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Should we use meshes or solid tube shelters when planting in Mediterranean semiarid environments?
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Abstract: Tree shelters in Mediterranean environments have a two-sided effect. They not only protect seedlings from browsing but also ameliorate microclimatic conditions, improving post-planting survival and growth. However, the ecophysiological basis of these effects is poorly understood. A factorial experiment combining light transmissivity and shelter type (solid tube vs mesh wall) was carried out to assess the impact of contrasting microclimatic characteristics on seedling performance and physiological stress levels of shelters in two Mediterranean shrubland species (Quercus coccifera and Rhamnus lycioides) planted in a semiarid site. Even though seedlings in solid tube shelters experienced higher temperature and were slightly more photoinhibited, they had higher predawn water potential and, in general, better survival and growth than in mesh wall shelters. However, these effects were species-specific, with Rh. lycioides more favoured by solid wall shelters than Q. coccifera. However, root growth cannot explain these interactions between species and shelter type on seedling survival. Since light transmission had a marginal effect compared with wall type, we proposed that the observed effects and interaction with species are not dependent on light intensity or temperature but on other microclimatic differences like air velocity or light quality and distribution. Further studies should assess the importance of these factors on post-planting growth and physiological stress levels, which can be critical for matching the correct tree shelters type for each species in plantations in semiarid environments.

Suggested Reviewers: Dugald C. Close
### Response to Reviewers:

**MANUSCRIPT NEFO S 17 00259**

Madrid, May 19 2018

**COVER LETTER TO Revision #1**

Dear Dr. Jacobs

Thank you very much for your email from April 19 concerning the review of the manuscript NEFO 17 00336 Should we use meshes or solid tube shelters when planting in Mediterranean semiarid environments?. We would like to thank the reviewers for their helpful comments, which we believe have improved the clarity and impact of the work. Virtually all the changes proposed by the reviewers have been incorporated in the new version. These changes are highlighted in red font in our revised manuscript, and references to previous or current manuscript position of the changes are presented to facilitate location and checking. Main changes consisted in presenting a different Figure 4 and correspondent post-hoc test in agreement with Reviewer#1 suggestion. Also minor changes in the text have been conducted accordingly. Please note that specific reviewer inquiries are in italics, and our responses are in bold.

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**COMMENTS FOR THE AUTHOR:**

Editor-in-Chief: Thank you for submitting your manuscript, which has now been appraised by an Associate Editor and two expert reviewers. Your manuscript will be acceptable after minor revision based on Associate Editor and reviewer comments below. Please provide a covering letter detailing your specific changes in response to each point raised by the Associate Editor and reviewers. Your revision is due within 6 weeks.

AE: The paper by Oliet et al. (NEFO-D-17-00259) describe a solid study with interesting results. Although much used in practice, not much have been published concerning physiological responses of seedlings to various plant tube materials and designs. Therefore this manuscript is interesting for readers of New Forests and is worth publishing. Both reviewers are positive with some comments that should be adressed before publication.

Thanks a lot for your positive inputs!

Despite the undoubted quality of the manuscript, I have relatively minor concerns about some points I would like the authors to consider First, I was not convinced that the authors have always used statistical post-hoc tests in appropriate manner. For instance in Figure 4, post-hoc tests have been made to compare the combined treatments species*shelter*light transmissivity but it is unclear if the interaction is significant or not. If not, please consider to show results in a different way (analyzing...
for instance the main effects only as in Fig 1 or interaction of the second order). Same remark for Table 4: the interaction Species*Shelter is only significant for root length (Table 3), so post-hoc tests are not appropriate when analyzing the influence of the treatments on the other variables. Please check this point in your different analyses and correct if necessary.

We agree with the reviewer about the appropriateness of using post-hoc test for species*shelter*light transmissivity combinations if this third order interaction is not significant. We have followed the Reviewer's suggestions in Figure 4 by analyzing the second order interaction for height and diameter, including in this figure the degree of signification of the ANOVA test for each of them. We have reworded the result section accordingly (Lines 320 to 322 of revised version).

With regard to Table 4, we have removed post-hocs letters for all variables except root length. New explanations are given at Table foot.

Second, the duration of the study is somewhat low: 16 months only from February 2014 to June 2015. I acknowledge that the first summer is often of a primary importance for plant survival but it also corresponds to a transplanting shock. To what extent this latter consideration could have influenced your results? Can you add some additional comments on this?

We have added some comments in the Introduction to clarify this question (Lines 100 to 102 of revised version).

Lastly, I have not well understood the method about roots measurements: what do you mean exactly when you indicate that "protruding roots from the plug" were analysed (P5)? I suppose it corresponds to the term "new root" introduced later in the paper. Can you clarify this point?

Some pictures (if available), showing for instance the types of shelter would be helpful for the reader I think.

We refer to the roots that emerge out of the plug. As they do not include new roots formed within the plug, we agree with the reviewer that "new root" can be confusing. Therefore, we have changed "new roots" for "protruding roots" along the text, and explained more the "protruding" issue by adding "out of the plug" (see line 210-211 of revised version).

We do not believe that pictures showing the types of shelter can be too helpful, providing the description given in Mat and Methods are clear enough, as they include transmissivity levels, colors, dimensions of the shelters and net holes dimensions.

Specific remarks

P1L50 benifited = favoured?
Done

P2L0 leporidae= rabbits and hare? It is easier to understand for the reader
Changed to rabbits and hares

P2L39-51 I particularly appreciated the good analysis of the literature!
Thanks!

P5 Were the protector stabilized by any particular system during the planation? Were there buried in the soil to assure stabilization?
Yes, the protector was stabilized with a stake and buried in the soil. We have added this detail in Mat and Meth.

