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1 **Influence of soil tillage on natural regulation of the cabbage root fly *Delia radicum***
2 **in brassicaceous crops.**

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11

12 **Abstract**

13 Ground dwelling predators provide regulation services of several insect pests.
14 Enhancing these services may be a step toward integrated crop protection. Many studies have
15 shown that soil tillage is deleterious to ground dwelling predators but pest regulation
16 processes and services have rarely been measured. We performed an experiment to study
17 whether simplifying soil tillage before the establishment of spring broccoli enhanced ground
18 dwelling predator populations and the control they provide on *Delia radicum*. The direct
19 effect of tillage on arthropods was assessed by comparing their emergence rates in plots
20 differing in soil tillage management. The natural regulation service was assessed by
21 comparing a control and an exclusion treatment in which predators were removed. The effect
22 of soil tillage on carabids, spiders and staphylinids did not match the gradient of disturbance
23 induced by tillage treatments. Tillage did not appear to affect the predators that likely
24 contribute to *D. radicum* regulation. Consistently, the number of pests suppressed and the root
25 injuries were unaffected by tillage treatments. The main deleterious effect of soil tillage was
26 on the emergence of those carabid species that overwinter partly as larvae, suggesting that
27 spring tillage could affect pest control in the following crops.

28 **Keywords**

29 ground dwelling predators; pest regulation service; cabbage root fly

30 **1 Introduction**

31 Farmers have long used soil tillage, mostly for weed management and refinement of
32 the soil structure to favor crop germination (El Titi, 2003). However, many studies have
33 shown that intensive tillage practices generally lead to a reduction in the abundance of soil-
34 inhabiting macroorganisms including potential predators of weed seeds or arthropod pests, i.e.
35 mainly spiders, carabid beetles and staphylinid beetles (Holland, 2004; Kendall, 2003). This
36 reduction results from direct effects (e.g. direct mortality from the tilling, desiccation;
37 Kendall, 2003) and/or from indirect effects, i.e. from different arthropods colonizing the field
38 after tillage (e.g. in response to modified plant cover, to alternative prey availability; Petersen,
39 2002; Thorbek and Bilde, 2004). To date, most studies did not distinguish between these
40 direct and indirect effects, mainly because it is difficult to design and carry out an experiment
41 allowing these two effects to be disentangled (Thorbek and Bilde, 2004).

42 The effects of soil tillage on ground dwelling predators depend on several traits of the
43 studied organisms including their development stage at tillage (Purvis and Fadl, 1996),
44 burrowing depth (Lorenz, 1995) or body size (Hatten et al., 2007). Therefore, carabids,
45 staphylinids and spiders respond differently to soil tillage: tillage generally does not have
46 much of an impact on staphylinids whereas carabids and spiders are more affected (Wardle,
47 1995). Consequently, soil tillage is likely to modify pest natural regulation processes because
48 ground dwelling predators abundance (Symondson et al., 2002) and traits (Wood et al., 2015)
49 are involved in their predation potential.

50 Many articles reported the effect of soil tillage on pest predation using sentinel preys
51 or predation cards (e.g. Petit et al., 2017; Tamburini et al., 2016), generally showing that
52 reduced tillage had a positive impact on predation potential. However, such methods only
53 give indications on the potential natural regulation obtained and have considerable limitations
54 for several pests (Zou et al., 2017). Direct measurements, e.g. using exclusion protocols (Luck

55 et al., 1988), are still needed to obtain a more realistic estimate of the effect of soil tillage on
56 the natural regulation provided by pest predators. There is also a need to go further and to test
57 whether tillage simplification leads to fewer injuries and/or damage to the crop in order to
58 assess the potential of soil tillage simplification as a conservation biological control practice
59 (Rusch et al., 2017).

