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Mesotrione dissipation and response of soil microbial communities in a soil amended with organic residues

Disipación de mesotriona en un suelo enmendado con residuos orgánicos y respuesta de las comunidades microbianas del suelo

Dissipação da mesotrione e resposta das comunidades microbianas em solos corrigidos com resíduos orgânicos

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ABSTRACT

The application of different organic residues as a soil amendment is an agricultural practice used to improve soil fertility by increasing the soil organic matter (OM). However, the OM from these residues can influence the behavior of pesticides applied jointly to the soil. Modification of the pesticide bioavailability in soils is of special interest since it can affect the activity and/or functioning of soil microbial community. Accordingly, the dissipation kinetics of mesotrione in unamended soil (S) and soils amended with sewage sludge (S+SS), green compost (S+C) and commercial pellets (S+P) and its possible effects on the soil microbial communities were studied. Soil biological parameters were determined as indicators of the soil microbial activity, functioning and structure: microbial biomass, dehydrogenase activity, respiration, and analysis of the phospholipid fatty acid (PLFA) profile extracted from the soil. Dissipation was more rapid in unamended soil than in amended soils and half-life (DT_{50}) values followed the order S+SS > S+C ≥ S+P > S. The biomass values increased in the amended soils with the exception of the P-amended soil. However, mesotrione had different effects on this parameter depending on the soil treatment. In general, dehydrogenase activity was stimulated by the addition of the amendment and herbicide to soil. Initially, respiration was higher in the unamended soil (control and treated soils) than the amended soils and mesotrione did not have any effect on this parameter. PLFAs analysis indicated that the overall structure of active microbial communities as well as the relative abundance of certain groups of microorganisms clearly changed according to the type of amendment and the incubation time, but remained unaffected by the application of mesotrione.

RESUMEN

El uso de diferentes residuos orgánicos como enmiendas del suelo es una práctica muy utilizada en la actualidad para mejorar su fertilidad como consecuencia de la aportación de materia orgánica (MO) de estos residuos. Sin embargo la MO aportada puede tener implicaciones en el comportamiento de los herbicidas aplicados simultáneamente al suelo con los residuos. De especial interés es la modificación de su biodisponibilidad ya que podría afectar en mayor o menor medida la actividad y funcionamiento de las comunidades microbianas del suelo. De acuerdo con esto se estudian en este trabajo la cinética de disipación del herbicida mesotriona en un suelo sin enmendar (S) y enmendado con lodo de depuradora (S+SS), compost verde (S+C) y una enmienda comercial en forma de pellets (S+P) y sus posibles efectos sobre las comunidades microbianas del suelo. Se determinaron los parámetros biológicos del suelo incluyendo la biomasa microbiana, la actividad deshidrogenasa, la respiración y el análisis del perfil de los ácidos grasos fosfolipídicos (PLFAs) extraídos del suelo como indicadores de la actividad, funcionamiento y estructura de los microorganismos del suelo. La disipación fue más rápida en el suelo sin enmendar que en los suelos enmendados y los valores de vida media (DT_{50}) siguieron el orden S+SS > S+C ≥ S+P > S. Los valores de biomasa aumentaron en los suelos enmendados con la excepción del suelo enmendado con P. Sin embargo, la mesotriona tuvo diferentes efectos

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en este parámetro dependiendo del tratamiento del suelo. En general, la actividad deshidrogenasa fue estimulada por la adición de la enmienda y del herbicida al suelo. Inicialmente la respiración fue mayor en el suelo sin enmendar (suelo control y suelo tratado con mesotriona) que en los suelos enmendados y la mesotriona no ejerció ningún efecto sobre este parámetro. El análisis de los PLFAs indicó cambios evidentes en la estructura total de las comunidades microbianas activas así como en la abundancia de ciertos grupos de microorganismos relacionados con el tipo de enmienda y el tiempo de incubación, pero no con la aplicación de mesotriona.

RESUMO

O uso de diferentes resíduos orgânicos coma condicionadores do solo é atualmente uma prática agrícola muito utilizada para melhorar a fertilidade do solo, através da contribuição para aumentar o seu teor de matéria orgánica (MO). No entanto, a MO fornecida pode afetar o comportamento dos herbicidas que são aplicados ao solo juntamente com estes resíduos. De particular interesse é a modificação da sua biodisponibilidade, uma vez que esta pode afetar, em maior ou menor medida, a atividade e funcionamento das comunidades microbianas do solo. Assim, o presente trabalho teve como objetivos o estudo da cinética de dissipação do herbicida mesotrione num solo sem condicionador (S) e num solo corrigido com lamas de ETAR (S+SS), composto verde (S+C) e com um condicionador comercial (S+P), assim como a sua possível influência sobre as comunidades microbianas do solo. Determinaram-se os seguintes parámetros biológicos: biomassa microbiana, atividade da desidrogenase, respiração e análise do perfil de ácidos gordos dos fosfolípidos (PLFAs) extraídos do solo como indicadores da atividade, funcionamento e estrutura dos microrganismos do solo. A dissipação foi mais rápida no solo sem condicionador do que nos solos corrigidos e os valores de vida média (DT_{50}) obedeceram à seguinte ordem S+SS > S+C ≥ S+P > S. Os valores da biomassa aumentaram nos solos corrigidos, com exceção do solo adicionado com P. No entanto, a mesotrione teve diferentes efeitos neste parâmetro consoante o tipo de tratamento do solo. Em geral, a atividade da desidrogenase foi estimulada pela adição ao solo do condicionador e do herbicida (com alguma exceção). No início, a respiração foi maior no solo sem condicionador (solo controlo e solo tratado com mesotrione) do que nos solo com condicionador e a mesotrione não teve qualquer tipo de efeito sobre este parámetro. A análise dos PLFAs indicou alterações evidentes na estrutura total das comunidades microbianas ativas, assim como na abundância de certos grupos de microrganismos relacionados com o tipo de condicionador e tempo de incubação, enquanto a aplicação da mesotrione não produziu qualquer efeito.

