Influence of different agricultural management practices on soil microbial community over dissipation time of two herbicides

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HIGHLIGHTS

- Agricultural practices affected herbicide dissipation and soil microbial structure.
- Repeated applications of herbicides accelerated the degradation of triasulfuron but not prosulfocarb.
- Higher content of organic matter resulted in increased herbicide residues in soils.
- Herbicides promoted the relative abundance of *Actinobacteria* and reduced fungi.
- The changes on soil microbiology produced by herbicides modify their degradation rates.

GRAPHICAL ABSTRACT
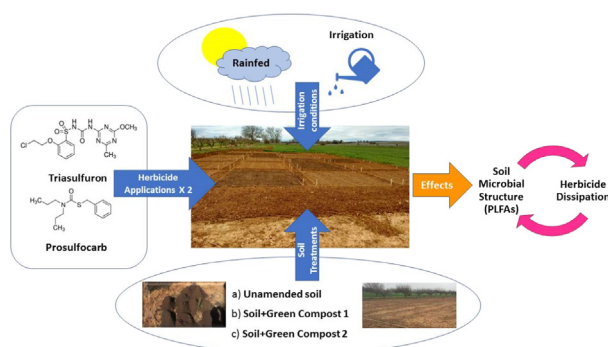
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ABSTRACT

Soil microbiology could be affected by the presence of pesticide residues during intensive farming, potentially threatening the soil environment. The aim here was to assess the dissipation of the herbicides triasulfuron and prosulfocarb, applied as a combined commercial formulation, and the changes in soil microbial communities (through the profile of phospholipid fatty acids (PLFAs) extracted from the soil) during the dissipation time of the herbicides under field conditions. The dissipation of herbicides and the soil microbial structure were assessed under different agricultural practices, such as the repeated application of herbicides (twice), in unamended and amended soils with two organic amendments derived from green compost (GC1 and GC2) and with non-irrigation and irrigation regimes. The results obtained indicate slower dissipation for triasulfuron than for prosulfocarb. The 50% dissipation time (DT_{50}) decreased under all conditions for the second application of triasulfuron, although not for prosulfocarb. The DT_{50} values for both herbicides increased in the GC2 amended soil with the highest organic carbon (OC) content. The DT_{50} values decreased for prosulfocarb with irrigation, but not for triasulfuron, despite its higher water solubility. The herbicides did not have any significant effects on the relative population of Gram-negative and Gram-positive bacteria during the assay, but the relative abundance of *Actinobacteria* increased in all the soils with herbicides. At the end of the assay (215 days), the negative effects of herbicides on fungi abundance were significant ($p < 0.05$) for all the treatments. These microbiological changes were detected in non-irrigated and irrigated soils, and were more noticeable after the second application of herbicides. *Actinobacteria* could be responsible for the modification of herbicide degradation rates, which tend

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to be faster after the second application. This study makes a useful contribution to the evaluation of the soil environment and microbiological risks due to the long-term repeated application of herbicides under different agricultural management practices.

1. Introduction

The structure of soil microbial communities and the changes produced in them by different environmental impacts is of great interest nowadays (Barra Caracciolo et al. 2015). Microbial activity is an accurate indicator of soil quality because soil microorganisms play a key role in organic matter (OM) decomposition and in the biogeochemical cycles that affect soil fertility (Pascual et al. 2000; García-Orenes et al. 2013). This microbial activity could be affected by the presence of pesticide residues in soil that pose a potential risk to soil ecology (Cycoń et al. 2013; Fang et al. 2018). Modern intensive farming involves the application of large quantities of pesticides during the crop growth period (Nyamwasa et al. 2018). In fact, the residues of herbicides, insecticides and fungicides have been detected in soils in agricultural areas across different countries in a broad range of concentrations (Li et al. 2014; Pose-Juan et al. 2015) which could modify soil microbial biodiversity. Residues of these agrochemicals depend on their dissipation in soils, being modified by different environmental factors (soil type, soil OM, weather, temperature, irrigation), pesticide formulation (individual or combined compounds), and application method (single or repeated application) (Arias-Estévez et al. 2008). This widespread use of pesticides could therefore lead to a potential decrease in soil microbial biodiversity, with a negative impact on crop yields (Baxter and Cummings 2008), which could be increased by the widespread loss of soil OM detected in recent years (Pascual et al. 2000).

A common practice used in agriculture to increase soil OM content involves the application of organic residues as soil amendments, with the aim being to improve soil fertility and stability, as well as stimulate microbial growth (Bastida et al. 2015). Organic residues of different origins (urban, agricultural or industrial) are generated in large quantities, and the improvement in soil properties due to their OM content has been well documented (Aranda et al. 2015; Bastida et al. 2015). However the combined application of pesticides and organic residues modifies the physicochemical behaviour of pesticides applied to soils, mainly through their adsorption-desorption (Marín-Benito et al. 2013, 2014). Changes in mobility or the formation of bound residues could occur depending on OM composition, which has implications regarding their bioavailability and total dissipation and consequences for overall soil microbial activity.

Studies on pesticide dissipation and its effects on soil microorganisms have been reported in unamended and amended soils. In general, these studies focus on the effect of a single compound with single application (Cycoń et al. 2013; Álvarez-Martín et al. 2016; Pose-Juan et al. 2017; Singh et al., 2018). They have been carried out in laboratory conditions, while few results have been reported from field experiments with more realistic environmental conditions (Herrero-Hernández et al. 2015). However, studies on the effects that combined application of pesticides have on their dissipation (Vischetti et al. 2008; Fang et al. 2018) and/or the effects of the repeated application of pesticides on soil microbial communities are scarcer in the literature (Baxter and Cummings 2008; Tortella et al. 2013; Fang et al. 2015; Wang et al. 2015). Soil microbial abundance and structure were evaluated through different approaches, and the part soil microorganisms play in the enhanced dissipation of pesticides after repeated applications has been reported. Nevertheless, these investigations have been scarcely assessed under field conditions using combined commercial formulations of pesticides, and most of these studies have been carried out in unamended soils (Kaur and Bhullar 2017; Kaur et al. 2017).

Triasulfuron (2-(2-chloroethoxy)-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino] carbonyl] benzene sulfonamide) is a mobile herbicide in soil due to its high water solubility and low hydrophobicity (EFSA 2015), while prosulfocarb (S-(phenylmethyl) dipropylcarbamothioate) is a hydrophobic herbicide with high adsorption, low mobility and a moderate persistence in soil (EFSA 2007a). These herbicides of the sulfonylurea and thiocarbamate group, respectively, are used on pre- and post-emergence in winter cereals (wheat, barley) and other crops (PPDB 2018). No study has been made of the combined long-term effect that triasulfuron and prosulfocarb have on their dissipation rates and soil microbial biomass and structure, although they are repeatedly applied to a broad range of crops. Compounds of the chemical groups sulfonamide and thiocarbamate, such as triasulfuron and prosulfocarb, are usually recommended in cereals for individual or joint use for controlling weeds in rainfed and irrigated cereal crops (Cirujeda and Taberner 2010; Bajya et al. 2015).

Relevant changes in soil microbial abundance, activity and structure have already been reported by the authors in a previous paper (García-Delgado et al. 2018) when triasulfuron and prosulfocarb were applied as a combined formulation in unamended and amended soils with green compost (GC) under field conditions. GC is the biodegradable organic residue from pruning in urban gardens and parks with an OM content higher than 15% (BOE 2013). The results were obtained for a single application of herbicides after the short-term dissipation of herbicides. Little is known about the other factors that could influence the dissipation of herbicides in soils and changes in the soil microbial structure under field conditions. Repeated herbicide application, different soil OC content from organic amendments, and/or irrigation may modify herbicide dissipation. The study of these factors would increase our knowledge on the effect herbicides have on soil microbial communities.

