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Root system of *Quercus suber* L. seedlings in response to herbaceous competition and different watering and fertilisation regimes

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Abstract Different management practices are commonly applied to increase pasture yield of Mediterranean open woodlands, but the consequences of increasing competition for soil resources with these practices on tree recruitment are still unknown. In a greenhouse study, belowground competition of *Quercus suber* L. seedlings growing together with natural (Oak_{NP}) or improved pasture (Oak_{IP}) was evaluated, and their root systems compared with those of seedlings growing in bare soil (Oak_{BS}). Two watering levels and two regimes of P_2O_5 fertilisation were also tested. Because of competition, the Oak_{IP} seedlings had their fine root mass density, coarse root length, and shoot mass reduced by up to 40, 36, and 39%, respectively, when compared to Oak_{NP} seedlings. Oak_{NP} and Oak_{BS} seedlings showed similar average root density parameters and shoot mass values, indicating that *Q. suber* seedlings growing with natural pasture is a low competitive system. High availability of water and phosphorus did not mitigate the strength of competition between herbaceous plants and oak seedlings, and favoured the pasture to the detriment of the trees. Our findings suggest that P_2O_5 fertilisation and irrigation practices performed to improve herbaceous productivity will negatively influence recruitment of *Q. suber* seedlings.

Keywords Cork oak · Dehesa · Improved pasture · Root density · Seedling recruitment

Introduction

Given the risk of environmental stress and herbivory, the seedling stage is an important and usually critical phase in the regeneration of trees. Under a Mediterranean-type climate, summer droughts have been identified as the main cause of early mortality of oaks, and competition for soil water by herbaceous plants is an additional cause of water limitation for oak seedlings (Gordon et al. 1989; Rey-Benayas et al. 2005). Some facilitation effects of neighbouring shrubs on seedling establishment, however, have been recently described (Plieninger et al. 2010). The coexistence of oak seedlings and herbaceous roots in the upper soil layers may impose competition for soil resources (Collet et al. 2006; Gea-Izquierdo et al. 2009; Marañón et al. 2009). A well-developed root system is a key factor for *Quercus ilex* L. and *Q. coccifera* L. to cope with summer drought; these species develop a deep tap root during the early stages

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of plant development (Tsakaldimi et al. 2005; Cubera et al. 2009a).

In western and south-western Mediterranean areas of the Iberian Peninsula there are more than 3.1 million hectares of savannah-like *Quercus* forests called "dehesas" (Moreno and Pulido 2009). Dehesas result from the partial clearance of oak forests to allow grazing, and consist of a mosaic of scattered, widely-spaced oak trees. Different management practices, namely agriculture, livestock husbandry, forestry and hunting activities are performed in dehesas. Pastures comprise 70–90% of cover and pasture yield and quality are increased through mineral fertilisation (mostly phosphate) and by introducing native and alien species with a high proportion of legumes (Olea et al. 2005). Introduced legume species may improve soil fertility and productivity since it enhances organic matter and N contents in the soil (Agbenin and Adeniyi 2005). The competition–stress–ruderal (C–S–R) model hypothesises that competition is of highest intensity and greatest importance in highly productive vegetation (Grime 1988). Little is known about the extent to which management practices that improve pasture yield affect oak seedling recruitment and consequently the natural regeneration and the long-term sustainability of this system. After some decades of intense pasture management practices (i.e. shrub-clearing, ploughing and sowing of pasture) and grazing activity, the sustainability of dehesas has been seriously questioned considering the over-aged oak trees, tree decline and the lack of seedling and sapling recruitment from seed origin (Costa et al. 2009; Moreno and Pulido 2009; Solla et al. 2009).

Given that soil water availability and phosphate content are among the main limiting factors for pasture production in dehesas (Moreno and Obrador 2007; Vázquez de Aldana et al. 2008), the aboveground and belowground growth of *Quercus suber* L. seedlings grown under different scenarios of competition with pasture and under different soil water and phosphorus availability was evaluated. The main objective of this work was to determine the spatial distribution of *Q. suber* roots with co-occurring herbaceous plants and how their patterns were reciprocally affected. Specifically, in a greenhouse experiment under controlled conditions the following hypotheses were tested: (i) *Q. suber* develops a deep root system during the early seedling stage irrespective of the presence of neighbouring herbaceous plants, (ii) *Q. suber* seedlings respond to increased competition for soil resources (with improved pasture vs natural pasture) by developing a deeper and/or denser rooting system, and (iii) the competitive effect of pasture on *Q. suber* seedlings will increase with increased soil resources availability.

