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2 **Role of soil texture and earthworm casts on the restoration of soil enzyme activities after**
3 **exposure to an organophosphorus insecticide**

4

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17 **Abstract**

18

19 Pesticides exert important effects on the soil fauna and health. However, little is known about
20 the interactions of soil, microorganisms and earthworms in the presence of pesticides and
21 about their respective roles in the soil biological activity. The aim of this study was to
22 evaluate the effect of the soil type on enzyme activities, measured in bulk soil and in casts of
23 two earthworm species, after exposure to the organophosphorus pesticide parathion. To this
24 aim, two endogeic earthworm species (*Apporectodea caliginosa* and *Allolobophora*
25 *chlorotica*) were cross-acclimated in two different soil textures (each representing the most
26 favorable soil environment for that species). Enzyme activities were measured as a soil
27 quality indicator in samples of bulk soil (collected at day 4 and day 7 of exposure to
28 parathion) and in earthworm casts (collected at day 7). A short exposure (4 days) to parathion
29 significantly (ANOVA, $p < 0.001$) inhibited carboxylesterases (25-43% of inhibition) and
30 alkaline phosphatase (~23% of inhibition). At day 7 of exposure, parathion impact on the
31 overall soil enzyme activities mainly depended on the soil texture. Indeed, activity inhibition
32 was higher (ANOVA, $p < 0.001$) in silt-clay soil (decrease by 37%) than in silt-loamy soil
33 (decrease by ~18%). Conversely, parathion effect was not influenced by earthworm
34 presence/absence and earthworm species. However, after soil exposure to parathion,
35 earthworms (both species) improved enzyme activity restoration in their casts.

36

37 **Keywords:** Soil extracellular enzymes; parathion; enzyme-based indexes; integrated
38 biomarker index; *Apporectodea caliginosa*; *Allolobophora chlorotica*.

39 **1. Introduction**

40 Pesticides are used in agriculture to control pests, weeds, and plant diseases. They include a
41 large panel of active ingredients that belong to different classes (e.g. insecticides, fungicides,
42 herbicides, nematicides), but that all raise environmental concerns (Köhler and Trieskborn,
43 2013). Pesticides are absorbed through surface runoff from the treated plants and accumulate
44 in the soil. As a result, the soil organisms are directly exposed to such molecules. Moreover,
45 pesticides affect soil health by modifying soil biota responsible for maintaining the soil
46 functions, thus contributing to the degradation of soil quality and fertility (Silva et al., 2019).
47 Soil biological functions require the activity of enzymes that contribute to soil health
48 maintenance (Abbas et al., 2021). These enzymes are mainly of microbial origin and are
49 implicated in many intracellular, cell-associated, and extracellular functions (Kiss et al., 1975;
50 Nannipieri et al., 1990; Utobo and Tewari, 2015). They play a pivotal role in the ecosystem
51 biogeochemical cycling (Luo et al., 2017) and in the soil biochemical functions. As microbial
52 enzyme activities are easy to monitor and change rapidly in response to ecological
53 disturbances, they are widely used as an early indicator of soil quality (Gil-Sotres et al., 2005;
54 Panettieri et al., 2013) and also as biomarkers of soil contamination by pesticides (Rao et al.,
55 2014; Baćmaga et al., 2015; Wolejko et al., 2020). Moreover, it has been demonstrated that
56 soil enzyme activities are influenced by the soil characteristics (including pH (Dick et al.,
57 2000)), agricultural activities, and land management (Medeiros et al., 2015), and that different
58 enzymes display different responses upon exposure to pesticides (Riah et al., 2014).

59 Besides the soil microflora, pesticides affect non-target soil invertebrates (Pelosi et al., 2015;
60 Gunstone et al., 2021), such as earthworms (Pelosi et al., 2014), and their related ecological
61 functions. Earthworms are commonly described as ecosystem engineers. They significantly
62 modify the soil physical (aggregate structure, porosity), chemical (nutrient supply and
63 cycling) and biological (soil fauna, microbial and enzyme activities) characteristics (Jones et

64 al., 1994; Edwards, 2004; Aira and Pearce, 2009; Blouin et al., 2013). They interact with soil
65 components and spread microorganisms in soil while creating their biogenic structures
66 (burrows, casts, and middens) (Brown et al., 2004; Blouin et al., 2013; Lemtiri et al., 2014).
67 Earthworms also influence the soil microbial composition by depositing casts that constitute a
68 microbial hotspot, due to their high carbon (C) concentration, and that harbor higher
69 enzymatic activities compared with the surrounding soil (Tao et al., 2009; Lipiec et al., 2016).
70 Many studies suggest that enzyme activities in casts are influenced by the earthworm diet
71 (Flegel and Schrader, 2000) and soil composition (Kizilkaya and Hepsen, 2004; Dempsey et
72 al., 2013). Soil also plays a pivotal role by affecting earthworm abundance and diversity in
73 cultivated and non-cultivated ecosystem (Singh et al., 2020). Clause et al. (2014)
74 demonstrated that the soil type where earthworms live is the main explanatory factor for the
75 modification of cast properties. Cast properties are also influenced by the earthworm species,
76 and this species-specific effect varies in function of the soil type (Clause et al., 2014).
77 Therefore, both soil texture and earthworm species are important factors that could mediate
78 the pesticide effects on the soil enzyme activities. In addition, it is known that extracellular
79 soil oxidase activities are important for the bioremediation of contaminated soils (Gianfreda
80 and Rao, 2004, 2008; Burns et al., 2013). However, the role of other enzymes in the
81 restoration of polluted soils has been scarcely investigated.

