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

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29 **Abstract**

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

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

37 *Results.* Acorn mass and macronutrients decreased remarkably < 2 weeks after emergence, with  
38 nitrogen and phosphorus remobilizing faster than potassium. Acorn removal at or 1 week after  
39 emergence inhibited seedling survival, growth and fine root formation, whereas removal from  
40 2-10 weeks after emergence had minor effects. Acorn macronutrient remobilization and effects  
41 of acorn removal on seedling performance were not reversed under high soil fertility. When  
42 acorns were removed  $\geq 2$  weeks after emergence, fertilization increased root surface and  
43 seedling nitrogen content.

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

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

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### 53 **Introduction**

54 Oaks are widely distributed in the northern hemisphere where they play important economic  
55 and ecological roles (Harper et al. 1970). Oaks produce large seeds (acorns), which implies high  
56 resource investment per seed (García-Cebrián et al. 2003) at the expense of producing fewer  
57 seeds than small seeded plants (Smith and Fretwell 1974; Coomes and Grubb 2003). Acorns  
58 contain large amounts of resources, which are translocated to support seedling development,  
59 especially during the early rapid growth stage after germination (Milberg and Lamont 1997;  
60 Merouani et al. 2001; Villar-Salvador et al. 2010; Yi et al. 2012; Jha et al. 2014). Knowledge of  
61 the ecological value of acorn reserves for oak seedling establishment is important for  
62 understanding oak stand regeneration (Esteso-Martinez et al. 2006). Studies addressing acorn  
63 mineral nutrient translocation during seedling development have mainly focused on nitrogen  
64 (García-Cebrián et al. 2003; Villar-Salvador et al. 2010; Yi and Liu 2014), and very few studies  
65 have analyzed the dynamics of other mineral nutrients (see Newton and Pigott 1991). The  
66 requirements of seedlings for specific mineral nutrients may vary with developmental stage as  
67 the capacity for seedlings to synthesize organic molecules changes over time (Taiz and Zeiger  
68 2010). This may result in varying patterns of acorn nutrient translocation through early seedling  
69 development.

70 Acorns are an important resource for many animals due to their high carbohydrate and fat  
71 content (Kabeya and Sakai 2003). Some of these animals also disperse and cache acorns and  
72 consequently contribute to the spread and regeneration of oak species (Gómez 2003; Pulido and

73 Díaz 2005; Muñoz et al. 2009). However, dispersers may partially or completely eat the acorn  
74 cotyledons during dispersal and recovery (Muñoz and Bonal 2007; Perea et al. 2011) but also  
75 after acorn germination and during seedling emergence (Bossema 1979; Chang and Zhang 2014;  
76 Zhang et al. 2016). Predation of acorn cotyledons after germination reduces seedling  
77 performance in most cases but this effect depends on the timing and intensity of cotyledon  
78 removal after germination (Andersson and Frost 1996; Bonfil 1998; Branco et al. 2002; García-  
79 Cebrián et al. 2003; Suszka 2006; Shi et al. 2017). Cotyledon predation reduces the amount of  
80 mineral nutrients available for seedling development. This hinders root development (Milberg  
81 and Lamont 1997; Suszka 2006), which in turn can potentially impair soil nutrient uptake and  
82 negatively affect seedling nutrient status (Marschner 2012). Root structure and size and plant  
83 nutrient status play important roles in seedling survival and growth (Davis and Jacobs 2005;  
84 Villar-Salvador et al. 2012; Grossnickle 2012; Oliet et al. 2013). However, the effect of  
85 complete cotyledon predation on seedling nutrition and root structure is poorly documented in  
86 *Quercus* species. Moreover, we are aware of no study that has analyzed how complete predation  
87 of cotyledons affects the remobilization of acorn mineral nutrients.

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

95 *Hakeas* species under contrasting soil nutrient levels.

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

## 109 **Material and Methods**

### 110 **Plant Material**

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

117 and 60% of humidity (Kormanik et al. 1998) until the experiment began the following April.