Materials and methods

Experimental design

A greenhouse study was conducted with three simulated vegetation systems: (i) oak seedlings growing in bare soil ($\mathrm{Oak}_{\mathrm{BS}}$); (ii) oak seedlings growing together with natural pasture ($\mathrm{Oak}_{\mathrm{NP}}$); and (iii) oak seedlings growing together with improved pasture ($\mathrm{Oak}_{\mathrm{IP}}$). Each of the three systems studied was subjected to two watering levels and two regimes of P fertilisation in a factorial design. Each treatment was replicated ten times. A total of 120 seedlings, comprising 3 systems × 2 watering levels × 2 P-fertilisation regimes × 10 replicates were used.

Plant and soil material

The plant material originated from a *Q. suber* savannah-like forest (Herdade dos Esquerdos, Southern Portugal, 39°07′ N, 7°29′ W; 314 m asl), where both improved pastures and unmanaged natural pastures were traditionally grown in adjacent fields. In November 2006, a random sample of acorns of *Q. suber* from numerous trees were collected and stored for 3 months in a sealed plastic bag in a cold chamber at 4°C. In February 2007, the acorns were weighed and germinated in trays with a mixture of peat and vermiculite 3:1 (v:v) and watered near field capacity. A week later, those acorns with emergent radicles were individually weighed and individually planted into cylindrical PVC pots (30 L volume; 1.50 m high, 0.16 m inner diameter) packed with natural soil. Previous research has shown that the size of the pot used would provide seedlings with unrestricted root growth during the first vegetative period (Chirino et al. 2008; Cubera et al. 2009a). Pots were filled with soil from the Portuguese *Q. suber* forest previously described. In accordance to the horizons observed in the soil profile of this forest, soil layers of 0–20, and >20 cm were collected separately and placed in the pots. The upper 20 cm layer had a pH of 5.3 and contained approximately

57% of sand, 34% of silt and 9% of clay and a total C and N averaging 1.2 and 0.1%, respectively. The lower 1.3 m layer had a natural soil with pH of 5.5 and contained about 96% of sand, 2% of silt and 2% of clay and a total C and N averaging 0.32 and 0.01%, respectively. The available P content of both soil layers (12.3 and 4.8 mg kg^{-1} respectively; Egnér–Riehm method) was qualified as moderately low, considering the range of values reported for Spanish dehesas and European soils (Moreno and Obrador 2007). A plastic mesh was placed at the bottom of the pots to prevent substrate movement and to facilitate water draining and root air pruning.

The upper layer of the soil of the Oak$_{NP}$ system included seeds of natural pasture with species such as *Vulpia myuros* (L.) C.C Gmelin, *V. ciliata* Dumort, *Rumex conglomerates* Murray, *Bromus hordeaceus* L., *Agrostis tenerrima* Trin., and *Brachypodium distachyon* (L.) Beauv. Two weeks before planting the germinated acorns in the pots, seeds of improved pasture were placed at the ground level in the Oak$_{IP}$ pots. Improved pasture (FERTIPRADO, Sementes e Nutrientes, Lda., Vaiamonte, Portugal, www.fertiprado.pt) included a mixture of *Trifolium subterraneum* L., *T. vesiculosum* Savi, *T. incarnatum* L., *Ornithopus compressus* L., *O. sativus* Brot., *Lotus subbiflorus* Lag., *Dactylis glomerata* L., and *Lolium multiflorum* Lam. Germinated seeds in the pots of the Oak$_{BS}$ system were removed. The pasture layers were visibly established in the pots around the middle of March. Pots were kept in natural daylight under a greenhouse shade that reduced solar radiation by 50% at the Centro Universitario de Plasencia, Cáceres (40°02′ N, 6°05′ W; 374 m asl).

Watering and fertilisation treatments

Initially, all pots were watered by hand to field capacity every 3 or 4 days until plants were well established. In June 2007, once oak seedlings had emerged after 4 months of growth, a drip-irrigation system was inserted in half of the pots (+W regime). Each of these pots received 300 mL of water every 2 days until October 2007, i.e. 18,900 mL per pot during the whole experiment. The other half of pots were watered on a single day with 2,000 mL in the middle of August (−W regime). The +W and −W watering regimes simulated a non-drought situation versus a typical Mediterranean summer drought, respectively. The soil water content was measured in the middle of August at 0, 30, 75 and 125 cm depths on 60 pots with a Delta–Theta ML2X probe, and measurements confirmed differences between both watering regimes (Fig. 1).

