Fall fertilization of Holm oak affects N and P dynamics, root growth potential, and post-planting phenology and growth

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Abstract
• *Introduction* For Holm oak (*Quercus ilex* L.), a flush
growing species, nutrient loading during the growing
season is difficult and can lead to a low nutrient status of
the seedlings. To provide insights about Holm oak nutrient
dynamics during fall in the nursery and subsequent planting
performance, a factorial nursery experiment was conducted
in a mild fall–winter area testing the effects of timing of
fertilization (early and late fall) and rate (two doses of a

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NPK fertilizer that applied 28 and 56 mg N per seedling),
followed by an experimental plantation.
• *Results* Biomass, allocation pattern, shoot N and K, and
root K were unaffected by both rate and timing of fall
fertilization. However, shoot P concentration of fall
fertilized plants was significantly increased, and root P
concentration was enhanced by applying fertilizer at either
the highest rate or during early fall. This revealed a
different nutrient dynamics during fall that was dependent
on the specific nutrient and plant component.
• *Discussion* Root growth potential was positively corre-
lated to nursery root P concentration. Six months after
planting, fall fertilized plants showed higher shoot biomass,
higher proportion of new leaves, and faster development,
producing leaves earlier compared with unfertilized plants.
• *Conclusion* It is concluded that early fall fertilization
promotes nutrient loading of P in Holm oak, with
significant effects on root growth potential and field growth
by means of a phenologically earlier development and a
higher aboveground biomass.

Keywords Late season fertilization · *Quercus ilex* · Nutrient
loading · Phosphorus · Root growth potential

1 Introduction

Fertilization strategies that promote nutrient loading during
seedling nursery culture have been recommended to
increase the performance of transplanted seedlings (Timmer
and Aidelbaum 1996; Salifu et al. 2008). Nutrient loaded
seedlings usually exhibit superior survival, growth, and
competitive ability over non-loaded cohorts when trans-
planted in a variety of habitats (Oliet et al. 2009a).
Remobilization of internal nutrient reserves to support

new growth is a key mechanism to explain the improved performance of nutrient-loaded plants (Salifu et al. 2008). Despite that traditional literature on seedling nutrient status and transplanting performance has focused on N, new studies have shown how other macronutrients, like P, are involved in planting response under Mediterranean conditions, probably through promoting root growth (Villar-Salvador et al. 2004; Oliet et al. 2005, 2009a), although this role needs to be clarified (South and Donald 2002; Warren et al. 2005).

Supply of extra nutrients during the hardening stage (fall fertilization) is a means of preventing nutrient dilution (Sung et al. 1997; Rikala et al. 2004). Many studies have shown positive effects of fall fertilization on nutrient loading of nursery seedlings (South and Donald 2002; Rikala et al. 2004; Islam et al. 2009) and subsequent outplanting performance (South and Donald 2002; Puértolas et al. 2003; Boivin et al. 2004) or frost resistance (Rikala and Repo 1997).

Fall fertilization is also appropriate in mild Mediterranean areas where the forest planting window ranges from November to February (Mollá et al. 2006) and where seedlings can remain in the nursery under warm temperatures for months, waiting to be planted. During this extended period, plants are relatively active (Fernández et al. 2008), and growth can very easily lead to nutrient dilution (Trubat et al. 2010).

Holm oak (Quercus ilex L.) is an important evergreen tree species widely used for forest restoration in Mediterranean ecosystems (Terradas and Savè 1999). However, their seedlings are vulnerable to transplanting stress and to summer drought, exhibiting high mortality and slow growth compared to other Mediterranean species (Bocio et al. 2004). As a species that retranslocates stored nutrients to support new growth (Silla and Escudero 2003), improving nutrient status of Holm oak seedlings in the nursery could lead to better growth and survival results in forestations. The role of retranslocation of stored nutrient in Holm oak is especially important on poor soils (Silla and Escudero 2003), which are common in many Mediterranean areas. High N (Villar-Salvador et al. 2004; Sanz-Pérez et al. 2007) and P (Sardans et al. 2006a) fertilization of Holm oak seedlings in the nursery can improve early field survival and growth after planting. However, some of the studies indicate that raising rates or using exponential regimes of fertilization do not considerably improve the nutrient status of Holm oak seedlings (Villar-Salvador et al. 2004; Oliet et al. 2009b), mostly due to its episodic growth through multiple flushes during nursery culture (Terradas and Savè 1999), which does not match any continuous fertilization regime. Along with Q. ilex, several Mediterranean forest species are in this case (Terradas and Savè 1999). Therefore, fall fertilization can be an interesting alternative to an exponential fertilization

regime as a way to improve nutrient loading in Holm oak and other similar species.

Studies about fall fertilization of Mediterranean species are scarce for both conifers and broadleaved sclerophyllous species (Puértolas et al. 2003; Trubat et al. 2010). Many questions concerning the implementation of this cultural practice arise. It is not clear whether Holm oak seedlings are physiologically active enough to absorb fall fertilizer and, if so, what would be the response to this nutrient loading in terms of morphology and post-planting performance. In addition, rate, formulation, and timing under the whole extended period comprising the fall and early winter weeks must be clearly defined. Characterizing how Holm oak seedlings respond to fall fertilization and subsequent effects on post-transplant performance will improve our understanding on how to manipulate nutrition at the nursery stage to enhance the success of this species under field conditions. In Q. ilex, the relationship between mineral nutrition and root growth remains unclear (Villar-Salvador et al. 2004), limiting the knowledge about how to manage the fertilization in this species to optimize seedling quality.

