Simulated predation of *Quercus variabilis* acorns impairs nutrient remobilization and seedling performance irrespective of soil fertility

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Abstract

Background and aims. Predators may partially or completely consume Quercus spp. acorns, but effects on nutrient remobilization and seedling performance are poorly understood. We investigated interactions between soil fertility and the removal of Quercus variabilis acorn cotyledons at different early developmental stages on seedling nutrition and development.

Methods. Seedlings were grown in two soils of contrasting fertility and the kinetics of acorn nitrogen, phosphorus and potassium remobilization, and seedling survival, growth and nutrient content were analyzed.

Results. Acorn mass and macronutrients decreased remarkably < 2 weeks after emergence, with nitrogen and phosphorus remobilizing faster than potassium. Acorn removal at or 1 week after emergence inhibited seedling survival, growth and fine root formation, whereas removal from 2-10 weeks after emergence had minor effects. Acorn macronutrient remobilization and effects of acorn removal on seedling performance were not reversed under high soil fertility. When acorns were removed ≥ 2 weeks after emergence, fertilization increased root surface and seedling nitrogen content.

Conclusions. Acorn nutrients are more important than soil nutrients during very early seedling development. Cotyledon damage at emergence impairs seedling performance despite no direct damage to the remainder of the seedling. This effect cannot be reverted by high soil fertility and has potential ecological and practical implications for oak regeneration.

Keywords: Acorn removal; Acorn reserves; Growth; Soil nutrients; Root structure; Survival
Introduction

Oaks are widely distributed in the northern hemisphere where they play important economic and ecological roles (Harper et al. 1970). Oaks produce large seeds (acorns), which implies high resource investment per seed (García-Cebrián et al. 2003) at the expense of producing fewer seeds than small seeded plants (Smith and Fretwell 1974; Coomes and Grubb 2003). Acorns contain large amounts of resources, which are translocated to support seedling development, especially during the early rapid growth stage after germination (Milberg and Lamont 1997; Merouani et al. 2001; Villar-Salvador et al. 2010; Yi et al. 2012; Jha et al. 2014). Knowledge of the ecological value of acorn reserves for oak seedling establishment is important for understanding oak stand regeneration (Esteso-Martínez et al. 2006). Studies addressing acorn mineral nutrient translocation during seedling development have mainly focused on nitrogen (García-Cebrián et al. 2003; Villar-Salvador et al. 2010; Yi and Liu 2014), and very few studies have analyzed the dynamics of other mineral nutrients (see Newton and Pigott 1991). The requirements of seedlings for specific mineral nutrients may vary with developmental stage as the capacity for seedlings to synthesize organic molecules changes over time (Taiz and Zeiger 2010). This may result in varying patterns of acorn nutrient translocation through early seedling development.

Acorns are an important resource for many animals due to their high carbohydrate and fat content (Kabeya and Sakai 2003). Some of these animals also disperse and cache acorns and consequently contribute to the spread and regeneration of oak species (Gómez 2003; Pulido and
However, dispersers may partially or completely eat the acorn cotyledons during dispersal and recovery (Muñoz and Bonal 2007; Perea et al. 2011) but also after acorn germination and during seedling emergence (Bossema 1979; Chang and Zhang 2014; Zhang et al. 2016). Predation of acorn cotyledons after germination reduces seedling performance in most cases but this effect depends on the timing and intensity of cotyledon removal after germination (Andersson and Frost 1996; Bonfil 1998; Branco et al. 2002; García-Cebrián et al. 2003; Suszka 2006; Shi et al. 2017). Cotyledon predation reduces the amount of mineral nutrients available for seedling development. This hinders root development (Milberg and Lamont 1997; Suszka 2006), which in turn can potentially impair soil nutrient uptake and negatively affect seedling nutrient status (Marschner 2012). Root structure and size and plant nutrient status play important roles in seedling survival and growth (Davis and Jacobs 2005; Villar-Salvador et al. 2012; Grossnickle 2012; Oliet et al. 2013). However, the effect of complete cotyledon predation on seedling nutrition and root structure is poorly documented in Quercus species. Moreover, we are aware of no study that has analyzed how complete predation of cotyledons affects the remobilization of acorn mineral nutrients.

