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Research paper Pesticide use and soil disturbance shape springtail communities in vineyards

Pierre Blondel^{a,*}, Benjamin Joubard^{a,b}, Adrien Rusch^a, Brice Giffard^{a,b}

^a INRAE, Bordeaux Sciences Agro, ISVV, UMR 1065, Santé et Agroécologie du Vignoble, Villenave d'Ornon, France ^b Bordeaux Sciences Agro, INRAE, UMR 1065, Santé et Agroécologie du Vignoble, F-33170 Gradignan, France

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ABSTRACT

Farming practices are known to affect soil fauna, which are essential for soil functioning. However, we lack quantitative assessment of the effect of several key farming practices, such as pesticide use or soil disturbance on several important soil taxa. In perennial crops such as vineyards, soil tillage and pesticide use are very intensive and may have major impacts on soil fauna. However, studies on such systems remain scarce. The aim of this study is to assess the response of springtail communities to soil management and pesticide use, while considering key physico-chemical parameters on 32 organic and conventional vineyards located in the southwest France. Our analyses revealed that soil organic matter and soil tillage had a positive impact on functional and taxonomic diversities of springtails. In addition, we found that the intensity of pesticide use and the diversity of active ingredients in particular, decreased the diversity of springtail communities. Surprisingly, soil copper concentration had no effect on abundance or diversity of springtail communities. Our study suggests that superficial tillage and less intensive pesticide applications can favor taxonomic and functional diversity of springtails, independently of certification schemes. Future studies should now investigate how these changes in community composition and diversity affect soil functioning.

1. Introduction

Worldwide vineyards are mostly concentrated in very homogeneous and conventionally managed landscapes, with major detrimental consequences on biodiversity and the environment (Andow, 1983; Matson et al., 1997; Nicholls et al., 2008; Ostandie et al., 2022). Harnessing biodiversity through nature-based solutions, such as increasing plant or habitat diversity across scales, appears as a promising way to build sustainable and resilient vinevard landscapes (Giffard et al., 2022; Paiola et al., 2020; Winter et al., 2018). Soil quality is of great importance in viticulture since the terroir concept strongly relates wine production to soil conditions (Giffard et al., 2022). Soil biodiversity is therefore essential for the functioning of vineyard socio-ecosystems through its key role in multiple ecosystem functions, such as the recycling of organic matter, nutrient availability and water infiltration (Giller, 1996; Matson et al., 1997; Wardle et al., 2004) which strongly influence plant growth and crop productivity (Wardle et al., 2004). However, we presently lack information about the status of soil quality in most vineyards across the globe and we have a limited understanding about how changes in farming practices affect soil biodiversity and

functioning.

Intensive soil management and pesticide use are known to deplete soil biodiversity and functioning in vineyards as in most agricultural systems (Beaumelle et al., 2023a; Giffard et al., 2022; Winter et al., 2018). Soil tillage and mowing have been found to positively affect springtail density (total number of individuals.m⁻²), while it appeared to be detrimental to epedaphic springtails (Buchholz et al., 2017). Extensive management of vegetation cover within no-tilled inter-rows is globally known to benefit biodiversity and ecosystem services (Nicholls et al., 2008; Rusch et al., 2016; Winter et al., 2018). A high frequency of pesticide applications is usually recorded in vineyards due to multiple pathogens and insect pests and with major potential consequences on biodiversity. For instance, the mean treatment frequency index in France, an indicator showing the number of doses of plant protection products applied per hectare (calculated by dividing the applied dose by the legal reference dose) was c.a. 15.3 in France, and 17.2 in Bordeaux, our study region (Simonovici, 2019). However, a majority of the documented impacts of pesticides on soil biodiversity have been observed in controlled experiments, focusing on a particular pesticide and limited number of taxa. Documentation of the consequences of multiple

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^{*} Corresponding author. E-mail address: pierre.blondel@inrae.fr (P. Blondel).

pesticide applications in farmers' fields remains scarce, and particularly for soil biodiversity (Beaumelle et al., 2023b). In addition, there is a growing concern regarding copper-based fungicides, widely used in both organic and conventional farming systems. To date, on-field quantification of the effects of copper-based fungicides on soil biodiversity remains rare (Karimi et al., 2021). Many studies concerning risk assessment of copper are done in a laboratory with copper salt, which favors the increase of copper availability through its effect on pH (Vázquez-Blanco et al., 2020). Moreover, the impact of both pesticide use and soil disturbance on belowground fauna is relatively unexplored. Very few studies compared different management treatments, such as organic and conventional fields, and showed contrasting responses depending on the taxa studied (Buchholz et al., 2017; Coll et al., 2011; Fiera et al., 2020; Ostandie et al., 2021; Pfingstmann et al., 2019). For example, Chang et al. (2013) observed the combined effects of ploughing and herbicide application on springtails but in an annual cropping system.

