Agroforest Syst (2014) 88:935–946
DOI 10.1007/s10457-014-9737-y

Restoring silvopastures with oak saplings: effects of mulch and diameter class on survival, growth, and annual leaf-nutrient patterns

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Received: 13 March 2014 / Accepted: 14 August 2014 / Published online: 23 August 2014

Abstract In Southwestern Spain, multifunctional silvopastoral systems consisting of pastureland and open oak woodlands are known as Dehesas. These, and other similar systems of the Mediterranean basin, are currently threatened by increasing intensive land use. As a consequence, oak regeneration is declining and is in need of adequate management and active restoration. Traditional restoration practices outplant one-year-old, nursery-produced oak seedlings grown in 250–350 cm^3 containers, but establishment and growth results are typically poor. This work examines holm oak (*Quercus ilex* L. subsp. *ballota* (Desf.) Samp.) grown in a non-conventional container size (24 l) and age (6–7 years) with three mulch treatments (control, stone, and straw). In an open Dehesa of SE Spain, 106 oak saplings were planted in March 2010, and survival, diameter at breast height (DBH), and leaf-nutrient concentrations were analyzed. Forty months after planting, all treatments showed high survival (81 %) but only straw-mulched saplings differed significantly (94 %) from control (74 %). DBH increased over time but showed no significant differences among mulch treatments. Saplings with high initial DBH showed the greatest growth and change in DBH at the end of the study period. Leaf-nutrient concentrations changed significantly in the year following outplanting. Bi-monthly foliar nutrient concentration data show decreases in P, K, Zn, and B and sharp increases in Ca and Fe. In this work, we provide some evidence concerning the viability of non-conventional oak size for restoring, regenerating, or building up new agroforestry or silvopastoral systems. A combination of saplings with more than 10 mm of DBH and straw mulch is recommended.

Keywords *Quercus ilex* subsp. *ballota* · Saplings · Dehesa · Mulch · Semi-arid · DBH · Spain

Electronic supplementary material The online version of this article (doi:10.1007/s10457-014-9737-y) contains supplementary material, which is available to authorized users.

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Introduction

Many forests of the Mediterranean region have been cleared and transformed into open agroforestry systems that are grazed (Pulido 2009). In Spain, these systems are called Dehesas and are internationally recognized for their ecological, socioeconomic, and cultural importance (Plieninger and Wilbrand 2001).

In recent decades, Dehesas and other silvopastoral systems of Europe are being threatened by mismanagement, including intensive land use or complete abandonment (Moreno and Pulido 2008). The consequences include altered disturbance regimes (from fire, pathogens, and invasive species) that lead to decreased seedling regeneration, tree senescence, and increased dieback, all of which may be further exacerbated by climate change (Eichhorn et al. 2006).

Because natural regeneration is currently decreased, facilitating oak regeneration is one method that could be used to restore the multifunctional silvopastoral benefits of Dehesas. Unfortunately, artificial regeneration efforts are plagued by high mortality because of poor seedling quality and challenging outplanting conditions, e.g. seasonal drought, herb competition, and overgrazing (Pausas et al. 2004; Plieninger et al. 2011). The *Quercus* genus rely on early development of a long tap root to escape drought, and studies indicate that the most commonly used nursery containers, 250–350 cm^3 in volume, are relatively shallow and may be limiting tap-root growth (Villar-Salvador et al. 2004; Chirino et al. 2008). Therefore, increasing stocktype size has been considered a way to improve survival and growth of container stock planted under dry conditions. This increased stocktype size has longer roots that promote deeper soil penetration before desiccation of upper soil layers due to drought (Oliet et al. 2012; Pinto et al. 2012). Some authors (Villar-Salvador et al. 2012) also point out that rapid root growth is commonly associated with high plant vigour, indicating an adequate nutrient status, high photosynthetic activity, and sufficient total carbohydrate reserves are mobilized to produce roots soon after planting. Pemán et al. (2006) revealed that root systems developed in larger container types were more efficient in water uptake, and this may influence holm oak establishment in the field. Similarly, other studies claim non-conventional container sizes offer efficiency in the field performance of other Mediterranean species (Oliet et al. 2012); however, very few outplanting studies have been tested using non-conventional volumes of containers for holm oak and other oak seedlings grown for restoration in Mediterranean environments (but see Tsakaldimi et al. 2005; González-Rodríguez et al. 2011). Furthermore, none have evaluated the effects on plant attributes within these non-conventional stocktypes (e.g. differences on height, diameter classes, biomass, etc.). In this sense, Fernández et al. (2011) illustrate the need for new silvicultural and economic viability studies on non-conventional stocktypes compatible with Dehesa uses.