82 Many laboratory studies found that microbial growth and enzymatic activities are decreased
83 upon soil exposure to pesticides (Pal et al., 2006; Sanchez-Hernandez et al., 2017), including
84 organophosphorus insecticides (Sanchez-Hernandez et al., 2017; Jaiswal et al., 2021). As the
85 biological interactions of earthworms, soil microorganisms, and contaminants are complex, it
86 is difficult to link soil enzyme activity variations to pesticide exposure. Results could be
87 influenced by the soil type and its characteristics (Wolejko et al., 2020), or earthworm
88 presence (Sanchez-Hernandez et al., 2018). To our knowledge, little is known about the

89 capacity of enzymes found in earthworm casts to restore polluted soils and about the soil type
90 role in such biological interactions when assessing undesirable environmental effects.
91 The hypothesis of this study was that the presence of endogeic earthworms in parathion-
92 contaminated soils reduces the impact of this organophosphorus pesticide on soil microbial
93 activity and nutrient cycling, evaluated by measuring the activity of different enzyme
94 involved in the C, nitrogen (N), and phosphorus (P) biogeochemical cycles, and microbial
95 activity (dehydrogenase). Two endogeic earthworm species, (*Aporrectodea caliginosa* and
96 *Allolobophora chlorotica*) and two soils with contrasting physicochemical properties were
97 used to determine whether the ecological services of endogeic earthworms (i.e., pollution
98 remediation) should be considered a generalized benefit. The agricultural soils corresponded
99 to the field soil where each earthworm species was collected in its natural environment.
100 Therefore, the study objective was to compare the impact of soil type and earthworm species
101 on soil enzyme activities measured in parathion-spiked soils and earthworm casts.

102

103 **2. Material and methods**

104 *2.1. Soils and earthworms*

105 The two different experimental soils used were Luvisols (USDA). They were sampled in two
106 different orchards, situated at 10 km distance, in Montfavet, near Avignon (southeastern
107 France). Both orchards had not been treated with pesticides for the last 15 years. The first soil
108 (Soil K, Fig.1A) was a silt loamy soil (23.4% clay, 57% silt, 19.6% sand, 28.3 g kg⁻¹ organic
109 matter, pH 8.3), dominated by *A. chlorotica*. The second soil (Soil G, Fig.1A) was a silt-clay
110 soil (38.3 % clay, 42.2% fine silt, 19.5% sand, 34 g kg⁻¹ organic matter, pH 8.5), dominated
111 by *A. caliginosa*. Healthy and adult *A. chlorotica* and *A. caliginosa* earthworms were
112 collected manually in their original orchard, washed with tap water, and divided in two groups
113 for cross-acclimation in a dark cold chamber (12 ± 1°C) for 5 days. These two endogeic

114 species are present worldwide, and exhibit different recovery capacities (Rault et al., 2008)
115 and cast production reduction (Jouni et al., 2018) upon exposure to parathion. Jouni et al.
116 (2018) suggested that *A. caliginosa* is the most sensitive between these species.
117 Soil samples from the two orchards were sieved at <2 mm, and the water content was adjusted
118 to 20-21% (approximately 81% of the maximum water holding capacity) with distilled water.
119 Wet soil samples were spiked with ethyl-parathion solutions to obtain a final concentration of
120 1 mg active ingredient (a.i.) kg⁻¹ wet soil, which refers to the usual application rate and
121 calculation of the predicted environmental concentration (Jouni et al., 2018). Ethyl-parathion
122 (parathion hereafter), an organophosphorus pesticide, is now banned in most developed
123 countries, but is still used in developing countries, and is a well-known pesticide model
124 (Sabzevari and Hofman, 2022). Control soils were prepared in the same conditions but
125 without pesticide. The wet polluted and unpolluted soils were then equally distributed in
126 plastic pots (100 g of soil/each).

127

128 2.2. *Experimental design*

129 After the acclimation period, earthworms were washed in tap water, blotted dried on filter
130 paper and weighed. For each experiment, two soils (G and K), two soil conditions (control
131 and with parathion), three earthworm conditions (earthworm-free, *A. caliginosa*, *A.*
132 *chlorotica*), and four replicates were used. Therefore, 48 pots were prepared (16 pots for each
133 earthworm condition). For the pots with earthworms, two individuals were placed in each pot
134 to obtain enough casts (total n=32 earthworms for each species). Pots were kept in a dark cold
135 chamber (12±1°C) for the entire experiment duration. Soils without earthworms were used as
136 controls. After 4 and 7 days, bulk soil samples were taken from each replicate and for each
137 condition. Casts were removed after 7 days. Bulk soil and cast samples were stored in plastic

138 tubes at -80°C, until analysis. The results on parathion toxicity in *A. caliginosa* and *A.*
139 *chlorotica* have been described in Jouni et al. (2018).

