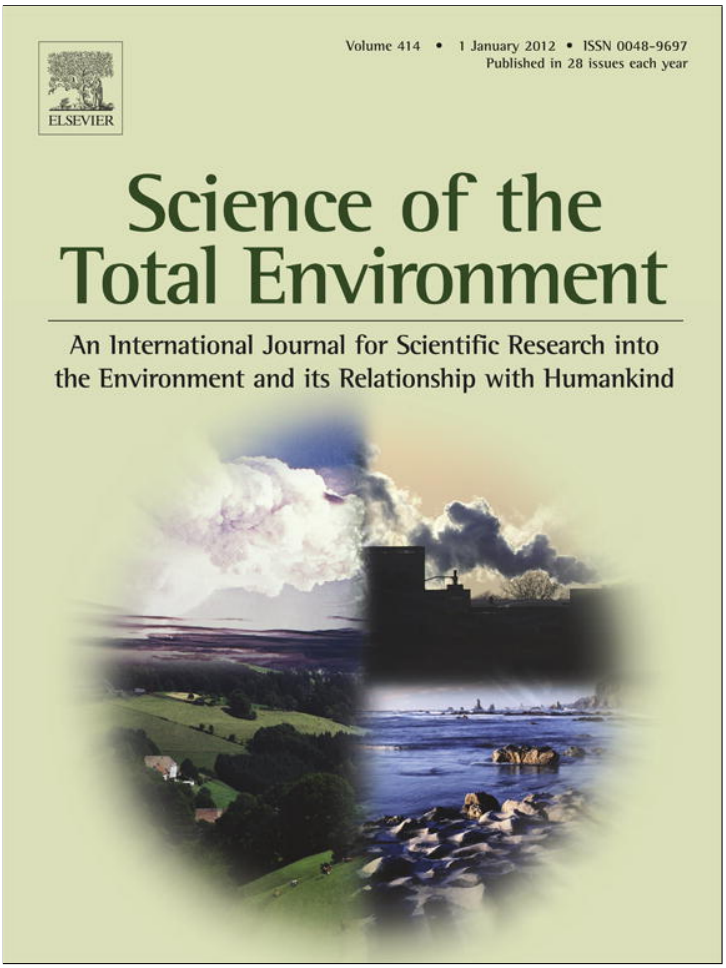

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Short-term effects of combined iprodione and vermicompost applications on soil microbial community structure

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ABSTRACT

The use of compost amendments to bioremediate potential organic pollutants in agricultural soils has recently become an increasingly important field of research. Although several fungicides have been extensively used to control a wide range of soil-borne fungal diseases, little is known about the impact of applying these pesticides on the structure and function of microbial communities in soils amended with vermicompost. The aim of this study was to evaluate the effect of a combined treatment of iprodione and vermicompost on soil microbiological parameters under laboratory conditions. The study was carried out on agricultural and grassland soils to identify the effect of iprodione application at field rate (FR) and 10-times FR (10FR) with and without vermicompost (VCH) on iprodione breakdown, fluorescein diacetate activity (FDA), total fatty acid methyl ester (FAME) profiles, total protein content, and protein profiles by using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Our results indicate that the addition of vermicompost decreased the iprodione breakdown at days 30 and 60 in non-sterilised agricultural soil and at 60 days in sterilised and non-sterilised grassland soil. Independent of vermicompost amended treatments, iprodione was found to mainly alter microbial communities after 30 days of incubation. On day 30, separation between communities treated with iprodione 10FR and iprodione 10FR + VCH treatments were well defined in both agricultural and grassland soils. Within each soil type, our results showed no difference in the total protein content. However, the protein content in the grassland soil was clearly higher than in the agricultural soil. SDS-PAGE gels revealed that the treatments applied to the agricultural soil using iprodione at the highest dosages (iprodione 10FR and iprodione 10FR + VCH) resulted in an alteration of the band pattern. In conclusion, the experiments revealed that the addition of vermicompost may decrease the breakdown of iprodione in soils. Furthermore, elevated dosages of iprodione may potentially affect the microbial community structure and diversity of the soil, which may lead to the deterioration of soil quality and fertility.

1. Introduction

Microorganisms are vital for maintaining soil quality and degrading organic matter and pollutants. The intensive use of agricultural chemicals has become an environmental concern due to their adverse effects on soil microorganisms, which may result in impairing soil fertility (Araújo et al., 2003; Pal et al., 2005). To control a wide range of soil-borne fungal diseases, a widely used fungicide iprodione (3-(3,5-dichlorophenyl)-N-iso-propyl-2,4-dioxoimidazoli dine-1-carboximide) has been introduced commercially. Although some microbial taxa may be able to use this fungicide as a source of energy, it may be toxic to other microorganisms (Monkiedje et al., 2002). In this respect, Wang et al. (2004) reported that iprodione altered the structural diversity of the soil by using denaturing gradient gel electrophoresis (PCR-DGGE). However, to the best of our knowledge, there is little information in the current literature about the effect of iprodione on key aspects of soil quality such as enzymatic activities or soil protein profiles.

Recently, the effects of the application of organic amendments on soil biological properties has become an interesting subject of research (Flieβbach et al., 1994; Albiach et al., 2000), as soil microbial communities may be affected by the application of pesticides (Pal et al., 2005). The addition of organic materials to soil has been reported to increase biomass C, basal respiration, the ratio of biomass C to total organic C, and the metabolic quotients, which indicate the activity of soil microorganisms (Pascual et al., 1997). An increase in microbial populations and activity after the addition of organic matter to soils has also been reported (Barakan et al., 1995; Zink and Allen, 1998; Arancon et al., 2006). In this respect, vermicomposts, which are stabilised organic materials produced by interactions between earthworms and microorganisms, have been suggested as a useful resource to remediate soils and to improve the carbon content and fertility (Field et al., 2004; Álvarez-Bernal et al., 2006).

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Although the addition of organic amendments has been shown to modify soil sorption, there is little information on how these amendments affect pesticide degradation (Delgado-Moreno and Peña, 2007). The existing information has shown that the application of vermicompost plays a positive role by increasing soil organic matter content, stimulating microbial activity, and enhancing degradation (Romero et al., 2010). Also, the addition of organic amendments was suggested to increase pesticide degradation rates due to the introduction of non-indigenous microbial populations that stimulate biodegradation (Fernández-Bayo et al., 2009). Also, the dissipation rates of herbicides or insecticides in soils amended with vermicompost has been studied (Fernández-Bayo et al., 2009).

Although it is widely accepted that chemicals sorbed onto soils are less accessible to microorganisms, which results in limiting their degradation (Koskinen et al., 2001), the relationship between fungicides and vermicompost has not been directly addressed. This relationship may be of interest, as a fungicide, such as iprodione, might act differently in soil with vermicompost addition. The aim of the present study is to evaluate the degradation rates of iprodione in two soils with different management histories, with and without vermicompost amendment. The present study allowed us to determine different effects of iprodione on various microbiological variables: particularly in respect to some of the adverse effects of iprodione on microorganisms in soils treated with vermicompost.