Studying taxa that are key soil indicators such as earthworms (Fusaro et al., 2018; Pelosi et al., 2014) or springtails (Gruss et al., 2019) has recently gained interest. The springtail community has been useful for studying soil quality (Roembke et al., 2006; Rusek, 1998) as they are present in most terrestrial ecosystems, are highly sensitive to environmental changes, and carry information about key ecological processes as decomposers, involved in organic matter cycling or regulation of plant-pathogenic fungi (Roembke et al., 2006; Sabatini and Innocenti, 2001; Yin et al., 2019). Using community functional metrics of soil taxa should provide a mechanistic understanding of the consequences of agricultural practices on soil functioning. However, such study has rarely been conducted on springtail communities and in agricultural systems (Joimel et al., 2021).

In the present study, we explored relative effects of soil management and pesticide use on springtail communities in organically and conventionally managed vineyards. We hypothesized that: (1) extensive vegetation management within fields as well as lower pesticide use will enhance functional and taxonomic diversity as grassy inter-rows could act as refuge for species and a source of feeding activity through higher organic matter content (Abad et al., 2021); (2) These effects will be more detrimental in the peak of use of pesticides and tillage treatments during the vine growth season as pesticides have negative effects on biodiversity (Beaumelle et al., 2023b) but intensity of applications could exacerbate these impacts (3) Soil conditions may dampen the negative effects of practices.

2. Materials and methods

2.1. Study area and vineyard characteristics

The study took place in 2021 in the long-term living lab BACCHUS located in vineyard-dominated landscapes in the southwest of France, Nouvelle-Aquitaine Region (Muneret et al., 2019; Ostandie et al., 2021). The study design consisted in a selection of sixteen pairs of two adjacent vineyards, one conventionally and one organically managed. All these vineyards differed in the inter-row management practices (herbaceous cover in all inter-rows, all inter-rows tilled, or alternating grassy and tilled inter-rows). Weed management under the rows was carried out mechanically (17/32 vines rows), using herbicides or a combination of herbicides and tillage.

We collected information about the number, type and quantity of pesticides applications in 2020 (the year before the beginning of our soil sampling), through interviews with winegrowers. Most vineyards used no or low herbicides hence we did not include these in our analysis. Most of the phytosanitary treatments in Bordeaux vineyards are fungicides (against downy and powdery mildew) and insecticides (against tortricid larvae and leafhoppers). Fungicides include sulfur-based and copperbased applications and other active synthetic substances (i.e. sulfur, metiram, difenoconazole, tetraconazole). Copper soil content related to its past use in vineyards was also analyzed separately (see below). TFI (Treatment Frequency Index) was calculated for each vineyard (total, and separately for fungicides and insecticides) using the following eq. (1):

$$TFI = \frac{\text{total amounts of active ingredients}}{\text{standard doses assigned to each use of the active ingredients}}$$
(1)

We also collected the intensity of soil disturbance in vine rows and tilled inter-rows as the number of tillage passes. For the 32 vineyards, we then computed 7 variables (with abbreviations used) in order to characterize the intensity of pesticide use and mechanical disturbance: total TFI (TFI_{total}), insecticide TFI (TFI_i), fungicide TFI (TFI_f), number of pesticide products used (nb.pp), management treatment (row: 3 different modalities with grassy inter-row, tilled inter-row and row), soil disturbance (Dis: 0 for no soil disturbance, 1 in case of mechanical soil disturbance), and intensity of soil disturbance (nb.w: number of tillage in rows and tilled inter-rows). The minimum age under viticulture (MAUV) which provides information about land-use history of each sampled field was calculated through aerial photographs and the maps from the Etat-Major (between 1820 and 1866). By default, we considered 156 years the maximum MAUV value (11/32 vineyards) when vinevards were already present in the maps of the Etat-Major. MAUV range between 18 and 156 years with a mean value of 87 years.

2.2. Soil sampling

In spring 2021, different sample locations were determined depending on the modalities of soil tillage management. Two or three samples per vineyard were analyzed based on whether the inter-row management was alternating or not. One soil sample for each management method was composed from 3 subsamples spaced 10 m apart (first subsample is 15 m distant from the row border) and taken on the first ten centimeters of soil. Each sample was then sieved at 5 mm stainless steel mesh and sent to a laboratory (AUREA AgroSciences) to obtain 4 indicator variables: the amount of total copper Cu (NF EN 13346) was extracted with aqua regia and analyzed via Inductively Coupled Plasma (ICP). Organic matter content (OM) and organic carbon content (OC) (NF ISO 14235) was obtained using sulphochromic oxidation of carbon followed by colorimetric dosage and the pH_{water} (NF ISO 10390) with glass electrode.

For springtails, three sampling sessions were conducted in 2021. First session "S1" was in early spring before the first mechanical and pesticide treatments (March 31th and April 2nd of 2021). The second "S2" in late spring during the period of pesticide use and soil management and before summer and hydric stress (May 4th and 5th), and the last "S3" was set in fall after harvest (October 26th and 27th). These sampling sessions were conducted in order to sample springtail community all along the seasonal growth of vine, from budburst until harvest. On each session, 3 subsamples, distant of 10 m, were collected on the vine row and adjacent inter-rows of different management (herbaceous cover and/or mechanically tilled). In each sampling point, ca. 300 mL of soil at the 0-10 cm depth was collected (758 samples in total, 260 in S1, 249 in S2 and 249 in S3). The difference in the number of samples is due to the loss of subsamples and variation of soil management in few plots. Then, each soil subsample was extracted by a Berlese-Tullgren system (ISO 23611-2), and the microarthropods collected in jars containing 70 % ethanol.