140

141 2.3. Soil enzyme activities

142 Extracellular enzymes activities involved in the C (carboxylesterase and β -glucosidase), N
143 (urease), and P (phosphatase) biogeochemical cycles (Balota and Chaves, 2010; Gougoulas et
144 al., 2014; Lessard et al., 2014) were used as biological indicators of the soil quality.
145 Dehydrogenase activity was used as a direct indicator of soil microbial activity because it
146 reflects the living microorganism biomass (von Mersi and Schinner, 1991; Shaw and Burns,
147 2006). Soil and cast water suspensions (1:25 w/v) were prepared according to Sanchez-
148 Hernandez et al. (2018) and homogenized using an orbital shaker at room temperature for 30
149 min. All enzyme activities were measured as previously described in Sanchez-Hernandez et
150 al. (2017). Briefly, carboxylesterase (EC 3.1.1.1) activity was measured using two different
151 substrates, 1-naphthyl butyrate and 4-nitrophenyl butyrate, because of the many enzyme
152 isoforms. The reaction mixture consisted of 140 μ l of 0.1 M Tris-HCl (pH=6.5), 100 μ l of
153 soil-water suspension, and 10 μ l of substrate (2.5 mM, final concentration). After incubation
154 in a thermostatically controlled orbital shaker (Elmi[®] Skyline DTS-2, 800 rpm) at 20°C for 1
155 h, microplates were centrifuged (2,500 g, 10°C, 10 min), and 150 μ l of each supernatant was
156 transferred to a new microplate. The product of naphthyl ester hydrolysis (1-naphthol) was
157 revealed by adding 75 μ l of a solution containing 2.5% (w/v) SDS in 0.1% Fast Red
158 ITR/2.5% Triton X-100, and incubating in the dark for 30 min until complete color
159 development. The naphthol–Fast Red ITR complex absorbance was read at 530 nm. For 4-
160 nitrophenyl butyrate hydrolysis, 4-nitrophenol formation was determined after addition of 75
161 μ l of a solution containing 2% (w/v) SDS and 2% (w/v) Tris-base to the microplate
162 containing 150 μ l of each supernatant, and absorbance was immediately read at 405 nm.

163 Enzyme activities were expressed as μmol of product per hour and gram of dry soil.
164 Calibration curves were made with 1-naphthol ($1.5\text{--}100\text{ nmol.ml}^{-1}$) and 4-nitrophenol ($5\text{--}100$
165 nmol.ml^{-1}) in the presence of soil-water suspensions to correct the adsorption of the
166 chromogenic substances onto soil colloids. Controls (substrate-free) and blanks (soil-free)
167 were used to correct the background absorbance and non-enzymatic hydrolysis of the
168 substrates, respectively.

169 Phosphatase (EC 3.1.3.2) and β -glucosidase (EC 3.2.1.21) activities were measured according
170 to Popova and Deng (2010) in a reaction medium that contained $100\ \mu\text{l}$ of soil:water
171 suspension, $100\ \mu\text{l}$ of distilled water, and $50\ \mu\text{l}$ of the respective substrate (4-nitrophenyl
172 phosphate or 4-nitrophenyl- β -D-glucanopyranoside; 5 mM final concentration) dissolved in
173 20 mM modified universal buffer ($\text{pH}=6.5$). After 4 h incubation (continuous shaking at
174 20°C), microplates were centrifuged ($2,500\text{ g}$, 10°C , 10 min), and $150\ \mu\text{l}$ of each supernatant
175 was transferred to a new microplate. The formed 4-nitrophenol was immediately ($<1\text{ min}$)
176 read at 405 nm after addition of $75\ \mu\text{l}$ of 0.5 M NaOH to the wells. Standard calibration
177 curves were made with 4-nitrophenol ($5\text{--}100\text{ nmol.ml}^{-1}$).

178 Urease (EC 3.5.1.5) activity was measured according to Schinner et al. (1996). The hydrolytic
179 reactions were performed in 10 ml tubes by mixing 1 ml of 80 mM urea and 1 ml of soil:
180 water suspension, and incubated (orbital shaking) at room temperature ($\sim 20^\circ\text{C}$) for 4 h .
181 Reactions were stopped by addition of 5 ml of cold 2 M KCl containing 10 mM HCl . Then,
182 tubes were agitated for 30 min to extract ammonium, and centrifuged ($4,500 \times \text{g}$, 10°C , 5
183 min). Supernatants ($150\ \mu\text{l}$) were transferred to microplate wells, and ammonium was
184 measured after addition of $75\ \mu\text{l}$ of $1:1\text{ (v:v)}$ $0.3\text{ M NaOH} : 1.06\text{ M}$ sodium salicylate
185 containing 4.6 mM sodium nitroprusside, followed by addition of $30\ \mu\text{l}$ of 39.1 mM sodium
186 dichloroisocyanurate. Microplates were left in the dark for 30 min for color development, and

187 absorbance was read at 690 nm. Urease activity was expressed as $\mu\text{g NH}_4^+\text{-N h}^{-1}\text{g}^{-1}$ dry soil
188 using a calibration curve made with NH_4Cl ($3.0\text{--}50 \mu\text{g NH}_4^+ \text{ml}^{-1}$).
189 Dehydrogenase (EC 1.1.1) activity was measured according to von Mersi and Schinner
190 (1991), using iodinitrotetrazolium chloride as the electron acceptor. The formation of reduced
191 iodinitrotetrazolium formazan was determined spectrophotometrically after 60 min
192 incubation at 40°C , and the results were expressed as $\mu\text{mol iodinitrotetrazolium formazan h}^{-1}\text{g}^{-1}$ dry soil.

