



Why do large, nitrogen rich seedlings better resist stressful transplanting conditions? A physiological analysis in two functionally contrasting Mediterranean forest species

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ABSTRACT

We analysed the physiological bases that explain why large and high nitrogen (N) concentration seedlings frequently have improved survival and growth relative to small seedlings in Mediterranean woodland plantations. Large seedlings of Aleppo pine (*Pinus halepensis* Mill.) and holm oak (*Quercus ilex* L.) with high N concentration ($L+$), and small seedlings with either high ($S+$) or low ($S-$) N concentration, were planted on two sites of different weed competition intensity that created contrasting stress conditions. Seedling survival, growth, gas exchange, N remobilization (N_R) and uptake (N_U), and water potential were assessed through the first growing season. Weeds reduced survival and growth, but seedling response to weed competition varied among phenotypes and between species. At the end of the first growing season, $L+$ Aleppo pine seedlings had higher survival than both small seedling types in presence of weeds but no differences were observed in absence of weeds. Mortality differences among phenotypes occurred in spring but not in summer. $L+$ Aleppo pines grew more than small Aleppo pines independently of weed competition. No holm oak seedling type survived in presence of weeds and no mortality differences among phenotypes were observed in absence of weeds, although $L+$ holm oak seedlings grew more than small seedlings. Mortality and growth differences in Aleppo pine were linked to marked physiological differences among phenotypes while physiological differences were small among holm oak phenotypes. $L+$ Aleppo pines had greater root growth, gas exchange, N_R , and N_U than small seedlings, irrespective of their N concentration. Seedling size in Aleppo pine had a greater role in the performance of transplanted seedlings than N concentration. The functional differences among oak phenotypes were small whereas they were large in pine seedlings, which led to smaller differences in transplanting performance in holm oak than in pine. This suggests that the nursery seedling quality improvement for planting in dry sites could depend on the species-specific phenotypic plasticity and functional strategy. Improved transplanting performance in large Aleppo pine seedlings relative to small seedlings was linked to greater gas exchange, root growth and N cycling.

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1. Introduction

Water stress, caused by weed competition and summer drought, are major factors that limit establishment of planted seedlings in abandoned Mediterranean croplands (Rey Benayas et al., 2005). Seedling transplanting performance also depends on seedling morphological and physiological attributes, which can be determined to a great extent by cultivation practices in the nursery

(van den Driessche, 1991a; Villar-Salvador et al., 2004a). Much evidence indicates that, for a given species, large seedlings frequently have improved survival and growth relative to small seedlings in Mediterranean environments (Puértolas et al., 2003; Villar-Salvador et al., 2008; Luis et al., 2009; Oliet et al., 2009; and references there in). Similar trends have also been reported in boreal and humid temperate environments, where large seedlings are more competitive against weeds than small seedlings (Lamhamedi et al., 1998; Noland et al., 2001; South et al., 2005). However, large seedlings can perform worse than small seedlings in very dry reforestation sites (Rose et al., 1993; Trubat et al., 2008). Several studies have also reported a positive correlation between transplanting performance of forest species and plant nitrogen (N) concentration (van den Driessche, 1988; Puértolas

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et al., 2003; Villar-Salvador et al., 2004a; Oliet et al., 2009). This indicates that seedling nutrient composition can play an important role in transplanting performance (Malik and Timmer, 1996; Salifu and Timmer, 2003), probably due to remobilization of internal N reserves (Millard et al., 2001; Silla and Escudero, 2003).

The above-mentioned relationships provide some insights into potential physiological mechanisms involved in the greater transplanting performance of larger-sized or high N concentration seedlings as compared to smaller-sized or lower N seedlings. Based on the transplant shock model of Burdett (1990), Villar-Salvador (2003) proposed a conceptual model that suggests that large seedlings and seedlings with high N concentration have higher net carbon gain because they have a greater photosynthetic surface area and a higher photosynthetic rate (Field and Mooney, 1986; Jose et al., 2003) than small seedlings or plants with low N concentration. Increase in carbon gain stimulates root growth (van den Driessche, 1991b) during the wet season, allowing seedlings to extract water and nutrients from a greater soil volume during the dry season. This allows plants to maintain high water potential and positive carbon balance (Burdett et al., 1983), which is critical to ensure plant survival during the dry season (Padilla and Pugnaire, 2007).

The objective of this study was to compare the survival, growth, and physiological performance of Aleppo pine (*Pinus halepensis* Mill.) and holm oak (*Quercus ilex* L.) seedlings of contrasting size and N concentration transplanted into two environments that differed in stress conditions. Distinct stressful transplanting conditions were achieved by experimentally manipulating weed density, which induced marked differences in soil water availability.

2. Materials and methods

2.1. Studied species

We selected Aleppo pine and holm oak because they are widely used in afforestation in the Mediterranean basin and they have different functional strategies to cope with environmental stresses. The holm oak is a shade-tolerant and slow growing species characteristic of late forest successional stages. By contrast, the Aleppo pine is a fast growing shade-intolerant, pioneer species (Zavala et al., 2000; Baquedano and Castillo, 2006). Holm oak has higher drought tolerance than Aleppo pine as it has lower osmotic potential at the turgor loss point (−3.2 vs. −2.2 MPa for holm oak and Aleppo pine, respectively) (Villar-Salvador et al., 1999, 2004b), and closes stomata at lower leaf water potential (Baquedano and Castillo, 2006).

