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Céline Pelosi, Lucile Toutous, François Chiron, Florence Dubs ...+6 more authors

Institutions: Institut national de la recherche agronomique, Centre national de la recherche scientifique, Agro ParisTech

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C. Pelosi[a,*], L. Toutous[a], F. Chiron[b], F. Dubs[c], M. Hedde[a], A. Muratet[d], J.-F. Ponge[e], S. Salmon[e], D. Makowski[f,g]

[a] INRA, UR251 PESSAC, F-78026 Versailles cedex, France
[b] Museum national d'Histoire naturelle, UMR 7204 MNHN-CNRS-UPMC. 55 rue Buffon, 75005 Paris, France
[c] IRD, UMR BIOEMCO, Centre France Nord, 93143 Bondy Cedex, France
[d] ODBU, Observatoire départemental de la Biodiversité urbaine, Direction de la Nature, des Paysages et de la Biodiversité, Conseil général de la Seine-Saint-Denis, Hôtel du Département, F-93006 Bobigny Cedex, France
[e] Muséum National d'Histoire Naturelle, CNRS UMR 7179, 4 Avenue du Petit-Château, 91800 Brunoy, France
[f] INRA, UMR211 Agronomie, BP 01, F-78850 Thiverval-Grignon, France
[g] AgroParisTech, UMR211 Agronomie, BP 01, F-78850 Thiverval-Grignon, France

* Corresponding author: UR 251 PESSAC INRA, Bâtiment 6, RD 10, 78026 Versailles cedex, France. Tel: (+33)1.30.83.36.07; Fax: (+33)1.30.83.32.59. E-mail address: celine.pelosi@versailles.inra.fr

Abstract

Agricultural intensification has led to reduced soil biodiversity in arable lands. The potential benefits from organic farming and from low-input cropping systems have not yet been precisely assessed. Earthworm, having important agro-ecological functions, may be affected by pesticide applications, especially those species living mainly in the surface soil layer. We used a five-year experimental database including conventional and organic cropping systems to establish simple relationships between the Treatment Frequency Index - a phytosanitary indicator of pesticide pressure - and the abundance of three important earthworm species. We found that insecticides were more harmful to earthworms than herbicides and fungicides, and that species living in the soil's surface layer were the most affected by pesticides. *Lumbricus castaneus* density could be quadrupled if the Treatment Frequency Index was halved, as is currently required by some European regulations. Our results thus demonstrate that a reduction in pesticide application would strongly increase earthworm population density in agricultural fields.

Keywords: Earthworm density; Treatment Frequency Index; Organic farming; Conventional cropping system; Pesticides

1. Introduction

Agricultural intensification has reduced soil biodiversity in cultivated fields (Bengtsson et al., 2005; Doran and Zeiss, 2000; Hubbard et al., 1999). Organic and low-input cropping systems have been proposed as alternatives to intensive agricultural practices to limit the impact of chemicals on human health and on the environment (Bengtsson et al., 2005; Hole et al., 2005). However, the effects of a reduction of pesticide applications on biodiversity and particularly on soil organisms need further investigation (Hole et al., 2005).

Earthworms represent a large proportion of soil biomass, i.e. up to 80% of fresh weight (Yasmin and D’Souza, 2010) and ensure important agro-ecological functions since they influence organic matter dynamics and soil structure (Edwards and Bohlen, 1996; Sims and Gerard, 1999). Earthworms are recognized as ecosystem engineers because they influence the availability of resources to other species (Jones et al., 1994) and have positive effects on organic matter dynamics and soil structure (Edwards and Bohlen, 1996). They are also considered as bioindicators of soil biological functioning (Paoletti, 1999). Bouché (1972) separated earthworms into three categories, based on morphological and behavioral characteristics. Epigeic species, e.g. *Lumbricus castaneus*, are litter-dwellers living and feeding on or near the soil surface. Anecic earthworms live in permanent vertical burrows within the soil and may emerge to feed on surface litter, e.g. *Lumbricus terrestris*. Endogeic species e.g., *Allolobophora chlorotica*, live in temporary horizontal burrows and feed on the soil. This species is geophagous since it gains its nutrients by eating the soil and the green morph is characterized by Bouché (1972) as more epigeic.

Laboratory studies have shown that earthworms are exposed to pesticides through ingestion or epidermal contact (Rodriguez-Castellanos and Sanchez-Hernandez, 2007; Yasmin and D'Souza, 2010). Little is known about the effects of earthworm exposure to pesticides in cultivated fields because most studies were conducted under laboratory conditions (Frampton et al., 2006; Yasmin and D'Souza, 2010) and cannot be easily extrapolated to field conditions (Lowe and Butt, 2007; Svendsen and Weeks, 1997). Field studies which compared earthworm communities in organic and conventional systems have shown variable results (Hole et al., 2005; Nuutinen and Haukka, 1990), mainly due to confounding factors such as variation in soil tillage, manure inputs or crop types (Hole et al., 2005). We decided to study the effect of pesticide use in agricultural fields involving conventional plowing and the same crop, i.e. winter wheat, on three earthworm species: *L. castaneus*, *L. terrestris* and *A. chlorotica* which are widespread in Europe and variably in contact with the soil surface and thus potentially exposed to pesticides (Römbke et al., 2004). These species belong to the three ecological categories mentioned above and are involved in the decomposition of surface and soil organic matter and in soil structure maintenance.

Pesticide risk assessment for human health and the environment has become a major concern for scientists, politicians and civil society (Pingault et al., 2009; Sattler et al., 2007). In Denmark the Treatment Frequency Index (TFI) was developed (Gravesen, 2003; Jørgensen, 1999; Jørgensen and Kudsk, 2006) for the assessment of pesticide pressure at different scales, from field to national level (Butault et al., 2011; Ferti Ouest 88, 2009). TFI is defined as the mean number of treatments per hectare with commercial products, weighted by the ratio of the dose used to the recommended dose (Pingault, 2007). TFI is easy to calculate and operational at different levels, since it allows the aggregation of very different substances to measure overall phytosanitary pressure (Butault et al., 2011). This indicator requires investigations on pesticide use in agricultural fields.

This study aims at i) establishing statistical relationships between the pressure indicator TFI and impact indicators for soil fauna, i.e. densities of three earthworm species variably in contact with the soil surface, ii) using these relationships to estimate threshold values of TFI leading to an effect on earthworm densities, and iii) using these relationships to estimate the effects of reduced pesticide use on earthworm densities.

2. Materials and methods

2.1. Sites and cropping systems

Field data were collected for 15 site-years with conventional cropping systems and 15 site-years with organic systems. A site-year is a unique combination of a site and a year. Study sites were located in two agricultural areas of the Ile-de-France region and sampled between 2005 and 2012 (Appendix A).