In addition to choosing the right high quality planting stock for an outplanting site, treatments that preserve, conserve, and promote soil moisture are desired. In that regard, mulches have been widely used as post-planting treatments in agriculture, forestry systems, and environmental restoration to improve seedling establishment or yields. As well, mulches can provide other advantages including: the reduction of competition with herbaceous vegetation and post-fire erosion; improvement of soil conditions, such as moisture, runoff, temperature, and available nutrients; improvement of soil physical properties, such as bulk density, porosity, and aggregate stability; and increased seedling survival and growth (Navarro-Cerrillo et al. 2005; Katra et al. 2008; Ceacero et al. 2012). In Mediterranean environments, studies have examined the use of different mulches (e.g. slash, black polyethylene, compost) in planting beds around oak seedlings (Navarro-Cerrillo et al. 2005; Larchevêque et al. 2006; Valdecantos et al. 2009; Ceacero et al. 2012). Elsewhere, the application of gravel, volcanic rocks, or small stones as mulch has also been used for crop production in dry areas and in forestry (Katra et al. 2008; Fuentes et al. 2009), but information on the use of stones or straw as mulch in the Mediterranean is currently scarce, especially for oak seedlings and saplings (Jiménez et al. 2007, 2013).

Seedlings with adequate nutrient reserves are better suited to translocate nutrients to maximize establishment and growth after outplanting, particularly on low-fertility sites. While several studies have investigated nutrient loading in seedlings to ameliorate low-fertility outplanting soils (Oliet et al. 2009), few have tracked post-planting leaf-nutrient data changes over time, particularly for holm oak seedlings. Intensive monitoring programs use time course foliar nutritional state as a development index for forests and to diagnose atmospheric and edaphic patterns or disturbances (Rautio et al. 2010). Other authors point out that nutrition profiles are the basis of the relationship between the growth and the element concentration in the plant, or in this case, seedling (Luyssaert et al. 2004). Time course nutrient data may provide additional information about plant growth, plant physiological status, and potentially the efficacy of post-planting treatments.

Based on the limitations of artificial regeneration on disturbed Dehesas, the aims of this study were three-fold. The objectives were: i) to evaluate the field performance of two different diameter classes of holm oak saplings cultivated in non-conventional nursery pots, ii) to analyse the effectiveness of the application of stone and straw mulches in the planting bed, and iii) to provide new data on changes over time in leaf-nutrient concentrations of holm-oak saplings growing under semiarid field conditions.

Materials and methods

Study area

The experiment was conducted in the Dehesas de Becerra (Guadix-Baza Basin, Granada), in the SE Iberian Peninsula (37°26′N and 3°5′W) at 1,000 m a.s.l. The climate is semi-arid Mediterranean with cold winters and hot, dry summers. Mean annual precipitation is 320 mm, with high inter-annual variability. The minimum and maximum mean monthly temperatures are −2 °C in January and 33 °C in July. In winter, the temperature can drop to as low as −15 °C and in summer it can exceed 40 °C. The area is in the xeric-oceanic bioclimate, mesomediterranean thermotype, and semiarid ombrotype. The soil is Petric Calcisol with a sandy clay loam texture and is very homogeneous throughout the assay area. A petrocalcic horizon with a high $CaCO_3$ content (40–60 %) is found at a 35–40 cm depth. The intense cultivation in the area created a markedly homogeneous soil profile with little differences between the Ap (0–25 cm) and Bw horizons (25–55 cm).

The study area was a flat, tilled agricultural zone with scattered holm oaks until 1993, when two contiguous pieces of land (996 ha, Cortijo del Conejo y Albarrán and 1,183 ha, Dehesas de Becerra) were bought by the Andalusian regional government and ploughing ceased. While the exact date is unknown, agriculture probably began near the end of the 19th century, when numerous public lands in the area were put up for auction. Before that, these public properties had mainly been alpha-grass (*Stipa tenacissim*a L.) steppes mixed with open holm-oak woodlands used by the local population to feed livestock, hunt, and gather firewood, as described by several romantic travellers or cadastres (Camarero & Campos 1991; López-Burgos 2000; Clemente 2002).

Plant material

Six- to 7-year-old Holm oak (*Q. ilex* L. subsp. *ballota* (Desf.) Samp.) saplings were acquired from a commercial nursery (Viveros Ponce Lajara S.L.). Nursery culture began with acorns sown directly into trays consisting of 45 cells that were each 450 ml in volume and 18 cm deep. After approximately 15 months, seedlings were transplanted into 3.5 l pots and grown for 2 years. Saplings were then selected and transplanted to 10 l pots and grown for another 1 or 2 years. Saplings were finally transplanted to 24 l pots and grown for another 2 years. The 24 l pots were 32 cm deep and 35 cm in diameter.

The nursery media was a mix of 50:50 (v:v) coconut fiber:peat (70 % blond peat and 30 % black peat). Controlled-release fertilizer (Osmocote® Plus, 15-9-11, 12–14 month release) was mixed in the growing media at a rate of 4 kg m^{-3}. Additional granular fertilizer (Floranid®, 16-7-15, plus micronutrients) was applied twice a year, both at the end of autumn and at the end of winter, at a rate of 3 g per pot (480 mg N sapling^{-1}). Since saplings were transplanted to 10 l pots, fertilizer was applied via drip irrigation at the same rate (480 mg N sapling^{-1}) during the growing season.

