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Abstract

1. Biological control is a key ecosystem service in arable lands, but its effectiveness varies according to environmental and biotic contexts. Cascading interactions between several trophic levels can affect natural enemies and their efficacy.
2. Here, we analysed how multitrophic interactions drive weed seed control under contrasting farming systems and landscapes. In particular, we analyse how the presence of higher-order predators and alternative prey affects the weed seed consumption by seed predators. We monitored 30 cereal fields organised into 15 pairs, each comprising one conventional and one conservation agriculture field, sampled along a gradient of proportion of conservation agriculture in the landscape.
3. We found that local and landscape management under conservation agriculture favours the presence of seed predators like carabids and rodents, higher-order predators like shrews and alternative animal prey. Weed seed predation is promoted by conservation agriculture through an increase in the number of seed predators. However, alternative animal prey reduces the efficacy of carabids to consume seeds, probably due to a prey switching behaviour. Similarly, shrews negatively affect the activity-density of carabids, resulting in an indirect negative effect on seed predation.
4. Synthesis and applications: Our study highlights that the implementation of conservation agriculture can improve the provision of biological control but the resulting effect may be partially limited by the increased complexity of trophic

interactions. The different trophic levels respond to local management and/or the surrounding landscape with cascading effects on the delivery of weed control. Our study highlights the importance of considering not only the direct effects of seed predators, but also the indirect effects of higher-order predators and alternative prey when predicting the level of weed biological control.

Keywords: conservation agriculture; carabids; rodents; alternative resources; higher-order predators; weed control; landscape composition; agroecology.

Second language abstract (français)

1. Les régulations biologiques constituent un service écosystémique clé dans les parcelles agricoles, mais leur efficacité peut varier en fonction des contextes environnementaux et biotiques. En effet, des interactions en cascade entre plusieurs niveaux trophiques peuvent affecter les ennemis naturels et leur efficacité.
2. Dans cet article, nous avons étudié comment les interactions multi trophiques déterminent les niveaux de prédation des graines d'adventices dans des systèmes agricoles et des paysages contrastés. En particulier, nous avons analysé comment la présence de prédateurs d'ordre supérieur et de proies alternatives affecte la consommation de graines d'adventices par les prédateurs de graines. Nous avons suivi 30 parcelles de céréales échantillonnées le long d'un gradient de proportion d'agriculture de conservation des sols dans le paysage. Les parcelles étaient organisées en 15 paires comprenant chacune une parcelle conduite en agriculture conventionnelle et une autre en agriculture de conservation des sols.
3. Nos résultats indiquent qu'une gestion en agriculture de conservation des sols, localement et dans le paysage, favorise la présence de prédateurs de graines d'adventices tels que les carabes et les rongeurs, et celle de prédateurs d'ordre supérieur tels que les musaraignes et de proies animales alternatives. La prédation des graines d'adventices est favorisée par l'agriculture de conservation des sols grâce à l'augmentation du nombre de prédateurs de graines. Cependant, les proies animales alternatives réduisent l'efficacité des carabes à consommer des graines, probablement en raison d'un comportement de changement de proie. De même, les musaraignes affectent négativement l'activité-densité des carabes, ce qui a un effet négatif indirect sur la prédation des graines.
4. Synthèse et applications : Notre étude souligne que l'agriculture de conservation des sols peut favoriser le contrôle biologique des adventices, mais cet effet peut être partiellement limité par la complexité des interactions trophiques. Les différents niveaux trophiques réagissent à la gestion locale et/ou au paysage environnant avec des effets en cascade sur la régulation des adventices. Notre étude montre l'importance de prendre en compte non seulement les effets directs des prédateurs de graines, mais aussi les effets indirects des prédateurs d'ordre supérieur et des

proies alternatives lorsqu'il s'agit de prédire le niveau de contrôle biologique des adventices.

Mots-clés : agriculture de conservation des sols ; carabes ; rongeurs ; ressources alternatives ; prédateurs d’ordre supérieur ; régulation biologique des adventices ; composition du paysage ; agroécologie.

1. Introduction

Biological control is an essential ecosystem service to prevent crop losses, reducing both pests and weeds in agroecosystems. This could contribute to the reduction of pesticide applications (Shields et al., 2019). However, biological control remains underused by farmers, possibly because its effectiveness varies over time and space, and is highly context-dependent. Agroecosystems are simultaneously visited by different functional groups influencing each other through interactions (Letourneau et al., 2009). Specifically, the efficacy of key natural enemies providing biological control may be inhibited by trophic interactions such as intraguild or higher-order predations (Finke & Denno, 2005; Martin et al., 2013) or by their consumption of prey other than the targeted pest (Prasad & Snyder, 2006). The efficacy of biological control thus results from complex trophic interactions, making the prediction of service provision difficult (Eisenhauer et al., 2019). Therefore, we need studies accounting for multitrophic levels that may have direct or indirect effects on the efficacy of biological control.

Among the wide array of biotic threats to crops, weeds usually have the greatest impact (Oerke, 2006). Weed biological control is mainly the result of top-down regulatory forces exerted by seed predators, thus reducing seed bank regeneration (Carbonne et al., 2020) and weed emergence (Blubaugh & Kaplan, 2016). In temperate climates, carabids and rodents are abundant weed seed predators (Kulkarni et al., 2015). However, the relationship between seed predator abundance and weed seed loss seems context-dependent (Saska et al., 2008). An important cause of this variation may be related to the different trophic interactions in which seed predators are involved that may affect their community (Carbonne et al., 2022b) and their foraging and feeding behaviours (de Heij & Willenborg, 2020). For example, carabids can be predated by higher-order predators, like birds or small mammals, but also by intraguild predators, with negative repercussions on biological control (Birthisel et al., 2014). Additionally, a significant proportion of carabid seed predators are generalist consumers which can feed on a plenty of prey (Frei et al., 2019). The presence of alternative prey (e.g. collembola, earthworms or aphids), which can substitute for the consumption of weed seeds, can affect carabid efficacy through prey switching (Carbonne et al., 2020; Gray et al., 2021). When alternative prey is much abundant or preferred, carabids may switch from seeds to other prey, indirectly dampening the biological control of weeds. The complexity of such

interactions that influence the efficacy of weed control requires substantial research. We need studies unravellig top-down vs. bottom-up effects to harness pest control services over time.

In agroecosystems, the species composition and the resulting interaction network are influenced by the intensity of field management with consequences on the provision of services (MacFadyen et al., 2009). For example, conservation agriculture, which is characterised by no-tillage and cover crops, improve the functional diversity of soil organisms, including seed predators, their predators and alternative prey (Henneron et al., 2015; Petit et al., 2017). At a landscape scale, the deployment of these systems could also play a major role in the composition of mobile species, but its role is poorly documented (Petit et al., 2020). By promoting in-field biodiversity, agroecological systems implemented locally or at landscape scales can complicate trophic cascades with potential indirect negative effects on service provision (Tschumi et al., 2018). Deciphering such complex relationships is necessary to understand why the implementation of agroecological practices does not always guarantee the promotion of biological control. This will contribute to guide the agroecological transition at large scales (Haan et al., 2020).

Here, we evaluated how trophic interactions drive the biological control of weed seeds in arable crops under conventional and conservation systems. The fields were positioned along a gradient of proportion of conservation agriculture in the landscape. Specifically, using structural equation modelling, we evaluated how local and landscape field management interact to explain the abundance of seed predators and their efficacy to consume seeds. We hypothesised that seed predators and seed consumption are promoted by local and landscape management in conservation agriculture. In addition, we also hypothesised that conservation agriculture promotes higher-order predators and alternative prey, which may reduce the efficacy of weed seed predators.