60 The main pest of brassicaceous vegetables in northwestern Europe is the cabbage root
61 fly *Delia radicum* L. (Diptera: Anthomyiidae), that may inflict 40-60% plant mortality
62 (Estorgues, 2005) on spring vegetables without insecticide protection. The flies lay their eggs
63 at the base of plant stems, on or slightly below ground (Hughes and Salter, 1959). Then the
64 larvae develop below ground, feeding on, and thus inflicting injuries to plant roots.
65 Metamorphosis occurs inside a pupa, also buried in the ground, near plant roots (Hughes and
66 Salter, 1959). The cabbage root fly suffers high mortality rates between egg and pupa, mostly
67 at the egg stage (about 80-90%; Hughes and Salter, 1959). Part of this mortality is due to
68 predation by ground dwelling arthropods. The most significant predators are probably
69 carabids (Andersen et al., 1983; Coaker and Williams, 1963) with a strong positive
70 relationship between their body size and their regulation potential (Finch, 1996). Fewer
71 staphylinid species are involved in *D. radicum* natural regulation but *Aleochara* (Gravenhorst)
72 species are particularly efficient egg predators (Andersen et al., 1983). Moreover, they
73 contribute to *D. radicum* natural regulation as parasitoids of the pupae (Hughes and Salter,
74 1959). Finally the role of spiders has never been reported but their significance as predators of
75 arthropod eggs could be greater than currently expected (Nyffeler et al., 1990).

76 The aim of the present study was to compare the levels of natural regulation of *D.*
77 *radicum* by ground dwelling predators under common tillage practices. We tested the
78 following hypotheses: 1) intensive soil tillage significantly decreases the abundance of ground
79 dwelling predators emerging inside the field; 2) The natural regulation provided by these

80 ground dwelling predators decreases with soil tillage intensity and root injuries consequently
81 increase. For this we monitored natural populations of *D. radicum* on broccoli *Brassica*
82 *oleracea* var. *italica* Plenck (Brassicales: Brassicaceae) in an exclusion experiment performed
83 under various tillage regimes.

84 **2 Materials and methods**

85 *2.1 Study site and experimental design*

86 The experiment was carried out in northwestern France during spring 2016, at the
87 INRA experimental station (“Domaine expérimental de la Motte”, UE 0787) of Le Rheu
88 (48°07'N, 1°47'W), inside a 6.3 ha field sown with Triticale the previous year. The field was
89 sown with an intercrop (mix Phacelia – buckwheat) in September 2015 and the intercrop was
90 mown and the residues removed in February 2016. In the five years preceding the experiment,
91 the field was under non-inversion tillage practices (i.e. no plowing). An area of 0.6 ha of the
92 study field was split into four blocks separated by 10m bare ground passageways. Each block
93 was split into three plots, each measuring 30m x 15m. Three tillage treatments were allocated
94 to the plots in a randomized complete block design along a gradient of tillage intensity:
95 plowed and harrowed, only harrowed or not tilled at all. Plowing was performed on March
96 21st using a four-bottom rollover moldboard plow equipped with skim coulters (Grégoire
97 Besson SAS, Sèvremoine, France; depth ~ 25 cm) and harrowing was performed on March
98 22nd using a rotary harrow (RABE Agri GmbH, Bad Essen, Germany; rotors at 420 rpm,
99 depth ~ 10 cm). On the 20th and 21st of April, the field was planted with broccoli (cv.
100 ‘Marathon’) plants at the stage of 2-3 true leaves, every 0.50 m in rows 0.75 apart. The field
101 was kept free of weeds at least until early June using herbicides (0.6 L/ha of metazachlore on
102 April 29th followed by 1kg/ha of pyridate on May 20th) and by manual weeding in the
103 experimental subplots on May 24th.

104 To minimize the homogenizing effect of ground dwelling arthropods migrating from
105 surrounding habitats after soil tillage (Thorbeck and Bilde, 2004), we excluded them in all
106 treatments. Two “predator treatments” were implemented per plot. The control “exclusion”
107 treatment, consisted of 25 m² square subplots surrounded by partially buried barriers (40cm
108 below ground and 20cm above ground, Greenborder, Nortene ®). The “removal” treatment
109 consisted of 9 m² square subplots in which 13 pitfall traps were added to remove the ground
110 dwelling arthropods. This resulted in 1.4 traps per m² in “removal” subplots, a density
111 exceeding most of the studies reporting efficient exclusion (e.g. 0.3 traps / m² on average in
112 Chiverton, 1987 and in Holland, 1998), to ensure that we obtained an efficient removal of
113 ground dwelling predators. Although subplot surfaces differed, plant density and plant
114 growing conditions were identical as space within and between rows were the same. The traps
115 were half-filled with water to which a few drops of odorless detergent were added and
116 renewed weekly. Exclusion barriers and pitfall traps were set up immediately after soil tillage,
117 on March 22nd and 23rd, to avoid arthropod immigration into experimental subplots.