In this study, we test the hypothesis that fall fertilization is a superior alternative to classical fertilization regimes applied to Holm oak seedlings. Our objectives were to test the effects of timing and rate of fall fertilization on macronutrient (N, P, and K) dynamics (uptake and retranslocation) in Q. ilex seedlings in the nursery and on the subsequent performance of outplanted seedlings. To this end, we followed a factorial design with timing (early and late fall) and rate of fertilization during the hardening period in a mild fall–winter area of Mediterranean Spain.

2 Materials and methods

2.1 Plant material, layout of fall fertilization experiment, and plant characterization

Holm oak acorns from "Alcarria-Serranía de Cuenca" provenance (ES-45-09; Ministerio de Agricultura, Pesca y Alimentación 2000) were winter-sown (February 24, 2005) into 300-ml containers (Plasnor trays, 45 containers per tray, 284 containers per square meter; Plasnor SA, Spain) filled with peat moss fertilized with 36 mg N, 27 mg P, and 45 mg K. Seedlings were raised at the CNM Forestal "El Serranillo" (Guadalajara, 40°40′ N, 3°10′ W, 650 ma.s.l., Spain) under operational conditions of the nursery: To avoid late spring frost damage, seedlings were raised in a greenhouse until mid-June 2005 and then moved outdoors under full sun. On September 20th, 15 trays full of culled seedlings (with no apparent diseases or damages) were moved to the University of Córdoba nursery (37°51′ N, 4° 50′ W, 123 ma.s.l., Spain) and placed under a shaded cloth

to apply fall fertilization treatments. Experimental design consisted in a 2×2 factorial with two factors (fertilizer rate and timing during the fall) plus a non-fall fertilized that served as a control (five treatments in total). Control mimics operative conditions for Holm oak nursery culture in Spain, which do not include any additional fertilizer supply along the whole fall period. Factor levels were: 28 and 56 mg of N per plant and early (EF) versus late fall (LF) application time. Total amounts of fertilizer were evenly split and applied weekly during 7 weeks through irrigation, according to the following timing: from October 17 to November 28 (EF) and from November 14 to December 26 (LF). The mentioned amounts of N were supplied via 20–20–20 (20 N, 8.7 P, 16.7 K) commercial water-soluble fertilizer (Scotts Co., Marysville, OH, USA) that included also amounts of P and K (12.2 and 23.4 mg of P and K, respectively, for the 28-mg N level and double for the 56-mg N level). Each fertilization treatment was applied to three trays. Trays were completely randomized on benches and their position rotated every 15 days to minimize edge effects. Supplemental irrigation was applied to field capacity based on gravimetric methods; watering to saturation was conducted when growing media decreased to 80% of the saturation point. No external rain was allowed by covering the experiment with a plastic cloth before a precipitation event happened. Temperature along treatment application was assessed with a Hobo H8 Pro Series (Onset Computer Co., Cape Cod, MA, USA). Temperature was measured at 1-min intervals and recorded as 10-min averages. Mean temperature from September 29 to December 31 was 13.7°C, absolute minimum 0.2°C, and absolute maximum 33.6°C. Mean temperatures during fertilization were 14.5°C and 10.1°C for the first (early fall) and second (late fall) fertilization periods, respectively. The number of accumulated chill hours with temperature ≤8°C was calculated. According to Fernández et al. (2008), Holm oak seedlings experienced some physiological changes and started the hardening process when they had been exposed to temperatures below 8°C for 288 h. In our study, this occurs on December 15, 11 days before the end of the late fall fertilization treatment application. Buds were set from the beginning of fall fertilization (October 17) and remained the same during the whole treatment application period.

To follow biomass and nutrient dynamics of the seedlings, two samplings were carried out during fall period. The first sample was taken when the EF fertilization was finished (November 28, intermediate sampling) and the second one after accomplishing the LF (January 10, final sampling). For each sampling, 15 plants per treatment (five plants per replicated tray) were randomly chosen. Height was measured in all plants from the root collar to the base of the terminal bud. Total leaf area per seedling was determined (in final sampling plants only) after scanning fresh leaves and measuring area with ImageJ 1.34s image analyzer software (University Health Network Research, Toronto, CA). After cutting plugs from shoots at root collar level, roots were carefully separated from the growing medium and washed. Both shoots and roots were then dipped in distilled water for 5 min prior to chemical analysis. Plant components were oven-dried (48 h at 65°C) and weighed for dry mass determination. Shoots and roots were composited by treatment replication (tray) for nutritional analysis (three composite samples per treatment). Nutrient analysis was conducted in the Laboratorio Agroalimentario de Córdoba (Junta de Andalucía, Spain) according to the Official Methods of Spanish Department of Agriculture (Ministerio de Agricultura, Pesca y Alimentación 1994): Kjeldahl procedure for N, colorimetry for P (blue-molybdate method), and K with flame photometry. Nutrient content (milligrams per seedling) was determined as the product between nutrient concentration and biomass.