KEY WORDS
Pesticide, compost, sewage sludge, pellets, microbial parameters and degradation

PALABRAS CLAVE
Pesticidas, compost, lodos de depuradora, pellets, parámetros microbianos y degradación

PALAVRAS-CHAVE
Pesticide, composto verde, lodo de esgoto, condicionador comercial, parâmetros microbianos e degradação

1. Introduction

The effect of crop pesticides on soil microbial communities has gained interest in recent years, as these communities have been recognized for their important role in numerous reactions relating to soil conservation and sustainability. However, divergent research findings are reported in the literature about these impacts. Some studies have reported that pesticide residues could serve as carbon or energy sources to microorganisms, but other studies have reported negative effects on soil microorganisms (Hussain et al. 2009). These effects, usually at an ecosystem level (outside the bacterial body) not a metabolic level (inside the bacterial body), would depend on the processes that affect the behavior of pesticides in soil, especially their adsorption-desorption, degradation and transport (Cheng 1990; Huang et al. 1995). These processes control their bioavailability and their possible interaction with microorganisms as well as the existence of an alternative pool of more labile OC, and thus the study of the factors controlling these processes is of special interest.

One of these factors is the soil organic matter (OM) content, which can be modified by the addition of different organic residues. This practice has been extended currently to improve

the fertility of agricultural soils, such as semi-arid Mediterranean soils due to their low OM content (García-Izquierdo and Lobo-Bedmar 2008) and at the same time to reduce landfill disposal. Some organic residues such as sewage sludge (Navarro et al. 2007; MARM 2009) and green waste from pruning, previously composted (Moreno Casco and Moral Herrero 2008) could be used as soil amendments and simultaneously applied with pesticides in agricultural practices. Several reports have determined the impact of organic residues on the pesticide sorption by soils (Briceño et al. 2007). However, there is scarce information about the response and functioning of soil microbial community when pesticides and organic residues are simultaneously applied.

The aim of this work was to evaluate the changes in soil microbial communities after the joint application of mesotrione, a herbicide with a wide range of use that has been introduced in recent years, and organic amendments to an agricultural soil. Three organic residues, sewage sludge (SS), compost from pruning residues (C), and a commercial residue in the form of pellets (P) were used as soil amendments. For this purpose, dissipation kinetics of mesotrione in unamended and amended soils was studied. In order to evaluate changes in global activity, abundance and functioning of the soil microbial community, this study determined the soil microbial biomass, dehydrogenase activity (DHA) and respiration at different times through the herbicide dissipation period. The phospholipids fatty acids profile (PLFA) was analyzed as indicator of the changes in soil microbial community structure.

2. Material and Methods

2.1. Herbicide and reagents

Mesotrione (2-(4-(methylsulfonyl)-2-nitrobenzoyl)-1,3-cyclohexanedione) is a herbicide used to control grass and broad-leaved weeds mainly in maize crops. It is a compound with low hydrophobicity (log Kow of 0.11) and its solubility in water is 160 mg L^{-1} (Tomlin 2000; PPDB 2014). Pure standard from PESTANAL® was supplied by Sigma-Aldrich Química SA (Madrid, Spain) (> 99% purity).

HPLC grade acetonitrile and anhydrous chloroform (> 99% purity) were supplied by VWR International Eurolab (Spain). Ammonium sulfate, potassium sulfate (≥ 99% purity), ninhydrin reagent solution, 2,3,5-triphenyltetrazolium chloride (TTC) and 2,3,5-triphenylformazan (TPF) were supplied by Sigma-Aldrich Química S.L. (Madrid, Spain).

2.2. Organic residues

Compost (C) consisting of green waste from pruning was supplied by the City Hall of Salamanca (Salamanca, Spain). Sewage sludge (SS) from a domestic waste treatment plant and stabilized by anaerobic digestion was supplied by Aqualia SA (Salamanca, Spain). Commercial pellet (P), a mixture of vegetal waste and animal manure by stabilized sterilisation under pressure, was supplied by TIMAC AGRO SA (Spain).

