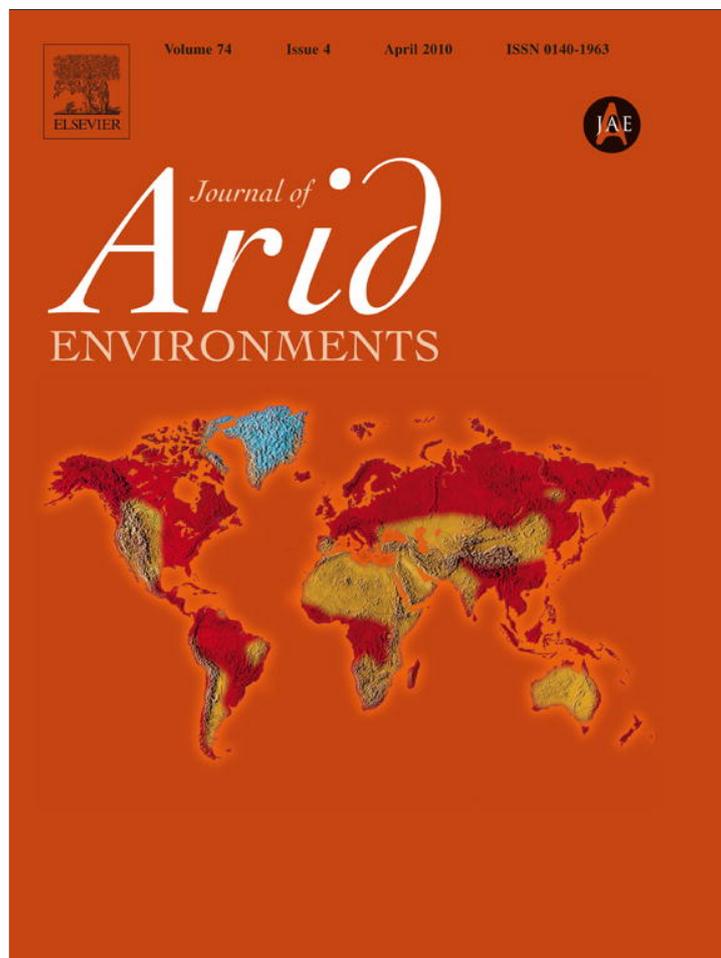


Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

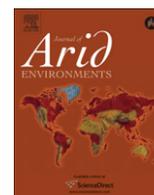
In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

Journal of Arid Environments

journal homepage: www.elsevier.com/locate/jaridenv

Nursery fertilization affects seedling traits but not field performance in *Quercus suber* L.

R. Trubat^{a,*}, J. Cortina^a, A. Vilagrosa^b^a Departament d'Ecologia and Multidisciplinary Institute for Environmental Studies, Universitat d'Alacant, Ap. 99, 03080 Alacant, Spain^b Fundación Centro de Estudios Ambientales del Mediterráneo (CEAM), Parque Tecnológico, C/Charles Darwin, 46980 Paterna, Spain

ARTICLE INFO

Article history:

Received 23 October 2008

Received in revised form

6 October 2009

Accepted 8 October 2009

Available online 4 November 2009

Keywords:

Functional attributes

Nutrient deprivation

Root growth potential

Seedling establishment

Seedling quality

ABSTRACT

The success of forest plantations in areas subjected to seasonal drought is strongly dependent on seedling traits. However, there is no consensus on the set of plant traits determining establishment success in these environments. We hypothesize that traits associated with nutritional stress, such as reduced biomass allocation and changes in root morphology, may promote seedling resistance to drought. We tested this hypothesis by assessing the effect of different fertilizer types and doses on the morphology and field survival of *Q. suber* seedlings. This hypothesis had been previously tested with five Mediterranean species in a semi-arid environment. Nutrient deprivation decreased total biomass accumulation and slenderness ratio (ratio between stem height and root collar diameter). Nitrogen deprivation increased biomass allocation and decreased root growth potential. Short-term seedling survival was not related to nutritional regimes, seedling size or root growth potential. Differences in stem height between control seedlings and those subjected to nutrient manipulations increased in the field as a result of shoot dieback and scarce growth. In contrast, differences in root collar diameter gradually vanished. Nutrient management in the nursery showed a strong potential for modifying the morphology of *Q. suber* seedlings but the relationship between these changes and seedling survival remains elusive.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

The establishment of forest plantations in areas subjected to seasonal drought is strongly limited by water availability (Alloza and Vallejo, 1999; Padilla and Pugnaire 2007; Pérez-Devesa et al., 2008). When irrigation is not feasible, plantation success can be greatly dependent upon seedling morpho-functional attributes. However, there is no consensus on the set of plant traits that determines establishment success in water-limited environments, or on the nursery methodologies needed to achieve these traits (Cortina et al., 2006). Several studies have shown that seedling survival under Mediterranean conditions can be positively affected by nursery practices that enhance seedling vigor (Oliet et al., 1997, 2004; Villar-Salvador et al., 2004) and drought resistance (Vilagrosa et al., 2003). In contrast, other studies suggest that nutrient-deficient seedlings may be better prepared to withstand transplant shock and summer drought (Seva et al., 2000; Trubat et al., 2004). This apparent disagreement may be the result of the differing

effects of varying intensities of summer drought, although evidence is inconclusive (Navarro et al., 2006).

Nursery cultivation regimes can strongly determine the functional characteristics of seedlings and their field performance (Burdett, 1990; Vilagrosa et al., 2003), affecting post-transplant rooting and early growth capacity (Landis, 1985; van den Driessche, 1984). Fertilization can be particularly relevant in Mediterranean areas, since nutrients ultimately influence seedling resistance to drought (van den Driessche, 1992). Fertilization may increase drought tolerance in several ways. For example, root growth potential and root hydraulic conductance increase with P and N availability (Singh and Sale, 2000; Trubat et al., 2006), and thus the ability to capture soil water may be enhanced in fertilized seedlings (Reinbott and Blevins, 1999). This can be crucial in degraded areas showing low soil fertility (Valdecantos et al., 2006). Nevertheless, N and P deficient plants commonly change their biomass accumulation and allocation patterns (Poorter and Nagel, 2000; Rubio et al., 2003), which may result in a decreased demand for water and a greater ability to endure drought. Nutrient deficiency may also enhance the accumulation of non-structural carbohydrates (Oliet et al., 2006), which may act as energy reserves and osmolites allowing seedlings to withstand transplant shock (Burdett, 1990).