In February 2010, 106 holm oak saplings grown in 24 l pots were taken at random from the nursery lot. At the same time, another 5 saplings were randomly assigned for biomass characterization at the laboratory. Saplings were separated into root, leaf, and stem tissues before drying in an oven at 70 °C for 48 h. Mean (± standard error) dry biomass of leaves, stems, and roots were 59.2 g (± 5.7), 260.0 g (± 27.1) and 160.1 g (± 24.0), respectively.

Experimental design

In March 2010, 106 planting sites were established following a complete random design. Planting holes were dug by an 80-horsepower backhoe excavator with a bucket of 50 × 80 cm. Seedlings were placed in the holes and excavated soil was carefully filled in using a hoe. Irrigation commenced immediately and was repeated 3 times during the first summer; saplings each received 50 l of water per event. To prevent damage from wild herbivores and livestock, all saplings were protected by wire mesh up to 1 m height.

Three mulch treatments (straw mulch, stone mulch, or a control) were randomly applied to each sapling following planting. For the straw mulch treatment (hereafter straw, $n = 36$), a 15–20 cm thick straw cover was spread over a 1 × 1 m area. The stone mulch treatment (hereafter stones, $n = 27$) covered a 1 × 1 m area and were exclusively limestone; the stones were 1 layer thick, approximately 20–40 cm in size (irregular shapes), and were collected on site. The control treatment (hereafter TC, $n = 43$) had no mulch. After outplanting, the saplings were classified into 2 diameter classes (class 1: 6–11 mm [$n = 50$], and class 2: 11–16 mm [$n = 56$]) to establish the effect of initial diameter at breast height (DBH) on survival and growth.

Survival, growth, and leaf-nutrient concentrations

Survival and growth DBH assessments were performed in April, July, and October from 2010 to 2013. Plants having any green foliage were counted as alive. In February 2010, prior to planting, leaf samples were randomly collected ($n = 9$) from the nursery stock to ascertain initial leaf-nutrient concentration levels. Post-planting, leaf samples were taken from the field every two months for a one-year period (April, June, August, October, and December 2010 and February 2011). A standardized process of foliar sampling can facilitate future comparisons with other studies. For this reason, we followed the methodology proposed by the International Cooperative Programme Forests expert panel on foliage and litterfall (Rautio et al. 2010).We randomly collected 5 replicates of leaves per treatment, discarding the tips of the shoots and damaged leaves, each one composed of a mixture from 3 oaks (45 oaks in total). Leaves and replicates were harvested consistently from the same individuals in each sampling period.

Leaf samples were transported in polyethylene bags to the Atarfe laboratory (Ministry of Agriculture, Fisheries and Rural Development of the Andalusia Regional Government), washed in distilled water, dried at 60 °C in a forced-air oven, and milled. Foliar nitrogen was determined by the Kjeldahl method. For other elements, subsamples were ashed overnight in porcelain crucibles at 450 °C. The residue was dissolved in HCl and diluted to analyse the tissue elements: Ca, Mg, Fe, Mn, Zn, and B by atomic-absorption spectrophotometry, K by flame-emission spectrophotometry (SpectrAA 220 FS Varian), and P by the classical Olsen method.

Statistical analysis

Statistical analyses were made to determine the effects of diameter classes and mulch treatments on survival (%), growth (mm), and leaf-nutrient concentration (% and ppm). Using the statistical package Statgraphics Plus 4.0, survival was analyzed by Kaplan–Meier survival curves, and the Logrank statistical test was employed for pair-wised curve comparisons. Growth was measured and tested in absolute DBH and change in DBH (ΔDBH, the individual difference between DBH recorded each sampling period and the previous one).

Using Statistix 8.0, repeated-measures analyses of variance (RM-ANOVAs) and one-way ANOVAs using a confidence level of 95 % were performed on the leaf-nutrient data and DBH measurements. Multiple comparison testing was done by Fisher's LSD measurement (equal variances). When the Bartlett test of variance equality was violated, the non-parametric Kruskal–Wallis test was used.

Results

Survival and growth

At the end of the study period (July 2013), mean sapling survival was 81 %. Survival was high (94 %) in the straw mulch treatment and was significantly higher than the control ($\chi^2 = 5.30$, $p = 0.021$) and stone mulch treatments ($\chi^2 = 5.74$, $p = 0.016$; Fig. 1). That had the same survival (74 %) and were not significantly different ($\chi^2 = 1.21$, $p = 0.270$). Similarly, no differences in survival were found between DBH diameter classes (80 % for both).

