

TEMPORAL DYNAMICS OF SOIL INVERTEBRATE COMMUNITIES IN A VINEYARD UNDER TREATMENT WITH PESTICIDES

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Abstract

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Phytosanitary treatments with pesticides are widely used to control pests and diseases in vineyards. An important part of the dispersed pesticide reaches the soil, affecting the fauna, producing quantitative and qualitative changes in the edaphic population's structure and physiological activities. This study aims to evaluate the temporal dynamics of the soil macro and mesofauna through different pesticide treatment periods, where fungicides are the dominant pest control agent. A field experiment was carried out in Boumerdes, a specific viticultural region in Algeria. Soil samples were taken during three periods, before, during, and after treatment with pesticides, using the quadrat method at three soil depths. During these three periods, the soil macro and mesofauna were observed and compared. The results showed significant differences in the composition of invertebrate communities that tended to disappear during pesticide application. The structure and diversity of 11 invertebrate classes, including Collembola, changed along the treatment gradient. The effects of sampling periods on occurrence, abundance, and taxon richness were consistently negative. These indices were significantly lower after pesticide application. Therefore, we can suppose that climatic factors and pesticide treatments are the main factors affecting the activity and density of all classes of invertebrates, but these effects vary between the functional groups of soil biota.

Key words: arthropods; collembolan; community structure; non-target effects; soil fauna; viticulture.

Introduction

Pest and disease control in vineyards is critical to maintaining yields and achieving high production levels and a good-quality crop. Production losses due to pests and diseases can be as high as 35%, and in some cases, the entire production may be compromised (Oerke, 2006). Applied pesticides should be toxic only to target organisms, biodegradable, and environmentally friendly to some extent (Rosell et al., 2008). Nevertheless, the repeated application of pesticide treatments has a negative impact on the environment and, in particular, on the soil (Arias-Estévez et al., 2008), causing effects on the soil ecosystem and heavy damage to invertebrate populations (De Silva et al., 2009).

Soil fauna plays an important role in the ecosystem, as it is involved in nutrient cycling, decomposition and mineralization of organic matter, maintenance of trophism, and biological control (Brygadyrenko, 2016; Frouz et al., 2013; Marichal et al., 2017). Pesticides can pose risks to the survival and reproduction of soil fauna, as demonstrated by ecotoxicity tests (Booth et al., 2003; Bart et al., 2019; Pelosi et al., 2014). It is recognized that pesticides and their metabolites could affect non-target organisms such as earthworms, springtails, spiders, and isopods (Pelosi et al., 2014; Paoletti, 1999). Therefore, macrofauna and

mesofauna are given significant protection and have been used as an ecotoxicological indicator tool in the risk assessment of pesticides (EFSA, 2007).

Springtails are the most abundant arthropods in soils and play an important role in organic matter decomposition (Petersen, 2002). They constitute an explicit community among other taxa as indicators of ecosystem functioning. The diversity, composition, and abundance of this fauna provide an indicator of the biological activity of the soil to qualify the litter and can be used as bioindicators of pollution (Cortet et al., 2003; Fiera et al., 2020a; Parisi et al., 2005; Seres et al., 2009). Numerous diagnostic studies of soil disturbance states concerning anthropogenic activity can be carried out by analyzing springtail communities (Fiera et al., 2020a; Frampton, van den Brink, 2007; Ghosal et al., 2018; Harta et al., 2020).

Therefore, it is assumed that the temporal variation of macro and mesofauna communities is affected by the different treatment periods and/or soil depth. This study aims to find patterns in invertebrate community composition related to the effect of pesticide treatment periods. For this purpose, the temporal variability of soil invertebrate community through different pesticide treatment periods was assessed in a vineyard where fungicides are the main pest control agent. The analysis of springtail

Table 1. Details of sampling periods and pesticides (typology, amount) used in the vineyards.

Management	March BT sampling		May DT sampling		August AT sampling	
	No.	Family	No.	Family	No.	Family
Tillage	0	-	1	-	0	-
Fungicides	0	-	24	Phosphonate Dinitrophenol Carbamate Phthalimide Inorganic substance Triazole Benzamide	+5	Carboxamide Strobilurin
Insecticides	0	-	0	-	+1	Organophosphorus

Notes: BT – before treatment; DT – during treatment; AT – after treatment.

communities in these soils will be highlighted with the treatment periods with plant protection products.

Material and methods

Presentation of the study regions

The study was conducted in a vineyard in the Boumerdes region in northern Algeria, 45 km east of Algiers. This vineyard region covers more than 11,000 ha of vineyards, an area that is constantly increasing (500 ha/year) (DAS, 2019). Pesticides are applied every 10–14 days from the end of April to mid-August, and fungicides are the most used pesticides for grapes (97% of all applications), while insecticides (3%) play a minor role.

Soil fauna sampling

The samples were collected at least 1 m within the plot from the boundaries between the vines' rows to avoid any border effect. Soil samples were taken using the quadrat method, which collects all individuals of all species present within a defined area (Bogyó et al., 2015; David et al., 1999; McCravy, 2018). Soil samples measuring 25 × 25 cm² and 10 cm depth were collected. The soil block was cut into three strata during extraction, depending on the depth: 0–10, 10–20, and 20–30 cm. In total, the fauna of 15 blocks, divided into five sampling points, was studied, thus making it possible to obtain the most representative sample possible of the fauna present. At the end of the rainy season, the first sampling (BT) was carried out just before the beginning of the treatment campaign with phytosanitary products (March). The treatments were carried out without interruption until the end of the sensitivity period (bunch closure). The second sampling (DT) was carried out at the end of this first treatment period (May). Subsequently, the treatments were reasoned according to the observations in the plot in relation to the pressure of pests and diseases. The last sampling (AT) corresponds to the end of all treatments with phytosanitary products (August) (Table 1).

