Title: Shrub influences on seedlings performance when restoring the slow-growing conifer *Pilgerodendron uviferum* in southern bog forests.

Running head: Shrub influences in southern bog forests

Authors: Jan R Bannister[a*], German Travieso[a], Nicole Galindo[a], Manuel Acevedo[b], Klaus Puettmann[c], Christian Salas-Eljatib[c,d]

Addresses: [a]Instituto Forestal, Oficina Chiloé, Chile; [b]Instituto Forestal, Sede Bio Bio, Chile; [c]Oregon State University, Corvallis, OR, USA; [c]Centro de Modelación y Monitoreo de Ecosistemas, Universidad Mayor, Santiago, Chile; [d]Laboratorio de Biometría, Universidad de La Frontera, Temuco, Chile;

*Corresponding author: Instituto Forestal, Oficina Chiloé, Nercón S/N, Castro Chile tel.: +56-65-2633641, jbannister@infor.cl

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Abstract

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* in Chiloe 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 (~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.

Keywords: 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.

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 benign croenvironments (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. 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. 2012*a*). 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 naturally 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. 2012*a*; 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 Chiloe Island, but elsewhere under similar conditions, especially within the context of climate change.

Material and methods

Study area

The study area was located near Lake Chaiguata (Tantauco Park, ~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 *P. uviferum*-dominated bog forests, most of which had burned in an extensive fire in 1942–43 (Holz & Veblen 2011). Southern Chiloé is in the south temperate biogeographic region of Chile (Bannister et al.

2012*b*). It has a cool-temperate climate with a strong oceanic influence, a mean annual temperature of 10º 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 (~13,000 yr. 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 m a.s.l.

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 drainage (slightly higher elevation), rare natural regeneration of broadleaf species like *T. stipularis, Nothofagus nitida, Drimys winteri,* and *Weinmannia trichosperma* (Fig. 2ab) 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 m^2), 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 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: µmol m-2 s-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 5 living seedlings per plot. Every 2 months for 1 year (April 2017-April 2018), the same 5 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 the 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 Kolgmorov-Smirnov and Levene's tests, respectively, as suggested by Salas-Eljatib (2002). If a model did not fulfil 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 non-parametric 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 modelling 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 a 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, USA).

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, ~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 ~81% in the summer (November-December) to ~97%–100% in the winter (May-August). The mean monthly photosynthetic

active radiation (PAR) during the day ranged from 179.0 (μmol/m^{2}s) in June to 739.5 (μmol/m^{2}s) in January. The monthly mean, minimal, and maximal temperatures are presented in Fig. 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 photosynthetic photon flux density (*PPFD*) available to seedlings, with significantly more light available for seedlings planted after shrub removal ($P \leq 0.01$).

After 4 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 (~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 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 *Gleichenia* 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 vitality. Our results were consistent with beneficial shrubs 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 non-significant 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 1999*a*). 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. (2012*a*). 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 1999*b*; Aerts et al. 2007; Padilla & Pugnaire 2006).

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 adventive roots of seedlings (Lamhamedi & Bernier 1994; DesRochers & Gagnon 1997) with a subsequent deformation of the root collars (DesRochers & Gagnon, 1997). Such 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 and 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 (non-significant 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).

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 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.

Acknowledgements

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ILLUSTRATIONS

TABLES

Table 1. Descriptive statistics of soil-related and micro environment-related variables by intervention levels. Values in brackets represent standard deviations. Different letters show significant differences among intervention levels ($P \leq 0.01$). OM: Organic matter; CEC: Cation exchange capacity; PPFD: Photosynthetic photon flux density

Variables	Intervention level	
	Shrub removal	Shrub retention
Soil-related		
Depth (cm)	24.3 ($\pm$ 10.02)	28.87 ($\pm$ 17.40)
pH	4.46 ($\pm$ 0.18)	4.54 ($\pm$ 0.17)
OM (%)	29.4 ($\pm$ 14.75)	22.8 ($\pm$ 4.66)
Ct (%)	17.16 ($\pm$ 8.43)	13.1 ($\pm$ 2.72)
Nt (%)	0.59 ($\pm$ 0.38)	0.352 ($\pm$ 0.13)
P (mg/kg)	6.88 ($\pm$ 1.86)	8.24 ($\pm$ 3.31)
C/N	31.2 ($\pm$ 4.09)	38.8 ($\pm$ 7.01)
CEC (cmol+/kg)	6.96 ($\pm$ 2.43)	6.052 ($\pm$ 0.97)
Micro environment-related		