* Corresponding author. Tel.: +34 96 590 95 64; fax: +34 96 590 36-25.
E-mail address: roman.trubat@ua.es (R. Trubat).

Seedling response to contrasting nutrient availability can be highly species-specific (Rubio et al., 2003). *Quercus suber* L. (cork oak), the mainstay of certain multiple-use agroforestry systems with great socio-economic and environmental value in the western Mediterranean region, seems to be highly responsive to changes in growing conditions (Chirino et al., 2008; Trubat et al., 2008), and thus it represents a suitable species for studying the relationship between seedling traits and field establishment. *Quercus suber* seedlings are especially prone to allocate large amounts of biomass aboveground and develop high slenderness ratios (Almeida et al., 2009). Thus, an excess nutrient supply could have deleterious effects on the ability of *Q. suber* to withstand drought and establish under field conditions. Despite evidence of nutrient limitations in Mediterranean areas (Valdecantos et al., 2006), little information is currently available on the nutrient status of *Q. suber* seedlings or on its potential implications for afforestation programmes.

We carried out a greenhouse and field experiment to explore the relationship between seedling morpho-functional traits and field performance in *Q. suber* seedlings. Our hypotheses are: (1) *Quercus suber* morphology is very sensitive to changes in nutrient availability during the nursery phase, and different fertilized seedlings may develop morpho-functional changes that improve their capacity to withstand harsh conditions despite the decrease in nutrient status; (2) nutritional hardening will affect plant morphology and nutritional status by reducing seedling size, increasing biomass allocation to belowground parts, and reducing the N-to-P ratio in leaves.

2. Materials and methods

2.1. Plant growth and experimental design

Quercus suber seedlings were grown from selected seeds in a public nursery (Santa Faç, Alicante, SE Spain; 38° 23' N, 0° 26' E, 50 m a.s.l.), under semi-arid climate (annual precipitation and average temperature of 353 mm and 18 °C, respectively). Seedlings were grown from February 2003 to February 2004 in 305-cm³ forest containers (Super-leach®) filled with a mixture of peat and coco-peat (1:1). Acorns were collected from local provenance in the Valencia region (E. Spain) by the Regional Forest Service (Generalitat Valenciana, Forest Seed Bank). Seedlings were cultivated in the open air and abundantly watered on alternate days with a modified Hoagland's solution containing 150 mg N L⁻¹ (as Ca(NO₃)₂ and KNO₃), 80 mg P L⁻¹ (as KH₂PO₄), and 100 mg K L⁻¹ (control), or similar solutions containing either no N (nitrogen-deficient seedlings, hereafter ND), or no P (phosphorus-deficient, hereafter PD). In ND and PD seedlings, the osmolarity of the nutrient solution was adjusted with KCl (Radin, 1984). Another set of seedlings was grown by adding 1.8 g L⁻¹ growing substrate of a commercial 14-9-15 (N:P:K) slow-release fertilizer, (Peters Professional™ Fertilizer; hereafter SRF).

In November 2003, an additional fertilization level was generated by imposing a short-duration N stress to half of the control seedlings, selected randomly. Nitrogen was excluded from the nutrient solution, while the supply rate of the other nutrients was kept constant (nutritional hardening treatment, hereafter NH). Nitrogen deprivation lasted from November 2003 to February 2004. Average temperature was 14.3 °C during the last phase of nursery culture (February 2004). Each treatment was replicated 39 times, as the total number of seedlings available for the experiment was 195.

2.2. Morphological, nutritional characteristics, and chlorophyll and carbohydrates determination

Measurements of stem height, root collar diameter (RCD), leaf area, belowground and aboveground biomass accumulation, and foliar nutrient content were carried out at the end of the nursery period (February 2004). Measurements were taken in 10 randomly selected seedlings per treatment. Leaf area was measured by scanning the leaves (EPSON Expression 1680 Pro scanner with transparency adapter) and analyzing the image by means of specific image analysis software (WinRhizo, Régent Instruments Inc., Québec, Canada). All biomass fractions were measured after drying at 65 °C for 48 h. The root weight ratio (RWR, g g⁻¹) was calculated as the ratio between root dry weight and total dry weight. The slenderness ratio was estimated as the ratio between stem height and root collar diameter. Specific root length (SRL, cm g⁻¹) was the ratio between root length and root dry weight. Dry leaves were fragmented by hand and digested in a heating block at 250 °C with a mixture of H₂SO₄ and H₂O₂ (1:1, v/v) for nutritional analyses. Total N concentration was determined by semi-micro Kjeldahl distillation (Tecator Kjeltex Auto 1030 Analyzer, Hogana, Sweden), and P and K concentration by ICP spectrometry (Perkin Elmer Optima 3000, Perkin Elmer Corp., Norwalk, CT, USA). A 130-mm² disk was cut from each leaf for pigment analysis. Each leaf disk was extracted for 24 h in the dark at 25 °C with dimethyl sulfoxide after which absorbance was measured and concentrations of chlorophyll *a* and chlorophyll *b* were computed using the equations of Lichtenthaler (1987). Samples of leaves were collected for non-structural carbohydrate determination in 5 seedlings per treatment. Fresh material was immediately frozen in liquid nitrogen and stored at -80 °C until analysis. Soluble sugars were extracted in ethanol (70%, v/v), and the residue was incubated in HCl (1.1%, v/v) for 30 min at 95 °C for starch extraction. Both soluble sugars and starch were determined colorimetrically at 635 nm with anthrone reagent.

2.3. Planting and field measurements

In February 2004, one 30 × 30-m plot was established in a previously cleared degraded shrubland in Serra Espadà (Chóvar, Castelló, E Spain; 38°91', 0°41'E, 540 m a.s.l.; 40° slope; 220° SW aspect). Twenty randomly selected seedlings per treatment were planted in 40 × 40 × 40-cm planting holes. The climate at the nearest weather station (Segorb HS meteorological station, Segorb, Castelló) is dry sub-humid thermo-Mediterranean, average annual rainfall is 485 mm and average temperature is 15.8 °C. Seedling survival, stem height, and RCD were monitored in all seedlings in May and December 2005, and in June 2006.