Animal dispersers cache acorns into microsites that vary in soil fertility (Sone et al. 2002). For instance, acorns may be cached under shrubs, where soil fertility is higher than in open sites (Moro et al. 1997; Verdú and García-Fayos 2003). High nutrient availability enhances seedling growth, vigor and drought tolerance of Quercus species (Jacobs et al. 2005; Oliet et al. 2009; Villar-Salvador et al. 2013). Soil fertility may also affect acorn nutrient remobilization. Villar-Salvador et al. (2010) showed that low soil fertility enhanced acorn N remobilization, but Milberg and Lamont (1997) found no significant differences in plant mass of large seeded
Hakeas species under contrasting soil nutrient levels.

The main objective of this study was to evaluate the interaction of simulated acorn cotyledon predation and soil fertility on seedling performance and acorn macronutrient remobilization. The oak species, *Quercus variabilis* Blume, which is an ecologically and economically valuable oak and one of the most important afforestation tree species in China (Zhang et al. 2002), was selected for study. The following hypotheses were tested: 1) complete cotyledon predation at the early developmental stage will reduce seedling performance, but no effect will be observed if predation occurs at the late seedling development stages; 2) high soil fertility reduces seedling dependence on acorn reserves by slowing macronutrient remobilization and ameliorating the negative effects of cotyledon predation. To test these hypotheses, we performed an experiment where we simulated cotyledon predation by removing the acorns at different times and grew the emerging seedlings under two contrasting soil fertility conditions. We measured seedling growth and nutrition, root structure and the kinetics of acorn nitrogen (N), phosphorus (P) and potassium (K) reduction.

**Material and Methods**

**Plant Material**

Mature *Q. variabilis* acorns were obtained from five open-pollinated mother trees in early September, 2012 from Sizuolou Forest Farm in Pinggu, Beijing, China (117°148′E, 40°282′N; elevation 316-467 m). Acorns from different trees were mixed and preprocessed as detailed in Li et al. (2014) to select viable acorns. Acorns were air-dried in a single layer on hydrophilic paper at ambient temperature for 24 h. As *Q. variabilis* acorns are recalcitrant (Li et al. 2014), acorns were stored in partially sealed polyethylene bags with a 100 μm wall thickness at 2°C.
and 60% of humidity (Kormanik et al. 1998) until the experiment began the following April.

**Experimental design and treatments**

After 6 months of storage, acorns of similar fresh weight (4.18±0.52 g) were selected for the study and their pericarps were manually stripped to exclude damaged acorns, therefore only seeds remained. A sample of 50 acorns without pericarps were weighed and then oven-dried at 70 °C for 48 h to determine initial acorn mass and moisture, N, P, and K content. The average acorn moisture content and mass was 37.8±4.7 %, and 2.67±0.39 g, respectively while the average acorn N, P, and K content was 23.73±1.22, 3.15±0.46 and 28.85±2.34 mg, respectively.

The remaining acorns without pericarps were immediately sown on 23 April 2013 at a depth of 1-2 cm in cylindrical hard plastic 983 ml containers (D60, Stuewe & Sons, Inc., USA; diameter and depth of 6.4 cm and 36.0 cm, respectively) (one acorn per container) filled with acid-washed sand to prevent any added nutrient supply (Villar-Salvador et al. 2010).

We designed an experiment with two soil fertility (SF) treatments and eight acorn removal times. The soil fertility treatments consisted of an unfertilized soil (low SF) for which no additional fertilizer was provided and a fertilized soil (high SF) that was supplied with fertilizer (20N-20P-20K with micronutrients, Peters Professional Scotts, USA). The high SF pots were fertilized weekly with 20 ml of the fertilization solution mixed using distilled water (2.5 g·L⁻¹) for 10 weeks commencing at the onset of emergence on 30 April. At the end of the study, each high SF pot had received 0.5 g of the fertilizer. The low SF pots were irrigated with 20 ml distilled water with care to avoid water contact with the acorn. The fertilization level for the high SF treatment is known to produce high quality *Q. variabilis* seedlings (Li et al. 2014). All seedlings were watered to field capacity on the morning of the day before fertilizing and again.
four days after. Following the fertilization period, seedlings were watered approximately weekly.