Eleven conventional and eleven organic sites were studied in 2012 in the department of Seine-et-Marne, east of Paris, on clay loamy soils with 70% silt, 25% clay and 5% sand and a neutral pH (Appendix A).

Four conventional site-years and four organic site-years were studied in 2005, 2006, 2007 and 2011 from a trial located in Versailles, 15 km south-west of Paris, on silty clay soils with 58% of silt, 17% of clay and 25% of sand and a neutral pH (Appendix A).

No significant differences of texture, pH, organic matter and C/N ratio were found ($p>0.05$) between organic and conventional systems (ANOVA, R version 2.15.3, 2013, data from Appendix A).

The climate in both study areas is oceanic temperate with a mean annual precipitation of 640 mm and a mean annual temperature of 10.4 °C.

A conventional plowing at 25-30 cm depth was performed in all fields, at a frequency ranging from every year to once every three years. The last plow was performed in 2010 or 2011, depending on the fields. All fields were cultivated with winter wheat at the time of sampling. The levels of mineral fertilizers applied were quite similar across conventional fields. No organic input was applied in organic and conventional fields in Versailles. In Seine-et-Marne, six organic fields and six conventional fields received organic inputs (poultry and cattle manure, vegetable wastes or vinasse depending on the field). Some farms included both fields under organic and conventional farming. This may explain why organic inputs were similar. Moreover, the number of organic applications and the density of the three earthworm species were not significantly correlated.

2.2. TFI calculation

Based on surveys conducted with field managers (farmers in Seine-et-Marne or the trial manager in Versailles), the name of the pesticides used, including insecticide, herbicide and fungicide treatments, the number of applications and the rate applied to the fields were used to calculate the TFI. The index was calculated over one year before each sampling date because mean Dissipation Time 50 (DT50) and mean DT90 in the field (i.e. time for respectively 50% and 90% disappearance of the active ingredients applied at specific initial concentrations in the field, in all our experimental fields), were respectively 2 and 8.5 months (PPDB, 2012) (Appendix B).

TFI was calculated using the following formula: $TFI_{field} = \Sigma\ (AD / HD)$, where AD is the amount of pesticide applied in a field per hectare and HD is the recommended rate per hectare (Ministère de l’Agriculture et de la Pêche, 2008). Four types of TFI were calculated, namely TFI Herbicide, TFI Insecticide, TFI Fungicide and TFI Total which is the sum of the three TFIs (Appendix C). TFIs are equal to 0 in organic fields because no chemical pesticides were applied.

2.3. Earthworm sampling method

Sampling was performed on each site on ten replicates in 2005, 2006, 2007 and 2011, and on three replicates in 2012 (see Appendix A for sites concerned) using both chemical extraction and hand-sorting of earthworms (Pelosi et al., 2009). After removing the vegetation on the ground surface, two applications of 3.2 l of a diluted expellant solution of allyl isothiocyanate (AITC) was applied to the soil at 10-min intervals within a 40 x 40 cm metal frame. AITC was first diluted with isopropanol (propan-2-ol) to obtain a 5 g l^{-1} solution (Pelosi et al., 2009; Zaborski, 2003). This solution was then diluted with water to reach a concentration of 0.1 g l^{-1}. After collecting emergent individuals during 20 minutes, a 40 cm x 40 cm x 20 cm-depth block of soil was excavated and remaining earthworms were hand-sorted from the soil. Earthworms were preserved in 4% formalin solution. All individuals (juveniles, sub-adults and adults) were counted. Sub-adults and adults were identified at species level according to the identification key of Sims and Gerard (1999). Juveniles were also identified at species level thanks to morphological characteristics of the species and to the specific form they take in formalin in comparison with that of identified adults. We focused on three earthworm species found in cultivated fields (Bouché, 1972). *Lumbricus castaneus*, which may be also found occasionally within the soil profile, is an epigeic species living mainly at the soil surface. *Lumbricus terrestris* is an anecic species feeding on the soil surface but living deeper in the soil. Sampled individuals of the third species, the endogeic *Allolobophora chlorotica* presented a green coloration which is more epigeic than the albinic form (Bouché, 1972). The green form of *A. chlorotica* is commonly found in the top 5 cm of the soil.

2.4. Statistical Analysis

The response variable was the density of earthworms per m^2. This variable was related to TFI using two statistical methods. The first method was based on Poisson log-linear regression. A Poisson model relating earthworm density to TFI was fitted for each species using the glm function of R (Venables and Ripley, 2002). A separate regression model was fitted for each type of TFI (corresponding to herbicide, insecticide, fungicide separately, and to all three pesticides together) leading to four different regression models per earthworm species. Each model relates the expected earthworm density to TFI as follows:

$$E(Y_i) = e^{\alpha_i + \beta_i TFI}$$

where Y_i is the density of earthworms of species i, E(.) is the expected value, α_i and β_i are two parameters corresponding to the log density for TFI=0 (i.e., maximum of the log density if β_i <0) and to the TFI effect respectively. Estimated parameter values, their standard deviations, and their associated p-values were used to analyze the effect of TFI on earthworm density and its interaction with the species. In order to assess the robustness of the results to the dataset characteristics, parameter estimation was repeated with a restricted dataset including the 22 sites located in Seine-et-Marne. The Akaïke Information Criterion (AIC) was computed for model with and without TFI variables and models including TFI showed better (i.e., lower) AIC values. The significance of the differences of the estimated TFI-effects across species was tested by including a species-effect and a TFI-effect (main effect and interaction) in the Poisson log-linear regression model, and by testing the significance of the interaction. The fitted models were used in three different ways. First, the models were used to estimate earthworm densities for low and high TFI values (equal to the 1st and 3rd quartiles of TFI data of our dataset respectively). Second, the models were used to calculate the TFI values leading to 50% and 75% of the earthworm densities obtained for TFI=0 (i.e, without pesticide application). Third, the models were used to assess the consequences of a reduction of 50% of the mean TFI values measured in France in 2006 according to Jacquet et al. (2006).

In the second method, a non-parametric technique was used to estimate the relationship between earthworm density and TFI. A polynomial quadratic regression was fitted locally using the loess function of R (Cleveland et al., 1992). With this approach, a quadratic function is fitted locally at each TFI value x using data weighted by their distance to x. As the quadratic function is applied locally, the overall relationship between density and TFI can take various shapes depending on the data distribution. Local regression was applied for each type of TFI and each earthworm species separately. Like Poisson regression models, fitted response curves were used to estimate earthworm densities for low and high TFI values and to estimate TFI thresholds.

