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Abstract

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

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

1. Introduction

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

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

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

Many laboratory studies found that microbial growth and enzymatic activities are decreased upon soil exposure to pesticides (Pal et al., 2006; Sanchez-Hernandez et al., 2017), including organophosphorus insecticides (Sanchez-Hernandez et al., 2017; Jaiswal et al., 2021). As the biological interactions of earthworms, soil microorganisms, and contaminants are complex, it is difficult to link soil enzyme activity variations to pesticide exposure. Results could be influenced by the soil type and its characteristics (Wolejko et al., 2020), or earthworm 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 constrasting 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 ß-glucosidase), N
143 (urease), and P (phosphatase) biogeochemical cycles (Balota and Chaves, 2010; Gougoulias 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 µl of 0.1 M Tris-HCl (pH=6.5), 100 µl of
153 soil-water suspension, and 10 µl 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 µl of each supernatant was
156 transferred to a new microplate. The product of naphthyl ester hydrolysis (1-naphthol) was
157 revealed by adding 75 µl 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 µl of a solution containing 2% (w/v) SDS and 2% (w/v) Tris-base to the microplate
162 containing 150 µl of each supernatant, and absorbance was immediately read at 405 nm.

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

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

Urease (EC 3.5.1.5) activity was measured according to Schinner et al. (1996). The hydrolytic reactions were performed in 10 ml tubes by mixing 1 ml of 80 mM urea and 1 ml of soil: water suspension, and incubated (orbital shaking) at room temperature (~20 °C) for 4 h. Reactions were stopped by addition of 5 ml of cold 2 M KCl containing 10 mM HCl. Then, tubes were agitated for 30 min to extract ammonium, and centrifuged (4,500 × g, 10°C, 5 min). Supernatants (150 µl) were transferred to microplate wells, and ammonium was measured after addition of 75 µl of 1:1 (v:v) 0.3 M NaOH : 1.06 M sodium salicylate containing 4.6 mM sodium nitroprusside, followed by addition of 30 µl of 39.1 mM sodium 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 µg NH_4^+-N $h^{-1}g^{-1}$ dry soil
188 using a calibration curve made with NH_4Cl (3.0–50 µg NH_4^+ ml^{-1}).
189 Dehydrogenase (EC 1.1.1) activity was measured according to von Mersi and Schinner
190 (1991), using iodonitrotetrazolium chloride as the electron acceptor. The formation of reduced
191 iodonitrotetrazolium formazan was determined spectrophotometrically after 60 min
192 incubation at 40°C, and the results were expressed as µmol iodonitrotetrazolium formazan $h^{-1}g^{-1}$
193 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

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

3. Results

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

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

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

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

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

3.3. Soil quality

In bulk soil, the IBRv2 score increased with the duration of exposure to parathion in Soil G (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 cast samples, the IBRv2 scores at day 7 (1.03 from Soil G and 2.02 from Soil K) were lower than in bulk soil samples.

4. Discussion

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

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

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

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

The activity of urease, an N-cycling enzyme, was not affected by parathion, in line with previous studies showing that exposure to chlorpyrifos does not modify urease activity (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 (Liepic 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

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

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

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

5. Conclusions

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

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

Acknowledgement

The authors are very grateful to the research structure ECCOREV and the regional grant Ref. SBPLY/19/180225/000077 from the government of Castilla-La Mancha (Spain) for their funding to support this work.

References

Abbas, S., Tariq, J.M., Quasim, A., Chaudhary, H.J., Rizwan, M., 2021. Alteration of plant physiology by the application of biochar for remediation of organic pollutants, in: Mirza Hasanuzzaman, M., Prasad, M.N.V. (Eds.), Handbook of Bioremediation. Academic Press, pp. 475-492. https://doi.org/10.1016/B978-0-12-819382-2.00030-2.

Aira, M., Piearce, T.G., 2009. The earthworm *Lumbricus terrestris* favours the establishment of *Lolium perenne* over *Agrostis capillaris* seedlings through seed consumption and burial. Appl. Soil Ecol. 41, 360-363.

Aira, M., Bybee, S., Pérez-Losada, M., Domínguez, J., 2015. Feeding on microbiomes: effects of detritivory on the taxonomic and phylogenetic bacterial composition of animal manures. FEMS Microbiol. Ecol. 91, fiv117.

