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RESEARCH PAPER

Acorn size is more important than nursery fertilization for outplanting performance of *Quercus variabilis* container seedlings

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Abstract

• ***Key message*** **Small acorns are usually discarded for seedling cultivation because they reduce plant quality. This, however, can potentially reduce genetic diversity of plantations. The use of small acorns will result in the production of a higher proportion of small seedlings containing low nutrient levels and having poor outplanting performance in oak container seedlings. Nursery fertilization partially offsets the negative effect of small acorns on seedling attributes in the nursery but not on outplanting performance.**

• ***Context*** Small acorns result in low-quality seedlings and so are usually discarded in artificial regeneration programs of oak species. This can potentially reduce genetic diversity of plantations. Nursery fertilization may compensate for the low quality of small-acorn seedlings.

• ***Aims*** To assess whether nursery fertilization interacts with *Quercus variabilis* acorn size to determine seedling morphology and nutrition in the nursery and outplanting performance.

• ***Methods*** Acorns of three size classes were used to cultivate seedlings with or without fertilization. Seedling emergence, nursery morphology and nutrient status, and outplanting survival and growth were measured.

• ***Results*** Small acorns represented 41% of the seed batch. Most acorn size variation occurred within trees rather than among trees. Smaller acorns were associated with lower emergence and resulted in smaller seedlings that had lower nutrient content levels. Nursery fertilization slightly increased seedling growth for all acorn sizes; it also strongly increased nutrient content, especially in small-acorn seedlings. Two years after planting, survival of small-acorn seedlings was 32% lower than the survival of medium- and large-acorn plants. Fertilization did not affect survival, but it did increase size, especially of small-acorn seedlings, though they did not achieve the growth of large-acorn seedlings.

• ***Conclusion*** Nursery fertilization increases growth and nutrient status, but not outplanting performance, of small-acorn seedlings.

Keywords Acorn size · Nursery fertilization · Nutrient concentration · Outplanting performance · Seedling growth · Survival

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Contributions of the authors WS designed the study, performed all the experimental work, ran the data analysis, and wrote the manuscript. PV performed data analysis and co-wrote the manuscript. GL supervised the work, participated in the experiment design, and co-worked in paper writing. XJ participated in data collection and interpretation.

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1 Introduction

Seed size strongly varies across plant species (Leishman et al., 1995). These differences have a strong phylogenetic basis but are also the result of opposing selective pressures related to dispersal mode, predation avoidance, and tree performance (Foster and Janson 1985; Venable and Brown 1988; Westoby et al. 1992; Carlo and Yang 2011). Seed size determines short-term seedling survival and growth, especially under stress conditions such as deep shade (Ke and Werger 1999; Quero et al. 2007), low soil fertility (Tungate et al. 2006), and low water availability (Rice et al. 1993; Ramírez-Valiente et al. 2009).

Acorn size varies widely among oak species (*Quercus* spp.; Ke and Werger 1999; Quero et al. 2007; González-Rodríguez et al. 2011a), and also within oak species (Ramírez-Valiente et al. 2009; Sage et al. 2011) and even within individual trees (Johnson et al. 2009). Larger acorns result in higher emergence rates (Pesendorfer 2014) and frequently larger seedlings (Rice et al. 1993; Navarro et al. 2006; Quero et al. 2007; Pesendorfer 2014). At a within-species level, seedlings grown from big acorns directly seeded in the field often have higher survival than seedlings grown from small acorns (Thcklin and McCreary 1991; Tripathi and Khan 1990; Rice et al. 1993; Bonfil 1998; González-Rodríguez et al. 2011a; Sage et al. 2011). The positive effect of acorn size on seedling performance is mainly explained by the *seedling size effect* (see Westoby et al. 1996; Leishman et al. 2000): large seeds result in large seedlings, which have greater capacity to resist stress than small seedlings. This can affect the survival of juvenile oak trees for many years after emergence (Ramírez-Valiente et al. 2009; Sage et al. 2011).

Oaks are important species in many forest communities in the northern hemisphere, where they play important economic and ecological roles (Johnson et al. 2009; Gil-Pelegrín et al. 2017). Artificial restoration of oak forests is important in many parts of the world, such as in northern China (Wang 2013), the Mediterranean basin (Villar-Salvador et al. 2004, 2013), and eastern US (Knoot et al. 2010). Artificial oak forest restoration can be achieved by direct seeding of acorns or by planting seedlings cultivated in the nursery. Direct seeding is cheaper than planting (Grossnickle and Ivetić 2017), but under harsh conditions, such as when soil is dry or of low quality, survival of plants grown from directly seeded acorns is frequently lower than that of nursery-grown seedlings (González-Rodríguez et al. 2011b; Li et al. 2012; Pemán et al. 2017). Therefore, seedling planting is the most commonly used forestation method in oak restoration projects. The strong effect of acorn size on seedling size and outplanting performance has important practical implications for the collection and selection of acorns used in oak forest restoration projects. If forestation is to be carried out through planting, small acorns are rarely used because they give rise to small seedlings. Similarly, in container nurseries, large acorns are difficult to seed into most standard forest containers.

