Basic and Applied Ecology 73 (2023) 18–26

Contents lists available at ScienceDirect

Basic and Applied Ecology

journal homepage: www.elsevier.com/locate/baae

RESEARCH PAPER

Glyphosate reduces the biodiversity of soil macrofauna and benefits exotic over native species in a tropical agroecosystem

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ARTICLE INFO

Keywords:
Banana
Biodiversity hotspot
Caribbean islands
Conservation
Martinique
Herbicide
Macro-arthropods
Tropical agroecosystems

ABSTRACT

Herbicides are the most applied pesticides in the world. Despite numerous laboratory studies demonstrating the toxic effect of herbicides on non-target organisms, the effect of herbicides on soil organisms in the field remains complex to understand and is still controversial. In order to understand how changes in agricultural practices aiming to reduce herbicide use could impact soil biodiversity, we studied the effect of the frequency of herbicide application on soil biodiversity in a tropical agroecosystem.

Our study was conducted on banana farms in Martinique, an island with a humid tropical climate belonging to the Caribbean biodiversity hotspot. Thirteen banana plots from five different farms were selected, ranging from plots receiving no herbicides to plots receiving 4–5 applications per year. Soil macro-arthropods were sampled using pitfall traps resulting in the collection of over 6,200 individuals. Of the 100 taxa that were differentiated, 75 could be identified to species level which allowed to assign each taxon to a trophic group and when possible to classify them according to whether they were introduced or native.

Macro-arthropod mean species richness was 21% lower in plots with the highest frequency of herbicide application. However, no conclusive effect of herbicides on macro-arthropod abundance was demonstrated. Mean species richness for different trophic groups also decreased with herbicide applications with decreases of 22% for predators, 17% for omnivores, 55% for herbivores, and 55% for decomposers in plots with 4–5 herbicide applications per year compared to plots with no herbicide use. Species composition of macro-arthropod communities varied significantly with herbicide applications. More specifically, we found that native species represented a higher proportion of individuals captured in plots where no herbicides were used; suggesting that agroecological practices implemented at the field level to reduce the frequency of herbicide use potentially play a relevant role in soil biodiversity conservation.

Introduction

Herbicides are the most frequently applied pesticides worldwide. In 2019, 2 million tonnes of herbicides were applied around the world, accounting for 50% of all pesticide applications (FAOSTAT, 2019), with glyphosate being the most widely used (Giesy et al., 2000). Despite relatively low toxicity compared to other pesticides (Gunstone et al., 2021), large volumes of glyphosate spread in the environment can pose a risk for human health and the environment (van Bruggen et al., 2018). In this respect, the effect of herbicides on non-target organisms has attracted the attention of many scientists. Research has mostly focused on vertebrates. Several studies have demonstrated herbicide toxicity on algae, birds, fish and amphibians (Tajnaiová et al., 2020; Galhano et al., 2011; Relyea, 2005). Less is known about the impact of herbicides on invertebrates (but see Fiera et al., 2020; Massoni et al., 2017), despite the key role these organisms play in the overall functioning and stability of ecosystems (Cardinale et al., 2012; Díaz et al., 2019; Tamburini et al., 2020).

Herbicides may have harmful effects on invertebrate communities, either directly by toxicity (Correia & Moreira, 2010; Stellin et al., 2018), or indirectly by changing and reducing the diversity of weed communities and thereby altering microclimatic conditions (Menezes & Soares, 2016; Shelton & Edwards, 1983). The effect of herbicides depends on the sensitivity of the taxon considered and its place in the food web

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https://doi.org/10.1016/j.baae.2023.10.001
Received 20 October 2022; Accepted 8 October 2023
Available online 13 October 2023

(Frampton, 2002). For predators, the direct toxicity of glyphosate on survival rates appears to be low in contrast to other more toxic herbicide molecules (Ward et al., 2022). However, glyphosate and its adjuvants may influence other life history traits, including reduced hunting activity, which could have a negative impact on bioregulation (Niedobová et al., 2019; Korenko et al., 2016). In contrast, for detritivores, the direct toxicity effects of glyphosate and 2–4D have been shown in the laboratory on the earthworm *Eisenia foetida* (Correia & Moreira, 2010). A recent study undertaken on a microcosm scale showed that glyphosate (Roundup 360®) induced a higher mortality rate, a lower reproduction rate and a lower body mass for several earthworm species (*Lumbricus terrestris* and *Octodrilus complanatus*) (Stellin et al., 2018). Evidence shows that contamination occurs either by ingestion of active substances or by epidermic penetration of the herbicide into the body (Menezes & Soares, 2016). The hazard of several herbicides via direct contact has thus been relatively well demonstrated by these *in vitro* laboratory studies. However, the results of *in situ* studies are few and far between. Given the complexity of the ecological systems studied, the effect of herbicides on invertebrate communities is sometimes difficult to interpret. Giesy et al. (2000) claimed that herbicides are non-toxic when they are used under recommended concentrations in the field. However, by removing vegetation herbicides can reduce habitats for herbivores, decomposers and, in turn, impact predators such as carabids and spiders (Brust, 1990; Brooks et al., 2005; Haughton, 2000). Some field studies have shown declining populations of isopods and carabids in agricultural systems with a high frequency of herbicide applications (Brust, 1990). However, there are some contrasting results. Indeed, other studies have shown no effect of herbicides, especially of glyphosate, on arthropod abundance or community composition in the field (Lindsay & French, 2004; Hagner et al., 2019; Nakamura et al., 2008). Most of these studies were field trials with short-term glyphosate application (Niemeyer et al., 2018), which limits the scope of the conclusions.

