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ORIGINAL ARTICLE

Burial-induced changes in the seed preferences of carabid beetles (Coleoptera: Carabidae)

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Abstract. Seeds of many species of plants may survive for a long time in the soil and germinate when brought to the surface, but whether they are subsequently eaten by seed predators is unknown. We examined the preferences of three species of carabids (Coleoptera: Carabidae) for 25 species of seeds and determined the difference in palatability between freshly dispersed and those buried for six years. The stability of their preferences was tested using a collection of seeds of different species, each of which was offered fresh or after being buried. Carabid beetles readily accepted previously buried seeds as food. In total, *Pseudoophonus rufipes* and *Amara littorea* ate more fresh seeds than previously buried seeds, while the opposite was true for *Harpalus affinis*. The seeds of some species were even more attractive to carabids after burial than in the fresh state. For all the species of carabids tested, the diet breadth was similar when the beetles were fed fresh or buried seeds, but the preferences for fresh and buried seed of particular species were correlated only in *P. rufipes* and *A. littorea*. We measured the seed characteristics (mass and viability) likely to be associated with the loss of attractiveness to carabids during burial. The change in carabid consumption was not related to changes in any of these characteristics. This finding indicates that factors responsible for variation in seed acceptability are complex. This study provides the first conclusive evidence that invertebrate seed predators will feed on seeds from seed banks, although they prefer fresh seeds.

INTRODUCTION

Seed mortality is an important factor in the population biology of plants (Harper, 1977; Larios et al., 2017), and an important component of seed mortality is post-dispersal seed predation, which typically occurs on the surface of soil where seed released from mother plants are exposed before germinating or entering the soil seed bank. While predation immediately following seed dispersal has been intensively studied (Kulkarni et al., 2017), little attention has been paid to predation of seeds that previously were buried in the soil for a period of time. Seeds may persist in the soil and remain viable for many years (Baskin & Baskin, 1998). Some species form only a transient seed bank, while seeds of other species remain alive in soil for many years (Thompson et al., 1997; Fenner & Thompson, 2005; Long et al., 2015). Plant species that form a persistent seed bank are typically characterised by a strong and impermeable seed testa (Gardarin & Colbach, 2015), which helps them to survive the soil conditions until they become favourable for germination. In soil, seed mortality is caused mainly by soil microbial pathogens or decomposers (Blaney & Kotanen, 2001; Schafer & Kotanen, 2004; Davis et al., 2006; Wagner & Mitschunas, 2008) but is also due to predation by earthworms (Eisenhauer et al., 2010), carabids (Kulkarni et al., 2015) and rodents (Hulme, 1998). Buried seeds are less likely to be found by surface active seed predators compared with seeds that remain exposed and uncovered (Hulme, 1988; White et al., 2007; Kulkarni et al., 2015; but see Ruzi et al., 2017).

A proportion of the seeds buried in soil eventually come to the surface as a result of soil mixing activities, tillage, freeze-thaw cycles and bioturbation. This provides an opportunity for the seed to germinate but also exposes it to surface predators for the second time. Predation may occur during this period, but very little is known about the predation of previously buried seeds. To date, this topic is considered in only two studies. Martinkova et al. (2006) studied the fate of the seeds of six species of weeds exposed to two species of ground beetle. Following burial for half a year, the consumption of the seed of four species did not change, one species (*Tripleurospermum inodorum* (L.) Schultz-Bip.) was accepted at a higher rate than when fresh and one was not eaten by the predators (*Taraxacum* agg., section Ruderalia). Koprdova et al. (2012) report different responses of invertebrate predators to previously buried, fresh, imbibed and germinating seed of *Brassica napus* ssp. *napus* L.

Predators use scent to locate (Kulkarni et al., 2017) and physical traits to evaluate the quality (Honek et al., 2007; Lundgren & Rosentratter, 2007) of seed prior to accepting it. Therefore, any change in the properties and traits of seed that occurs during burial may affect the likelihood of it being eaten by predators (Martinkova et al., 2006). A study that includes the seed of more species of plants and predators of seeds and compares the consumption of fresh seed with that seed buried for some time is warranted to elucidate the extent of predator pressure on seed released from the seed bank.