Invertebrates recovery

The macrofauna present in the samples was sorted manually and placed in 75° ethanol or 4° formalin in the laboratory. After the

recovery of the macrofauna, the samples were placed in Berlese extractors for 10 days. The recovered mesofauna was preserved in 75° ethanol and subsequently examined under a binocular microscope for correct identification. The samples were first separated into broad taxonomic groups before being determined to species level for Collembola. Due to the large number of specimens collected, identifying all individuals to species level was not possible. Mesofauna and macrofauna in the samples were identified to analyze the effects of pesticide treatment periods on all invertebrate communities. Only springtails were identified to species.

Data analysis

Total soil faunal abundance, species richness, Shannon (H'), Hill, and Simpson (D) diversity indices, as well as the equitability index (E) were used to assess species diversity within the soil macrofauna and mesofauna communities. The use of three indices, Shannon Wiener, Simpson, and Hill, concomitantly allows for a more comprehensive analysis of information regarding community structure. The indices are calculated using the following formulae:

$$H' = - \sum_{i=1}^s P_i \log_2 P_i \quad (1),$$

where P_i is the proportional abundance ($P_i = n_i/N$), n_i is the number of individuals of each i species in the sample, and N is the total number of individuals.

$$E = H' / H'_{\max} \quad (2),$$

where $H'_{\max} = \log S$ (S = total number of species).

$$D = \frac{\sum n_i(n_i - 1)}{N(N - 1)} \quad (3),$$

$$\text{Hill} = \lambda^{(-1)/e^{H'}} \quad (4),$$

where $\lambda = D$ (Simpson index).

Concerning Collembola, in addition to abundance, species richness, Shannon diversity indices (H'), and equitability index

Table 2. Effects of pesticide treatment on soil invertebrate abundance (mean $\pm$ SD).

Treatment		BT			DT			AT		
Depth		P1	P2	P3	P1	P2	P3	P1	P2	P3
Collembola	Entomobryomorpha	14.2 ± 6.14	10.6 ± 6.69	26.6 ± 17.18	0.2 ± 0.44	0 ± 0	0.2 ± 0.44	5 ± 7	3 ± 3.74	5.4 ± 7.4
	Poduromorpha	23.2 ± 15.2	20.8 ± 21.13	41 ± 54.39	0.4 ± 0.89	0.8 ± 1.78	0 ± 0	18.8 ± 22.02	5.2 ± 6.14	1.8 ± 3.03
Diptera	Dicellurata	1.2 ± 1.78	1.2 ± 1.3	1.8 ± 1.78	0 ± 0	0 ± 0	0.2 ± 0.44	0.4 ± 0.54	0.2 ± 0.44	0.2 ± 0.44
	Rhabdura	1.6 ± 2.07	1.4 ± 2.6	1 ± 1	0 ± 0	0.2 ± 0.44	0.2 ± 0.44	0.2 ± 0.44	0.8 ± 1.09	0 ± 0
Symphyla		0.4 ± 0.54	0.4 ± 0.89	0.4 ± 0.54	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.44	0 ± 0	0.4 ± 0.89
Paupropoda		12.6 ± 6.58	11 ± 6.7	2.8 ± 3.7	0 ± 0	0 ± 0	0 ± 0	0.8 ± 1.3	2.2 ± 4.38	1.8 ± 2.68
	Mesostigmata	133 ± 206.38	47.8 ± 63.06	30.2 ± 15.31	4.33 ± 5.27	2.66 ± 2.19	6.5 ± 3.57	13 ± 15.81	5.6 ± 7.56	11 ± 14.84
Acari	Ixodida	14.8 ± 13.59	23 ± 16.38	7 ± 7.81	22 ± 18.74	1 ± 0.54	1 ± 7.15	1 ± 4.61	1 ± 3.049	1 ± 2.68
	Oribate	21 ± 40.91	0 ± 0	0 ± 0	5.33 ± 5.63	0.33 ± 0.44	0 ± 0	0 ± 0	1.33 ± 1.78	1 ± 0.89
	Holothyrida	94.6 ± 174.82	72 ± 104.56	14.2 ± 15.38	19.33 ± 19.57	1.33 ± 1.3	0 ± 0	1 ± 1.34	1.33 ± 1.78	2 ± 1.78
	Prostigmata	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 0.89
Insecta	Metastigmata	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2 ± 1.78
	Hemiptera	15.6 ± 16.53	3.2 ± 2.38	1 ± 0.44	2.33 ± 1.67	0.5 ± 0.44	0.5 ± 0.44	0.66 ± 0.54	4 ± 5.36	1.33 ± 1.3
	Diptera	0.8 ± 0.44	2.2 ± 1.92	0.66 ± 0.89	0 ± 0	0 ± 0	1 ± 0.89	2.66 ± 2.3	5.66 ± 3.71	0.33 ± 0.44
	Psocoptera	0.4 ± 0.89	0 ± 0	0 ± 0	0 ± 0	0.5 ± 0.44	0 ± 0	0 ± 0	0.33 ± 0.44	0 ± 0
Arachnida	Opiliones	3 ± 4.24	2.2 ± 1.64	0.4 ± 0.54	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.44	0.6 ± 1.34	4 ± 7.38
	Pseudoscorpionida	0.2 ± 0.44	0 ± 0	0 ± 0	0 ± 0	0.6 ± 0.89	0 ± 0	0 ± 0	0.4 ± 0.54	0 ± 0
Chilopoda		1 ± 1.22	0.8 ± 0.83	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0.6 ± 0.89	0.4 ± 0.89	0.2 ± 0.44
Diplopoda		1.6 ± 1.51	0.6 ± 1.34	0.4 ± 0.54	0 ± 0	0 ± 0	0 ± 0	1.2 ± 2.68	0.8 ± 1.3	1.2 ± 1.78
	Coleoptera	0.4 ± 0.54	0 ± 0	0.66 ± 0.54	0.33 ± 0.44	0 ± 0	0.5 ± 0.44	0.33 ± 0.44	0.66 ± 0.89	0.33 ± 0.44
Insecta	Formicidae	4.2 ± 9.39	0.8 ± 1.78	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Arachnida	Araneae	0.4 ± 0.89	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.4 ± 0.89	0 ± 0
Clitellata	Haplotoxida	0.2 ± 0.44	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2 ± 1.09	1 ± 0.54b	1 ± 0.44
Malacostraca	Isopoda	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.6 ± 1.34	0 ± 0

Notes: P1 – depth 0–10 cm; P2 – depth 10–20 cm; P3 – depth 20–30 cm; BT – before treatment; DT – during treatment; AT – after treatment.