Agricultural landscapes generally support the spread of invasive alien species (Lonsdale, 1999). This effect can be related to direct introduction (Hulme et al., 2008), or the creation of a disturbance regime that tends to promote introduced species that are opportunist in their foraging and habitat preferences (Alpert, 2006; Stavert et al., 2017). However, there have so far been few studies on the role played by herbicides currently widely used in conventional agriculture in promoting introduced species. A few studies have focused on this topic for vascular plants (Ellis et al., 2012), but to our knowledge no studies have been conducted on soil invertebrates. This point is particularly important in the context of the 6th mass extinction (Ceballos & Ehrlich, 2018), and especially in the face of declining insect populations in different parts of the world (Hallmann et al., 2017; Semmens et al., 2016). Indeed, it seems important that agricultural areas should play a role in the conservation of species (land sharing) and that this service is not only maintained in natural protected areas (land sparing) (Tscharntke et al., 2012). Reducing herbicides in cropping systems could be a lever for increasing biodiversity and thus making these systems useful in the conservation of certain invertebrate species.

Our study set out to determine the effect of herbicides on soil macro-arthropod communities taking a field approach in farmers' plantations in the context of the Lesser Antilles. Banana agroecosystems are a suitable field for our research because banana is a semi-perennial crop whose plots are cultivated for 8 to 10 years; it is thus possible to test the effect of herbicides over a longer period of time than in trials conducted with single applications of herbicides, or over short periods of time. Moreover, farmers are increasingly converting to new agroecological practices. Indeed, an increasing number of farmers have reduced or stopped the use of herbicides on their own initiative before being obliged to by regulations. This all creates a favourable context for observing the effect of different levels of herbicide applications on soil macro-arthropod communities.

Based on earlier studies highlighting herbicide toxicity on macro-arthropods, we expected to find a higher abundance and diversity of macro-arthropods in plots with no herbicide use (hypothesis 1). Considering that macro-arthropods are preserved when the ecosystem is not disturbed and when non-crop habitats are maintained, we expected to find a different species composition in macro-arthropod communities depending on the frequency of herbicide applications (hypothesis 2). In plots with herbicides, we expected to find a high abundance of species that were able to adapt to the herbicide disturbance, such as introduced species having undergone a high introduction effort by exchanges of crops and inputs, and which often proliferate (Blackburn et al., 2015; Blackburn & Duncan, 2001). It is likely that such species are more adapted to agricultural disturbance. We thus expected that introduced species would outcompete native species and be more abundant in plots with herbicide applications (Manchester & Bullock, 2000). In addition, we expected herbicides to affect the food web through a bottom-up effect, by removing the primary resource (weeds), hence the abundance of herbivores and detritivores should decrease the most, followed by predators, which should be affected by a decrease in the abundance of prey (hypothesis 3). To test these hypotheses, macro-arthropod communities were sampled using pitfall traps in banana fields with different herbicide application frequencies.

Materials and methods

The study sites

The study was conducted in banana fields in central Martinique (Lesser Antilles), on the Atlantic coast. The climate in Martinique is tropical humid, with a mean annual temperature of 26°C and a mean annual rainfall of 2406mm (Average of 1981–2010mm from the Lezarde meteorological station, located in the vicinity of the sampling area). The relief is generally mountainous, especially in the North and South of the island. The centre is characterized by a relatively flat relief with small escarpments (Germa, 2010).

Based on interviews with farmers about their crop management, we selected farms with contrasting weed management leading to different levels of herbicide application in the field. Interviews resulted in the selection of 13 plots on 5 farms (between 2 and 3 plots per farm). Six plots were free of herbicides and seven plots had regular herbicide applications. Seven plots were selected for the herbicide modality, making the design slightly unbalanced, as some plots were too narrow or too steep to allow for a sufficient number of traps to be placed under good conditions (Table 1). In the six plots without herbicide, weed management was only mechanical, carried out either with a brush-cutter or with a rotary cutter, in both cases the residues were left in the field. In the other seven plots, herbicide applications could be classed in two categories: four plots had two to three herbicide applications per year and three plots had four to five herbicide applications per year (herbicides/year). Therefore, herbicide use could be divided into three categories, i.e. 0 herbicide applications per year, 2 or 3 herbicide applications per year, 4 or 5 herbicide applications per year; this was retained for the statistical analyses (Table 1).

The plots were banana monocultures (cultivar 'Grande Naine', Cavendish sub-group, grown worldwide for dessert banana export), planted at a density of 1800 plants per hectare. Apart from weed management, all the plots were under similar management practices, representative of those used in the banana production area in Martinique. No insecticides were used in these plots and all of them were preceded by a fallow period.

Sampling and identification of soil macro-arthropods

Macro-arthropods were sampled using pitfall traps. The protocol was designed to achieve an equal number of sampling units between plots with and without herbicide application. Consequently, 48 pitfall traps were distributed in the six herbicide-free plots (hence, eight traps per plot). Then, 48 more pitfall traps were distributed throughout the seven

Table 1
Summary of plot characteristics, relative to the number of samples, pedoclimatic factors and herbicide application frequencies identified during interviews with farmers. The herbicide molecules used are mainly glyphosate and glufosinate. Means are indicated in the grey line.

Fields	Sample number	Area (ha)	Age (year)	Altitude (m)	Soil			Herbicide applications/year	Herbicide molecules
					Organic matter (g/kg)	pH	C/N		
RC-LG	8	1.2	4	180	93	5.2	10.7	0	–
RC-CR	8	0.5	4	202	64.7	5.9	10.4	0	–
RC-BL	8	0.3	4	212	107	6.8	10.9	0	–
TB-GP	8	3.6	1	164	66	6.8	10.6	0	–
TB-GV	8	3.3	1	253	96.1	6.1	12.3	0	–
TB-DT	8	3.8	1,5	193	67.3	5.7	10.4	0	–
Mean		2.1	2.5	200	82.4	6.1	10.9		
GL-C3	8	1.2	5	18	79.7	6.5	12.7	3	2 Glyphosates, 1 Glufosinate
GL-C2	8	0.4	5	30	66.5	7.1	10.8	2	1 Glyphosates, 1 Glufosinate
DS-MR	8	2.1	2	75	40.3	6.7	10.5	2	2 Glyphosates
DS-TY	4	1.2	4	94	34.6	6.6	9.4	2	2 Glyphosates
Mean		1.2	4	54	55.3	6.7	10.9		
MT-RV	8	0.5	5	76	31.1	5.4	9.5	4	3 Glyphosates, 1 Glufosinate
MT-BM	4	0.8	5	43	25.7	5.1	8.9	5	4 Glyphosates, 1 Glufosinate
GL-C4	8	1.4	5	10	84.8	6.5	13.3	4	3 Glyphosates, 1 Glufosinate
Mean		0.9	5	43	47.2	5.7	10.6		

