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Abstract Forest restoration projects with Holm oak (*Quercus ilex*) have had limited success, mostly due to water stress after planting and poor plant quality. Recent studies indicated that large and nutrient rich plants perform better in Mediterranean areas, suggesting that late-season fertilization may improve plant quality and field performance. The purpose of this study was to assess the effect of late-season fertilization on the quality of Holm oak seedlings, as determined by morphological, nutritional, and water relations analyses. We grew Holm oak nursery seedlings under 5 different late-season fertilization regimes and then analyzed morphological characteristics, nutritional status, and water relations parameters of the fertilization groups at the end of the nursery period. We also analyzed the effect of fertilization on nutritional status by use of vector nomograms. Our results indicated that late-season NPK fertilization improved shoot and root growth, and the overall nutritional status of seedlings. The lack of late-season fertilization leads to nutrient deficiency in plants, whilst the application of imbalanced fertilization treatments may trigger nutrient luxury consumption and nutrient dilution, pointing out the importance of NPK proportions in the fertilizer. Moreover, late-season fertilization with nitrogen might improve the drought resistance of seedling by enhancing their osmotic adjustment.

Keywords Autumn fertilization · Nutrient loading · Nursery culture · *Quercus ilex* · Osmotic adjustment · Vector analysis

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Introduction

Forest restoration projects with Mediterranean evergreen oaks and other sclerophyllous species have had limited success because of low survival and growth rates after planting (Cortina et al. 2011; Pausas et al. 2004; Valdecantos et al. 2006; Villar-Salvador et al. 2004a). Water stress during the first drought period after planting, usually the first summer, is the main cause of seedling mortality in plantations of Holm oak (*Quercus ilex*) (Villar-Salvador et al. 2004a, 2012). Poor soils, inadequate site preparation, competing vegetation, and low plant quality also limit field performance (del Campo et al. 2010; Palacios et al. 2009; Pardos et al. 2005; Sanz-Pérez et al. 2007; Valdecantos et al. 2006).

Assessment of plant quality has traditionally considered morphological parameters (Grossnickle 2012). Recent studies have used shoot height and root collar diameter as quality indicators for nursery seedlings of Mediterranean woody species, because these parameters are easily measured and are often related to field performance (Navarro-Cerrillo et al. 2006; Tsakaldimi et al. 2013). The European Union uses these parameters in its regulations regarding recommended plant materials for restoration of Mediterranean species (Alía et al. 2005). However, there is controversy about which seedling traits are truly related to establishment success, especially under severe drought conditions (Cortina et al. 2006). Many studies of Mediterranean species indicated that large seedlings perform better than small seedlings (Cuesta et al. 2010; Puértolas et al. 2003; Tsakaldimi et al. 2013; Villar-Salvador et al. 2004a, 2012). However, under arid conditions small seedlings have higher survival rates (Trubat et al. 2008, 2011). The nutritional status of Mediterranean seedlings is important for field performance, and N-rich plants have higher survival rates than N-poor plants (Cuesta et al. 2010; Oliet et al. 2009; Puértolas et al. 2003; Villar-Salvador et al. 2004a), however Oliet et al. (2006) reported a reduced survival with increasing leaf N and P concentration of *Q. ilex* nursery seedlings. An ecophysiological model proposed by Villar-Salvador et al. (2012) suggested that large and N-rich seedlings have higher growth rates during the wet season immediately after planting than small or N-poor seedlings, and that large and N-rich seedlings had better root development, maintenance of high water potential, and positive net photosynthesis during the dry season. This indicates that it is necessary to consider nutritional and physiological parameters in assessing the quality of Mediterranean seedlings.

Adjustment of nursery fertilization level is a powerful tool that allows modification of the morphology, physiology, and nutrient status of seedlings and may also improve survival rate after planting (Puttonen 1997). Nutrient deprivation during the late-season has been used for nursery culture of some Mediterranean species, and this method improves field performance under arid conditions (Trubat et al. 2008, 2011). Late-season fertilization has been applied to many other species, and this technique can improve physiological status, growth, and survival after planting (Boivin et al. 2004; Colombo et al. 2003; Islam et al. 2009; Salifu and Timmer 2003). More recently, late-season fertilization has been used in Mediterranean species, because seedlings grow during the autumn when the temperatures are mild (Fernández et al. 2008) and this growth may lead to nutrient dilution if seedlings are not fertilized during this period. Some studies of late-season fertilization of Holm oak nursery seedlings have reported net positive effects. For example, Oliet et al. (2011) reported that fertilization increased the P concentration of seedling tissues, root growth capacity, and field performance. Andivia et al. (2011, 2012a, b) reported that late-season fertilization increased seedling size, nutrient content, root growth capacity, and tolerance to frosts, but had no effect on survival and growth after planting. Seedling survival and growth in forest plantation is extremely dependent on water, carbon and nutrient balance after planting (Grossnickle 2012; Villar-Salvador et al. 2012), which are conditioned by plant-site interaction, so the production of high quality seedlings requires matching of plant attributes to site conditions (Oliet et al. 2013). As multiple stress and resource limitations, especially water, frosts and soil nutrients are presented along the Mediterranean region, it is necessary to completely characterize the effect of late-season fertilization on Holm oak nursery seedlings in order to determine the optimal dose and timing of fertilization.