In this paper, we investigate the response of three carabid species in terms of seed consumption of the seed of 25 species of herbaceous plants provided fresh and after being buried in soil for six years. In this study, we address three questions: (1) Is the consumption by carabid predators higher or lower when offered previously buried seed compared to fresh seed? As a previous study (Martinkova et al., 2006) shows that the patterns in the change in consumption of six species of seed is variable, we do not predict the rate and direction of carabid response to previously buried seed. Instead, using a wider range of species of seed, we explore what proportion of the different species of seed becomes less or more preferred after burial. (2) How does the diet breadth change when carabids are offered fresh or previously buried seed? In theory, an animal may adjust its diet breadth in response to a change in the available range and quality of food (Sexton et al., 2017). It is likely that the size of such an adjustment depends on the degree of food specialization of particular species. The guild of carabid granivores is quite diverse and includes unspecialized omnivores that accept a wide range of different seeds (have a broad seed diet breadth), while other species show marked preferences (have narrow seed diet breadth) (e.g., Talarico et al., 2016). We hypothesize that the diet breadth will remain the same or become broader in generalists because seed that loses its morphological or chemical defence will become more acceptable to predators. Generalists enlarge the range of acceptable food or "replace" species of seed that loses its attractiveness or decays. In contrast, we expect that the diet breadth of a specialist would tend to become narrower as preferred seed is more likely lose its attractiveness during burial in contrast to non-preferred seed, which becomes more attractive. (3) Is the change in consumption after burial related to a change in seed properties? Seed transformation during burial may include changes in biological, chemical and physical properties. At the population level, seed mass varies with time spent in soil (authors' unpubl. data) because the proportion of dead seeds increases or their seed coats are eroded. These changes in seed characteristics may have consequences for their attractiveness to predators. As it is known that changes occur in the seed material used in this study (authors' unpubl. data), we test whether the change in consumption is related to change in seed viability or mass due to burial. We hypothesize that the change in consumption will be greater for the seed of plants that produce a high proportion of dead seed. The response in terms of a decrease in consumption due to seed mass can be more variable and more difficult to predict, depending on the cause of the reduction in seed mass, as mentioned above.

MATERIALS AND METHODS

Seed material

We used seed of 25 common herbaceous plants that differ in size, morphology, taxonomic position and persistence in soil and are likely to be available to carabid seed predators (Table 1) (Thompson et al., 1997; Grime et al., 2007). Shortly after dispersal from mother plants, these seeds differ in attractiveness for carabid seed predators (Honek et al., 2007). Seed was collected from stands of wild herbaceous plants in 10 × 10-km area centred at 50.088N and 14.274E (surroundings of Prague – Ruzyně, western Czech Republic). The seed was collected from mother plants at full ripeness in July–October 2005 and October 2006, dried at room temperature for 20 days and then stored at 5°C until buried. The seed of each species were each placed in a separate bag. The nylon fabric bags were made to contain approximately 1000 seeds mixed with sieved soil. Mixing with soil is important as it prevents the seed amassing into large clumps and its subsequent degradation (Van Mourik et al., 2005). Finely sieved soil facilitates the separation of the seed when the bags are dug up. For the entire experiment (including seed burial and preference trials), soil was obtained from one place at one time at a depth of 0.6 m and sieved through 0.05-mm mesh. This prevented the soil being contaminated with seed from the natural soil seed bank.

At the beginning of November in the years when the seed was collected (2005 and 2006, Table 1), the bags were buried at a depth of 20-cm in grassland in the grounds of the Crop Research Institute at Prague – Ruzyně (50.08581N, 14.29727E). To facilitate recovery, the bags were connected by a nylon cord to a label on the surface of the ground. Six years after burial, the bags were dug up in late April. The seeds were separated from the soil, dried at 25°C and 40% r.h., and then stored at –20°C until used in the experiments. Samples of fresh seeds used in this experiment (a control) were stored at –20°C from the beginning of the experiment. Hereafter, we refer to freshly frozen control seeds, as "fresh" seeds, and seeds dug up after six years, as "buried" seeds.

Seed quality

In this study, we measured two seed properties/traits presumed to change during burial: seed viability and seed mass. Viability of seeds was estimated by means of the imbibed seed crush test, the results of which are highly correlated with those of the classical tetrazolium chloride test (Borza et al., 2007). Twenty seeds per cohort were left to absorb water for 3 days and then crushed with the tips of a pair of forceps. If cotyledons or embryos appeared or the seed did not collapse, the seed was considered to be viable; if not, the seeds were considered to be dead. Seed mass was measured using seeds cleaned of dust and fine soil particles in an ultrasound cleaner (Sonorex RK 31, Bandelin Electronic, Berlin, Germany), submerged in water for 2 min and dried in an oven for 24 h at 75°C. The average seed mass was determined based on five batches of 20 seeds per species and cohort using an analytical balance (CP225D-0CE, Sartorius AG, Göttingen, Germany) with a precision of 0.00001 g. The fresh seeds and buried seeds are henceforth referred to as "cohorts".

Table 1. Species of seeds used in this study, their dry mass (control seeds) and use in the experiments with three species carabids. The plant nomenclature follows Kubát et al. (2002). Carabid species: PR – *Pseudoophonus rufipe*s; HA – *Harpalus affinis*; AL – *Amara littorea*.