Phosphate fertilisation was undertaken in June 2007 at the start of the second growth flush, according to Oliet et al. (2009). In a previous research fertilization during February did affect the acorn germination rate and slightly damaged the young radicles of oaks (unpublished results). Fertilisation treatments consisted of surface application of 36 kg ha^{-1} of P_2O_5 (added as $Na_2HPO_4 \cdot 2H_2O$) following the recommended fertilisation management practices (+F) (Olea et al. 2005), and application of 0 kg ha^{-1} of P_2O_5 (unfertilised pots, −F).

Plant and soil measurements

On August 2007, 2 days before watering the −W plants and coinciding with the maximum water stress status of the non-watered plants, the predawn water potential was measured in five *Q. suber* seedlings of each system and treatment (60 plants) by a Scholander pressure chamber. The measurements obtained confirmed differences at the plant level between +W and −W treatments (Fig. 2). In November 2007, coinciding with the end of the vegetative period, all potted herbaceous plants were clipped to ground level to estimate the herbaceous production, and seedlings survival was registered. Seedlings shoots were cut at

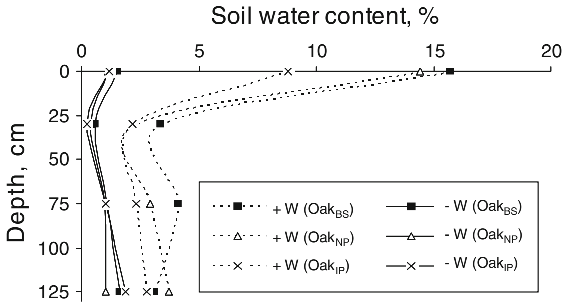

Fig. 1 Soil water content profile inside pots in the middle of August 2007 at 0, 30, 75 and 125 cm depth in which *Q. suber* seedlings were grown in bare soil (*Oak$_{BS}$*), together with natural pasture (*Oak$_{NP}$*) or together with improved pasture (*Oak$_{IP}$*) and submitted to field capacity (+*W*) and water stress (−*W*) ($n = 5$)

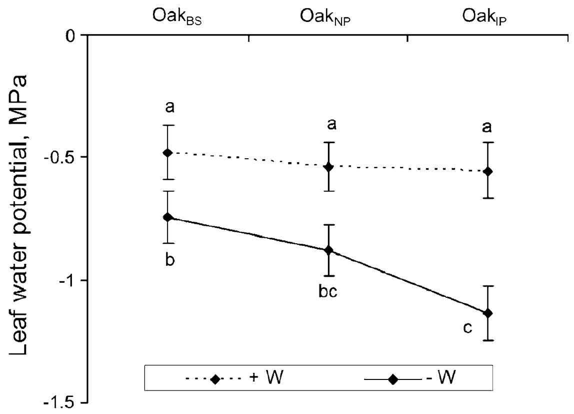

Fig. 2 Leaf water potential values at predawn, in the middle of August 2007 in *Q. suber* seedlings growing in bare soil (Oak_{BS}), together with natural pasture (Oak_{NP}) or together with improved pasture (Oak_{IP}) and submitted to field capacity ($+W$) and water stress ($-W$). *Vertical bars* indicate standard errors and different *letters* show significant differences between +W and −W treatments and among systems ($P < 0.05$, LSD test) ($n = 10$)

the cotyledon insertion point, and pasture and seedlings roots were harvested and washed according to Cubera et al. (2009a). All pasture roots were classified as fine roots, whereas oak roots were distinguished in two classes, namely fine roots (FR, diameter <2 mm) and coarse roots (CR, diameter ≥2 mm). Maximum rooting depth (MRD) of pasture and of *Q. suber* coarse roots was recorded. To morphologically characterise oak and herbaceous roots, five replicates per system and treatment were randomly selected (60 pots). Oak roots were identified by their black colour, while grass roots were white. The roots were scanned separately using an Epson expression 10000 XL (Epson America, Inc., USA). For each 10 cm-deep cylinder, the following root variables were obtained through the image analysis software WinRhizo Pro v.2007d (Régent Instruments Inc., Quebec, Canada): fine and coarse root length, and mean diameter of roots. After scanning, the plant materials (including non-scanned samples) were oven-dried and weighed. Data were expressed as root mass density (RMD; mg dm^{-3} of soil) and root length density (RLD; cm dm^{-3} of soil).

At the end of the experiment, soil samples were collected from the surface (0–10 and 10–20 cm) in each pot, sieved (<2 mm) and air-dried for the determination of available P. Available P was determined by the molybdenum blue method after extraction with AL solution (0.4 M acetic acid and 0.1 M ammonium lactate) (Egnér et al. 1960).

