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Interaction between humus form and herbicide toxicity to Collembola (Hexapoda)

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Abstract

Laboratory experiments were conducted using intact collembolan communities, exposed to Madit D®, a phenylurea herbicide (active ingredient isoproturon). Effects were investigated using two distinct humus types, an acid Dysmoder and a neutral Eumull. Within two weeks, no effect of the herbicide was displayed by the Eumull population, while the Dysmoder population was stimulated. When animals were able to escape from the herbicide through a perforated wall separating two compartments filled with natural soil, the behavior of collembolan communities exhibited interactive (non-additive) effects of humus type and herbicide application. The combination of an acid soil (supposedly providing greater tolerance to organic pollutants) with a neutral soil, increased biodiversity of Collembola, but caused the disappearance of some acido-sensitive species, pointing to complex relationships between pesticides, soils and soil organisms. Parallel experiments with single species demonstrated that at the recommended dose Madit D® may cause avoidance effects, but no toxicity. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Herbicides; Humus; Collembola; Communities; Isoproturon

1. Introduction

Numerous studies have assessed the impact of herbicides on soil fauna, but contradictory results have been obtained, depending on animal groups studied, chemical nature of the herbicide and additives, soil type and rate of application (Rapoport and Cangioli, 1963; Edwards, 1965; Edwards and Lofty, 1969; Curry, 1970; Van der Drift, 1970; Fratello et al., 1985; Mallow et al., 1985; Prasse, 1985; Chalupský, 1989; Krogh, 1991). Before standardized laboratory tests were developed, some authors claimed that there was no direct effect of herbicides on soil animals and that most increases or decreases of populations were indirect effects, mostly caused by vegetation changes (Curry, 1970; Edwards and Thompson, 1973; Chalupský, 1989). Nevertheless, under standardized conditions without interference from vegetation, herbicides differ strongly in toxic as well as stimulatory effects when applied at field doses (Eijsackers, 1978; Subagja and Snider, 1981; Eijsackers, 1991; Van Gestel et al., 1992). Despite fruitful efforts to standardize methods (Riepert and Kula, 1996; Crouau et al., 1999) some uncertainty is inherent in the representativeness of ecotoxicological studies for field conditions, given what we know about avoidance and dispersion behavior of Collembola (Ulber, 1979; Duelli et al., 1990; Mebes and Filser, 1997; Alvarez et al., 2000). Acute toxicity levels given by standardized indices such as LOEC or EC_{50} (Reinecke, 1992; Forbes and Forbes, 1994; Riepert and Kula, 1996; Crouau et al., 1999) may be irrelevant for risk assessment if field animals are killed

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by moving to untreated but unfavorable microsites at doses far below recommended levels. The reversibility of local changes in habitat use, induced by pesticides, can also be questioned, given the large variation in recovery processes from one species to another (Alvarez et al., 2000). Given what we know about the behavior of whole animal communities compared to single species facing experimental stresses, and strong interactions between animal species (Haukka, 1987; Hågvar, 1990; Ponge, 1999; Salmon and Ponge, 1999), community level effects must be suspected, which are not taken into account in standardized tests using mass cultures of single species (Van Gestel et al., 1992). Thus, there is a need for field and laboratory-based tests using communities (Frampton, 1997; Urzelai et al., 2000; Cortet and Poinsot-Balaguer, 2000) if we want to become more realistic in ecological risk assessment (Van Straalen and Løkke, 1997; Van Straalen and Van Rijn, 1998; Suter et al., 2000).

From our knowledge of the biochemical properties of soils at varying levels of acidity and organic matter content (Coulson et al., 1960; Davies et al., 1964; Lindqvist, 1983; Petersen and Persson, 1987; White, 1994), it may be hypothesized that organisms living in acid organic soils are more tolerant of phenolic and terpenic compounds than organisms living in neutral organo-mineral soils (Hågvar and Abrahamsen, 1984; Poinsot-Balaguer et al., 1993; Ponge, 1993; Loranger et al., 2001). Thus, the effects of pesticides on non-target organisms can be expected to vary according to soil features, in particular acidity, organic matter, clay and water content, not only because of the impact of these factors on the fate of organic compounds (Mortland et al., 1986; Müller-Wegener, 1987; Laszlo, 1987; Stehouwer et al., 1993; Akhouri et al., 1997), but also because of different tolerance levels of different soil biocenoses. The latter point has remained unstudied until now.

In the present study, the influence of soil humus type on the response of Collembola (springtails) to a herbicide was investigated. Collembola were chosen because of their abundance and diversity in most soils (Petersen and Luxton, 1982; Ponge et al., 1997; Hopkin, 1997) and the marked shifts in species composition caused by changes in acidity (Hågvar and Abrahamsen, 1984; Ponge, 1993; Chagnon et al., 2000; Loranger et al., 2001). In addition to laboratory bioassays in semi-natural conditions, using two undisturbed acid and neutral soils, attraction or avoidance of a herbicide by Collembola was assessed on impregnated filter paper, using two different species coming from the same soils.

2. Materials and methods

2.1. Laboratory tests using complete communities

Experiments were performed on natural soils, with undisturbed animal communities, to which a herbicide (Madit D®, with isoproturon as the active molecule) was applied at doses and in the same way as in conventional agriculture. In order to test simultaneously the effects of herbicide application and of acid humus inoculation, two different soils, the hemorganic A horizon from a neutral (pH 7.5) Eumull and the holorganic O horizon from an acid (pH 4.3) Dysmoder (Brêthes et al., 1995), were placed together in two-compartment boxes, with or without the addition of herbicide to one or both compartments. Other experiments used the same soil in both compartments, with and without herbicide.

The neutral hemorganic Eumull was considered to be a reference for agricultural soils, harboring more complete faunal communities due to organic matter input and the absence of pesticide use and deep ploughing (Ponge, 2000b). Previous studies had shown that agricultural soils and forest soils did not differ fundamentally when soil-dwelling communities were considered (Ponge, 1993). The acid organic Dysmoder was used as a possible source for the introduction of more tolerant strains of soil animals in agricultural soils.

Boxes made of molded polystyrene (175 mm × 115 mm × 65 mm $l \times w \times h$) were used for these experiments. A 2 mm thick wall made of polymethylmethacrylate was perforated (400 holes, 2 mm diameter each) then inserted in the central part of each box, dividing it into two connected compartments 650 ml each. Small arthropods (including springtails) and worms (nematodes, enchytraeids, small epigeic earthworms) were expected to cross the wall easily, following preliminary experiments using defaunated soil (data not shown). There was no contact between the soils present in the two compartments. Thus, only animals and gases could move between compartments.

OF (fragmented) and OH (humified) organic horizons of a Dysmoder humus profile (10 cm deep) were collected in a mixed forest stand (Senart forest, 20 km southwest of Paris), made of sessile oak (*Quercus petraea* (Mattus.) Liebl.) and Scots pine (*Pinus sylvestris* L.), then gently homogenized by hand in a plastic sheet before being added to the appropriate compartments. Stones, large roots and large pieces of wood were removed during the homogenizing process. The same method was used for collecting the A (organo-mineral) horizon of a calcic Eumull profile in a hornbeam stand (*Carpinus betulus* L.) in the park adjacent to the laboratory (close to the Senart forest). Compartments were filled with humus, leaving only the top 1 cm free of soil, thus each compartment held approximately 500 ml of soil.