## 118 **Experimental design and treatments**

119 After 6 months of storage, acorns of similar fresh weight ( $4.18 \pm 0.52$  g) were selected for the  
120 study and their pericarps were manually stripped to exclude damaged acorns, therefore only  
121 seeds remained. A sample of 50 acorns without pericarps were weighed and then oven-dried at  
122  $70$  °C for 48 h to determine initial acorn mass and moisture, N, P, and K content. The average  
123 acorn moisture content and mass was  $37.8 \pm 4.7$  %, and  $2.67 \pm 0.39$  g, respectively while the  
124 average acorn N, P, and K content was  $23.73 \pm 1.22$ ,  $3.15 \pm 0.46$  and  $28.85 \pm 2.34$  mg, respectively.  
125 The remaining acorns without pericarps were immediately sown on 23 April 2013 at a depth of  
126 1-2 cm in cylindrical hard plastic 983 ml containers (D60, Stuewe & Sons, Inc., USA; diameter  
127 and depth of 6.4 cm and 36.0 cm, respectively) (one acorn per container) filled with acid-  
128 washed sand to prevent any added nutrient supply (Villar-Salvador et al. 2010).

129 We designed an experiment with two soil fertility (SF) treatments and eight acorn removal  
130 times. The soil fertility treatments consisted of an unfertilized soil (low SF) for which no  
131 additional fertilizer was provided and a fertilized soil (high SF) that was supplied with fertilizer  
132 (20N-20P-20K with micronutrients, Peters Professional Scotts, USA). The high SF pots were  
133 fertilized weekly with 20 ml of the fertilization solution mixed using distilled water ( $2.5 \text{ g} \cdot \text{L}^{-1}$ )  
134 for 10 weeks commencing at the onset of emergence on 30 April. At the end of the study, each  
135 high SF pot had received 0.5 g of the fertilizer. The low SF pots were irrigated with 20 ml  
136 distilled water with care to avoid water contact with the acorn. The fertilization level for the  
137 high SF treatment is known to produce high quality *Q. variabilis* seedlings (Li et al. 2014). All  
138 seedlings were watered to field capacity on the morning of the day before fertilizing and again



139 four days after. Following the fertilization period, seedlings were watered approximately  
140 weekly.

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

152 A total of 240 germinating acorns were randomly distributed into the 16 treatments.  
153 Containers were placed in a greenhouse equipped with partial climatic control at Beijing  
154 Forestry University near Jiufeng Mountain, Beijing (39°54'N, 116°28'E). The day/night  
155 temperatures in the greenhouse were approximately 28/16 °C, while the mean air relative  
156 humidity was 85.3%. A black shade screen was fixed outside of the greenhouse resulting in an  
157 average daily light level inside the greenhouse of 820  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . In mid-October, all  
158 seedlings were moved outside to accelerate hardening. Experimental treatments were  
159 completely randomized into 12 trays (20 containers per tray). To eliminate edge effects,  
160 container positions were rotated once a week.

## 161 **Measurement of morphology and tissue nutrient concentration**

162 After removal, acorns were gently cleaned with distilled water, and oven-dried at 70°C for 48  
163 h to determine the remaining mass and the N, P and K content. When all leaves had abscised  
164 (22 November), 10 seedlings per treatment (only five and six seedlings remained from treatment  
165 0 for which acorns were removed at shoot emergence for low SF and high SF, respectively)  
166 were randomly sampled for morphological and nutrient analyses. Seedlings were washed gently  
167 to free the growing medium and each plant was excised at the root collar to separate into root  
168 and shoot sections. Seedling height was measured as the length of the stem (from the cotyledon  
169 insertion point to the tip of the apical bud) and stem diameter (RCD) was measured slightly (2  
170 mm) above the cotyledon insertion point. The roots were divided into sections based on taproot  
171 depth: 0-12, 12-24 and 24-36 cm. Root sections were scanned (Epson Expression 1640XL,  
172 Canada) and then analyzed with an image analysis system (WinRHIZO, Regent Instruments  
173 Canada Inc.). Then, the stem and all roots were oven-dried at 70°C for 48 h to determine their  
174 mass. Each of the individual organ samples and previously harvested acorn samples were  
175 subsequently ground, sieved through a 0.25 mm screen, and wet-digested using the H<sub>2</sub>SO<sub>4</sub>-  
176 H<sub>2</sub>O<sub>2</sub> method of (Oyama et al. 1991). A standard Kjeldahl digestion with water distillation  
177 (UDK-152, VelpScientifica, Italy) was used to measure total N. P was determined with a UV-  
178 visible spectrophotometer (Agilent 8453, USA) and K was quantified with an atomic emission  
179 photometry (SpectrAA 220 Atomic Absorption Spectrometer, VARIN, USA).

