INFLUENCE OF DIFFERENT AGRICULTURAL MANAGEMENT PRACTICES ON SOIL MICROBIAL COMMUNITY OVER DISSIPATION TIME OF TWO HERBICIDES

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1   **ABSTRACT**

2   Soil microbiology could be affected by the presence of pesticide residues during

3   intensive farming, potentially threatening the soil environment. The aim here was to

4   assess the dissipation of the herbicides triasulfuron and prosulfocarb, applied as a

5   combined commercial formulation, and the changes in soil microbial communities

6   (through the profile of phospholipid fatty acids (PLFAs) extracted from the soil) during

7   the dissipation time of the herbicides under field conditions. The dissipation of

8   herbicides and the soil microbial structure were assessed under different agricultural

9   practices, such as the repeated application of herbicides (twice), in unamended and

10   amended soils with two organic amendments derived from green compost (GC1 and

11   GC2) and with non-irrigation and irrigation regimes. The results obtained indicate

12   slower dissipation for triasulfuron than for prosulfocarb. The 50% dissipation time

13   (DT_{50}) decreased under all conditions for the second application of triasulfuron,

14   although not for prosulfocarb. The DT_{50} values for both herbicides increased in the GC2

15   amended soil with the highest organic carbon (OC) content. The DT_{50} values decreased

16   for prosulfocarb with irrigation, but not for triasulfuron, despite its higher water

17   solubility. The herbicides did not have any significant effects on the relative population

18   of Gram-negative and Gram-positive bacteria during the assay, but the relative

19   abundance of *Actinobacteria* increased in all the soils with herbicides. At the end of the

20   assay (215 days), the negative effects of herbicides on fungi abundance were significant

21   ($p < 0.05$) for all the treatments. These microbiological changes were detected in non-

22   irrigated and irrigated soils, and were more noticeable after the second application of

23   herbicides. *Actinobacteria* could be responsible for the modification of herbicide

24   degradation rates, which tend to be faster after the second application. This study makes

25   a useful contribution to the evaluation of the soil environment and microbiological risks

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26   due to the long-term repeated application of herbicides under different agricultural

27   management practices.

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31   **1. Introduction**

32   The structure of soil microbial communities and the changes produced in them

33   by different environmental impacts is of great interest nowadays (Barra Caracciolo et al.

34   2015). Microbial activity is an accurate indicator of soil quality because soil

35   microorganisms play a key role in organic matter (OM) decomposition and in the

36   biogeochemical cycles that affect soil fertility (Pascual et al. 2000; García-Orenes et al.

37   2013). This microbial activity could be affected by the presence of pesticide residues in

38   soil that pose a potential risk to soil ecology (Cycoń et al. 2013; Fang et al. 2018).

39   Modern intensive farming involves the application of large quantities of pesticides

40   during the crop growth period (Nyamwasa et al. 2018). In fact, the residues of

41   herbicides, insecticides and fungicides have been detected in soils in agricultural areas

42   across different countries in a broad range of concentrations (Li et al. 2014; Pose-Juan

43   et al. 2015) which could modify soil microbial biodiversity. Residues of these

44   agrochemicals depend on their dissipation in soils, being modified by different

45   environmental factors (soil type, soil OM, weather, temperature, irrigation), pesticide

46   formulation (individual or combined compounds), and application method (single or

47   repeated application) (Arias-Estévez et al. 2008). This widespread use of pesticides

48   could therefore lead to a potential decrease in soil microbial biodiversity, with a

49   negative impact on crop yields (Baxter and Cummings 2008), which could be increased

50   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

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76 assessed under field conditions using combined commercial formulations of pesticides,

77 and most of these studies have been carried out in unamended soils (Kaur and Bhullar

78 2017; Kaur et al. 2017).

79 Triasulfuron (2-(2-chloroethoxy)-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)

80 amino] carbonyl] benzene sulfonamide) is a mobile herbicide in soil due to its high

81 water solubility and low hydrophobicity (EFSA 2015), while prosulfocarb (S-

82 (phenylmethyl) dipropylcarbamothioate) is a hydrophobic herbicide with high

83 adsorption, low mobility and a moderate persistence in soil (EFSA 2007a). These

84 herbicides of the sulfonylurea and thiocarbamate group, respectively, are used on pre-

85 and post-emergence in winter cereals (wheat, barley) and other crops (PPDB 2018). No

86 study has been made of the combined long-term effect that triasulfuron and prosulfocarb

87 have on their dissipation rates and soil microbial biomass and structure, although they

88 are repeatedly applied to a broad range of crops. Compounds of the chemical groups

89 sulfonamide and thiocarbamate, such as triasulfuron and prosulfocarb, are usually

90 recommended in cereals for individual or joint use for controlling weeds in rainfed and

91 irrigated cereal crops (Cirujeda and Taberner 2010; Bajya et al. 2015).

92 Relevant changes in soil microbial abundance, activity and structure have

93 already been reported by the authors in a previous paper (García-Delgado et al. 2018)

94 when triasulfuron and prosulfocarb were applied as a combined formulation in

95 unamended and amended soils with green compost (GC) under field conditions. GC is

96 the biodegradable organic residue from pruning in urban gardens and parks with an OM

97 content higher than 15% (BOE 2013). The results were obtained for a single application

98 of herbicides after the short-term dissipation of herbicides. Little is known about the

99 other factors that could influence the dissipation of herbicides in soils and changes in

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100   the soil microbial structure under field conditions. Repeated herbicide application,

101   different soil OC content from organic amendments, and/or irrigation may modify

102   herbicide dissipation. The study of these factors would increase our knowledge on the

103   effect herbicides have on soil microbial communities.