Selection of acorns by size for the purposes of oak forest restoration may have long-term disadvantages, such as by reducing genetic diversity (Burgarella et al. 2007). Therefore, it may be desirable to use small acorns in the nursery, even if they result in low-quality seedlings. It may be possible to compensate for lower seedling quality by using fertilization in the nursery. This can strongly increase seedling growth, nutrient status, and outplanting performance (Villar-Salvador et al. 2004, 2013; Oliet et al. 2009; Salifu et al. 2009). Little information exists on the effect of acorn size on the outplanting survival and growth of nursery-grown seedlings (see Ramírez-Valiente et al. 2009), as to whether or not nursery fertilization can compensate for the low quality of small-acorn seedlings.

The objectives of this study were to (*i*) assess whether the size of acorns used to cultivate seedlings in a nursery determines their outplanting performance and (*ii*) examine whether nursery fertilization interacts with acorn size to determine seedling performance, both at the nursery stage and after planting. We hypothesized that (*i*) large acorns result in large seedlings, which show higher outplanting performance than small-acorn seedlings and (*ii*) nursery fertilization offsets the reduced growth and nutrient status of using small-acorn seedlings in the nursery and on subsequent outplanting performance. To test these hypotheses, we performed an experiment where *Quercus variabilis* Blume seedlings were grown in the nursery from acorns in three size classes, and seedlings were either left unfertilized or were fertilized. Seedling emergence, growth, and mineral nutrient status were measured at the end of the nursery growing season, and outplanting survival and growth were monitored for 2 years. We used *Q. variabilis* because it is dominant in many Chinese forests and is widely used in afforestation in China (Zhang et al. 2002).

2 Material and methods

2.1 Acorn collection and classification by size

In September 2013, acorns were collected from 20 *Q. variabilis* trees located at the Yuntaishan National Forest Farm in Jiaozuo, Henan province, China (113° 22′ E, 35° 26′ N; elevation 1022–1225 m). Damaged acorns were removed by water flotation immediately after collection as detailed in Li et al. (2014). Then, acorns were air-dried on paper in a ventilated house at ambient temperature for 24 h. A subsample of 80 acorns from each tree was randomly selected, and the fresh weight of each individual acorn was determined; the fresh weight values ranged from 1.48 to 7.71 g in all the sampled population of acorns (see Appendix A). Hereafter, the remaining acorns from all trees were mixed into one batch and stored in 34 × 24 cm polyethylene bags (wall thickness, 100 μm) that were permeable to carbon dioxide and oxygen, yet impermeable to moisture. Bags were stored at 2–5 °C in a refrigerator (Kormanik et al. 1998; Li et al. 2014) until the experiment began in April 2014.

Before sowing in the next spring, 1200 acorns from the batch of mixed acorns were randomly chosen, and their fresh weight, length, and width were determined. A K-means cluster analysis approach (Wagstaff et al. 2001) was performed in SPSS 18.0 for Windows (IBM, Chicago, IL, USA) to determine three acorn size categories that best separated the acorn population into three groups according to their fresh weights. Based on this information, all the acorns of the batch of mixed acorns (this includes the randomly chosen 1200 acorns) were then separated into three category sizes: small (< 3.51 g), medium (3.51–4.83 g), and large (≥ 4.83 g). Mean lengths were 21.3 mm for large acorns, 20.6 mm for medium ones, and 19.4 mm for small ones; the corresponding mean widths were 20.8, 18.3, and 16.9 mm.

Fifty acorns of each size class were randomly sampled and oven-dried at 70 °C for a minimum of 48 h to allow determination of initial acorn mass as well as nitrogen (N), phosphorus (P), and potassium (K) concentrations. For N, P, and K determination, each acorn was ground up, sieved through a 0.25-mm screen, and wet-digested using the H_2SO_4–H_2O_2 method. Standard Kjeldahl digestion with water distillation was performed to allow determination of total N using a distillation unit (UDK-159, VelpScientifica, Italy). P concentration was determined with a UV-visible spectrophotometer (Agilent 8453, USA), while K concentration was quantified with an atomic emission spectrometer (SpectrAA 220, VARIN, USA). Acorn N, P, and K content was calculated as the product of acorn N, P, K concentration and acorn mass. Acorn moisture content (MC) was calculated based on percent fresh weight as:

$$MC = \frac{(FW - DW)}{FW} \times 100\ \% \qquad (1)$$

where FW is acorn fresh weight (before oven drying) and DW is acorn dry weight (hereinafter referred to as acorn mass).

2.2 Nursery experiment

On 23 April 2014, 400 acorns of each size class were sown in cylindrical, hard plastic D60 containers (Stuewe & Sons, Corvallis, USA), one acorn per container, at a depth of 1–2 cm. Twenty containers were arranged in a tray (37.6 cm long × 30.0 cm wide), and 20 trays were prepared for each acorn class. Container diameter was 6.4 cm and depth was 36.0 cm, resulting in a volume of 983 ml. Growing medium was a 3:1 (*v*:*v*) mixture of peat (pH 6.0, screening 0–6 mm, Pindstrup seeding, Ryomgaard, Denmark) and perlite (5 mm diameter, Xinyang Jinhualan Mining Co., Henan, China). Trays were placed inside the greenhouse of Beijing Forestry University near Jiufeng Mountain, Beijing (39° 54′ N, 116° 28′ E). Mean air temperature in the greenhouse was 28.5 °C during the day and 16.5 °C at night, while the mean air relative humidity was 84.7%. A black shade screen was extended outside of the greenhouse, resulting in an average daily light level of 820 μmol m^{-2} s^{-1} inside the greenhouse.