2.4. Root growth potential test

Root growth potential (RGP) was measured on 9 seedlings per treatment in March 2004. Seedlings were transplanted to 3-L PVC pots filled with vermiculite, and watered with a standard nutrient solution every 2 days. The growth chamber provided day conditions of 20 °C for 16 h with a photosynthetic photon flux density of 400 μmol m⁻² s⁻¹. The number of newly elongated white roots longer than 5 mm was counted on each seedling at the end of a 21-day period. As both variables, new roots and seedling size, were highly correlated (Pearson bivariate $r = 0.791$, $N = 45$, $p < 0.001$), here we only present the results for new white roots.

2.5. Statistical analyses

We used one-way analysis of variance (ANOVA) with treatments as a fixed factor and 5 levels to evaluate their effect on seedling

Table 1

Foliar nutrient concentrations (mg g^{-1}) of *Quercus suber* seedlings grown under standard nutritional conditions (Control), under late-season N deprivation (N), under a nitrogen-deficient solution (ND), under a phosphorus-deficient solution (PD), and with slow-release fertilizer (SRF).

	Control	NH	ND	PD	SRF	F	p <
N (mg g^{-1})	12.9 ± 0.5a	11.1 ± 0.2c	5.7 ± 0.4d	11.5 ± 0.3b	11.3 ± 0.5b	37.7	0.01
P (mg g^{-1})	0.66 ± 0.03a	0.72 ± 0.02a	0.78 ± 0.05a	0.30 ± 0.01b	0.70 ± 0.02a	4.8	0.01
K (mg g^{-1})	3.31 ± 0.08a	2.95 ± 0.04a	4.91 ± 0.10b	3.24 ± 0.14a	2.57 ± 0.09a	10.7	0.01

Data are means ± SE ($N = 10$ plants). Different letters indicate significant differences at $p < 0.05$.

morphology at the end of the nursery phase, and after planting. When these analyses showed a significant treatment effect, we carried out pairwise post-hoc comparisons using Tukey's HSD test at $p = 0.05$. As heterogeneous first summer mortality resulted in an unbalanced design, we randomly selected 6 seedlings per treatment for post-summer seedling size analyses. RGP was log-transformed to meet requirements of normality and homoscedasticity. Analyses of survival frequency data were based on Pearson's χ^2 tests. We used Pearson correlation analysis to evaluate the degree of covariation between root growth potential and seedling size. The relationship between seedling size and summer mortality was evaluated using logistic regression. All statistical analyses were performed with the SPSS 9.0 package (SPSS, Chicago, IL).

3. Results

3.1. Nursery measurements

Foliar concentrations of P and N were greatly reduced in seedlings without P or N in the nutrient solution (Table 1). Foliar P concentration was above 0.66 mg g^{-1} in control seedlings and decreased to 0.3 mg g^{-1} in PD seedlings. Nitrogen concentration ranged from 12.9 mg g^{-1} in control seedlings to less than 5.7 mg g^{-1} in ND seedlings. Potassium concentration was higher in ND plants than in other seedlings. NH resulted in a significant but weak decrease in foliar N concentration as compared to control seedlings. Seedlings from NH and ND presented significantly lower chlorophyll contents (both chl. *a* and chl. *b*) (Table 2). Nonstructural carbohydrate concentrations were decreased in NH and ND treatments (Table 2).

Seedlings receiving slow-release fertilizer showed higher stem height and root collar diameter than other treatments, while ND and PD seedlings showed an important decrease with respect to the other treatments (Table 3). NH resulted in a trend towards lower stem height and root collar diameter, but the differences were not statistically significant compared to the control treatment. The proportion of dry weight allocated below- and aboveground was significantly affected by the nutritional regimes; ND, NP and NH showed significant differences in the multiple mean comparisons.

Table 2

Non-structural carbohydrate and chlorophyll content in the leaves of *Quercus suber* seedlings grown under standard nutritional conditions (Control), under late-season N deprivation (N), under a nitrogen-deficient solution (ND), under a phosphorus-deficient solution (PD), and with slow-release fertilizer (SRF).

	TNC ($\mu\text{mol g}^{-1}$)	Chlorophyll content ($\mu\text{g cm}^{-2}$)
Control	10.16 ± 0.09b	51.6 ± 3.62c
NH	10.06 ± 0.01ab	36.3 ± 2.64ab
ND	10.01 ± 0.06a	21.1 ± 2.72a
PD	10.27 ± 0.05b	49.5 ± 3.38bc
SRF	10.17 ± 0.02b	30.1 ± 3.21abc
F	3.64	6.13
p	0.029	0.002

Data are means ± SE ($N = 5$ plants). Different letters indicate significant differences at $p < 0.05$.

Both the number of leaves per plant and the leaf area were lower in the ND, NH, and NP treatments than in the control treatment. ND and NP showed a significant increase in SRL as compared to control and SRF seedlings. SRF seedlings showed an important increase in SLA with respect to control seedlings, while ND seedlings showed a significant decrease compared to the other treatments.

The slenderness index was significantly lower in ND seedlings, followed by PD and NH seedlings ($F_{4,45} = 24.0$, $p < 0.001$; Fig. 1). Control and SRF seedlings showed the highest slenderness ratio.

Only ND seedlings differed from the other treatments in the RGP test ($F_{4,40} = 13.2$, $p < 0.001$; Fig. 2). RGP was positively related to seedling size ($r = 0.507$, $N = 45$, $p < 0.001$, and $r = 0.724$, $N = 45$, $p < 0.001$ for the comparisons between RGP and root collar diameter and stem height, respectively).

3.2. Field measurements

Rainfall was especially high after planting (Fig. 3). Field survival was high before the summer (>95%) and was not affected by the treatments (data not shown). Post-summer survival, October 2004, was highest for the seedlings receiving slow-release fertilizer, and lowest for the NH seedlings, but the overall effect of the nutritional regimes was statistically marginal (Pearson's $\chi^2 = 8.0$, $p = 0.098$, power = 7.88; Fig. 4). Differences in survival rates between control, and ND and PD seedlings were small, despite substantial differences in their morphology and nutrient content. Differences in survival rates between the different treatments were reduced in spring 2006, 16 months after planting (Pearson's $\chi^2 = 4.2$, $p = 0.411$).