Species	Family	Year of burial	Dry mass [mg ± SE]	PR	HA	AL
Amaranthus powellii S. Watson	Amaranthaceae	2006	0.494 ± 0.013	x	x	x
Amaranthus retroflexus L.	Amaranthaceae	2006	0.494 ± 0.007	x	x	x
Campanula trachelium L.	Campanulaceae	2006	0.134 ± 0.005	x	x	
Capsella bursa-pastoris (L.) Med.	Brassicaceae	2005	0.143 ± 0.017	x	x	x
Chenopodium album agg.	Amaranthaceae	2006	0.583 ± 0.008	x	x	x
Chenopodium glaucum L.	Amaranthaceae	2006	0.186 ± 0.005	x	x	x
Chenopodium polyspermum L.	Amaranthaceae	2006	0.285 ± 0.009	x	x	x
Crepis biennis L.	Asteraceae	2005	0.743 ± 0.034	x		
Geum urbanum L.	Rosaceae	2005	2.177 ± 0.073	x		
Hyoscyamus niger L.	Solanaceae	2006	0.613 ± 0.023	x	x	x
Hypericum perforatum L.	Hypericaceae	2006	0.115 ± 0.003	x	x	x
Lavandula angustifolia Mill.	Lamiaceae	2006	0.907 ± 0.025	x	x	x
Leonurus cardiaca L.	Lamiaceae	2005	0.672 ± 0.061	x	x	x
Lycopus europaeus L.	Lamiaceae	2005	0.262 ± 0.005	x	x	x
Persicaria lapathifolia (L.) Delarbre	Polygonaceae	2006	2.023 ± 0.028	x	x	x
Plantago lanceolata L.	Plantaginaceae	2006	0.256 ± 0.009	x	x	x
Plantago major L.	Plantaginaceae	2006	0.236 ± 0.006	x	x	x
Plantago media L.	Plantaginaceae	2006	1.407 ± 0.020	x		
Portulaca oleracea L.	Portulacaceae	2006	0.154 ± 0.005	x	x	x
Silene noctiflora L.	Caryophyllaceae	2006	1.037 ± 0.008	x	x	x
Silene vulgaris (Moench) Garcke	Caryophyllaceae	2006	0.642 ± 0.015	x	x	x
Thlaspi arvense L.	Brassicaceae	2005	1.167 ± 0.025	x	x	x
Tripleurospermum inodorum (L.) Schultz-Bip.	Asteraceae	2005	0.287 ± 0.012	x	x	x
Urtica dioica L.	Urticaceae	2005	0.153 ± 0.010	x	x	x
Urtica urens L.	Urticaceae	2006	0.389 ± 0.010	x	x	x

Predators

In this study, we used three species of ground beetles (Coleoptera: Carabidae) abundant in arable fields: *Pseudoophonus rufipes* (DeGeer) (body length = 13.8 mm, dry body mass = 31.3 mg), *Harpalus affinis* (Schrank) (body length = 10.2 mm, dry body mass = 14.1 mg) and *Amara littorea* C.G. Thomas (body length = 7.7 mm, dry body mass = 6.7 mg). Body length was taken from Hůrka (1996), and dry body mass was calculated from the body length using the formula of Jarošík (1989). The differences in carabid body size enabled the testing of the effects of (i) the carabid seed size ratio that appears to be important in determining the seed preferences of carabid beetles (Honek et al., 2007) and (ii) the diversity of body sizes of carabid seed predators in natural communities in arable fields. The carabids were collected by means of pitfall traps in the same area as the seeds in June and July 2014 (*P. rufipes*) and 2015 (*H. affinis* and *A. littorea*). To standardize hunger, the beetles were starved for 24 h prior to the experiments. According to previous experiments (Honek et al., 2003, 2007) the carabid species selected accept a range of the different species of seeds used in preference experiments.

Preference experiments

The preferences of the carabid beetles were examined by means of multi-choice cafeteria experiments. Petri dishes measuring 25 cm in diameter with a 0.8-cm deep layer of sieved soil at the bottom served as experimental arenas. The seeds were mounted on a tin tray filled with white modelling clay (Plasticina JOVI®, Barcelona) and pressed into the layer of soil so that the clay surface was flush with the soil surface (Honek et al., 2003; Saska et al., 2014). Trays filled each with thirty fresh or thirty buried seeds of a particular species were placed in a Petri dish and arranged in two concentric circles. Each Petri dish was then considered an experimental replicate and contained the full available range of different species of seed for a particular cohort presented simultaneously (Table 1). Five replicates (dishes) with fresh seed and five replicates with buried seed, each with three beetles per dish, were used for *P. rufipes*, four replicates with fresh seed and four replicates with buried seed, each with four beetles for *H. affinis* and four replicates with fresh seed and four replicates with buried seed, each with seven beetles for *A. littorea*. The difference in number of replicates was because the availability (abundance in the open) of the carabids differed. The number of beetles per tray differed because their body mass differed. The objective of this was to standardize the expected overall seed exploitation. The beetles removed and consumed the seed in the tin trays and the remaining seeds were counted daily. Trays were replaced if more than 15 seeds per tray were consumed. The experiments each lasted for four days.