118 2.2 *Data collection*

119 2.2.1 *Soil tillage and ground dwelling arthropods emergence*

120 Emerging arthropods abundance was monitored using emergence tents (60cm x 60cm
121 x 60cm, MegaView Science Co.). Two tents were set up in each plot, immediately after tillage
122 (between the 22nd and the 29th of March), so that there was a total of eight tents per tillage
123 treatment. The lateral flaps of the emergence traps were buried vertically to a depth of about
124 10-15cm, as in Hanson et al. (2016), to avoid immigration or emigration of ground dwelling
125 arthropods. Each tent included one pitfall and one aerial collector to collect ground dwelling
126 and climbing or flying arthropods, respectively, emerging inside the tent. Both collectors were
127 half-filled with water containing a few drops of odorless detergent. The trapping period began
128 on March 30th and the traps were collected weekly until June 27th. Carabids, staphylinids and

129 spiders were sorted and counted. The cumulative numbers of carabids, staphylinids and
130 spiders trapped per tent from March 30th to June 27th were used as response variables in data
131 analysis. Carabids were identified at the species level based on Roger et al. (2016) because: i/
132 their sensibility to soil tillage has been shown to depend on their overwintering stage (Purvis
133 and Fadl, 1996) and ii/ they are thought to contribute much to *D. radicum* natural regulation
134 (Coaker and Williams, 1963). Their overwintering stage (only as adults vs. partly or only as
135 larvae) was assigned following Ribera et al. (1999) and the public database carabids.org
136 (Homburg et al., 2014); and their mean body size was taken from Roger et al. (2016) (Table
137 S1 in Supplementary materials). For each tent, mean carabid body length was calculated using
138 community weighted means (CWM).

139 2.2.2 *Soil tillage and Delia radicum natural regulation*

140 *Delia radicum* egg laying was monitored using felt traps strapped at the base of
141 broccoli stems (Freuler and Fischer, 1983). Four traps were set up in each removal subplot at
142 planting, aligned along a diagonal to have two traps close to the barriers and two in the center
143 of the subplots. They were recorded weekly until June 20th. Eggs laid on the traps were
144 counted, removed and the trap was then replaced around the same plant. Plants used for egg
145 monitoring were not used for any other measurement. The cumulated number of eggs laid per
146 plant throughout the experiment (i.e. from April 21st to June 20th) was used as a response in
147 data analysis.

148 When eggs had theoretically reached the pupal stage (about 300 degree days between
149 egg laying and pupation; Collier and Finch, 1985; i.e. about 27 days after the end of egg
150 laying in our experiment), soil samples (12cm in diameter, 13.5 ± 0.4 cm in depth) were taken
151 from around plant roots (including the root system after cutting the broccolis at the base of
152 plant stem) and the number of pupae was counted after washing the samples through a 1mm x
153 1mm square mesh sieve. Ten samples were taken in every subplot on June 30th, on randomly

154 selected plants, making a total of 240 samples, i.e. 40 per combination of tillage treatment –
155 predator treatment. The same plants were used to assess root injuries caused by *D. radicum*
156 larvae feeding following the qualitative ordinal notation proposed by Dosdall et al. (1994): 0
157 = no root damage; 1 = small feeding channels on the root comprising less than 10% of the
158 root surface area; 2 = 11-25%; 3 = 26-50%; 4 = 51-75%; and 5 = 76-100% of the tap root
159 surface area injured. In figures, this qualitative ordinal scale was transformed into a
160 continuous variable representing the fraction of the tap root surface injured (using the median
161 of each class) to facilitate the interpretations.