The aim here was therefore to assess the changes in soil microbial communities during the dissipation of two herbicides continuously applied under different agronomical practices. A combined commercial formulation of triasulfuron and prosulfocarb (Aurus Plus®) was applied twice to an unamended soil and one amended with two organic amendments derived from green compost (GC1 and GC2) and with non-irrigation and irrigation regimes. The effect of these factors on the dissipation of herbicides (DT_{50}) and on the soil microbial structure was studied under field conditions. The study makes a useful contribution to the evaluation of environmental and microbiological risks due to the combined long-term application of herbicides under different management practices in agriculture.

2. Materials and methods

2.1. Herbicides

The commercial formulation of triasulfuron (TSF) (2-(2-chloroethoxy)-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino] carbonyl] benzene sulfonamide) (20% p/p) and prosulfocarb (PSC) (S-(phenylmethyl) dipropylcarbamothioate) (80% p/v) (Auros Plus®, Syngenta Agro S.A., Madrid, Spain) was used in the field study. Analytical standards of both herbicides (purity > 98.9%) were supplied by Sigma Aldrich Química S.A. (Madrid, Spain). Water solubility is 815 and 13.0 mg L^{-1} and log K_{ow} is −0.59 and 4.48 for triasulfuron and prosulfocarb, respectively (PPDB 2018).

2.2. Organic residues

Two green composts formed by composted vegetal residues were used. They were supplied by the local authority (GC1) and by the nursery "El Arca" (GC2) from Salamanca (Spain). Their main physicochemical characteristics on a dry weight basis were determined by standard methods (Sparks et al., 1996) and are as follows: pH 7.33 and 7.58, organic carbon (OC) content 9.80% and 24.1%, dissolved organic carbon (DOC) content 0.353% and 0.700%, total N 1.04% and 1.10%, C/N ratio 9.42 and 21.9, and ash percentage 74.5% and 53.0% for GC1 and GC2, respectively.

2.3. Experimental set-up

A field experiment was conducted in an agricultural soil (sandy clay loam soil, *Typic Haploxerept*) in the Muñovela experimental farm belonging to IRNASA-CSIC (Salamanca, Spain). An experimental layout of randomized complete blocks was designed in February 2016, with six treatments and three replicates per treatment (18 plots of 9 m^2) corresponding to unamended soil (6 plots, S) and soil amended with GC1 (6 plots, S + GC1) or GC2 (6 plots, S + GC2) at the rate of 120 and 180 t ha^{-1} on a dry weight basis, respectively. For each soil treatment, three plots received only natural rainfall, while other three plots received weekly 2.8 mm (I). In March 2016, the commercial formulation of triasulfuron and prosulfocarb (Auros Plus®) was applied to 18 experimental plots at doses of 250 g a.i. ha^{-1} and 11.25 kg a.i. ha^{-1}, respectively, corresponding to 2.5 times the maximum agronomic dose for both herbicides recommended for heavy soils with a greater adsorption capacity. The increase in the soil's capacity for adsorbing the herbicides after an organic amendment supports the use of doses higher than those recommended to maintain the efficacy of the compounds. Once the herbicide half-lives (DT$_{50}$) were achieved in all plots (after 68 days), the herbicides were applied again at the same doses in May 2016. A check was made prior to the application of the herbicides to ensure that no amounts of these compounds were detectable in the soil samples. This was as expected, because the plots had no history of triasulfuron and prosulfocarb application in the previous five years. Additionally, eighteen control plots (six by soil treatment) did not receive herbicide application, but nine of them received irrigation.

Weather conditions were recorded throughout the experiment (215 days) by a meteorological station located on site (Fig. S1 in Supplementary material). Air temperature ranged from 1.7 °C to 14.6 °C (mean air temperature 8.3 °C) and from 10.5 °C to 26.3 °C (mean air temperature 19.1 °C) during the first (0–68 days) and the second (69–215 days) period of the application, respectively. Cumulative precipitation and additional irrigation were 139.2 mm and 11.2 mm during the first application, and 46.6 mm and 58.8 mm during the second application of herbicides, respectively. Total cumulative precipitation and additional irrigation were 185.8 mm and 70 mm, respectively.

Table 1
Characteristics of unamended and GC amended soils, non-irrigated or irrigated (I) after the first and the second application of herbicides in the field plots (sampling times corresponding to 13 days and 84 days after the beginning of the experiment).

	pH	OC[a] (%)	DOC[b] (mg kg^{-1})	N (%)	C/N
Soil	7.10–6.71	1.41–0.98	0.057–0.045	0.12–0.08	11.8–12.2
Soil-I	7.12–6.77	1.22–1.05	0.051–0.041	0.11–0.10	11.1–10.5
Soil + GC1	7.69–7.59	2.29–2.09	0.084–0.106	0.19–0.21	12.1–9.95
Soil + GC1-I	7.55–7.51	2.17–2.07	0.060–0.088	0.20–0.20	10.8–10.4
Soil + GC2	7.24–7.47	4.81–3.75	0.322–0.324	0.43–0.33	11.2–11.4
Soil + GC2-I	7.18–7.41	5.87–4.32	0.482–0.413	0.46–0.37	12.8–11.7

[a] Organic carbon.
[b] Dissolved organic carbon.

2.4. Soil sampling and herbicide extraction and analysis

Surface soil samples from 0 to 10 cm were collected to determine herbicide dissipation at different times between 0 and 68 days (first herbicides' application) and between 69 and 215 days (second herbicides' application) and to determine soil microbial structure at 0, 28, 69, 97, 124 and 215 days. Soil samples were collected, processed and characterized following the methods described by Marín-Benito et al. (2018a) and García-Delgado et al. (2018) (complete information is included in Supplementary material). Soil characteristics are included in Table 1. Soil samples were sieved (<2 mm) and moisture content of the bulk sample was determined. Duplicate subsamples of moist soil (6 g) were extracted with methanol (12 mL) by shaking and sonication. The determination of the herbicides in the soil extracts was performed by HPLC-MS (Waters Assoc., Milford, MA, USA). The molecular ions [*m/z*] 402.8 (triasulfuron) and 252.4 (prosulfocarb) were monitored and the retention times were 6.1 min and 14.1 min, respectively. A detailed description of the herbicide extraction and analytical methods is included in Supplementary material.

2.5. Soil microbial community by PLFA analysis

The soil microbial community composition of the soil samples was determined using phospholipid fatty acid (PLFA) analysis, as described in Frostegård et al. (1993). Lyophilized soils samples were extracted with a one-phase chloroform-methanol-phosphate buffer solvent by sonication. Extracts were purified by SPE and polar lipids were transesterified with methanol-KOH. Finally, hexane extracts containing the resultant fatty acid methyl esters were analyzed by gas chromatography. Quantification was performed using an Agilent 7890 gas chromatograph (Agilent Technologies, Wilmington, DE, USA) equipped with a 25-m Ultra 2 (5% phenyl)-methylpolysiloxane column (J&W Scientific, Folsom, CA, USA) and a flame ionization detector. PLFAs were identified using bacterial fatty acid standards and software from the Microbial Identification System (Microbial ID, Inc., Newark, DE, USA). Specific PLFAs (Zelles, 1999) were used as biomarkers to quantify the relative abundances of Gram negative (monounsaturated fatty acids and cyclopropyl 17:0 and 19:0) and Gram positive (iso and anteiso saturated branched fatty acids) bacteria, *Actinobacteria* (10-methyl fatty acids) and fungi (18:2 ω6 cis).