During cultivation, half the plants of each acorn size class were left unfertilized (F0), while the other half was fertilized (F+). Experimental design was completely random, with five replications for each of the six treatments (three acorn size classes × two fertilization treatments). Each replication was formed by two trays. Trays were rotated every week to minimize edge effects. Each F+ plant was fertilized weekly with 20 ml of a solution prepared with a 20N-20P-20K fertilizer containing micronutrients (Peters Professional, ICL Specialty Fertilizers, Scotts, USA) dissolved in distilled water at a 2.5 g L^{-1} concentration. Fertilization lasted 10 weeks and started after emergence finished, which occurred around 22 May 2014. By the end of the nursery cultivation stage, each F+ pot had received 0.5 g of fertilizer (100 mg N), which is a recommended fertilization rate (or standard method) for producing high-quality *Q. variabilis* seedlings (Li et al. 2014). To maintain the same moisture content in the growing medium than the F+ pots, F0 plants were irrigated with 20 ml distilled water on the same day that F+ plants were fertilized. During acorn germination and seedling emergence, the substrates were watered with tap water every 2 days. After emergence finished, seedlings were irrigated with tap water as needed, about twice weekly. From early September to mid-October, the amount of irrigation was reduced to once a week to induce hardening. When seedling emergence started, each tray was evaluated every 3 days for seedling emergence, which was defined as the first visible sign of a shoot (following Tecklin and McCreary 1991).

In mid-October 2014, all seedlings were moved outside to accelerate hardening. When leaves had fallen (3 December 2014), 40 seedlings per treatment (eight seedlings per replication) were randomly sampled: their shoots were cut at the cotyledon insertion point, and roots were carefully washed from the substrate. All seedling parts were oven-dried at 70 °C for at least 48 h and subsequently weighed. To assess seedling tissue nutrient concentration, the individuals of a replicate were combined into a composite sample and separated by organ type. N, P, and K concentration was determined as described above for acorns.

The remaining seedlings were stored outdoors under snow cover until planting in early spring of the following year. Monthly average air temperature during outdoor storage was 0.1 °C in December 2014 and −0.8, −0.5, and

9.7 °C during January, February, and March 2015, respectively.

2.3 Field experiment

In mid-March 2015, seedlings representing all treatments were planted on abandoned fenced cropland at the experimental site of the Beijing Forestry University near Jiufeng Mountain, Beijing (39° 54′ N, 116° 28′ E). The plot was at 112 m a.s.l. and had a slope $< 2\%$. The depth of soil varied between 45 and 60 cm. The surface soil (0–30 cm) was loam (60.4% sand, 31.9% silt, and 7.7% clay) with a pH of 7.7, and soil organic carbon was 0.71%. Average total N was 844 mg kg^{-1}; available P, 112 $\text{mg}\cdot\text{kg}^{-1}$; and available K, 373 $\text{mg}\cdot\text{kg}^{-1}$. The soil was tilled before planting. The climate at the site is characterized by hot, rainy summers and cold, dry winters. The mean annual air temperature was 12.5 °C during 2015 and 2016, while the minimum air temperature during the same period was − 15 °C, and the maximum 36 °C. Annual rainfall was 628.9 mm.

The field experiment was a randomized complete block design with five replications. Each block measured 5 × 9 m and was separated from adjacent blocks by an unplanted buffer zone, 1.5 m wide. Ten seedlings were randomly selected from each nursery replication per treatment and were planted in single parallel rows (10 seedlings × 5 replications × 6 treatments). Seedlings were planted in holes measuring approximately 0.40 × 0.40 × 0.40 m, which were manually dug with a spacing of 1 × 1 m between seedlings. Weeds were removed by hand when necessary.

Survival was assessed in October 2015 and 2016. On 10 November 2016, the stems of all living seedlings were cut at the root collar, washed with tap water and oven-dried at 70 °C for at least 48 h to determine their mass.

2.4 Data analysis

Cumulative emergence versus time for each treatment was modeled using logistic linear regression of the form

$$Y_x = Y_0 + \frac{a}{1 + \left(\frac{x}{b}\right)^c} \tag{2}$$

where Y_x is the total percentage (%) of seedling emergence at time x (days) after sowing; Y_0, the asymptotic value for the model; and a, b, and c, the shape parameters.

Effects of acorn size and fertilization on seedling morphology, nutrient status, and field stem mass were analyzed using a generalized linear model, followed by Duncan's test for multiple comparisons. Normality and variance homogeneity requirements were met, and no data transformation was necessary except for field stem mass, which was Log_{10} transformed. A generalized nonlinear model for binomial distribution and a logit link function were carried out to analyze the effects of acorn size and fertilization on seedling emergence in the nursery, as well as on outplanting survival. Statistical analyses were performed using SPSS 18.0 for Windows (IBM, Chicago, IL, USA), and graphs were created using SigmaPlot 12.5 (Systat Software, San José, CA, USA).

