

## Remobilization of acorn nitrogen for seedling growth in holm oak (*Quercus ilex*), cultivated with contrasting nutrient availability

PEDRO VILLAR-SALVADOR,<sup>1,2</sup> NORBERTO HEREDIA<sup>1</sup> and PETER MILLARD<sup>3</sup>

<sup>1</sup> Departamento de Ecología, Facultad de Ciencias, Universidad de Alcalá, ctra N-II 33,500 Alcalá de Henares, 28871 Madrid, Spain

<sup>2</sup> Corresponding author (pedro.villar@uah.es)

<sup>3</sup> The Macaulay Institute, Craigiebuckler, Aberdeen AB15 8QH, UK

Received July 16, 2009; accepted November 10, 2009; published online December 18, 2009

**Summary** The relative contribution of nitrogen (N) reserves from seeds or uptake by the roots to the growth and N content of young seedlings has received little attention. In this study, we investigated the contribution of N from the acorn or uptake by the roots to the N content of holm oak (*Quercus ilex* L.) seedlings and determined if remobilization of acorn N was affected by nutrient availability in the growing media. *Q. ilex* seedlings were cultivated for 3 months, until the end of the second shoot flush of growth, with three N fertilization rates: 8.6 mM N, 1.4 mM N or no fertilization. Fertilizer N was enriched in <sup>15</sup>N. Between 62 and 75% of the N contained in high and low fertilized seedlings, respectively, at the end of the second flush of growth was derived from the acorn. However, the dependence on acorn N was greater during the early root growth and first shoot flush of growth and decreased during the second shoot flush of growth, with root uptake contributing 32–54% of plant new N in this latter developmental stage in high and low fertilized plants, respectively. Fertilization rate did not affect the amount of N taken up during the earliest developmental stages, but it increased it during the second shoot flush of growth. Fertilization increased the mass of the shoot segment formed during the second shoot flush of growth and reduced the root mass, with no effect on whole plant growth. Remobilization of acorn N was faster in unfertilized plants than in fertilized plants. It is concluded that the holm oak seedlings depend greatly upon acorn N until the end of the second shoot flush of growth, that significant root N uptake starts at the beginning of the second shoot flush of growth and that acorn N remobilization is a plastic process that is accelerated under extremely low substratum nutrient content.

**Keywords:** <sup>15</sup>N labelling, plant nutrition, seed, soil fertility.

### Introduction

Resources stored in seeds are essential for the early development of seedlings, and cotyledon damage can impair seedlings' performance (Bonfil 1998, Kennedy et al. 2004, Hanley and

May 2005). Seed size determines the capacity of seedlings to withstand adverse environmental conditions. Seedlings grown from large seeds have greater performance in poor soils, deep shade and competitive environments than seedlings derived from small seeds (Leishman and Westoby 1994, Moles and Westoby 2004). This is in part attributed to the greater independence of seedlings grown from large seeds from soil resources (Milberg and Lamont 1997, Kitajima 2002). Although much work has been done on the ecological and evolutionary implications of seed size and the metabolic changes in germinating seeds (Bewley and Black 1994), much less is known on how seedlings use seed and soil nutrients to build up their nutrient reserves. The contribution of seed and soil nitrogen (N) to the seedling N pool only has been studied in *Juglans regia* and *Helianthus annuus* (Maillard et al. 1994, Lehmeier et al. 2005). Most studies on the role of seeds on seedling growth have either excised the cotyledons or grown seedlings without nutrients (Meiners and Handel 2000, Kitajima 2002, Hanley and May 2005). However, these approaches cannot distinguish either the role of specific seed resources (for instance N or carbohydrates) on seedling development or the transition between the use of seed and external resources.

Remobilization of N from roots and stems in deciduous woody species and from non-senescent leaves in evergreen species provides most of the N required for initial seasonal growth (Nambiar and Fife 1991, Millard 1996). The amount of N remobilized for spring growth depends on the amount of stored N in the previous season, while current soil fertility has a minor role (Millard and Neilsen 1989, Millard and Proe 1993, Dyckmans and Flessa 2001, Milla et al. 2005). This is because N remobilization for spring growth of established trees is a source-driven process (Millard et al. 2001). In contrast, little is known about how environmental factors, such as soil nutrient availability, influence remobilization of cotyledon resources. *Quercus robur* seedlings became more dependent on acorn nutrients under competition with herbs (Frost and Rydin 1997). Instead, the type of soil did not influence the amount of N and P remobilized from *Q. robur* acorns (Newton and Pigott 1991).