Contrary to Poisson regression, the non-parametric method does not rely on any assumption about the probability distribution of the data. However, non-parametric methods generally produce less accurate estimated values with small datasets. Results obtained with the two methods were compared in order to assess the robustness of the conclusions to the statistical technique used to analyze the data.

3. Results

A. chlorotica, L. castaneus, and *L. terrestris* densities ranged from 0 to 135, 105, and 44 individuals m^{-2}, respectively, and the TFI Total in conventional sites ranged from 1.6 to 7.0 (mean = 4.1) (Fig. 1). When TFI Total was 0, mean values of earthworm densities were 25.0 ± 37.8, 7.5 ± 27.0, and 5.6 ± 12.4 individuals m^{-2} for *A. chlorotica, L. castaneus*, and *L. terrestris* respectively (Fig. 1).

Estimated values of β_i i.e., TFI effect on earthworm density, are presented in Table 1. The values of this parameter correspond to the effects of a one-unit increase of TFI on the log earthworm density. TFI Total, TFI Herbicide, TFI Insecticide, and TFI Fungicide exerted significant negative effects on earthworm densities for the three considered species (Table 1). TFI effect differed significantly between the three species for all TFI categories ($p<0.05$), and was invariably higher for *L. castaneus* for all the TFIs compared to *L. terrestris* and *A. chlorotica*. TFI effects were always the lowest for *A. chlorotica*, effects on *L. terrestris* being intermediate (Table 1).

The effect of TFI Insecticide was the largest for all three species (Table 1). For *L. castaneus*, TFI Fungicide had a stronger effect than TFI Herbicide while the opposite result was obtained for *L. terrestris* and *A. chlorotica* (Table 1). In order to assess the robustness of the results to the dataset characteristics, parameter estimation was repeated with a dataset restricted to the 22 sites located in Seine-et-Marne. Results obtained with the full and the restricted datasets are compared in Table 1. The results obtained for *A. chlorotica* and *L. terrestis* show that the TFI effect is still significant (p<0.05) even when the data obtained in the trial are excluded from the analysis. The ranking of these species are similar with the full and the restricted datasets for all type of TFI. In addition, results obtained with the restricted dataset confirm that TFI Insecticide had a stronger effect on earthworm density than the other types of TFI. It was not possible, to fit the model for *L. castaneus* with the restricted dataset because only one non-zero data was included in this dataset for this species.

With the Poisson regression model, densities of *L. castaneus* reached values below 1 individual m^{-2} when TFI Total, TFI Herbicide, TFI Insecticide, and TFI Fungicide were 2.8, 1.7, 0.9, and 1.0 respectively. Estimated density for *L. terrestris* reached values below 1 individual m^{-2} when TFI Total, TFI Herbicide, and TFI Insecticide were 5.8, 2.9, and 1.9, respectively but did not reach values below 1 individual m^{-2} for TFI Fungicide values considered in our dataset (Fig. 2). Estimated densities of *A. chlorotica* were always above 1 individual m^{-2} for the observed TFI values, but strongly decreased at high TFI. *A. chlorotica* densities were 23.7%, 18.6%, 31.8% and 43.4% of the maximum estimated density values when TFI Total, TFI Herbicide, TFI Insecticide, and TFI Fungicide reached the highest values reported in the dataset (Fig. 2).

Results obtained with the two statistical methods were similar (Table 2). Earthworm densities estimated for TFI = 0 using Poisson and non-parametric regressions were almost identical. When TFI Total, TFI Herbicide, TFI Insecticide, and TFI Fungicide were 4.5, 2.4, 1.0, and 1.0 respectively (i.e. the third quartiles of TFI in the dataset), Poisson and non-parametric models again showed similar earthworm density values (Table 2). Differences were greater for the density estimates obtained for *A. chlorotica* and for the third quartile of TFI Herbicide and Fungicide. In this case, the Poisson models led to a lower estimate of *A. chlorotica* density for the third quartile of TFI Herbicide and to higher estimated density for the third quartile of TFI Fungicide (Table 2) compared to non-parametric estimated values. Standard errors of non-parametric models were higher than those obtained with Poisson models, due to the relatively small size of our dataset (Table 2).

Using both models, threshold values to maintain 50% and 75% of the maximum density of the three species were calculated for the four TFI categories (Table 3). Each species showed different threshold values for the four TFI categories, *L. castaneus* showing the lowest thresholds (except in one case, for TFI Insecticide with the non-parametric model, where thresholds for 50% of the maximum density for *L. castaneus* and *L. terrestris* were 0.3 and 0.2 respectively) and *A. chlorotica* usually the highest ones (except for TFI Total and TFI Herbicide with the non-parametric model, for which thresholds for *L. terrestris* were higher than those of *A. chlorotica*). Intermediate threshold values were obtained for *L. terrestris*. The lowest threshold values were usually obtained for TFI Insecticide (except for 50% of *A. chlorotica* maximum density with the non-parametric model, where thresholds of TFI Insecticide and TFI Fungicide were 0.9 and 0.5 respectively).

According to Jacquet et al., (2011), mean TFI Total in 2006 in France was 3.8, which corresponds to an estimated density of 0.5 ± 0.2, 2.3 ± 0.4, and 11.4 ± 0.9 individuals m^{-2} for *L. castaneus*, *L. terrestris*, and *A. chlorotica* respectively, using Poisson models (Table 4). According to the fitted models, 50% reduction of the TFI target (proposed by the French government for 2018, Butault et al., 2011) would increase *L. castaneus*, *L. terrestris, and A. chlorotica* densities by a factor 3.8, 1.4, and 1.5 according to Poisson models and by a factor

4.8, 1.5, and 1.5 according to the non-parametric method, compared to values estimated for TFI in 2006 (Table 4). Pesticide reduction objectives set up in Denmark in 2009 are close to French target values for 2018 and lead to similar earthworm densities (Table 4).

4. Discussion

Earthworm density values found in this study were in the same range as values reported by other authors in plowed cultivated fields (Berry and Karlen, 1993; Schmidt et al., 2001). We found that densities were higher for *A. chlorotica* than for *L. castaneus* and *L. terrestris,* a well-known relationship between soil invertebrates of increasing size (Petersen and Luxton, 1982). Moreover, earthworms, especially anecics and epigeics, may be less abundant because plowing affects earthworms directly through mechanical damage or exposure to predation as well as indirectly through consequent changes in the soil environment. These changes include destruction of burrows, loss of surface organic matter, and changes in soil physical conditions such as water content and temperature (Chan, 2001; Edwards and Bohlen, 1996).