162 2.3 Data analysis

163 We tested the effect of soil tillage on ground dwelling predators abundance and on
164 carabid traits and we tested the effects of soil tillage, predator treatment and of their
165 interaction on the number of *D. radicum* eggs laid, on the number of pupae and on root
166 injuries. All statistical analyses were performed using R software (R core team, 2017). We
167 used generalized linear mixed models with a distribution appropriate to the type of the
168 response variable (functions ‘lmer’, ‘glmer’ or ‘glmer.nb’ of the package ‘lme4’; Bates et al.,
169 2015). Considered distributions were: negative binomial (response variables: cumulative
170 density of carabids, staphylinids and spiders; number of *D. radicum* eggs per plant; number of
171 *D. radicum* pupae per plant), binomial (response variable: proportion of carabids
172 overwintering partly as larvae) and Gaussian (response variable: carabid body length). In
173 every model, the experimental block was added as a random factor. Finally, the qualitative
174 ordinal variable of root injuries was analyzed with a cumulative link mixed model (function
175 ‘clmm’, package ‘ordinal’; Christensen, 2015). The significance of the fixed effects was
176 tested using type II Wald chi-square tests, except for root injuries where a likelihood-ratio chi-
177 square test was used (function ‘Anova’, package ‘car’; Fox and Weisberg, 2011 and package
178 ‘RVAideMemoire’; Hervé, 2017). When the effect of tillage treatment was significant,

179 pairwise comparisons of the estimated marginal means of each treatment were performed
180 using the Tukey method (function ‘emmeans’ from the ‘emmeans’ package; Lenth, 2017).

181 **3 Results**

182 *3.1 Soil tillage and ground dwelling arthropods emergence*

183 Between March 30th and June 27th, we trapped 380 spiders, 5171 staphylinids and
184 1069 carabids in the emergence tents (see Table S2 and Figure S1 in Supplementary materials
185 for details on species composition and emergence dynamic). The cumulative number of
186 carabid beetles and the cumulative number of spiders trapped per tent did not differ between
187 tillage treatments (Table 1) but tillage treatment had a significant effect on the number of
188 staphylinids, which was significantly lower in the “no-tillage” treatment than in the other two
189 (Table 1). There was no effect of tillage treatment on the CWM carabid body length but the
190 proportion of carabids overwintering at least partly as larvae was significantly lower in the
191 tents set up in the “only harrowed” treatment than in the two others (Table 1). *Trechus* gr.
192 *quadristriatus* (85.9% ± 1.4%) was highly dominant in this group of carabid species that
193 overwintered at least partly as larvae.

194 *3.2 Soil tillage and Delia radicum natural regulation*

195 The number of eggs laid per plant did not differ significantly between tillage
196 treatments ($\chi^2 = 4.52$, $df = 2$, $P = 0.104$). We collected significantly more pupae in the
197 “removal” than in the “exclusion” treatment ($\chi^2 = 6.26$, $df = 1$, $P = 0.012$; Figure 1A). There
198 was no significant effect of tillage treatment on the number of collected pupae ($\chi^2 = 1.97$, $df =$
199 2 , $P = 0.372$) and the interaction between the removal and tillage treatments was not
200 significant ($\chi^2 = 0.81$, $df = 2$, $P = 0.667$). The number of pupae was reduced on average by
201 22.7% in the presence of ground-dwelling predators.

202 *D. radicum* larvae inflicted significantly more root injuries in the “removal” than the
203 “exclusion” treatment ($\chi^2 = 11.43$, $df = 1$, $P < 0.001$; Figure 1B) but there was again no

204 significant difference among soil tillage treatments ($\chi^2 = 0.73$, $df = 2$, $P = 0.693$) and no
205 interaction between these two factors ($\chi^2 = 3.51$, $df = 2$, $P = 0.173$).