Aboveground growth was very low before and after the first summer. Moreover, some treatments (NH, PD, SRF) showed some degree of shoot dieback as reflected in a decrease in stem height that was particularly patent during the first summer (Table 4). Treatments had a significant effect on stem height and root collar diameter before the summer ($F_{4,91} = 22.8$, $p < 0.001$, and $F_{4,91} = 8.3$, $p < 0.001$, respectively), and on stem height, but not on root collar diameter, after the summer ($F_{4,25} = 4.9$, $p = 0.005$, and $F_{4,25} = 1.6$, $p = 0.206$). Size differences among treatments were maintained before the summer, with ND seedlings showing the lowest stem height and root collar diameter, and control and SRF seedlings showing the highest stem height. Differences were less evident after the first summer, when only the stem height of control seedlings was significantly higher than those of the other treatments. We should note, however, that the power of the analysis decreased after the summer as a result of seedling mortality.

4. Discussion

Foliar N concentration in seedlings receiving no N was below the levels that have been commonly associated with N deficiency for other Mediterranean forest species (Bonneau, 1988; Robert et al., 1996; Trubat et al., 2006). Similarly, foliar P levels were very low in seedlings receiving no P, and these seedlings were likely to be P deficient (Cornelissen et al., 1997; Oliveira et al., 1996). Foliar potassium concentration was low in all seedlings except those

Table 3
Morphological characteristics of *Quercus suber* seedlings grown under standard nutritional conditions (Control), under late-season N deprivation (N), under a nitrogen-deficient solution (ND), under a phosphorus-deficient solution (PD), and with slow-release fertilizer (SRF).

	Control	NH	ND	PD	SRF	F	p
Height (cm)	31.52 ± 0.38ab	28.08 ± 0.91bc	11.14 ± 0.14d	26.52 ± 0.19c	35.94 ± 0.53b	65.17	<0.001
RCD (mm)	5.83 ± 0.11ab	5.52 ± 0.06ab	3.64 ± 0.06c	4.99 ± 0.11b	6.18 ± 0.14a	17.37	<0.001
Root dry weight (g)	4.90 ± 0.37a	3.22 ± 0.45b	3.17 ± 0.47b	2.71 ± 0.46b	5.06 ± 0.54a	5.56	0.01
Shoot dry weight (g)	1.38 ± 0.09a	1.03 ± 0.09b	0.49 ± 0.09d	0.76 ± 0.07c	1.58 ± 0.12a	26.01	<0.001
Leaf area (cm ²)	116.81 ± 4.80c	85.42 ± 6.12b	38.12 ± 3.46a	61.44 ± 3.51ab	121.16 ± 5.62c	27.39	<0.001
No. leaves	59.4 ± 6.24c	44.1 ± 3.06b	15.2 ± 1.29a	16.2 ± 1.67a	70.2 ± 6.76c	32.66	<0.001
RWR (g g ⁻¹)	0.64 ± 0.02b	0.62 ± 0.02b	0.82 ± 0.01a	0.64 ± 0.03b	0.59 ± 0.02b	16.62	<0.001
SLA (cm ² g ⁻¹)	88.42 ± 1.21bc	84.931 ± 72a	83.51 ± 1.13ab	82.63 ± 1.24ab	92.17 ± 2.07c	7.02	<0.001
SRL (cm ² mg ⁻¹)	2.31 ± 0.24	2.15 ± 0.29	3.91 ± 0.359	2.86 ± 0.31	2.35 ± 0.16	1.94	0.134

Data are means ± SE (N = 10 plants). Different letters indicate significant differences at p < 0.05.

receiving no N. This fact probably resulted from luxury consumption (Marschner, 1995). Both ND and PD seedlings were small, but the effect of nutrient deficiency was much stronger in ND seedlings, which may not have been suitable for commercial purposes (RD 1356/1998 for 26 June 1998; BOE 153). Our results suggest that *Q. suber* acorns cannot supply seedlings with enough N and P to sustain growth for 1 year, and these results are consistent with N and P budget estimations in acorns and seedlings. Acorns may contain substantial amounts of P (Gea-Izquierdo et al., 2006). Scarce information is available on the N content of *Q. suber* acorns, but J. Oliet (University of Córdoba, 2007; personal communication) showed that N concentration in *Quercus ilex* acorns was 0.95 mg g⁻¹. Considering an average *Q. suber* acorn dry weight of 3.3 g for this area (M. Pérez-Devesa, CEAM Foundation personal communication), acorns could supply 9.5 mg g⁻¹ of the 1st year requirements for N and P. The same observation has been reported for other oaks (García-Cebrián et al., 2003; Newton and Pigott 1991). The effect of NH on the nutritional status was weak. Slow-release fertilizer produced seedlings with N, P and K concentrations similar to control seedlings. In addition, seedling biomass was similar in control and SRF seedlings, suggesting either that the seedlings subjected to both treatments were not nutrient-limited, or that the increased nutrient availability resulting from SRF application had vanished by the end of the nursery period. Different fertilization regimes significantly affected TNC status in the leaves. Nutrient limitation generally promotes carbohydrate accumulation in leaves of actively growing plants (Bollmark et al., 1999; Sanz-Pérez et al., 2007; Waring et al., 1985). Several studies have reported that during the growing season the starch concentration was lower in

current foliage from high fertility sites than from low fertility sites. According to these findings, the relationship between starch and nitrogen concentrations may be positive during the dormant season and negative during the growing season (Birk and Matson, 1986; Ludovici et al., 2002). In our study, the nitrogen-deficient treatments (ND and NH) decreased the non-structural carbohydrate concentration in leaves. When growth declined in autumn, the larger leaf area and possibly higher photosynthetic capacity allowed the control, NP, and SRF seedlings to accumulate TNC faster than the NH and ND seedlings. The generally high chlorophyll content observed in all but the ND treatments was due to nitrogen-supply availability. Several studies indicate that N fertilization increases leaf area and chlorophyll content under optimum conditions (Kathju et al., 2001).

ND, NH, and PD resulted in a decrease in the number of leaves and the whole plant leaf area. Reductions in leaf area in nutrient-deficient plants have been observed elsewhere (Trubat et al., 2008; Yates et al., 2002) and may result in a decrease in the overall plant transpiration rate, which would enhance a drought-avoidance strategy (Nielsen and Orcutt, 1996), improving water use efficiency under drought (Miller and Stoner, 1979). NH, ND, and NP also showed a significant reduction in SLA. These variations can be the consequence of several anatomical changes, such as a reduction in cell diameter, an increase in cell wall thickness, an increase in the proportion of dense tissues, and an increase in cuticular waxes (Castro-Díez et al., 1997).