The herbicide Madit D® comprised 50% (w/v) isoproturon. It was applied at a rate of 3 l ha^{-1}, which is the rate prescribed for the current control of annual grasses and broad-leaved weeds. For that purpose, each compartment receiving the herbicide (1 dm^{-2}) was sprayed with 1.5 ml of a diluted solution containing 3 μl of the herbicide. Thus, 1.5 mg of isoproturon was applied to each compartment, corresponding to a nominal concentration of 5.6 mg kg^{-1} dry weight for the Eumull and 23 mg kg^{-1} dry weight for the Dysmoder. Boxes were then closed and randomly placed in a dark chamber at constant temperature (15 °C) for 2 weeks.

The following ten treatments were applied, with five replicates each, representing all possible combinations of herbicide treatment (with or without) and humus type (Eumull or Dysmoder):

- Eumull/Eumull (E/E)
- Dysmoder/Dysmoder (D/D)
- Eumull + Herbicide/Eumull + Herbicide (EH/EH)
- Eumull + Herbicide/Eumull (EH/E and E/EH)
- Dysmoder + Herbicide/Dysmoder + Herbicide (DH/DH)
- Dysmoder + Herbicide/Dysmoder (DH/D and D/DH)
- Eumull/Dysmoder (E/D and D/E)
- Eumull+Herbicide/Dysmoder+Herbicide (EH/DH and DH/EH)
- Eumull + Herbicide/Dysmoder (EH/D and D/EH)
- Eumull/Dysmoder + Herbicide (E/DH and DH/E)

The experiment allowed several comparisons to be made, which were treated separately for the sake of clarity. Untreated collembolan communities (E/E and D/D) were used for comparisons between the two animal communities. Soils treated with isoproturon but with the same soil in both compartments (EH/EH and DH/DH) were compared with untreated soils (E/E and D/D, respectively) to assess the effects of herbicide application alone. Experimental boxes with the two compartments, having received different treatments (herbicide or humus type), were used to study more complex interactions, involving either the same humus type (EH/EH, EH/E, DH/DH, DH/D) or two different humus types (E/D, EH/DH, EH/D, E/DH) in the same box.

At the end of the experiment, the twin boxes were thoroughly voided and the soil samples corresponding to the 100 compartments were separately extracted in Berlese funnels. Soil samples (500 ml) were gently spread on a large size wire net (5 mm mesh size, 20 cm diameter), in order to avoid possible barrier effects of surface-applied pesticides during extraction (Krogh, 1991). During the 10-day extraction, the soil was gently heated by 25 W bulb lamps in order to avoid too rapid desiccation. Animals collected under the desiccating soil were preserved in 95% ethyl alcohol before being sorted under a dissecting microscope. Collembola were mounted in chloral lactophenol (25 ml lactic acid, 50 g chloral hydrate, 25 ml phenol) and identified to species under a phase contrast microscope at 400× magnification. Gisin (1960) was used as a reference book but several more recent identification keys and diagnoses were used additionally, including Zimdars and Dunger (1994), Jordana et al. (1997), Fjellberg (1998) and Bretfeld (1999).

The basic data consisted of animal densities and number of species per compartment. An additional index was calculated as the ratio between the number of species and the number of individuals. It was referred to as the relative richness. Comparisons between treatments were done at the species level or at the community level, using Kruskal–Wallis tests for comparisons of means when replicates of compared treatments were independent (not in the same boxes) or Friedman tests for paired comparisons when two compartments within the same box were compared (Sokal and Rohlf, 1995). When the two compartments received the same treatment, values were averaged for each box and used for comparisons with other treatments. Given that extraction efficiency could vary from one humus type to another (Macfadyen, 1957), comparisons between Eumull and Dysmoder should be made with care. Thus, most conclusions were based on comparison between treatments applied to the same humus type. Despite the (apparent) factorial design of the experiment, data could not be analyzed by multi-way ANOVA, since (i) humus types were too different in their chemical and biological properties to expect additive effects of humus type and herbicide, (ii) compartments within a box were not independent from each other.

2.2. Laboratory tests using single species

Two collembolan species, *Heteromurus nitidus* (Entomobryidae), living in the Eumull, and *Folsomia manolachei* (Isotomidae), living in the Dysmoder, were previously reared on an artificial substrate used for standardized tests (Riepert and Kula, 1996), made of a mixture of 10% sphagnum peat, 20% kaolinite and 70% fine quartz sand. The pH of the artificial substrate was brought to 6.2 by adding 0.45% calcium carbonate. The substrate was moistened to 50% of water holding capacity by adding deionized water. Cattle dung powder was given as additional food when necessary. Cultures were kept in the dark at 15 °C. Before each experimental run, animals were conditioned for 20 min to light (artificial light) and temperature (20 °C) used in the experiment. Animals were individually introduced into 8 cm diameter petri dishes, on the bottom which was partially covered by two half-disks of 7 cm diameter Whatman filter paper at a distance of 0.5 cm from each other. One half-disk was impregnated with deionized water and used as a control, the other was impregnated with the test solution at varying concentration. Fifteen petri dishes (replicates) were placed under a Sharp® fluorescent illuminator normally used for scanning documents, each with an animal placed in the space between two half-disks at the beginning of the trial. Since subterranean springtails such as *Heteromurus nitidus* are highly sensitive to light (Salmon and Ponge, 1998), control experiments were done with water on both half-disks in order to verify the absence of light gradients under the illuminator. One hour after the introduction of test animals, their position on one or the other half-disk was checked in each petri dish. Data were total number of animals either on water or on test solution. The significance of attractive or repulsive effects of test solutions was assessed by a sign-test, based on binomial distribution (Rohlf and Sokal, 1995). Animals used for an experimental run were kept for further experiments with at least 48 h interval between two successive runs with the same animal. In the meantime, animals were kept on uncontaminated artificial substrate with food added.

3. Results

3.1. Untreated collembolan communities

Thirty-nine species were identified in the material extracted at the end of the experiment (Table 1). Ten were common to both soils (ubiquitous species), nine were found in the neutral Eumull (pH 7.5) only (Eumull species), 16 in the acid Dysmoder (pH 4.3) only (Dysmoder species), and six could not be attributed unambiguously to one or other humus type, since they were found only in boxes with the two different soils (dubious species). Based on our extraction results, five times as many animals and twice as many species were present in the acid than in the neutral soil (Table 2, Fig. 1). Thus, the relative richness of the Eumull was higher than that of the Dysmoder. Abundance and number of ubiquitous species were higher in the Dysmoder than in the Eumull. Among the common species (species present in more than 10 compartments) ubiquitous relative to humus type, all but one either did not express significant differences in their densities between humus types or were more abundant in the Dysmoder, the exception being *Dicyrtoma fusca* which was more abundant in the Eumull (Table 2). *Parisotoma notabilis*, although classified as ubiquitous, was nearly absent in the Eumull, although it was the second most abundant species in the Dysmoder, after *Isotomiella minor*, which dominated both communities.