Holm oak (*Quercus ilex* L.) is a late succession evergreen tree that dominates many types of woodland in the western Mediterranean basin, and it is widely used for afforestation. Fertilization in the nursery strongly improves the out-planting performance of holm oak and of other Mediterranean species (Villar-Salvador et al. 2004, 2008). An efficient fertilization programme should promote plant nutrient loading while minimizing fertilizer leaching from nurseries. Efficient fertilization can be achieved by matching fertilization rates to plant uptake capacity (Salifu and Timmer 2003a). However, if seedlings rely on seed reserves for a long time to support their growth, as can occur in large-seeded species (Kitajima 2002), early fertilization or the use of fertilized substratum will reduce fertilization efficiency. Therefore, to implement efficient fertilization practices, it is important to know when the transition from seed mineral nutrient reserves to soil nutrient uptake takes place.

The objectives of the study were to study (i) the contribution of the acorn and soil N to the N content of *Q. ilex* seedlings during their early development, (ii) the timing and rate of soil N uptake and (iii) if acorn N remobilization depends on nutrient availability in the substratum. To address these objectives, we carried out an experiment where soil was enriched in  $^{15}\text{N}$  to quantify N uptake and its distribution in the plant. This was studied until seedlings finished the second shoot flush of growth.

## Materials and methods

### Experimental design

Acorns were collected in November 2006 from a pure holm oak stand near Romancos (Guadalajara, Spain, latitude  $40^{\circ}42' \text{ N}$ ,  $2^{\circ}50' \text{ W}$ , longitude  $2^{\circ}50'2.48'' \text{ W}$ ). To reduce variability and growth differences due to seed size, acorns were obtained from a single tree, and only those with a fresh weight between 3.1 and 3.9 g, which represented the upper and lower quartiles of acorn fresh weight, were used in the study. Acorns were washed with tap water, dried with blotting paper and stored in polyethylene bags at  $+1^{\circ}\text{C}$  until used.

In mid-February 2007, acorns were germinated for 15 days in trays containing vermiculite moistened with deionized water. When the length of most radicles ranged 5–30 mm, 350 acorns were transplanted into 305-ml pots (Super-Leach<sup>TM</sup>, Industrias Bardi S.A.L., Navarra, Spain) (Day 0, 2 March 2007). The growing medium was vermiculite previously washed twice with deionized water. Acorns were wrapped with aluminium foil before transplanting to prevent nutrient absorption from the growing medium. The experiment was carried out in a glasshouse, which means maximum and minimum temperatures were 28 and  $15^{\circ}\text{C}$ , respectively, and the photosynthetic photon flux density was 40% of the ambient.

Seedlings were cultivated under one of three N fertilization rates: 8.6 mM N ( $F_{+}$ ), 1.4 mM N ( $F_{-}$ ) and no fertilization ( $F_0$ ). Nitrogen was supplied as  $\text{KNO}_3$  enriched to

5.414 atom%  $^{15}\text{N}$  (Sigma Aldrich Química, Madrid, Spain). The  $F_{+}$  nutrient solution also contained 1 mM  $\text{CaSO}_4$ , 0.7 mM  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ , 1.3 mM  $\text{MgCl}_2$  and 100 p.p.m. of a micronutrient mixture (Kanieltra, Hydro Agri, Oslo, Norway). The  $F_{-}$  fertilizer solution had the same salts as the  $F_{+}$  fertilizer solution but diluted six times with deionized water. Fertirrigation started on the same day that acorns were transplanted. Plants were fertirrigated every 2–3 days with 20–45 ml of each nutrient solution depending on seedling size and on greenhouse temperature. At the end of the experiment,  $F_{+}$  and  $F_{-}$  plants had received 172 and 29 mg N, respectively, which can be considered as optimal and suboptimal levels, respectively, for growing holm oak seedlings in the nursery (Villar-Salvador et al. 2004).  $F_0$  plants were irrigated with deionized water. Pots were randomly arranged in space.

