Effects of pesticides on organic matter recycling and microarthropods in a maize field: use and discussion of the litterbag methodology

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Abstract

The effects of two herbicides (atrazine and alachlore) and two insecticides (fipronil and carbofuran) on organic matter decomposition and soil mesofauna were evaluated in a maize field under normal agriculture conditions. Soil mesofauna were studied using the litterbag method. Near infrared reflectance spectrophotometry was used to study the effects on decomposition parameters (litter mass and nitrogen dynamic). The effects on soil microarthropods varied depending on both taxon and pesticide, with fipronil having a particularly strong effect. Differences were also observed between treatments for organic matter decomposition parameters, especially with the use of alachlore. Therefore, the analysis of microarthropods and the decomposition parameters provides complementary information on the effects of these chemical treatments. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

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1. Introduction

The effects of chemicals, particularly pesticides, on microarthropods have been well studied. However, the majority of the previous studies have been laboratory based. Of those experiments carried out in the field, few have been under real agricultural use conditions (i.e., normal pesticide doses and other agricultural practices). Further, few have focused on the relation between microarthropod effects and the dysfunction of soil biological processes (see reviews in [4] and [6]).

In the present study, we use and evaluate the litterbag methodology [5], in combination with near infrared reflectance spectrometry analysis [7], to analyse the effects of different pesticides on decomposition parameters (litter mass loss and nitrogen dynamic) in relation to the colonisation of the litterbags by microarthropods.

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2. Materials and methods

2.1. Study site and field experiments

The site is located on an experimental farm in La Côte Saint André (Department of Isère, Rhône-Alpes region, France). Experimentation was carried out in a 1600 m^2 field of cultivated maize (*Zea mays* L.) [2,3]. Maize had been cultivated in this field for 8 years, with the exception of 1995 when tobacco was introduced. The soil is a typical hapludalf (Orthic luvisol). The two-leaf step was observed on 16 May, and the six-maize-leaf step was reached on 6 June 1997. Nitrogen (240 kg ha^{-1}), phosphorus (45 kg ha^{-1}) and potassium (300 kg ha^{-1}) were added to the field. The

maize was harvested between 20 and 24 October 1997, and was regularly irrigated during the summer.

The field was divided into two sections. The first section was used for the 'herbicide test', and the second for the 'insecticide test'. Each section was divided into 12 elementary plots in a randomised block design. Each plot measured 12.8*20 m, and contained 16 maize rows.

Atrazine (chloro-2 éthylamino-4 isopropylamino-6 triazine-1,3,5) and alachlore [chloro-2*N*- (diethyl-2,6 phenyl) *N*-methoxymethyl acetamide] were compared on the herbicide section. Each herbicide was tested on four plots. The remaining four plots were used as a control. No insecticides and fungicides were used in the herbicide area. However, as the testing area was too big to eliminate arable weed flora by hand, another herbicide, pyridate [0-(6-chloro-3 phenyl-4-pyridazinyl) carbothiolate *n*-octyl], was used. This was sprayed all over the herbicide section, including the control plots. Atrazine (1 kg ha^{-1}) and alachlore (2.4 kg ha^{-1}) were sprayed after sowing and before the appearance of the first maize leaves. Pyridate (0.9 kg ha^{-1}) was sprayed at the six-maize-leaf step. These are the normal recommended concentrations in agriculture [1].

The insecticide section was used to test carbofuran (*N*-methylcarbamate de dimethyl-2,2 dihydro-2,3 benzofurannyle-7) and fipronil [5- amino-1-(2,6 dichloro-x,x,x,-trifluro-*p*-tolyl)-4-trifluoromethylsulfinyl-pyrazole-3-carbonitrile]. Each insecticide was tested on four plots. Carbofuran (0.6 kg ha^{-1}) was added to the soil in microgranule form just before sowing. Fipronil (0.2 kg ha^{-1}) was sprayed on the topsoil before sowing and then added to the soil by harrowing. The harrowing was carried out on all the insecticide sections, including the four control plots. The entire insecticide section was also sprayed with two herbicides: atrazine (1 kg ha^{-1}) and dimethanamide [1.44 kg ha^{-1}; chloro-*N* (1-methyl-2-methoxyethyl)-*N*- (2,4-dimethyl-thien-3 yl) acétamide].