3.2. Collembolan communities with herbicide and the same humus in both compartments

Laboratory effects of Madit D® on collembolan communities can be assessed by comparing untreated

Table 1
List of collembolan species found in the experiments with complete communities

	Eumull	Dysmoder	Ubiquitous	Dubious
Allacma fusca (Linné, 1758)	0	0	0	1
Allacma gallica (Carl, 1899)	0	1	0	0
Arrhopalites caecus (Tullberg, 1871)	1	0	0	0
Arrhopalites sericus (Gisin, 1947)	0	1	0	0
Dicyrtoma fusca (Lucas, 1842)	0	0	1	0
Dicyrtomina minuta (Fabricius, 1783)	0	0	0	1
Entomobrya nivalis (Linné, 1758)	0	1	0	0
Folsomia candida (Willem, 1902)	1	0	0	0
Folsomia manolachei (Bagnall, 1939)	1	0	0	0
Friesea truncata (Cassagnau, 1958)	0	0	1	0
Heteromurus major (Monniez, 1889)	0	1	0	0
Heteromurus nitidus (Templeton, 1835)	1	0	0	0
Isotomiella minor (Schäffer, 1896)	0	0	1	0
Isotomurus palustris (Müller, 1776)	1	0	0	0
Kalaphorura burmeisteri (Lubbock, 1873)	1	0	0	0
Lepidocyrtus lanuginosus (Gmelin, 1788)	0	0	1	0
Lepidocyrtus lignorum (Fabricius, 1781)	0	0	1	0
Lipothrix lubbocki (Tullberg, 1872)	0	1	0	0
Megalothorax minimus (Willem, 1900)	0	1	0	0
Mesaphorura betschi (Rusek, 1979)	0	1	0	0
Mesaphorura jevanica (Rusek, 1996)	0	0	1	0
Mesaphorura leitzaensis (Jordana, 1993)	0	0	1	0
Mesaphorura macrochaeta (Rusek, 1976)	1	0	0	0
Mesaphorura yosii (Rusek, 1967)	0	1	0	0
Micranurida pygmaea (Börner, 1901)	0	1	0	0
Neanura muscorum (Templeton, 1835)	0	1	0	0
Oncopodura crassicornis (Shoebotham, 1911)	0	0	0	1
Onychiurus jubilarius (Gisin, 1957)	0	0	0	1
Orchesella cincta (Linné, 1758)	0	0	1	0
Paratullbergia callipygos (Börner, 1902)	0	1	0	0
Parisotoma notabilis (Schäffer, 1896)	0	0	1	0
Pogonognathellus flavescens (Tullberg, 1871)	0	0	0	1
Proisotoma minima (Absolon, 1901)	0	1	0	0
Pseudosinella alba (Packard, 1873)	0	1	0	0
Sminthurinus aureus (Lubbock, 1862)	1	0	0	0
Sphaeridia pumilis (Krausbauer, 1898)	0	0	1	0
Stenaphorura denisi (Bagnall, 1935)	1	0	0	0
Tomocerus minor (Lubbock, 1862)	0	1	0	0
Willemia anophthalma (Börner, 1901)	0	1	0	0
Xenylla tullbergi (Börner, 1903)	0	1	0	0
Xenyllodes armatus (Axelson, 1903)	0	0	0	1

0: absence, 1: presence.

experimental boxes with those in which the herbicide was added to both compartments (Table 2, Fig. 1). There were no significant effects of the herbicide on the abundance or on the species richness of collembolan communities, whatever the humus type, but two species, namely *Megalothorax minimus* and *Sphaeridia pumilis*, reacted positively to the addition of the herbicide in the Dysmoder. Both species were found exclusively in herbicide-treated boxes.

Possible avoidance or attraction effects could be tested when the herbicide was applied to only one compartment. No such effect was detected in Eumull (Table 3), but a small, although significant effect was perceived in Dysmoder (Table 4). In Dysmoder,

Table 2
Results of treatments in which both 550 ml compartments of experimental boxes received or did not receive the herbicide

	Eumull	Eumull + Herbicide	Dysmoder	Dysmoder + Herbicide
Dicyrtoma fusca	1.9 ± 0.7 a	2.3 ± 0.3 a	0 b	0.1 ± 0.1 b
Folsomia manolachei	0.6 ± 0.3	0.3 ± 0.1	0	0
Friesea truncata	1.5 ± 0.3 b	1.3 ± 0.3 b	14.6 ± 0.9 a	18.4 ± 3.4 a
Heteromurus major	0 b	0 b	1.7 ± 0.7 a	3.2 ± 0.5 a
Isotomiella minor	10.7 ± 1.6 b	9.9 ± 1.3 b	46.8 ± 6.3 a	56.7 ± 10.0 a
Isotomurus palustris	3.7 ± 1.6 a	4.5 ± 2.2 a	0 b	0 b
Lepidocyrtus lanuginosus	0 b	0.1 ± 0.1 b	7.0 ± 1.0 a	3.9 ± 1.3 a
Lepidocyrtus lignorum	0 b	0.2 ± 0.2 b	2.8 ± 0.6 a	3.3 ± 1.1 a
Lipothrix lubbocki	0 b	0 b	2.8 ± 0.4 a	2.7 ± 0.3 a
Megalothorax minimus	0 b	0 b	0 b	0.9 ± 0.2 a
Mesaphorura leitzaensis	0	0.1 ± 0.1	0.1 ± 0.1	0.7 ± 0.3
Micranurida pygmaea	0	0	0.2 ± 0.2	0.4 ± 0.2
Neanura muscorum	0 b	0 b	3.3 ± 1.0 a	3.4 ± 2.4 ab
Orchesella cincta	0.1 ± 0.1	0	0.6 ± 0.4	0
Paratullbergia callipygos	0	0	0.2 ± 0.1	0.9 ± 0.4
Parisotoma notabilis	0.2 ± 0.2 b	0.2 ± 0.2 b	16.7 ± 0.9 a	14.4 ± 2.6 a
Proisotoma minima	0 b	0 b	7.9 ± 1.6 a	8.7 ± 1.9 a
Sphaeridia pumilis	0 b	0.1 ± 0.1 b	0 b	3.4 ± 1.9 a
Stenaphorura denisi	0.4 ± 0.2	0.3 ± 0.2	0	0
Tomocerus minor	0 b	0 b	1.2 ± 0.3 a	1.3 ± 0.4 a
Total abundance	19.9 ± 2.2 b	19.9 ± 3.8 b	106.3 ± 7.5 a	122.8 ± 13.0 a
Total number of species	5.0 ± 0.6 b	4.9 ± 0.6 b	10.3 ± 0.3 a	11.7 ± 0.7 a
Relative richness	0.27 ± 0.08 a	0.28 ± 0.03 a	0.10 ± 0.01 b	0.10 ± 0.01 b
Abundance of Eumull species	5.5 ± 1.9 a	5.5 ± 2.5 a	0 b	0 b
Number of Eumull species	2.5 ± 0.4 a	1.7 ± 0.2 a	0 b	0 b
Abundance of ubiquitous species	14.4 ± 1.8 b	14.3 ± 1.8 b	88.7 ± 6.3 a	100.9 ± 12.7 a
Number of ubiquitous species	2.5 ± 0.3 b	3.1 ± 0.4 b	5.5 ± 0.3 a	5.9 ± 0.4 a
Abundance of Dysmoder species	0 b	0 b	17.6 ± 1.7 a	21.9 ± 4.1 a
Number of Dysmoder species	0 b	0 b	4.8 ± 0.4 a	5.8 ± 0.3 a

Data are numbers of animals per compartment averaged over five replicates with standard errors. Significant differences among treatments ($P < 0.05$) are indicated by different letters.

