Lipid content drives weed seed consumption by ground beetles (*Coleopterea, Carabidae*) within the smallest seeds

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Summary

Seed predation by natural predators can contribute to the regulation of the weed seedbank, hence has been acknowledged as a promising way to significantly reduce the use of chemicals and mechanical weeding to manage arable weeds. However, the complex aspects that determine seed species preference are not yet fully understood, especially, the relationship between weed seed traits and the feeding behaviour of seed predator species. In this study, we examine predation on 42 weed species by four ground beetle species as a function of two major seed traits: seed mass and seed lipid content. We used an _original experimental design to create a statistically independent trait value distribution for evaluating the separate and interactive effects of these two traits. Our results show that all the ground beetles species consumed weed seeds, but at different rates. The smallest weed seeds with high lipid content were among the most consumed. While seed mass might be limiting for ground beetles, due to ease of handling or the thickness of the seed coat, they may preferentially consume seeds rich in lipids to increase energy uptake. To our knowledge, this study is the first to reveal that two major seed traits drive seed predation by ground beetles.

Keywords: agroecology, biological control, carabid beetles, seed predation, seed mass, trade-off.

Introduction

Weeds may compete strongly with crops and reduce yields, so weed management remains one of the major challenges in agriculture (Oerke, 2006). For decades, weeds have been mainly managed by herbicides. However, the negative consequences of herbicide use on human health (Wilson & Tisdell, 2001) and on the environment (Geiger *et al.*, 2010), and the recurrent emergence of weed resistance (Heap, 2014) require us to consider alternative weed control strategies. Ecological intensification based on the use of natural enemies such as seed predators has been suggested as a way to reduce the dependence on current intensive practices such as agrochemicals (Bommarco *et al.*, 2013; Gaba *et al.*, 2014a). The majority of arable weeds are annuals producing large numbers of seeds that persist in the seedbank. Post-dispersal seed predation may therefore be a key process for regulating the weed seedbank (Westerman *et al.*, 2003; Pannwitt

et al., 2017) and has received much attention. Seed loss due to predation in cereals was estimated at 32–70% of the seedbank (Westerman *et al.*, 2003), and granivorous ground beetles are often considered as major weed seed predators, able to regulate weeds in arable fields (Honek *et al.*, 2003; Bohan *et al.*, 2011; Kulkarni *et al.*, 2017). However, for efficient top-down weed regulation by seed-eating animals, a full understanding of the various factors that control food uptake by seed predators is required, in particular the relationship between weed seed traits, such as seed size and nutrient content, and the feeding behaviour of seed predators. However, these factors interact and are still largely unexplored (Moles *et al.*, 2003; Wang & Chen, 2009).

In our study, we investigated the influence of two major seed traits, seed mass (strongly correlated to seed size) and seed lipid content in the feeding prefer-ences of four common ground beetle species of Wes-tern European arable farmlands. Seed mass and seed lipid content are positively correlated with seed energy content (Bretagnolle *et al.*, 2016), which is an impor-tant determinant of seed preferences in rodents and arthropods in general (Brust & House, 1988; White *et al.*, 2007), including ground beetles (Petit *et al.*, 2014). However, the influence of these two major traits on seed preferences is not straightforward. In the sky-lark (*Alauda arvensis*), for instance, a trade-off in for-aging strategies was found between smaller seeds and seeds with high lipid content (Gaba *et al.*, 2014b).

Our aim in this study was, therefore, to assess seed preferences in ground beetles quantitatively and disen-tangle the effects of seed size and seed lipid content in their seed preferences. Seed selection was investigated through three different components of consumption (as response variables): (i) total number of seeds, (ii) total biomass of seeds and (iii) total lipid biomass of seeds. We selected a sample of 42 weed species to avoid any correlation between the two explanatory variables (seed mass and seed lipid content). The statis-tically independence between these two variables allows testing whether beetles trade-off or prioritise weed seed consumption according to nutritional value (lipid intake) or quantity (biomass). We predicted (i) that carabid beetles would show preference for the seeds with lowest seed mass among the set of seeds, since larger seeds would be more difficult to handle (Honek *et al.*, 2007; Petit *et al.*, 2014) and (ii) that within the smallest seeds, beetles would prefer those with high lipid content. Finally, we expected ground beetles to maximise energy uptake and, therefore, maximise total lipid biomass (the product of seed lipid content and seed mass), rather than the number of seeds or the total biomass.