P5 L41-42 See above remark about the roots
It has been already clarified. See above answer to your remarks.

P7L5 You should call up Fig1 here
Done

P7L17 Table 3 to be replaced by Table 1
You are right. Done

P7L29 Can you indicate how was VPD determined (several formulas are possible)?
VPD was determined using Rosenberg formula:
Rosenberg ND, Blad BL, Berma SB (1983) Microclimate: the biological environment. 2nd Edition. John Willey and Sons. USA. 495 pp.
We have included this reference.

P7L38 Indicate (Table 2)
Reviewer #2: The work by Oliet et al. was aimed at improving the knowledge of the ecophysiological mechanisms occurring in plants growing in tree-shelter after transplanting in arid environments. They compared the effect of two types of shelter - plastic tube and mesh - in combination with three different gradients of light transmissivity on seedlings of two species usually planted in arid environments for restoration purposes (Quercus coccifera and Rhamnus lycoides). Despite in international literature the studies on the effect of tree-shelter on early seedling development are pretty spread, most of them are focused on describing the shelter effect against browsing and/or on survival and seedling early morphology. Few experiments are designed to understand the ecophysiological basis to explain such findings. This is the strength point of this study, which was planned and carried out accurately. I enjoyed reading the manuscript by Oliet et al; all sections of the manuscript are well written, materials and methods are correct, and results are interesting and properly presented and discussed. Thus, my recommendation is to accept the manuscript.

Just check citations in the text vs reference list (e.g. in page 2 Dupraz and Bergez 1999 and Mariotti et al. 2015 are cited but not present in reference list).

Thanks a lot for your comments. We have incorporated mentioned references in the list.
Should we use meshes or solid tube shelters when planting in Mediterranean semiarid environments?

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Abstract

Tree shelters in Mediterranean environments have a two-sided effect. They not only protect seedlings from browsing but also ameliorate microclimatic conditions, improving post-planting survival and growth. However, the ecophysiological basis of these effects are poorly understood. A factorial experiment combining light transmissivity and shelter type (solid tube vs mesh wall) was carried out to assess the impact of contrasting microclimatic characteristics on seedling performance and physiological stress levels of shelters in two Mediterranean shrubland species (*Quercus coccifera* and *Rhamnus lycioides*) planted in a semiarid site. Even though seedlings in solid tube shelters experienced higher temperature and were slightly more photoinhibited, they had higher predawn water potential and, in general, better survival and growth than in mesh wall shelters. However, these effects were species-specific, with *R. lycioides* more favoured by solid wall shelters than *Q. coccifera*. However, root growth cannot explain these interactions between species and shelter type on seedling survival. Since light transmission had a marginal effect compared with wall type, we proposed that the observed effects and interaction with species are not dependent on light intensity or temperature but on other microclimatic differences like air velocity or light quality and distribution. Further studies should assess the importance of these factors on post-planting growth and physiological stress levels, which can be critical for matching the correct tree shelters type for each species in plantations in semiarid environments.
Introduction

Animal browsing is an important threat to the successful establishment of planted seedlings (Burney and Jacobs 2018). The incidence of browsing is highly dependent on the ecological characteristics of the reforested area that affects the animal specific composition and their abundance. Landscapes such as cropland matrixes tend to support high amounts of rabbits, hares and other generalist species (Calvete et al. 2004) that can be very detrimental for young plantations and result in major economic losses and delays in the restoration process. Among the most common system to protect seedlings is the use of individual tree shelters (Devine and Harrington 2008). Tree shelters are usually plastic tubes enclosing seedlings shoots to preclude browsing. Broadly speaking, two types of tree shelters are commercially available: solid tubes and meshes. Solid tubes are made in a continuous plastic wall, while meshes are open nets that allows free air circulation throughout the seedling. Solid tubes can also be ventilated by several holes. These characteristics have a strong influence on the environmental conditions around the protected plant (Bergez and Dupraz 2009). Therefore, tree shelters not only play a mere physical barrier role, but also can affect plant establishment and growth in additional ways by the changes in temperature, light, vapor pressure deficit or others (Dupraz and Bergez 1999; Oliet and Jacobs 2007; Pemán et al. 2010; Puértolas et al. 2010; Mariotti et al. 2015). There are numerous studies from different geographical areas analyzing the response of planted seedlings to the use of solid tube shelters in relation to the micro-environmental conditions inside (Del Campo et al. 2006; Jacobs 2011; Close et al. 2009; Bellot et al. 2002; Bergez and Dupraz, 2000). As expected, plant response is species- and environment-specific (Oliet et al. 2003; Padilla et al. 2011 Devine and Harrington 2008; Defaa et al. 2015). In semiarid areas, the use of solid tubes has proven to be on average beneficial for survival and growth (Piñeiro et al. 2013). On the contrary, the number of studies analyzing the effect of meshes is much lower (but see Ward et al. 2000; Devine and Harrington 2008), despite this type of protector is broadly used in operational plantations (Taylor et al. 2006; Van Lerberghere 2014). Unlike solid tubes, meshes allows air circulation, which precludes greenhouse effect, while reducing radiation incidence on the leaves. Although these effects could be beneficial for plant establishment in harsh areas, we are only aware of two studies addressing plant response to both types of tree shelters under semiarid Mediterranean conditions (Close et al. 2009; Padilla et al. 2011). Results from these studies are opposite, probably due to different site conditions and species, which reinforces the necessity of improving the knowledge of physiological basis for that responses.