Friesea truncata decreased in abundance in the treated compartment (DH/D) compared to the control (D/D), while its abundance in the untreated compartment (D/DH) was not different from the control. The case of the ubiquitous species *Sphaeridia pumilis* was somewhat different, since it was absent in the control (D/D), but present in both treated (DH/D) and untreated (D/DH) compartments (Table 4). This resulted in an increase by one in the number of ubiquitous species, as well as in the relative richness index.

3.3. Interaction between humus type and herbicide in Eumull communities

The combination of Dysmoder with Eumull caused an increase in the relative richness of the collembolan Eumull community (Table 5). This increase was due to the addition of Dysmoder species to the Eumull population (Fig. 1). This addition was counterbalanced by a strong decrease in the abundance and number of Eumull species, from 5.5 and 2.5, respectively, to only one individual (and thus one species) per compartment. The net result was a decrease in the total abundance and an increase in the total number of species of Collembola per compartment. The relative richness index increased accordingly. At the species level (Table 5), *Isotomurus palustris*, an Eumull species, was negatively affected by the presence of Dysmoder, its population collapsing to less than one individual per compartment. The observed decrease in the total abundance of Collembola was mainly due to a collapse in the population of *Isotomurus palustris*, the

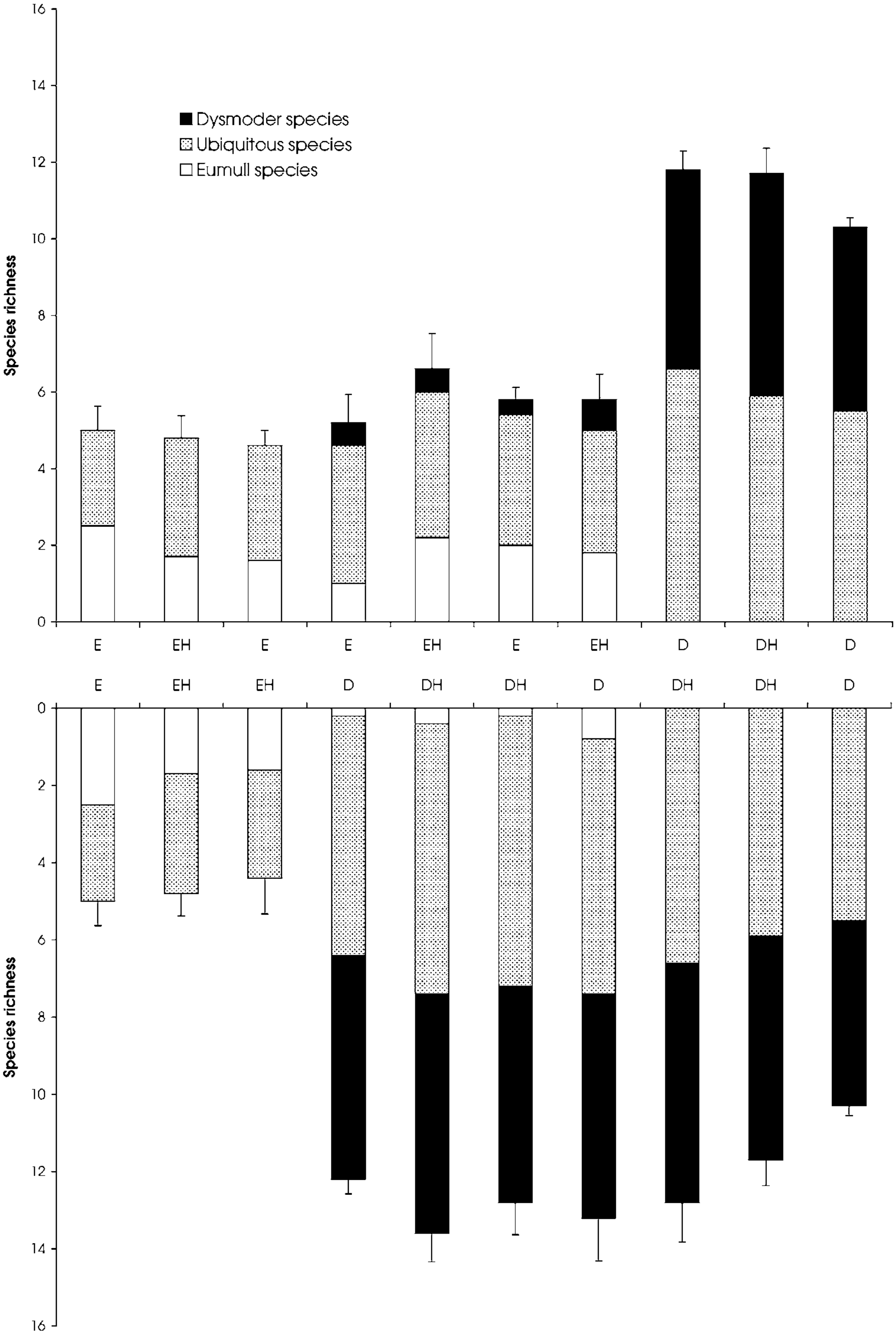

Fig. 1. Species richness of Collembola in the adjacent compartments of experimental twin boxes. E, Eumull soil; EH, Eumull soil treated with isoproturon; D, Dysmoder soil; DH, Dysmoder soil treated with isoproturon. Bars indicate standard errors (five replicates).