Data analysis

To summarise the vertical distribution of fine root density of herbaceous plants and the total root density of cork oak seedlings, we used the concept of Gale and Grigal (1987) model, i.e. reporting the depths above which 50 and 95% of the RMD were located in the pots (d_{50} and d_{95}, respectively). The cumulative root density within each soil layer was calculated and expressed as a fraction of the total root density. A non-linear regression was used to fit the function $fc = 1 - \beta^d$ to the profile of the cumulative root fraction (fc) from the soil surface to depth (d, cm). In the equation, β is the fitted "extinction coefficient". The values for d_{50} and d_{95} were then calculated from the following equations: $d_{50} = Ln\ (0.5)/Ln\ (\beta)$ and $d_{95} = Ln\ (0.05)/\mathrm{Ln}\ (\beta)$, respectively. Three-way ANCOVAs were used to examine the effects of the different systems ($\mathrm{Oak_{BS}}$; $\mathrm{Oak_{NP}}$; $\mathrm{Oak_{IP}}$), watering (+W and −W) and fertilisation (+F and −F) treatments on the MRD of coarse root, d_{50} and d_{95}. Initial acorn fresh mass was used as a statistical covariate.

In order to detect differences of RMD and RLD of herbaceous roots, as well as fine and coarse root RMD, RLD and average root diameter of oak seedlings between treatments (between-subject factor) and depths (within-subject factor), different analyses were applied. First, normality of data was checked through the Kolmogorov–Smirnov test, and independence of the root parameters at different depths was assessed through the Mauchley's sphericity test. Mauchley's test revealed dependence among values of different depths. Consequently, multivariate analyses of variance (MANOVA) were applied to analyse differences of the previous root parameters between treatments. Wilks' lambda statistic was used to obtain the signification levels of differences between treatments at the whole profile and at different depths. Least significant differences (LSD) test was used to determine differences between means when significant MANOVA results occurred. Linear regression analyses were made to relate acorn biomass with seedling root traits. The STATISTICA 6.0 software (StatSoft Inc., Tulsa, OK, USA) was used to perform all statistical analyses.

Results

Herbaceous roots

In −W−F pots, pasture production varied significantly from natural to improved pastures, yielding 1,540 and 2,400 kg ha^{-1} $year^{-1}$ of aboveground biomass, respectively ($P < 0.001$; Table 1). Mean values of RMD and RLD for improved pasture were more than sevenfold higher ($P < 0.001$; Table 1) than for natural pasture (13.8 and 1.9 mg dm^{-3} respectively, and 118.6 and 15.1 cm dm^{-3} respectively), and MRD was also higher in improved pasture than in natural pasture (75 and 42 cm respectively; Table 1). In both pasture types most of the roots were located in the uppermost soil layer (0–10 cm), with root density decreasing sharply with increasing depth (Fig. 3). Values of d_{50} and d_{95} did not vary between pasture types (Table 1). Watering positively and significantly affected improved pasture yield (increased by 1.26 fold for +W−F with respect to the −W−F pots; 2,400 and 3,020 kg ha^{-1} respectively) and RLD (increased by 2.2 fold; 118.6 and 256.8 cm dm^{-3} respectively; Table 1). Contrary to expectations, watering had no effect on the natural pasture yield and on the natural pasture root density (Table 1). Fertilisation also had no significant effect on pasture yield, or on pasture root density. However, the effect of watering on improved pasture root density was reinforced with fertilisation, resulting in significant Watering × Fertilisation interactions for RMD and RLD ($P < 0.01$ in both cases; Table 1). Watering and fertilisation, individually or together, reinforced differences between natural pasture and improved pasture in aboveground production and root density (Table 1). Watering produced a slightly shallower rooting profile, similarly affecting to both types of pasture (Fig. 4).

The soil water content decreased significantly with the presence of improved pasture compared to natural pasture, although the differences were significant in +W pots and not in −W pots ($P = 0.008$ and $P = 0.36$; respectively; Fig. 1).

Seedlings roots

Differences in soil water content between systems were detected at the seedling level; a lower leaf water potential (Ψ_{pd}) was measured in Oak_{IP} than in Oak_{BS} seedlings, when no watering was applied ($P = 0.01$; Fig. 2). Although 100% of seedling survival was recorded for the three systems in −W−F pots, at the end of the experiment, shoot biomass was significantly reduced with the presence of improved pasture (Table 2). In −W−F pots, coarse root density decreased sharply with depth regardless of the three simulated vegetation systems tested (Fig. 5a) and MRD of coarse root was significantly higher in Oak_{BS} than in Oak_{IP} (0.71 vs 0.51 m; $P = 0.03$) whereas Oak_{NP} showed an intermediate value (0.56 m), not significantly different from the previous ones. The fine roots of seedlings reached the bottom of the pots (1.5 m) and showed an irregular profile with depth