Both aboveground and belowground accumulations were reduced by low nutrient availability. This effect has been widely described in the literature (Rubio et al., 2003). Root weight ratio followed the expected direct relationship with nutrient supply (Poorter and Nagel, 2000). An increase in RWR is predicted to be

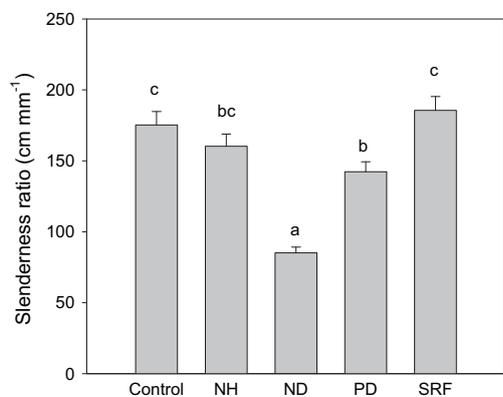


Fig. 1. Slenderness ratio of *Quercus suber* seedlings grown under standard nutritional conditions (Control), under late-season N deprivation (N), under a nitrogen-deficient solution (ND), under a phosphorus-deficient solution (PD), and with slow-release fertilizer (SRF). Data are means ± SE (N = 10 plants). Different letters indicate significant differences at p < 0.05.

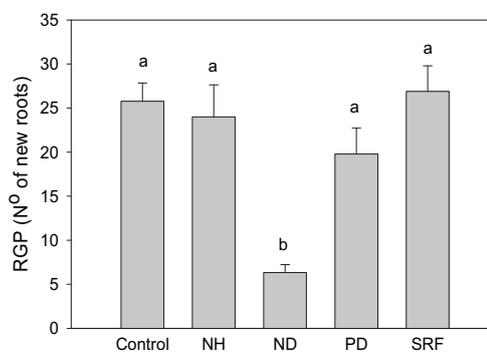


Fig. 2. Root growth potential (RGP) of *Quercus suber* seedlings grown under standard nutritional conditions (Control), under late-season N deprivation (N), under a nitrogen-deficient solution (ND), under a phosphorus-deficient solution (PD), and with slow-release fertilizer (SRF). Data are means ± SE (N = 10 plants). Different letters indicate significant differences at p < 0.05.

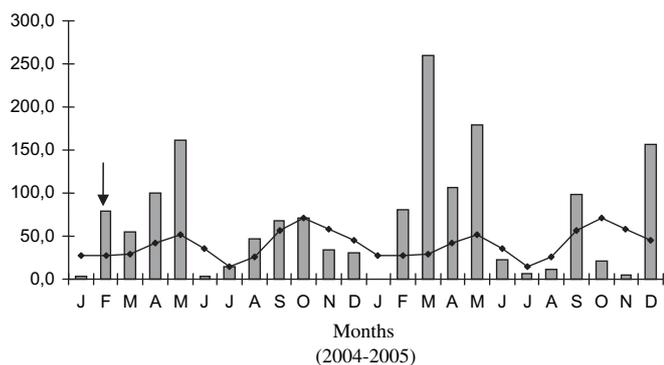


Fig. 3. Survival (%) of *Quercus suber* seedlings grown under standard nutritional conditions (Control), under late-season N deprivation (N), under a nitrogen-deficient solution (ND), under a phosphorus-deficient solution (PD), and with slow-release fertilizer (SRF), 8 months (black bar) and 16 months (white bar) after outplanting in a degraded shrubland.

a better strategy for maintaining growth under water-limiting conditions, because it can increase water and nutrient absorption and return carbon and nutrient contents to a more favorable balance for storage (Vilela et al., 2003). ND and NP treatments showed a significant increase in specific root length, a trait that has been related to increased exploitation and uptake efficiency (Comas and Eissenstat, 2002; Fitter, 1991).

Seedling survival was high before the summer, 4 months after planting (>95% in all treatments; data not shown). This is common in Mediterranean forest plantations and suggests that transplant shock may not be a major source of mortality in well-implemented plantations (Cortina et al., 2004; Maestre et al., 2003). The range of seedling survival rates observed after the first summer was similar to those found in other *Q. suber* plantations, except in NH seedlings, which was very low (Cortina et al., 2009). Seedling survival was not related to seedling size and morphology at the treatment level. For example, the correlation between seedling dry weight in the nursery and post-summer survival was not significant when considering all planted seedlings ($r = 0.525$, $N = 5$, $p = 0.363$), nor at the individual seedling level (Nagelkerke pseudo- $R^2 = 0.004$, $\chi^2 = 0.254$, $p = 0.614$ for the logistic regression between pre-summer stem height and summer mortality). Indeed, the survival rate of ND seedlings (50%) was similar to the rate shown by control seedlings (55%) in May 2005, despite substantial differences in size and nutritional status. In contrast, in control and NH seedlings, which showed small differences in size and foliar nutrient concentration, survival rates differed by almost 50%. Field survival

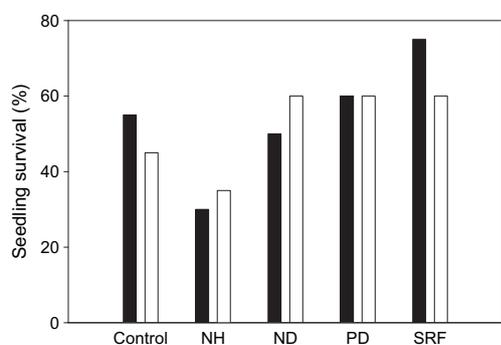


Fig. 4. Monthly precipitation between January 2004 and December 2005 at the weather station nearest to the experimental plot (Segorb HS, Castelló). The line indicates the average rainfall for the last 30 years. The arrow indicates the month of planting.

Table 4

Root collar diameter (mm) and stem height (cm) of *Quercus suber* seedlings grown under standard nutritional conditions (Control), under late-season N deprivation (N), under a nitrogen-deficient solution (ND), under a phosphorus-deficient solution (PD), and with slow-release fertilizer (SRF), planted in a degraded shrubland.

