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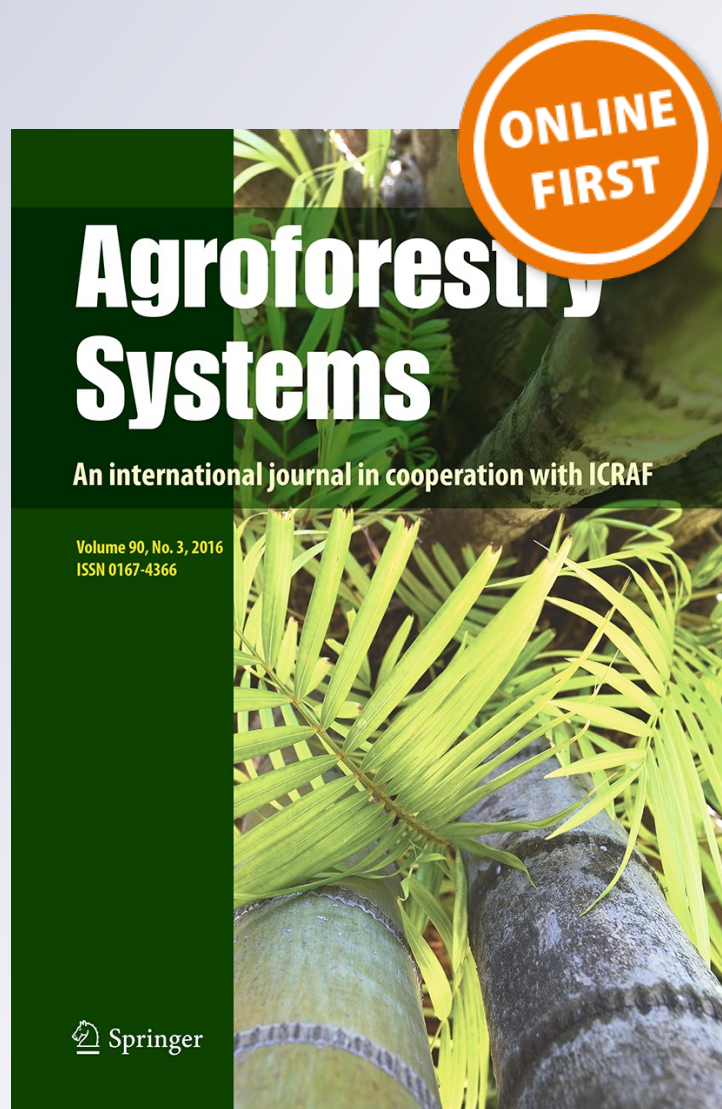
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Competition effects of grazing-modified herbaceous vegetation on growth, survival and water relations of lenga (*Nothofagus pumilio*) seedlings in a temperate forest of Patagonia, Argentina

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Abstract In this study, we determined the competition effects of herbaceous vegetation on survival, growth, and plant water relations of planted lenga (*Nothofagus pumilio*) seedlings under field and nursery conditions in western Patagonia, Argentina. In the field, thirty (1.5 × 1.5 m) plots were randomly set in a grazing-free enclosure built in a typical grazing-degraded lenga canopy gap mainly colonized by non-native herbs and grasses (*empastado*). Herbaceous aerial and root biomass was removed in half of the plots (non-competition treatment, NCT), while the others (competition treatment, CT), remained undisturbed. Four similarly-sized lenga seedlings were planted per plot. In the nursery, 120 lenga seedlings were planted in individual pots containing soil of the

field study site, and set to a factorial experiment including two competition levels (CT and NCT) and two watering regimes: normal (simulating average rains during the growing season, 500 mm, NW), and high (equivalent to 1000 mm, HW). During three growing seasons, we determined seedling survival, growth, and plant and soil water status of both experiments. Higher survival and growth, and better plant water status values were obtained in NCT as compared to CT in both experiments. In the nursery, HW did not improve survival and growth as compared to NW. In restoration trials implying grazing-degraded areas, increases in lenga seedling survival and growth could be achieved by reducing nearby competition of grasses and herbs, while extra watering appears unnecessary. However, limitations in the experimental design (pseudo-replication), limits generalization of results to other forest ecosystems with similar structural and functional characteristics.

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Seedling performance · Regeneration · Water potential

Introduction

Ungulates have a strong influence in forest ecosystem processes, including nutrient cycling, primary productivity, and disturbance regimes. These processes may have, in turn, direct and indirect effects on forest

regeneration and vegetation dynamics (Hobbs 1996). Direct grazing and trampling damages on vegetative and reproductive parts of plant have been widely studied (Ammer 1996). Indirect effects, such as interspecific interactions, including competition or facilitation among grazing-tolerant species and tree seedlings, habitat modification, and effects on nutrient availability, have received proportionately less attention (Weisberg and Bugmann 2003).

In forest gaps dominated by herbaceous cover, survival, growth, and emergence of tree seedlings may be conditioned by competition or facilitation interactions. In some cases, herbaceous vegetation may physically impede seed contact with the top-soil, precluding germination and emergence of tree seedlings (Vandenberghé et al. 2006). However if seeds reach the top soil and germination occurs, herbs may facilitate initial tree seedling survival (Vandenberghé et al. 2006). Later, herbs may begin to compete with tree seedlings for either below (water and nutrients) or above-ground (light) resources (Heinemann and Kitzberger 2006).

In Patagonian Andean forests, introduced domestic livestock has produced one of the most relevant anthropogenic disturbances (Vázquez 2002). In the understory of these forests, livestock has affected species composition, causing a decline in native species richness and a concomitant increase of non-native species (Blackhall et al. 2008; Relva and Veblen 1998). Grazing, browsing, and trampling disturbances open gaps in which non-native species establish, especially in areas under low canopy cover (Vázquez 2002).