2.2. Seedling cultivation

Seeds originating from inland Spain provenances (Alcarria-Serranía de Cuenca and Alcarria for holm oak and Aleppo pine, respectively) were sown in Plasnor 190/300-45 trays (45 cavities of 300 ml per tray; Plasnor, Legazpi, Spain) with unfertilized *Sphagnum* peat (Vapopeat XL, Vapo, Jyväskylä, Finland). We cultivated 1080 seedlings of three phenotypes in both species that differed in their morphology and N concentration (Table 1): (1) large seedlings with high N concentration (*L+*), (2) small seedlings with high N concentration (*S+*), and (3) small seedlings with low N concentration (*S−*). Phenotypes were achieved by varying the length of the growing period and fertilization rate. *L+* seedlings were sown on December 15, 2004 and fertilized twice weekly with a fertilizer solution of 100 ppm N from late May to mid December 2005. Small seedlings were sown on March 21, 2005 and fertilized weekly with 100 ppm N from late May until mid July 2005. Subsequently, fertilization of small seedlings was withheld for 3

weeks until early August and resumed until the end of October 2005, with seedlings being fertilized every 2 weeks with 50 ppm N. Half of the seedlings sown in March then received no fertilization until the end of the cultivation (*S−* seedlings) while the remainder were heavily fertilized once per week with 200 ppm N (*S+* seedlings). Fertilization was done with a Peters Professional® 20-7-19: N-P₂O₅-K₂O fertilizer (Scotts, USA). Furthermore, *L+* and *S+* seedlings were weekly sprayed with a foliar fertilizer (N-P-K: 8-8-6, Wuxal AA, Schering España S.A., Spain) at a 250 ml l^{−1} concentration for 3 weeks in December 2005. Seedlings were arranged in four random blocks and they were initially grown in a glass greenhouse in which temperature ranged from 4 to 25 °C and radiation was approximately 50% of that outside. In mid May 2005, seedlings were moved outside. Seedlings were kept well watered according to their requirements by irrigating them every 1–3 days. Cultivation was completed in the third week of December 2005.

2.3. Morphology and N concentration measurements following cultivation

At the end of the cultivation period, 12 and 16 seedlings per phenotype in Aleppo pine and holm oak, respectively, were randomly sampled and their shoots were cut at the cotyledon insertion point and root plugs were cleaned from the peat. Shoot height was measured as the length of the stem and stem diameter was measured at the cotyledon insertion point. Then, all seedling parts were washed and oven dried at 60 °C for 48 h to measure their mass.

To assess tissue N concentration, sampled seedlings of each phenotype were randomly distributed into four groups and their shoots and roots were pooled separately and finely ground. N concentration was determined by Kjeldahl analysis with K-SeSO₄-Se₂Cu₄ as catalyst in a Tecator DS-40 digestion system (FOSS Tecator, Sweden) and a SAN++ auto-analyser (Skalar, Netherlands).

2.4. Field study site and plantation experimental design

The field study site was located in Guadalajara, central Spain (40°39'N, 3°10'W) at 650 m a.s.l. on a flat river terrace with a poorly developed entisol soil from alluvium material. The climate is Mediterranean continental with mean annual precipitation and temperature of 400 mm and 13.4 °C, respectively. Spring (March to late May) is usually mild and wet while a pronounced dry and hot season occurs from June to mid September. Spring (01 March to 31 May) rainfall and mean maximum air temperature in the study year (2006) were 119 mm and 20.9 °C, respectively, while summer (01 June to 31 September) rainfall and mean maximum air temperature were 51 mm and 31.5 °C, respectively.

Seedlings were transplanted to the field in December 22, 2005. The experimental design consisted of a split-plot with five blocks. Each block was split into two main plots, which were randomly assigned to each weed competition treatment (presence and absence of weeds). In the weed treatment plot, the natural weed community was allowed to develop while in the other plot, competing weeds were removed with herbicide (Oxyfluorfen 24%, Inteike, TradeCorp, Madrid, Spain) at planting and at mid-summer along with manual hoeing when necessary. Within each main plot, six subplots were randomly assigned to each of the six treatments resulting from the combination of two species × three seedling phenotypes. Each subplot consisted of a row of 12 seedlings. Distance between seedlings within a row was 1 m and distance between subplots was 2 m. Soil was ripped to 50 cm depth before planting. Dominant species in the weed plots were wild oat (*Avena sterilis*), brome grass (*Bromus* spp.), shepherd's purse (*Capsella bursa-pastoris*), common lambsquarters (*Chenopodium album*), wall barley (*Hordeum murinum*), corn poppy (*Papaver rhoeas*), little hogweed (*Portulaca oleracea*), annual bastard cabbage (*Rapistrum*

Table 1

Morphological attributes, N concentration and N content of the three seedling phenotypes of Aleppo pine and holm oak at planting. Data are means \pm 1 SE; $n = 16$ for morphological attributes and $n = 4$ for N data. L+: large seedlings with high N concentration; S+: small seedlings with high N concentration; S–: small seedlings with low N concentration.