Constructive characteristics of tree shelters are variable. Apart from different heights to adapt to herbivory size (Van Lerberghere 2014), other characteristics such as ventilation, color or light transmissivity affect micro-environmental conditions inside. Ventilation reduces air overheating during midday (Bergez and Dupraz 2009). Light transmissivity affects the amount of incident radiation, with effects on the intensity of
stress and on plant growth response that could be crucial for plant survival under harsh conditions (Oliet et al. 2003). For example, photoinhibition can occur in shade tolerant species when protected by highly transmissive solid wall shelters (Puértolas et al. 2010), and some studies reveal a species-specific response of resources allocation to shoot or root as a function of shade tolerance (Jiménez et al. 2005; Puértolas et al. 2010; Vázquez de Castro et al. 2014). This could explain differences in survival between species with contrasted functional traits planted in Mediterranean environments under a gradient of light transmissivity (Oliet et al; 2003 and 2015). However, these studies have only compared gradients of light transmissivity within solid wall tube shelters, but the characteristics of mesh shelters (higher ventilation, different quality of the transmitted light) might interact with light transmission to determine the effects on survival and performance.

The objective of our experiment is to compare the effect of both types of tube shelters (solid and mesh) on two species (Quercus coccifera L. and Rhamnus lycioides L.) under semiarid Mediterranean conditions. We assessed first year after planting survival, growth and physiological stress levels (water potential and photochemical efficiency) and under a gradient of light transmissivity (40, 60 and 80 %) for both types of shelters. Testing a gradient of light transmissivity for both types of guards will help to characterize the tree shelter ecophysiological system by assessing the relative contribution of different environmental variables on seedling response during establishment. Despite the duration of the study is relatively short, it includes post planting summer, which under harsh Mediterranean conditions is the most critical period in terms of survival (Villar-Salvador et al. 2012). The selected species are sprouting shrubs widespread in the western Mediterranean Basin. In semi-arid environments, they are considered keystone species affecting community composition and ecosystem function (Maestre and Cortina, 2003). Q. coccifera is widely used in afforestations in the semiarid areas of the Mediterranean basin (Maestre and Cortina, 2004; Sackali and Ozturk 2004), although frequently accounts for low planting success (Baquedano and Castillo 2006). So far, the use of Rh. lycioides has been constrained to small scale or experimental plantations (Trubat et al. 2011; Chirino et al. 2013).

Semiarid areas of the Mediterranean Basin are among the most challenging zones for the establishment of woody vegetation. Numerous biotic and abiotic factors negatively affect survival of planted young trees in these zones. Summer drought combined with excess of radiation and high temperatures (Martínez-Ferri et al. 2000; Niinemets and Keenan 2014) can reduce post summer survival to very low levels (Villar-Salvador et al. 2012). Besides, predation by small mammals, birds or ungulates constitutes another major source of failure (Leverkus et al. 2013). All these factors dramatically reduce the efficiency of restoration efforts. In addition, the current scenario of climate change, with higher probabilities of extreme, harsh summers in these areas (Giorgi and Lionello 2008), suggests that the success of restoration programs in dry Mediterranean environments will require improvements in planting techniques (Cortina et al. 2011; Vallejo et al. 2012). Our study could contribute to improve establishment success of key woody species in these challenging areas by a better management of tree shelters.

Materials and methods

Study site and plant material
The study site is located in an old cropland of central Spain in Toledo province (39°39'8"N, 3°28'5"W, elevation 660 m a.s.l.). The slope of the planting area is North aspect with a moderate 14% steep. Soils are mostly Inceptisols (Gómez-Miguel and Badía-Villlas, 2016). In accordance with a sample from study site, soils are deep (0 to 110 cm) of mostly loamy texture (22.4-35.2% sand, 44.0-48.7 % silt and 19.7-29.1 % clay). Horizons are light-colored highly basic (pH from 7.9 to 8.3) and calcic, with organic matter ranging from 2.14% (upper) to 0.86% (deepest horizon). Maximum electric conductivity of deepest horizon is low (170 µS·cm⁻¹). Permeability is high for the first two horizons (0-30 cm) and moderate below this depth, according to Gandullo (1985). The climate is Mediterranean semiarid, with mean annual precipitation of 418 mm and mean annual temperature of 14.2°C. Summers are very hot and dry, with drought periods lasting four months, mean maximum temperature of 33°C in July and absolute maximum temperature reaching 43°C. Winters are cold with frequent frosts.

Temperature can drop to -11°C and the mean minimum temperature is 0.9°C in January (Ninyerola et al. 2005). During the planting year (2014) annual rainfall was much lower than average (252 mm), with a prolonged dry period from May to September of accumulated rainfall as low as 32 mm (data from National Agency of Meteorology, Agriculture and Environment Department, Spanish Government).

Seedlings of Q. coccifera and Rh. lycioides were raised from seeds of provenance region ES29 Montes de Toledo (Alía-Miranda et al. 2009) and cultivated in 200 cm³ cells (plant density 370 m⁻² Plasnor, Spain). After one year in the nursery and prior to planting, seedling height, root collar diameter, total biomass and root biomass were 22.1±1.5 cm, 5.5±0.4 mm, 8.1±0.4 g and 5.0±0.4 g, respectively, for Q. coccifera, and 22.9±1.5 cm, 3.8±0.3 mm, 4.7±0.6 g and 1.4±0.2 g, respectively, for Rh. lycioides (n=10). These values fall within the recommended ranges for both species according to Pemán et al. 2013.