206 **4 Discussion**

207 Arthropod emergence in the three tillage treatments tested did not fit the expected
208 gradient of disturbance. Our results are therefore not in agreement with the general consensus
209 that ground dwelling arthropod abundance decreases when soil tillage intensity increases
210 (Rusch et al., 2017; Wardle, 1995). However, most studies published to date did not
211 distinguish between the direct and indirect effects of soil tillage. The discrepancy between our
212 results and previous reports may then come from the removal of indirect effects in our
213 experimental setup. Using similar methods on the same taxa, Thorbek and Bilde (2004) also
214 found that the direct effects of soil tillage were minimal. Nevertheless, among carabids we
215 showed that the proportion of species overwintering at least partly as larvae was lowest when
216 plots had been only harrowed. This suggests that overwintering larvae are more sensitive than
217 adults to the rotary harrow, but not if the field is previously plowed. The higher sensibility of
218 larvae had already been pointed out in carabids (Purvis and Fadl, 1996) but our results further
219 indicate that the direct effects of soil tillage probably depend on the sequence of applied
220 practices: the negative effect of harrowing was suppressed when applied after plowing. The
221 deepest tillage operation (i.e. often plowing) is thus not necessarily the most injuring and
222 could even have a protective effect on overwintering larvae against more damaging practices.
223 This is in line with the fact that some overwintering larvae live at a shallow depth, as shown
224 for *Pseudoophonus rufipes* for instance (10-15cm in Hartke et al., 1998), and thus could have
225 been buried by plowing (Roger-Estrade et al., 2001) and protected from the harrow. Finally, it
226 should be noted that some effects of tillage such as modified soil structure (Bronick and Lal,
227 2005) and modified soil organic matter distribution in the tilled horizon (Balesdent et al.,
228 2000) are not immediate. They may have consequences on microorganisms (Bronick and Lal,

229 2005) and on higher-order trophic levels (such as the predators monitored here) in the long
230 term that we could not assess due to the short duration of tillage treatments differentiation. To
231 date the response, over several years, of ground dwelling predators following tillage changes
232 remains largely unexplored (but see Wardle et al., 1999).

233 Regardless of tillage practice, we found that the number of pupae per plant and the
234 intensity of root injuries were reduced in the presence of locally emerging ground dwelling
235 predators. On the other hand, the number of pests suppressed or the level of root injuries did
236 not differ between tillage treatments. The ground dwelling predators that overwintered inside
237 the field therefore provided the same level of regulation in every tillage condition. This is
238 consistent with the limited effect of soil tillage on the emergence of the ground dwelling
239 arthropods. Especially, soil tillage had no effect on two characteristics of carabid communities
240 which potentially determine their predation potential: i/ body size, which is a key factor in
241 prey – predator relationships in general (Brose et al., 2006) and in this biological system in
242 particular (Finch, 1996); and ii/ their abundance, especially at the beginning of egg laying,
243 which is probably the appropriate time for *D. radicum* regulation (Mesmin et al., 2019).
244 Conversely, the two features of ground dwelling arthropods emergence that were impacted by
245 soil tillage were unlikely to lead to different levels of predation. First, the observed effect on
246 overwintering traits of carabids was related to the massive emergence of *T. gr. quadristriatus*,
247 a species that emerged too late to contribute to *D. radicum* egg predation. Secondly, although
248 emerging staphylinids were not determined to species, there were very probably few
249 *Aleochara* among them as only 63 *D. radicum* pupae out of 1713 were parasitized by
250 *Aleochara* spp. (results not shown), a weak abundance that is usual in the region studied (e.g.
251 Lamy et al., 2016). Other potential *D. radicum* predators may also have rapidly dispersed by
252 air after emergence as most staphylinids have good flying abilities (Levesque and Levesque,
253 1995).

254 To conclude, soil tillage before crop establishment did not have a significant impact on
255 the natural regulation of *D. radicum* in spring broccoli. This finding is consistent with the fact
256 that the ground dwelling predators that are likely to provide the service in spring did not
257 suffer from soil tillage. However, we showed that shallow tillage affected the species that
258 overwinter partly as larvae, suggesting that the natural regulation services these species
259 provide later in summer and autumn crops could be weakened. Furthermore, the harmlessness
260 of plowing vs. the deleterious effect of harrowing on these taxa raises the question of the
261 relative impact, on ground dwelling predators, of one deep soil disturbance vs. the multiple
262 shallow disruptions that can be necessary to control weeds in no-plow systems.