In general, pooled DBH values increased over time in all treatments (omitted data). Saplings with straw mulch tended to have higher mean DBH and change in DBH for each sampling period, but RM-ANOVAs yielded no significant differences among mulch treatments ($p = 0.830$; Table 1). Because the "mulch × period" interaction was also not significant, we can assume that the significant differences found with regard to sampling period were independent of the mulch treatment. Similarly, we found the same results

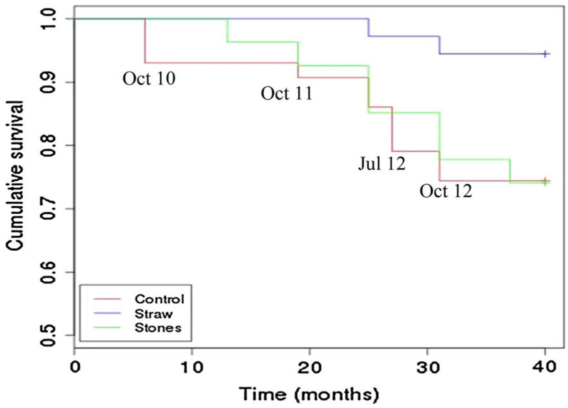

Fig. 1 Kaplan–Meier cumulative survival curves for holm oak saplings subjected to straw, stones, and no (control) mulch treatments

Table 1 RM-ANOVAs results showing differences in breast height diameter (DBH) and change in DBH (ΔDBH) for factors mulch treatment (T), sampling period (P), and the interaction terms (T × P), and diameter class (DC) with regard to sampling period

	DBH (mm)			ΔDBH (mm)		
	df	F	p	df	F	p
Treatment (T)	2	0.19	0.830	2	2.40	0.097
Period (P)	10	81.67	**0.001**	9	25.62	**0.000**
T × P	20	0.55	0.946	18	0.56	0.928
	DBH (mm)			ΔDBH (mm)		
Diameter class (DC)	1	4.29	**0.040**	1	6.35	**0.014**
Period	10	238.80	**0.000**	9	24.35	**0.000**
DC × P	10	7.63	**0.000**	9	1.91	**0.047**

p<0.05 are showed in bold

with regard to change in DBH. In contrast, RM-ANOVAs revealed statistical differences among diameter classes for DBH and change in DBH (Table 1). The highest DBHs were found in saplings of the largest diameter class ($p = 0.040$) although this was time dependent in terms of interaction ($p < 0.001$). Saplings belonging to the largest diameter class (class 2) showed the most significant values in the entire sampling period (Fig. 2). Similarly, we found significant differences in ΔDBH (Table 1) with regard to the diameter class ($p = 0.014$) but it was also time dependent in terms of interaction ($p = 0.047$). Finally, the total ΔDBH was statistically different

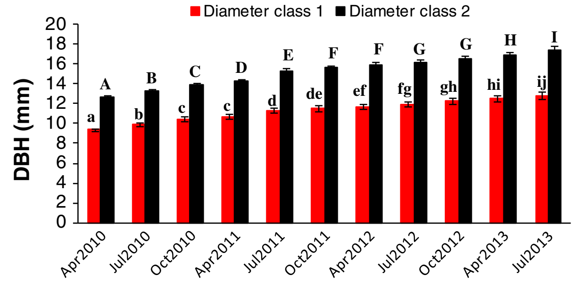

Fig. 2 Mean values of diameter at breast height (DBH) ± SE sorted by diameter classes during the study period. *Different letters* indicate significant differences among sampling periods following RM-ANOVA results (LSD test). Diameter class 1 = 6–11 mm and diameter class 2 = 11–16 mm

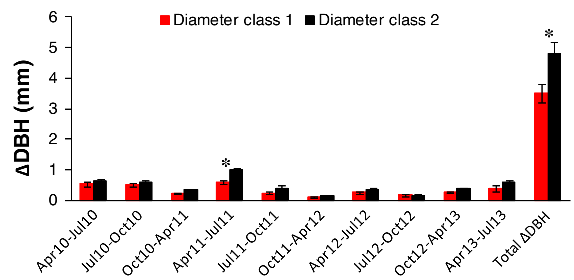

Fig. 3 Mean values for periodic growth increments in diameter at breast height (ΔDBH) ± SE sorted by diameter classes during the study period. *Asterisks* indicate significant differences between diameter classes following RM-ANOVA results (LSD test). Diameter class 1 = 6–11 mm and diameter class 2 = 11–16 mm

between diameter classes ($p = 0.011$) (Fig. 3), but not among mulch treatments ($p = 0.389$).

Leaf-nutrient concentrations

For all saplings, leaf-nutrient concentrations changed significantly during the year-long study period. With regard to the initial nursery leaf-nutrient concentrations, P, K, Zn, and B concentrations decreased ($p \leq 0.044$), while Ca and Fe increased ($p < 0.001$; Table 2).