Some characteristics of these organic residues were determined in samples previously air dried, homogenized and sieved (< 2 mm) (**Table 1**). The pH was determined in a residue/water suspension (1/2.5 w/v ratio). Organic carbon (OC) content was determined by oxidation (Walkley-Black method). Dissolved organic carbon (DOC) was determined in a suspension of residue (1/100 w/v ratio) in Milli-Q ultrapure water after residue shaking (24 h at 20 ºC), centrifugation (20 min at 10000 rpm), and filtering (Minisart NY 25 filter 0.45 m, Sartorius Stedim Biotech, Germany) using a Shimadzu 5050 (Shimadzu, Columbia, MD, USA) organic carbon analyzer. Total N was determined by the Kjeldahl method.

Table 1. Characteristics of organic residues given on a dry weight basis

Organic residue	pH	OC (%)	DOC (mg/g)	N (%)	C/N
Compost (C)	7.08	8.06	1.02	0.79	10.2
Sewage sludge (SS)	6.73	27.0	21.7	4.76	5.67
Pellets (P)	7.83	22.2	15.2	1.75	12.7

2.3. Unamended and amended soil

The soil sample used in all experiments was taken from the surface horizon (0-30 cm) on a farm located in Toro (Zamora, Spain). It is a Typic Xerorthent (Soil Survey Staff 2006) and its texture was classified as sandy loam (83.4% sand, 5.96% silt and 10.7% clay).

The amended soils were prepared by uniformly mixing soil with C, SS or P *in situ* at a rate of 50 t ha^{-1} (dry weight) on 15 October 2012. Unamended soil is termed S hereafter, whilst soil amended with SS, C, or P is termed S+SS, S+C and S+P. Soil samples of all treatments (~ 30 kg) were collected from the field (0-30 cm) and incubated undisturbed under environmental conditions in 60 x 40 x 25 cm trays at the IRNASA (Salamanca, Spain) over the experimental period. Samples of unamended and amended soils were sieved (< 2 mm) prior to their use in dissipation experiments.

Characteristics of unamended and amended soils were determined using standard analytical methods (MAPA 1986) and are included in **Table 2**. The DOC was determined in soil extracts (1/2 w/v ratio) as previously indicated for organic residues.

Table 2. Characteristics of unamended and amended soils given on a dry weight basis

Soil	pH	OC (%)	N (%)	C/N	DOC (mg/g)
S	6.29	0.49	0.04	12.2	0.05
S+C	7.05	1.50	0.18	8.33	0.07
S+SS	6.16	2.20	0.35	6.28	0.68
S+P	7.86	1.73	0.13	13.3	0.59

2.4. Dissipation studies

The herbicide dissipation experiment was conducted in accordance with SETAC guidelines (Lynch 1995). The standard compound was added to unamended or amended soils (500 g) to obtain a pesticide concentration of 2 mg kg^{-1} dry soil. Samples of each soil treatment were prepared in duplicate and they were incubated at 20 ºC in the dark. The soil moisture content, determined by the gravimetric method, was previously adjusted to 40% of the maximum soil water-holding capacity (26.8% for S; 22.5% for S+C; 29.4% for S+SS; 27.8% for S+P), and it was maintained by adding sterile (autoclaved at 120 ºC during 20 min) Milli-Q ultrapure water as necessary. A sterilized unamended soil sample was also prepared by autoclaving soil at 120 ºC for 1 h on three consecutive days. Sterilized soil was treated with the herbicide and incubated as indicated above, and these samples were used as controls to check the chemical degradation

of mesotrione. Finally, soils for microbiological control were prepared by adding only sterile Milli-Q ultrapure water. All soils were thoroughly stirred with a sterilized spatula, and all the steps were performed in a sterile cabinet. Soil samples were taken at day 0 for pesticide analysis, and thereafter repeatedly at different time intervals (up to 99 days).

2.5. Extraction and determination of mesotrione

Duplicate 5 g samples of each duplicate treatment (500 g of unamended or amended soil) were taken at each sampling time and shaken at 20 ºC for 2 h with 10 mL of methanol:water (1:1) in glass tubes. The samples were then sonicated for 1 h and centrifuged at 5045 g for 15 min, and the pesticide extracts were filtered in a Minisart NY 25 filter (Sartorius Stedim Biotech, Germany) to remove particles > 0.45 µm. For the determination of the herbicide, a volume of the extract was transferred to a glass vial for analysis. The recoveries of the extraction method were determined by spiking three unamended and amended soil samples with a solution of pesticide to a final concentration of 2 mg kg^{-1} and performing the extraction procedure as described above. The mean recovery values were 76% for S, 74% for S+C, 74% for S+SS, and 73% for S+P.

Mesotrione was quantified by HPLC with diode array (DAD) and mass spectrometer (MS) detectors (Waters Associates, Milford, MA), and Empower software as the data acquisition and processing system. The analytical column was a Luna PFP(2) (150 × 4.6 mm i.d., 3.0 µm) (Phenomenex, Torrance, CA, USA). The mobile phase was 90:10 (v/v) acetonitrile/water (1% formic acid). The flow rate of the mobile phase was 0.4 mL min^{-1} and the sample injection volume was 10 µL. The retention time was 5.6 min. Quantitative analysis was performed using the peak area of the compound obtained from the total ion chromatogram (TIC) in SIM mode. The molecular ions (m/z) corresponding to mesotrione in the positive ionization mode $[M]^+$ was 340.0. Calibration curve was performed with herbicide standards with concentrations between 0.5 and 25 µg mL^{-1} with correlation coefficient being > 0.99. Limit of detection (LOD) and limit of quantification (LOQ) were > 0.0018 and > 0.020 µg mL^{-1}, respectively.