Material and methods

Weed seed selection

We selected 42 weed species belonging to the weed flora of the Long-Term Social-Ecological Research 'Zone Atelier Plaine & Val de Sèvre' (LTSER ZA-PVS; Bretagnolle *et al.*, 2018; see species lists in Munoz *et al.*, 2017), Deux-Sèvres, France. We selected the weed species such that their mass and lipid content were uncorrelated (Table 1). The trait values (seed mass, in mg, and lipid content, in %) of each species were obtained from Bretagnolle *et al.* (2016). For the 42 species selected, the lipid content and mass were uncorrelated (Spearman correlation test: $r_s = -0.19$, *P*-value = 0.22). All seeds were obtained from Her-biseed® (Twyford, the UK). The frequency of occur-rence *in natura* of the 42 weed species was estimated from weed surveys performed in 2013, 2014 and 2015 in arable fields (492 fields in total) at the study site (see details on the survey protocol in Bretagnolle *et al.*, 2018). Seventy per cent of the 42 selected weed species were among the most frequent species, being in the top quartile of the weed species distribution for the LTSER ZA-PVS. Seed mass and seed lipid content could also be correlated with other traits driving seed predation. Seed coat hardness, for instance, may be a major driver of predation by carabids. However, too few data of seed coat hardness were available to prop-erly control for this trait in our experiment. Neverthe-less, we used available data on seed coat thickness to investigate for potential confounding effects with seed mass and/or seed lipid. Despite a thick seed coat may not necessarily mean a harder seed, this was however the best proxy available when the experiment was implemented. We failed to detect any significant corre-lation between seed coat thickness and seed lipid con-tent, but found a positive relationship with seed mass (Figure S1). This supports that our design allowed dis-sociating handling mechanisms (seed mass, seed coat thickness) and nutrient content (seed lipid content).

Ground beetles

We selected four ground beetle species for the experi-ment, ranging in size (see Fig. 1A for body weights) from *Pseudophonus rufipes* (De Geer; 15 mm), through *Harpalus dimidiatus* (P. Rossi; 13 mm) and *Poecilus cupreus* (Linnaeus; 12 mm) to *Harpalus affinis* (Schrank; 10 mm; body mass: author's own data). *Poecilus cupreus* belongs to the tribe Pterostichini, which are not typical seed eaters, while the other three species are Harpalini – which are typical seed eaters. Adult ground beetles were collected in the LTSER ZA-PVS using

Table 1 Weed seed species used in the experiment with seed mass (mg) and seed lipid content (%)

Clade	Order	Family	Species	EPPO	Mass (mg)	Lipid (%)
Malvids	Malvales	Malvaceae	*Abutilon theophrasti* Medik.	ABUTH	0.90	15.0
Commelinids	Poales	Poaceae	*Alopecurus myosuroides* Hud.	ALOMY	2.21	7.70
Eudicots	Caryophyllales	Amaranthaceae	*Amaranthus retroflexus* L.	AMARE	0.50	7.50
Campanulids	Asterales	Asteraceae	*Ambrosia artemisiifolia* L.	AMBEL	3.99	20.3
Asterids	Ericales	Primulaceae	*Anagallis arvensis* L.	ANGAR	0.50	20.8
Commelinids	Poales	Poaceae	*Avena fatua* L.	AVEFA	12.00	8.80
Commelinids	Poales	Poaceae	*Bromus sterilis* L.	BROST	6.42	1.90
Campanulids	Asterales	Asteraceae	*Centaurea cyanus* L.	CENCY	3.90	22.0
Eudicots	Caryophyllales	Chenopodiaceae	*Chenopodium album* L.	CHEAL	0.72	8.55
Eudicots	Caryophyllales	Chenopodiaceae	*Chenopodium hybridum* L.	CHEHY	1.69	9.10
Eudicots	Caryophyllales	Chenopodiaceae	*Chenopodium polyspermum* L.	CHEPO	0.30	15.2
Campanulids	Asterales	Asteraceae	*Cirsium arvense* (L.) Scop.	CIRAR	1.30	19.3
Campanulids	Asterales	Asteraceae	*Crepis capillaris* (L.) Wallr.	CVPCA	0.24	31.0
Asterids	Solanales	Solanaceae	*Datura stramonium* L.	DATST	6.75	22.2
Commelinids	Poales	Poaceae	*Digitaria sanguinalis* (L.) Scop.	DIGSA	0.51	4.80
Commelinids	Poales	Poaceae	*Echinochloa crus-galli* (L.) P. Beauv.	ECHCG	0.40	3.70
Fabids	Malpighiales	Euphorbiaceae	*Euphorbia helioscopia* L.	EPHHE	2.34	32.6
Eudicots	Caryophyllales	Polygonaceae	*Fallopia convolvulus* (L.) A. Löve	POLCO	6.90	1.50
Eudicots	Ranunculales	Papaveraceae	*Fumaria officinalis* L.	FUMOF	3.17	29.6
Asterids	Gentianales	Rubiaceae	*Galium aparine* L.	GALAP	6.60	3.20
Malvids	Geraniales	Geraniaceae	*Geranium dissectum* L.	GERDI	2.40	28.1
Malvids	Geraniales	Geraniaceae	*Geranium pusillum* L.	GERPU	0.70	27.9
Campanulids	Asterales	Campanulaceae	*Legousia speculum-veneris* (L.) Chaix	LEGSV	0.19	33.3
Fabidees	Malpighiales	Linaceae	*Linum usitatissimum* L.	LIUUT	6.50	32.8
Commelinids	Poales	Poaceae	*Lolium multiflorum* Lam.	LOLMU	2.90	1.80
Campanulids	Asterales	Asteraceae	*Matricaria perforate* Mérat	MATIN	0.36	16.6
Commelinids	Poales	Poaceae	*Panicum capillare* L.	PANCA	0.30	4.87
Eudicots	Ranunculales	Papaveraceae	*Papaver rhoeas* L.	PAPRH	0.20	40.5
Asterids	Lamiales	Plantaginaceae	*Plantago media* L.	PLAME	0.53	11.9
Commelinids	Poales	Poaceae	*Poa annua* L.	POAAN	0.30	4.00
Eudicots	Caryophyllales	Polygonaceae	*Polygonum aviculare* L.	POLAV	1.30	4.20
Eudicots	Caryophyllales	Polygonaceae	*Polygonum lapathifolium* (L.) *Delarbre*	POLLA	1.19	3.80
Commelinids	Poales	Poaceae	*Setaria viridis* (L.) P. Beauv.	SETVI	0.80	3.10
Malvidees	Brassicales	Brassicaceae	*Sinapis arvensis* L.	SINAR	2.20	22.9
Asterids	Solanales	Solanaceae	*Solanum nigrum* L.	SOLNI	0.70	35.0
Campanulids	Asterales	Asteraceae	*Sonchus asper* (L.) Hill	SONAS	0.28	26.3
Eudicots	Caryophyllales	Caryophyllaceae	*Stellaria media* L.	STEME	0.40	5.30
Campanulids	Apiales	Apiaceae	*Torilis arvensis* (Huds.)	TOIAR	2.60	20.8
Fabids	Fabales	Fabaceae	*Trifolium pratense* L.	TRFPR	2.60	8.55*
Asterids	Lamiales	Plantaginaceae	*Veronica hederifolia* L.	VERHE	4.00	14.6
Asterids	Lamiales	Plantaginaceae	*Veronica persica* Poir.	VERPE	0.96	23.0
Fabids	Malpighiales	Violaceae	*Viola arvensis* Murray	VIOAR	0.46	33.3