Table 1 Mean values of yield and root measurements of natural and improved pasture (NP and IP, respectively) growing in combination with *Q. suber* seedlings submitted to watering (+W, watered; −W, unwatered) and phosphorous fertilisation (+F, fertilised; −F, unfertilised) treatments

Systems	Treatments	Yield (kg ha^{-1})	RMD (mg dm^{-3})	RLD (cm dm^{-3})	d_{50} (cm)	d_{95} (cm)	MRD (cm)
NP	−W−F	1,540a	1.9a	15.1a	8.6ab	37.3ab	42.0ab
	+W−F	1,400a	1.0a	10.7a	11.9ab	51.5ab	26.1a
	−W+F	1,120a	0.6a	4.4a	15.3ab	66.3ab	27.2a
	+W+F	1,320a	1.7a	11.4a	6.8ab	29.4ab	26.0a
IP	−W−F	2,400b	13.8b	118.6c	7.9ab	34.3ab	75.0c
	+W−F	3,020cd	26.9b	256.8d	3.0a	13.0a	57.2abc
	−W+F	2,650bc	11.0ab	89.4b	13.8b	59.6b	82.1c
	+W+F	3,230d	76.6c	610.3e	5.2ab	22.4ab	81.7c

The bare soil (BS) system is not included since it does not contain any herbaceous plants. Within columns, different letters indicate differences among treatments ($P < 0.05$, LSD test)

RMD root mass density, *RLD* root length density, d_{50} and d_{95} depth for 50 and 95% cumulative root fractions, respectively *MRD* maximum rooting depth

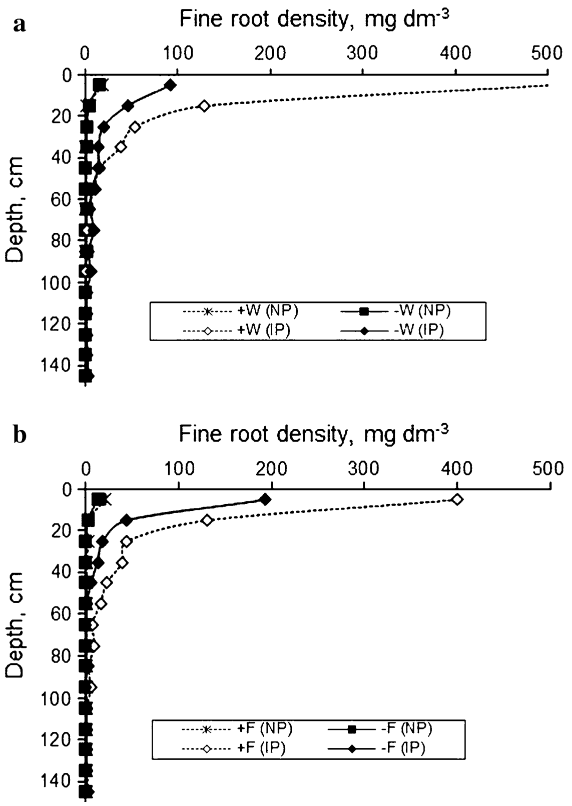

Fig. 3 Variation in depth of the fine RMD of natural (*NP*) and improved pastures (*IP*) submitted to different watering (**a**) and fertilisation (**b**) treatments. The bare soil (BS) system is not included since it does not contain any herbaceous roots

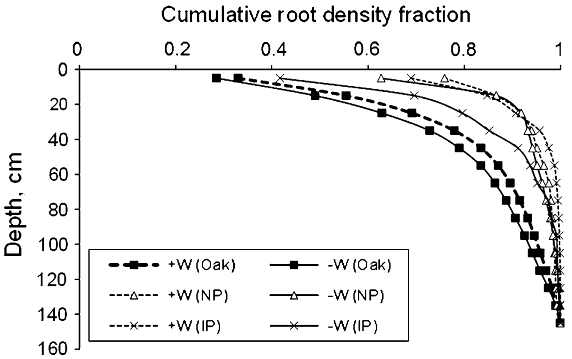

Fig. 4 Cumulative root densities of *Q. suber* seedlings (*Oak*), natural pasture (*NP*) and improved pasture (*IP*) submitted to different watering regimes

regardless of the three systems tested (Fig. 5b). In −W−F pots, Oak_{NP} seedlings showed values similar to those of fine RMD, RLD, and shoot mass of Oak_{BS} seedlings (Table 2) but the presence of improved pasture significantly reduced coarse RMD and RLD as well as fine RMD of oaks around 70% with respect to the presence of natural pasture ($P = 0.002$; Fig. 5b). The mean root diameter was also reduced by the presence of improved pasture when compared with bare soil (Table 2). Oak_{IP} registered significantly lower d_{50} and d_{95} values than Oak_{NP} seedlings (Table 2), indicating a shallower root system for Oak_{IP} than for Oak_{NP}. In the upper soil layers (0–20 cm) and only in the case of IP, pasture showed a greater fine root density than seedlings (Figs. 3, 5b).