2.6. Data analysis

The dissipation kinetics for triasulfuron and prosulfocarb were fitted to a single first-order (SFO) or first-order multicompartment (FOMC) models and values for the time to 50% dissipation (DT$_{50}$) were estimated using the Excel Solver add-in package (FOCUS, 2006). More details about the fitting of the dissipation kinetics are included in Supplementary material.

Analysis of variance (ANOVA) was used to evaluate the effects of the different treatments (green compost application, repeated herbicide application and irrigation) on herbicide dissipation. Standard deviation (SD) was used to indicate variability among replicates. Fisher's least significant difference (LSD) method, at a confidence level of 95%, was determined with IBM SPSS Statistics v24 software package (SPSS Inc. Chicago, USA).

Data of PLFAs were submitted for the analysis of variance (ANOVA) by previous Levene variance homogeneity test to determine significant differences between treatments at each sampling time. Means were compared by either Tukey or Games–Howell post hoc test based on whether or not variance homogeneity was met, respectively (*p* < 0.05). Pearson correlation coefficients between the remaining concentration and percentages of herbicides, and microbial biomass and structure were determined to elucidate how variables are related to each other. ANOVA and correlation analyses were carried out using the IBM SPSS Statistics v24 software package (SPSS Inc. Chicago, USA). Principal

component analysis (PCA) was performed, with PAST v3.15 software (Hammer et al. 2001), to determine the most meaningful variables and the global impact of the herbicides and soil treatments on soil microbial community. In addition to PCA, PERMANOVA analysis was performed to determine the significance of herbicides application, sampling time, soil treatments and their interactions.

3. Results and discussion

3.1. Herbicide dissipation after repeated application in soils, soil amendment, and irrigation conditions

The dissipation kinetics of herbicides after the first and second application fit the SFO model for most of the treatments, and only in four plots the dissipation kinetics provide a better fit for the FOMC model (Figs. S2 and S3 in Supplementary material). Other authors also report that the SFO equation is the model that best fits triasulfuron and prosulfocarb dissipation in the field (Rouchaud et al. 1997; Sarmah et al. 2000). The DT_{50} values were used to compare the dissipation rates of triasulfuron and prosulfocarb under the different conditions studied (herbicide type, repeated dose, soil amendment, and irrigation) (Tables 2 and 3). The dissipation curves show a continuous decrease in triasulfuron and prosulfocarb concentrations over time. It is faster for prosulfocarb than for triasulfuron for all the treatments studied, as indicated by the lower DT_{50} values. The faster dissipation of prosulfocarb could be due to processes of adsorption, biodegradation, mineralization, and/or volatilization, as reported previously (Marín-Benito et al., 2018b; Braun et al., 2017; EFSA, 2007a).

The DT_{50} values for both herbicides after the first application were consistent with those determined in a previous work, when they were applied as a single or combined formulation in unamended or GC-amended soils, albeit in a lower dose than in this study (Marín-Benito et al., 2018a). The repeated application of triasulfuron has an effect on its persistence, with higher dissipation rates, and DT_{50} values 1.3 to 3 times lower than after the first application (Table 2). These results

indicate that the persistence of triasulfuron was lower after the second application. This is consistent with the remaining percentages of triasulfuron at 68 days after the first application, ranging between 14%–51%, and at 69 days after the second application (corresponding to 138 days after the first application), being between 3%–17% in the soils with the different treatments. Other authors have also reported accelerated dissipation after repeated pesticide applications due to the faster metabolism caused by enhanced biodegradation, which could lead to a reduction in pesticide efficacy in some cases (Baxter and Cummings 2008; Fang et al. 2018).

The DT_{50} values for prosulfocarb decreased by up to 1.3 and 1.6 times after its second application in irrigated S and S + GC2 soils, respectively, but the DT_{50} values were, in general, similar for all the other soil treatments (Table 3). However, the remaining percentages of prosulfocarb at 68 days after the first application were lower (0%–3%) than those at 69 days after the second application (corresponding to 138 days after the first application) (2%–21%). These results indicate that the amounts of prosulfocarb remaining after the second application decreased more slowly over time than after the first application, without fully dissipating. Rouchaud et al. (1997) have reported that repeated prosulfocarb application to a barley crop enhanced soil biodegradation. However, the decrease in prosulfocarb concentrations after the second application and over the course of 50% dissipation was slower than after the first application, and this resulted in a higher persistence of the herbicides in the soil at the end of the assay (215 days) (Fig. S3).

The DT_{50} values for the dissipation of triasulfuron and prosulfocarb after the two applications were higher in S + GC2 than in S or S + GC1. The DT_{50} values increased by 3.1–1.8 times and by 2.0–1.9 times compared to the unamended soil. These results are related to the higher OC content in S + GC2 than in S + GC1, which could help increase the persistence of these herbicides in the top soil, decreasing its leaching (Marín-Benito et al., 2018b). In fact, a significant and positive correlation ($R^2 = 0.828$, $p < 0.001$) was found between the DT_{50} values of triasulfuron and prosulfocarb and OC content. Furthermore, the adsorption of both herbicides by S + GC2 could occur with the possible

Table 2
Dissipation parameters and goodness of fit for triasulfuron after repeated application (1 and 2) in unamended or amended soils under non-irrigated or irrigated conditions calculated by fitting the SFO or FOMC models.

Sample plot	Non-irrigated soils				Irrigated soils			
	k (days^{-1})	DT_{50} (days)	χ^2	R^2	k (days^{-1})	DT_{50} (days)	χ^2	R^2
Soil								
A -1	0.023	30.3 ± 1.84	7.3	0.95	0.026	26.2 ± 0.89	12.9	0.89
-2	0.045	15.5 ± 0.46	8.6	0.97	0.039	17.6 ± 0.12	8.5	0.97
B -1	0.020	34.1 ± 0.05	7.7	0.94	0.030	23.3 ± 0.90	13.2	0.89
-2	0.046	15.0 ± 0.02	12.1	0.97	0.047	14.9 ± 0.15	9.6	0.97
C -1	0.026	27.2 ± 0.50	10.7	0.91	0.026	26.8 ± 0.95	10.4	0.92
-2	0.042	16.6 ± 0.71	10.7	0.97	0.043	16.3 ± 0.50	8.3	0.98
Mean -1		30.5 ± 3.46c				25.4 ± 1.87bc		
-2		15.7 ± 0.82a				16.3 ± 1.35a		
Soil+GC1								
A -1	0.023	30.0 ± 0.19	6.7	0.96	0.024	28.6 ± 2.18	14.9	0.84
-2	0.038	18.3 ± 0.28	12.5	0.96	0.033	20.7 ± 0.46	17.5	0.89
B -1	0.019	36.0 ± 2.32	12.6	0.83	0.023	30.1 ± 0.75	10.0	0.90
-2	0.031	22.5 ± 0.11	9.8	0.97	0.030	23.1 ± 0.73	7.5	0.97
C -1	0.029	24.1 ± 1.99	14.7	0.87	0.025	27.4 ± 0.56	13.5	0.87
-2	0.032	21.7 ± 0.01	9.1	0.98	0.034	20.5 ± 0.72	10.3	0.97
Mean -1		30.0 ± 5.95c				28.7 ± 1.35bc		
-2		20.8 ± 2.23ab				21.4 ± 1.45ab		
Soil+GC2								
A -1	0.341–8.818[a]	59.2 ± 3.61	5.8	0.93	0.008	91.1 ± 8.40	1.7	0.97
-2	0.030	23.0 ± 1.39	10.1	0.83	0.021	32.6 ± 1.71	6.3	0.97
B -1	0.008	90.5 ± 3.96	2.9	0.94	0.010	72.4 ± 0.44	4.6	0.92
-2	0.020	34.5 ± 8.94	9.7	0.86	0.033	21.0 ± 1.21	5.3	0.99
C -1	0.518–17.68[a]	55.1 ± 2.97	4.1	0.97	0.010	72.7 ± 9.85	4.9	0.86
-2	0.024	28.4 ± 0.32	8.7	0.97	0.028	25.0 ± 0.46	8.0	0.97
Mean -1		68.3 ± 17.6d				78.7 ± 10.7e		
-2		28.6 ± 5.75bc				26.2 ± 5.89bc		

[a] Dissipation parameters (α and β) calculated from fitting experimental data to the FOMC model. Different letters in DT_{50} values indicate significant differences among samples and treatments (LSD = 7.963, $p < 0.05$).