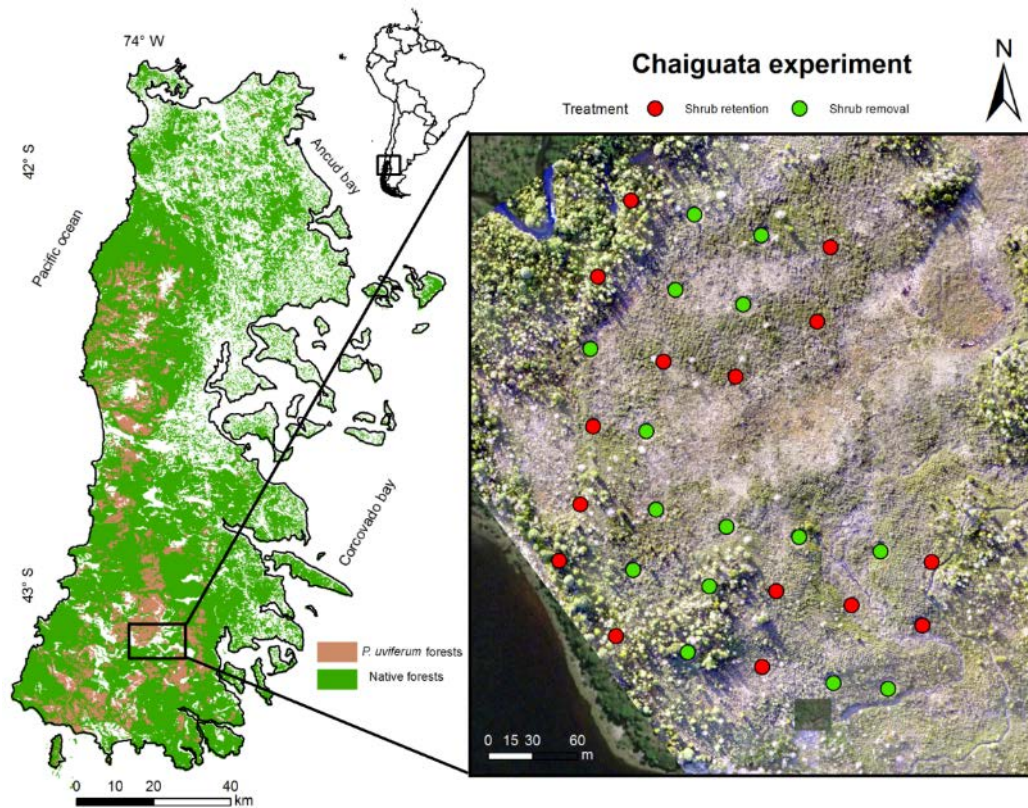|		
PPFD (%)	82.87 (± 13.35)^A	44.87 (± 11.90)^B
Annual depth of water table (cm)	9.66 (± 9.22)	13.49 (± 7.16)
Minimum depth of water table (cm)	5.62 (± 7.59)	4.25 (± 6.38)