	RCD, May 2004 (mm)	RCD, October 2004, (mm)	Height, May 2005 (cm)	Height, October 2005 (cm)
Control	4.7 ± 0.3a	5.5 ± 0.4a	31.3 ± 2.2a	31.1 ± 3.2a
NH	4.2 ± 0.2a	4.3 ± 0.8a	23.0 ± 1.5b	13.7 ± 3.1b
ND	3.1 ± 0.2b	3.0 ± 0.3a	11.6 ± 1.0c	10.8 ± 2.2b
PD	4.2 ± 0.2a	4.3 ± 0.4a	24.8 ± 1.6b	10.0 ± 2.8b
SRF	4.6 ± 0.2a	4.6 ± 0.2a	28.1 ± 1.6ab	17.5 ± 3.1b

Data are means and standard error of $N = 17$ – 20 seedlings per treatment (05/2004) and 15 seedlings per treatment (10/2004). Different letters indicate significant differences for a given sampling date (Tukey HSD, $p < 0.05$).

was not related to the slenderness ratio, which was positively related to seedling size, nor to the proportion of biomass allocated belowground, which was higher only in ND seedlings. These results suggest that size and morphology are not strong indicators of field performance in this species. These results are thus in disagreement with those reported by Seva et al. (2000) and Villar-Salvador et al. (2004), who found that bigger seedlings of Mediterranean trees planted in dry sub-humid areas performed better in the field than smaller ones. In a recent review on this topic, Navarro et al. (2006) found that the frequency of studies showing no significant effect of initial seedling size on field survival was higher than the frequency of studies showing either a positive or a negative effect. These contrasting results are not clearly related to differences in drought intensity (Navarro et al., 2006) and may reflect complex species × environment interactions.

The negative results of late-season NH were unexpected. Suppressing N during late stages of nursery growth resulted in small changes in foliar nutrient concentrations and stem height, but it generated a strong reduction in aboveground biomass accumulation. This suggests that, under the mild climatic conditions in the nursery, growth was not completely arrested during winter in well fertilized seedlings. After outplanting (May 2004), stem height gradually decreased in NH seedlings, as reflected in the significant differences between NH and control seedlings which appeared even before the onset of summer drought. Thus, results suggest that NH seedlings failed to take advantage of the relatively favorable climatic conditions present in spring, and were unable to withstand summer drought. This result is particularly puzzling, as morphophysiological changes in NH seedlings were less severe than changes experimented by ND and PD seedlings. In addition, in a previous study we found that late-season N deprivation had a positive effect on field performance of 5 Mediterranean woody species planted in a semi-arid area (Trubat et al., 2008). In this study, nutritional hardening resulted in a wide range of reductions in seedling size, suggesting that this trait may not be entirely responsible for the contrasted results obtained in both studies. Late-season N deprivation has been recommended as it may arrest late-season growth, promote carbohydrate storage, foster the accumulation of other essential nutrients, and improve seedling establishment (Tan and Hogan, 1997). However, our results show that this technique may have a negative impact on *Q. suber* seedling performance in the field, suggesting that further studies are needed before this technique can be incorporated into nursery protocols for this species.

RGP may be a suitable indicator of field performance, as the formation of new roots is critical for seedling establishment (Kaushal and Aussenac, 1989). RGP in *Q. suber* seedlings was well related to seedling size, and probably to short-term vitality, but not to field performance (Pearson correlation between RGP and field

survival $r = 0.196$, $N = 5$, $p = 0.752$). Thus, this technique cannot be recommended as an indicator of field performance for this species. This is somewhat surprising since seedling initial response and, particularly, seedling rooting ability are crucial for ensuring a summer water supply and successful establishment under Mediterranean conditions (Fonseca, 1999; Reader et al., 1993). According to Simpson and Ritchie (1997), the ability of seedling vigor to predict field performance may be low when planting site conditions are mild, or when they are harsh but seedlings are highly resistant to stress.

Interestingly, the residuals of the linear relationship between stem height in nursery seedlings and RGP were significantly affected by the treatments ($F_{4,40} = 4.232$, $p = 0.006$), suggesting that nutrient regimes had an effect on RGP beyond that resulting from seedling size. Resource storage probably played a role in determining RGP in these seedlings.

In conclusion, nutrient management in the nursery showed a strong potential to modify morpho-functional characteristics of *Q. suber* seedlings, and thus it represents a powerful tool to manipulate seedling quality. However, field performance was not related to initial seedling size or root growth potential, suggesting that other factors may be stronger drivers for field performance in this species. According to our results, standard fertilization seems the best option for producing high quality *Q. suber* seedlings.

Acknowledgements

This study was funded by the Government of the Region of Valencia (Generalitat Valenciana), the Bancaixa Foundation, the European project CREOAK (QLRT-2001-01594) and the projects SUROS (MICINN, REN2002-11326-E), GRACCIE (MICINN, Consolider-Ingenio 2010, CSD2007-00067) and ESTRES (MARM, O63/SGTB/2007). We are grateful to Marian Perez, Adolfo Miravet and Oret Subericultura SA for their help. JC acknowledges support from the Secretaría de Estado de Universidades e Investigación, Ministerio de Educación y Ciencia (PN Ayudas para la Movilidad), and thanks the Ecological Restoration Institute and the School of Forestry, Northern Arizona University for their hospitality.

