depth of the water table, minimum depth of the water table), and plant performance (i.e. mortality and browsing). We fitted linear regression models for each of

the response variables, using intervention levels as predictor variables (i.e. included in the model as dummy variables). The statistical significance of the difference in response to the intervention levels was based on the variance estimator of its regression coefficient. The normality and homoscedasticity of the model residuals were assessed by the Kolmogorov-Smirnov and Levene tests, respectively, as suggested by Salas-Eljatib (2002). If a model did not fulfill these assumptions (in our case: soil water content, PPFD%, mean depth of the water table, minimum depth of the water table, phosphorus concentration), we computed the nonparametric Wilcoxon test instead.

To assess the growth variables at the individual seedling level, we fitted mixed-effects models for each response variable (i.e. pai_d or pai_h) using initial size (i.e. diameter or height) and light exposure (using PPFD as a proxy) as predictor variables and a dummy variable to represent the intervention levels. These models were fitted using maximum likelihood in a mixed-effects modeling framework that accounted for the nested structure of the data (i.e. seedlings are nested within plots, which are nested within intervention levels). As pointed out by Salas-Eljatib et al. (2008), these statistical models are suitable for representing the hierarchical structure of the data and allowing appropriate statistical inferences by avoiding the pseudoreplication problem (Huber 1981). These analyses were carried out with the “car” (Fox & Weisberg 2019) and the “nlme” (Pinheiro & Bates 2000) packages, both implemented in R (R Development Core Team 2018).

Finally, to compare the differences between the two intervention levels in the water table and the Fv/Fm values of seedlings throughout the year (April 2017–April 2018), we performed variance analyses of repeated measurements, fitted using the method of maximum likelihood, to model variance and covariance structure with a 95% level of confidence (Oliver et al. 2000). These analyses were carried out using the PROC MIXED procedure (SAS Institute, Inc., Cary, NC, U.S.A.).

Results

Microhabitat Conditions of the Study Site

The weather during the year (April 2017–April 2018) in which we took soil moisture and seedling stress measurements was characterized by temperate-wet site conditions, with an annual mean temperature of 9.3°C (± 3.22 SD), a mean maximum temperature of 21.8°C (± 6.87 SD), and a mean minimum temperature of -3.0°C (± 2.23 SD). The mean air relative humidity was 89.7% (± 7.10 SD) and the mean soil water content was 158.6% (± 2.45 SD). According to the data collected from the nearest official weather station (Butalcura station, Agrometeorological Network, INIA, Chile, approximately 96 km from the study site), the annual precipitation between May 2017 and April 2018 was 2,391 mm. Soil water content was constant during the year (154–161%) and air relative humidity ranged from approximately 81% in the summer (November–December) to approximately 97–100% in the winter (May–August). The mean monthly photosynthetic active radiation (PAR)

during the day ranged from 179.0 ($\mu\text{mol m}^{-2} \text{second}^{-1}$) in June to 739.5 ($\mu\text{mol m}^{-2} \text{second}^{-1}$) in January. The monthly mean, minimal, and maximal temperatures are presented in Figure 3.

Influence of Intervention Levels on Growing Conditions and Seedling Performance

The retention or removal of shrubs did not have a significant effect on soil-related variables (Table 1). In terms of the micro-environment, the intervention levels tested in the study only had a significant effect on the percentage of PPFD available to seedlings, with significantly more light available for seedlings planted after shrub removal ($p \leq 0.01$).

After four growing seasons, the periodic annual increment in root collar diameter (pai_d) and height (pai_h) of each seedling was significantly affected by the three predictor variables: intervention level (pai_d and pai_h , $p < 0.001$), light exposure (pai_d : $p < 0.05$ and pai_h : $p < 0.01$), and initial size (pai_d and pai_h , $p < 0.05$). *Pilgerodendron uviferum* seedlings presented wide ranges in pai_d for both intervention levels (shrub removal: 0–3.95 mm/year; shrub retention: 0–2.25 mm/year). However, pai_d was significantly higher for seedlings established in the shrub removal intervention level ($p \leq 0.01$). In contrast, pai_h was significantly higher ($p \leq 0.01$) for seedlings in the areas where shrub cover was retained (0–12.75 cm/year) than for seedlings in areas with shrub removal (0–5.75 cm/year). Despite the slow growth rates, seedling mortality was surprisingly low (approximately 2–4%) and showed no significant difference between the two intervention levels. However, browsing was significantly higher ($p \leq 0.01$) for seedlings established after shrub removal, compared with seedlings in the area where shrubs had been retained, at 82.3 vs. 53.1%, respectively (Fig. 4).

Overall, all seedlings had fairly low N and P foliar concentrations (Fig. 5). However, seedlings established in open conditions after shrub removal had significantly higher N and P foliar concentrations and N:P ratios than seedlings growing in areas where shrubs were retained (N: $p \leq 0.01$; P and N:P: $p \leq 0.05$).

Impact of Selected Micro-Environmental Conditions on Seedling Performance

Overall, across all plots, there was a positive relationship between light (measured as %PPFD) and pai_d of seedlings, but there was a negative relationship with pai_h (Fig. 6). Furthermore, lower %PPFD was related to a higher percentage of healthy seedlings (with <10% of dead foliage) and higher Fv/Fm values.

