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PLANT-MICROBE-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

Selective predation on acorn weevils by seed-caching Siberian chipmunk *Tamias sibiricus* in a tripartite interaction

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Abstract

Although food-hoarding animals benefit plant seeds by generating predation pressure on granivorous insects, we lack experimental evidence of whether the tripartite interactions maintain a mutualistic relationship between the third trophic level and primary producers. Relying on the behavior of shelling, Siberian chipmunks (*Tamias sibiricus*) selectively consumed weevil larvae infested in acorns of Mongolian oak (*Quercus mongolica*) but chose the non-infested acorns to scatter-hoard. Shelling not only reduced volatile emission from acorns but also decreased cache loss to pilferers, weevil larvae and fungi, allowing *T. sibiricus* to gain more rewards from their caches. Moreover, shelling by *T. sibiricus* enhanced acorn germination and seedling establishment of *Q. mongolica*, possibly due to the diminishment of the negative effects of weevil infestation on acorn viability. Here, we show that both food-hoarding animal *T. sibiricus* and oak *Q. mongolica* can be conditionally benefited from selective predation on weevil larvae inside acorns. Our results highlight the need to integrate the mutualisms between the third and first trophic level into the tripartite interaction model. We suggest that more efforts should be made in the tripartite interactions of food-hoarding animals, seeds and granivorous insects.

Keywords Hoarding animal · Weevil infestation · Cache loss · Acorn selection · Seedling recruitment · Tripartite interaction

Introduction

Pre-dispersal seed predation by insects has been recognized as a major source of seed crop losses in various plant species, imposing strong selective pressure on life-history traits of many plant genera (Herrera 1995; Carlson and Holsinger 2010; Xiao et al. 2016). In temperate deciduous forests, insects including *Curculio* weevils (Coleoptera: Curculionidae), tortricid moths (Lepidoptera: Tortricidae) and cynipids (Hymenoptera: Cynipoidea) attack the dominant tree species including oaks (*Quercus*) and hickories (*Carya*) (Lombardo and McCarthy 2008; Wang et al. 2008; Yi and Zhang 2008). Among these granivorous insects, weevils (*Curculio*) appear to be the main seed predators that cause damage to acorns of many oak species worldwide (Bell and Clark 2016; Higaki 2016; Xiao et al. 2016; Bogdziewicz et al. 2018).

Natural history observations show that adult females of the genus *Curculio* use their long rostrum to drill a hole in the pericarp of an acorn at its early stage of development. After the eggs are oviposited through the hole in late summer, the hatched larvae consume the meat in acorns for 2–6 weeks before exiting the acorns (Venner et al. 2011; Pélisson et al. 2012). Previous studies have shown that weevils may cause severe damage to a significant proportion of the acorn crop in many oak species, resulting in loss of > 70% of the acorn crop (Wang et al. 2008; Espelta et al. 2009; Xiao et al. 2016). The high level of infestation by

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weevils is expected to impact the oak reproductive output because the tunnels and granular frass in the acorn left by the feeding larvae have the potential to prohibit acorn germination and seedling establishment (Weckerly et al. 1989; Bonal et al. 2007; Lombardo and McCarthy 2009). Moreover, consumption of acorn cotyledons by weevil larvae reduces a considerable amount of reserves that are essential for early development of 1-year seedlings (Bonal and Muñoz 2008, 2009; Hou et al. 2010; Bartlow et al. 2018). An increasing body of literature has shown that acorns damaged by weevils experience lower germination compared to the uninfested ones (Bonal and Muñoz 2007; Guo et al. 2009; Hou et al. 2010), indicating that pre-dispersal acorn predation by granivorous insects is one of the most decisive factors constraining the natural regeneration of oaks (Nakamura et al. 2013; Udaka and Sinclair 2014; Xiao et al. 2016).

Plants can attract specific predators and parasitoids to control or kill the phytophagous insects infested in their propagules (Dixon et al. 1997; Bonal and Muñoz 2007; Madeira et al. 2013). The interactions between plants, phytophagous insects and predators represent a typical tri-trophic system, in which the third level (predators) has an indirect positive effect on the first level (plants) (Wootton 1994; Bonal and Muñoz 2007; Grinath et al. 2015). Most oak species (e.g., red and white oaks) produce large acorns containing high nutrient amounts that are attractive to a number of seed predators including rodents, jays and ungulates (Bonal and Muñoz 2007; Neuschulz et al. 2014; Yi and Wang 2015; Zhang et al. 2016a). Several studies have reported that infested acorns are avoided by mammalian and avian seed consumers (Bossema 1979; Hubbard and McPherson 1997; Bonal and Muñoz 2007), possibly due to their lower nutritional value and/or reduced palatability. Other studies have found that small mammals appear to be unable to distinguish between infested and uninfested seeds and handle them similarly (Semel and Andersen 1988; Weckerly et al. 1989; Sone et al. 2016), indicating that seed predators relying heavily on acorns for long-term energy requirements may have the potential to control weevil infestation in oaks in the tripartite interactions. Other observations have shown that rodents and jays can distinguish between the infested and non-infested acorns and prefer to consume the infested acorns (Johnson et al. 1993; Perea et al. 2012a, b; Yi et al. 2012; Zhang et al. 2014), possibly because weevils in the infested acorns ameliorate the negative dietary effects of acorn tannins on food-hoarding animals. These observations collectively indicate that infestation of acorns is likely to be a significant factor influencing the foraging behavior of many seed consumers. Although predation on weevils by granivores may reduce infestation of oaks (Bonal and Muñoz 2007; Perea et al. 2012a), to our knowledge, no evidence has shown that this suppression of weevils directly benefits seedling establishment of oaks. Moreover, we lack experimental evidence of whether the tripartite interaction forms a conditional mutualism between the third level (food-hoarding animals) and the first one (oak acorns) though predators often suppress herbivores and indirectly benefit plants in the tri-trophic interactions (Terborgh and Estes 2010; Estes et al. 2011; Visser et al. 2011).