To quantify the remobilization of cotyledon N, P and K reserves during seedling development, acorns were removed from the seedlings at different developmental stages. Germinating acorns were labeled and randomly allocated to one of the following eight treatments that differed in the acorn removal time: Control, where acorns remained intact throughout the study; acorns were removed at the time of emergence when the shoot had reached a length of 5-7 cm and around four leaves had formed but not expanded (0); and acorns removed 1, 2, 4, 6, 8, or 10 week(s) after emergence, respectively. At week 1 after emergence, seedlings had four completely unfolded leaves. Between week 2 to 10 seedlings were in the rapid growth period according to Yang et al. (2012). Most of the seedlings had four to six expanding new leaves at week 2; their first shoot flush completed at week 8; and were beginning their second shoot flush of growth at week 10.

A total of 240 germinating acorns were randomly distributed into the 16 treatments. Containers were placed in a greenhouse equipped with partial climatic control at Beijing Forestry University near Jiufeng Mountain, Beijing (39°54´N, 116°28´E). The day/night temperatures in the greenhouse were approximately 28/16 °C, while the mean air relative humidity was 85.3%. A black shade screen was fixed outside of the greenhouse resulting in an average daily light level inside the greenhouse of 820 μmol·m⁻²·s⁻¹. In mid-October, all seedlings were moved outside to accelerate hardening. Experimental treatments were completely randomized into 12 trays (20 containers per tray). To eliminate edge effects, container positions were rotated once a week.
Measurement of morphology and tissue nutrient concentration

After removal, acorns were gently cleaned with distilled water, and oven-dried at 70°C for 48 h to determine the remaining mass and the N, P and K content. When all leaves had abscised (22 November), 10 seedlings per treatment (only five and six seedlings remained from treatment 0 for which acorns were removed at shoot emergence for low SF and high SF, respectively) were randomly sampled for morphological and nutrient analyses. Seedlings were washed gently to free the growing medium and each plant was excised at the root collar to separate into root and shoot sections. Seedling height was measured as the length of the stem (from the cotyledon insertion point to the tip of the apical bud) and stem diameter (RCD) was measured slightly (2 mm) above the cotyledon insertion point. The roots were divided into sections based on taproot depth: 0-12, 12-24 and 24-36 cm. Root sections were scanned (Epson Expression 1640XL, Canada) and then analyzed with an image analysis system (WinRHIZO, Regent Instruments Canada Inc.). Then, the stem and all roots were oven-dried at 70°C for 48 h to determine their mass. Each of the individual organ samples and previously harvested acorn samples were subsequently ground, sieved through a 0.25 mm screen, and wet-digested using the H₂SO₄-H₂O₂ method of (Oyama et al. 1991). A standard Kjeldahl digestion with water distillation (UDK-152, VelpScientifica, Italy) was used to measure total N. P was determined with a UV-visible spectrophotometer (Agilent 8453, USA) and K was quantified with an atomic emission photometry (SpectrAA 220 Atomic Absorption Spectrometer, VARIN, USA).

Data Calculations

The relative proportion of initial acorn mass and/or nutrient content decrease ($R_i$) between two consecutive acorn removal times ($i$ and $j$) was calculated using Eq. (1):
\[ R_i = 100 \times \frac{(Y_j - Y_i)}{Y_0}, \quad i = 1,2,4,6,8,10; \ j = 0,1,2,4,6,8 \]  
\[
\text{Total} = 100 \times \frac{(Y_0 - Y_i)}{Y_0}
\]

where \( Y_0 \) was the initial acorn mass or nutrient content measured in the sample of 50 acorns measured prior the experiment, \( Y_i \) was the remaining mass or nutrient content at time \( i \) and \( Y_j \) was the remaining mass or nutrient at time \( j \). The total decrease in mass and nutrient content at a specific time \( i \) was calculated as the proportional decrease in the initial acorn mass or N, P or K content occurred up to a specific sampling moment.