(E), the Berger–Parker dominance index (d) was used to express the proportion of individuals represented by the most abundant species for each period, given by the following formula:

$$d = N_{\max}/N \quad (5),$$

where $N_{\max}$ is the number of individuals of the numerically dominant species.

Species constancy (C), or frequency of occurrence, is the number of samples in which a given species is found relative to the total number of samples. Six classes of constancy were considered: the species is rare if $C \leq 17\%$, incidental if $17\% < C \leq 34\%$, accessory if $34\% < C \leq 51\%$, regular if $51\% < C \leq 68\%$, constant if $68\% < C \leq 85\%$, and omnipresent if $C > 85\%$.

Statistical analysis

We used some statistical methods to identify the changes in the invertebrate communities in relation to the period and depth of sampling.

By using the Shapiro–Wilk and Levene’s tests, we found that the normality of data and homogeneity of variance are violated. Thus, we chose to use the Friedman test (a nonparametric alternative to the analysis of variance [ANOVA]). Assumptions for the Friedman test are all met (continuous data, random sample, group measured on three different occasions). Friedman test was completed by the Wilcoxon rank test. Statistical analyses were performed by using IBM SPSS Version 26.

Results

Overall response of the invertebrate community

Considering the temporal dynamics of the community structure, the soil macro and mesofauna communities in March, May, and August were clearly different from each other. In March, a total of 3354 individuals were counted before the pesticide treatment (BT), including 3295 taxa of mesofauna and 59 taxa of macrofauna. In May, a strong decrease in the mesofauna was recorded with 224 individuals and almost no macrofauna during pesticide application (DT). Then, an increase was observed after the pesticide treatment, where we counted 39 and 480 individuals for the macro and mesofauna, respectively. Among the different invertebrates present in the soil, we recorded for the phylum Arthropoda, the classes Collembola, Diplura, Insecta, Arachnida, Diplopoda, Chilopoda, Symphyla, Pauropoda, Acari, and Malacostraca, and only one class of the phylum Annelida, which is Clitellata. Among the different classes present in the soil, the mites represented 68.24% of the total fauna in the first sampling period (BT), followed by the class Collembola (20.34%). Mesostigmata recorded the highest percentage (31.46%) in the Acari class, and Poduromorpha were the most abundant (12.68%) in the Collembola class (Table 2).

The results of the Friedman test for the effects of sampling periods and soil depth on the abundance of meso and macrofauna (Table 3) showed significant differences between sampling periods ($p = 0.00005$ and $p = 0.0004$ for meso and macrofauna, respectively), but there was no significant difference in the depth ($p = 0.229$ and $p = 0.422$ for meso and macrofauna, respectively).

Table 3. p -Values obtained from Friedman test of the effects of sampling periods and depth on the total abundance of the soil meso and macrofauna communities.

Friedman test	Mesofauna		Macrofauna	
	χ^2	p -Value	χ^2	p -Value
Depth	2.945	0.229	1.724	0.422
Periods	19.600	0.000055*	15.244	<0.05
DT–BT	-	<0.05	-	<0.05
AT–BT	-	<0.05	-	0.276
AT–DT	-	<0.05	-	<0.05

Notes: *Significantly different groups according to the Wilcoxon test ($p < 0.05$). Given are χ^2 estimates and associated nonparametric significances. BT – before treatment; DT – during treatment; AT – after treatment.

Thus, the negative effects on soil fauna are explained by the periods, not by the depth.

In mesofauna community, Wilcoxon test identified three statistically significant different groups (Table 4) related to the sampling months, separating periods of low (DT: 44.8 ± 49.635 individuals), intermediate (AT: 96 ± 85.55), and higher abundance (BT: 659 ± 334.774) (values are presented as mean $\pm$ SD).

In the macrofauna community, Wilcoxon test identified significant differences in the abundance between the community during the treatment period (DT: 0.4 ± 0.89 individuals) and the community before (BT: 11.8 ± 12.07) and after (AT: 7.8 ± 8.01) treatment (values are presented as mean $\pm$ SD).

Concerning the groups’ richness, the lowest richness was observed in the middle of the treatment period (Table 4). Friedman test showed no significant differences for the mesofauna, but there was a significant difference for the macrofauna ($p = 0.029$); thus, March was significantly richer than May ($p = 0.042$).

In addition to higher number of orders and abundance in the two non-treatment periods (BT and AT), the fauna showed a homogeneous structure, verified by higher values of biological diversity (Hc) and equitability (E) and lower Simpson’s values (D). Although the average diversity was higher during this period, these indices did not significantly differ for both communities (macro and mesofauna) (Table 4). Nevertheless, the Hill index of the macro and mesofaunal community varied significantly ($p = 0.029$ and $p = 0.036$, respectively) between March and May. This led to the conclusion that samples collected in March, before pesticide application, had greater diversity of macro and mesofaunal communities (Table 4).

Individual responses of the invertebrate orders

The abundance of most invertebrates was reduced through sampling periods. Entomobryomorpha, Poduromorpha, Mesostigmata, Ixodida, and Hemiptera were the most affected groups of mesofauna and showed markedly reduced number. Moreover, abundances of the orders of the soil macrofauna differed among sampling months. However, the mesofauna community abundance exhibited more pronounced decrease than that of macrofauna and was affected by the sampling periods.

Table 4. Effects of periods (BT, DT, AT) on the total abundance, richness, evenness, and diversity of the soil macro and mesofauna communities.