2. Materials and methods

a. Experimental design.

A field survey was conducted in 15 pairs of cereal fields (25 fields with winter wheat) within a 66 km radius around Dijon in France (47° 19' 19.369" N 5° 2' 29.328" E, Fig. S1). This area is dominated by arable land with significant forest cover and some grasslands. All field sizes ranged from 1.94 to 38.7 ha, with the exception of two experimental fields (Table S1). The

mean distance between two nearest sampled field pairs was 9.6 km. Within each pair, biological measures were conducted in two adjoining fields with an average distance between the two sampled zones of 123.6 m (SD=121.1). Within each field, measures were carried out at three sample points spaced 15 m apart and located 20-50 m from the field margins (Fig. S2) except for the two experimental fields. This distance allows sampling in the field core and reduces the influence of field edges. Two field sampling sessions were conducted in May and June 2021. Given the measurements taken in the field, our study did not require any special permission or ethical approval.

b. Local and landscape management.

Fields were selected to cover a gradient of proportion of conservation agricultural ("%CSA") lands in the landscape. The landscape was documented using the French Graphic Parcel Register, superimposed on the CORINE Land Cover map 2000 in ArcMap 10.8. The land use and the management system of the parcels were recorded through field and farmer surveys within a circle of 1 km radius around focal fields. It is a relevant scale for detecting the responses of seed predators to the landscape (Veres et al., 2013). The %CSA ranged from 3.1 to 37.5% and was not correlated with other landscape descriptors (Table S2) ensuring no confounding effects.

Each pair consists of one field managed with conventional agriculture ("CVA") and another with CSA. CSA fields are characterised by no tillage nor superficial soil management for at least 4 years and by the introduction of a cover crop during the period between crops. Conventional fields are tilled during the intercropping period with a rare use of a mouldboard plough. In the two systems, weed management is based on rational use of chemical weed control during the crop and intercropping period (Table S1). Fungicides and insecticides are used following locally developed integrated management practices.

c. Seed-predators: carabids and rodents.

Carabids (Coleoptera: Carabidae) were sampled using three pitfall traps per field. Each trap was composed of a plastic beaker (10 cm height, 8 cm diameter), filled with 150ml of a preservative solution of salt-saturated water and a few drops of odourless soap. A plastic cover was placed above each trap to avoid rain flooding. The traps were exposed for seven days between 3-12 May and 2-9 June 2021. Carabids were identified at the species level

(Coulon et al., 2011). Carabids collected in the three traps in each field were pooled to give a field total per sampling session. For the analyses, we considered the carabid activity-density for granivorous and omnivorous species only following Homburg et al. (2014). We caught 1204 (May) and 632 (June) omnivores dominated by *Poecilus cupreus* and 1370 (May) and 1217 (June) granivores dominated by species of the genera *Harpalus* (Table S4).

Rodents were captured using "INRA traps" (16 cm long, 5 cm height, 5 cm width - BTTM company, Fig. S3) connected to 500 ml boxes containing hay, a gel water ball and a food pellet (beef-based cat food and peanut butter) to meet the needs of the captured small mammals. The traps were placed in two lines of 25 traps spaced at 3 m intervals. The two lines were placed near a tractor wheel path and separated by 20 - 30 m. The traps were exposed for three consecutive days and the presence of captures was checked every 24 hours. Rodent sampling on all fields was conducted over a single period between 26 May and 10 June 2021. Captured individuals were carefully identified, marked, and released directly into the field with minimal handling; therefore, our study did not require ethical approval. All captures for the three days were pooled to obtain a total abundance per field. We captured a total of 123 rodents (Table S5).

d. Higher-order predators of carabids: shrews and spiders.

We included shrews (Soricidae: Sorex) in higher-order predators as they are insectivores that frequently consume beetles, e.g., about 60% of the spring diet consists of beetles for *Sorex araneus* (Rudge, 1968). We captured 18 shrews using the same protocol used for rodents.

Moreover, we included a selection of spider taxa that are likely to consume carabids according to expert opinion. Spiders were collected using the same protocol used for carabids. Spiders were identified at the species level and pooled to obtain a total abundance per field. We captured 587 (May) and 411 (June) spiders (Table S6).

e. Invertebrate animal resources: collembola, aphids and earthworms.

Animal resource availability was described using collembola, aphids and earthworms that are consumed by carabids in arable land (Frei et al., 2019).

Collembola and aphids were collected using suction sampling between the June 2 and June 4. Suction sampling was performed using a 'Vortis' insect suction sampler (Burkard

193 Manufacturing Co Ltd) on windless and rainless days when the temperature was above 15°C.
194 Due to bad weather conditions, the May sampling session could not be completed. 'Vortis'
195 samples were taken at each of the three sampling points, for each of which two suctions were
196 performed. The vortis is set at maximum speed starting from the top of the vegetation and
197 gradually descending to the soil surface, where the vacuum is maintained for 10 seconds. All
198 captures were pooled to obtain the total abundances of collembola and aphids per field. We
199 captured a total of 21047 collembola and 2376 aphids in June (Table S7).

200 To estimate the abundance of earthworms, we collected a soil volume of 18 L with a shovel
201 (15 cm height, 35 cm length, 35 cm width) in April 2021 for each field in the same area as the
202 other records. Soil samples were sorted and all earthworms were counted and classified into
203 four functional groups: endogeneous, epigeneous, strict-anecic and epi-anecic. We selected
204 epigeneous, strict-anecic and epi-anecic earthworms because they are more available on the
205 ground surface and susceptible to be consumed by carabids (Jelaska & Symondson, 2016).
206 These three groups were pooled to obtain a total abundance per field. We counted a total of
207 270 earthworms (Table S7).

208 ***f. Plant resources.***

209 Plant resources were described using the availability of weed seeds and the weed flora. Weed
210 seeds were collected and counted using the same suction sampling as those used to capture
211 collembola and aphids. All weed seeds were pooled to obtain a total abundance per field. We
212 collected a total of 321 seeds in June (Table S7).

213 Weed communities were surveyed between 7-9 June in a 50 × 40 m (2000 m^2) area located
214 near the three sampling points. All plants were recorded following a W-shaped walking path
215 and identified at the species level (Jauzein, 1995). The abundance of each species was
216 estimated using a gradual scale of abundance: [0.1] found once, [0.75-1.25] less than 1; [1.75-
217 2.25] 1–2; [2.75-3.25] 3–20; [3.75-4.25] 21–50 and [4.75-5.25] more than 50 individuals per
218 m^2. Only plants in the flowering and fructification stages were retained as these are the ones
219 that have or will rapidly provide resources for seed predators. All abundance indices of all
220 weeds were summed to obtain a total index per field (Table S7).