Data analysis

The difference in overall seed consumption by individual carabid species was tested using GLM with Poisson distribution of errors (GLM-p) with counts of consumed seeds as the response variable and seed cohort (fresh vs. buried) as the factor. To investigate whether the diet breadth changed depending on provision of fresh or buried seeds, a modification of the standardized Levins' niche breadth index B_A was computed (Krebs, 2009) for each carabid species and seed cohort as follows:

$$B_A = \frac{\frac{1}{\sum p_i^2} - 1}{n - 1}$$

where p_i is the proportion of total seed consumption made up of species of seed *i*, and *n* is the number of species of seeds. Diet breadth is narrow (low values of B_A) if a species accepts a low number of different species of seeds. Broad diet breadth (high values of B_A) means that a carabid accepts high numbers of different species of seeds. To classify the seed in terms of its attractiveness to carabids, consumption of each species of seed was standardized relative to the consumption of the most preferred species (the consumption of which was set at 1). Species were arbitrarily (Krebs, 2009) classified as preferred if the standardized consumption was > 0.2, consumed if > 0.05 but < 0.2, and rejected if < 0.05.

The change or stability in overall preferences was initially tested using Pearson correlation test for the complete dataset (species combined) and for each species of carabid separately (Crawley, 2007), assuming that a significant correlation indicates that the overall carabid preferences remains stable for both cohorts. Overlap of the diet breadth O_{jk} was computed according to Pianka (1973) as follows:

$$O_{jk} = \frac{\sum p_{ij}\, p_{ik}}{\sqrt{\sum p_{ij}^2\, p_{ik}^2}}$$

where p is the proportion of total seed consumption made up of seed species i, and j and k are the seed cohorts of fresh or buried seeds, respectively. The effect of cohort (fresh or buried) on consumption of individual species of seeds was further tested using the mixed effect model (R package lme4; Bates et al., 2015). Poisson distribution of errors was used as seeds of the most consumed species were replenished. The models included different species of seeds, burial (fresh vs. buried) and their interaction as fixed terms and replicate (Petri dish) as a random term. The analysis was repeated for each species of carabid separately. The significance of differences in the consumption of particular species of seeds between cohorts was assessed based on the confidence intervals of the differences. A difference was considered significant if the confidence interval of the difference excluded zero (Zar, 1999).

To test the hypothesis that particular seed traits affected the change in seed consumption, we calculated the differences in seed mass and seed viability of fresh and buried cohorts. As the decrease in seed mass and viability may be correlated and thus describe the same thing, we first estimated the strength of their relationship. Indeed, the two traits were correlated (Fig. S1), and an R^2 value that was not very high (0.42) and scattered along the line indicated that some species of seeds that lost mass were still viable. Using the change in both traits for explaining the change in seed consumption of the different cohorts is justified. All analyses were performed in R 3.3.1 (R Core Team, 2017).

RESULTS

Preferences for fresh seeds

The different species of carabid differed in their preferred species of fresh seeds (Fig. 1) and diet breadth. *Pseudoophonus rufipes* was the least choosy and preferred (standardized consumption of 0.2 or higher) 21 out of 25 species of seeds, resulting in a diet breadth of $B_A = 0.687$. The most preferred species of seeds in the fresh state were *U. urens*, *L. angustifolia* and *H. niger* (Fig. 1). *Harpalus affinis* preferred 15 out of 22 species of seeds ($B_A = 0.658$), and the most preferred fresh seeds were *U. urens*, *L. angustifolia* and *C. album* (Fig. 1). *Amara littorea* was the species with most restricted choice and preferred 4 out of 21 species of seeds ($B_A = 0.248$). The most preferred seeds for this carabid were *C. bursa-pastoris*, *T. arvense* and *T. inodorum* (Fig. 1).