plots with herbicide application (see Table 1 for sample distribution between treatments). The pitfall traps were 7cm in diameter and filled with a 50/50 solution of demineralized water/monopropylene glycol, which allows good conservation of invertebrates and is not toxic for non-target animals (Weeks & McIntyre, 1997). We added some droplets of soap to the solution to reduce surface tension and prevent macro-arthropods from floating and escaping. The traps were kept in the plots for a week. The sampling campaign for all the plots lasted from 1 to 23 July 2019. Once in the laboratory, the macro-arthropods were transferred to 70% ethanol. They were then identified mostly to species or morpho-species level, otherwise we stopped at the family level. When the taxa were juveniles, we stopped at the class level and they were considered only in the abundance analysis and not in the diversity analysis. For the three major macro-arthropod groups in our dataset (ants, spiders and diplopods), which were also the best-known groups, we collected information on their geographical distribution from the literature and from local specialists of each group. In the end, we assigned a status to each species based on its distribution: either native, introduced, or unknown if knowledge about its distribution was insufficient. A similar approach was taken to assign a trophic group to each species, the types being omnivores, predators, herbivores and decomposers (see Appendix A: Table S1).

In each sampling position, two kinds of measurements were taken in the vicinity of the pitfall trap. Litter was sampled in a 25×25 cm^2 quadrat. Litter was sorted in the laboratory in order to remove soil aggregates stuck to decaying leaves, then placed in an oven at 60°C for 48 h and weighed to estimate the mass of litter per square metre. Below the quadrat of litter, soil was sampled in order to carry out a chemical analysis: organic matter was measured by a sulphochromic oxidation method, C/N by the Dumas method, pH by water extraction and exchangeable soil cations (K_2O, MgO, CaO) were extracted by shaking the test sample in an ammonium acetate solution and determined by atomic absorption spectrometry.

Data analyses

All analyses were carried out with R software (Version 1.4.1106). Except for species accumulation curves plotted at the treatment level using the specaccum function, all analyses are conducted at the level of the pitfall trap. The abundance of soil macro-arthropods was obtained by counting the number of individuals per pitfall. Species richness was calculated by summing the number of different species trapped per pitfall. Evenness and the Shannon index were calculated as per the Hill (1973) formula using the 'VEGAN' package (Oksanen et al., 2013).

To test the effect of herbicides on the macro-arthropod's community while considering the pedoclimatic differences between the plots, we constructed models integrating pedoclimatic variables (altitude, organic matter, C/N, K_2O, MgO, CaO and pH of soil) as co-variables. To reduce the number of co-variables, and avoid collinearity between them, the coordinates of each banana field on the two dimensions of a principal component analysis (PCA) conducted on the pedoclimatic variables (Dim1, Dim2) were integrated in the statistical models as:

Y~Herbicides+Dim1+Dim2+(Herbicides: Dim1) + (Herbicides: Dim2) +
(Herbicides: Dim1: Dim2) + (Dim1: Dim2)

Herbicide application frequencies (Herbicides) were included in the models as a categorical variable accounting for the three categories of treatment frequency (i.e. 0, 2–3 and 4–5 herbicide applications per year). For each response variable (Y), a link function was chosen according to the distribution of the data. For total abundance and omnivore abundance a negative binomial function was used to deal with overdispersion. For decomposer, herbivore and predator abundances a Poisson function was used. For the evenness, Shannon and richness data a Gaussian function was used. For the data on the proportion of native and non-native species abundance a binomial function was used. Tukey tests, from the 'TUKEYC' package (Faria et al., 2021) were then used to build groups with the same mean and indicate letters on the graphics.

To select the best statistical model, we proceeded in a similar way for the response variables studied to test the different hypotheses. First, for each dependant variable, the most parsimonious model was selected according to the Akaike Information Criterium (AIC), using the 'dredge' function of the 'MUMIN' package (Barton and Barton, 2015). Then each selected model established with only fixed effects was compared to similar models integrating random effects. These random effects were used to consider the hierarchical structure of the error (random effect placed either on the plot, the farm, the plot and the farm or on the plot nested in the farm). Model comparisons was done by comparing the AIC of the five models using the 'anova' function. When the mixed models (glmer) explained a larger part of the variance and had an AIC lower than 5 compared to the model with fixed effects, only models with fixed effects were retained. In the end five models only were implemented with the plot as a random effect: models for abundance of herbivores, richness of herbivores, evenness of detritivores and proportion of native spiders.

To test the second hypothesis (H2), i.e., the effect of herbicides on community composition, non-metric multidimensional scaling (NMDS) was conducted on the community matrix. An analysis of similarity (ANOSIM) was then performed to test for the differences between the three herbicide application frequencies. In order to consider variability induced by pedoclimatic conditions, the PCA coordinates of the pedoclimatic variables (Dim1, Dim2) were fitted to the NMDS analysis, using the envfit function from the 'VEGAN' package. The significance of all correlations between PCA coordinates of the pedoclimatic variables and species composition was assessed using permutation tests (n=999 permutations).

Results

Multivariate analyses of pedoclimatic variables in the banana plots

The first dimension of the PCA (Dim1) explained 59.4% of total inertia mostly representing soil chemical properties. The contribution of the variables CaO, MgO, K_2O and C/N to this dimension was 22.6%, 20.4%, 17.5%, and 16%, respectively (see Appendix A: Fig. S1). The second dimension (Dim2) explained 20.7% of total inertia representing mostly altitude and soil organic matter, contributing 58.9% and 30% to this dimension, respectively (see Appendix A: Fig. S1). Banana plots with no herbicide use were positioned at the top of the second dimension where altitude and soil organic matter were high (Table 1). Banana plots with herbicide use (2–3 and 4–5 herbicides/year) were mostly positioned at the bottom of the second dimension where altitude and soil organic matter were low (Table 1).

