Fertilizer location modifies root zone salinity, root morphology, and water-stress resistance of tree seedlings according to the watering regime in a dryland reforestation

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Abstract

There is a direct relationship between soil nutrient concentration in localized zones and root proliferation and elongation under well-watered conditions. However, in field studies under semiarid conditions this relationship can change due to higher salt accumulation and soil dryness that affect root growth, water stress resistance, and seedling survival. We assessed the effect of different locations of fertilizer placement in the soil profile and water availability on root zone salinity, root development and ecophysiological responses of *Quillaja saponaria* Mol. after outplanting. A single dose (6 g L^{-1}) of controlled-release nitrogen fertilizer (CRF$_N$) was placed at 0 cm (top layer), 15 cm (middle layer), or 30 cm (bottom layer) depth in the containers in a greenhouse, in addition to an unfertilized treatment (control). After 6 months, seedlings were transplanted to the field and subjected to weekly watering regimes (2 L plant^{-1} and unwatered). Morphological and ecophysiological parameters were periodically measured on seedlings, as well as soil electrical conductivity (EC). After 1 year, the shoot : root ratio of unwatered seedlings decreased as a function of CRF$_N$ placement depth, which was attributed to lower shoot growth and not to greater root growth. The root morphology of the bottom layer treatment was negatively affected by high EC in unwatered seedlings. Greater total root length and root volume of the middle layer treatment was found only when well-watered; however, this did not contribute to improve physiological responses against water stress. The lowest EC and the highest photochemical efficiency, net photosynthesis, and stomatal conductance were shown by unfertilized seedlings, independent of water availability. Our findings suggest that varying depth of CRF$_N$ placement does not contribute significantly to improve root growth under water restriction. Water supplements, independently of the CRF$_N$ location in the substrate, contribute to decrease root zone salinity, and consequently, improve root volume growth.

Key words: nutritional patches / root plasticity / salt concentration / seedling ecophysiology / South American dryland forest

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1 Introduction

Inorganic fertilization and watering regime are the most common culture practices applied to forest tree seedlings to improve quality attributes, field performance and survival under water-stressed conditions (*Luis* et al., 2009; *Villar-Salvador* et al., 2012; *Cortina* et al., 2013). Morphological root development of tree species established under severely dry conditions is of particular importance because this allows moisture to reach the deepest soil layers (*Padilla* and *Pugnaire*, 2007; *Paula* and *Pausas*, 2011), and consequently maintains higher productivity rates, better seedling water status (*Giliberto* and *Estay*, 1978; *Armas* et al., 2010; *Lopes* and *Reynolds*, 2010), and shoot growth rates during summer drought (*Ovalle* et al., 2015).

An effective strategy to promote root proliferation and growth is localized nutrient application (*Drew*, 1975; *Li* et al., 2014; *Zhan* et al., 2014). The underlying principle is the ability of the root system to adjust its architecture, morphology and physiology to the spatial and temporal distribution of resources in the soil profile to optimize their acquisition (*Jackson* et al., 1990; *Hodge*, 2004; *Lambers* et al., 2006; *Lynch*, 2013). A recent study of crop species showed a positive relationship between deep phosphorus application and watering, and the development of deep roots (*Zhan* et al., 2014), reflecting an interaction between nutrients and water with important implications for water use efficiency (*Song* et al., 2010). However, *Chapman* et al. (2011) suggested that deep rooting ability is determined by water availability rather than by nutrient supply.

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In environments with prolonged exposure to dry soils, such as Mediterranean-type ecosystems, root growth and functionality as well as plant survival may be adversely affected (*Padilla* and *Pugnaire*, 2007; *Grossnickle*, 2012). An increase in dryness in the soil profile affects the extension and exploration of lateral and fine roots into deeper soil layers due to greater penetration resistance because of lower soil matric potential (*Whitmore* and *Whalley*, 2009; *Bengough* et al., 2011; *Jin* et al., 2015). Increased stem water stress generates total or partial reduction of root growth potential (*Tinus*, 1996; *Villar-Salvador* et al., 2004), root hydraulic conductivity (*Hernández* et al., 2009), stomatal conductance (*Jacobs* et al., 2004) and transpiration rates, which decrease the diffusion rate of nutrients from soil to root surface, reducing both nutrient uptake by roots and root-to-shoot transport (*Alam*, 1999).

Application of inorganic nutrients under drought or sub-irrigation regimes tends to produce excessive accumulation of fertilizer salts and/or specific ion toxicity (*e.g.*, Na^+ and Cl^-) in the rhizosphere (*Hu* and *Schmidhalter*, 2005; *Bumgarner* et al., 2015), decreasing soil osmotic potential and altering soil pH (*Jacobs* et al., 2003). An electrical conductivity (EC) 2.5–2.6 dS m^{-1} has been identified as potentially harmful for fertilized tree seedlings (*Timmer* and *Parton*, 1984; *Landis* et al., 1989; *Jacobs* et al., 2003). Under saline conditions, anatomical and morphological changes to the root system may occur (*Jacobs* and *Timmer*, 2005; *Hu* and *Schmidhalter*, 2005; *Tattini* et al., 2006) such as lower root apical meristem activity (*Drew*, 1975) and inhibition of lateral root growth and changes in vertical root growth orientation (*Galvan-Ampudia* and *Testerink*, 2011). Functional root traits are also affected by high salinity (*Bernstein*, 2013), highlighting the reduced absorption of phosphorus (*Rewald* et al., 2013).

Controlled-release nitrogen fertilizer (CRF_N) is a nutritional source widely used in culture and plantation of forest tree seedlings in diverse forest ecosystems (*Shaviv*, 2001). The technology associated with CRF_N helps to reduce salinity risks because nutrients are contained inside thermosensitive polymer-coated granules, and thus nutrient release is gradual (*Sharma*, 1979; *Rose* et al., 2004). However, as polymer-coated permeability depends on soil temperature, the nutrient release rate is accelerated under warm conditions (*Huett* and *Gogel*, 2000), increasing the risk of saline stress and root damage (*Handreck* and *Bunker*, 1996; *Jacobs* et al., 2003). This not only shows the potential impacts of CRF_N location, but also suggests that morphological root development is conditioned by the degree of interference between fertilizer and the zone of active root growth, especially under low water availability.

There have been several studies with localized root fertilization treatments with crop species (*Hordeum vulgare, Triticum aestivum, Vicia faba, Zea mays*; *Drew*, 1975; *Li* et al., 2014; *Jin* et al., 2015) and tree seedlings of temperate and boreal forests (*Pseudotsuga menziesii, Cedrus atlantica, Quercus rubra*; *Jacobs* et al., 2003; *Boukcim* et al., 2006; *Bumgarner* et al., 2015) grown in nurseries or greenhouses. The effects on root growth have generally been positive because the environmental conditions are more favorable than those in semiarid areas. However, there is not enough information regarding CRF_N application criteria in semiarid environments, despite the risk that high soil temperatures may affect root development. In addition, it is necessary to understand the interaction between salinity and water stress (*Munns*, 2002; *Hu* and *Schmidhalter*, 2005) to optimize the CRF_N and watering management at nursery and post-planting stages under stressful conditions (*Jacobs* et al., 2004; *Oliet* et al., 2004).

In the present study we hypothesized that field water shortage restricts the positive effect of localized nutrient concentration on root growth and, consequently, influences seedling performance under drought conditions in the Mediterranean zone of central Chile. Our objective was to assess the post-planting impact of different locations of inorganic fertilizer placement in the soil profile and water availability on root zone salinity, root development and ecophysiological responses of *Quillaja saponaria* Mol. (Quillajaceae) seedlings. We chose this species because (1) it is a representative evergreen tree species of South American Mediterranean forests (30–38°W) (*Donoso*, 1982), (2) it has been extensively used for forest restoration with irregular outcomes (*Becerra* et al., 2013; *Ovalle* et al., 2015), and (3) it develops a strong tap root as one of the main survival strategies to long drought periods (*Giliberto* and *Estay*, 1978).