2.2. Field sampling and laboratory procedures

The litterbag method was used [5]. Four and a half grams of uncontaminated dried maize leaves were introduced in 12*12 cm nylon bags. The mesh of each bag was 4 mm. Thirty bags were placed on the topsoil at the centre of each plot, in the seventh and eighth rows (15 bags in each row, in the middle of the row, with a gap of 30 cm between each bag). The bags were introduced immediately following sowing, onto bare soil. The bags were then sprayed with the same doses of pesticides as the soil, except for carbofuran plots. This insecticide was introduced directly into the soil and not on the topsoil, where the bags were placed. The bags of carbofuran plots were therefore not treated. Five bags per plot were sampled at different dates: 20 May (after 18 days), 18 June (47 days), 2 July (61 days), 12 August (102 days), 11 September (132 days) and 3 October (154 days). The samples were placed in plastic bags and taken directly to the laboratory.

Mesofauna was extracted from the litterbags using the Berlese method, for four of the sampling dates (respectively, 18, 61, 102 and 134 days). Five groups were counted and identified: Collembola, Oribatida, Gamasida, 'other mites' and 'other arthropods'.

Leaves from the bags were dried (24 h, 60 ºC) and sieved (0.5 mm) to eliminate soil particles and little stones. Following this, they were milled with a Cyclotec-1093 (1 mm mesh filter). The resulting samples were analysed using a near infrared spectrophotometer (NIRS system 6500). Each sample was illuminated by a monochromatic radiation source. For each sample, two reflectance measures were carried out at each 2 nm between 400 and 2500 nm (from visible to infrared). Reflectance (R) was transformed into absorbency (A), using the following equation: $A = \log(1/R)$. The spectra obtained (total of 720) were analysed using ISI software [9]. Abnormal spectra ('outliers') were detected using first derivatives, and samples were eliminated as a result.

Following this, samples representative of the entire population of spectra were selected for chemical analyses (ISI software; [7]). Initial maize samples were also analysed. The quantity of ash residue was calculated for 48 samples (550 ºC for 3 h), and nitrogen content for 49 samples (using the Kjeldahl method). Using the ash and nitrogen content obtained chemically as reference values, a calibration equation was calculated for each parameter (ash and N). These equations allowed ash and N content to be estimated for all the samples. To estimate the quality of the calibration equations, the determination coefficient (r^2), standard error of calibration (SEC) and standard error of cross validation (SECV) were calculated. For a mean ash content of 62.48%, r^2, SEC and SECV were 0.98, 3.077 and 5.099, respectively. For a mean N content of 3.713%, they were 0.952, 0.351and 0.494, respectively. These coefficients were judged acceptable. For each sample, the percentage of remaining litter mass (LMR) was calculated using the ash content and litter dry weight, and the N concentration ([N]) was calculated from the estimated N and ash content.

For each section (herbicide and insecticide), and for each sampling month, the differences between treatments were compared, based on an ANOVA (Statview software) of the calculated parameters LMR and [N] and the microarthropod abundances. Microarthropod abundance was expressed as mean number of individuals per bag.

3. Results

3.1. Litter mass remaining

The results for the remaining litter mass show few significant differences between the treatments in the herbicide section during the experiment. However, in July (61

Table 1
Litter mass remaining and nitrogen concentrations in litter maize residues for the various treatments in the herbicide part and insecticide part and the various decomposition days. LMR, percentage of litter mass remaining; [N], nitrogen concentration; Atra, atrazine; ala, alachlore; Hco, control herbicide; fip, fipronil; carb, carbofuran; Ico, control insecticide. Results are expressed as mean values with standard deviations. * $P < 0.05$; ** $P < 0.01$