Field experiment

The site was cross subsoiled prior to planting at a 60 cm depth with two rippers 1 m apart to reduce soil compaction. Subsoiling was conducted following contour lines separated by two meters and perpendicular directions. Seedlings were planted on January 11, 2014 every 1 m along contour lines (spacing was 2 x 1 m), after manually opening holes (0.3 m x 0.3 m x 0.3 m) in the junction of the subsoiling furrows. No weed control were conducted during the experiment, as the seed bank of weeds was weak in this area, specially under the arid conditions of the planting year. Both species were alternated within each planting row, and shelter treatments were randomly assigned to each seedling. The experimental design was a 2 x 2 x 3 factorial design, with the following factors and levels: (1) species (Q. coccifera vs. Rh. lycioides), (2) tree shelter type (solid wall vs. mesh) and (3) light transmissivity of the solid plastic or mesh (values around 40, 60 or 80%). The solid wall tube shelters were made from plastic material supplied by Repsol Química (Spain). Additives were added to the copolymer base to reach the light transmissivities tested in this experiment, maintaining the red/far red ratio around 1 (neutral shade) (Vázquez de Castro et al. 2014). Hand-made tubes using the plastic sheets were circular, single-walled tubes, 50 cm tall × 10 cm wide, with four ventilation holes facing each other of 2.5 cm width and situated at 18 and 36 cm in height. Minimum value of 40% light transmissivity was considered as a target when designing shelters that promote biomass allocation to roots and improve water balance of Mediterranean seedlings (Vázquez de Castro et al. 2014). Solid plastic tubes...
were stabilized by fixing a plastic stake with clamps and burying the shelter in the soil. Plastic meshes were chosen among available polyethylene products in the market. Mesh of 80% was a 60 cm tall × 15 cm wide cylindrical blue net with holes 8 × 8 mm (Protec Blaunet model, Projar SA, Spain). 60 % light transmissivity mesh was also a 60 cm tall × 15 cm wide cylindrical black net with holes 2.4 × 2.4 mm (P40 model, Projar S.A., Spain). And 40 % light transmissivity mesh was a was a 60 cm tall × 12 cm wide cylindrical black net with holes 4 × 3 mm (V8 model, Improfort Limited, Spain). Meshes were stabilized by using two plastic stakes. Actual light transmissivity of solid tubes and meshes under field conditions was determined in several daily cycles of photosynthetically active radiation (PAR) measurements with two or three sensor replicates (QSO-SUN, Onset, USA) per shelter type connected to a U12 data-logger (Onset, USA). Values were registered every 10-15 minutes. Light transmissivity was averaged along the mean daily cycle. Mean transmissivity percentages were 77 (named solid tube 80 %), 58 (named solid tube 60 %) and 36 (named solid tube 40 %) % for solid tubes and 83 (plastic mesh 80 %), 56 (plastic mesh 60 %) and 46 (plastic mesh 40 %) % for meshes. A total of 300 seedlings per species were planted, of which 50 seedlings were randomly assigned to each combination of type of shelter × transmissivity. As experimental plot was small and homogeneous, arrangement of treatments was fully randomized, and no blocking or grouping as a mean to control experimental error was necessary.

Monitoring plant response and microclimatic conditions for shelter types

Seedlings survival was measured four times along the study, from June 2014 to February 2015. Some apparently dead seedlings resprouted after measurements and were accounted as live in the following assessment. Height and basal stem diameter were measured on every plant at the end of October 2014. Seedlings biomass and root development were evaluated from five randomly chosen seedlings per treatment and species (60 plants in total) that were destructively harvested on February 28, 2015. Using small hand tools, root systems were carefully excavated from soil up to a depth of 70 cm and taking care to retain roots > 1 mm diameter. Shoots were separated from the roots at the root collar and all parts were frozen until processing. Roots protruding out of the plug were excised and washed free from soil with tap water. Leaves and protruding roots were scanned and leaf area and root length measured with an image analyzer (ImageJ V1.48®, National Institutes of Health, USA). After these measurements, dry mass of each component (leaves, stem, plug roots and protruding roots) was determined by oven drying them at 65 °C for 48 h and weighing.

Physiological measurements took place in two consecutive sunny days of June (14 and 15) and July (15 and 16) 2014 in five seedlings per shelter type, transmissivity and species (60 seedlings in total, 30 per day). A small window was opened in the solid or mesh wall of the shelters to facilitate sampling for water potential and chlorophyll fluorescence measurements; the window was otherwise closed. Shoot xylem water potential was measured at predawn ($\Psi_{pd}$). A 3-7 cm healthy twig of the upper third of the plant was excised between 05.00 and 07.00 AM, wrapped in aluminum foil, kept in sealed polyethylene bags and stored refrigerated in an ice box. Water potential was measured within 3 h using a pressure chamber (Model 1000®, PMS Instruments Company, USA). To check potential confounding effect of time since twig excising and
measurement on water potential, both variables were plotted and no significant
correlation was found (data not shown). Chlorophyll fluorescence was evaluated on the
same plants. The ratio of variable to maximum fluorescence ($F_v/F_m$) as a surrogate of
maximum photochemical efficiency of photosystem II was measured through the
opened window with a fluorometer (FMS, Hansatech Instruments, UK). A fully
expanded leaf of the upper third of the seedling was chosen. Prior to $F_v/F_m$
measurements, that were done at predawn (07:00 AM) and midday (13:30 PM) leaves
were dark acclimated for 30 min (Kalaji et al. 2014).

To assess the effect of light transmissivity of tube shelters on internal microclimate
conditions, air temperature and relative humidity (RH) data logger sensors (U23-001
Onset, USA) were installed in the shelters from 14 to 25 June 2014. All this period was
cloudless. Sensors were randomly installed inside two shelters of each shelter type ×
light transmissivity combination (12 sensors in total), attached to a stake at a height
between both pairs of ventilation holes for solid tubes and the same height for meshes.
Temperature and RH were recorded every 15 min. Vapor pressure deficit (VPD) was
calculated from temperature and RH data following Rosenberg et al. (1983) method.