In +W pots, Ψ_{pd} was similar in Oak_{BS}, Oak_{NP} and Oak_{IP} ($P = 0.79$; Fig. 2). However, a System × Watering interaction tendency was observed for coarse RMD ($P < 0.001$) and fine RLD ($P = 0.07$), indicating differences of seedlings growth depending on watering and on systems (Table 2). The water supply treatment (+W) significantly increased shoot biomass and coarse root density of Oak_{BS}. Watering significantly reduced d_{50} and d_{95} values in unfertilized Oak_{BS} and Oak_{NP} seedlings but not in Oak_{IP} seedlings (Table 2). Interestingly, fine RMD of Oak_{BS} and Oak_{NP} seedlings were better related to acorn biomass under −W pots than under +W pots [$r = 0.64$ ($P = 0.003$) and $r = 0.56$ ($P = 0.01$) for Oak_{BS}, and $r = 0.46$ ($P = 0.04$) and $r = 0.16$ ($P = 0.5$) for Oak_{NP}, respectively]. In the uppermost soil layer (0–20 cm depth), fertilisation increased the soil P concentration (from 8.43 to 15.11 $\mu\text{g g}^{-1}$ in −F and +F pots) irrespective of the three vegetation systems tested. However, the effects of fertilisation on seedling were weak and did not significantly affect the shoot biomass in any of the systems. In Oak_{BS} and when no water was applied, the coarse RMD decreased significantly with fertilisation while that of Oak_{IP} and Oak_{NP} remained unaffected. The oak seedlings developed a shallower root system with fertilisation, regardless of the system ($d_{50} = 25$ cm and $d_{95} = 108$ cm in −F pots vs $d_{50} = 22$ cm and $d_{95} = 96$ cm in +F pots, $P = 0.05$). Overall, fertilisation did not change the pattern of differences between oak seedlings growing in different systems. ANCOVAs showed that fertilisation increased the coarse RMD and the fine RLD in watered seedlings (significant Watering × Fertilisation interaction; $P = 0.006$ and 0.023, respectively). In the three systems, the effect of both watering and fertilisation promoted a shallower rooting profile when compared to −W−F seedlings ($d_{50} = 27$ cm and $d_{95} = 117$ cm in −W−F pots vs $d_{50} = 22$ cm and $d_{95} = 95$ cm in +W+F pots, $P < 0.001$).

Table 2 Mean values of shoot biomass and root measurements of *Q. suber* seedlings growing in bare soil (Oak$_{BS}$), together with natural pasture (Oak$_{NP}$), and together with improved pasture (Oak$_{IP}$)

Systems	Treatments	Shoot biomass (g)	RMD (mg dm^{-3})		RLD (cm dm^{-3})		Average root diameter (mm)	d$_{50}$ (cm)	d$_{95}$ (cm)
			Fine	Coarse	Fine	Coarse			
Oak$_{BS}$	−W−F	4.0b	69.4bcd	213.1de	93.2ab	2.9d	0.62de	27.8cd	120.1cd
	+W−F	9.2e	77.3d	265.6f	121.1c	2.6bcd	0.61de	21.4ab	92.3ab
	−W+F	4.1b	79.6d	175.3bc	82.1ab	2.8bcd	0.60cd	23.1ab	99.8ab
	+W+F	7.6de	79.8d	270.6f	112.3bc	3.0d	0.68ef	24.1bc	104.2bc
Oak$_{NP}$	−W−F	4.3bc	78.2d	205.7cde	109.2bc	2.8cd	0.52ab	29.3d	126.8d
	+W−F	6.6cd	73.3cd	204.1cde	95.8abc	2.3abcd	0.63de	22.3abc	96.2abc
	−W+F	4.7bc	58.7abc	204.6cde	73.0a	2.2abc	0.61d	20.0ab	86.2ab
	+W+F	7.5de	57.6ab	226.5e	85.8ab	2.4bcd	0.70f	18.5a	79.9a
Oak$_{IP}$	−W−F	2.6a	47.0a	135.1a	89.2ab	1.8a	0.47a	22.9abc	98.8abc
	+W−F	4.5bc	55.1ab	158.4ab	70.7a	2.1ab	0.73f	22.5ab	97.2ab
	−W+F	2.0a	56.1ab	130.1a	85.4ab	2.1ab	0.55bc	24.5bcd	106.1bcd
	+W+F	4.5bc	55.6ab	182.6bcd	105.6bc	2.5bcd	0.57bcd	23.7bc	102.4bc