221 ***g. Weed seed predation.***

Weed seed predation cards (Westerman et al., 2003) were used to estimate seed predation rates. We selected three seeds covering different sizes, shapes and oil contents (Gaba et al., 2019): *Alopecurus myosuroides* and *Galium aparine* that can lead to significant losses in cereal yield production, and *Viola arvensis,* which is a model seed that is often used to measure predation rates. We glued 20 seeds of each weed species on separate cards (95 × 40 mm card of sandpaper) using SADER ® WOOD PRO D3 diluted with two-thirds of water. At each sample point, and for each seed species we exposed one card with a vertebrate exclosure cage (1 cm^2 wire mesh) to measure predation by invertebrates and another card without cage to measure total (invertebrate + vertebrate) weed seed predation. A total of 18 cards per field were exposed over seven days simultaneously with the pitfall traps. The seed predation rate was estimated using the number of seeds removed from the seed cards. We pooled the records obtained for the three weed species to calculate the invertebrate and total predation rate per field and session (Table S3).

h. Statistical analysis.

We developed two piecewise structural equation models (SEM). The first SEM aimed at explaining invertebrate weed seed predation by quantifying the indirect effect of local management (CVA *vs.* CSA) and the %CSA in landscape through trophic interactions involving higher-order predators, carabid seed-predators and resources (animal and plant) (Fig. 1 A). In the second SEM we aimed to explain total weed seed predation using the same conceptual diagram in addition to which we added the rodent seed-predators (Fig. 1 B). Separate SEMs were performed for the May and June sampling sessions. As the data for the May session are not complete (no plant and animal index), the corresponding SEM is provided as additional material (Fig. S4 and Table S8). The small mammal survey is common to both sessions, as their sampling covered both months.

As different methods have been used to quantify trophic resources and since some are much more abundant than others, we have performed a Min-Max normalisation in order to give each resource the same weight by bounding them between 0 and 1. The animal resource index used was the sum of the abundances of collembola, aphids and earthworms after log-transformation and Min-Max normalisation. The plant resource index was the sum of the abundances of weed seed and weed plants after log-transformation and Min-Max normalisation.

To reduce SEM complexity, the explanatory variables for each individual model in the SEMs were selected in several steps. First, we applied a model selection based on the AIC and we kept all selected variables in the best models (delta AIC < 2). For complex models that explain predation rates, separate selections were made sequentially. In the second step, we improved the fit of individual models by performing a step-by-step backward simplification. The resulting SEMs were further simplified by removing non-significant relationships in cases where this improved the overall AIC of the SEMs. These sequential simplifications resulted in the elimination of some variables initially included (Fig. 1).

We used generalised linear mixed effects models within piecewise SEMs with the binomial family (link = "logit") for predation rates and the poisson family (link = "log") for counts. We used linear mixed-effects models for the analysis of animal and plant resource indexes. The random structure of all models included the pair identity (1:15) to take account of the dependence of the paired fields. We also included in some models a field observation-level random effect (1:30) to correct overdispersion. The model residuals were visually checked for normality and homoscedasticity using DHARMa (Hartig, 2022). Collinearity in the models was low (variance inflation factor for non-interaction effects < 3). The overall fit of our piecewise SEM was assessed with D-separation tests on Fisher's C statistic (Lefcheck, 2016) and AIC. For the SEM explaining total seed predation in June, a significant missing path was detected and added. The P-values and model parameters were recovered from the 'summary.psem ' procedure (package 'PiecewiseSEM'). The stability of model parameters and the absence of overfitting of the SEMs was checked by simplifying the SEMs by removing variables not involved in direct/indirect effects on seed predation (Fig. S5 and Table S9). In addition, we checked the stability of the linear models alone and included in the SEM.

All analyses and figures were conducted in R version 4.2.1 (R Core Team, 2022) and we used packages lme4 (procedures 'glmer' and 'lmer'); MuMIn ('dredge'; Bartoń, 2022), PiecewiseSEM ('psem'; Lefcheck, 2016) and ggeffect for the figures.

3. Results

a. Invertebrate weed seed predation

281 The SEM for invertebrate seed predation in June represented our data well, with no significant
282 missing paths detected (Fig. 2.A; Fisher's C = 16.56, P = 0.867). Variation in invertebrate seed
283 predation rates was well-explained (R^2m = 27%; R^2c = 98%).

284 Local management significantly affected the granivore carabid activity-density (p=0.017; Table
285 1) and animal resources (p=0.014; Table 1). CSA fields had higher animal resources and
286 granivore carabids compared to conventionally managed fields (Fig. 3.A and B, respectively).
287 Seed predation rates were affected by an interaction effect between granivore carabids and
288 animal resources (p-value = 0.002; Table 1). We detected a positive relationship between seed
289 predation rates and granivore carabids that decreased in intensity as animal resources
290 increased (Fig. 4.A). In contrast to the June session, in May we detect a positive relationship
291 between invertebrate seed predation rates and omnivorous carabids, which are much more
292 abundant at this period (Fig. S4 and Table S8).

293 The abundance of shrews was significantly affected by %CSA in the landscape in interaction
294 with the local management (p=0.026; Table 1; Fig. 3.D). In CSA fields, increasing the %CSA in
295 landscape has a positive effect on shrew abundance, while no relationship was detected for
296 conventional fields. In addition, the increase in shrew abundance has a negative effect on
297 granivorous carabids (p=0.018; Table 1; Fig. 3.E). Spider abundance was positively correlated
298 with %CSA (Table 1).

299 Consequently, local field management and %CSA in the landscape indirectly affected
300 invertebrate weed seed predation rates through positive and negative cascading effects. CSA
301 fields favoured granivore carabids that were, in turn, positively associated with weed seed
302 predation. However, local field management and the deployment of CSA in the landscape
303 favoured animal resources and shrew abundance, respectively, which in turn reduced the
304 efficacy and the activity-density of carabids.

305 ***b. Total weed seed predation rates***

306 The SEM for total weed seed predation in June represented our data well (Fig. 3.B; Fisher's C
307 = 29.28, P = 0.503). SEM very well explained the variation in the seed predation rates (R^2m =
308 59%; R^2c = 98%).

309 The local management directly affected the total seed predation rates (p<0.001; Table 1; Fig.
310 4.B) with higher predation in CSA fields than in conventional fields. Moreover, local

311 management indirectly affected seed predation through animal resources and rodent
312 abundances. Animal resources, which were significantly higher in CSA than in conventional
313 fields, were negatively correlated to total seed predation rates ($p<0.001$; Table 1; Fig. 4.C). The
314 abundance of rodents was significantly affected by the %CSA in the landscape in interaction
315 with local management ($p=0.001$; Table 1; Fig. 3.C). In fields managed with conventional
316 agriculture, increasing the %CSA in the landscape has a positive effect on rodent abundance,
317 while no relationship was detected for CSA fields. The increased abundance of rodents has a
318 positive effect on the total seed predation rate ($p=0.007$; Table 1; Fig. 4.D). Contrary to the
319 June session, in May we only detect a positive trend effect of omnivore carabids on total
320 predation rates (Fig. S4 and Table S8).

321 Consequently, local management and %CSA in the landscape directly affected total weed seed
322 predation rates, but also indirectly through two cascading effects: a negative one mediated
323 by animal resources and a positive one mediated by rodent abundance.

324 ## 4. Discussion

325 Our results reveal that the implementation of conservation agriculture – both at the local and
326 landscape scales - can improve the weed control service by promoting the presence of seed
327 predators. This increase in seed predation appears to be partially limited by the complexity of
328 the multitrophic interactions including seed predators, higher-order predators and alternative
329 prey. These different trophic levels all respond to local management and/or the surrounding
330 landscape with cascading effects on the delivery of weed control. Compared to conventional
331 agriculture, we found that conservation agriculture favours the density of carabids and
332 rodents that consume weed seeds but also promotes the presence of higher-order predators
333 and alternative animal prey that reduce carabid efficacy to consume seeds.

