Research Article

Single and combined toxicity of the pesticides abamectin and difenoconazole on soil microbial activity and *Enchytraeus crypticus* population

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Abstract
Besides being toxic to enchytraeids, pesticides may also affect microbial communities, which are the main diet of enchytraeids. This study aimed to analyze the individual and combined effects of the insecticide Kraft® 36 EC (abamectin) and the fungicide Score® 250 EC (difenoconazole) to soil microbial communities and *Enchytraeus crypticus* populations. The abamectin and difenoconazole effects to the microbial community metabolism, as revealed by qCO_2 increase in the first two periods of exposure, might indicate an acute effect of pesticides, which might result in lowered microbial biomass once microorganisms spend more energy in detoxification processes than in microbial growth. *E. crypticus* juvenile production was not affected at the different conditions tested. However, the importance of microorganisms on the enchytraeids diet was ratified. Besides, it is important to emphasize that only one recommended dose of the pesticides was tested in this study. Hence, other situations (e.g. pesticides over application or a slower process of pesticides degradation) may result in a different scenario of effects and should be further investigated.

Keywords Pesticides · Abamectin · Difenoconazole · Soil microorganisms · Soil ecotoxicology · *Enchytraeus crypticus*

1 Introduction

Conventional agriculture is largely based on the use of agrochemicals, such as pesticides, which are used to protect plants from diseases and pests [3]. Despite the importance of such products in preventing and eliminating agricultural pests, they have been a reason for concern as they can affect nontarget organisms [39]. Once applied, pesticides can reach the terrestrial environment and become bioavailable for assimilation by soil organisms, such as microorganisms and enchytraeids [33], which are of great importance for several ecological services (e.g. organic matter decomposition, energy and nutrients cycling, soil structure and degradation of pollutants).

The insecticide Kraft® 36 EC (active ingredient: abamectin, from the avermectin group) and the fungicide Score® 250 EC (active ingredients: difenoconazole, from the triazole group) are widely used pesticides at

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the conventional agriculture (Silva 2016). Abamectin is a broadly spread active ingredient used worldwide, not only in the agriculture but also as pharmaceuticals in both human and animal protection [4]. Its effects on the survival and reproduction of soil organisms such as collembolans, mites and earthworms [18, 30], as well as changes in the behavior of earthworms [36], have been reported.

In the case of the fungicide difenoconazole, although it seems to be less toxic to soil organisms, when compared to abamectin, their use is of great concern because of its persistence in the environment, which is confirmed mainly by its high chemical stability and low biodegradability [47]. The effects of triazole fungicides on microbial indicators have been assessed [38, 51]; however, studies assessing the effects of such compounds on enchytraeids as well as on other soil invertebrates such as worms and collembolans are rare [19, 23, 30].

Apart from the exposure to pesticides, natural stress conditions such as space and food limitation may be also a factor of concern for the terrestrial organism development and, combined to the chemical exposure, may change the organisms' response increasing their sensitivity to potentially toxic substances [31]. Due to enchytraeids sensitivity and importance, they are frequently used as bioindicators in acute and chronic experiments for soil quality and ecotoxicological assessment [8], but the above-mentioned natural stress conditions are not taken into consideration.

Enchytraeid population densities may vary with physical, chemical and biological specific soil conditions, besides regional variation [16] and the presence of contaminants, such as copper and zinc [24, 31, 32]. Moreover, most existing studies have evaluated the effects of enchytraeids on microbial communities [41, 45] and no studies investigated the opposite relation. The diet of the enchytraeids is mainly composed of fungal hyphae, usually present in finely divided plant materials, and bacteria [7]. Thus, their diet is directly dependent on microbial communities, which are the basis of the soil organic matter (SOM) [15]. Therefore, negative effects to soil microorganisms may affect enchytraeid populations.

Therefore, the effects of both pesticides to soil organisms need to be further investigated, especially considering different exposure situations such as trophic interaction or organism's resource limitations. Furthermore, using natural soil from the tropics is also of great importance since very scarce studies are available for tropical environments. Hence, the aim of the present study was to determine the individual and combined effects of the recommended doses of the insecticide (Kraft® 36 EC) and the fungicide (Score® 250 EC) on: (1) soil microbial communities at different periods of exposure; (2) *Enchytraeus crypticus* population at different initial densities; and (3) the interactive effect of microorganisms with the *Enchytraeus crypticus* population upon pesticide application.