2 Material and methods

2.1 Plant material and fertilization applied under controlled conditions

Seeds of *Quillaja saponaria* were collected from a forest located in Cuesta Zapata (33°23'S–71°16' W), central Chile. Germination and initial growth phase took place at Vivero y Jardín Pumahuida Ltda, Santiago, Chile. Seedlings selected ($n = 240$) for the study were homogeneous in size (6–8 cm in height and 1.0–1.5 mm in stem diameter), thus, differences in initial seedling sizes were minor (*MacFarlane* and *Kobe*, 2006). Selected seedlings were transplanted in 400 cm^3 black polyethylene bags at a research site at the Pontificia Universidad Católica de Chile (33°29'S–70°36'W) and grown for 6 months starting in January 2011. The potting substrate consisted of a mixture of loam soil, leaf mold and compost at a ratio of 2 : 1 : 1. A chemical analysis showed a nutritional composition of 0.47% N, 0.18% P, 0.38% K, 0.64% Mg, and 1.94% Ca. Chemical soil properties were: pH 7.0, EC 1.5 dS m^{-1}, and 13.9% organic matter. All seedlings were watered three times a week (200 cm^3) and covered with a Rashell mesh to avoid excess radiation.

The experiment consisted of a completely randomized design with four treatments of depth of CRF_N placement. Each treatment was applied to 60 replicates (240 plants in total). The experimental unit and the sampling unit was the individual plant. The conditions in the greenhouse site were homogeneous in luminosity, temperature, slope, and airflow. Treatments consisted of a single dose of 6 g L^{-1} or 17.10 g $plant^{-1}$ of CRF_N [6–7 kg m^{-3} substrate recommended by the manufacturer for nurseries and by the author based on previous nurs-

ery tree studies (*Jacobs* et al., 2003; *Bustos* et al., 2008)] placed at 0 cm (top layer), 15 cm (middle layer), and 30 cm (bottom layer) depth in the container, and an unfertilized control (Fig. 1). The CRF_N used was Basacote® Plus (COMPO) 15 N (8% NH_4^+ and 7% NO_3^-)–8 P_2O_5–12 K_2O–2 MgO, with a 12-month release period (estimated for a constant temperature of 21°C). The nitrogen contribution of the CRF_N dose was equivalent to 0.90 g N L^{-1} or 2.56 g N $plant^{-1}$.

2.2 Field site description and plantation

Seedlings were planted at Quebrada de la Plata (33°29'S–70°52'O; 490 m asl), central Chile. The study area has a semi-arid Mediterranean climate characterized by prolonged summer drought (6–8 months) and marked rainfall seasonality with an annual average of 330 mm, a mean temperature of 15°C and 67% relative humidity (*Di Castri* and *Hajek*, 1976). The summer drought lasted for 8 months during the present study, with maximum mean temperatures of 30°C and absolute lack of rain for 5 months (Fig. 2). The soil texture was characterized as clay loam with abundant gravel, pH 6.0, EC 0.2 dS m^{-1} and 3.1% organic matter. The soil nutritional composition was 14 mg P kg^{-1} and 274 mg K kg^{-1}.

Plantation was performed in July 2011. The plot was fenced using a protective mesh of 1.50 m height to prevent herbivore attacks. Manually opened planting holes (0.40 m wide × 0.60 m deep) were distributed 1.5 m × 2.0 m apart to prevent the wet bulbs of plants from influencing the water availability of neighbor plants (observations by trial pit in the experimental plot). To minimize the loss of fine roots and facilitate the work at the time of extraction, each plant was introduced into a 0.40 m wide × 0.65 cm deep permeable bag. An initial establishment period was defined as the first 90 days from planting (July–September 2011), in which 2 L water (plant · week)$^{-1}$ were manually applied to each seedling to overcome transplant shock.
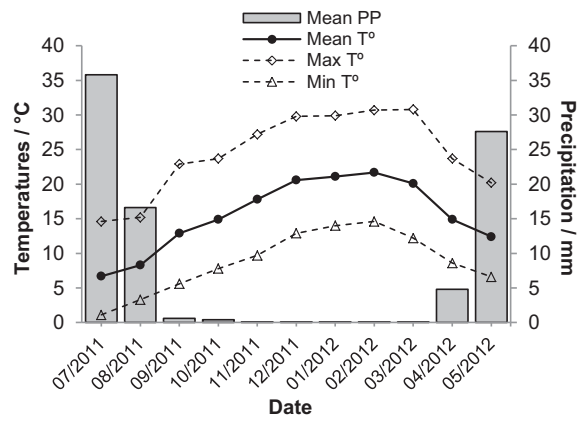

Figure 2: Monthly temperature (mean, minimum, and maximum; lines) and mean precipitation (bars) during the field experiment period, 2011–2012 growing season (source: Estación Pudahuel, Dirección Meteorológica de Chile).

2.3 Field treatments and experimental design

All fertilization treatments in the field were planted under two watering regimes: 2 L (plant · week)$^{-1}$ (W+) applied on one day and unwatered (W–), representing moderate and severe water stress, respectively, following an operational criterion commonly used in reforestation (*Luna*, 2006; *Valenzuela*, 2007). For the W– it was not possible to control the water received from rain or fog during the dry season, although these contributions were negligible due to the severe drought season 2011–2012 (Fig. 2). The experimental design for the field phase was a 4 × 2 factorial (eight treatments). Each treatment considered 15 replicates (120 plants in total) randomly assigned to each planting spot. The experimental unit and sampling unit was an individual plant. The study site conditions were homogeneous in luminosity, temperature, and airflow.
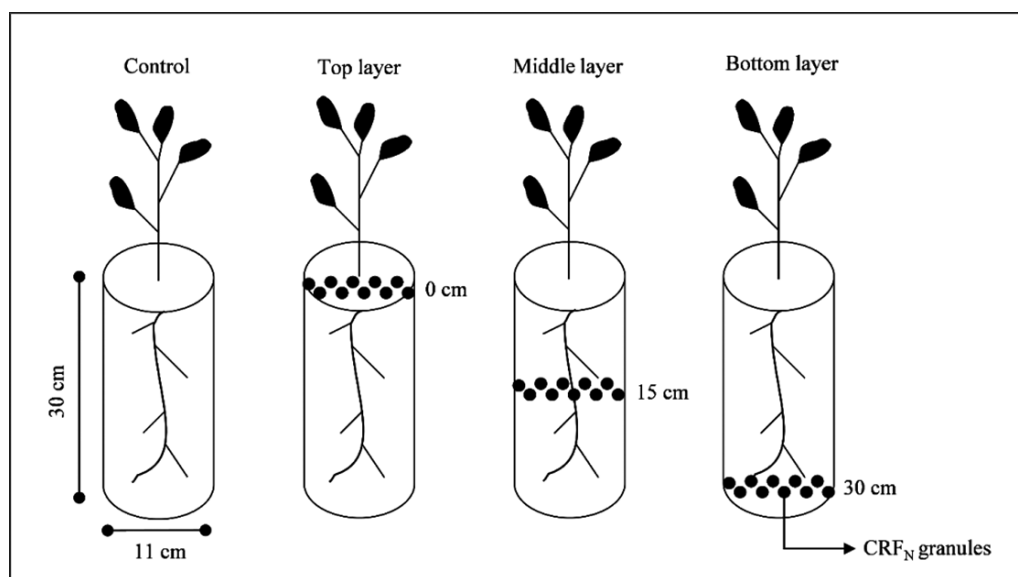

Figure 1: Schematic presentation of the experiment with different depths of controlled-release nitrogen fertilizer (CRF_N) placement in *Q. saponaria* seedlings.