## 180 **Data Calculations**

181 The relative proportion of initial acorn mass and/or nutrient content decrease ( $R_i$ ) between two  
182 consecutive acorn removal times ( $i$  and  $j$ ) was calculated using Eq. (1):

183  $R_i = 100 \times (Y_j - Y_i) / Y_0, i = 1, 2, 4, 6, 8, 10; j = 0, 1, 2, 4, 6, 8$  (%) (1)

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

189  $Total = 100 \times (Y_0 - Y_i) / Y_0$  (%) (2)

### 190 **Statistical Analyses**

191 The effects of acorn removal time, soil fertility, and their interaction on acorn mass and nutrient  
192 decrease, and seedling shoot and root morphology were analyzed using two-way ANOVA (IBM  
193 18.0, SPSS Statistic, Inc., Chicago, IL, USA). Multiple comparison of means was conducted  
194 using a Duncan test at  $\alpha=0.05$ . Normality and variance homogeneity requirements were met  
195 and no data transformation was necessary. A generalized nonlinear regression model with  
196 binomial distribution and a logit link function were also carried out to estimate the differences  
197 in seedling survival among experimental treatments. We also performed linear regression  
198 analysis to assess the relationship between nutrient and mass decrease. An Analysis of  
199 Covariance was performed to determine whether acorn N, P, and K decreased at the same rate.  
200 The total acorn mass decrease was the covariate, the dependent variable was the total nutrient  
201 decrease and the type of nutrient was the independent variable with three levels (N, P and K).  
202 The reduction differences among nutrients were assessed by the interaction between the  
203 independent variable and the covariate.

204 All statistical analyses were performed using IBM 18.0 for Windows (SPSS Statistic, Inc.,

205 Chicago, IL, USA) and graphs were produced using SigmaPlot 12.5 for microcomputers (Systat  
206 Software, Inc., USA).

## 207 **Results**

### 208 **Seedling emergence**

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

### 212 **Acorn mass and nutrient depletion**

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

219 Acorn nutrient content responded to acorn removal time and varied by specific nutrient (Fig.  
220 1 and Table 1). At emergence, acorn N, P, and K content decreased by 11.3, 14.6, and 12.5%,  
221 respectively. The highest proportion of N, P, and K reduction (39.2, 38.9 and 28.2%,  
222 respectively) occurred during the first week following emergence. At emergence and during the  
223 first two weeks after emergence, initial acorn N, P and K decreased by 78.4, 77.8 and 57.9%,  
224 respectively. In contrast, from week 3 to week 6 after emergence, the decrease of acorn N and  
225 P slowed substantially (9.7 and 13.9% for N and P, respectively) while acorn K continued to  
226 decrease rapidly and more than 20% of the initial K content was lost in this period. Six weeks

227 after emergence, the total decline of acorn N and P leveled off whereas K continued to decrease.  
228 At the end of the experiment, the total decrease in initial acorn N, P, and K was 88.3, 91.8 and  
229 87.8%, respectively.

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

### 235 **Seedling survival, morphology and nutrition**

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

242 Acorn removal time and soil fertility treatment each affected seedling root and shoot mass,  
243 but there was no interaction (Table 2). Seedlings for which acorns were removed at emergence  
244 and at week 1 after emergence had significantly lower root and stem mass compared to control  
245 seedlings (Fig. 4). No impact on root or shoot mass was observed for seedlings for which acorns  
246 were removed two weeks after emergence or later. Acorn removal at emergence and one week  
247 later resulted in a higher shoot to root mass ratio than for control seedlings. This was due to a  
248 larger impact of acorn removal on root mass than on stem mass. Plants grown under high soil

249 fertility were larger and had higher S/R than seedlings grown under low soil fertility.