To answer this question, we used a tripartite interaction between Mongolian oak *Quercus mongolica*, weevils *Curculio arakawai* and Siberian chipmunks *Tamias sibiricus*, in which *T. sibiricus* ubiquitously shell *Q. mongolica* acorns before caching (Yi et al. 2012). Shelling helps *T. sibiricus* to selectively consume weevils in the infested acorns and then choose the non-infested acorns for scatter-hoarding. Moreover, shelling by *T. sibiricus* is also expected to change emission of volatile compounds from acorns and may alter cache detectability by hoarders and pilferers because seed odor plays an important role in affecting cache pilfering (Yi et al. 2016a). In this context, *T. sibiricus* is expected to gain more rewards from their caches and the buried acorns have more opportunities to survive and establish seedlings. Our experiments may provide first evidence that predation on weevil larvae maintains a delicate mutualism between oaks and food-hoarding animals in a tripartite interaction.

Materials and methods

Study site and animal handling

The study was conducted in the Dongfanghong Forestry Center (mean elevation of 750 m, 45°58′N, 129°08′E) in the Dailing District, Yichun City, Heilongjiang Province, northeast China. To trap *T. sibiricus* and *Apodemus peninsulae* for enclosure experiments, SJL601 steel-framed live-traps (9 cm × 10 cm × 25 cm, manufactured by Sichuan Shujile Company, Sichuan, China) baited with peanuts and carrots were placed in forests at 5-m intervals along four transects at 8:00 a.m. All traps were pre-baited for 1 day and protected from predators by wrapping with steel mesh. Traps were checked twice daily in the early morning and late afternoon for 6 consecutive days. Trapping stopped when encountering bad weather (e.g., heavy rainfall). The captured animals were transported to the animal housing room and kept in frame cages (30 cm × 40 cm × 50 cm) individually at a range of temperatures (day 15–20 °C, night 10–15 °C) and natural photoperiod (about 14 light hours). They were provided with nesting boxes, carrots, peanuts, seeds and water ad libitum. No animals died during the field trapping.

Enclosures

We conducted behavioral experiments in separate enclosures (10 × 10 × 2.5 m) established in an open, non-forested area

(Fig. S1; Yi et al. 2016b). Brick enclosures were covered with concrete and were extended about 2.5 m above ground and 0.5 m below the soil surface. The top of the enclosure was covered with plastic nettings to prevent access to predators. An artificial burrow was provided at one corner of each enclosure, and an arena was established at the center of each enclosure. The ground in enclosures was paved with bricks to create 64 shallow pits (brick length × width × depth: 24 × 12 × 6 cm) evenly spaced in an 8 by 8 grid. A piece of iron sheet was laid beneath each of the pits to prevent animal burrowing. These small shallow pits were filled with fine sand to allow scatter-hoarding animals to bury acorns. We also established artificial caches in these shallow pits to study cache pilferage by acorn-eating animals (*T. sibiricus* and *A. peninsulae*).

Scatter-hoarding of the infested and non-infested acorns by Siberian chipmunks in the enclosures

To test how *T. sibiricus* selectively consumed weevil-damaged acorns and cache non-infested acorns after removing the pericarps of *Q. mongolica* acorns, we provided each of eight chipmunks with 15 paired infested and non-infested acorns. Water flotation and visual inspection were used to determine whether the acorns were infested or not (Xiao et al. 2003), because X-ray equipment was unavailable in the field. Different from previous tagging methods (Cao et al. 2016; Chen et al. 2017), a 1-cm-long nail was inserted into acorns for easy identification (Yi and Wang 2015). However, acorns with apparent emergence holes were discarded for experiments. Selection of acorns by each chipmunk was tested in a separate enclosure. In the morning (0700 h), 15 paired infested and non-infested acorns were placed at the seed station in each enclosure. Then, an individual *T. sibiricus* was introduced into each enclosure and allowed to eat and cache freely for 11 h. At 1800 h, we live trapped *T. sibiricus* and searched the whole enclosure to record the number of each type of acorns that were removed, eaten, or scatter-hoarded. Seed fates were categorized into intact in situ (IIS), eaten in situ (EIS), eaten after removal (EAR), intact after removal (IAR), scatter-hoarded (SH), and larder-hoarded (LH) (Gu et al. 2017). We also presented 3 g of live weevils to each of 13 indoor caged chipmunks, to test whether ad lib-feeding *T. sibiricus* would use weevils for food.