2. Materials and methods

2.1. Soils and vermicompost

Two soils with different management histories were used in this study: a silty loam soil from an agricultural plot located at INTA Manfredi in Córdoba City, Argentina and a loamy soil from natural grassland located at Ciudad Universitaria in Córdoba city, Argentina. Samples of both agricultural and grassland soils were collected from the surface layer (0–15 cm). The agricultural soil had mainly been cultivated with soybean and maize, which received a minimum amount of inorganic fertiliser without irrigation. The grassland soil had not received any pesticide treatment in the 10 months prior to the study. The characteristics of these two soils are shown in Table 1.

The vermicompost (VCH) was prepared from horse manure inoculated with *Eisenia fetida* (initial density: 7 g worms per 100 g horse manure) in a 3.0 m^2 vermicomposting bed according to the procedure described by Benitez et al. (2002). During the vermicomposting period (10 months), the moisture content of the substrate was kept at 80–85% by irrigation. The finished product was air dried, mechanically homogenised and passed through a 2 mm mesh. Then, it was placed in a storage room under dark and dry conditions. The vermicompost had the following properties: pH 7.7; organic C content of 370 g kg^{-1}; total N content of 18.4 g kg^{-1}; a C/N ratio of 25; humic substances content of 44.7 g kg^{-1}; humic acid content of 30.7 g kg^{-1}; fulvic acid content of 14 g kg^{-1}; an electrolytic conductivity of 1.43 dS m^{-1} and ash content of 630 g kg^{-1}. The soil samples were placed in microcosms containing about 500 g (dry weight) of soil. The amended soils were then left undisturbed for 10 days to allow for an equilibrium to be reached in the system.

Table 1
Chemical characteristics in agricultural and grassland soils prior iprodione and vermicompost addition.

Soil parameter	Agricultural soil	Grassland soil
% Clay (<0.002 mm)	17.2	20.1
% Silt (0.002–0.02 mm)	56.8	45.7
% Sand (0.02–2.0 mm)	26.0	34.2
pH	5.90	6.59
Bulk density (g cm^{-3})	1.60	1.20
Organic matter (SOM)	2.17	8.53
Total C (%)	1.26	4.95
Total N (%)	0.124	0.397
C/N ratio	10.2	12.5
N-NO_3 (ppm)	147.0	202.0
S-SO_4^{2-} (ppm)	11.7	28.3
P (ppm)	64.7	74.9
Electric conductivity (dS m^{-1})	2.6	2.6

2.2. Addition of iprodione and soil sampling

Iprodione (Rovral 50 WP) was applied as an aqueous solution to each container of the preincubated soils at concentrations of 0, 0.83 and 8.30 mg kg^{-1} and 0, 1.10 and 11.0 mg kg^{-1} for the agricultural and grassland soils, respectively. The first concentration of iprodione corresponds to concentrations used in the field (FR) and the second to 10 fold this dose (10FR). The 10 FR dose is recommended for laboratory tests to assess the side effects of pesticides on soil microflora (Sommerville, 1987). The conversion of the field application to milligrams of iprodione per kilogram of soil was calculated assuming an even distribution of the fungicide in the 0–15 cm soil layer. After iprodione application, the soils were vigorously homogenised and then incubated under static conditions for 90 days. The soils were incubated in the dark, and soil moisture was adjusted to 60% of water holding capacity (WHC). Microbiological studies were conducted for both agricultural and grassland soils, with and without vermicompost amendment. Except for soil protein analysis, soil sub-samples for microbiological studies were taken at 1 (26 h after application), 7, 30, 60 and 90 days after fungicide application. There were three replicate samples of each treatment.

2.3. Iprodione analysis

The iprodione breakdown study was carried out under both sterile and non-sterile conditions. Soil samples for iprodione breakdown were taken at 1 (26 h after application), 7, 30, 60 and 90 days after fungicide application. The iprodione remaining in soil samples was determined according to the procedure described by Wang et al. (2004). Briefly, 1 g of a soil sub-sample was extracted with 2 ml acetonitrile, previously filtered to 0.2 μm, and analysed using HPLC (Perkin Elmer) with a C-18 reverse-phase column. The mobile phase consisted of acetonitrile and water (80:20; v/v) with a flow rate of 1.0 ml min^{-1}: Iprodione was detected using a UV–vis detector at 216 nm. In order to improve analyte peak separation, the oven temperature was programmed to 27 °C.

2.4. Soil microbial enzyme

Fluorescein diacetate hydrolysing activity (FDA) was measured according to the method of Adam and Duncan (2001). Briefly, 2 g of soil sub-samples were mixed with 20 ml phosphate buffer (pH 7.6) in 50 ml Erlenmeyer flasks. Then, 0.2 ml of a solution of fluorescein diacetate (1 mg ml^{-1}) in acetone was added and incubated at 28 °C for 30 min and shaken. The reaction was immediately quenched by adding chloroform/methanol 2:1 v/v (15 ml). The suspension was subsequently centrifuged at 2000 rpm for 10–15 min, and the optical density of the clear supernatant was measured at 490 nm. Values for FDA hydrolysis were obtained using a calibration curve relating optical density to fluorescein concentration (ranging from 0 to 10 μg ml^{-1}).

2.5. Soil community fatty acid profiles

Analysis of whole-soil FAME profiles were used to detect changes in microbial communities in the disturbed soils with pesticides and vermicompost (Cavigelli et al., 1995). Ten gram sub-samples were weighed into ashed glass test tubes. Extraction of lipids and

saponification was performed by adding 5 ml of 3.25 M NaOH dissolved in methanol and heating at 80 °C for 1 h. Samples were vortexed for 20 s every 20 min. Extraction mixtures were neutralised with 10 ml of 3.25 M HCl in methanol; 3 ml of hexane were added to each sample. Extracts were centrifuged at 1000 rpm for 20 min. The organic phase was separated using a Pasteur pipette into a clean ashed glass tube. The hexane was evaporated almost to dryness under nitrogen gas and then transferred to labelled vials for injection into a gas chromatograph (Clarus 500 Perkin Elmer) equipped with flame ionisation detector (FID) and an Elite-5 capillary column. Methyl nonadecanoate was used as a quantitative internal standard. The separated fatty acid methyl-esters were identified and quantified by chromatographic retention time using a standard bacterial acid methyl ester mix (Supelco, Supelco UK, Poole, Dorset, UK). The fatty acid methyl esters described here use the standard nomenclature for lipid markers, A:BωC; where A is the number of carbon atoms, B is the number of double bonds, and ωC indicates the number of carbon atoms from the aliphatic end of the molecule and the first unsaturated bond. Isomers are denoted with the suffixes c (cis) or t (trans). Methyl branching is described by the prefixes i (iso) and a (anteiso), while methyl and cyclopropyl groups by Me and cy, respectively. The branched fatty acids i15:0, a15:0, i16:0, i17:0, and a17:0 were chosen to represent Gram (+) bacteria. The monoenoic and cyclopropane unsaturated fatty acids 16:1ω9, 16:1ω11, cy17:0, 18:1ω9c, 18:1ω9t, and cy19:0 were chosen to represent Gram (−) bacteria. The polyenoic 18:2ω6,9 was used as an indicator of fungal biomass.