104   The aim here was therefore to assess the changes in soil microbial communities

105   during the dissipation of two herbicides continuously applied under different

106   agronomical practices. A combined commercial formulation of triasulfuron and

107   prosulfocarb (Aurus Plus®) was applied twice to an unamended soil and one amended

108   with two organic amendments derived from green compost (GC1 and GC2) and with

109   non-irrigation and irrigation regimes. The effect of these factors on the dissipation of

110   herbicides (DT_{50}) and on the soil microbial structure was studied under field conditions.

111   The study makes a useful contribution to the evaluation of environmental and

112   microbiological risks due to the combined long-term application of herbicides under

113   different management practices in agriculture.

114

115   **2. Materials and methods**

116   *2.1. Herbicides*

117   The commercial formulation of triasulfuron (TSF) (2-(2-chloroethoxy)-N-[[(4-

118   methoxy-6-methyl-1,3,5-triazin-2-yl) amino] carbonyl] benzene sulfonamide) (20% p/p)

119   and prosulfocarb (PSC) (S-(phenylmethyl) dipropylcarbamothioate) (80% p/v) (Auros

120   Plus®, Syngenta Agro S.A., Madrid, Spain) was used in the field study. Analytical

121   standards of both herbicides (purity > 98.9%) were supplied by Sigma Aldrich Química

122   S.A. (Madrid, Spain). Water solubility is 815 and 13.0 mg L^{-1} and log K_{ow} is -0.59 and

123   4.48 for triasulfuron and prosulfocarb, respectively (PPDB 2018).

124

125 *2.2. Organic residues*

126   Two green composts formed by composted vegetal residues were used. They

127 were supplied by the local authority (GC1) and by the nursery "El Arca" (GC2) from

128 Salamanca (Spain). Their main physicochemical characteristics on a dry weight basis

129 were determined by standard methods (Sparks et al., 1996) and are as follows: pH 7.33

130 and 7.58, organic carbon (OC) content 9.80% and 24.1%, dissolved organic carbon

131 (DOC) content 0.353% and 0.700%, total N 1.04% and 1.10%, C/N ratio 9.42 and 21.9,

132 and ash percentage 74.5% and 53.0% for GC1 and GC2, respectively.

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134 *2.3. Experimental set-up*

135   A field experiment was conducted in an agricultural soil (sandy clay loam soil,

136 *Typic Haploxerept*) in the Muñovela experimental farm belonging to IRNASA-CSIC

137 (Salamanca, Spain). An experimental layout of randomized complete blocks was

138 designed in February 2016, with six treatments and three replicates per treatment (18

139 plots of 9 m^2) corresponding to unamended soil (6 plots, S) and soil amended with GC1

140 (6 plots, S+GC1) or GC2 (6 plots, S+GC2) at the rate of 120 and 180 t ha^{-1} on a dry

141 weight basis, respectively. For each soil treatment, three plots received only natural

142 rainfall, while other three plots received weekly 2.8 mm (I). In March 2016, the

143 commercial formulation of triasulfuron and prosulfocarb (Auros Plus[®]) was applied to

144 18 experimental plots at doses of 250 g a.i. ha^{-1} and 11.25 kg a.i. ha^{-1}, respectively,

145 corresponding to 2.5 times the maximum agronomic dose for both herbicides

146 recommended for heavy soils with a greater adsorption capacity. The increase in the

147 soil´s capacity for adsorbing the herbicides after an organic amendment supports the use

148 of doses higher than those recommended to maintain the efficacy of the compounds.

149 Once the herbicide half-lives (DT$_{50}$) were achieved in all plots (after 68 days), the

150 herbicides were applied again at the same doses in May 2016. A check was made prior

151 to the application of the herbicides to ensure that no amounts of these compounds were

152 detectable in the soil samples. This was as expected, because the plots had no history of

153 triasulfuron and prosulfocarb application in the previous five years. Additionally,

154 eighteen control plots (six by soil treatment) did not receive herbicide application, but

155 nine of them received irrigation.

156 Weather conditions were recorded throughout the experiment (215 days) by a

157 meteorological station located on site (Fig. S1 in Supplementary material). Air

158 temperature ranged from 1.7 ℃ to 14.6 ℃ (mean air temperature 8.3 ℃) and from 10.5

159 ℃ to 26.3 ℃ (mean air temperature 19.1 ℃) during the first (0-68 days) and the second

160 (69-215 days) period of the application, respectively. Cumulative precipitation and

161 additional irrigation were 139.2 mm and 11.2 mm during the first application, and 46.6

162 mm and 58.8 mm during the second application of herbicides, respectively. Total

163 cumulative precipitation and additional irrigation were 185.8 mm and 70 mm,

164 respectively.

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166 *2.4. Soil sampling and herbicide extraction and analysis*

167 Surface soil samples from 0 to 10 cm were collected to determine herbicide

168 dissipation at different times between 0 and 68 days (first herbicides´ application) and

169 between 69 and 215 days (second herbicides´ application) and to determine soil

170 microbial structure at 0, 28, 69, 97, 124 and 215 days. Soil samples were collected,

171 processed and characterized following the methods described by Marín-Benito et al.

172 (2018a) and García-Delgado et al. (2018) (complete information is included in

173 Supplementary material). Soil characteristics are included in Table 1. Soil samples were

174 sieved (<2 mm) and moisture content of the bulk sample was determined. Duplicate

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175 subsamples of moist soil (6 g) were extracted with methanol (12 mL) by shaking and

176 sonication. The determination of the herbicides in the soil extracts was performed by

177 HPLC-MS (Waters Assoc., Milford, MA, USA). The molecular ions [m/z] 402.8

178 (triasulfuron) and 252.4 (prosulfocarb) were monitored and the retention times were 6.1

179 min and 14.1 min, respectively. A detailed description of the herbicide extraction and

180 analytical methods is included in Supplementary material.