250 Acorn removal time and soil fertility interacted to affect root morphology, both for total root  
251 surface area and root surface area partitioned by depth (Table 2). Under low SF, root surface  
252 area was lower than for the control seedlings only in seedlings for which acorns were removed  
253 at emergence (Fig. 5). For the rest of the acorn removal times, low SF plants had significantly  
254 higher root surface area than for control seedlings. In contrast, under high SF, root surface area  
255 for the seedlings for which acorns were removed up to 4 weeks after emergence was  
256 significantly lower than the root surface area of control seedlings. Acorns that were removed 4  
257 weeks or later after emergence had similar total root surface area compared with control  
258 seedlings (Fig. 5).

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

269 Seedling N content was significantly affected by the interaction between removal time and soil  
270 fertility (Table 3). Under low SF, N content was lower in seedlings for which acorns were

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

## 278 **Discussion**

### 279 **Acorn nutrient reserve decrease**

280 Most of the initial mass and nutrient content of *Quercus variabilis* acorns decreased within the  
281 first two weeks after emergence. Similar rapid changes in mass and N content have also been  
282 reported for *Q. robur* L. (García-Cebrián et al. 2003). Most acorn mass decrease was likely due  
283 to respiration (Kitajima 2003) and carbohydrate and nutrient remobilization as indicated by  
284 acorn nutrient content keeping pace with acorn mass decrease (Fig. 2). It is possible that some  
285 nutrients could have been leached from acorns but the intercept values in regressions of total  
286 nutrient reduction against total mass reduction were slightly negative for N and K or slightly  
287 positive (1,9%) for P, which suggests that leaching was negligible.

288 Seedlings were mostly comprised of the tap root at emergence, so most nutrients remobilized  
289 from the acorn at this stage were used to support tap root growth and stored in the tap root.  
290 Previous studies have also shown that the taproot is a main nutrient storage organ during oak  
291 seedling establishment (Johnson et al. 2009; Villar-Salvador et al. 2010). Maximum reduction  
292 of acorn N, P and K occurred during the first week after emergence (Fig. 1), probably due to

293 strong sink strength increase linked to foliage unfolding and stem elongation. Root uptake at  
294 this stage may not meet the nutrient demand of fast elongating shoots even in high fertilized  
295 plants (Johnson et al. 2009; Villar-Salvador et al. 2010), which makes oak seedlings very  
296 dependent on acorn reserves. Consistent with this idea, the majority of nutrients were  
297 translocated from the acorns within 2 weeks after emergence (accounting for 78.4, 77.8 and  
298 57.9 % of initial acorn N, P and K, respectively). At latter development stages (> 2 weeks),  
299 seedlings usually increase fine root growth (Johnson et al. 2009), which allows nutrient uptake  
300 to meet a high portion of the seedling nutrient demand (Villar-Salvador et al. 2010). This can  
301 explain the reduction in acorn nutrient loss over time.

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

313 The remobilization rate of acorn mineral nutrients was independent of soil fertility, which,  
314 does not support our second hypothesis. This lack of response was likely due to poor fine root



315 development at early seedling developmental stages (Harmer 1990; Johnson et al. 2009).  
316 Contrary to our findings, Villar-Salvador et al. (2010) reported that acorn N in *Q. ilex* L. was  
317 depleted faster in seedlings cultivated under extreme low soil fertility.

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

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

333 Acorn removal two weeks after emergence had little effect on seedling survival, growth and  
334 nutrient uptake. In *Q. robur*, acorn removal up to 21 days after emergence still reduced seedling  
335 growth (García-Cebrián et al. 2003). Differences between studies may be due to acorn pericarp  
336 stripping in our experiment, which may have accelerated water imbibition and consequently

337 emergence, but also may be a function of acorn size, species differences, and variable  
338 environmental conditions.