2.6. Total soil protein content and protein profiles

Total protein quantification and the subsequent sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) were only conducted on 0 FR and 10 FR dosages of iprodione from samples with 30 days after application, with and without vermicompost amendment. Soil protein was extracted according to the snap freeze method (Singleton et al., 2003). For each soil sample, 1 g (at 60% WHC) was weighed into a microcentrifuge tube, and 100 μl of protease inhibitor cocktail (SIGMA P2714) and 1 ml of extraction buffer were added. The buffer solution contained 50 mM Tris–HCl, 10% sucrose, 2 mM dithiothreitol, 4 mM EDTA, and 0.1% Brij 58 (pH 7.58). The solution containing the soil sample was mixed and subjected to four repeated cycles of snap freezing in liquid nitrogen, and it was subsequently thawed to 25 °C. The solution was centrifuged at 15,000 rpm for 30 m at 4 °C, and a supernatant was then collected. Protein concentration was obtained by adding 1.2 ml acetone at 4 °C. The supernatant was then discarded and the white protein pellet was retained. Total protein determination of each soil extract was performed according to Lowry et al. (1951) assay procedures, which use bovine serum albumin as a standard.

Protein concentrations were also used to perform SDS-PAGE electrophoresis by using a minislab (28575-00 Moldel, Cole-Palmer, Vernon Hills, IL) according to the method of Laemmli (1970). The loading gel was 3% acrylamide in a 2.5 M Tris–HCl, pH 6.8 and 10% SDS. The resolving gel was prepared using 10% acrylamine in a 3.5 M Tris–HCl, pH 8.8 and 10% SDS. The electrode buffer was Tris-glycine (3.0 g Tris base, 14.4 g glycine, 5 ml 20% SDS, and 14 μl β-mercaptoethanol in 1 L water). Electrophoresis was conducted at a constant current of 20 mA for 2–3 h. After electrophoresis, the gel was fixed with a solution of acetic acid (5% v/v) and methanol (50% v/v) for 30 min and then stained with silver nitrate (0.1% w/v) for 20 min at 4 °C. The gel was developed with a sodium carbonate solution (2% w/v) mixed with formaldehyde solution (0.04% v/v) and stored in acetic acid (1% v/v) at 4 °C. After electrophoresis, gels were stained with the Silver Stain Kit (Bio-Rad) and then scanned using an optical densitometry system according to Kim and Barbeau (1991). The qualitative differences between soil extracts were estimated by calculating the number and relative-mobility values of protein bands. The molecular weight of the protein bands was determined by simultaneous running of standard molecular-weight proteins purchased from Bio-Rad Co (Hercules, C.A.).

2.7. Data analysis

A two-way analysis of variance (ANOVA) was carried out to establish the differences of iprodione breakdown, enzyme activity and total protein content, which uses INFOSTAT/Professional 2007 p.1 (F.C.A.—Universidad Nacional de Córdoba, Argentina).

Mean separation was accomplished using Fisher's protected least significant difference (LSD) test. Shifts in community FAME profiles over time were analysed by principal component analysis (PCA) to reduce the dimensionality. The first three components of the PCA were analysed using two-way multivariate analysis of variance (MANOVA) to determine statistical differences in the microbial community structure profiles. In addition, correlations between FAME concentrations and PC coordinates were calculated to identify FAMEs whose gradients were represented by PCs 1 and 2.

3. Results and discussion

3.1. Iprodione breakdown

It has been shown that when microbial dissipation is the primary contributor to iprodione degradation, applications of a pesticide to the soil frequently lead to the enrichment of the degrading microbial populations, which is followed by an accelerated disappearance of the chemical (Athiel et al., 1995). In our study, the dissipation of iprodione in both agricultural and grassland soils was similar during the 90 days incubation period (Table 2). However, in general, the dissipation of iprodione in sterilised soil was slower than in non-sterilised soil. After 90 days, the quantity of remaining iprodione varied from 18.5% to 36.5% in non-sterilised soil and 32.5% to 78.5% in sterilised soil. Thus, in our study, the dissipation of iprodione under non-sterilised conditions was always faster than under sterilised conditions and independent of soil type. These results suggest that the biological dissipation of the fungicide is more important than chemical dissipation in both agricultural and grassland soils, and these results are consistent with those previously reported by Wang et al. (2004). In their study, the results showed that the dissipation of iprodione was influenced by high temperatures and sterile soil conditions combined with biological and chemical influences.

In the current experiment, addition of vermicompost had only a limited and transient effect on the dissipation of iprodione. In the majority of the incubation period, the statistical interaction between iprodione dosages and vermicompost application was not significant for the two soils analysed. Our results indicate that addition of vermicompost decreased the iprodione breakdown at days 30 and 60 in non-sterilised agricultural soils. Similar results were observed at 60 days in sterilised and non-sterilised grassland soils. However, the addition of vermicompost to sterilised agricultural soil did not affect iprodione breakdown. These results suggest that vermicompost application can reduce iprodione availability to microorganisms, which is the result of sorption of the organic amendment or a toxic effect of iprodione on the microbial population. Although previous studies have shown that vermicompost addition can alter the dissipation rate of pesticides in soils, there is inconsistent information about the effect of vermicompost on pesticide degradation. Delgado-Moreno and Peña (2007) reported that compost and vermicompost from olive cake favoured the biological degradation of bensulphuron during the first week of incubation. However, in their study the addition of vermicompost to soil did not modify the dissipation rate for either chlorsulphuron or prosulphuron.

Table 2
Iprodione remaining (%) in agricultural and grassland soils under sterilized and non-sterilized conditions.