334 ### *a. Multitrophic interactions drive weed seed predation:*

335 Our results indicate that the contribution of granivore carabids to the predation of weed seeds
336 could be affected by trophic interactions involving higher-order predators and alternative prey
337 like animal resources. Invertebrate seed predation was positively affected by omnivorous and
338 granivorous carabids, respectively, in May and June. This difference may result from
339 omnivores being twice as abundant in May as in June. The increase in shrew abundance has a
340 negative effect on granivorous carabids for both periods, resulting in an indirect negative

effect on weed seed consumption in June. This negative effect was expected given the insectivorous diet of shrews and their important consumption of large invertebrates (6-10 mm) encompassing many carabid species (Rudge, 1968). Predation by insectivorous vertebrates, in particular shrews or birds, can negatively influence coleopteran insect populations (Mooney et al., 2010. This negative effect can indirectly lead to a reduction in the effectiveness of biological control (Grass et al., 2017; Martin et al., 2013) including weed seed predation by carabids (Birthisel et al., 2014). Moreover, insectivorous predators can also affect the foraging activity of carabids, and thus their efficacy to consume seeds (de Heij & Willenborg, 2020). For example, Blubaugh et al. (2017) showed that exposure to mouse cues reduced the activity-density of a seed-eating carabid, *Harpalus pensylvanicus*, but increased its seed consumption, which is not observed in our study. Charalabidis et al. (2019) also observed an increase in seed consumption by *H. affinis* when exposed to chemical cues from *Pterostichus melanarius*, a potential intraguild predatory carabid. Therefore, the effectiveness of weed seed biological control is modulated by the presence of higher-order and intraguild predators that can simultaneously affect the populations and behaviour of seed-eating carabids, making the prediction of service provision complex.

Our SEM analyses showed that the weed seed predation by carabids was reduced by the availability of animal prey. This negative effect was already observed for collembola and aphid prey (Carbonne et al., 2020) and could be related to carabid prey switching behaviour (Frank et al., 2011). Animal prey, indeed, can be consumed by a large number of carabid species, including species classified as granivorous such as *Pseudoophonus rufipes* (Frei et al., 2019). The preferential consumption of one type of prey by the predator may indirectly and positively affect another type of prey that will be less targeted by the predator. This pattern of indirect positive interactions between prey can be termed "apparent commensalism or mutualism" (Chailleux et al., 2014). Consumption of animal prey could be more attractive to some species of carabids and lead to a satiation of individuals that will consume fewer seeds. Furthermore, the high availability of animal prey may reduce the encounter rate between seeds and carabids through a dilution effect (Evans, 2008). Consequently, the presence of animal prey may indirectly preserve seeds by reducing their consumption by carabids. This observation seems to be confirmed by the clear negative effect of animal resources on total seed predation (without cage). However, this effect is not detected through an interaction with carabids,

probably because their contribution was masked by that of rodents, whose consumption on seed cards is often important (Westerman et al., 2003).

Although seeds and animal prey appear to be concurrent prey in late spring, it is possible that earlier in the growing season, animal prey may play a major role in the maintenance and development of carabid populations in fields (Lundgren, 2009). The presence of alternative prey may satisfy the dietary requirements of certain carabid species and permit their rapid field colonisation and temporal persistence in fields (Symondson et al., 2002). Therefore, the trophic interactions identified in our study can vary over the course of the cropping season, depending on the dynamics of the different trophic levels. Due to incomplete data in May, we could not assess the role of alternative prey during this period. However, the preharvest period sampled in June corresponds to a key period for seed predation, as seed predators are particularly active and weed seed rain is quite intense. Future research should extend this analysis over several time replications over the cropping season and over several years to verify the evolution of the trophic cascades described. Furthermore, given the high complexity of interactions tested in trophic ecology SEMs (Redlich et al., 2021), ours included, we recommend that future research increases the number of field replications performed.

b. Local and landscape management modulate multitrophic interactions:

The conservation system favoured granivorous carabids in May and June, but also animal resources compared to conventionally managed fields. These results are in accordance with the previous observations of Henneron et al. (2015) showing that long-term CSA increased the number of many organisms including earthworms, herbivores and granivore arthropods. The absence of tillage is particularly favourable to earthworms, but also to certain carabid species, whose larvae are present in the soil and are particularly sensitive to autumn tillage (Müller et al., 2022). Tillage affects carabids directly by physical killing, but also indirectly by influencing food resources and soil physical properties such as soil structure, water balance, and plant residues (Müller et al., 2022). Moreover, the presence of cover crops in CSA could favour arthropods, including carabids, by providing a favourable microhabitat and greater food resources (Kulkarni et al., 2015).

At the landscape scale, we found that the %CSA in the landscape, in interaction with local management, favoured shrew and rodent abundances. Small mammals have a high dispersal

capacity that allows them to colonise and move between arable fields. CSA fields may represent a relatively more stable and suitable habitat for small mammals than conventional fields (Ruscoe et al., 2022), and can therefore be source habitats for neighbouring fields. The positive effect of %CSA on rodents was stronger in fields locally managed with conventional than CSA. Rodent populations in low-disturbed fields, like those in CSA, could become established over the long term, and therefore their abundance could be less dependent on source habitats in the surrounding landscape. In contrast, rodent populations in highly disturbed conventional fields may not be able to establish, and observed abundances could mainly depend on the colonisation from surrounding source habitats (Ruscoe et al., 2022), such as CSA fields for example. The increased abundance of rodents has a positive effect on total seed predation in June but not in May, possibly because animal resources were not included in May, which may have affected the final model. Moreover, rodents are also a concern for farmers in CSA because they may generate crop damage (Ruscoe et al. 2022) particularly *Microtus sp.* (voles) which are dominant in our surveys and damage the crop at the time of sowing, germination or crop establishment. However, Apodemus species can also cause damage by entering the field during the pre-harvest period and feeding on the seeds on the ground or on the ears.

5. Conclusion

The simultaneous consideration of multitrophic groups, especially alternative prey and higher-order predators in addition to seed predators, appears essential to understand the intensity of weed seed control. Our study shows that CSA and its deployment in the landscape support carabids and rodents and thus indirectly promotes the predation of weed seeds. However, this increase in seed predation is partially limited by promoting insectivorous predators, such as shrews, which can consume and negatively affect carabid populations and their seed consumption. The presence of alternative prey, moreover, can lead to prey-switching behaviour of carabids, which may shift from seeds to other food resources. Therefore, conservation practices may improve overall weed biocontrol, but the resulting effect may be partially attenuated by the associated increased complexity of multitrophic interactions.

Data accessibility. Data available via the Data INRAE repository https://doi.org/10.57745/51CZ5X (Carbonne et al., 2022a).