2.4 Field samplings, harvest and measurements

Shoot height (S_H, cm), stem diameter (S_D, mm), and survival (number of living plants) were evaluated monthly (n = 15). Pre-dawn xylem water potential (Ψ_W), chlorophyll fluorescence (F_v/F_m), net photosynthetic rate (P_n, μmol m^{-2} s^{-1}), and stomatal conductance (g_S, mmol m^{-2} s^{-1}) were assessed every 2 months between July 2011 and May 2012. Pre-dawn Ψ_W was obtained from terminal parts of 5 cm-long lignified twigs from five randomly selected seedlings per treatment, with three or four mature leaves (second growing season). Measurements were carried out only in predawn hours (04:00-07:00) using a Scholander pressure probe (Model 1000, PMS Instruments, Inc., Corvallis, OR, USA). Chlorophyll fluorescence, expressed as maximal photochemical efficiency (Fv/Fm), was measured with 12 randomly selected seedlings at peak radiation (12:00–14:00) using a portable modulated chlorophyll fluorometer (Model OS-30 Opti-Sciences Inc., Hudson, NH, USA). Each measurement represented three readings of different leaves (mature leaves exposed to direct radiation) from the same plant. Each leaf was obscured for 20–25 min prior to measurement so that all components of the electron transport chain were fully oxidized and the energy dissipation mechanisms had descended to a basal level (*Maxwell* and *Johnson*, 2000), after which the optical fiber was installed in the darkened area of the leaf and the light pulse that determined minimum fluorescence (F_0) was applied. A saturating actinic light pulse (approx. 8000 μmol^{-1} s^{-2}) was applied for 0.8 s to obtain maximum fluorescence (F_m). Variable fluorescence (F_v) was calculated as the difference between F_m and F_0, while the ratio between F_v and F_m (F_v/F_m) gave the maximum photochemical efficiency of PSII. To obtain g_S and P_n values (n = 5) an infrared gas analyzer (IRGA) Model CI-340 (CID Inc., WA, USA) was used, and the measurement was taken at the same time and following the same sampling protocol described for F_v/F_m.

Eleven months after outplanting (May 2012), a random sample (n = 12) from each treatment was unearthed to analyze the effect of fertilization treatments on S_H, S_D, shoot dry mass (S_{DM}, g), shoot/root ratio (g g^{-1}), total root length (total R_L, m), total root volume (total R_V, cm^3), and root dry mass (R_{DM}, g g^{-1}). Roots were separated from the substrate by applying abundant water at low pressure to avoid loss of fine roots. Each plant was divided into shoots and roots by cutting at the cotyledon scar. Roots were grouped according to their diameter as fine (< 1 mm), medium (1–2 mm) and coarse (> 2 mm) roots. Morphological root variables were quantified using a high-resolution scanner (1200 DPI resolution, Epson Perfection 4490 Scanner, Nagano, Japan) and the image analysis software WinRHIZO Basic® (Regent Instruments Inc., Quebec, Canada). S_{DM} and R_{DM} data were obtained by drying in a forced air oven at 65°C until constant weight. The shoot : root ratio was calculated as the quotient between S_{DM} and R_{DM}. Concentrations of macro (N, P, K, Ca, Mg in %) and micronutrients (Cu, Zn, Mn in mg kg^{-1}) were analyzed based on composite leaf samples of three seedlings per treatment (n = 4), which were dried in an oven at 65°C for 72 h. Nitrogen concentration was determined with the Kjeldahl digestion process, and P, K, Ca, and Mg concentrations after wet-ashing. Electrical conductivity measurements (Hanna Instruments DIST 3 Conductivity Meter Tester, Woonsocket, RI, USA) were obtained from five samples from each soil layer (15 soil samples per treatment). Samples were dried in a forced-air oven at 70°C for 72 h. Subsequently, a sample of 5 g of dry soil (< 2 mm) was diluted in 50 cm^3 of distilled water until a homogeneous solution was obtained and after 24 h the EC was registered.

2.5 Statistical analyses

Simple Pearson correlation models were used to determine relations among variables. Prior to testing, compliance with normality, homogeneity of variance and linearity assumptions were verified. Variables that did not meet normality assumptions (S_H, S_D, R_{DM}) were transformed logarithmically (pre-transformation values are presented). Data were subjected to a two-way analysis of variance performed with the general linear ANOVA model (GLM) to evaluate the effects of depth of CRF_N placement and watering regimes and interaction effects. Treatments with significant differences (P < 5%) were identified with the Tukey multiple comparison test. To analyze survival, the non-parametric Chi-square test was applied based on the Kaplan-Meier method with a log-rank test (Mantel-Cox). Statistical analyses were carried out using the SPSS v. 17.0 program (SPSS Inc., Chicago, IL, USA).

3 Results

3.1 Electric conductivity in the root zone

After the first year post-planting, EC in the root zone differed significantly between fertilizer locations, between watering regimes, and the interaction between these two factors (Table 1). Among all treatments, EC varied approx. in the range of 0.5 to 2.0 dS m^{-1}. Fertilized and unwatered seedlings concentrated the higher EC values; the top layer treatment had significantly higher EC values. The unfertilized seedlings (control) had the lowest EC values for both watering regimes (Fig. 3). Unwatered seedlings (W–) had an average increase of EC of 50% in all fertilizer location treatments with respect to W+, while the unwatered control only increased 19% (Fig. 3).

3.2 Root morphological traits

Total R_V, total R_L, R_{DM}, and the shoot : root ratio differed significantly between CRF_N locations, between watering regimes and the interaction between these two factors, whereas S_{RL} differed significantly only between watering regimes and the interaction between factors (Table 1). Under both watering regimes the highest total R_V was observed in middle layer treatment (Fig. 4a). With respect to the effects of EC relative to each soil layer under W– conditions, the highest salinity value coincided with the lowest total R_V (bottom layer treatment). However, the lowest salinity value did not coincide with the highest total R_V (control). For both watering regimes the top and middle layer treatments showed similar EC levels, how-

Table 1: Two-way ANOVA results for main treatment effects (fertilizer location and watering regime) and their interaction on morphology, ecophysiology, foliar nutrients and soil properties measured in *Q. saponaria* seedlings. Ψ_W data were analyzed based on Ψ_W averages of the driest months (September, November, March, and April). Abbreviations are defined in the 'Material and methods' section.

Variables	Fertilizer location (FL)		Watering regime (W)		Interaction FL × W	
	F value	*P* value	*F* value	*P* value	*F* value	*P* value
MORPHOLOGY						
S_H / cm	8.383	< 0.001	9.808	0.002	0.377	0.769
S_D / mm	268.170	< 0.001	416.232	< 0.001	237.781	< 0.001
S_{DM} / g	77.416	< 0.001	34.243	< 0.001	0.654	0.582
Total R_L / m	9.108	< 0.001	28.450	< 0.001	10.517	< 0.001
Total R_V / cm^3	14.462	< 0.001	21.280	< 0.001	5.321	0.002
R_{DM} / g	23.575	< 0.001	52.134	< 0.001	10.892	< 0.001
Shoot/root ratio / g g^{-1}	18.032	< 0.001	118.972	< 0.001	10.151	< 0.001
S_{RL} / cm g^{-1}	1.628	0.187	38.032	< 0.001	7.491	< 0.001
ECOPHYSIOLOGY						
Pre-dawn Ψ_w / MPa	2.986	0.048	48.824	< 0.001	0.531	0.594
F_v/F_m	2.355	0.076	17.560	< 0.001	3.054	0.031
g_S / mmol m^{-2} s^{-1}	99.588	< 0.001	17.954	< 0.001	1.599	0.209
P_n / μmol m^{-2} s^{-1}	56.736	< 0.001	60.344	< 0.001	1.107	0.361
FOLIAR NUTRITION						
N / %	1.357	0.280	0.226	0.639	10.806	< 0.001
P / %	1.245	0.315	11.955	0.002	8.468	0.001
K / %	626.401	< 0.001	17720.548	< 0.001	138.481	< 0.001
SOIL PROPERTIES						
EC / dS m^{-1}	103.397	< 0.001	358.251	< 0.001	28.284	< 0.001

ever, the total R_V of the former was significantly lower than the latter (Fig. 4a).