2.6. Soil biochemical analysis

At the beginning of the dissipation experiments (0 days), and at 28 and 91 days, soil biomass-N, dehydrogenase activity (DHA), respiration and the phospholipid fatty acid (PLFA) were measured in unamended and amended soils (control – untreated, and herbicide treated soils).

Microbial biomass-N was extracted using the chloroform fumigation-extraction technique (Vance et al. 1987). Ninhydrin-reactive N released by fumigation was converted to biomass-C using a conversion factor of 20.6 (Joergensen and Brookes 1990).

Soil DHA was determined following the Tabatabai method (Tabatabai 1994). The method is based on the extraction and colorimetric determination of the intensely colored TPF produced from the reduction of colorless TTC in soils.

Soil respiration was determined by measuring O_2 absorption by microorganisms in 50 g portions of soil incubated at 25 ºC for 96 h, using an OxiTop Control BM6 containers with OxiTop Control OC 110 measurement system (WTW, Weilheim, Germany).

PLFA analysis was performed following the extraction and fractioning methodology described by Zelles (1997, 1999). Determination of PLFA was carried by GC with an Agilent 7890 equipment with flame ionization detector (FID) (Agilent Technologies, Wilmington, DE, USA). The identification and quantification of the fatty acids was performed with the Sherlock® 6.0 software (MIDI Inc., Newark, DE, USA). Specific PLFAs were used as biomarkers to quantify the relative abundances of particular microbial groups within the group of 53 fatty acids detected in the samples, on the basis that they have been isolated from organisms within these microbial groups with some specificity.

2.7. Data analysis

The dissipation kinetics for mesotrione was fitted to a single first-order (SFO) kinetic model ($C = C_0\ e^{-kt}$) or a first order multicompartment (FOMC) model ($C = C_0 / ((t / \beta) + 1)^{\alpha}$), known also as the Gustafson and Holden model. C is the pesticide concentration (mg kg^{-1}) at time t, C_0 is the initial pesticide concentration, k (day^{-1}) is the dissipation rate, α is a shape parameter determined by the coefficient of variation of k values and β is a location parameter. For the selection of the kinetic model that best describes the dissipation results, FOCUS work group guidance recommendations were followed (FOCUS 2006). The coefficient of determination (r^2) and the chi-square (χ^2) test were calculated as indicators of the goodness of fit. Values for the time to 50% dissipation, or DT_{50} values, were used to characterize the decay curves and compare variations in dissipation rates. The parameters of the kinetic models were estimated using the Excel Solver add-in package (FOCUS 2006).

Analysis of variance (ANOVA) was used to evaluate the effects of the different treatments on the soil DHA, microbial biomass and respiration. Standard deviation (SD) was used to indicate variability among replicates. Standard error (SE), at a confidence level of 95%, was determined with SPSS Statistics 22.0 software for windows (SPSS Inc. Chicago, USA).

PLFA data were analysed using multivariate (PCA and RDA) analyses with the CANOCO 4.5 program (Microcomputer Power, Ithaca, NY). Four replicates of unamended and SS- and C-amended soils without mesotrione (control) treatment and with mesotrione (M) treatment, after 0, 28 and 91 days of incubation time, were coded as dummy variables and used as independent variable in the multivariate analyses. Significance in RDA analyses was tested by Monte Carlo permutation tests (999 unrestricted permutations) for the first canonical axis as well as for the sum of all canonical axes.

3. Results and Discussion

3.1. Dissipation kinetics of mesotrione in unamended and amended soils

The decrease in the concentrations of mesotrione (expressed as a percentage of the herbicide initially applied) in unamended soil, sterile unamended soil and amended soils is shown in **Figure 1**. At the end of the incubation period, the mesotrione was almost totally dissipated in the S, S+C and S+P soils. However, in the S+SS soil, the percentage of mesotrione extracted from the soil was about 40% at 99 days, indicating a slower dissipation rate.

Data for residual concentrations of the herbicide as a function of time were fitted to SFO and FOMC models and kinetic parameters were calculated for each soil treatment. The dissipation kinetics fitted the SFO model better than the FOMC model (χ^2 error values were lower than those corresponding to the FOMC model). Kinetic parameters for the SFO fit are presented in **Table 3**. Previous studies have also reported the dissipation curves of mesotrione in unamended soils fitted to a SFO model (Dyson et al. 2002; Chaabane et al. 2008).

The DT_{50} value for the dissipation of mesotrione (9.7 days) in unamended soil was in the same range as that reported in the literature (4.5-34 days) for unamended soils (Dyson et al. 2002; Chaabane et al. 2008; PPDB 2014).

