

Using field and nursery treatments to establish *Quercus suber* seedlings in Mediterranean degraded shrubland

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Cork oak (*Quercus suber* L.) is a suitable species for restoring Mediterranean ecosystems due to its capacity to resprout after wildfires and its economic importance for the use of cork. Several studies have focused on improving the seedling quality and abiotic conditions at the outplanting site to favour the field performance of *Q. suber*, however, most studies have been conducted by independently testing treatments. The aim of this study was to assess the combined effect of three techniques that focused on reforestation success with *Q. suber* in Mediterranean degraded shrubland: (i) a nursery technique to improve root system development, such as the use of deep containers to develop a longer tap root, combined with two field techniques such as (ii) the use of tree shelters to diminish solar radiation stress, and (iii) shrubland treatments to reduce competition for soil water and nutrients. For this purpose, 1-year-old *Q. suber* seedlings were grown in two containers types: a shallow container (CCS-18) and a deep container (CCL-30). Seedlings were established in a degraded shrubland at three experimental sites in the Calderona mountain range of Castellón, Spain. A factorial design was combined based on container type (CCS-18 and CCL-30), shrubland management (undisturbed shrubland and cleared shrubland in strips) and tree shelters (vegetable fibre tree shelters and no tree shelters). After 2 years of monitoring, the outplanting results indicated that using: (i) a deep container produced a longer taproot, but did not favour better survival or better field performance of seedlings; (ii) tree shelters improved the microweather conditions around seedlings, particularly by reducing excess incoming solar radiation; (iii) cleared shrubland strips reduced competition for soil water by favouring a higher water potential, better maximum photochemical PSII efficiency and higher survival rates for the seedlings established into cleared sites. The results indicate that the cleared shrubland treatment effects overlap the effects of using deep containers and tree shelters. This, in turn, reveals that shrubland clearing is the most suitable technique for favouring the introduction of a resprouter species like *Q. suber* into ecosystems characterized by predominantly degraded shrublands.

Keywords: Deep Container, Tree Shelters, Cleared Shrubbyland, Ecological Restoration

Introduction

Different biotic and abiotic factors constrain the success of reforestation projects in dry and semiarid degraded areas (Vallejo et al. 2012, Oliet et al. 2015). Stress conditions due to both intense solar radiation, and water and nutrient deficits in introduced seedlings, is one of the main factors to affect the success of forest restoration projects in dry ecosystems (Cortina et al. 2009). Previous studies have analyzed several treatments aimed at reducing these stressful conditions during site outplanting. Some of these treatments have focused on improving root system structure and growth to increase water uptake and to avoid water stress (Chirino et al. 2008, Mariotti et al. 2015b). Other studies have reduced incoming solar radiation stress (Bellot et al. 2002) or competition with co-existing vegetation by shrubland management techniques (Pérez-Devesa et al. 2008, Bashan & Bar-Massada 2017). However, in most studied cases, the different reforesta-

tion techniques followed to improve seedling introduction into degraded shrubland have been analyzed individually and have not been combined.

In Spain, species of the *Quercus* genus are very important for the forest restoration of Mediterranean ecosystems. Cork oak (*Quercus suber* L.) is a very interesting typical resprouter Mediterranean species for restoring fire-prone ecosystems (Leite et al. 2018). Like other *Quercus* species, cork oak develops a long taproot during early development in the nursery (Chirino et al. 2005, Pemán et al. 2006). Therefore, using shallow containers and low volumes physically limits its root system growth (Domínguez et al. 2006, Puértolas et al. 2010). Moreover, field conditions impose other limitations for seedlings to be established in the countryside. Tree shelters have been used to improve microclimatic conditions and protect against herbivory in outplanted seedlings (Puértolas et al. 2010, Defaa et al. 2015, Mariotti et al. 2015a). Photo-

synthetic photon flux density, air temperature and relative humidity are climate variables that are affected by the use of tree shelters (Devine & Harrington 2008). Previous studies have shown that tree shelters help improving seedling survival and growth (Vallejo et al. 2006, Andrews et al. 2010). Tree shelter design (height, ventilation and the materials used) determines the protective effect on seedlings (Bellot et al. 2002, Puértolas et al. 2010, García 2016). For this reason, the use of tree shelters built according to an unusual design of vegetable fibres was considered herein.

Degraded ecosystems are characterized by shrubland vegetation dominated by seeder species that well tolerate drought conditions and have a high degree of competitive characteristics (Pausas & Keeley 2014). Under these conditions, shrubland management techniques to reduce the competitiveness of standing vegetation have been recommended in some studies (Pérez-Devesa et al. 2008), but the selected shrubland management technique (i.e., type, extension and width of clearing strips) must be carefully designed (Madrigal et al. 2014). The use of cleared shrubland strips in parallel with contour curves is a recommended technique for preventing soil erosion and facilitating the outplanting performance of seedlings. This technique is applied to strips no wider than a few metres. It is a selective treatment that helps to conserve juvenile or adult individuals of important species in cleared strips, thereby favouring wildlife feeding and sheltering (Serrada et al. 2005, Masso et al. 2015).

In this context, we hypothesize that the combined effects of several nursery and field techniques, all of which focus on reducing abiotic stress, would better improve the field performance of seedlings in restoration projects compared to each technique individually applied. To test this hypothesis, the objective of this study was to assess the combined effects of using deep containers for nursery culture, tree shelters and cleared shrubland strips with *Quercus suber* seedlings in early outplanting stages. For this purpose, the following effect were studied: (i) deep containers during nursery culture on seedling morphological characteristics; (ii) vegetable fibre tree shelters to improve the microclimatic conditions around seedlings and diminish solar radiation stress; (iii) cleared shrub-

lands strips to reduce competition for soil water between the introduced seedlings and the co-existing vegetation in restoration areas.

Material and Methods

Study area and experimental sites

The experimental sites are located in the Calderona mountain range in Castellón, Spain, where altitude ranges between 576 and 727 m a.s.l., with predominantly west-facing slopes between 13-40%. Sandy soils have developed from rock acidic sandstone. The climate is dry subhumid thermomediterranean, with an average annual rainfall of 485 mm and an average temperature of 15.2 °C (Segorbe HS weather station, data 1961-1990 – Pérez Cueva 1994). The dominant vegetation is degraded shrubland composed mainly of *Cistus monspeliensis* L., *Cistus salviifolius* L., and *Ulex parviflorus* Pourr. At the less degraded sites, individuals of *Quercus coccifera* L., *Rhamnus alaternus* L., *Pistacia lentiscus* L., and *Arbutus unedo* L. are also present. On the tree layer, some isolated individuals of *Pinus halepensis* M., and *Quercus suber* L., can be found. The herbaceous layer is dominated by *Brachypodium retusum* (Pers.) Beauv. In this study area, the three experimental sites were Puntal de la Bella, El Sapo and Tristán, which presented different geographical localizations, altitudes and west-facing slopes (Tab. 1).

Experiment design

In order to assess the combined effect of several techniques that focus on reducing abiotic stress in outplanting, a three-factor experimental design was implemented whose factors were: container type (CT_i), shrubland management (SM_i) and tree shelters (T_i).