Plants were submitted to watering (+W, watered; −W, unwatered) and phosphorous fertilisation (+F, fertilised; −F, unfertilised) treatments. Within columns, different letters indicate differences among treatments ($P < 0.05$, LSD test)

RMD root mass density, *RLD* root length density, *d$_{50}$* and *d$_{95}$* depth for 50 and 95% cumulative root fractions, respectively

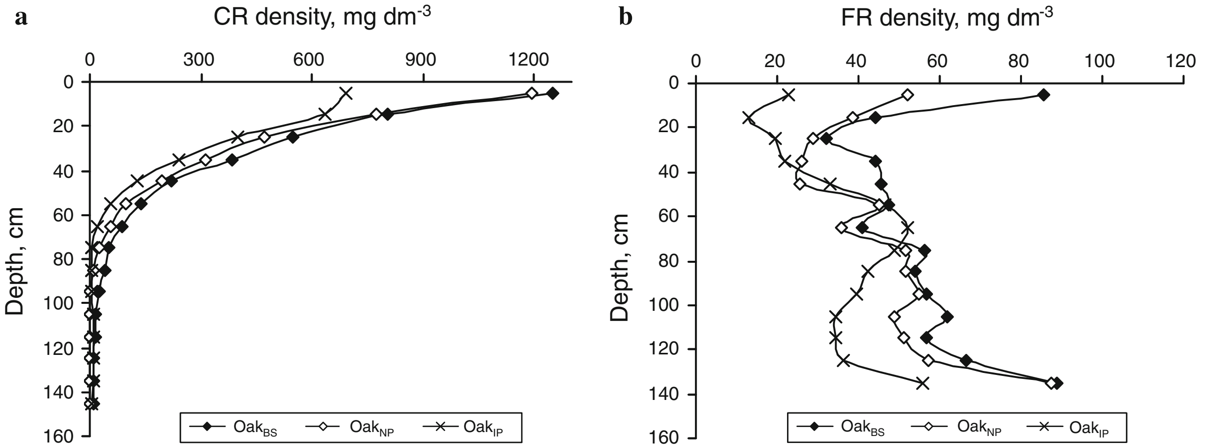

Fig. 5 Variation in depth of coarse root (*CR*) mass densities (**a**) and fine root (*FR*) mass densities (**b**) of *Q. suber* seedlings growing under −W−F treatment in bare soil (*Oak$_{BS}$*), together with natural pasture (*Oak$_{NP}$*) or together with improved pasture (*Oak$_{IP}$*)

Discussion

Deep taproot of *Q. suber* trees during the early stage

Q. suber seedlings developed a deep taproot irrespective of the system and treatments, probably as a common mechanism found in other oak species (Danner and Knapp, 2001; Cubera et al. 2009a). When growing in bare soil (Oak$_{BS}$), both the root and shoot biomass of seedlings were reduced under drought conditions. This mass reduction was previously reported in other trees (Pardos et al. 2001; Marañón et al. 2004; Chiatante et al. 2006) and can be explained by the stress-induced closure of stomata resulting in decreased CO$_2$ assimilation (Joffre et al. 1999). Shoot mass was more affected by drought than the root system, since the water shortage decreased seedling shoot mass and RMD by 56 and 19%, respectively; the limited availability of water favoured the allocation of photosynthetate to the roots, in accordance with literature relative to *Q. ilex* (Canadell and Rodá 1991).

Unwatered seedlings developed a deeper rooting system than watered ones. Padilla et al. (2007) reported that different Mediterranean shrub species responded to water shortage by developing deeper root systems. Similarly, in *Q. robur* under water stress conditions, it was observed that the root morphology changed from short, thick roots to a profusion of long, thin roots that penetrated deeper into the soil (Osonubi and Davies 1981).

The importance of seed weight in governing the fitness of a tree has been supported by extensive empirical evidence, i.e. larger seeds promote early germination and favor growth (Solla et al. 2011; Vivas et al. 2012). The rapid development of a deep taproot requires of large seeds. We found that acorn biomass positively influenced fine RMD, especially when the seedlings grew alone under water-stressed conditions. The dependence between the growth of one-year-old oak seedling and seed reserve has been observed to be stronger when resources as light are more scarce (Quero et al. 2007), and we confirmed the same trend when the soil water availability is scarce.