Scatter-hoarding of the infested and non-infested acorns in the field

To test whether small rodents differently handled the infested and non-infested acorns in the field, we placed 15 paired infested and non-infested acorns in each of 10 seed stations (1 m × 1 m) scattered 30 m apart in a broad-leaved deciduous forest with abundant *T. sibiricus*. Each acorn was labeled for easy relocation by attaching onto a small plastic tag (Wróbel and Zwolak 2017). When the tagged acorn was buried by the scatter-hoarding animals, the attached tag facilitates recovery of the buried acorns by researchers (Zhang et al. 2017). Seed consumption and seed removal were checked every day for 10 days until all acorns were removed from the seed stations. Then, we searched the area around each seed station (radius = 15 m) to locate the removed acorns. Seed fates were categorized into eaten in situ (EIS), eaten after removal (EAR), intact after removal (IAR), scatter-hoarded (SH), and missing (M). The shelled acorns referred to those that were pericarp-removed by *T. sibiricus*.

Volatile emission from the intact and shelled acorns

To test if shelling by *T. sibiricus* affected volatile profiles of mature acorns, we selected nine intact and nine shelled acorns of *Q. mongolica* with similar sizes for measurement. The shelled acorns were randomly collected from the scatter-hoards of *T. sibiricus* in the enclosure experiments, while the intact acorns were randomly selected from a composite sample collected from the field. For the intact acorns, three of them were initially sealed in each of three gas chromatography (GC) headspace vials (Daobang Technology, Nanjing, China) and stored at 4°C for 24 h before headspace analyses. A solid phase microextraction (SPME) device was used for sampling (extraction temperature: 50 °C; balance period: 15 min; extraction period: 30 min). The same procedure was applied for the shelled acorns. Then, volatile compounds were analyzed using an Agilent 7697A headspace autosampler + Agilent 7890-5975 GC–MS (Agilent Technologies. Inc. USA) in the Environmental Protection Department of Jiangxi Province (Nanchang, China) (Yi et al. 2016a). Blanks were not measured because we intended to compare the differences between the intact and shelled acorns at the same condition. The volatiles were separated using GC on an HP-5MS column (30 m length, 0.32 mm internal diameter, 0.25 μm film thickness; Agilent Technologies. Inc. UAS) running a temperature program (4 min hold at 40 °C, 5 °C per min to 100 °C, 10 °C per min to 200 °C and 2 min hold; helium carrier gas at constant flow rate of 1 ml per min). The volatile compounds were putatively identified from the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) mass spectral database because retention time of each component was not measured with their standards. Internal standards were also not used. Therefore, seed volatile concentration was expressed as abundance of normalized peak area proportion of a given putative compound.

Pilferage of the intact and shelled acorns

To test whether shelling by *T. sibiricus* reduced cache pilferage or recovery, we prepared artificial caches buried with intact or chipmunk-shelled acorns (i.e., pericarp removed by *T. sibiricus*) and then recorded pilferage rates by individual *T. sibiricus* and another sympatric hoarding animal Korean wood mice *A. peninsulae* in the enclosures. To do so, 32 intact and 32 shelled acorns were individually buried in randomly selected pits in the 8 × 8 grid in each of the enclosures. *T. sibiricus* (6♀, 6♂) or wood mice (5♀, 7♂) were individually introduced into the enclosures for free searching for the buried acorns. For *T. sibiricus*, artificial caches were created in the morning and the pilferage rate was checked in the afternoon. For *A. peninsulae*, however, caches were established in the evening and the pilferage rate was checked next morning. The pilfering rates of the intact and shelled acorns were recorded 8 h after cache establishment by the experimenters. We also established ten paired caches containing intact and shelled acorns in each of 30 plots (1 m × 1 m) scattered in a broad-leaved deciduous forest. These artificial caches were checked every day for 10 days to test if cache pilfering rates differed between the intact and shelled acorns.

Effect of shelling on microbial attack on acorns

To test if shelling by *T. sibiricus* reduced cache loss to microbial attack, we randomly selected 20 intact acorns and 20 chipmunk-shelled acorns free of weevils to test. Twenty flowerpots (diameter = 20 cm, height = 15 cm) each prepared with forest soil were divided into two groups, and two acorns of each treatment (intact and shelled) were randomly sown 1 cm deep in each of the ten pots. Forest soil was directly collected under the *Q. mongolica* trees in the field and randomly placed into the 20 flowerpots. All the pots were kept in natural conditions for 50 days to check the possibility of microbial attack on acorns. Acorns covered by hyphae were considered attacked by microbes; however, germinating acorns and those free of hyphae were regarded as healthy.