Macro-arthropods in banana plots

Of the 6211 individuals trapped, 103 species and morphospecies were identified. Insects were represented by seven different taxonomic orders, including Isoptera, Diptera, Dermaptera, Orthoptera, Hemiptera, Coleoptera and Hymenoptera (Fig. 1). The most represented taxon was ants. Indeed, 39 species of ants were recorded with great abundance levels in the banana plantations of Martinique. The most abundant species found were the two species of the Myrmicinae subfamily; *Wasmannia auropunctata* and *Solenopsis geminata*. In addition, 13 different spider species and morphospecies, from seven families, were recorded in all the samples. We also identified 10 species and morphospecies of diplopods from four different orders (Fig. 1). Chilopoda were not numerous in our samples, no Geophilomorpha were trapped, and only 4 Scolopendromorpha were found in all the samples.

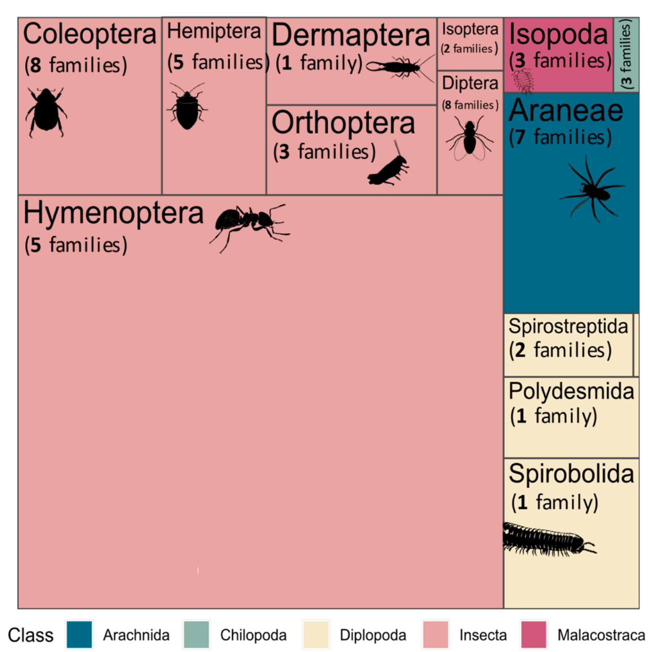

Fig. 1. Treemap of mean species richness of macro-arthropods per pitfall trap, according to their taxonomic orders. The area of the rectangle represents the species richness per pitfall trap for the given taxonomic order. The number of taxonomic families, found in all the traps, is given in brackets.

Effect of herbicide on soil macro-arthropod diversity and abundance

Overall, the species accumulation curves for plots with and without herbicide use reached saturation (see Appendix A: Fig. S2). Alpha diversity, corresponding to local diversity (in each plot), decreased with herbicide use (plots without herbicides: $\alpha = 12.08$, plots with herbicides: $\alpha = 9.85$), whereas beta diversity, corresponding to inter-plot diversity, was high in plots with herbicides compared to plots without herbicide use; i.e. 69.15 and 59.96 respectively. Reducing herbicide applications did not significantly affect litter mass (g/m^2) in banana plots ($p > 0.05$, see Appendix A: Fig. S3).

Reducing the frequency of herbicide applications positively affected the local diversity of soil arthropod communities in a significant way (Table 2, Fig. 2). More specifically, the mean species richness per sampling unit was 16% and 21% lower in plots with 2–3 and 4–5 herbicides/year, respectively, compared to plots with no herbicide use (Fig. 2B). In the same way, the Shannon index and Evenness decreased significantly in line with the frequency of herbicide applications. The Shannon index in plots with 2–3 and 4–5 herbicides/year decreased by 9% and 39%, respectively compared to plots without herbicides (Fig. 2C). Evenness in plots with 2–3 and 4–5 herbicides/year decreased by 2% and 36%, respectively, compared to plots without herbicides (Fig. 2D). The pedoclimatic variables had either a direct or indirect impact on macro-arthropod diversity (see interaction terms in Table 2), but the single variable of herbicide frequency still explained most of the variance, as shown by the chi-square values (Table 2). Herbicide applications and pedoclimatic variables (Dim1 and Dim2) had a significant effect on the total abundance of soil macro-arthropods. The latter averaged 75.0±11 (mean ± SE) in plots with no herbicide use and 81.9±13.9 in plots with 4–5 herbicides/year. Plots with 2–3 herbicides/year had the lowest abundance (46.8±5.7).

Effect of herbicides on the composition of soil macro-arthropod communities

Pedoclimatic factors greatly influenced the composition of macro-arthropod communities, as shown by the high squared correlation coefficient (R^2) when pedoclimatic factors (i.e. PCA coordinates on Dim1 and Dim2) were fitted to the NMDS analysis (Dim1 $R^2 = 0.32$, Dim2 $R^2 = 0.45$, p=0.001, Fig. 3B). However, herbicides also played a significant structuring role in soil macro-arthropod communities, as shown by the significant dissimilarity of their communities depending on herbicide applications (ANOSIM p=0.001, Fig. 3A). The macro-arthropod community in plots with no herbicide use was significantly but moderately different from plots with 2–3 herbicides/year (ANOSIM R=0.26), and very different from plots with 4–5 herbicides applications per year (ANOSIM R=0.46). However, arthropod communities did not differ greatly between plots with 2–3 and 4–5 herbicide applications per year (ANOSIM R=0.13).