3 Results

3.1 Acorn properties and seedling emergence

Acorn size varied more than five-fold in *Q. variabilis* in this study. Large acorns accounted for a small proportion (18.3%) of all acorns, while there were similar proportions of medium (40.6%) and small (41.1%) acorns (Table 1). Small acorns had 58% of the mass of large ones, while medium acorns had 76% of the mass of large ones. Most variation in acorn fresh weight (76.8%) occurred within trees, while only 0.4% was explained by differences among trees (Tables 2 and 3 in Appendix A). MC of *Q. variabilis* acorns was $> 50\%$, and it increased significantly with size; large acorns had 5% higher MC than small ones. Nutrient content in acorns increased significantly with size (Table 1). Small acorns contained around 45% of the total N and K content and only 32% of the total P content in large acorns. Medium acorns contained intermediate amounts of the three nutrients.

Acorn size significantly affected seedling emergence ($\chi^2 = 201$, $p < 0.001$): final emergence for small acorns was less than half the emergence of large and medium acorns ($> 93\%$, Fig. 1). Seedlings derived from large and medium acorns emerged at similar speed, between days 9 and 27 after sowing, which was faster than small acorns. The time needed for emergence to level off was similar in seedlings from each of the three acorn-size categories.

3.2 Seedling morphology and nutrient status in the nursery

Acorn size and nursery fertilization synergistically increased seedling growth, but there was no interaction between them. Compared to seedlings grown from large and medium acorns, seedlings from small acorns had the lowest values for stem mass ($F = 48.9$, $p < 0.001$), root mass ($F = 7.4$, $p = 0.003$), and total mass ($F = 12.1$, $p < 0.001$; Fig. 2a). Whole-plant mass was 18–23% smaller for small-acorn seedlings than for other seedlings. Conversely, small-acorn seedlings had the highest root-to-shoot ratios ($F = 27.5$, $p < 0.001$; Fig. 2b). Seedlings derived from medium or large acorns were similar in root mass and total mass. However, seedlings from medium acorns had lower stem mass and higher root-to-shoot ratio than

Table 1 Physical characteristics, nutrient content, and moisture content of large, medium, and small *Quercus variabilis* acorns. Fresh weight, length, and diameter measurements were based on data for 220 large, 487 medium, and 493 small acorns. Results for other parameters were based on data for 50 acorns of each size class. All parameters were measured after mixing the acorns of the 20 mother trees and acorn processing by water flotation and air drying

Variables	Acorn size class		
	Small	Medium	Large
Proportion in the batch (%)	41.1	40.6	18.3
Acorn fresh weight (g)	2.88 ± 0.09^{c}	4.18 ± 0.10^{b}	5.52 ± 0.27^{a}
Acorn mass (g)	1.35 ± 0.02^{c}	1.77 ± 0.11^{b}	2.32 ± 0.10^{a}
Acorn moisture content (%)	53.2 ± 1.1^{c}	55.9 ± 0.7^{b}	58.7 ± 0.9^{a}
Nitrogen content (mg acorn^{-1})	13.1 ± 0.2^{c}	21.2 ± 1.4^{b}	29.1 ± 1.2^{a}
Phosphorus content (mg acorn^{-1})	1.74 ± 0.08^{c}	3.75 ± 0.29^{b}	5.38 ± 0.25^{a}
Potassium content (mg acorn^{-1})	13.1 ± 0.3^{c}	21.6 ± 1.4^{b}	30.7 ± 1.7^{a}

Means with different superscript in the same row differ with $p < 0.05$ according to Duncan's test

seedlings from large acorns. Fertilization increased stem mass ($F = 28.3$, $p < 0.001$) but not root mass ($F = 1.16$, $p = 0.29$) or total seedling mass ($F = 3.11$, $p = 0.09$; Fig. 2). Consequently, fertilization led to 15% smaller root-to-shoot ratio ($F = 18.3$, $p < 0.001$).

Acorn size interacted with nursery fertilization to increase seedling concentration of N (fertilization × acorn size, $F = 71.6$, $p < 0.001$; Fig. 3a), P ($F = 3.8$, $p = 0.037$; Fig. 3b), and K ($F = 19.3$, $p < 0.001$; Fig. 3c), as well as N content ($F = 68.8$, $p = 0.002$) and K content ($F = 44.7$, $p = 0.031$). However, this interaction was not found for seedling P content ($F = 14.5$, $p = 0.323$; Fig. 3). Among F0 seedlings, concentrations of N, P, and K were in an average of 37.6%, 43.4%, and 33.2% lower in the small-acorn seedlings than in those derived from acorns in the other two size categories; similarly, content of N, P, and K was 58.1%, 62.1%, and 55.5% lower for small-acorn seedlings than for large-acorn seedlings. Fertilization increased nutrient concentration and content in seedlings cultivated from all acorn sizes, and the increase was greatest in small-acorn seedlings (Fig. 3). As a result,

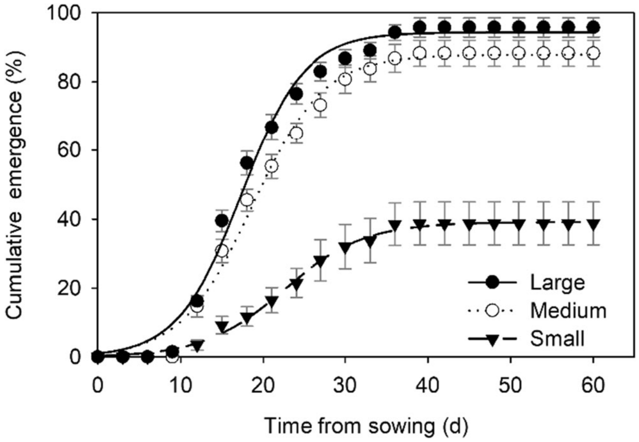

Fig. 1 Cumulative emergence of *Quercus variabilis* seedlings grown from three acorn size classes. Emergence was calculated as the mean of 20 trays for each acorn size (20 acorns per tray). Curves show the logistic regression models adjusted to the raw data