	Days of incubation after iprodione treatment									
	Day 1		Day 7		Day 30		Day 60		Day 90	
	Non-sterilized	Sterilized	Non-sterilized	Sterilized	Non-sterilized	Sterilized	Non-sterilized	Sterilized	Non-sterilized	Sterilized
Agricultural soil										
Iprodione FR	97.7 ± 1.4	99.7 ± 0.3	91.5 ± 1.5	98.5 ± 0.5	71.0 ± 2.0	78.5 ± 6.5	36.5 ± 1.5	82.0 ± 3.0	21.5 ± 2.5	78.5 ± 1.5
Iprodione 10FR	98.7 ± 1.3	99.3 ± 0.7	91.0 ± 1.0	96.5 ± 1.5	54.0 ± 2.1	77.5 ± 7.5	29.5 ± 5.5	83.0 ± 6.0	18.5 ± 2.5	54.5 ± 0.5
Iprodione FR + VCH	99.0 ± 1.0	99.0 ± 0.6	86.0 ± 1.0	96.0 ± 2.1	80.5 ± 4.4	90.5 ± 1.5	70.6 ± 5.4	69.5 ± 3.5	36.5 ± 8.5	49.0 ± 5.0
Iprodione 10FR + VCH	99.7 ± 0.3	99.3 ± 0.7	85.5 ± 0.5	98.5 ± 0.6	87.6 ± 4.5	95.0 ± 3.0	47.5 ± 2.5	68.0 ± 2.0	28.0 ± 3.0	66.5 ± 3.5
LSD	3.6	1.9	4.2	5.1	13.6	20.5	11.5	15.3	19.0	12.3
Significant level										
Vermicompost (VCH)	NS	NS	**	NS	**	NS	**	NS	NS	NS
Iprodione	NS	NS	NS	NS	NS	NS	*	NS	NS	NS
VCH x Iprodione	NS	NS	NS	NS	*	NS	NS	NS	NS	**
Grassland soil										
Iprodione FR	99.7 ± 0.3	99.3 ± 0.7	62.5 ± 0.6	97.5 ± 0.5	43.3 ± 4.8	62.0 ± 4.0	29.1 ± 2.9	31.3 ± 2.3	22.5 ± 6.5	46.5 ± 6.5
Iprodione 10FR	99.7 ± 0.3	99.4 ± 0.6	89.0 ± 3.0	97.5 ± 0.5	44.5 ± 1.5	87.5 ± 1.5	35.5 ± 3.5	69.5 ± 3.5	23.0 ± 2.0	32.5 ± 0.5
Iprodione FR + LCC	99.3 ± 0.7	99.0 ± 0.9	75.0 ± 2.1	94.6 ± 2.5	45.0 ± 2.0	70.7 ± 4.3	35.5 ± 1.5	68.0 ± 1.0	20.3 ± 2.0	58.0 ± 3.0
Iprodione 10FR + LCC	99.0 ± 1.0	99.3 ± 0.7	67.5 ± 1.5	98.0 ± 0.9	52.1 ± 7.0	81.5 ± 3.5	49.3 ± 2.8	73.0 ± 3.0	34.0 ± 4.0	76.5 ± 1.5
LSD	2.1	2.5	7.7	5.5	17.3	14.4	10.6	9.6	13.4	14.4
Significant level										
Vermicompost (VCH)	NS	NS	NS	NS	NS	NS	*	***	NS	**
Iprodione	NS	NS	**	NS	NS	**	*	***	NS	NS
VCH x Iprodione	NS	NS	**	NS	NS	NS	NS	**	NS	*

NS, not significant ($P > 0.05$); FR, field rate; LSD, least significant difference.
* Significant level: $P \leq 0.05$.
** Significant level: $P \leq 0.01$.
*** Significant level: $P \leq 0.001$.

3.2. Soil microbial activity

In this study, microbial activity in soil was measured using FDA hydrolysis, a method widely used as whole microbial activity indicator (Das et al., 2007). The results show that after 1, 60 and 90 days of incubation, microbial activity was not affected either by iprodione applications or vermicompost amendment. However, significant differences in microbial activity were observed at days 7 and 30 in both agricultural and grassland soils (Table 3). The effect of vermicompost on microbial activity was inconsistent and small, in relation to the amount of vermicompost that was added. At day 30 of incubation of agricultural soil, the microbial activity in the soil amended

Table 3
Microbial activity (μg fluorescein g^{-1} soil) in agricultural and grassland soils treated with iprodione either with and without vermicompost.

	Days of incubation after iprodione treatment				
	Day 1	Day 7	Day 30	Day 60	Day 90
Agricultural soil					
Control	0.91 ± 0.04	1.06 ± 0.07	1.00 ± 0.02	0.68 ± 0.01	0.62 ± 0.02
Iprodione FR	1.01 ± 0.07	1.30 ± 0.02	1.07 ± 0.04	0.65 ± 0.02	0.62 ± 0.03
Iprodione 10FR	0.83 ± 0.05	1.20 ± 0.05	1.36 ± 0.04	0.68 ± 0.09	0.61 ± 0.01
Control + VCH	0.88 ± 0.02	1.08 ± 0.02	0.48 ± 0.01	0.60 ± 0.01	0.63 ± 0.01
Iprodione FR + VCH	0.88 ± 0.04	1.03 ± 0.03	0.48 ± 0.01	0.65 ± 0.02	0.62 ± 0.02
Iprodione 10FR + VCH	0.95 ± 0.04	1.18 ± 0.03	0.54 ± 0.01	0.59 ± 0.02	0.61 ± 0.01
LSD	0.14	0.13	0.09	0.12	0.05
Significant level					
Vermicompost (VCH)	NS	*	***	NS	NS
Iprodione	NS	*	***	NS	NS
VCH X Iprodione	NS	*	***	NS	NS
Grassland soil					
Control	0.99 ± 0.13	1.67 ± 0.06	0.94 ± 0.06	0.96 ± 0.02	0.90 ± 0.09
Iprodione FR	0.92 ± 0.05	1.10 ± 0.17	0.74 ± 0.02	0.81 ± 0.07	0.99 ± 0.10
Iprodione 10FR	1.09 ± 0.04	0.91 ± 0.15	0.69 ± 0.02	0.81 ± 0.05	0.90 ± 0.07
Control + VCH	1.08 ± 0.04	0.92 ± 0.07	1.04 ± 0.12	1.06 ± 0.16	1.03 ± 0.08
Iprodione FR + VCH	1.01 ± 0.04	0.75 ± 0.10	0.69 ± 0.02	0.93 ± 0.02	0.85 ± 0.05
Iprodione 10FR + VCH	1.06 ± 0.01	1.05 ± 0.15	0.65 ± 0.05	0.97 ± 0.07	0.93 ± 0.02
LSD	0.21	0.40	0.18	0.24	0.23
Significant level					
Vermicompost (VCH)	NS	*	NS	NS	NS
Iprodione	NS	*	***	NS	NS
VCH X Iprodione	NS	*	NS	NS	NS

NS, not significant ($P > 0.05$); FR, field rate; LSD, least significant difference.
* Significant level: $P \leq 0.05$.
** Significant level: $P \leq 0.01$.
*** Significant level: $P \leq 0.001$.

with vermicompost decreased in comparison to the control treatment. A similar situation has been observed when other authors compared the effect of vermicompost on dehydrogenase activity (Romero et al., 2010). However, our results partially disagree with the findings of Masciandaro et al. (1997), who found that the microbial activity, which was estimated using dehydrogenase, β-glucosidase, and BAA-protease, was increased by vermicompost. The significantly lower microbial activity observed in vermicompost-treated soils could be due to an inhibitory effect resulting from the introduction of non-native soil microorganisms from the vermicompost, which may have stimulated competition among microorganisms (Arancon et al., 2006).

Gianfreda and Bollag (1996) reported that at recommended field rates pesticides usually did not exhibit serious and appreciable effects on the activity of enzymes. However, at elevated dosages, pesticides can decrease or inhibit soil enzyme activity.