Treatment	Abundance			Friedman test				
	BT	DT	AT	χ^2	p	DT- BT	AT- BT	AT-DT
Mesofauna								
Shannon's index (H')	2.53 ± 0.59	1.51 ± 1	1.96 ± 1.19	3.26	0.196	-	-	-
E	0.7 ± 0.16	0.6 ± 0.36	0.6 ± 0.33	0.31	0.854	-	-	-
Simpson's d index (D)	0.25 ± 0.12	0.46 ± 0.32	0.39 ± 0.34	2.21	0.331	-	-	-
Hill	0.34 ± 0.04	0.61 ± 0.23	0.49 ± 0.29	6.63	0.036*	0.043*	0.225	0.068
Richness (S)	12.4 ± 0.54	5.4 ± 3.28	8.4 ± 5.31	5.44	0.066	-	-	-
Macrofauna								
Shannon's index (H')	1.44 ± 0.29	0 ± 0	1.04 ± 0.96	5.77	0.056			
E	0.88 ± 0.14	0 ± 0	0.55 ± 0.5	5.33	0.069			
Simpson's index (D)	0.21 ± 0.21	1 ± 0	0.55 ± 0.4	5.77	0.056			
Hill	0.48 ± 0.21	1 ± 0	0.81 ± 0.18	7.11	0.029*	0.043*	0.080	0.109
Richness (S)	3.4 ± 1.14	0.2 ± 0.44	2.2 ± 2.04	7.11	0.029	0.042*	0.131	0.102

Notes: Abundance, richness, and diversity data are averaged across sampling events. Values are means ± SD. Given are χ^2 estimates and associated nonparametric significances of Friedman test. In the case of abundance and richness, * indicates significantly different groups according to the Wilcoxon test ($p < 0.05$). BT – before treatment; DT – during treatment; AT – after treatment.

Table 5. Collembola species abundance found in the experimental location (mean ± SD), constancy values (C), and their corresponding categories.

Species	BT			DT			AT		
	Mean abundance (±SD)	C (%)	Category	Mean abundance (±SD)	C (%)	Category	Mean abundance (±SD)	C (%)	Category
<i>Onychiuridae</i> sp.	49.4 ± 50.78	80.00	Constant	1.2 ± 1.78	13.33	Rare	18.2 ± 12.73	60.00	Regular
<i>Protaphorura armata</i>	34.6 ± 27.28	80.00	Constant	0 ± 0	-	-	7.2 ± 9.85	13.33	Rare
<i>Ceratophysella armata</i>	1 ± 0.7	26.67	Accidental	0 ± 0	-	-	0.4 ± 0.89	6.67	Rare
<i>Cryptopygus thermophilus</i>	0.4 ± 0.54	13.33	Rare	0.2 ± 0.44	6.67	Rare	0 ± 0	-	-
<i>Folsomia quadrioculata</i>	5.2 ± 4.54	46.67	Accessory	0 ± 0	-	-	4.2 ± 5.76	20.00	Accidental
<i>Isotomiella minor</i>	43 ± 20.96	100	Omnipresent	0.2 ± 0.44	6.67	Rare	9 ± 12.44	46.67	Accessory
<i>Proisotoma minuta</i>	0.8 ± 1.3	13.33	Rare	0 ± 0	-	-	0.2 ± 0.44	6.67	Rare
<i>Lepidocyrtus cyaneus</i>	1.4 ± 2.07	20.00	Accidental	0 ± 0	-	-	0 ± 0	-	-
<i>Entomobrya</i> sp.	0.6 ± 0.54	20.00	Accidental	0 ± 0	-	-	0 ± 0	-	-

Notes: BT – before treatment; DT – during treatment; AT – after treatment.

Overall response of the springtail community

In total, 886 individuals and only nine species of springtails (Table 5) were sampled and identified over the three study periods

in the vineyard. An overall effect on springtails was observed; the composition of the soil springtail community differed significantly among the three sampling periods. Friedman test (Table 6) clearly showed a negative effect of sampling periods ($p =$

8.781E-06) on the abundances of springtails in the sampled soils. In contrast, the depth did not affect the overall springtail community ($p = 0.368$).

The six constancy classes considered were observed (Table 5). Five constancy classes were identified before the treatment (BT). Regular species were absent; only one species (*Isotomiella minor*) was omnipresent with $C = 100\%$ and two species were constant (*Onychiuridae* sp. and *Protaphorura armata*). Four classes of constancy were also identified after the treatment (AT). However, during this period, no springtail species was identified as omnipresent or constant. The three species identified in March (DT) were rare: *Onychiuridae* sp. (13.33%), *Cryptopygus thermophilus*, and *Folsomia quadrioculata* (6.67% for each species).

The soil springtail community showed significantly higher diversity in March than May ($p = 0.043$). In addition to the high species diversity in March, we highlight a high uniformity of community structure, supported significantly ($p = 0.043$) by higher equitability (E) (Table 7). Richness differed significantly among the three periods ($p = 0.008$). The highest values were observed in March (before the pesticide treatment) and the lowest in May (during treatment). Significant differences in the Berger-Parker Index were also observed for collembolan community ($p = 0.036$). This index shows that the relative preponderance of the most dominant morphospecies (*Onychiuridae* sp.) was higher in the posttreatment period. The lowest value for this index (highest diversity) was observed before the start of the treatment (Table 7).

Individual responses of springtail taxa

Among the springtail species inventoried during the first, second, and third experimental periods, four species had a frequency of occurrence higher than 40% during the pretreatment period: *Onychiuridae* sp., *Isotomiella minor*, and *Protaphorura armata*, contributing 36.21, 31.52, and 25.36% of the total Collembola abundance, respectively. These three species represented more than 93% of all soil springtails caught and *Folsomia quadrioculata* showed an abundance of 3.81%.

The curves in Figure 1 have a hyperbolic shape, which shows a strong inequality among species. The most abundant species (more than 50 individuals per species) were very few. The rare species were numerous. This series of diagrams shows that

Onychiuridae sp. and *Isotomiella minor* were the most abundant species during all three periods, occupying the first and second positions, respectively. *Protaphorura armata* occupied the third position during the BT and AT periods, while during the DT period, *Cryptopygus thermophilus* occupied this position.

Discussion

Temporal patterns of macrofaunal and mesofaunal abundance and diversity vary by time period. Previous studies have found that temperature and precipitation are the critical factors determining soil faunal abundance (Irmeler, 2006). Thus, the combination of high temperature and lack of precipitation significantly reduces the abundance of soil invertebrate communities (Wu, Wang, 2019). At the same time, in our study, faunal abundance and diversity were lowest in May than in August (warmer and drier months). This result reflects the fact that pesticide treatment can be a mediator of the changes in soil macro and mesofaunal communities.