Within Patagonian Andean forests, *Nothofagus pumilio* (Poepp. et Endl.) Krasser, locally known as *lenga*, is one of the most important tree species from both the ecological and the economical points of view. In Argentina, these forests cover a strip about 2000 km long (in north to south direction), and 80–100 km wide. Within this long latitudinal distribution gradient, *lenga* grows under a cool-temperate environment, with a typical Mediterranean climate prevailing in the north-central area of its distribution (about 65 % of total annual precipitation falls in autumn and winter). A sub-polar oceanic climate, in which precipitation is evenly distributed throughout the year, dominates instead the southern region in which *lenga* thrives (Dimitri 1972; Donoso 1995) (Fig. 1). In these two types of climates, natural *lenga* stands regeneration may begin either in large-scale openings, as a consequence of catastrophic wind-storms events, or in small scale gap-openings

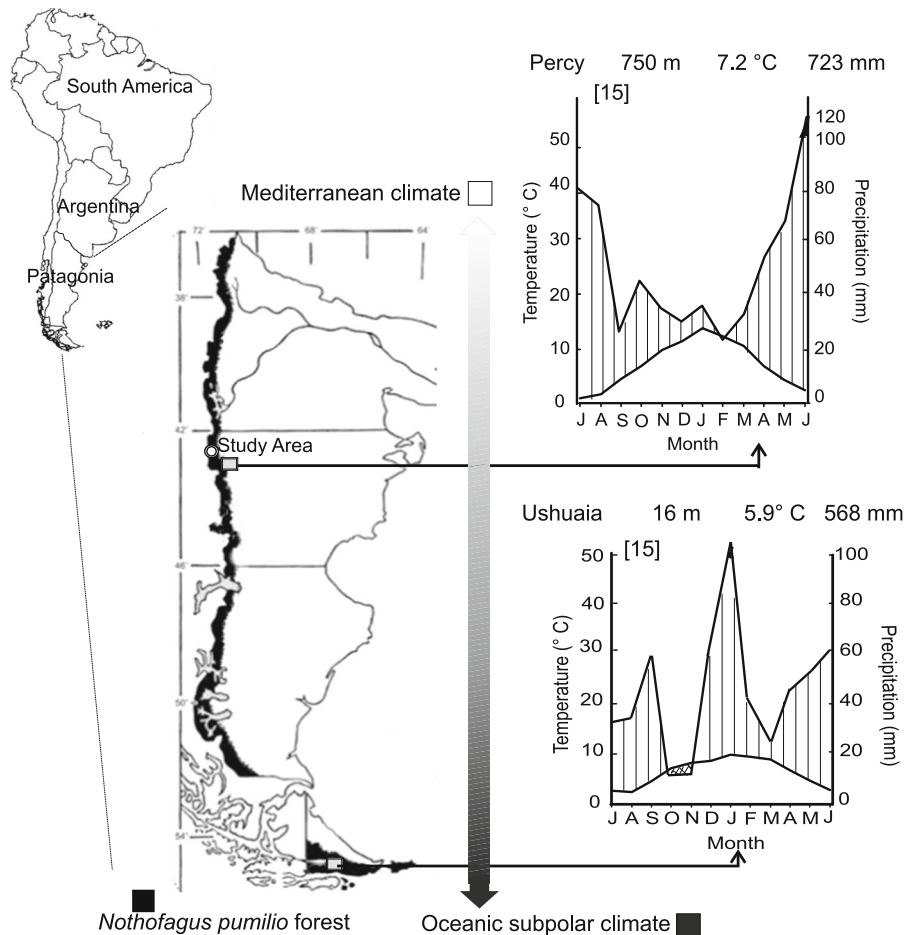
(Veblen 1992). In the north-central area of *lenga* distribution, natural regeneration generally occurs in small to medium-size canopy gaps (25–1000 m²), usually produced by the fall of one, or several, senescent trees (Veblen 1992). In this area of distribution, the success of natural *lenga* regeneration is conditioned by a set of environmental and biotic factors, such as the availability of viable seed (Cuevas 2002) and appropriate soil microsites for seed germination and seedling survival (safe sites, sensu Harper 1997). Grazing disturbance by exotic herbivores, moreover, adds another detrimental factor that negatively affects, or even precludes, *lenga* stands regeneration (Bava and Rechene 2004; Vázquez 2002). In these grazing-disturbed *lenga* canopy gaps, herbaceous non-native species establish (Sánchez-Jardón et al. 2014), conforming what it is locally known as *empastados*, which are permanent pastures generally composed of non-native grasses, grass-like species, and herbs. These *empastados* may last for several years, even for a long time after livestock has been removed. While *empastados* may be a good alternative for livestock production, they may negatively affect native *lenga* forest regeneration (Rusch 1992). These adverse effects in *lenga* regeneration may be augmented by the Mediterranean conditions prevailing in this north-central area of its distribution. In fact, water deficit during the growing season appears as crucial for *lenga* germination and early seedling survival (Rusch 1992). In areas where the objective is to restore former *lenga* forest stands, it is necessary to scientifically determine which of these factors or mechanisms impede its natural regeneration. The objectives of this study were therefore to: (1) determine, under field conditions in a typical *empastado* within a *lenga* forest canopy gap, the effects of herbaceous cover on growth, soil and plant water relations and survival of planted *lenga* seedlings; and (2) determine, under controlled conditions in a nursery, the combined effects of herbaceous cover and watering regimes on *lenga* seedlings growth, plant water status and survival.

Materials and methods

Study area

Part of this study was carried out at Huemules experimental site, located 25 km northwest of Esquel

Fig. 1 Distribution of lenga (*Nothofagus pumilio*) forests in Patagonia, Argentina (redrawn from Dimitri 1972). The two climate-diagrams (in the format of Walter and Lieth 1967) indicated extremes in the seasonal distribution of annual precipitation, showing a typical mediterranean climate in the northern and central area of lenga distribution (Percy meteorological station, near the study area), and gradually changing to an Oceanic sub-polar climate down south as approaching the city of Ushuaia. The location of the field experiment (*study area*) is indicated



city (Chubut, Argentina), in the $42^{\circ}49'57''$ SL parallel and $71^{\circ}27'46''$ WL meridian, at about 1100 m a.s.l. (Fig. 1). Isolated records (Hahn 1992) and estimations of previous studies (Jobbágy et al. 1995; López Bernal et al. 2012) showed that, in the area, mean precipitation is around 1050 mm year, and mean annual temperature is 6 °C (Haufe 1992). During the growing season (September to March), rains reach about 400–500 mm (Hahn 1992). This precipitation represents an intermediate value for the different environments in which lenga stands develop, which comprise a wide range of precipitation conditions that goes from 500 to 3000 mm/year (Veblen et al. 1977). Soils of the area derive from volcanic ashes, which are rich in nutrients and organic matter, and possess high water retention (Marcolin et al. 1989).

The study site is a lenga forest which was used up to about 50 years ago for timber production by

implementing selective cuts of the best lenga specimens (high-grading). Livestock management in this site has been extensive and seasonal (summer pasturage). The field experiment was established in January 2010 in an *empastado* lenga gap used for livestock grazing since about 30 years ago. A 700 m² cattle enclosure was built, within which this experiment was established (Fig. 2).