At days 7 and 30 of incubation, iprodione application significantly altered soil microbial activity. In general, the application of iprodione to agricultural soils tended to increase microbial activity. However, the opposite response was observed when iprodione was applied to grassland soil. This apparent contradiction could be explained by different sensitivities of microbial populations in agricultural and grassland soils. In this respect, Perucci (1992) reported a close relationship between FDA hydrolytic kinetics and soil microbial biomass. Thus, the

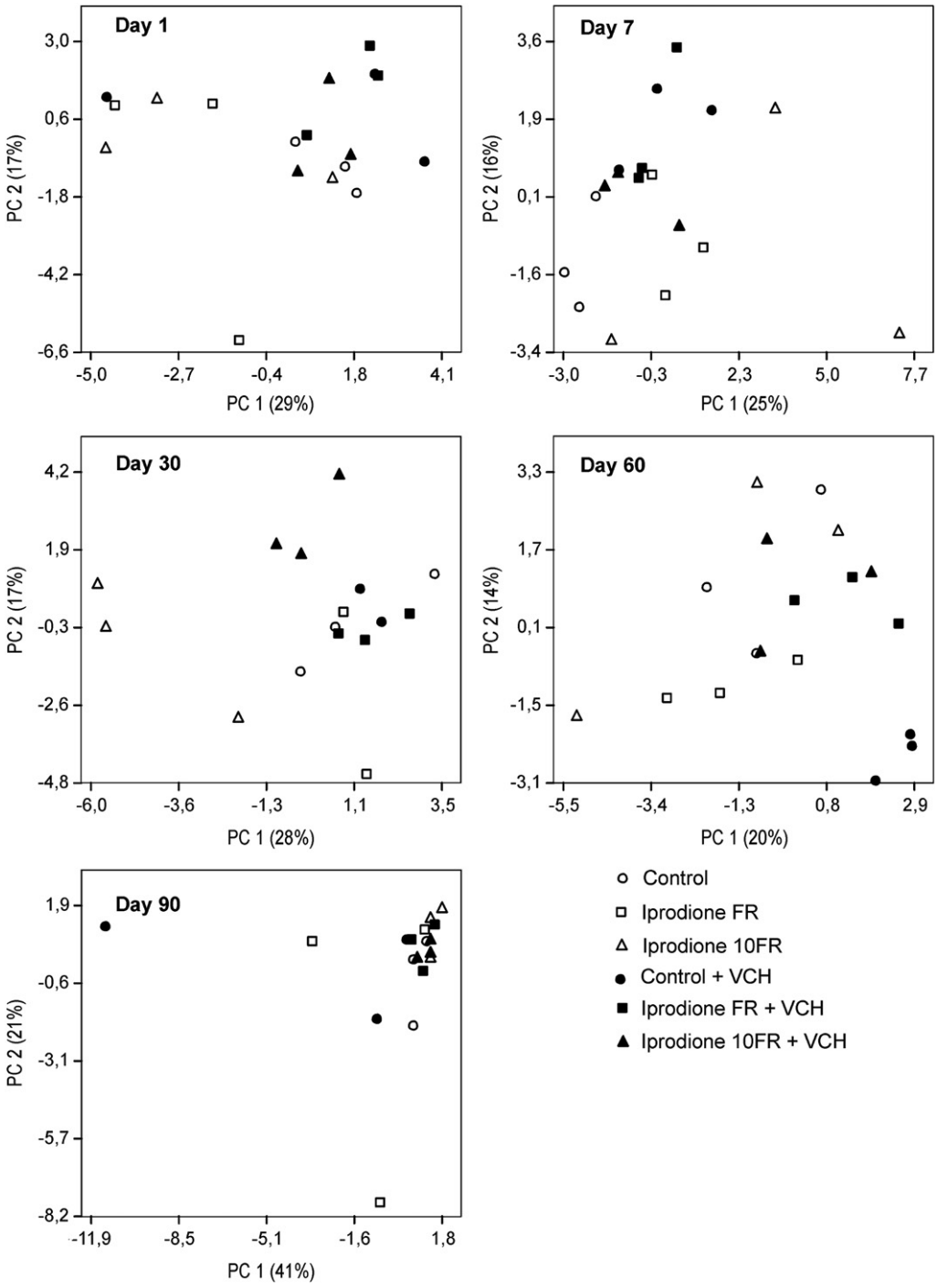

Fig. 1. Principal components analysis of microbial community FAME profiles in agricultural soil in response to iprodione with and without the addition of vermicompost. FR, field rate; VCH, vermicompost.

decrease in microbial activity observed in the grassland soil may be related to the reduction in the soil microbial biomass and the inhibitory effect of iprodione on the synthesis of the enzymes involved in FDA hydrolysis. It is possible that microbial communities with less sensitivity to iprodione may increase microbial activity. The increase in FDA values observed in the agricultural soil in our study could have resulted from the degradation of iprodione as a substrate by microorganisms. This effect of fungicides on soil enzyme activities has also been reported by other authors. For example, Chen and Edwards (2001) reported that the fungicide chlorothalonil decreased dehydrogenase activity in a silt loam soil, but this activity was increased in a sandy loam soil.

3.3. Soil microbial community structure

The FAME profiles consisted of 26 fatty acids, which were identified from each day of incubation in both agricultural and grassland soils. Previous similar results (Klose et al., 2006), showed that soil samples were dominated by several saturated fatty acids (16:0 and 18:0), branched fatty acids (principally a17:0 and i17:0) and monounsaturated fatty acids (18:1ω9). However, the grassland soil in our study showed a higher content of several saturated and cyclic fatty acids in comparison with the agricultural soil.

Changes in community structure determined by PCA in response to iprodione application, vermicompost amendment and time are