2.3. Springtail community

Springtails were then mounted with a drop of lactic acid under a binocular magnifying glass (Nikon SMZ1270) and determined at the species or genus levels under microscope (Nikon ECLIPSE Ni). In total, 2106 springtails were collected and 1917 identified, representing 68 species among 40 genera (Hopkin, 2007).

The 3 subsamples for each session, each management treatment and

each vineyard were summed to create a dataset "Season" with 254 values of abundance for each springtail species. We also summed the abundance of each species observed in S1, S2 and S3 at the plot level (row and inter-row within each vineyard) and obtained a "Year" dataset with the total abundance of each species, with 87 values.

In these two datasets, species richness, total abundance and Shannon diversity index were calculated. Biological quality index based on collembola (QBSc index) is used to assess functional diversity based on ecomorphological traits (Parisi, 2001; Gruss et al., 2019). It was calculated from the functional criteria of the identified species and EMI-score associated (Supplementary Table S1). When it was impossible to calculate a QBSc (undetermined individuals, highly damaged), the corresponding samples were removed from the statistical analysis (3/254 samples). When the identification at the species level was not possible, only the genus was indicated and the EMI-score of the genus of the most abundant species in our dataset was used.

This score varies in our case between 1 and 33 and is associated with the sum of score of 6 different criteria mentioned in Gruss et al. (2019).

(2)

braces "{}" of the formula (see below).

We used multi-model inferential statistics to explore the relative importance of each explanatory variables on the several response variables. The models were constructed via the function "glmmTMB" (R package glmmTMB v1.1.5) (Brooks et al., 2024). Most of the explanatory variables were log-transformed (Cu, OM, TFIs, MAUV, and nb.w) and the paired-field was set as a random variable. Then, all models of each combination were compared with "dredge" function (R package MuMIn v1.47.1) (Bartoń, 2024). The same model structure was used for "season" and "year" datasets with the same explanatory variables, except the season effect only included in models using the "season" dataset. For each response variable (QBSc, Shannon diversity, species richness and abundance), we obtained 24 configurations of explanatory variables for each response variable in both "Year" and "Season" models. Each dredge function compared 128 possible combinations of explanatory variables (from the null model to the model with all variables for each model combination) for "season" dataset, and 64 for the "year" dataset. The following eq. (2) depicts the possible combination:

 $Y = logCu + \{logOM \text{ or } logOC\} + \{logTFI_{total} \text{ or } logTFI_{i} \text{ or } logTFI_{f} \text{ or } lognb.pp\} + logMAUV + pH_{w}$

 $+ \{row \text{ or } lognb.w \text{ or } Dis\} + season (only for Season dataset) + (1|paired.field)$

Briefly, each criterion has a value from 0 to 6. These criteria are related to pigmentation (0: fully pigmented to 6: no pigmentation), structures on cuticules (0: well-developed chaeta or scales, and presence of trichobotria presence to 6: low number of chaeteae, other structures present only in selected parts of the body), the number of ocelli in the eyespot (0: 8 + 8 ocelli to 6: no ocelli), the size of antennae (0: longer than the head to 6: much shorter than the head), legs (0: well-developed legs to 6: reduced legs or with reduced claw and mucro), and furcular development (0: well-developed furcula or 6: furcula reduced in residual form).

2.4. Statistical analyses

2.4.1. Preliminary analyses

Firstly, we checked for differences between explanatory variables (organic matter, organic carbon, TFIs, pH_{water}, number of soil disturbances) within treatments (grassy inter-row, tilled inter-row and vine row) through Kruskal-Wallis tests (R package *rstatix* v0.7.2) (Kassambara, 2023). Copper concentration did not differ between our 3 treatments (vine rows, grassy inter-rows and tilled inter-rows) within the 32 vineyards (KS test: H = 1.17; df = 2; P > 0.05). As well as for copper content, OM and OC showed no significant differences between the three treatments (KS test: H = 2.16, df = 2; and H = 2.17, df = 2; P > 0.05 respectively for OM and carbon contents). Concerning pH, no significant differences were observed between our three treatments (KS test: H = 0.83, df = 2, P > 0.05). In total 42 rows were mechanically disturbed (17 vine-rows and 25 tilled inter-rows).