194

195 *2.4. Data analysis*

196 Data (enzyme activities) were first scaled and centered and then analyzed using principal
197 component analysis (PCA) and the 'ade4' package in R. Differences between ellipses were
198 tested with a between-class analysis. Enzyme activities were compared between soils,
199 earthworms and pesticide conditions with one-way analysis of variance (ANOVA) followed
200 by the Tukey HSD post-hoc comparison test using the XLSTAT software (version 2013.3.01).
201 The integration of soil enzyme activities into numerical indexes of microbial diversity allows
202 assessing the deterioration of soil quality by pesticides. Three numerical indexes to assess the
203 impact of parathion, soil, and earthworm species on soil enzyme activities were used:
204 Geometric Mean (GMean) index (Hinojosa et al., 2004), Integrated Biological Responses
205 version 2 (IBRv2) index (Sanchez et al., 2013), and Treated-Soil Quality Index (T-SQI)
206 (Mijangos et al., 2010). High GMean index values indicate high microbial functional diversity
207 (Lessard et al., 2014). The IBRv2 index is a modified version of the original IBR index
208 (Beliaeff and Burgeot, 2002) and is based on the deviation between a disturbed (polluted soil)
209 and a non-disturbed (unpolluted soil) state (Sanchez et al., 2013). It integrates the global
210 response of several biomarkers and allows a quick visualization of parathion effect on soil
211 enzyme activities to evaluate the soil quality. Higher IBRv2 index absolute values indicate

212 higher deviation from the control soil. The T-SQI index was proposed by Mijangos et al.
213 (2010) as an integrative enzymatic index of soil pollution. This index measures the magnitude
214 and direction (increase or inhibition) of changes induced by an environmental stressor on soil
215 enzyme activities compared with a reference (here the corresponding unpolluted bulk soil).
216 For further details concerning the numerical index calculation, see Sanchez-Hernandez et al.
217 (2017).

218

219 **3. Results**

220 *3.1. Effect of soil type, earthworm species, and parathion exposure time on bulk soil enzyme* 221 *activities*

222 PCA showed significant differences in enzyme activities in function of the soil type in all
223 conditions tested (Fig. 1B). In control bulk soil samples (no parathion), microbial functional
224 diversity (GMean index) was similar in samples with/without earthworms and with *A.*
225 *caliginosa* and with *A. chlorotica* (Fig. 2A-B). The GMean index was significantly decreased
226 in both polluted soils (4 and 7 days of exposure to parathion) compared with control soils
227 (Fig. 2A-B). However, at day 7, the GMean indexes were much lower in polluted bulk soil
228 samples from Soil G than Soil K (decrease by $36.7 \pm 4.0\%$ and $17.8 \pm 1.0\%$, respectively,
229 compared with control soils) (Fig. 2B). Changes in soil enzyme activities were observed very
230 rapidly, already at day 4 of exposure. Specifically, carboxylesterase and phosphatase activities
231 were significantly reduced in both soil types after 4 days of exposure to parathion (Table 1).
232 On the other hand, β -glucosidase enzyme activity was significantly decreased only in the silt-
233 loamy soil (Soil K) but not in the silt-clay soil (Soil G). After 7 days, sunray plots showing
234 the T-SQI score distribution for the different enzymes clearly indicated that in Soil G most
235 enzyme activities were decreased in polluted bulk soil samples (compared with control),
236 except for dehydrogenase activity that did not seem to be much affected by parathion

237 exposure (at day 4 the activity reduction was not significant) (Fig. 3A). Conversely in Soil K,
238 carboxylesterase and phosphatase activities were fully recovered at day 7, and β -glucosidase
239 activity was partially recovered (Fig. 3B).

240

241 *3.2. Effect of soil type, earthworm species, and parathion exposure on cast enzyme activities*

242 In earthworm casts (both species), the GMean values were not different in control and
243 polluted samples (Fig. 2C). The GMean values were significantly higher in casts collected
244 from control and polluted Soil K samples compared with the corresponding unpolluted
245 ($p < 0.0001$) and polluted bulk soil samples ($p < 0.0001$) (Fig. 2C). Conversely, the GMean
246 values were comparable in cast samples collected from Soil G (control or polluted soil) and in
247 control soil (Fig. 2C). The T-SQI score distribution for the different enzymes at day 7
248 indicated that all the tested enzymes had recovered their initial activity, except for β -
249 glucosidase that was still reduced in casts from both soil types (Fig. 3A-B).

250

251 *3.3. Soil quality*

252 In bulk soil, the IBRv2 score increased with the duration of exposure to parathion in Soil G
253 (from 4.06 at day 4 to 6.00 at day 7), but not in Soil K (~3.8 at day 4 and day 7) (Fig. 3C). In
254 cast samples, the IBRv2 scores at day 7 (1.03 from Soil G and 2.02 from Soil K) were lower
255 than in bulk soil samples.

256

257 **4. Discussion**

258 *4.1. Effect of soil type, earthworm species, and exposure time on bulk soil enzyme activities*

259 Our results suggest that soil enzyme activities are affected by parathion exposure with a time-
260 dependent response modulated by the soil texture. First, the observed activity change (at day 4
261 and 7 of exposure) was very rapid compared with previous works. For example, chlorpyrifos

262 (another organophosphorus pesticide) induced changes in soil enzyme activities after 2 weeks
263 (Sanchez-Hernandez et al., 2017) and 45 days (Tejada et al., 2011) of exposure. Specifically,
264 Tejada et al. (2011) did not observe any effect after 3 days of exposure, but only after 45 days.
265 These differences could be explained by the higher toxicity of parathion compared with
266 chlorpyrifos (Kumar et al., 2018). Parathion effect is consistent with previous studies showing
267 that soil carboxylesterase, phosphatase and β -glucosidase are inhibited by organophosphorus
268 pesticides and might be used as indicators of contaminated soils (Sanchez-Hernandez et al.,
269 2017). Carboxylesterase inhibition is due to the direct interaction of the organophosphorus
270 pesticide with soil carboxylesterases. This mechanism involves phosphorylation of the active
271 site of serine hydrolases, leading to a covalent and stable 'enzyme-inhibitor' complex
272 (Wheelock et al., 2008). The observed decrease in phosphatase and β -glucosidase activities is
273 consistent with the findings by Sanchez-Hernandez et al. (2017), although we cannot
274 conclude on a direct inhibition by parathion. These results suggest that β -glucosidase activity
275 variation could be used as an early indicator of changes in the soil physical-chemical and
276 biological properties (Monreal and Bergstrom, 2000).