The water table on the study site and the Fv/Fm values varied through the year for both intervention levels (the interaction between intervention level and month: $p < 0.05$ for water table, and $p < 0.01$ for Fv/Fm). The precipitation during the sampled period (April 2017–April 2018) was concentrated in winter, with a peak in August (510.8 mm). However, the water table began to rise significantly in June, peaked in September, and then slowly decreased. The decreasing trend was even stronger starting in February. Water table was always higher in plots

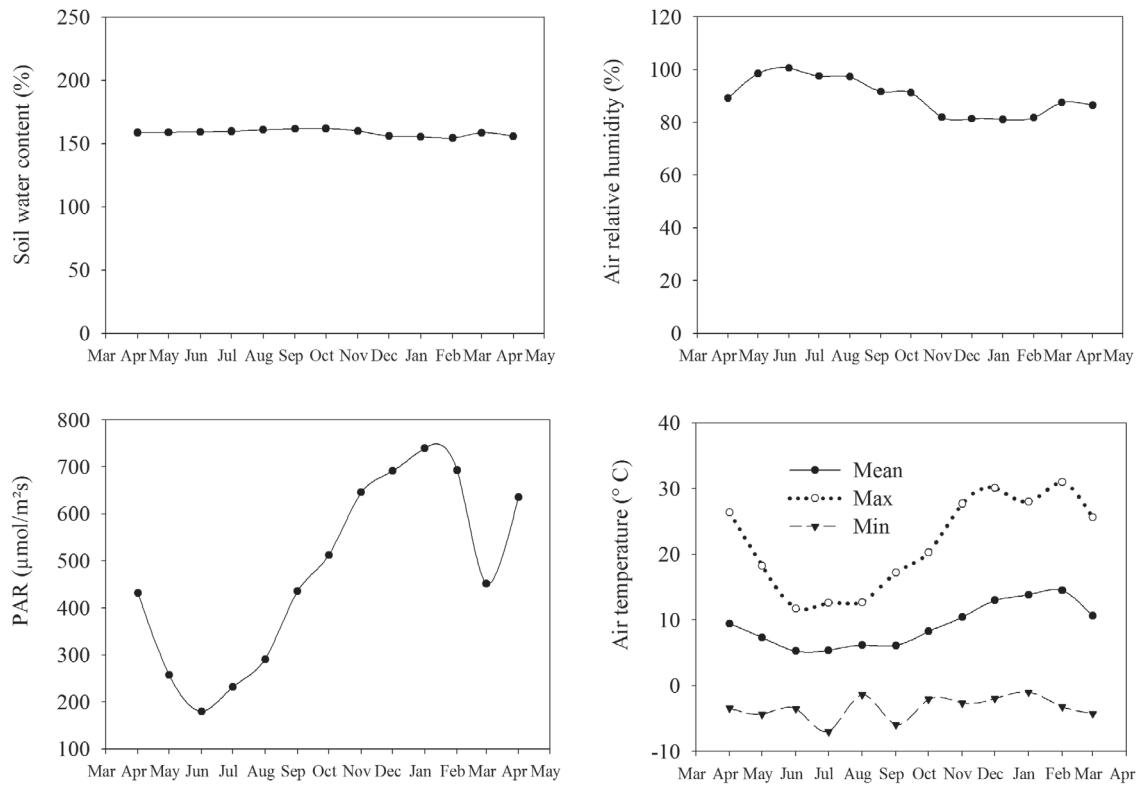


Figure 3. Variation of micro-environmental variables through the year 2017/2018 on the study site. Data were captured and averaged from two data loggers located in experimental units with different intervention levels.

Table 1. Descriptive statistics of soil-related and micro-environment-related variables by intervention levels. Values in brackets represent SDs. Different letters show significant differences among intervention levels ($p \leq 0.01$). CEC, cation exchange capacity; OM, organic matter; PPFD, photosynthetic photon flux density.

Variables	Intervention Level	
	Shrub Removal	Shrub Retention
Soil-related		
Depth (cm)	24.3 (± 10.02)	28.87 (± 17.40)
pH	4.46 (± 0.18)	4.54 (± 0.17)
OM (%)	29.4 (± 14.75)	22.8 (± 4.66)
Ct (%)	17.16 (± 8.43)	13.1 (± 2.72)
Nt (%)	0.59 (± 0.38)	0.352 (± 0.13)
P (mg/kg)	6.88 (± 1.86)	8.24 (± 3.31)
C/N	31.2 (± 4.09)	38.8 (± 7.01)
CEC (cmol+/kg)	6.96 (± 2.43)	6.052 (± 0.97)
Micro-environment-related		
PPFD (%)	82.87 (± 13.35) ^A	44.87 (± 11.90) ^B
Annual depth of water table (cm)	9.66 (± 9.22)	13.49 (± 7.16)
Minimum depth of water table (cm)	5.62 (± 7.59)	4.25 (± 6.38)

where shrubs were removed, but differences were not significant (Fig. 7). At the same time, physiological stress of seedlings also varied in similar fashion in both intervention levels, with

significant decreases of Fv/Fm values during August–October and February–April (>physiological stress) and significant increases during October–February (<physiological stress). However, Fv/Fm values were always higher in seedlings growing in areas where shrubs had been retained, especially in the middle of the winter (June–August) and summer (February). However, differences between intervention levels for Fv/Fm values in seedlings were only significant in August 2017, February 2018, and April 2018 (Fig. 7).