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182 *2.5. Soil microbial community by PLFA analysis*

183   The soil microbial community composition of the soil samples was determined

184 using phospholipid fatty acid (PLFA) analysis, as described in Frostegård et al. (1993).

185 Lyophilized soils samples were extracted with a one-phase chloroform-methanol-

186 phosphate buffer solvent by sonication. Extracts were purified by SPE and polar lipids

187 were transesterified with methanol-KOH. Finally, hexane extracts containing the

188 resultant fatty acid methyl esters were analyzed by gas chromatography. Quantification

189 was performed using an Agilent 7890 gas chromatograph (Agilent Technologies,

190 Wilmington, DE, USA) equipped with a 25-m Ultra 2 (5% phenyl)-methylpolysiloxane

191 column (J&W Scientific, Folsom, CA, USA) and a flame ionization detector. PLFAs

192 were identified using bacterial fatty acid standards and software from the Microbial

193 Identification System (Microbial ID, Inc., Newark, DE, USA). Specific PLFAs (Zelles,

194 1999) were used as biomarkers to quantify the relative abundances of Gram negative

195 (monounsaturated fatty acids and cyclopropyl 17:0 and 19:0) and Gram positive (iso

196 and anteiso saturated branched fatty acids) bacteria, *Actinobacteria* (10-methyl fatty

197 acids) and fungi (18:2 ω6 cis).

198

199 *2.6. Data analysis*

200   The dissipation kinetics for triasulfuron and prosulfocarb were fitted to a single

201   first-order (SFO) or first-order multicompartment (FOMC) models and values for the

202   time to 50% dissipation (DT_{50}) were estimated using the Excel Solver add-in package

203   (FOCUS, 2006). More details about the fitting of the dissipation kinetics are included in

204   Supplementary material.

205   Analysis of variance (ANOVA) was used to evaluate the effects of the different

206   treatments (green compost application, repeated herbicide application and irrigation) on

207   herbicide dissipation. Standard deviation (SD) was used to indicate variability among

208   replicates. Fisher´s least significant difference (LSD) method, at a confidence level of

209   95%, was determined with IBM SPSS Statistics v24 software package (SPSS Inc.

210   Chicago, USA).

211   Data of PLFAs were submitted for the analysis of variance (ANOVA) by

212   previous Levene variance homogeneity test to determine significant differences between

213   treatments at each sampling time. Means were compared by either Tukey or Games–

214   Howell post hoc test based on whether or not variance homogeneity was met,

215   respectively ($p < 0.05$). Pearson correlation coefficients between the remaining

216   concentration and percentages of herbicides, and microbial biomass and structure were

217   determined to elucidate how variables are related to each other. ANOVA and

218   correlation analyses were carried out using the IBM SPSS Statistics v24 software

219   package (SPSS Inc. Chicago, USA). Principal component analysis (PCA) was

220   performed, with PAST v3.15 software (Hammer et al. 2001), to determine the most

221   meaningful variables and the global impact of the herbicides and soil treatments on soil

222   microbial community. In addition to PCA, PERMANOVA analysis was performed to

223   determine the significance of herbicides application, sampling time, soil treatments and

224   their interactions.

225

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

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250   between 14% - 51%, and at 69 days after the second application (corresponding to 138

251   days after the first application), being between 3% - 17% in the soils with the different

252   treatments. Other authors have also reported accelerated dissipation after repeated

253   pesticide applications due to the faster metabolism caused by enhanced biodegradation,

254   which could lead to a reduction in pesticide efficacy in some cases (Baxter and

255   Cummings 2008; Fang et al. 2018).

256   The DT_{50} values for prosulfocarb decreased by up to 1.3 and 1.6 times after its

257   second application in irrigated S and S+GC2 soils, respectively, but the DT_{50} values

258   were, in general, similar for all the other soil treatments (Table 3). However, the

259   remaining percentages of prosulfocarb at 68 days after the first application were lower

260   (0%-3%) than those at 69 days after the second application (corresponding to 138 days

261   after the first application) (2%-21%). These results indicate that the amounts of

262   prosulfocarb remaining after the second application decreased more slowly over time

263   than after the first application, without fully dissipating. Rouchaud et al. (1997) have

264   reported that repeated prosulfocarb application to a barley crop enhanced soil

265   biodegradation. However, the decrease in prosulfocarb concentrations after the second

266   application and over the course of 50% dissipation was slower than after the first

267   application, and this resulted in a higher persistence of the herbicides in the soil at the

268   end of the assay (215 days) (Fig. S3).

269   The DT_{50} values for the dissipation of triasulfuron and prosulfocarb after the two

270   applications were higher in S+GC2 than in S or S+GC1. The DT_{50} values increased by

271   3.1-1.8 times and by 2.0-1.9 times compared to the unamended soil. These results are

272   related to the higher OC content in S+GC2 than in S+GC1, which could help increase

273   the persistence of these herbicides in the top soil, decreasing its leaching (Marín-Benito

274   et al., 2018b). In fact, a significant and positive correlation ($R^2 = 0.828$, $p<0.001$) was

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275   found between the DT_{50} values of triasulfuron and prosulfocarb and OC content.