Data processing and statistical analysis

Post summer (October 2014) and 13 months (February 2015) after planting survival
data were analyzed using a generalized lineal model based upon a binomial errors
distribution with a logit link function. Full model included shelter type, light
transmissivty, species, and all interactions among factors as predictors. Post hoc
comparisons among treatments for last measurement (February 2015) were done using
Bonferroni correction for paired comparisons.

In case of plant development (height, diameter, biomass and root length), a general
lineal model with three main fixed factors as per survival were applied by running a
three ways analysis of variance (ANOVA). Physiological data were analyzed similarly,
although a fourth fixed factor (summer month, June and July) was included in the
model. Non-normal data (basal stem diameter in October 2014 and all data from
February 2015 plant excavation) were previously converted to logarithmic forms to
fulfill normality and variance homogeneity requirements. When ANOVA showed
significance, differences among means were identified using a Tukey post-hoc test.
Differences were considered statistically significant if $P < 0.05$. Results are given as
mean ± SE throughout the paper.

Data from temperature and relative humidity inside tube shelters were averaged per
sensor and time to represent a mean daily cycle.

All the statistical analyses were performed using software R version 3.1.1 (R Core Team
2014 Vienna, Austria). Figures were produced using Sigmaplot, Version 12.0 (Sigma
Plot 2012, Inc., San Jose, CA, USA).

Results

Physiological response to shelters (maximum photochemical efficiency and water
potential) and microclimate in summer.

Maximum photochemical efficiency ($F_v/F_m$) at both pre-dawn and midday was
significantly affected by all factors of the study during June and July. Besides,
significant interactions were found between month of measurement and shelter type (mesh or solid tube) and those two factors and species for predawn \( F_v/F_m \) (Table 1). Seedlings growing in solid tubes showed lower values of both predawn and midday \( F_v/F_m \) than those in meshes, although differences were lower for predawn (0.02) than for midday (0.06) (Figure 1). A drop in \( F_v/F_m \) was found with increasing light transmissivity, with plants growing in lightest tubes (80%) having lowest values of both pre-dawn and midday maximum photochemical efficiency. In addition fluorescence plants response to light transmissivity was higher for midday \( F_v/F_m \), as seedlings in 60% light transmissivity shelters had also minimum values as per lightest shelters (Figure 1). On average, \( F_v/F_m \) dropped from June to July (data not shown) and, by species, predawn and midday \( F_v/F_m \) for \( Q. \) coccifera was higher (0.83 ± 0.00 and 0.72 ± 0.01) than that of \( Q. \) coccifera (0.77 ± 0.00) and 0.69 ± 0.01, respectively).

Predawn water potential during summer months was only affected by shelter type (Table 1). Seedlings in meshes were significantly more water stressed, with values of -3.11 ± 0.12 MPa, while plants growing in solid tubes were more hydrated (-2.32 ± 0.11 MPa).

Averaged temperature in solid wall shelters during the daylight period in late June was higher than that in meshes, with maximum differences (pooling the three light transmissivity levels) reaching 7.3°C at 13:15 h solar time (Figure 2). Temperature differences among light transmissions were minor, with the 80% shelter tending to have higher temperatures than the rest (1.1°C, data not shown). Differences in RH among type of shelters and light transmissivities were minimum (Figure 2). As a consequence, VPD inside shelters follow the same pattern as temperature, with solid tubes having maximum VPD differences (after averaging by light transmissivity) of 1.86 kPa at 13:15 h solar time (Figure 2).

**Survival and growth**

Post summer survival (October 2014) was significantly affected by type of shelter and light transmissivity, although shelter type interacted with species (Table 2). One year after planting (February 2015), the interaction between shelter type and transmissivity became also significant (Table 2). Survival was almost double in solid wall shelters (84 ± 3%) than in meshes (45 ± 4%) for \( Rh. \) lycioides (Figure 3). In contrast, for \( Q. \) coccifera only the mesh of 80% light transmissivity had significantly lower survival (Figure 3). In February 2015, this combination of shelter type and transmissivity had the lower survival in both species (Figures 3). Overall survival of \( Rh. \) lycioides in February 2015 was slightly superior to \( Q. \) coccifera (68 ± 3 versus 60 ± 4%, respectively), but survival in the most favorable shelter (solid wall tube 60%) was higher for \( Rh. \) lycioides (89 ± 4.6%) than for \( Q. \) coccifera (75 ± 5.8%).

Post summer height was significantly affected by all factors and by almost every second order interactions among them (P<0.001), with the exception of species × shelter type (P = 0.07). Seedlings growing under 40 and 60% light transmissivity grew similarly in height, while a detrimental effect of height growth appears when plants grew under maximum light transmissivity level. Besides, this pattern is much more intense for seedlings growing in meshes and for \( Rh. \) lycioides (Figure 4A). Thus, differences ranged between almost same height across transmissivities for \( Q. \) coccifera and plants growing in solid tubes, to a significant depletion in height under 80%
transmissivity for Rh. lycioides and those growing in meshes (Figure 4A). Post-summer basal stem diameter (BSD) was significantly affected by shelter type ($P<0.001$) and by a light transmissivity × shelter type interaction ($P=0.03$). BSD of plants within solid tubes were on average 18% larger than that in meshes with no differences between light transmissivity within shelter types except for the 80% mesh, which were significantly smaller for Rh. lycioides and almost significant for Q. coccifera (Figure 4B). No significant third order interaction appeared among three factors for height or diameter. Cross values for this three factors combination are presented in Supplementary material.