2.4.2. Taxonomic diversity, functional diversity and abundance of springtails

Using GLMM, we explained the QBSc value, the Shannon index, the species richness and the abundance of springtails for each type of row and inter-row. These variables were explained by environmental soil variables (OM, OC, pH, Cu), variables related to the intensity of practices (TFI_{total}, TFI_f, nb.pp., row, Dis, nb.w), season (3 periods: S1, S2 and S3) and the minimum age under viticulture (MAUV). For all variables, we checked for outliers and collinearity between variables, (Zuur et al., 2007; Zuur et al., 2009) in order to not compute collinear variables in a same combination model. Thus, we selected only one parameter in each

For the "Season" models, QBSc and species richness followed a Quasi-Poisson family link function (since we observed overdispersion in Poisson models) whereas a Negative-Binomial family link function was applied for abundance (overdispersion observed in model residuals using Poisson and Quasi-Poisson families). For the "Year" models, we also used a Negative-Binomial family link for the abundance due to residual overdispersion but a Gaussian family link for species richness and Shannon index and a Quasi-Poisson family link function for the QBSc (due to residual overdispersion using Poisson family).

For each configuration of each response variable, the best models were selected comparing second-order variant of Akaike Information Criterion (AICc) (Burnham and Anderson, 2002). Results show the best models for each response variable and for both "season" and "year" datasets regarding the AICc, and we provided the results of all models with Δ AICc \leq 2 compared to the best model as higher Δ AICc provided less support (Burnham and Anderson, 2002) and increased drastically the number of candidate models. These models are in a summarized table in the Results section and complete tables of results are available in the Supplementary Material. All final models were checked with the R package *DHARMa* v0.4.6 (Hartig and Lohse, 2022) through QQ-plot of residuals and a plot of residuals against predicted values, checking for deviations, dispersion and outliers.

2.4.3. Community composition

Afterwards, we explored the variation in the springtail community matrix using abundance but also presence/absence of species by correspondence analysis. We then performed a Principal Coordinate Analysis (PCoA) ("pcoa" in R package *ape* v5.6–2) (Paradis and Schliep, 2019) with Bray-Curtis distance ("vegdist" in R package *vegan* v2.6–4) (Oksanen et al., 2024), and a Permutational Multivariate Analysis of Variance (PERMANOVA) ("adonis2" in R package *vegan* v2.6–4) (Oksanen et al., 2024) using Bray-Curtis distance for abundance data and Jaccard for presence/absence. We projected it on the PCoA to estimate the potential effects of the environmental gradients to our community ordination. Environmental variables were used to assess their potential effects on community composition. For these analyses few individuals were determined only at the genus level due to lack of observed characteristics. We choose to assign these few individuals to

the dominant species of the genus (*Entomobrya* sp. to *E. lanuginosa*, *Isotoma* sp. to *I. viridis*, *Isotomodes* sp. to *I. productus*, *Lepidocyrtus* sp. to *L. lanuginosus*, *Proisotoma* sp. to *P. minuta*, *Pseudachorutes* sp. to *P. boerneri*, *Pseudosinella* sp. to *P. alba*, *Sminthurinus* sp. to *S. niger* and *Tomocerus* sp. to *T. minutus*). For 6 genera, the number of individuals determined at the species level was very low and we assigned all individuals at the genus level (*Ceratophysella* sp., *Hypogastrura* sp., *Isotomurus* sp., *Mesaphorura* sp., *Paratullbergia* sp., *Sminthurides* sp.). We then only kept for further analyses, species or genera that were observed on at least 10 % of our samples (9 occurrences for 87 values). All statistical analyses were made using R (R version 4.2.2 (2022-10-31 ucrt)) (R Core Team, 2022).

3. Results

3.1. Species diversity of springtail community

Among the 1917 identified springtails (for 2106 individuals collected), we observed a dominance of *Cryptopygus thermophilus* observed in 30 of the 32 vineyards and representing ca. 22 % of our identified springtails, followed by *Entomobrya lanuginosa* (29 over 32, ca. 9 %) and *Lepidocyrtus lanuginosus* (26 over 32, ca. 8 %). Most of the collected species represented <1 % of the total abundance with 48 species and 12 genera (Supplementary Fig. S1). We identified between 25 and 161 springtails per vineyards (S1, S2 and S3 summed).

Mean densities per plot varied between 310 ± 382 individuals.m⁻² (mean \pm SD) in the end of spring (S2) and 1249 ± 1917 individuals.m⁻² in Autumn (S3). The mean density was intermediate in the beginning of the spring (S1): 734 ± 1238 individuals.m⁻². Mean densities in the vine rows or the grassy or tilled inter-rows were lower in spring than autumn (respectively, 663 ± 730 , 690 ± 623 and 591 ± 603 in S1; 1246 ± 2323 , 1210 ± 1349 and 1302 ± 1988 individuals.m⁻² in S3). The mean density was higher in grassy inter-rows in the end of spring (S2) with 384 ± 508 individuals.m⁻² compared to 328 ± 291 individuals.m⁻² in tilled inter-rows and 228 ± 293 individuals.m⁻² within vine rows. Mean richness (\pm SD) was 8.16 ± 4.18 per plot with a minimum value of 0 species in a row and a maximum value of 21 species observed in tilled

Table 1

inter-row of an organic vineyard. Shannon index varied from 0 to 2.77 with a mean at 1.69 \pm 0.55. QBSc mean value was 73.48 \pm 46.39 with a maximum at 215.