Factor container type (CT_i): a CCS-18 container (shallow container: 18 cm deep; volume of 353 cm³) and a CCL-30 container (deep container: 30 cm deep; volume of 589 cm³). Both employed container types were made of high-density polyethylene were cylindrically shaped and open-bottomed, and had a 5 cm diameter and a cultivation density of 318 seedlings m⁻² (Fig. S1 in Supplementary material). The shallow container represented the container widely used for culture in nurseries of the genus *Quercus* and other Mediterranean species,

while the deep container represented a technological innovation (Chirino et al. 2008). Both containers were manufactured by CETAP-Antonio Matos Lda. (Forestry Containers Manufacturer Company, Espinho, Portugal) and the CCL-30 deep container is patented by this company (Ref. no. 9976, Boletim Propiedade Industrial no. 11-2004, Portugal).

Factor tree shelters (T_i): vegetable fibre tree shelters (VFT). A panel of vegetable fibre was composed of wicker branches (*Salix purpurea*), 0.50 m wide and 1.00 m long. Wicker branches (length: 1.00 m; diameter: 0.3-0.8 cm) were woven with a wire at four height levels (20, 40, 60 and 80 cm) to guarantee a stable structure. To build tree shelters, the vegetable fibre panel was placed in an upright position, whose ends were joined to form a cylinder (20 cm diameter, 1.00 m high). A bamboo cane (height: 1.00 m, diameter: ~1.2 cm) was needed to close the panel and form a cylinder to provide vertical support. In the field, tree shelters were buried in soil at a depth of 10-15 cm, along with three other bamboo canes, to confer tree shelters more stability and to avoid the flag effect (Fig. S2, Supplementary material). The opposite experimental treatment was to not use tree shelters (NVFT).

Factor shrubland management (SM_i): cleared shrubland strips (CSs), 3 m wide, were made running perpendicularly to the hillside's main slope. CSs were alternated with undisturbed shrubland strips (USs), 5 m wide (Fig. S3, Supplementary material). The clearing of strips at the experimental sites was done with a STIHL FS410 C-E® brush cutter (Andreas Stihl Ltd., Stihl House, Surrey, UK) using bush knives and grinding. At each site, four 3-metre wide strips of CSs and four 5-metre strips of USs were prepared. In USs, a 1-metre diameter circle was cleared to plant *Q. suber* seedlings.

In each strip (USs and CSs), the other experimental treatments were performed according to the three-factor design. Twelve planting holes (60 × 60 × 60 cm) were made every 3 m on the centre line of each strip. Holes were made with the help of a pneumatic hammer. No soil was removed from holes to avoid inverting soil horizons. Ninety-six one-year old *Quercus suber* L. seedlings (12 seedlings × 2 container types × 2 shrubland management treatments × 2 tree shelters type as treatment) were planted at each experimental site in January 2011. Thus at each site, there were 48 seedlings per level of container type factor, 24 seedlings per level of shrubland management factor and 24 seedlings per level of tree shelters factor. Twelve seedlings were used to analyze the interactions between factors.

Plant material and nursery culture

Cork oak (*Quercus suber* L.) seedlings were grown for a 1-year period in two container types: CCS-18 and CCL-30. Cultivation

Tab. 1 - Geographical coordinates, altitude, slope and slope-facing of the experimental sites.

Variable	Experimental sites		
	Puntal de la Bella	El Sapo	Tristán
Latitude	39° 41' N	39° 43' N	39° 44' N
Longitude	00° 23' W	00° 27' W	00° 28' W
Altitude (m a.s.l.)	576	722	727
Slope (%)	13	40	20
Slope-facing	270° W	340° NW	210° SW

was carried out at the *Centro de Investigación y Experimentación Forestal* (CIEF) in Quart de Poblet (Valencia, Spain – 39° 28' N, 00° 31' W; 70 m a.s.l), with a mean annual rainfall of 462 mm and a mean annual temperature of 16.9 °C (Pérez Cueva 1994). The cork oak acorns from the Calderona mountain range were supplied by the Regional Government Forest Service (Banc de Llavors, Quart de Poblet) and were seeded in January 2010. The substrate was a mixture of limed peat (60%) and coconut peat (40%). A slow-release fertilizer (Plantacote plus®, N-P-K: 14-8-14; approaches longevity of 12 months at a mean temperature of 21 °C) was mixed with the substrate at a substrate dose of 1 g L⁻¹. The watering regime was weekly (2 days week⁻¹), with 15 mm applied in autumn, winter and spring, and 25 mm in summer.

Seedling morphology and biomass

At the end of nursery culture after 12 months of cultivation, morphological characterization was carried out. Fifteen seedlings per treatment were randomly sampled, and stem height and basal diameter were measured. Seedlings were cut at the cotyledon insertion point and separated into four fractions: leaves, stem, fine roots (diameter < 2 mm) and taproot (diameter > 2 mm). The dry weight of each fraction was determined after oven drying at 65 °C for 48 h. Shoot dry weight (DW_s), root dry weight (DW_r), seedling dry weight (SM_r) were calculated. Two morphological indices were also calculated: root/shoot ratio (DW_r/DW_s) and root weight ratio (DW_r/SM_r).

Vegetable fibre tree shelters: inside and outside environmental conditions

In order to compare the environmental conditions inside and outside the vegetable fibre tree shelters (VFT), three climatic variables were monitored. In one set in the nursery, one Ho8-032-08 HOBO® sensor with a data-logger (Onset Computer Corporation, USA) was placed inside three VFT to measure air temperature (T^a, °C) and relative humidity (RH, %). Photosynthetic photon flux density (PPFD, μmol m⁻² s⁻¹) was measured using a Ha-Li HOBO® sensor (Onset Computer Corporation, USA). Simultaneously under open site conditions, the same variables (T^a, RH and PPFD) were measured by similar sensors. Vapour pressure deficit (VPD, kPa) was determined from T^a and HR. These measurements were taken in the nursery in August 2010 on 7 sunny days.

Rainfall, air temperature and relative humidity in the study area

The outplanting period lasted 2 years: 2011, the wetter year with an annual rainfall of 528.5 mm, a mean air temperature of 16.0 °C and 68.8% relative humidity (CEAMET data, the weather station in Al-turas, Castellón). April (105.4 mm), March (101.2 mm) and November (85.7 mm) were

the wettest months, while August (4 mm) was the driest month (Fig. S4, Supplementary material). In summer, accumulated rainfall came to 49.8 mm. There were 16 rainfall events, for which more than 5 mm were recorded (minimum: 7.9 mm and maximum: 11.7 mm). However, 2012 was a less rainy year (397.8 mm) given the 25% drop compared to the annual rainfall of 2011, and the 18% drop compared with the region's average annual (485 mm). The average temperature was 16.1 °C, while relative humidity was 62.2%. February (0.2 mm), May (0.8 mm), December (2.5 mm), August (4.2 mm) and July (5.0 mm) were the least rainy months, when rainfall did not exceed 5 mm month⁻¹. The wettest month was October (172.8 mm – Fig. S4, Supplementary material) due to extraordinary rainfall (84.5 mm). The summer of 2012 was very dry (14 mm). Although 10 rainy events were reported, none exceeded 4 mm. Consequently, there were 120 days (June 21 to September 20) with no significant rainfall (> 5 mm).