 Statistical Analyses

The effects of acorn removal time, soil fertility, and their interaction on acorn mass and nutrient decrease, and seedling shoot and root morphology were analyzed using two-way ANOVA (IBM 18.0, SPSS Statistic, Inc., Chicago, IL, USA). Multiple comparison of means was conducted using a Duncan test at \( \alpha=0.05 \). Normality and variance homogeneity requirements were met and no data transformation was necessary. A generalized nonlinear regression model with binomial distribution and a logit link function were also carried out to estimate the differences in seedling survival among experimental treatments. We also performed linear regression analysis to assess the relationship between nutrient and mass decrease. An Analysis of Covariance was performed to determine whether acorn N, P, and K decreased at the same rate.

The total acorn mass decrease was the covariate, the dependent variable was the total nutrient decrease and the type of nutrient was the independent variable with three levels (N, P and K). The reduction differences among nutrients were assessed by the interaction between the independent variable and the covariate.

All statistical analyses were performed using IBM 18.0 for Windows (SPSS Statistic, Inc.,
Chicago, IL, USA) and graphs were produced using SigmaPlot 12.5 for microcomputers (Systat Software, Inc., USA).

Results

Seedling emergence

Total emergence was 91.7%. Emergence began 10 days after acorn sowing and extended for up to 71 days. According to the logistic function, it took 20 days for 50% shoot emergence, and 30 days for 90% shoot emergence (see Supplementary material Fig A).

Acorn mass and nutrient depletion

Acorn mass significantly decreased through time with no effect due to soil fertility (Table 1). At emergence, 0.53 g of the acorn mass was consumed by seedling development, which represented around 20% of the initial acorn mass (Fig. 1). On week 1 and 2 after emergence, acorn initial mass had decreased by 45.8 and 69.1%, respectively. Subsequently, the decrease of acorn mass slowed and acorns only lost 15% of their initial mass over the next 8 weeks. Ten weeks after shoot emergence, 84.1% of the initial acorn mass had been reduced.

Acorn nutrient content responded to acorn removal time and varied by specific nutrient (Fig. 1 and Table 1). At emergence, acorn N, P, and K content decreased by 11.3, 14.6, and 12.5%, respectively. The highest proportion of N, P, and K reduction (39.2, 38.9 and 28.2%, respectively) occurred during the first week following emergence. At emergence and during the first two weeks after emergence, initial acorn N, P and K decreased by 78.4, 77.8 and 57.9%, respectively. In contrast, from week 3 to week 6 after emergence, the decrease of acorn N and P slowed substantially (9.7 and 13.9% for N and P, respectively) while acorn K continued to decrease rapidly and more than 20% of the initial K content was lost in this period. Six weeks
after emergence, the total decline of acorn N and P leveled off whereas K continued to decrease. At the end of the experiment, the total decrease in initial acorn N, P, and K was 88.3, 91.8 and 87.8%, respectively.

The total acorn nutrient decrease was highly and positively correlated with the total acorn mass decrease (Fig. 2). The velocity of nutrient decrease varied with nutrient type. According to the ANCOVA, the slopes of the acorn N, P and K decrease were not parallel as indicated by the significant interaction between the covariate and the factor (nutrient type) on nutrient total decrease (see Supplementary material Table A).

**Seedling survival, morphology and nutrition**

Acorn removal at emergence strongly reduced seedling survival at the end of the growing season compared with control seedlings ($\chi^2=78.6, P<0.001$). Acorn removal at week 1 after emergence also decreased seedling survival, but the reduction was less intense as removal at emergence. Seedlings for which acorns were removed at week 2 after emergence or later had almost full survival at the end of the study, not differing from the control seedlings. Soil fertility did not significantly affect seedling survival ($\chi^2=0.07, P=0.80$; Fig. 3).