	Aleppo pine			Holm oak		
	L+	S+	S–	L+	S+	S–
Plant height (cm)	18 \pm 1.6 ^a	7.5 \pm 0.3 ^b	7.5 \pm 0.4 ^b	15 \pm 1.1 ^a	11 \pm 0.7 ^b	11.5 \pm 0.9 ^b
Plant diameter (mm)	3.1 \pm 0.1 ^a	2.2 \pm 0.1 ^b	2.2 \pm 0.1 ^b	4.0 \pm 0.2 ^b	3.3 \pm 0.2 ^b	3.5 \pm 0.3 ^b
Shoot mass (g)	4.0 \pm 0.3 ^a	0.9 \pm 0.1 ^b	1.1 \pm 0.1 ^b	1.9 \pm 0.2 ^a	1.1 \pm 0.1 ^b	1.1 \pm 0.2 ^b
Root mass (g)	2.6 \pm 0.3 ^a	1.0 \pm 0.1 ^b	1.3 \pm 0.1 ^b	2.1 \pm 0.2 ^a	1.3 \pm 0.2 ^b	1.8 \pm 0.3 ^{ab}
Plant N concentration (mg g ⁻¹)	22 \pm 1.2 ^a	19 \pm 0.8 ^a	9 \pm 0.2 ^b	18 \pm 0.3 ^a	13 \pm 0.3 ^b	9 \pm 0.5 ^c
Plant N content (mg)	154 \pm 10 ^a	37 \pm 3 ^b	22 \pm 2 ^b	71 \pm 9 ^a	32 \pm 3 ^b	27 \pm 2 ^b

In each species, seedling phenotypes with different letter indicate significant differences at $P < 0.05$ after a post hoc Tukey's test.

rugosum), Russian thistle (*Salsola kali*), and medusahead (*Taeniathesum caput-medusae*).

2.5. Effect of weeds on seedling microclimate

Incident photosynthetic photon flux density (PPFD) at seedling mid-height was measured at midday in May 23 on a clear day using a quantum photo/radiometer (HD 9021, Delta OHM, Casselle Di Selvazzano, Italy) on three seedlings in each subplot. Air vapour pressure deficit (VPD) was quantified in late spring (May 25) and in mid-summer (August 9) from the air relative humidity and temperature measures taken next to five seedlings per subplot using a portable gas exchange system (LCpro+ System, ADC BioScientific, Hoddesdon, UK). Soil water content in the 0–60 cm soil profile was measured monthly from April to September by Time Domain Reflectometry (TDR, Tektronix, Beauverton, CO, USA) according to methodology in Cassel et al. (1994) in each subplot. In May 31, following flowering of the weed community, we measured weed height and aboveground mass within three 50 \times 50 cm quadrants per block after drying at 50 °C for 2 days.

2.6. Transplant mortality and shoot growth

Seedling mortality and shoot growth was recorded at the end of spring (June 1) and after summer drought (October 30) of 2006. Shoot growth was measured as the stem volume increase and calculated as the difference between the stem volume at the end and at the beginning of each experimental period (spring and summer). Stem volume was calculated from the height and diameter of the stem assuming a cone shape. Diameter was measured at the cotyledon insertion point.

2.7. Gas exchange and water potential measurements in the field

We compared the net photosynthesis rate (A), stomatal conductance to water vapour (g_s), and leaf water potential (Ψ) of seedling phenotypes in spring (May 25 and 26), before summer drought onset, and in mid-summer (August 9 and 10), the hottest and driest time of 2006. Seedlings measured in May were different from those used in July, since the former were extracted for root growth and N cycling measurements (see Section 2.8). In spring, after the first shoot flush of growth and when leaves were mature, A and g_s were measured in the morning (9:00–11:00 h solar time) and at midday (12:00–14:00 h solar time) on current year shoots. Ψ was measured at pre-dawn and at midday on current year leaves (in holm oak) or twigs (in Aleppo pine). In summer, we did not measure all treatments because no holm oak seedlings survived in the weed plots. In plots with no weeds, pre-dawn Ψ was measured only in Aleppo pine, while midday Ψ was measured in both species. In the weed plots, summer A and g_s in the morning and at midday, and midday Ψ in Aleppo pine were only measured in L+ and S+ phenotypes due to low survival of S– seedlings. Gas exchange was

measured with a portable gas exchange system (LCpro+ System, ADC BioScientific, Hoddesdon, UK). Shoots were enclosed in the conifer chamber-type until measurement stabilization (3–4 min.). Ψ was measured with a Scholander-type pressure chamber. In all cases, we measured in one randomly selected seedling per subplot. In spring, air temperature and VPD at measuring time were 29.3 \pm 0.4 °C and 3.1 \pm 0.11 kPa (mean \pm 1 SE) in the morning and 37.1 \pm 0.5 °C and 5.5 \pm 0.12 kPa at midday. In summer, air temperature and VPD were 29.3 \pm 0.5 °C and 3.0 \pm 0.16 kPa in the morning and 41.0 \pm 0.5 °C and 6.8 \pm 0.22 kPa at midday.