FIGURES

Figure 1. Study area in Chiloé Island, North Patagonia (left), and location of the 30 experimental units near Lake Chaiguata (right).
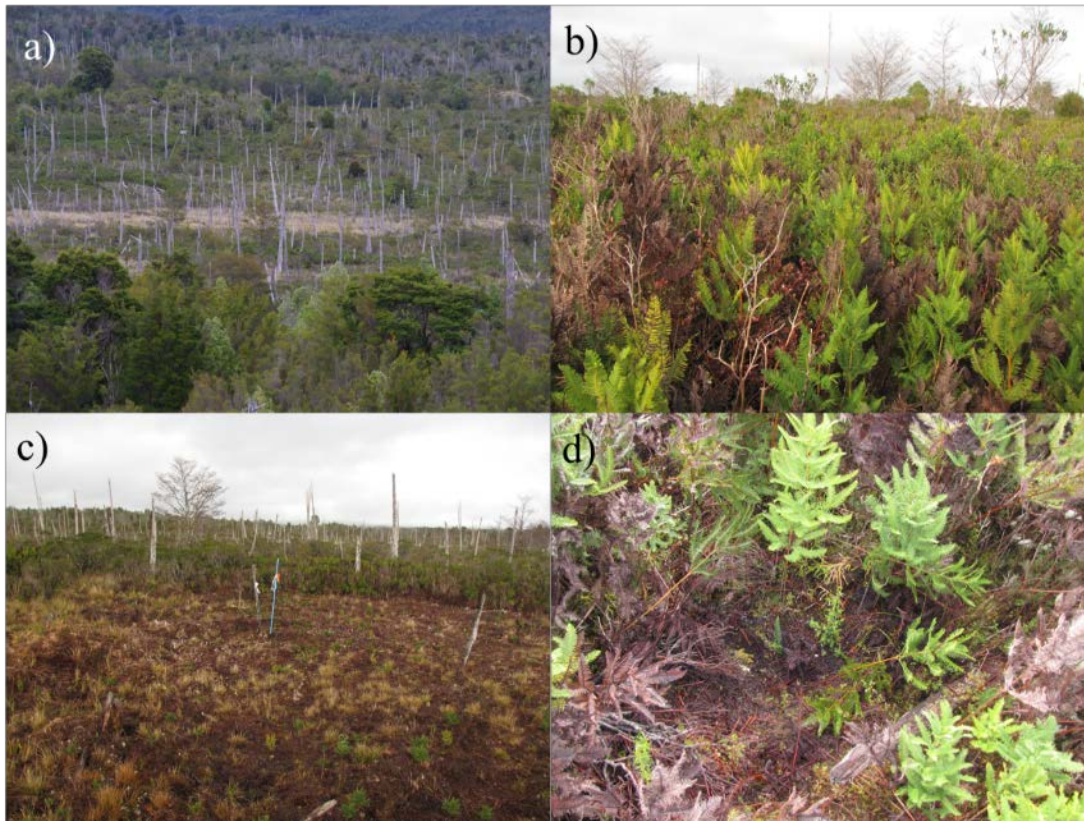
Figure 2. (a) Landscape previously dominated by forests of *P. uviferum* on Chiloé Island that had burned previously, in 1942-43; (b) secondary native shrublands of *Bacharis* sp. and *Gleichenia* sp. that have grown since the fires and hamper tree seedlings planting; (c) and *Pilgerodendron uviferum* seedlings planted under two different intervention levels, i.e. shrub removal (c) and shrub retention (d).