Seedling performance

Seedling survival and growth in stem height (H_s) and basal diameter (D) at the experimental sites were measured for 100% individuals in the experimental plots in January 2011 after plantation, and in June and November 2011 and 2012. The relative growth rate (RGR) was calculated by the differences in the average stem height (H_s) and basal diameter (D) of seedlings between the last measurement (t₂) and the first measurement (t₁) using $RGR = [\ln(H_s t_2) - \ln(H_s t_1)] / (t_2 - t_1)$. To determine the effect of the experimental treatments on seedling water stress, soil moisture and several ecophysiology variables were measured during the first outplanting year (2011). One part of the measures was taken at the end of spring (June), and coincided with the high soil water content conditions (low water stress). The others were taken in summer (August) and coincided with the period with the lowest soil water content conditions (maximum water stress). Five seedlings per experimental treatment and site were randomly selected for this purpose. In each seedling, the following were measured: (1) water potential at midday (Ψ_{md}, -MPa) using a Sholander pressure chamber (Soil Moisture 3005®, Soil moisture Equipment Corp., Santa Barbara, CA, USA); (2) leaf chlorophyll content (LCC, no units) with SPAD 502® (Spectrum Technology, Inc. Aurora, USA); (3) maximum quantum yield of Photosystem II (PSII; Fv/Fm under dark conditions) at predawn (Fv/Fm_{pd}) and midday (Fv/Fm_{md}) by a fluorimeter PAM 2100® (Heinz Walz GmbH, Germany). Simultaneously, soil water content (SWC, %) at a 15- to 20-cm depth was measured in all the selected seedlings for ecophysiological measurements. For this purpose, an EC-5 probe and an instant reader ProCheck (both manufactured by Decagon Devices, Inc., USA) were used. The intention was to

assess the effect of the experimental treatments shrubland management (CSs and USs) and tree shelters (VFT and NVFT) on the climate variables. Air temperature (T^a, °C), relative humidity (RH, %) and photosynthetic photon flux density (PPFD, μmol m⁻² s⁻¹) were monitored in both CSs and USs, and in the seedlings with VFT and without vegetable fibre tree shelters (NVFT). For these measures, HOBO® sensors (Onset Computer Corporation, USA) model Ho8-032-08, with a datalogger for T^a and HR, and Ha-Li for the PPFD measurements, were used.

Statistical analyses

The statistical analysis was carried out with the SPSS® statistical software, v. 21.0 (SPSS Inc. Chicago, Illinois, USA). The data on the seedling morphological characteristics at the end of the nursery culture were compared by a t-test, when the variables fulfilled theoretical assumptions. A non-parametric test (W by Wilcoxon) was run for the variables that did not match theoretical assumptions. To test the environmental conditions (T^a, RH, VPD, and PPFD) inside and outside the vegetable fibre tree shelters (VFT), an analysis of variance (one-way ANOVA, Tukey's HSD post-hoc test) was carried out. The comparison of the T^a, RH, VPD, and PPFD data in the field was made by a two-factor ANOVA (Tukey's HSD post-hoc test) using shrubland management (SM_r) and tree shelters (T_r) as fixed factors. The data of relative growth ratio in stem height (H_s-RGR) and basal diameter (D-RGR), soil water content (SWC), water potential at midday (Ψ_{md}), leaf chlorophyll content (LCC) and maximum quantum yield of Photosystem II (Fv/Fm_{pd} and Fv/Fm_{md}) were compared by a GLM univariate (three-way ANOVA, Tukey's HSD post-hoc test) at each site to analyze the effect of the experimental factors: container type (CT_r), shrubland management (SM_r), and tree shelters (T_r). Data were transformed whenever necessary to assure the assumptions of the ANOVA. The seedling survival data were analyzed by the Kaplan-Meier test (Log Rank-Mantel-Cox) to each experimental factor.

Results

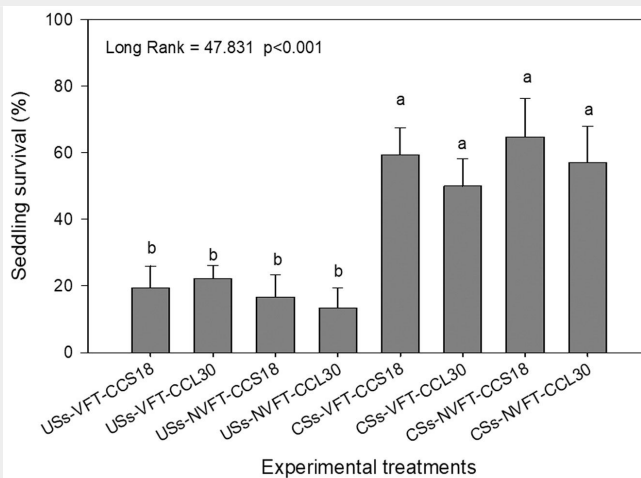
Morphological characteristics of seedlings

The analysis of the morphological characteristics of seedlings at the end of the culture period in the nursery indicated several differences between seedlings (Tab. 2). The seedlings grown in the deep CCL-30 container presented higher stem height (p = 0.009), longer taproot (p < 0.001), higher shoot dry weight (p = 0.016) and seedling dry weight (p = 0.019 – Tab. 2) values than the seedlings grown in CCS-18. Two tendencies were observed. The seedlings in CCL-30 tended to present higher taproot dry weight (root > 2 mm; p = 0.081) and root dry weight (p = 0.076 – Tab. 2) values than

Tab. 2 - Seedling morphological characteristics (mean ± standard error) at the end of the nursery culture. The results of t-test or Wilcoxon test for differences between container types are reported, along with their statistical significance. (DW_S): Shoot dry weight; (DW_R): root dry weight; (SM_T): seedling dry weight; (***) : $p < 0.001$; (**) : $p < 0.01$; (*) : $p < 0.05$; (ns): non-significant.

Variables	Units	Container types		Test applied	Significance
		CCS-18	CCL-30		
Stem height	cm	51.26 ± 3.60	66.46 ± 4.91	W by Wilcoxon	170.0 **
Root collar diameter	mm	6.70 ± 0.37	7.47 ± 0.48	W by Wilcoxon	194.0 ^{ns}
Taproot length	cm	17.28 ± 0.14	29.01 ± 0.22	W by Wilcoxon	120.0 ***
Taproot dry weight (>2 mm)	g	4.28 ± 0.51	5.95 ± 0.70	t-test	-1.811 ^{ns (1)}
Shoot dry weight	g	7.69 ± 0.94	12.66 ± 1.65	W by Wilcoxon	175.0 *
Root dry weight	g	4.73 ± 0.53	6.51 ± 0.81	t-test	-1.843 ^{ns (1)}
Seedling dry weight	g	12.42 ± 1.37	19.18 ± 2.41	W by Wilcoxon	176.0 *
DW_R/DW_S ratio	$g\ g^{-1}$	0.64 ± 0.05	0.56 ± 0.04	W by Wilcoxon	202.0 ^{ns}
DW_R/SM_T ratio	$g\ g^{-1}$	0.38 ± 0.02	0.35 ± 0.02	t-test	1.290 ^{ns}

Fig. 1 - Seedling survival by the combined effect of the three experimental treatments. (CSs): cleared shrubland strips; (USs): undisturbed shrubland strips; (VFT): vegetable fibre tree shelters; (NVFT): without vegetable fibre tree shelters; (CCS-18): shallow container; (CCL-30): deep container.



those grown in CCS-18. No significant differences between container types were found for the remaining analyzed morphological variables ($p > 0.05$ - Tab. 2).