Discussion

Influence of Intervention Levels on Growing Conditions and Seedling Performance

As found for Mediterranean and tropical ecosystems around the world (e.g. Castro et al. 2004; Gómez-Aparicio et al. 2004; Aerts et al. 2007; Galindo et al. 2017), our results highlight the benefits of facilitation by using shrubs as nurse plants in restoration efforts in temperate ecosystems characterized by extremely wet conditions (waterlogged, disturbed bog forests). In our study, the retention of secondary *Bacharis* sp. and *Glechonia* sp. shrubs had a nurse-plant effect on *P. uviferum* seedlings by providing partial shade, for example, which may protect seedlings from drying out and therefore reduce physiological stress. In addition, the shrubs protected seedlings from browsing, which led to increased height growth and

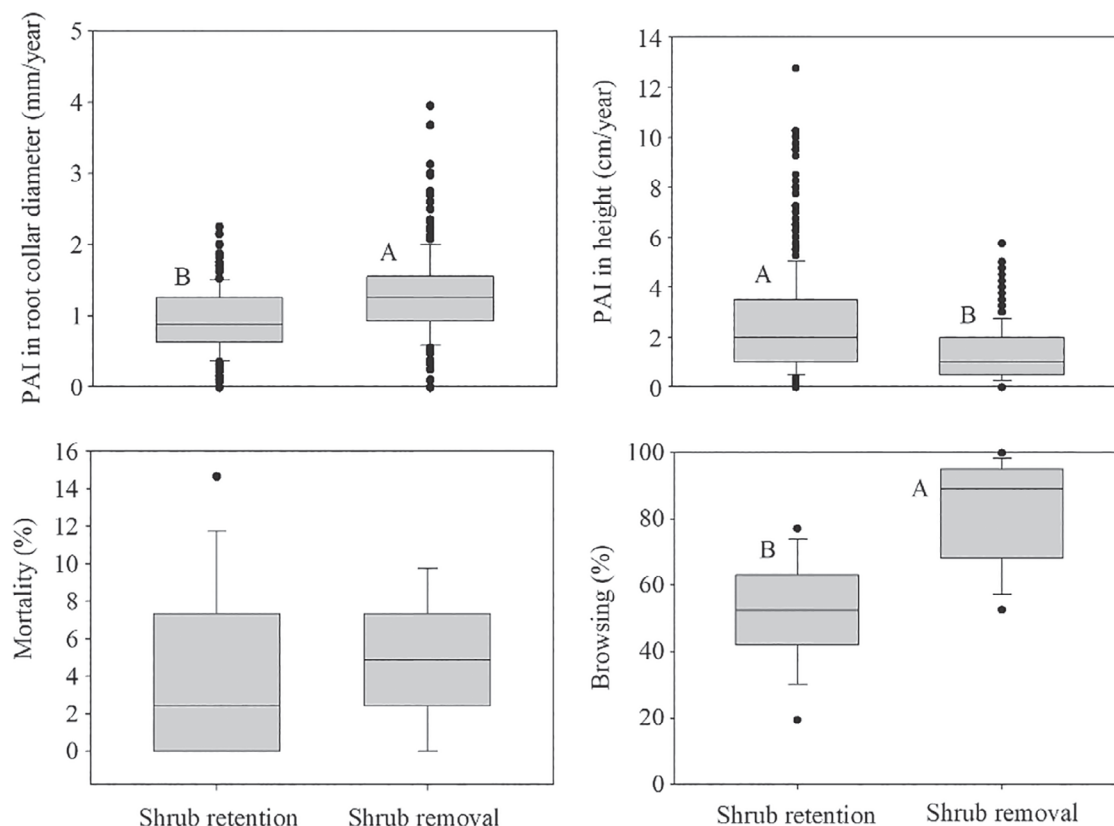


Figure 4. Periodic annual increment (*pai*) in root collar diameter, height, mortality rate, and browsing percentage of *Pilgerodendron uviferum* seedlings established under two intervention levels (i.e. shrub retention and shrub removal). Different letters show statistically significant differences among intervention levels ($p \leq 0.01$).

vitality. Our results were consistent with beneficial shrub effects found in other ecoregions with waterlogged conditions, like those reported for *Pinus sylvestris* seedlings in boreal peatlands of Finland, especially in elevated microsites with deeper water tables (Holmgren et al. 2015). However, the impact of shrubs on seedling growth through soil water changes can also be negative, especially when tree species have shallow and slow-growing root systems, and the dominant factor influencing the performance of planted seedlings is light, which is the case of *Picea mariana* in moist sites of Canada (Lamhamedi & Bernier 1994). In this context, whether the shrub effect is positive or negative is ultimately species dependent (Blanco-García et al. 2011).

In contrast, shrub removal created more open microsite conditions, which had negative effects on seedlings' general performance. The removal of shrubs did not have a short-term effect on soil nutrients, likely due to the slow decomposition rates in these ecosystems (Péli et al. 2016). However, shrub removal instantly changed other growing conditions, principally by creating a higher light environment. In our study *P. uviferum* showed low mortality rates in both intervention levels (2–4%) and a consistent, but nonsignificant trend that mortality was lower in areas where shrubs were retained. As highlighted in several studies of the interaction between competition and herbivory, short-term mortality rates were less influenced

by growing conditions (e.g. Saunders & Puettmann 1999a). However, this trend may be reversed over time. Nonetheless, the low mortality rates reported here confirmed the persistence and capacity of *P. uviferum* for restoration efforts in areas with harsh site conditions, as was previously shown on undisturbed sites by Bannister et al. (2012a). Furthermore, by removing the shrub layer, we reduced hiding cover and created opportunities for Pudu deer (*Pudu puda*), which prefer open conditions for browsing (Burger et al. 2019). This supports findings from studies in other forest ecosystems about the browsing protection conferred by nurse shrubs, which benefits planted seedlings (Saunders & Puettmann 1999b; Padilla & Pugnaire 2006; Aerts et al. 2007).