277 Clay content, organic matter (Bandick and Dick, 1999) and total organic carbon (Eivazi and
278 Tabatabai, 1988) are key factors that display a strong positive correlation with β -glucosidase
279 activity (Turner et al., 2002). Therefore, the significant inhibitory effect of parathion on β -
280 glucosidase activity in Soil K (silt-loamy soil), compared with Soil G (silt-clay soil), could be
281 related to the soil texture. Specifically, Soil G higher clay and organic matter contents could
282 contribute to β -glucosidase activity stability even in the presence of parathion.

283 The activity of urease, an N-cycling enzyme, was not affected by parathion, in line with
284 previous studies showing that exposure to chlorpyrifos does not modify urease activity
285 (Sanchez-Hernandez et al., 2017), except after longer exposure time (Tejada et al., 2011).

286 Dehydrogenase activity, which reflects the presence of viable cells, is mostly used as an
287 indicator of soil microbial activity (von Mersi and Schinner, 1991). Here, dehydrogenase
288 activity was higher in the unpolluted bulk soil samples from Soil K than Soil G, and increased
289 only in Soil K samples after 4 days of exposure to parathion. As previous studies showed a
290 negative correlation between dehydrogenase activity and soil water content (Wolińska and
291 Stepniewska, 2012), its higher activity in Soil K could be attributed to its lower capacity to
292 retain water (silt-loamy soil) compared with Soil G (silt-clay soil).

293 Lastly, neither earthworm presence nor the species influenced the enzyme activities in bulk
294 soil samples. This was unexpected because earthworms modify the soil biological properties
295 (Jones et al., 1994; Blouin et al., 2013). The most surprising observation concerns the full
296 recovery of carboxylesterase activity in Soil K bulk soil samples after 7 days of exposure, as
297 indicated by the T-SQI values, independently of earthworm presence/absence. Previous
298 studies have shown that soil carboxylesterase activities can be increased in the presence of
299 *Lumbricus terrestris*, but not in earthworm-free soil (Sanchez-Hernandez et al., 2015). Thus,
300 three hypotheses could explain our results. First, the biomass and density of the endogeic
301 species investigated in the present work were lower compared with previous works (Dempsey
302 et al. (2013) used 90 *L. terrestris* individuals/m⁻², Sanchez-Hernandez et al. (2015) used 4 *L.*
303 *terrestris* individuals/250g soil). Second, the experiment short duration (7 days compared with
304 12 weeks in Sanchez-Hernandez et al. (2015)) might have been insufficient to allow
305 microorganism spreading by earthworms and soil microcosm colonization. Third,
306 carboxylesterase activity recovery could be due to its direct excretion from soil
307 microorganisms. This suggests that endogeic species, such as *A. caliginosa* and *A. chlorotica*,
308 may not be involved in carboxylesterase secretion from their gut, unlike what shown for the
309 anecic earthworm *L. terrestris* (Sanchez-Hernandez et al., 2009). Consequently, in Soil K,
310 carboxylesterase activity recovery in polluted bulk soil samples after 7 days of exposure could

311 stabilize its global quality by scavenging parathion molecules, compared with Soil G
312 (Sanchez-Hernandez et al., 2015). In agreement, the IBRv2 indexes showed that overall,
313 health status was better in Soil K (silt-loamy) than in Soil G (silt-clay) after exposure to
314 parathion.

315

316 *4.2. Effect of soil type and earthworm species on cast enzyme activities*

317 Our results showed that in earthworm casts, enzyme activities were similar (Soil G) or higher
318 (Soil K) than in their corresponding bulk soil samples, except for β -glucosidase. Moreover,
319 enzyme activities were not different in casts collected from polluted and control soils,
320 independently of the earthworm species. Soil texture was the most important factor to explain
321 the differences in cast enzyme activities in control soils and after parathion exposure. This is
322 consistent with previous works showing that the effect of soil type is higher than that of
323 earthworm species on cast properties (Clause et al., 2014). Casts are enriched in nutrients and
324 harbor larger microbial populations and biomass than the surrounding soil (Sheehan et al.,
325 2008; Lipiec et al., 2016). During the intestinal passage, earthworm mucus facilitates organic
326 matter mineralization and humification and promotes microbial activity (Huang and Xia,
327 2018) and consequently enzyme activities in casts. The observed increase in cast
328 dehydrogenase activity compared with undigested soil (bulk soil) confirmed previous results
329 and indicates that earthworms and their active microbiomes enhance the cast metabolic
330 capacity through organic matter oxidation (Lipiec et al., 2016). Moreover, depending on the
331 cast properties (pH, moisture) or total N and organic C content, different enzyme activity
332 profiles can be obtained (Bowles et al., 2014). For instance, it has been shown that N-cycling
333 enzyme activities increase with C availability, while C-cycling enzyme activities increase
334 with N availability (Bowles et al., 2014). Therefore, the significant decrease in β -glucosidase
335 activity observed in casts from polluted soils could reflect a reduction in N availability and/or

336 a decrease in cellulose content following organic matter breakdown during soil ingestion
337 (Nozaki et al., 2009).