3.2. Descriptive analysis of the environmental variables

The mean TFI_{total} (\pm SD) of our vineyards was 13.10 \pm 6.13 (range: 3.47–26.74) with a mean of 9.18 \pm 4.48 and 17.03 \pm 4.97 for organic and conventional vineyards, respectively. Most of the treatments were fungicides (about 80 % of TFI_{total}) whereas insecticides represent ca. 15 % of TFI_{total}. The number of pesticides used (nb.pp) ranged between five and twenty-one.

Total soil copper content varied between 24.68 mg.kg⁻¹ and 340.22 mg.kg⁻¹ with a mean at 114.25 \pm 68.10 mg.kg⁻¹. OM content ranged from 1.12 % to 5.43 % with a mean at 2.32 \pm 0.84 % while OC range from 0.65 % to 3.16 % with a mean at 1.35 \pm 0.49 %. The pH value ranged from 5.80 to 8.26 with a mean at 7.09 \pm 0.79. About half of samples showed slightly acidic values. Among disturbed rows and interrows, the number of soil disturbance varied between 1 and 10 with a mean at 3.59 \pm 1.61.

3.3. Effects of season, pesticide use, soil management and characteristics on springtail community

3.3.1. Seasonal dataset

We found a significant effect of season in all models with $\Delta AICc \leq 2$ for QBSc, Shannon diversity index, richness and abundance (Table 1, Supplementary Table S2). Shannon diversity, species richness, QBSc and abundances of springtail were significantly lower in the end of spring (S2) than in autumn (Fig.1, Fig.2, Supplementary Figs. S2 and S3) notably in vine rows and tilled inter-rows. The mean values of all response variables (Shannon index, richness, QBSc and abundance) were slightly higher in autumn (S3) than in the beginning of spring (S1), even if this increase was only significant for springtail abundance.

There was a significant and consistent effect of the content of OM (or organic carbon) on QBSc, richness and Shannon diversity index but not on abundance (Table 1, Supplementary Fig. S4 and Table S2). The

Main results of the effects of viticultural practices and soil variables on springtail community indexes over the 3 seasons (seasonal models in the upper part) or for the total year dataset (year models in the lower part). Significant relationships are shown in bold: " x_1^* of x_2 " means x_2 models with this variable or group of variables selected over the total number of best models and x_1^* models with a significant effect of the explanatory variable. No x_1^* value means that the variable has been selected in the best models, but its effect was never significant (see Supplementary Tables S4 to S11 for the detailed results of all models). Cells with "+" indicate a positive effect of explanatory variables (columns) on springtail community indexes (lines), and cells with "-" indicate a negative effect. Empty cells indicate that the variable was not selected in the best models and cells with a "X" indicate that the variable was not tested and relevant in the set of models (seasonal effect in the year models).

	Number of best models	Copper	Organic matter	pH	Pesticides	MAUV	Disturbance (tillage)	Season
Seasonal models								
Abundance	14	+	+	+	-	+	+	$\mathrm{S2} < \mathrm{S1} < \mathrm{S3}$
		(2)	(3)	(12)	(5* of 13)	(1)	(2)	(14* of 14)
QBSc	16		+	-	-	-	+	$\mathrm{S2} < \mathrm{S1} < \mathrm{S3}$
			(9* of 15)	(1)	(1* of 8)	(11)	(9* of 15)	(4* of 16)
								S2 < (S1; S3)
								(4* of 16)
Richness	37		+	+	-	-	+	S2 < (S1; S3)
			(10* of 29)	(6)	(1* of 22)	(8)	(15)	(37* of 37)
Shannon index	15	-	+	+/-	-	-	+	S2 < (S1; S3)
		(2)	(10* of 14)	(2)	(2)	(2)	(13)	(15* of 15)
Year models								
Abundance	12	+	+	+	-	+	+	Х
		(1)	(1)	(6)	(5)	(2)	(1)	
QBSc	13		-	-	-	-	+	Х
			(4)	(3)	(13* of 13)	(6)	(10)	
Richness	10				-	-	+	Х
					(8)	(1)	(5)	
Shannon index	7		-		-	-	+	Х
			(4)		(2* of 7)	(7* of 7)	(4)	



Fig. 1. Effect of the session (sampling sessions S1, S2 and S3) and of soil management within the vineyards (grassy and tilled inter-rows and vine rows) on springtail QBS-c. S1 refers to early spring session, S2 to late spring and S3 to autumn. A facet wrap was applied based on the nature of the management: grassy inter-rows (green boxes), tilled inter-rows (brown boxes) and vine rows (purple boxes). S2 shows a significant decrease of the QBS-c value compared to S1, indicating a negative effect (*P*-value <0.01) in late spring (Table 1, Supplementary Table S2).

relationships between community variables and OM content were always positive with an increase of springtail diversity and abundance with higher values of organic matter (and carbon) content in soil (Fig. 4). The increase of pH value had a significant positive effect only for abundance (Table 1, Supplementary Table S7) and a slight positive effect on species richness (Table 1). We found no significant effect of copper soil concentration on Shannon index, richness, QBSc and abundance. Soil copper concentration was only selected in very few models with Δ AICc ≤ 2 (Table 1). The MAUV had a consistent and negative effect on QBSc and species richness (Table 1, Supplementary Tables S2 and S4). Disturbance, measured as the number of tillage or the presence/ absence of mechanical disturbance, and pesticides used had the most important and significant effects on springtail community (Table 1).