Repeated measures analysis revealed mulch treatment differences for Ca, Fe, and Zn ($p \leq 0.035$); time was significant for all nutrients ($p < 0.001$); and the time × period interaction was significant for N, Ca, Mg, Fe, Mn, and Zn ($p \leq 0.044$; Table 3). In general, we found the lowest N and P concentrations in late

Table 2 Leaf nutrient concentrations (mean, ±SE, and range) before outplanting (February 2010) and at the end of the study period (February 2011)

Nutrients	February 2010	February 2011	*p value*
N	1.55 ± 0.02 (1.44–1.74)	1.56 ± 0.04 (1.39–2.02)	0.878
P	0.11 ± 0.00 (0.08–0.14)	0.08 ± 0.00 (0.08–0.10)	**0.001**
K	0.51 ± 0.01 (0.37–0.62)	0.42 ± 0.00 (0.37–0.46)	**<0.001**
Ca	0.63 ± 0.04 (0.34–0.89)	2.39 ± 0.10 (1.79–3.24)	**<0.001**
Mg	0.25 ± 0.01 (0.16–0.32)	0.28 ± 0.01 (0.23–0.38)	0.203
Fe	84.21 ± 4.42 (57.00–114.00)	130.27 ± 9.12 (54.00–183.00)	**<0.001**
Mn	168.86 ± 17.62 (63.00–304.00)	128.20 ± 10.84 (49.00–229.00)	0.111
Zn	37.35 ± 1.85 (22.00–49.00)	30.26 ± 2.75 (12.00–55.00)	**0.044**
B	51.71 ± 3.21 (31.00–74.00)	39.60 ± 2.51 (16.00–54.00)	**0.007**

Kruskal–Wallis all-pairwise comparisons tests were applied. Macronutrient (N, P, K, Ca, Mg) unit is % dry weight and micronutrient (Fe, Mn, Zn, B) unit is $\mu g\ g^{-1}$ (ppm) dry weight

$p<0.05$ are showed in bold

summer and early fall; conversely, the highest values of Mg, Fe, and B were observed at this time. K concentrations decreased similarly for all treatments during the sampling period, whereas Ca concentrations increased (Fig. 4).

At the end of the study period (February 2011), significant differences were found for Mg, Fe, and Zn concentrations among mulch treatments. The control treatment Mg concentration was significantly higher than the mulch treatments; however, stone mulch Fe and Zn concentrations were significantly higher than the control and straw mulch (Fig. 4).

Discussion

Survival

Forty months after planting large, non-conventional holm oak saplings, survival was 81 %. This is high considering that seedling survival is generally low (<50 %) in dry and semiarid areas when 1-year-old seedlings from smaller stock sizes of 250–350 cm^3 are used (Andivia et al. 2012). Similarly, these survival rates were higher than those found by Tsakaldimi et al. (2005) in Greece (581 mm of annual rainfall). In that study, they observed 30–65 % survival 2 summers after outplanting of holm oak seedlings grown in 482 cm^3 paper pots and 275, 450, and 650 cm^3 rigid re-usable plastic containers. In southern Spain (609 mm of rainfall), González-Rodríguez et al. (2011) observed second year survival for both 1-year-old, 400-cm^3 container seedlings and 3-year-old, 3 l saplings in containers to be 45 and 18 %, respectively. Unlike this study, however, none of these experiments applied post-planting irrigation. To our knowledge, no other studies report field survival data for non-conventional holm oak stock used in restoration.

Straw mulch significantly improved sapling survival (94 %) compared to the control treatment (74 %). This is similar to the first year results of Navarro-Cerrillo et al. (2005) and Ceacero et al. (2012) and their use of a black polyethylene mulch treatment. Larchevêque et al. (2006) also had similar second year results with the use of compost mulch. Fuentes et al. (2009) found that stone mulch under semiarid field conditions increased survival of wild olive seedlings when compared to conventional planting techniques. However, Valdecantos et al. (2009, 2011) observed no differences in survival when slash mulch and air-dried biosolids were applied on the topsoil surrounding the planting holes. In contrast, other studies (Fuentes et al. 2007; Chaar et al. 2008) observed negative effects when sewage sludge and black polyethylene mulch were applied for different oak seedlings. While we did observe differences in survival, the combination of larger sapling size and post-planting irrigation—a treatment not used in the previously mentioned studies—may have reduced the expected mulch effects on growth.

Sapling survival decreased only slightly following the first and second summers after outplanting (October 2010 and 2011). We attribute this high survival

Table 3 RM-ANOVAs results showing differences in leaf nutrients concentrations due to mulch treatments (T), changes over time (P) and the interaction terms (T × P). Significant differences are indicated in bold