Seedling establishment from the intact and shelled acorns

To test if shelling by the chipmunks influenced seedling establishment of acorns, we randomly selected 50 intact acorns in a composite sample for germination experiments. Moreover, 50 shelled non-infested acorns were selected because chipmunk-shelled acorns in caches appeared to be free of weevils (Yi et al. 2012). This deployment allowed us to know the joint effects of pericarp removal and weevil elimination by shelling on acorn germination. Five intact acorns were evenly sown 1 cm deep in each of ten flowerpots (diameter = 20 cm, height = 15 cm) filled with forest soil collected for the field. The same procedure was applied for the shelled acorns as well. All the containers were kept in room conditions (14:10 light:dark cycle, 22–25 °C, 40% humidity). All flowerpots were randomly moved every 5 days to eliminate any possibility of a position effect on acorn germination and seedling growth in the experiment. Acorn germination was checked every day for 35 days to test if it was affected by shelling by *T. sibiricus*. The proportion of germination was determined after 50 days of cultivation to test if shelling by *T. sibiricus* benefited seedling establishment. Leaf number, dry mass of shoots and roots were measured at the end of cultivation to test if shelling affected seedling development. We also buried ten paired intact and shelled acorns in each of ten rodent-proof plots (1 m × 1 m) scattered in a broad-leaved deciduous forest in 2015. All plots were checked in the next spring to test if shelling by *T. sibiricus* influenced seedling establishment from the two types of acorns in the field.

Data analysis

Data analyses were performed by *base, lme4, vegan* and *survival* packages in R software (3.4.1) (R Core team 2014). Generalized linear mixed models (GLMMs) were applied with seed fates as response variables (e.g., scatter-hoarded versus all other fates), and rodent individuals as random effects for data from enclosures (Table S1). GLMMs were also applied with seed fates as response variables (e.g., scatter-hoarded versus all other fates), and stations as random effects for data from fields (Table S2). We performed non-metric multidimensional scaling (NMDS) to visualize the differences in the volatile profiles between the intact and shelled acorns, and ADONIS to test the statistical significance of the difference (Table S3). Kruskal–Wallis test was performed to test the difference of each putative compound between the intact and shelled acorns (Table S4). For GLMMs with cache pilferage rates from enclosures as response variables (pilfered vs not pilfered), we used acorn type and rodent species as fixed effects, and rodent individual nested within rodent species as random effects (Table S5). GLM was performed with cache pilferage rates from field as response variables (pilfered vs not pilfered) and acorn type as the only fixed effect (Table S5). Chi-square tests were used to test if shelling decreased the possibility of microbial attack. Seedling establishment (proportion of germinated seeds) was analyzed by generalized linear model (GLM) with acorn type as fixed effect (Table S6). Cox proportional hazards regression model was also performed for testing the difference of germination rate between acorn types. Seedling growth data including germinate time, shoot height, leaf number, dry mass of shoots, dry mass of roots and the root to shoot ratio were tested by MANOVA (Table S7).

Results

Scatter-hoarding of uninfested and infested acorns

In the enclosures, *T. sibiricus* showed no preference for either non-infested or infested at the seed stations ($z = -1.57$, $P = 0.117$), as seen from the acorns that remained intact in situ (Fig. 1a). However, more infested acorns were eaten in situ (EIS) by *T. sibiricus* compared to the non-infested acorns ($z = -6.826$, $P < 0.001$). Acorns were all shelled before scatter- or larder-hoarding by *T. sibiricus*. Interestingly, *T. sibiricus* preferred to scatter-hoard non-infested acorns over infested acorns ($z = 334.50$, $P < 0.001$). Although a few acorns were larder-hoarded, no difference was found between larder-hoarding of the infested and non-infested acorns ($z = 0.484$, $P = 0.629$). Moreover, we found acorns in the scatter-hoards were universally shelled by *T. sibiricus* and were free of infestation. We found no weevil larvae either at seed station or in the scatter-hoards and larders of *T. sibiricus*. Moreover, all individuals in the cages consumed 3 g of larvae within 10 min, albeit they were fed ad lib. Consistent with the results in the enclosures, non-infested acorns in the field were more likely to be removed and scatter-hoarded by food-hoarding animals ($z = 2.665$, $P = 0.008$) (Fig. 1b). However, the infested acorns were more likely to be consumed at seed stations by food-hoarding animals ($z = -7.464$, $P < 0.001$) (Fig. 1b).