We found that the variable related to the disturbance effect (Dis) showed the higher impact on response variables: disturbance increased the QBSc values (Fig.3) and had a non-significant but also positive effect on Shannon diversity (Table 1). All our four response variables showed higher values in mechanically disturbed plots compared to non-disturbed plots (rows with herbicides and grassy inter-rows). The plot position itself (row; inter-row) did not show any effect on Shannon index, QBSc, richness and abundance. Pesticides-related parameters TFI_{total} , TFI_{f} and nb.pp. showed consistent, significant and negative effects on QBSc, richness and abundance but not on Shannon diversity (Table 1).

3.3.2. Year dataset

OM was selected in the best models for QBSc and Shannon index

(Supplementary Tables S8 and S9). It surprisingly showed negative but non-significant effects for these two variables. pH was also selected with a positive but non-significant effect on springtail abundance (Table 1, Supplementary Tables S3 and S11).

Copper was not selected in the best models for any response variable (only one model for springtail abundance). MAUV was negatively correlated with our variables with a significant effect on Shannon diversity index (Table 1, Supplementary Fig. S5). It was also present in the selected models for QBSc but the effect was not significant. Disturbance was more relevant than the total number of tillage in all models for the year dataset. This variable has been selected in a large part of the best models for species richness, QBSc and springtail abundance (Table 1).

Regarding pesticide variables, the number of pesticide products (nb. pp) was the best explanatory parameter for all variables, except for abundance, and we found consistent negative effects of TFI variable or nb.pp. on all springtail community variables. Nb.pp. influenced negatively and significantly QBSc (Fig. S5). For Shannon diversity and species richness, nb.pp. was also selected in models with a non-significant but negative effect. For abundance, TFI_{insecticides} was selected as an explanatory variable in the models with a negative but non-significant effect (Supplementary Table S11).

3.4. Multivariate analysis

Correspondence analysis (CA) of the species that were present at least in 10 % of our plots showed a percentage of explained variance of 21.5 % by first and second dimensions (Supplementary Fig. S6). The



Fig. 2. Effect of the session (sampling sessions S1, S2 and S3) and of soil management within the vineyards (grassy and tilled inter-rows and vine rows) on springtail abundance. S1 refers to early spring session, S2 to late spring and S3 to autumn. A facet wrap was applied based on the nature of the management: grassy inter-rows (green boxes), tilled inter-rows (brown boxes) and vine rows (purple boxes). S2 shows a significant decrease of abundance value compared to S1, indicating a negative effect (*P*-value <0.001) in late spring (Table 1, Supplementary Table S2).



Fig. 3. Relationship between seasonal QBS-c values and organic matter content (log values). No-disturbed (0) rows and inter-rows are represented in red dot and lines, disturbed (1) rows and inter-rows values are represented by blue dots and lines. Three different types of lines representing the 3 seasons (S1: early spring, S2: late spring and S3: autumn with S1 in solid lines, S2 in dotted lines and S3 in large dotted lines). Higher functional diversity was found in disturbed modalities for every season. S2 presented significantly lower functional diversity in both disturbed and undisturbed rows and inter-rows compared to S1 and S3.



Fig. 4. Relationship between the number of phytosanitary products applied (log values) among vineyards and QBS-c values. The main graphic represents raw data (empty dots) and the top-right insert represents the significant relationship, i.e., the prediction and standard errors from the Year QBSc Generalized Linear Mixed Model (Supplementary Table S3).

PERMANOVA results for springtails matrix showed a significant effect of logTFI_{total} (P = 0.022 and 0.027), logTFI_{fungicides} (P = 0.035 and 0.022) and pH (P = 0.006 and 0.019) in both abundance and presence/absence matrices respectively (Supplementary Fig. S7). These results indicated a difference in community composition linked to these variables or a difference in composition variance. However, its projection on PCoA axis did not show any significant result, that could be attributed to the low eigenvalue of the axes or the high abundance of *Cryptopygus thermophilus* among vineyards because this projection is made on 2 dimensions ordination. The importance of the effect of these 3 variables are low ($r^2 = 0.034$ for pH, 0.014 for logTFI_{total} and 0.002 for logTFI-fungicides) but consistent with other results shown on community variables. Results were similar using presence-absence matrix, where lognb. pp. ($r^2 = 0.021$) was also significant (P = 0.029).