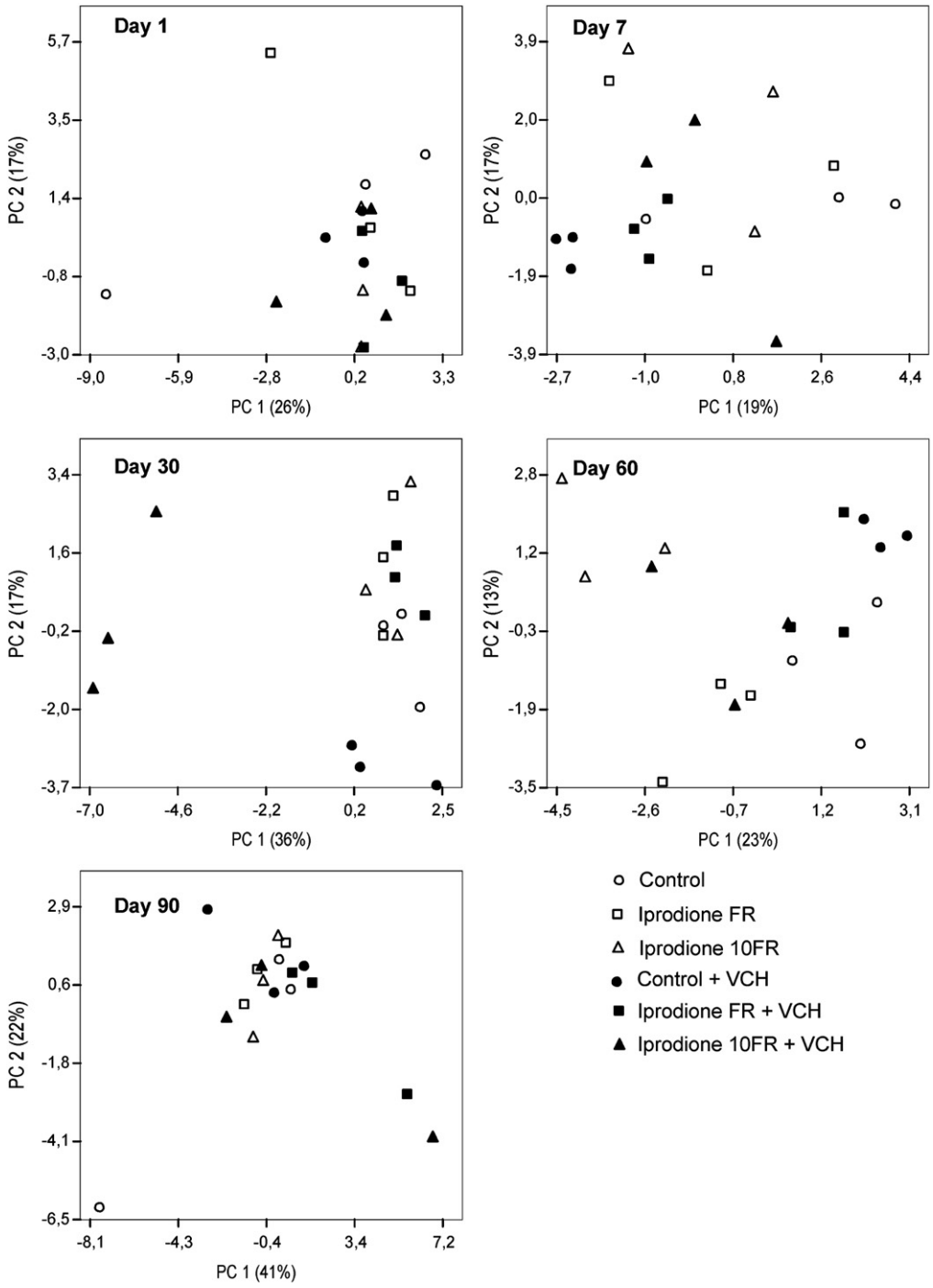

Fig. 2. Principal components analysis of microbial community FAME profiles in grassland soil in response to iprodione with and without the addition of vermicompost. FR, field rate; VCH, vermicompost.

presented in Figs. 1 and 2. At days 1 and 7, the PCA from the agricultural soil showed an unclear separation between treatments (Fig. 1). However, at day 30, separation among communities from samples of iprodione 10FR and iprodione10FR + VCH treatments were well defined. At day 60, PC analysis clearly distinguished Control + VCH treatment from the remaining treatments. At day 90, PC analysis did not reveal any significant relationship between treatments and variation in FAME profiles. The microbial communities in the grassland soil showed a similar trend (Fig. 2). At days 1 and 90, PC analysis could not clearly discriminate between treatments. MANOVA also indicated that iprodione application had a significant impact on the FAME community profiles of both agricultural and grassland soils (Table 5). This effect was very clear at day 30. Vermicompost affects microbial community of soils principally at day 60. At the end of the incubation period, MANOVA did not reveal significant differences between treatments. This result suggests that changes in the microbial

Table 4
Community FAMEs significantly correlated with Principal Components 1 and 2 of PC plots. Correlation between FAME concentrations and PC scores are indicated in parentheses. N. D., no detected.

Days of incubation	Agricultural soil		Grassland soil	
	PC 1	PC 2	PC 1	PC 2
Day 1	i16:0 (0.78)	i15:0 (0.70)	14:0 (0.76)	3OH 12:0 (0.85)
	18:2w6,9 (0.77)	cy19:0 (0.70)	i16:0 (0.65)	2OH 10:0 (0.73)
	18:1w9 (0.75)	15:0 (0.59)	i17:0 (0.64)	13:0 (0.69)
	2OH 12:0 (0.73)	3OH 12:0 (0.53)	16:1w9 (0.59)	a15:0 (0.60)
	13:0 (0.65)	a15:0 (0.53)	a15:0 (0.58)	
	3OH 12:0 (0.65)	11:0 (0.51)	i15:0 (0.55)	
	3OH 14:0 (0.50)		16:0 (0.54)	
	18:0 (−0.82)	17:0 (−0.53)		
	a15:0 (−0.67)		18:0 (−0.87)	2OH 16:0 (−0.57)
	cy17:0 (−0.65)		cy19:0 (−0.82)	i17:0 (−0.55)
	12:0 (−0.64)		11:0 (−0.63)	16:0 (−0.49)
	20:0 (−0.52)		cy17:0 (−0.57)	
			2OH 16:0 (−0.49)	
Day 7	16:0 (0.97)	13:0 (0.68)	i17:0 (0.82)	N.D.
	2OH 16:0 (0.72)	3OH 14:0 (0.49)	cy17:0 (0.79)	
			cy19:0 (0.69)	
	18:1w9 (−0.93)	cy17:0 (−0.78)	18:0 (0.56)	a15:0 (−0.74)
	18:2 (−0.86)	a15:0 (−0.64)		13:0 (−0.65)
	15:0 (−0.75)	i16:0 (−0.63)	18:2w6,9 (−0.61)	20:0 (−0.58)
	16:1w9 (−0.62)		i16:0 (−0.58)	12:0 (−0.50)
	2OH 12:0 (−0.55)		16:0 (−0.56)	i16:0 (−0.45)
	17:0 (−0.51)			
	2OH 10:0 (−0.49)			
Day 30	15:0 (0.72)	2OH 12:0 (0.55)	cy19:0 (0.75)	18:2 (0.81)
	cy19:0 (0.71)	cy17:0 (0.52)	17:0 (0.68)	18:1w9 (0.75)
	20:0 (0.70)	2OH 16:0 (0.51)	18:0 (0.60)	
	17:0 (0.68)	16:1w9 (0.49)	i16:0 (0.57)	cy17:0 (−0.77)
	a15:0 (0.67)		16:0 (0.52)	3OH 14:0 (−0.70)
	2OH 14:0 (0.56)			i17:0 (−0.58)
	16:1w9 (0.52)		12:0 (−0.95)	a15:0 (−0.50)
			2OH 10:0 (−0.93)	2OH 14:0 (−0.49)
	2OH 10:0 (−0.91)	i15:0 (−0.71)	11:0 (−0.93)	
	12:0 (−0.86)	14:0 (−0.66)	2OH 12:0 (−0.90)	
	11:0 (−0.77)	3OH 14:0 (−0.59)	14:0 (−0.88)	
	18:0 (−0.66)	18:2w6,9 (−0.57)	13:0 (−0.69)	
	18:2w6,9 (−0.54)	18:1w9 (−0.57)	i15:0 (−0.68)	
	18:1w9 (−0.54)	i17:0 (−0.53)	3OH 12:0 (−0.68)	
			18:1w9 (−0.53)	
Day 60	i16:0 (0.90)	2OH 12:0 (0.77)	cy19:0 (0.86)	i16:0 (0.54)
	2OH 14:0 (0.75)	3OH 12:0 (0.73)	i17:0 (0.74)	
	a15:0 (0.68)	16:0 (0.48)	12:0 (0.66)	2OH 16:0 (−0.64)
	18:0 (0.59)		18:1w9 (0.66)	17:0 (−0.52)
	20:0 (0.51)		18:0 (0.65)	2OH 14:0 (−0.48)
			3OH 12:0 (0.57)	
	16:0 (−0.76)	13:0 (−0.66)	17:0 (0.51)	
	cy17:0 (−0.63)		16:1w9 (0.49)	
			16:0 (−0.94)	
			2OH 12:0 (−0.65)	
Day 90	18:1w9 (0.76)	16:0 (0.86)	18:1w9 (0.89)	11:0 (0.89)
	18:0 (0.73)		18:2w6,9 (0.89)	16:0 (0.69)
			18:0 (0.83)	20:0 (0.68)
	2OH 10:0 (−0.97)	i16:0 (−0.89)	i16:0 (0.51)	
	13:0 (−0.96)	18:2w6,9 (−0.87)		12:0 (−0.85)
	3OH 12:0 (−0.95)	16:1w9 (−0.81)	3OH 12:0 (−0.95)	16:1w9 (−0.72)
	14:0 (−0.91)	i17:0 (−0.71)	13:0 (−0.95)	cy17:0 (−0.58)
	a15:0 (−0.86)	cy17:0 (−0.52)	14:0 (−0.87)	2OH 12:0 (−0.51)
	i15:0 (−0.80)	15:0 (−0.52)	cy19:0 (−0.86)	2OH 10:0 (−0.51)
	2OH 14:0 (−0.72)		2OH 12:0 (−0.83)	
	12:0 (−0.67)		2OH 10:0 (−0.82)	
	2OH 12:0 (−0.61)		2OH 14:0 (−0.81)	
	cy19:0 (−0.54)		a15:0 (−0.69)	