Consumption and preferences for seeds after burial

Summed across seed species, *P. rufipes* and *A. littorea* consumed fewer buried seeds than fresh seeds (GLM-p, *P. rufipes*: $\chi^2_8 = 127$, $P << 0.001$; *A. littorea*: $\chi^2_6 = 8.66$, $P = 0.003$), while *H. affinis* consumed more buried than fresh seeds (GLM-p, $\chi^2_6 = 17.9$, $P < 0.001$). In *P. rufipes* and *H. affinis*, respectively, the diet breadths were similar ($B_A = 0.698$ for both species) and slightly broader in *A. littorea* ($B_A = 0.364$). Diet breadths of all the species of carabids largely overlapped between groups when fed fresh and buried seeds (O = 0.863 for *P. rufipes*, 0.801 for *H. affinis*, and 0.727 for *A. littorea*).

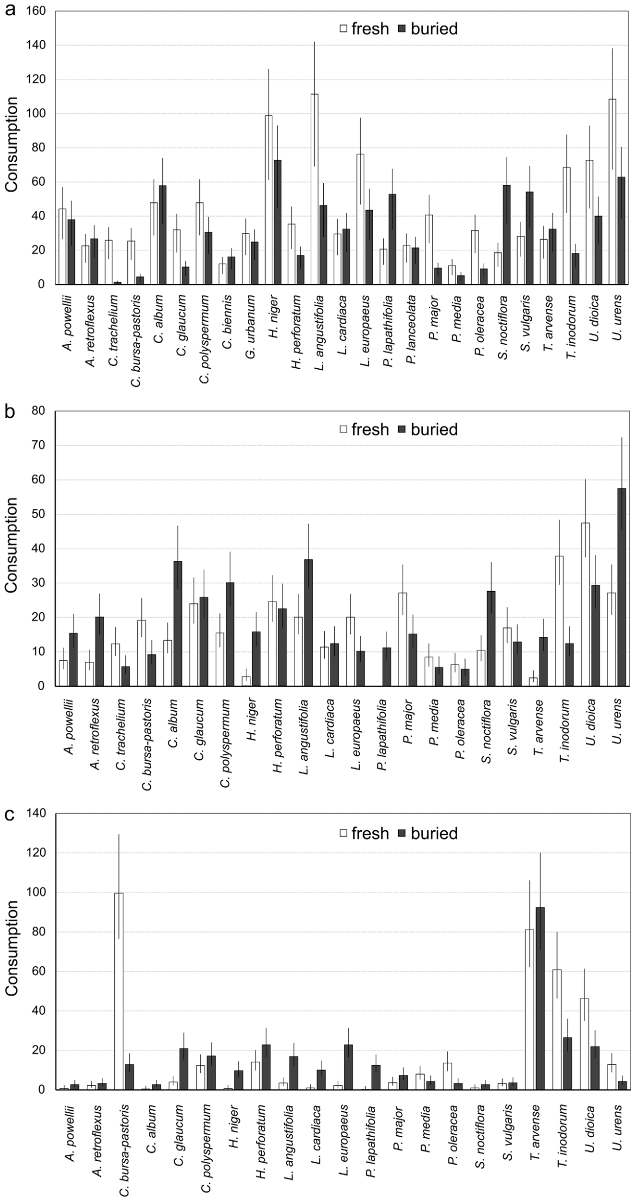

Fig. 1. Preferences for seeds of three species of carabids based on consumption in multi-choice experiments. Error bars indicate 95% confidence intervals. a – *P. rufipes*; b – *H. affinis*; c – *A. littorea*.

The overall preferences for seeds were correlated ($P < 0.05$) for fresh and buried seeds when data for all three species of carabids were combined, and similar results were obtained for *P. rufipes* and *A. littorea* (Table 2). In contrast, the preferences were not correlated (at $P < 0.05$) in *H. affinis* (Table 2). Low values of the correlation coefficients (close to 0.5) were significant only for *P. rufipes* and *A. littorea*, indicating that the consumption of fresh and buried seeds does vary. The presence of this variation was con-

Table 2. Correlation of carabid preferences for fresh and buried seeds.

Model	r	*t*	df	P-value
Species combined	0.707	8.109	66	<< 0.001
P. rufipes	0.524	2.948	23	0.007
H. affinis	0.356	1.701	22	0.104
A. littorea	0.596	3.232	19	0.004

firmed by the significant interaction of species of seeds and cohort in all mixed effect models (Table 3). Plots of the confidence intervals for differences in mean consumption between cohorts (Fig. 2) also reveal that the response of carabids to buried seeds largely depended on the species of seeds.

Regressing the changes in consumption on the changes in seed properties (viability and mass) did not reveal any significant trends (Fig. 3). The absence of a relationship between change in seed characteristics and carabid consumption indicates that the decline in viability or seed mass does not influence carabid preferences. In fact, each of the carabid species preferred the seed of at least one species of buried seed of which all were dead, over fresh seed. Obvious examples were *S. vulgaris* in the case of *P. rufipes*, *A. retroflexus* and *A. powellii* in the case of *H. affinis*, and *C. glaucum* in the case of *A. littorea* (Fig. 2).