The purpose of the present study is to assess the effect of different autumn fertilization treatments on the morphological, nutritional, and water relations characteristics of Holm oak nursery seedlings.

Materials and methods

Plant material and experimental conditions

These experiments were conducted in an outdoor nursery of the University of Huelva, under a white shade-cloth that reduced outside radiation by 50 %. The nursery is located in Palos de la Frontera (Southwest of the Iberian Peninsula, 37°12′04″N, 6°55′07″W) and has a coastal Mediterranean climate, with mild autumn temperatures and rare frosts during the winter. Acorns of *Quercus ilex* ssp. *ballota* (Desf.) Samp. from the Spanish provenance "Sierra Morena Occidental" were used to cultivate about 1,000 healthy plants. Pre-germinated acorns were sowed in 24 Plasnor® trays (45 cavities of 300 cm^3) at the end of February 2007 and placed in the nursery. Cavities were filled with sphagnum peat Kekkilä® B0. During the entire nursery period, plants were growing at environmental conditions (Fig. 1), watered with tap water as needed and trays were rotated weekly to avoid microsite effects.

Fertilization experiment

Seedlings were growing with no fertilization from the sowing date (end of February) to the end of March (about 1 month after seedling emergence). Afterwards, a constant fertilization regime was applied from 26 March to the end of September (28 weeks, Fig. 1). During this time, each seedling received a total of 70.0 mg N, 30.5 mg P, and 58.1 mg K. Fertilizer was applied weekly using a 20-20-20 water-soluble fertilizer (Peters Professional®) at a solution rate of 125 ppm N, 54 ppm P, and 104 ppm K. A liquid-dispenser (Hirschmann Laborgeräte ceramus classic, Eberstadt, Germany) was used to provide each plant with the exact weekly amount of the fertilizer solution (20 ± 1 ml).

After the 28 weeks, 600 seedlings were randomly selected and distributed to 20 Plasnor® trays with 45 cavities of 300 cm^3 (30 plants per tray) and five different fertilization treatments were established (Table 1), with 120 seedlings (four trays) per fertilization group. These treatments used different amounts of $(NH_4)_2SO_3$, $Ca(NO_3)_2$, KH_2PO_4, NaH_2PO_4, KCl, $MgSO_4$, $CaCl_2$ solutions and a mixture of micronutrients so that there were different doses of N, P and K, but similar doses of other macro- and micronutrients. The five treatment groups were: *C* (control treatment), low doses of N, P and K, accordingly to low nutrient treatments applied during the late-season in some Mediterranean nurseries, but with avoidance of total nutrient deprivation; *+NPK*, fertilization of N, P, and K at the same level as the previous 28 weeks; *+N*, doses of N at the same level as the previous 28 weeks and P and K levels as in the control group; *+P*, doses of P at the same level as the previous

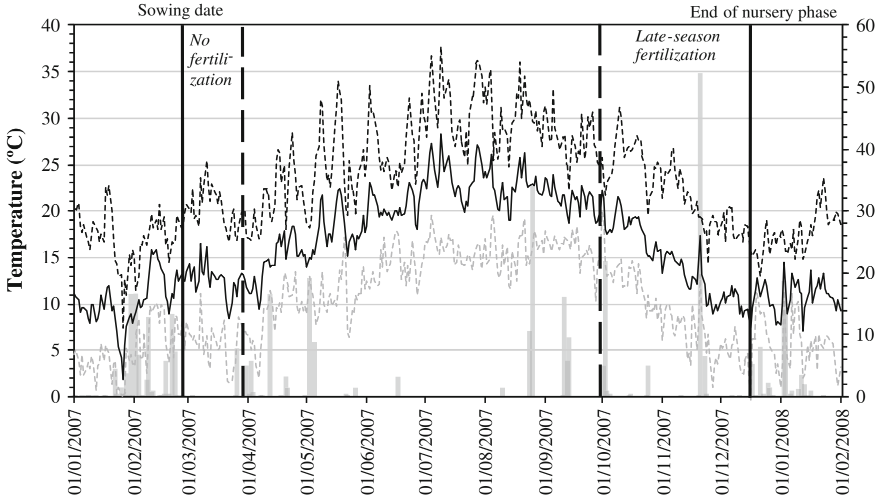

Fig. 1 Daily maximum temperature (*black dashed line*), minimum temperature (*grey dashed line*), mean temperature (*solid black line*) and precipitation (*grey bars*) at the nursery location during the experimental period. Sowing date and the end of the experimental period are defined by *vertical solid lines*. The different fertilization regimes during the nursery phase are shown in the figure (*no fertilization, constant fertilization,* and *late-season fertilization*), with *dashed lines* indicating the end of the specific period

28 weeks and N and K levels as in the control group; and +*K*, doses of K at the same level as the previous 28 weeks and N and P levels as in the control group. These fertilization treatments were applied weekly from 1 October to 17 December (12 weeks), using the same liquid-dispenser as in the previous 28 weeks. At week-41, 1 week after the end of the 40 weeks of the fertilization experiment (28 weeks at baseline fertilization and 12 weeks at altered fertilization), seedling morphology, nutrient status, and water relations parameters were measured.