Authors' contributions. Benjamin Carbonne participated in the design of the study, carried out the statistical analyses and drafted the manuscript; Sandrine Petit and Lucile Muneret conceived and coordinated the study, participated in data analysis and helped draft the manuscript; Benjamin Carbonne, Lucile Muneret, Emilien Laurent, Emeline Felten, Chantal Ducourtieux, Nicolas Henon, Annick Matejicek and Bruno Chauvel collected field data. Emilien Laurent, Emeline Felten and Benjamin Carbonne carried out GIS analyses. All authors gave their final approval for publication and agreed to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

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References

Bartoń, K. (2022). *MuMIn: Multi-Model Inference. R package version 1.47.*

Birthisel, S. K., Gallandt, E. R., & Jabbour, R. (2014). Habitat effects on second-order predation of the seed predator *Harpalus rufipes* and implications for weed seedbank management. *Biological Control*, *70*, 65–72. https://doi.org/10.1016/J.BIOCONTROL.2013.12.004

Blubaugh, C. K., & Kaplan, I. (2016). Invertebrate Seed Predators Reduce Weed Emergence Following Seed Rain. *Weed Science*, *64*(01), 80–86. https://doi.org/10.1614/WS-D-15-00111.1

Blubaugh, C. K., Widick, I. v., & Kaplan, I. (2017). Does fear beget fear? Risk-mediated habitat selection triggers predator avoidance at lower trophic levels. *Oecologia*, *185*(1), 1–11. https://doi.org/10.1007/s00442-017-3909-1

Carbonne, B., Muneret, L., Laurent, E., Felten, E., Ducourtieux, C., Henon, N., Matejicek, A., Chauvel, B., Petit, S. (2022a). Data for : Conservation agriculture affects multitrophic interactions driving the efficacy of weed biological control. Recherche Data Gouv, V4, https://doi.org/10.57745/51CZ5X

Carbonne, B., Bohan, D. A., Foffová, H., Daouti, E., Frei, B., Neidel, V., Saska, P., Skuhrovec, J., & Petit, S. (2022b). Direct and indirect effects of landscape and field management intensity on carabids through trophic resources and weeds. *Journal of Applied Ecology*, *59*(1), 176–187. https://doi.org/10.1111/1365-2664.14043

Carbonne, B., Petit, S., Neidel, V., Foffova, H., Daouti, E., Frei, B., Skuhrovec, J., Řezáč, M., Saska, P., Wallinger, C., Traugott, M., & Bohan, D. A. (2020). The resilience of weed seedbank regulation by carabid beetles, at continental scales, to alternative prey. *Scientific Reports*, *10*(1), 19315. https://doi.org/10.1038/s41598-020-76305-w

Chailleux, A., Mohl, E. K., Teixeira Alves, M., Messelink, G. J., & Desneux, N. (2014). Natural enemy-mediated indirect interactions among prey species: Potential for enhancing biocontrol services in agroecosystems. *Pest Management Science*, *70*(12), 1769–1779. https://doi.org/10.1002/ps.3916

Charalabidis, A., Dechaume-Moncharmont, F.-X., Carbonne, B., Bohan, D. A., & Petit, S. (2019). Diversity of foraging strategies and responses to predator interference in seed-eating carabid beetles. *Basic and Applied Ecology*, *36*, 13–24. https://doi.org/10.1016/j.baae.2019.02.003

Coulon, J., Pupier, R., Quéinnec, E., Ollivier, E., & Richoux, P. (2011). *Coléoptères carabiques, compléments et mise à jour, vol 1 et 2. Faune de France 94 et 95. Fédération Francaise Des Societes De Sciences Naturelles.* (Federation Francaise Des Societes De Sciences Naturelles, Ed.).

de Heij, S. E., & Willenborg, C. J. (2020). Connected carabids: network interactions and their impact on biocontrol by carabid beetles. *BioScience*, *70*(6), 490–500. https://doi.org/10.1093/biosci/biaa039

Eisenhauer, N., Schielzeth, H., Barnes, A. D., Barry, K. E., Bonn, A., Brose, U., Bruelheide, H., Buchmann, N., Buscot, F., Ebeling, A., Ferlian, O., Freschet, G. T., Giling, D. P., Hättenschwiler, S., Hillebrand, H., Hines, J., Isbell, F., Koller-France, E., König-Ries, B., … Jochum, M. (2019). A multitrophic perspective on biodiversity–ecosystem functioning research. *Advances in Ecological Research*, *61*(August), 1–54. https://doi.org/10.1016/bs.aecr.2019.06.001

Evans, E. W. (2008). Multitrophic interactions among plants, aphids, alternate prey and shared natural enemies - A review. *European Journal of Entomology*, *105*(3), 369–380. https://doi.org/10.14411/eje.2008.047

Finke, D. L., & Denno, R. F. (2005). Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. *Ecology Letters*, *8*(12), 1299–1306. https://doi.org/10.1111/j.1461-0248.2005.00832.x

Frank, S. D., Shrewsbury, P. M., & Denno, R. F. (2011). Plant versus prey resources: Influence on omnivore behavior and herbivore suppression. *Biological Control*, *57*(3), 229–235. https://doi.org/10.1016/j.biocontrol.2011.03.004

Frei, B., Guenay, Y., Bohan, D. A., Traugott, M., & Wallinger, C. (2019). Molecular analysis indicates high levels of carabid weed seed consumption in cereal fields across Central Europe. *Journal of Pest Science*, *92*(3), 935–942. https://doi.org/10.1007/s10340-019-01109-5

Gaba, S., Deroulers, P., Bretagnolle, F., & Bretagnolle, V. (2019). Lipid content drives weed seed consumption by ground beetles (Coleopterea, Carabidae) within the smallest seeds. *Weed Research*, *59*(3), 170–179. https://doi.org/10.1111/wre.12354

Grass, I., Lehmann, K., Thies, C., & Tscharntke, T. (2017). Insectivorous birds disrupt biological control of cereal aphids. *Ecology*, *98*(6), 1583–1590. https://doi.org/10.1002/ecy.1814

Gray, C., Ma, A., McLaughlin, O., Petit, S., Woodward, G., & Bohan, D. A. (2021). Ecological plasticity governs ecosystem services in multilayer networks. *Communications Biology*, *4*(1), 75. https://doi.org/10.1038/s42003-020-01547-3

Haan, N. L., Zhang, Y., & Landis, D. A. (2020). Predicting landscape configuration effects on agricultural pest suppression. *Trends in Ecology and Evolution*, *35*(2), 175–186. https://doi.org/10.1016/j.tree.2019.10.003

Hartig, F. (2022). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.5, https://CRAN.R-project.org/package=DHARMa

Henneron, L., Bernard, L., Hedde, M., Pelosi, C., Villenave, C., Chenu, C., Bertrand, M., Girardin, C., & Blanchart, E. (2015). Fourteen years of evidence for positive effects of conservation agriculture and organic farming on soil life. *Agronomy for Sustainable Development*, *35*(1), 169–181. https://doi.org/10.1007/s13593-014-0215-8

Homburg, K., Homburg, N., Schäfer, F., Schuldt, A., & Assmann, T. (2014). Carabids.org - a dynamic online database of ground beetle species traits (Coleoptera, Carabidae). *Insect Conservation and Diversity*, *7*(3), 195–205. https://doi.org/10.1111/icad.12045

Jauzein, P. (1995). *Flore des champs cultivés* (INRA éditions).

Jelaska, L. Š., & Symondson, W. O. C. (2016). Predation on epigeic, endogeic and anecic earthworms by carabids active in spring and autumn. *Periodicum Biologorum*, *118*(3), 281–289. https://doi.org/10.18054/pb.2016.118.3.4709

Kulkarni, S. S., Dosdall, L. M., & Willenborg, C. J. (2015). The Role of Ground Beetles (Coleoptera: Carabidae) in Weed Seed Consumption: A Review. *Weed Science*, *63*(2), 355–376. https://doi.org/10.1614/WS-D-14-00067.1

Lefcheck, J. S. (2016). piecewiseSEM : Piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, *7*(5), 573–579. https://doi.org/10.1111/2041-210X.12512

Letourneau, D. K., Jedlicka, J. A., Bothwell, S. G., & Moreno, C. R. (2009). Effects of natural enemy biodiversity on the suppression of arthropod herbivores in terrestrial

ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, *40*(1), 573–592. https://doi.org/10.1146/annurev.ecolsys.110308.120320

Lundgren, J. G. (2009). Chapter 16: Non-prey foods and biological control of arthropods. In *Relationships of natural enemies and non-prey foods* (Progress i, pp. 279–307). Springer Netherlands.