Natural pasture and tree seedling: a low competitive system

In −W−F pots, the natural pasture yield was equivalent to 1,538 kg ha^{-1} year^{-1} of aboveground biomass and fell within the range reported for natural open grassland in Iberian dehesas (Olea et al. 2005; Cubera et al. 2009b) confirming that the production in greenhouse was similar to those of real conditions. The presence of this pasture significantly reduced the mean root diameter of *Q. suber* seedlings but did not affect the shoot biomass, root density, rooting profile and leaf water potentials when compared to values obtained from seedlings grown alone. Results show a scarce competition of the oak seedling and the natural pasture system. The trees presented a much deeper root system than the herbaceous plant species, showing a relatively low fine RMD in the upper layers of the soil, while roots of the natural pasture did not reach deep layers. This root pattern would likely contribute to reduce belowground competition, thus falling into the general category of "niche separation" (Casper and Jackson 1997) and would confirm our first hypothesis, i.e. *Q. suber* develop a deep root system during the early seedling stage irrespective of the presence of neighbouring herbaceous plants. The coexistence of two or more species that result from reduced resource competition by the vertical partitioning of belowground space between herbaceous species and mature woody plants has been previously described in dehesas of *Q. ilex* (Moreno et al. 2005). In fact, the natural pastures of Iberian dehesas only use water from the first 50 cm depth (Cubera and Moreno 2007), leaving an important amount of available soil water to be consumed by the long taproots of trees.

Reinforced belowground tree-pasture competition did not originate denser or deeper root systems of seedlings

In −W−F pots, the pasture yield and pasture root density increased in the improved pasture compared to natural pasture. The aboveground biomass production was 1.6-fold higher, and the improved pasture root density increased by 7.2 times in comparison to the natural pasture. Interestingly, the rooting profile of Oak$_{IP}$ seedlings became shallower and less dense with respect to Oak$_{NP}$. Several publications have reported increased root density or depth with increased competition for soil resources (Welker et al. 1991; Mulia and Dupraz 2006). Here, we failed to confirm the second hypothesis. The decrease of the root biomass of Oak$_{IP}$ was partially compensated by some changes in the root morphology. The ratio of fine to coarse seedling roots significantly increased with the introduction of improved pasture, and Oak$_{IP}$ seedlings developed a finer root system (lower average root diameter) than Oak$_{NP}$ seedlings especially in the layer situated between 10 and 40 cm (data not shown).

Increasing soil resources reinforced the strength of competition

The root density of the improved pasture increased twofold in response to watering whereas the root density of the seedlings of Oak$_{IP}$ did not vary significantly. Moreover, differences in tree seedlings growth of Oak$_{IP}$ with respect to Oak$_{BS}$ were more acute in watered than in unwatered seedlings. Results indicate that water availability increased the strength of pasture competition for soil resources in detriment to the oak seedlings. Watering also affected the MRD of pasture, which became shallower while the rooting depth of $\mathrm{Oak}_{\mathrm{IP}}$ seedlings did not vary. Hence, under +W conditions, the competition for the uppermost soil resources (mostly nutrients) probably increased while the competition for deeper resources (mostly water) probably diminished. Indeed, under well-watered conditions, all seedlings showed high leaf water potential (good hydric conditions) irrespective of the three vegetation systems studied.

Although P being the primary limiting nutrient in dehesa soils (Vázquez de Aldana et al. 2008), natural pasture, improved pasture and oak seedlings did not generally respond to P fertilisation. The effects of fertilisation were only evident in +W+F pots. Apart of the direct positive effect of increasing available water, watering could have helped to N and/or P mineralisation and to P diffusion in soil (Jackson et al. 1988; Aponte et al. 2010). With fertilisation, the root density of improved pasture increased significantly while that of the seedling of $\mathrm{Oak}_{\mathrm{IP}}$ only increased slightly. With fertilisation, the rooting profile of the improved pasture was deeper while that of the seedling of $\mathrm{Oak}_{\mathrm{IP}}$ system did not vary. In consequence, also fertilisation seemed to increase the competition between improved pasture and oak seedlings. Previous findings agree with the Grime's C–S–R model, which states that the intensity of competition increases as stress declines (Grime 1988), and confirm our third and last hypothesis.

As main conclusions, at the early stages of seedling development, the deep root system developed by cork oak seedlings enables the tree to quickly reach deep moist soils, thereby allowing the seedlings to minimise belowground competition with the herbaceous layer. Management practices conducted to increase pasture yield produce a significant decrease in root and shoot growth of cork oak seedlings, potentially compromising survival of the trees. Finally, fertilisation or irrigation practices will not mitigate the strength of competition for resources between herbaceous plants and seedlings, and will favour the pasture to the detriment of the trees. As a next step, a field trial has been installed to test the performance and survival of *Q. suber* seedlings under native and improved pastures of Iberian dehesas.

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