The findings that seedlings established in open conditions had higher root collar diameter growth, but slower height growth was surprising. This apparently contradictory result may be related to the impact of shrub removal on other vegetation. For example, increased shrub cover was related to decreased bryophyte cover in boggy conditions in the Western Eurasian tundra (Pajunen et al. 2011). Thus, open conditions with more light may have resulted in increased growth of *Sphagnum* on our study site. Such growth can facilitate the formation of adventitious roots of seedlings (Lamhamedi & Bernier 1994; DesRochers & Gagnon 1997) with a subsequent deformation of the root collars (DesRochers & Gagnon 1997). Such

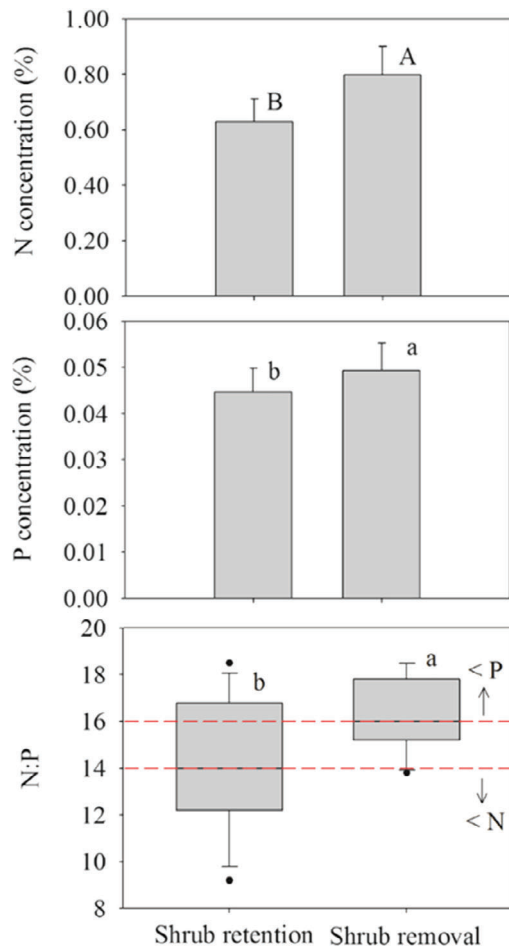


Figure 5. Foliar N and P concentrations and N/P ratios in seedlings of *Pilgerodendron uviferum* under two intervention levels. Dashed lines and arrows indicate P and N limitation (Koerselman & Meuleman 1996). Different letters show significant differences among intervention levels (lower case letters: $p \leq 0.05$; capital letters: $p \leq 0.01$).

deformations at the root collar could explain, at least partially, the higher root collar diameter of seedlings established after shrub removal in our experiment. Also, in upland conditions, Bannister et al. (2013) found that 2 years after planting, *Pilgerodendron uviferum* seedlings established in gaps had higher maximum rates of CO_2 assimilation, suggesting photosynthetic acclimation to high irradiance environments. However, these seedlings did not have higher growth rates (shoot and basal area growth). Therefore, it was assumed that the higher C costs of seedlings adapted to full light may not pay off in the environment of North Patagonia, where sunny conditions prevail only for a short time in summer. Consequently, nutrient availability was hypothesized to be the main factor limiting growth (Bannister et al. 2013). In this study, we did not find a significant relationship between intervention levels and soil nutrition in the short term (in the 4 years since planting), even though seedlings established in areas with shrub removal had higher N and P foliar concentrations. It is noteworthy that foliar nutrient concentrations, especially P, were at relatively low levels

(sensu Koerselman & Meuleman 1996), which also suggests that, in the waterlogged conditions, nutrient availability rather than light availability may be the most important factor limiting growth.

Impact of Selected Micro-Environmental Variables on Seedling Performance

Almost 80 years after the fire that killed the overstory trees, shrub cover was not homogeneous in the study area. Thus, the intervention levels applied in this study resulted in a wide range of light conditions. While light varied between the two intervention levels and even within intervention levels, increased light led to lower height growth. The proportion of healthy seedlings was also lower and the physiological stress of seedlings was higher in areas with more light. Only plots with mean %PPFD lower than 40% had seedlings that were not in the physiological stress zone (sensu Maxwell & Johnson 2000). In agreement with our hypothesis, and previous studies in other areas of the world (Castro et al. 2004; Padilla & Pugnaire 2006; Aerts et al. 2007; Galindo et al. 2017), our results suggest that leaving shrubs provides better micro-environmental conditions for seedling growth.

However, the physiological stress of seedlings was not uniform during the year. We found two clear temporal patterns. First, physiological stress was lower in seedlings with shrub retention, but the nurse effect of shrubs was more effective in the rainiest and warmest months (i.e. August and February). We hypothesize that this is due to the protection that shrubs provide by regulating solar radiation and air and soil temperature (Castro et al. 2004; Padilla & Pugnaire 2006; Holmgren et al. 2015), but unfortunately this cannot be tested with our data. Second, in general the highest physiological stress was not in the driest and wettest months but in late summer and late winter, suggesting that during this time, the nurse-plant effect of shrubs was not as effective. This pattern could be partially explained by the variations in PAR, temperature, and water table (Castro et al. 2004; Padilla & Pugnaire 2006; Holmgren et al. 2015). In both intervention levels, the physiological stress of seedlings significantly increased during a month (October) characterized by higher water table, low PAR, and low mean minimal temperatures. Furthermore, this time of the year overlaps with the time between bud break and the middle of the shoot elongation period, during which plants have been shown to exhibit high sensitivity to water stress (Lamhamedi & Bernier 1994). In contrast, the higher physiological stress exhibited in late summer was during a period characterized by lower water table, high PAR, and high mean maximal temperatures. In general, physiological stress of seedlings also exhibited “delayed effect” after periods of high (May–August) and low (November–February) precipitation. For example, the water table was always higher in areas where shrubs had been removed (nonsignificant trend), and in these areas, it began to rise in June and to decrease in February. Thus, our results support the global findings that shading can ameliorate thermal and water stress and thus result in positive interactions among plants (Holmgren et al. 2012).