Nutrient		Treatment (T)	Period (P)	T × P
N	**df**	2	4	8
	F	0.89	8.38	2.19
	p	0.437	**<0.001**	**0.044**
P	**df**	2	5	10
	F	0.27	28.26	0.78
	p	0.765	**<0.001**	0.645
K	**df**	2	5	10
	F	0.73	56.22	0.72
	p	0.500	**<0.001**	0.705
Ca	**df**	2	5	10
	F	11.74	143.28	12.55
	p	**0.001**	**<0.001**	**<0.001**
Mg	**df**	2	5	10
	F	0.75	43.69	12.04
	p	0.492	**<0.001**	**<0.001**
Fe	**df**	2	5	10
	F	4.46	34.60	4.74
	p	**0.035**	**<0.001**	**<0.001**
Mn	**df**	2	5	10
	F	3.30	20.40	2.79
	p	0.072	**<0.001**	**0.006**
Zn	**df**	2	5	10
	F	5.08	8.42	3.08
	p	**0.025**	**<0.001**	**0.003**
B	**df**	2	5	10
	F	1.73	31.40	1.21
	p	0.218	**<0.001**	0.303

rate to the applied post-planting irrigation and higher than usual precipitation (466 mm) that occurred from October 2010 to September 2011. Following this period, however, survival clearly decreased (Fig. 1). From October 2011 to September 2012, rainfall was lower than usual (163 mm) and temperatures were extremely cold, reaching an absolute low of −13.7 °C in February 2012. The combination of these factors likely contributed to the decreased survival.

Growth

No statistical differences in DBH or ΔDBH were detected among mulch treatments; although, saplings with straw mulch tended to show the highest mean values. Fuentes et al. (2007) had similar results with holm oak seedlings and found no significant differences in height or root-collar diameter among sewage sludge mulch treatments. Other authors (Samyn and De Vos 2002) did not record the best growth with organic mulch (bark, paper, wood chips, sawdust, or corncobs) under different climate conditions or for different species, finding that the control treatment had intermediate growth among the mulch treatments. According to Valdecantos et al. (2009), the application of slash mulch negatively influenced the relative growth rate in diameter of holm oak seedlings. Chaar et al. (2008) found little advantage in using black-polyethylene mulch for *Quercus suber* seedlings; only when the mulch was used with tree shelters was there a slight height growth benefit. Some studies, however, found higher growth in holm oak seedlings treated with different kinds of mulch (polyethylene, compost, sewage sludge, or slash; Navarro-Cerrillo et al. 2005; Larchevêque et al. 2006; Valdecantos et al. 2011; Ceacero et al. 2012). Most of these authors used 1-year-old seedlings grown in trays, and therefore special consideration should be made before comparing these with our results.

Our analysis of diameter class showed that saplings with the largest initial DBH (diameter class 2) showed significantly higher values of absolute DBH and total ΔDBH at the end of the sampling period (Fig. 2 and 3). This was not the case for the periodic incremental ΔDBHs taken during the study, which were very low due to slow growth. More than 3 growing seasons were needed to compile significant data between diameter classes. During the first few years following planting, analyses indicate that diameter classes seemed to have more influence on growth than the mulch treatments. Villar-Salvador et al. (2004) show comparable results with large oak seedlings frequently having higher absolute growth after outplanting than smaller seedlings. Furthermore, Villar-Salvador et al. (2012) proposes a seedling establishment model that advocates large seedlings with adequate nutrient reserves for improved survival and growth on Mediterranean plantations.

Leaf-nutrient concentrations

The factors that determine the concentration of elements in leaves are extremely varied: type of leaf,