276   Furthermore, the adsorption of both herbicides by S+GC2 could occur with the possible

277   formation of bound residues and a potential decrease in bioavailability and

278   biodegradation (Gennari et al. 2002; Said-Pullicino et al. 2004). The relationship

279   between the adsorption and degradation of these herbicides has also been reported in

280   previous works (Nègre et al., 2006; Said-Pullicino et al., 2004). A significant and

281   positive correlation was also found here between the K_d determined for triasulfuron (S,

282   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

283   prosulfocarb (S, 21.6 ± 5.55 mL g^{-1}; S+GC1, 24.7 ± 7.62 mL g^{-1} and S+GC2, $57.1 \pm$

284   2.09 mL g^{-1}) (Marín-Benito et al., 2018b) and the DT_{50} values determined for the first

285   and second dissipation kinetics ($R^2 = 0.665$, $p < 0.02$, triasulfuron) and ($R^2 = 0.860$,

286    $p < 0.001$, prosulfocarb).

287   Additional irrigation did not significantly modify the dissipation rates of

288   triasulfuron after the two applications in S-I or S+GC1-I, but it decreased in S+GC2-I

289   after the first application. Similarly, Sarmah et al. (2000) have reported that the DT_{50}

290   values of triasulfuron in an unamended soil under field conditions remained unchanged

291   when the soil received 89 mm of irrigation compared to the non-irrigated soil. The DT_{50}

292   values of prosulfocarb were similar in irrigated soils after the first herbicide application.

293   However, the dissipation rates increased in S-I and to a greater extent in S+GC2-I after

294   the second herbicide application. Irrigation could lead to a higher potential degradation

295   and/or leaching of herbicides through the soil profile.

296   The additional evaluation of the influence of overall weather conditions on the

297   dissipation of triasulfuron and prosulfocarb revealed the possible effect of temperature

298   for explaining the accelerated degradation of triasulfuron after the second application.

299   The average temperature differed during the two dissipation periods, increasing by 10.8

300   $^{\circ}$C after the second herbicide application. This result is consistent with the increase in

301   the degradation rate of 2.58 times when the temperature increases by 10 $^{\circ}$C, as

302   determined by the European Food Safety Authority (EFSA 2007b). Dinelli et al. (1998)

303   have reported that temperature had an effect on the degradation rate of triasulfuron, and

304   the DT_{50} value decreased three times when temperature increased from 10 $^{\circ}$C to 20 $^{\circ}$C.

305   Stork (1995) has observed that triasulfuron degradation rates increased with soil

306   temperature, but they were not affected by soil water content. Temperature had no effect

307   for prosulfocarb after the second application of herbicide because faster dissipation

308   occurred only in irrigated treatments of S-I and S+GC2-I and could be explained by

309   other processes, as previously indicated.

310

311   *3.2. Effect of herbicides residues, organic amendments and irrigation regimes on the*

312   *soil microbial structure*

313   The total microbial population behaved in a similar way towards herbicides in

314   both irrigated and non-irrigated soils. The amounts of herbicide residues, in total ($\mu g\ g^{-}$

315   ^{1}) or relative concentration (%), were positively related to the total microbial population

316   ($nmol\ g^{-1}$) (Tables 4 and 5). It means a decrease in this population while the dissipation

317   of herbicides occurs. The toxicity of pesticides towards soil microorganisms is well

318   described in the literature, mainly in high doses (El Azhari et al., 2018; Fang et al.,

319   2018; Franco-Andreu et al., 2016a; Kalia and Gosal, 2011; Wang et al., 2015).

320   However, previous studies do not provide consistent results on the toxicity of

321   triasulfuron in soil microbiology between laboratory and field scale. Lupwayi et al.

322   (2004) and Pose-Juan et al. (2017) report that triasulfuron has no toxic effects on

323   microbial biomass at field and laboratory scale, respectively. In contrast, Sofo et al.

324   (2012) have reported toxic effects at laboratory scale for an agronomic dose or higher.

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325 A recent study at field scale using a lower dose of triasulfuron than in this work agreed

326 with the latter, indicating a decrease in microbial biomass due to the toxic effects of

327 triasulfuron, and more so a combination of triasulfuron and prosulfocarb in the

328 unamended and GC amended soils (García-Delgado et al., 2018). Therefore, there is

329 evidence of the toxicity of triasulfuron and prosulfocarb towards soil microbiota.

330 Additionally, our results indicated that the bacteria/fungi ratio was negatively correlated

331 with the herbicide residues, i.e. the soils had more bacteria and a lower population of

332 fungi with respect to the initial situation. This effect has also been observed in soils

333 fumigated with imazethapyr, herbicide with the same mechanisms of action than

334 triasulfuron (Zhang et al., 2010).

335   The presence of triasulfuron and prosulfocarb reveal different effects on

336 microbial structure in both irrigated and non-irrigated soils during the field assay. The

337 most abundant microorganisms in the irrigated and non-irrigated soils were Gram-

338 negative bacteria followed by Gram-positive bacteria, *Actinobacteria,* and finally fungi

339 (Figs. 1 and 2). A different composition of the microbial structure could be expected

340 due to the different irrigation regime and organic amendment management, as reported

341 by Franco-Andreu et al. (2016a, 2016b) and Sun et al. (2017). These authors reported

342 that non-irrigated soils have been related to a higher proportion of Gram-positive

343 bacteria than in irrigated soils because of the higher rigidity of cell walls compared to

344 Gram-negative bacteria. However, the application of organic amendments may reduce

345 this phenomenon (Franco-Andreu et al., 2016b) because of the higher water retention by

346 the OM from organic amendments. In this study, the dominance of Gram-negative

347 bacteria irrespective of irrigation could also be explained by the fact the drought

348 conditions of non-irrigated soils were not extreme, as was the case in the above

349 references. The cumulative rainfall during the assay was 185.8 mm and irrigation was

350 70 mm (Fig. S1 in Supplementary material), so the irrigated soils received a total of

351 255.8 mm of water, 38% more than the non-irrigated soils.