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

### 349 **Conclusions**

350 Acorn reserves are critical to the performance of *Q. variabilis* seedlings up to the second week  
351 after emergence. Simulated predation of acorns during this period reduced seedling survival,  
352 growth, nutrient content and the proportion of deep roots. High soil nutrient supply does not  
353 alleviate the negative effect of acorn removal on seedling performance, indicating that seedlings  
354 rely on acorn nutrients for early development. Removal of the acorn later than two weeks after  
355 emergence had little effect on seedling development. K remobilization was about 20 % slower  
356 than N and P remobilization during this period and nutrient remobilization was independent of  
357 soil fertility. Future research should address the importance of early acorn cotyledon predation  
358 on *Q. variabilis* performance and acorn nutrient translocation under field situations (i.e.,

359 following natural vs. artificial acorn stratification) and its impact on recruitment of oak  
360 seedlings. A series of experiments using a finer scale of acorn removal times, matching the  
361 growth dynamics of *Quercus* seedlings, could also clarify the relationship between acorn  
362 nutrient remobilization and seedling growth.

### 363 **Compliance with ethical standards**

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

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508

509

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

Source	Acorn mass	Acorn nutrient decrease		
	decrease	N	P	K
<b>RT</b>	248 (<0.001)	259 (<0.001)	240 (<0.001)	121 (<0.001)
<b>SF</b>	0.060 (0.807)	0.215 (0.644)	0.032 (0.858)	0.001 (0.971)
<b>RT×SF</b>	0.904 (0.505)	0.280 (0.961)	0.024 (1.000)	1.532 (0.159)

513

514 **Table 2** Effects of acorn removal time (RT), soil fertility (SF), and their interaction (RT×SF)  
 515 on *Q. variabilis* root and stem mass, ratio of stem to root mass (S/R), total root surface area and  
 516 partitioning of total root surface in depth (ratios of top-root, mid-root, bottom-root surface area  
 517 to total surface area, respectively) (n=10), indicated by *F* and (*P*) values derived from ANOVA  
 518 analyses

Source	Root mass	Stem mass	S/R	Total root surface area	Root surface area partitioning		
					Top	Mid	Bottom
<b>RT</b>	19.8 (<0.001)	10.8 (<0.001)	10.6 (<0.001)	65.5 (<0.001)	56.1 (<0.001)	33.0 (<0.001)	12.4 (<0.001)
<b>SF</b>	5.33 (0.022)	30.4 (<0.001)	6.50 (0.012)	3.64 (0.060)	1.04 (0.311)	14.9 (<0.001)	17.2 (<0.001)
<b>RT × SF</b>	1.67 (0.121)	1.11 (0.362)	1.32 (0.246)	9.07 (<0.001)	4.85 (<0.001)	3.51 (0.002)	4.53 (<0.001)

519

520 **Table 3** Effects of acorn removal time (RT), soil fertility (SF), and their interaction (RT×SF)  
 521 on *Q. variabilis* seedling N, P and K content (n=10), indicated by *F* and (*P*) values derived from  
 522 ANOVA analyses

Source	Seedling nutrient content		
	N	P	K
<b>RT</b>	12.5 (<0.001)	12.9 (<0.001)	13.8 (<0.001)
<b>SF</b>	117 (<0.001)	10.9 (0.001)	1.45 (0.231)
<b>RT × SF</b>	2.78 (0.010)	1.59 (0.144)	1.84 (0.084)

523

524 **Figure captions**

525 **Fig. 1** Relative and total proportion of *Q. variabilis* acorn mass, N, P and K decrease (% ratio  
526 to initial) at specific acorn removal times. The relative decrease represents the proportion of  
527 initial acorn mass or N, P or K content that is reduced between two consecutive times. Total  
528 decrease at a specific time reflects the proportional decrease of initial acorn mass or N, P or K  
529 content occurred up to a specific sampling moment. In both cases, the initial values were from  
530 a sample of 50 additional acorns on a per replicate basis. 0 – acorn removed at emergence when  
531 the shoot had reached a length of 5-7 cm and leaves were starting to expand; 1, 2, 4, 6, 8 and  
532 10 are the week after emergence at which the acorn was removed after emergence. The mean  
533 and SE are shown for each removal time. Bars with different letters differ statistically according  
534 to Duncan's test  $\alpha=0.05$