2.2 Root growth potential and planting experiments

For root growth potential (RGP) determination, five seedlings per tray (15 seedlings per treatment) were randomly chosen in the nursery and individually transplanted to 3-L pots containing perlite on January 17, 2006. Seedlings were randomly arranged in a greenhouse that maintained temperature between 15°C and 30°C (19°C on average). PAR radiation was registered during the experiment with a Ha-Li sensor (EIC SL, Madrid, Spain) placed at plant height. Average PAR radiation (±SE) was 4.2 (0.2) mol m^{-2} day^{-1}, with minimum and maximum values of 1.6 and 5.4 mol m^{-2} day^{-1}, respectively, along the 28 days of experiment. Absolute maximum PAR value at midday ranged between 134 and 400 μmol m^{-2} s^{-1}. Seedlings were watered every 2–4 days to keep the substrate at field capacity. After 28 days, seedlings were lifted, cleaned from the medium, and all new roots protruding from the root plug were excised and dried at 65°C for at least 48 h and weighed. The RGP of each seedling was determined as the total dry mass of new roots.

To evaluate the field performance of plants under different fertilization treatments, seedlings were outplanted at "Finca de Rabanales" (37°55′ N, 4°43′ W, 140 ma.s.l., Córdoba University, Spain). The soil of the plot was sandy loam (69% sand, 20% loam, and 11% clay) with a pH of H_2O of 6.8 and organic matter content of 1.5%. The soil was plowed at 40-cm depth and then flattened with a harrow. Seedlings were planted manually in 0.35×0.30× 0.40-m holes 1.5 m apart. We planted 60 plants for each of the five treatments (20 plants from each nursery tray, 300 plants in total). Plants of each treatment were fully randomized across the plot. Experimental plantation was framed with Holm oak seedlings at the same spacing to avoid

edge effects. The plantation was done in March 2006. Plants were protected with a 60-cm height, 15-cm diameter, 0.8× 0.8-cm gap size flexible mesh (Protec Blaunet, Projar, Spain). Weed competition was avoided by mowing mechanically two times during the study period.

Height and basal stem diameter were measured in each of the 300 plants at two dates: March (25–30) and June (20–28) 2006. Aboveground biomass (stem and leaves) was estimated in a subsample (21 plants per treatment, 105 plants in total) in March and June (1–11 for both months) 2006. Stem biomass (main stem plus branches) was estimated using the stem volume as a predictor. A biomass–stem volume model was adjusted with data collected in the final nursery sampling (R^2=0.82, P<0.05). Leaf biomass was estimated from leaf area as follows. Leaf area was measured by the intersect method (Poorter et al. 2004) in all leaves using acetate sheets with a grid of 7 mm. A previous test of the accuracy of the method showed a very high R^2 (0.96, P< 0.01). Leaves were classified in old and young leaves. After the June measurement, we took one young and one old leaf for each of the 105 plants. After scanning and area calculation using ImageJ software, leaves were dried at 70°C during at least 48 h and weighed. Leaf mass per area (LMA, grams per square meter) of each leaf was calculated as the dry mass divided by the leaf area. The LMA of the leaves (young and old) was used to calculate the leaf biomass of each plant as the sum of all products leaf area × LMA.

A monthly estimation of phenology state and survival was done on all plants. We used five phenological states to characterize the development stage (0—plants with leaf buds; 1—swollen leaf buds; 2—growing leaves; 3—leaves formed but not fully expanded; 4—leaves fully expanded).

2.3 Data analysis

All data were analyzed by a two-way analysis of variance (ANOVA) with a hanging control group (Ministry of Forestry and Range 2007). The two fixed factors were: fertilizer rate (two doses of a NPK fertilizer that applied 28 and 56 mg N per seedling) and timing (early versus late) in the fall. With this ANOVA procedure, F and P values for the control group correspond to the contrast to compare control mean with the average of all the other treatments while maintaining the two-way analysis for the factors. Root growth potential data were previously log-transformed to meet ANOVA assumptions of equal variance, though data were reported as original means with standard errors. For N concentration in roots, as ANOVA interaction was significant, differences between means were identified using Fisher's least significant difference (LSD) test, adjusting the overall α level by Bonferroni correction. Regression analysis was used to explore relationships among the variables. In particular, regression analysis of

RGP over root [P] at the final sampling of the nursery experiment was conducted on a tray basis after averaging all RGP values from each tray. Effects were considered significant when P<0.05. SPSS Statistical Package version 14.00 (SPSS Inc., Chicago, IL, USA) was employed for data analysis.

3 Results

3.1 Morphological and nutritional attributes

Shoot height, shoot, root and total biomass, and shoot-to-root ratio were not significantly affected by fertilization treatments in any sampling date. On average, height growth along the 6-week period between samplings was low (mean ± SE), shifting from 9.5±0.3 to 9.9±0.3 cm, and total dry weight and shoot-to-root ratio ranged from 2.1±0.1 g and 0.67±0.03 (intermediate sampling) to 2.6±0.1 g and 0.63±0.05 (final sampling), respectively (data not shown per treatment). No significant differences were also found for root collar diameter and leaf area at final sampling (3.1±0.4 mm and 33.0± 1.6 cm², respectively, on average).