For all biomass and growth traits measured after plant excavation in February 2015 (13 months after planting), no significant effect was found for light transmissivity (Table 3). However, leaf area and shoot biomass was significantly bigger in solid wall tubes than in meshes (64 and 33% respectively) (Tables 3 and 4). Protruding roots length was also greater in solid tubes and affected by the interaction of this factor with species: it was 78% longer in Q. coccifera and not significantly different from meshes in Rh. lycioides (Tables 3 and 4). Shoot:root ratio (g·g$^{-1}$), specific leaf area and specific root length of seedlings were unaffected by shelter type or light transmissivity. Most of the evaluated traits from excavation were species-specific: Q. coccifera leaf area was 65% higher, while protruding roots length, shoot:root ratio and specific root length were 36, 60 and 40% lower than those of Rh. lycioides. Nevertheless, data from plant excavation 13 months after planting show high variability levels (Table 4), precluding the declaration of additional significant responses to tested factors.

Discussion

Unexpectedly, seedlings in solid wall tubes showed a higher survival and growth for almost every combination of light transmissivity and species. Microclimatic conditions inside mesh shelters (T, VPD) during the hot Mediterranean summer were expected to decrease heat and water stress compared to solid wall shelters. In fact, chlorophyll fluorescence data suggests a higher degree of photoinhibition in solid tubes with differences deepening from the beginning (June) to midsummer (July). Maximum photochemical efficiency is more sensitive to thermal than to hydric stress, as shown in a wide variety of woody species that presented significant reduction in F$_{v}$/F$_{m}$ under higher temperatures (Matías et al. 2017; Methy et al. 1997). However, values were always close to 0.8, which is considered the optimum value (Bjorkman and Demmig, 1987) and the differences between both shelter types were small. Deactivation of reaction centers of photosystem II is part of the acclimation process to avoid photodamage when photosynthesis is impaired (Demmig-Adams and Adams, 1992). The small changes observed here might only reflect slight differences in this deactivation but with no impact on plant capacity to survive and grow.

In contrast, differences in predawn water potential were in agreement with seedling performance. Average predawn water potential registered in summer for both species in plastic mesh (-3.1 MPa) indicates a moderate level of water stress. Q. coccifera shows drastic reduction of assimilation rate with $\psi_{pd}$ values below -2 MPa (Baquedano and Castillo 2006) which implies reduction of root growth. Even though these values are higher than the critical water potential values (-6 MPa) inducing fatal
Several hypotheses can be formulated to explain the lower $\psi_{pd}$ in meshes. Air velocity inside solid wall shelters is negligible, even for ventilated tubes (Bergez and Dupraz 2000). Restricted air movement creates a thicker and less conductive boundary layer that reduces foliar water loss and improves hydric status. This effect has been demonstrated in previous works with solid wall shelters under controlled conditions (Kjelgren and Rupp, 1997; Bergez and Dupraz, 1997), where water losses of seedlings in tube shelters was much lower than in unprotected ones. Even though mesh shelters might restrict air movement, they allow some air circulation through leaves. This would decrease leaf-air boundary layer conductance (Lambers, 2008), accelerating soil water depletion in the root-zone. Within this hypothesis the increase in transpiration demand due to air movement in meshes would be more intense than the higher VPD inside solid wall shelters provoked by temperature.

Alternatively, increased air velocity also induces stomatal closure and hence decreased carbon gain, which along with lower air temperature during spring in mesh shelters could explain the observed reduction in growth in this type of shelters. Besides the effects related to differences in air movement between mesh and tube shelters, growth reduction could be linked to light quality and distribution. Even though the experimental factorial design allowed the comparison of both types of shelters with similar total radiation levels, light inside tubes was exclusively diffuse while in meshes there was a mixture of direct and indirect radiation. Moreover, leaves in meshes were exposed not only to those patches of different light quality and intensity, but also to relatively rapid changes as the sun moved during the day or the leaves were shaken by wind. These rapid changes similar to sunflecks within or beneath canopies can decrease photosynthetic efficiency and water use efficiency, as the capacity of photoacclimation to contrasting light conditions cannot cope with such rapid changes (Townsend et al. 2017). Potentially, these two negative effects on carbon gain described above could impair root growth, which is essential for summer survival in dry environments (Padilla and Pugnaire, 2007), and has been linked to survival rates in $Q. coccifera$ across a range of different tree shelters (Bellot et al. 2003). However, unlike the latter study, no relationship between root growth and survival was observed here. For $Rh. lycioides$, even though survival was much higher in tubes, root length was similar to mesh shelters, while $Q. coccifera$ roots grew less in mesh shelters but only survival was impaired for the 80% transmissivity.

This species-specific effect of the type of shelter on seedling performance confirms that the effect of tube shelters on seedling performance depend on the ecophysiological features of the species (Puértolas et al. 2010; Vazquez de Castro et al. 2014). These previous studies pointed towards shade tolerance as the main trait explaining plant responses to tree shelters. Shade tolerant species were more benefited from protection, as root growth was less negatively affected by the reduction in light compared to intolerant species. Moreover, light reduction during summer could explain increased survival in $Q. ilex$ (Puértolas et al. 2010). Also, $Rh. lyciodes$ benefits from shade in semiarid plantations (Soliveres et al. 2008). However, our results suggest that other characteristics might also contribute to explain differential effects across species. Lack of knowledge on the physiological characteristics of $Rh. lycioides$ makes difficult to understand the basis of these differences. It seems that the higher overall survival
rates of this species compared to Q. coccifera, which are coincident with previous studies (Trubat et al. 2008, 2011), could be linked to faster root growth. Adults from Rh. lycioides show higher assimilation rates than Q. coccifera during spring, which could explain larger growth and root development (Bellot et al. 2004). The reason for the clear differences in survival between mesh and tube shelters within Rh. lycioides, which are less evident than in Q. coccifera, are not easy to explain with the current information available. Further studies should investigate which functional attributes other than shade tolerance determine seedling response to tube shelters.