Aira, M., Pérez-Losada, M., Domínguez, J., 2019. Microbiome dynamics during cast ageing in the earthworm *Aporrectodea caliginosa*. Appl. Soil Ecol. 139, 56-63.

Baćmaga, M., Borowik, A., Kucharski, J., Tomkiel, M., Wyszkowska, J., 2015. Microbial and enzymatic activity of soil contaminated with a mixture of diflufenican + mesosulfuron-methyl + iodosulfuron-methyl-sodium. Environ. Sci. Pollut. R. 22, 643–656. https://doi.org/10.1007/s11356-014-3395-5

Balota, E.L., Chaves, J.C.D., 2010. Enzymatic Activity and Mineralization of Carbon and Nitrogen in Soil Cultivated with Coffee and Green Manures. Revista Brasileira de Ciência Do Solo 34, 1573–83. http://doi:10.1590/S0100-06832010000500010.

408 Bandick, A.K., Dick, R.P., 1999. Field management effects on soil enzyme activities. Soil
409 Biol. Biochem. 31, 1471–1479.
410 Beliaeff, B., Burgeot, T., 2002. Integrated biomarker response: a useful tool for ecological
411 risk assessment. Environ. Toxicol. Chem. 21, 1316–1322.
412 Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt K.R., Dai,
413 J., Dendooven, L., Peres, G., Tondoh, J.E., Cluzeau, D., Brun, J-J., 2013. A review of
414 earthworm impact on soil function and ecosystem services: earthworm impact on
415 ecosystem services. Eur. J. Soil Sci. 64, 161-182.
416 Bowles, T.M., Acosta-Martínez, V., Calderón, F., Jackson, L.E., 2014. Soil enzyme activities,
417 microbial communities, and carbon and nitrogen availability in organic agroecosystems
418 across an intensively-managed agricultural landscape. Soil Biol. Biochem. 68, 252-262.
419 https://doi.org/10.1016/j.soilbio.2013.10.004.
420 Brown G.G., Doube B.M., Edwards C.A., 2004. Functional interactions between earthworms,
421 microorganisms, organic matter, and plants. In Edwards C.A., (ed.), CRC Press, Boca
422 Raton, FL, USA, pp. 213-239.
423 Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein,
424 M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment:
425 current knowledge and future directions. Soil Biol. Biochem. 58, 216-234.
426 Capowiez, Y., Dittbrenner, N., Rault, M., Triebskorn, R., Hedde, M., Mazzia, C., 2010.
427 Earthworm cast production as a new behavioural biomarker for toxicity testing. Environ.
428 Pollut. 158, 388-393.
429 Clause, J., Barot, S., Richard, B., Decaëns, T., Forey, E., 2014. The interactions between soil
430 type and earthworm species determine the properties of earthworm casts. Appl Soil Ecol.
431 83, 149-158.

Dempsey, M.A., Fisk, M.C., Yavitt, J.B., Fahey, T.J., Balser, T.C., 2013. Exotic earthworms alter soil microbial community composition and function. Soil Biol. Biochem. 67, 263-270.

Dick, W. A., Cheng, L., Wang, P., 2000. Soil Acid and Alkaline Phosphatase Activity as PH Adjustment Indicators. Soil Biol. Biochem. 32, 1915–19. http://doi:10.1016/S0038-0717(00)00166-8.

Drake, H. L., Horn, M. A., 2007. As the worm turns: the earthworm gut as a transient habitat for soil microbial biomes. Annu. Rev. Microbiol. 61, 169-189. http://doi:10.1146/annurev.micro.61.080706.093139

Edwards, C. A., 2004. The importance of earthworms as key representatives of the soil fauna. Earthworm ecology 2, 3-11.

Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20, 601–606.

Flegel, M., Schrader, S., 2000. Importance of food quality on selected enzyme ac- tivities in earthworm casts (Dendrobaena octaedra, Lumbricidae). Soil Biol. Biochem. 32, 1191-1196.

Gianfreda, L., Rao, M.A., 2004. Potential of extra cellular enzymes in remediation of polluted soils: a review. Enzyme Microb. Technol. 35, 339-354.