Table 3

Dissipation parameters and goodness of fit for prosulfocarb after repeated application (1 and 2) in unamended or amended soils under non-irrigated or irrigated conditions calculated by fitting the SFO or FOMC models.

Sample/plot	Non-irrigated soils				Irrigated soils			
	k (days^{-1})	DT$_{50}$ (days)	χ^2	R^2	k (days^{-1})	DT$_{50}$ (days)	χ^2	R^2
Soil								
A -1	0.085	8.2 ± 0.42	14.9	0.96	0.070	9.9 ± 0.99	12.1	0.96
-2	0.067	10.3 ± 0.02	13.7	0.97	0.106	6.5 ± 0.86	11.4	0.98
B -1	0.059	11.8 ± 0.34	14.2	0.94	0.076	9.1 ± 0.62	8.4	0.98
-2	0.060	11.6 ± 0.32	14.9	0.94	0.090	7.7 ± 0.03	14.9	0.97
C -1	0.080	8.6 ± 0.37	14.6	0.93	0.077	9.0 ± 0.71	13.4	0.96
-2	0.069	10.0 ± 0.41	14.4	0.95	0.097	7.2 ± 0.45	15.0	0.95
Mean -1		9.5 ± 1.97ab				9.3 ± 0.49ab		
-2		10.6 ± 0.85b				7.1 ± 0.60a		
Soil + GC1								
A -1	0.055	12.5 ± 0.43	13.9	0.96	0.064	10.8 ± 0.27	11.5	0.97
-2	0.051	13.5 ± 0.03	11.7	0.96	0.073	9.5 ± 0.24	14.2	0.94
B -1	0.060	11.5 ± 0.08	9.2	0.98	0.071	9.8 ± 0.48	5.1	0.99
-2	1.284–12.91[a]	9.2 ± 0.54	11.4	0.98	0.070	9.9 ± 0.19	14.9	0.87
C -1	0.077	9.1 ± 0.56	10.5	0.98	0.080	8.7 ± 0.13	9.3	0.99
-2	1.461–16.0[a]	9.7 ± 0.72	14.5	0.95	0.061	11.5 ± 0.47	13.7	0.97
Mean -1		11.0 ± 1.75b				9.8 ± 1.05ab		
-2		10.8 ± 2.35b				10.3 ± 1.06ab		
Soil+GC2								
A -1	0.033	21.0 ± 1.19	11.9	0.94	0.033	21.2 ± 0.06	14.7	0.93
-2	0.051	13.6 ± 0.02	14.9	0.94	0.063	11.2 ± 0.00	14.8	0.97
B -1	0.037	18.6 ± 0.34	9.7	0.97	0.039	17.6 ± 0.42	11.0	0.97
-2	0.046	15.0 ± 0.32	13.5	0.86	0.063	11.1 ± 0.73	15.0	0.95
C -1	0.036	19.1 ± 0.70	9.0	0.97	0.039	17.9 ± 0.41	9.4	0.97
-2	0.021	32.4 ± 0.97	13.3	0.88	0.055	12.6 ± 0.20	14.4	0.92
Mean -1		19.6 ± 1.27c				18.9 ± 2.00c		
-2		20.3 ± 10.5c				11.6 ± 0.84b		

[a] Dissipation parameters (α and β) calculated from fitting experimental data to the FOMC model. Different letters in DT$_{50}$ values indicate significant differences among samples and treatments (LSD = 3.227, p < 0.05).

formation of bound residues and a potential decrease in bioavailability and biodegradation (Gennari et al. 2002; Said-Pullicino et al. 2004). The relationship between the adsorption and degradation of these herbicides has also been reported in previous works (Nègre et al., 2006; Said-Pullicino et al., 2004). A significant and positive correlation was also found here between the K_d determined for triasulfuron (S, 0.31 ± 0.01 mL g^{-1}; S + GC1, 0.38 ± 0.09 mL g^{-1} and S + GC2, 0.67 ± 0.03 mL g^{-1}) or for prosulfocarb (S, 21.6 ± 5.55 mL g^{-1}; S + GC1, 24.7 ± 7.62 mL g^{-1} and S + GC2, 57.1 ± 2.09 mL g^{-1}) (Marín-Benito et al., 2018b) and the DT$_{50}$ values determined for the first and second dissipation kinetics (R^2 = 0.665, p < 0.02, triasulfuron) and (R^2 = 0.860, p < 0.001, prosulfocarb).

Additional irrigation did not significantly modify the dissipation rates of triasulfuron after the two applications in S—I or S + GC1-I, but it decreased in S + GC2-I after the first application. Similarly, Sarmah et al. (2000) have reported that the DT$_{50}$ values of triasulfuron in an unamended soil under field conditions remained unchanged when the soil received 89 mm of irrigation compared to the non-irrigated soil. The DT$_{50}$ values of prosulfocarb were similar in irrigated soils after the first herbicide application. However, the dissipation rates increased in S—I and to a greater extent in S + GC2-I after the second herbicide application. Irrigation could lead to a higher potential degradation and/or leaching of herbicides through the soil profile.

The additional evaluation of the influence of overall weather conditions on the dissipation of triasulfuron and prosulfocarb revealed the possible effect of temperature for explaining the accelerated degradation of triasulfuron after the second application. The average temperature differed during the two dissipation periods, increasing by 10.8 °C after the second herbicide application. This result is consistent with the increase in the degradation rate of 2.58 times when the temperature increases by 10 °C, as determined by the European Food Safety Authority (EFSA 2007b). Dinelli et al. (1998) have reported that temperature had an effect on the degradation rate of triasulfuron, and the DT$_{50}$

value decreased three times when temperature increased from 10 °C to 20 °C. Stork (1995) has observed that triasulfuron degradation rates increased with soil temperature, but they were not affected by soil water content. Temperature had no effect for prosulfocarb after the second application of herbicide because faster dissipation occurred only in irrigated treatments of S—I and S + GC2-I and could be explained by other processes, as previously indicated.