References

- Alloza, J.A., Vallejo, R., 1999. Relación entre las características meteorológicas del año de plantación y los resultados de las plantaciones. *Ecología* 13, 173–187.
- Almeida, H., Merouani, H., Costa e Silva, F., Trubat, R., Khaldi, A., Stiti, B., El Alami, L., Vallejo, R., 2009. Manipulation of cork oak regeneration materials and nurseries techniques. In: Aronson, J., Pereira, J.S., Pausas, J.G. (Eds.), *Cork Oak Woodlands in Transition: Ecology, Adaptive Management, and Restoration of an Ancient Mediterranean Ecosystem*. Island Press.
- Birk, E.M., Matson, P.A., 1986. Site fertility affects seasonal carbon reserves in loblolly pine. *Tree Physiology* 2, 17–27.
- Bollmark, L., Sennerby-Forsse, L., Ericsson, T., 1999. Seasonal dynamics of nitrogen supply rate on nitrogen and carbohydrate reserves in cutting-derived *Salix viminalis* plants. *Canadian Journal of Forest Research* 29, 85–94.
- Bonneau, M., 1988. Le diagnostic foliaire. *Revue Forestière Française* 40, 19–28.
- Burdett, A.N., 1990. Physiological processes in plantation establishment and the development of specifications for forest planting stock. *Canadian Journal of Forest Research* 20, 415–427.
- Castro-Díez, P., Villar-Salvador, P., Pérez-Rontomé, C., Maestro-Martínez, M., Montserrat-Martí, G., 1997. Leaf morphology and leaf chemical composition in three *Quercus* (Fagaceae) species along a rainfall gradient in NE Spain. *Trees* 11, 127–134.
- Chirino, E., Vilagrosa, A., Hernandez, E.I., Matos, A., Vallejo, R., 2008. Effects of a deep container on morpho-functional characteristics and root and root colonization in *Quercus suber* L. seedlings for reforestation in Mediterranean climate. *Forest Ecology Management* 256, 779–785.
- Comas, L.H., Eissenstat, D.M., 2002. Linking fine roots traits to maximal potential growth rate among 11 mature temperate tree species. *Functional Ecology* 18, 388–397.
- Cornelissen, J.H.C., Werger, M.J.A., Castro-Díez, P., van Rheenen, J.W.A., Rowland, A.P., 1997. Foliar nutrients in relation to growth, allocation and leaf traits in seedlings of a wide range of woody plant species and types. *Oecologia* 111, 460–469.
- Cortina, J., Bellot, J., Vilagrosa, A., Catarla, R., Maestre, F., Rubio, E., Martínez, J.M., Bonet, A., 2004. Restauración en semiárido. In: Vallejo, V.R., Alloza, J.A. (Eds.), *Avances en el Estudio de la Gestión del Monte Mediterráneo*. Fundación CEAM, Valencia, pp. 345–406.
- Cortina, J., Navarro, R.M., del Campo, A., 2006. Evaluación del éxito de la reintroducción de especies leñosas en ambientes Mediterráneos. In: Cortina, J., Peñuelas, J.L., Puértolas, J., Vilagrosa, A., Savé, R. (Eds.), *Calidad de planta forestal para la restauración en ambientes Mediterráneos*. Estado actual de conocimientos. Organismo Autónomo Parques Nacionales. Ministerio de Medio Ambiente, Madrid, pp. 67–69.
- Cortina, J., Pérez-Devesa, M., Vilagrosa, A., Abourouh, M., MessaoudèneBerrahmouni, N., Silva, L.N., Khaldi, A., 2009. Artificial regeneration of cork oak. In: Aronson, J., Pereira, J.S., Pausas, J.G. (Eds.), *Cork Oak Woodlands in Transition: Ecology, Adaptive Management, and Restoration of an Ancient Mediterranean Ecosystem*. Island Press.
- Fitter, A.H., 1991. Characteristics and functions of root systems. In: Waisel, Y., Eshel, E., Kafkafi, U. (Eds.), *Plant Roots, the Hidden Half*, first ed. Marcel Dekker, New York, USA.
- Fonseca, D., 1999. Manipulación de las características morfoestructurales de plantones de especies forestales mediterráneas producidos en vivero. Implicaciones sobre su viabilidad y adaptación a condiciones de campo en ambiente semiárido. MSc thesis, IAMZ, Zaragoza.
- García-Cebrián, F., Estesó-Martínez, J., Gil-Pelegrín, E., 2003. Influence of cotyledon removal on early seedling growth in *Quercus robur* L. *Annals of Forest Science* 60, 69–73.
- Gea-Izquierdo, G., Canellas, I., Montero, G., 2006. Acorn production in Spanish holm oak woodlands. *Investigación Agraria: Sistemas y Recursos Forestales* 15, 339–354.
- Kathju, S., Burman, U., Garg, B.K., 2001. Influence of nitrogen fertilization on water relations, photosynthesis, carbohydrate and nitrogen metabolism of diverse pearl millet genotypes under arid conditions. *Journal of Agricultural Science* 137, 207–318.
- Kaushal, P., Aussenac, G., 1989. Transplanting shock in Corsican pine and cedar of Atlas seedlings: internal water deficits, growth and root regeneration. *Forest Ecology Management* 27, 29–40.
- Landis, T.D., 1985. Mineral nutrition as an index of seedling quality. In: Duryea, M.L. (Ed.), *Evaluating Seedling Quality: Principles, Procedures, and Predictive Abilities of Major Tests*. Forest Research Laboratory. Oregon State University, Corvallis, pp. 29–48.
- Lichtenthaler, H.K., 1987. Chlorophyll and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymology* 148, 350–382.
- Ludovici, K.H., Allen, H.L., Albaugh, T.J., Dougherty, P.M., 2002. The influence of nutrient and water availability on carbohydrate storage in loblolly pine. *Forest Ecology Management* 159, 261–270.
- Maestre, F.T., Cortina, J., Bautista, S., Bellot, J., Vallejo, V.R., 2003. Small-scale environmental heterogeneity and spatio-temporal dynamics of seedling establishment in a semiarid degraded ecosystem. *Ecosystems* 6, 630–643.
- Marschner, H., 1995. *Mineral Nutrition of Higher Plants*, second ed. Academic Press, London.
- Miller, P.C., Stoner, W.A., 1979. Canopy structure and environmental interactions. In: Solbrig, O.T., Jain, S., Johnson, G.B., Raven, P.H. (Eds.), *Topics in Plant Population Biology*. Columbia University Press, New York, USA, pp. 438–458.
- Navarro, R.M., Villar-Salvador, P., del Campo, A., 2006. Morfología y establecimiento de los plantones. In: Cortina, J., Peñuelas, J.L., Puértolas, J., Vilagrosa, A., Savé, R. (Eds.), *Calidad de planta forestal para la restauración en ambientes Mediterráneos*. Estado actual de conocimientos. Organismo Autónomo Parques Nacionales. Ministerio de Medio Ambiente, Madrid, pp. 67–69.
- Newton, A.C., Pigott, C.D., 1991. Mineral nutrition and mycorrhizal infection of seedling oak and birch. I. Nutrient uptake and the development of mycorrhizal infection during seedling establishment. *New Phytologist* 117, 37–44.
- Nielsen, E.T., Orcutt, D.M., 1996. *Physiology of Plants Under Stress: Abiotic Factors*. Wiley, New York.
- Oliet, J.A., Planelles, R., López, M., Artero, F., 1997. Efecto de la fertilización en vivero sobre la supervivencia en plantación de *Pinus halepensis* Mill. *Investigaciones Agrarias: Sistema y Recursos Forestales* 8, 207–228.
- Oliet, J., Planelles, R., Segura, M.L., Artero, F., Jacobs, D.F., 2004. Mineral nutrition and growth of containerized *Pinus halepensis* seedlings under controlled-release fertilizer. *Scientia Horticulturae* 103, 113–129.
- Oliet, J., Valdecantos, A., Puértolas, J., Trubat, R., 2006. Influencia del estado nutricional y el contenido en carbohidratos en el establecimiento de las plantaciones. In: Cortina, J., Peñuelas, J.L., Puértolas, J., Vilagrosa, A., Savé, R. (Eds.), *Calidad de planta forestal para la restauración en ambientes Mediterráneos*. Estado actual de conocimientos. Organismo Autónomo Parques Nacionales. Ministerio de Medio Ambiente, Madrid, pp. 109–111.
- Oliveira, G., Martins-Louçao, M.A., Correia, O., Catarino, F., 1996. Nutrient dynamics in crown tissues of cork oak (*Quercus suber* L. *Trees* 10, 247–254.
- Padilla, F.M., Pugnaire, F.I., 2007. Rotting depth and soil moisture control Mediterranean woody seedling survival during drought. *Functional Ecology* 21, 489–495.
- Pérez-Devesa, M., Cortina, J., Vilagrosa, A., Vallejo, R., 2008. Shrubland management to promote *Quercus suber* L. establishment. *Forest Ecology Management* 255, 374–382.
- Poorter, H., Nagel, O., 2000. The role of biomass allocation in the growth response of plants to different levels of light, CO₂, nutrients and water: a quantitative review. *Australian Journal of Plant Physiology* 27, 595–607.