Seedling survival and post-outplanting growth

The seedlings in the cleared shrubland strips (CSs), combined with vegetable fibre tree shelter (VFT) or without VFT (NVFT), and in container CCS-18 or CCL-30, maintained the highest survival values (Long Rank = 47.831; $p < 0.001$ - Fig. 1), with survival values ranging between 50-59%. A second analysis was run to consider only field treatments (*i.e.*, shrubland management and use of tree shelters). It confirmed that CSs was the main treatment (Long Rank =

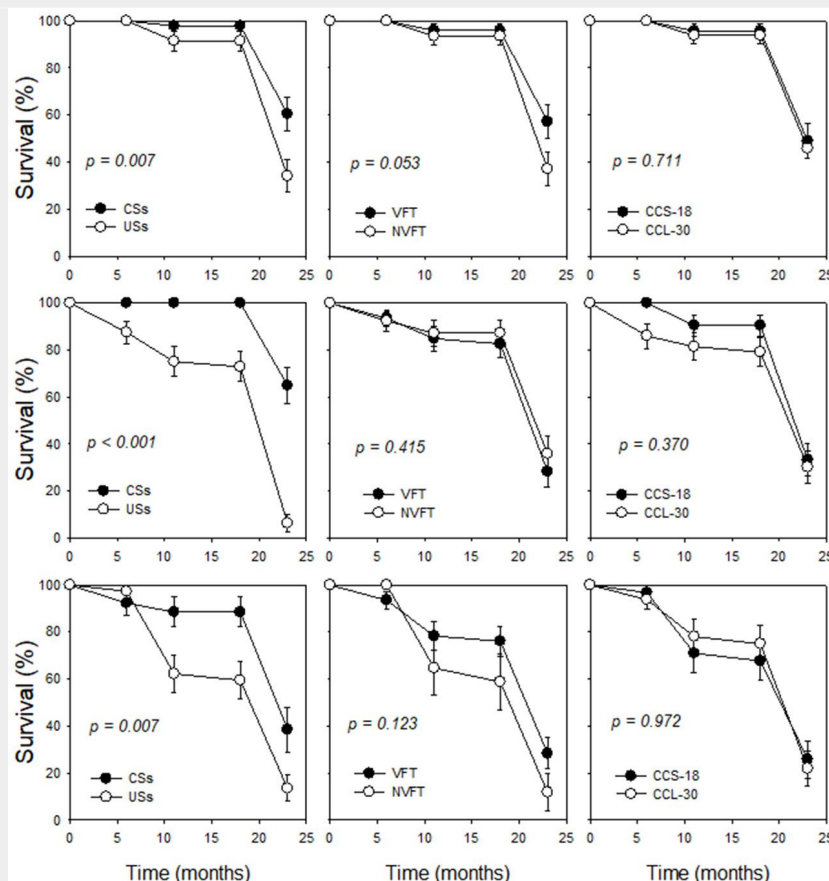


Fig. 2 - Seedling survival by the experimental factors at each site. The results are shown per experimental site: Puntal de la Bella (above), El Sapo (middle) and Tristán (below). Data at 6, 11, 18 and 23 months after planting (mean ± standard error). Kaplan Meier test results (Log Rank, p-value). Abbreviations: cleared shrubland strips (CSs); undisturbed shrubland strips (USs); vegetable fibre tree shelters (VFT); without vegetable fiber tree shelters (NVFT); shallow container (CCS-18); deep container (CCL-30).

Tab. 3 - Relative growth ratio (RGR) in stem height (H_s) and root collar diameter (D) of outplanted seedlings. Results per experimental site of the GLM univariate, three-way ANOVA. Factors: shrubland management (SM_f), tree shelters (T_f), and container type (CT_f). Mean \pm SE, effect of the main factors and interactions between factors (F value). (***) : $p < 0.001$; (**) : $p < 0.01$; (*) : $p < 0.05$; (ns): non-significant.

Factors	Levels/ F values	Puntal de la Bella		El Sapo		Tristán	
		H-RGR	D-RGR	H-RGR	D-RGR	H-RGR	D-RGR
Shrubland management (SM_f)	CSs	0.057 \pm 0.001	0.013 \pm 0.001	0.035 \pm 0.001	0.110 \pm 0.002	0.010 \pm 0.002	0.010 \pm 0.003
	USs	0.073 \pm 0.001	0.007 \pm 0.001	0.085 \pm 0.001	0.005 \pm 0.003	0.007 \pm 0.002	0.005 \pm 0.001
	F value	1.177 ^{ns}	13.486 ^{***}	7.349 ^{**}	3.591 ⁽¹⁾	0.732 ^{ns}	1.607 ^{ns}
Tree shelters (T_f)	VFT	0.084 \pm 0.001	0.008 \pm 0.001	0.006 \pm 0.001	0.006 \pm 0.001	0.009 \pm 0.002	0.007 \pm 0.002
	NVFT	0.046 \pm 0.001	0.012 \pm 0.001	0.003 \pm 0.001	0.010 \pm 0.004	0.006 \pm 0.001	0.010 \pm 0.003
	F value	6.267 [*]	5.275 [*]	0.820 ^{ns}	1.727 ^{ns}	0.403	1.111 ^{ns}
Container type (CT_f)	CCS-18	0.072 \pm 0.001	0.009 \pm 0.002	0.007 \pm 0.001	0.007 \pm 0.002	0.008 \pm 0.002	0.009 \pm 0.002
	CCL-30	0.059 \pm 0.001	0.011 \pm 0.001	0.005 \pm 0.001	0.009 \pm 0.002	0.009 \pm 0.002	0.006 \pm 0.002
	F value	0.740 ^{ns}	0.797 ^{ns}	0.590 ^{ns}	0.116 ^{ns}	0.008 ^{ns}	0.265 ^{ns}
Interactions among treatments	$SM_f \times T_f$	0.003 ^{ns}	1.024 ^{ns}	8.864 [*]	1.861 ^{ns}	0.258 ^{ns}	1.228 ^{ns}
	$SM_f \times CT_f$	0.345 ^{ns}	1.363 ^{ns}	0.265	0.108 ^{ns}	0.052 ^{ns}	0.050 ^{ns}
	$T_f \times CT_f$	0.034 ^{ns}	0.000 ^{ns}	1.316	0.086 ^{ns}	0.021 ^{ns}	0.023 ^{ns}
	$SM_f \times T_f \times CT_f$	1.112 ^{ns}	0.014 ^{ns}	0.629	2.083 ^{ns}	0.211 ^{ns}	0.254 ^{ns}

46.391; $p < 0.001$ – Fig. S5, Supplementary material) to affect seedling survival. This analysis demonstrated that using tree shelters did not affect seedling survival. The temporal seedling survival dynamics by the experimental factors at each site ratified that neither factor tree shelters (VFT vs. NVFT), nor factor container (CCS-18 vs. CCL-30) affected seedling survival. This analysis reported that, once again, the seedlings grown in CSs maintained the highest survival values ($p < 0.01$ – Fig. 2) at the three experimental sites. The seedlings grown in VFT only reflected a higher survival tendency ($p = 0.053$) at the Puntal de la Bella site. The factor container had no effect on seedling survival (Fig. 2). The differences among years associated with rainfall regimes showed that 2012 was less rainy (397.8 mm) than 2011 (528.5 mm), but this did not affect the differences between treatments, and the seedlings in CSs maintained the highest survival values (Fig. 2).