The three ecological categories of earthworms, represented by the three studied species, were all reduced in number by pesticides but *L. castaneus* seemed to be the most sensitive species, followed by *L. terrestris* and finally *A. chlorotica*. Estimated earthworm densities reached zero at the highest TFI values reported in our dataset, except for *A. chlorotica*. Apart from the work of Rault et al. (2007), who demonstrated that *L. castaneus*, together with *L. terrestris*, exhibited the strongest physiological reaction to pesticide exposure when compared with endogeic and endo-anecic species, little is known about the sensitivity of *L. castaneus* to pesticides. However, according to Culy and Berry (1995) and other authors (Edwards and Bohlen, 1996; Lofs-Holmin, 1981; Römbke et al., 2004; Tu et al., 2011; Van Gestel, 1992), earthworms feeding on or near the soil surface are more affected by pesticides than those feeding deeper in the soil. This may be explained by the fact that most pesticides applied in cultivated fields stay in the top 2.5 cm (Van Gestel, 1992). *L. castaneus* has a higher exposure to pesticides than the two other species considered in this study, in particular when compared with *A. chlorotica*. *L. terrestris,* although living in deep soil layers, feeds on surface organic matter such as leaf litter while *A. chlorotica* lives in the first five centimeters of the soil, feeding on soil organic matter in the mineral layer. Our study suggests that the more time an earthworm species spends on or near the soil surface, the more it is affected by pesticide application.

Different assumptions may be made to explain lower earthworm densities with increasing pesticide applications. Firstly, some compounds may have lethal effects (acute toxicity), even at recommended rates, due for instance to accumulation of maximum-rate treatments (Fayolle and Stawiecki, 1990; Ruppel and Laughlin, 1977). Moreover, pesticides may affect earthworm fecundity and growth (chronic toxicity), leading to a long-term decrease in earthworm abundance (Yasmin and D'Souza, 2010). Furthermore, earthworms, being reactive to the quality of their environment, can move away to avoid unsuitable conditions (Mathieu et al., 2010). Individuals could thus escape from polluted fields. Epigeic species seem to have better dispersal capabilities than anecic species, which in turn disperse better than endogeics (Eijsackers, 2010). For instance, Eijsackers (2010) showed that the epigeic *L. rubellus* dispersed much better than the endogeic *A. chlorotica* and even than the anecic *L. terrestris.* Finally, the number of pesticide applications is likely to be positively related to the amount of field traffic which could add negative effects on earthworm populations (Hansen and Engelstad, 1999), especially on epigeic densities.

According to our results, insecticides seem to be the most harmful to the three earthworm species, followed by herbicides and then by fungicides. Jänsch et al. (2006) reviewed 92 studies dealing with insecticides, of which 60 revealed depressive effects of insecticides on earthworm densities. Tu et al. (2011) reported that a single application of insecticides i.e., carbaryl and imidacloprid, applied at “manufacturer’s suggested dose” significantly inhibited the activity of earthworm communities in the field. Our results confirm that insecticide use has to be decreased in first instance, prior to herbicides and fungicides, if we want to alleviate most pesticide effects harmful to earthworms living in contact with the soil surface.

Concerning herbicides, Jänsch et al. (2006) and others (Edwards and Bohlen, 1996; Lee, 1985) reported no effect on Lumbricidae. In our study, TFI Herbicide reduced densities of species in contact with the soil surface. Herbicides may cause community reduction by reducing sources of organic matter (leaf and root litter) on which earthworms feed. Authors who studied herbicide effects on earthworms have shown relatively few harmful responses of earthworms but they generally worked under laboratory conditions, not taking into account repeated applications, cocktail (synergistic) effects, and indirect effects like interactions between pesticides and natural stress factors which may have deleterious effects on earthworm populations (Holmstrup et al., 2010; Santos et al., 2011; Zhou et al., 2011).

Finally, in our study, fungicides seemed to have a smaller effect on *L. terrestris* and *A. chlorotica* densities than insecticides and herbicides. Jänsch et al. (2006) highlighted the harmful effect of fungicides on Lumbricidae in 50% of the reviewed studies i.e. 106 publications dealing with fungicides. Tu et al. (2011) explained that some fungicides they tested and that occurred in our study sites, i.e., thiophanatemethyl, propiconazole, chlorothalonil, and azoxystrobin, did not show significant toxicity to earthworms when applied only once, but their toxicity increased with application frequency.

Other factors than TFI are likely to influence earthworm density in agricultural fields, especially soil tillage and fertilization. It was not possible to investigate the effect of these factors in details because the experimental design was not appropriate to test the effect of tillage and fertilization. As tillage practices were similar in all site-years, it was not possible to use our dataset to estimate tillage effect. The effect of fertilization type (organic vs. nonorganic) was tested in our paper but, as no detailed information on the quantity of each type of fertilizer applied by the farmers was available, it was not possible to include fertilizer dose as a covariable in our statistical model. In addition, even if information on fertilizer doses had been available, the inclusion of this information in the statistical model would be difficult because each type of organic fertilizer has its own characteristics. Risk of confounding effect can never be considered equal to zero, but this risk is limited in our dataset because the selected fields differ mainly by their TFI values.

The average TFI Total for French farms specializing in field crops was 3.8 in 2006. The objective of reducing the use of pesticides by 50%, planned in the "Ecophyto2018 plan" in France (Jacquet et al., 2011), would mean that all French agriculture should convert to integrated farming, which will require significant effort (Butault et al., 2011). Integrated farming involves the limitation of pesticide and mineral fertilizer use by means of alternative cultural techniques, e.g. organic manures, longer rotations, and accurate monitoring of diseases and deficiencies. In the studied fields, approximately the same number of organic inputs was used in conventional and organic cropping systems. Moreover, at the rates applied in the studied sites (data not shown), mineral fertilizers are generally not harmful to earthworms (Whalen et al., 1998) and may even be indirectly beneficial to them by increasing the quantity of crop residues returned to the soils (Edwards et al., 1995). In the case of a 50% reduction in pesticide use in 2018, TFI Total would be in the range 1.9 - 2.5 according to Jacquet et al. (2011). Our models show that, if the Treatment Frequency Index was halved compared to the value obtained in 2006, as is currently required by some European regulations, then the densities of *L. castaneus, L. terrestris*, and *A. chlorotica* would increase by a factor 4.8, 1.5, and 1.5 respectively. An increase in density of these three earthworm species would favor the provision of ecosystem services such as biogeochemical cycling, soil structure maintenance and water infiltration.