Table 3
Experiments with Eumull in the two compartments of experimental boxes

	Eumull/Eumull	Eumull/Eumull + Herbicide	Eumull + Herbicide/ Eumull
Dicyrtoma fusca	1.9 ± 0.7	1.4 ± 0.5	1.8 ± 0.6
Folsomia manolachei	0.6 ± 0.3	0.8 ± 0.4	1.6 ± 0.5
Friesea truncata	1.5 ± 0.3	1.6 ± 0.7	1.8 ± 0.9
Heteromurus major	0	0	0
Isotomiella minor	10.7 ± 1.6	10.0 ± 2.1	8.2 ± 3.6
Isotomurus palustris	3.7 ± 1.6	13.6 ± 12.4	20.4 ± 17.9
Lepidocyrtus lanuginosus	0	0	0
Lepidocyrtus lignorum	0	0	0
Lipothrix lubbocki	0	0	0
Megalothorax minimus	0	0	0
Mesaphorura leitzaensis	0	0	0
Micranurida pygmaea	0	0	0
Neanura muscorum	0	0	0
Orchesella cincta	0.1 ± 0.1	0	0
Paratullbergia callipygos	0	0	0
Parisotoma notabilis	0.2 ± 0.2	0	0
Proisotoma minima	0	0	0
Sphaeridia pumilis	0	0.2 ± 0.2	0.2 ± 0.2
Stenaphorura denisi	0.4 ± 0.2	0	0
Tomocerus minor	0	0	0
Total abundance	19.9 ± 2.2	28.8 ± 13.7	35.6 ± 22.4
Total number of species	5.0 ± 0.6	4.6 ± 0.4	4.6 ± 0.9
Relative richness	0.27 ± 0.08	0.28 ± 0.08	0.31 ± 0.09
Abundance of Eumull species	5.5 ± 1.9	15.4 ± 12.4	23.4 ± 17.8
Number of Eumull species	2.5 ± 0.4	1.6 ± 0.2	1.6 ± 0.5
Abundance of ubiquitous species	14.4 ± 1.8	13.4 ± 2.4	12.0 ± 4.8
Number of ubiquitous species	2.5 ± 0.3	3.0 ± 0.3	2.8 ± 0.5
Abundance of Dysmoder species	0	0	0
Number of Dysmoder species	0	0	0

Data are numbers of animals per compartment averaged over five replicates with standard errors. The slash sign separates the considered compartment (left) and the adjacent compartment (right). When both compartments were given the same treatment, corresponding data were pooled, otherwise they were considered as distinct treatments. Differences between treatments are not significant ($P > 0.05$).

second most abundant species of the Eumull after *Isotomiella minor*. The decrease in the number of Eumull species was due, not only to the collapse of the *Isotomurus palustris* population, but also to the disappearance of several other less abundant Eumull species, among them *Heteromurus nitidus* (data not shown).

The observed collapse in Eumull species (both in abundance and number) under the influence of the presence of Dysmoder was less pronounced when the herbicide was added, regardless of the compartment chosen for this addition (Table 5, Fig. 1). Nevertheless, the abundance of *Isotomurus palustris* remained always at a very low level, compared to Eumull alone (with or without herbicide, see Table 2). An increase in the number of ubiquitous species was observed in Eumull when it was combined with Dysmoder plus herbicide. As already mentioned, Dysmoder species appeared, too, in Eumull when this was combined with Dysmoder, irrespective of the application of the herbicide.

3.4. Interaction between humus type and herbicide in Dysmoder communities

An increase in the total number of species was observed in Dysmoder combined with Eumull, compared

Table 4
Experiments with Dysmoder in the two compartments of experimental boxes

	Dysmoder/Dysmoder	Dysmoder/Dysmoder + Herbicide	Dysmoder + Herbicide/ Dysmoder
Dicyrtoma fusca	0	0	0
Folsomia manolachei	0	0	0
Friesea truncata	14.6 ± 0.9 a	18.6 ± 6.5 ab	7.4 ± 1.9 b
Heteromurus major	1.7 ± 0.7	3.0 ± 1.0	2.8 ± 0.4
Isotomiella minor	46.8 ± 6.3	40.6 ± 6.4	32.6 ± 7.3
Isotomurus palustris	0	0	0
Lepidocyrtus lanuginosus	7.0 ± 1.0	5.8 ± 1.0	5.2 ± 1.5
Lepidocyrtus lignorum	2.8 ± 0.6	2.8 ± 0.7	4.2 ± 1.2
Lipothrix lubbocki	2.8 ± 0.4	2.2 ± 0.7	2.2 ± 1.0
Megalothorax minimus	0	2.2 ± 2.2	1.0 ± 0.5
Mesaphorura leitzaensis	0.1 ± 0.1	0.6 ± 0.6	0.4 ± 0.2
Micranurida pygmaea	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
Neanura muscorum	3.3 ± 1.0	1.0 ± 0.3	2.2 ± 1.7
Orchesella cincta	0.6 ± 0.4	1.0 ± 0.4	0.6 ± 0.4
Paratullbergia callipygos	0.2 ± 0.1	0.4 ± 0.2	0.8 ± 0.4
Parisotoma notabilis	16.7 ± 0.9	16.6 ± 3.9	14.6 ± 4.0
Proisotoma minima	7.9 ± 1.6	6.2 ± 1.6	5.4 ± 2.7
Sphaeridia pumilis	0 b	3.2 ± 2.0 a	2.8 ± 1.2 a
Stenaphorura denisi	0	0	0
Tomocerus minor	1.2 ± 0.3	1.2 ± 0.8	1.8 ± 0.7
Total abundance	106.3 ± 7.5	106.6 ± 6.5	85.2 ± 16.6
Total number of species	10.3 ± 0.3	11.8 ± 0.5	12.8 ± 1.0
Relative richness	0.10 ± 0.01 b	0.11 ± 0.01 ab	0.19 ± 0.05 a
Abundance of Eumull species	0	0	0
Number of Eumull species	0	0	0
Abundance of ubiquitous species	88.7 ± 6.3	89.2 ± 5.5	67.8 ± 12.8
Number of ubiquitous species	5.5 ± 0.3 b	6.6 ± 0.2 a	6.6 ± 0.2 a
Abundance of Dysmoder species	17.6 ± 1.7	17.4 ± 3.5	17.4 ± 4.4
Number of Dysmoder species	4.8 ± 0.4	5.2 ± 0.6	6.2 ± 1.0

Data are numbers of animals per compartment averaged over five replicates with standard errors. The slash sign separates the considered compartment (left) and the adjacent compartment (right). When both compartments were given the same treatment, corresponding data were pooled, otherwise they were considered as distinct treatments. Significant differences between treatments ($P < 0.05$) are indicated by different letters.

to Dysmoder alone (Table 6, Fig. 1). At the species level, the only effect of the presence of Eumull was a strong decrease in the abundance of *Neanura muscorum*, a Dysmoder species (Table 1).

The herbicide interacted with the humus type by increasing the number of ubiquitous species in Dysmoder combined with Eumull, when the application occurred in both compartments (Fig. 1, Table 6). The abundance of Dysmoder species in Dysmoder combined with Eumull decreased by 50% when the herbicide was applied to the Dysmoder compartment. At the species level, the earlier mentioned decrease in the abundance of *Neanura muscorum* when Dysmoder was combined with Eumull, disappeared in the presence of herbicide, whatever the compartment chosen for the application (Table 6). *Sphaeridia pumilis*, which was absent from Dysmoder in the absence of Eumull (Table 6) or of herbicide (Table 2), was present when the acid humus was combined with Eumull, and the abundance of this species increased in the presence of herbicide, whatever the compartment chosen for its application. Another stimulatory effect of the herbicide was observed also on *Lepidocyrtus lignorum*, the population of which doubled when the herbicide was applied to both compartments.