Gianfreda, L., Rao, M.A., 2008. Interactions between Xenobiotics and Microbial and Enzymatic Soil Activity. Crit. Rev. Environ. Sci. Technol. 38, 269-310. http://doi:10.1080/10643380701413526

Gil-Sotres, F., Trasar-Cepeda, M., Leirós, C., Seoane, S., 2005. Different Approaches to Evaluating Soil Quality Using Biochemical Properties. Soil Biol. Biochem. 37, 877–87. http://doi:10.1016/j.soilbio.2004.10.003.

Gougoulias, C., Clark, J.M., Shaw, L.J., 2014. The Role of Soil Microbes in the Global

457 Carbon Cycle: Tracking the below-Ground Microbial Processing of Plant-Derived
458 Carbon for Manipulating Carbon Dynamics in Agricultural Systems. J. Sci. Food Agric.
459 94, 2362–71. http://doi:10.1002/jsfa.6577.
460 Gunstone, T., Cornelisse, T., Klein, K., Dubey, A., Donley, N., 2021. Pesticides and Soil
461 Invertebrates: A Hazard Assessment. Front. Environ. Sci. 9, 643847.
462 http://doi:10.3389/fenvs.2021.643847, ISSN=2296-665X
463 Hinojosa, M.B., Garcia-Ruiz, R., Vineglia, B., Carreira, J.A., 2004. Microbiological rates and
464 enzyme activities as indicators of functionality in soils affected by the Aznalcollar toxic
465 spill. Soil Biol. Biochem 36, 1637-1644.
466 Huang, K., Xia, H., 2018. Role of earthworms'mucus in vermicomposting system:
467 biodegradation tests based on humification and microbial activity. Sci. Total Environ.
468 610, 703-708.
469 Jaiswal, D.K., Krishna, R., Singh, S., Belwal, T., Verma, J.P., Yadav, J., 2021. Toxicity of
470 Organophosphate Pesticide on Soil Microorganism: Risk Assessments Strategies. In:
471 Singh, K.P., Jahagirdar, S., Sarma, B.K. (eds) Emerging Trends in Plant Pathology.
472 Springer, Singapore. https://doi.org/10.1007/978-981-15-6275-4_12
473 Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. Oikos 69,
474 373-386.
475 Jouni, F., Sanchez-Hernandez J.C., Mazzia C., Jobin M., Capowiez Y., Rault, M., 2018.
476 Interspecific Differences in Biochemical and Behavioral Biomarkers in Endogeic
477 Earthworms Exposed to Ethyl-Parathion. Chemosphere 202, 85–93.
478 http://doi:10.1016/j.chemosphere.2018.03.060.
479 Kah, M., Beulke, S., Brown, C.D., 2007. Factors Influencing Degradation of Pesticides in
480 Soil. J. Agric. Food Chem. 55, 4487–4492. http://doi:10.1021/jf0635356

Kaur, R., Singh, D., Kumari, A., Sharma, G., Rajput, S. and Arora, S., 2021. Pesticide residues degradation strategies in soil and water: a review. Int. J. Environ. Sci. Technol. 1-24. https://doi.org/10.1007/s13762-021-03696-2

Kiss, S., Drăgan-Bularda, M., Rădulescu, D., 1975. Biological Significance of Enzymes Accumulated in Soil. Advances in Agronomy, Academic Press 27, 25-87.

Kizilkaya, R., Hepsen, S., 2004. Effect of biosolid amendment on enzyme activities in earthworm (Lumbricus terrestris) casts. J. Plant. Nutr. Soil Sci. 167, 202-208.

Kumar, S., Kaushik, G., Dar, M.A., Nimesh, S., Lopez-Chuken, U.J., Villareal-Chiu, J.F., 2018. Microbial degradation of organophosphate pesticides: a review. Pedosphere 28, 190-208.

Köhler, H.R., Triebskorn, R., 2013. Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond? Science 341, 759-765.

Lemtiri, A., Colinet, G., Alabi, T., Cluzeau, D., Zirbes, L., Haubruge, É., Francis, F., 2014. Impacts of earthworms on soil components and dynamics. A review. Biotechnol. Agron. Soc. Environ. 18, 121-133.

Lessard, I., Sauvé, S., Deschênes, L., 2014. Toxicity Response of a New Enzyme-Based Functional Diversity Methodology for Zn-Contaminated Field-Collected Soils. Soil Biol. Biochem. 71, 87–94. http://doi:10.1016/j.soilbio.2014.01.002.

Lipiec, J., Frąc, M., Brzezińska, M., Turski, M., Oszust, K., 2016. Linking microbial enzymatic activities and functional diversity of soil around earthworm burrows and casts. Front. Microbiol. 7, 662–669.