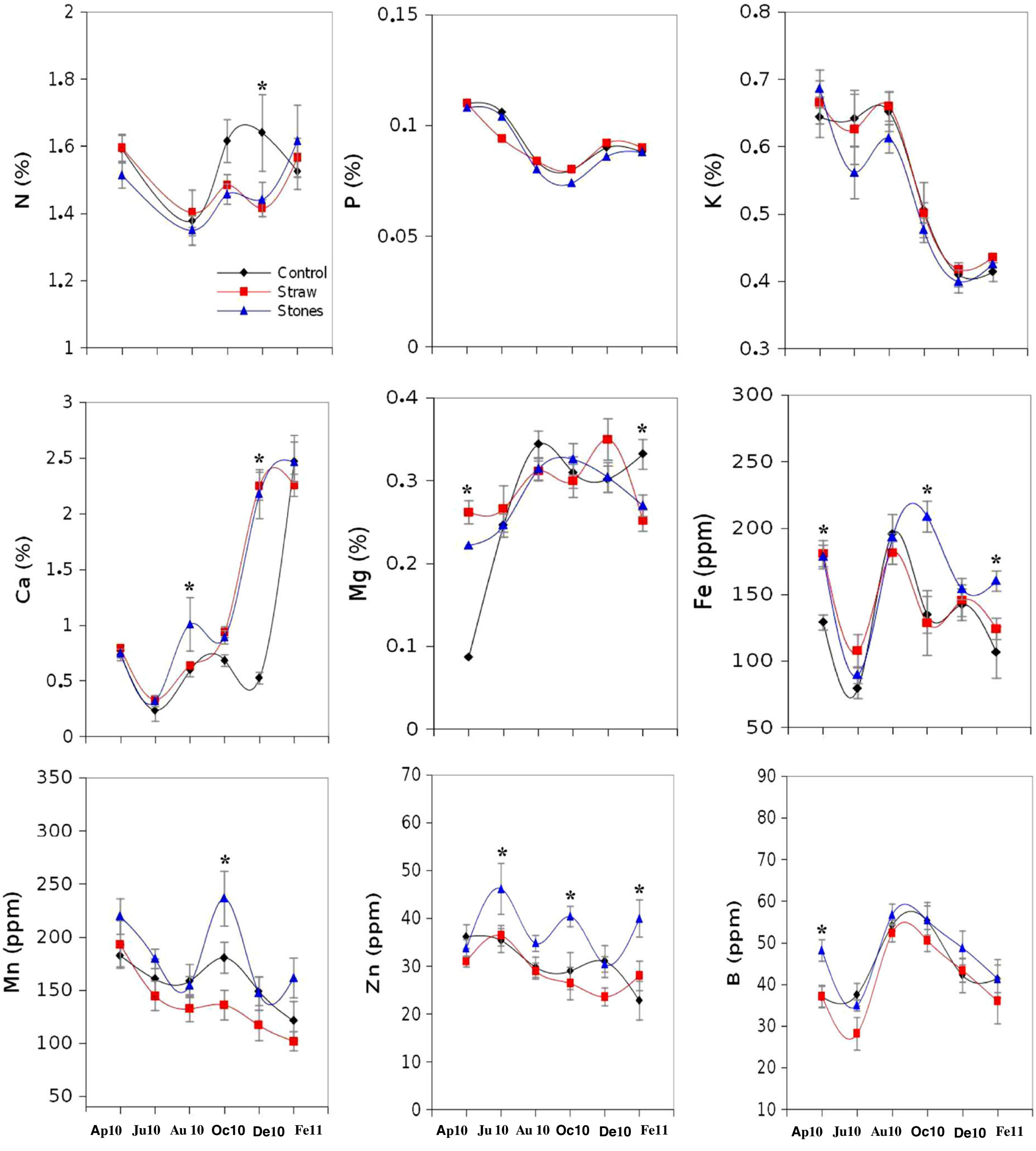

Fig. 4 Temporal pattern of leaf nutrient concentrations (mean ± SE) sorted by mulch treatments applied in the plantation bed of the saplings. *Asterisks* indicate significant differences among treatments following the RM-ANOVA results (LSD test)

age of the plant, season of the year, availability of nutrients in the soil, climate conditions, and soil properties (Bussotti et al. 2000). This is further complicated when seedlings are newly planted—their nutrients are sourced from nursery culturing and the initial contact of roots to the planting site soils. Few studies show foliar nutrient data in holm oaks seedlings grown under field conditions (Pardos et al. 2005; Valdecantos et al. 2006; Fuentes et al. 2007; Jiménez et al. 2007, 2013) and none examine trends over time.

Moreover, critical or optimal nutrient levels for holm oak are yet to be defined.

Initially, N, P, and K concentrations found in our saplings were very similar to those reported by other authors for older, mature holm oaks (König et al. 2010; Bussotti et al. 2003; Sardans et al. 2011). The P concentration, however, was higher than those reported by Moreno and Obrador (2007) and Mediavilla et al. (2011), but was lower than those found by Sardans et al. (2008). Compared with other oak species, our Mn concentrations were lower than those reported in adults (Santa Regina 2000; Orgeas et al. 2002; Bussotti et al. 2003) while Ca and Fe concentrations were similar to different stocktype sizes (Stefan et al. 1997; Valdecantos et al. 2006). No B values were available in the literature for adult *Quercus* species, but the B concentration in our study was higher than shown in the Classification Values for European Foliar Data (König et al. 2010).

A few authors have published nutrient values for holm oak seedlings as well as those that have been outplanted for nearly a decade. Our N and P sapling values were higher than those found by Pardos et al. (2005) and Valdecantos et al. (2006). Compared with Jiménez et al. (2007), our N and P values were lower while Ca and Mg concentrations were similar. Valdecantos et al. (2011) and Larchevêque et al. (2006) reported similar K and Zn concentrations, respectively. Nine years after outplanting, on the same experimental site as this study, Jiménez et al. (2013) found similar P, Ca, and Zn; higher N, K, Mg, and Mn; and lower Fe concentrations. This high variability in leaf-nutrient concentrations indicate that standards for each nutrient according to the growth phases (seedling, sapling, adult tree) are needed and that new studies should be formulated in this way.

After the saplings were outplanted, nutrient concentrations fluctuated with growing season. The lowest N and P concentrations were found in summer or in early autumn, most likely as sapling growth diluted stored nutrients obtained in the nursery. In adult holm oaks, Sabaté et al. (1995) found that N and P leaf concentrations were lower during summer and higher during autumn or spring. This indicates the importance of N and P acquired by plants during the preceding growing season. Silla and Escudero (2003) observed that, during the period prior to resumption of shoot growth, plants usually accumulate nutrients to meet the needs of spring growth. Other authors (Escudero and Mediavilla 2003) indicated than N reabsorption was associated with leaf senescence and, in oaks, most leaf abscission occurred during the spring just before the expansion of the new leaf biomass. It is thus likely that the resources resorbed from senescing leaves would be used to promote the growth of young leaves. Santa Regina (2000), studying *Q. pyrenaica*, also found the highest N concentration in April, pointing out that the contents of this element decrease throughout the vegetative cycle and retranslocate it to perennial tissues occurs before total abscission.