Table 5
Experiments with Eumull communities adjacent to Dysmoder

	Eumull/Eumull	Eumull/ Dysmoder	Eumull/ Dysmoder + Herbicide	Eumull+Herbicide/ Dysmoder + Herbicide	Eumull + Herbicide/ Dysmoder
Dicyrtoma fusca	1.9 ± 0.7	1.2 ± 0.4	1.2 ± 0.5	1.4 ± 0.5	1.0 ± 0.3
Folsomia manolachei	0.6 ± 0.3 ab	0.0 ± 0.0 b	1.0 ± 0.0 a	0.4 ± 0.2 ab	1.0 ± 0.8 ab
Friesea truncata	1.5 ± 0.3	1.8 ± 0.8	1.8 ± 0.7	1.4 ± 0.4	1.2 ± 0.5
Heteromurus major	0	0.2 ± 0.2	0	0.2 ± 0.2	0.4 ± 0.2
Isotomiella minor	10.7 ± 1.6	8.4 ± 1.5	10.8 ± 2.6	9.8 ± 2.4	7.4 ± 2.1
Isotomurus palustris	3.7 ± 1.6 a	0.6 ± 0.2 b	1.0 ± 0.8 ab	0.8 ± 0.2 b	0.4 ± 0.2 b
Lepidocyrtus lanuginosus	0	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
Lepidocyrtus lignorum	0	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0
Lipothrix lubbocki	0	0	0	0.2 ± 0.2	0.2 ± 0.2
Megalothorax minimus	0	0	0.4 ± 0.4	0	0
Mesaphorura leitzaensis	0	0	0	0.4 ± 0.2	0
Micranurida pygmaea	0	0	0	0	0
Neanura muscorum	0	0.2 ± 0.2	0	0.2 ± 0.2	0.2 ± 0.2
Orchesella cincta	0.1 ± 0.1	0.2 ± 0.2	0	0	0.2 ± 0.2
Paratullbergia callipygos	0	0.2 ± 0.2	0.4 ± 0.4	0	0
Parisotoma notabilis	0.2 ± 0.2	0.6 ± 0.4	0.2 ± 0.2	0	0.4 ± 0.2
Proisotoma minima	0	0	0	0	0
Sphaeridia pumilis	0	0.4 ± 0.4	0.2 ± 0.2	0.8 ± 0.6	0
Stenaphorura denisi	0.4 ± 0.2	0.4 ± 0.2	0.2 ± 0.2	0.4 ± 0.2	1.0 ± 0.4
Tomocerus minor	0	0	0	0	0
Total abundance	19.9 ± 2.2	15.2 ± 2.6	18.2 ± 3.0	17.0 ± 2.6	16.8 ± 5.0
Total number of species	5.0 ± 0.6	5.8 ± 0.7	6.0 ± 0.3	6.6 ± 0.9	5.8 ± 0.7
Relative richness	0.27 ± 0.04 b	0.40 ± 0.03 a	0.36 ± 0.05 ab	0.41 ± 0.06 ab	0.44 ± 0.10 ab
Abundance of Eumull species	5.5 ± 1.9 a	1.0 ± 0.0 b	2.6 ± 1.1 ab	2.2 ± 0.6 ab	5.6 ± 3.6 ab
Number of Eumull species	2.5 ± 0.4 a	1.0 ± 0.0 b	2.0 ± 0.5 ab	2.2 ± 0.6 ab	1.8 ± 0.4 ab
Abundance of ubiquitous species	14.4 ± 1.8	13.0 ± 2.4	14.6 ± 2.3	14.2 ± 2.4	10.4 ± 2.4
Number of ubiquitous species	2.5 ± 0.3 b	3.6 ± 0.7 ab	3.4 ± 0.2 a	3.8 ± 0.7 ab	3.2 ± 0.5 ab
Abundance of Dysmoder species	0 b	0.6 ± 0.2 a	0.8 ± 0.5 ab	0.6 ± 0.2 a	0.8 ± 0.4 ab
Number of Dysmoder species	0 b	0.6 ± 0.2 a	0.4 ± 0.2 ab	0.6 ± 0.2 a	0.8 ± 0.4 ab

Data are means of five replicates with standard errors. The slash sign separates the considered compartment (left) and the adjacent compartment (right). When both compartments were given the same treatment, corresponding data were pooled, otherwise they were considered as distinct treatments. Significant differences between treatments ($P < 0.05$) are indicated by different letters.

3.5. Laboratory assays on single species

Experiments with *Heteromurus nitidus*, a typical Eumull species (Ponge, 1993; Salmon and Ponge, 1999), showed it to be indifferent to the presence of isoproturon until a concentration of $0.5\,\mathrm{mg\,l^{-1}}$ was reached (Fig. 2). Above this threshold, the animals avoided the herbicide, but the departure from indifference was significant only above $5\,\mathrm{mg\,l^{-1}}$. The other species, *Folsomia manolachei*, coming from Dysmoder soil, exhibited a weak (insignificant) attraction to the herbicide at concentrations below $0.05\,\mathrm{mg\,l^{-1}}$, then the response abruptly turned to avoidance, which became significant above $0.5\,\mathrm{mg\,l^{-1}}$.

At the beginning of the experiment with complete collembolan communities, the concentration of isoproturon in the soil solution varied from $2.7\,\mathrm{mg\,l^{-1}}$ for Eumull to $4.4\,\mathrm{mg\,l^{-1}}$ for Dysmoder, as ascertained from the amount of herbicide applied at the beginning of the experiment and the amount of water present in the soil. Compared to filter paper experiments, this corresponded to a concentration where *Heteromurus nitidus* shifted from indifference to avoidance (Fig. 1) and to a range of concentration where *Folsomia manolachei* clearly avoided the herbicide.