Morphological parameters

Morphological parameters were measured on 12 randomly selected plants from each treatment (three plants per tray). Height (*H*) was measured as the distance from the terminal bud to the cotyledon insertion point, and diameter (*D*) was measured 0.5 cm above the cotyledon insertion point. Shoots were cut from plugs at the cotyledon insertion point, and roots were carefully separated from the growing medium using a water stream. Then, samples were washed with distilled water, oven-dried at 65 °C during 48 h, and the dry mass (±0.0001 g) of leaves (*LM*), stems (*StM*), and roots (*RM*) were determined. The following values were then calculated: shoot dry mass ($SM = StM + LM$), total dry mass ($TM = SM + RM$), shoot:root ratio ($S/R = SM/RM$), sturdiness (*H/D*), Dickson's quality index [$DQI = \mathrm{TM}/((\mathrm{H/D}) + (\mathrm{S/R}))$], leaf mass fraction ($LMF = LM/TM$), stem mass fraction ($StMF = StM/TM$), and root mass fraction ($RMF = RM/TM$).

Nutrient status

Nutrient analysis of the leaves, stems, and roots of the same plants were also performed. Dry plant material from each fraction (leaves, stems and roots) was pooled per tray, and

Table 1 Amounts of N, P, K fertilization given to each seedling of the five different late-season fertilization treatments (last 12 weeks), and the total nutrients received during the fertilization experiment (40 weeks)

Treatment	Nutrient content given in the last 12 weeks (mg)			Total nutrient content given during 40 weeks (mg)		
	N	P	K	N	P	K
C	1.5	0.3	0.5	71.5	30.8	58.6
+NPK	30.0	13.1	24.9	100.0	43.6	83.0
+N	30.0	0.3	0.5	100.0	30.8	58.6
+P	1.5	13.1	0.5	71.5	43.6	58.6
+K	1.5	0.3	24.9	71.5	30.8	83.0

ground to finally obtain four replicates of each fraction per treatment. N was determined using an elemental analyzer (Termo Finnigan 1112 Series EA, Milan, Italy), and P, K, Mg, and Fe were measured with an ICP-OES Jobin–Yvon Ultima 2 (Tokyo, Japan) after digestion with HCl (Temminghoff and Houba 2004). Nutrient contents are expressed as the product of concentration (mg g^{-1}) and dry mass (g).

The effect of late-season fertilization treatment on growth was analyzed by vector nomograms, diagrams that display plant mass (*z*-axis), nutrient concentration (*y*-axis), and nutrient content (*x*-axis). The vector analysis was conducted with the mean value per treatment of leaf mass, leaf nutrient concentration and content of the same plants used for assessing the morphological and nutritional status of plants. The magnitude and direction of a vector show the effect of a fertilization treatment on growth dilution, deficiency, luxury uptake, toxicity, and nutrient interactions of seedlings (Salifu and Jacobs 2006; Timmer and Armstrong 1987). All responses are expressed relative to the control (*C*) treatment, which was normalized to 100. Groupings (Fig. 3a) were done based on the direction and magnitude of vectors, because they diagnose the seedling nutritional status. Group 1 consists of nutrients whose concentration and content increased when the fertilization treatment was applied, but without a significant increment in plant biomass (all nutrients in +*P* and +*K* treatments). Groups 2 and 3 consist of nutrients whose concentration and content increased, together with a significant increment in plant biomass (all nutrients in +*NPK* treatment, and N and P in +*N* treatment). As nutrient concentration, content and biomass increments were higher in the +*NPK* treatment than in the +*N* treatment, one group per treatment was established (groups 2 and 3, respectively). Groups 4 and 5 were established for the rest of nutrients of the +*N* treatment. The group 4 consists of nutrients whose concentration decreased, whilst the nutrient content and plant biomass was increased (K and Mg). Finally, Fe is grouped in the group 5, reduction in nutrient concentration and content with increasing plant biomass.

Water relations

Water relations parameters were measured in four plants of each treatment group (one plant per tray, randomly selected) after the end of the fertilization treatment (week-41). Seedlings had no visual signs of shoot elongation with the last flush occurred in early November. Seedlings were well watered the morning before and kept under environmental conditions in the nursery. Water relations parameters (water potential at the turgor loss point [Ψ_{tlp}], osmotic potential at saturation [Ψs_{sat}], relative water content at the turgor loss point [RWC_{tlp}], the maximum bulk elasticity modulus (ε_{max}), and symplasm volume fraction at the turgor loss point [S_{tlp}]) were calculated from pressure–volume (P–V) curves, by following the free-transpiration method described by Koide et al. (1989). P–V curves were obtained by measuring the weight and water potential of seedling shoots at constant time intervals until values close to −5.0 MPa were reached. The shoot water potential was measured with a pressure chamber (Model 1000; PMS Instruments, Corvallis, OR).

Data analysis

A one-way ANOVA was used to analyze the effect of fertilization treatment on morphological characteristics, nutritional status, and water relations parameters. Plant mass ratios and shoot:root ratio were log-transformed to achieve homocedasticity. Differences between treatments were assessed by the least significant difference (LSD) method, and a *p* value less than 0.05 was considered significant. The relations between water relations parameters were assessed by calculation of Pearson's correlation coefficient. All statistical analyses were conducted in R (ver. 2.15.2).