We found a significant effect of the periods on soil invertebrates, although the interaction of periods and pesticide treatments showed a clearer trend on soil meso and macrofauna. For example, higher abundance and diversity in summer compared to spring depended on the presence of pesticide. Managing vine-

Table 6. p -Values obtained from Friedman test of the effects of sampling periods and depth on the total abundance of Collembola community.

Friedman test	Total abundance	
	χ^2	p -Value
Depth	2.000	0.368
Periods	23.286	8.78E-06*
DT–BT		0.001*
AT–BT		0.008*
AT–DT		0.008*

Notes: *Significant differences ($p < 0.05$). Given are χ^2 estimates and associated no-parametric significances. BT – before treatment; DT – during treatment; AT – after treatment.

Table 7. Effects of sampling periods (BT, DT, AT) on the total abundance, richness, evenness, and diversity of the Collembola community.

Treatment	H'		E		d		S	
BT	0.67 ± 0.11		1.74 ± 0.31		0.5 ± 0.1		6.2 ± 1.3	
DT	0 ± 0		0 ± 0		1 ± 0		0.6 ± 0.54	
AT	0.63 ± 0.41		1.03 ± 0.86		0.61 ± 0.32		2.6 ± 1.94	
Friedman test	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Periods	6.42	0.040*	7.68	0.021*	6.631	0.036*	9.57	0.008*
DT–BT		0.043*		0.043*		0.043*		0.041*
AT–BT		0.686		0.138		0.345		0.042*
AT–DT		0.068		0.068		0.068		0.066

Notes: *Significant differences ($p < 0.05$). Abundance, richness, and diversity data are averaged across sampling events. Values are means $\pm$ SD. Given are χ^2 estimates and associated nonparametric significances of Friedman test. BT – before treatment; DT – during treatment; AT – after treatment.

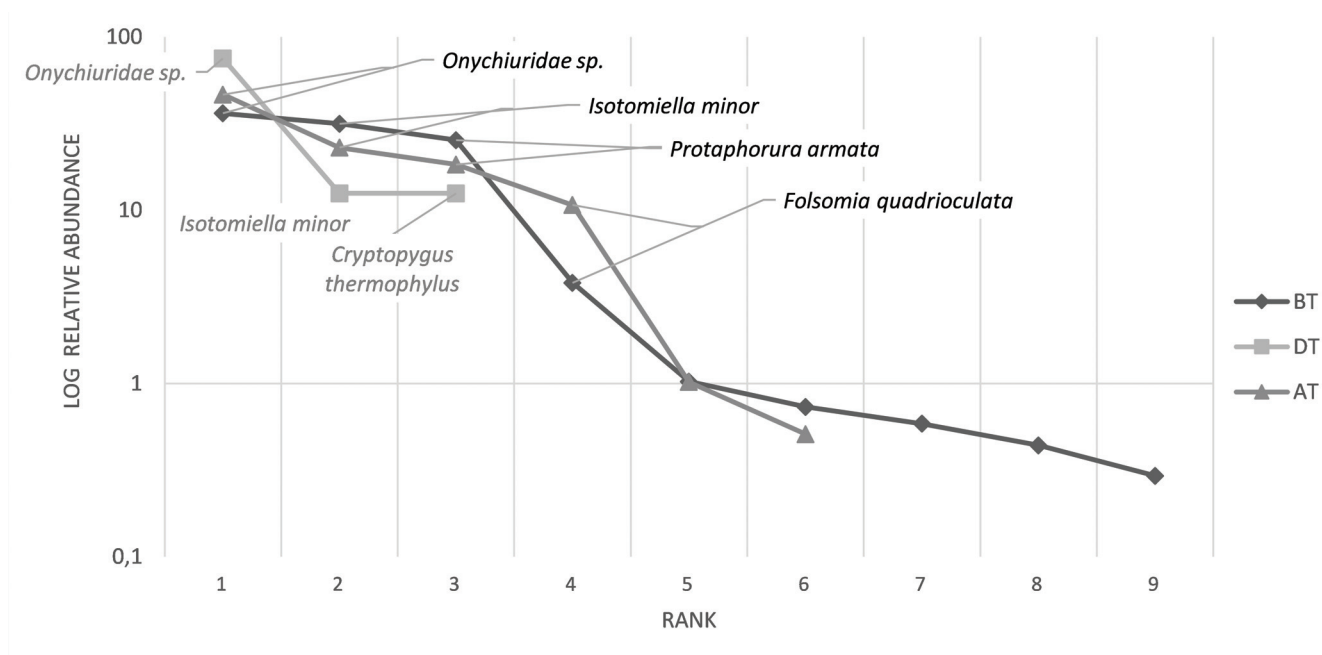


Fig. 1. Rank–abundance curves in the log of faunal diversity of the fauna observed in the three treatments.

yard soils is known to largely affect soil fauna: chemicals (pesticides) and tillage can seriously reduce soil invertebrate populations (El Titi, 2003; Paoletti, 1999).

This suggests that soil fauna of the vineyard was disturbed by the agronomic practices adopted (pesticides and tillage) and/or by the climatic factors. Presumably, pesticide addition (especially fungicides) to vineyard soils resulted in a consistently lower diversity/abundance of meso and macrofauna, as found in earlier studies. Several studies (Bart et al., 2019; Booth et al., 2003; De Silva et al., 2009; Vogelweith, Thiéry, 2018) have shown that fungicides are potentially dangerous for earthworm reproduction, thus explaining the near absence of this taxon in our samples. Pesticides affect the abundance of several arthropod species, especially in vineyard soils (Pennington et al., 2018).