MacFadyen, S., Gibson, R., Polaszek, A., Morris, R. J., Craze, P. G., Planqué, R., Symondson, W. O. C., & Memmott, J. (2009). Do differences in food web structure between organic and conventional farms affect the ecosystem service of pest control? *Ecology Letters*, *12*(3), 229–238. https://doi.org/10.1111/j.1461-0248.2008.01279.x

Martin, E. A., Reineking, B., Seo, B., & Steffan-Dewenter, I. (2013). Natural enemy interactions constrain pest control in complex agricultural landscapes. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(14), 5534–5539. https://doi.org/10.1073/pnas.1215725110

Mooney, K. A., Gruner, D. S., Barber, N. A., van Bael, S. A., Philpott, S. M., & Greenberg, R. (2010). Interactions among predators and the cascading effects of vertebrate insectivores on arthropod communities and plants. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(16), 7335–7340. https://doi.org/10.1073/pnas.1001934107

Müller, P., Neuhoff, D., Nabel, M., Schiffers, K., & Döring, T. F. (2022). Tillage effects on ground beetles in temperate climates: a review. *Agronomy for Sustainable Development*, *42*(4), 65. https://doi.org/10.1007/s13593-022-00803-6

Oerke, E.-C. (2006). Crop losses to pests. *The Journal of Agricultural Science*, *144*(01), 31–43. https://doi.org/10.1017/S0021859605005708

Petit, S., Muneret, L., Carbonne, B., Hannachi, M., Ricci, B., Rusch, A., & Lavigne, C. (2020). Landscape-scale expansion of agroecology to enhance natural pest control: A systematic review. In *Advances in Ecological Research* (Vol. 63, pp. 1–48). https://doi.org/10.1016/bs.aecr.2020.09.001

Petit, S., Trichard, A., Biju-Duval, L., McLaughlin, Ó. B., & Bohan, D. A. (2017). Interactions between conservation agricultural practice and landscape composition promote weed seed predation by invertebrates. *Agriculture, Ecosystems & Environment*, *240*, 45–53. https://doi.org/10.1016/j.agee.2017.02.014

Prasad, R. P., & Snyder, W. E. (2006). Polyphagy complicates conservation biological control that targets generalist predators. *Journal of Applied Ecology*, *43*(2), 343–352. https://doi.org/10.1111/j.1365-2664.2006.01129.x

R Core Team. (2022). *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.*

576 Redlich, S., Martin, E. A., & Steffan-Dewenter, I. (2021). Sustainable landscape, soil and crop
577 management practices enhance biodiversity and yield in conventional cereal systems.
578 *Journal of Applied Ecology*, *58*(3), 507–517. https://doi.org/10.1111/1365-2664.13821

579 Rudge, M. R. (1968). The Food of the Common Shrew *Sorex araneus L.* (Insectivora:
580 Soricidae) in Britain. *Journal of Animal Ecology*, *37*(3), 565–581.
581 https://www.jstor.org/stable/3075

582 Ruscoe, W. A., Brown, P. R., Henry, S., van de Weyer, N., Robinson, F., Hinds, L. A., &
583 Singleton, G. R. (2022). Conservation agriculture practices have changed habitat use by
584 rodent pests: implications for management of feral house mice. *Journal of Pest Science*,
585 *95*(1), 493–503. https://doi.org/10.1007/s10340-021-01370-7

586 Saska, P., van der Werf, W., de Vries, E., & Westerman, P. R. (2008). Spatial and temporal
587 patterns of carabid activity-density in cereals do not explain levels of predation on
588 weed seeds. *Bulletin of Entomological Research*, *98*(2), 169–181.
589 https://doi.org/10.1017/S0007485307005512

590 Shields, M. W., Johnson, A. C., Pandey, S., Cullen, R., González-Chang, M., Wratten, S. D., &
591 Gurr, G. M. (2019). History, current situation and challenges for conservation biological
592 control. *Biological Control*, *131*(December 2018), 25–35.
593 https://doi.org/10.1016/j.biocontrol.2018.12.010

594 Symondson, W. O. C., Sunderland, K. D., & Greenstone, M. H. (2002). Can generalist
595 predators be effective biocontrol agents? *Annual Review of Entomology*, *47*(1), 561–
596 594. https://doi.org/10.1146/annurev.ento.47.091201.145240

597 Tschumi, M., Ekroos, J., Hjort, C., Smith, H. G., & Birkhofer, K. (2018). Predation-mediated
598 ecosystem services and disservices in agricultural landscapes. *Ecological Applications*,
599 *28*(8), 2109–2118. https://doi.org/10.1002/eap.1799

600 Veres, A., Petit, S., Conord, C., & Lavigne, C. (2013). Does landscape composition affect pest
601 abundance and their control by natural enemies? A review. *Agriculture, Ecosystems and*
602 *Environment*, *166*, 110–117. https://doi.org/10.1016/j.agee.2011.05.027

603 Westerman, P. R., Hofman, A., Vet, L. E. M., & van der Werf, W. (2003). Relative
604 importance of vertebrates and invertebrates in epigeaic weed seed predation in
605 organic cereal fields. *Agriculture, Ecosystems and Environment*, *95*, 417–425.
606 https://doi.org/10.1016/S0167-8809(02)00224-4

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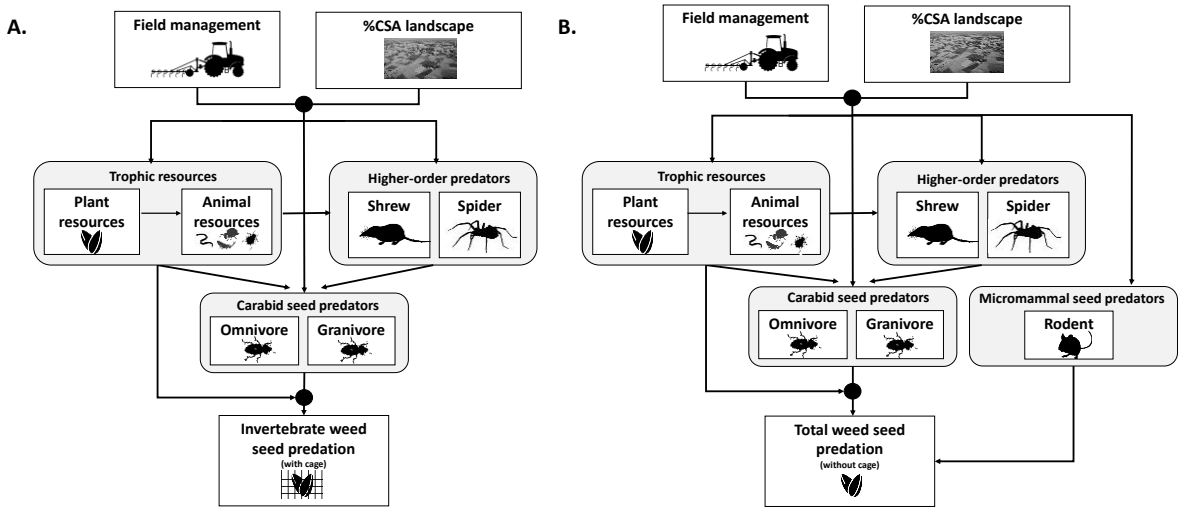

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610 **Figure 1:** Conceptual casual diagram for SEM explaining A. invertebrate weed seed
611 predation and B. total weed seed predation. The diagram shows all initial tested
612 relationships. The black circles (●) show tested two-way interactions.