DISCUSSION

This paper presents information on the consumption of seeds from the soil seed bank by carabids. Because of interspecific variation in the response of carabids to fresh and buried seed, it is difficult to generalize the results. Carabids prefer similar species of seeds in both fresh and buried states but consume smaller amounts of buried seed. The exception was *H. affinis*, which consumed more buried than fresh seed. The diet breadth remained the same or very similar when fresh of buried seeds were offered, regardless of the degree of food specialization of the carabid. This study thus revealed that carabid predation of previously buried seed is substantial and potentially an important component of the population dynamics of plants.

Using a modification of Levins' index (Krebs, 1999), we quantified the diet breadths of the three species of carabids. We expected that if it changed, there would be opposite changes in the diet breadth of generalists and specialists. However, a large change was not found, and all the carabids preferred similar number of species of the array provided. This finding may be related to the fact that for all the species, regardless of the similarity in their diet breadths and existing correlations between their preferences for fresh and buried seed, the actual number of fresh and buried seeds of a particular species consumed was significantly different, but the direction of change was both negative and positive. *A. littorea* is known to prefer seeds of *Brassicaceae* (Honěk et al., 2007), which was also confirmed in this study, as it consumed more seed of *C. bursa-pastoris* and *T. arvense* followed by *T. inodorum* of the family *Asteraceae*. The consumption of seed of *C. bursa pastoris* dramatically decreased after burial. In fact, the decrease in overall

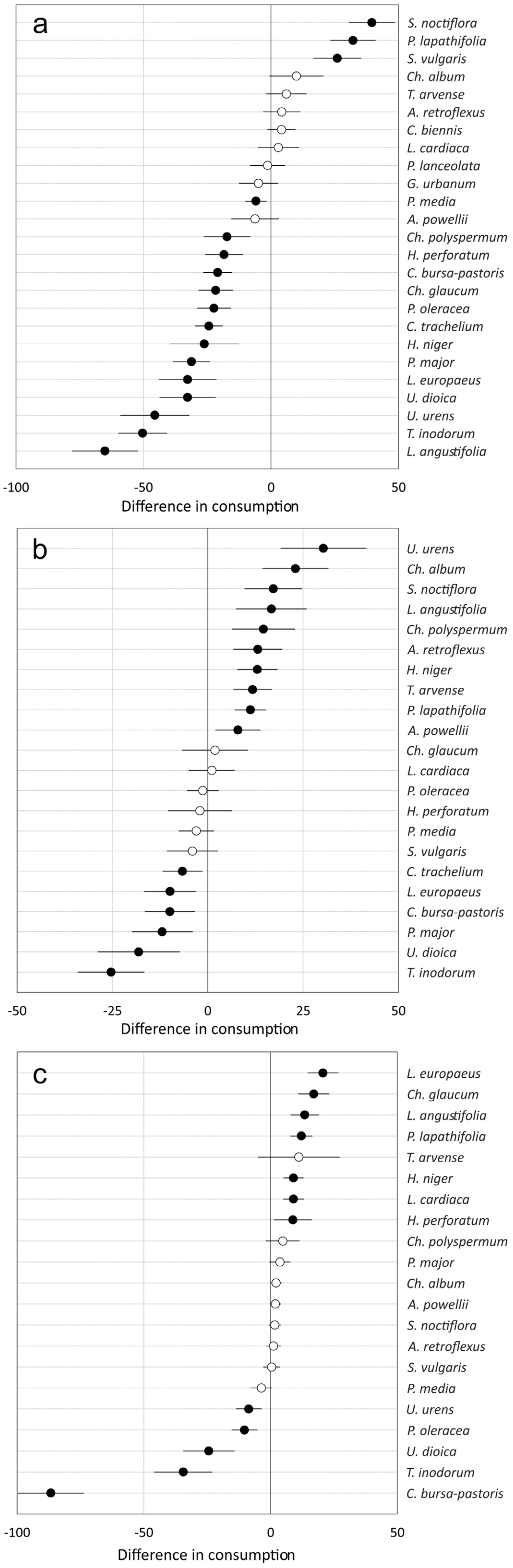

Fig. 2. Mean difference in consumption of fresh and buried seeds by three carabid beetles in multi-choice experiments. Points located on the right of the y-axis indicate seed species that were consumed more after 6 years in soil compared with the control; points located on the left of the y-axis indicate seed species in which burial for 6 years resulted in a reduction in consumption. Solid circles indicate species for which the mean difference was significant; open circles indicate species for which the mean difference was not significant. Horizontal lines indicate 95 % confidence intervals of the mean difference. a – *P. rufipes*; b – *H. affinis*; c – *A. littorea*.