Table 5
Summary of effects according to analysis of variance.

	Days of incubation after iprodione treatment				
	Day 1	Day 7	Day 30	Day 60	Day 90
Agricultural soil					
Vermicompost (VCH)	*	NS	NS	**	NS
Iprodione	NS	NS	*	NS	NS
VCH X Iprodione	NS	***	NS	*	NS
Grassland soil					
Vermicompost (VCH)	NS	*	***	*	NS
Iprodione	NS	NS	***	***	NS
VCH X Iprodione	NS	NS	***	NS	NS

NS, not significant ($P>0.05$).
* Significant level: $P\leq0.05$.
** Significant level: $P\leq0.01$.
*** Significant level: $P\leq0.001$.

community structure associated with iprodione application and vermicompost amendment were not permanent. At days 7, 30 and 60, PC analysis separated Control + VCH from the remaining treatment. Furthermore, at day 30, the treatment with the highest dosage of iprodione and vermicompost (Iprodione 10FR + VCH) showed a clear separation. These results are in good accordance with those previously obtained by Wauchope et al. (1992), who found an inhibitory effect of iprodione on the growth of several populations of soil microorganisms. More recently, Wang et al. (2004) researched the effect of iprodione on soil bacterial community, as estimated by PCR-DGGE technique, and they reported a similar change in the band pattern of fungicide treatment in comparison with the control treatment. Previous findings and the results obtained in the current experiment indicate that iprodione alters microbial communities mainly at 30 days of incubation, although this effect may return to initial values. These results, in respect to the addition of vermicompost amendments, contribute novel information on its impact on soil microbial communities and its relationship to the dissipation of pesticide.

In this study, community FAME with the highest correlation scores along the PC1 and 2 are shown in Table 4. Taking into account the entire incubation time, neither component was clearly associated with a particular group of microorganisms or type of individual FAME. However, the results clearly show that the microbial communities changed from the initial moment of incubation. In general, both agricultural and grassland soils showed the highest correlations with several saturated (18:0, 12:0 and 14:0), branched (i16:0 and i17:0), hydroxylated (2OH 10:0, 2OH 12:0), monounsaturated (18:1ω9) and cyclic fatty acids (cy17:0 and cy19:0). At day 30, the agricultural soil treated with iprodione (Iprodione 10FR and Iprodione 10FR + VCH) showed a positive correlation with saturated and hydroxylated fatty acids (11:0, 12:0 and 2OH 10:0). At day 30, PCs of the grassland soil treated with iprodione showed a strong correlation with similar fatty acids observed in the agricultural soil. These results are in good agreement with a recent study reported by Miñambres et al. (2010), who observed an increment of saturated fatty acids in soil treated with iprodione. Further, there are studies that indicate that saturated fatty acids (11:0, 12:0, 16:0 and 18:0) are ubiquitous and commonly found in high amounts in all microorganism populations (Leckie, 2005).

3.4. Total soil protein content and protein profiles

The extraction of soil protein may be used to monitor the response of soil microbial communities to contaminants or to other environmental changes such as anoxia, drought and the application of organic matter (Singleton et al., 2003). Recently, Taylor and Williams (2010) postulated that the capacity to resolve the dynamics of the proteome of soil microbial communities will help in the investigation of the function and activity of soil microbiota. However, there are very few reports on the effect of contaminants on total soil protein content and/or protein profiles. Soil protein was extracted according to the snap freeze method reported by Singleton et al. (2003). These authors reported that the repeated cycles of snap freezing in liquid nitrogen and thawing to ambient temperature increases the amount of recuperated soil protein. This is the reason why we used the snap freezing method to extract soil protein. To our knowledge, this is the first report concerning the alteration of protein profiles in soils due to pesticide pollution. Within each soil type, our results showed that there were no differences observed in soil protein content between iprodione and vermicompost combined treatments (Fig. 3). However, the protein content in the grassland soil was clearly higher than in the agricultural soil. In fact, the protein extracts of the grassland soil were much darker in comparison to the lighter extracts from the agricultural soil, which contained a lower content of organic matter. Similar results have been observed by other authors, who reported that the coloration of protein extract may be related to the microbial biomass and diversity of the soil (Taylor and Williams, 2010).

Relatively high molecular weight protein bands were evident between 97 and 53 kDa and lower molecular weight bands between 19 and 36 kDa (Fig. 4). SDS-PAGE gels and densitometers also revealed that the treatments with iprodione at the highest dosages (iprodione 10FR and iprodione 10FR + VCH), when applied to the agricultural soil, altered the band pattern. In control treatment of the agricultural soil, two clear bands were detected at the top of the gel in correspondence to 53 kDa. However, these bands were not detected in iprodione 10FR and iprodione 10FR + VCH treatments. Singleton et al. (2003) observed that the increases or shifts in