Luo, L., Meng, H., & Gu, J. D., 2017. Microbial extracellular enzymes in biogeochemical cycling of ecosystems. J. Environ. Manage. 197, 539-549.

Medeiros, E.V., de Alcantara Notaro K., de Barros J.A., da Silva Moraes, W., Silva, A.O., Moreira, K.A., 2015. Absolute and Specific Enzymatic Activities of Sandy Entisol from

506 Tropical Dry Forest, Monoculture and Intercropping Areas. Soil Tillage Res. 145, 208-
507 2015. http://doi:10.1016/j.still.2014.09.013.
508 Medina-Sauza, R. M., Álvarez-Jiménez, M., Delhal, A., Reverchon, F., Blouin, M., Guerrero-
509 Analco, J. A., Cerdan, C.R., Guevara, R., Villain, L., Barois, I., 2019. Earthworms
510 building up soil microbiota, a review. Front. Environ. Sci. 7.
511 https://doi.org/10.3389/fenvs.2019.00081.
512 Mijangos I., Albizu I., Epelde L., Amezaga I., Mendarte S., Garbisu C., 2010. Effects of
513 liming on soil properties and plant performance of temperate mountainous grasslands. J.
514 Environ. Manage.91, 2066-2074.
515 Monreal, C.M., Bergstrom, D.W., 2000. Soil enzymatic factors expressing the influence of
516 land use, tillage system and texture on soil biochemical quality. Can. J. Soil Sci. 80, 419-
517 428.
518 Nannipieri, P., Grego, S., Ceccanti, B., 1990. Ecological Significance of the Biological
519 Activity in Soil. Edited by G. Bollag, J.M. and Stotzky. Eds.; Soil. New York,: Marcel
520 Dekker.
521 Nozaki, M., Miura, C., Tozawa, Y., Miura, T., 2009. The contribution of endogenous
522 cellulase to the cellulose digestion in the gut of earthworm (Pheretima hilgendorfi:
523 Megascolecidae). Soil Biol. Biochem. 41, 762-769.
524 https://doi.org/10.1016/j.soilbio.2009.01.016.
525 Pal, R., Chakrabarti, K., Chakraborty, A., Chowdhury, A., 2006. Degradation and effects of
526 pesticides on soil microbiological parameters-A review. Int. J. Agric. Res. 1, 240-258.
527 Panettieri, M., Lazaro, L., López-Garrido, R., Murillo, J.M., Madejón, E., 2013. Glyphosate
528 Effect on Soil Biochemical Properties under Conservation Tillage. Soil Tillage Res. 133,
529 16–24. http://doi:10.1016/j.still.2013.05.007.
530 Paoletti, M.G., 1999. The Role of Earthworms for Assessment of Sustainability and as

531 Bioindicators. Agric. Ecosyst. Environ. 74, 137–55. http://doi:10.1016/S0167-
532 8809(99)00034-1.
533 Pelosi, C., Barot, S., Capowiez, Y., Hedde, M., Vandenbulcke, F., 2014. Pesticides and
534 earthworms. A review. Agron. Sustain. Dev. 34, 199–228.
535 https://doi.org/10.1007/s13593-013-0151-z
536 Pelosi, C., Bertrand, M., Thénard, J., Mougin, C., 2015. Earthworms in a 15 years agricultural
537 trial. Appl. Soil Ecol. 88, 1–8. https://doi.org/10.1016/j. apsoil.2014.12.004.
538 Popova, I.E., Deng, S., 2010. A high-throughput microplate assay for simultaneous
539 colorimetric quantification of multiple enzyme activities in soil. Appl. Soil Ecol. 45, 315–
540 318.
541 Rao, M.A., Scelza, R., Acevedo, F., Diez, M.C., Gianfreda, L., 2014. Enzymes as useful tools
542 for environmental purposes. Chemosphere 107, 145–162.
543 Rault, M., Collange, B., Mazzia, C., Capowiez, Y., 2008. Dynamics of acetylcholinesterase
544 activity recovery in two earthworm species following exposure to ethyl-parathion. Soil
545 Biol. Biochem. 40, 3086-3091.
546 Riah, W., Laval, K., Laroche-Ajzenberg, E., Mougin, C., Latour, X., and Trinsoutrot-Gattin,
547 I., 2014. Effects of Pesticides on Soil Enzymes: A Review. Environ. Chem. Lett. 12,
548 257–73. http://doi:10.1007/s10311-014-0458-2.
549 Sabzevari, S., Hofman, J., 2022. A worldwide review of currently used pesticides' monitoring
550 in agricultural soils. Sci. Total Environ. 812, 152344.
551 http://doi:10.1016/j.scitotenv.2021.152344.
552 Sanchez, W., Burgeot, T., Porcher, J.-M., 2013. A novel Integrated Biomarker Response
553 calculation based on reference deviation concept. Environ. Sci. Pollut. R. 20, 2721–2725.
554 Sanchez-Hernandez, J.C., Mazzia, C., Capowiez, Y., Rault, M., 2009. Carboxylesterase
555 activity in earthworm gut contents: Potential (eco)toxicological implications. Comp.