338 Nevertheless, the most interesting result of our study was the restoration of enzyme
339 activities in casts collected from polluted soils at day 7. This suggests that the soil transit
340 through the earthworm gut might allow reducing parathion negative impact on the soil
341 enzyme activities. Besides the higher microbial activities in casts, the earthworm microbiome
342 capacity to feed on pesticides and use them as a C source cannot be neglected. In addition, the
343 earthworm gut could act as a biological filter where some ingested microorganisms may be
344 digested, favored, or selected (Drake and Horn, 2007; Wüst et al., 2011; Aira et al., 2015).
345 Therefore, modifications of microbial communities during their transit in the gut might
346 contribute to the microbial composition differences between dejected casts and ingested soil.
347 Moreover, the ingested microorganisms could provide exoenzymes that enhance organic
348 matter degradation in the gut and favor nutrient assimilation (Medina-Sauza et al., 2019).
349 Altogether, these mechanisms could promote parathion degradation and/or metabolization,
350 resulting in enzyme activity restoration in casts.

351 Overall, earthworm presence promoted the maintenance of different enzyme activities and the
352 catabolic potential in cast compared with bulk soil samples. This effect was comparable with
353 both earthworm species; however, parathion direct impact on earthworms may limit their
354 contribution to the microbial activity spatial diversity in the surrounding soil. Indeed, in our
355 previous work (Jouni et al., 2018), we highlighted a species-specific response to parathion,
356 suggesting that *A. caliginosa* is more sensitive. Specifically, inhibition of *A. caliginosa*
357 enzyme activities was correlated with a significant decrease in body weight and a strong
358 decrease in cast production. Therefore, the higher sensitivity of *A. caliginosa* behavioral
359 responses (e.g. cast production) may have ecologically consequences on soil fertility and
360 degradation in terms of quantity of cast produced (Capowiez et al., 2010).

361

362 **5. Conclusions**

363 Exposure to parathion caused fast changes in total soil enzyme activities in bulk soils and
364 casts. Enzyme activities were more affected by parathion in silt-clay soil (Soil G) than in silt-
365 loamy soil (Soil K). Soil G higher clay content, by physical capturing and protecting organic
366 matter, microorganisms, nutrients, exogenous compounds and enzymes, could enhance the
367 contact between soil components at different scales, thus increasing the pesticide effect on
368 soil enzyme activities.

369 Casts collected from polluted soils exhibited a significant restoration of soil quality. It should
370 be noted that casts were collected at day 7 of exposure and could have undergone some
371 ageing, resulting in bacterial community changes. However, Aira et al. (2019) showed that
372 cast collected between day 0 and day 7 are younger samples compared with casts collected
373 from day 15 onwards. It should be interesting to measure the enzyme activity recovery
374 dynamics in fresh casts collected at different time points (from 0 to 7 days). Nutrients also
375 should be thoroughly investigated in casts to better correlate specific enzyme activities with
376 C, N and P content in function of the soil texture. Lastly, as modifications of the microbial
377 communities during transit in the earthworm gut contribute to the microbial composition of
378 dejected casts, it will be important to investigate the pesticide effects on earthworm digestive
379 functions. Analysis of the soil microbial diversity fractions that were maintained or
380 suppressed during digestive transit in the earthworm gut could allow better understanding the
381 biological interactions of earthworms, soil microorganisms, and contaminants.

382

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387

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605

606 **Figure captions**

607 Fig. 1. Differences in the two soils used for this study. (A) Soil texture triangle for Soil G and
608 Soil K. (B) Principal component analysis results of bulk soil enzyme activities in the two
609 soils.

610

611 Fig. 2. Geometric Mean (GMean) indexes of enzyme activities in control and polluted
612 (parathion) bulk soil samples after 4 days (A) and 7 days (B) of exposure (mean \pm SD, n = 8)
613 in the different conditions (earthworm presence/absence and species). (C) GMean index of
614 enzyme activities in bulk soil (mean \pm SD, n = 16) and cast (mean \pm SD, n = 8) samples from
615 control and polluted soils after 7 days. Different letters denote significant differences between
616 treatments ($p < 0.001$). EW, earthworms.

617

618 Fig. 3. (A-B) Sunray plots showing the distribution of the Treated-Soil Quality Index (T-SQI)
619 scores calculated for each enzyme activity measured in polluted bulk soil (dotted lines) and
620 cast (continuous lines) samples from Soil G (A) and Soil K (B) after 7 days of exposure to
621 parathion compared with control values (gray thin dotted lines at zero). CbE, total
622 carboxylesterases; Phtase, phosphatase; Glu, β -glucosidase; Ure: urease; Dehy:
623 dehydrogenase. (C) Integrated Biological Responses version 2 (IBRv2) scores calculated for
624 bulk soils after 4 and 7 days of exposure and in casts collected after 7 days of exposure to
625 parathion. IBRv2 values are the sum of the deviations between reference (control bulk soil)
626 and parathion-exposed soils (n = 19).