4. Discussion

4.1. Pesticides and minimum age under viticulture

We found negative effects of pesticide use on several response variables, independently of the type of variables used to assess pesticide use (either the number of pesticides used or the total TFI). Pesticide use negatively impacts seasonal and year QBSc as well as species richness, abundance (seasonal dataset) and Shannon diversity (year dataset) of springtails, confirming partially the first hypothesis concerning negative impact of pesticide use. These results are in accordance with a recent meta-analysis (Beaumelle et al., 2023b) that found a higher effect of pesticide use on both soil richness and diversity than on abundance. The negative effect of the number of products used is an interesting and original result suggesting the importance of potential cocktail effects on springtail communities compared to the existing body of knowledge

often focusing on the effect of one particular pesticide. This result is in line with recent studies which also reported detrimental effect of multiple substances on soil fauna with possible synergistic effects (Beaumelle et al., 2023b; Panico et al., 2022). Concerning phytosanitary products, Petersen (2002) reported that some species such as Lepidocyrtus cyaneus are able to rapidly recolonize after pesticide use. Wiles and Frampton (1996), also reported intraspecific variation in insecticide toxicity as well as it could also change dominance structure of springtails community (Endlweber et al., 2006). Because we have a large diversity of species, the effects could be dampened by the dynamics and rapid colonization of ruderal species such as Lepidocyrtus cyaneus. Further investigations and a more replicated sampling effort are needed to discriminate species that are sensitive to phytosanitary products as it has probably a higher impact on community diversity than in the total abundance dominated by few species. Moreover, our multivariate analyses revealed that pesticide use (TFI total, TFI fungicides and number of phytosanitary products) significantly influenced the springtail community, even if these effects were weak. It would be helpful to study the effects of particular active substances commonly used in vineyards (i.e. sulfur, metiram, zoxamide, pyrethroids).

There is also a harming effect of pesticide metabolites, which could remain in the field and accumulate with the age of vineyards that should be considered for future studies. We found slight but consistent negative effects of the minimum age, since the field is conducted in vineyards, on diversity. Contamination by pesticides and their metabolites, even those that are today forbidden, could influence and explain the observed current community. Indeed, previous studies, analysing pesticides and their metabolites, revealed that they could be detected decades after their application (Chiaia-Hernandez et al., 2017). We found that the minimum age since the field is conducted in vineyards negatively affected QBSc (seasonal and year models) and Shannon diversity (only in year model). This effect of minimum age under viticulture is in accordance with a previous study by Simoni et al. (2013) on croplands, which observed higher springtail densities in young organic fields compared to old organic and conventional fields contaminated by pesticides or copper accumulation.

4.2. Copper concentration

Contrary to our expectations, we found no effect of total copper content on springtail communities. Our samples comprise a wide range of copper content, reflecting values observed (17–491 mg Cu_{total}.kg⁻¹) by El Hadri et al. (2012). Copper availability for organisms is strongly influenced by the amount of total copper in soil. It could bind to organic ligands and enhance OM by protecting it from degradation (Daoust et al., 2006; Fernández-Calviño et al., 2008; Parat et al., 2002; Sauve et al., 1997). The pH highly influences copper speciation and solubility (Sauve et al., 1997) with an increase in solubility in low pH conditions. Springtails are also known to be tolerant to high copper contamination in soils. Ardestani and Van Gestel (2013) suggested the presence of a homeostatic regulation of copper with their study on Folsomia candida. We then suggest that this absence of copper effect is probably due to its low availability among our samples because of pH and OM content values in our samples. Indeed, previous study on Eisenia fetida and Folsomia fimetaria showed that old contaminated soil were less toxic than newly spiked soils with copper, attributed to the low availability of ancient copper (Scott-Fordsmand et al., 2000).

4.3. Soil disturbance

We expected a strong effect of soil disturbance with an increase of functional and species diversities as well as their abundance in grassy inter-rows compared to tilled ones. Our analyses revealed that disturbance enhanced both functional and taxonomic diversity of springtails. This result is in line with Buchholz et al. (2017) who also found higher densities under tillage management compared to a permanent green cover, without affecting species diversity.

Tillage is often associated with an increase of water erosion or soil compaction (Costantini et al., 2018), and could also have detrimental effects on soil biodiversity (Giffard et al., 2022). For example, Betancur-Corredor et al. (2022) observed that reduced and no tillage promoted densities of springtails by 35 %. However, we found no relation between mean springtail density and the management of the inter-row. Möth et al. (2023) observed higher mean densities in cover crop inter-rows compared to tilled inter-rows. They also observed lower mean densities in May and June (compared to September). Nevertheless, tillage in inter-rows can enhance soil compaction and reduce pore space, which lead to lower abundance of euedaphics life-form species (Larsen et al., 2004). At the opposite, Dittmer and Schrader (2000), also observed a positive effect of soil compaction on Sminthurinus aureus and Folsomia fimetaria, respectively epedaphics and hemiedaphic species. Tillage has different effects on soil compaction and at different depths which could counteract the negative effect of soil compaction associated with machine load. However, in our study we observed positive effect of soil disturbance by a binary parameter while the number of passes was not significant. Several tractor passes during the season could be detrimental as wheel load and passes frequency are the main contributors of soil compaction (Lagacherie et al., 2006; van Dijck and van Asch, 2002). We then found a positive effect of soil tillage in springtail communities in our vineyard system, even if we cannot exclude that it has a long-term negative effect of OM content and then also contributes to the decrease of species diversity and abundance.