352   In the case of non-irrigated soils, the application of herbicides did not have any

353 significant effects on the relative populations of Gram-negative and Gram-positive

354 bacteria during the assay (Fig. 1). However, herbicides tended to increase the relative

355 abundance of *Actinobacteria* after the second application (69 – 215 days) in S-H,

356 S+GC1-H and S+GC2-H (Fig. 1). Baxter and Cummings (2008) have described the

357 changes in soil microbial structure after three consecutive applications of the herbicide

358 bromoxynil and, what's more, at high doses. The change in bacteria structure prompted

359 a significant decrease in the Gram-negative/total Gram-positive (sum of Gram-positive

360 group and *Actinobacteria*) bacteria ratio between treatments with and without herbicides

361 in S+GC1 and S+GC2 at the end of the assay (Fig. S4 in Supplementary material).

362 Similar results were found in soils fumigated with imazethapyr (Zhang et al., 2010).

363   Results indicated that the residual concentrations of herbicides were positively

364 correlated with the relative percentage of Gram-positive bacteria and fungi, and

365 negatively correlated with *Actinobacteria* (Table 4). Therefore, the dissipation of

366 herbicides negatively affected Gram-positive bacteria and fungi, whereas it enhanced

367 the relative population of *Actinobacteria*. So, fungi behaved in the opposite way to

368 *Actinobacteria*. After the first application of herbicides (28 days), the relative

369 population of fungi in S-H and S+GC1-H was significantly lower than in S and S+GC1,

370 although S+GC2 and S+GC2-H did not record significant differences (Fig. 1). At the

371 end of the assay (215 days), the negative effects of herbicides on fungi abundance were

372 significant ($p < 0.05$) for all the treatments, and produced a generalized increase in the

373 bacteria/fungi ratio (Fig. S4 in Supplementary material). This suggests that fungi were

374 sensitive to the herbicides triasulfuron and prosulfocarb. The opposite effect between

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375   fungi and bacteria (Santás-Miguel et al., 2018) was because of the significant increase

376   in *Actinobacteria*, the minimal effects of herbicides on Gram-negative and Gram-

377   positive bacteria, and the significant decrease in fungi (Fig. 1).

378   The irrigation of soils enhanced the effects of herbicides in unamended and

379   amended soils. The relative abundance of Gram-negative bacteria between the first and

380   second herbicide applications (28 days) was lower in S+GC2-I-H than in S+GC2-I, but

381   there were no significant differences between S-I and S-I-H or between S+GC1-I and

382   S+GC1-I-H (Fig. 2). The same behaviour was found after the second herbicide

383   application at 69 and 97 days. At the end of the assay (215 days), nonetheless, the

384   decrease in Gram-negative abundance was significant ($p < 0.05$) for all the soil

385   treatments with herbicides (S-I-H, S+GC1-I-H and S+GC2-I-H). The effect on Gram-

386   positive bacteria was only significant ($p < 0.05$) at the end of the assay in S+GC2-I,

387   where the presence of herbicides increased the relative population of Gram-positive

388   bacteria.

389   The relative percentage of Gram-negative bacteria was negatively correlated

390   with the prosulfocarb residue (Table 5). In contrast, the relative percentage of Gram-

391   positive bacteria was positively correlated with the prosulfocarb residue. Therefore, the

392   presence of prosulfocarb could induce the substitution of Gram-positive bacteria by

393   Gram-negative bacteria. The same phenomenon could be found between *Actinobacteria*

394   (negatively correlated with the herbicide residues) and fungi (positively correlated with

395   the herbicide residues). The relative population of *Actinobacteria* was significantly

396   higher with herbicides in all the soil treatments (S-I-H, S+GC1-I-H and S+GC2-I-H) as

397   happened in non-irrigated soils. Despite the significant differences found during the

398   assay for all the treatments, the ratio Gram-negative/total Gram-positive bacteria (Fig.

399   S5 in Supplementary material) only recorded significant differences between S+GC2-I

17

400 and S+GC2-I-H. In this soil, as in non-irrigated soils, the application of herbicides

401 decreased this ratio accordingly with the increased persistence of herbicides (higher

402 DT_{50}).

403   In contrast to *Actinobacteria,* the effects of herbicides on fungi followed the

404 opposite trend by decreasing their relative abundance over time in all soil treatments.

405 Moreover, the fungal decrease was clearly reflected in the bacteria/fungi ratio (Fig. S5

406 in Supplementary material). The increase in this ratio at the end of the assay again

407 reflected the bacteria – fungi antagonism. The negative effects of some herbicides on

408 fungi have been previously reported (Martin-Laurent et al., 2003; Wu et al., 2014).

409 However, other studies have shown the negative effects that some herbicides, including

410 triasulfuron and prosulfocarb, have on fungi at the beginning of the incubation period,

411 although the fungal population subsequently recovered (Cycoń et al., 2013; García-

412 Delgado et al., 2018; Wang et al., 2015; Zhang et al., 2010). In this study, the

413 significant decline ($p < 0.05$) in the relative abundance of fungi could be related to the

414 consecutive applications of high doses of a mixture of two herbicides. In addition,

415 between 0 and 69 days the GC-amended soils (S+GC1 and S+GC2) tended to decrease

416 the relative abundance of fungi with respect to S, as previously described by García-

417 Delgado et al. (2018) and Pose-Juan et al. (2017).

418   The microbiological changes in soils after the second application of herbicides

419 could be responsible for the change in their degradation rates, which tend to be faster

420 after the second application when the relative population of *Actinobacteria* is enhanced.