Biochem. Physiol. Part C, 150, 503-511.

Sanchez-Hernandez, J.C., Notario del Pino, J., Domínguez, J., 2015. Earthworm-induced carboxylesterase activity in soil: assessing the potential for detoxification and monitoring organophosphorus pesticides. Ecotoxicol. Environ. Saf. 122, 303–312.

Sanchez-Hernandez, J.C., Marco Sandoval, and Antoine Pierart., 2017. Short-Term Response of Soil Enzyme Activities in a Chlorpyrifos-Treated Mesocosm: Use of Enzyme-Based Indexes. Ecological Indicators 73, 525–35. http://doi:10.1016/j.ecolind.2016.10.022.

Sanchez-Hernandez, J.C., Notario del Pino, J., Capowiez, Y., Mazzia, C., Raul,t M., 2018. Soil enzyme dynamics in chlorpyrifos-treated soils under the influence of earthworms. Sci. Total Environ. 612, 1407-1416.

Schinner, F., Kandeler, E., Ohlinger, R., Margesin, R., 1996. Methods in Soil Biology. Springer-Verlag, Berlin Heidelberg, New York.

Shaw, L.J., Burns, R.G., 2006. Enzyme activity profiles and soil quality. In: Bloem, J., Hopkins, D.W., Benedetti, A. (Eds.), Microbiological Methods for Assessing Soil Quality. CAB International, Oxfordshire, UK, pp. 158–182.

Sheehan, C., Kirwan, L., Connolly, J., Bolger, T., 2008. The Effects of earthworm functional diversity on microbial biomass and the microbial community level physiological profile of soils. Eur. J. Soil Biol. 44, 65–70.

Silva, V., Mol, H.G.J., Zomer, P., Tienstra, M., Ritsema, C.J., Geissen, V. 2019. Pesticide residues in European agricultural soils – A hidden reality unfolded. Sci. Total Environ. 653, 1532-1545, ISSN 0048-9697.

Singh, S., Sharma, A., Khajuria, K., Singh.J., Vig, A.P., 2020. Soil properties changes earthworm diversity indices in different agro-ecosystem. BMC Ecol. 20, 1-14. https://doi.org/10.1186/s12898-020-00296-5

Tao, J., Griffiths, B., Zhang, S., Chen, X., Liu, M., Hu, F., Li, H., 2009. Effects of