2 Materials and methods

2.1 Soil collection and spiking

The characterization of the soil was performed at the Environmental Geotechnics Laboratory of the Sao Carlos School of Engineering (EESC/USP). Soil was collected at a depth of 0–15 cm in a grassland area of the Center for Water Resources and Environmental Studies (CRHEA/EESC/USP), Itirapina, SP, Brazil (coordinates 22°10′10″S 47°53′56″W), and characterized as loamy sand (35% clay, 21% silt and 46% sand). The selected area had no history of pesticide contamination [36], and the main soil properties are: pH (H_2O/KCl) 5.52/5.94; organic matter (%) 11; and cation exchange capacity (meq/100 g) 3.52. After sampling, soil was sieved (2 mm), dried and defaunated at 65ºC for 24 h, to avoid the presence of other mesofauna individuals. However, in order to ensure the presence of microorganisms, an elutriate solution was inoculated in the soil test. The solution was prepared according to the procedures described by Jensen and Scott-Fordsmand [20], using the soil from the same area of study.

After 5 days of the microbial community inoculation, the soil was spiked with the insecticide dose recommended to control the infestation of the acari *Tetranychus urticae*, and the fungicide dose recommended to control the fungi *Mycosphaerella fragariae*, in strawberry plantations at tropical regions [27, 28]. The recommended dose (RD) for the insecticide Kraft® 36 EC was 0.02 mg abamectin kg^{-1} dry soil, while for the fungicide Score® 250 EC it was 0.04 mg difenoconazole kg^{-1} dry soil. For the treatment in which the mixture of both pesticides was assessed, the same RD was used. Soil moisture was maintained at 50% of the soil water holding capacity (WHC) [37].

Two issues were mandatory for the choice of the pesticide concentrations used in the experiment. First, realistic scenarios of pesticides used in strawberry plantations were intended. Second, in order to have the opportunity to analyze the relationship between the effects to the microorganisms and to the enchytraeid species, it was essential not to have a drastic effect of the pesticide to the microbial communities. See detailed experimental design described below.

2.2 Experimental procedures

All experiments (microbial analysis and enchytraeid bioassays) were performed using the same spiked soil. Thus, soil spiking, as described above, was performed at the same

time and the total amount of soil was prepared considering six periods of exposure (0, 7, 14, 28, 56 and 84 days after soil spiking), four treatments: Control (no pesticide), Kraft (RD), Score (RD) and Kraft (RD) + Score (RD) and both microbial and enchytraeid assessments (Fig. 1). For each condition, five replicates were used giving 120 replicates. Each replicate consisted of 500 g of soil (dry basis) placed in plastic containers (approx. 9 cm diameter and 10 cm height), which were maintained at 23ºC, 16 h:8 h (light:dark) photoperiod (approx. 4,000 lx) and weekly opened for soil moisture adjustment and gas exchange. After each period of exposure, replicates were sacrificed and soil was sampled for microbial and enchytraeid assessments as described in detail below.

2.2.1 Microbial analysis

From each plastic container, soil was sampled in order to analyze the microbial communities in all periods of exposure (0, 7, 14, 28, 56 and 84 days after soil contamination) and treatments (Control, Kraft, Score and Kraft + Score). The indicators used were: microbial biomass carbon (MBC) [mg C kg^{-1}soil], soil basal respiration (SBR) [mg CO_2–C g^{-1} solo h^{-1}], metabolic quotient (qCO_2) [mg C–CO_2 g^{-1} MBC h^{-1}] and β-glucosidase enzyme (BG) [mg ρ-nitrophenol g^{-1} dry soil h^{-1}].

The chloroform fumigation–extraction method was used for MBC estimation [13, 44], with kec coefficient (correction for the C extracted from the microbial biomass after fumigation) of 0.33 [43]. The SBR was estimated by incubating the non-fumigated samples of the microbial biomass carbon tests with KOH 0.3 M followed by titration with HCl 0.1 M [14, 17]. The metabolic quotient was provided through the ratio between the SBR and the MBC [2]. The BG was determined by colorimetric analysis of the ρ-nitrophenol released after the soil incubation with ρ-nitrophenyl-glucoside solution for 1 h at 37ºC [9, 35].