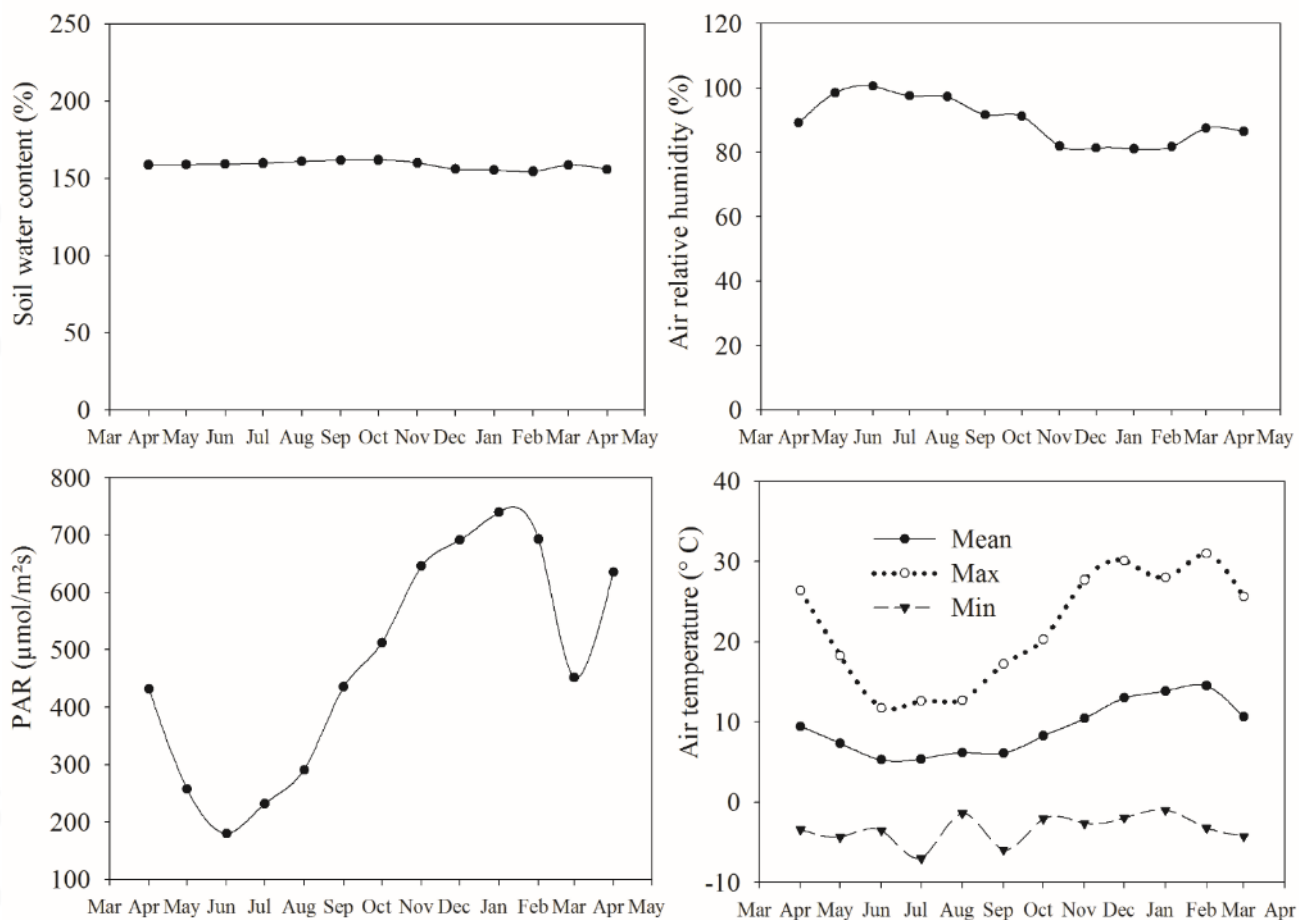
Figure 3. Variation of micro-environmental variables through the year 2017/18 on the study site. Data were captured and averaged from two data loggers located in experimental units with different intervention levels.
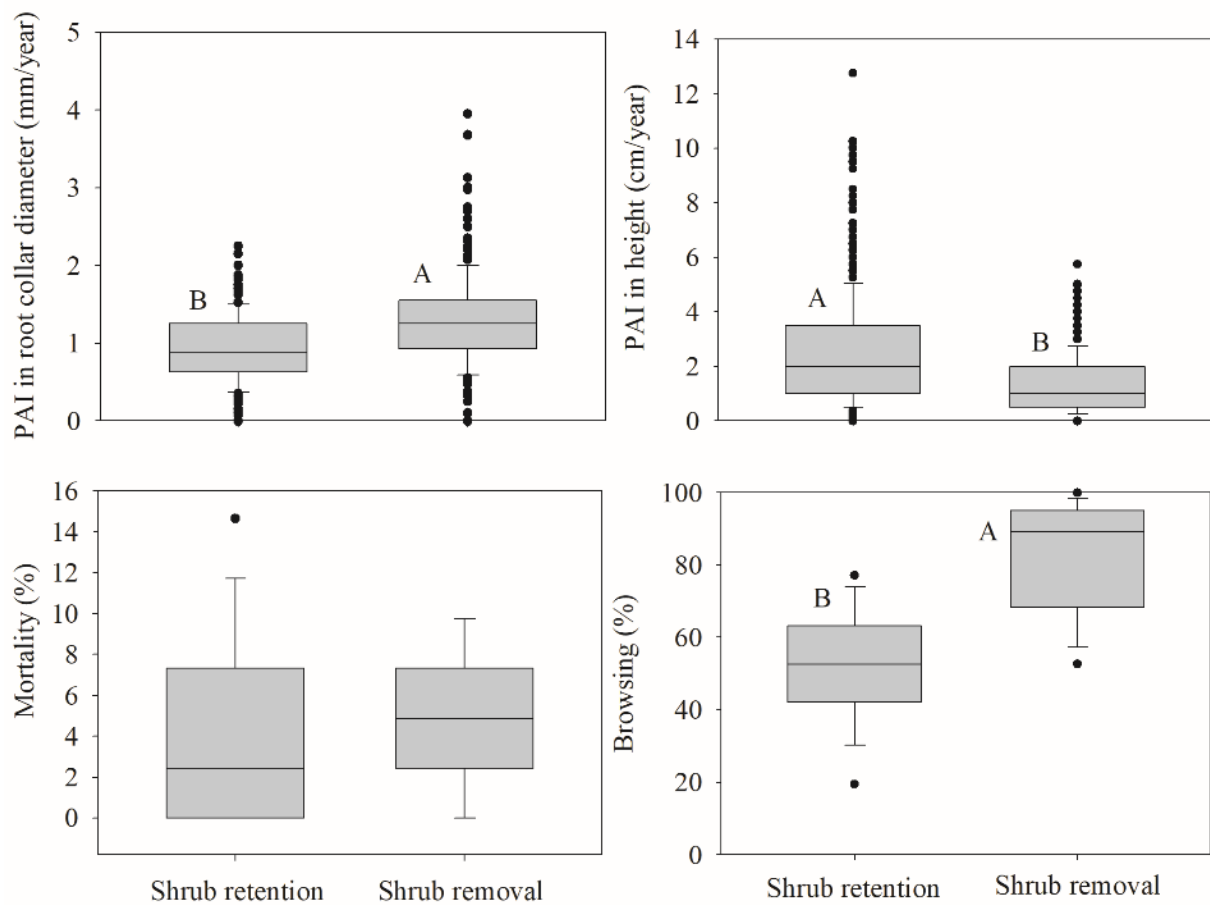
Figure 4. Periodic annual increment (*pai*) in root collar diameter, height, mortality rate, and browsing percentage of *P. uviferum* seedlings established under two intervention levels (i.e. shrub retention and shrub removal). Different letters show statistically significant differences among intervention levels (*P* ≤ 0.01).