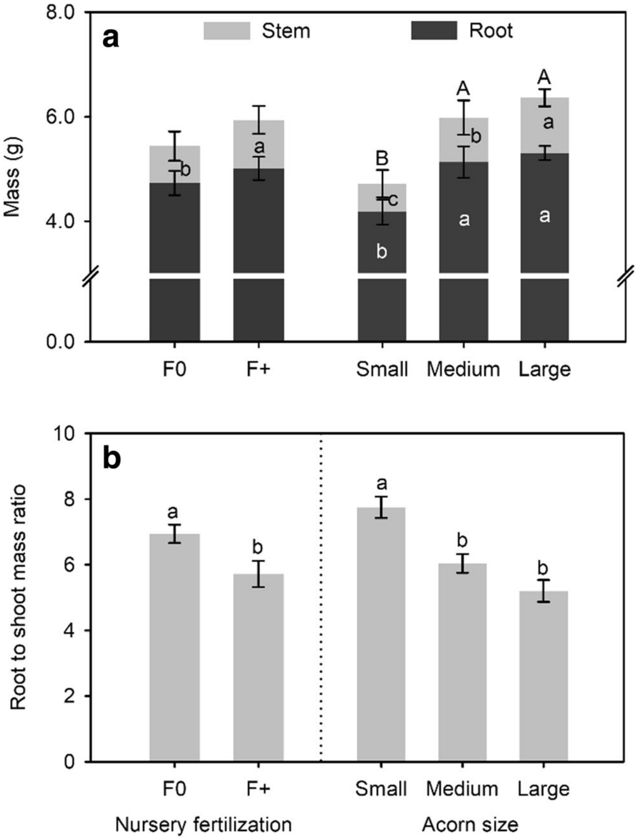

Fig. 2 Main effects of soil fertility and acorn size on the stem, root, and total seedling mass (**a**) as well as the root-to-shoot mass ratio (**b**) (mean ± SE; $n = 40$) of *Quercus variabilis* seedlings after the first growing season in the nursery. Bars with different uppercase letters differ statistically for total mass, while bars with different lowercase letters differ statistically for other variables. Statistical analysis was performed using Duncan's test ($\alpha = 0.05$)

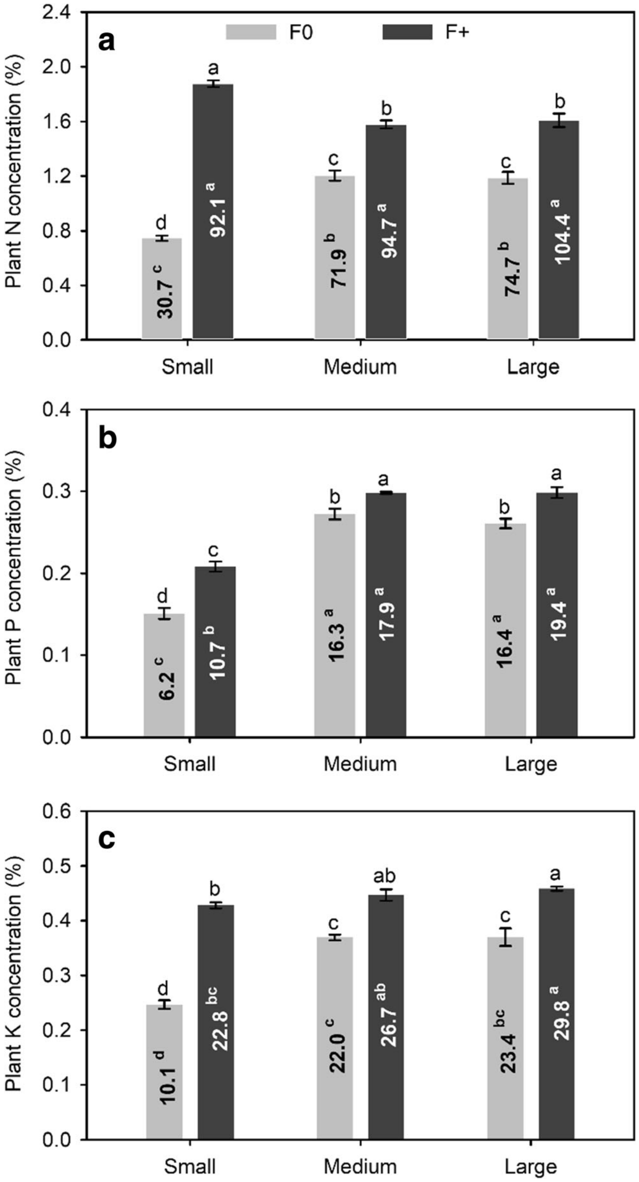

Fig. 3 Effects of nursery fertilization and acorn size on plant N (**a**), P (**b**), and K (**c**) concentration (mean ± SE; $n = 5$) of *Quercus variabilis* seedlings after the first growing season in the nursery. The number inside each column refers to the mean plant content of N, P, or K (mg). Bars and numbers marked with different letters differ statistically according to Duncan's test ($\alpha = 0.05$)

F+ small-acorn seedlings had the highest N concentration but similar K concentration as other F+ seedlings. F+ small-acorn seedlings had lower P concentration than other F+ seedlings, but the differences were smaller than those observed among F0 seedlings.