Dissipation was greater in the unamended soil than in the amended soils. The sequence of DT50 values followed the order S+SS > S+C ≥ S+P > S (**Table 3**). The higher mesotrione sorption coefficients of amended soils could explain the slower dissipation rate in these soils (Abaunza 2014). The higher OC content of SS-amended soil could explain both the higher sorption of mesotrione by this soil and the higher DT_{50} value. Previous studies in the literature reported a correlation between the adsorption and dissipation of mesotrione and the soil OC content (Dyson et al. 2002; Chaabane et al. 2008). The influence of sorption on the dissipation kinetics of pesticides in soils has been observed in many studies, due to a decrease in the bioavailability

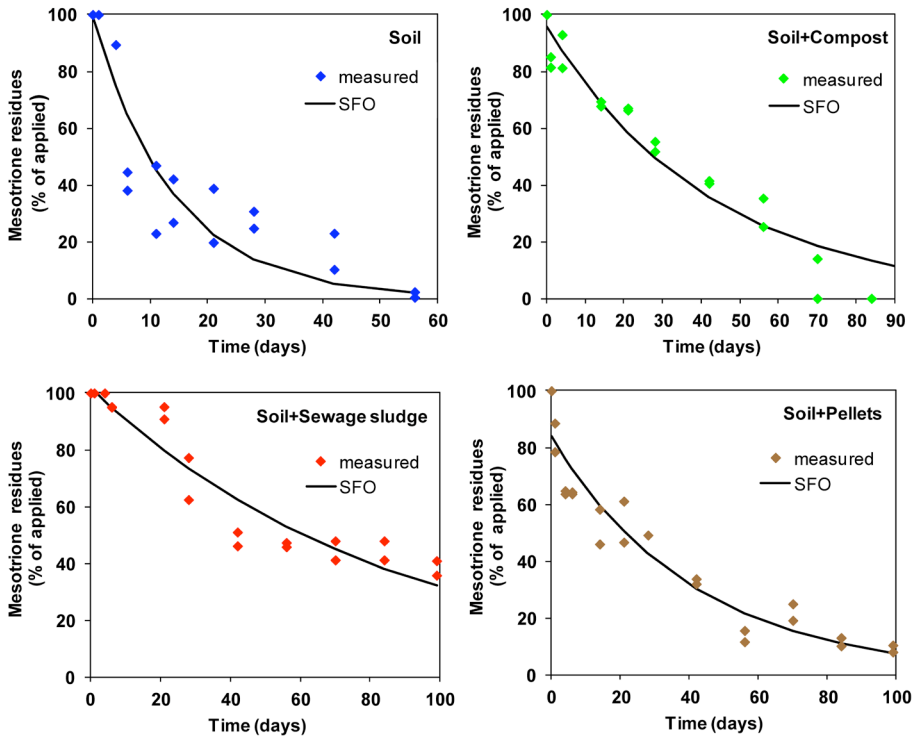

Figure 1. Dissipation kinetics of mesotrione in unamended and amended soils (n=2). Experimental mesotrione residue data (% of applied) (solid symbols) and fitted data to SFO model of mesotrione residues (% of applied) (lines).

Table 3. Kinetics parameters for the dissipation of mesotrione in unamended and amended soils obtained from fitting kinetics to a single first order (SFO) model

Soil sample	k ($days^{-1}$)	DT_{50} (days)	r^2	χ^2
Soil	0.071	9.7	0.93	14.4
Sterilised soil	0.028	24.3	0.92	13.5
Soil+C	0.024	29.4	0.96	11.2
Soil+SS	0.012	59.1	0.97	7.8
Soil+P	0.024	28.5	0.96	13.0

and biodegradation of these compounds sorbed by soil (Marín-Benito et al. 2012, 2014).

Dissipation experiment of mesotrione carried out in unamended soil after sterilsation showed that dissipation was slower than for non-sterilised soil (DT_{50} = 24.3 days). Mesotrione can be degraded following both abiotic and biotic ways. It has been reported that mesotrione affects the metabolism of soil bacteria, for example, Olchanheski et al. (2014) observed that some bacterial enzymes are involved in the degradation of mesotrione and that this herbicide had toxic effects on bacterial cells.

3.2. Soil microbial biomass

The microbial biomass-C values obtained at different times during the incubation period, for unamended and amended soils either untreated (controls) or treated with mesotrione are shown

in Figure 2. Microbial biomass-C reflects the size of the microbial community. In general, the biomass values increased in the amended soils (SE=82.38, P<0.0001) with the exception of the P-amended soil. Amendments had a stimulating effect on soil microbial biomass, acting as additional carbon sources. In the unamended soil and the C- and P-amended soils, there was a decrease in the biomass values at 91 days of incubation. However, SS amendment increases the amount of OC and DOC available for microorganisms and the biomass increases over the incubation period. OC of the SS amendment is labile (García et al. 1993) and it decomposes rapidly with release of nutrients available for soil microbial biomass.

After treatment with the herbicide mesotrione in the unamended and amended soils at the beginning and after 28 days of incubation time, biomass values were not significantly different with one exception. There was a significant difference in the biomass values of unamended and SS amended soils treated with mesotrione at the end of the incubation period (SE=25.45, P<0.0001). Alexander (1981) reported that herbicide impacts may be both negative and positive and may not affect all soil microorganisms at the same time. Reduction of soil microbial biomass with the application of different herbicides has been found in previous papers (Perucci et al. 2000; Vischetti et al. 2002; Singh and Ghoshal 2010).