### Plant sampling and processing

*Quercus ilex* seedlings experience a rhythmic pattern of shoot growth during their cultivation in the nursery with most seedlings experiencing two or three flushes of shoot growth (Puértolas et al. 2009). Between shoot growth flushes, there are periods where no apparent shoot elongation occurs. In this experiment, plants were harvested periodically when they reached specific development stages. We used this criterion rather than time because most important functional changes in plants occur at specific ontogenetic or phenological stages (Sloan and Jacobs 2008), and it facilitates the comparison with other species and the application of cultivation management recommendations. Development stages were:

- (1) **Seedlings only have roots:** Before shoot emergence, seedling mass was made up exclusively of roots, with the taproot reaching the bottom of the container, and a small shoot primordium (<0.5 cm) emerging above soil. Seedlings reached this stage on Days 13–17 after sowing.
- (2) **First shoot flush of growth completed:** Shoot elongation had ceased, and all leaves were completely unfolded and mature. Most seedlings reached this stage on Days 53–56 after sowing.
- (3) **Second shoot flush of growth completed:** As in Stage 2 but in the second shoot flush of growth. Most seedlings reached this stage on Days 83–90 after sowing.

At each development stage, 12 seedlings of the  $F_{+}$  and  $F_{-}$  treatments and four seedlings of the  $F_0$  treatment were harvested. Plants were divided into shoots, roots and acorns. Roots were washed from the growing media with tap water, and shoots were separated into first flush segment and second flush segment. Acorns were separated into cotyledons and pericarp, which was discarded. Then shoot segments, roots and cotyledons were rinsed twice in deionized water, dried at  $50^{\circ}\text{C}$  for 48–72 h and weighed. Plant fractions of all  $F_0$  seedlings and of six randomly selected  $F_{+}$  and  $F_{-}$  seedlings were ground in a ball mill (PM100, Retsch, Haan, Germany) for N concentration and  $^{15}\text{N}$  isotope abundance. At the beginning of the experiment, we randomly sampled 30 acorns to

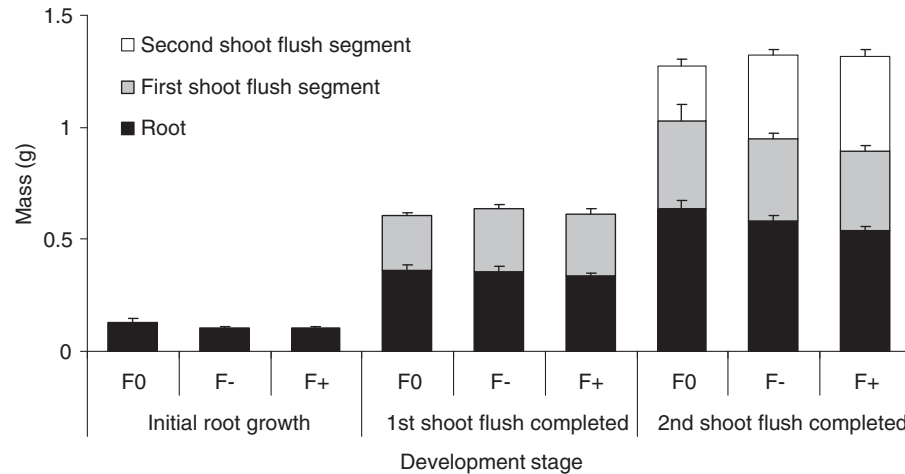


Figure 1. Growth of plant parts at different developmental stages in *Q. ilex* seedlings cultivated with contrasting nutrient availability. Data are means  $\pm$  1 SE. The seedlings were unfertilized ( $F_0$ ), or provided with low ( $F^-$ ) or high ( $F^+$ ) fertilizer applications ( $n = 4-12$ ).

determine their mass after removing the pericarp. Then cotyledons were randomly assigned to six groups, each one of five cotyledons, to determine N concentration and  $^{15}\text{N}$  isotope abundance. Total N concentration was determined with an elemental analyser (EA-3010 Eurovector, Milan, Italy), while  $^{15}\text{N}$  isotope abundance (atom%) was measured with a continuous flow isotope ratio mass spectrometer (CF-IRMS Isochrom, Micromass, UK) after combustion of the samples in an elemental analyser (EA 1108-CHNS, Carlo Erba, Milan, Italy).