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Table 1. Results from ANOVA (Snedecor F and associated probability P) test for the effects of Month of measurement (June and July), Species (*Q. coccifera* and *Rh. lycioides*), Shelter type (solid tube and plastic mesh) and Transmissivity (80-60-40 %) during 2014 summer on predawn water potential ($\Psi_{pd}$) and leaf fluorescence at predawn ($F_{v/F_{m_{pd}}}$), midday ($F_{v/F_{m_{md}}}$).

|                     | $\Psi_{pd}$ | $F_{v/F_{m_{pd}}}$ | $F_{v/F_{m_{md}}}$ |
|---------------------|-------------|-------------------|-------------------|
|                     | df | F   | $P>F$ | F   | $P>F$ | F   | $P>F$ |
| Month (M)           | 1  | 0.02 | 0.89  | 6.10 | **0.01** | 5.40 | **0.02** |
| Species (S)         | 1  | 1.80 | 0.18  | 60.94 | **<0.001** | 5.08 | **0.02** |
| Shelter Type (ST)   | 1  | 22.49 | **<0.001** | 6.11 | **0.01** | 19.80 | **<0.001** |
| Transmissivity (T)  | 2  | 0.15 | 0.85  | 6.30 | **0.002** | 3.64 | **0.02** |
| Month × Species     | 1  | 0.11 | 0.73  | 0.01 | 0.93   | 2.30 | 0.13   |
| Month × Shelter Type| 1  | 0.001 | 0.97  | 10.83 | **0.001** | 0.58 | 0.44   |
| Species × Shelter Type| 1 | 0.47 | 0.49  | 0.01 | 0.92   | 0.40 | 0.52   |
| Month × Transmissivity| 2 | 0.17 | 0.83  | 0.12 | 0.88   | 0.14 | 0.86   |
| Species × Transmissivity| 2 | 1.17 | 0.31  | 2.64 | 0.07   | 0.72 | 0.48   |
| Shelter Type × Transmissivity| 2 | 0.01 | 0.99  | 0.37 | 0.68   | 0.98 | 0.37   |
| M × S × ST          | 1  | 0.01 | 0.93  | 4.75 | **0.03** | 3.49 | 0.06   |
| M × S × T           | 2  | 0.250 | 0.77 | 0.08 | 0.91   | 0.53 | 0.58   |
| M × ST × T          | 2  | 0.12 | 0.88  | 0.41 | 0.66   | 0.11 | 0.89   |
| S × ST × T          | 2  | 1.05 | 0.35  | 0.71 | 0.49   | 0.24 | 0.78   |
| M × S × ST × T      | 2  | 0.16 | 0.85  | 1.24 | 0.29   | 0.65 | 0.52   |

Significant values ($P<0.05$) are highlighted in bold
Table 2. Contrasts of the generalized linear model effects for survival in October 2014 and February 2015 of *Q. coccifera* and *Rh. lycioides* species planted with solid tubes or meshes (Shelter type) under three levels of light transmissivity (40- 60- 80%).

|                     | October 2014 | February 2015 |
|---------------------|--------------|---------------|
|                     | df | Wald $\chi^2$ | $P > \chi^2$ | Wald $\chi^2$ | $P > \chi^2$ |
| Species (S)         | 1  | 6.07          | **0.014**     | 3.04          | 0.081         |
| Shelter type (ST)   | 1  | 31.67         | **0.000**     | 38.86         | **0.000**     |
| Transmissivity (T)  | 2  | 7.25          | **0.027**     | 12.18         | **0.002**     |
| Species × Shelter Type | 1  | 11.00         | **0.001**     | 14.12         | **0.000**     |
| Species × Transmissivity | 2  | 1.17          | 0.556         | 0.90          | 0.637         |
| Shelter type × Transmissivity | 2  | 3.76          | 0.153         | 7.37          | **0.025**     |
| S×ST×T              | 2  | 0.60          | 0.742         | 0.21          | 0.898         |

Significant values ($P<0.05$) are highlighted in bold.
Table 3

Table 3. Results from ANOVA (Snedecor F and associated probability P) test for the effects of Species (*Q. coccifera* and *Rh. lycoides*), Shelter type (solid tube and plastic mesh) and Transmissivity (80-60-40 %) on morphological traits of seedlings excavated in February 2015

|                | Leaf Area | Protruding Roots length | Shoot weight | Shoot/Root | Specific Leaf Area | Specific Root Length |
|----------------|-----------|-------------------------|--------------|------------|--------------------|-----------------------|
|                | df        | F  | P  | F  | P  | F  | P  | F  | P  | F  | P  | F  | P  |
| Species (S)    | 1         | 12.59 | **0.001** | 6.68 | **0.01** | 2.48 | 0.12 | 83.30 | **0.001** | 0.007 | 0.97 | 16.43 | **0.001** |
| Shelter type (ST) | 1       | 23.86 | < **0.001** | 3.88 | **0.05** | 8.14 | **0.006** | 0.81 | 0.37 | 1.13 | 0.29 | 1.02 | 0.31 |
| Transmissivity (T) | 2       | 0.57 | 0.56 | 0.13 | 0.87 | 1.34 | 0.26 | 1.83 | 0.16 | 0.13 | 0.87 | 0.62 | 0.54 |
| Species × Shelter type | 2   | 0.03 | 0.86 | 4.15 | **0.04** | 0.03 | 0.86 | 0.57 | 0.45 | 0.16 | 0.68 | 1.29 | 0.26 |
| Species × Transmissivity | 1  | 0.31 | 0.73 | 0.30 | 0.74 | 0.06 | 0.93 | 0.09 | 0.91 | 1.05 | 0.35 | 0.46 | 0.63 |
| Shelter type × Transmissivity | 2   | 1.66 | 0.19 | 0.95 | 0.39 | 0.45 | 0.63 | 2.7 | 0.07 | 1.54 | 0.22 | 0.27 | 0.78 |
| S × ST × T     | 2         | 2.07 | 0.13 | 2.35 | 0.08 | 1.43 | 0.24 | 1.20 | 0.30 | 2.66 | 0.08 | 1.5 | 0.21 |