The growth response of seedlings to experimental treatments differed per site. The seedlings grown in CSs at Puntal de la Bella presented a higher D -RGR than those grown in USs ($p < 0.001$ – Tab. 3). In contrast, the seedlings grown in USs (El Sapo) gave higher H_s -RGR values than those grown in CSs ($p < 0.01$ – Tab. 3). Regarding D -RGR, a tendency ($p < 0.1$) was observed. The seedlings with VFT (Puntal de la Bella) had higher H_s -RGR ($p = 0.05$) and lower D -RGR ($p < 0.05$) values than those grown without VFT (Tab. 3). The factor container had no effect on the RGR (H_s and D) of seedlings. At Tristán, no treatment affected the RGR of seedlings ($p > 0.05$ – Tab. 3). An interaction between SM_f and T_f was observed at the El Sapo site in H RGR. A posterior analysis (one-way ANOVA) indicated that the seedlings grown in CSs had higher H_s -RGR values than those grown in USs ($F = 8.067$; $p = 0.006$). T_f did not affect this interaction.

Effects of shrubland management and tree shelters on seedlings' microclimatic conditions in the field

The measurements taken in the nursery indicated that VFT brought about no change in the T^a inside tree shelter ($p > 0.05$ – Tab. 4), but a significant decrease in RH (about 7%) and a significant drop in PPFd (about 59% of external sunlight) were observed. VPD significantly increased by 15% in relation to the external environmental conditions (Tab. 4).

At the experimental sites, shrubland management affected only the PPFd values. The seedlings grown in CSs had higher PPFd values (43% in June and 25% in August) than those grown in USs ($p < 0.001$ – Tab. 5). The use of tree shelters (VFT) significantly lowered the RH values (5.3% in June and 4.0% in August) and PPFd (54% in June and 31% in August – Tab. 5) in relation to the NVFT treatment. An interaction between SM_f and T_f was observed. A later analysis indicated that the seedlings under treatments CSs+NVFT had higher PPFd values than those subject to the other treatments in both June and August ($p < 0.001$ – Tab. 5). In June, USs+VFT had the lowest PPFd values, while CSs+VFT and USs+NVFT gave intermediate results. No significant differences were observed between them. In August, no significant differences in the

three treatments were noted (CSs+VFT, USs+VFT and Uss+NVFT – Tab. 5).

Soil water content and plant ecophysiology response

The analysis of the effect of experimental treatments (SM_f , T_f and CT_f) on soil water content (SWC), water potential at midday (Ψ_{md}), leaf chlorophyll content (LCC), and maximum quantum yield of Photosystem II (Fv/Fm at predawn and midday) per experimental site gave different results across sites. When spring ended (June 2011), the shrubland management factor had less effect on the analyzed variables. Only at the Tristán site the seedlings grown in CSs had higher SWC than those in USs ($p = 0.036$ – Fig. 3C), which favoured less stress in the seedlings in CSs (Ψ_{md} , $p = 0.015$ – Tab. S1, Supplementary material). A similar tendency ($p < 0.1$ – Tab. S1) was observed at the El Sapo site for SWC regarding the shrubland management factor.

All the SWC values were higher than 17%, which did not directly limit seedlings' physiological activity. Under these optimal soil moisture conditions, using vegetable fibre tree shelters or not, was the experimental factor with the stronger effect on water potential and Fv/Fm in seedlings. At the Puntal de la Bella site, the seedlings grown with VFT presented lower water stress

Tab. 4 - Comparison in the nursery of the environmental conditions inside and outside the vegetable fibre tree shelters (VFT). Data of air temperature (T^a), relative humidity (RH) and photosynthetic photon flux density (PPFD). The t-test results (mean \pm standard error). (***) : $p < 0.001$; (ns): non-significant.

Variable	Units	Outside VFT	Inside VFT	t-test
T^a	$^{\circ}C$	26.91 \pm 0.10	27.05 \pm 0.10	-1.050 ^{ns}
RH	%	72.61 \pm 0.42	67.88 \pm 0.43	8.752 ^{***}
VPD	kPa	1.10 \pm 0.02	1.32 \pm 0.02	-7.634 ^{***}
PPFD	$\mu mol\ m^{-2}\ s^{-1}$	1.07 \pm 0.02	0.44 \pm 0.02	-59.697 ^{***}

Tab. 5 - Effect of the experimental treatments' shrubland management (SM_f) and tree shelters (T_f) on climatic variables air temperature (T^a), relative humidity (RH), vapour pressure deficit (VPD) and photosynthetic photon flux density ($PPFD$). Measurements taken in June 2011 (above) and August 2011 (below). Results of the GLM univariate (two-way ANOVA). Factors: shrubland management and tree shelters; F value, Tukey's HSD post hoc test. (***): $p < 0.001$; (**): $p < 0.01$; (*): $p < 0.05$; (ns): non-significant.

Period	Factors	Param.	T^a	RH	VPD	$PPFD$
June 2011	Shrubland management (SM_f)	CSs	22.6 ± 0.2	69.8 ± 0.6	1.08 ± 0.03	523.1 ± 23.3 ^a
		USs	22.5 ± 0.2	70.1 ± 0.6	1.04 ± 0.02	298.5 ± 16.7 ^b
		F value	0.37 ^{ns}	1.64 ^{ns}	0.68 ^{ns}	61.35 ^{***}
	Tree shelters (T_f)	VFT	22.4 ± 0.1	68.1 ± 0.6 ^b	1.08 ± 0.02	258.1 ± 15.3 ^b
		NVFT	22.6 ± 0.2	71.9 ± 0.7 ^a	1.04 ± 0.03	563.6 ± 23.6 ^a
		F value	0.92 ^{ns}	18.48 ^{***}	0.92 ^{ns}	118.05 ^{***}
	$SM_f \times T_f$ interactions	CSs × VFT	22.3 ± 0.2	68.2 ± 0.9	1.07 ± 0.03	351.9 ± 25.8 ^b
		CSs × NVFT	22.9 ± 0.2	71.4 ± 1.0	1.10 ± 0.04	694.4 ± 36.6 ^a
		USs × VFT	22.5 ± 0.2	68.0 ± 0.8	1.09 ± 0.03	164.7 ± 15.0 ^c
		USs × NVFT	22.4 ± 0.2	72.3 ± 0.9	0.99 ± 0.03	432.8 ± 28.0 ^b
F value		3.20 ^{ns}	0.52 ^{ns}	3.98 ^{ns}	64.21 ^{***}	
Aug 2011	Shrubland management (SM_f)	CSs	22.9 ± 0.2	77.4 ± 0.9	0.9 ± 0.04	646.1 ± 30.1 ^a
		USs	22.3 ± 0.3	80.2 ± 1.0	0.9 ± 0.05	483.3 ± 28.9 ^b
		F value	0.38 ^{ns}	0.24 ^{ns}	1.68 ^{ns}	15.25 ^{***}
	Tree shelters (T_f)	VFT	22.2 ± 0.3	80.0 ± 1.1 ^a	0.87 ± 0.05	461.1 ± 26.2 ^b
		NVFT	22.9 ± 0.2	77.6 ± 0.9 ^b	0.85 ± 0.04	668.3 ± 32.0 ^a
		F value	0.92 ^{ns}	10.17 ^{**}	0.92 ^{ns}	25.12 ^{***}
	$SM_f \times T_f$ interactions	CSs × VFT	22.3 ± 0.4	80.5 ± 1.4	0.8 ± 0.07	472.5 ± 34.9 ^b
		CSs × NVFT	23.5 ± 0.3	74.4 ± 1.0	0.9 ± 0.05	819.6 ± 44.7 ^a
		USs × VFT	22.1 ± 0.2	79.6 ± 1.5	0.9 ± 0.08	449.7 ± 39.2 ^b
		USs × NVFT	22.6 ± 0.4	80.8 ± 1.4	0.8 ± 0.06	517.0 ± 42.4 ^b
F value		3.20 ^{ns}	0.58 ^{ns}	3.98 ^{ns}	18.14 ^{***}	