Results

Morphological attributes and biomass partitioning

Our results indicate that the nature of the 12 week fertilization treatment had a significant effect on the morphology of seedlings (Table 2). In particular, seedlings in the +*NPK* and +*N* groups were larger and had greater above-ground biomass and lower *RMF* than those in the other 3 groups (Fig. 2). Above-ground biomass increased 52–85 % (in the +*N* and +*NPK* groups, respectively) relative to seedlings in the other groups. Similarly, root biomass was 13–29 % greater in the +*N* and +*NPK* groups, respectively (Fig. 2b) and *S/R* was also greater in seedlings of these groups (0.388–0.408) than in seedlings in the other groups (0.260–0.304), with seedlings of the *C* group showing the lowest *S/R* values. Fertilization treatment also affected *H/D* and *DQI*. In particular, seedlings in the +*K* group had higher *H/D* values (6.19 ± 0.58) than those in the other groups (mean value 4.76 ± 0.33). The *DQI* of the five groups were: 1.79 ± 0.14 (+*NPK*), 1.67 ± 0.11 (+*N*), 1.39 ± 0.13 (+*P*), 1.18 ± 0.18 (+*K*), and 1.37 ± 0.15 (*C*). Statistical analysis indicated that the *DQI* values of seedlings in the +*N* and +*NPK* groups were significantly different from that in the +*K* group.

Nutritional analysis

Late-season fertilization significantly affected shoot nutrient concentration and content, especially of leaves, but had no effect on root nutrient status (Tables 3 and 4). In particular, seedlings in the +*NPK* group had higher leaf concentrations of N and Mg than leaves in the other groups (Table 4). Seedlings in the +*N* group had higher leaf concentration of N than seedlings in the *C* and +*P* groups (Table 4), although these differences were significant at a level of $p = 0.052$ and $p = 0.078$ (calculated from the multiple comparison test), respectively. Leaves in the +*NPK* group had 150–200 % of the nutrient content of leaves in the other groups, and leaves in the +*N* group had 50–70 % of the N nutrient content of leaves in the +*K*, +*P*, and *C* groups. Fertilization only had a significant effect on the N and

K content of the stem. Seedlings in the +*N* and +*NPK* groups had 175 and 210 %, respectively, of the N content of stem in the other groups. Furthermore seedlings of +*NPK* group had higher stem K content (3.74 ± 0.25 mg) than those in the other groups (mean value 2.39 ± 0.20 mg).

Vector nomograms (Fig. 3a) indicated the presence of luxury nutrient consumption in the +*P* and +*K* groups because of the nutrient accumulation (concentration and content) without a significant increase in plant biomass (arrow 1, Fig. 3b). Plants in the +*NPK* group had increased leaf biomass, leaf nutrient concentration, and leaf nutrient content, indicating a nutrient deficiency in the control group (arrow 2, Fig. 3b). Seedlings in the +*N* group responded differently, in that leaf concentration and content of N and P increased, indicating that these nutrients were limiting in the *C* group (arrow 3, Fig. 3b). K and Mg may be characterized as a dilution effect due to the decreased nutrient concentration with increased growth (arrow 4, Fig. 3b), and Fe leaf concentration and contents decreased in spite of growth increase (arrow 5, Fig. 3b).

Water relations

The analysis of water relations indicated that *Q. ilex* seedlings responded to late-season N fertilization by decreasing Ψ_{tlp} and Ψs_{sat} (Table 5). Both osmotic potentials were significantly lower in seedlings in the +*N* and +*NPK* groups, although seedlings in the +*P* group had Ψ_{tlp} values that were between those of the +*NPK* and +*N* groups and the *C* and +*K* groups. However, no significant differences in the RWC_{tlp}, ε_{max}, and S_{tlp} were found among fertilization groups. RWC_{tlp} was negative related with Ψ_{tlp} ($r = -0.513$, $p = 0.05$), Ψs_{sat} ($r = -0.648$, $p = 0.009$) and S_{tlp} ($r = -0.831$, $p < 0.001$), and positive related with ε_{max} ($r = 0.828$, $p < 0.001$). Analysis of correlations also showed a positive relationship between Ψ_{tlp} and Ψ_{Ssat} ($r = 0.663$, $p = 0.007$), as well as a negative relationship between Ψ_{Ssat} and ε_{max} ($r = -0.837$, $p < 0.001$).

Discussion

Our results indicate that late-season N fertilization increased shoot growth and plant biomass of nursery-grown Holm oak seedlings. These results differ from those of Oliet et al. (2011), but are consistent with previous studies conducted at the same nursery (Andivia et al. 2011, 2012a, b). This discrepancy could be explained by differences in meteorological and other conditions at the two nurseries (Mollá et al. 2006). In particular, low temperatures during the nursery phase inhibits shoot growth (Fernández et al. 2008), so late-season fertilization in nurseries at colder locations might be expected to have less effect on seedling growth. The mild autumn temperatures in the nursery of the present study (Fig. 1) may have led to our observation of increased growth following autumn fertilization. In contrast, Oliet et al. (2011) reported no effect of late-season fertilization on root biomass. We found that the two high-N groups had greater increase in shoot biomass than root biomass, leading to higher *S/R* of these seedlings relative to plants in the low-N groups. Consequently, more biomass was partitioned to the roots of plants in the low-N groups, whereas the high-N groups had more biomass invested in the shoot, especially the stem. On the other hand, seedlings in the +*K* group had the greatest *H/D* values and the lowest *DQI* values, because of an unbalanced shoot growth due to the reduced *D* growth.