The results regarding the composition of macro-arthropod communities depending on the range of species origins showed that the effect of herbicides on the proportion of individuals belonging to native species differed depending on the taxon considered. Spiders and ants had a similar response, i.e., the proportion of native species tended to decrease significantly with increasing frequency of herbicide use (55% and 75% lower in plots with 4–5 applications per year compared to plots with no herbicide use; Fig. 4 and Table 3). However, very few native diplopod species were present in the plots (84% of all individuals sampled in plots belonged to introduced species) and their proportion was not significantly affected by the frequency of herbicide use. Pedoclimatic variables (Dim2) had a significant effect on the proportion of individuals belonging to native species for ants and diplopods only, but not for spiders (Table 3).

Table 2
Results of LM and GLM p-values to test for the effects of herbicide applications and pedoclimatic factors on the abundance and diversity metrics of soil macro-arthropod communities. "-" indicates that the variable was not selected in the most parsimonious model.

	Abundance			Richness			Shannon			Evenness		
	DF	Chi^2	P	DF	F	P	DF	F	P	DF	Chi^2	P
Herbicides/year	2	13.965	0.001	2	4.129	0.019	2	21.384	$2.84e^{-8}$	2	37.639	$3.5e^{-6}$
Dim1	1	8.772	0.003	1	0.002	0.967	1	2.383	0.126	1	3.904	0.067
Dim2	1	2.017	0.156	1	0.426	0.515	1	0.971	0.327	1	3.755	0.079
Herbicides/year: Dim1	2	2.477	0.290	3	2.753	0.047	3	7.212	0.0002	–	–	–
Herbicides/year: Dim2	2	0.493	0.782	3	2.569	0.05	3	2.807	0.044	2	22.57	0.0003

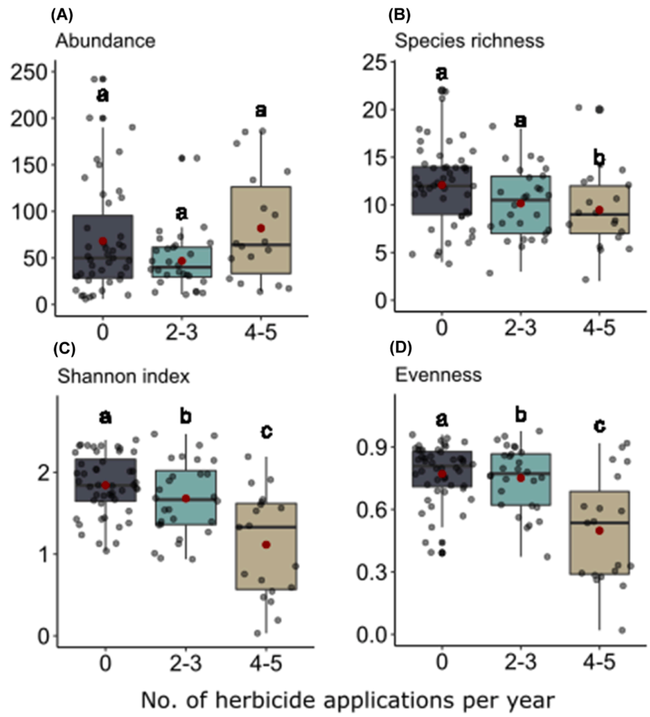

Fig. 2. Boxplots showing abundance (A) and diversity metrics: Species richness (B), Shannon index (C) and Evenness (D) of soil macro-arthropods according to the number of herbicide applications per year in banana plots. The bold horizontal bars indicate the median, the box indicates the first and the third quartile and whiskers indicate the minimum and maximum excluding the outliers. Red points indicate means. Grey points indicate samples; n=48 for 0 herbicides/year, n=24 for 2–3 herbicides/year and n=20 for 4–5 herbicides/year). Lowercase letters indicate significant differences after Tukey post-hoc test (p < 0.05), depending on herbicide applications per year in banana plots.

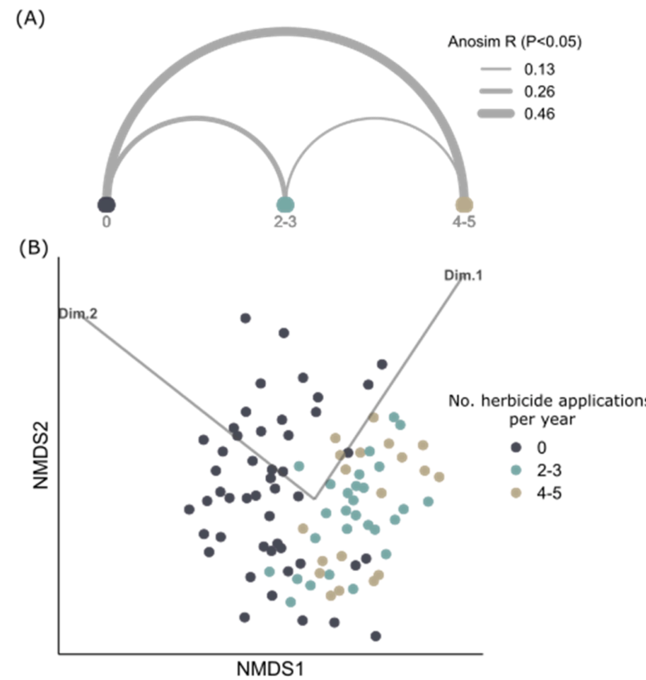

Fig. 3. (A) Arcplot representing the results of a similarity analysis (ANOSIM) comparing soil macro-arthropod community species composition depending on herbicide applications per year in banana plots. The thickness of the arc represents the R statistic. The higher the R is, the more dissimilar the communities are. (B) NMDS plot of species composition of soil macro-arthropod communities according to herbicide applications per year. Vectors represent relationships between macro-arthropod communities and pedoclimatic factors represented by the PCA coordinates; Dim1 and Dim2 (squared correlation coefficient R^2 = 0.32 and 0.45 respectively, p=0.001).