The presence of a high amount of calcium carbonate in the soil may have influenced both Ca and K nutrient patterns over time. After outplanting, the K concentration decreased for all treatments while the Ca concentration increased. According to Jiménez et al. (2007), the decreases in K concentration could be linked to the high amounts of Ca in the soil and the increased availability of Ca in the soil solution; in that study, the observed correlation between exchangeable-K content and calcium carbonate in the soil was significant and negative. This negative relationship has also been shown in foliar nutrient concentrations of other *Quercus* and *Pinus* species (Oliveira et al. 1996; López-Serrano et al. 2005; Valdecantos et al. 2006).

The seasonal pattern of the Mg concentration was highly variable during the sampling period. López-Serrano et al. (2005) reported similar results for *Pinus halepensis*, and Orgeas et al. (2002) observed comparable trends for *Quercus suber* but pointed out that leaf age was the most important factor in nutrient dynamics. Yet other studies failed to discern a clear seasonal pattern in adult cork oaks (Oliveira et al. 1996).

Several micronutrients showed declines over the study period which may have been influenced by soil pH. In general, the Mn declined during the sampling period for all treatments. The Fe concentration showed fluctuations, but at the end of the study period, declined with respect to initial measurements. This decline was probably due to the lower solubility of the Fe–Ca and Mn–Ca compounds frequent in limestone media, which is related to increased soil pH (Marschner 1995). In this sense, Bussotti et al. (2003) found the opposite was true for adult holm oaks growing in acidic soils where Fe and Mn increased during the study period (from June to April). Because Zn also becomes less available with increased soil pH, we suspect this contributed to generally decreasing Zn concentrations over time (Marschner 1995).

At the end of the sampling period, mulch treatments had only significantly influenced Mg, Fe, and Zn nutrient concentrations. Stone mulch significantly increased Fe and Zn values compared with the straw mulch and control treatments. However, the control treatment yielded significantly higher Mg concentrations than either of the other mulch treatments.

Few studies have analyzed changes in foliar nutrient concentrations in adult holm oaks over time (Sabaté et al. 1995; Bussotti et al. 2003; Silla and Escudero 2003), and no published data is available concerning the same information regarding seedlings or saplings. Overall, our results showed that temporal patterns of foliar nutrient concentration were similar among straw, stone, and control mulch treatments. We suspect irrigation during the first growing season may have counteracted any major potential mulch effects. This may complicate data interpretation beyond the first year, but significant mulch treatment differentiation at the end of the monitoring period may be indicative of future long-term trends and potential mulch effects.

Conclusions

Survival among holm oaks 40 months after outplanting was high (81 %). Only straw mulch, however, significantly improved survival (94 %) with regard to the control (74 %). Most mortality occurred after dry and cold years, and no differences in survival were found between diameter classes.

No differences in DBH, ΔDBH, and total ΔDBH were found in saplings influenced by mulch treatments, although those with straw mulch tended to show higher mean values. On the contrary, saplings with the highest initial DBH showed significantly greater values of DBH and total ΔDBH at the end of the sampling period. During the study period, sapling growth was slow and ΔDBHs were very low; more than three growing seasons were needed to compile significant data between diameter classes. These diameter classes appeared to have more influence than the mulch treatments, at least until the last measurement was made.

Mulch treatment did not have clear effects on foliar nutrient concentrations with regard to the control. Compared to the initial nutrient status in the nursery, P, K, Zn, and B concentrations decreased while Ca and Fe increased by the end of the study period. Nutrient data interpretation is somewhat complicated with the available literature's variable results; however, this is the first study to report temporal data in foliar macro- and micronutrients of outplanted holm oak saplings. Although the use of non-conventionally sized saplings implies a high economic cost, the payoff is in improved survival and increased trajectory for new Dehesa or open woodland restoration. This allows concomitant livestock activity and consequently reduces rent loss by livestock exclusion. On the basis of this study, a combination of large saplings with more than 10 mm of DBH and straw mulch is recommended.

Acknowledgments We thank the Environmental Council (Junta de Andalucía) for financial support provided by Convenio de Colaboración no. 372 and the members of the Forest Management for Conservation research group (PAIDI AGR-245) from the IFAPA and EEZ-CSIC for their invaluable assistance. M.N. Jiménez and A. Sánchez-Miranda were sponsored by the Operating Programme of the European Social Found for Andalucía (2007–2013). We thank David Nesbitt and R. Kasten Dumroese for assistance with English revisions.

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