3.2. Effect of herbicides residues, organic amendments and irrigation regimes on the soil microbial structure

The total microbial population behaved in a similar way towards herbicides in both irrigated and non-irrigated soils. The amounts of herbicide residues, in total (µg g^{-1}) or relative concentration (%), were positively related to the total microbial population (nmol/g) (Tables 4 and 5). It means a decrease in this population while the dissipation of herbicides occurs. The toxicity of pesticides towards soil microorganisms is well described in the literature, mainly in high doses (El Azhari et al., 2018; Fang et al., 2018; Franco-Andreu et al., 2016a; Kalia and Gosal, 2011; Wang et al., 2015). However, previous studies do not provide consistent results on the toxicity of triasulfuron in soil microbiology between laboratory and field scale. Lupwayi et al. (2004) and Pose-Juan et al. (2017) report that triasulfuron has no toxic effects on microbial biomass at field and laboratory scale, respectively. In contrast, Sofo et al. (2012) have reported toxic effects at laboratory scale for an agronomic dose or higher. A recent study at field scale using a lower dose of triasulfuron than in this work agreed with the latter, indicating a decrease in microbial biomass due to the toxic effects of triasulfuron, and more so a combination of triasulfuron and prosulfocarb in the unamended and GC amended soils (García-Delgado et al., 2018). Therefore, there is evidence of the toxicity of triasulfuron and prosulfocarb towards soil microbiota. Additionally, our results indicated that the bacteria/fungi ratio was negatively correlated with the herbicide residues,

Table 4
Pearson correlation coefficients of non-irrigated soils between population of Gram−, Gram+, Actinobacteria, fungi and their corresponding relative concentrations, total biomass, ratio Gram−/ total Gram+, ratio bacteria/fungi, remaining concentrations of triasulfuron (TSF) and prosulfocarb (PSC), and remaining percentage of TSF and PSC. Significant correlations were denoted by asterisks and blond font.

No irrigation	Gram− (nmol/g)	Gram+ (nmol/g)	Actinobacteria (nmol/g)	Fungi (nmol/g)	Biomass (nmol/g)	Gram−/Gram+	Bacteria/fungi	Gram− (%)	Gram+ (%)	Actinobacteria (%)	Fungi (%)	[TSF]	[PSC]	TSF (%)	PSC (%)
Gram− (nmol/g)	1														
Gram+ (nmol/g)	0.960***	1													
Actinobacteria (nmol/g)	0.936***	0.952***	1												
Fungi (nmol/g)	0.576***	0.575***	0.506***	1											
Biomass (nmol/g)	0.986***	0.989***	0.966***	0.604***	1										
Gram−/Gram+	−0.313*	−0.535***	−0.551***	−0.257	−0.455**	1									
Bacteria/fungi	−0.169	−0.149	−0.077	−0.740***	−0.177	0.168	1								
Gram− (%)	−0.288*	−0.510***	−0.505***	−0.422**	−0.435**	0.970***	0.020	1							
Gram+ (%)	0.535***	0.733***	0.600***	0.301*	0.634***	−0.789***	−0.390**	−0.765***	1						
Actinobacteria (%)	−0.318**	−0.344*	−0.087	−0.504***	−0.331*	−0.243	−0.952***	−0.187	−0.256	1					
Fungi (%)	0.172	0.145	0.041	0.669***	0.158	0.067	−0.247	−0.053	0.02	−0.401**	1				
[TSF]	0.652***	0.651***	0.609***	0.478***	0.659***	−0.261	−0.223	−0.212	0.412**	−0.330*	0.283*	1			
[PSC]	0.602***	0.597***	0.573***	0.368**	0.605***	−0.220	−0.362*	−0.217	0.341**	−0.207	0.301*	0.811***	1		
%TSF	0.614***	0.624***	0.570***	0.535***	0.639***	−0.196	−0.362*	−0.219	0.359**	−0.378**	0.406**	0.895***	0.867***	1	
%PSC	0.627***	0.612***	0.574***	0.498***	0.628***	−0.196	−0.370	−0.219	0.337*	−0.290*	0.404**	0.863***	0.919***	0.930***	1

$*\ p < 0.050.$
$**\ p < 0.010.$
$***\ p < 0.001.$

Table 5
Pearson correlation coefficients of irrigated soils between population of Gram−, Gram+, Actinobacteria, fungi and their corresponding relative concentrations, total biomass, ratio Gram−/ total Gram+, ratio bacteria/fungi, remaining concentrations of triasulfuron (TSF) and prosulfocarb (PSC), and remaining percentage of TSF and PSC. Significant correlations were denoted by asterisks and blond font.

Irrigation	Gram− (nmol/g)	Gram+ (nmol/g)	Actinobacteria (nmol/g)	Fungi (nmol/g)	Biomass (nmol/g)	Gram−/Gram+	Bacteria/fungi	Gram− (%)	Gram+ (%)	Actinobacteria (%)	Fungi (%)	[TSF]	[PSC]	TSF (%)	PSC (%)
Gram− (nmol/g)	1														
Gram+ (nmol/g)	0.937***	1													
Actinobacteria (nmol/g)	0.865***	0.963***	1												
Fungi (nmol/g)	0.763***	0.652***	0.520***	1											
Biomass (nmol/g)	0.977***	0.986***	0.939***	0.738***	1										
Gram−/Gram+	0.019	−0.311*	−0.436**	0.163	−0.176	1									
Bacteria/fungi	−0.420**	−0.230	−0.120	−0.710***	−0.342*	−0.481**	1								
Gram− (%)	−0.088	−0.413**	−0.505***	−0.071	−0.291*	0.963***	−0.302*	1							
Gram+ (%)	0.305*	0.595***	0.617***	−0.011	0.460**	−0.854***	0.425**	−0.844***	1						
Actinobacteria (%)	−0.620***	−0.400**	−0.183	−0.705***	−0.497***	−0.652***	0.665***	−0.492***	0.250	1					
Fungi (%)	0.433**	0.273	0.134	0.906***	0.380*	0.447**	−0.856***	0.232	−0.355**	−0.723***	1				
[TSF]	0.782***	0.778***	0.701***	0.667***	0.795***	−0.035	−0.476**	−0.151	0.247	−0.491***	0.413**	1			
[PSC]	0.670***	0.733***	0.731***	0.420**	0.716***	−0.230	−0.248	−0.293**	0.373**	−0.211	0.188	0.795***	1		
%TSF	0.775***	0.782***	0.750***	0.623***	0.797***	−0.087	−0.474**	−0.198	0.262	−0.403**	0.390**	0.904***	0.903***	1	
%PSC	0.770***	0.811***	0.777***	0.601***	0.809***	−0.183	−0.360*	−0.277	0.350**	−0.346*	0.316*	0.875***	0.942***	0.952***	1

$*\ p < 0.050.$
$**\ p < 0.010.$
$***\ p < 0.001.$

i.e. the soils had more bacteria and a lower population of fungi with respect to the initial situation. This effect has also been observed in soils fumigated with imazethapyr, herbicide with the same mechanisms of action than triasulfuron (Zhang et al., 2010).