Acorn removal time and soil fertility treatment each affected seedling root and shoot mass, but there was no interaction (Table 2). Seedlings for which acorns were removed at emergence and at week 1 after emergence had significantly lower root and stem mass compared to control seedlings (Fig. 4). No impact on root or shoot mass was observed for seedlings for which acorns were removed two weeks after emergence or later. Acorn removal at emergence and one week later resulted in a higher shoot to root mass ratio than for control seedlings. This was due to a larger impact of acorn removal on root mass than on stem mass. Plants grown under high soil
Acorn removal time and soil fertility interacted to affect root morphology, both for total root surface area and root surface area partitioned by depth (Table 2). Under low SF, root surface area was lower than for the control seedlings only in seedlings for which acorns were removed at emergence (Fig. 5). For the rest of the acorn removal times, low SF plants had significantly higher root surface area than for control seedlings. In contrast, under high SF, root surface area for the seedlings for which acorns were removed up to 4 weeks after emergence was significantly lower than the root surface area of control seedlings. Acorns that were removed 4 weeks or later after emergence had similar total root surface area compared with control seedlings (Fig. 5).

Under low SF, control seedlings did not colonize the bottom of the containers and roots were evenly distributed in the upper and middle portion of the containers (0-24 cm). Surprisingly, seedlings for which acorns were removed at emergence only had roots in the upper portion of the container (depth <12cm) (Fig. 5). For subsequent acorn removal times, there were fewer roots in the upper and middle of the containers and the roots elongated to the bottom of the container. Under high SF, roots of the control seedlings grew to the bottom of the containers, whereas seedlings for which acorns were removed at emergence had 85% of their roots concentrated in the upper part of the containers and no roots were found in the lowest portion of the containers. Removal of acorns later than 4 weeks following emergence did not affect root partitioning in depth compared to control seedlings.

Seedling N content was significantly affected by the interaction between removal time and soil fertility (Table 3). Under low SF, N content was lower in seedlings for which acorns were
removed at emergence compared to control seedlings and the rest of the removal treatments (Fig. 6). Under high SF, seedling N content was significantly lower in seedlings for which acorns were removed at emergence and 1 week after emergence compared to plants of the control and the remainder of the treatments. Under both soil SF conditions, P and K content were lower in seedlings for which acorns were removed at shoot emergence compared to control seedlings and the other acorn removal treatments (Fig. 6). For seedling P and K content, there was no significant interaction between removal time and soil fertility.

Discussion

Acorn nutrient reserve decrease

Most of the initial mass and nutrient content of *Quercus variabilis* acorns decreased within the first two weeks after emergence. Similar rapid changes in mass and N content have also been reported for *Q. robur* L. (García-Cebrián et al. 2003). Most acorn mass decrease was likely due to respiration (Kitajima 2003) and carbohydrate and nutrient remobilization as indicated by acorn nutrient content keeping pace with acorn mass decrease (Fig. 2). It is possible that some nutrients could have been leached from acorns but the intercept values in regressions of total nutrient reduction against total mass reduction were slightly negative for N and K or slightly positive (1.9%) for P, which suggests that leaching was negligible.

Seedlings were mostly comprised of the tap root at emergence, so most nutrients remobilized from the acorn at this stage were used to support tap root growth and stored in the tap root. Previous studies have also shown that the taproot is a main nutrient storage organ during oak seedling establishment (Johnson et al. 2009; Villar-Salvador et al. 2010). Maximum reduction of acorn N, P and K occurred during the first week after emergence (Fig. 1), probably due to
strong sink strength increase linked to foliage unfolding and stem elongation. Root uptake at this stage may not meet the nutrient demand of fast elongating shoots even in high fertilized plants (Johnson et al. 2009; Villar-Salvador et al. 2010), which makes oak seedlings very dependent on acorn reserves. Consistent with this idea, the majority of nutrients were translocated from the acorns within 2 weeks after emergence (accounting for 78.4, 77.8 and 57.9 % of initial acorn N, P and K, respectively). At latter development stages (> 2 weeks), seedlings usually increase fine root growth (Johnson et al. 2009), which allows nutrient uptake to meet a high portion of the seedling nutrient demand (Villar-Salvador et al. 2010). This can explain the reduction in acorn nutrient loss over time.