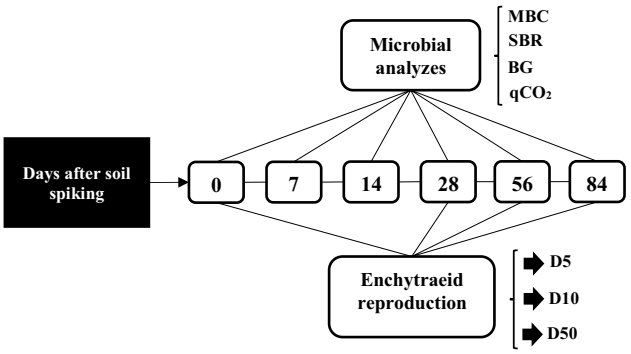

Fig. 1 Schematic representation of the experimental design. Analyses performed for the microbial communities were: microbial biomass carbon (MBC); soil basal respiration (SBR); metabolic quotient (qCO_2) and β-glucosidase enzyme (BG), which were performed in all periods of exposure. For the enchytraeid reproduction assessment, tests were performed at days 0, 28, 56 and 84 after soil spiking. Tests with the enchytraeids were performed with different initial densities: D5 (five initial adults); D10 (10 initial adults); and D50 (50 initial adults).

2.2.2 Enchytraeid reproduction test with different initial population densities

The University of Coimbra (Portugal) gently donated the first individuals of *E. crypticus* in 2014. Since then, the organisms are maintained in well-established cultures at the Center for Water Resources and Environmental Studies (CRHEA/EESC/USP). In the laboratory, organisms were kept in a bacterial agar substrate at 20 ± 1ºC, photoperiod cycle of 16 h:8 h (light:darkness), and fed on rolled oats twice a week. Organisms from the cultures are periodically tested, in order to ensure their sensitivity to chemical substances, using boric acid as reference substance.

The *E. crypticus* reproduction experiments were performed considering four periods of exposure (0, 28, 56 and 84 days after soil spiking) in order to guarantee the stability of the microbial communities. Hence, after each period of exposure, for each treatment (Control, Kraft, Score and Kraft + Score), soil was sampled from the test container and the enchytraeid experiments were performed considering three different initial densities of organisms (5, 10 and 50 initial adults). Thus, 5, 10 and 50 adult organisms (well-developed clitellum) were placed in plastic vessels of approx. 5 cm diameter and 6 cm height, containing 20 g of the treated soil per replicate and 5 replicates per treatment, giving 20 vessels per density and 60 vessels per exposure period. The enchytraeids were exposed during 21 days at 20 ± 1ºC and photoperiod cycle of 16 h:8 h (light:darkness). Vessels were weekly opened to replenish soil water and allow gas exchanges. In order to analyze possible relations between enchytraeid and the microbial communities, microorganisms added before soil spiking were the only source of food during the experiments. Organisms were tested following an adaptation of the OECD 220 (2016) guideline.

The instantaneous rate of increase (RI) was used to evaluate the different population densities of enchytraeids under the same parameter [42]. The RI is a dimensionless indicator that represents a logarithmic relation between the initial and the final population of the test (RI = (ln(nt/no))/t, where *nt* and *no* are the population sizes at the end and start of the experiment, respectively, and *t* is the time in days). The following diagram shows a schematic representation of the experimental design (Fig. 1).

2.3 Pesticide chemical analyses

Stock solutions used to prepare the different pesticide treatments were chemically analyzed to confirm nominal test concentrations. Concentrations of abamectin and difenoconazole were measured through LC/MS/MS (liquid chromatography–tandem mass spectrometry) and ID-LC/MS/MS (isotope dilution–liquid chromatography–tandem mass spectrometry), respectively. Chemical analysis was conducted by an external laboratory (Merieux NutriSciences) following standard guidelines in accordance with DIN EN ISO/IEC 17,025: 2005. The detection limits of the pesticide chemical analyses were 0.1 µg/L for abamectin and 5 ng/L for difenoconazole with analytical recoveries of 116% and 115%, respectively.

2.4 Statistical analyses

Three-way analysis of variance (ANOVA) and Tukey post hoc tests were performed to compare the interactions between different pesticides and periods of exposure. Each microbial indicator was considered as a dependent variable, and the periods of exposure after the contamination and the different pesticides were the independent variables. For the enchytraeid reproduction tests, the RI was the dependent variable. Pearson correlation coefficients were also determined for each period of exposure to correlate the microbial parameters and the RI. All analyses were performed using *SigmaPlot*® 11.0 and *IBM SPSS Statistics*® 22 software. The threshold for statistical significance was fixed at 5% for all analyses.