#### Calculations and data statistical analysis

We used a mixing isotope model to disentangle the contribution of acorn N and soil N to the total plant N. We assumed that fertilizer was the only source of labelled N, and unlabelled N in organs came exclusively from the acorn N. The fraction of labelled N (fertilizer N,  $f_F$ ) in an organ or the whole seedling was calculated following the same procedure as in Maillard et al. (1994):

$$f_F = \frac{A_p - A_a}{A_F - A_a} \quad (1)$$

where  $A_p$ ,  $A_a$  and  $A_F$  are the  $^{15}\text{N}$  isotope abundance of the plant organ, acorn and the fertilizer, respectively. Some N from the fertilizer was translocated to the acorns during the study, but acorn  $^{15}\text{N}$  enrichment was four to six times less than that in plant organs and one order of magnitude less than that of the fertilizer. Therefore, we used the mean  $^{15}\text{N}$  isotope abundance of the acorns at the beginning of the study, which was 0.3675% ( $n = 6$ ), as the  $A_a$  value for  $f_F$  calculations in all development stages. The amount of labelled N ( $N_F$ ) in an organ or the whole seedling was calculated as

$$N_F = f_F \times \text{organ N concentration} \times \text{organ mass (mg)} \quad (2)$$

The amount of unlabelled N (acorn N,  $N_a$ ) in the plant was calculated as

$$N_a = (\text{organ N concentration} \times \text{organ mass}) - N_F \text{ (mg)} \quad (3)$$

Data were analysed by a two-way analysis of variance using a Type III sum of squares. The two factors considered were development stage (one to three levels depending on the organ) and fertilization rate (three levels). Both absolute and relative  $N_a$  data were considered. We used relative  $N_a$  to remove potential biases due to different initial acorn N content on  $N_a$ , and it was calculated as the ratio between  $N_a$  and initial acorn N content. Initial acorn N content was estimated as the content of the N remaining in the acorn at each sampling date plus  $N_a$ . We assumed N leaching to be negligible. The Levene's test was used to check data homoscedasticity. Variables with heteroscedastic data were  $\log_{10}$ -transformed. Statistical analyses were performed with the Statistica 6.0 Package (StatSoft, Inc., Tulsa, OK, USA)

#### Results

Whole plant, root and first shoot flush segment mass increased through development stages. Fertilization increased the mass of the second flush segment and reduced the root mass, with  $F_0$  seedlings having lower and higher mass, respectively, than the remaining treatments, which did not differ between them (Figure 1). Fertilization had no effect either on whole plant mass or on the mass of the first shoot flush segment (Table 1).

Plant N content increased through development stages ( $F = 453$ ;  $P < 0.001$ ), and fertilization increased it ( $F = 3.67$ ;  $P = 0.035$ ) (Figure 2). At the end of the study, plant N content of

Table 1. Results of ANOVAs on the effect of the developmental stage and the fertilization rate on the mass and the amount of labelled (fertilizer) and unlabelled (acorn) N content in the plant and in several organs of *Q. ilex* seedlings. Data are *F* values. \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ , \*\*\*\* $P \leq 0.065$ , ns:  $P > 0.065$ .

	Whole plant	Roots	First shoot flush segment	Second shoot flush segment
<b>Mass</b>				
Development stage	1414***	612***	21.3***	
Fertilization	0.074 ns	4.34*	0.057 ns	7.99**
Development stage $\times$ Fertilization	0.81 ns	0.82 ns	0.56 ns	
<b>Unlabelled N</b>				
Development stage	410***	118***	0.31 ns	
Fertilization	3.01 ns****	1.33 ns	1.35 ns	1.45 ns
Development stage $\times$ Fertilization	1.45 ns	0.68 ns	0.54 ns	
<b>Labelled N</b>				
Development stage	561***	389***	3.91 ns****	
Fertilization	65.3***	94.1***	6.9*	5.68*
Development stage $\times$ Fertilization	13.2***	15.0***	0.73 ns	