Although shoot N of intermediate sampling date was significantly affected by fertilization, N concentration of shoots was not significantly different between treatments at the end of the culture (Table 1 and Fig. 1a). An interaction between both factors for final root N appeared to significantly separate the treatments (Table 1 and Fig. 1b). Concentration of P in shoots was not affected at the end of culture by the rate or fertilization regime. Only non-fertilized plants resulted in a lower P concentration in this plant component (Table 1 and Fig. 1c). The response of root P to both rate and timing of fall fertilization was more intense and significant than that of shoots (Table 1 and Fig. 1d). Plants fertilized in early fall concentrated 0.12 mg more P per gram of dry root tissue than when fertilized in late fall. In addition, roots of *Q. ilex* showed to be highly sensitive to fertilizer rate as root P concentration (at the end of the culture, final sampling) increased 0.18 mg g^{-1} when the fertilization rate of P increased from 12.2 to 24.4 mg (Table 1 and Fig. 1d). As for shoot N, K concentration of shoots decreased during fall (Fig. 1a) irrespective of treatment. Within fertilized plants, shoot K values shifted from 3.62±0.10 mg g^{-1} at mid-fall (intermediate sampling) to 3.22±0.10 mg g^{-1} at the end of fall (final sampling), with no significant differences among fertilization rate or timing. Only non-fall fertilized (control) seedlings concentrated significantly less K in shoots than fertilized plants (Table 1 and Fig. 1e), but, again, as observed for N in shoots, this effect vanished at the end of the culture period. Likewise, root K concentration was not significantly affected by fall fertilization, although the pattern was somewhat different,

Table 1 ANOVA P values for nutrient concentration (mg g^{-1} of N, P, and K) in shoots and roots of $Q.\ ilex$ for intermediate and final sampling dates

	Control[a] $P>F$	Fertilizer rate (R) $P>F$	Fertilizer timing (T) $P>F$	$R \times T$ $P>F$
Intermediate sampling				
Shoot N	0.011	1.000	1.000	0.341
Root N	0.570	0.595	0.047	0.176
Shoot P	0.000	0.471	0.002	0.471
Root P	0.104	0.746	0.024	0.127
Shoot K	0.035	0.346	0.748	0.429
Root K	0.627	0.856	0.499	0.750
Final sampling				
Shoot N	0.452	0.850	0.353	0.850
Root N	0.077	0.002	0.316	0.040
Shoot P	0.001	0.628	0.165	0.628
Root P	0.001	0.001	0.014	0.682
Shoot K	0.076	0.942	0.942	0.612
Root K	0.286	0.055	0.749	0.413

[a] P values for the control group correspond to the contrast to compare control mean with the average of all the other treatments means. $n=3$ composite samples (five seedlings per replicated tray, three trays) per treatment

with an almost significant trend to concentrate more K in roots of seedlings fertilized with 56 mg of N ($P=0.055$, Table 1 and Fig. 1f).

Nutrient N and K content in the seedlings (24.2 ± 1.2 and 14.2 ± 0.6 mg per seedling on average, respectively) were not affected, while P content was significantly influenced ($P=0.048$) by fall fertilization. Control seedlings receiving no fertilization in fall contained 1.4 ± 0.1 mg of P, while fall fertilized plants accumulated 2.1 ± 0.1 mg on average. Differences in plant P content within fertilized treatments appeared to be not significant (data not shown).

3.2 Root growth potential and outplanting performance

Fall fertilization affected root growth potential as the control had significantly lower root growth than the mean of fertilized treatments ($P=0.003$): Holm oaks fall fertilized produced 42.4 mg of new roots dry mass on average, while the control only reached 20.2 mg (Fig. 2, inset). A significant relationship between P concentration in roots and root growth potential was found among all the attributes tested at final sampling ($P=0.002$, Fig. 2). Thus, an increased root P concentration after fertilization at early fall or at higher rates in the nursery preceded a higher amount of newly grown roots at RGP test. No significant correlation was found between shoot height at test planting and RGP itself (data not shown).

Regarding outplanting performance, seedlings did not differ in height, stem basal diameter, or aerial biomass in March (just after planting, data not shown). In June, significant differences in basal stem diameter for fertilizer timing appeared (Table 2). Additionally, we found significant differences in total plant leaf area, leaf, and stem biomass in June (Table 2 and Fig. 3a, b), with control plants having lower values than fertilized ones. Moreover, fertilized plants showed a higher proportion of new leaves (biomass-based) than control plants (Fig. 3c). There was no effect of fertilization on the percentage of 1-year-old leaves abscised during spring (data not shown). About half ($44\pm26\%$) of the 1-year-old leaves were replaced in spring.

Phenology was also significantly affected by fall fertilization. In April, control plants without fertilization showed a slower phenological development ($P<0.001$, Table 2): Only 19% of plants were in state 4 (fully expanded leaves, Fig. 3d), whereas plants fertilized with 56 mg of N showed a higher proportion of plants (58%) at state 4. Fertilized plants showed a high proportion of plants at states 3 and 4 (between 76% and 81%), while control plants at these states only reached 52% (Fig. 3d).