Effect of herbicides on soil macro-arthropod trophic groups

The abundance of individuals in different trophic groups showed a contrasting response in the banana plots depending on herbicide applications (Fig. 5). The abundance of predators, herbivores and decomposers decreased significantly in plots with 4–5 herbicides/year (54%, 8% and 23%, respectively) while omnivores significantly increased with 55% in plots with 4–5 herbicides/year compared to plots with no herbicide use. However, the diversity (mean species richness, Shannon index and evenness) of all trophic groups significantly decreased in plots with 4–5 herbicides/year. More specifically, the mean species richness of omnivores, predators, herbivores and decomposers was 17%, 22%, 55% and 55% lower, respectively, in plots with 4–5 herbicides/year compared to plots with no herbicide use. Shannon index and evenness followed the same trend and decreased significantly in plots with 4–5 herbicides/year compared to plots with no herbicide use (Fig. 5). The pedoclimatic variables (Dim1 and Dim2) only had a significant effect on omnivore abundance, predator abundance, decomposer abundance, and the mean species richness (Table 4).

Discussion

Diversity loss due to herbicide applications: A cascading effect

Recent studies documented a massive decline of insects (Hallmann et al., 2017; Semmens et al., 2016). Industrial farming practices, such as intensive use of pesticides, in addition to habitat loss, were clearly in focus as the likely causes of this decline (Sánchez-Bayo & Wyckhuys, 2019; Dudley & Alexander, 2017). Our results come with clear evidence that herbicide applications cause a significant decrease in soil macro-arthropod diversity in farmers' banana fields in Martinique. The mean species richness was 21% lower when herbicides were applied at a high frequency. Moreover, our results showed that the diversity of all trophic groups (omnivores, predators, herbivores and decomposers) decreased with herbicide applications: e.g., 17%, 22%, 55% and 55% lower mean species richness, respectively, in plots with high herbicide applications compared to plots with no herbicide use (Fig. 5). These

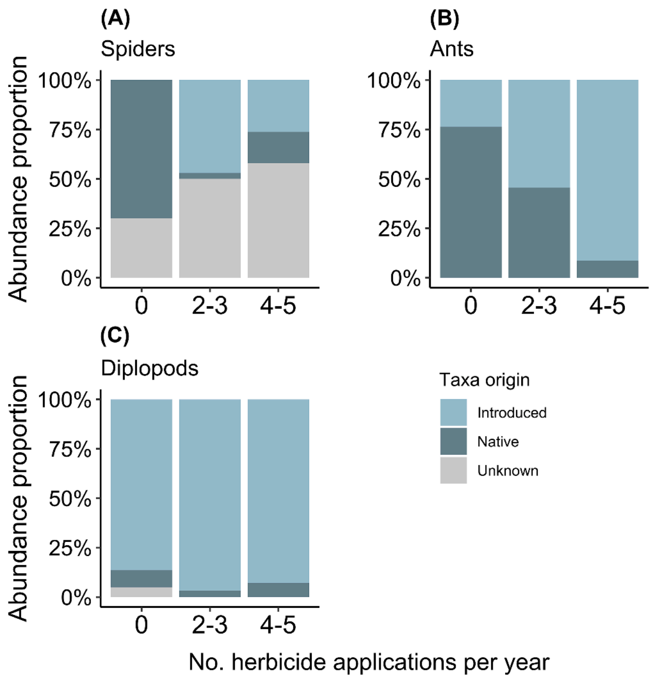

Fig. 4. Proportions of native and introduced species abundance for spiders (A) ants (B) and diplopods (C) depending on herbicide applications per year in banana plots. Species of unknown origin are assigned to the unknown category.

results conflict with the findings of Hagner et al. (2019) who showed that there was no effect of herbicide (glyphosate) on soil trophic groups in a controlled field study. Our study, carried out *in situ*, in cultivated fields, and on very diverse invertebrate communities (more than 100 species), does not allow us to draw conclusions on a possible direct toxicity of herbicides. It would require manipulative experiments on a large number of species to unravel this question. However, our results suggest that the effect of herbicides on macro-arthropod diversity could be mediated by a bottom-up trophic effect. Indeed, although a modification of the micro-habitat may have played a role, we believe that it is probably through the suppression of an important primary resource in the soil food web (ie. weeds and weed residues) that herbicides influenced macro-arthropod diversity in this study (Menezes & Soares, 2016; Brust, 1990; Cortet & Poinsot-Balaguer, 2000). Indeed, disturbing or removing that primary resource could influence detritivores and herbivores as well as predators that feed on mesofauna, triggering a cascading effect throughout the food web (Dyer & Letourneau, 2003) that may even reach vertebrates living in the agroecosystem. It has been shown that populations of lizards, birds and frogs declined at the same time when a declining trend in arthropods was spotted (Lister & Garcia, 2018).

Herbicide effects on macro-arthropod abundance depend on the trophic group: A pendulum effect

Unlike diversity, total macro-arthropod abundance did not display the same downward trend with the frequency of herbicide use. Our analysis on trophic groups showed that predator, herbivore and decomposer abundances decreased significantly, and drastically, with herbicide applications. These results are consistent with other studies, suggesting that predator (such as spiders, carabids and beetles), herbivore and decomposer abundances are higher in organic farming fields due to a large amount of weeds and a consistent soil cover when herbicides are not used (Coulis, 2021; Dassou & Tixier, 2016). Indeed, herbivores are directly affected by the suppression of weeds, leading to a decrease in their abundance and thus a decline in predator abundance. Conversely, omnivore abundance increases significantly with herbicide applications, probably because they are generalists occupying vacant niches, with a broader range of food preferences (Sánchez-Bayo & Wyckhuys, 2019). Orthoptera (Gryllidae/Grylloidea), for example, were very abundant in plots with high herbicide applications: 4±0.9 individuals per trap in plots with 4–5 herbicide/year compared to 0.6±0.1 in plots with no herbicide use. This finding supports other studies that have shown their great abundance at disturbed sites (Báldi & Kisbenedek, 1997). Given that plots receiving herbicides undergo suppression of the soil cover, crickets could be favoured and proliferate under such conditions. This pendulum effect (decrease in one group and increase in another at the same time) explains the inconclusive effect of herbicide applications on the abundance of the overall community.