421 These organisms are known to be good degraders of complex substrates (Pose-Juan et

422 al., 2017).

423 *3.3. Relationship between soil microbial structure, herbicides, organic amendments and*

424 *irrigation regimes*

425   The global impact of herbicides on soil microbial community was assessed by

426   PCA including the results in non-irrigated (Fig. 3 A, B and C) and irrigated (Fig. 3 D, E

427   and F) soils. A PERMANOVA analysis (Table S1 in Supplementary material) testing

428   for the significance of the effects on the relative abundances of PLFAs of herbicide

429   application, sampling time, soil treatments and their respective interactions revealed the

430   statistical significance of the three factors in non-irrigated and irrigated soils.

431   With respect to non-irrigated soils, unamended and amended soils recorded

432   different PCA profiles. The soil treatment was not significant, but the application of

433   herbicides was statistically significant in all three soil treatments (S, S+GC1 and

434   S+GC2) (Table S1). The application of herbicides in soil without compost (S-H) clearly

435   enhanced the abundance of *Actinobacteria* and reduced the relative abundance of fungi

436   and Gram-negative bacteria. S+GC1-H and S+GC2-H were related to a low relative

437   abundance of fungi and a high relative abundance of *Actinobacteria* and Gram-negative

438   bacteria. In contrast, amended soils without herbicides were related to a high relative

439   abundance of fungi and Gram-positive bacteria. In non-irrigated soils, therefore,

440   herbicides had a clearly negative effect on fungi and Gram-positive bacteria, while

441   promoting the relative abundance of *Actinobacteria,* irrespective of compost use. S-H

442   was related to high relative abundance of Gram-negative bacteria, mainly at 124 – 215

443   days, while S+GC1-H and S+GC2-H recorded a closer relationship with Gram-negative

444   bacteria than S+GC1 and S+GC2 (Fig. 3 A, B and C). In a laboratory assay, soil

445   fumigated with another sulfonylurea herbicide, azimsulfuron, enhanced microbial

446   diversity by detecting different Gram-negative bacteria that were not found in a non-

447   fumigated soil (Valle et al., 2006). S+GC1-H and S+GC2-H produced slower

448   triasulfuron degradation than S-H, so the microbial shift may be deeper in the former.

449   The microbiology of non-irrigated soils was exposed to three clear stress factors,

450   namely, high doses of triasulfuron and prosulfocarb, two consecutive applications, and

451   drought conditions. In this respect, the toxic effects of pesticides can be enhanced, with

452   negative effects on microbial composition, enzyme activity and pesticide degradation

453   (Franco-Andreu et al., 2016a). This could be the cause of the significant impact

454   triasulfuron and prosulfocarb have on the microbial structure of unamended and GC-

455   amended soils, as reported here. In contrast, a previous study under similar conditions

456   but with lower herbicide doses revealed an impact on the microbial structure of

457   unamended soil but not on the microbial structure of GC-amended soil (García-Delgado

458   et al., 2018). Therefore, the capacity of organic amendments to buffer the effects of

459   herbicides on the microbial structure could be limited by herbicide doses or consecutive

460   applications.

461   The three soil treatments in irrigated soils had significant effects (Table S1 in

462   Supplementary material) on the soil microbial structure. Unamended and GC1 amended

463   soils (with and without herbicides) had a similar distribution in PCA with no significant

464   relationship with any factor (Fig. 3 D and E). In contrast, GC2 amended soils (with and

465   without herbicides) had a strong relationship with *Actinobacteria* and a weak one with

466   fungi (Fig. 3F). The negative effects of GC or other organic amendments on fungal

467   abundance has previously been reported at both laboratory scale (Pose-Juan et al., 2017)

468   and field scale (García-Delgado et al., 2018).

469   The application of herbicides in irrigated soils had some similarities with non-

470   irrigated soils. The soil microbial structure of irrigated soils after herbicide application

471   shifted towards a higher proportion of *Actinobacteria* and a lower relative amount of

472   fungi, as was the case in non-irrigated soils. The presence or absence of herbicides and

473   their interaction over time was significant in unamended soil. S-I was related to a high

20

474   proportion of Gram-positive bacteria and fungi. In contrast, S-I-H evolved from points

475   related to fungi towards a clear relationship with *Actinobacteria* and Gram-negative

476   bacteria at the end of the assay. The herbicide factor was not significant in S+GC1

477   treatment. However, the time factor and the herbicide * sampling time interaction were

478   significant. In fact, the evolution of S+GC1-I-H (dark blue symbols in Fig. 3) tends to

479   be more closely related to *Actinobacteria* and less so to fungi over time. In contrast, the

480   changes in microbial structure over time in S+GC1 seem to be related to the variation in

481   the proportion of Gram-negative and Gram-positive bacteria because of the dispersion

482   of points in component 1 and the low values of points in component 2, being closely

483   related to fungi and *Actinobacteria*. The application of herbicides in GC2-amended soils

484   and their interaction with sampling time were significant. PCA showed a clear

485   differentiation between S+GC2-I and S+GC2-I-H. S+GC2-I was related to a high

486   relative abundance of Gram-positive bacteria, mainly at 215 days. In contrast, S+GC2-I-

487   H was related to a high relative abundance of *Actinobacteria* and Gram-negative

488   bacteria. In addition, there was a clearly negative relationship with the abundance of

489   fungi in both, S+GC2-I and S+GC2-I-H treatments, as described above.