The TFI is used by farmers as an index for adjusting the quantity of pesticides to the prevailing pest level in fields. In our study context, this index is convenient for calculating and comparing pesticide usage between fields. Compared to the commonly used index 'total amount of pesticides', it provides relevant information on environmental risks due to pesticides usage as doses applied are standardized to the recommended application rate. Also, TFI accounts for all active molecules composing pesticides and is weighted by the percentage of the treated area. However, it does not account for the chemical and toxic properties of some specific substances composing pesticides which can influence pesticide's effect on the environment. Moreover, different pesticide regimes may lead to similar TFI because pesticides have different homologated doses. There is thus no direct relationship between the number of applied doses and TFI values. Assessing environmental effect of each substance in a single statistical model would have been interesting but it was impossible due to over-parameterization issues.

To evaluate the risks on biological populations linked to pesticide application, ecotoxicological laboratory experiments use different indicators based on survival and reproduction as endpoints, e.g. concentrations affecting 50% of exposed individuals (LC_{50} for survival or EC_{50} for reproduction) or No Observed Effect Concentration (NOEC), but they are insufficient to extrapolate these effects to natural conditions (Baveco and De Roos, 1996). The final outcome, in terms of the damage inflicted on natural populations, depends on ecological relationships between earthworm species and the local physical, chemical and biological properties of the site (Baveco and De Roos, 1996). Here, relationships between pressure and impact bioindicators were calculated from doses applied according to current farming practices. These relationships may help in establishing objectives for reducing pesticide use according to required earthworm densities. An interesting perspective could be to explore whether these relationships would be maintained in fields involving different types of management, such as other crops and other types of tillage management.

5. Conclusion

This study demonstrated a negative relationship between TFI, a common and easily-calculated pesticide pressure indicator, and an impact bioindicator, earthworm density. Using data collected in agricultural fields, we found that earthworm species living in close contact with the soil surface were the most affected by pesticide application. The statistical relationships established in this paper are useful for predicting the effect of an increase or a decrease of TFI of three different families of pesticides on three different earthworm species. We found that a 50% reduction of pesticide use is likely to lead to large increases (i.e. up to 4.8 times more) in earthworm density and that insecticide use has to be managed as a priority to reduce the most negative effects on earthworm populations living in contact with the soil surface.

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Table 1. Estimated values of the parameter β_i corresponding to the TFI effect, obtained with Poisson regression models (standard errors of estimated values in brackets), using the four TFI categories and the three earthworm species. TFI means Treatment Frequency Index. All estimated values were significantly different from zero ($p<0.05$). Full dataset designates data from all sites (in Seine-et-Marne and Versailles). Restricted dataset designates data from the 22 sites located in Seine-et-Marne.

TFI	Earthworm species	Estimated value of β_i	
		Full dataset	Restricted dataset
Total	*A. chlorotica*	-0.21 (0.02)	-0.23 (0.03)
	L. castaneus	-0.73 (0.12)	-
	L. terrestris	-0.31 (0.06)	-0.36 (0.11)
Herbicide	*A. chlorotica*	-0.38 (0.04)	-0.39 (0.04)
	L. castaneus	-1.17 (0.19)	-
	L. terrestris	-0.64 (0.11)	-0.56 (0.17)
Insecticide	*A. chlorotica*	-0.63 (0.09)	-0.91 (0.13)
	L. castaneus	-2.14 (0.44)	-
	L. terrestris	-0.84 (0.22)	-2.0 (0.68)
Fungicide	*A. chlorotica*	-0.33 (0.07)	-0.54 (0.11)
	L. castaneus	-1.82 (0.37)	-
	L. terrestris	-0.36 (0.16)	-0.93 (0.41)

Table 2. Estimated values of earthworm densities and standard errors (in brackets) obtained with Poisson and non-parametric models for two TFI values (1st and 3rd quartile for each TFI). These values are listed for each TFI category and each earthworm species. TFI means Treatment Frequency Index.

Earthworm species	TFI	TFI value	Earthworm density (Poisson model)	Earthworm density (non-parametric model)
Allolobophora chlorotica	Total	0.0	25.0 (1.2)	25.0 (7.5)
		4.5	9.8 (0.9)	9.9 (12.5)
	Herbicide	0.0	25.3 (1.2)	25.1 (7.2)
		2.4	10.3 (0.8)	16.8 (13.0)
	Insecticide	0.0	21.7 (1.0)	21.8 (6.4)
		1.0	11.6 (1.0)	11.7 (11.9)
	Fungicide	0.0	20.5 (1.0)	22.3 6.6)
		1.0	14.7 (1.0)	6.6 (11.5)
Lumbricus castaneus	Total	0.0	7.6 (0.7)	7.5 (5.1)
		4.5	0.3 (0.1)	0.3 (8.6)
	Herbicide	0.0	7.3 (0.7)	7.2 (5.0)
		2.4	0.4 (0.2)	0.6 (8.9)
	Insecticide	0.0	5.8 (0.5)	5.8 (4.4)
		1.0	0.7 (0.3)	0.4 (8.1)
	Fungicide	0.0	5.9 (0.5)	6.1 (4.5)
		1.0	1.0 (0.3)	0.0 (7.8)
Lumbricus terrestris	Total	0.0	5.9 (0.6)	5.5 (2.4)
		4.5	1.5 (0.3)	2.2 (4.0)
	Herbicide	0.0	6.1 (0.6)	5.9 (2.3)
		2.4	1.3 (0.3)	0.7 (4.2)
	Insecticide	0.0	4.8 (0.5)	5.0 (2.1)
		1.0	2.1 (0.4)	1.3 (3.8)
	Fungicide	0.0	4.4 (0.5)	4.7 (2.1)
		1.0	3.1 (0.5)	1.7 (3.7)

Table 3. Threshold values of TFI Total, TFI Herbicide, TFI Insecticide and TFI Fungicide to obtain 50% and 75% of the maximum estimated earthworm density i.e., when TFI=0, for the three earthworm species. TFI means Treatment Frequency Index.

			Thresholds values of TFI			
Earthworm species	% of the maximum estimated earthworm density	Statistical method	Total	Herbicide	Insecticide	Fungicide
A. chlorotica	50%	Poisson model	3.3	1.8	1.1	2.1
		Non-parametric method	3.4	3.1	0.9	0.5
	75%	Poisson model	1.4	0.8	0.4	0.9
		Non-parametric method	1.4	0.7	0.2	0.2
L. castaneus	50%	Poisson model	0.9	0.6	0.3	0.4
		Non-parametric method	1.3	0.9	0.3	0.3
	75%	Poisson model	0.4	0.2	0.1	0.2
		Non-parametric method	0.6	0.4	0.1	0.1
L. terrestris	50%	Poisson model	2.2	1.1	0.8	1.9
		Non-parametric method	4.0	1.7	0.2	0.5
	75%	Poisson model	0.9	0.4	0.3	0.8
		Non-parametric method	2.5	1.1	0.1	0.2

Table 4. Density predictions of the three earthworm species as a function of the mean TFI Total in France in 2006 (Jacquet et al., 2011), the Denmark objective for 2009 (Pingault et al., 2009) and the France objective for 2018 (Jacquet et al., 2011), using Poisson regression and non-parametric models. TFI means Treatment Frequency Index.