3 Results and discussion

3.1 Microbial indicators

All the microbial indicators are presented as percentage of the Control treatment at day 0 (Fig. 2). However, absolute values were used for statistical analysis. microbial biomass carbon, which represents the sum of the active and inactive fractions of soil microorganisms, declined at the beginning of the experiment for both Control and the pesticide-spiked soils (Fig. 2a). The reduced microbial biomass in the Control treatment when compared to the initial stage might indicate that the recolonization by the inoculated microbial community was yet taking place. Three-way ANOVA indicated the factors time after soil spiking ($p < 0.001$), Kraft ($p = 0.030$), Kraft associated with Score ($p = 0.034$), Score associated with time ($p = 0.010$), and the interaction of the three factors ($p = 0.010$) as the source of variation for MBC. Hence, as MBC showed to be significantly higher in the Control treatment after 84 days of exposure (Fig. 2a), results suggest that chronic effects might be more relevant than acute effects for the total microbiota. After 28 days of the soil spiking, MBC was significantly higher for the Kraft treatment when compared to Control soil (Fig. 2a). This is not a surprising response since abamectin is a chemical with neither bacterial nor antifungal activity, except under very high doses of exposure [4].

The possible toxic effects of the fungicide Score on soil fungal communities were not reflected in a reduction in microbial biomass over time, although at least 30% reduction on MBC, when compared to the Control group after 84 days of exposure, was observed. There is still no consensus about the dominance of either fungi or bacteria in the soil microbial biomass composition. The share between these two groups shifts according to pH and land use intensity because of the adaptation of fungi in acidic conditions and the prevalence of bacteria in nutrient-rich environments [10]. Recently, Malik et al. [26] have proposed the clear dominance of bacteria over fungi biomass. On the other hand, it is reported the dominance of fungi on soil microbial respiration [1]. Some studies have also reported that toxic effects on soil fungal communities may result in less competition for degradation of organic compounds, which favors the growth of heterotrophic bacterial communities [50].

In the present study, the higher amount of β-glucosidase (Fig. 2d) in the soils treated with fungicide alone or combined with the insecticide indicates the occurrence of a microbial succession, and the favoring of bacteria over the fungi dead biomass is a mechanistic explanation for this consistent result. Furthermore, the enzymatic activity showed to be a more sensitive parameter than MBC for the fungicide treatment. Fungicide Score consistently increased soil BG enzyme activity ($p < 0.001$), but with lower variation, when compared to the other treatments, over time (Fig. 2d).

Regarding soil basal respiration, the results of the present study differ from other studies that characterized difenoconazole [34] and other triazole pesticides [21] as compounds that do not affect basal respiration at low doses (Fig. 2b). On the other hand, for the abamectin and other ivermectin compounds there is no consensus and they have been described as compounds that may inhibit [5], do not affect [11] or even stimulate [25] microbial respiration [40].

In contrast to MBC, the Metabolic Coefficient increase 7 days after soil spiking indicates an acute effect of the pesticides tested on the microbial community (Fig. 2c). This qCO2 increase is indicative of stress to the microbial community, which might have spent more energy to sustain basic functions instead of promoting microbial growth [34]. After 7 days of pesticide amendment, both pesticides caused stress in the microbial community