A major finding of our study was that acorn N and P reserves decreased more rapidly than K reserves at early development stages. Similar to our findings, Newton and Pigott (1991) observed that acorn K was remobilized slower than acorn N and P during early development of Q. robur seedlings. In contrast to our findings, however, Newton and Pigott (1991) found that N was translocated faster than P. They suggested that seedling growth rate drives the mineral nutrient demand and that acorn K reserves are relatively immobile or are little utilized. Potassium availability has a lower impact on growth than that of N and P, which form part of essential plant metabolites (Marschner 2012). Likewise, demand of specific macronutrients differs through ontogeny (Landis 1989; Marschner 2012) and seedlings likely prioritize N and P during the rapid growth stages, while K becomes more important during stress hardening stages latter after emergence.

The remobilization rate of acorn mineral nutrients was independent of soil fertility, which, does not support our second hypothesis. This lack of response was likely due to poor fine root
development at early seedling developmental stages (Harmer 1990; Johnson et al. 2009).

Contrary to our findings, Villar-Salvador et al. (2010) reported that acorn N in *Q. ilex* L. was
depleted faster in seedlings cultivated under extreme low soil fertility.

**Effects of acorn removal and soil fertility on seedling performance**

Consistent with our hypothesis, the impact of acorn removal on *Q. variabilis* seedling
performance decreased with time. Acorn removal within two weeks after emergence, which
deprived seedlings from more than 85 % of acorn nutrient reserves, produced low vigor-
seedlings that consistently had low survival, growth and nutrient content even under high
nutrient supply. Hanley and Fegan (2007) and García-Cebrián et al. (2003) also reported that
seed removal during the seedling establishment phase caused a long-term growth inhibition. In
contrast, Sonesson (1994), did not observe any reduction in the growth of *Q. robur* seedlings
after removing the cotyledons one week after seedlings were 5-10 cm tall. High soil fertility
could not revert the poor seedling performance caused by early acorn removal after emergence
(Figs. 3-6). This result strongly supports that acorn nutrient reserves are indispensable for
emergence and subsequent early growth of *Q. variabilis*. The poor performance of seedlings
for which acorns were removed earlier than 2 weeks after emergence may be due to poor
development of fine lateral roots (Harmer 1990) as suggested by the poor root development in
the middle and lower parts of the containers in these seedlings (Figs. 4-5).

Acorn removal two weeks after emergence had little effect on seedling survival, growth and
nutrient uptake. In *Q. robur*, acorn removal up to 21 days after emergence still reduced seedling
growth (García-Cebrián et al. 2003). Differences between studies may be due to acorn pericarp
stripping in our experiment, which may have accelerated water imbibition and consequently
emergence, but also may be a function of acorn size, species differences, and variable environmental conditions.

The results of this study have both ecological and practical implications for oak regeneration. Cotyledon consumption by scatter hoarding animals (e.g., jays and mice) during seedling emergence impairs seedling survival and growth despite a lack of direct damage to the remainder of the seedling. This may hinder the effectiveness (Schupp et al. 2010) of acorn dispersers and jeopardize recruitment if consumer populations are high. The impact of this process in the field must be tested in future studies. From a practical point of view, nursery fertilization of Q. variabilis seedlings should be delayed until 2 weeks after emergence. Because acorn nutrient supply during this period accounts for 55.4, 51.5, and 52.4 % of the total plant demand in the first year and because the poor development of fine roots hinders uptake of nutrients.

Conclusions

Acorn reserves are critical to the performance of Q. variabilis seedlings up to the second week after emergence. Simulated predation of acorns during this period reduced seedling survival, growth, nutrient content and the proportion of deep roots. High soil nutrient supply does not alleviate the negative effect of acorn removal on seedling performance, indicating that seedlings rely on acorn nutrients for early development. Removal of the acorn later than two weeks after emergence had little effect on seedling development. K remobilization was about 20 % slower than N and P remobilization during this period and nutrient remobilization was independent of soil fertility. Future research should address the importance of early acorn cotyledon predation on Q. variabilis performance and acorn nutrient translocation under field situations (i.e.,
following natural vs. artificial acorn stratification) and its impact on recruitment of oak
seedlings. A series of experiments using a finer scale of acorn removal times, matching the
growth dynamics of *Quercus* seedlings, could also clarify the relationship between acorn
nutrient remobilization and seedling growth.