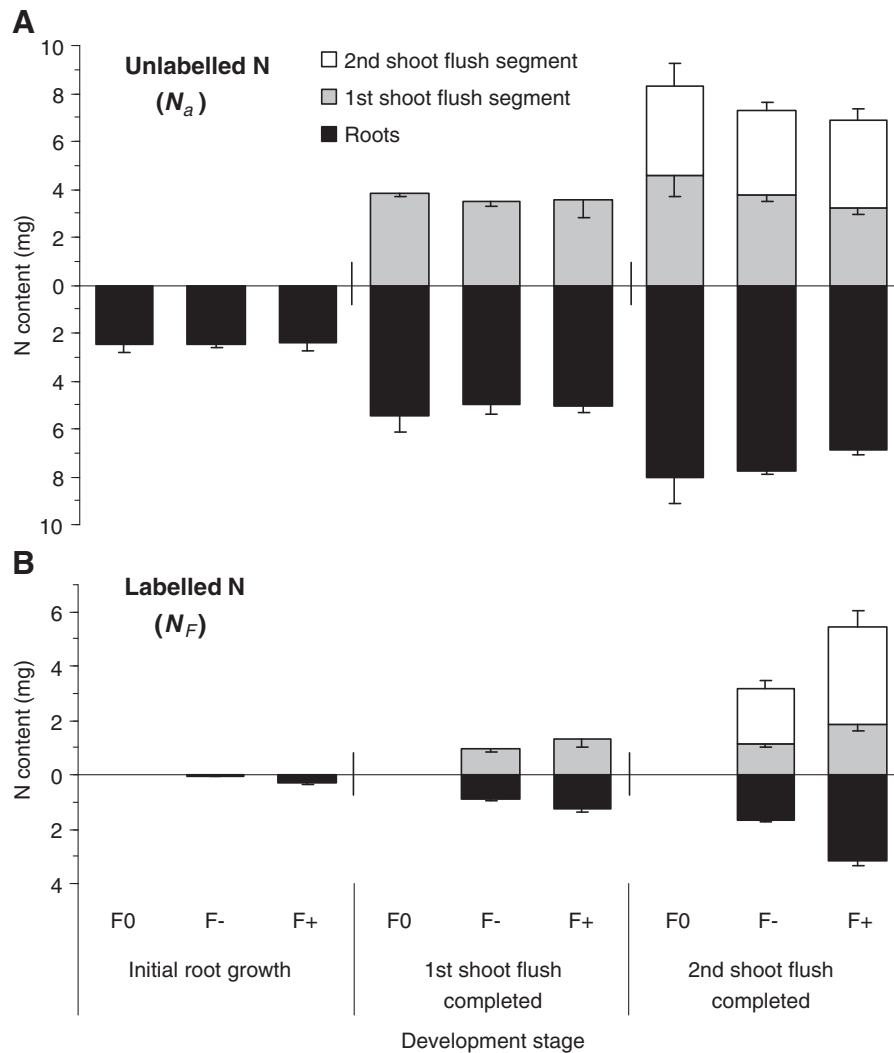


Figure 2. Amount of unlabelled N ( $N_a$ , recovered from the acorn, upper figure) and labelled N ( $N_F$ , recovered from the fertilizer, lower figure) at different development stages in plant parts of *Q. ilex* seedlings cultivated with contrasting nutrient availability. Data are means  $\pm$  1 SE. The seedlings were unfertilized ( $F_0$ ), or provided with low ( $F^-$ ) or high ( $F^+$ ) fertilizer applications ( $n = 4-6$ ).

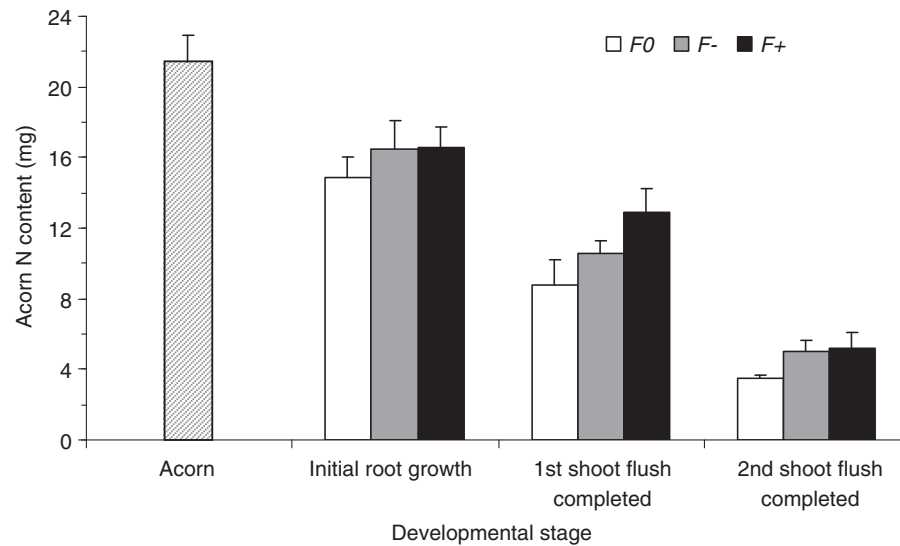


Figure 3. Acorn N content at different development stages in *Q. ilex* seedlings cultivated with contrasting nutrient availability. Data are means  $\pm$  1 SE. The seedlings were unfertilized ( $F_0$ ), or provided with low ( $F^-$ ) or high ( $F^+$ ) fertilizer applications ( $n = 4-6$ ).

the fertilization treatments were  $F^+ = 22 \pm 1.2$  mg,  $F^- = 20 \pm 0.5$  mg and  $F_0 = 16 \pm 0.8$  mg (mean  $\pm$  1 SE).