581 Earthworms on Soil Enzyme Activity in an Organic Residue Amended Rice-Wheat
582 Rotation Agro-Ecosystem. Appl. Soil Ecol. 42, 221–26.
583 http://doi:10.1016/j.apsoil.2009.04.003.
584 Tejada, M., Gómez, I., del Toro, M., 2011. Use of organic amendments as a bioremediation
585 strategy to reduce the bioavailability of chlorpyrifos insecticide in soils. Effects on soil
586 biology. Ecotoxicol. Environ. Saf. 74, 2075–2081.
587 Turner, B. L., Hopkins, D. W., Haygarth, P. M. and Ostle, N., 2002. Beta-glucosidase activity
588 in pasture soils. Appl. Soil Ecol. 20, 157–162.
589 Utobo, E.B., Tewari, L., 2015. Soil Enzymes As Bioindicators Of Soil Ecosystem Status.
590 Appl. Ecol. Environ. Res. 13, 147–69. http://doi:10.15666/aeer/1301.
591 Von Mersi, W., Schinner, F., 1991. An Improved and Accurate Method for Determining the
592 Dehydrogenase Activity of Soils with Iodonitrotetrazolium Chloride. Biol. Fertil. Soils
593 11, 216–20. http://doi:10.1007/BF00335770.
594 Wheelock, C.E., Phillips, B.M., Anderson, B.S., Miller, J.L., Miller, M.J., Hammock, B.D.,
595 2008. Applications of carboxylesterase activity in environmental monitoring and toxicity
596 identification evaluations (TIEs). Rev. Environ. Contam. Toxicol. 195, 117-178.
597 Wołejko, E., Jabłońska-Trypuć, A., Wydro, U., Butarewicz, A., Łozowicka, B., 2020 Soil
598 biological activity as an indicator of soil pollution with pesticides – A review. Appl. Soil
599 Ecol. 147, 103356. https://doi.org/10.1016/j.apsoil.2019.09.006.
600 Wolińska, A., Stepniewska, Z., 2012. Dehydrogenase Activity in the Soil Environment. In
601 (Ed.), Dehydrogenases. IntechOpen. https://doi.org/10.5772/48294
602 Wüst, P.K., Horn, M.A., Drake, H.L., 2011. Clostridiaceae and Enterobacteriaceae as Active
603 Fermenters in Earthworm Gut Content. The ISME Journal 5, 92–106.
604 http://doi:10.1038/ismej.2010.99.
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 ± SD, n = 8)
613 in the different conditions (earthworm presence/absence and species). (C) GMean index of
614 enzyme activities in bulk soil (mean ± SD, n = 16) and cast (mean ± 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

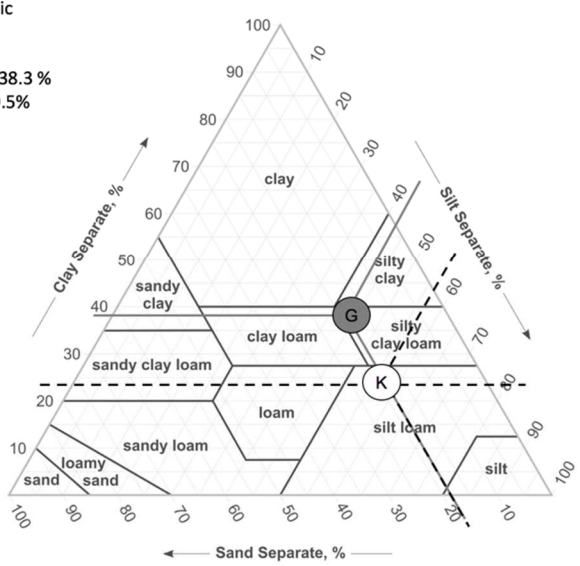
635 - Earthworm species did not influence enzyme activities in control and polluted soils

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)