Field experiment

Within the enclosure, 10 square, 1 m² subplots were randomly set, and frequency and percentage of cover of all vascular plant species surveyed according to Damascos and Rapoport (2002). It is important to mention that within lenga *empastados*, most of the plant species found were herbs, grasses, and only a few shrubs, while the presence of tree seedlings (i.e. lenga)

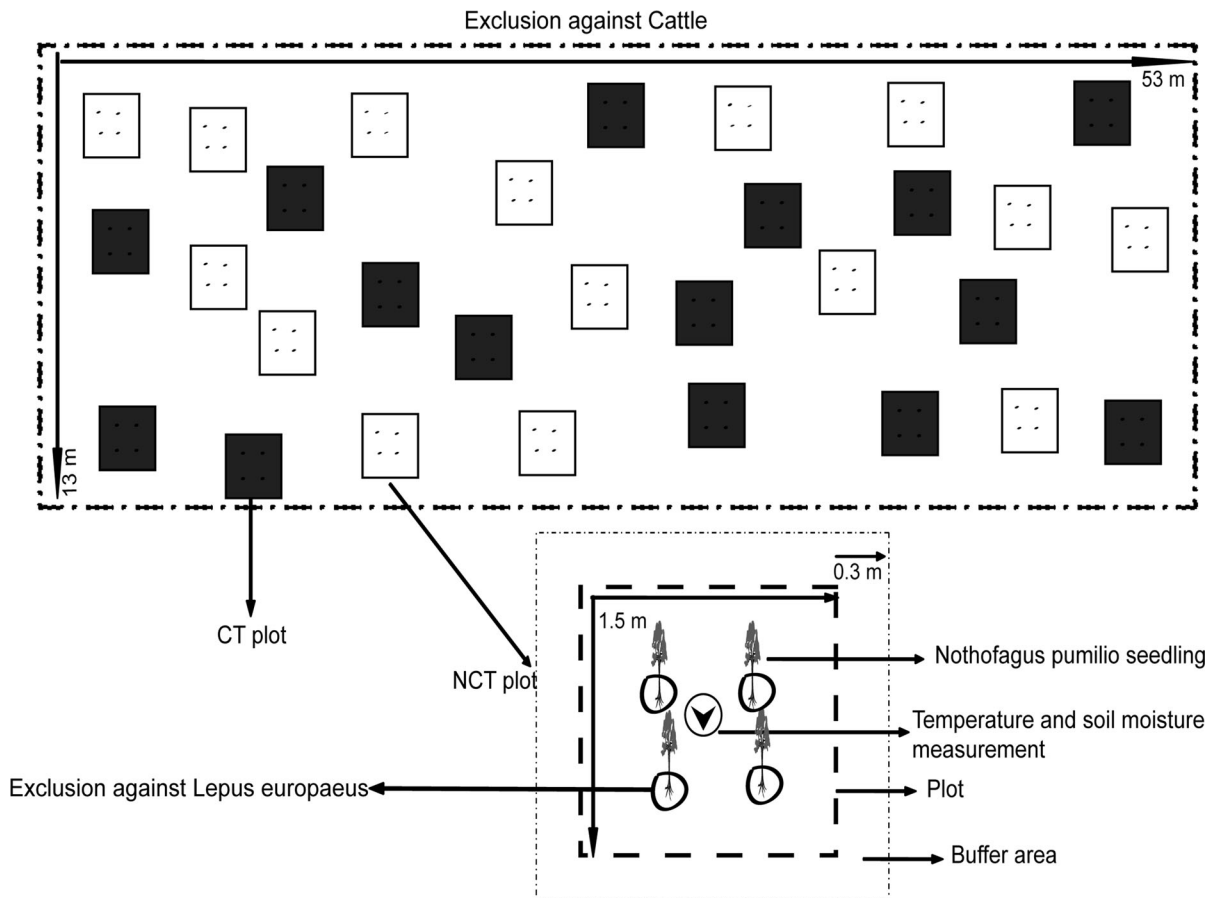


Fig. 2 Layout of the sampling units established, detailing the study plots in the field experiment carried out in Huemules site in Patagonia, Argentina

were nil. In June 2010, thirty 1.5×1.5 m plots were randomly installed within the enclosure. Half of them ($n = 15$) were left undisturbed (competition treatment or CT from now on), while in the other 15 plots, the herbaceous and/or grass cover was removed (non-competition treatment or NCT from now on). Grass and herbaceous vegetation removal in NCT included aerial and root biomass. Aerial biomass was removed manually, while root extraction was done by digging the upper soil layers. This practice was done trying to cause the less possible disturbance to the upper soil layer. After that, four 1-year-old nursery grown lenga seedlings with similar above- and belowground biomass, were planted in every plot of both CT and NCT treatments (Fig. 2). At the beginning of the experiment, mean height of CT seedlings was 16.24 ± 0.39 cm, and stem diameter 3.19 ± 0.12 mm, and NCT seedlings height was 16.56 ± 0.45 , and stem diameter 3.12 ± 0.11 mm.

No significant differences were recorded between treatments.

After plantation, every seedling of each treatment was also protected with a metallic mesh to avoid browsing by the European hare (*Lepus europaeus* Pallas). During the rest of the experiment newly established herbs and grasses were manually removed from the NCT plots to maintain the non-competition condition. We acknowledge that this experimental design lacks of replications (Hurlbert 1984) so that careful considerations should be given when trying to extrapolate or generalize this results to other lenga forests.

Soil moisture and temperature were measured as follows: four soil temperature sensors (1-Wire/iButton) were placed at 5 cm soil-depth (two sensors in CT and two in NCT plots) during the first two growing seasons, and moved to different plots at each reading

date. Soil volumetric water content was measured in the center of 10 randomly selected plots per treatment, using a portable Time Domain Reflectometer (IMKO Trime FM-3). The readings were taken three times during the first growing season (December 23, 2010 (early summer), February 8, 2011 (mid-summer), and March 22, 2011 (late summer), and twice during the second growing season (January 25, 2012 (mid-summer), and March 23, 2012 (late summer), (Fig. 2). Rainfall data of the last 15 growing seasons (from September to March, 1997–2013) were taken from the nearby Río Percy Meteorological station (42°51'30" SL, 71°25'47" WL, 750 m a.s.l). Initial height and stem diameter growth were measured at the beginning of the experiment. Seedling height was measured from the base of the stem to the bud of the extended apical branch. Stem diameter was measured at 3 cm from the stem base; a pain t mark was made on each seedling at this height so that subsequent measurements could be taken at the same place. Height and stem diameter growth were further measured in all seedlings, in late summer of 2011, 2012, 2013 and 2014. Branches water potential was measured in 10 randomly selected seedlings per treatment plot. The measurements were taken at predawn (from 05:00 to 06:00 h AM), and at about midday (from 12:00 to 02:00 h PM), using a pressure chamber (Model 4 MPa, Bio-Control, USA). The readings were taken the same times as mentioned for soil moisture. Seedlings survival was measured for all seedlings in late summer of 2011, 2012, 2013 and 2014, respectively.