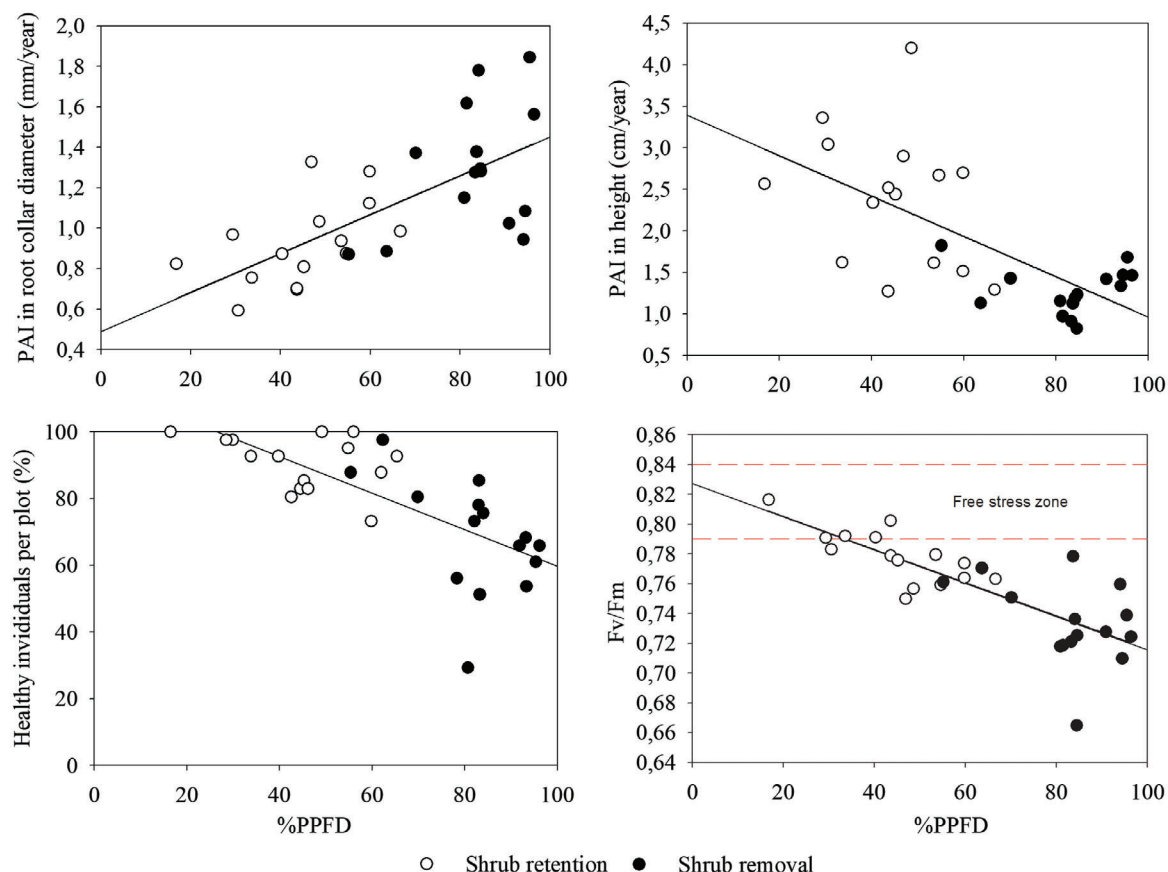


Figure 6. Overall trends between mean periodic annual increment (*pai*) in diameter (mm/year), *pai* in height (cm/year), vitality (healthy seedlings: <10% of dead foliage), Fv/Fm values, and light availability (represented by the photosynthetic photon flux density). Each dot represents a sample plot. Dashed red lines indicate the free physiological stress zone according to Maxwell and Johnson (2000).

Implications for Large-Scale Forest Restoration With Slow-Growing Conifers

In the temperate forests of south Chile, various initiatives to restore forests by establishing slow-growing conifers such as *Fitzroya cupressoides* and *Pilgerodendron uviferum* that are able to grow in restrictive sites have been initiated in the last decades (Lara et al. 2008; Bannister 2015). When restoring fire-disturbed *P. uviferum* forests, however, foresters have struggled with the question of whether the dense shrub cover on sites should be retained or removed before planting. Our study at an operational scale provides the first scientific support that *P. uviferum* can tolerate extreme wet conditions, but suffers from physiological stress when grown in open conditions. Thus, shrub removal is not only an expensive treatment, but also negatively influences restoration efforts that involve planting *P. uviferum* seedlings. Instead, retaining the existing shrub layer apparently facilitates early seedling performance by promoting better microsite conditions and reducing browsing levels. In addition, although open conditions with higher presence of *Sphagnum* bogs could be safe sites for germination of *P. uviferum* seeds (Bannister et al. 2014), safe sites for germination are not necessarily safe sites for seedling growth and survival (Schupp 1995). Seedlings may be overgrown by

bryophytes, and this can be an important mortality factor for slow-growing tree seedlings in bogs (Hörnberg et al. 1997). Therefore, seedlings with faster height growth will have a better chance to stay above the upward growth of *Sphagnum* (Ohlson 1995).

Previous studies in these disturbed bog forests have shown that *P. uviferum* has a limited seed dispersal potential and an effective seedling recruitment distance of <20 m. Consequently, restoration plantings are an important tool within the range of potential restoration approaches (Bannister et al. 2014; Bannister 2015). However, one of the main challenges in forest restoration is to develop practical techniques for implementing restoration at large scales (Hobbs & Norton 1996; Lamb et al. 2005).