Table 3. Effect of seed species and burial on consumption (GLMM) and standardized consumption (LME) by three carabid beetles.

Term	*P. rufipes*		*H. affinis*		*A. littorea*	
	χ^2 (df)	P-value	χ^2 (df)	P-value	χ^2 (df)	P-value
*Seed * Burial* [1]	1028.4 (24)	<< 0.001	450.1 (21)	<< 0.001	785.3 (20)	<< 0.001
Burial [2]	1.9 (1)	0.168	1.4 (1)	0.236	0.7 (1)	0.420
Seed [3]	3020.7 (24)	<< 0.001	932.2 (21)	<< 0.001	3357.7 (20)	<< 0.001

[1] Deletion test: *Seed* * *Burial* + (1|*Dish*) vs. *Seed* + *Burial* + (1|*Dish*); [2] Deletion test: *Seed* + *Burial* + (1|*Dish*) vs. *Seed* + (1|*Dish*); [3] Deletion test: *Seed* + *Burial* + (1|*Dish*) vs. *BURIED* + (1|*Dish*).

consumption of buried seed is mainly driven by this species. Conversely, seven species of seeds were significantly more consumed after burial, but their consumption was still notably low. In contrast, *P. rufipes* consumed the seed of different families before and after burial. Before burial, *L. angustifolia* (Lamiaceae), *U. dioica* (Urticaceae) and *H. niger* (Solanaceae) were the most preferred. After burial, *H. niger*, *U. urens* (Urticaceae) and *S. noctiflora* (Caryophyllaceae) were the most eaten. Overall, more species of buried seeds were consumed less than fresh seeds, and the total consumption after burial decreased. *Harpalus affinis* was exceptional in consuming more species of seeds after burial than in a fresh state. Seeds of *U. dioica*, *T. inodorum* and *P. major* (Plantaginaceae) were the most preferred in a fresh state, while *U. urens*, *L. angustifolia* and *C. album* were the most consumed after burial. Why the response of this species is different from that of the other two species of carabids is difficult to explain.

One interesting finding is that the seed of *P. lapathifolia* (Polygonaceae) was preferred by all species of carabids more after burial than fresh. In contrast, the seeds of *T. inodorum* and *C. bursa-pastoris* were consistently consumed less after burial than fresh. Many other species of seeds showed the same direction of change in consumption for two species of carabids. Seed qualities responsible for changes in preference are likely to be perceived and evaluated accordingly.

In this study, we a priori hypothesized that decreases in seed mass and viability are traits that explain the patterns in the change in seed attractiveness. However, our data do not support this hypothesis as the change in consumption was not correlated with decreases in seed mass or viabil-