At all development stages, the amount of unlabelled N ( $N_a$ ) in the seedlings was higher than the amount of labelled N ( $N_F$ ) (Figure 2). At the end of the first flush, 82 and 77% of the plant N in  $F^-$  and  $F^+$  seedlings, respectively, was  $N_a$ , whereas at the end of the second flush this proportion was 75 and 62%, respectively. The amount of  $N_a$  allocated to the shoot and to the root was similar, but the amount of  $N_F$  allocated to the shoot was higher than that allocated to the root. Between the end of the first and the second shoot flushes of growth,  $N_F$  contributed 32 and 54% of the new N incorporated into  $F^-$  and  $F^+$  seedlings, respectively.

Fertilization marginally reduced  $N_a$  and the relative  $N_a$  ( $F = 6.56$ ;  $P < 0.003$ ) at the plant level but not at any organ level (Table 1 and Figure 2 for  $N_a$ ; relative  $N_a$  data not shown). The amount of  $N_F$  recovered in both the whole seedling and in the different organs increased through development and with fertilization. However, increase in  $N_F$  at the whole plant and in roots due to fertilization was only observed during the second flush of growth (Figure 2) but not during earlier development stages (interaction development stage  $\times$  fertilization, Table 1).

The mean N content of the acorn at the beginning of the study was  $21 \pm 1.5$  mg (mean  $\pm$  1 SE,  $n = 6$ ). Acorn N content decreased as plants grew ( $F = 73.7$ ,  $P < 0.001$ ), and at the end of the second flush, 76% of acorn N on average had been remobilized. However, acorn N was depleted faster in  $F_0$  than in  $F^-$  or  $F^+$  seedlings ( $F = 3.26$ ;  $P = 0.049$ ), which reduced acorn N at a similar rate (Figure 3). At the end of the second flush, the proportion of the initial acorn N remaining in  $F_0$ ,  $F^-$  and  $F^+$  acorns were 17, 25 and 26%, respectively.

## Discussion

At the end of the second shoot flush of growth, 3 months after shoot emergence, most of the N accumulated in holm oak seedlings came from the acorn. Only 25–38% of the N recovered in the plant had been taken up by the roots. This pattern differs from that in *J. regia*, which can derive some 60% of the seedling N content from root uptake by 40 days after shoot emergence (Maillard et al. 1994). Holm oak seedlings relied almost only on acorn N during very early development stages, as roots took up little N during early root growth and in the first shoot flush of growth, and root uptake was independent of fertilization rate. In contrast, during the second flush of shoot growth, roots took up N in greater quantities, and high-fertilized seedlings absorbed more N than low-fertilized plants, explaining their higher plant N content at the end of the study. The lower growth of the second shoot flush segment together with higher root growth in non-fertilized plants as compared with fertilized plants is a typical response of plants growing with low nutrient availability (Aerts 1994) and, therefore, might be indicative of the increasing dependence of plants on fertilizer N during the second shoot flush growth.

The contribution of the acorn to the seedling N remained high until the end of the second shoot flush of growth. This pattern was not observed for the deciduous oak, *Q. robur*, in which acorn N reserves are depleted at the end of the first shoot flush of growth, and new shoot flushes of growth rely entirely on soil N (García Cebrián et al. 2003). This difference between oak species might be related to foliar habit, with deciduous species growing faster and concentrating more N in their foliage than evergreen species (Cornelissen et al. 1996), thereby demanding a higher N supply during early development stages.

The pattern of use of acorn and soil N in *Q. ilex* seedlings has important ecophysiological and practical implications. Our data explain the low morphological and physiological plasticity of holm oak seedlings to fertilization (Valladares et al. 2000, Villar-Salvador et al. 2004). The great dependence on acorn resources potentially makes holm oak seedlings strongly independent of external resources during their early development (see Milberg and Lamont 1997). We also demonstrate why fertilization during nursery cultivation is not necessary until the beginning of the second shoot flush of growth. In addition, a high tissue N content, as observed in high-fertilized plants in comparison with low- and non-fertilized seedlings, may enhance out-planting performance (Salifu and Timmer 2003b, Oliet et al. 2009).