As for microarthropods, their responses to fungicide application vary considerably. For example, Gan and Wickings (2017) reported that monthly applications of chlorothalonil (fungicide) at a medium preventive (MP) dose significantly reduced overall microarthropod abundance.-

The results show that viticultural soil management practices (pesticides, tillage) can strongly influence the temporal dynamics of soil fauna, in agreement with other studies (Burgio et al., 2016; Pryke et al., 2013; Sáenz-Romo et al., 2019). Sharley et al. (2008) found that tillage in vineyards significantly reduces the population of several taxa. Indeed, tillage is known to have a detrimental effect on functional diversity (Fiera et al., 2020b; Sánchez-Moreno et al., 2015). Tillage can affect soil arthropods in different ways. For example, they can be directly affected by being killed by mechanical damage or burial and indirectly through habitat disturbance or deterioration that leads to reduced densities in vineyards (Sharley et al., 2008) and other crops (Thorbeck et al., 2017). However, further experiments are needed to explore the mechanisms responsible for changes in fauna.

This work shows that springtails are less susceptible to the negative effect of periods. Previous work has shown that springtails can exhibit large population sizes despite pesticide application and high management intensity (Fiera et al., 2020b; Framp-ton, van den Brink, 2007). Thus, these arthropods may be less affected by agricultural practices than other soil organisms such as earthworms or epigeous predators (Faber et al., 2017; Holland et al., 1994). This is explained by a greater negative effect on Collembola predators than on the Collembola themselves, leading to a classical resurgence and rapid recolonization after the end of treatments.

The current study shows that monitoring the total abundance of springtails in the field does not detect species-specific effects: the rarest taxa were not affected by the variation of sampling month. The present research highlights the strong influence of periods and intensive vineyard management on the composition of springtail communities: the number of individuals for both species *Protaphorura armata* and *Isotomiella minor* was higher in March than in May and August. In line with our hypothesis, pesticide addition, particularly after application, tended to affect the abundance and/or composition of Collembola. Therefore, these results are in line with previous conclusions that targeted monitoring of species that are representative, easily identified, and sensitive to chemical treatments may be more appropriate. These results suggest that these two species (*Protaphorura armata* and *Isotomiella minor*) are potential candidates as indicators for identifying the risks of pesticides to soil invertebrates and should be routinely tested as representatives of soil arthropods.

It should be noted that these species have lower densities during the posttreatment period, because they are directly affected by the addition of pesticides, especially insecticides, as their morphological and physiological characteristics are similar to those of the Insecta (Peck, 2009; Zortéa et al., 2018). Studies

have shown the high toxicity of organophosphates to springtails (Natal-da-Luz et al., 2012; Santos et al., 2012), which could explain the significant reduction in numbers of some species after pesticide application. Other studies in vineyards have shown that mechanical disturbance through frequent tillage is not always harmful to springtails (Buchholz et al., 2017; Pfingstmann et al., 2019). Indeed, springtails seem to be more sensitive to the intensity of pesticide management than to tillage.

Considering the effects of month and pesticide on community structure, abundance, and diversity of soil fauna, we found that pesticides have a strong effect on the soil meso and macrofauna communities. As a result, our hypothesis was supported. The results suggest more attention should be focused on the pesticide treatments in different seasons when monitoring soil fauna diversity.

However, more detailed studies with more sampling dates per season are needed to clarify the underlying processes. It is also important to note that methods for calculating an ecomorphological index show that edaphic species may be more sensitive to various factors. For example, above-ground active springtail communities are probably more exposed to pesticide treatments than active soil species. In addition, species with a low dispersal capacity are expected to take longer to recover.

Conclusion

This study explored how soil meso and macrofauna vary among different periods of sampling in the context of pesticide application. It is relevant to note from the experimental results that the richness of 11 classes of invertebrates consisting of 24 orders was reduced following sampling periods. Our results suggest that vineyard treatments with pesticides (mainly fungicides) can restructure the soil community by affecting the abundance and diversity of non-target organisms; the most important effects occur directly after application. These treatment effects, however, vary between species, which was confirmed by springtails in this study. The latter showed clear composition, species richness, abundance, and diversity responses, thus estimating pesticide risks. However, whether these changes lead to a definitive decline in numbers remains speculative and requires further research. Field studies and experiments under controlled conditions are needed to disentangle the effects of environmental variables and fungicide toxicity on the viticultural soil faunal community.