Today, after almost 10 years of studying these ecosystems, we have learned much about how to establish *P. uviferum* seedlings in fire-disturbed bog and upland forests and how to facilitate their early performance. Some studies have found, for example, that seedlings are best planted on natural mounds or small elevated areas in the landscape to avoid waterlogging conditions (Bannister et al. 2013; Holmgren et al. 2015). In upland areas, planting seedlings beneath existing canopies of other tree species appears to be a good option to promote better

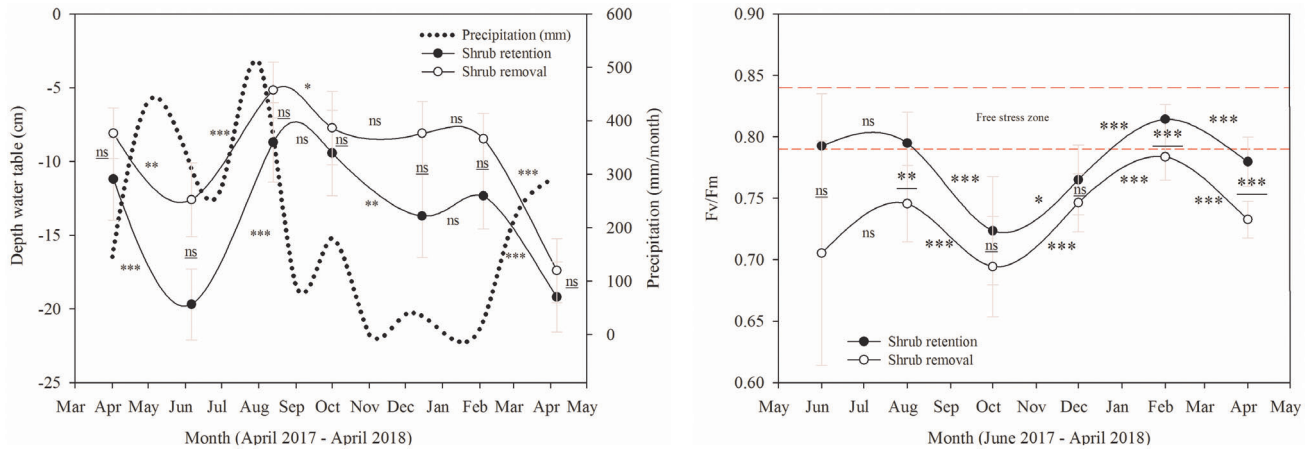


Figure 7. Depth of the water table (left) and Fv/Fm values of *Pilgerodendron uviferum* seedlings (right) for both intervention levels throughout the year 2017–2018. Dashed red lines indicate the free physiological stress zone according to Maxwell and Johnson (2000). Ns, nonsignificant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

nutrition and faster growth of *P. uviferum* seedlings (Bannister et al. 2013). In this study, our results suggest that the retention of shrubs on planting sites in fire-disturbed bogs will offer more suitable environments for the growth of *P. uviferum* seedlings. Planting in open areas could be also a valid option (as indicated by the low mortality rates found), but it should be noted that the early performance of this species will be lower in open areas than under the protection of shrubs.

One of the most challenging questions for future research in these ecosystems is the viability of restored *P. uviferum*-dominated forests in the context of global warming. Moist substrates comprising *Sphagnum* or mineral soil are the best substrates for the germination of *P. uviferum* seedlings. In contrast, dry conditions in the same substrates decreased seed germination by more than a third (Bannister et al. 2014). Chiloé Island is located in the northern part of the distribution of *P. uviferum*. At this latitude (41°S–43°S) simulation models predict significant climatic changes, with a predicted increase in the summer mean temperature of 2–4°C and an estimated reduction of 30–50% in summer precipitation by the end of this century (Universidad de Chile 2006). These predicted conditions, i.e. dryer and warmer microsites, are not ideal for the germination and growth of *P. uviferum* seedlings. Therefore, in the context of climate change, relying on the natural regeneration dynamics of *P. uviferum* could present problems in the northern part of its geographical distribution. On the other hand, previous studies have shown that positive interactions between shrubs and trees facilitate a vegetation shift from a moss-dominated to a tree-dominated system in the boreal ecosystems of northern Europe, in response to climate warming and drying (Gunnarsson & Rydin 1998; Kapfer et al. 2011; Holmgren et al. 2015). Our results suggest a similar pattern by highlighting that shrub cover has a positive influence on seedlings' early performance on our sites. However, more long-term ecological research is needed to clarify how these ecosystems of North Patagonia will develop in the context of climate warming in the next decades.

Acknowledgments

We thank the administration and staff of the Tantauco Park for constant help and support in the field. We also thank many field assistants that helped us under difficult conditions in the field. This is a contribution to the forest restoration research program of Instituto Forestal. This work was supported by FONDECYT Initiation into Research No. 11160191 and MINAGRI.