Total R_L was positively and significantly affected by the middle layer treatment in well-watered seedlings (W+), exceeding by more than twice the treatment with the lowest value (bottom layer/W–; Fig. 4b). Under W+, CRF_N location treatments (independent of placement depth) had significantly higher total R_L compared to W–, while control treatments showed no differences for any of the watering regimes. With respect to the total R_L by diameter class, thin roots (30.56 ± 3.51 m, $P < 0.1\%$) and medium roots (4.76 ± 0.42 m; $P < 0.1\%$) were significantly greater in the middle layer/W+ treatment compared to thick roots. Thick roots were significantly greater than fine and medium roots in the bottom layer/W+ treatment (1.31 ± 0.07 m; $P < 0.1\%$; Fig. 4b).

The R_{DM} results showed an opposite trend to that observed for total R_V and total R_L. Under W–, the middle layer (21.51 ± 3.20 g) and bottom layer treatments (24.30 ± 2.50 g) had significantly higher values than the top layer treatment.

As well, under W–, the thin root dry mass showed significantly higher values ($P < 0.1\%$) in the middle (3.53 ± 0.25 g) and bottom layer treatments (3.47 ± 0.25 g). A significant increase of thick root dry mass ($P < 0.1\%$) was observed in the bottom layer/W– treatment (15.60 ± 1.15 g), coinciding with the response shown by total R_L for the same soil layer (Fig. 4b). The highest shoot : root ratio was produced by the top layer/W+ treatment (Fig. 4c). The shoot : root ratio was affected by W–, showing a 35% decrease with respect to watered seedlings. The lowest shoot : root ratio was observed for the bottom layer/W– treatment, with a ratio less than 1. Under both watering regimes we observed a decrease of shoot : root ratio according to depth of CRF_N placement (Fig. 4c).

3.3 Shoot growth

S_H and S_{DM} differed significantly between CRF_N locations and between watering regimes. For S_D, there was a significant effect of the principal factors and the interaction between these was also significant (Table 1). S_H growth did not show
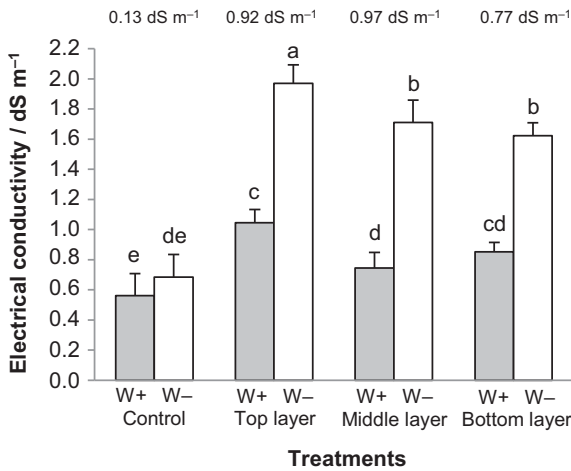
Figure 3: Electrical conductivity (EC, dS m^{-1}) in the root plug of *Q. saponaria* seedlings cultivated under different depths of CRF_N placement (control, top layer, middle layer, bottom layer), and two contrasting watering regimes [W+: 2 L (plant · week)$^{-1}$; W–: unwatered]. Numbers above the bars indicate the differences in EC between the two watering regimes under each fertilizer location treatment. The evaluation was made at the end of dry season after outplanting (May 2012). Mean values ± SE (*n* = 15) with different letters indicate significant differences at *P* < 5% (Tukey's HSD test).

significant differences among CRF_N location treatments, but there were significant differences between CRF_N location treatments and control. Well-watered seedlings had significantly higher S_H values than unwatered seedlings (Table 2).

The highest S_D increase (21.45 ± 1.23 mm) was found in the bottom layer/W+ treatment. Under W+ and W– regimes the control treatment had the lowest S_D (11.15 ± 1.51 mm and 10.11 ± 1.45 mm, respectively). A significant positive correlation (Pearson *R* = 0.72; *P* < 0.1%) between S_D and R_{DM} was found, which indicates that more than 50% of root biomass variation was attributed to increased S_D (Fig. 5). The highest S_{DM} was found in the middle layer/W+ treatment (Table 2).

3.4 Physiology, nutrition and survival

At the end of first dry season after outplanting (April 2012), Ψ_w differed significantly between fertilizer location and watering regimes (Table 1). All treatments experienced a strong pre-dawn Ψ_w decrease during the dry months (September 2011 to April 2012) of the first year of *Q. saponaria* establishment (Fig. 6). The top layer treatment showed the highest pre-dawn Ψ_w (Table 2). However, there was a significant difference only with the middle layer treatment. The W+ regime had a significantly higher pre-dawn Ψ_w than the W– (Table 2). There was a rapid water status recovery in all treatments after the first rain (May 13, 2012; Fig. 2). This response resulted in strong pre-dawn Ψ_w increases, reaching values from –0.91 to –1.59 MPa (*P* > 5%) (Fig. 6).

The F_v/F_m differed significantly between watering regimes and the interaction between the main factors (Table 1). Under the W– regime F_v/F_m was significantly lower in all CRF_N location treatments than in the control treatment (Fig. 7). The con-
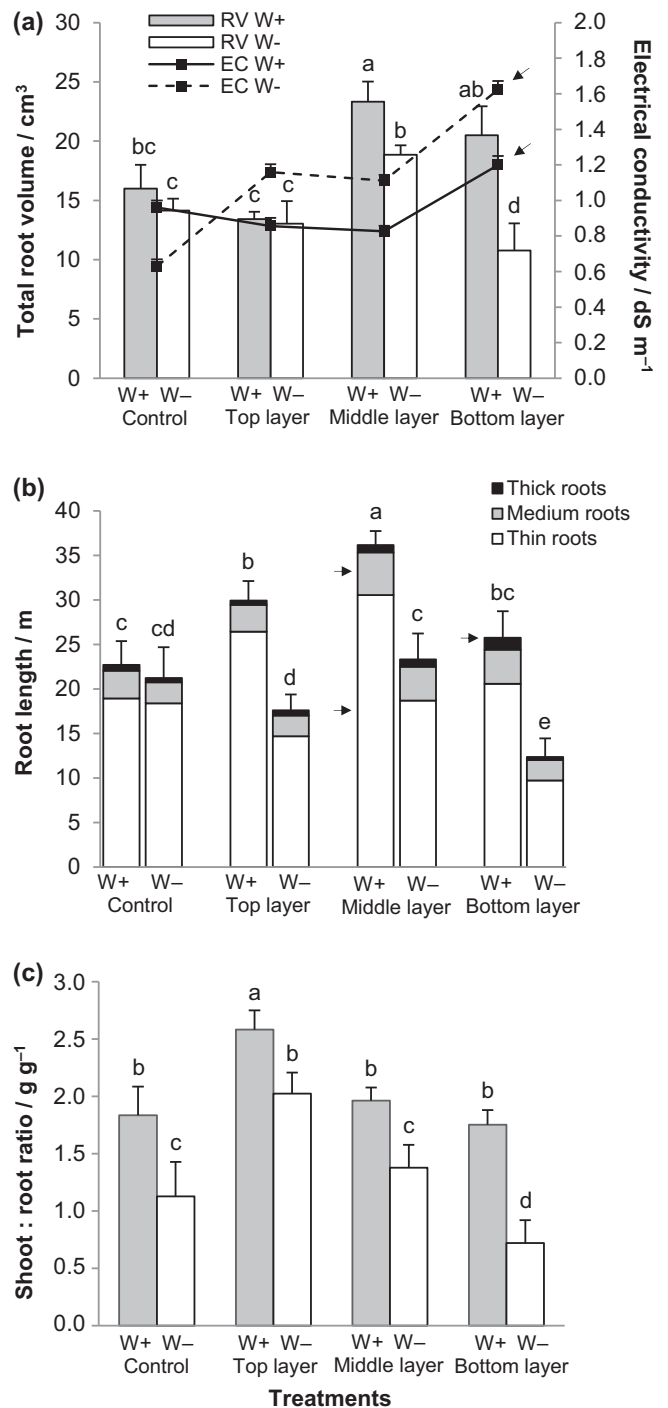