References

- Arias-Estévez, M., López-Periago, E., Martínez-Carballo, E., Simal-Gándara, J., Mejuto, J. C. & García-Río L. (2008). The mobility and degradation of pesticides in soils and the pollution of groundwater resources. *Agric. Ecosyst. Environ.*, 123(4), 247–260. DOI: 10.1016/j.agee.2007.07.011.
- Bart, S., Barraud, A., Amossé, J., Péry, A. R. R., Mougin, C. & Pelosi C. (2019). Effects of two common fungicides on the reproduction of *Aporrectodea caliginosa* in natural soil. *Ecotoxicol. Environ. Saf.*, 181(1), 518–524. DOI: 10.1016/j.ecoenv.2019.06.049.
- Bogýó, D., Magura, T., Nagy, D.D. & Tóthmérész B. (2015). Distribution of millipedes (Myriapoda, diplopoda) along a forest interior – forest edge – grassland habitat complex. *Zookeys*, 510, 181–195. DOI: 10.3897/zookeys.510.8657.
- Booth, L.H., Bithell, S.L., Wratten, S.D. & Heppelthwaite V.J. (2003). Vineyard pesticides and their effects on invertebrate biomarkers and bioindicator species in New Zealand. *Bull. Environ. Contam. Toxicol.*, 71(6), 1131–1138. DOI: 10.1007/s00128-003-8879-9.
- Brygadyrenko, V. (2016). Effect of canopy density on litter invertebrate community structure in pine forests. *Ekológia (Bratislava)*, 35(1), 90–102. DOI: 10.1515/eko-2016-0007.
- Buchholz, J., Querner, P., Paredes, D., Bauer, T., Strauss, P., Guernion, M., Scimia, J., Cluzeau, D., Burel, F., Kratschmer, S., Winter, S., Potthoff, M. & Zaller J.G. (2017). Soil biota in vineyards are more influenced by plants and soil quality than by tillage intensity or the surrounding landscape. *Scientific Reports*, 7(1), 1–12. DOI: 10.1038/s41598-017-17601-w.
- Burgio, G., Marchesini, E., Reggiani, N., Montepaone, G., Schiatti, P. & Sommaggio D. (2016). Habitat management of organic vineyard in Northern Italy: The role of cover plants management on arthropod functional biodiversity. *Bull. Entomol. Res.*, 106(6), 759–768. DOI: 10.1017/S0007485316000493.
- Cortet, J., Joffre, R., Elmholt, S. & Krogh P.H. (2003). Increasing species and trophic diversity of mesofauna affects fungal biomass, mesofauna community structure and organic matter decomposition processes. *Biol. Fertil. Soils*, 37(5), 302–312. DOI: 10.1007/s00374-003-0597-2.
- DAS (2019). *Directorate of agricultural services*. Tizi-Ouzou: DAS National Agricultural Statistics Services.
- David, J.F., Devernay, S., Loucougaray, G. & Le Floch E. (1999). Belowground biodiversity in a Mediterranean landscape: relationships between saprophagous macroarthropod communities and vegetation structure. *Biodivers. Conserv.*, 8, 753–767. DOI: 10.1023/A:1008842313832.
- De Silva, P.M.C.S., Pathiratne, A. & van Gestel C.A.M. (2009). Influence of temperature and soil type on the toxicity of three pesticides to *Eisenia Andrei*. *Chemosphere*, 76(10), 1410–1415. DOI: 10.1016/j.chemosphere.2009.06.006.
- EFSA (2007). Opinion of the Scientific Panel on Plant protection products and their residues (PPR) related to the revision of Annexes II and III to Council Directive 91/414/EEC concerning the placing of plant protection products on the market - Fate and Behaviour in t. *EFSA Journal*, 5(2), 1–44. DOI: 10.2903/j.efsa.2007.448.
- El Titi, A. (2003). Non-inversion tillage in integrated farming concepts: prospects and constraints of cropping systems in the southwest of Germany. In L. Garcia-Torres, J. Benites, A. Martinez-Vilela & A. Holgado-Cabrera (Eds.), *Conservation agriculture* (pp. 211–219). Dordrecht: Springer. DOI: 10.1007/978-94-017-1143-2_26.
- Faber, F., Wachter, E. & Zaller J.G. (2017). Earthworms are little affected by reduced soil tillage methods in vineyards. *Plant, Soil and Environment*, 63(6), 257–263. DOI: 10.17221/160/2017-PSE.
- Fiera, C., Ulrich, W., Popescu, D., Buchholz, J., Querner, P., Bunea, C.I., Strauss, P., Bauer, T., Kratschmer, S., Winter, S. & Zaller J. G. (2020a). Tillage intensity and herbicide application influence surface-active springtail (Collembola) communities in Romanian vineyards. *Agric. Ecosyst. Environ.*, 300, 107006. DOI: 10.1016/j.agee.2020.107006.
- Fiera, C., Ulrich, W., Popescu, D., Bunea, C.I., Manu, M., Nae, I., Stan, M., Markó, B., Urák, I., Giurginca, A., Penke, N., Winter, S., Kratschmer, S., Buchholz, J., Querner, P. & Zaller J.G. (2020b). Effects of vineyard inter-row management on the diversity and abundance of plants and surface-dwelling invertebrates in Central Romania. *J. Insect Conserv.*, 24(1), 175–185. DOI: 10.1007/s10841-019-00215-0.
- Frampton, G.K. & van den Brink P.J. (2007). Collembola and macroarthropod community responses to carbamate, organophosphate and synthetic pyrethroid insecticides: Direct and indirect effects. *Environ. Pollut.*, 147(1), 14–25. DOI: 10.1016/j.envpol.2006.08.038.
- Frouz, J., Livečková, M., Albrechtová, J., Chroňáková, A., Cajthaml, T., Pižl, V., Háněl, L., Starý, J., Baldrian, P., Lhotáková, Z., Šimáčková, H. & Cepáková Š. (2013). Is the effect of trees on soil properties mediated by soil fauna? A case study from post-mining sites. *For. Ecol. Manag.*, 309, 87–95. DOI: 10.1016/j.foreco.2013.02.013.
- Gan, H. & Wickings K. (2017). Soil ecological responses to pest management in golf turf vary with management intensity, pesticide identity, and application program. *Agric. Ecosyst. Environ.*, 246, 66–77. DOI: 10.1016/j.agee.2017.05.014.
- Ghosal, A., Hati, A., Mal, S., Mukherjee, A. & Mukherjee A. (2018). Impact of some new generation insecticides on beneficial rhizospheric microorganisms in rice maize cropping system. *International Journal of Current Microbiology and Applied Sciences*, 7(05), 666–676. DOI: 10.20546/ijcmas.2018.705.081.