Data sources are from Bretagnolle *et al.* (2016) except for * Kew Gardens Seed Information Database. Taxonomy obtained from Tela Botanica (http://www.tela-botanica.org/site:botanique).

pitfall traps placed in arable fields between April and August 2016. The capture frequency of the four selected species is among the highest capture rates in the LTSER ZA-PVS (Marrec *et al.*, 2015; Deroulers, 2017; Deroulers & Bretagnolle, 2018), *P. cupreus* being the most frequent species at the study site (Marrec *et al.*, 2015). Traps consisted of plastic cups (diameter: 8.5 cm, depth: 7 cm) and were buried with the lip at ground level. Lumps of soil were placed at the bottom of the traps to provide shelter for the arthropods trapped and the traps were checked daily to avoid cannibalism. Once brought to the laboratory, the ground beetles were kept individually in plastic boxes (diameter: 5.5 cm, height: 6.5 cm) at room temperature. They were fed *ad libitum* every two days on a diet consisting of a weed seed mixture and crushed cat biscuits (Purina®, Friskies®). Ground beetles have different emergence periods depending on the species, so the experiments were timed to match their life cycles, with *H. dimidiatus* and *P. cupreus* in spring, *H. affinis* in late June and *P. rufipes* in August.
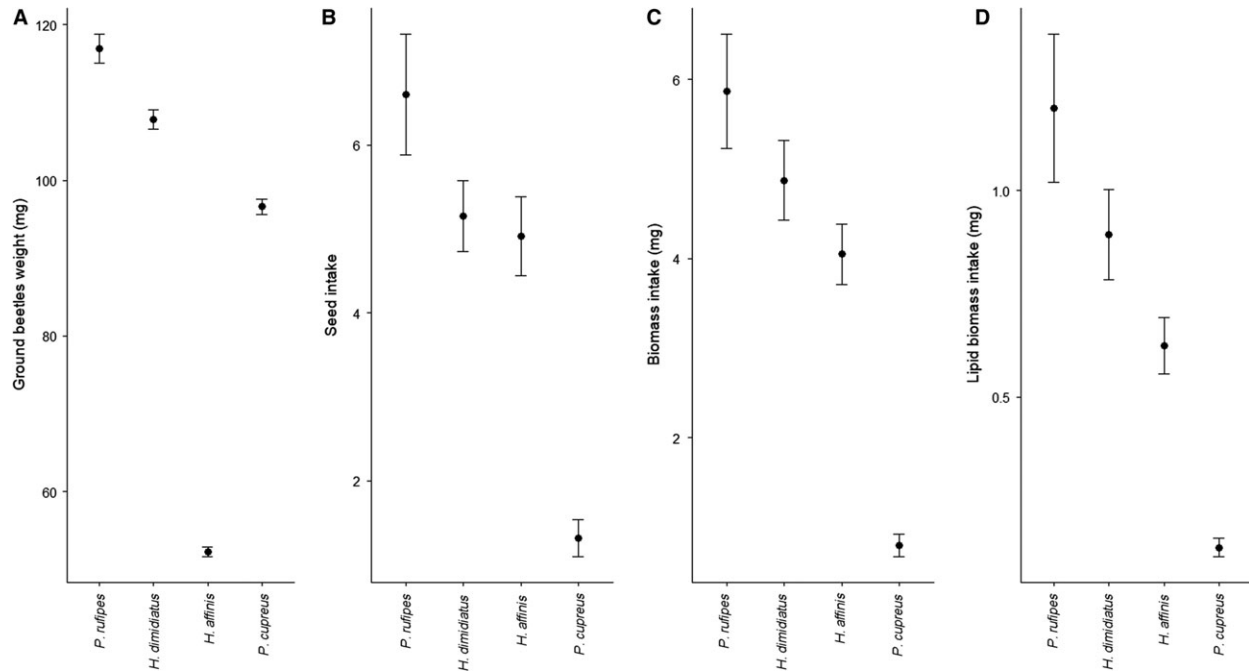
Fig. 1 Body weight (A), numerical seed intake (B), total biomass ingested (C) and total lipid biomass ingested (D) for the four beetle species studied. For each dot, the mean of the five individual beetles for the 42 seed species, are shown with their standard errors.