than those in NVFT ($p = 0.016$ – Fig. 3D), expressed as higher Ψ_{md} values. This result gave higher Fv/Fm_{pd} values ($p = 0.019$ – Fig. 3G) in the seedlings with VFT. At the Tristán site, the seedlings with VFT gave higher Ψ_{md} values ($p = 0.014$ – Fig. 3F) and higher Fv/Fm_{pd} ; $p = 0.018$ – Fig. 3I) than those without VFT. A similar result for Fv/Fm_{pd} ($p = 0.003$ – Fig. 3H) was observed at the El Sapo site.

The container type factor (CT_f) had little effect on the analyzed variables. Only at the Puntal de la Bella did the seedlings cultivated in CCL30 have higher LCC values than those in CCS-18 ($p = 0.041$ – Tab. S1, Supplementary material). Two interactions between factors were observed: an interaction between SM_f and CT_f in LCC ($p = 0.007$ – Tab. S1) at Puntal de la Bella, and another interaction between factors $T_f \times CT_f$ in Ψ_{md} ($p = 0.049$ – Tab. S1) at Tristán. In both cases, the one-way ANOVA did not find any effects of these factors separately ($p < 0.05$). The results of the GLM univariate three-way ANOVA (factors: tree shelters, container type and shrubland management) are shown in Tab. S1 and Tab. S7 in the Supplementary material.

As expected, at mid-summer the SWC values notably lowered in relation to the June values. However, shrubland management was the factor that most affected the variables analyzed at all the sites. At Puntal de la Bella, the seedlings grown in CSs had higher SWC ($p = 0.010$ – Fig. 4A) and, consequently, presented less water stress (Ψ_{md} , p

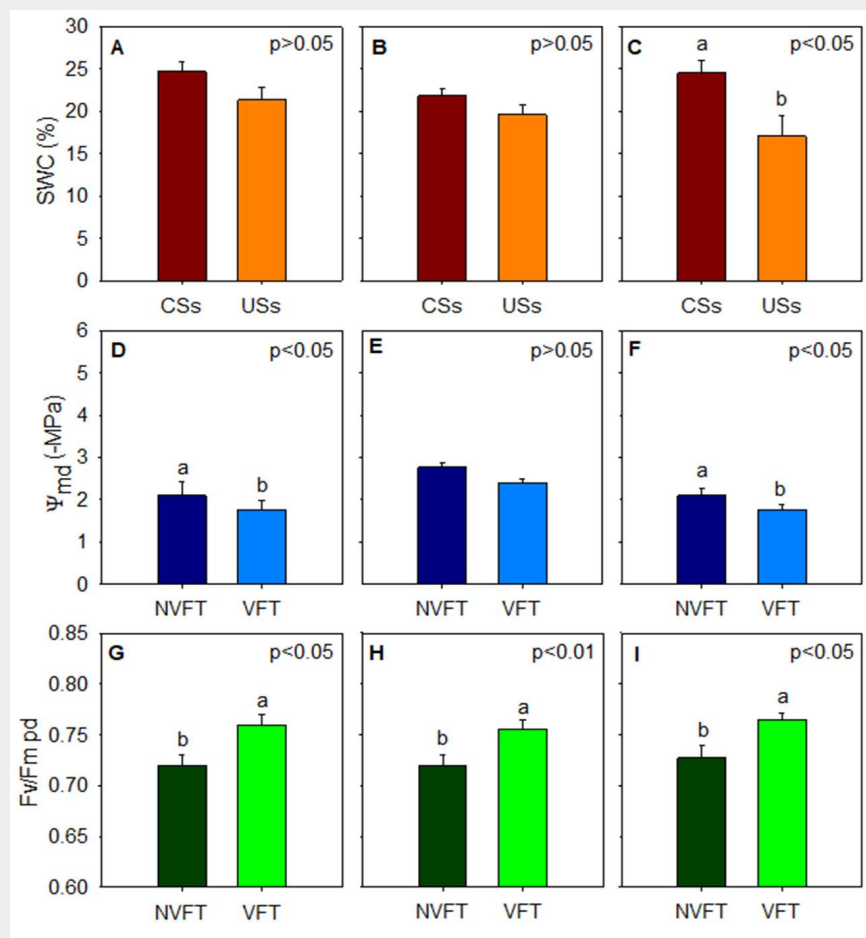
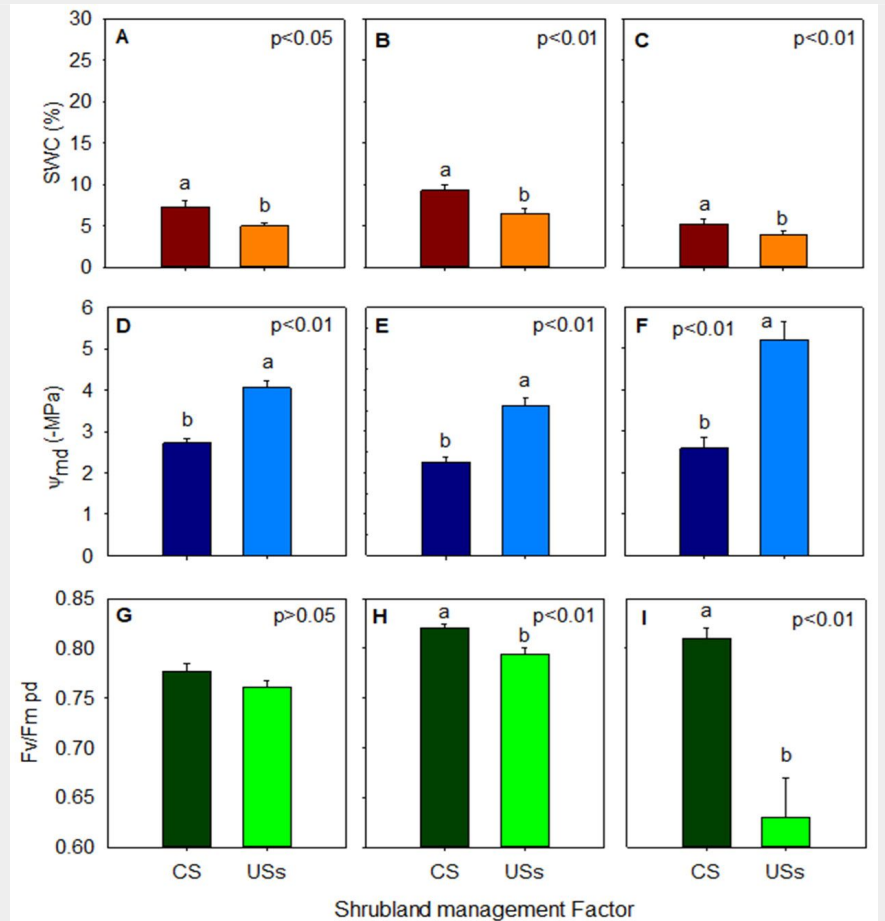