Figure 4: Root volume (*n* = 12) and EC by layer of root plug (*n* = 15) (a), root length (*n* = 12) (b), and shoot/root ratio (*n* = 12) (c) of *Q. saponaria* seedlings cultivated under different depths of CRF_N placement and two contrasting watering regimes [W+: 2 L (plant · week)$^{-1}$ and W–: unwatered]. The evaluation was made at the end of the dry season after outplanting (May 2012). Mean values ± SE with different letters indicate significant differences at *P* < 5% (Tukey's HSD test). Arrows show the significantly highest value (*P* < 5%).

trol treatment showed the most stable F_v/F_m values to changes in water availability, with a slight difference between the two watering regimes. The top layer/W+ treatment had the

Table 2: Shoot morphology and physiology of two-year-old *Q. saponaria* seedlings grown under different depths of CRF_N placement in the container (6 g L^{-1} Basacote Plus) and contrasting watering regimes [W+: 2 L (plant · week)$^{-1}$ and W–: unwatered]. Data were collected at the end of dry season after outplanting (May 2012). Values are means ± SE (*n* = 5). Means with different letters indicate significant differences at *P* < 5% (Tukey's HSD test). Abbreviations are defined in the Material and methods section.

| Variables	Fertilizer location				Watering regime	
	Control	**Top layer**	**Middle layer**	**Bottom layer**	**W+**	**W–**
SHOOT MORPHOLOGY						
S_H / cm	50.91 ± 2.09 B	60.24 ± 3.41 AB	63.44 ± 1.76 A	56.88 ± 2.56 AB	60.76 ± 1.31 a	54.97 ± 1.32 b
S_{DM} / g	17.25 ± 1.03 C	25.96 ± 1.35 B	30.45 ± 0.89 A	17.97 ± 0.45 C	25.03 ± 0.59 a	20.78 ± 0.43 b
PHYSIOLOGY						
Pre-dawn Ψ_w / MPa	−4.76 ± 0.61 AB	−4.29 ± 0.74 A	−5.11 ± 0.72 B	−4.87 ± 0.59 AB	−3.83 ± 0.67 a	−5.68 ± 0.70 b
g_S / mmol m^{-2} s^{-1}	113.60 ± 8.23 A	61.65 ± 2.84 B	30.58 ± 2.47 C	38.42 ± 4.73 C	69.01 ± 2.45 a	53.12 ± 0.88 b
P_n / μmol m^{-2} s^{-1}	12.80 ± 0.76 A	10.14 ± 0.54 B	6.05 ± 0.45 C	6.63 ± 0.48 C	10.54 ± 0.39 a	7.27 ± 0.12 b
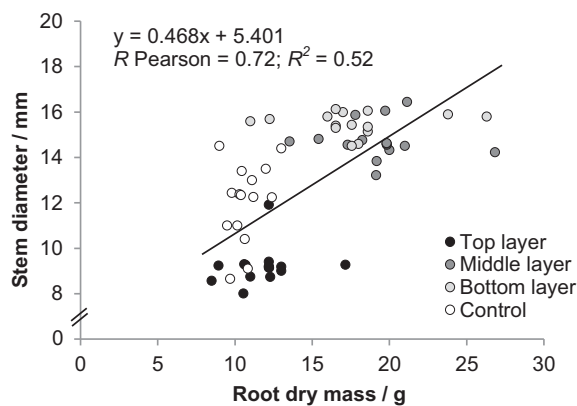
Figure 5: Relationship between stem diameter (S_D, mm) and root dry mass (R_{DM}, g) of *Q. saponaria* seedlings. Points represent mean values of watering treatments for each CRF_N placement treatment ± SE (*n* = 60).
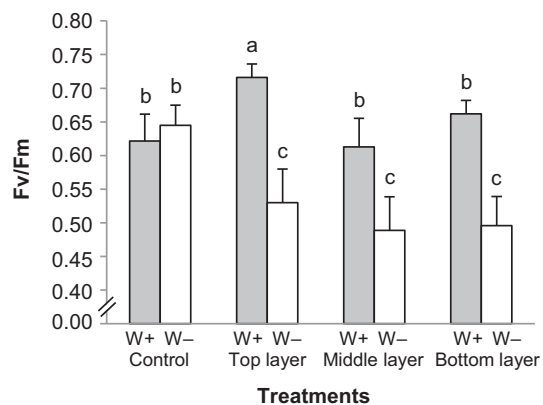

Figure 7: Maximum photochemical efficiency of PSII (chlorophyll fluorescence; F_v/F_m) in *Q. saponaria* seedlings cultivated under different depths of CRF_N placement (control, top layer, middle layer, bottom layer), and contrasting watering regimes [W+: 2 L (plant · week)$^{-1}$ and W–: unwatered]. The evaluation was made at the end of dry season after outplanting (April 2012). Mean values ± SE (*n* = 12) with different letters indicate significant differences at *P* < 5% (Tukey's HSD test).
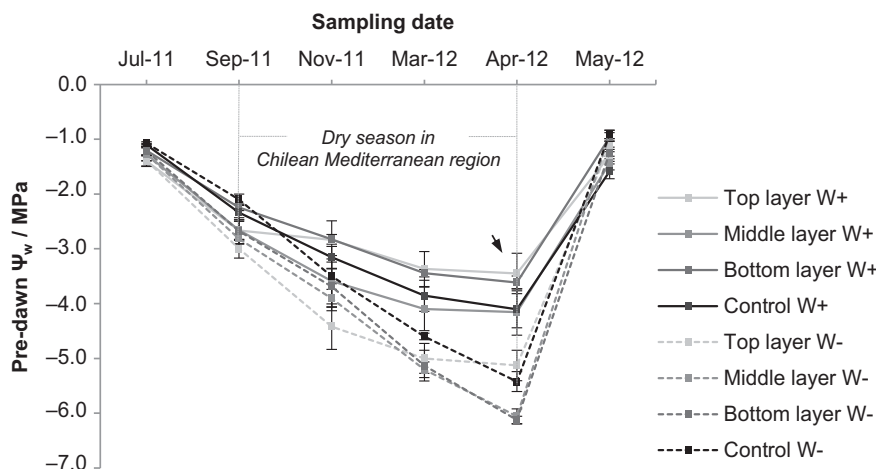

Figure 6: Pre-dawn xylem water potential (pre-dawn Ψ_w, MPa) of *Q. saponaria* seedlings cultivated under different depths of CRF_N placement (control, top layer, middle layer, bottom layer), and contrasting watering regimes [W+: 2 L (plant · week)$^{-1}$; W–: unwatered] during the first year after outplanting (2011/2012). Each point represents the mean value ± SE (*n* = 5). The arrow indicates significant differences (*P* < 5%, Tukey's HSD test) of the final measurement of the dry season (April 2012). The area between the double-dashed lines shows the dry period (severe drought), which strongly affects the plant water status.

closest value to the optimum range at the end of the dry season (April 2012). All F_v/F_m values were below the optimal reference range 0.80–0.83 recommended by *Maxwell* and *Johnson* (2000).