Earthworm species	Statistical method	Mean TFI Total in France in 2006 (TFI Total = 3.8)	Denmark objective for 2012 (TFI Total = 1.7)	France objective for 2018 (TFI Total = 1.9)
A. chlorotica	Poisson model	11.4 ± 0.9	17.6 ± 0.8	16.8 ± 0.8
	Non-parametric method	11.6 ± 11.2	17.8 ± 10.1	17.2 ± 10.5
L. castaneus	Poisson model	0.5 ± 0.2	2.2 ± 0.4	1.9 ± 0.4
	Non-parametric method	0.5 ± 7.6	2.6 ± 6.9	2.4 ± 7.2
L. terrestris	Poisson model	2.3 ± 0.4	3.5 ± 0.4	3.3 ± 0.4
	Non-parametric method	3.0 ± 3.6	4.7 ± 3.3	4.6 ± 3.4

Figure1

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Fig.1

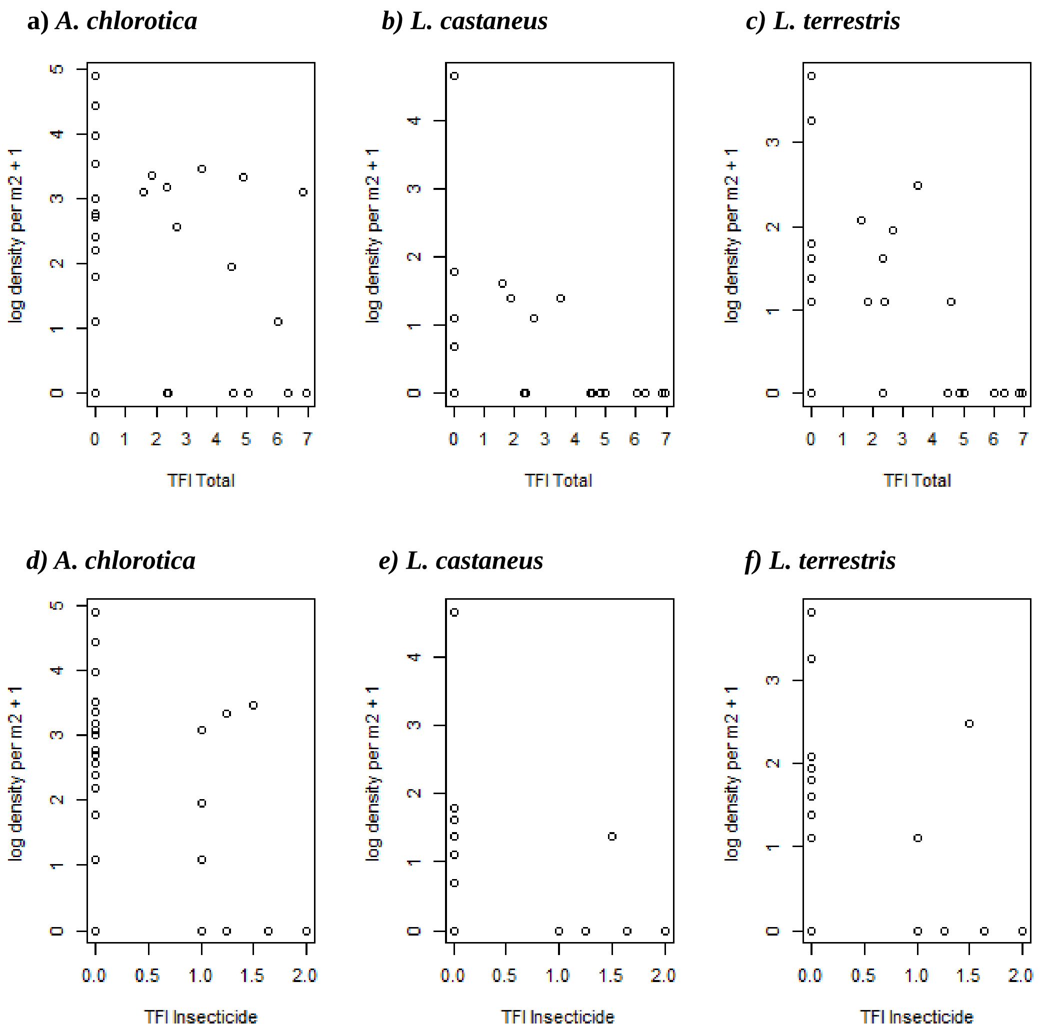

Figure2
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Fig. 2

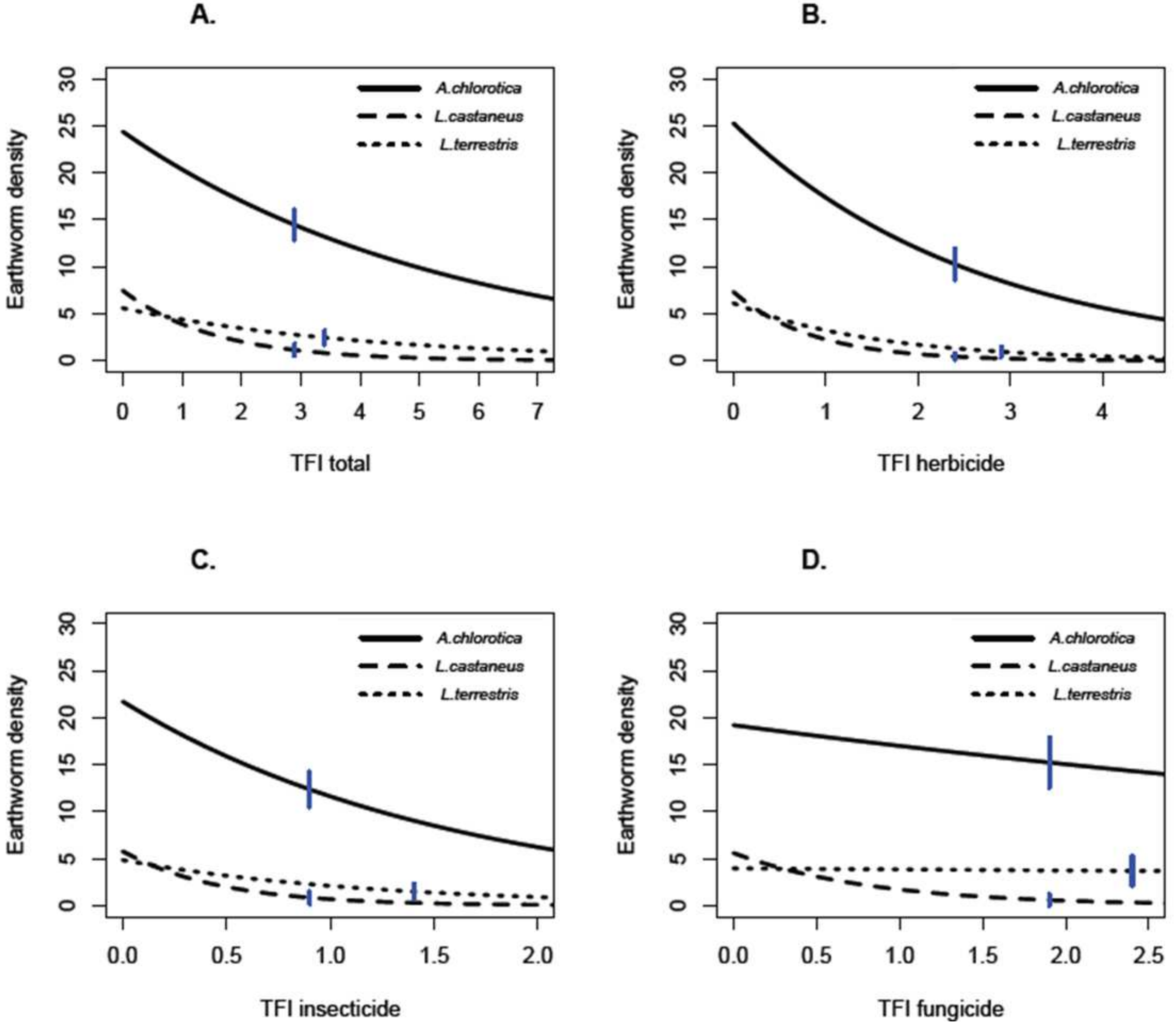

Fig. 1. Densities of the three earthworm species as a function of TFI Total (a, b, c) and of TFI insecticide (d, e, f) on the 30 site-years. TFI means Treatment Frequency Index. Earthworm densities were expressed as log(density per m^2 + 1).

Fig. 2. Poisson regressions of densities of the three earthworm species as a function of TFI Total (A), TFI Herbicide (B), TFI Insecticide (C), and TFI Fungicide (D). TFI means Treatment Frequency Index. Vertical bars are 95% confidence intervals.

AppendixA

Appendix A: Site and soil characteristics of the twenty-two plots in Seine-et-Marne and the eight plots in Yvelines (same crop i.e. winter wheat).

Plot name	Site	Year of soil sampling	GPS Coordinates		Cropping system	Clay (g kg^{-1})	Silt (g kg^{-1})	Sand (g kg^{-1})	Organic matter (g kg^{-1})	CaCO3 (g kg^{-1})	C/N ratio	pH
Org1	Seine-et-Marne	2012	N 48°79,909'	E 3°13,267'	Organic	261.0	646.0	26.0	19.3	1.0	10.2	7.4
Org2	Seine-et-Marne	2012	N 48°66,961'	E 3°17,820'	Organic	242.0	696.0	16.0	19.5	1.0	9.8	7.8
Org3	Seine-et-Marne	2012	N 48°67,099'	E 3°17,904'	Organic	299.0	653.0	12.0	19.5	1.0	9.8	7.5
Org4	Seine-et-Marne	2012	N 48°68,602'	E 2°78,570'	Organic	174.0	664.0	65.0	22.7	1.0	11.9	7.8
Org5	Seine-et-Marne	2012	N 48°43,157'	E 3°32,136'	Organic	193.0	284.0	77.0	25.7	1.0	11.2	8.5
Org6	Seine-et-Marne	2012	N 48°70,640'	E 2°67,897'	Organic	178.0	733.0	33.0	32.7	1.0	13.6	7.3
Org7	Seine-et-Marne	2012	N 48°76,876'	E 3°15,272'	Organic	194.0	751.0	25.0	23.2	1.0	10.8	7.5