A shift in community composition: Herbicides may modify agroecosystem functioning

Beyond the decrease in local richness, our results showed that macro-arthropod community composition was drastically modified by herbicide use. Such a change in community composition may alter ecosystem functioning and its stability (Chapin et al., 2000). Many of the macro-arthropods found in plots with reduced herbicide applications provide services within the agroecosystem (pest bioregulation, soil structuring and nutrient cycling). Indeed, predator biodiversity can play an important role in pest regulation. For instance, in banana agroecosystems several predators, such as ants and earwigs, feed on *Cosmopolites sordidus* (Mollot et al., 2014), the banana weevil for which chlordecone, an organochlorine insecticide, was used until 1993 (Cabidoche et al., 2009). A richer and more abundant predator community can therefore boost conservation biological control and help reduce insecticide use. Moreover, decomposers, such as diplopods and isopods,

Table 3
Results of GLM p-values to test for the effects of herbicide applications and pedoclimatic factors on native species abundance proportions. "-" indicates that the variable was not selected in the most parsimonious model.

	Spiders			Ants			Diplopods		
	DF	Chi^2	p	DF	Chi	p	DF	Chi^2	p
					Native proportion				
Herbicides/year	2	9.6	0.008	2	18.6	9.e-5	2	2.2	0.322
Dim1	–	–	–	–	–	–	–	–	–
Dim2	1	0.8	0.371	1	5.8	0.016	1	3.9	0.047
Herbicides/year: Dim1	–	–	–	–	–	–	–	–	–
Herbicides/year: Dim2	–	–	–	–	–	–	2	7.8	0.02
					Introduced proportion				
Herbicides/year	2	13.0	0.001	2	18.6	9.e-5	2	2.2	0.322
Dim1	–	–	–	–	–	–	–	–	–
Dim2	1	4.2	0.041	1	5.8	0.016	1	3.9	0.047
Herbicides/year: Dim1	–	–	–	–	–	–	–	–	–
Herbicides/year: Dim2	–	–	–	–	–	–	2	7.8	0.02

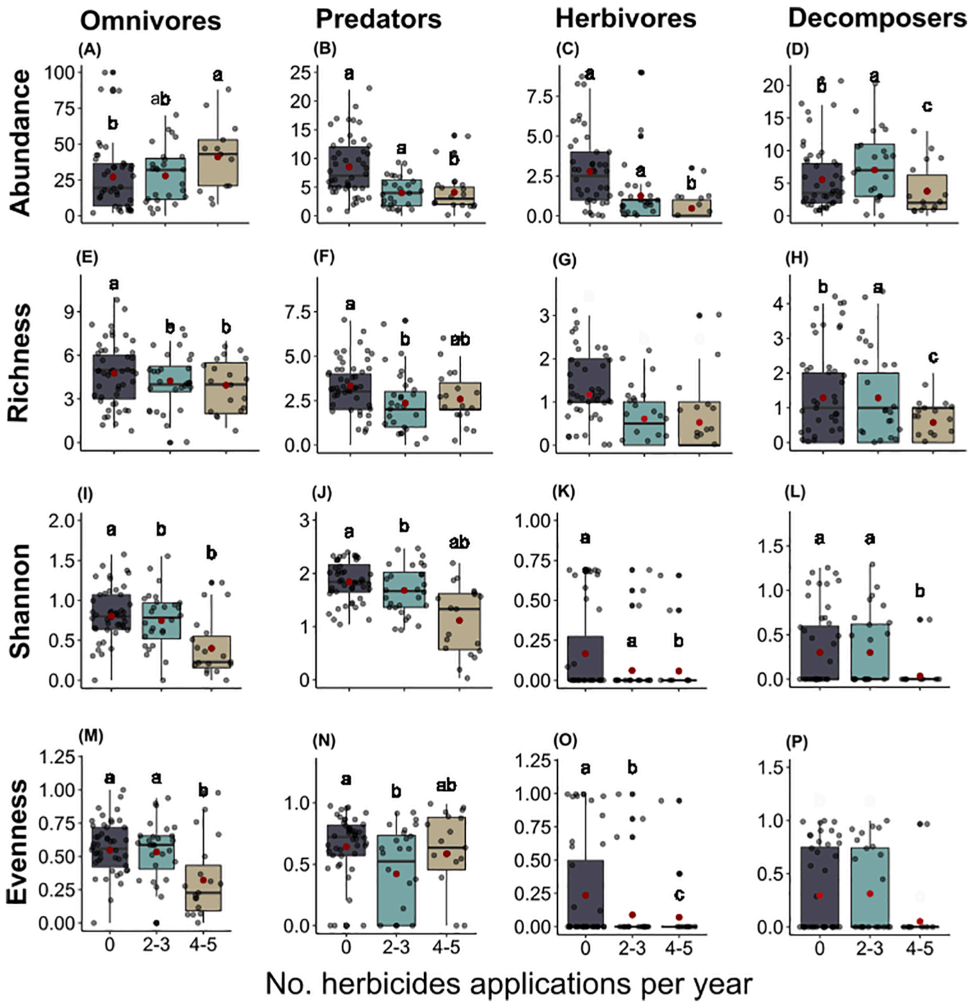

Fig. 5. Boxplots of abundance (A, B, C, D) and diversity metrics: Species richness (E, F, G, H), Shannon index (I, J, K, L) and Evenness (M, N, O, P) for soil macro-arthropod trophic groups depending on herbicide applications per year in banana plots. The bold horizontal bars indicate the median, the box indicates the first and the third quartile and whiskers indicate the minimum and maximum excluding the outliers. Red points indicate means. Grey points indicate samples; n=48 for 0 herbicides/year, n=24 for 2–3 herbicides/year and n=20 for 4–5 herbicides/year. Lowercase letters indicate significant differences after Tukey post-hoc test ($p <$ 0.05), depending on herbicide applications per year in banana plots.

Table 4
Summary of LM and GLM p-values to test for the effects of herbicide applications and pedoclimatic factors on the abundance and diversity metrics of soil macro-arthropod trophic groups. "-" indicates that the variable was not selected in the most parsimonious model.