The g_S and P_n differed significantly among fertilizer location and watering regimes, but not in the interaction between the main factors (Table 1). The control treatment showed a significantly higher g_S value than the other fertilizer treatments (Table 2). The g_S values were higher in the W+ than the W– treatment. The control treatment had the highest P_n values, while the middle and bottom layer treatments had the lowest values for this variable. Finally, P_n was higher in the W+ treatments than the W– treatment (Table 2).

The foliar nutrition variables (N, P, K) differed significantly in the interaction between the main factors (Table 1). Nutrient concentrations varied slightly among treatments and all had within-normal ranges (Table 3). Potassium was the only nutrient to show significant differences, especially in the middle layer/W+ treatment, when its concentration was greater than the other treatments (Table 3).

After the first dry season (11 months after planting), the survival rate was 100% in all treatments (data not shown) except for the bottom layer/W– treatment, which reported a slight mortality of 20% by the end of the dry season.

4 Discussion

4.1 Influence of localized fertilization and water availability on root morphological traits

Our findings suggest that the effects of localized CRF_N on the development of root morphological traits of *Q. saponaria* seedlings were highly dependent on the watering regime (Table 1), because watering is effective in washing excess soil salts at the level of the root zone (Fig. 3). Therefore, these results partially confirm our hypothesis that field water shortage restricts the positive effect of localized nutrient concentration on root growth. For example, we found more root growth after outplanting under the middle layer treatment with watering, because it maintained moderate EC levels (< 1.5 dS m^{-1}; Fig. 4a,b). The results of watering seedling were consistent with several studies that found positive responses of root growth from localized nutrient application (*Drew*, 1975; *Pregitzer* et al., 1993; *Hodge*, 2004; *Boukcim* et al., 2006; *Nan*

et al., 2013). *Jacobs* et al. (2003) found the opposite when they applied a similar CRF_N dose to that of our study in the middle layer of containerized *Pseudotsuga menziesii* seedlings. The authors reported a marked decrease in the growth of white and lateral roots, which prevented root penetration into the deepest layer of the container. These differences are due to species- and nutrient-specific plasticity responses to localized nutritional enrichment (*Li* et al., 2014).

Soil localized fertilization to promote root growth is not recommended under water shortage, especially for deep-rooted species in dryland ecosystems that require rapid and early root development (*Padilla* and *Pugnaire*, 2007; *Paula* and *Pausas*, 2011). Edaphic factors impeding root growth and penetration are mainly physical limitations such as compaction, water stress, hypoxia (*Bengough* et al., 2011), and chemical limitations caused by over-fertilization, which generates toxic conditions due to high levels of salinity in the rhizosphere (*Jacobs* and *Timmer*, 2005; *Bernstein*, 2013). Saline stress can affect the activity of the apical meristem (*Drew*, 1975), which directly affects lateral root growth, and therefore soil nutrient uptake ability in poor soils (*Bernstein*, 2013). In our study, despite not having applied high fertilization doses, we found high salt concentration values in the treatments under W– (Fig. 3), which we attribute to lower washing effect of salts in the soil profile. However, these EC values do not exceed the critical EC range (> 2.5 dS m^{-1}) described for Northern Hemisphere conifers (*Timmer* and *Parton*, 1984; *Landis* et al., 1989; *Jacobs* et al., 2003). Furthermore, negative effects on root morphological traits of *Q. saponaria* were observed even under moderate levels of salinity (2.0 dS m^{-1}), particularly in total R_L (Fig. 4b). In contrast, total R_L did not decrease in the control/W– treatment, which can be attributed to a low average EC value (0.7 dS m^{-1}). The higher EC values (1.6 dS m^{-1}) of the bottom layer/W– treatment reduced total R_V (Fig. 4a), probably because the lower soil layer had higher salt concentrations due to the fertilizer-rich water accumulation from the upper layer, but the negative effects disappeared when watering was applied (*Zhan* et al., 2014).

Under water-restricted conditions, fertilizer placement in the top layer is a questionable practice because of the presence of higher surface temperatures and water evaporation, which results in a higher nutrient release rate and more salt accumulation (*Jacobs* and *Timmer*, 2005). However, there is no consensus about the most appropriate fertilizer location in containers or planting holes, generating discussions about whether the dose should be homogeneously mixed with the

Table 3: Foliar nutritional status of two-year-old *Q. saponaria* seedlings grown under different CRF_N placements (6 g L^{-1} Basacote Plus) and contrasting watering regimes [W+: 2 L (plant · week)$^{-1}$ and W–: unwatered]. Data were collected at the end of dry season after outplanting (May 2012). Values are means ± SE ($n = 4$). The mean with asterisk indicates a significant difference at $P < 5$ (Tukey's HSD test).

Fertilizer location	Control		Top layer		Middle layer		Bottom layer	
Watering regime	W+	W–	W+	W–	W+	W–	W+	W–
N / %	2.23 ± 0.07	1.93 ± 0.08	2.12 ± 0.08	1.94 ± 0.08	1.78 ± 0.06	2.34 ± 0.05	2.17 ± 0.08	2.20 ± 0.09
P / %	0.17 ± 0.02	0.15 ± 0.01	0.16 ± 0.01	0.17 ± 0.02	0.14 ± 0.01	0.20 ± 0.03	0.16 ± 0.01	0.19 ± 0.01
K / %	1.92 ± 0.01	1.20 ± 0.03	1.93 ± 0.02	1.33 ± 0.01	2.33 ± 0.03*	1.45 ± 0.01	2.19 ± 0.02	1.31 ± 0.01

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soil (*Jacobs* et al., 2003; *Oliet* et al., 2004) or applied at the bottom of the planting hole (*Carlson* and *Preisig*, 1981).

Although intensified water stress (W– treatment) negatively affected the root development of *Q. saponaria*, the response of R_{DM} contrasts with the trend followed by other morphological variables affected by water (Fig. 5). We found 29% more dry mass in W– than in W+, particularly in thin roots (Fig. 4c). The opposite has been reported in high (*Eucalyptus* spp.) and low water-demand species (*Quercus* spp.), in which water stress generates a strong decrease in total dry mass compared to watered plants (*Villar-Salvador* et al., 1999; *Graciano* et al., 2005).

4.2 Influence of localized fertilization on ecophysiological performance and survival under severe drought

Inorganic fertilization promotes greater fine root production (*Domenicano* et al., 2011) and longer lateral roots (*Boukcim* et al., 2001), which leads to increased root hydraulic conductance (*Hernández* et al., 2009), better water status, and increased plant productivity, even under moderate water stress conditions (*Luis* et al., 2009; *Villar-Salvador* et al., 2012). In our study, the positive influence of the middle and bottom layer treatments on morphological traits did not necessarily result in better ecophysiological responses to the intensification of water stress. This was shown by low pre-dawn Ψ_w, F_v/F_m, g_S, and P_n values, except for the control (Table 2). The greater water stress resistance shown by the control treatment can be attributed to the absence of fertilization, which attenuates the possible negative effects of increasing salinity in the root zone and osmotic stress. *Armas* et al. (2010) confirmed that an increase in soil salinity affected the physiological activity of plants, independent of the depth of roots. For example, *Jacobs* et al. (2003) showed that low F_v/F_m values with *Pseudotsuga menziesii* seedlings were attributable to saline toxicity produced by a high dose of CRF (24 g) applied in the growth medium. *Tattini* et al. (2006) subjected *Myrtus communis* and *Pistacia lentiscus* to saline conditions (200 mM NaCl solution) and found a significant decrease in F_v/F_m, although less pronounced in *P. lentiscus*.