Nursery experiment

The nursery experiment was conducted at the Patagonian Andes Forest Research and Extension Center (CIEFAP), in Esquel. This experiment was installed in order to mimic the field experiment, but under controlled conditions and adding another factor (irrigation). In June 2009, 120 two-years-old nursery grown, and similarly sized lenga seedlings (whose mean height was 31.1 ± 1.1 cm, and their stem diameter 2.7 ± 0.1 mm), were planted in individual polyethylene pots (17 cm wide and 25 cm deep). Previous to planting, all pots were filled with top-soil taken from experimental site. In half of the pots (60), this top-soil contained the original herbs and grasses present in the field, and was set as competition treatment (CT). The rest of the pots were filled with the

same top-soil, but after manually removing all above- and below-ground biomass of herbs and grasses, and set as non-competition treatment (NCT). All pots were placed on an iron table, under a cover of a plastic shade cloth (30 % shading), and protected by a 100 μ m transparent polyethylene roof cover to avoid rainfall to reach the pots and interfere with the treatments. A factorial design was established with two levels of competition (CT and NCT), and two watering regimes (normal, NW, and high, HW). The water added to NW (equivalent to 500 mm) was set up to mimic the average rainfall received under natural conditions by the experimental lenga stand during the growing season (from September to March) at Huemules site (Hahn 1992). The high watering (HW) treatment, instead, received twice as much the average precipitation (1000 mm). Irrigation for all treatments was performed daily only during the two growing seasons. Height and stem diameter growth were measured with the same methodology as mentioned for the field experiment, in late summer of 2010 and 2011. Plant water potential was measured with the same methodology as mentioned for the field experiment, in the branches of five randomly selected seedlings per treatment combination. The readings were taken during one growing season. Midday branch water potential was measured three times, in January 7, 2011 (early summer), February 3, 2011 (mid-summer), and March 19, 2011 (late summer). Predawn branch water potential was measured only on the last two sampling dates. Seedlings survival was measured in all seedlings for two growing season post-planting, in late summer of 2010 and 2011.

Data analyses

Growth, both in field and the nursery, was calculated as the difference between the measured values (height in cm and stem diameter in mm) at the end of each growing season compared to those obtained in the previous growing season. A linear mixed-effects model (LME) with restricted maximum likelihood estimation (REML) (Di Rienzo et al. 2011) was fitted to investigate the effect of herbaceous cover treatments on seedling growth in the field and nursery experiments. Field data of competition treatments (NCT and CT), and years after planting (1, 2, 3, and 4) were considered as fixed effects. The time after planting (years) was considered a fixed effect because

the post-planting period is especially important to evaluate the establishment of field seedlings (Kozłowski and Davies 1975). In this model, the plot containing four seedlings ($N = 30$) was included as a random factor. In the nursery, the fixed effects were the herbaceous cover treatment (NCT and CT) and watering treatments (HW and NW), and years from planting (1 and 2) was included as a random factor. Bonferroni a posteriori test was applied. One-way ANOVA was carried out to test the competition effects on water potential, soil temperature and soil moisture in the field experiment, because we had no repeated measure. Normality of residuals (Shapiro–Wilk's test) and homogeneity of variance (Levene's test) were tested for these variables. A factorial analysis was used to test for main effects and interactions between competition and irrigation treatments on water potential in the nursery experiment. Survival was compared for the different treatments using contingency tables and a Chi square Test (Sokal and Rohlf 1973). For all data presented, statistically significant differences were set at $p \leq 0.05$. Response variables are reported as mean values with standard errors (mean \pm SE).

Results

The plant survey showed that 100 % of frequency and about 70 % of cover of the understory in the experimental forest gap corresponded to non-native herbaceous and grass species (Table 1). These species were *Poa pratensis* and *Trifolium repens*, with less abundance and frequency of *Taraxacum officinale*, *Rumex acetosella* and *Veronica serpyllifolia* (Table 1). About 25 % cover corresponded to native herb species such as *Osmorhiza chilensis*, *Vicia nigricans*, *Acaena ovalifolia*, *Potentilla chilensis*, *Viola maculata*, *Acaena pinnatifida*, and *Calceolaria biflora*. The native shrub stratum was very low, mainly represented by *Ribes cucullatum* (10 % of frequency and 2 % of cover). Other shrubs, such as *Berberis serratodentata* and *Ribes magellanicum*, showed very low frequency and cover <1 % (Table 1).

Field experiment

Soil temperature was similar in CT and NCT plots along both seasons, respectively. These temperatures

averaged 8 °C at the beginning of the growing season and 11 °C at the end of it, with peaks of 14 °C in mid-summer of both growing seasons measured (Fig. 3a). Soil moisture showed higher values for NCT plots than for CT plots, with values marginally significant (Schabenberger and Pierce 2002) for the sampling dates of early ($p = 0.08$) and mid-summer ($p = 0.06$) of 2010–2011 growing season, and for late summer ($p = 0.06$) of 2011–2012 growing season. These values, instead, were significantly different ($p = 0.04$) for the sampling dates of late summer of 2010–2011 growing season, when soil moisture was higher for CT plots than for NCT (Fig. 3b).

The average rainfall during the growing season for the last 15 years (from September to March) was 277 mm. In the first growing season evaluated (2010–2011), precipitation reached 240 mm; in the second season it reached 265 mm, while for the third growing season, it was 315 mm (Fig. 4).

Seedling performance

Height growth showed a significantly decreasing trend over time (Fig. 5a; Table 2). Related to stem diameter growth, consistently higher values were found for NCT seedlings as compared to CT seedlings at all dates. However this trend was not statistically significant. This variable showed the highest growth in the second year, and the lowest in the fourth year after seedling establishment (Fig. 5b; Table 2).

Predawn water potential values showed a consistent trend, presenting less negative values for NCT seedlings as compared to CT seedlings, although showing only significant marginal differences for early summer of 2009–2010 growing season (Fig. 5a; Table 3). Values of midday seedling branch water potentials, instead, were always less negative for NCT as compared to CT seedlings at all sampling dates, although only significantly different in mid-summer of 2010–2011 growing season and in late summer of 2011–2012 growing season (Fig. 6b; Table 3).