Compliance with ethical standards

Conflict of Interest: The authors declare that they have no conflict of interest.

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Table 1 Effects of acorn removal time (RT), soil fertility (SF), and their interaction (RT×SF) on *Q. variabilis* acorn relative mass and nutrient (N, P and K) decrease during and after shoot emergence (n=15) indicated by $F$ and ($P$) values derived from ANOVA analyses.

<table>
<thead>
<tr>
<th>Source</th>
<th>Acorn mass decrease</th>
<th>Acorn nutrient decrease</th>
<th>N</th>
<th>P</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>248 (&lt;0.001)</td>
<td>259 (&lt;0.001)</td>
<td>240</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.060 (0.807)</td>
<td>0.215 (0.644)</td>
<td>0.032</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>0.807 (0.971)</td>
<td>0.904 (0.505)</td>
<td>0.024</td>
<td>1.532</td>
<td></td>
</tr>
<tr>
<td>RT×SF</td>
<td>0.505 (0.961)</td>
<td>(1.000)</td>
<td>0.159</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2 Effects of acorn removal time (RT), soil fertility (SF), and their interaction (RT×SF) on *Q. variabilis* root and stem mass, ratio of stem to root mass (S/R), total root surface area and partitioning of total root surface in depth (ratios of top-root, mid-root, bottom-root surface area to total surface area, respectively) (n=10), indicated by *F* and (*P*) values derived from ANOVA analyses

<table>
<thead>
<tr>
<th>Source</th>
<th>Root mass</th>
<th>Stem mass</th>
<th>S/R</th>
<th>Total root surface area</th>
<th>Root surface area partitioning</th>
</tr>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>Top</td>
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<td>RT</td>
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<td>10.6</td>
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<td></td>
<td>(&lt;0.001)</td>
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<tr>
<td>SF</td>
<td>5.33</td>
<td>30.4</td>
<td>6.50</td>
<td>3.64</td>
<td>1.04</td>
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<tr>
<td></td>
<td>(0.022)</td>
<td>(&lt;0.001)</td>
<td>(0.012)</td>
<td>(0.060)</td>
<td>(0.311)</td>
</tr>
<tr>
<td>RT × SF</td>
<td>1.67</td>
<td>1.11</td>
<td>1.32</td>
<td>9.07</td>
<td>4.85</td>
</tr>
<tr>
<td></td>
<td>(0.121)</td>
<td>(0.362)</td>
<td>(0.246)</td>
<td>(&lt;0.001)</td>
<td>(&lt;0.001)</td>
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</tbody>
</table>
Table 3 Effects of acorn removal time (RT), soil fertility (SF), and their interaction (RT×SF) on *Q. variabilis* seedling N, P and K content (n=10), indicated by *F* and *(P)* values derived from ANOVA analyses

<table>
<thead>
<tr>
<th>Source</th>
<th>Seedling nutrient content</th>
<th>N</th>
<th>P</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td></td>
<td>12.5</td>
<td>12.9</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.001)</td>
<td>(&lt;0.001)</td>
<td>(&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td></td>
<td>117</td>
<td>10.9</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.001)</td>
<td>(0.001)</td>
<td>(0.231)</td>
<td></td>
</tr>
<tr>
<td>RT × SF</td>
<td></td>
<td>2.78</td>
<td>1.59</td>
<td>1.84</td>
</tr>
<tr>
<td></td>
<td>(0.010)</td>
<td>(0.144)</td>
<td>(0.084)</td>
<td></td>
</tr>
</tbody>
</table>
**Figure captions**

**Fig. 1** Relative and total proportion of *Q. variabilis* acorn mass, N, P and K decrease (%, ratio to initial) at specific acorn removal times. The relative decrease represents the proportion of initial acorn mass or N, P or K content that is reduced between two consecutive times. Total decrease at a specific time reflects the proportional decrease of initial acorn mass or N, P or K content occurred up to a specific sampling moment. In both cases, the initial values were from a sample of 50 additional acorns on a per replicate basis. 0 – acorn removed at emergence when the shoot had reached a length of 5-7 cm and leaves were starting to expand; 1, 2, 4, 6, 8 and 10 are the week after emergence at which the acorn was removed after emergence. The mean and SE are shown for each removal time. Bars with different letters differ statistically according to Duncan’s test $\alpha=0.05$