LITERATURE CITED

- Aerts R, Negussie A, Maes W, November E, Hermy M, Muys B (2007) Restoration of dry afromontane forest using pioneer shrubs as nurse-plants for *Olea europaea* ssp. *cuspidata*. *Restoration Ecology* 15:129–138
- Avendaño-Yáñez ML, Sánchez-Velásquez LR, Meave JA, Pineda-López MR (2014) Is facilitation a promising strategy for cloud forest restoration? *Forest Ecology and Management* 329:328–333
- Bannister JR (2015) Recuperar bosques no es solo plantar árboles: lecciones aprendidas luego de 7 años restaurando bosques de *Pilgerodendron uviferum* (D. Don) Florin en Chiloé. *Anales del Instituto de la Patagonia* 43:35–51
- Bannister JR, Donoso PJ (2013) Forest typification to characterize the structure and composition of old-growth evergreen forests on Chiloé Island, North Patagonia (Chile). *Forests* 4:1087–1105
- Bannister JR, Donoso PJ, Bauhus J (2012a) Persistence of the slow growing conifer *Pilgerodendron uviferum* in old-growth and fire-disturbed southern bog forests. *Ecosystems* 15:1158–1172
- Bannister JR, Vidal OJ, Teneb E, Sandoval V (2012b) Latitudinal patterns and regionalization of plant diversity along a 4,270-km gradient in continental Chile. *Austral Ecology* 37:500–509
- Bannister JR, Coopman R, Donoso P, Bauhus J (2013) The importance of microtopography and nurse canopy for successful restoration planting of the slow-growing conifer *Pilgerodendron uviferum*. *Forests* 4: 85–103
- Bannister JR, Wagner S, Donoso P, Bauhus J (2014) The importance of seed trees in the dioecious conifer *Pilgerodendron uviferum* for passive restoration of fire disturbed southern bog forests. *Austral Ecology* 39:204–213
- Bannister JR, Kremer K, Carrasco-Farías N, Galindo N (2017) Importance of structure for species richness and tree species regeneration niches in old-growth Patagonian swamp forests. *Forest Ecology and Management* 401:33–44

- Blanco-García A, Sáenz-Romero C, Martorell C, Alvarado-Sosa P, Lindig-Cisneros R (2011) Nurse-plant and mulching effects on three conifer species in a Mexican temperate forest. *Ecological Engineering* 37:994–998
- Brooker RW, Maestre FT, Callaway RM, Lortie CL, Cavieres LA, Kunstler G, et al. (2008) Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology* 96:18–34
- Burger A, Bannister JR, Galindo N, Vargas-Gaeste R, Vidal OJ, Schlegel B (2019) Browsing evidence of the native and near-threatened *Pudu pudu* deer in restoration plantings on Chiloé Island, Chile. *Gayana Botanica* 76:24–33
- Bustamante-Sanchez MA, Armesto JJ, Halpern CB (2011) Biotic and abiotic controls on tree colonization in three early successional communities of Chiloé Island, Chile. *Journal of Ecology* 99:288–299
- Callaway RM, Walker LR (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology* 78:1958–1965
- Castro J, Zamora R, Hódar JA, Gómez JM (2002) Use of shrubs as nurse plants: a new technique for reforestation in Mediterranean mountains. *Restoration Ecology* 10:297–305
- Castro J, Zamora R, Hódar JA, Gómez JM, Gómez-Aparicio L (2004) Benefits of using shrubs as nurse plants for reforestation in Mediterranean mountains: a 4-year study. *Restoration Ecology* 12:352–358
- DesRochers A, Gagnon R (1997) Is ring count at ground level a good estimation of black spruce age? *Canadian Journal of Forest Research* 27:1263–1267
- Di Castri F, Hajek E (1976) Bioclimatología de Chile. Vicerrectoría Académica de la Universidad Católica de Chile, Santiago, Chile
- Díaz MF, Bigelow S, Armesto JJ (2007) Alteration of the hydrologic cycle due to forest clearing and its consequences for rainforest succession. *Forest Ecology and Management* 244:32–40
- Donoso PJ, Soto DP, Coopman RE, Rodríguez-Bertos S (2013) Early performance of planted *Nothofagus dombeyi* and *Nothofagus alpina* in response to light availability and gap size in a high-graded forest in the south-central Andes of Chile. *Bosque* 34:23–32
- Duncan RS, Chapman CA (2003) Tree-shrub interactions during early secondary forest succession in Uganda. *Restoration Ecology* 11:198–207
- Fox J, Weisberg S (2019) An R companion to applied regression. Third edition. Sage, Thousand Oaks, California
- Galindo V, Calle Z, Chará J, Armbrrecht I (2017) Facilitation by pioneer shrubs for the ecological restoration of riparian forests in the Central Andes of Colombia. *Restoration Ecology* 25:731–737
- García-Garduño JA (2016). Estudio experimental para el seguimiento de plantaciones de *Quercus suber* L. de diferentes regiones de procedencia presentes en Castilla y León. Master thesis. Universidad de Valladolid, Palencia, Spain
- Gavinet J, Prévosto B, Fernandez C (2016) Do shrubs facilitate oak seedling establishment in Mediterranean pine forest understory? *Forest Ecology and Management* 381:289–296
- Gómez-Aparicio L, Zamora R, Gómez JM, Hódar JA, Castro J, Baraza E (2004) Applying plant facilitation to forest restoration: a meta-analysis of the use of shrubs as nurse plants. *Ecological Applications* 14:1128–1138
- Gunnarsson U, Rydin H (1998) Demography and recruitment of scots pine on raised bogs in eastern Sweden and relationships to microhabitat differentiation. *Wetlands* 18:133–141
- Hobbs RJ, Norton DA (1996) Towards a conceptual framework for restoration ecology. *Restoration Ecology* 4:93–110
- Holmgren M, Gómez-Aparicio L, Quero JL, Valladares F (2012) Non-linear effects of drought under shade: reconciling physiological and ecological models in plant communities. *Oecologia* 169:293–305
- Holmgren M, Lin CY, Murillo JE, Nieuwenhuis A, Penninkhof J, Sanders N, et al. (2015) Positive shrub–tree interactions facilitate woody encroachment in boreal peatlands. *Journal of Ecology* 103:58–66
- Holz A (2010) Climatic and human influences on fire regimes and forest dynamics in temperate rainforests in southern Chile. PhD thesis. University of Colorado, Boulder
- Holz A, Veblen TT (2011) The amplifying effects of humans on fire regimes in temperate rainforests in western Patagonia. *Palaeogeography, Palaeoclimatology, Palaeoecology* 311:82–92
- Hörnberg G, Ohlson M, Zackrisson O (1997) Influence of bryophytes and microrelief conditions on *Picea abies* seed regeneration patterns in boreal old-growth swamp forests. *Canadian Journal of Forest Research* 27:1015–1023
- Huber PJ (1981) Robust statistics. Wiley, New York
- Kapfer J, Grytnes JA, Gunnarsson U, Birks HJB (2011) Fine-scale changes in vegetation composition in a boreal mire over 50 years. *Journal of Ecology* 99:1179–1189
- Koerselman W, Meuleman AFM (1996) The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology* 33:1441–1450
- Lamb D, Erskine PD, Parrotta JA (2005) Restoration of degraded tropical forest landscapes. *Science* 310:1628–1632
- Lamhamedi MS, Bernier PY (1994) Ecophysiology and field performance of black spruce (*Picea mariana*): a review. *Annals of Forest Science* 51:529–551
- Lara A, Echeverría C, Thiers O, Huss E, Escobar B, Tripp K, Zamorano C, Altamirano A (2008) Restauración ecológica de coníferas longevas: el caso del alerce (*Fitzroya cupressoides*) en el sur de Chile. Pages 39–56. In: González-Espinoza M, Rey Benayas JM, Ramírez-Marcial N (eds) Restauración de bosques en América Latina. Fundación Internacional para la Restauración de Ecosistemas (FIRE), México
- Maxwell K, Johnson GN (2000) Chlorophyll fluorescence, a practical guide. *Journal of Experimental Botany* 51:659–668
- Ohlson M (1995) Growth and nutrient characteristics in bog and fen populations of Scots pine (*Pinus sylvestris*). *Plant and Soil* 172:235–245
- Oliver JC, Rosel J, Murray L (2000) Análisis de medidas repetidas mediante métodos de máxima verosimilitud. *Psicothema* 12:403–407
- Padilla FM, Pugnaire FI (2006) The role of nurse plants in the restoration of degraded environments. *Frontiers in Ecology and the Environment* 4:196–202
- Pajunen AM, Oksanen J, Virtanen R (2011) Impact of shrub canopies on understorey vegetation in western Eurasian tundra. *Journal of Vegetation Science* 22:837–846
- Parent S, Messier C (1996) A simple and efficient method to estimate microsite light availability under a forest canopy. *Canadian Journal of Forest Research* 26:151–154
- Péli ER, Nagy J, Cserhalmi D (2016) Decomposition rate, and carbon and nitrogen dynamics of *Sphagnum* litter: lessons from a peat bog. *Polish Journal of Ecology* 64:231–240
- Pérez CA, Armesto JJ, Torrealba C, Carmona MR (2008) Litterfall dynamics and nitrogen use efficiency in two evergreen temperate rainforests of southern Chile. *Austral Ecology* 28:591–600
- Pinheiro J, Bates D (2000) Mixed-effects models in S and S-PLUS. Springer Science & Business Media, New York
- Putz F, Canham C (1992) Mechanisms of arrested succession in shrublands: root and shoot competition between shrubs and tree seedlings. *Forest Ecology and Management* 49:267–275
- R Development Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
- Sadzawka R, Carrasco M, Grez R, Mora M, Flores H, Neaman A (2006) Métodos de análisis de suelos recomendados para los suelos de Chile. Revisión 2006. Serie Actas INIA. Instituto de Investigaciones Agropecuarias, Santiago, Chile
- Salas-Eljatib C (2002) Ajuste y validación de ecuaciones de volumen para un relicto del bosque de Roble-Laurel-Lingue. *Bosque* 23:81–92
- Salas-Eljatib C, Stage AR, Robinson AP (2008) Modeling effects of overstory density and competing vegetation on tree height growth. *Forest Science* 54:107–122
- Sánchez-Velásquez LR, Quintero-Gradilla S, Aragón-Cruz F, Pineda-López MR (2004) Nurses for *Brosimum alicastrum* reintroduction in secondary tropical dry forest. *Forest Ecology and Management* 198:401–404