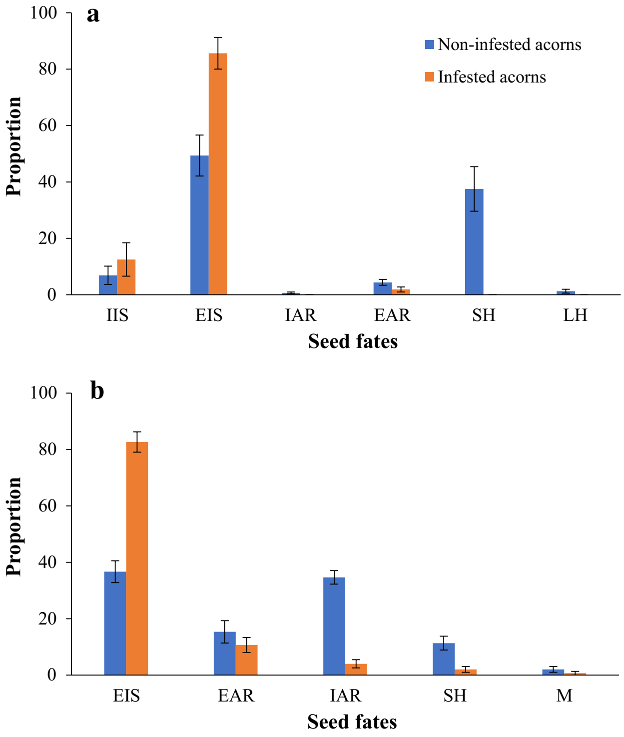

Fig. 1 Seed fates of the infested and non-infested acorns in **a** enclosure experiments ($n = 8$) and **b** field experiments ($n = 10$). IIS, EIS, EAR, IAR, SH, LH, and M stand for seeds intact in situ, eaten in situ, eaten after removal, intact after removal, scatter-hoarded, larder-hoarded, and missing, respectively. Data are expressed as mean ± SE

Effects of shelling on volatile emission and cache pilferage

We detected some putative volatile compounds both in the intact and shelled acorns of *Q. mongolica* (Table 1). NMDS of the different compounds based on Bray–Curtis distance is presented in Fig. 2. Probably due to small sample size, the difference in the volatile profiles of intact and shelled acorns was not statistically different (ADONIS, $F_{1,4} = 3.267$, $P = 0.1$). However, Kruskal–Wallis test showed significant differences in several compounds between the two types of acorns (Table S4). Shelled acorns were less likely to be pilfered than the intact non-infested acorns in the enclosures ($z = -2.952$, $P = 0.003$) (Fig. 3a). Moreover, *T. sibiricus* and *A. peninsulae* showed different pilfering abilities in the enclosures ($z = 2.025$, $P = 0.043$). In the field, however, we found no significant difference in the pilferage rates between the intact and infested acorns ($z = -0.977$, $P = 0.329$) (Fig. 3b).

Effects of shelling on microbial attack and seedling establishment

Shelling by *T. sibiricus* significantly reduced the possibility of microbial attack on acorns of *Q. mongolica*. Intact acorns were more likely to be attacked by fungi and bacteria compared to the shelled acorns (15 vs 45%; $\chi^2 = 8.067$, $df = 1$, $P = 0.005$). Although the shelled acorns did not germinate faster ($F = 2.379$, $P = 0.128$) (Fig. 4a), they showed higher germination rate (Cox regression, $z = 0.476$, $P < 0.001$) and proportion of germination (48 vs 88%, $z = 3.992$, $P < 0.001$) than the intact acorns (Fig. 4b). We also found that shelling benefited seedling growth of *Q. mongolica* in the lab experiments, as seen from the differences in shoot height ($F_{1,66} = 14.007$, $P < 0.001$), leaf number ($F_{1,66} = 6.0352$, $P = 0.017$), dry mass of shoots ($F_{1,66} = 10.276$, $P = 0.002$), dry mass of roots ($F_{1,66} = 8.7607$, $P = 0.004$), and the root to shoot ratio ($F_{1,66} = 11.275$, $P = 0.001$) (Table 2). In congruence with the increase in seedling establishment by shelling in the lab, we found that seedling establishment from the shelled acorns was higher than that from the intact acorns in the field ($z = 6.043$, $P < 0.001$).

Discussion

Our results showed that *T. sibiricus* selectively consumed the infested acorns along with the weevil larvae inside. Meanwhile, the non-infested acorns were more likely to be

Table 1 Volatile compounds emitted from the intact ($n = 3$) and shelled acorns ($n = 3$) of *Quercus mongolica*