All seedlings survived of the first growing season. Seedling survival, however, was 93, 90 and 86 % for CT of the second, third and fourth growing seasons, respectively. These values were significantly lower than those of NCT, which showed no mortality in the second and third years after planting, and presented a 97 % survival in the fourth growing season (χ^2 : 6.32, p : 0.01, χ : 5.68, p : 0.01, χ : 6.79, p : 0.009 for the

Table 1 Frequency and cover of the species registered in the forest gap “empastado” were the field experiment was installed

Species	Frequency (%)	Cover average (SE) (%)
<i>Poa pratensis</i> L. ^a	100	44.9 (8.2)
<i>Trifolium repens</i> L. ^a	100	36 (6.8)
<i>Taraxacum officinale</i> L. ^a	50	6.8 (2.4)
<i>Rumex acetosella</i> L. ^a	50	1.4 (0.4)
<i>Osmorhiza chilensis</i> Hook. & Arn.	40	10.7 (4.9)
<i>Bromus</i> sp.	40	10 (6.7)
<i>Vicia nigricans</i> Hook. & Arn.	40	8.7 (1.5)
<i>Acaena ovalifolia</i> Ruiz & Pav.	40	6 (3.7)
<i>Potentilla chilensis</i> (L.)	30	11.3 (4.1)
<i>Veronica serpyllifolia</i> L. ^a	30	0.7 (0.3)
<i>Viola maculata</i> Cav., Mabb.	10	7
<i>Acaena pinnatifida</i> Ruiz & Pav.	10	4
<i>Ribes cucullatum</i> Hook. & Arn. ^b	10	3
<i>Calceolaria filicaulis</i> Closs.	10	2

Native herbs are unmarked

^a Exotic herbs^b Shrubs

second, third and fourth growing season, respectively).

Nursery experiment

Seedling performance

During the two seasons considered, seedling variations in both height and diameter showed similar patterns for all treatment combinations. At the end of both growing seasons, and for both watering levels, only NCT seedlings increased in height, while CT seedlings showed withered apical sprouts, with negative or no growth at all. During the first growing season (2009–2010), NCT seedlings grew, in height (0.80 ± 0.44 cm) significantly more than CT seedlings (-0.91 ± 0.85 cm). NCT seedlings grew 1.67 ± 0.17 mm in diameter, while CT seedlings grew 1.03 ± 0.18 mm. During the second growing season (2009–2011), NCT seedlings grew significantly more in height and diameter (1.20 ± 0.66 cm, and 2.68 ± 0.20 mm) than CT seedlings, which showed negative values (-1.42 ± 0.74 cm and 1.0 ± 0.14 mm, respectively). Different watering regimes, instead, did not produce significant increases. No significant interactions were found between factors (Fig. 5c, d; Table 2).

Predawn water potential values showed a similar pattern during the two sampling dates, although their values were not significantly different (Fig. 6c; Table 4).

Branch midday water potential was significantly less negative for HW seedling than for NW. The more negative values corresponded to CT-NW seedlings compared to NCT-HW at all sampling dates. The other two treatments (CT-HW and NCT-LW) showed intermediate values for all readings. No significant interactions were found between factors (Fig. 6d; Table 4).

Seedling survival in the first year was high (above 90 %) and showed no significant statistical differences between treatments. On the second season, however, seedling survival was 80 % for HW and 52 % for NW (χ : 10.7 p: 0.001), and 77 % for NCT and 55 % for CT (χ : 6.26, p: 0.01). Considering treatments combination, survival was 87 % for NCT-HW seedlings, dropping to only 37 % for CT-NW seedlings. The other two combination treatments showed intermediate values (73 and 67 % for CT-HW and NCT-NW, respectively).

Discussion

Since this study was carried out in only one lenga *empastado* exclosure, its results should be carefully considered when trying to extrapolate them to other lenga canopy gaps also disturbed by cattle grazing. In pristine lenga forests, however, the opening of canopy gaps due to the fall of senescent trees increases understory herbaceous cover in the first successional stages after this natural disturbance (Veblen et al. 1996). During this stage, the colonization of obligate

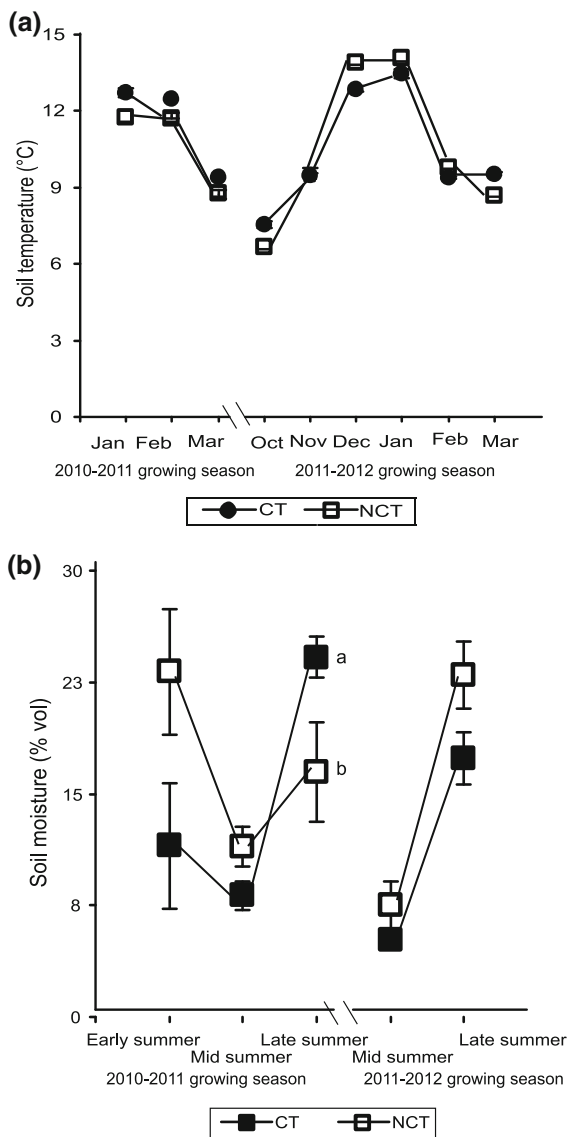


Fig. 3 Soil temperature (a) and moisture (b) (Mean \pm SE). Data registered during 2010–2011 and 2011–2012 growing seasons at field experiment. Different letters indicate significant differences (Bonferroni test, at $p < 0.05$)

heliophilous species occurs, with low cover of exotic species (Damascos and Rapoport 2002). The course of succession allows then lenga recovery in the newly created canopy gap (Veblen et al. 1996). But if a similar forest gap is, or has been disturbed by domestic livestock grazing, its understory is covered by non-native herbs species. Thus, in lenga gaps intensively grazed, non-native species may start dominating total understory cover, which in turn severely preclude lenga regeneration (Bava and Puig 1992; Quinteros

et al. 2012; Rusch 1992; Vázquez 2002). In the lenga gap studied, some understory native species were observed. The dominants, however, were non-natives, such as *P. pratensis*, *T. repens* and *T. officinale* which are associated to areas under high livestock impact, as it has been mentioned for other grazing-disturbed lenga forests of the region (Quinteros et al. 2012; Sánchez-Jardón et al. 2010, 2014). The presence of very few lenga seedlings in the gap analyzed confirms that grazing of *empastados* change vegetation structure and composition of the gaps in which they are produced, and that these changes may have long-time lasting effects, precluding lenga natural regeneration. Contrasting to this, Rechene (1995) found a density of 10.8 ± 3.6 lenga saplings/m² in a nearby allotment which remained under pristine conditions and showed no signs of long-term grazing.