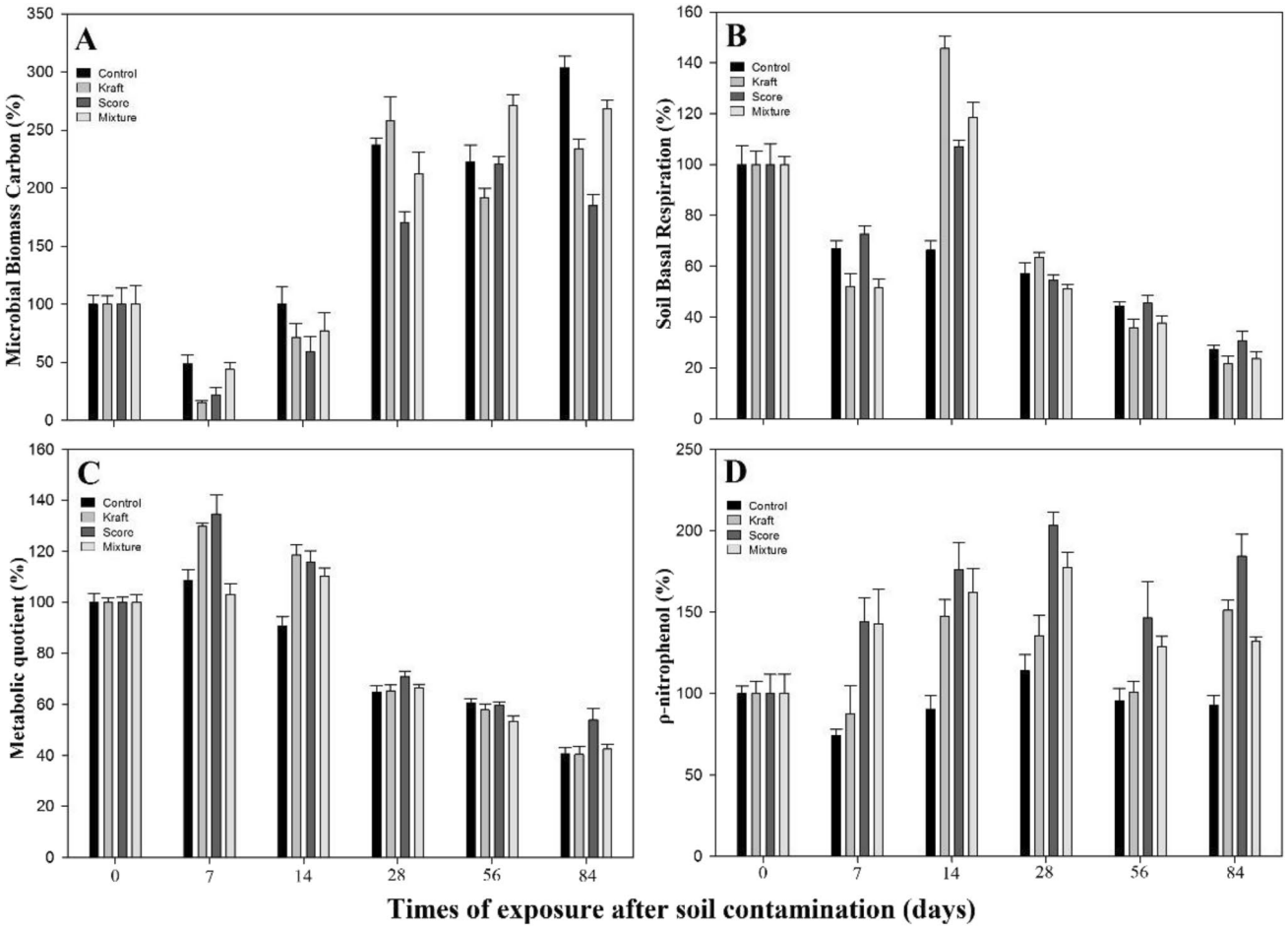

Fig. 2 Variations and standard errors of A) microbial biomass carbon [mg C kg^{-1} soil], B) soil basal respiration [mg CO_2-C g^{-1} solo h^{-1}], C) metabolic quotient [mg C-CO_2 g^{-1} MBC h^{-1}] and D) β-glucosidase enzyme [mg ρ-nitrophenol g-1 dry soil h-1], in 6 periods of exposure after soil spiking with recommended doses of pesticides Kraft® 36 EC, Score® 250 EC and the mixture of both products. For statistical significance, results of the two-way ANOVA are available at the supplementary materials

as revealed by the increase in qCO_2 ($p < 0.001$). Interestingly, the interaction of the pesticides caused effect only at 14 days of exposure when compared to the Control ($p < 0.001$, Fig. 2c). This effect might be, for instance, due to interaction of excipients or chemicals that could reduce their availability to the microbial community. In general, time after pesticide amendment interacted with all the other experimental factors ($p < 0.001$), being the lowest values of qCO_2 observed in the three last periods after exposure (days 28, 56 and 84).