2.8. Root growth and N cycling

In May 31 2006, the seedlings used for gas exchange measurements were extracted by carefully digging a 50 cm deep hole using an excavator with a 0.04 m³ bucket. Seedlings were separated into old and new shoots and roots and senescent leaves, washed with tap water, rinsed in distilled water, and oven dried at 60 °C for 48 h, and weighed for biomass determinations. Most seedlings had no senescent leaves, or if present they remained attached to the stem in both species. Roots protruding out of the root plug were designated as new roots while those in the root plug were classified as old roots.

Seedling fractions were finely ground and N concentration was determined by Kjeldahl analysis as described above. The N content was calculated as the product of the N concentration and the mass of the fraction. The N remobilized (N_R) from old tissues and net N uptake (N_U) from soil was estimated following similar methodology in Silla and Escudero (2003):

$$N_R = N_p - N_o - N_s \text{ (mg)} \quad (1)$$

where N_p , N_o , and N_s are the seedling N content at the end of the cultivation, N content in old roots and shoots, and N content in senescent leaves, respectively. N_U was quantified as

$$N_U = (N_o + N_n) - N_p - N_s \text{ (mg)} \quad (2)$$

where N_n is the N content in new shoots and roots.

Standardization of N_R by seedling N content at planting was used as a measure of N remobilization efficiency.

2.9. Data analysis

Although the experimental design included species as a factor, we performed statistical analyses separately for each species due to strong heterogeneity of variance, which could not be corrected by data transformation. The effect of weed competition and seedling type on PPFD were analysed by two-way ANOVA, and the effect of weed competition in air temperature and VPD were analysed by one-way ANOVA. Soil water content was analysed by repeated measures ANOVA. Differences in seedling attributes at the end of nursery cultivation were analysed by one-way ANOVA. Spring,

summer, and cumulative mortality were analysed using a generalized linear model with a binomial distribution and a logit link function with weed treatment, and seedling type as the factors. Shoot growth was analysed by a split-plot analysis with measures over time (Gómez and Gómez, 1984). New root production, N remobilization, and N uptake were analysed by split-plot analysis. A , g_s , and Ψ of both species in spring (May) and of Aleppo pine in summer were analysed by a split-plot analysis with measures over time. In summer, as no $S-$ Aleppo pine seedling was measured in the weed competition plots, seedling type was nested to weed competition for Aleppo pine. Holm oak A and g_s in summer were analysed by repeated measures ANOVA, whereas midday Ψ was analysed by ANOVA. Data were checked for normality and homogeneity of variances, and were transformed when necessary to correct deviations from these assumptions. All statistical analyses were performed with the Statistica 6.0. Package (StatSoft Inc., Tulsa, OK, USA).

3. Results

3.1. Functional characteristics of phenotypes following cultivation

The $L+$ phenotype of Aleppo pine seedlings had greater height, stem diameter, shoot mass, and root mass than $S+$ and $S-$ seedlings, which did not significantly differ between them (Table 1). N concentration of $L+$ seedlings was more than twice than that of $S-$ seedlings. $S+$ seedlings had twice the N concentration of $S-$ seedlings. No significant difference in N concentration was observed between $L+$ and $S+$ seedlings. N content was higher for $L+$ seedlings compared to the other two phenotypes.

$L+$ holm oaks were taller than $S+$ and $S-$ seedlings, but had similar shoot diameter. Shoot mass in $L+$ seedlings was greater than in $S+$ and $S-$ seedlings, while root mass of $L+$ seedlings was greater than in $S+$ seedlings, but was similar to that of $S-$ seedlings. $S+$ and $S-$ holm oak seedlings did not significantly differ in size. $L+$ and $S-$ holm oak seedlings had the highest and lowest N concentration, respectively, while $S+$ seedlings had intermediate values. N content was higher in $L+$ than in $S+$ and $S-$ seedlings, which did not significantly differ between them (Table 1).

3.2. Seedling microenvironment in the field

Mean height and aboveground biomass of the weed community was 65 ± 5 cm and 0.22 ± 0.02 kg m⁻², respectively. Weeds reduced PFD by 25% (Table 2), but this did not significantly differ among seedling types (data not shown). Air temperature was lower in presence than in absence of weeds, although these differences were not statistically significant. Mean VPD was unaffected by presence or absence of weeds. Soil water content diminished from spring through summer ($P < 0.001$). Soil water content was reduced by ca. 32% in spring by presence of weeds but differences between weed and no weeds plots dissipated in mid-summer (date \times weed competition interaction, $P < 0.001$).