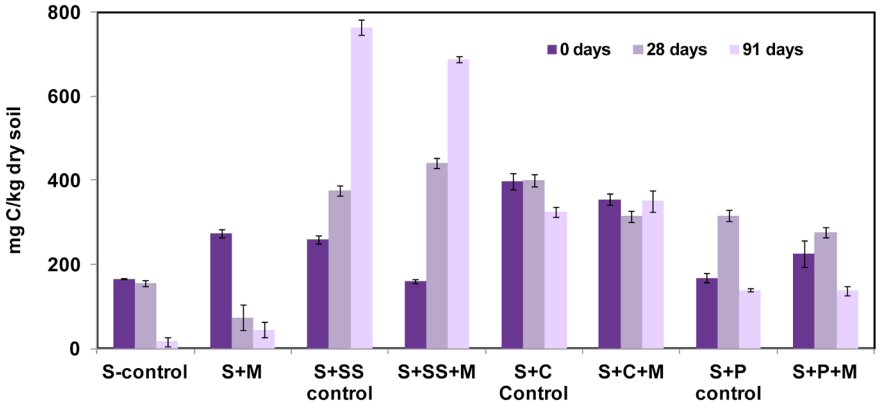

Figure 2. Soil microbial biomass-C for unamended and amended soils, untreated (control) and treated with mesotrione (M) at different sampling times. Bars indicate the standard deviation of the mean (n=2).

3.3. Soil dehydrogenase activity

The DHA values for unamended and amended soils either untreated (controls) or treated with mesotrione over the incubation period are presented in Figure 3. At the beginning, DHA mean values were significantly higher in C- and P-amended soils than in the unamended one (SE=8.15, P<0.0001), indicating the positive effect of the amendment on soil microbial activity (Herrero-Hernández et al. 2011; Marín-Benito et al. 2012, 2014). The addition of compost and pellets to the soil stimulated the DHA due to both the greater available OC content in the amended soil and to the presence of new soil microbial populations introduced with the amendment. However in the SS-amended soil, DHA was significantly lower than in unamended soil, possibly due to a certain toxic effect of the SS amendment to bacteria as reported by Malara and Oleszczuk (2013). The higher DHA in C- and P-amended soils is also consistent with the higher dissipation rates of mesotrione when compared with the SS-amended soil.

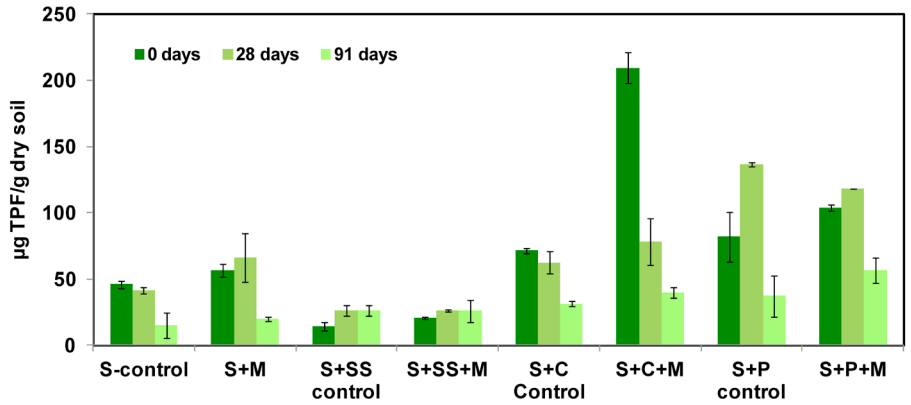

Figure 3. Soil dehydrogenase activity for unamended and amended soils, untreated (control) and treated with mesotrione (M) at different sampling times. Bars indicate the standard deviation of the mean (n=2).

DHA values were significantly higher at the beginning than at the end of the incubation time for the unamended soil and the C- and P-amended soils (SE=15.02, P<0.011). In SS-amended soil, DHA increased at 28 days and then remained practically constant at the end of the incubation time, indicating the stabilization of microbial activity.

DHA values in the soils treated with mesotrione were, in general, higher than in the control soils (without herbicide) at time 0 days, although differences were only significant for the C- and P-amended soil (SE=8.15, P<0.0001), indicating the soil microbial activity was stimulated by the addition of the herbicide to the soil. This effect has also been reported for other fungicides or herbicide-amendment-soil combinations in the literature (Herrero-Hernández et al. 2011; Marín-Benito et al. 2012, 2014). In the soils treated with mesotrione, DHA decreased at the end of the incubation similarly to the control soils. In the SS-amended soil treated with mesotrione, DHA was practically constant in all measurements, and measurements were similar to those of the SS-amended soil without herbicide (control soil), indicating that a certain toxic effect of the herbicide or its metabolites could harmfully alter soil microbial communities.