The presence of triasulfuron and prosulfocarb reveal different effects on microbial structure in both irrigated and non-irrigated soils during the field assay. The most abundant microorganisms in the irrigated
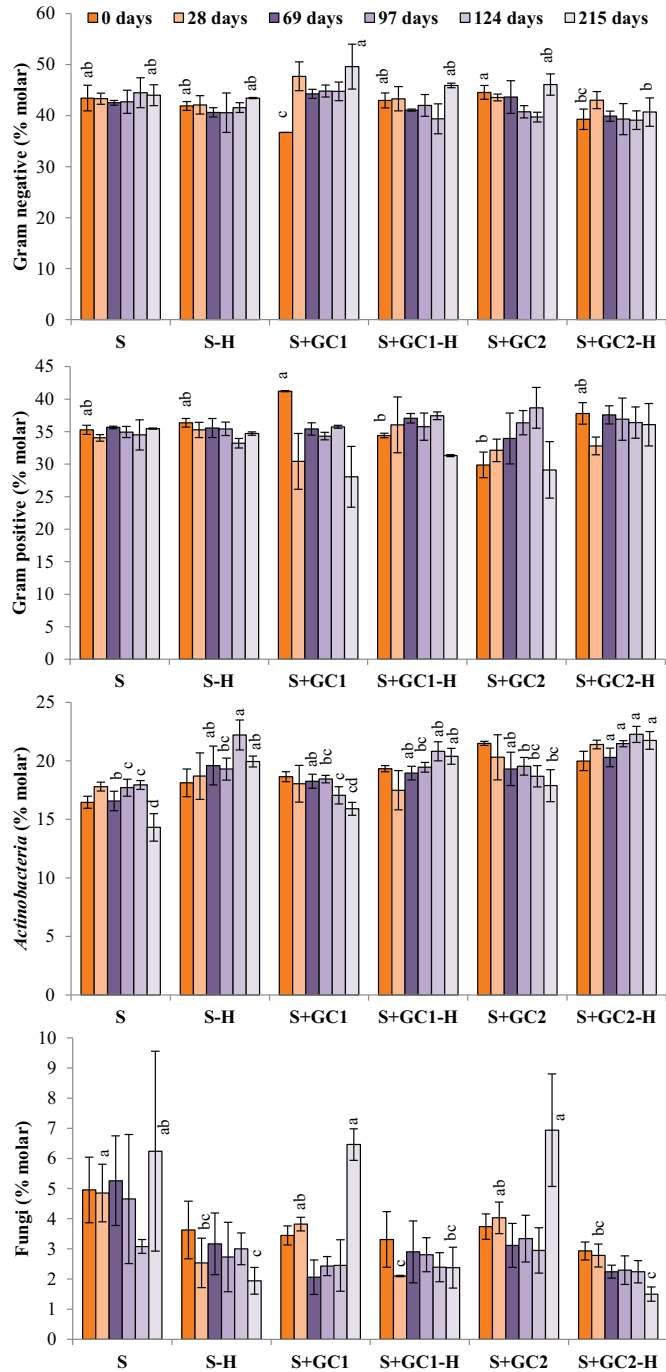

Fig. 1. Relative abundance (%mol) of PLFAs specifically diagnostics of Gram− and Gram+ bacteria, *Actinobacteria* and fungi in the non-irrigated soils. Vertical bars represent the standard deviation of three replicates. Different letters indicate significant differences among treatments at the same sampling time (Tukey post hoc test, *p* < 0.05). S: unamended soil; S + GC1: amended soil with green compost 1; S + GC2: amended soil with green compost 2; H: herbicides application. The first application of herbicides is denoted by brown colour and the second application by purple colour.
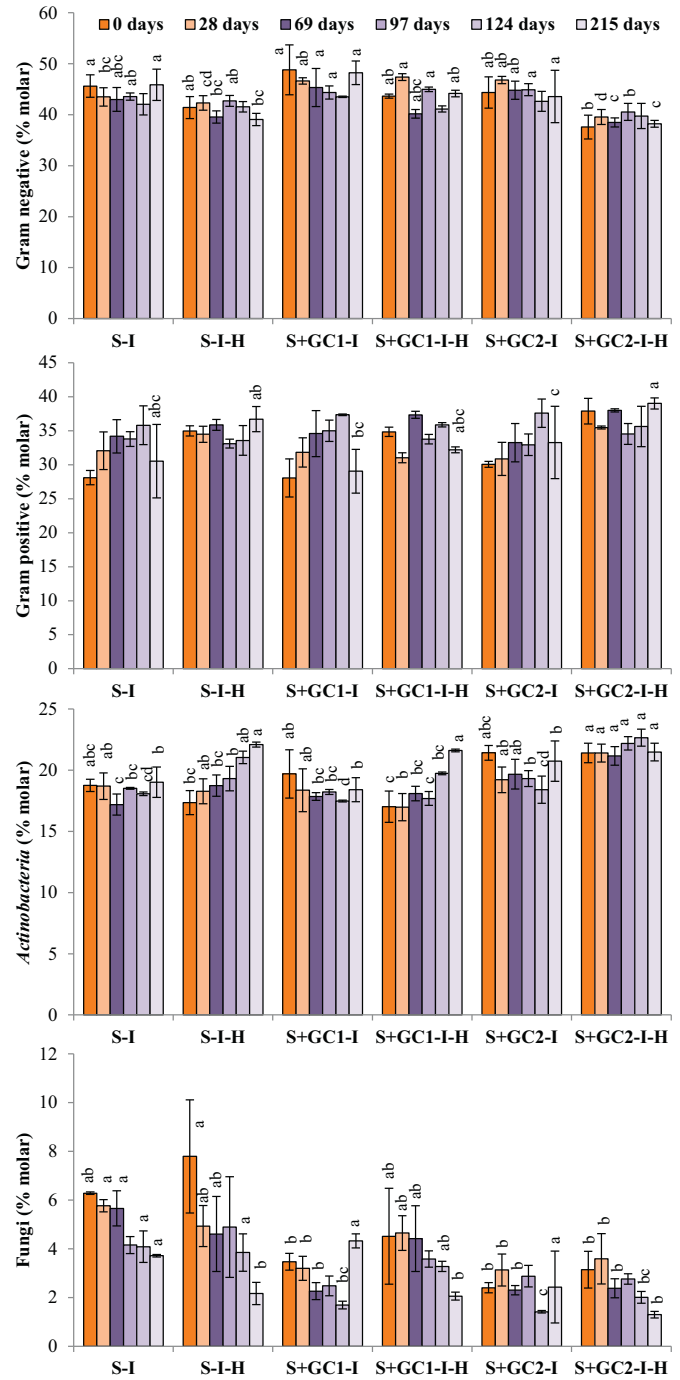

Fig. 2. Relative abundance (%mol) of PLFAs specifically diagnostics of Gram− and Gram+ bacteria, *Actinobacteria* and fungi in the irrigated soils. Vertical bars represent the standard deviation of three replicates. Different letters indicate significant differences among treatments at the same sampling time (Tukey post hoc test, *p* < 0.05). S: unamended soil; S + GC1: amended soil with green compost 1; S + GC2: amended soil with green compost 2; H: herbicides application; I: irrigation. The first application of herbicides is denoted by brown colour and the second application by purple colour.

and non-irrigated soils were Gram-negative bacteria followed by Gram-positive bacteria, *Actinobacteria,* and finally fungi (Figs. 1 and 2). A different composition of the microbial structure could be expected due to the different irrigation regime and organic amendment management, as reported by Franco-Andreu et al. (2016a, 2016b) and Sun et al. (2017). These authors reported that non-irrigated soils have been related to a higher proportion of Gram-positive bacteria than in irrigated

soils because of the higher rigidity of cell walls compared to Gram-negative bacteria. However, the application of organic amendments may reduce this phenomenon (Franco-Andreu et al., 2016b) because of the higher water retention by the OM from organic amendments. In this study, the dominance of Gram-negative bacteria irrespective of irrigation could also be explained by the fact the drought conditions of non-irrigated soils were not extreme, as was the case in the above references. The cumulative rainfall during the assay was 185.8 mm and irrigation was 70 mm (Fig. S1 in Supplementary material), so the irrigated soils received a total of 255.8 mm of water, 38% more than the non-irrigated soils.

In the case of non-irrigated soils, the application of herbicides did not have any significant effects on the relative populations of Gram-negative and Gram-positive bacteria during the assay (Fig. 1). However, herbicides tended to increase the relative abundance of *Actinobacteria* after the second application (69–215 days) in S—H, S + GC1-H and S + GC2-H (Fig. 1). Baxter and Cummings (2008) have described the changes in soil microbial structure after three consecutive applications of the herbicide bromoxynil and, what's more, at high doses. The change in bacteria structure prompted a significant decrease in the Gram-negative/total Gram-positive (sum of Gram-positive group and *Actinobacteria*) bacteria ratio between treatments with and without herbicides in S + GC1 and S + GC2 at the end of the assay (Fig. S4 in Supplementary material). Similar results were found in soils fumigated with imazethapyr (Zhang et al., 2010).