Putative volatile compounds	Peak area (%)		Retention time (min)
	Intact acorns	Shelled acorns	
Acetoin	2.76 ± 0.73^{a}	0.29 ± 0.17^{b}	3.29
1-Butanol, 3-methyl-	10.17 ± 2.78^{a}	0.72 ± 0.33^{b}	3.83
1-Butanol, 2-methyl-	5.82 ± 1.83^{a}	0.22 ± 0.04^{b}	3.92
2,3-Butanediol	4.86 ± 0.64^{a}	0.14 ± 0.06^{b}	4.98
2-Hexanone	0.19 ± 0.19^{a}	0.25 ± 0.19^{a}	5.12
3-Hexanol	0.25 ± 0.13^{a}	2.17 ± 1.14^{a}	5.37
2-Hexanol	0.58 ± 0.30^{a}	0.33 ± 0.19^{a}	5.50
1,5-Dimethyl-6-oxa-bicyclo[3.1.0]hexane	0.51 ± 0.17^{a}	0.18 ± 0.11^{a}	5.93
Bicyclo[4.2.0]octa-1,3,5-triene	16.85 ± 7.00^{a}	15.83 ± 8.55^{a}	8.53
Benzene, tert-butyl-	4.63 ± 0.19^{a}	0.16 ± 0.03^{b}	13.92
2,6-Octadien-1-ol, 3,7-dimethyl-, propanoate	1.49 ± 0.07^{a}	0.75 ± 0.17^{b}	14.13
Cyclopropanecarboxylic acid, pentadecyl ester	0.29 ± 0.25^{a}	0.37 ± 0.18^{a}	15.14
Acetophenone	0.54 ± 0.39^{a}	0.24 ± 0.07^{a}	15.53
Trans-linalool oxide	0.43 ± 0.19^{a}	0.21 ± 0.07^{a}	15.80
Phenylethyl alcohol	1.89 ± 1.00^{a}	2.89 ± 1.38^{a}	17.40
Azulene	2.60 ± 0.23^{a}	0.35 ± 0.04^{b}	20.25
Decanal	0.24 ± 0.06^{a}	0.24 ± 0.07^{a}	21.16
Cyclohexanone, 2-acetyl-	0.40 ± 0.23^{a}	0.29 ± 0.06^{a}	22.08
1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol	0.12 ± 0.07^{a}	0.16 ± 0.04^{a}	23.92
1-Tridecene	0.23 ± 0.02^{a}	0.66 ± 0.25^{a}	24.40
Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	3.00 ± 1.71^{a}	2.85 ± 0.18^{a}	29.31
Total	57.84	29.29	

Different lowercase letters in the same row indicate statistically significant at $P < 0.05$ level by Kruskal–Wallis test. Data are expressed as mean ± SE

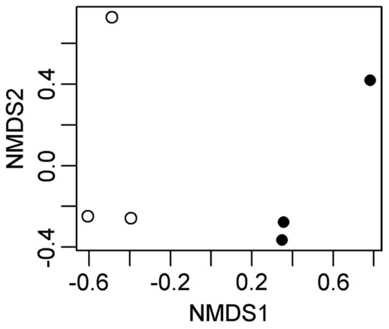

Fig. 2 Non-metric multidimensional scaling (NMDS) ordination of volatile composition of the shelled (hollow points) and intact (solid points) acorns based on Bray–Curtis distance. Stress is close to 0 due to small sample size

scatter-hoarded following shelling by *T. sibiricus*. Moreover, acorns scatter-hoarded by *T. sibiricus* were universally shelled and free of infestation. According to a tri-trophic symbiotic relationship hypothesis proposed by Johnson et al. (1993), herbivores would preferentially select acorns containing weevil larvae, although this model was rejected by several studies (Dixon et al. 1997; Hubbard and McPherson 1997). Our study provided evidence that *T. sibiricus* selectively consume infested acorns but cache non-infested acorns in a tripartite interaction, supporting the results of Steele et al. (1996) and Xiao et al. (2003) on caching mammals. Other mammal herbivores have been reported to consume weevil larvae in oak acorns; however, they also damage a large proportion of uninfested acorns (Semel and Andersen 1988; Weckerly et al. 1989; Bonal and Muñoz 2007). Our results showed that selective predation on weevil larvae by *T. sibiricus* increased scatter-hoarding of non-infested acorns of *Q. mongolica*, which has a potential to benefit acorn survival and natural regeneration of oaks. Moreover, if selective predation on the infested acorns is sufficient to compensate for the large amounts of non-infested acorns consumed, the increase in germination rate due to shelling might be an acceptable price to pay for minimizing the number of infested acorns that are cached.

We also found that the shelled acorns were less likely to be attacked by microbiota than the intact acorns, possibly due to three reasons: (1) the microbes on the pericarps were removed following pericarp removal; (2) the saliva of chipmunks can inhibit propagation of microbes; and (3) frass left by weevil larvae in the intact acorns may contain abundant

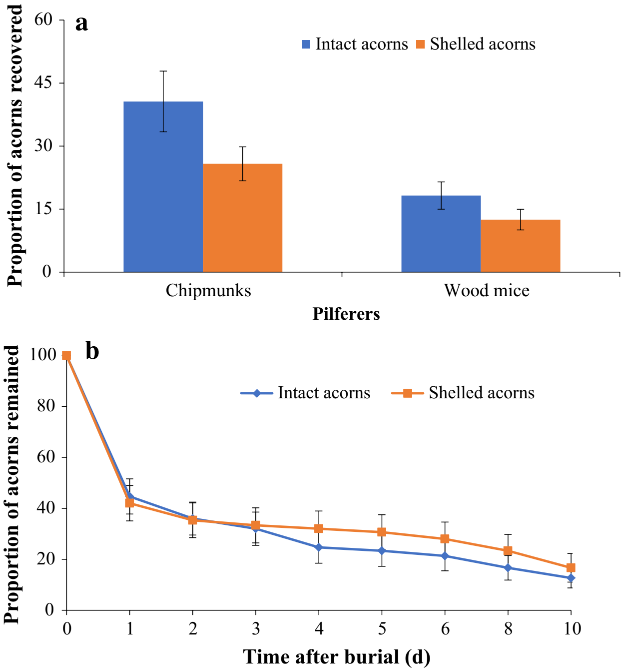

Fig. 3 Effects of shelling on cache pilferage in (**a**) the enclosures by chipmunks (n = 12) and wood mice (n = 12) and (**b**) field experiments (n = 30). Data are expressed as mean ± SE