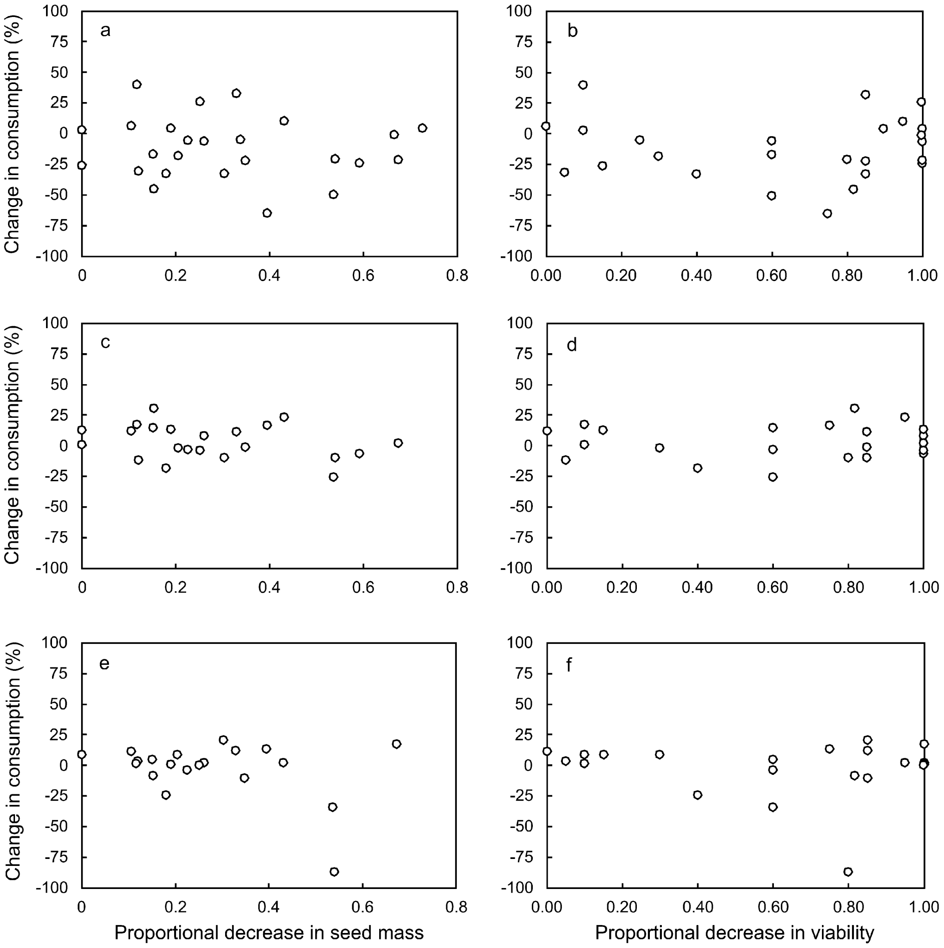

Fig. 3. Relationship of the change in consumption of buried seeds relative to fresh seeds with proportional change in seed mass (a, c, e) and viability (b, d, f) after 6 years burial in soil. a–b – *P. rufipes*; c–d – *H. affinis*; e–f – *A. littorea*.

ity. Changes in qualities not evaluated in this study were potentially involved in the changes in carabid preferences. Seeds in the soil are under continuous attack by soil-living microorganisms (Blaney & Kotanen, 2001, 2002; Dalling et al., 2011) and under the influence of soil chemistry (Long et al., 2009) which, in combination, may alter the chemical, biochemical and mechanical properties of the seed coat, including coat hardness and the semiochemicals present on the surface (Davis et al., 2008, 2016; Tiansawat et al., 2014). The cues that carabids use for selecting seeds are not fully understood. Carabid beetles use visual, tactile and olfactory cues when searching for food (Bauer & Kredler, 1993) and the cue that predominates can be deduced from carabid morphology, with those that have large eyes orientating visually and those with small eyes and few ommatidia using mainly olfactory cues (Bauer & Kredler, 1993). The morphology of the species used in this study indicate they may respond primarily to olfactory cues. Indeed, carabids locate seeds using the smells they produce when germinating (Kulkarni et al., 2017), which explains why more granivorous carabid beetles are caught by pitfall traps surrounded by seed (Honek & Martinkova, 2001). In this study, the change in preference is most likely due to changes in the (bio)chemical composition of the seed surface, which is faster in species in which the seed persists for only a short period than those that persist for a long period. This is attributed to the fact that short lived seeds rely more on chemical than mechanical defence (Davis et al., 2008). However, the hardness of the testa of seeds also decreases with time spent in soil (Tieu & Egerton-Warburton, 2000; Davis et al., 2016; but see Zalamea et al., 2015). Decreasing the strength of this mechanical protection facilitates the crushing and eating of seeds by carabids. We did not measure the toughness of the testa in this study, but noted a difference in a number of seed species during the viability assessment. In the crush test, the testa of buried seed was markedly thinner, softer or more fragile than that of fresh seed. The best example was *P. lapathifolia*, which was preferred more after being buried for a prolonged period due to the weakening of the (originally) thick testa. In some cases, carabids preferred even dead exhumed seeds to fresh seeds. Carabids thus may scavenge dead plant material and have no effect on plant population dynamics. The changes that seeds undergo in soil are very complex, and selecting only one seed characteristic as the explanatory variable for change in carabid preference might be misleading.

In conclusion, this study showed the effect of carabids on the mortality of previously buried seeds. Compared with fresh seeds, the consumption of buried seeds increased or decreased depending on both the species of seed and carabid. Determining to what extent the predation of buried seeds may have on the population biology of herbaceous species of plants requires further study.

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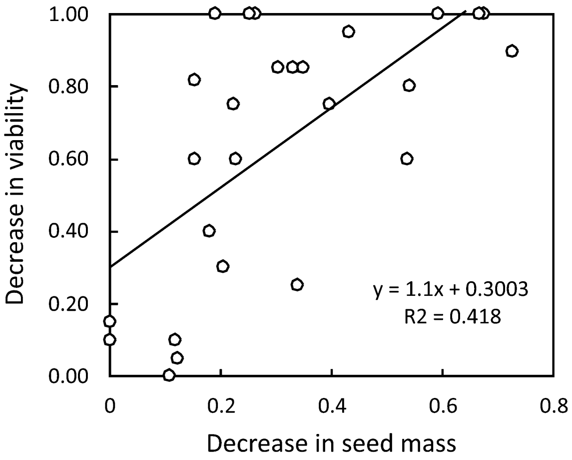

Fig. S1. Correlation between the changes in seed mass and viability after burial in soil for six years.