	18 days	47 days	61 days	102 days	132 days	154 days
Herbicide part						
LMR atrazine	67 ± 9	61 ± 4	51 ± 8	36 ± 13	19 ± 9	20 ± 10
LMR alachlore	64 ± 8	60 ± 5	45 ± 8	33 ± 9	21 ± 9	21 ± 12
LMR H-control	67 ± 5	60 ± 5	51 ± 7	39 ± 13	23 ± 11	18 ± 8
ANOVA	ns	ns	atra > ala** Hco > ala*	ns	ns	ns
[N] atrazine	0.60 ± 0.097	0.81 ± 0.12	0.92 ± 0.1	1.11 ± 0.17	1.14 ± 0.23	1.02 ± 0.15
[N] alachlore	0.67 ± 0.09	0.85 ± 0.1	1.02 ± 0.11	1.19 ± 0.32	1.13 ± 0.3	1.16 ± 0.3
[N] H-control	0.59 ± 0.072	0.85 ± 0.091	0.93 ± 0.092	1.20 ± 0.25	1.27 ± 0.52	1.0098 ± 0.21
ANOVA	ala > atra* ala > Hco**	ns	ala > atra** ala>Hco**	ns	ns	ala > Hco*
Insecticide part						
LMR fipronil	69 ± 4	63 ± 4	59 ± 9	40 ± 14	27 ± 13	29 ± 16
LMR carbofuran	73 ± 10	62 ± 5	53 ± 7	34 ± 15	21 ± 10	19 ± 7
LMR I-control	68 ± 5	63 ± 8	55 ± 8	42 ± 13	27 ± 12	26 ± 14
ANOVA	carb > fip* carb > Ico*	ns	fip > carb*	ns	ns	fip > carb*
[N] fipronil	0.60 ± 0.068	0.82 ± 0.13	0.95 ± 0.22	1.12 ± 0.25	1.09 ± 0.22	1.05 ± 0.14
[N] carbofuran	0.59 ± 0.072	0.80 ± 0.14	0.98 ± 0.18	1.15 ± 0.27	1.30 ± 0.32	1.06 ± 0.14
[N] I-control	0.60 ± 0.088	0.77 ± 0.11	0.94 ± 0.16	1.07 ± 0.13	1.14 ± 0.34	0.98 ± 0.23
ANOVA	ns	ns	ns	ns	carb > fip*	ns

decomposition days), decomposition was faster in the alachlore plots in comparison to the atrazine and H-control plots.

In the insecticide section, differences were observed from early on in the experiment. A greater decomposition was seen in the fipronil and I-control plots in comparison to the carbofuran plots, after 18 decomposition days. In July (61 decomposition days) and October (154 decomposition days), however, the observed decomposition was significantly greater in the carbofuran plots than in the fipronil plots.

3.2. N concentration

In the herbicide section, significant differences were observed in the N concentrations of the litter. Concentrations of N were higher in the alachlore plots than in the atrazine plots in May (18 decomposition days) and July (61 decomposition days), and greater than the H-control plots in May, July and October (154 decomposition days). Few significant differences were observed in the insecticide section. A greater N concentration is seen in the carbofuran plots in comparison to the fipronil plots in September alone (132 decomposition days) (Table 1).

3.3. Microarthropod abundance

In the herbicide section, the only significant difference was observed for the Collembola group in October, after 154 decomposition days. At this point, a greater abundance was found in the plots than in the alachlore H-control and atrazine plots (Fig. 1).

In the insecticide section, greater differences between treatments were observed after 61 and 102 decomposition days, with higher abundancies in I-control and carbofuran compared to fipronil for Oribatida. This was also observed for the 'other arthropods' group, but only at 61 days. The taxon 'other mites' was also significantly more abundant in carbofuran plots than in fipronil plots after 102 days (Fig. 2).

4. Discussion

4.1. Herbicide section

In this section, maize leaves treated with alachlore–pyridate mixtures decomposed faster, and had higher N concentrations than in the H-control and atrazine plots. Therefore, at the end of the experiment, after 6 months of decomposition, significant differences were observed in N dynamics. These results could indicate a higher microbiological activity in alachlore plots. This may be explained by the use of herbicides by micro-organisms, as it is well known that some species can use herbicides as a source of energy [8,11]. This hypothesis is confirmed by a higher observed microbial biomass in the soil after four study months on the same plots [2].