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272

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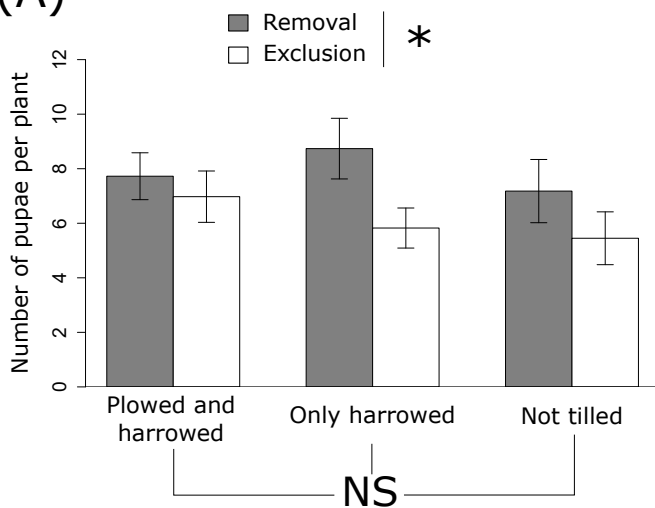
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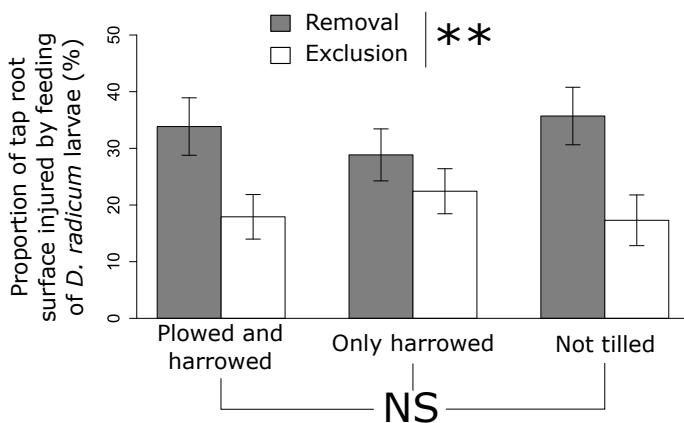
412 Table 1: Values and significance of Type-II Wald chi square tests performed on the
413 cumulative number of carabids, spiders and staphylinids trapped per tent from March 30th to
414 June 27th and on two functional traits of carabids trapped during this period: the proportion of
415 each overwintering stage and the community weighted mean body length. The back-
416 transformed estimated marginal means (\pm SE) are given for each tillage treatment (PH:
417 plowed and harrowed, OH: only harrowed, \emptyset : not tilled) and the results of pairwise
418 comparisons are shown when chi square tests were significant: tillage treatments denoted with
419 different letters are significantly different ($p < 0.05$).
420

421 Figure 1: Mean number (\pm SE) of pupae collected per plant at harvest (A) and mean
422 proportion (\pm SE) of the tap root surface attacked by *D. radicum* larvae (B) in all tillage
423 treatments. Grey bars indicate the removal treatment and white bars the exclusion treatment.
424 The asterisks and “NS” indicate the significance of differences between treatments
425 (**<0.010<*<0.050<NS).

(A)



(B)



Response variable		χ^2	df	P	Tillage	Estimate (\pm SE) and group	
Abundance	Carabids	5.68	2	0.058	PH	59.9 \pm 9.4	-
					OH	37.1 \pm 6.3	-
					Ø	44.6 \pm 7.6	-
	Spiders	5.67	2	0.059	PH	12.5 \pm 2.4	-
					OH	16.0 \pm 3.2	-
					Ø	24.0 \pm 4.7	-
	Staphylinids	19.87	2	< 0.001	PH	259.4 \pm 24.7	a
					OH	268.7 \pm 26.8	a
					Ø	168.1 \pm 17.2	b
Functional traits of carabids	Proportion of carabids overwintering only as adults	44.33	2	< 0.001	PH	0.3 \pm 0.1	a
					OH	0.7 \pm 0.1	b
					Ø	0.4 \pm 0.1	a
	Body length	2.33	2	0.311	PH	4.1 \pm 0.2	-
					OH	4.0 \pm 0.2	-
					Ø	4.4 \pm 0.2	-