Org8	Seine-et-Marne	2012	N 48°76,566'	E 3°14,826'	Organic	170.0	765.0	32.0	19.5	1.0	10.7	7.5
Org9	Seine-et-Marne	2012	N 48°64,506'	E 3°04,909'	Organic	227.0	689.0	19.0	23.7	1.1	10.0	7.6
Org10	Seine-et-Marne	2012	N 48°29,850'	E 2°87,968'	Organic	256.0	347.0	175.0	26.0	1.7	11.6	7.6
Org11	Seine-et-Marne	2012	N 48°84,641'	E 3°10,906'	Organic	165.0	774.0	24.0	19.0	1.0	10.7	7.9
Org12	Yvelines	2005	N 48°48'	E 2°08'	Organic	174.0	605.0	222.0	16.7	0.9	9.8	7.2
Org13	Yvelines	2006	N 48°48'	E 2°08'	Organic	174.0	605.0	222.0	16.7	0.9	9.8	7.2
Org14	Yvelines	2007	N 48°48'	E 2°08'	Organic	174.0	605.0	222.0	16.7	0.9	9.8	7.2
Org15	Yvelines	2011	N 48°48'	E 2°08'	Organic	174.0	605.0	222.0	17.1	< 1	10.1	7.4
Conv1	Seine-et-Marne	2012	N 48°61,808'	E 2°96,832'	Conventional	204.0	704.0	28.0	18.1	1.0	9.9	7.9
Conv2	Seine-et-Marne	2012	N 49°03,467'	E 2°84,154'	Conventional	213.0	723.0	12.0	18.2	5.5	9.9	8.1
Conv3	Seine-et-Marne	2012	N 49°06,166'	E 2°94,686'	Conventional	180.0	756.0	10.0	16.9	1.1	9.9	7.9
Conv4	Seine-et-Marne	2012	N 48°45,583'	E 3°14,232'	Conventional	221.0	658.0	43.0	20.7	7.9	9.9	8.3
Conv5	Seine-et-Marne	2012	N 48°43,775'	E 3°04,751'	Conventional	160.0	580.0	135.0	16.1	1.0	10.5	6.4
Conv6	Seine-et-Marne	2012	N 48°50,036'	E 3°12,826'	Conventional	228.0	667.0	25.0	16.0	1.0	9.9	8.2
Conv7	Seine-et-Marne	2012	N 49°02,709'	E 2°98,335'	Conventional	298.0	648.0	7.0	17.6	1.0	9.4	7.4
Conv8	Seine-et-Marne	2012	N 48°40,579'	E 3°32,293'	Conventional	270.0	457.0	123.0	26.9	7.5	10.9	8.2
Conv9	Seine-et-Marne	2012	N 48°79,928'	E 3°13,529'	Conventional	209.0	692.0	23.0	17.3	1.0	10.4	7.2
Conv10	Seine-et-Marne	2012	N 48°68,684'	E 2°78,558'	Conventional	197.0	646.0	55.0	18.3	1.0	10.2	7.1
Conv11	Seine-et-Marne	2012	N 48°84,380'	E 3°10,896'	Conventional	244.0	662.0	41.0	23.8	5.9	10.4	8.0
Conv12	Yvelines	2005	N 48°48'	E 2°08'	Conventional	180.0	605.0	216.0	18.1	0.9	10.0	7.4
Conv13	Yvelines	2006	N 48°48'	E 2°08'	Conventional	180.0	605.0	216.0	18.1	0.9	10.0	7.4
Conv14	Yvelines	2007	N 48°48'	E 2°08'	Conventional	180.0	605.0	216.0	18.1	0.9	10.0	7.4
Conv15	Yvelines	2011	N 48°48'	E 2°08'	Conventional	180.0	605.0	216.0	17.8	< 1	10.5	7.3