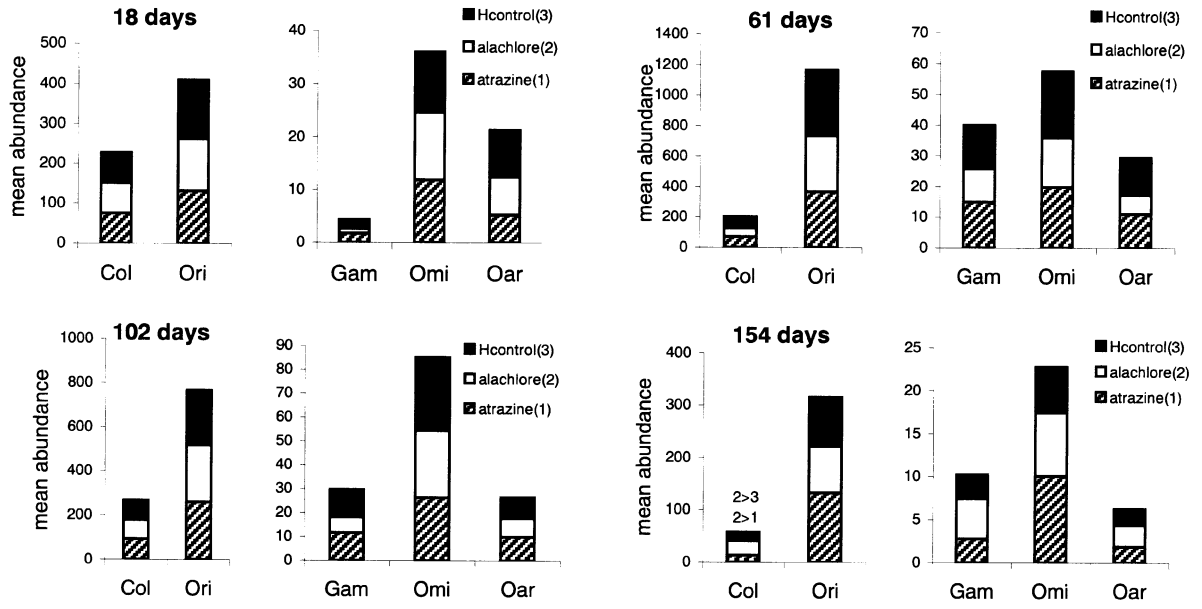


Fig. 1. Mean abundance of microarthropods (number of individuals/litterbag) in the herbicide section for each treatment (atrazine, alachlore, H-control) and each sampling date (18, 61, 102, 154 days). Col, Collembola; Ori, Oribatida; Gam, Gamasida; Omi, other mites; Oar, other arthropods. Significant differences between treatments (ANOVA, $P < 0.05$) are noted on the concerned histograms.

No differences were observed between the atrazine and H-control plots. However, as atrazine was used on this field during the preceding cultivation seasons, selection processes may have acted on some fungi or bacteria.

It is difficult to relate the results of the decomposition parameters for the herbicide section to the results of the microarthropods fauna observed in the litterbags. Differences were observed between the alachlore plots and other