Experimental design

All experiments were carried out in climatic chambers (Vötsch Industrietechnik®) to control the temperature and humidity and avoid any confounding factors. Humidity was set at 70%, temperature at 25°C and photoperiod for summer (17 h/7 h; day/night). These climatic conditions are the most appropriated for ground beetles' predation activity (Honek *et al.*, 2003; Eskelson *et al.*, 2011; Petit *et al.*, 2014). Before starting any tests, we standardised the level of hunger among all the individuals using a 72 h fasting period. The experiment duration was 24 h (see Deroulers, 2017 (chap3) and Deroulers & Bretagnolle, 2018 for details of standard protocols).

We used plastic boxes (11 cm × 23 cm × 8 cm) with rounded corners and 1 mm × 1 mm mesh covered openings in the lid to allow good air circulation in the box. Weed seeds were placed in a 64 cm² area, corresponding to 25% base area of the plastic box and covered with sand (300 μm grain size, chocolate colour, Le marchand de sable, Challans 85) to hide them. Moistened cotton wool and a paper shelter (5 × 4 cm) were placed in the plastic box. The tests were carried out with a single individual and 50 seeds of a single weed species. This number was set to match the average seed density of 2000 seeds m⁻² (see below), estimated using soil cores in the LTSER ZA-PVS (Powolny, 2012). The consumption rate per weed species and per ground beetle species were quantified based on the

consumption rates of five different individual ground beetles per species. A total of 210 individuals were used in the experiments; each individual was used only once, except for a few individuals of *H. affinis*, which were used twice. After each experiment, the number of remaining seeds was counted; missing seeds and those of which >50% was consumed were considered eaten (Honek *et al.*, 2003). The boxes were washed (water and liquid potassium soap) to remove the odours of the previous ground beetles that might be detected by the next ground beetles (Kielty *et al.*, 1996). For similar reasons, the sand, cotton wool and shelter were used only once. After all experiments, we released all the ground beetles. In total, 840 experiments (42 weed species × 5 individual beetles × 4 ground beetle species) were performed and 10 500 seeds were offered to each ground beetle species (42 weed species × 50 seeds × 5 individuals).

Statistical analyses

The statistical analyses were repeated for three different metrics of seed consumption that were considered as separate response variables: the total numerical intake of seeds (i.e. number of seeds eaten per experiment), total seed biomass (i.e. the product of the number of seeds eaten and the seed mass) and total lipid biomass (i.e. the product of total seed biomass and seed lipid content). We used generalised linear models (GLM) with Poisson error for numerical intake and

linear models (LM) for total seed biomass and total lipid biomass intakes with seed mass (mg), seed lipid content (%) and their interaction as explanatory variables. Seed mass and seed lipid content were centred and reduced for all analyses (i.e. z-score transformation), such that the model coefficients could be compared between species and between factors. All analyses were performed using R version 3.1.3 (R Core Team, 2017). We used the ANOVA (type II) function from the car R library (Fox & Weisberg, 2011).