Summer drought was very severe; therefore, survival 7 months after planting was very low (between 5% and 10%). The scant surviving plants did not exhibit a clear pattern for the different fertilization treatments.

4 Discussion

4.1 Morphological and nutritional attributes

Shoot growth during fall in the nursery could lead to imbalanced seedling morphology; in our study, shoot growth biomass was negligible, probably due to the effect of temperature inhibiting growth during the fall (Fernández et al. 2008). Additionally, no effect of fall fertilization on biomass allocation pattern between shoot and root was detected. Similarly, in other fall fertilization experiments with *Picea mariana* Mill. B.S.P. and *Pinus resinosa* Ait., final plant shoot-to-root ratio was unaffected by fall

Fig. 1 N, P, and K concentration (mean ± SE) in shoots (**a**, **c**, **e**) or roots (**b**, **d**, **f**) of Holm oak (*Q. ilex*) as affected by fall fertilization: no fertilization (control), fertilizer rate (28 versus 56 mg of N), and timing (early versus late fall, *EF* and *LF*, respectively) at two sampling periods (intermediate and final). Level 28 mg of N also included 12.2 and 23.4 mg of P and K, respectively, and double amount for level 56 mg N. *Asterisks on control bars* indicate significance of the contrast that compares control mean with the average of all the other treatment means. Within a factor level (fertilizer rate or timing) and sampling period (intermediate or final), when no *letters are given next to the bars*, no significant treatment effect was apparent from the factorial ANOVA treatment (*capital letters* for comparison among intermediate sampling treatments). For root N concentrations, two-way interactive effect is presented (see Table 1), precluding main effects comparisons. For this plant component, the *same letters within a sampling period* reflect no significant N differences among treatments according to Fisher LSD post hoc test. See Table 1 for sample size
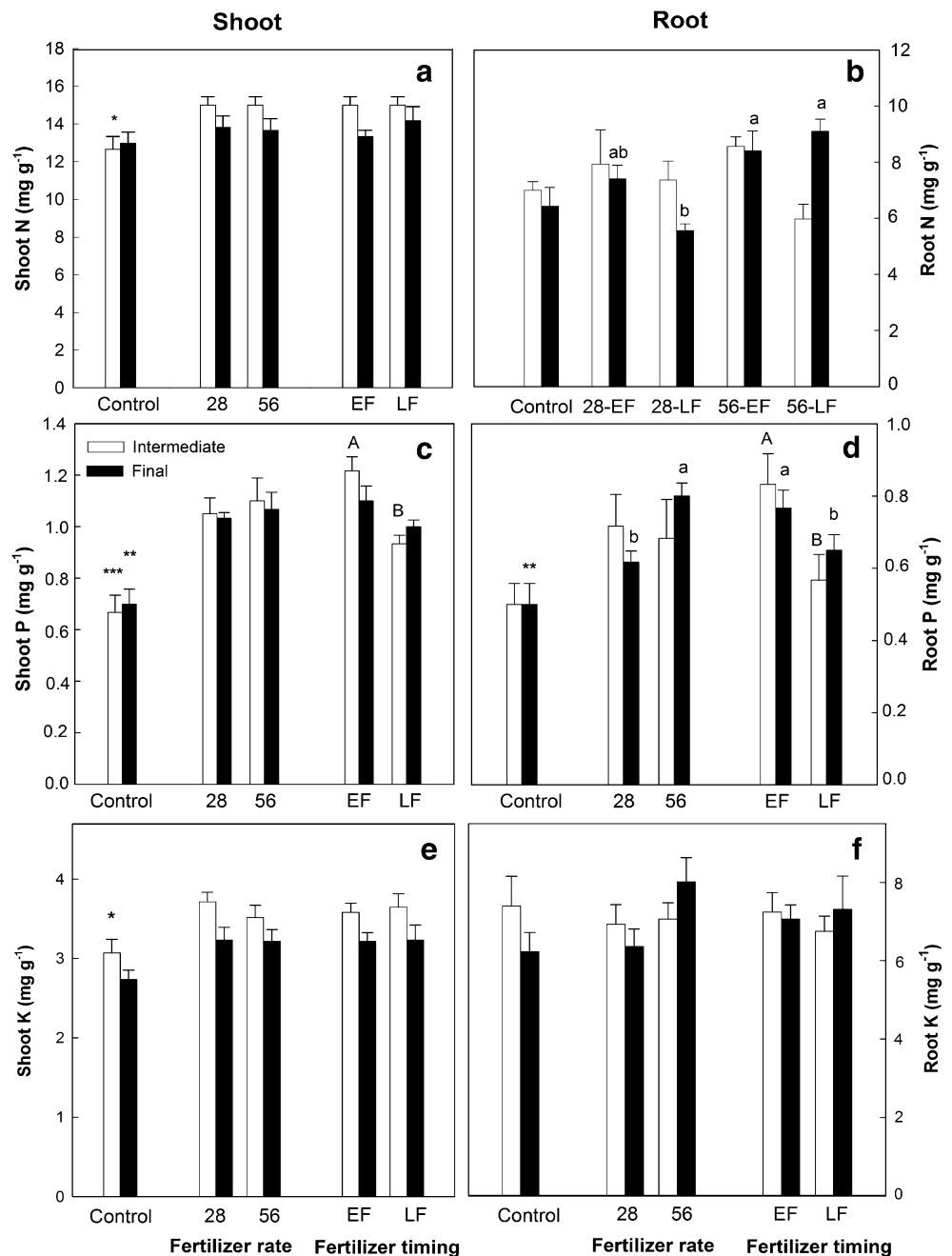

treatment (Boivin et al. 2004; Islam et al. 2009, respectively). Some of the mentioned studies were bare root stock where no limits to root expansion exist. However, in containerized Holm oak nursery culture, root confinement effect occurs at the end of the growing season under this cell size (Oliet et al. 2009b), impairing root growth response to additional fertilizer supply during fall.