- Radin, J.W., 1984. Stomatal responses to water stress and to abscisic acid in phosphorus-deficient cotton plants. *Plant Physiology* 75, 372–377.
- Reader, R.J., Jalili, A., Grime, J.P., Spencer, R.E., Matthews, N., 1993. A comparative study of plasticity in seedling rooting depth in drying soil. *Journal of Ecology* 81, 543–550.
- Reinbott, T.M., Blevins, D.G., 1999. Phosphorus nutritional effects on root hydraulic conductance, xylem water flow and flux of magnesium and calcium in squash plants. *Plant and Soil* 209, 263–273.
- Robert, B., Bertoni, G., Sayag, D., Masson, P., 1996. Assessment of mineral nutrition of cork oak through foliar analysis. *Communications in Soil Science and Plant Analysis* 27, 2091–2109.
- Rubio, G., Zhu, J.M., Lynch, J.P., 2003. A critical test of the two prevailing theories of plant response to nutrient availability. *American Journal of Botany* 90, 143–152.
- Sanz-Pérez, V., Castro-Díez, P., Valladares, F., 2007. Growth versus storage: responses of Mediterranean oak seedlings to changes in nutrient and water availabilities. *Annals of Forest Science* 64, 201–210.
- Seva, J.P., Valdecantos, A., Vilagrosa, A., Cortina, J., Bellot, J., Vallejo, V.R., 2000. Seedling morphology and survival in some Mediterranean tree and shrub species. EC Report EUR 19303, Brussels. In: Balabanis, P., Peter, D., Ghazi, A., Tsogas, M. (Eds.), *Mediterranean Desertification. Research Results and Policy Implications*, Vol. 2, pp. 397–406.
- Simpson, D.G., Ritchie, G.A., 1997. Does RGP predict field performance? A debate. *New Forests* 13, 249–273.
- Singh, D.K., Sale, P.W.G., 2000. Growth and potential conductivity of white clover roots in dry soil with increasing phosphorus supply and defoliation frequency. *Agronomy Journal* 92, 868–874.
- Tan, W., Hogan, G.D., 1997. Physiological and morphological responses to nitrogen limitation in jack pine seedlings: potential implications for drought tolerance. *New Forests* 14, 19–31.
- Trubat, R., Cortina, J., Vilagrosa, A., 2004. Estado nutricional y establecimiento de especies leñosas en ambiente semiárido. *Cuadernos de la SECF* 17, 245–251.
- Trubat, R., Cortina, J., Vilagrosa, A., 2006. Plant morphology and root hydraulics are altered by nutrient deficiency in *Pistacia lentiscus* L. *Trees* 20, 334–339.
- Trubat, R., Cortina, J., Vilagrosa, A., 2008. Short-term nitrogen deprivation increases field performance in nursery seedlings of Mediterranean woody species. *Journal of Arid Environments* 72, 879–890.
- Valdecantos, A., Cortina, J., Vallejo, V.R., 2006. Nutrient status and field performance of tree seedlings planted in Mediterranean degraded areas. *Annals of Forest Science* 63, 249–256.
- van den Driessche, R., 1984. Relationship between spacing and nitrogen fertilization of seedlings in the nursery, seedling mineral nutrition and outplanting performance. *Canadian Journal of Forest Research* 14, 431–436.
- van den Driessche, R., 1992. Changes in drought resistance and root growth capacity of container seedlings in response to nursery drought, nitrogen and potassium treatments. *Canadian Journal of Forest Research* 22, 740–749.
- Vilagrosa, A., Cortina, J., Gil-Pelegrín, E., Bellot, J., 2003. Suitability of drought-pre-conditioning techniques in Mediterranean climate. *Restoration Ecology* 1 (1), 208–216.
- Vilela, A.E., Rennella, M.J., Ravetta, D.A., 2003. Response of tree-type and shrub-type *Prosopis* (Mimosaceae) taxa to water and nitrogen availabilities. *Forest Ecology Management* 186, 327–337.
- Villar-Salvador, P., Planelles, R., Enrique, E., Peñuelas, J.R., 2004. Nursery cultivation regimes, plant functional attributes, and field performance relationships in the Mediterranean oak *Quercus ilex* L. *Forest Ecology Management* 196, 257–266.
- Waring, R.H., McDonald, A.J.S., Larson, S., Ericsson, T., Wiren, A., Arwidson, E., Ericson, A., Lohammar, T., 1985. Differences in chemical composition of plants grown at constant relative rates with stable mineral nutrition. *Oecologia* 66, 157–160.
- Yates, E.J., Ashwath, N., Midmore, D.J., 2002. Responses to nitrogen, phosphorus, potassium and sodium chloride by three mangrove species in pot culture. *Trees* 16, 120–125.