- Harta, I., Simon, B., Vinogradov, S. & Winkler D. (2020). Collembola communities and soil conditions in forest plantations established in an intensively managed agricultural area. *J. For. Res.*, 32, 1819–1832. DOI: 10.1007/s11676-020-01238-z.
- Holland, J. M., Frampton, G. K., Çilgi, T. & Wratten S.D. (1994). Arable acronyms analysed – a review of integrated arable farming systems research in Western Europe. *Ann. Appl. Biol.*, 125(2), 399–438. DOI: 10.1111/j.1744-7348.1994.tb04980.x.
- Irmeler, U. (2006). Climatic and litter fall effects on collembolan and oribatid mite species and communities in a beech wood based on a 7 years investigation. *Eur. J. Soil Biol.*, 42 (1), 51–62. DOI: 10.1016/j.ejsobi.2005.09.016.
- Marichal, R., Praxedes, C., Decaëns, T., Grimaldi, M., Oszwald, J., Brown, G. G., Desjardins, T., da Silva, M. L., Feijoo Martinez, A., Oliveira, M. N. D., Velasquez, E. & Lavelle P. (2017). Earthworm functional traits, landscape degradation and ecosystem services in the Brazilian Amazon deforestation arc. *Eur. J. Soil Biol.*, 83, 43–51. DOI: 10.1016/j.ejsobi.2017.09.003.
- McCravy, K. (2018). A review of sampling and monitoring methods for beneficial arthropods in agroecosystems. *Insects*, 9(4), 170. DOI: 10.3390/insects9040170.
- Natal-da-Luz, T., Moreira-Santos, M., Ruepert, C., Castillo, L.E., Ribeiro, R. & Sousa J.P. (2012). Ecotoxicological characterization of a tropical soil after diazinon spraying. *Ecotoxicology*, 21(8), 2163–2176. DOI: 10.1007/s10646-012-0970-8.
- Oerke, E. (2006). Crop losses to pests. *J. Agric. Sci.*, 144(1), 31–43. DOI: 10.1017/S0021859605005708.
- Paoletti, M.G. (1999). The role of earthworms for assessment of sustainability and as bioindicators. *Agric. Ecosyst. Environ.*, 74(1–3), 137–155. DOI: 10.1016/S0167-8809(99)00034-1.
- Parisi, V., Menta, C., Gardi, C., Jacomini, C. & Mozzanica E. (2005). Microarthropod communities as a tool to assess soil quality and biodiversity: a new approach in Italy. *Agric. Ecosyst. Environ.*, 105(1–2), 323–333. DOI: 10.1016/j.agee.2004.02.002.
- Peck, D.C. (2009). Long-term effects of imidacloprid on the abundance of surface- and soil-active non-target fauna in turf. *Agric. For. Entomol.*, 11(4), 405–419. DOI: 10.1111/j.1461-9563.2009.00454.x.
- Pelosi, C., Barot, S., Capowiez, Y., Hedde, M. & Vandenbulcke F. (2014). Pesticides and earthworms. A review. *Agronomy Sustainable Development*, 34(1), 199–228. DOI: 10.1007/s13593-013-0151-z.
- Pennington, T., Reiff, J. M., Theiss, K., Entling, M.H. & Hoffmann C. (2018). Reduced fungicide applications improve insect pest control in grapevine. *BioControl*, 63(5), 687–695. DOI: 10.1007/s10526-018-9896-2.
- Petersen, H. (2002). General aspects of collembolan ecology at the turn of the millennium Proceedings of the Xth international Colloquium on Apterygota, České Budějovice 2000: Apterygota at the Beginning of the Third Millennium. *Pedobiologia*, 46(3–4), 246–260. DOI: 10.1016/s0031-4056(04)70140-7.
- Pfingstmann, A., Paredes, D., Buchholz, J., Querner, P., Bauer, T., Strauss, P., Kratschmer, S., Winter, S. & Zaller J. (2019). Contrasting effects of tillage and landscape structure on spiders and springtails in vineyards. *Sustainability (Switzerland)*, 11(7), 1–14. DOI: 10.3390/su1102095.
- Pryke, J.S., Roets, F. & Samways M.J. (2013). Importance of habitat heterogeneity in remnant patches for conserving dung beetles. *Biodivers Conserv.*, 22(12), 2857–2873. DOI: 10.1007/s10531-013-0559-4.
- Rosell, G., Quero, C., Coll, J. & Guerrero A. (2008). Biorational insecticides in pest management. *J. Pestic. Sci.*, 33(2), 103–121. DOI: 10.1584/jpestics.R08-01.
- Santos, M.J.G., Ferreira, M.F.L., Cachada, A., Duarte, A.C. & Sousa J.P. (2012). Pesticide application to agricultural fields: Effects on the reproduction and avoidance behaviour of *Folsomia candida* and *Eisenia andrei*. *Ecotoxicology*, 21(8), 2113–2122. DOI: 10.1007/s10646-012-0963-7.
- Sáenz-Romo, M.G., Veas-Bernal, A., Martínez-García, H., Campos-Herrera, R., Ibáñez-Pascual, S., Martínez-Villar, E., Pérez-Moreno, I. & Marco-Mancebón V.S. (2019). Ground cover management in a Mediterranean vineyard: Impact on insect abundance and diversity. *Agric. Ecosyst. Environ.*, 283, 106571. DOI: 10.1016/j.agee.2019.106571.
- Sánchez-Moreno, S., Castro, J., Alonso-Prados, E., Alonso-Prados, J. L., García-Baudín, J. M., Talavera, M. & Durán-Zuazo V.H. (2015). Tillage and herbicide decrease soil biodiversity in olive orchards. *Agronomy Sustainable Development*, 35(2), 691–700. DOI: 10.1007/s13593-014-0266-x.
- Seres, A., Posta, K., Bakonyi, G., Nagy, P., Kiss, I., Fábrián, M., Répási, V. & Nosek J.N. (2009). Collembola decrease the nitrogen uptake of maize through arbuscular mycorrhiza. *Ekológia (Bratislava)*, 28(3), 242–247. DOI: 10.4149/ekol_2009_03_242.
- Sharley, D.J., Hoffmann, A.A. & Thomson L.J. (2008). The effects of soil tillage on beneficial invertebrates within the vineyard. *Agricultural and Forest Entomology*, 10(3), 233–243. DOI: 10.1111/j.1461-9563.2008.00376.x.
- Thorbek, A.P., Bilde, T., Thorbek, P. & Bildett T. (2017). Reduced numbers of generalist arthropod predators after crop management. *J. Appl. Ecol.*, 41(3), 526–538. DOI: 10.1111/j.0021-8901.2004.00913.x.
- Vogelweith, F. & Thiéry D. (2018). An assessment of the non-target effects of copper on the leaf arthropod community in a vineyard. *Biol. Control.*, 127, 94–100. DOI: 10.1016/j.biocontrol.2018.08.011.
- Wu, P. & Wang C. (2019). Differences in spatiotemporal dynamics between soil macrofauna and mesofauna communities in forest ecosystems: the significance for soil fauna diversity monitoring. *Geoderma*, 337, 266–272. DOI: 10.1016/j.geoderma.2018.09.031.
- Zortéa, T., dos Reis, T.R., Serafini, S., de Sousa, J.P., da Silva, A.S. & Baretta D. (2018). Ecotoxicological effect of fipronil and its metabolites on *Folsomia candida* in tropical soils. *Environ. Toxicol. Pharmacol.*, 62, 203–209. DOI: 10.1016/j.etap.2018.07.011.