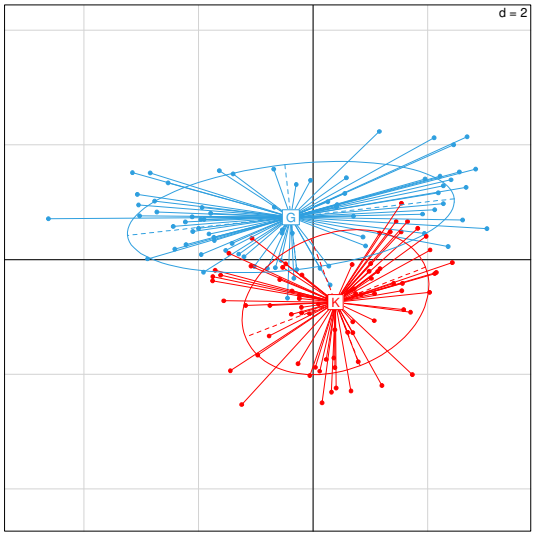


Figure 2

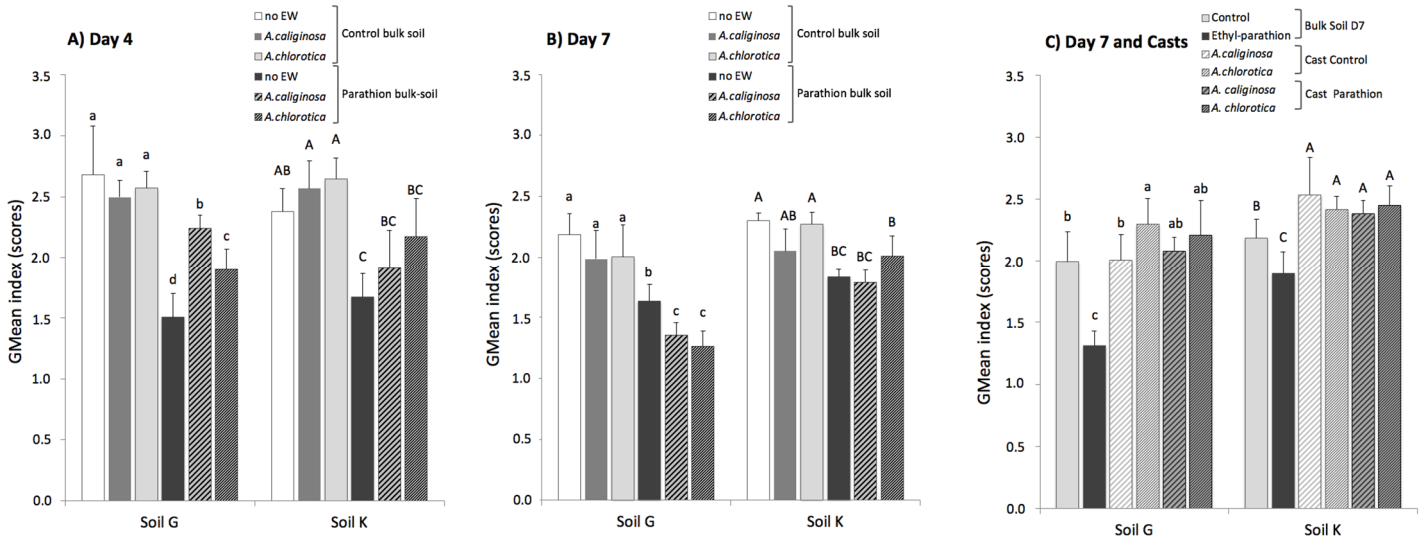

A) Day 4
no EW
A.caliginosa
A.chlorotica
Control bulk soil
no EW
A.caliginosa
A.chlorotica
Parathion bulk-soil
GMean index (scores)
Soil G
Soil K
B) Day 7
Control bulk soil
Parathion bulk soil
C) Day 7 and Casts
Control
Ethyl-parathion
Bulk Soil D7
A.caliginosa
A.chlorotica
Cast Control
A. caliginosa
A. chlorotica
Cast Parathion