Shoot height and diameter are the most common morphological characteristics used for assessment of plant quality (Alía et al. 2005; Grossnickle 2012). Tsakaldimi et al. (2013)

Table 2 One-way ANOVA of the effect of late-season fertilization treatments on the morphological, and water relations parameters of Holm oak seedlings

Parameter	*F*	*P*
Height	10.68	<0.001
Diameter	10.64	<0.001
Leaf mass	11.63	<0.001
Stem mass	13.27	<0.001
Root mass	11.42	<0.001
Total mass	5.01	0.002
H/D	2.97	0.027
S/R	10.95	<0.001
DQI	2.52	0.074
Leaf mass fraction	5.63	<0.001
Stem mass fraction	7.85	<0.001
Root mass fraction	11.78	<0.001
Ψ_{tlp}	3.48	0.048
$\Psi_{\mathrm{S_{sat}}}$	4.89	0.019
$\mathrm{RWC_{tlp}}$	0.68	0.620
$\mathrm{S_{tlp}}$	0.41	0.796
ε_{max}	1.13	0.396

H/D sturdiness, *S/R* shoot:root ratio, *DQI* Dickson's quality index, Ψ_{tlp} osmotic potential at turgor loss point, Ψs_{sat} osmotic potential at saturation, RWC_{tlp} relative water content at the turgor loss point, S_{tlp} symplasm volume fraction at the turgor loss point, ε_{max} maximum bulk elasticity modulus

recently suggested that root collar diameter could be an accurate predictor of second-year field survival of *Q. ilex* seedlings, based on the established positive relationship between plant diameter and root volume in *Quercus* species (Jacobs et al. 2005, 2009). Thus, some recent studies have reported a positive association of Holm oak seedling size and plantation survival (Cuesta et al. 2010; Villar-Salvador et al. 2004a, 2012). Taken together with the reported increment in plant biomass and diameter of seedlings fertilized with the +*N* and +*NPK* late-season treatments, this suggests that autumn N and, mainly, N-P-K fertilization of Holm oak seedlings could be an effective nursery practices to improve plant morphological characteristics related to field performance.

Our results indicate that late-season fertilization of *Q. ilex* nursery seedlings also modified plant nutritional status. In particular, the application of high-dose N, P and K (i.e. +*NPK* treatment) increased overall nutrient content and N concentration in leaves, in agreement with other studies (Andivia et al. 2012b; Oliet et al. 2011), and avoided the nutrient dilution effect in roots and stem despite increased biomass. Vector nomogram analysis provides a comprehensive and accurate analysis of nutrient status and interactions of growing plants (Salifu and Jacobs 2006). Analysis of our results by a vector nomogram (Fig. 3b) indicates different plant responses in nutrient levels to fertilization treatments. The nutritional status of plants in groups +*P* and +*K* shows that nutrient rates in the *C* treatment were at sufficiency levels, because the addition of these elements led to nutrient luxury consumption (no increments in biomass but in concentration). However, when +*N* treatment was applied growth was stimulated, and our results evidence that N and P were at deficiency levels in the *C* treatment, while the rest of the elements fell within dilution because nutrient supplied did not meet the increment in nutrient demand with growth. P did not show a dilution effect in the +*N* group, despite it was applied at a low rates in this fertilization treatment. The increment in P leaf concentration promoted by increasing N rates has been previously described by this species (Andivia et al. 2011, 2012b; Villar-Salvador et al. 2004a), and may be related with its higher P uptake efficiency