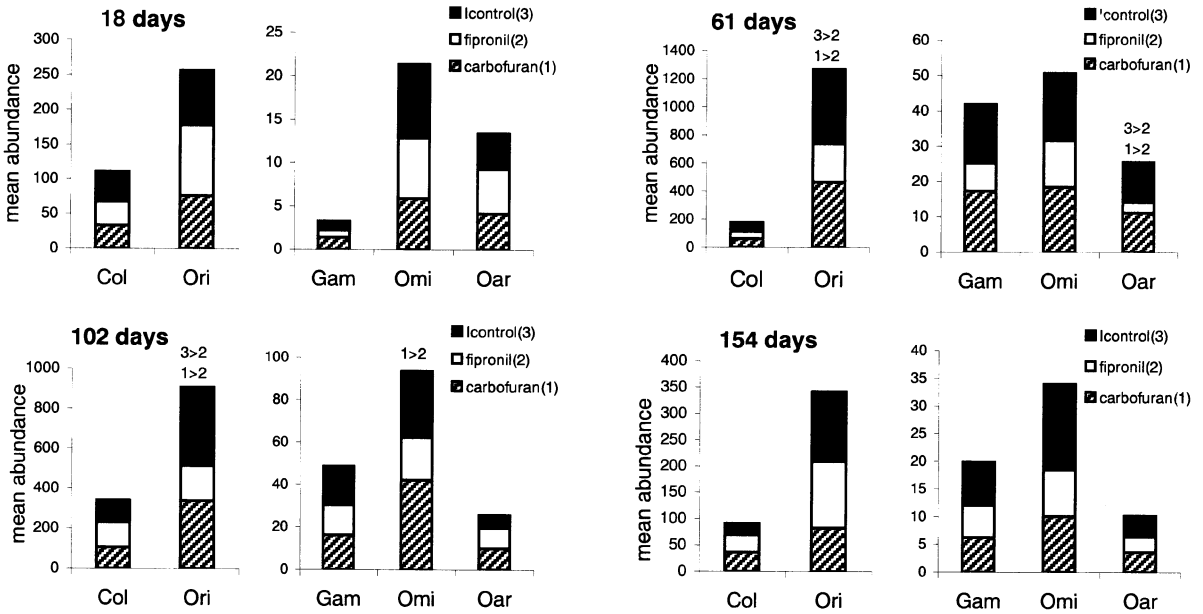


Fig. 2. Mean abundance of microarthropods (number of individuals/litterbag) in the insecticide section for each treatment (fipronil, carbofuran, I-control) and each sampling date (18, 61, 102, 154 days). Col, Collembola; Ori, Oribatida; Gam, Gamasida; Omi, other mites; Oar, other arthropods. Significant differences between treatments (ANOVA, $P < 0.05$) are noted on the relevant histograms.

plots, but only for the microphytophagous group of collembola after 154 days. However, this greater abundance of collembola may be as a result of an increase in micro-organisms, which constitute their main food source. No differences between treatments were observed for earthworms in the soil [2].

4.2. Insecticide section

Two phases could be observed in the decomposition process. At the first sampling date (18 days), a reduced level of litter decomposition was observed in the carbofuran plots when compared to I-control and fipronil. This did not result from microbial activity, as no differences were observed in the N concentration dynamics. These differences may instead have been induced by a reduction in the activity of soil macrofauna rather than mesofauna: no differences were observed in the mesofauna at this sampling interval, but a less significant earthworm abundance was observed in the carbofuran plots during the experiment [2].

Differences between the fipronil and carbofuran plots were observed from July onwards. However, no significant differences were observed between the decomposition parameters of the carbofuran and I-control plots, or of the fipronil and I-control plots. In comparison with the I-control plot, the results show an increase in the decomposition observed in the carbofuran plot, and a slight decrease in the decomposition in the fipronil plot. The decrease in decomposition observed in the fipronil plots may be explained by the high toxicity for the microarthropods, especially for the most important and numerous oribatida group, after 61 and 102 decomposition days. The tendency towards an increase in decomposition in the carbofuran plots may result from the application method of the carbofuran, as it was introduced directly into the soil. The litter, which was not directly contaminated, may have acted as a refuge area for microarthropods and micro-organisms, causing an increase in the decomposition parameters [10].

4.3. Methodology considerations

Our results show the potential of litterbags for pesticide assessment in the field based on functional parameters. This approach allows comparisons of the differences between treatments to be made. In addition, biological activity can be controlled by using different mesh sizes. For example, in our study the mesh size was 4 mm which allowed the action of microfauna, mesofauna and macrofauna, e.g. earthworms.

Further, by studying both microarthropods and functional parameters, we have obtained complementary information, which has increased the validity of the measurable responses.

The NIRS method has been shown to be well suited for the determination of ash and N content. The calibration method allowed us to reduce the number of chemical analyses required (only 51 samples were used for chemical analyses on a total of 720 initial samples). Many samples, therefore, can be analysed in a short time, at a high resolution. The cost is also reduced, as chemical analyses are more time consuming than NIRS analyses. Further, this method is non-destructive, and samples can be re-used for further analysis. As the spectra contains all the biochemical information, nothing is lost.

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