Field experiment

Low moisture and high temperatures in the soil during the growing season have been reported as limiting factors for lenga regeneration in forest gaps (Rusch 1992). Our results showed water content was higher only in CT plots in March during the first growing season, when rains occurred 3 days before the reading. This could be due to increased water retention by the roots of grasses in the first centimeters of soil depth right after rainfall. For the subsequent measurements, soil moisture values were slightly higher in NCT plots as compared to CT plots (marginally significant values, Schabenberger and Pierce 2002). Other studies carried out in forest stands of *Quercus* sp. and *Fagus* sp. grown under Mediterranean conditions, showed that high herbaceous cover causes high water uptake, leaving less available water in the soil (Coll et al. 2004; Davis et al. 1999; Rey Benayas et al. 2003).

The precipitation in three growing seasons analyzed represented 86, 95, and 113 % of the long term mean for the growing season, respectively. It is interesting to note, however, that rains were below normal at the beginning of the three growing seasons analyzed. These data give a general idea of the rainfall pattern in the area and correspond to the Percy meteorological station, which is the nearest the study area (located about 5 km to the East). However, it is important to mention that this meteorological station is located at 750 m a.s.l., and its precipitation may be considerably lower than that received in the study

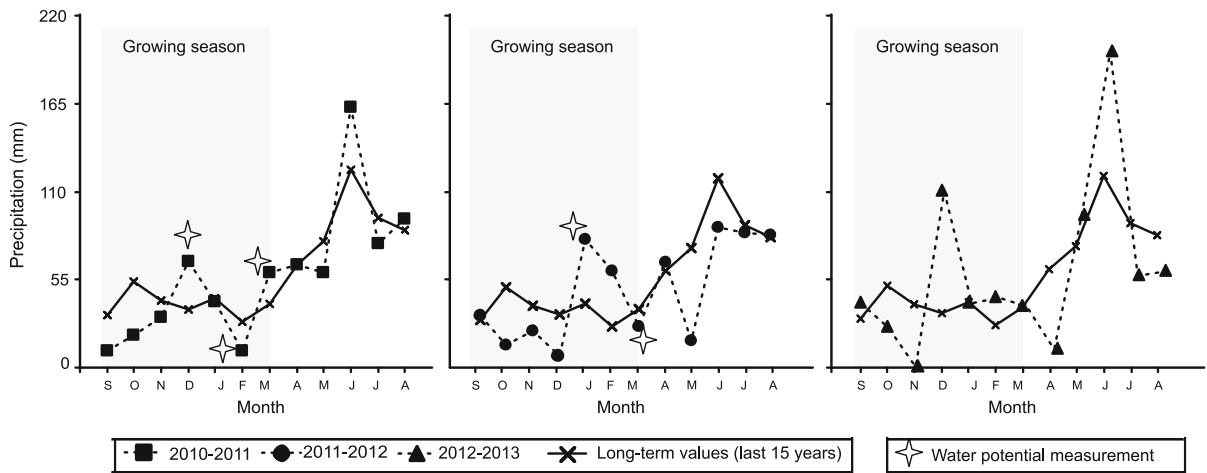


Fig. 4 Precipitation (long-term and for the studied periods of 2010–2011, 2011–2012 and 2012–2013) registered at Percy meteorological station, located 5 km to the east of Huemules study site in Patagonia, Argentina

area. It should be taken into account, however, that the sum of all precipitation during each seasons considered were within the average values for this mesic site, and that lenga seedlings may have not suffered from water stress.

Seedlings performance

This study was carried out in the early stages of lenga seedlings establishment, in a grazing-disturbed forest gap where light is not a limiting factor, but in which root competition among lenga seedlings and herbaceous or grass vegetation may be important. Former studies around the world have established that root competition between tree seedlings and herbaceous and grass vegetation may be severe in the upper soil horizons (Coll et al. 2004). In our study and under field conditions, lenga seedling survival was high in general. This high survival rate was also noticed by another study carried out in different areas of Patagonia showing similar mediterranean type of climate (Urretavizcaya et al. 2012). This study showed that in grazing-free areas, lenga seedlings may survive and develop even in areas in which annual precipitation only reaches 500 mm (Urretavizcaya et al. 2012). This may be in part due to the fact that under pristine conditions, lenga presents mechanism to avoid soil water stress (Henn et al. 2014; Martínez Pastur et al. 2011; Peri et al. 2009) and that roots of native understory species do not compete for this resource

with lenga seedlings. This assertion may be corroborated by the fact that in the southernmost part of its distribution, the understory of pristine lenga forests is mainly composed of sparse native herbaceous and shrubs species with low cover and diversity (Lencinas et al. 2008). Contrasting with this and in the *empastado* that comprise our study site, it was evident that competition affected survival in the second, third and fourth growing seasons, and NCT showed significantly higher survival than CT. These results are in close agreement with those reported by Heinemann and Kitzberger (2006), in other lenga forest grown under a similar Mediterranean climate. Furthermore, Dezzotti et al. (2003) showed similar effects on seedling survival by removing herbaceous vegetation around tree seedlings of the related *Nothofagus dombeyi*, *N. obliqua* and *N. nervosa* grown of northern Patagonia. Decreasing competition from nearby herbaceous species also showed higher growth rates for seedlings of *Quercus* sp., a related genus of the same *Fagales* order that grows under a similar Mediterranean climate of the northern Hemisphere (Davis et al. 1999; Rey Benayas et al. 2003). Apart from its effect on seedling survival, our study showed that competition effect was more visible on seedlings diameter growth than on height growth.