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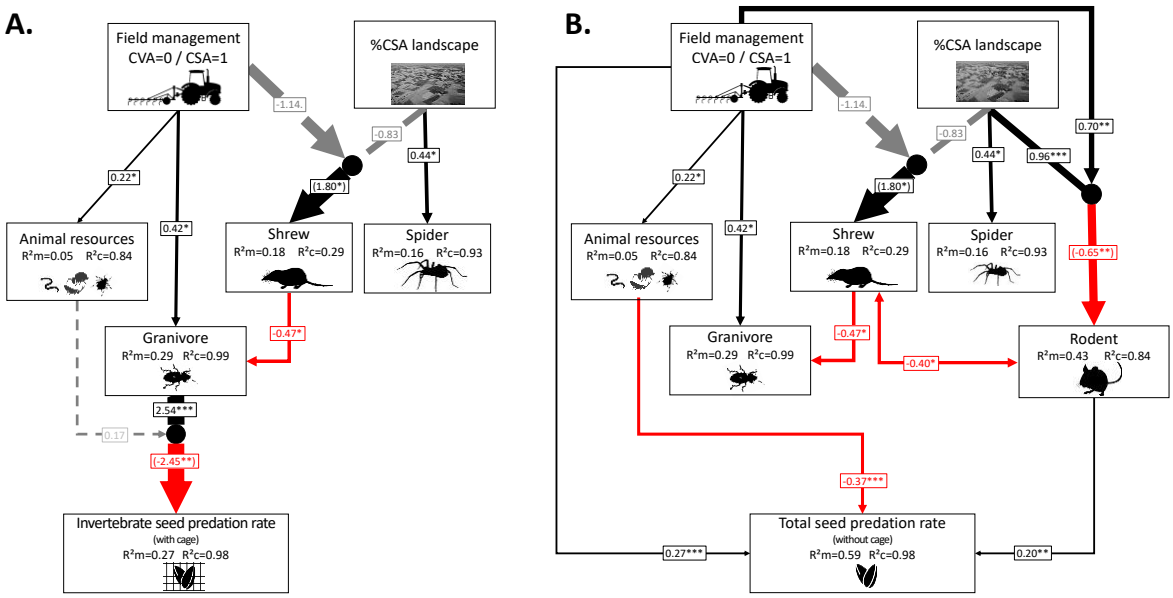

Figure 2: SEMs for the June sampling session including cascading effects of 'Field management' (CVA: conventional and CSA: conservation agriculture) in interaction with the '%CSA landscape' (the proportion of conservation agriculture in the landscape) on (A) invertebrate and (B) total weed seed predation. The indirect cascading effects considered seeds predators: 'granivore' carabid activity-density (for A and B) and 'rodent' abundance (for B); 'shrew' abundance as potential higher-order predators and 'animal resources' as alternative prey. The black and red arrows denote significant positive and negative effects, respectively. The grey dotted arrows indicate non-significant effects. Bidirectional arrows show causal correlations between variables. Standardised coefficients are shown for each path. The black circles (●) show interactions and the numbers in parentheses show interaction coefficients. The numbers of asterisks indicate the level of significance (p<0.1, *p < 0.05, **p < 0.01, ***p < 0.001).

Table 1: Parameter estimates from SEMs for the June session. The response variables were distinguished according to whether they were present in both SEMs or only one of the two SEMs. For each response variable we reported marginal (R^2m) and conditional (R^2c) r-squared. For each predictor with reported unscaled estimate, standard error (SE), degrees of freedom (DF), critical ratio value (Crit. Value), P-value (p) and scaled estimate. Highlighted are predictors with p≤ 0.05.

SEM	Response	R^2m	R^2c	Predictor	Unscale est.	SE	DF	Crit. Value	p	Scaled est.	
Common to both SEMs	Animal resources	0.05	0.84	**Field management**	**0.22**	**0.08**	**14**	**7.98**	**0.014**	**0.22**	*
	Shrews	0.18	0.29	Field management	-2.57	1.40	30	-1.84	0.065	-1.14	.
				CSA%	-0.10	0.08	30	-1.30	0.194	-0.83	
				Field management x %CSA landscape	**0.19**	**0.09**	**30**	**2.22**	**0.026**	**1.80**	*
	Granivorous carabids	0.29	0.99	**Field management**	**1.32**	**0.55**	**30**	**2.40**	**0.017**	**0.42**	*
				Shrews	**-0.64**	**0.27**	**30**	**-2.36**	**0.018**	**-0.47**	*
	Spiders	0.16	0.93	**%CSA landscape**	**0.04**	**0.02**	**30**	**2.28**	**0.023**	**0.44**	*
SEM A: Invertebrate seed predation (with cage)	Invertebrate seed predation	0.27	0.98	**Granivorous carabids**	**0.07**	**0.02**	**30**	**3.30**	**0.001**	**2.54**	***
				Animal resources	0.71	0.44	30	1.61	0.108	0.17	
				Granivorous carabids x Animal resources	**-0.03**	**0.01**	**30**	**-3.17**	**0.002**	**-2.45**	**
SEM B: Total seed predation (without cage)	Rodents	0.43	0.84	**Field management**	**1.54**	**0.53**	**30**	**2.89**	**0.004**	**0.70**	**
				%CSA landscape	**0.11**	**0.03**	**30**	**4.12**	**<0.001**	**0.96**	***
				Field management x %CSA landscape	**-0.07**	**0.02**	**30**	**-3.20**	**0.001**	**-0.65**	**
	Total seed predation	0.59	0.98	**Management**	**1.17**	**0.27**	**30**	**4.39**	**<0.001**	**0.27**	***
				Animal resources	**-1.60**	**0.34**	**30**	**-4.68**	**<0.001**	**-0.37**	***
				Rodent	**0.08**	**0.03**	**30**	**2.68**	**0.007**	**0.20**	**
	~~Shrew			**~~Rodent**	**-0.40**	-	**30**	**-2.26**	**0.016**	**-0.40**	*