Despite low field performance, the physiological status recovered rapidly at the beginning of the wet season (May 2012), showing high pre-dawn Ψ_w values (Fig. 6). A similar response was found in *Quercus rubra* seedlings after the watering replacement, which could be associated with a rapid recovery of photosynthetic activity (*Jacobs* et al., 2009). In our study, lower physiological activity did not affect the feasibility of planting. On the contrary, survival was over 80% for all treatments by the end of the first year post-planting, even under unwatered conditions.

On the other hand, lower photochemical efficiency of PSII in all fertilized and unwatered seedlings was observed. However, the above 0.5 values show that *Q. saponaria* is able to keep stable levels of F_v/F_m despite low water availability in the field (Fig. 7). Other studies with Mediterranean tree species reported an exponential decrease of F_v/F_m to values close to 0.20 when seedlings were exposed to drought (*Vilagrosa* et al., 2003).

5 Conclusions

The combined effect of localized fertilization and water restriction after outplanting generates moderate salinity that in turn negatively affects the root morphology and physiological responses of *Q. saponaria* seedlings, in accordance with our hypothesis. Therefore, the technique of varying the depth of CRF_N placement is not recommended for deep-rooted species growing under dry soil conditions that require rapid and early root development as a survival strategy. On the other hand, low salinity conditions presented by unfertilized seedlings improve the ecophysiological responses at the end of the dry season. We suggest that water supplements applied to fertilized seedlings contribute to decreasing root zone salinity, and consequently, improve root volume growth in early establishment under dry conditions.

References

Alam, S. (1999): Nutrient Uptake by Plants under Stress Conditions, in Pessarakli, M. (ed.): Handbook of Plant and Crop Stress. Marcel Dekker, New York, NY, USA, pp. 285–314.

Armas, C., *Padilla, F. M.*, *Pugnaire, F. I.*, *Jackson, R. B.* (2010): Hydraulic lift and tolerance to salinity of semiarid species: consequences for species interactions. *Oecologia* 162, 11–21.

Becerra, P. I., *Cruz, G.*, *Ríos, S.*, *Castelli, G.* (2013): Importance of irrigation and plant size in the establishment success of different native species in a degraded ecosystem of central Chile. *Bosque* 34, 23–24.

Bengough, A. G., *McKenzie, B. M.*, *Hallett, P. D.*, *Valentine, T. A.* (2011): Root elongation, water stress, and mechanical impedance: a review of limiting stresses and beneficial root tip traits. *J. Exp. Bot.* 62, 59–68.

Bernstein, N. (2013): Effects of Salinity on Root Growth, in Eshel, A., Beeckman, T. (eds.): Plant Roots: The Hidden Half, 4rd Ed. CRC Press, FL, USA, pp. 787–805.

Boukcim, H., *Pages, L.*, *Plassard, C.*, *Mousain, D.* (2001): Root system architecture and receptivity to mycorrhizal infection in seedlings of *Cedrus atlantica* as affected by nitrogen source and concentration. *Tree Physiol.* 21, 109–115.

Boukcim, H., *Pagès, L.*, *Mousain, D.* (2006): Local NO_3^- or NH_4^+ supply modifies the root system architecture of *Cedrus atlantica* seedlings grown in a split-root device. *J. Plant Physiol.* 163, 1293–1304.

Bumgarner, M. L., *Salifu, K. F.*, *Mickelbart, M. V.*, *Jacobs, D. F.* (2015): Effects of fertilization on media chemistry and *Quercus rubra* seedling development under subirrigation. *HortScience* 50, 454–460.

Bustos, F., *González, M. E.*, *Donoso, P.*, *Gerding, V.*, *Donoso, C.* (2008): Effects of different doses of slow-release fertilizer (Osmocote®) in the development of coigüe, raulí and ulmo seedlings. *Bosque* 29, 155–161 (in Spanish).

Carlson, W. C., *Preisig, C. L.* (1981): Effects of controlled-release fertilizers on the shoot and root development of Douglas-fir seedlings. *Can. J. Forest Res.* 11, 230–242.

Chapman, N., *Whalley, W. R.*, *Lindsey, K.*, *Miller, A. J.* (2011): Water supply and not nitrate concentration determines primary root growth in *Arabidopsis*. *Plant Cell Environ.* 34, 1630–1638.

Cortina, J., *Vilagrosa, A.*, *Trubat, R.* (2013): The role of nutrients for improving seedling quality in drylands. *New Forest.* 44, 719–732.

Di Castri, F., *Hajek, E.* (1976): Bioclimatología de Chile, Bioclimatología de Chile. Santiago de Chile, Chile.

Domenicano, S., *Coll, L.*, *Messier, C.*, *Berninger, F.* (2011): Nitrogen forms affect root structure and water uptake in the hybrid poplar. *New Forest.* 42, 347–362.

Donoso, C. (1982): Reseña ecológica de los bosques Mediterráneos de Chile. *Bosque* 4, 117–146.

Drew, M. (1975): Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot in barley. *New Phytol.* 75, 479–490.

Galvan-Ampudia, C. S., *Testerink, C.* (2011): Salt stress signals shape the plant root. *Curr. Opin. Plant Biol.* 14, 296–302.

Giliberto, J., *Estay, H.* (1978): Seasonal water stress in some chilean matorral shrubs. *Bot. Gaz.* 139, 236–240.

Graciano, C., *Guiamét, J. J.*, *Goya, J. F.* (2005): Impact of nitrogen and phosphorus fertilization on drought responses in *Eucalyptus grandis* seedlings. *Forest Ecol. Manag.* 212, 40–49.

Grossnickle, S. C. (2012): Why seedlings survive: influence of plant attributes. *New Forest.* 43, 711–738.

Handreck, K., *Bunker, K.* (1996): Fertilizers and hot weather. *Aust. Hort.* 94, 83–85.

Hernández, E., *Vilagrosa, A.*, *Luis, V. C.*, *Llorca, M.*, *Chirino, E.*, *Vallejo, V. R.* (2009): Root hydraulic conductance, gas exchange and leaf water potential in seedlings of *Pistacia lentiscus* L. and *Quercus suber* L. grown under different fertilization and light regimes. *Environ. Exp. Bot.* 67, 269–276.

Hodge, A. (2004): The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytol.* 162, 9–24.

Hu, Y., *Schmidhalter, U.* (2005): Drought and salinity: a comparison of their effects on mineral nutrition of plants. *J. Plant Nutr. Soil Sci.* 168, 541–549.

Huett, D. O., *Gogel, B. J.* (2000): Longevities and nitrogen, phosphorus, and potassium release patterns of polymer-coated controlled-release fertilizers at 30°C and 40°C. *Commun. Soil Sci. Plan.* 31, 959–973.

Jackson, R. B., *Manwaring, J. H.*, *Caldwell, M. M.* (1990): Rapid physiological adjustment of roots to soil enrichment. *Nature* 344, 58–59.

Jacobs, D. F., *Rose, R.*, *Haase, D. L.* (2003): Development of Douglas-fir seedling root architecture in response to localized nutrient supply. *Can. J. Forest Res.* 125, 118–125.

Jacobs, D. F., *Rose, R.*, *Haase, D. L.*, *Alzugaraya, P. O.* (2004): Fertilization at planting impairs root system development and drought avoidance of Douglas-fir (*Pseudotsuga menziesii*) seedlings. *Ann. For. Sci.* 61, 643–651.

Jacobs, D. F., *Timmer, V. R.* (2005): Fertilizer-induced changes in rhizosphere electrical conductivity: relation to forest tree seedling root system growth and function. *New Forest.* 30, 147–166.

Jacobs, D. F., *Salifu, K. F.*, *Davis, A. S.* (2009): Drought susceptibility and recovery of transplanted *Quercus rubra* seedlings in relation to root system morphology. *Ann. For. Sci.* 66, 1–12.