Branch midday water potential indicated a better water status for NCT seedlings as compared to CT seedlings for the whole sampling period. It is interesting to note that in the field, significant

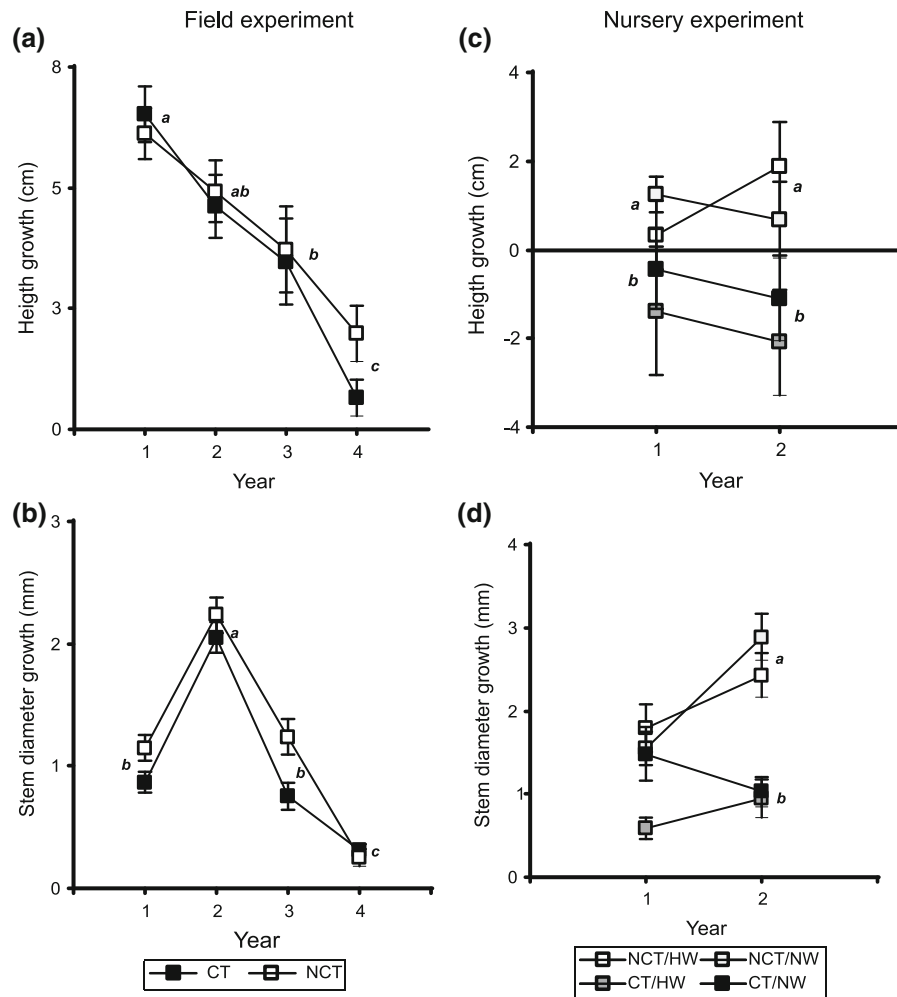


Fig. 5 Lenga seedling height and stem diameter growth (Mean \pm SE) under field (a, b) and nursery conditions (c, d). These measurements were taken at the end of each growing season. In the field experiment, growth was analyzed considering the competition treatment (CT competition treatment, NCT non-competition treatment) and year after planting (a, b).

In the nursery experiment, growth was determined for CT/NW competition treatment and normal watering, CT/HW competition treatment and high watering, NCT/NW non-competition treatment and normal watering, NCT/HW non-competition treatment and high watering. Different letters indicate significant differences (Bonferroni test at $p < 0.05$)

differences in midday water potential were found when accumulated rains during the month previous to the readings was below the 15 year of average values. As mentioned before, Peri et al. (2009) found that lenga has mechanisms to avoid plant water stress. This was evidenced by the less negative water potential values measured in NCT as compared to CT (see Fig. 6b; Table 4).

Seedlings used in the experiment were produced under greenhouse in ferti-irrigated containers. This enrichment prior to planting may have caused a

significant increases in seedlings growth right after field planting in relation to the rest of the evaluated period, as pointed out by Owston et al. (1992). It has been pointed out that lenga seedlings are well adapted to high light levels if soil water is not a limiting factor. Of course, if soil water is limiting, they reduce their photosynthetic rate, and then their growth (Peri et al. 2009). Another factor to be considered is temperature. It has been demonstrated that in lenga forests, slightly higher ambient temperatures may be found in gaps than within the forest canopy. However, these slightly

Table 2 Analysis of variance to test for the effects of the competition [competition treatment (CT) and non- competition treatment (NCT)] and time after planting (years: 1–4), on height and stem diameter growth in Huemules field

experimental site. In the nursery experiment, the effects of the competition (CT and NCT) and watering [high watering (HW) and normal watering (NW)] were analyzed

	DF effect	DF error	Height growth		Stem diameter growth	
			F	p-level	F	p-level
Fields						
Competition	1	28	0.11	0.74	2.03	0.16
Year	3	424	22.9	<0.001*	113.3	<0.001*
Competition × year	3	424	0.74	0.52	2.29	0.08
Nursery						
Competition	1	191	8.82	0.003*	33.02	0.001*
Watering	1	191	0.34	0.55	2.62	0.10
Competition × watering	1	191	0.36	0.55	1.46	0.22

* Significant differences (Bonferroni test, at $p < 0.05$)

Table 3 Analysis of variance for the effects of the competition [competition treatment (CT) and no competition treatment (NCT)] on water potential at predawn and midday in Huemules field experimental site for early, mid and late summer, respectively

Growing season	Data	Water potential	Effect	Df effect	Df error	F	p-level
2010–2011	Early summer	Predawn	Competition	1	18	4.05	0.07
		Mid-day		1	18	0.13	0.73
	Mid summer	Predawn		1	18	0.84	0.37
		Mid-day		1	18	6.44	0.02*
	Late summer	Predawn		1	18	1.96	0.17
		Mid-day		1	18	0.54	0.47
2011–2012	Mid summer	Predawn	Competition	1	18	0.24	0.63
		Mid-day		1	18	0.62	0.44
	Late summer	Predawn		1	18	2.62	0.12
		Mid-day		1	18	12.26	0.002*

* Significant differences (Bonferroni test, at $p < 0.05$)

higher temperatures do not represent any constraint for survival and growth of lenga seedlings (Piper et al. 2013; Tercero-Bucardo et al. 2007).

Nursery experiment

In nursery conditions, NCT seedlings grew more compared to CT seedlings. Competition treatment affected seedlings height growth, which also showed bud desiccation. During the second growing season, CT seedlings showed negative growth values. While this may appear first hand as unlikely to occur, it is important to mention that when exposed to some

environmental stress (in this case competition for water), lenga seedlings may experience the death of the apical meristem. Should this occur, a lateral bud may take its place and then the seedling resumes its growth in height (Barthélémy et al. 1999). The death of the apical meristem under high irradiance and water stress has also been reported for the related *Nothofagus nervosa* and *N. obliqua* by Varela et al. (2010). Furthermore, our results were somehow similar to those reported for some species of the related *Quercus* genus, in which high grass cover affected height growth and produced seedling desiccation (Koukoura and Menke 1995). Our results also coincide with those