Differences in uptake and traslocation patterns according to the nutrient considered, fertilization rate, and timing along the hardening period provide important keys for optimizing nutrient load of Holm oak via fall fertilization. In this experiment, nutrient uptake and translocation

processes were significantly affected by fall fertilization, although this effect depends on the nutrient and plant component (shoot versus root) considered. Shoot N and K concentrations at the end of the hardening period were not affected by fall fertilization, and both elements experienced dilution during fall. In fall fertilized *Pinus taeda*, N and K also experienced dilution during fall regardless of fertilization treatment (Sung et al. 1997). However, N and K in roots experienced dilution only at no or at the lowest rates of fertilization, although differences were only significant for root N. This indicates that root dilution of these nutrients could be prevented by increasing the amount of

Fig. 2 Relationship between root P concentration at final nursery sampling and root growth potential (*RGP*) of Holm oak (*Q. ilex*) measured as dry mass of new roots (mean ± SE) protruding the plug. Each *dot* represents averaged RGP of five seedlings from a tray over root P concentration of five composite roots from the same tray (*n*=15 trays). *Inset* RGP (mean ± SE, *n* =15 plants per treatment) of Holm oak as affected by fall fertilization (see Fig.1 for treatment descriptions). *Asterisks on control bar* indicate significance of the contrast that compares control mean with the average of all the other treatments means (*P*=0.003)
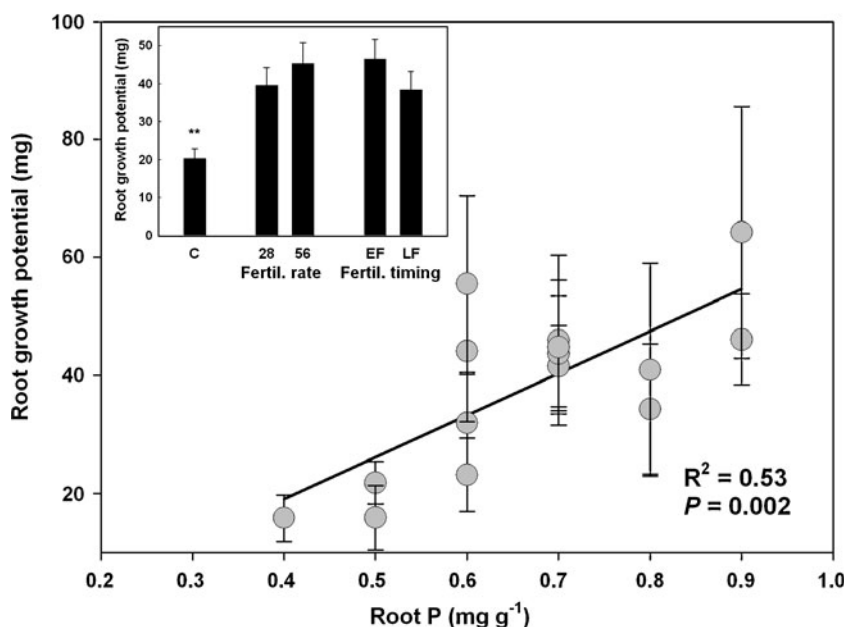
fertilizer applied during the hardening period (Boivin et al. 2004). Significant differences in N concentration by fall fertilization for root, but not for shoot, indicate that N uptake and storage in roots responds to different concentrations of N in the growing media, but N translocation to shoots is almost null as no differences in N concentration based on treatments were found. Fall fertilization affected more clearly P dynamics: Concentration in roots was significantly affected by fertilizer rate and timing (Fig. 1d), with the highest concentration values at the maximum fertilizer rate applied in early fall. This indicates that P uptake efficiency is higher at the beginning of fall. With regards to shoot, a significant response to fall fertilization (on average) occurred, indicating a partial translocation to the shoots of the supplied P in the fall, which is not

observed for N and K. As for conifers, P uptake during periods of growth cessation results in greater levels that could be available for future retranslocation (Folk and Grossnickle 2000).