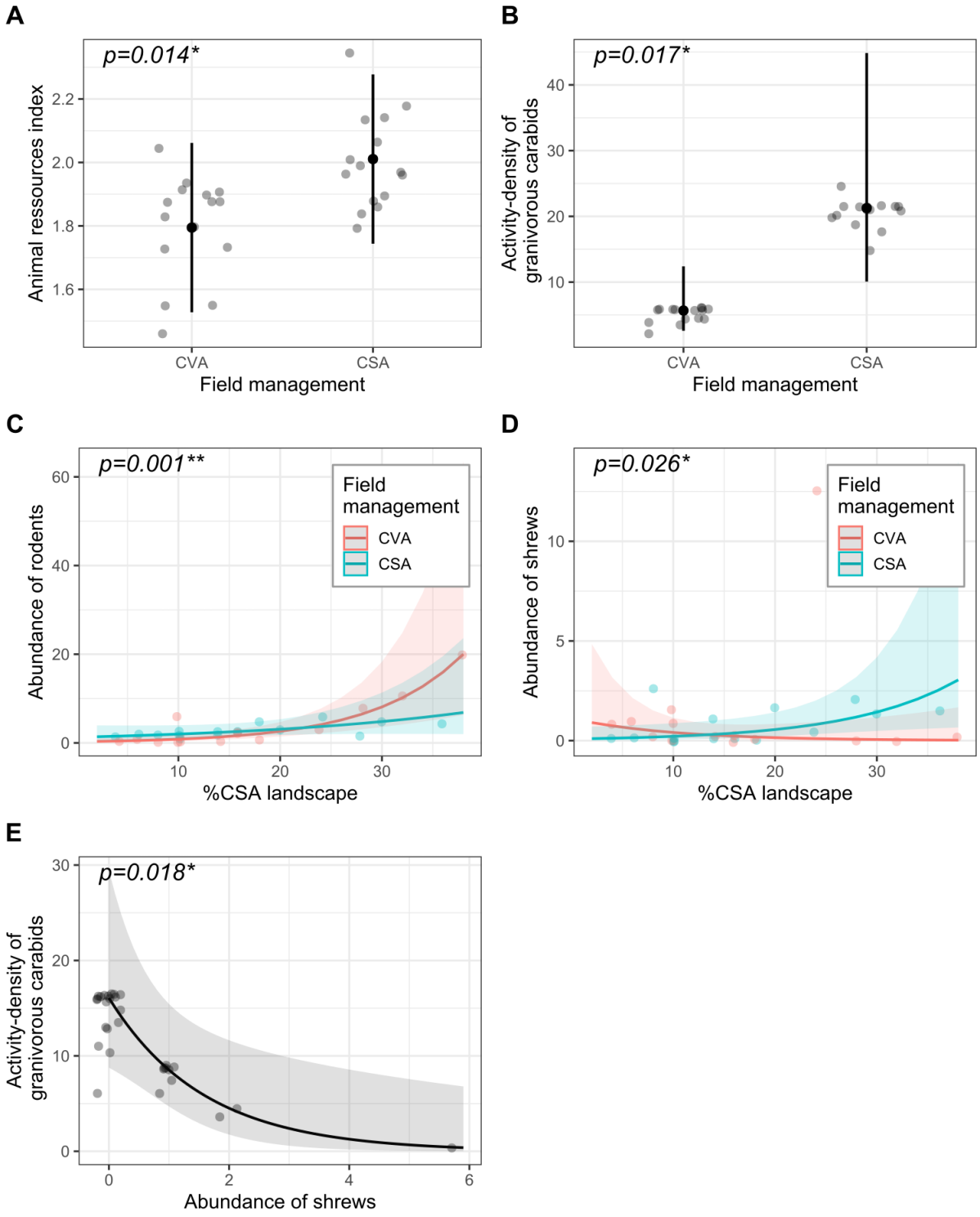

Figure 3: Fixed-effect predictions, with the associated 95% confidence intervals and partial residual points, for the relationships between: the local management (CVA: conventional and CSA: conservation agriculture) and (A) the animal resources index and (B) the activity-density of granivore carabids; the interaction effect between the local field management and the percentage of conservation agriculture in landscape (%CSA

643 landscape) and (C) the rodent abundance and (D) the shrew abundance; (E) the granivore
644 carabids and the shrew abundance. For each of these relationships, we have reported
645 the corresponding p-value.

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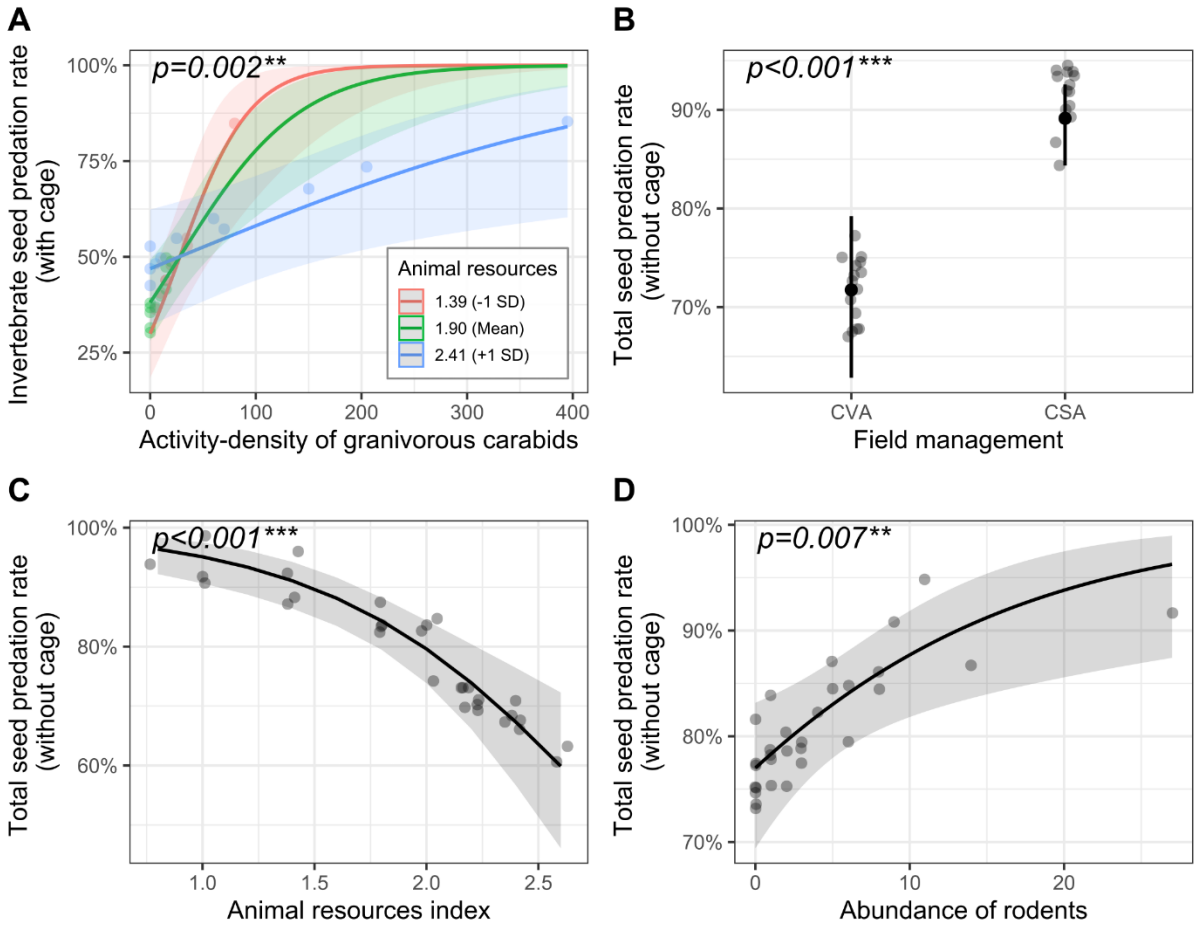

Figure 4: Fixed-effect predictions, with the associated 95% confidence intervals and partial residual points, for the relationships between: (A) the invertebrate seed predation rates and the interaction between granivorous carabids and animal resources; the total seed predation rates and: (B) the local management, (C) the animal resources and (D) the rodent abundance. “p” corresponds to p-value.