535

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

539

540 **Fig. 3** *Q. variabilis* seedling survival at the end of the growing season for different experimental  
541 treatments. C – control seedlings without acorn removal; 0 – acorn removed at emergence when  
542 the shoot had reached a length of 5-7 cm and leaves were starting to expand; 1, 2, 4, 6, 8 and 10  
543 indicate the week at which the acorn was removed after emergence. Data are unweighted  
544 marginal means  $\pm 1$  SE

545

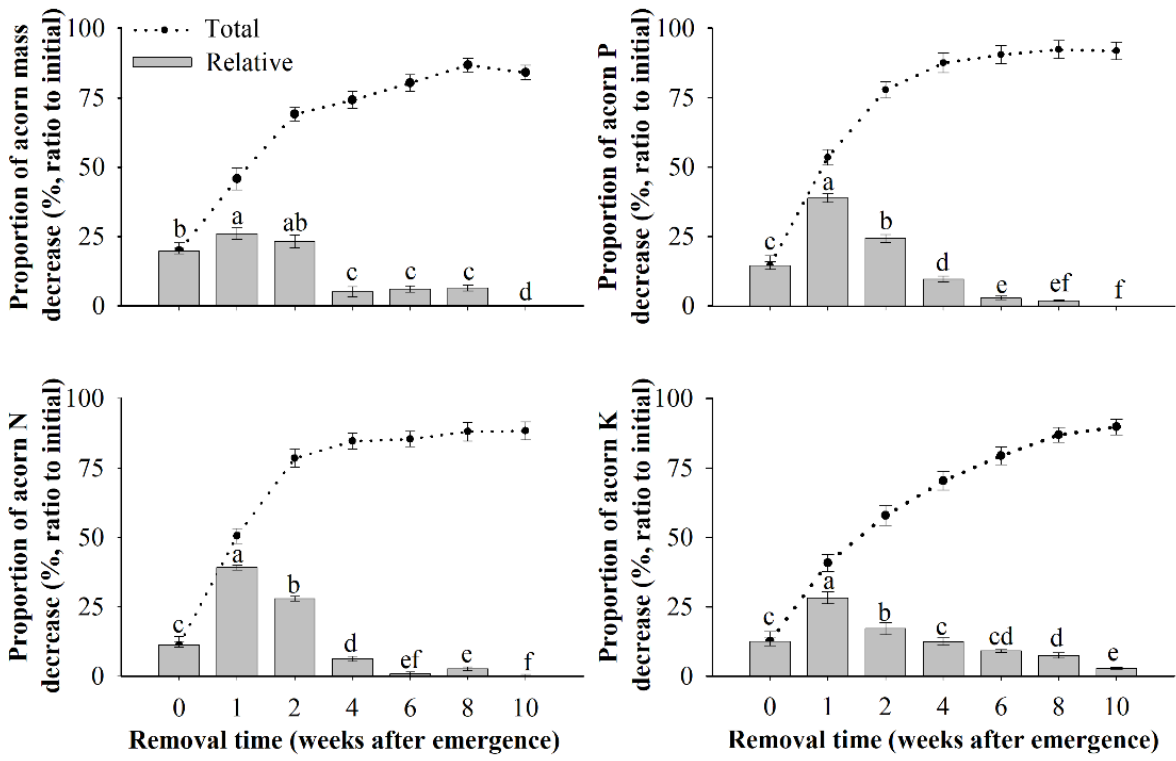
546 **Fig. 4** Effects of soil fertility and acorn removal time (weeks after emergence) on *Q. variabilis*  
547 seedling root mass, stem mass, and ratio of stem to root mass (S/R) (mean and SE) at the end  
548 of the growing season. Bars with different letters differ statistically according to Duncan's test  
549  $\alpha = 0.05$  (L and H means low and high soil fertility, respectively; see Figure 3 for explanations  
550 of removal time axis)