3.3. Field mortality and growth

Seedling mortality was lower in Aleppo pine than in holm oak and lower in spring than in summer. In both species, weed competition strongly increased mortality in spring and in summer ($P < 0.001$ for both species in both seasons) (Fig. 1). All holm oak seedlings died in the weed plots by summer. In Aleppo pine, $L+$ and $S-$ seedlings had the lowest and highest spring mortality, respectively ($P < 0.001$). No significant differences were observed in summer mortality among Aleppo pine seedling phenotypes. Cumulative mortality in $L+$ Aleppo pine seedlings during the whole first

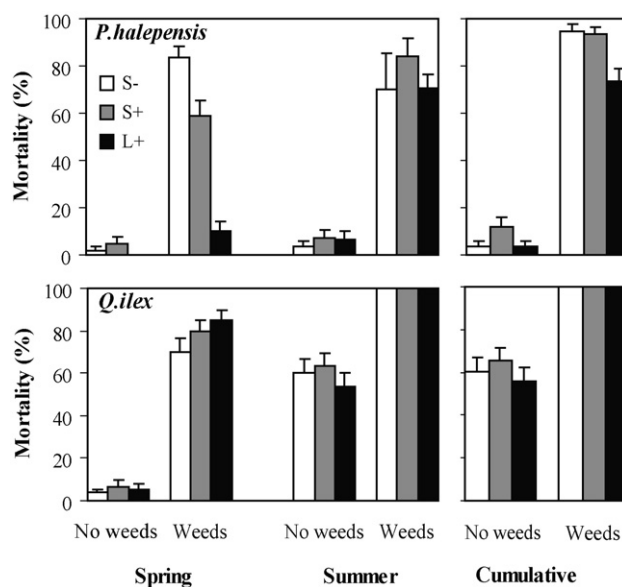


Fig. 1. Mortality of Aleppo pine (above) and holm oak (below) seedlings transplanted with weed and without weed competition during spring, summer and the whole first growing season (cumulative). In holm oak, no seedling survived in the herb plots during summer. Data are means \pm 1 SE. $L+$: large seedlings with high N concentration; $S+$: small seedlings with high N concentration; $S-$: small seedlings with low N concentration.

growing season was lower than either $S+$ or $S-$ seedlings in presence of weeds, whereas no significant differences among seedling phenotypes was found in absence of weeds (seedling type \times weed competition interaction, $P = 0.013$). In holm oak, no significant differences in mortality were observed among seedling phenotypes throughout the first growing season.

Both species grew more in summer than in spring ($P < 0.040$). Weeds reduced seedling growth in both species ($P < 0.042$) (Fig. 2). $L+$ Aleppo pines grew more than small Aleppo pine seedlings, and this difference was greater in absence than in presence of weeds (seedling type \times weed competition interaction, $P = 0.003$). $L+$ holm oak seedlings grew more than small holm oaks in absence of weeds, but no significant differences among seedling types was observed in presence of weeds (seedling type \times weed competition interaction, $P = 0.005$). Holm oak seedlings showed negative growth in spring in presence of weeds. In absence of weeds, $L+$ holm oak seedlings grew more than small holm oaks in summer but no difference was observed in spring (seedling type \times season interaction, $P < 0.001$). In both species, $S+$ seedlings had greater growth than $S-$ seedlings but differences were not statistically significant.

In Aleppo pine, $L+$ seedlings had greater new root mass in the field than either $S+$ or $S-$ seedlings, and this difference was greater in presence than in absence of weeds (seedling type \times weed competition interaction, $P = 0.04$; Fig. 2). Neither weed competition nor seedling phenotype affected new root growth in holm oak.

3.4. Seedling physiological performance in the field

Weeds reduced gas exchange and Ψ in both species ($P \leq 0.05$; Fig. 3). For instance, pre-dawn Ψ in spring without weeds was higher than -0.8 MPa in both species, but was -2.3 ± 0.3 and -3.3 ± 0.3 MPa for Aleppo pine and holm oak, respectively, in the weed plots. In Aleppo pine, reduction in A and g_s by weeds was greater at midday than at morning in summer (time of measure \times weed competition interaction, $P = 0.03$) but not in spring. Midday A was negative in summer in presence of weeds. In spring, $L+$ Aleppo pines in presence of weeds had higher A and g_s than $S+$ and $S-$ seedlings, which did not significantly differ between

Table 2Seedling microclimate in presence and absence of weeds, in spring and summer. Values are means \pm SE.

	Spring		Summer	
	Weeds	No weeds	Weeds	No weeds
Photosynthetic photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	1665 \pm 442 ^b	2184 \pm 318 ^a		
Air temperature ($^{\circ}\text{C}$)	36.7 \pm 0.4	40.1 \pm 0.9	37.7 \pm 0.5	41.3 \pm 0.5
Vapour pressure deficit (kPa)	4.3 \pm 0.18	4.4 \pm 0.19	4.5 \pm 0.55	5.0 \pm 0.29
Soil water content (%)	10.3 \pm 0.5 ^b	14.9 \pm 0.5 ^a	8.1 \pm 0.4 ^c	9.5 \pm 0.5 ^{bc}

Means with different superscript letters are significantly different at $P \leq 0.05$ after a Tukey's post hoc test.

them. However, seedling phenotypes did not significantly differ in absence of weeds (seedling type \times weed competition interaction, $P < 0.04$). By contrast, in summer, *L+* Aleppo pines had higher A and g_s than small Aleppo pines in absence of weeds, but not in presence of weeds (Fig. 3). Neither in spring nor in summer did Ψ significantly differ among Aleppo pine seedling types. In holm oak, seedling types did not significantly differ in Ψ or gas exchange, both in spring and in summer.