We found evidence that remobilization of acorn N in *Q. ilex* seedlings is a plastic process that is enhanced at very low soil fertility (Figure 3). This result is consistent with the higher  $N_a$  in  $F_0$  plants as compared with fertilized plants' seedlings. Milberg and Lamont (1997) did not find any clear pattern to the amount of nutrient remobilized from seeds in four Australian woody species after cultivating them in two soils of contrasted fertility. Similarly, N and P remobilization from *Q. robur* acorns was independent of the soil type in which seedlings had grown (Newton and Pigott 1991). Our results do not concur with those that demonstrated that N remobilization from storage to support the spring growth of temperate tree species is not influenced by nutrient availability (Millard and Neilsen 1989, Millard and Proe 1993, Dyckmans and Flessa 2001). However, in accordance with our findings, greater N remobilization from storage organs in Mediterranean oak saplings occurred when soil N supply could not meet shoot growth N demand in spring (Silla and Escudero 2003). Increase in growth rate accelerated remobilization of acorn N reserves in *Q. robur* seedlings (Newton and Pigott 1991). However, in our study fertilization treatments did not differ in growth so we suggest that the faster acorn N remobilization in non-fertilized plants compared to fertilized plants can be a response for maximizing acorn N use efficiency at very low nutrient availability.

#### Acknowledgments

The authors are grateful to J. Cuadrado and the Botanical Garden Juan Carlos I of the Universidad de Alcalá for their technical support. This study was supported by a contract of the Government of the Community of Madrid to N.H. and by projects AGL2006-12609-C02-01/FOR, CGL2007-60533/BOS (Spanish Ministry of Science and Education) and S-0505/AMB/0355-REMEDINAL (Madrid Government).

#### References

Aerts, R. 1994. The effect of nitrogen supply on the partitioning of biomass and nitrogen in plant species from heathlands and fens: alternatives in plant functioning and scientific approach. *In* A Whole Plant Perspective on Carbon–Nitrogen Interactions. Eds.

- J. Roy and E. Garnier. SPB Academic Publishing bv, The Hague, pp 247–265.
- Bewley, J.D. and M. Black. 1994. Seeds: physiology of development and germination. Plenum, New York.
- Bonfil, C. 1998. The effects of seed size, cotyledon reserves, and herbivory on seedling survival and growth in *Quercus rugosa* and *Q. laurina* (Fagaceae). *Am. J. Bot.* 85:79–87.
- Cornelissen, J.H.C., P. Castro-Díez and R. Hunt. 1996. Seedling growth, allocation and leaf attributes in a wide range of woody plant species and types. *J. Ecol.* 84:755–765.
- Dyckmans, J. and H. Flessa. 2001. Influence of tree internal N status on uptake and translocation of C and N in beech: a dual  $^{13}\text{C}$  and  $^{15}\text{N}$  labelling approach. *Tree Physiol.* 21:395–401.
- Frost, I. and H. Rydin. 1997. Effects of competition, grazing and cotyledon nutrient supply on growth of *Quercus robur* seedlings. *Oikos* 79:53–58.
- García Cebrián, F., J. Esteso Martínez and E. Gil Pelegrín. 2003. Influence of cotyledon removal on early seedling growth in *Quercus robur* L. *Ann. For. Sci.* 60:69–73.
- Hanley, M.E. and O.C. May. 2005. Cotyledon damage at the seedling stage affects growth and flowering potential in mature plants. *New Phytol.* 169:243–250.
- Kennedy, P.G., N.J. Hausmann, E.H. Wenk and T.E. Dawson. 2004. The importance of seed reserves for seedling performance: an integrated approach using morphological, physiological, and stable isotope techniques. *Oecologia.* 141:547–554.
- Kitajima, K. 2002. Do shade-tolerant tropical tree seedlings depend longer on seed reserves? Functional growth analysis of three *Bignoniaceae* species. *Funct. Ecol.* 16:433–444.
- Lehmeier, C., R. Schäufele and H. Schnyder. 2005. Allocation of reserve-derived and currently assimilated carbon and nitrogen in seedlings of *Helianthus annuus* under subambient and elevated  $\text{CO}_2$  growth conditions. *New Phytol.* 168:613–621.
- Leishman, M.R. and M. Westoby. 1994. Hypotheses on seed size: tests using the semiarid flora of western New South Wales, Australia. *Am. Nat.* 143:890–906.
- Maillard, P., E. Deléens, F.A. Daudet, A. Lacoite and J.S. Frossard. 1994. Carbon and nitrogen partitioning in walnut seedlings during the acquisition of autotrophy through simultaneous  $^{13}\text{CO}_2$  and  $^{15}\text{NO}_3$  long-term labelling. *J. Exp. Bot.* 45:203–210.
- Meiners, S.J. and S.N. Handel. 2000. Additive and non-additive effects of herbivory and competition on tree seedling mortality, growth, and allocation. *Am. J. Bot.* 87:1821–1826.
- Milberg, P. and B.B. Lamont. 1997. Seed/cotyledon size and nutrient content play a major role in early performance of species on nutrient-poor soils. *New Phytol.* 137:665–672.
- Milla, R., P. Castro-Díez, M. Maestro-Martínez and G. Montserrat-Martí. 2005. Relationships between phenology and the remobilization of nitrogen, phosphorus and potassium in branches of eight Mediterranean evergreens. *New Phytol.* 168:167–178.
- Millard, P. 1996. Ecophysiology of the internal cycling of nitrogen for tree growth. *Z. Pflanzen. Bodek.* 159:1–10.
- Millard, P. and G.H. Neilsen. 1989. The influence of nitrogen supply on the uptake and remobilisation of stored N for the seasonal growth of apple trees. *Ann Bot.* 63:301–309.
- Millard, P. and M.F. Proe. 1993. Nitrogen uptake, partitioning and internal cycling in *Picea sitchensis* (Bong.) Carr. as influenced by nitrogen supply. *New Phytol.* 125:113–119.
- Millard, P., A. Hester, R. Wendler and G. Baillie. 2001. Interspecific defoliation responses of trees depend on sites of winter nitrogen storage. *Funct. Ecol.* 15:535–543.
- Moles, A.T. and M. Westoby. 2004. Seedling survival and seed size: a synthesis of the literature. *J. Ecol.* 92:372–383.