3.3 Outplanting performance

Outplanting survival was 25–30% lower for the small-acorn seedlings than for the other seedlings at the end of the first ($\chi^2 = 1894$, $p < 0.001$) and second growing season ($\chi^2 = 2383$, $p < 0.001$; Fig. 4a). Medium and large-acorn seedlings had similar survival. In contrast to acorn size, nursery fertilization had no effect on seedling survival (see Table 4 in Appendix B for complete statistical analysis of the survival data).

Stem mass at the end of the second growing season was significantly affected by the interaction between nursery fertilization and acorn size ($F = 13.98$, $p < 0.001$; see Table 5 in Appendix B for complete statistical analysis). Stem mass was significantly smaller for small-acorn seedlings than for medium- and large-acorn seedlings, which did not differ significantly from each other. Nursery fertilization was associated with greater stem mass, and this effect was greatest for small-acorn seedlings (Fig. 4b). Nursery fertilization explained 3.4% of stem mass variance, while acorn size explained 46.3%. In other words, seedling growth after outplanting was much more strongly affected by acorn size than by nursery fertilization.

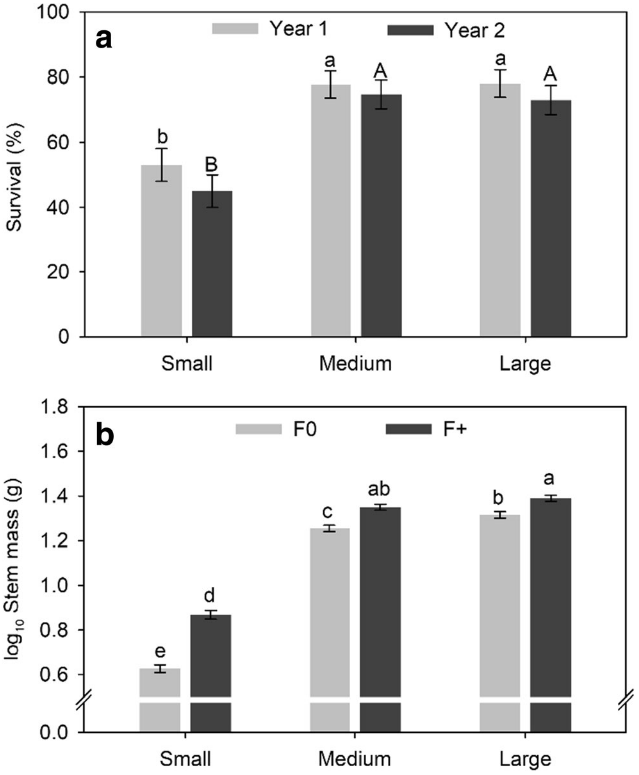

Fig. 4 Field performance of *Quercus variabilis* seedlings in relation to acorn size and fertilization regime used to cultivate the plants in the nursery. **a** Effects on survival during the first and second years (mean ± SE; $n = 100$). Means with different letters differ statistically according to Duncan's test ($\alpha = 0.05$). Lowercase letters refer to survival at the end of year 1; uppercase letters, survival at the end of year 2. **b** Effects on stem mass at the end of the second growing season (mean ± SE). Means with different letters differ statistically according to Duncan's test ($\alpha = 0.05$)

4 Discussion

Our results show that acorn size strongly affects *Quercus variabilis* seedlings in the short term by increasing seedling emergence, growth, and nutrient content in the nursery and in the mid-term by increasing seedling outplanting survival and growth. Irrespective of acorn size, nursery fertilization also increased seedling mineral nutrient content and growth, particularly in small-acorn seedlings. However, fertilization affected seedling attributes much less than acorn size, and it did not offset the poor outplanting performance of small-acorn plants.

Most acorn size variation occurred at the within-tree level, suggesting strong non-genetic maternal effects for this trait. Many studies have shown that seed size variation within species occurs mostly within plants rather than among plants or populations (Willis and Hulme 2004; Pizo et al. 2006). Our finding is consistent with the hypothesis that seed mass variation is largely related to seed position within branches, pods, and fruits (Wulff 1986; Mendez 1997), and it indicates that most variation in *Q. variabilis* acorn size is due to physiological or morphological constraints on resource allocation to seeds within individuals (Moles and Leishman 2008).

4.1 Effects of acorn size and nursery fertilization on seedling emergence and functional attributes

In our study, acorn size strongly increased seedling emergence, growth and nutrient status of *Q. variabilis* plants at the end of nursery cultivation. The use of small acorns resulted in low seedling emergence, which is similar to the findings for other oak species (Tripathi and Khan 1990; Thcklin and McCreary 1991; Pesendorfer 2014; Sánchez-Montes de Oca et al. 2018). It is possible that small acorns in our study stored insufficient resources (Table 1) to support germination and emergence (Flint and Palmblad 1978; Tripathi and Khan 1990), akin to the way in which experimental reduction of cotyledon reserves results in poor seedling emergence (Giertych and Suszka 2011; Liu et al. 2012; Shi et al. 2017). In addition, small acorns in our study had lower MC than medium-sized and large acorns (Table 1). Acorn moisture determines germination, and moisture reduction of only 8–10% from optimum values can hinder germination (Sung et al. 2006; Bonner 2008; Ganatsas and Tsakaldimi 2013). However, MC was high (> 50%) in all acorn size classes in our study, and the greatest difference in MC among the three acorn size classes was 5.5 percentage points, which seems too low to explain the observed large reduction in emergence for small-acorn seedlings (see Connor and Sowa 2003; Ganatsas and Tsakaldimi 2013). Low emergence of small acorns may also be explained by higher concentrations of inhibitory substances (i.e., tannins, polyphenols, abscisic acid, and other chemical inhibitors or plant hormones involved in seed germination and seedling emergence) (Liu et al. 2012).