N_U was greater than N_R in absence of weeds in both species, but in the presence of weeds N_R was greater than N_U (Fig. 4). This was a result of a reduction in N_U by weeds in both species ($P < 0.001$ for Aleppo pine and $P = 0.07$ for holm oak) with no changes in N_R . N uptake by *L+* Aleppo pine seedlings in absence of weeds was greater than in small seedlings, which did not significantly differ between them. However, in presence of weeds there were no differences in N_U among pine phenotypes (seedling type \times weed competition interaction, $P = 0.03$). N_R was not affected by weed presence, but differed among seedling types, being highest in *L+* seedlings and lowest in *S-* seedlings ($P < 0.001$). Relative N_R was similar in *L+* and *S+* Aleppo pines, and higher than in *S-* Aleppo pines ($P < 0.001$). *L+* holm oaks absorbed more N than small seedlings, which did not significantly differ between them ($P = 0.02$). Neither weed competition nor seedling type affected the absolute or relative N_R in holm oak.

In Aleppo pine, N concentration in new shoots did not significantly differ among seedling types in absence of weeds (it varied from $14.0 \pm 0.6 \text{ mg g}^{-1}$ in *S-* to $13.0 \pm 0.9 \text{ mg g}^{-1}$ in *S+*). In competi-

tion with weeds, *S+* and *S-* Aleppo pine seedlings had highest and lowest N concentrations in new shoots (15 ± 1.5 and $9 \pm 0.7 \text{ mg g}^{-1}$, respectively), whereas *L+* seedlings showed intermediate values ($11 \pm 1.1 \text{ mg g}^{-1}$) (seedling type \times weed competition interaction, $P = 0.006$). In holm oak, N concentration in new shoots did not significantly differ among phenotypes, neither in presence nor in absence of weeds (data not shown).

4. Discussion

Weed competition resulted in significantly greater water stress as indicated by lower seedling Ψ in weed plots. This impaired seedling physiological performance and reduced survival and growth in both species, as found in previous studies (Gordon et al., 1989; Löf, 2000; Picon-Cochard et al., 2006). However, seedling phenotypes had marked differences in response to the stressful conditions created by weeds, which differed between species. Large Aleppo pines had greater survival and growth than small seedlings (irrespective of N concentration) in weed plots, which is consistent with other research (Villar-Salvador et al., 2008; Luis et al., 2009; Oliet et al., 2009; and references there in). Holm oak seedlings had 100% mortality in competition with weeds. By contrast, under low and mild stressful conditions as those imposed in the plots without weeds, seedling types did not differ in mortality but growth of larger seedlings was greater than in small seedlings in both species. Our results indicated that performance differences among Aleppo pine seedling phenotypes are higher when environmental stress

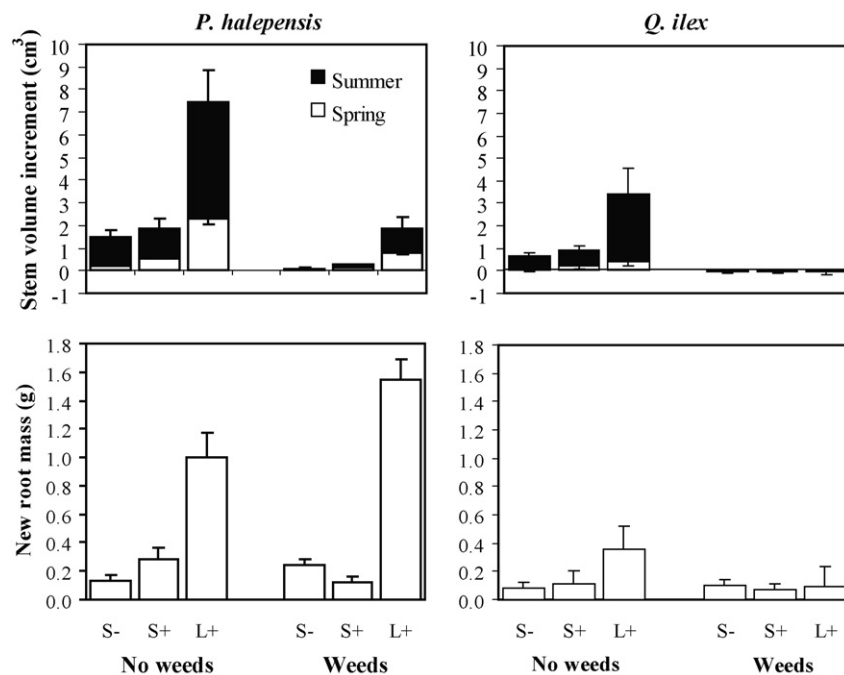


Fig. 2. Shoot growth, measured as the stem volume increment, during spring and summer (above) and new root growth in the field (below) during spring of Aleppo pine (left) and holm oak (right) seedlings, transplanted with and without weed competition. Data are means \pm 1 SE; $n = 5$. *L+*: large seedlings with high N concentration; *S+*: small seedlings with high N concentration; *S-*: small seedlings with low N concentration.