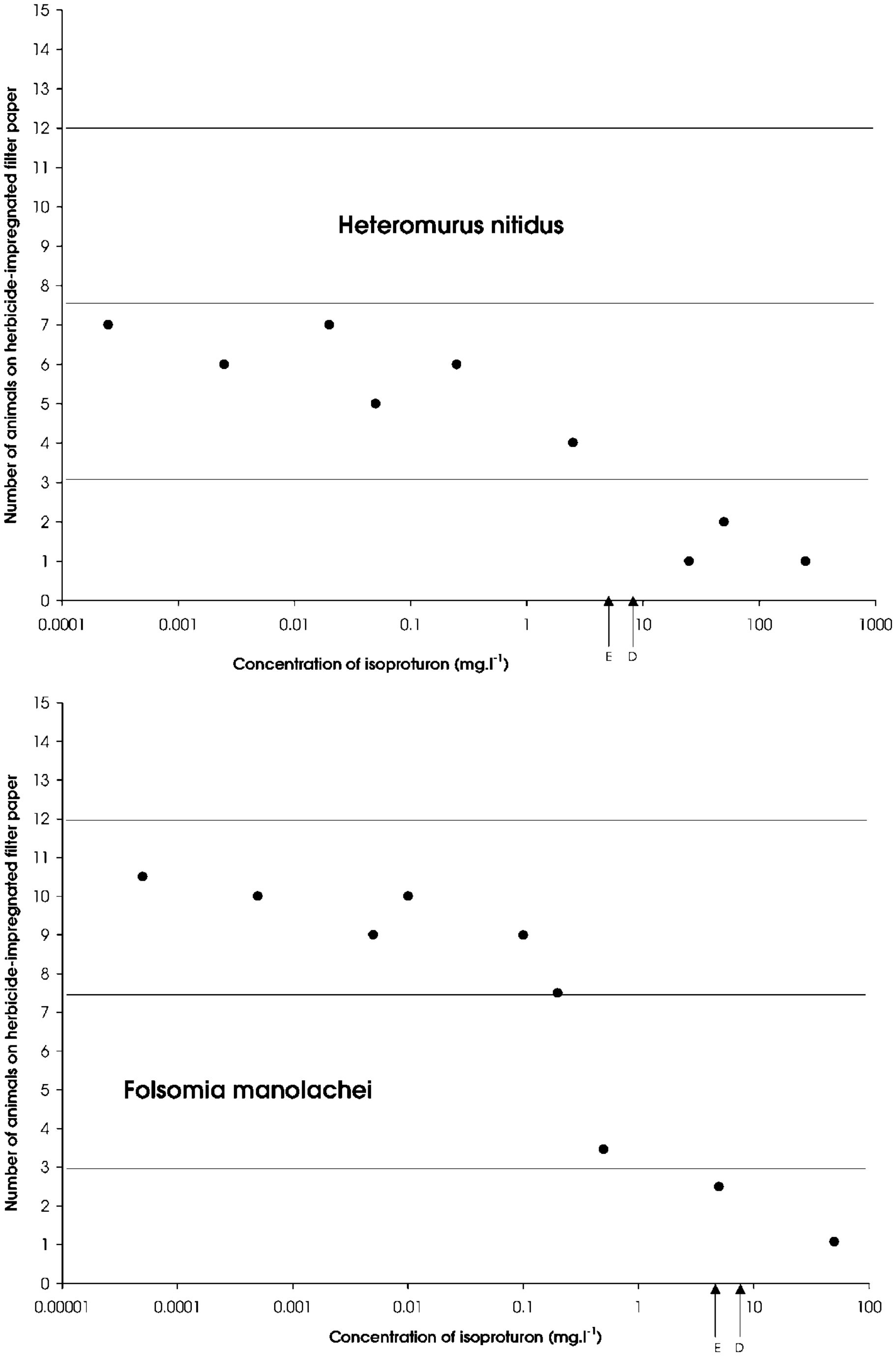

Fig. 2. Results of choice experiments with *Heteromurus nitidus* (Eumull population) and *Folsomia manolachei* (Dysmoder population), showing the number of animals found on impregnated filter paper after 1 h (15 replicates with one individual each) plotted as a function of the concentration of the herbicide (logarithmic scale). Arrows (E for Eumull and D for Dysmoder) indicate the concentration of the soil solutions in experiments with complete collembolan communities. Horizontal lines indicate absence of choice (7.5 individuals on each side) and its confidence interval at the 95% threshold level.

Table 6
Experiments with Dysmoder communities adjacent to Eumull

	Dysmoder/ Dysmoder	Dysmoder/ Eumull	Dysmoder/ Eumull + Herbicide	Dysmoder + Herbicide/Eumull + Herbicide	Dysmoder + Herbicide/ Eumull
Dicyrtoma fusca	0	0	0.4 ± 0.4	0	0.2 ± 0.2
Folsomia manolachei	0	0	0.2 ± 0.2	0.2 ± 0.2	0
Friesea truncata	14.6 ± 0.9 a	15.0 ± 4.8 ab	14.8 ± 3.6 ab	16.4 ± 4.8 ab	8.4 ± 1.9 b
Heteromurus major	1.7 ± 0.7	2.2 ± 0.9	3.8 ± 0.7	3.6 ± 0.7	2.2 ± 0.4
Isotomiella minor	46.8 ± 6.3	63.8 ± 12.8	45.6 ± 12.3	54.8 ± 9.3	67.8 ± 8.0
Isotomurus palustris	0	0.2 ± 0.2	1.0 ± 1.0	0.2 ± 0.2	0
Lepidocyrtus lanuginosus	7.0 ± 1.0	4.8 ± 0.7	7.8 ± 3.0	6.6 ± 1.3	5.2 ± 1.7
Lepidocyrtus lignorum	2.8 ± 0.6 b	2.6 ± 0.4 b	3.6 ± 0.8 ab	5.0 ± 0.5 a	3.6 ± 0.9 ab
Lipothrix lubbocki	2.8 ± 0.4 a	2.8 ± 1.0 ab	3.6 ± 0.9 a	2.4 ± 0.7 ab	1.4 ± 0.4 b
Megalothorax minimus	0	0	0.2 ± 0.2	0.2 ± 0.2	0.8 ± 0.4
Mesaphorura leitzaensis	0.1 ± 0.1	0.6 ± 0.6	0.2 ± 0.2	0.4 ± 0.2	0.8 ± 0.4
Micranurida pygmaea	0.2 ± 0.2	0.2 ± 0.2	0.6 ± 0.2	0.4 ± 0.2	0.2 ± 0.2
Neanura muscorum	3.3 ± 1.0 a	0.6 ± 0.2 b	4.6 ± 3.1 a	3.0 ± 1.4 ab	1.0 ± 0.3 ab
Orchesella cincta	0.6 ± 0.4	0.4 ± 0.2	0.8 ± 0.8	1.0 ± 0.4	0.2 ± 0.2
Paratullbergia callipygos	0.2 ± 0.1	1.2 ± 0.6	0.6 ± 0.4	0.8 ± 0.4	0.6 ± 0.2
Parisotoma notabilis	16.7 ± 0.9	19.6 ± 4.3	16.0 ± 3.4	18.8 ± 1.8	18.4 ± 4.1
Proisotoma minima	7.9 ± 1.6 a	7.4 ± 1.5 ab	9.8 ± 3.6 a	7.4 ± 1.4 a	2.4 ± 0.7 b
Sphaeridia pumilis	0.0 ± 0.0 c	1.2 ± 0.6 bc	5.2 ± 2.3 ab	4.8 ± 1.2 a	3.4 ± 1.0 ab
Stenaphorura denisi	0	0	0	0	0
Tomocerus minor	1.2 ± 0.3	1.2 ± 0.6	0.4 ± 0.4	1.2 ± 0.4	1.0 ± 0.4
Total abundance	106.3 ± 7.5	127.8 ± 18.1	121.0 ± 29.5	127.6 ± 13.7	118.0 ± 14.7
Total number of species	10.3 ± 0.3 b	12.2 ± 0.4 a	13.8 ± 1.1 a	13.8 ± 0.7 a	13.0 ± 0.8 a
Relative richness	0.10 ± 0.01	0.10 ± 0.01	0.13 ± 0.02	0.11 ± 0.01	0.11 ± 0.01
Abundance of Eumull species	0	0.2 ± 0.2	1.6 ± 1.1	0.4 ± 0.2	0.2 ± 0.2
Number of Eumull species	0	0.2 ± 0.2	0.8 ± 0.4	0.4 ± 0.2	0.2 ± 0.2
Abundance of ubiquitous species	88.7 ± 6.3	108.0 ± 17.6	94.4 ± 22.7	107.8 ± 12.7	108.0 ± 14.0
Number of ubiquitous species	5.5 ± 0.3 c	6.2 ± 0.2 bc	6.6 ± 0.4 ab	7.0 ± 0.0 a	7.0 ± 0.3 ab
Abundance of Dysmoder species	17.6 ± 1.7 a	19.6 ± 1.2 ab	24.2 ± 6.9 a	19.2 ± 2.3 a	9.6 ± 1.7 b
Number of Dysmoder species	4.8 ± 0.4	5.8 ± 0.2	5.8 ± 0.7	6.2 ± 0.7	5.6 ± 0.8