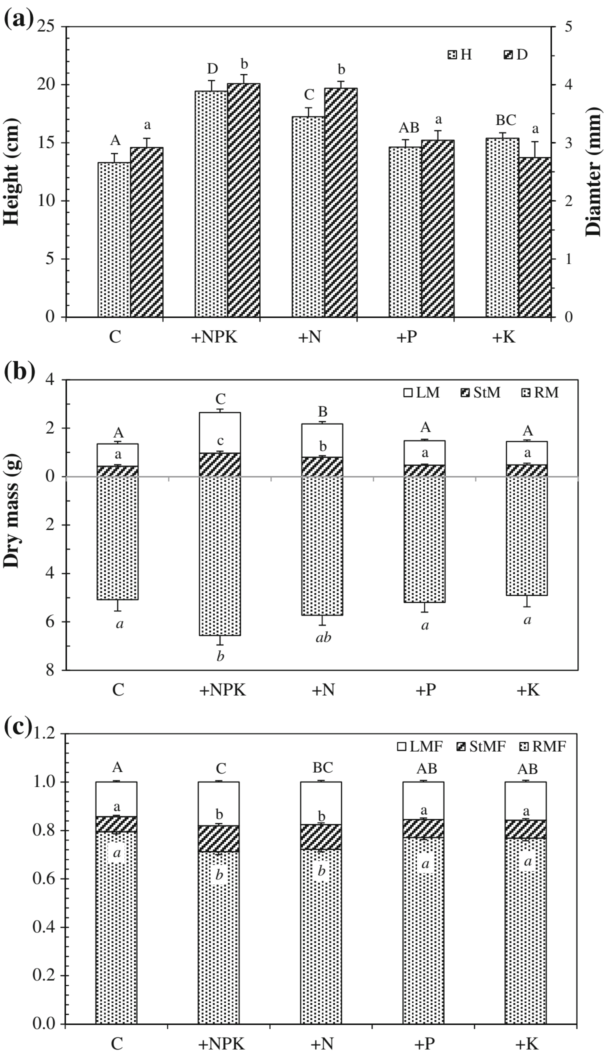

Fig. 2 Effect of late-season fertilization treatments on growth of Holm oak seedlings. **a** height and diameter, **b** *LM* leaf mass, *StM* stem mass, and *RM* root mass, **c** *LMF* leaf mass fraction, *StMF* stem mass fraction, and *RMF* root mass fraction. *Different letters* indicate significant differences ($p < 0.05$) (*capital letters* for *LM*, *LMF* and height, *lower-case letters* for *StM*, *StMF* and diameter, and *italicized lower-case letters* for *RM* and RMF)

at the beginning of fall (Oliet et al. 2011). On the other hand, when N, P and K were applied at proportional rates (+*NPK* treatment) growth, nutrient concentrations and content increased, in spite of N was applied at the same rates as in the +*N* treatment. This indicates that the proportion of N, P and K in the fertilizer could be more important than the total doses (Fernández et al. 2003; Oliet et al. 2013). As we stressed above P concentration is fostered by N fertilization in *Q. ilex* seedlings, but also N and K leaf concentration are affected by N-P-K rates (Andivia et al. 2011, 2012a), and similar positive effects of increasing N and K rates in the fertigation solution have been reported for others Mediterranean species, such as *Ceratonia siliqua* (Planelles 2004). Our results point out that lack of autumn fertilization limits plant growth and nutrient loading, at least under the conditions of our nursery. Fertilization with low nutrient doses during the late-season has been used for nursery culture of some Mediterranean species, and positive effects of this fertilization on field performance have been reported under arid environments, probably

Table 3 One-way ANOVA of the effect of late-season fertilization treatments on nutrient concentrations and contents of leaves (L), stems (St), and roots (R) of Holm oak seedlings

Parameter	Fraction	*F*	*P*
[N]	L	20.82	<0.001
	St	1.81	0.179
	R	1.96	0.152
[P]	L	2.99	0.145
	St	1.00	0.457
	R	1.07	0.405
[K]	L	1.74	0.202
	St	1.66	0.243
	R	0.59	0.677
[Mg]	L	4.76	0.014
	St	1.35	0.325
	R	0.70	0.604
[Fe]	L	2.12	0.136
	St	0.98	0.464
	R	0.12	0.974
N content	L	46.87	<0.001
	St	5.97	0.004
	R	1.12	0.384
P content	L	13.93	<0.001
	St	2.36	0.124
	R	0.62	0.655
K content	L	9.82	<0.001
	St	3.64	0.048
	R	0.16	0.956
Mg content	L	9.55	<0.001
	St	1.36	0.320
	R	0.36	0.832
Fe content	L	18.60	<0.001
	St	1.49	0.282
	R	0.13	0.971

due to the strong influence of decreased shoot:root ratio (Trubat et al. 2008, 2011). Shoot elongation of *Q. ilex* seedlings continues during October and November in nurseries that have mild autumns (Andivia et al. 2011). Thus, lack of fertilization or imbalanced fertilization during this important phase could lead to nutrient deficiency, dilution or luxury consumption, as indicated by our vector nomogram analysis (Fig. 3).

Larger and N-rich plants have greater root growth capacity (Mollá et al. 2006; Villar-Salvador et al. 2004a, 2012). This is even more important under Mediterranean conditions, where rapidly developing roots provide improved tolerance to summer water stress and survival (Palacios et al. 2009; Tsakaldimi et al. 2005; Vallejo et al. 2000). Although under arid conditions that limit root growth immediately after planting, a conservative water use strategy can be more important to promote survival, and small seedlings with low shoot:root ratios may achieve a greater success (Cortina et al. 2013; Oliet et al. 2013). Recently, a growing body of evidences indicates that larger and N-rich seedlings perform

Table 4 Effect of late-season fertilization treatments on nutrient concentrations of leaves, stems, and roots of Holm oak seedlings