Results indicated that the residual concentrations of herbicides were positively correlated with the relative percentage of Gram-positive bacteria and fungi, and negatively correlated with *Actinobacteria* (Table 4). Therefore, the dissipation of herbicides negatively affected Gram-positive bacteria and fungi, whereas it enhanced the relative population of *Actinobacteria*. So, fungi behaved in the opposite way to *Actinobacteria*. After the first application of herbicides (28 days), the relative population of fungi in S—H and S + GC1-H was significantly lower than in S and S + GC1, although S + GC2 and S + GC2-H did not record significant differences (Fig. 1). At the end of the assay (215 days), the negative effects of herbicides on fungi abundance were significant ($p < 0.05$) for all the treatments, and produced a generalized increase in the bacteria/fungi ratio (Fig. S4 in Supplementary material). This suggests that fungi were sensitive to the herbicides triasulfuron and prosulfocarb. The opposite effect between fungi and bacteria (Santás-Miguel et al., 2018) was because of the significant increase in *Actinobacteria*, the minimal effects of herbicides on Gram-negative and Gram-positive bacteria, and the significant decrease in fungi (Fig. 1).

The irrigation of soils enhanced the effects of herbicides in unamended and amended soils. The relative abundance of Gram-negative bacteria between the first and second herbicide applications (28 days) was lower in S + GC2-I-H than in S + GC2-I, but there were no significant differences between S—I and S-I-H or between S + GC1-I and S + GC1-I-H (Fig. 2). The same behaviour was found after the second herbicide application at 69 and 97 days. At the end of the assay (215 days), nonetheless, the decrease in Gram-negative abundance was significant ($p < 0.05$) for all the soil treatments with herbicides (S-I-H, S + GC1-I-H and S + GC2-I-H). The effect on Gram-positive bacteria was only significant ($p < 0.05$) at the end of the assay in S + GC2-I, where the presence of herbicides increased the relative population of Gram-positive bacteria.

The relative percentage of Gram-negative bacteria was negatively correlated with the prosulfocarb residue (Table 5). In contrast, the relative percentage of Gram-positive bacteria was positively correlated with the prosulfocarb residue. Therefore, the presence of prosulfocarb could induce the substitution of Gram-positive bacteria by Gram-negative bacteria. The same phenomenon could be found between *Actinobacteria* (negatively correlated with the herbicide residues) and fungi (positively correlated with the herbicide residues). The relative population of *Actinobacteria* was significantly higher with herbicides in all the soil treatments (S-I-H, S + GC1-I-H and S + GC2-I-H) as happened in

non-irrigated soils. Despite the significant differences found during the assay for all the treatments, the ratio Gram-negative/total Gram-positive bacteria (Fig. S5 in Supplementary material) only recorded significant differences between S + GC2-I and S + GC2-I-H. In this soil, as in non-irrigated soils, the application of herbicides decreased this ratio accordingly with the increased persistence of herbicides (higher DT_{50}).

In contrast to *Actinobacteria,* the effects of herbicides on fungi followed the opposite trend by decreasing their relative abundance over time in all soil treatments. Moreover, the fungal decrease was clearly reflected in the bacteria/fungi ratio (Fig. S5 in Supplementary material). The increase in this ratio at the end of the assay again reflected the bacteria – fungi antagonism. The negative effects of some herbicides on fungi have been previously reported (Martin-Laurent et al., 2003; Wu et al., 2014). However, other studies have shown the negative effects that some herbicides, including triasulfuron and prosulfocarb, have on fungi at the beginning of the incubation period, although the fungal population subsequently recovered (Cycoń et al., 2013; García-Delgado et al., 2018; Wang et al., 2015; Zhang et al., 2010). In this study, the significant decline ($p < 0.05$) in the relative abundance of fungi could be related to the consecutive applications of high doses of a mixture of two herbicides. In addition, between 0 and 69 days the GC-amended soils (S + GC1 and S + GC2) tended to decrease the relative abundance of fungi with respect to S, as previously described by García-Delgado et al. (2018) and Pose-Juan et al. (2017).

The microbiological changes in soils after the second application of herbicides could be responsible for the change in their degradation rates, which tend to be faster after the second application when the relative population of *Actinobacteria* is enhanced. These organisms are known to be good degraders of complex substrates (Pose-Juan et al., 2017).

3.3. Relationship between soil microbial structure, herbicides, organic amendments and irrigation regimes

The global impact of herbicides on soil microbial community was assessed by PCA including the results in non-irrigated (Fig. 3A, B and C) and irrigated (Fig. 3D, E and F) soils. A PERMANOVA analysis (Table S1 in Supplementary material) testing for the significance of the effects on the relative abundances of PLFAs of herbicide application, sampling time, soil treatments and their respective interactions revealed the statistical significance of the three factors in non-irrigated and irrigated soils.

With respect to non-irrigated soils, unamended and amended soils recorded different PCA profiles. The soil treatment was not significant, but the application of herbicides was statistically significant in all three soil treatments (S, S + GC1 and S + GC2) (Table S1). The application of herbicides in soil without compost (S—H) clearly enhanced the abundance of *Actinobacteria* and reduced the relative abundance of fungi and Gram-negative bacteria. S + GC1-H and S + GC2-H were related to a low relative abundance of fungi and a high relative abundance of *Actinobacteria* and Gram-negative bacteria. In contrast, amended soils without herbicides were related to a high relative abundance of fungi and Gram-positive bacteria. In non-irrigated soils, therefore, herbicides had a clearly negative effect on fungi and Gram-positive bacteria, while promoting the relative abundance of *Actinobacteria,* irrespective of compost use. S—H was related to high relative abundance of Gram-negative bacteria, mainly at 124–215 days, while S + GC1-H and S + GC2-H recorded a closer relationship with Gram-negative bacteria than S + GC1 and S + GC2 (Fig. 3A, B and C). In a laboratory assay, soil fumigated with another sulfonylurea herbicide, azimsulfuron, enhanced microbial diversity by detecting different Gram-negative bacteria that were not found in a non-fumigated soil (Valle et al., 2006). S + GC1-H and S + GC2-H produced slower triasulfuron degradation than S—H, so the microbial shift may be deeper in the former.