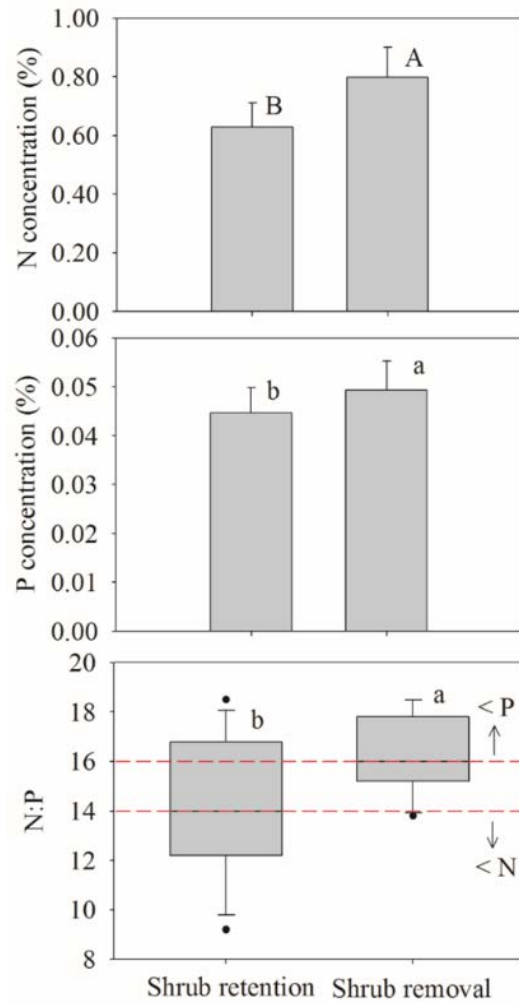
Figure 5. Foliar N and P concentrations and N/P ratios in seedlings of *P. uviferum* under two intervention levels. Dashed lines and arrows indicate P and N limitation (Koerselman & Meuleman 1996). Different letters show significant differences among intervention levels (lower case letters: $P \leq 0.05$; capital letters: $P \leq 0.01$).
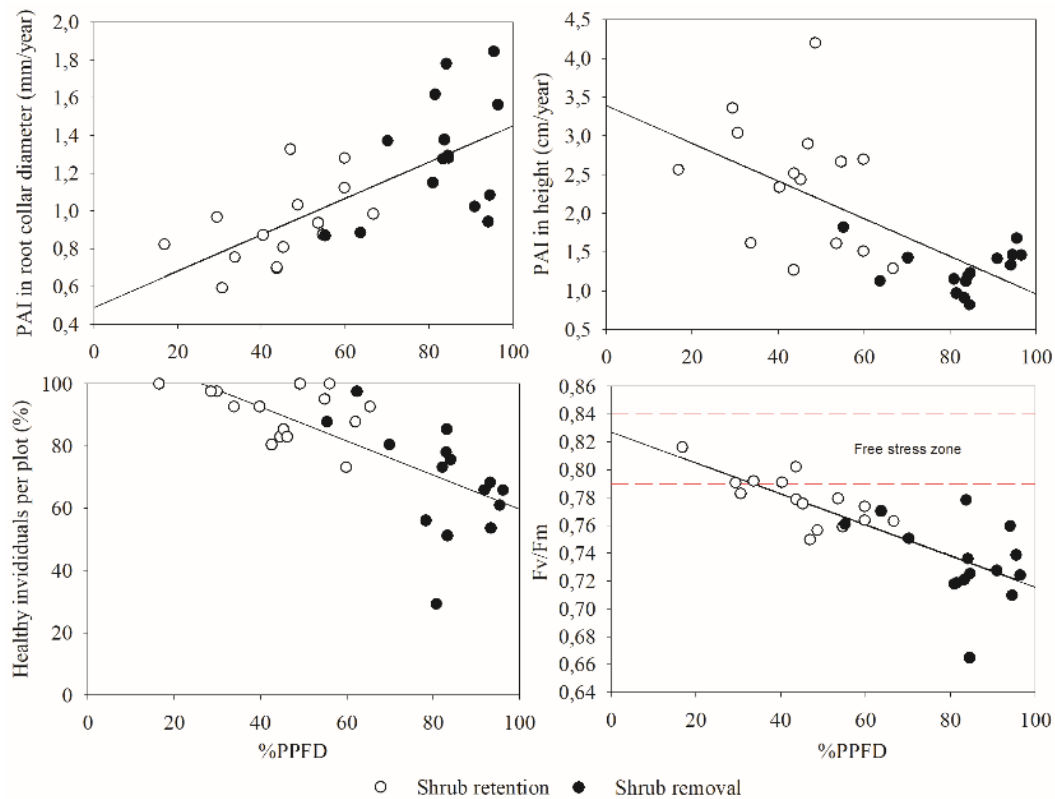
Figure 6. Overall trends between mean periodic annual increment (*pai*) in diameter (mm/year), *pai* in height (cm/year), vitality (healthy seedlings: <10% of dead