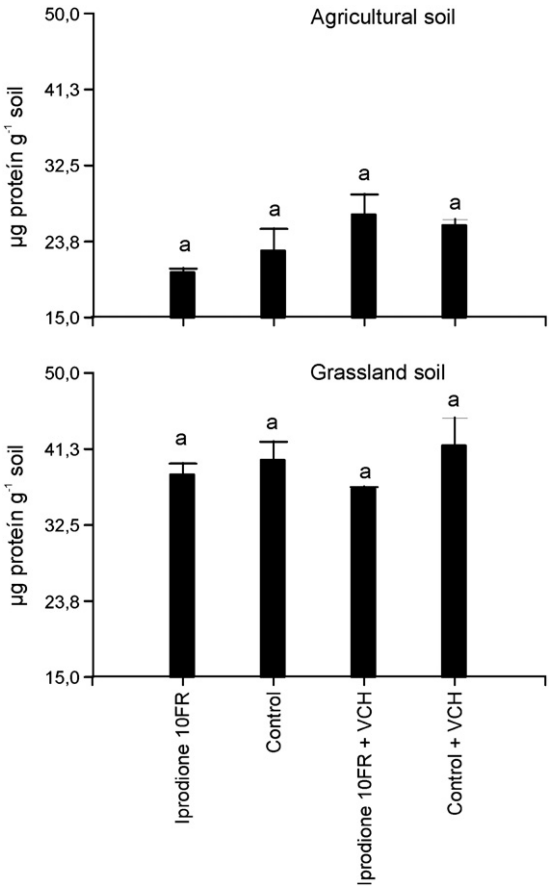

Fig. 3. Total protein content (µg protein g^{-1} soil) extracted from agricultural and grassland soils treated with iprodione with and without the addition of vermicompost. FR, field rate; VCH, vermicompost. Bars topped by the same letter are not significantly different according to Fisher's protected LSD test ($P\leq0.05$).

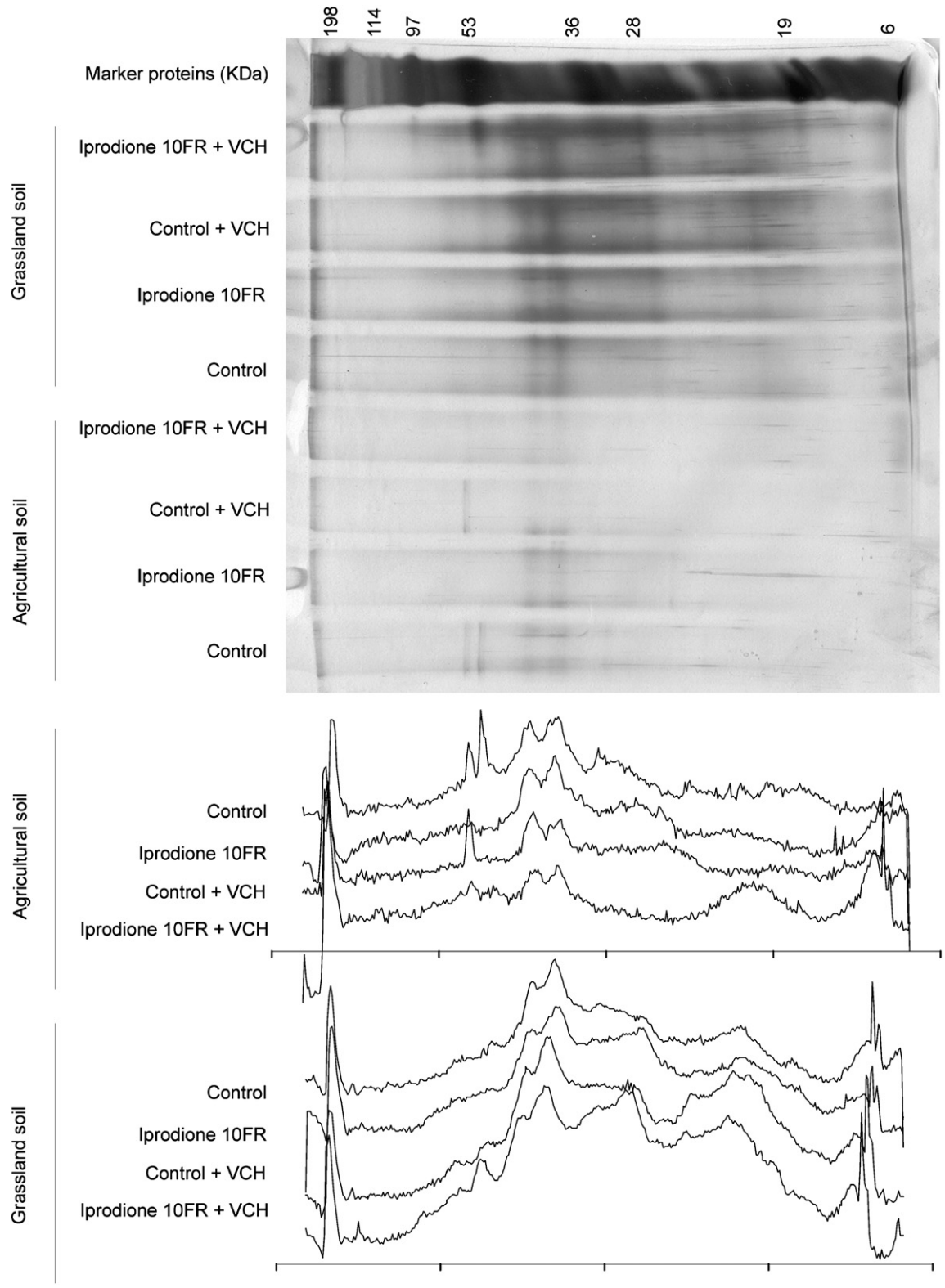

Fig. 4. SDS-PAGE profiles and densitometer scans of proteins from agricultural and grassland soils treated with iprodione with and without the addition of vermicompost. FR, field rate; VCH, vermicompost.

microbial communities contained different protein levels. According to these authors, the change in the band pattern observed in agricultural soil supports the idea that the application of this fungicide may result in the loss of iprodione-sensitive microbial biomass. In contrast, combination of iprodione and vermicompost treatments applied to the grassland soil in our study produced very similar protein patterns on the SDS-PAGE gel. It was surprising to find that the band pattern of the grassland soils were much darker in comparison to those obtained from the agricultural soil. One possible explanation for the smearing of proteins is that the grassland soil is composed of abundant microbial biomass, which yields a highly diverse protein pool and makes it difficult to detect well defined bands using SDS-PAGE.

In support of this observation, Masciandaro et al. (2008) showed that proteins may be in a free form or in complexes with colloids and humic substances. While enzyme activity in soils may be easily estimated, the direct determination of the extracellular protein concentration is difficult to study due to the extractant selection and the presence of interferences. Further, Taylor and Williams (2010) reported that high protein richness and diversity in soils may contribute to the smearing and low resolution of bands on gels.

4. Conclusion

To our knowledge, this is the first report about alterations in the protein profiles in soils due to pesticide pollution. The experiments showed that the addition of vermicompost may decrease the breakdown of iprodione. Land-use history was not related to dissipation rates, and biological degradation was found to be the main component affecting the dissipation of the fungicide. However, there was no clear relationship between the response of microbial activity and the combined iprodione and vermicompost treatments. Thus, elevated dosages of iprodione application may potentially affect soil microbial community structure and diversity, which may lead to the deterioration of soil quality and fertility. The results also confirm that SDS-PAGE is an appropriate and sensitive method to detect soil disturbances produced by the application of pesticides. However, further work is required to optimise the extraction of soil proteins and to confirm the validity of the soil protein profile method.

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