Generally, Holm oak is a conservative species and exhibits low phenotypic plasticity to resource availability (Valladares et al. 2000), although this response may vary with the nutrient considered, as shown by Sardans et al. (2006b) for P. In this study, shoot growth was minimum. Therefore, low N and K uptake capacity must be explained mostly by a weak sink demand. N uptake has been shown to be greatly dependent upon ontogeny (Imsande and Touraine 1994), and low N uptake amounts are expected during hardening (Silla and Escudero 2003). Finally, the ability to take up and translocate nutrients during fall is

Table 2 ANOVA P values for different variables of biomass allocation and phenological state for plants of *Q. ilex* under different treatments of fertilization and growing in the field for 4 months (June)

	Control[a] $P > F$	Fertilizer rate (R) $P > F$	Fertilizer timing (T) $P > F$	$R \times T$ $P > F$
Height	0.945	0.987	0.891	0.663
Basal stem diam	0.594	0.387	0.017	0.732
Plant area	0.014	0.242	0.148	0.017
Leaf biomass	0.012	0.111	0.143	0.027
Stem biomass	0.010	0.078	0.039	0.959
Shoot biomass	0.007	0.079	0.080	0.083
New leaves (%)	0.012	0.006	0.354	0.809
Apr phen state	<0.001	0.209	0.674	0.842

Height (maximum stem height, centimeters), basal stem diam (basal stem diameter, millimeters), plant area (square centimeters), leaf, stem and shoot biomass (grams), Apr phen state (phenological state in April, see legend of Fig. 3)

[a] *P* values for the control group correspond to the contrast to compare control mean with the average of all the other treatments means. *n*=60 plants per treatment for height, basal stem diameter, and phenological stage in April and 21 plants per treatment for plant area, leaf, stem, and shoot biomass
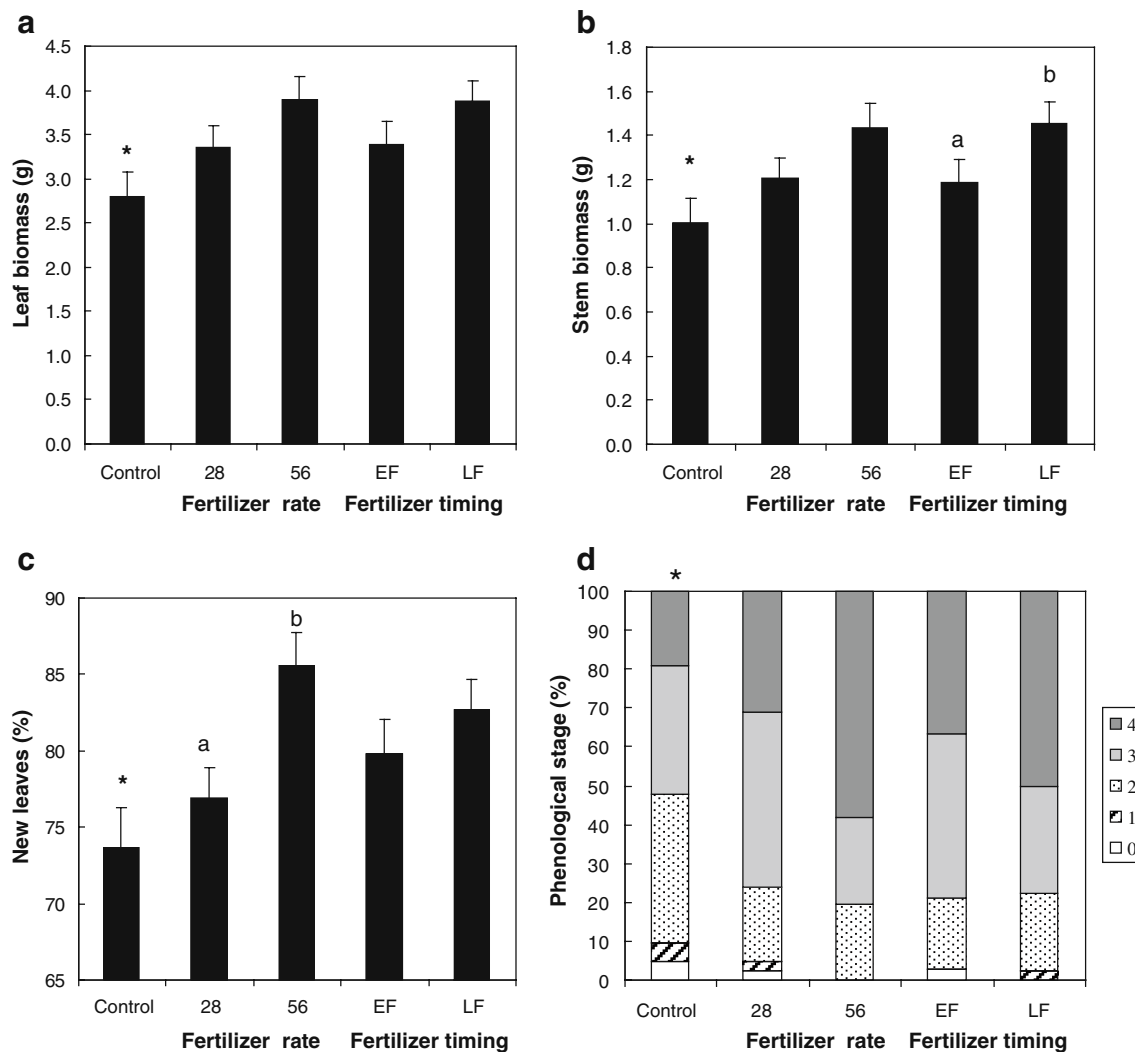
Fig. 3 Leaf biomass (**a**), stem biomass (**b**), and proportion of new leaves (biomass-based) (**c**) in June and proportion of plants under different phenological stages in April (**d**) of planted Holm oak (*Q. ilex*) as affected by treatments of fall fertilization in the nursery (see Fig. 1 for treatment descriptions). Phenological stages *0*—plants with leaf buds; *1*—swollen leaf buds; *2*—growing leaves; *3*—leaves formed but not completely expanded; *4*—leaves completely expanded. *Asterisks on control bars* indicate significance of the contrast that compares control mean with the average of all the other treatments means ($P<0.05$). Within a factor level, when no *letters are given next to the bars*, no significant effect was apparent from the ANOVA treatment. See Table 2 for sample size