Seedling growth and nutrient concentration were consistently lower in small-acorn seedlings than in medium or large acorns, which did not differ significantly from each other. This suggests that there is an acorn size threshold below which seedling nutrient status and growth strongly decrease. Poor growth and nutrition of small-acorn seedlings may reflect the smaller amount of resources contained within small acorns (Shi et al. 2017, 2018a) (Table 1).

In accordance with our first hypothesis, nursery fertilization increased the growth and mineral nutrient status of small-acorn seedlings but did not completely compensate for it. Fertilization exerted these effects by strongly increasing plant nutrient concentration, especially N, and also slightly increasing growth. Nevertheless, acorn size was a much stronger determinant than fertilization of *Q. variabilis* seedling functional attributes. It is possible that a higher fertilization rate than the one used in this study (100 mg N plant^{-1} growing season^{-1}) could have increased growth further. Fertilization can be less effective for early oak growth—irrespective of acorn size because seedlings strongly depend on acorn reserves during early life stages (Villar-Salvador et al. 2010; Shi et al. 2018a).

In contrast to its small effect on growth, fertilization was effective at increasing the nutrient content of small-acorn seedlings, reducing the gap in N, P, and K status between small-acorn seedlings and other seedlings. One explanation for the strong nutritional improvement is that the slower growth of small-acorn seedlings translates to less dilution of nutrients with plant growth (Salifu and Timmer 2003). Another potential explanation is that plants will not efficiently absorb nutrients from fertilizer while still drawing from acorn resources (Villar-Salvador et al. 2010; Shi et al. 2018a). Accordingly, it is likely that such resources were exhausted earliest in small-acorn seedlings.

Large- and medium-sized acorns produced seedlings with larger roots, but the proportion of mass allocated to roots was smaller in these plants than in those grown from small acorns. As a result, the root-to-shoot ratio decreased with increasing acorn size, a trend that was probably amplified because seedling roots were confined to small rooting volumes (Climent et al. 2011). Our results are in line with those of Navarro et al. (2006), who showed that acorn weight negatively correlated with the root-to-shoot ratio in *Quercus ilex* L.

4.2 Effects of acorn size and fertilization on outplanting performance

Seedlings derived from small acorns showed consistently lower survival and growth than other seedlings, and this effect lasted for at least two growing seasons. Large seedlings frequently show higher outplanting performance than small ones (see Cuesta et al. 2010; Grossnickle and MacDonald 2018). Villar-Salvador et al. (2012) suggested that larger plants show higher outplanting performance than smaller ones because they can mobilize more resources to support high growth, accelerating seedling establishment, and improving resistance to drought. Similar to our findings, Ramírez-Valiente et al. (2009) reported that *Q. suber* L. seedling survival and growth after planting were positively related to initial seedling size, which in turn was determined by acorn size.

A low root-to-shoot ratio may cause drought stress immediately after transplanting (Grossnickle 2012). Nevertheless, the outplanting performance of medium- and large-acorn seedlings was not hindered by their lower root-to-shoot ratio (Fig. 2b). This may be attributed to high new root production after transplanting (Villar-Salvador et al. 2012), which may offset the increased water stress vulnerability of plants with a low root-to-shoot ratio, although new root growth was not assessed after planting in this study.

Surprisingly, nursery fertilization had no effect on outplanting survival. This contrasts with previous reports showing positive effects of nursery fertilization on outplanting survival of Mediterranean oaks (Villar-Salvador et al. 2004; Villar-Salvador et al. 2013). In contrast to its lack of survival effects in our study, fertilization increased seedling growth by 26% on average. This growth increase was two-fold greater for small-acorn seedlings than for other seedlings (Fig. 4). The disproportionately higher growth in F+ small-acorn seedlings is consistent with their disproportionately higher nutrient accumulation during nursery cultivation.

The ability of fertilization to increase nutrient accumulation and the ability of acorn size to increase outplanting performance highlight the importance of seedling nutrient storage and high resource mobilization for high outplanting growth. At the same time, small-acorn seedlings remained smaller than medium- and large-acorn seedlings even after 2 years. This suggests that, despite the well-established ability of fertilization to improve oak seedling outplanting growth (Villar-Salvador et al. 2004, 2010, 2013; Li et al. 2014; Shi et al. 2017), acorn size has a stronger effect on outplanting performance than nursery fertilization.