In addition to the pesticides effects, the experimental conditions may have influenced the microbial indicators since similar variations in the controls of the different treatments were also observed. A possible explanation for this response is that the microorganisms were not yet stabilized in the three first periods of exposure (0–14 days). In the three last periods (28–84 days), the values of all parameters indicated a microbial stabilization, which is expected due to the limitation of space [22, 46], followed by a general growth of microbial communities and decreasing of metabolic rates, possibly due to processes of pesticides degradation (Fig. 2).

The decomposition rate of pesticides is normally expressed by their half-life (time to degrade 50% of the compound) and depends on many factors [49]. In the experimental conditions of the present study—laboratory at 23ºC, aerobic clay loam soil, similar pesticide doses and periodic UV radiation, the expected half-life of abamectin is 3–14 days [4] and for difenoconazole is 33–55 days [48]. Thus, it is reasonable to consider a substantial reduction in the effects of Kraft from the third analysis (day 14) and Score from the fifth analysis (day 56).

Finally, although no deleterious effects on MBC, metabolic indicators (SBR and qCO_2) or β-glycosidase enzyme were observed in spiked soils, the control treatment showed a greater stability, in relation to the variation of

the parameters tested over time. The MBC in the control increased continuously until it surpassed the other treatments in the last analysis (day 84). Also, only in the control soil the MBC did not decrease after the recovery in the day 7. Similarly, the SBR, the qCO2 and the β-glycosidase enzyme had less variation in the control than in the pesticides treated soils.

3.2 Enchytraeid reproduction in different initial densities

All validity criteria were fulfilled according to the guideline used (survival ≥ 80% and reproduction ≥ 50 juveniles in the control treatment) [37]. The soil pH was 6.21 at the test start with no significant variation within time.

For all densities analyzed, the differences between treatments were not statistically significant (Fig. 3). This indicates that the pesticides did not impair the enchytraeid reproduction under the experimental conditions, which has already been described for similar active ingredient in low doses [18] and when exposed to standard density conditions (10 initial adults–D10) [30].

The RI of the *E. crypticus* was not significantly different between treatments at the same time of exposure, and few significant differences were noted between times of exposure for the same treatment. For the lowest density tested (5 initial organisms), the RI of the *E. crypticus* showed a general continuous growth trend from day 0 to day 84 for all treatments. For the standard condition, the RI variation in the control treatment was smaller, when compared to the pesticide treated soils, although not resulting in significant differences between treatments or between periods of exposure. Only one exception was observed for the Score treatment, in which the RI at day 28 was significantly higher when compared to the organisms exposed at day 56. For the highest enchytraeid density (50 initial