Significant values (*P*<0.05) are highlighted in bold
Table 4. Morphological traits by species (*Q. coccifera* and *Rh. lycioides*) as affected by shelter type (plastic mesh or solid tube) of seedlings excavated in February 2015.

|                       | *Q. coccifera*                      | *Rh. lycioides*                   |
|-----------------------|-------------------------------------|----------------------------------|
|                       | Plastic mesh | Solid tube | Plastic mesh | Solid tube |
| Leaf Area (cm²)       | 60.60 ± 10.9 | 127.03 ± 20.43 | 36.15 ± 10.93 | 77.25 ± 19.0 |
| Protrud. roots length (mm)¹ | 82.93 ± 14.60b | 147.50 ± 20.77a | 171.47 ± 49.33a | 189.30 ± 56.83a |
| Shoot weight (g)      | 3.44 ± 0.47  | 5.11 ± 0.80  | 2.98 ± 0.93  | 4.39 ± 1.20  |
| Shoot/Root ratio (g·g⁻¹) | 0.58 ± 0.0   | 0.59 ± 0.0   | 1.40 ± 0.23  | 1.55 ± 0.27  |
| Specific leaf area (cm²·g⁻¹) | 74.07 ± 15.37 | 58.48 ± 5.23 | 72.84 ± 15.27 | 63.09 ± 6.5  |
| Specific root length (cm·g⁻¹) | 159.59 ± 25.97 | 180.80 ± 55.63 | 323.94 ± 63.80 | 246.09 ± 46.83 |

¹ As Species × Shelter type was significant for this variable (see Table 3), different letters following mean values ± SE denote differences among combination treatments after Tukey's post hoc test.
Figures

Figure 1

Figure 1. Summer predawn (A) and midday (B) photochemical efficiency ($F_v/F_m$) as affected by shelter type (plastic mesh or solid tube) and light transmissivity (T40-T60-T80 %). Data are averaged by species and month of measurement (June and July 2014). Different letters between Shelter type and among Transmissivity levels denote differences among levels of factors after Tukey’s post hoc test. Error bars represent ± SE.
Figure 2. Temperature, relative humidity (RH) and vapor pressure deficit (VPD) during a mean daily cycle (GMT+2) of June 2014 within different combinations of shelters types and light transmissivity. Data from two sensors per shelter type and transmissivity, 11 days of measurements and 15 minutes frequency are averaged to one point per hour.
Figure 3. Survival of *Q. coccifera* (top) and *Rh. lycioides* (bottom) along the study period as affected by type of shelter (plastic mesh and solid tube) and transmissivity (40%, 60% and 80%). Letters at the end of each treatment denote significant differences during last measurement (February 2015).
Figure 4. Post summer height (A) and basal diameter (B) of tested species (*Rh. lycioides* and *Q. coccifera*) in October 2014 as affected by shelter type (plastic mesh or solid tube) and light transmissivity (40-60-80 %). Different letters denote differences among levels of treatments after Tukey’s post hoc test. Error bars represent ± SE. *P* values for second order interaction are shown on top of each subfigure.
A

\[ P_{\text{Species } \times \text{Trasmissivity}} < 0.001 \]

\[ P_{\text{Shelter type } \times \text{Trasmissivity}} < 0.001 \]

Height (cm) Oct. 2014

Q. coccifera  R. lycioides  Plastic mesh  Solid tube

40%  60%  80%
B

$P_{\text{Species } \times \text{ Transmissivity}} = 0.5$

$P_{\text{Shelter type } \times \text{ Transmissivity}} = 0.03$

| Species            | Diameter (mm) Oct. 2014 |
|--------------------|--------------------------|
| Q. coccifera       | 4                        |
| R. lycioides       | 5                        |
| Plastic mesh       | 3                        |
| Solid tube         | 2                        |
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Title: Should we use meshes or solid tube shelters when planting in Mediterranean semiarid environments?

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Supplementary material. Post summer height and basal diameter of tested species (*Rh. lycioides* and *Q. coccifera*) in October 2014 as affected by shelter type (plastic mesh or solid tube) and light transmissivity (40-60-80 %).

|                     | Q.coccifera          | Rh. lycioides         |
|---------------------|----------------------|-----------------------|
|                     | Plastic Mesh         | Solid Tube            | Plastic Mesh         | Solid Tube            |
| Height (cm)         |                      |                       |                      |                       |
| Transmissivity 40 % | 17.60 ± 0.72         | 18.58 ± 0.73          | 24.91 ± 2.03         | 26.95 ± 2.03          |
| Transmissivity 60 % | 17.52 ± 0.82         | 18.40 ± 0.70          | 26.74 ± 1.98         | 29.81 ± 1.86          |
| Transmissivity 80 % | 12.57 ± 1.62         | 18.17 ± 0.87          | 10.08 ± 1.39         | 23.58 ± 1.75          |
| Diameter (mm)       |                      |                       |                      |                       |
| Transmissivity 40 % | 3.60 ± 0.2           | 4.47 ± 0.1            | 3.56 ± 0.1           | 3.9 ± 0.3             |
| Transmissivity 60 % | 3.83 ± 0.1           | 4.26 ± 0.2            | 3.70 ± 0.2           | 4.02 ± 0.2            |
| Transmissivity 80 % | 3.31 ± 0.3           | 4.40 ± 0.2            | 3.17 ± 0.2           | 4.41 ± 0.2            |