5 Conclusions and practical implications

The results of this study demonstrate that the size of the acorn used for nursery cultivation strongly affects outplanting performance of oak seedlings. Best results can be obtained using large or medium acorns together with nursery fertilization. The fertilization rate used in this study only partially offsets the low seedling growth and nutrition and poor outplanting performance of seedlings derived from small acorns. Acorn sorting would increase production cost. However, cultivation costs would also be higher if these acorns are not removed, resulting in a higher proportion of small seedlings that might have to be culled after lifting. Therefore, future studies should address whether higher fertilization rates can further improve nursery growth and nutrition and the outplanting performance of small-acorn seedlings. Because oaks are also cultivated in bareroot nurseries in many regions (Pemán et al. 2017), these studies should be extended to the bareroot production system, where seedlings derived from the small acorns might be exposed to stronger competition due to root intermingling. In the meantime, our results suggest that medium and large acorns should be used in nursery production of *Q. variabilis* seedlings. These acorns should be collected from many trees distant from one another in order to maximize genetic diversity (see recommendations in Burgarella et al. 2007). Small *Q. variabilis* acorns can be left in the forest for animal feeding, helping maintain ecological balance in the forest ecosystem. In years of low acorn production, large acorns with fresh weight ≥ 4.8 g can be left in the forest, given that our results show these acorns to improve plant nursery growth and nutrition and outplanting performance to a similar extent as medium acorns.

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Data availability The datasets generated and analyzed in the current study are available in the Mendeley repository (Shi et al. 2018b). Datasets are not peer-reviewed. Shi et al. (2018b) Data from: Acorn size is more important than nursery fertilization for outplanting performance of *Quercus variabilis* container seedlings. V2. Mendeley Digital Repository. [dataset]. https://doi.org/10.17632/krgxdd2rtp.2

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Appendix A: Acorn weight variation within *Quercus variabilis* trees

Table 2 Mean, minimum and maximum fresh weight of 80 acorns randomly sampled from each of 20 *Quercus variabilis* mother trees, and the proportion of each acorn size class for each mother tree

Tree no.	Acorn fresh weight (g)			Proportion of acorns in each class (%)		
	Mean	Min	Max	Large	Medium	Small
1	4.006	1.993	6.672	21.3	47.5	31.3
2	4.093	2.025	7.026	23.8	52.5	23.8
3	4.115	1.481	6.210	26.3	47.5	26.3
4	3.707	2.109	6.522	11.3	42.5	46.3
5	3.623	1.912	6.457	8.8	37.5	53.8
6	3.969	1.918	6.303	23.8	37.5	38.8
7	3.991	1.536	7.144	22.5	43.8	33.8
8	3.963	1.671	7.687	25.0	31.3	43.8
9	3.957	1.705	7.619	23.8	31.3	45.0
10	3.867	2.095	7.000	15.0	40.0	45.0
11	3.893	1.811	6.964	26.3	35.0	38.8
12	3.559	2.058	6.263	7.5	36.3	56.3
13	3.645	1.882	6.321	8.8	41.3	50.0
14	3.907	2.456	7.081	25.0	38.8	36.3
15	3.822	1.866	7.712	21.3	37.5	41.3
16	3.739	2.049	6.301	7.5	51.3	41.3
17	3.675	2.307	7.448	18.8	37.5	43.8
18	3.691	2.119	6.949	8.8	43.8	47.5
19	3.595	2.004	5.801	15.0	41.3	43.8
20	3.824	2.546	6.301	20.0	42.5	37.5

Table 3 Statistical analysis of among- and within-tree effects on *Quercus variabilis* acorn fresh weight

Variable/Interaction	SS	df	MS	F	p	η^{2} *
Among-trees effect	5.492	19	0.289	1.553	0.060	0.004
Within-tree effect	1036.423	2	518.211	2784.453	<0.001	0.768
Among × Within-tree effect	20.778	38	0.547	2.938	<0.001	0.015
Error	286.608	1540	0.186			

* Calculated as $SS_{between}$ / SS_{total}.

Appendix B: Statistical analysis of the effects of nursery fertilization and size of acorns for seedling cultivation on *Quercus variabilis* seedling survival and growth after planting.

Table 4 Statistical results on the effects of acorn mass (AS), nursery fertilization (NF), field Blocks and their interactions on the survival of *Quercus variabilis* seedling one and two years after planting

Variable/Interaction	df	Survival after year 1		Survival after year 2	
		χ^2	*p*	χ^2	*p*
Acorn mass (AS)	2	1894	**<0.001**	2383	**<0.001**
Nursery fertilization (NF)	1	0.78	0.38	0.009	0.92
Block	4	4845	0.30	304	0.55
AS × NF	2	0.41	0.81	0.85	0.65
AS × Block	8	536	0.72	556	0.69
NF × Block	4	256	0.63	182	0.77
AS × NF × Block	8	839	0.40	135	0.99

Table 5 Statistical results on the effects of acorn mass (AS), nursery fertilization (NF), field Blocks and their interactions on stem mass of *Quercus variabilis* seedling at the end of the second out-planting season

Variable/Interaction	df	SS	MS	F	p
Intercept	1	219	2194	32035	**<0.001**
Acorn mass (AS)	2	10.52	52.6	768	**<0.001**
Nursery fertilization (NF)	1	0.78	0.78	114	**<0.001**
Block	4	0.04	0.01	1.53	0.196
AS × NF	2	0.19	0.096	13.98	**<0.001**
AS × Block	8	0.07	0.01	1.19	0.31
NF × Block	4	0.06	0.02	2.19	0.072
AS × NF × Block	8	0.11	0.01	2.06	**0.043**
Error	160	10.96	0.01		

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