Results

All four ground beetle species consumed weed seeds, though at different rates and with different preference patterns (Figs 1B and 2). *Pseudophonus rufipes* ate the largest amount of seeds with a grand total of 1396 seeds consumed (13.3% of the total number of seeds offered to this species, all experiments taken together). Then, *H. dimidiatus* and *H. affinis* consumed, respectively, 1081 (10.3%) and 1031 (9.8%) seeds. Seed consumption was far lower for *P. cupreus* with a total of only 277 seeds (2.6%). Ranking seed consumption using the total biomass or total lipid biomass intake was similar (Fig. 1C and D) but with a greater difference between *H. dimidiatus* and *H. affinis* (1023.57 mg and 850.01 mg respectively). Therefore, apart from *H. affinis*, consumption increased with increasing beetle size (Fig. 1), hence with increasing presumed food requirement. The five most consumed weed seeds among the 42 weed seed species used in the experiment were *Chenopodium polyspermum* L. (346 seeds consumed in total) followed by *Plantago media* L. (314), *Chenopodium album* L. (305), *Viola arvensis* Murray (265) and *Sonchus asper* (L.) Hill. (245). Only two weed seeds were not consumed at all: *Galium aparine* L. and *Veronica hederifolia* L. (Fig. 2).

Pairwise correlations indicated that the two *Harpalus* species and *P. rufipes* were similar in their patterns of seed preferences (Fig. 2; *H. dimidiatus* and *H. affinis*; $r_s = 0.64$, $P < 0.0001$; *H. dimidiatus* and *P. rufipes*; $r_s = 0.83$, $P < 0.0001$; *H. affinis* and *P. rufipes*, $r_s = 0.63$, $P < 0.0001$). *Poecilus cupreus*, however, showed a distinct pattern of seed preference (Fig. 2). In addition to showing far smaller seed consumption, *P. cupreus* also differed in seed preferences. *Pseudophonus rufipes*, *Harpalus dimidiatus* and *Harpalus affinis* consumed more than five seeds of 14, 18 and 15 different weed species respectively (Table S1 shows consumption of each species). For each carabid species, we found strong correlations between the seed numerical intake rank and the ranks of seed biomass intake and lipid biomass intake when the weed species were ranked in decreasing order based on seed intake

(Figure S2). The weed seed species with the highest numerical intake rates were also the species with the highest total biomass intake ($r_s = 0. 54$, $P < 0.0001$), and the highest total lipid biomass intake ($r_s = 0.51$, $P < 0.001$). However, there was also strong variability between pairs of seed consumption metrics, indicating that analysing the three metrics separately provided useful information.

The number of seeds eaten was strongly and negatively correlated with seed mass unit in all four species of beetles (Figure S3), while there was a significant positive correlation with seed lipid content for two of these four species (*P. rufipes, H. dimidiatus*). These were the two largest species of beetles for which there was also a significant positive correlation between seed mass and lipid content, but numerical seed intake was mainly dependent on seed mass (Table 2), with seed lipid content having no effect for smaller seeds. As expected, biomass intake was positively correlated with seed mass, at least for the three seedeaters (excluding *P. cupreus*). For *P. rufipes* and *H. dimidiatus*, biomass intake was also positively correlated with seed lipid content and the interaction between seed mass and lipid content (seed lipid biomass; Table 2; Fig. 3). The most interesting results were for lipid biomass intake (Table 2). For all four species, seed mass had a negative effect, lipid content had a positive effect and the interaction between these two factors was significantly negative on lipid biomass intake (Table 2). Therefore, for all species of beetles, the highest lipid biomass intake was for smaller seeds with high seed lipid content (Fig. 3 columns 4 and 5). However, the relative magnitudes (as indicated by the coefficients) of the effects of the two factors differed between the four species. For *P. rufipes*, the largest species, the lipid content coefficient was twice that of the seed mass and interaction coefficients, so maximising lipid biomass intake was achieved by eating the seeds with the highest lipid content (Fig. 3). In this species, small seeds were ingested preferentially (maximum seed size preferred was only 2.5 mg). The relative importance of seed lipid content decreased gradually from *P. rufipes* to *P. cupreus* (Fig. 3, second column from right), with a decrease in the coefficient (Table 2) in the model while seed size coefficient remained approximately stable. Therefore, small seed size and high seed lipid content were the main drivers of choice in all four species, but with decreasing body size of the carabid beetle, seed lipid content became less important than seed size.