627

628

629

630

631 **Highlight**

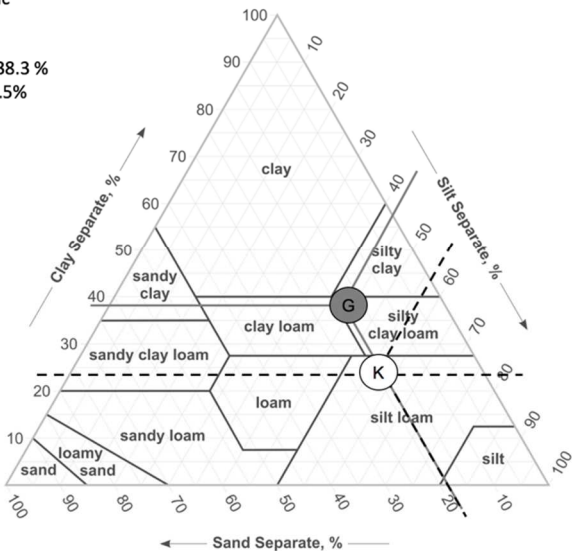
- 632 - Parathion exposure induced a decrease in bulk soil enzyme activities
- 633 - Bulk soil enzyme activity changes were influenced by the soil type
- 634 - After parathion exposure, soil enzyme activities were restored in earthworm casts
- 635 - Earthworm species did not influence enzyme activities in control and polluted soils

Figure 1

A)

Soil-K is a silt loamy soil
 23.4% clay, 57% silt, 19.6% sand, 28.3 g kg⁻¹ organic matter, pH 8.3

Soil-G is a silt-clay soil
 38.3% clay, 42.2% fine silt, 19.5% sand, 34 g kg⁻¹ organic matter, pH 8.5



B)