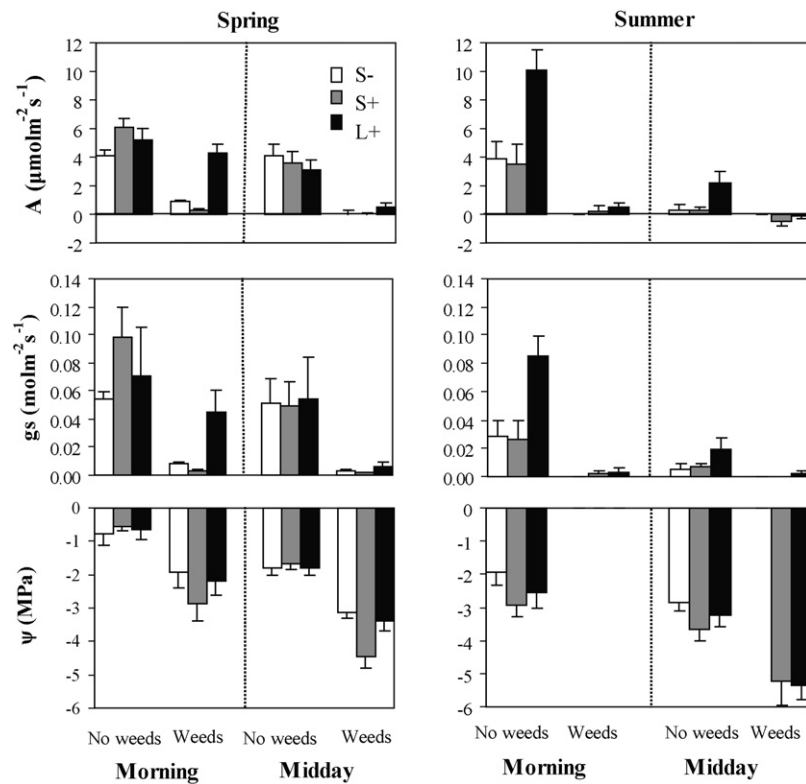


Fig. 3. Morning and midday net photosynthesis rate (A), stomatal conductance to water vapour (g_s) and water potential (Ψ) in both spring and summer in three Aleppo pine seedling phenotypes, transplanted with and without weed competition. Data are means \pm 1 SE; $n=5$. $L+$: large seedlings with high N concentration; $S+$: small seedlings with high N concentration; $S-$: small seedlings with low N concentration.

increases, as reported in Aleppo pine plantations in eastern Spain (Del Campo García et al., 2007), but they disappear under very strong stressful conditions as those created in the weed plots in summer.

4.1. Physiological differences among seedling phenotypes after transplanting

P. halepensis phenotypes had marked physiological differences, while differences among holm oak phenotypes were very small. Thus, we focussed this section on Aleppo pine. Maintenance of photosynthetic capacity is essential for surviving under resource limiting conditions (Johnson and Smith, 2005). Large Aleppo pine seedlings had higher A and g_s than small seedlings under low and moderate stress conditions, which might explain the reduced spring mortality of large Aleppo pine seedlings in comparison to seedlings in weed plots. In contrast, under severe drought, net photosynthesis rate was very low or negative and did not differ among Aleppo pine phenotypes, which can explain the high summer mortality and the absence of differences in summer mortality among phenotypes in the weed plots.

Production of large and deep roots is important for overcoming water stress (Grossnickle, 2005; Padilla and Pugnaire, 2007). Large seedlings produced more new roots than small seedlings at the onset of summer drought. Interestingly, large seedlings have more root growth than small seedlings in competition with weeds, indicating a greater capacity to allocate resources to roots in response to stress. However, greater root development did not provide any advantage in Ψ or N concentration indicating that greater g_s and A in large Aleppo pines cannot be ascribed to differences in Ψ (Kolb and Stone, 2000; Luis et al., 2009) or foliar N concentration (Samuelson et al., 2001; Maier et al., 2002). Increased g_s , hence A , in large seedlings compared to small seedlings may be attributed to greater

hydraulic conductivity linked to new root growth (Grossnickle and Russell, 1990; Hubbard et al., 2001; Sayer et al., 2005) or changes in root specific hydraulic conductance (Trubat et al., 2006). Teskey et al. (1983) showed that partial root removal depressed g_s in *Abies amabilis* independent of leaf water potential.

Nitrogen remobilization and uptake support plant growth (Millard, 1996; Salifu and Timmer, 2003; Silla and Escudero, 2003). Aleppo pine phenotypes differed in N cycling, with large seedlings remobilizing and absorbing more N than small seedlings and also having greater shoot and root growth. This highlights the importance of N cycling for growing in high-competition or nutrient-poor sites. Increased remobilization in large seedlings, which contained more N than small seedlings at planting, and the absence of any competition effect on remobilization support the contention that N remobilization is driven by source rather than sink strength (Millard et al., 2001). This response contrasts with that in *Picea mariana* where weed competition stimulated N remobilization (Malik and Timmer, 1996). It is remarkable that seedlings with high tissue N concentration (*i.e.* $L+$ and $S+$ seedlings) remobilized more N than seedlings with low tissue N concentration not only because they had higher N content but also because they remobilized a higher proportion of their stored N, as indicated by higher relative N_R in large Aleppo pines. This may increase N use efficiency of high N concentration seedlings in low-fertility transplanting sites. Similar to our findings, Millet et al. (2005) observed a positive relationship between N uptake and sapling size. Differences in N uptake between large and small seedlings can be attributed to differences in the size and architecture of the new root system (Chapin, 1991), growth, which determines sink strength (Nambiar and Fife, 1991), or transpiration, which determines nutrient mass flow. Reduction in N uptake due to competition may be due either to lower seedling growth, which reduces sink strength or to lower soil N and water availability (Nambiar and Fife, 1991; Picon-Cochard et al., 2006).