Figure 3

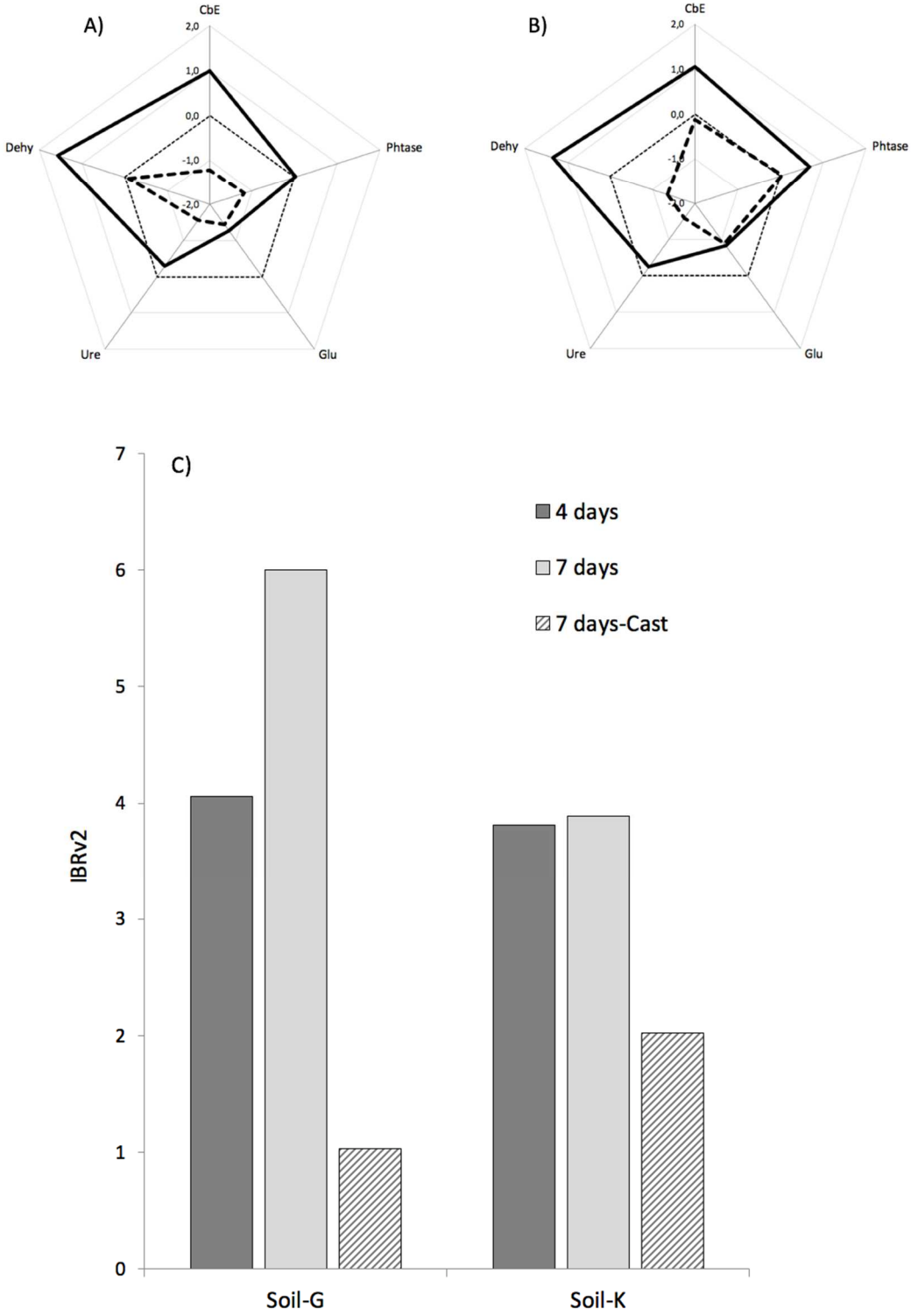

A)
CbE
2,0
1,0
0,0
-1,0
-2,0
Dehy
Phtase
Ure
Glu
B)
CbE
2,0
1,0
0,0
Dehy
Phtase
Ure
Glu
C)
4 days
7 days
7 days-Cast
IBRv2
7
6
5
4
3
2
1
0
Soil-G
Soil-K

Table 1: Soil enzyme activities (mean ± 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 Day 4	Parathion Day 4	Activity inhibition (%) at day 4	*P-value*
Carboxylesterase 4-NPB	*G*	15.11 ± 2.52 (a)	11.24 ± 3.80 (b)	-25.6	*<0.0001*
(µmol h^{-1} g^{-1} dry soil)	*K*	14.40 ± 4.06 (A)	8.17 ± 2.80 (B)	-43.3	*<0.0001*
Carboxylesterases 1-NB	*G*	1.92 ± 0.76 (a)	1.20 ± 0.28 (b)	-37.4	*<0.0001*
(µmol h^{-1} g^{-1} dry soil)	*K*	2.55 ± 0.62 (A)	1.56 ± 0.64 (B)	-37.2	*<0.0001*
Phosphatase	*G*	1.61 ± 0.38 (a)	1.25 ± 0.49 (b)	-22.4	*0.009*
(µmol h^{-1} g^{-1} dry soil)	*K*	0.90 ± 0.12 (A)	0.68 ± 0.12 (B)	-24.6	*<0.0001*
β-Glucosidase	*G*	1.34 ± 0.19	1.34 ± 0.35	0	*1*
(µmol h^{-1} g^{-1} dry soil)	*K*	1.40 ± 0.12 (A)	1.00 ± 0.29 (B)	-28.4	*<0.0001*
Dehydrogenase	*G*	76.5 ± 14.4	61.7 ± 18.7	-19.3	*0.136*
(nmol INTF h^{-1} g^{-1} dry soil)	*K*	110.7 ± 27.6 (A)	141.2 ± 24.0 (B)	+21.6%	*0.0005*
Urease	*G*	66.42 ± 5.45	61.63 ± 8.51	-7.2	*0.398*
(µg $NH4^+$-N h^{-1} g^{-1} dry soil)	*K*	62.12 ± 6.06	55.14 ± 7.72	-11.2	*0.046*