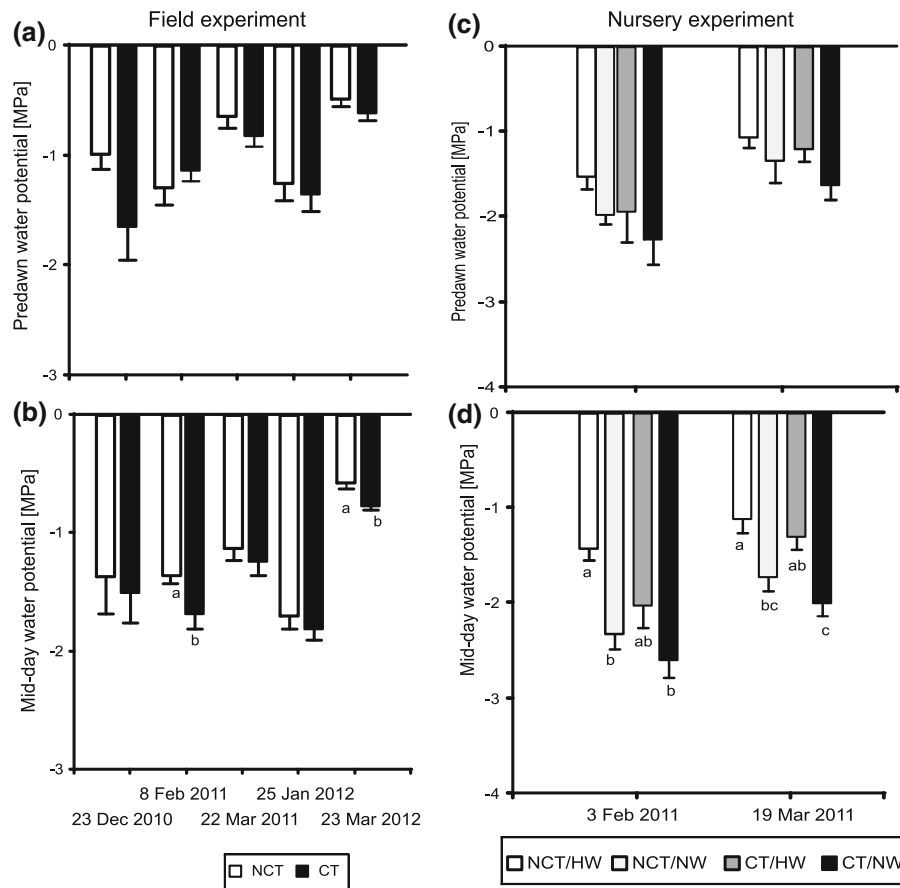


Fig. 6 Values of branch water potential of lenga seedlings (Mean \pm SE) at predawn and mid-day, in the field (**a**, **b**) and in the nursery experiments (**c**, **d**). In the field experiment, it was determined in seedlings under competition treatment (CT) and non-competition treatment (NCT) during two growing seasons. In the nursery, it was determined under CT/NW competition

treatment and normal watering, CT/HW competition treatment and high watering, NCT/NW non-competition treatment and normal watering, NCT/HW non-competition treatment and high watering, during one growing season. Different letters indicate significant differences (Bonferroni test, at $p < 0.05$)

reported for *Fagus* sp. seedlings, in which the absence of belowground competition produced greater diameters and height growth rates (Coll et al. 2004). In the nursery, the use of containers forced the grass and seedling roots to develop in a limited exploitable soil volume (Coll et al. 2004). This fact may increase the effect of competition treatment as compared to the field experiment. Extra irrigation, however, showed no extra effects on seedling growth. With regard to this, we could not support the hypothesis that in *empastados*, the lack of enough soil moisture during the growing season could be highly detrimental for lenga seedling establishment as suggested by Rusch (1992). In the same way as in growth, seedling survival was significantly higher in NCT than CT seedlings.

Differences were recorded in water potential in relation to levels of irrigation and competition treatments; however the interaction of both treatments was not significant. By relating water status with growth, it can be noted that lenga seedlings in absence of competition and with a high irrigation regime (NCT-HW) did not show better growth than NCT-NW seedlings. This may indicate that lenga seedlings may grow satisfactorily under field conditions with normal precipitation regimes, providing a planting site free of competing herbs and grasses (NCT-NW). This corroborates what Urretavizcaya et al. (2012) found in the drier sites of lenga distribution. They found that in these sites, lenga seedlings planted in restoration trials may growth satisfactorily, provided the site was free of

Table 4 Analysis of variance for the effects of the competition [competition treatment (CT) and non-competition treatment (NCT)] and watering [high watering (HW) and normal

watering (NW)] treatments, on water potential at predawn and midday in nursery experiment. These data are for the 2011–2012 growing season

Data	Water potential	Effect	Df effect	Df error	F	p-level
Early summer	Mid-day	Competition	1	16	10.74	<0.01*
		Watering	1	16	0.67	0.42
		Competition × watering	1	16	0.34	0.56
Mid summer	Predawn	Competition	1	16	2.16	0.16
		Watering	1	16	2.68	0.12
		Competition × watering	1	16	0.09	0.77
	Mid-day	Competition	1	16	6.61	0.02*
		Watering	1	16	18.69	<0.01*
		Competition × watering	1	16	0.87	0.36
Late summer	Predawn	Competition	1	16	1.43	0.24
		Watering	1	16	3.98	0.06
		Competition × watering	1	16	0.16	0.69
	Mid-day	Competition	1	16	3.11	0.09
		Watering	1	16	24.85	<0.01*
		Competition × watering	1	16	0.15	0.70

* Significant differences (Bonferroni test, at $p < 0.05$)

grazing by exotic grazers and that browsing by the European hare. This lack of response to above-normal irrigation levels have been reported for other *Nothofagus* species, showing that they are adapted to low or moderate water deficits without significant changes in growth parameters (Varela et al. 2010). This may explain, in part, why lenga is adapted to live in a wide range of precipitation regimes (Urretavizcaya et al. 2012; Veblen et al. 1977).

Our study was aimed at understanding some of the mechanisms by which *empastados* limit the development of lenga seedlings. To be conclusive, however, our results should be corroborated with field studies in other lenga environments. The formation of *empastados* in central and northern Patagonia is of particular concern because livestock pressure is increasing. In Patagonian forests, restoration and maintenance of former ecosystem functions and services require the knowledge about the mechanisms involved in the interaction lenga-herbs. In this sense and for successfully restoring grazing damaged lenga forest stands, other aspects should be considered. Among them, the effects of *empastados* on lenga seed germination and early seedling dynamics.

Previous observations showed that passive restoration alone (such as the removal of grazing in

empastados), was ineffective to allow the restoration of former lenga forests in the short term. In these areas, instead, active restoration practices (by planting lenga seedlings) seems to be necessary to accelerate the process of lenga forest stands recovery. The success in these restoration activities (i.e. increases in lenga seedling survival and growth), however, could be enhanced by simply reducing nearby competition of grasses and herbs. Irrigation, instead, may not be necessary to improve lenga seedlings performance, providing that rains during the growing season are within the average for the site.

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