foliage), Fv/Fm values, and light availability (represented by the photosynthetic photon flux density). Each dot represents a sample plot. Dashed red lines indicate the free physiological stress zone according to Maxwell and Johnson (2000).
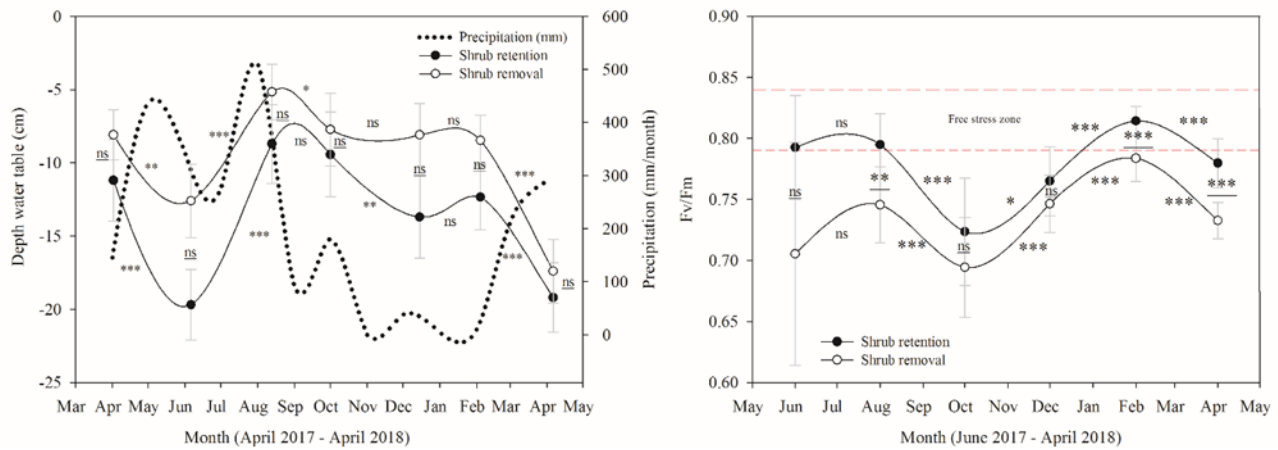
Figure 7. Depth of the water table (left) and Fv/Fm values of *P. uviferum* seedlings (right) for both intervention levels throughout the year 2017-2018. Dashed red lines indicate the free physiological stress zone according to Maxwell and Johnson (2000). Ns: non-significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.