- Nambiar, E.K.S. and D.N. Fife. 1991. Nutrient retranslocation in temperate conifers. *Tree Physiol.* 9:185–207.
- Newton, A.C. and C.D. Pigott. 1991. Mineral nutrition and mycorrhizal infection of seedling oak and birch. *New Phytol.* 117:37–44.
- Oliet, J., M. Tejada, K. F. Salifu, A. Collazos and D.F. Jacobs. 2009. Performance and nutrient dynamics of holm oak (*Quercus ilex* L.) seedlings in relation to nursery nutrient loading and post-transplant fertility. *Eur. J. Forest Res* 128:253–263.
- Puértolas, J., K. Disante, D. Fuentes, A. Valdecantos, J. Monerri, J. Cortina, L.F. Benito and J.L. Peñuelas. 2009. Ritmos de acumulación de biomasa en *Quercus ilex* durante el primer año de cultivo en contenedor: efectos maternos y ambientales. In *Proceedings of the 5° Congreso Forestal Español*, Ref. 5CFE01-278. Ed. Junta de Castilla y León. Sociedad Española de Ciencias Forestales, Spain.
- Salifu, K.F. and V.R. Timmer. 2003a. Optimizing nitrogen loading of *Picea mariana* seedlings during nursery culture. *Can. J. For. Res.* 33:1287–1294.
- Salifu, K.F. and V.R. Timmer. 2003b. Nitrogen retranslocation response of young *Picea mariana* to nitrogen-15 supply. *Soil Sci. Soc. Am. J.* 67:309–317.
- Silla, F. and A. Escudero. 2003. Uptake, demand and internal cycling of nitrogen in saplings of Mediterranean *Quercus* species. *Oecologia.* 136:28–36.
- Sloan, J.L. and D. Jacobs. 2008. Carbon translocation patterns associated with new root proliferation during episodic growth of transplanted *Quercus rubra* seedlings. *Tree Physiol.* 28:1121–1126.
- Valladares, F., E. Martínez-Ferri, L. Balaguer, E. Pérez-Corona and E. Manrique. 2000. Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy? *New Phytol.* 148:79–91.
- Villar-Salvador, P., R. Planelles, E. Enríquez and J. Peñuelas Rubira. 2004. Nursery cultivation regimes, plant functional attributes, and field performance relationships in the Mediterranean oak *Quercus ilex* L. *For. Ecol. Manag.* 196:257–266.
- Villar-Salvador, P., F. Valladares, S. Domínguez-Lerena, B. Ruiz-Díez, M. Fernández-Pascual, A. Delgado and J.L. Peñuelas. 2008. Functional traits related to seedling performance in the Mediterranean leguminous shrub *Retama sphaerocarpa*: insights from a provenance, fertilization, and rhizobial inoculation study. *Environ. Exp. Bot.* 64:145–154.