Discussion

The aim of this study was to understand how seed mass and seed lipid content govern the feeding
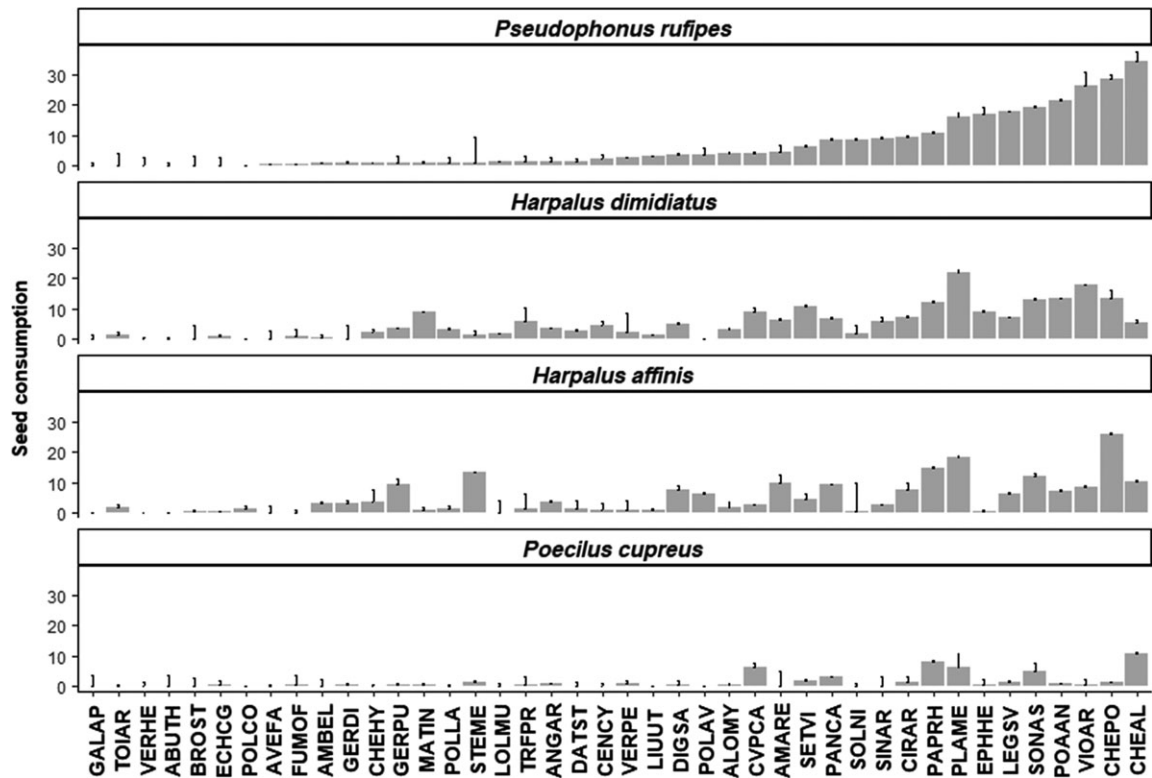
Fig. 2 Seed consumption of *P. rufipes, H. dimidiatus, H. affinis* and *P. cupreus* of the 42 weed seed species ranked in increasing order of numerical seed intake by *P. rufipes,* the species which consumed the highest number of seeds. From top to bottom, ground beetles are ranked from the largest to the smallest species. For each seed and beetle, histogram represents the mean for the five individuals, with standard error.

preferences of four common ground beetles of different body sizes. We used an original experimental design to create statistically independent trait value distributions for evaluating the separate and interactive effects of these two traits, which are usually negatively correlated (Bretagnolle *et al.*, 2016). As expected, weed seed consumption varied with both seed and ground beetle species. We expected that ground beetles should be limited by seed mass (i.e. seed size) and that, among the smallest seeds, ground beetles should consume more seeds with higher lipid content to ensure higher energy uptake. Our results confirmed these expectations, revealing a preferential intake of small seed species even for *P. cupreus,* which showed the lowest seed consumption rate. Preferential seed intake of the smallest seeds is consistent with previous findings in ground beetles (Honek *et al.*, 2007; Petit *et al.*, 2014), and other seed predators, which showed that seed mass strongly influenced seed predation mainly because of physical constraints (Wang & Chen, 2009; Gaba *et al.*, 2014b). *Harpalus affinis* was found to consume more species of weed seeds than the other ground beetle species tested (see also Honek *et al.*, 2007), and the results for *P. rufipes* also seem in agreement with other studies

(Goldschmidt & Toft, 1997; Hartke *et al.*, 1998; Harrison & Gallandt, 2012).

Handling larger seeds is generally more time-consuming (Heredia & Detrain, 2005) and requires more energy than for the smaller seeds (Pirk & Casenave, 2011). The positive relationship between seed mass and seed coat thickness (Davis *et al.*, 2008; Figure S1) may partially explain the preferential intake for smaller seeds by ground beetles. We observed that at least for the larger seeds, carabid beetles remove the coat and ingested only the seed itself. Seed coat hardness and physical structure may also affect seed consumption. The two weed species that were not consumed at all are also among the largest (diameter: 3–4 mm for *Galium aparine* L. and 2.5 to 3.5 mm for *Veronica hederifolia* L.) and the heaviest among our set (the 4th and 7th heaviest seeds), with a thorny or scarred coat (see www2.dijon.inra.fr/hyppa), presumably making their consumption more difficult for ground beetles. The consumption of *G. aparine* seed was found to be <0.01 seeds per day in another study (Petit *et al.*, 2014), while 0.45 ± 0.28 seeds per day were consumed in winter wheat in field preference experiments (Honek *et al.*, 2003). Clear preference for some seeds, for

Table 2 Outputs of the models for (A) numerical seed intake (B) biomass intake and (C) lipid mass intake as explained by seed mass, seed lipid content and their interaction