Fraction	Treatment	N	P	K	Mg	Fe
Leaves	C	9.84 ± 0.50^{a}	0.95 ± 0.30	5.99 ± 2.36	2.78 ± 0.67^{a}	0.40 ± 0.11
	+NPK	13.21 ± 0.81^{b}	1.83 ± 0.28	11.65 ± 2.39	5.41 ± 0.62^{b}	0.64 ± 0.03
	+N	11.30 ± 0.21^{a}	1.16 ± 0.13	4.63 ± 1.93	2.56 ± 0.52^{a}	0.21 ± 0.05
	+P	9.66 ± 0.80^{a}	1.28 ± 0.29	7.56 ± 2.55	3.00 ± 0.42^{a}	0.45 ± 0.02
	+K	10.43 ± 1.24^{a}	1.10 ± 0.38	6.66 ± 3.11	3.26 ± 0.85^{ab}	0.51 ± 0.12
Stem	C	5.42 ± 0.31	0.79 ± 0.11	5.40 ± 0.40	1.78 ± 0.51	0.28 ± 0.10
	+NPK	4.96 ± 0.56	1.08 ± 0.17	3.82 ± 0.38	2.09 ± 0.55	0.31 ± 0.12
	+N	4.87 ± 0.86	0.80 ± 0.14	3.66 ± 0.52	2.35 ± 0.76	0.25 ± 0.12
	+P	3.71 ± 0.41	0.80 ± 0.07	4.36 ± 0.32	1.51 ± 0.47	0.14 ± 0.09
	+K	5.55 ± 0.37	1.04 ± 0.17	4.71 ± 0.44	3.01 ± 0.51	0.34 ± 0.10
Roots	C	4.30 ± 0.35	1.07 ± 0.17	6.97 ± 1.64	2.23 ± 0.59	0.34 ± 0.10
	+NPK	4.12 ± 0.24	0.92 ± 0.25	5.10 ± 1.47	2.07 ± 0.64	0.25 ± 0.15
	+N	4.24 ± 0.29	0.84 ± 0.13	5.05 ± 1.42	2.65 ± 0.61	0.26 ± 0.08
	+P	4.42 ± 0.45	1.08 ± 0.28	6.65 ± 2.27	2.87 ± 0.95	0.26 ± 0.10
	+K	5.33 ± 0.38	1.43 ± 0.24	7.50 ± 1.49	3.41 ± 0.14	0.34 ± 0.09

Values indicate means (mg g^{-1}) ± standard errors. Different letters show significant differences ($p < 0.05$) between late-season fertilization treatments for each nutrient and fraction

better in Mediterranean areas (Oliet et al. 2013; references therein). In this sense and based in our results, late-season fertilization is an efficient tool to promote growth and nutrient loading in *Q. ilex* seedlings. Nevertheless, late-season fertilization regime should be redefined to match plant attributes to environmental conditions of arid areas.

Water parameters derived from the pressure–volume curves were similar to those obtained in other surveys involving *Q. ilex* (Castro-Díez and Navarro 2007; Corcuera 2003; Corcuera et al. 2002; Villar-Salvador et al. 2004b). The physiological characterization of seedling at the end of the nursery period indicates that seedling subjected to late-season fertilization with higher N doses could have a higher drought tolerance capacity by decreasing both osmotic potentials (Ψ_{tlp} and Ψs_{sat}). Although there were not significant differences between fertilization treatments for ε_{max}, there was a negative relationship between ε_{max} and Ψs_{sat}. This relationship agrees with other studies that reported an association between high cell-wall rigidity (high ε_{max}) and low Ψ_{tlp} and Ψs_{sat} in xerophytic Mediterranean oaks (Corcuera et al. 2002; Villar-Salvador et al. 2004b). Plants with lower Ψ_{tlp} can maintain gas exchange and assimilation at lower water potential (Lambers et al. 2008) because protoplasm dehydration tolerance is enhanced by a lower Ψs_{sat} (Koide et al. 1989; Villar-Salvador et al. 2004b). Nevertheless, we found no differences for S_{tlp}, so these differences in osmotic adjustment were a consequence of the higher solute accumulation of nutrient loaded plants during the slow growing period in the fall hardening phase (Fernández et al. 2008; Mollá et al. 2006; Villar-Salvador et al. 2004b). Osmotic adjustment did not occur under water stress, but may be associated with ontogenetic changes and/or cold hardening (Bigras et al. 2001). Late-season fertilization improves cold tolerance in *Q. ilex* nursery seedlings (Andivia et al. 2011, 2012b). Considering the physiological differences, we expect that seedlings in the +*N* and +*NPK* groups would be more tolerant to frost and water stress immediately after planting, because osmotic adjustment and solute accumulation are involved in tolerance to these stressors (Savé et al. 1999; Tognetti et al. 1998).