551

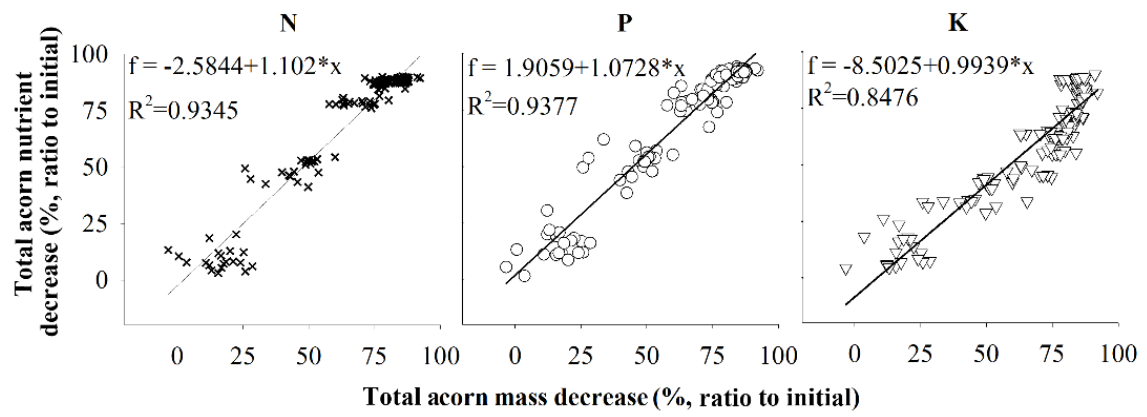
552 **Fig.5** The interaction effects of *Q. variabilis* acorn removal time and soil fertility on total root  
553 surface area (cm<sup>2</sup>) and the partitioning of total root surface at different depths (top: 0~12 cm,  
554 mid: 12~24 cm, bottom-: 24~36 cm) at the end of the experiment. See Figure 3 for explanations  
555 of removal time axis. Data are means  $\pm$  SE. Means with different letters differ statistically  
556 according to Duncan's test  $\alpha = 0.05$

557

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



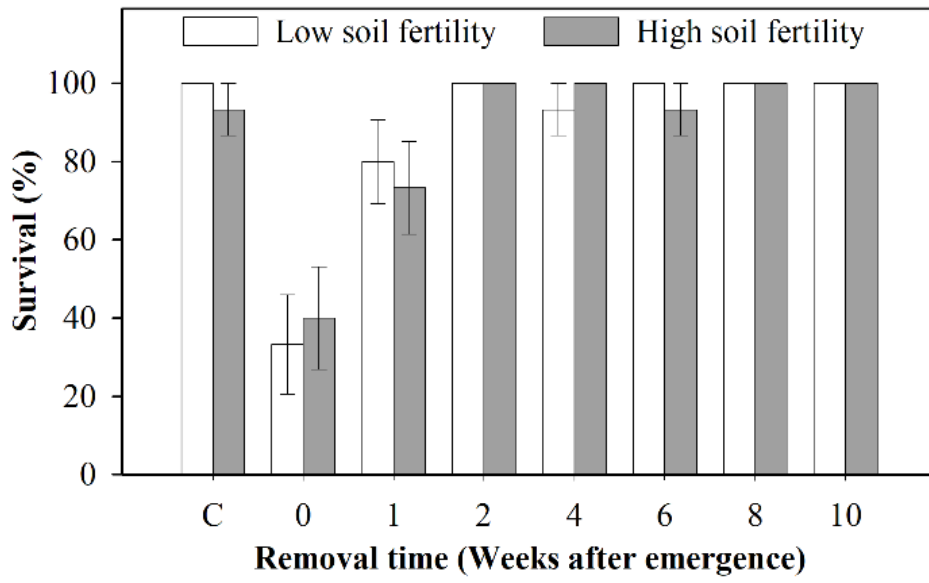
565 Figure 2



566



567 Figure 3



568