3.4. Soil microbial respiration

The respiration values for unamended and amended soils either untreated (controls) or treated with mesotrione over the incubation

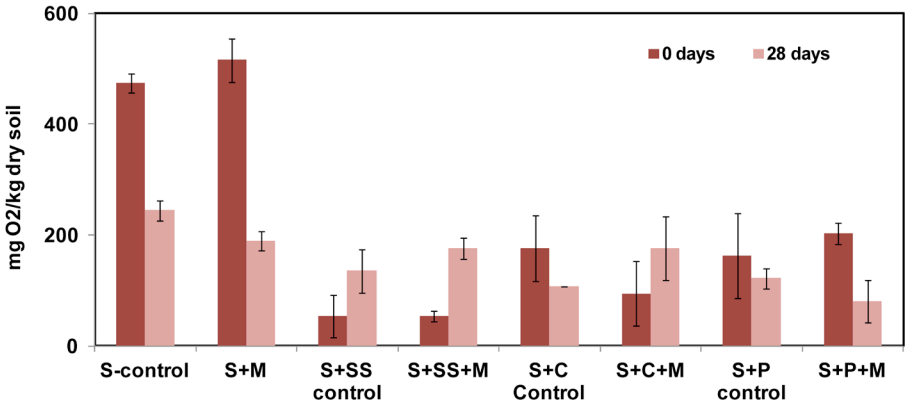

Figure 4. Soil respiration for unamended and amended soils, untreated (control) and treated with mesotrione (M) at different sampling times. Bars indicate the standard deviation of the mean (n=2).

period are presented in Figure 4. Initially, respiration was higher in the unamended soil (control and treated soils) than in the amended soils (SE=44.95, P<0.0001). However in previous reports on the effect of different organic amendments (municipal solid waste compost, sewage sludge) on microbial activity, soil respiration increased after application of organic amendments (Jorge-Mardomingo et al. 2013; Mattana et al. 2014). The decrease observed in this work could be due to the respiration measurement at the beginning of the incubation period, although a problem of oxygen transport could have also occurred.

The application of mesotrione did not have any effect on soil respiration for unamended and amended soils. After 28 days of incubation, there were no significant differences between control and treated soils for unamended and amended soils. In the soils treated with mesotrione, respiration increased in the SS- and C-amended soils and decreased in the unamended and P-amended soils after 28 days of incubation.

3.5. Phospholipid fatty acid profile analysis (PLFAs)

PLFA profiles are often used to study microbial diversity in complex communities (Zelles 1999). The method was used for overall microbial community analysis and for the study of specific groups. Relative abundances of each PLFA detected in the samples as well as those of the microbial groups were grouped to form two data matrices of, respectively, 3 816 data points (53 PLFAs x 3 soil samples x 2 herbicide treatments (control and M application) x 3 incubation times x 4 replicates) and 288 data points (4 microbial groups x 3 soil samples x 2 herbicide treatments (control and M application) x 3 incubation times x 4 replicates).

An indirect analysis (PCA) of the data was initially used to summarize the variation in relative abundances of PLFAs across all the 72 samples analysed. Figure 5a shows the biplot resulting from this PCA analysis. The first PCA axis explained 49.5% of the variance in the data and the second axis accounted for 28.3%, each surpassing the relative amount of the total variability that should be explained in order to be considered significant under the null model of random variation, as calculated by the broken-stick model (Legendre and Legendre 1998). Further examination of the PCA biplot, focusing on the disposition the centroids of the 18 dummy independent variables projected *post hoc* into the ordination space, reveals that the first principal component was related to soil amendments, as the PLFAs profile of the S+C samples were very different from those of the S and S+SS samples. The second principal component was related to time of incubation, showing that the more pronounced differences in the profile of PLFAs occur between S+C samples taken at day 0 and after 28 or 91 days of incubation. The presence of mesotrione did not appreciably modify the PFLAs profiles of the samples, as shown by the very close position of each pair of centroids representing treatments with (M) or without (control) mesotrione. Statistically significant effects of soil amendments, herbicide application and incubation time on the PLFA profiles were assessed by redundancy analysis (RDA). As expected from the separations observed in the PCA biplot (Figure 5a), RDAs revealed statistical significance for the effects of soil amendments (F-ratio=68.069, P=0.001) and time of incubation (F-ratio=11.680, P=0.001) but not for the effect of herbicide application (F-ratio=0.312, P=0.806).

Regarding the relative abundance of particular microbial groups (Gram-positive and Gram-negative bacteria, actinobacteria and fungi), the first PCA axis accounted for most of the variance (70.8%) and clearly separated S+C samples collected after 28 and 91 of incubation from the remaining samples (Figure 5b). The second PCA axis accounted for only 16.3% of the variability. As calculated by the broken-stick model (Legendre and Legendre 1998), only the fraction of variability explained by the first axis surpassed the values predicted by the null model, indicating that the first axis describes non-random, interpretable variation in the data while the second does not. S+C samples collected after 28 and 91 days had the highest relative abundance of Gram-negative bacteria,