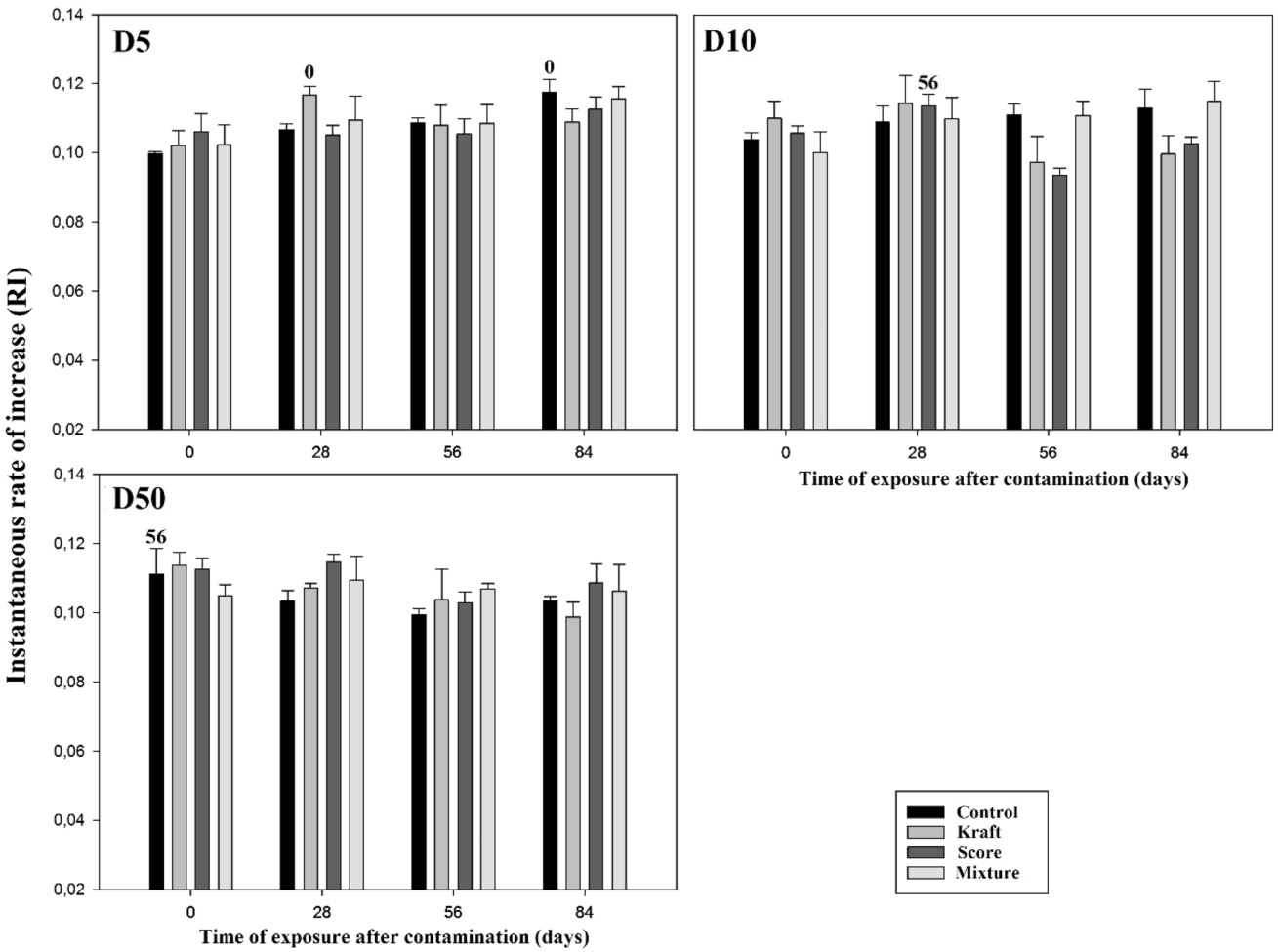

Fig. 3 Instantaneous rate of increase and standard errors of *Enchytraeus crypticus* in initial densities of 5 (D5), 10 (D10) and 50 (D50) adults, in tests started after 0, 28, 56 and 84 days from soil contamination with recommended doses of pesticides Kraft® 36 EC, Score® 250 EC and the mixture of both. Numbers above bars indicate significant differences between the RI of the respective period of exposure and the IR of the period 0 or 56 (for the same treatment and density)

adults–D50), the only significant difference was observed for the control treatment (day 0), which showed a higher RI when compared to organisms exposed after 56 days of the contamination (Fig. 3).Other authors have studied whether the *E. crypticus* sensitivity could be density dependent and they have not found a significant interaction between the two parameters when organisms were exposed to copper [31]. However, when the second generation of the organisms was exposed to the same density conditions, the sensitivity to copper showed an opposite pattern of response in regard to density, which means lower toxicity for higher density of organisms [32].

Other studies indicate that the enchytraeid reproduction is expected to decrease with the density increase [12, 31], which did not occur here. The current results suggest that a density-dependent decrease in reproduction may not be so evident under circumstances of food limitation, since this factor was excluded in the other studies. This hypothesis was raised by Kramarz et al. [24] and is particularly important because food abundance is a limiting factor for oligochaete distribution [29].

3.3 Relationship between microorganisms and enchytraeid reproduction

Correlation analysis was performed in order to understand whether a relation between enchytraeids RI and microorganisms as food source could be assumed (Table 1). Strong positive correlations between MBC and RI were found to be significant. Besides, strong correlation coefficients, both negative and positive, were also observed between other microbial indicators and RI. Although such correlations were not constant among the microbial indicators, treatments, densities and or the periods of exposure, the importance of the microorganisms for the enchytraeid reproduction could be observed. Results obtained for the RI in the present study were about two times lower than that found by Menezes-Oliveira et al. [31]. The main difference between the two studies is the lack of food supply in the present work, which suggests that feeding is a limiting factor of the test. This was especially important at the first analysis, when the microbial communities were probably not stabilized yet.

Hence, whereas other studies indicate that the increase in the enchytraeid populations usually decreases MBC because of their microbial consumption [6, 45], our results suggest that the microbial biomass is a relevant factor for enchytraeid reproduction for the same reason. For a better understanding, other concentrations capable of impairing microbial biomass should be tested. Besides that, studies qualifying the microbial communities could also be important.