Fig. 3 - Measurements taken in spring of soil water content (SWC, %), water potential at midday (Ψ_{md} , -MPa), leaf chlorophyll content (LCC, units SPAD), and maximum efficiency of photosystem PSII (Fv/Fm) at predawn per experimental site. Results of the GLM univariate, three-way ANOVA; factors: tree shelters (T_f), container type (CT_f) and shrubland management (SM_f). Mean ± standard error. Values followed by the same letter were not significantly different ($p > 0.05$). Results shown by experimental sites: Puntal de la Bella (panels A, D and G), El Sapo (panels B, E and H) and Tristán (panels C, F and I).

Fig. 4 - Measurements taken in summer of soil water content (SWC, %), water potential at midday (Ψ_{md} , -MPa), and maximum efficiency of photosystem PSII (Fv/Fm) at predawn and midday per experimental site. Results of the GLM univariate, three-way ANOVA; factors: tree shelters (T_f), container type (CT_f) and shrubland management (SM_f). Mean \pm standard error. Values followed by the same letter are not significantly different ($p > 0.05$). Results shown by the experimental site Puntal de la Bella (panels A, D and G), El Sapo (panels B, E and H) and Tristán (panels C, F and I).



= 0.001 – Fig. 4D) and higher Fv/ $F_{m,md}$ values ($p = 0.014$ – Tab. S2, Supplementary material) than those in USs. Similar results were observed at El Sapo site in SWC ($p < 0.01$ – Fig. 4B), Ψ_{md} ($p < 0.001$ – Fig. 4E), and Fv/Fm at predawn ($p < 0.01$ – Fig. 4H). At the Tristán site, the seedlings grown in CSs had higher SWC ($p < 0.001$ – Fig. 4G), presented lower water stress ($p < 0.001$ – Fig. 4H) and higher Fv/Fm at both predawn ($p < 0.001$ – Fig. 4I) and midday ($p < 0.001$ – Tab. S2) than the seedlings in USs. At Puntal de la Bella, the seedlings grown in CCL-30 tended to show higher LCC ($p = 0.071$ – Tab. S2) than those grown in CCS-18. At El Sapo, an interaction between SM_f and T_f was observed ($p = 0.008$ – Tab. S2). One-way ANOVA indicated that the seedlings grown in CSs+VFT had lower LCC values ($p = 0.008$) than for the other treatments, and no significant differences appeared among them. Another interaction between factors $SM_f \times T_f$ was observed at Tristán ($p = 0.022$ – Tab. S2). The one-way ANOVA reported that the seedlings in USs had higher LCC ($p = 0.043$) than those in CSs.

Discussion

Effect of deep container on the morphological characteristics and seedling field performance

The effect of increasing container volume on seedling morphology development is widely documented (Prieto-Ruiz et al.

2007, Gil & Díaz 2016). However, the effect of increased container depth on seedling morphology may have different responses according to species characteristics (Chirino et al. 2005, Kostopoulou et al. 2011, De la Fuente et al. 2017). In this study, a deep container was used to produce seedlings with a longer taproot and a higher DW_r/DW_s ratio to favour not only deep root system development, but also the rapid colonization of deeper soil layers before the first summer. At the end of the nursery culture, the seedlings cultivated in a deep container (CCL-30) presented a longer taproot and a tendency to have higher root dry weight values. They also showed longer stem height and higher shoot dry weight values. Consequently, no significant difference in the DW_r/DW_s ratio was observed (Tab. 2). Previous studies that employed containers with similar depths in *Quercus coccifera*, *Q. ilex* (Chirino et al. 2005) and *Q. suber* (Chirino et al. 2008) reported the production of seedlings with no significant differences in height and basal diameter compared to those grown in standard containers. This also allowed to produce seedlings destined to dry ecosystems with a higher DW_r/DW_s ratio in the seedlings grown in deep containers (Chirino et al. 2005, 2008, Mariotti et al. 2015b), which favours better root growth and greater resistance to Mediterranean water stress characteristics (Peman et al. 2006, De la Fuente et al. 2017). Other studies that have

employed deep containers produced seedlings with a longer taproot (Trinidad et al. 2015) and a higher stem height (Mariotti et al. 2015c). However, effects on stem height, diameter and other morphological characteristics have not always been observed (Trinidad et al. 2015, De la Fuente et al. 2017).

Relative resources allocation to roots or leaves is considered a key factor in species strategies in relation to the use of water (De la Riva et al. 2014) and seedling survival in the field (Villar-Salvador et al. 2013). In our study, we expected the seedlings grown in CCL-30 to develop a deeper root system over a shorter time period, which would promote higher survival rates, more growth (H_r -RGR, D -RGR) and a better physiological status than the seedlings grown in short CCS-18 containers. Our results did not meet the expected results because using deep containers did not lead to any differences in seedling survival (Fig. 2), growth (Tab. 3) or ecophysiological status (*i.e.*, water potential and maximum quantum yield of Photosystem II – Tab. S1, Tab. S2 in Supplementary material) compared to the seedlings in CCS-18. This result may be due to the fact that both seedling types had a similar DW_r/DW_s ratio upon planting. Some previous studies have also reported no effects on water status in different container types (Oliet et al. 2012).

Effect of vegetable fibre tree shelters on microclimatic conditions, seedling survival and growth

The design of tree shelters (height, ventilation and material used to build them) affects the micro-meteorological conditions inside tree shelters (Bellot et al. 2002, Puértolas et al. 2010, Mechergui et al. 2013). Former studies have shown that using tree shelters in relation to external micro-meteorological conditions increases air temperature (Devine & Harrington 2008, Pemán et al. 2010, Mechergui & Pardos 2017). In contrast, the VFT in our study did not increase temperature in relation to the external environmental conditions (Tab. 4). The used material and tree shelter design, i.e., built with small wicker shoots, allowed air to flow through lateral ribs, which favours good ventilation and avoids overheating. These results are consistent with those obtained by Bellot et al. (2002), who used tree shelters made by a network of *Stipa tenacissima* L. leaves. Tree shelters also reduced the PPFD, which has been widely documented. For example, Bellot et al. (2002) reported a reduction in PPFD of between 70% and 80%, Jiménez et al. (2005) reported a 78% reduction, while Devine & Harrington (2008) indicated one of 15-54% for radiation at an open site. In our study, VFT reduced PPFD by 59%.