	Pseudophonus rufipes			Harpalus dimidiatus			Harpalus affinis			Poecilus cupreus		
	Estimate (±SD)	z value	P	Estimate (±SD)	z value	P	Estimate (±SD)	z value	P	Estimate (±SD)	z value	P
Numerical seed intake												
(Intercept)	1.28 (±0.06)	21.89	**<0.001**	1.25 (±0.05)	25.28	**<0.001**	1.06 (±0.06)	18.17	**<0.001**	−0.86 (±0.18)	−4.89	**<0.001**
Seed mass	−1.51 (±0.09)	−16.41	**<0.001**	−1.14 (±0.08)	−14.73	**<0.001**	−1.46 (±0.09)	−16.26	**<0.001**	−2.4 (±0.27)	−9.00	**<0.001**
Seed lipid content	0.53 (±0.06)	9.40	**<0.001**	0.23 (±0.05)	4.55	**<0.001**	−0.05 (±0.06)	−0.90	0.373	−0.04 (±0.18)	−0.22	0.823
Seed mass × Seed lipid content interaction	0.59 (±0.08)	7.065	**<0.001**	0.19 (±0.08)	2.59	**0.001**	−0.03 (±0.09)	−0.28	0.783	−0.31 (±0.26)	−1.21	0.228
Biomass intake												
(Intercept)	6.27 (±0.61)	10.36	**<0.001**	5.21 (±0.43)	12.25	**<0.001**	4.16 (±0.34)	12.24	**<0.001**	0.82 (±0.13)	6.49	**<0.001**
Seed mass	1.61 (±0.64)	2.51	**0.013**	1.39 (±0.45)	3.08	**0.002**	0.58 (±0.36)	1.60	0.112	−0.20 (±0.13)	−1.52	0.131
Seed lipid content	2.55 (±0.61)	4.20	**<0.001**	1.24 (±0.43)	2.90	**0.004**	−0.12 (±0.35)	−0.36	0.721	0.04 (±0.13)	0.32	0.746
Seed mass × Seed lipid content interaction	2.72 (±0.67)	4.06	**<0.001**	2.26 (±0.47)	4.80	**<0.001**	0.76 (±0.38)	2.01	**0.046**	0.17 (±0.14)	1.20	0.231
Lipid mass intake												
(Intercept)	118.87 (±14.5)	8.20	**<0.001**	88.33 (±7.69)	11.49	**<0.001**	75.23 (±8.3)	9.10	**<0.001**	22.58 (±5.42)	4.16	**<0.001**
Seed mass	−68.25 (±15.4)	−4.44	**<0.001**	−52.34 (±8.14)	−6.43	**<0.001**	−53.25 (±8.8)	−6.08	**<0.001**	−21.23 (±5.75)	−3.70	**<0.001**
Seed lipid content	103.40 (±14.6)	7.10	**<0.001**	73.66 (±7.7)	9.54	**<0.001**	49.48 (±8.31)	5.96	**<0.001**	22.62 (±5.5)	4.15	**<0.001**
Seed mass × Seed lipid content interaction	−61.75 (±16.1)	−3.84	**<0.001**	−48.74 (±8.5)	−5.72	**<0.001**	−44.73 (±9.17)	−4.88	**<0.001**	−23.49 (±6.0)	−3.91	**<0.001**

All explanatory variables were reduced and centred to allow comparisons between beetle species and parameters. Significant values are bold.
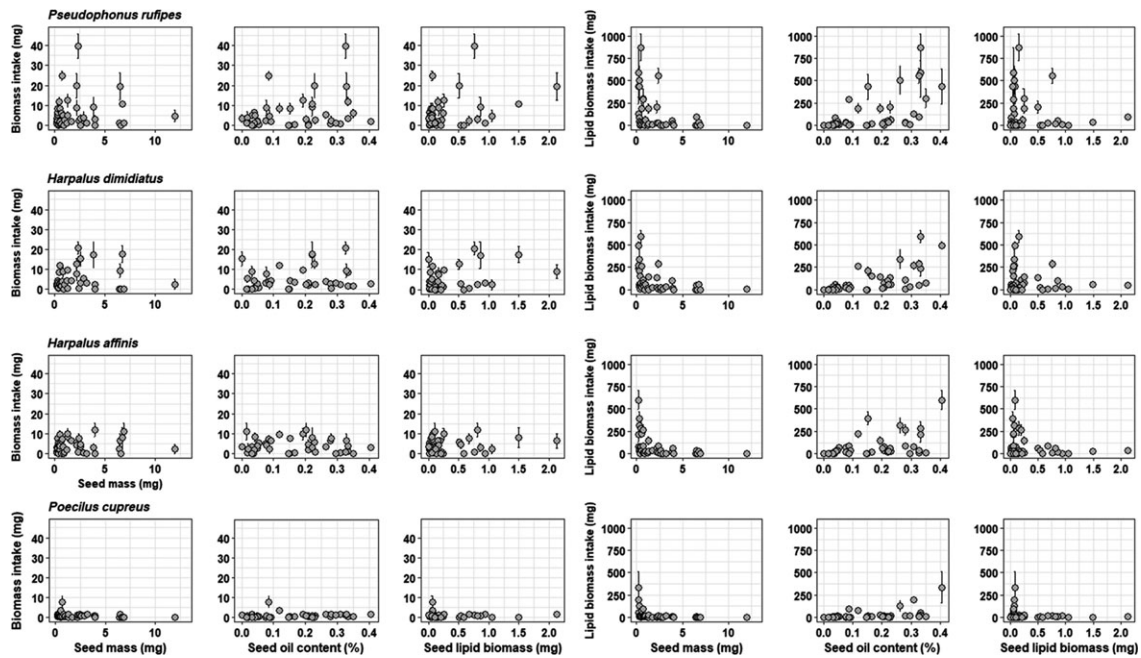
Fig. 3 Total biomass intake (three left graphs) and total lipid biomass intake (three right graphs) as a function of, from left to right, seed mass, seed lipid content, and seed total lipid biomass, for each of the four ground beetles.