4 Conclusions

Pesticides effect on microbial community metabolism, as revealed by qCO_2 increase, indicated an acute effect. This first stress on microbial community might result in lowered microbial biomass once microorganisms spend more energy in detoxification processes than in microbial growth. Moreover, although the enchytraeid juvenile production was not significantly affected at the different conditions tested, strong correlations were evidenced between RI and SBR. Therefore, our results suggest a food web response due to the importance of the limitation of substrate after the acute phase of pesticides. Despite the importance of pesticides use in developing countries, their application should strictly follow the recommendations, to avoid endangering the environment. When the recommendations are not followed, the effects in field scenarios may differ from those reported here. Tests using other nontarget organisms are important, because the sensitivity of the organisms might differ due to differentiated modes of action of the pesticides.

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Table 1 Pearson correlation coefficients between instantaneous rate of increase in enchytraeids and microbial indicators

Density	Treatment	Day 0				Day 28				Day 56				Day 84			
		MBC	SBR	qCO_2	BG	MBC	SBR	qCO_2	BG	MBC	SBR	qCO_2	BG	MBC	SBR	qCO_2	BG
D5	Control	−0.59	−0.22	0.16	−0.22	0.52	0.53	0.06	−0.47	0.15	0.95**	0.25	−0.08	0.91*	0.80	0.85*	0.12
	Kraft	0.01	0.12	0.07	−0.48	−0.20	0.83*	0.36	−0.01	−0.45	0.49	0.63	0.58	0.96**	0.05	−0.13	−0.04
	Score	0.62	0.66*	−0.11	−0.25	0.39	−0.27	−0.47	0.44	−0.51	−0.57	−0.57	0.00	0.19	0.15	0.02	0.17
	Mixture	−0.49	−0.09	0.32	0.09	0.29	−0.52	−0.53	−0.09	0.81*	0.48	0.65	0.82*	−0.55	−0.78	−0.88*	0.17
D10	Control	0.08	−0.31	−0.26	−0.08	0.28	0.57	0.22	−0.58	0.12	−0.54	−0.37	−0.18	−0.36	−0.13	−0.04	0.76
	Kraft	0.16	0.89**	0.58	−0.38	0.25	−0.31	−0.19	0.25	−0.18	−0.68	−0.54	−0.30	−0.22	0.82*	0.82*	−0.15
	Score	0.02	−0.11	−0.32	−0.31	0.12	0.65	0.19	−0.38	0.86*	0.76	0.59	0.65	0.37	−0.87*	−0.76	−0.23
	Mixture	0.80	0.66	−0.66	0.22	−0.48	−0.24	0.43	0.21	−0.16	0.50	0.46	0.67	−0.53	−0.41	−0.20	−0.77
D50	Control	−0.50	0.42	0.49	0.12	−0.14	0.78	0.60	−0.72	0.91*	0.30	−0.89*	0.56	−0.48	0.61	0.55	−0.17
	Kraft	−0.12	−0.97**	−0.65	0.50	−0.41	−0.67	0.23	0.18	−0.09	0.08	0.12	−0.87*	0.18	−0.92*	−0.88*	0.43
	Score	0.07	0.30	0.33	0.04	0.18	−0.63	−0.32	−0.30	0.82*	0.67	0.43	0.99**	0.28	−0.72	−0.63	−0.04
	Mixture	0.64	0.38	−0.68	−0.14	0.65	0.63	−0.37	0.05	−0.31	0.69	0.60	0.90*	−0.11	−0.51	−0.68	−0.06

[a] Pearson correlation coefficients (r-values) between instantaneous rate of increase in *E. crypticus* in initial densities of 5 (D5), 10 (D10) and 50 (D50), in tests started after 0, 28, 56 and 84 days of soil contamination with recommended doses of the pesticides Kraft® 36 EC, Score® 250 EC and the mixture of both, and the following microbial indicators: microbial biomass carbon, soil basal respiration, metabolic quotient and β-glucosidase enzyme

Significant correlations are indicated by * ($P < 0.05$) and ** ($P < 0.01$)

MBC microbial biomass carbon, *SBR* soil basal respiration, *qCO_2* soil metabolic quotient, *BG* β-glucosidase enzyme

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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