Regarding survival, several studies have indicated that using tree shelters significantly increases seedling survival (Vallejo et al. 2006, Defaa et al. 2015). Although Valkonen (2008) and Oliet et al. (2016) observed only marginal improvement, other authors did not find this relation (Oliet et al. 2012). The use of VFT in our study did not increase seedling survival, although a tendency ($p = 0.053$ - Fig. 2) was observed at Puntal de la Bella. Other studies have indicated that using tree shelters also favours an increased growth height (Defaa et al. 2015, Oliet et al. 2016, Mechergui & Pardos 2017). We report the effect of tree shelters (VFT) only at the Puntal de la Bella site, where H_s -RGR increased and D -RGR lowered (Tab. 3). For the ecophysiological seedling response, the effects of using VFT were observed only in spring, mainly on the Fv/Fm_{pd} values at the three experimental sites. This last result agrees with Puértolas et al. (2010) and Ceacero et al. (2012), who reported that tree shelters improved the physiological characteristics of seedlings. However, other studies like that Pemán et al. (2010) did not find any differences in Fv/Fm either with or without tree shelters.

Effect of shrubland management on seedling survival and field performance

Studies on the competitive or facilitative effect of some shrub species on seedling survival and field performance have been well-documented (Pérez-Devesa et al. 2008, Rey et al. 2016, Macek et al. 2018, Santana et al. 2018). In summer, under lower SWC conditions, shrubland manage-

ment (CSs treatment) was the factor that contributed more to diminish water stress, and gave better maximum quantum yields status of Photosystem II and seedling performance. Our study indicated that the seedlings grown in CSs had higher PPFD (Tab. 5) and also higher SWC (Tab. S1, Supplementary material), which favoured less water stress (expressed as higher Ψ_{md} - Fig. 4), as well as higher Fv/Fm values at both predawn (Fig. 4) and midday (Tab. S1, Tab. S2, Supplementary material). This better water and physiological status probably led to higher seedling survival in CSs than for the seedlings grown in USs. Our results agree with those of Pérez-Devesa et al. (2008), who reported that the seedlings planted in the middle line of CSs presented more soil moisture, their survival was 22% higher, they displayed better growth for height and root collar diameter, and had higher Fv/Fm values at both predawn and midday than those planted in USs (control treatment). However, differences in seedling survival have not always been observed with these treatments (Valdecantos et al. 2009, Herrero-Jáuregui et al. 2016). The seedlings in CSs presented a higher H_s -RGR at El Sapo, which is consistent with the results reported in *Pistacia lentiscus* by Valdecantos et al. (2009) for H_s -RGR, and by Madrigal et al. (2014) in *Pinus halepensis* growth in Guadalajara, Spain. Another effect of the CSs treatment was its higher PPFD (43% in June and 25% in August) than for the seedlings in USs. This result is consistent with Pérez-Devesa et al. (2008), who obtained 30% higher PPFD, and also coincides with the increased chlorophyll content in *Pinus* reported by Cambrón-Sandoval et al. (2011). It is very important to indicate that, for our experimental sites, high PPFD values at midday in summer can cause radiation stress for seedlings (Cortina et al. 2009, Granados et al. 2016). The year 2012 was relatively dry (Fig. S4, Supplementary material) as 120 days went by with no significant rainfall (< 5 mm). Thus, mortality of almost 100% was expected (Vallejo & Alloza 1998). However, the survival values of the seedlings planted in the CSs remained between 39% and 64%.

Overall, our study results indicated that in both spring and summer, at the Tristán site the CSs treatment (shrubland management factor) and the use of vegetable fibre tree shelters (tree shelters factor) had a stronger effect on the ecophysiological response of seedlings (Fig. 3, Fig. 4). Tristán was the only site that was SW slope-facing. This slope-facing type presents more solar exposure, higher water stress and more stress by PPFD. Under those most unfavourable conditions compared to the other sites, both the studied treatments contributed to a better seedling water status and to improved seedling performance. We speculate that a better response of both experimental treatments for sites with more unfavourable conditions could be expected.

Conclusions

According to our starting hypothesis, we expected the combined use of deep containers, vegetable fibre tree shelters and shrubland clearing to improve the field performance of *Q. suber* seedlings to a greater extent than when applying only one treatment. Our results did not ratify this hypothesis, but highlighted the fact that shrubland clearing was the main treatment to improve *Q. suber* performance. The effects of the cleared shrubland treatment overlapped the functional improvement of deep containers and tree shelters. Using deep containers and tree shelters had no significant effect on seedling survival, despite improvements in microclimatic conditions and differences in the root system developed by seedlings, as observed at the end of the nursery culture. Accordingly, we conclude that shrubland clearing is the most suitable technique to favour the introduction of *Q. suber* into ecosystems where shrublands dominate as a result of degraded forests.

List of abbreviations

The following abbreviations have been used throughout the text:

- Ct_i: Factor container type;
- T_i: Factor tree shelters;
- VFT: Vegetable fibre tree shelters;
- NVFT: Without vegetable fibre tree shelters;
- Sm_i: Factor shrubland management;
- CSs: Cleared shrubland strips;
- USs: Undisturbed shrubland strips;
- T^a: Air temperature;
- RH: Relative humidity;
- PPFD: Photosynthetic photon flux density;
- VPD: Vapour pressure deficit;
- H_s: Stem height;
- D: Basal diameter;
- H_s-RGR: Relative growth ratio in stem height;
- D-RGR: Relative growth ratio in diameter;
- DW_s: Shoot dry weight;
- DW_r: Root dry weight;
- SM_i: Seedling dry weight;
- DW_r/DW_s: Root/shoot ratio;
- DW_r/SM_i: Root weight ratio;
- Ψ_{md} : Water potential at midday;
- LCC: Leaf chlorophyll content;
- Fv/Fm_{pd} : Maximum quantum yield of Photosystem II at predawn;
- Fv/Fm_{md} : Maximum quantum yield of Photosystem II at midday;
- SWC: Soil water content.

Author contributions

JM-R, ECh, VC, JM and AV conceived and designed the experiment. JM-R, ECh, VC and JM performed the experiment and took field measurements. ECh and OF carried out the statistical analysis. JM-R, ECh, AV and OF contributed to the data analysis and data interpretation. JM-R, ECh and AV wrote and edited the manuscript.

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Supplementary Material

Fig. S1 - Details of the used containers.

Fig. S2 - Panel of vegetable fiber composed per wicker branch (*Salix purpurea*).

Fig. S3 - View of a cleared shrubland strips (CSs) and undisturbed shrubland strips (USs) on both sides.

Fig. S4 - Ombroclimatic diagram. Monthly mean rainfall and temperature.

Fig. S5 - Seedling survival by the combined effect of the two experimental treatments.

Tab. S1 - Measures taken in June 2011 of soil water content (SWC, %), water potential at midday (Ψ_{md} , -MPa), leaf chlorophyll content (LCC, units SPAD), and maximum efficiency of photosystem PSII (F_v/F_m at predawn and midday) per experimental site.

Tab. S2 - Measures taken in August 2011 of soil water content (SWC, %), water potential at midday (Ψ_{md} , -MPa), leaf chlorophyll content (LCC, units SPAD), and maximum efficiency of photosystem PSII (F_v/F_m at predawn and midday) per experimental site.

Link: Munoz-Rengifo_3095@suppl001.pdf