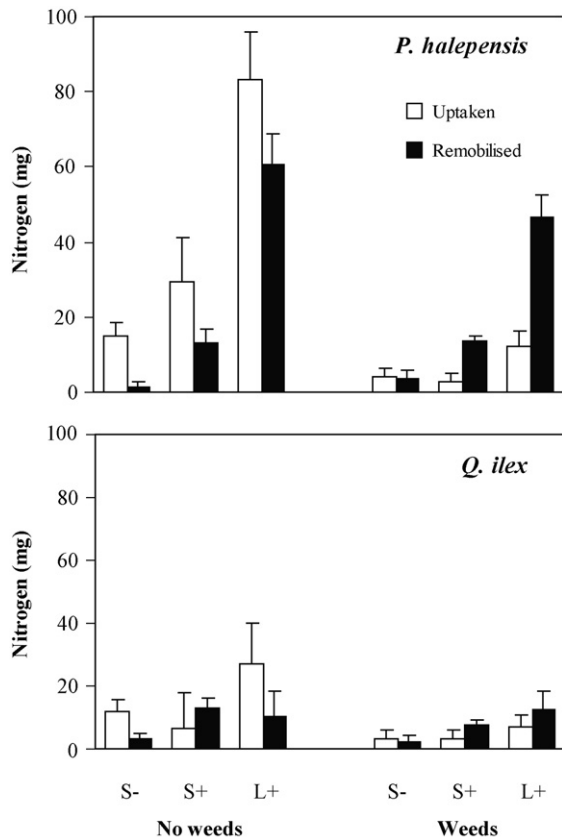


Fig. 4. N remobilization and uptake in three seedling phenotypes of Aleppo pine (above) and holm oak (below), transplanted with (right) and without (left) weed competition. Data represent means \pm 1 SE; $n=5$. L+: large seedlings with high N concentration; S+: small seedlings with high N concentration; S-: small seedlings with low N concentration.

However, we did not measure soil nutrient concentrations to support this assertion.

Many fertilization experiments have shown relationships between transplanting survival and seedling functional attributes (e.g. van den Driessche, 1988; Villar-Salvador et al., 2004a,b; Oliet et al., 2009). However, since plant size and nutrition covary in fertilization studies, the specific contribution of morphology and nutrient concentration on seedling establishment cannot be disentangled. Both plant size and N concentration can independently determine seedling transplanting performance by affecting the carbon and water economy of plants (Field and Mooney, 1986; Lamhamedi et al., 1998), the concentration of osmotically active compounds (Gebre et al., 1998) or the amount of N for remobilization (Millard, 1996; Silla and Escudero, 2003). Although our experimental design is incomplete (due to the lack of large seedlings with low N concentration), it provides two results that support the hypothesis that seedling size in Aleppo pine plays a more important role in seedling post-transplanting performance than N concentration. Firstly, the two small seedling types had similar survival, gas exchange, Ψ , shoot, and absolute new root growth in spite of the twofold difference in their N concentration at transplanting. Secondly, large and small seedlings with the same tissue N concentration (L+ and S+ seedlings) showed remarkable differences in survival, growth, and physiological performance.

4.2. Differences between holm oak and Aleppo pine

Whereas Aleppo pine phenotypes had marked differences in physiological and transplanting performance, holm oak pheno-

types showed much smaller differences. Distinctions between species might be explained by the smaller functional differences among holm oak phenotypes than in Aleppo pine phenotypes, which indicate lower phenotypic plasticity in the former species to growing conditions in the nursery. This result conforms with the general trend that pioneer species tend to have higher phenotypic plasticity than late-successional species (Bazzaz, 1979; Valladares et al., 2000). Our results therefore suggest that improvement in transplanting performance on dry sites with nursery practices might depend on species functional plasticity to environmental conditions and resource availability, and the strategy to cope with drought stress. Future studies should further investigate this hypothesis.

5. Conclusions

Large Aleppo pine seedlings had greater survival and growth than small seedlings, while differences among holm oak phenotypes were minor. Improved transplanting survival of large Aleppo pines seedlings depended on the degree of environmental stress experienced by seedlings, with large seedlings having better performance under low and moderate stress conditions; however, no difference may occur between seedling types under severe conditions. Improved survival and growth of larger Aleppo pines was attributed to higher gas exchange, root growth and N cycling, but not to water potential. Optimal seedling attributes for Mediterranean dry environments should promote high photosynthesis and nutrient remobilization during the wet season to ensure suitable carbon balance during the dry season. Seedling size at transplanting had a greater influence on post-transplanting survival than N concentration in Aleppo pine. These findings provide a physiological basis for understanding differences in survival and growth of planted stock in Mediterranean forest plantations and improve our understanding of the role of specific functional attributes on performance of planted seedlings.

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