	Abundance				Richness				Shannon				Evenness			
	Omn	Pred	Herb	Dec	Omn	Pred	Herb	Dec	Omn	Pred	Herb	Dec	Omn	Pred	Herb	Dec
Herbicides/year	7.1e-7	0.001	4.7e^{-7}	2.2e^{-16}	0.251	0.02	0.179	0.001	0.003	0.004	0.239	0.04	0.001	0.015	0.04	0.277
Dim1	0.009	0.002	0.8	6.9e^{-14}	0.972	–	–	0.407	0.09	–	–	–	0.318	–	0.06	–
Dim2	0.198	0.761	0.06	3.2e^{-13}	0.413	–	–	0.01	0.99	–	–	–	–	–	–	0.868
Herbicides/year: Dim1	0.287	0.001	1.1e^{-9}	0.001	–	–	–	0.06	–	–	–	–	0.002	–	–	–
Herbicides/year: Dim2	0.309	0.001	2.7e^{-10}	1.02e^{-10}	0.004*	–	–	0.041*	0.001*	–	–	–	–	–	–	0.015*

make an important contribution to litter comminution and its decomposition (Coulis et al., 2016). Indeed, studies have shown that they are not only facilitators of microbial decomposition, but also able to chemically break down organic matter with their endogenic enzymes (Joly et al., 2020; Griffiths et al., 2021). Increasing decomposer populations in agroecosystems can consequently enhance nutrient cycling and plant growth.

Management implications

On the one hand, our results show an increase in non-native species in plots treated with glyphosate, suggesting a synergy between two important factors in the decline of global biodiversity (pesticides and invasive alien species) (Maxwell et al., 2016). Thus, pesticides may have an even greater effect on biodiversity than previously thought via this feedback loop on invasive alien species. In our study, the removal of vegetation cover and residues by herbicides probably favoured non-native species with greater dispersal capabilities and tolerance to local microclimatic conditions than native species that evolved in a tropical island environment that was primarily forested and whose microclimate was probably highly buffered. On the other hand, a cessation or reduction of herbicides could play a role in conserving soil macro-arthropod biodiversity. The herbicide-free banana fields that were monitored in this study were part of former farms that were much more heavily treated than they are today, according to the practices in effect twenty years ago. Our results have therefore indirectly shown that an ecological restoration of environments disturbed by intensive agriculture is possible. Nevertheless, it is important to point out that a return of soil biodiversity following the cessation of herbicides is not systematic. Indeed, the use of ploughing as an alternative method of weed control can be even more detrimental to soil biology than herbicides, as has been observed in organic vineyards in the south of France for example (Coll et al., 2011). In our case, the weed control was done by mowing the vegetation cover which is a practice that allows to preserve the soil and is therefore particularly interesting to preserve the soil biodiversity.

Our results strongly suggest that a decrease in herbicide use also favoured the return of a greater proportion of native species, whose conservation could be at stake. In the current context, with an extinction rate of 2.5% per year, it is crucial to seek to conserve macro-arthropods, before reaching a loss of biodiversity that leads to irreversible destabilization of the ecosystem (Sánchez-Bayo & Wyckhuys, 2019). Proponents of land sparing advocate input intensification in agriculture to maximize yields and to avoid the destruction of natural habitats (Phalan et al., 2011). However, in the context of the Caribbean Biodiversity Hotspot (Marchese, 2015; Sieber et al., 2018), the remaining primary natural habitats account for only 11% of total land area (Myers et al., 2000). The rate of endemism is very high and some species are sometimes endemic to a single mountain or small locality. It therefore seems preferable to maximize areas that can serve as habitats for potentially threatened species. Thus, by reducing harmful agricultural practices, such as herbicide use, large agricultural areas could provide useful habitats for potentially threatened species that are restricted in distribution. Ultimately, our results showed that a conservation strategy of the land sharing type (Tscharntke et al., 2012) could be effective and constitute a complementary lever to nature reserves, especially in a tropical island context.

Conclusion

Our research showed a significant decrease in soil macro-arthropod diversity with herbicide applications in banana fields. Moreover, this diversity loss was observed for all trophic groups. The latter strongly suggests that the effect of herbicides was related to a cascading effect on the food web due to removal of the primary trophic resource. Our results also showed that species composition inside macro-arthropod communities varied significantly with herbicide applications. The abundance of native spiders and ants was greater in herbicide-free plots. This finding suggests that reducing herbicide applications may contribute to the conservation of native species, which is a crucial issue in the context of a worldwide decline in insects. Furthermore, the farmers involved in our study were technically able to turn a new leaf and reduce their herbicide use. As a consequence, our study clearly suggests a return of biodiversity in their plots, which is a major ecological benefit in the functioning and stability of their agroecosystems.

Data availability statement

Data available via https://doi.org/10.18167/DVN1/ROYNXM.

CRediT authorship contribution statement

Meryem El jaouhari: Conceptualization, Formal analysis, Investigation, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. **Gaëlle Damour:** Conceptualization, Formal analysis, Investigation, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. **Philippe Tixier:** Conceptualization, Formal analysis, Investigation, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. **Mathieu Coulis:** Conceptualization, Formal analysis, Investigation, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing.

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.

Acknowledgments

We should like to express our sincere thanks to the farmers who took time to listen to us and answer our questions and who allowed us to carry out this study on their farms. We are very grateful to Thibault Ramage (ant specialist), Lilou Leonetti and Patrick Maréchal (spider specialists) for their valuable help in identifying macro-arthropods and determining their distribution in the Caribbean. We are grateful to Damien Beillouin for his help with the statistical analysis. We should like to thank Laurent Gervais for his help in selecting the banana plantations. We also thank Christiane Mauriol, Jérôme Sainte-Rose and Roger Rieux for their valuable help during field sampling.

This work was made possible thanks to funding provided by the Sustainable Banana Plan II project co-financed by Europe (EAFRD fund) and the territorial community of Martinique (CTM).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.baae.2023.10.001.

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