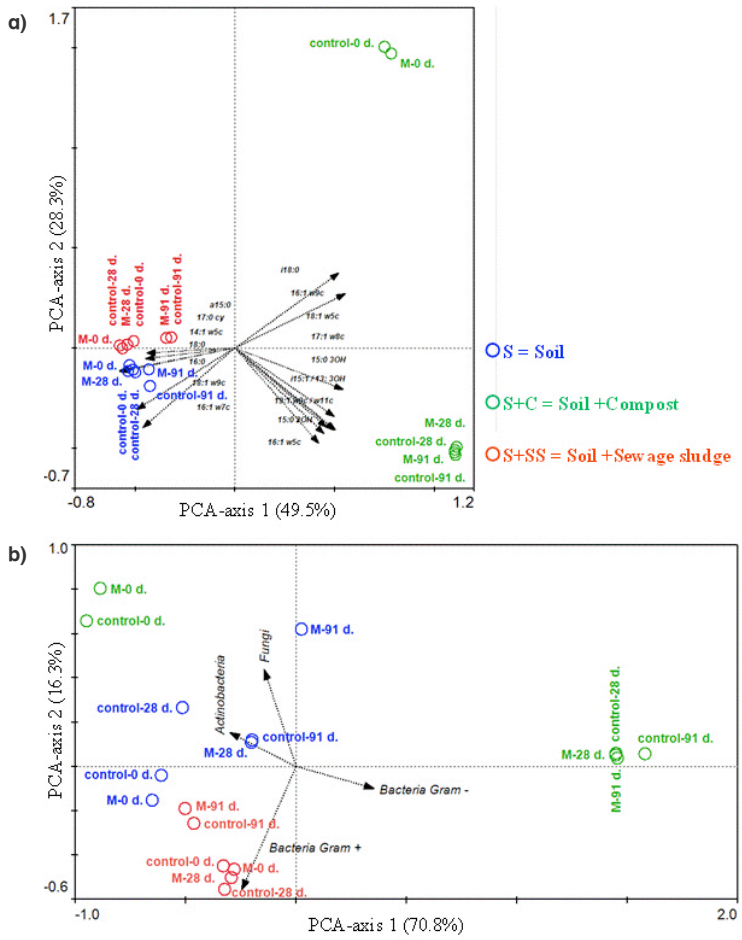

Figure 5. Biplot representations of the results of PCA analyses performed on (**a**) the matrix with relative abundances of individual PLFAs and on (**b**) the matrix with relative abundances of microbial groups. Circles represent the centroids of four replicates for each sampling time (0, 28 and 91 days), mesotrione application (control and M), and soil samples (S, S+C, and S+SS).

whereas the S+C samples collected just after mesotrione application (0 days of incubation) tended to have the highest relative abundances of actinobacteria and fungi. The presence of mesotrione did not appreciably alter the relative abundances of these microbial groups. As before, RDAs revealed statistical significance for the effects of soil amendments (F-ratio=28.792, P=0.001) and time of incubation (F-ratio=13.546, P=0.001) but not for the effect of herbicide application (F-ratio=0.270, P=0.765).

Similar to our results obtained by PLFA analyses, mesotrione application at field-rate was found not to affect the microbial community structure of a Chernozem soil as assessed by PCR-DGGE analyses, although higher doses of this herbicide induced changes on the function and structure of the soil microbial community (Crouzet et al. 2010, 2013). In our experiment, however, major differences in the microbial community structure existed in function of soil amendments and time of incubation. While the microbial community structures of the unamended soil and SS-amended soil were notably similar to each other, with little shifts along time, the C-amended soil samples had a markedly different microbial community structure, which shifted sharply over the incubation period (**Figure 5a**). This shift was characterised by increasing

the relative abundance of Gram-negative bacteria as well as by decreasing the relative abundance of actinobacteria with respect to the samples collected at the beginning (0 days of incubation) of the experiment (**Figure 5b**). These findings suggest that Gram-negative bacteria significantly increase in response to compost application in soil. This is probably a stimulation effect by the surplus of organic substrates and nutrients released in soil with the application of compost rather than a direct effect of bioactive substances contained in compost, because Gram-negative bacteria are fast-growing microorganisms that utilize a range of carbon sources and can adapt quickly to a variety of environmental conditions (Ponder and Tadros 2002). Toxicity of the SS amendment to bacteria (Malara and Oleszczuk 2013) could explain why changes in the microbial community structure were not seen in the SS-amended soil, but additional work is necessary to confirm this.

4. Conclusions

The results provide information on the intensity and nature of the response of soil microbial communities to two agricultural practices (herbicide and amendments application to soil). Mesotrione applied to soils dissipated faster from unamended soils than from amended soils. Analyses of the impacts of mesotrione and amendment application on the broad-scale microbial parameters (microbial biomass, dehydrogenase activity and soil respiration) yielded inconsistent results, suggesting that it is important to assess the impacts at a narrow-scale. Our PLFA results indicate that both the overall structure of active microbial communities and the relative abundance of certain groups of microorganisms clearly changed according to the type of amendment and the time of incubation, but remained unaffected by the application of mesotrione at the concentration assayed. Although it may be suggested that amendments could reduce oxygen availability to metabolic degradation of mesotrione, further work is required to evaluate the impact of increasing doses of herbicide on soil microbial communities in order to provide a better understanding of the responses of soil microbial communities under different scenarios. At the same time the study of other agricultural "inputs", such as the application of soil amendments, is necessary to further the understanding of the responses of soil microbial communities.

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