Jin, K., *Shen, J.*, *Ashton, R. W.*, *White, R. P.*, *Dodd, I. C.*, *Parry, M. A. J.*, *Whalley, W. R.* (2015): Wheat root growth responses to horizontal stratification of fertiliser in a water-limited environment. *Plant Soil* 386, 77–88.

Lambers, H., *Shane, M. W.*, *Cramer, M. D.*, *Pearse, S. J.*, *Veneklaas, E. J.* (2006): Root structure and functioning for efficient acquisition of phosphorus: Matching morphological and physiological traits. *Ann. Bot.* 98, 693–713.

Landis, T., *Tinus, R.*, *McDonald, S.*, *Barnett, J.* (1989): The Container Tree Nursery Manual: Volume 4, Seedling Nutrition and Irrigation. USDA, Washington, DC, USA.

Li, H., *Ma, Q.*, *Li, H.*, *Zhang, F.*, *Rengel, Z.*, *Shen, J.* (2014): Root morphological responses to localized nutrient supply differ among crop species with contrasting root traits. *Plant Soil* 376, 151–163.

Lopes, M. S., *Reynolds, M. P.* (2010): Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. *Funct. Plant Biol.* 37, 147–156.

Luis, V. C., *Puértolas, J.*, *Climent, J.*, *Peters, J.*, *González-Rodríguez, Á. M.*, *Morales, D.*, *Jiménez, M. S.* (2009): Nursery fertilization enhances survival and physiological status in Canary Island pine (*Pinus canariensis*) seedlings planted in a semiarid environment. *Eur. J. Forest Res.* 128, 221–229.

Luna, G. (2006): Evaluación de parámetros fisiológicos y de crecimiento en plantas de *Quillaja saponaria* Mol. bajo condiciones de déficit hídrico. Tesis de grado. Facultad de Ciencias Forestales, Universidad de Chile, Santiago de Chile, Chile.

Lynch, J. P. (2013): Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. *Ann. Bot.* 112, 347–357.

MacFarlane, D. W., *Kobe, R. K.* (2006): Selecting models for capturing tree-size effects on growth–resource relationships. *Can. J. Forest Res.* 36, 1695–1704.

Maxwell, K., *Johnson, G. N.* (2000): Chlorophyll fluorescence—a practical guide. *J. Exp. Bot.* 51, 659–68.

Munns, R. (2002): Comparative physiology of salt and water stress. *Plant Cell Environ.* 25, 239–250.

Nan, H., *Liu, Q.*, *Chen, J.*, *Cheng, X.*, *Yin, H.*, *Yin, C.*, *Zhao, C.* (2013): Effects of nutrient heterogeneity and competition on root architecture of spruce seedlings: implications for an essential feature of root foraging. *PLoS One* 8, DOI: 10.1371/journal.pone.0065650.

Oliet, J., *Planelles, R.*, *Segura, M. L.*, *Artero, F.*, *Jacobs, D. F.* (2004): Mineral nutrition and growth of containerized *Pinus halepensis* seedlings under controlled-release fertilizer. *Sci. Hortic.* 103, 113–129.

Ovalle, J. F., *Arellano, E. C.*, *Ginocchio, R.* (2015): Trade-Offs between drought survival and rooting strategy of two South American Mediterranean tree species: implications for dryland forests restoration. *Forests* 6, 3733–3747.

Padilla, F. M., *Pugnaire, F. I.* (2007): Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. *Funct. Ecol.* 21, 489–495.

Paula, S., *Pausas, J. G.* (2011): Root traits explain different foraging strategies between resprouting life histories. *Oecologia* 165, 321–331.

J. Plant Nutr. Soil Sci. 2016, *179*, 223–233

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Pregitzer, K., *Hendrick, R. L.*, *Fogel, R.* (1993): The demography of fine roots in response to water and N. *New Phytol.* 125, 575–580.

Rewald, B., *Shelef, O.*, *Ephrath, J. E.*, *Rachmilevitch, S.* (2013): Adaptive Plasticity of Salt-Stressed Root Systems, in Ahmad, P., Azooz, M. M., Prasad, M. N. V. (eds.): Ecophysiology and Responses of Plants under Salt Stress. Springer, New York, NY, USA, pp. 169–202.

Rose, R., *Haase, D. L.*, *Arellano, E.* (2004): Fertilizantes de entrega controlada: potencial para mejorar la productividad de la reforestación. *Bosque* 25, 89–100.

Sharma, G. (1979): Controlled-release fertilizers and horticultural applications. *Sci. Hortic.* 11, 107–129.

Shaviv, A. (2001): Advances in controlled release of fertilizers. *Adv. Agron.* 71, 1–49.

Song, C.-J., *Ma, K.-M.*, *Qu, L.-Y.*, *Liu, Y.*, *Xu, X.-L.*, *Fu, B.-J.*, *Zhong, J.-F.* (2010): Interactive effects of water, nitrogen and phosphorus on the growth, biomass partitioning and water-use efficiency of *Bauhinia faberi* seedlings. *J. Arid Environ.* 74, 1003–1012.

Tattini, M., *Remorini, D.*, *Pinelli, P.*, *Agati, G.*, *Saracini, E.*, *Traversi, M. L.*, *Massai, R.* (2006): Morpho-anatomical, physiological and biochemical adjustments in response to root zone salinity stress and high solar radiation in two Mediterranean evergreen shrubs, *Myrtus communis* and *Pistacia lentiscus*. *New Phytol.* 170, 779–794.

Timmer, V. R., *Parton, W. J.* (1984): Optimum nutrient levels in a container growing medium determined by a saturation aqueous extract. *Commun*. *Soil Sci. Plan.* 15, 607–618.

Tinus, R. W. (1996): Root growth potential as an indicator of drought stress history. *Tree Physiol.* 16, 795–799.

Valenzuela, L. (2007): Evaluación de un ensayo de riego y fertilización de Quillay (*Quillaja saponaria* Mol.) en la comuna de San Pedro, Provincia de Melipilla, Región Metropolitana. Tesis de grado. Facultad de Ciencias Forestales, Universidad de Chile, Santiago de Chile, Chile.

Vilagrosa, A., *Cortina, J.*, *Gil-Pelegrin, E.*, *Bellot, J.* (2003): Suitability of drought-preconditioning techniques in Mediterranean climate. *Rest. Ecol.* 11, 208–216.

Villar-Salvador, P., *Ocaña, L.*, *Peñuelas, J. L.*, *Carrasco, I.* (1999): Effect of water stress conditioning on the water relations, root growth capacity, and the nitrogen and non-structural carbohydrate concentration of *Pinus halepensis* Mill. *Ann. For. Sci.* 56, 459–465.

Villar-Salvador, P., *Planelles, R.*, *Enríquez, E.*, *Peñuelas, J.* (2004): Nursery cultivation regimes, plant functional attributes, and field performance relationships in the Mediterranean oak *Quercus ilex* L. *Forest Ecol. Manag.* 196, 257–266.

Villar-Salvador, P., *Puértolas, J.*, *Cuesta, B.*, *Peñuelas, J. L.*, *Uscola, M.*, *Heredia-Guerrero, N.*, *Rey Benayas, J. M.* (2012): Increase in size and nitrogen concentration enhances seedling survival in Mediterranean plantations. Insights from an ecophysiological conceptual model of plant survival. *New Forest.* 43, 755–770.

Whitmore, A. P., *Whalley, W. R.* (2009): Physical effects of soil drying on roots and crop growth. *J. Exp. Bot.* 60, 2845–57.

Zhan, A., *Chen, X.*, *Li, S.* (2014): The combination of localized phosphorus and water supply indicates a high potential for savings of irrigation water and phosphorus fertilizer. *J. Plant Nutr. Soil Sci.* 177, 884–891.