example, *Viola arvensis* Murray and *Chenopodium album* L. have been found in other studies as well (Petit *et al.*, 2014).

Our results further showed a higher consumption of those seed species with high lipid content. Provided seed size was suitable for consumption, ground beetles consumed seeds with higher lipid content. Number of seed intake was positively correlated with seed lipid content for the two largest ground beetles, while these were not correlated for the two smallest beetles (*H. affinis* and *P. cupreus*). Though the four carabid beetles tested in this study clearly preferred small seeds, we found that seed lipid content also drives the consumption rate. Many small weed seeds were not consumed at all, so there are other seed characteristics that discourage ground beetles from consuming them, such as lack of lipids or the presence of chemical compounds. In addition, by quantifying the statistical interaction between seed mass and seed lipid content, and using a weed seed selection design avoiding correlation between seed mass and seed lipid content, we found that both *Harpalus* species and *P. rufipes* prioritised seed lipid content over seed size. The seed families we tested were however unequally distributed (60% of the families were represented by a single species). Using the weed seed clade as a proxy for the phylogenetic factor (factor with 6 levels), we detected no significant effect on the seed mass ($F_{5,36} = 0.487$, $P = 0.78$), but there was a significant effect on the seed lipid content ($F_{5,36} = 6.22$, $P = 0.0003$). The

phylogenetic signal in our seed data set therefore calls for further studies.

Our results partly support the hypothesis that largest-bodied beetles would have access to a wider set of weed seed species (Honek *et al.*, 2003). *Poecilus cupreus* showed both the lowest total consumption and the smallest range of weed seeds consumed, suggesting that it is not actually a seed eater. This ground beetle species is known to be omnivorous and seems to be a specialist of few weed seeds (Hanski & Cambefort, 1991). Since ground beetles make use of olfactory cues to detect weed seeds, soaked seeds would be easier to detect (Kulkarni *et al.*, 2017) in addition to being softer and, therefore, easier to ingest (Law & Gallagher, 2015).

Top-down regulation of weeds resulting from the activity of granivorous species is potentially an important ecosystem function (Losey & Vaughan, 2006; Bohan *et al.*, 2011; Gaba *et al.*, 2014a). The involvement of beetles has not yet been studied quantitatively, and the potential for weed seedbank regulation has not been compared between beetles and other seed eaters, such as birds and small mammals (but see Brust & House, 1988; Honek *et al.*, 2003). It is likely that the diversification of seed predators based on their size and metabolic requirements may result in complementary seed predation due to their different preferences for seed size and seed lipid content. Few studies have investigated the effects of seed traits on weed seed consumption by granivorous species such as skylarks

(*Alauda arvensis*), a winter seed feeder and a common farmland specialist bird (Robinson, 2004; Eraud *et al.*, 2015). Skylarks mostly eat seeds that are as small as the seeds selected by carabid beetles, that is <2 mg (Green, 1978; Eraud *et al.*, 2015). The seed species most commonly eaten by ground beetles and skylarks include *Chenopodium* spp. Skylarks also trade off size and lipid content, showing a preference for smallest weed seeds with high lipid content (Gaba *et al.*, 2014b). Conversely, a preference for the largest seeds, including hard-shelled seeds, was found for the North American deer mouse *Peromyscus maniculatus* (Brust & House, 1988; see also Moles *et al.*, 2003). For a consistent assessment of the potential for weed seedbank regulation, it may, therefore, be useful to compare foraging preferences of the main seed eaters in farmland landscapes over a range of fields, since seed availability in fields may affect weed seed consumption rate.

Acknowledgements

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Relationship between seed coat thickness and (A) seed mass and (B) seed oil content.

Table S1 Summary of numerical seed intake, total biomass ingested and total lipid mass ingested for the ground beetle species.

Figure S2 The rank of the weed species based on seed intake as a function of the rank of the species based on biomass intake (A) and lipid mass intake (B) for the four ground beetles.

Figure S3 Seed intake as a function of seed mass, seed lipid content and seed total lipids, for the four ground beetles.