Appendix B: Active ingredients, DT50 and DT90 (mean Dissipation Time 50 and 90 in the field i.e. time for respectively 50% and 90% disappearance of the active ingredients applied at specific initial concentrations in the field) of the pesticides applied in the fifteen conventional fields i.e., eleven fields in Seine-et-Marne and four fields in Yvelines. DT50 and DT90 are mean DT50 and DT90 of all pesticides used in the field, respectively (PPDB, 2012).

Plot name	Site	Active ingredients	DT50 (days)	DT90 (days)
Conv1	Seine-et-Marne	Sulcotrione, Nicosulfuron, Prosulfuron, Chlorantraniliprole	12.4	58.4
Conv2	Seine-et-Marne	Boscalid, Pyroxsulam, Isoproturon, Pendimethaline, Mesosulfuron-methyl-sodium, Iodosulfuron-methyl-sodium	57.6	238.5
Conv3	Seine-et-Marne	Phendimediphame, Ethofumesate, Triflusulfuron-methyl, Metamitrone, Lenacile, Clomazone, Flusilazole	36.0	153.8
Conv4	Seine-et-Marne	Clodinafop-propargyl, Cloquintocet-mexyl, Bromoxynil, Ioxynil, Diflufenican, Ethofumesate, Phenmediphame, Metamitrone, Cyproconazole, Azoxystrobine, Difenoconazole, Fenpropidine	64.2	198.8
Conv5	Seine-et-Marne	Nicosulfuron, Prosulfuron, Mesomitrone, Lambda-cyhalothrine, S-metolachlore, Benoxacor, Chlortoluron, Betacyfluthrine, Pyrimicarbe	20.9	101.6
Conv6	Seine-et-Marne	Fluroxypyr, Clopyralid, CPA, 2,4,-D, Diflufenicanil, Chlortoluron, Prochloraze, Cyproconazole, Propiconazole, Chlorothalonil, Epoxiconazole, Fenpropimorphe, Pyraclostrobine	60.4	540.7
Conv7	Seine-et-Marne	Glyphosate, Ethofumesate, Phenmediphame, Lenacile, Clethodime, S-metolachlore, Propiconazole,Difenoconazole, Epoxiconazole, Fenpropidine, Cypermethrine	54.8	265.1
Conv8	Seine-et-Marne	Pendimethaline, Flurochloridone, Chlortoluron, Diflufenicanil, Ioxynil, Bromoxynil, Difenoconazole, Fenpropidine, Lambda-cyhalothrine, Pyrimicarbe	68.6	212.7
Conv9	Seine-et-Marne	Mesosulfuron-methyl-sodium, Iodosulfuron-methyl-sodium, Fluroxypyr, Metsulfuron-methyle, Tribenuron-methyle, Isoproturon, Spiroxamine, Prothioconazole, Cyproconazole, Propiconazole, Chlorothalonil, Epoxiconazole, Fenpropimorphe, Prochloraze,	52.8	376.6
Conv10	Seine-et-Marne	Tau-fluvalinate, Chortoluron, Bifenox, Ioxynil, Mecoprop-p, Pyroxsulam, Cloquintocet-mexyl, Florasulam, Propiconazole, Cyproconazole, Chlorothalonil, Epoxiconazole, Fenpropidine, Prochloraze, Cypermethrine	65.4	467.6
Conv11	Seine-et-Marne	Chlortoluron, Bifenox, Ioxynil, Mecoprop-p, Phenmediphame, Desmediphame, Ethofumesate, Metamitrone, Lenacile, Triflusulfuron-methyl	30.4	159.1
Conv12	Yvelines	Pendimethaline, Aclonifen, Pyrimethanil, Chlorothalonil	61.0	265.0
Conv13	Yvelines	Pendimethaline, Aclonifen, Azoxystrobine	117.0	427.1
Conv14	Yvelines	Boscalid, Lambda-cyhalothrine	71.5	238.5
Conv15	Yvelines	Glyphosate, Diflufenicanil, Isoproturon	116.7	51.0

Appendix C: TFI values in the fifteen conventional plots i.e., eleven plots in Seine-et-Marne and four plots in Yvelines. TFI means Treatment Frequency Index.

Plot name	Site	TFI Total	TFI Herbicide	TFI Insecticide	TFI Fungicide
Conv1	Seine-et-Marne	2.4	1.4	1.0	0.0
Conv2	Seine-et-Marne	6.3	2.3	2.0	2.0
Conv3	Seine-et-Marne	4.6	3.6	0.0	1.0
Conv4	Seine-et-Marne	7.0	4.5	1.3	1.2
Conv5	Seine-et-Marne	5.0	3.4	1.6	0.0
Conv6	Seine-et-Marne	2.4	1.4	0.0	1.0
Conv7	Seine-et-Marne	6.0	4.5	1.0	0.5
Conv8	Seine-et-Marne	4.9	2.6	1.3	1.0
Conv9	Seine-et-Marne	6.9	3.4	1.0	2.5
Conv10	Seine-et-Marne	4.5	2.8	1.0	0.7
Conv11	Seine-et-Marne	2.4	2.4	0.0	0.0
Conv12	Yvelines	2.7	1.7	0.0	1.0
Conv13	Yvelines	1.9	1.7	0.0	0.2
Conv14	Yvelines	3.5	0.0	1.5	2.0
Conv15	Yvelines	1.6	1.6	0.0	0.0