both species- and nutrient-dependent, as shown in some fall fertilization studies with several conifer species (Sung et al. 1997; South and Donald 2002; Boivin et al. 2004; Islam et al. 2009).

4.2 Root growth potential and outplanting performance

The production of large deep root system during the wet season before the onset of drought is essential for summer survival under Mediterranean conditions (Padilla and Pugnaire 2007). In our experiment, fertilizing in fall promoted RGP of Holm oak seedlings. New root growth can be enhanced by either the role of nutrients (either N or P) due to the activation of photosynthesis and/or by the use

of root reserves (Heiskanen and Rikala 1998; Warren et al. 2005, respectively). For some other *Quercus* species, stored carbohydrates in roots could promote initial new root proliferation (Jacobs et al. 2009), whereas current photosynthates assume a greater role as new leaves mature and the flush terminates (Sloan and Jacobs 2008). In our RGP test, Holm oak seedlings' shoot growth was not activated yet and only at the end of the test terminal did buds start to swell (data not shown). Under these circumstances, root traits like carbohydrate or P reserves could trigger new root proliferation during the first stages of dormancy exit. In our experiment, the relationship between fertilization and RGP is via P concentration in roots, which appeared positively correlated to new root proliferation. In previous studies, fertilized Holm oaks

grew more new root tissues after planting than seedlings receiving a low fertilizer rate irrespective of soil fertility (Oliet et al. 2009b). This indicates a relative independence of root growth on external resources (current assimilates or nutrients taken up) during early establishment.

The response of root growth and survival after planting to P concentration in roots has been observed in earlier studies with other Mediterranean species, like *Acacia salicina* Lindl. and *Pinus halepensis* Mill. (Oliet et al. 2005, 2009a, respectively) and also with Holm oak (Sardans et al. 2006a). However, the mechanisms by which P could promote root extension after planting are still debated (Folk and Grossnickle 2000; Landis and Van Steenis 2004). In Holm oak, previous studies showed a significant effect of fertilization on root growth potential; although this effect could not be associated to any nutrient in particular, mortality after planting showed a significant and negative relationship with root P (Villar-Salvador et al. 2004; Mollá et al. 2006), i.e., less seedlings died when roots had more P.

Our findings agree with those of Villar-Salvador et al. (2004) showing significantly less shoot growth of outplanted non-fertilized *Q. ilex* seedlings than those cultivated at either low or high fertilization levels in the nursery. Interestingly, our results also showed that fertilization resulted in a faster development, producing new leaves earlier than in control plants. Higher N levels in spring have also been shown to accelerate bud burst of *Picea abies* L. (Floistad and Kohmann 2004). Bud bursting earlier in spring can be an interesting advantage for seedlings planted under mild Mediterranean conditions, where summer drought occurs at the very beginning of summer. However, in contrast to Villar-Salvador et al. (2004), we did not find any effect of fertilization treatment on survival. Summer of 2006 was very dry and showed high maximum temperatures. It may explain why the fertilization treatments did not show any effect under these stressful conditions.

The large amount of 1-year-old leaves that were replaced during spring (i.e., leaf longevity around 1 year, from spring 2005 to spring 2006) contrasts the measured leaf longevity of Holm oak adult trees, which was estimated in more than 2 years (Mediavilla et al. 2008). As Silla and Escudero (2003) pointed out, leaf N retranslocation from old leaves can be a supply for new growth. Although nutrients for new growth can be supplied by soil uptake and/or nutrient retraslocation, Silla and Escudero (2003) found a strong nitrogen retranslocation up to 90% due to the high asynchrony between nitrogen demand by growing tissues and soil supply.

5 Conclusions

Our approach of applying nutrients at two different times in the fall has been proven to be effective in showing the differences between N, P, and K dynamics in the seedlings. The uptake efficiency of P decreases as fall progresses, while N and K absorption were independent of application time. Fall fertilization of Holm oak in the nursery has been proven to be effective for P (and to a lesser extent N) loading proposals, with different responses as a function of fertilizer regime and plant component (shoot versus root) considered. Our results also show that root growth potential of P-loaded seedlings was higher, suggesting an important role of this element in boosting root growth. In addition, field growth of nutrient-loaded seedlings was increased by means of a faster phenological development and a higher aboveground biomass. We recommend, for mild autumn nurseries raising Holm oak seedlings, to apply nutrients (P in particular) during early fall and increase the rates of N and K applied to values higher than those used in this experiment.

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