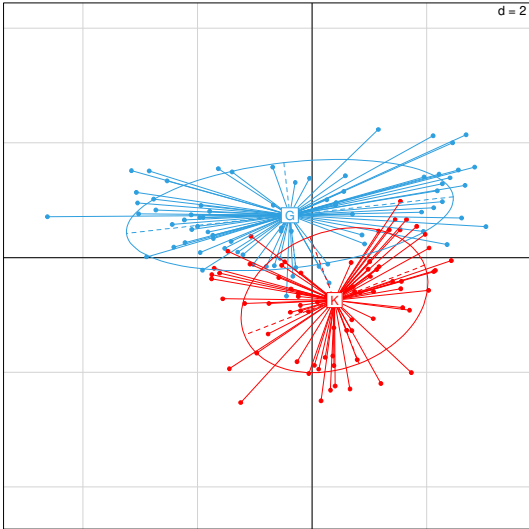


Figure 2

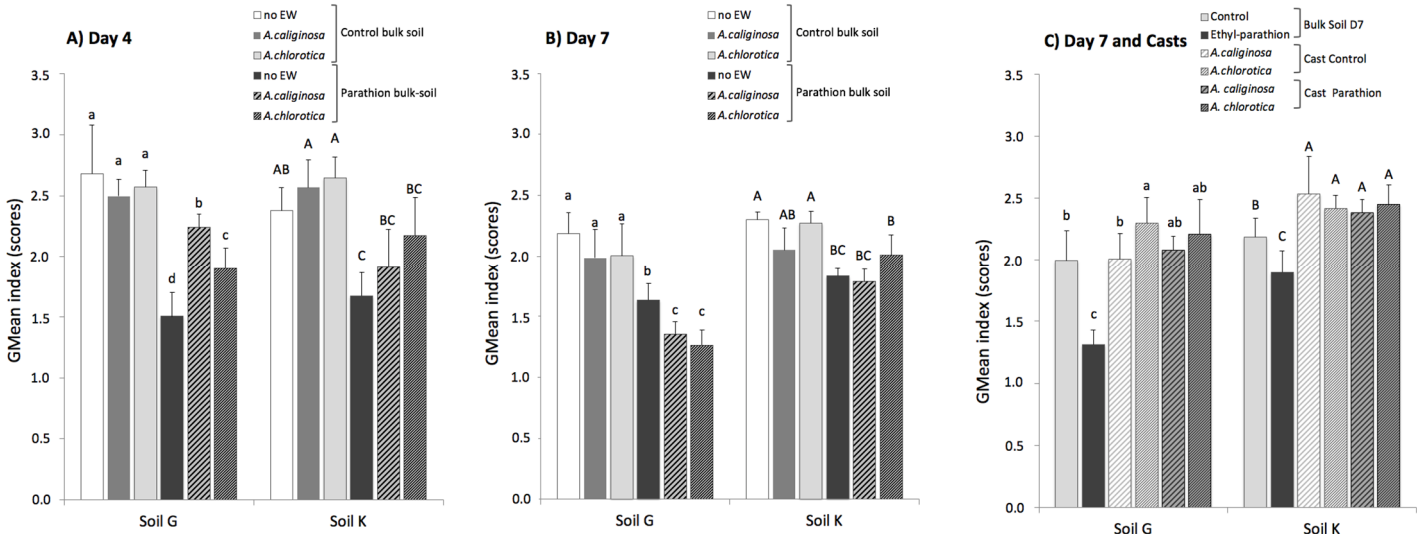


Figure 3

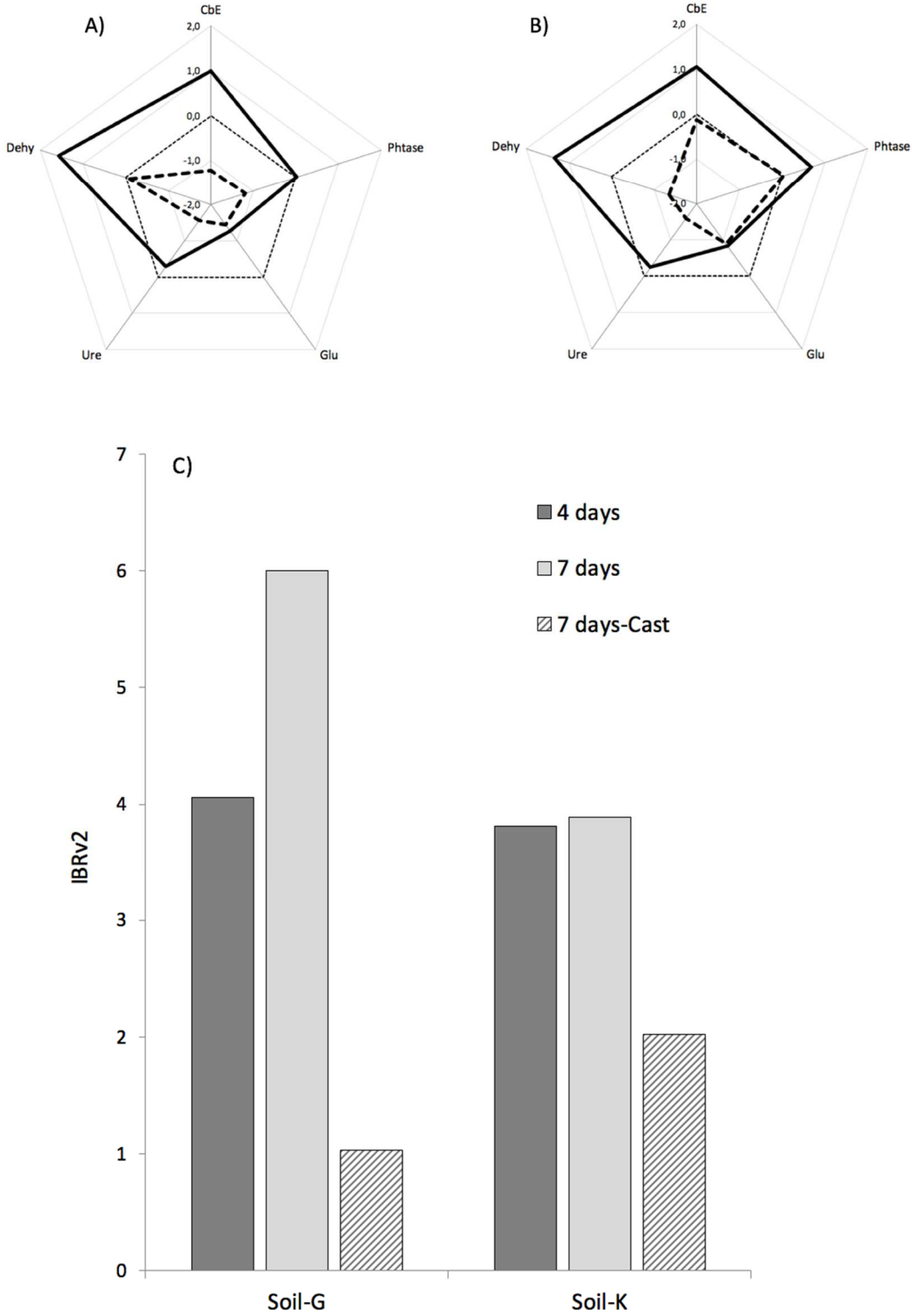


Table 1: Soil enzyme activities (mean \pm SD, n = 19) measured after 4 days of exposure to parathion (1 mg a.i. kg⁻¹ dry soil). Different letters denote significant differences between treatments. 4-NPB, 4-nitrophenyl butyrate; 1-NB, 1-naphthyl butyrate; INTF, iodonitrotetrazolium formazan.

Enzyme activities	Soil	Control	Parathion	Activity	<i>P</i> -value
		Day 4	Day 4	inhibition (%) at day 4	
Carboxylesterase 4-NPB ($\mu\text{mol h}^{-1} \text{g}^{-1}$ dry soil)	<i>G</i>	15.11 \pm 2.52 (a)	11.24 \pm 3.80 (b)	-25.6	<0.0001
	<i>K</i>	14.40 \pm 4.06 (A)	8.17 \pm 2.80 (B)	-43.3	<0.0001
Carboxylesterases 1-NB ($\mu\text{mol h}^{-1} \text{g}^{-1}$ dry soil)	<i>G</i>	1.92 \pm 0.76 (a)	1.20 \pm 0.28 (b)	-37.4	<0.0001
	<i>K</i>	2.55 \pm 0.62 (A)	1.56 \pm 0.64 (B)	-37.2	<0.0001
Phosphatase ($\mu\text{mol h}^{-1} \text{g}^{-1}$ dry soil)	<i>G</i>	1.61 \pm 0.38 (a)	1.25 \pm 0.49 (b)	-22.4	0.009
	<i>K</i>	0.90 \pm 0.12 (A)	0.68 \pm 0.12 (B)	-24.6	<0.0001
β -Glucosidase ($\mu\text{mol h}^{-1} \text{g}^{-1}$ dry soil)	<i>G</i>	1.34 \pm 0.19	1.34 \pm 0.35	0	1
	<i>K</i>	1.40 \pm 0.12 (A)	1.00 \pm 0.29 (B)	-28.4	<0.0001
Dehydrogenase (nmol INTF h ⁻¹ g ⁻¹ dry soil)	<i>G</i>	76.5 \pm 14.4	61.7 \pm 18.7	-19.3	0.136
	<i>K</i>	110.7 \pm 27.6 (A)	141.2 \pm 24.0 (B)	+21.6%	0.0005
Urease ($\mu\text{g NH}_4^+\text{-N h}^{-1} \text{g}^{-1}$ dry soil)	<i>G</i>	66.42 \pm 5.45	61.63 \pm 8.51	-7.2	0.398
	<i>K</i>	62.12 \pm 6.06	55.14 \pm 7.72	-11.2	0.046