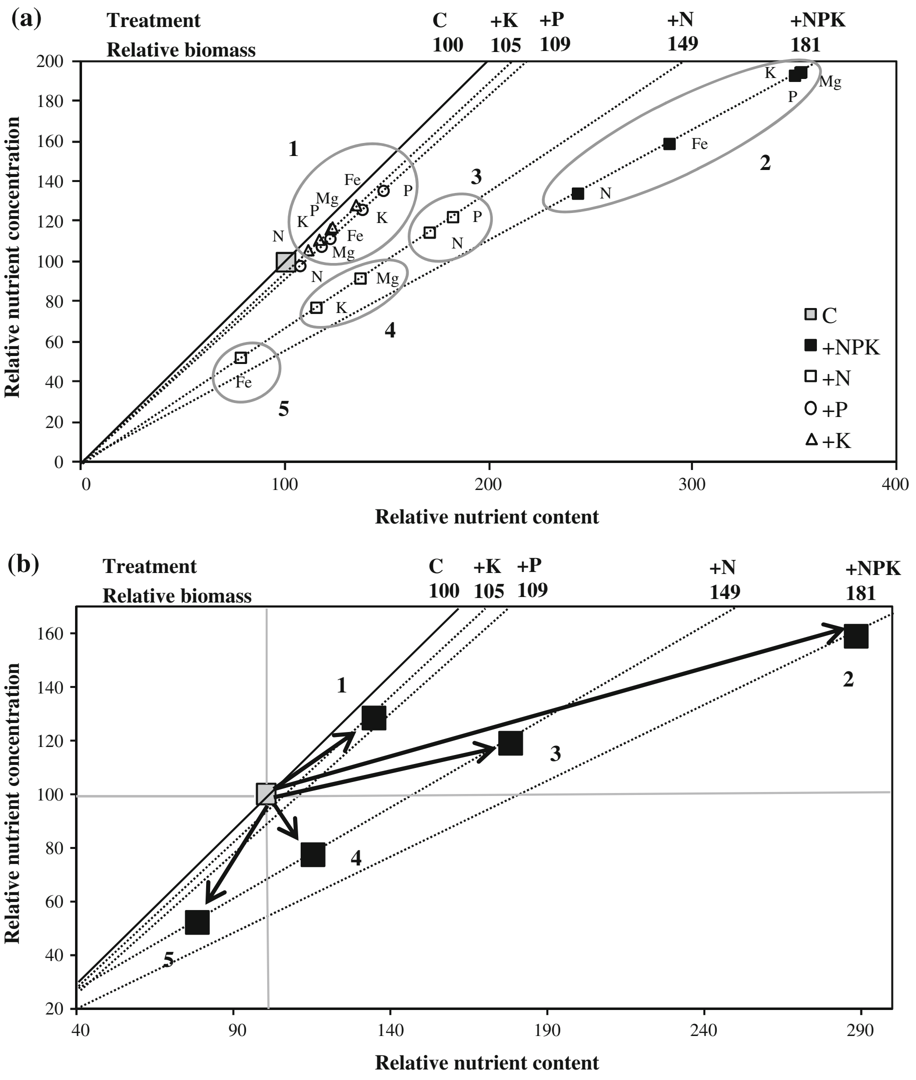

Fig. 3 Graphical analysis (**a**) and vector nomogram (**b**) of relative changes in leaf dry mass (*diagonal lines*), nutrient content (*x*-axis), and nutrient concentration (*y*-axis) of Holm oak seedlings given different fertilization treatments. Seedlings in the *C* group (*grey square*) were used as reference (100 value for leaf dry mass, nutrient content, and nutrient concentration). Nutrients in *circles* (**a**) are schematically represented in **b** (*bottom*) with the same numbers. The nutritional response is characterized by the vector direction and magnitude (**b**)

The lack of differences in RWC_{tlp} among fertilization treatments might be due to, at the time of measuring, plants had only suffered a moderate cold period, they had not suffered any water stress, and any fertilization treatment did not show severe nutrient deficiency. Therefore, further studies should evaluate water relations under non-optimum environmental conditions in order to better assess seedling performance attributes and potential (Folk and Grossnickle 1997). Nevertheless, seedlings in the +*N* and +*NPK* groups had greater capacity to maintain open stomata and cell turgor at lower water potentials (lower

Table 5 Effect of late-season fertilization treatments on water relations parameters of Holm oak seedlings

Parameter	C	+NPK	+N	+P	+K
Ψ_{tlp} (MPa)	-2.77 ± 0.02^{a}	-3.07 ± 0.07^{b}	-3.00 ± 0.08^{b}	-2.88 ± 0.14^{ab}	-2.67 ± 0.09^{a}
Ψs_{sat} (MPa)	-1.54 ± 0.17^{a}	-2.03 ± 0.09^{b}	-2.11 ± 0.08^{b}	-1.86 ± 0.06^{a}	-1.58 ± 0.15^{a}
RWC_{tlp} (%)	91.97 ± 1.42	93.29 ± 0.51	93.92 ± 0.70	91.58 ± 2.61	89.65 ± 3.27
S_{tlp} (%)	17.84 ± 1.96	19.82 ± 2.13	20.58 ± 0.48	24.34 ± 7.65	24.16 ± 4.95
ε_{max} (MPa)	19.93 ± 5.38	28.91 ± 5.83	34.59 ± 6.26	23.91 ± 6.59	20.72 ± 7.20

Values indicate means (mg g^{-1}) ± standard errors. Different letters indicate significant differences ($p < 0.05$) between late-season fertilization treatments

Ψ_{tlp} Osmotic potential at turgor loss point, Ψs_{sat} osmotic potential at saturation, RWC_{tlp} relative water content at the turgor loss point, S_{tlp} symplasm volume fraction at the turgor loss point, ε_{max} maximum bulk elasticity modulus

Ψ_{tlp}). This indicates that their photosynthetic capacity and carbon gain could be maintained under conditions of high water stress, and might foresee a greater root growth after planting and improved field performance (Cuesta et al. 2010; Villar-Salvador et al. 2012).

Conclusions

Our results indicate that late-season NPK fertilization of Holm oak seedlings increases shoot and root growth, biomass partitioning to the leaves and stem, and the overall nutritional status. Vector nomogram analysis pointed out that lack of late-season fertilization may lead to nutrient deficiency, whilst the application of imbalanced fertilization treatments might trigger nutrient dilution and luxury consumption. However these effects may depend on nursery conditions during the autumn and winter, so the fertilization treatments described here may require modification for nurseries with colder temperatures.

Our results also indicate that late-season N fertilization improves the drought tolerance and osmotic adjustment of seedlings, although further studies should be conducted to corroborate the better performance of these improved seedlings under stressful environmental conditions.

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