**Fig. 2** Linear regressions between the total decrease (%, relative to initial mass and nutrient content values) in *Q. variabilis* acorn N, P and K content and acorn mass. Calculation of total decrease is explained in the legend of Figure 1. Each point represents an individual

**Fig. 3** *Q. variabilis* seedling survival at the end of the growing season for different experimental treatments. C – control seedlings without acorn removal; 0 – acorn removed at emergence when the shoot had reached a length of 5-7 cm and leaves were starting to expand; 1, 2, 4, 6, 8 and 10 indicate the week at which the acorn was removed after emergence. Data are unweighted marginal means ±1 SE
**Fig. 4** Effects of soil fertility and acorn removal time (weeks after emergence) on *Q. variabilis* seedling root mass, stem mass, and ratio of stem to root mass (S/R) (mean and SE) at the end of the growing season. Bars with different letters differ statistically according to Duncan’s test $\alpha = 0.05$ (L and H means low and high soil fertility, respectively; see Figure 3 for explanations of removal time axis).

**Fig. 5** The interaction effects of *Q. variabilis* acorn removal time and soil fertility on total root surface area (cm$^2$) and the partitioning of total root surface at different depths (top: 0–12 cm, mid: 12–24 cm, bottom: 24–36 cm) at the end of the experiment. See Figure 3 for explanations of removal time axis. Data are means ± SE. Means with different letters differ statistically according to Duncan’s test $\alpha = 0.05$.

**Fig. 6** The interaction of acorn removal time and soil fertility on plant N content (upper portion) and the main effects of acorn removal time (weeks after emergence) and soil fertility on plant P and K content (lower portion) of *Q. variabilis* seedlings analyzed at the end of the growing season. See Figure 3 for explanations of removal time axis. Data are means ± SE. Bars with different letters differ statistically within each main effect according to Duncan’s test $\alpha = 0.05$. 
Figure 1

Proportion of acorn mass decrease (% ratio to initial) vs. removal time (weeks after emergence). The data shows a significant increase in mass decrease over time, with peaks at specific removal times indicated by different letters. The graph on the left compares Total vs. Relative decrease, while the graph on the right focuses on acorn P and K. Significant differences are highlighted by different letters above the bars.
Figure 2

- **N**: \( f = -2.5844 + 1.102x \)
  \( R^2 = 0.9345 \)

- **P**: \( f = 1.9059 + 1.0728x \)
  \( R^2 = 0.9377 \)

- **K**: \( f = -8.5025 + 0.9939x \)
  \( R^2 = 0.8476 \)
Figure 3

![Bar chart showing survival (%) of organisms with low and high soil fertility over different removal times in weeks after emergence.](chart.png)
Figure 4

The figure shows the effects of soil fertility and removal time on root mass, stem mass, and S/R ratio. The data is represented with error bars and letters indicate significant differences among treatments.

- **Root mass (g)**: Bars for L and H soil fertility, with different letters indicating significant differences.
- **Stem mass (g)**: Bars for different removal times, with different letters indicating significant differences.
- **S/R** ratio: Bars for different removal times, with different letters indicating significant differences.

The x-axis represents soil fertility (L, H) and removal time (0, 1, 2, 4, 6, 8, 10).
Figure 5

[Graph showing root surface area and root partitioning across different removal times and fertility levels, with letters indicating significance levels.]
Figure 6

The figure shows the effect of soil fertility and removal time on plant N content (top) and plant P content (bottom). The data is presented for low and high fertility conditions. The graphs display columns representing different soil fertility levels (L, H) and removal times (0, 1, 2, 4, 6, 8, 10) with error bars indicating variability. The letters (a, b, c) above the bars denote statistical significance groups, with different letters indicating significant differences.