490   After herbicide application, all the soils, irrespective of irrigation conditions,

491   tended to enhance the relative population of *Actinobacteria*, mainly after the second

492   application of herbicides, when their DT_{50} values were lower than the first one. The

493   remaining triasulfuron concentration 69 days after the second application recorded

494   lower concentrations than in the first application. Dissipation therefore increased in the

495   second application, when *Actinobacteria* increased their relative abundance. In contrast,

496   the remaining concentrations of prosulfocarb were higher after the second application. It

497   therefore seems clear that a high relative abundance of *Actinobacteria* plays a key role

498   in herbicide dissipation, being positive in the case of triasulfuron and negative in the

499   case of prosulfocarb degradation.

500

501   **4. Conclusions**

502   The dissipation of triasulfuron and prosulfocarb in an agricultural soil under field

503   conditions is influenced by the type and amount of green compost applied to the soil

504   and by the irrigation regime. Two consecutive applications of triasulfuron increase the

505   dissipation rate of this herbicide, although in the case of prosulfocarb it produces an

506   accumulation of residual herbicide after the second application. A positive correlation

507   between the amounts of herbicide residues and the total microbial population led to a

508   decrease in the microbial population during the dissipation of herbicides and to a certain

509   toxicity of herbicides for the microbial community. The microbial structure of

510   unamended and GC-amended soils is modified after two consecutive applications of the

511   herbicides triasulfuron and prosulfocarb. Herbicides increase the relative population of

512   *Actinobacteria* and reduce the relative population of fungi compared to the initial

513   situation in all the conditions studied. *Actinobacteria* seems to be responsible for the

514   increase in the of degradation rate of triasulfuron after the second application.

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522   **References**

523 Álvarez-Martín, A., Hilton, S.L., Bending, G.D., Rodríguez-Cruz, M.S., Sánchez-

524   Martín, M.J., 2016. Changes in activity and structure of the soil microbial

525   community after application of azoxystrobin or pirimicarb and an organic

526   amendment to an agricultural soil. Appl. Soil Ecol. 106, 47–57.

527   doi:10.1016/j.apsoil.2016.05.005

528 Aranda, V., Macci, C., Peruzzi, E., Masciandaro, G., 2015. Biochemical activity and

529   chemical-structural properties of soil organic matter after 17 years of amendments

530   with olive-mill pomace co-compost. J. Environ. Manage. 147, 278–285.

531   doi:10.1016/j.jenvman.2014.08.024

532 Arias-Estévez, M., López-Periago, E., Martínez-Carballo, E., Simal-Gándara, J.,

533   Mejuto, J.C., García-Río, L., 2008. The mobility and degradation of pesticides in

534   soils and the pollution of groundwater resources. Agric. Ecosyst. Environ. 123,

535   247–260. doi:10.1016/j.agee.2007.07.011

536 Bajya, D.R., Parween, T., Lakharan, M.C., Razaaza, S.K., 2015. Efficacy of new

537   formulations of triasulfuron on weeds in wheat (*Triticum aestivum*) and their

538   residual effects on succeeding maize (*Zea mays*). Indian J. Agron. 60, 57–60.

539 Barra Caracciolo, A., Bustamante, M.A., Nogues, I., Di Lenola, M., Luprano, M.L.,

540   Grenni, P., 2015. Changes in microbial community structure and functioning of a

541   semiarid soil due to the use of anaerobic digestate derived composts and rosemary

542   plants. Geoderma 245–246, 89–97. doi:10.1016/j.geoderma.2015.01.021

23

543   Bastida, F., Selevsek, N., Torres, I.F., Hernández, T., García, C., 2015. Soil restoration

544   with organic amendments: Linking cellular functionality and ecosystem processes.

545   Sci. Rep. 5, 15550. doi:10.1038/srep15550

546   Baxter, J., Cummings, S.P., 2008. The degradation of the herbicide bromoxynil and its

547   impact on bacterial diversity in a top soil. J. Appl. Microbiol. 104, 1605–1616.

548   doi:10.1111/j.1365-2672.2007.03709.x

549   BOE, 2013. Real Decreto 506/2013, de 28 de junio, sobre productos fertilizantes, BOE.

550   Braun, K.E., Luks, A.K., Schmidt, B., 2017. Fate of the14C-labeled herbicide

551   prosulfocarb in a soil and in a sediment-water system. J. Environ. Sci. Heal. - Part

552   B Pestic. Food Contam. Agric. Wastes 52, 122–130.

553   doi:10.1080/03601234.2016.1248140

554   Cirujeda, A., Taberner, A., 2010. Chemical control of herbicide-resistant *Lolium*

555   *rigidum* Gaud. in north-eastern Spain. Pest Manag. Sci. 66, 1380–1388.

556   doi:10.1002/ps.2031

557   Cycoń, M., Wójcik, M., Borymski, S., Piotrowska-Seget, Z., 2013. Short-term effects of

558   the herbicide napropamide on the activity and structure of the soil microbial

559   community assessed by the multi-approach analysis. Appl. Soil Ecol. 66, 8–18.

560   doi:10.1016/j.apsoil.2013.01.014

561   Dinelli, G:, Vicari, A., Accinelli, C., 1998. Degradation and side effects of three

562   sulfonylurea herbicides in soil. J. Environ. Qual. 27, 1459-1464. doi:

563   10.2134/jeq1998.00472425002700060023x

564   EFSA, 2015. Conclusion on the peer review of the pesticide risk assessment of the

565   active substance triasulfuron. EFSA J. 13, 3958. doi:10.2903/j.efsa.2015.3958

566   EFSA, 2007a. Conclusion regarding the peer review of the pesticide risk assessment of

567   the active substance clomazone finalised : 27 July 2007. EFSA Sci. Rep. 111, 1–81.