Another indirect and positive effect of tillage has been already suggested in vineyard studies; tillage has negative effects on arthropod predators compared to cover inter-rows and the predation of springtails could be then lower (Buchholz et al., 2017). At the opposite, tilled interrows may favor the burial of resources deeper in the soil, which can enhance hemi- and euedaphic life forms which are more prone to be parthenogenetic than epedaphics forms (Petersen, 1978). We suggest that the positive effect of disturbance on springtail diversity promotes rstrategist species with parthenogenetic strategy of reproduction (Chernova et al., 2010). These species are relatively small ones, poorly pigmented with few ocelli, which also characterize species with high QBSc values like *Mesaphorura macrochaeta, Isotomiella minor* or *Parisotoma notabilis* (Chahartaghi et al., 2006). Further analysis investigating how the distribution of several functional traits (e.g., using community-weighted trait means) respond to farming practices would be interesting to confirm these observations.

4.4. Seasonal effect

We found low springtails density among the studied vineyards, especially during the season where vineyards are the most managed. This observation contrasts with other studies conducted in vineyards (Buchholz et al., 2017; Möth et al., 2023; Renaud et al., 2004). In our study system, the peak of pesticide use and disturbance associated with tillage overlaps with the end of spring and summer periods that show an increase of thermal and hydric stresses for soil communities. This is in accordance with previous results (Hodkinson et al., 1998; Kardol et al., 2011) who reported that summer desiccation is a major threat for springtails. Rusek (1989) also reported that biomass dynamics decrease during dry summer, which corroborates with our results. In our study system, this seasonal effect is related to the period of peak of pesticide use, confirming our second hypothesis. This seasonal aspect could be an important factor as it occurred during the treatment period and warming days, which could be negative and synergistic effects (Sørensen and Holmstrup, 2005).

4.5. Soil parameters: Organic matter and pH

As expected in our third hypothesis, we found positive effects of pH and OM content on springtail community indexes. Previous studies showed that pH has direct effect on springtail abundance by its influence on fecundity and longevity (Hutson, 1978; Rusek, 1998). These results are in accordance with our study; but further investigation on Collembola biology are needed, as these effects probably remain speciesdependent. The OM content is a food supplier for springtails, which could explain the observed increase of Shannon diversity, species richness and QBSc with higher OM content in our samples. OM will favor the growth and diversity of microbial communities: more complex structures and metabolites to degrade will probably favor various species instead of a large abundance of individuals. Moreover, some species are associated with important quantity of decaying OM such as Proisotoma minuta (Hopkin, 2007). Potapov et al. (2016) explored the relationships between trophic niches of collembolans linked to their life-form or groups based on Δ^{13} C and Δ^{15} N values. *Poduromorpha* are mostly euedaphics species and of highest ranking in trophic positions compared to epedaphics species, closer to primary producers feeding on micro-algae and first stages of litter decomposition. Hemiedaphics and euedaphics species are more prone to consume microorganisms associated with higher organic matter contents. We may also hypothesize that pH and OM content increase can dampen negative effects of soil management, compaction, or negative pesticides effects. These soil variables also influence copper bioavailability in soil which could explain the lack of significant effects regarding soil copper contamination.

4.6. Springtail communities

Within our samples, 48 of the 68 species were below the 1 % threshold of total abundance. This means that our samples were largely dominated by few species common and abundant in our vineyard soils. This trend was also observed in previous studies. Our samples were largely dominated by *Cryptopygus thermophilus*, then *Entomobrya*

lanuginosa and *Lepidocyrtus lanuginosus* which, gathered, accounted for ca. 40 % of identified individuals. Renaud et al. (2004) also observed high abundance of *Entomobrya lanuginosa*, *Cryptopygus thermophilus*, *Sphaeridia pumilis*, *Lepidocyrtus cyaneus* or also *Ceratophysella denticulata*, which were common in our samples. Buchholz et al. (2017) observed a high representation of *Parisotoma notabilis* in their soil core samples, which was highly represented in our vineyard soils (4th in abundance).

5. Conclusion

Our study investigated the effect of soil management and pesticide use on springtail communities in vineyards. Soil organic matter and soil disturbance enhances both functional and taxonomic diversities whereas the intensity of pesticide use had consistent and negative effects. Enhancing soil organic matter appears as a key aspect for promoting springtail communities and more generally soil biodiversity in vineyard systems. This could be properly managed through organic matter inputs and adapted soil tillage practices (e.g., conservation tillage) reducing soil disturbance. Our study also emphasizes the importance of reducing the use of pesticides, both in terms of frequency and applied doses, due to their detrimental effects on the springtail communities. However, further analyses focusing on cocktail effects and specific substances are recommended to design efficient strategies for protecting soil biodiversity in vineyards.

CRediT authorship contribution statement

Pierre Blondel: Writing – original draft, Methodology, Formal analysis, Data curation. **Benjamin Joubard:** Writing – review & editing, Methodology, Conceptualization. **Adrien Rusch:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Brice Giffard:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.apsoil.2024.105694.

Data availability

Data will be made available on request.

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