The microbiology of non-irrigated soils was exposed to three clear stress factors, namely, high doses of triasulfuron and prosulfocarb, two
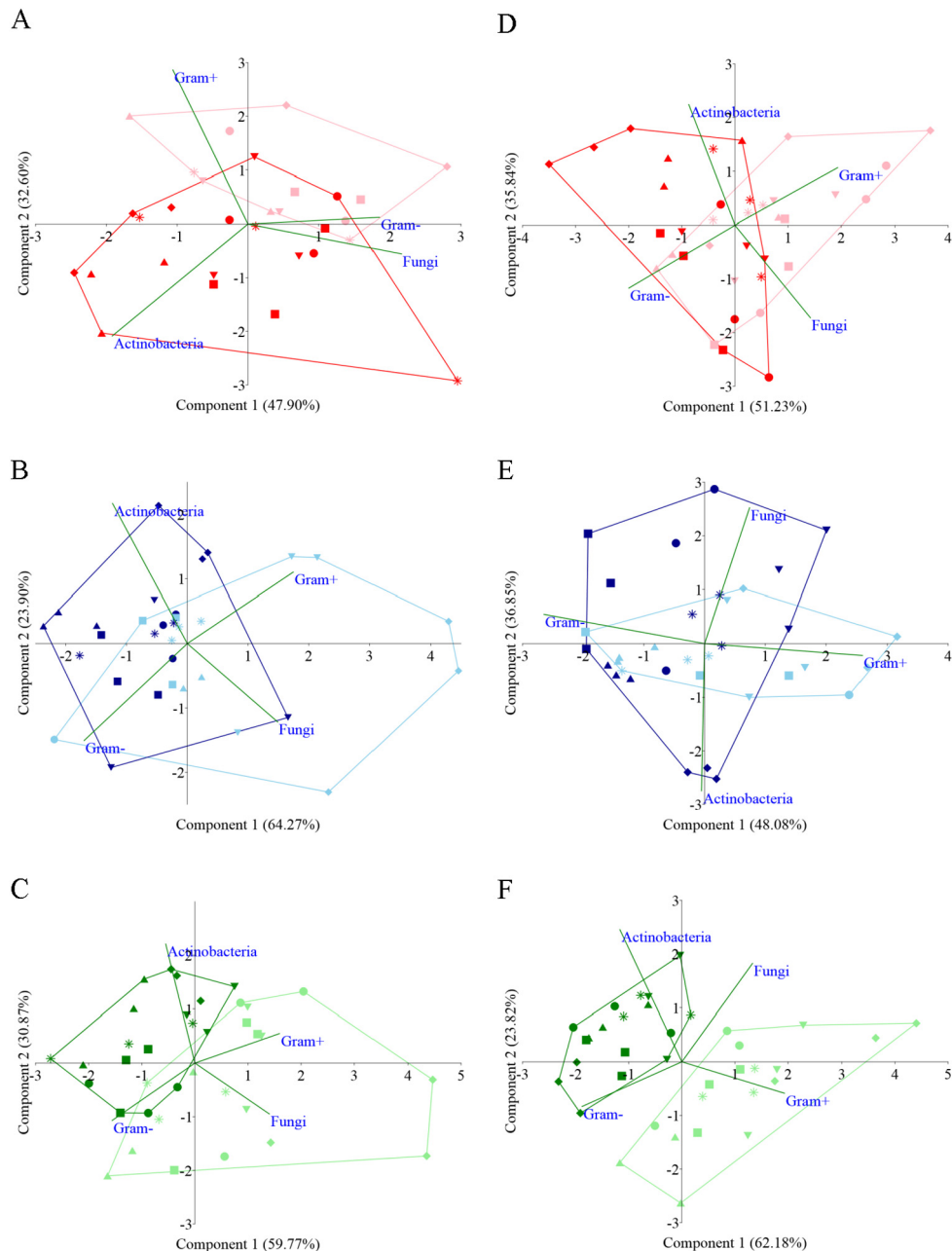
Fig. 3. Principal components analysis (PCA) of non-irrigated (A, B, C) and irrigated (D, E, F) soils showing loading scores for Gram− and Gram+ bacteria, *Actinobacteria* and fungi, and the scores of sampling times (0 days: circle; 28 days: inverse triangle; 69 days: square; 97 days: star; 124 days: triangle; 215 days: diamond) on the two main components. The application of herbicides is denoted by dark colors, the non-application of herbicides by light colors in unamended soil (Red), GC1-amended soil (Blue) and GC2-amended soil (Green). Percent variability explained by each principal component is shown in parentheses after each axis legend.

consecutive applications, and drought conditions. In this respect, the toxic effects of pesticides can be enhanced, with negative effects on microbial composition, enzyme activity and pesticide degradation (Franco-Andreu et al., 2016a). This could be the cause of the significant impact triasulfuron and prosulfocarb have on the microbial structure of unamended and GC-amended soils, as reported here. In contrast, a previous study under similar conditions but with lower herbicide doses revealed an impact on the microbial structure of unamended soil but not on the microbial structure of GC-amended soil (García-Delgado et al., 2018). Therefore, the capacity of organic amendments to buffer the effects of herbicides on the microbial structure could be limited by herbicide doses or consecutive applications.

The three soil treatments in irrigated soils had significant effects (Table S1 in Supplementary material) on the soil microbial structure.

Unamended and GC1 amended soils (with and without herbicides) had a similar distribution in PCA with no significant relationship with any factor (Fig. 3D and E). In contrast, GC2 amended soils (with and without herbicides) had a strong relationship with *Actinobacteria* and a weak one with fungi (Fig. 3F). The negative effects of GC or other organic amendments on fungal abundance has previously been reported at both laboratory scale (Pose-Juan et al., 2017) and field scale (García-Delgado et al., 2018).

The application of herbicides in irrigated soils had some similarities with non-irrigated soils. The soil microbial structure of irrigated soils after herbicide application shifted towards a higher proportion of *Actinobacteria* and a lower relative amount of fungi, as was the case in non-irrigated soils. The presence or absence of herbicides and their interaction over time was significant in unamended soil. S—I was related

to a high proportion of Gram-positive bacteria and fungi. In contrast, S-I-H evolved from points related to fungi towards a clear relationship with *Actinobacteria* and Gram-negative bacteria at the end of the assay. The herbicide factor was not significant in S + GC1 treatment. However, the time factor and the herbicide * sampling time interaction were significant. In fact, the evolution of S + GC1-I-H (dark blue symbols in Fig. 3) tends to be more closely related to *Actinobacteria* and less so to fungi over time. In contrast, the changes in microbial structure over time in S + GC1 seem to be related to the variation in the proportion of Gram-negative and Gram-positive bacteria because of the dispersion of points in component 1 and the low values of points in component 2, being closely related to fungi and *Actinobacteria*. The application of herbicides in GC2-amended soils and their interaction with sampling time were significant. PCA showed a clear differentiation between S + GC2-I and S + GC2-I-H. S + GC2-I was related to a high relative abundance of Gram-positive bacteria, mainly at 215 days. In contrast, S + GC2-I-H was related to a high relative abundance of *Actinobacteria* and Gram-negative bacteria. In addition, there was a clearly negative relationship with the abundance of fungi in both, S + GC2-I and S + GC2-I-H treatments, as described above.

After herbicide application, all the soils, irrespective of irrigation conditions, tended to enhance the relative population of *Actinobacteria*, mainly after the second application of herbicides, when their DT_{50} values were lower than the first one. The remaining triasulfuron concentration 69 days after the second application recorded lower concentrations than in the first application. Dissipation therefore increased in the second application, when *Actinobacteria* increased their relative abundance. In contrast, the remaining concentrations of prosulfocarb were higher after the second application. It therefore seems clear that a high relative abundance of *Actinobacteria* plays a key role in herbicide dissipation, being positive in the case of triasulfuron and negative in the case of prosulfocarb degradation.

4. Conclusions

The dissipation of triasulfuron and prosulfocarb in an agricultural soil under field conditions is influenced by the type and amount of green compost applied to the soil and by the irrigation regime. Two consecutive applications of triasulfuron increase the dissipation rate of this herbicide, although in the case of prosulfocarb it produces an accumulation of residual herbicide after the second application. A positive correlation between the amounts of herbicide residues and the total microbial population led to a decrease in the microbial population during the dissipation of herbicides and to a certain toxicity of herbicides for the microbial community. The microbial structure of unamended and GC-amended soils is modified after two consecutive applications of the herbicides triasulfuron and prosulfocarb. Herbicides increase the relative population of *Actinobacteria* and reduce the relative population of fungi compared to the initial situation in all the conditions studied. *Actinobacteria* seems to be responsible for the increase in the of degradation rate of triasulfuron after the second application.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.07.395.

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