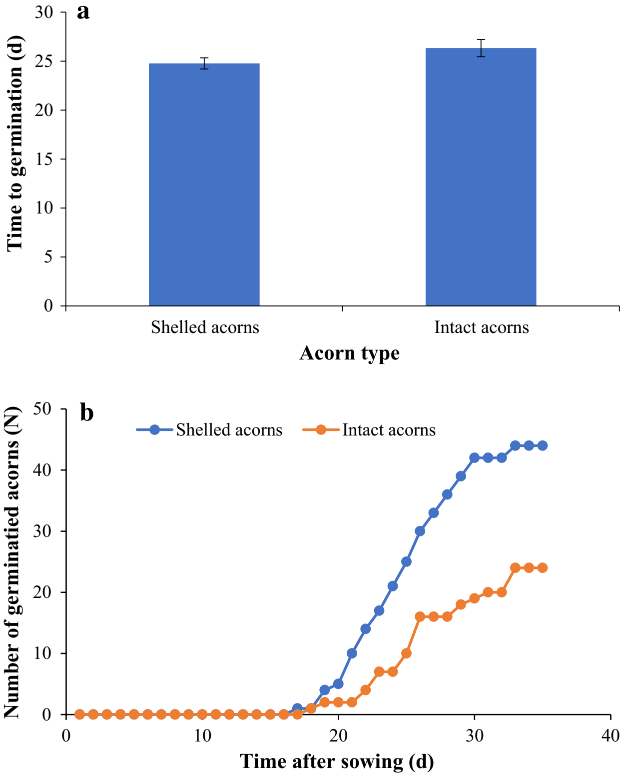

Fig. 4 Effects of shelling on acorn germination of *Quercus mongolica* in the lab (n = 50). **a** Time to germination; **b** accumulated germinated acorns. Data are expressed as mean ± SE

Table 2 Performance of seedlings from the intact ($n = 24$) and shelled ($n = 44$) acorns of *Quercus mongolica*

	Intact acorns	Shelled acorns
Seedling height (cm)	8.72 ± 0.54^{a}	11.06 ± 0.35^{b}
Leaf number (N)	3.88 ± 0.26^{a}	4.48 ± 0.11^{b}
Shoot dry mass (g)	0.29 ± 0.04^{a}	0.44 ± 0.03^{b}
Root dry mass (g)	0.95 ± 0.07^{a}	0.74 ± 0.04^{b}
Root to shoot ratio	8.02 ± 2.48^{a}	1.88 ± 0.13^{b}

Lowercase letters in the same row indicate statistically significant at $P < 0.05$ level. Data are expressed as mean ± SE

microbes (Xu et al. 2016). If the buried acorns both in the lab and field can be regarded as the caches established by *T. sibiricus*, intact acorns apparently suffer much more cache loss than the shelled acorns due to weevil infestation. Therefore, preferring to store non-infested acorns over infested acorns may help *T. sibiricus* gain more rewards from their caches due to the fact that the infested acorns will cause great loss to caches because of the perishability of the infested seeds (Steele et al. 1996).

We detected some putative volatile compounds from the intact and shelled acorns of *Q. mongolica* based on headspace analyses (Table 1). Several common volatiles (e.g., butanol and hexanal) have also been detected in acorns of *Q. robur* (Colville et al. 2012). However, our volatile profiles were different from previous studies on *Q. robur*, *Q. serrata*, and *Q. myrsinaefolia*, possibly because desiccated acorns rather than fresh acorns were used in those studies (Akimoto et al. 2004; Colville et al. 2012). We also showed that shelling by *T. sibiricus* tended to decrease volatile emission from acorns of *Q. mongolica*. Seeds emitting weak odor have been evidenced to reduce the possibility of being pilfered by animals (Hollander and Longland 2012; Paulsen et al. 2013, 2014; Yi et al. 2016a). Intact acorns in the artificial caches in our study were more likely to be pilfered than the shelled acorns in the enclosures, possibly due to the pericarp odor. Moreover, intact acorns appeared to be recovered faster than the shelled acorns in the field despite no statistical difference (Fig. 3). We also observed that *T. sibiricus* often roll the shelled acorns in their mouth before caching, which has been assumed to be an adaptive saliva-clumping process to confer protection against pilferage in the least chipmunk *Neotamias minimus* (Jenkins and Devenport 2014). Although we lack the solid evidence that the lower cache loss rate is totally attributed to decreased emission of several volatile components in the shelled acorns, our observations collectively indicate that shelling by *T. sibiricus* may increase survival rate of caches containing shelled acorns, as they were free of infestation and showed lower level of pilferage. Some may argue that the ability of *T. sibiricus* to find their own cache will also be reduced following acorn shelling. However, *T.*