Data are means of five replicates with standard errors. The slash sign separates the considered compartment (left) and the adjacent compartment (right). When both compartments were given the same treatment, corresponding data were pooled, otherwise they were considered as distinct treatments. Significant differences between treatments ($P < 0.05$) are indicated by different letters.

4. Discussion

The 2-week laboratory experiment on complete collembolan communities did not reveal effects of the application of isoproturon on the Eumull population. On the contrary, in several treatments the Dysmoder population showed an increase in the abundance of two species, the Symphypleone *Sphaeridia pumilis* and the Neelipleone *Megalothorax minimus*. Both were totally absent from the untreated soil; thus the herbicide can be suspected of favoring egg hatching, for instance by terminating diapause, or by decreasing egg predation. Although no clear-cut explanation can be found, it should be noted that *Sphaeridia pumilis* is known for its temporary disappearance, eggs being the only resting stage during unfavorable seasons (Blancquaert et al., 1982). A higher predation pressure can be suspected in Dysmoder compared to Eumull (Salmon and Ponge, 1999), which could explain why such effects were not shown in Eumull. Thus, direct as well as indirect effects of the herbicide could explain the observed phenomenon, although literature data cannot support either one or the other hypothesis.

Avoidance effects were revealed by choice experiments on filter paper, at the same concentration as that employed for the community experiment with natural soils. Such a phenomenon was only poorly revealed in soil when animals were allowed to cross a perforated wall to reach a compartment without herbicide. Among the more abundant species, only the predator *Friesea truncata* was suspected of having escaped from the herbicide-treated Dysmoder compartment. This species decreased in density in the treated Dysmoder soil when combined with Eumull, but in this case, no corresponding increase was observed in the Eumull counterpart, pointing to the ability of the Dysmoder population of *Friesea truncata* to cross the perforated wall to escape from the herbicide-treated compartment, but its inability to survive in Eumull.

Most marked effects were displayed by the Eumull community when Dysmoder was present in the neighboring compartment. A strong depressive effect was observed on the epigeic species *Isotomurus palustris*, although it was not affected at all by the herbicide. At first sight, this could be due to attraction to Dysmoder as well as to toxicity. Attraction to the Dysmoder compartment can be ruled out since only a few individuals of this Eumull species were found in Dysmoder when adjacent to Eumull, thus no compensatory increase occurred in the Dysmoder. Since *Isotomurus palustris* normally lives aboveground (Ponge, 1993), we can suspect that it did not withstand the Dysmoder smell in our experimental boxes, where volatile compounds (fungal odours, terpenes) probably abound. It has been demonstrated that the odor of the micromycete *Trichoderma* was avoided by the collembolan *Onychiurus sinensis* (Sadaka-Laulan et al., 1998), and that some compounds isolated from fungal odorous could be repellent to Collembola, too (Bengtsson et al., 1991), but no toxic effect mediated by olfactory compounds has been demonstrated so far.

Reverse effects of Eumull upon the Dysmoder community were quite different. No toxic effect of Eumull was observed, except a decrease in the abundance of the neanurid *Neanura muscorum*. Colonization of the Eumull compartment by animals coming from the Dysmoder was demonstrated. In the Dysmoder compartment, the population was stimulated to a great extent, probably above numbers actually found at the end of the experiment if Dysmoder animals found in the Eumull compartment (colonizing individuals) are added.

Despite weak effects of the herbicide alone, the combination of herbicide and humus treatments exhibited non-additive effects. The disappearance of toxic effects of Dysmoder upon herbicide application could result from an inactivation of the toxic components of the Dysmoder smell (if any) by isoproturon or adjuvant compounds. In the absence of any knowledge on the formulation of Madit D®, this information being not given by the producer, no clear hypotheses can be drawn. If we take into account the rapid transformation of isoproturon in the soil (Verschueren, 2001), mainly resulting from demethylation, hydrolysis and hydroxylation of the phenylurea moiety within a few weeks, a number of different chemicals, each with its own sorptive properties, may have appeared within the 2 weeks of our experiment. When Dysmoder was combined with Eumull, Dysmoder species were not eliminated by the herbicide, which otherwise would have resulted in a decrease in their number. Nevertheless, we observed a decrease in the abundance of species commonly but exclusively living in acid soils, such as the epigeic *Lipothrix lubbocki* and the endogeic *Proisotoma minima* (Ponge, 1993; Ponge, 2000a), in addition to the already mentioned depressive effect on the ubiquitous *Friesea truncata*. Since *Lipothrix lubbocki* and *Proisotoma minima* were never retrieved in the adjacent Eumull compartment, it appears that either (i) they were directly or indirectly affected by the herbicide without being able to escape it or (ii) they escape Dysmoder compartments but were killed in Eumull compartments. Such an effect was not displayed by the application of herbicide to Dysmoder in the absence of Eumull, which could give support to the second hypothesis.

Methods used in the present study allow the measurement of subtle effects of herbicides on motile non-target organisms, such as soil microarthropods, in particular when toxicity, attraction and avoidance are combined. Doses at which the herbicide was applied are well below the thresholds given for the toxicity of isoproturon. For instance, nitrification is not inhibited below $300\,\mathrm{mg\,l^{-1}}$ (Verschueren, 2001, nominal data per unit of soil not given), which is not far from the concentration of the undiluted herbicide ($500\,\mathrm{mg\,l^{-1}}$). Despite low concentration of the herbicide, perturbations of soil animal communities were displayed, especially when two distinct humus types were put together in the same boxes, pointing on non-additive effects of humus type and herbicide application. At the low doses used for our experiment on collembolan communities, avoidance rather than toxic effects can be suspected, supported by experimental results with single species, but toxic effects of one humus type upon the other, as well as detoxifying effects of the herbicide were registered, too, pointing to complex relationships between pesticides and soils when living organisms are involved (Torstensson, 2000). We conclude that further experiments are necessary before the inoculation of organisms living in acid soils, suggested as a trade-off for the negative impact of pesticides on biodiversity (Forbes and Forbes, 1994), could be applied successfully to agricultural soils.

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