- Saunders MR, Puettmann KJ (1999a) Effects of overstory and understory competition and simulated herbivory on growth and survival of white pine seedlings. *Canadian Journal of Forest Research* 29:536–546
- Saunders MR, Puettmann KJ (1999b) Use of vegetational characteristics and browsing patterns to predict deer damage in eastern white pine (*Pinus strobus*) plantations. *Northern Journal of Applied Forestry* 16: 96–102
- Schupp EW (1995) Seed-seedling conflicts, habitat choice, and patterns of plant recruitment. *American Journal of Botany* 82:399–409
- Soto DP, Donoso PJ, Puettmann KJ (2014) Mortality in relation to growth rate and soil resistance varies by species for underplanted *Nothofagus* seedlings in scarified shelterwoods. *New Forests* 45:655–669
- Soto DP, Donoso PJ, Salas C, Puettmann KJ (2015) Light availability and soil compaction influence the growth of underplanted *Nothofagus* following partial shelterwood harvest and soil scarification. *Canadian Journal of Forest Research* 45:998–1005
- Svriz M, Damascos MA, Zimmermann H, Hensen I (2013) The exotic shrub *Rosa rubiginosa* as a nurse plant. Implications for the restoration of disturbed temperate forests in Patagonia, Argentina. *Forest Ecology and Management* 289:234–242
- Universidad de Chile (2006) Estudio de la variabilidad climática en Chile para el siglo XXI. Informe Final. Departamento de Geofísica, Santiago, Chile
- Villagrán C (1988) Late quaternary vegetation of southern Isla Grande de Chiloé, Chile. *Quaternary Research* 29:294–306

Coordinating Editor: Fernando Valladares

Received: 14 May, 2019; First decision: 27 July, 2019; Revised: 11 November, 2019; Accepted: 24 November, 2019; First published online: 22 December, 2019