sibiricus have been found to re-visit their own caches for reinforcement of their memory and rely on spatial memory for cache recovery (Yang et al. 2012; Zhang et al. 2016b). Moreover, recent studies have shown that *T. sibiricus* tend to better remember caches with weak odor (Li et al. 2018). Therefore, we can expect that *T. sibiricus* will gain more rewards for their own caches containing shelled acorns than intact acorns because hoarding animals show cache recovery advantages over naïve animals (Vander Wall et al. 2006).

We found no difference in the time to germination between the shelled acorns and intact acorns in our lab, which can not be explained by the increased water intake by cotyledons or decreased potential germination inhibitors (Liu et al. 2012). The high water content (~57%) in *Q. mongolica* acorns may have already guaranteed their early germination in autumn (Yi and Wang 2016). However, the shelled acorns showed a higher proportion of germination and seedling establishment than the intact acorns both in the lab and field. The most possible reason may be that a few intact acorns randomly selected by the experimenters must have been partially damaged or embryo-killed by weevil larvae, which will decrease acorn germination. We were unable to attribute this to the sampling bias because shelling by *T. sibiricus* will generate two concurrent effects: pericarp removal and elimination of infested acorns. Our previous study has shown that weevil infestation significantly decreases acorn viability and seedling establishment of *Q. mongolica* (Yi and Zhang 2008). Although we lack direct evidence how pericarp removal benefits acorn germination and seedling establishment of *Q. mongolica*, pericarp removal has been shown to promote acorn germination and seedling establishment of another oak species *Q. aliena* var. *acuteserrata*, mainly due to the release of mechanical restriction of germination inhibitors in the pericarp (Liu et al. 2012). Therefore, we can predict that the behavior of shelling by *T. sibiricus* is beneficial for acorn germination and seedling recruitment of *Q. mongolica*. Moreover, seedlings from the shelled acorns performed better than those produced from the intact acorns, as indicated by seedling height and seedling biomass. Given that the intact acorns have been partially damaged by weevil larvae, the reduced reserves in the cotyledons could explain the low performance of seedlings from the intact acorns (Hou et al. 2010). Although shelling benefited seedling establishment, some may doubt that the rapid germination of acorns of white oaks (e.g., *Q. mongolica*) may cause great loss to food-hoarding animals according to the perishability hypothesis (Hadj-Chikh et al. 1996). However, *T. sibiricus* appear to re-visit their own caches to prune the emerged radicles from the germinating acorns (Yang et al. 2012), which has been shown to retard acorn germination but not germination proportion of acorns. Moreover, acorns of *Q. mongolica* with 18-cm taproots only lose <20% of their dry mass after germination in autumn (Yi's unpublished data), indicating that rapid germination in autumn causes little variations of acorn dry mass of white oaks (Yi et al. 2013). In this case, *T. sibiricus* are expected to gain more rewards relying on the behavior of shelling on acorns of *Q. mongolica*. Collectively, both scatter-hoarding animals and oak acorns are expected to be conditionally benefited from the behavior of shelling by *T. sibiricus*.

Although predation on acorn weevils by herbivores has been well studied in the tripartite interactions (Johnson et al. 1993; Steele et al. 1996; Bonal and Muñoz 2007), to our knowledge, no study has evidenced how this effect influences both the first (oaks) and the third trophic (scatter-hoarding animals) levels. The null hypothesis that shelling by *T. sibiricus* has no effect on cache loss and seedling establishment could not be rejected on the basis of our study. We show that shelling of acorns and concomitant predation on weevil larvae by *T. sibiricus* not only potentially benefit acorn survival and seedling establishment but also help food-hoarding animals gain more rewards from their caches, representing a novel mutualistic interaction between scatter-hoarding animals and oaks in a tripartite interaction. Given the fact that scatter-hoarding animals prefer to cache non-infested over infested acorns (Steele et al. 1996; Perea et al. 2012a, b; Yi et al. 2012; Zhang et al. 2014), predation on weevils is expected to play a crucial role in the tripartite interaction between oaks, weevils and seed-eating animals. Although we lack solid evidence that *T. sibiricus* clearly regulates weevil abundance, it seems a likely determinant of weevil abundance in the current year. It can be expected that the behavior of shelling and predation on weevil larvae will provide more benefits to food-hoarding animals and oaks in lean crop years with high weevil infestation due to high predation on weevils. We also suggest that more efforts should be made in the interactions of food-hoarding animals, seeds and infested granivorous insects.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interests.

Data accessibility Data deposited in the College of Life Science, Jiangxi Normal University.

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