568   EFSA, 2007b. Opinion on a request from EFSA related to the default Q 10 value used

569   to describe the temperature effect on transformation rates of pesticides in soil.

570   EFSA J. 622, 1–32. doi:10.2903/j.efsa.2008.622

571   El Azhari, N., Dermou, E., Barnard, R.L., Storck, V., Tourna, M., Beguet, J., Karas,

572   P.A., Lucini, L., Rouard, N., Botteri, L., Ferrari, F., Trevisan, M., Karpouzas, D.G.,

573   Martin-Laurent, F., 2018. The dissipation and microbial ecotoxicity of

574   tebuconazole and its transformation products in soil under standard laboratory and

575   simulated winter conditions. Sci. Total Environ. 637–638, 892–906.

576   doi:10.1016/j.scitotenv.2018.05.088

577   Fang, H., Han, L., Zhang, H., Deng, Y., Ge, Q., Mei, J., Long, Z., Yu, Y., 2018.

578   Repeated treatments of ciprofloxacin and kresoxim-methyl alter their dissipation

579   rates, biological function and increase antibiotic resistance in manured soil. Sci.

580   Total Environ. 628–629, 661–671. doi:10.1016/j.scitotenv.2018.02.116

581   Fang, H., Lian, J., Wang, H., Cai, L., Yu, Y., 2015. Exploring bacterial community

582   structure and function associated with atrazine biodegradation in repeatedly treated

583   soils. J. Hazard. Mater. 286, 457–465. doi:10.1016/j.jhazmat.2015.01.006

584   FOCUS, 2006. Guidance document on estimating persistence and degradation kinetics

585   from environmental fate studies on pesticide in EU registration. Report of the

586   FOCUS Work Group on Degradaton Kinetics, EC Document Reference

587   Sanco/10058/2005 version 2.0.

588   Franco-Andreu, L., Gómez, I., Parrado, J., García, C., Hernández, T., Tejada, M.,

589   2016a. Behavior of two pesticides in a soil subjected to severe drought. Effects on

590   soil biology. Appl. Soil Ecol. 105, 17–24. doi:10.1016/j.apsoil.2016.04.001

591   Franco-Andreu, L., Gómez, I., Parrado, J., García, C., Hernández, T., Tejada, M.,

592   2016b. Soil biology changes as a consequence of organic amendments subjected to

593   a severe drought. L. Degrad. Dev. 905, 897–905. doi:10.1002/ldr.2663

594   Frostegård, Å., Bååth, E., Tunlid, A., 1993. Shifts in the structure of soil microbial

595   communities in limed forests as revealed by phospholipid fatty acid analysis. Soil

596   Biol. Biochem. 25, 723–730.

597   García-Delgado, C., Barba, V., Marín-Benito, J.M., Igual, J.M., Sánchez-Martín, M.J.,

598   Rodríguez-Cruz, M.S., 2018. Simultaneous application of two herbicides and green

599   compost in a field experiment: Implications on soil microbial community. Appl.

600   Soil Ecol. 127, 30–40. doi:10.1016/j.apsoil.2018.03.004

601   García-Orenes, F., Morugán-Coronado, A., Zornoza, R., Scow, K., 2013. Changes in

602   soil microbial community structure influenced by agricultural management

603   practices in a mediterranean agro-ecosystem. PLoS One 8, e80522.

604   doi:10.1371/journal.pone.0080522

605   Gennari, M., Ambrosoli, R., Nègre, M., Minati, J.L., 2002. Bioavailability and

606   biodegradation of prosulfocarb in soil. J. Environ. Sci. Heal. - Part B Pestic. Food

607   Contam. Agric. Wastes 37, 297–305. doi:10.1081/PFC-120004471

608   Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: Paleontological Statistics

609   software package for education and data analysis. Palaeontol. Electron. 4, 1–9.

610   doi:10.1016/j.bcp.2008.05.025

611   Herrero-Hernández, E., Marín-Benito, J.M., Andrades, M.S., Sánchez-Martín, M.J.,

612   Rodríguez-Cruz, M.S., 2015. Field versus laboratory experiments to evaluate the

613   fate of azoxystrobin in an amended vineyard soil. J. Environ. Manage. 163, 78–86.

614   doi:10.1016/j.jenvman.2015.08.010

615   Kalia, A., Gosal, S.K., 2011. Effect of pesticide application on soil microorganisms.

616   Arch. Agron. Soil Sci. 57, 569–596. doi:10.1080/03650341003787582

617   Kaur, P., Bhullar, M.S., 2017. Effect of repeated application of pendimethalin on its

618   persistence and dissipation kinetics in soil under field and laboratory conditions.

619   Environ. Technol. 1–9. doi:10.1080/09593330.2017.1415378

620   Kaur, P., Randhawa, S.K., Duhan, A., Bhullar, M.S., 2017. Influence of long term

621   application of butachlor on its dissipation and harvest residues in soil and rice. Bull.

622   Environ. Contam. Toxicol. 98, 874–880. doi:10.1007/s00128-017-2070-1

623   Li, Y., Niu, J., Shen, Z., Zhang, C., Wang, Z., He, T., 2014. Spatial and seasonal

624   distribution of organochlorine pesticides in the sediments of the Yangtze Estuary.

625   Chemosphere 114, 233–240. doi:10.1016/j.chemosphere.2014.04.112

626   Lupwayi, N.Z., Harker, K.N., Clayton, G.W., Turkington, T.K., Rice, W.A.,

627   O'Donovan, J.T., 2004. Soil microbial biomass and diversity after herbicide

628   application. Can. J. Plant Sci. 84, 677–685. doi:10.4141/P03-121

629   Marín-Benito, J.M., Barba, V., Ordax, J.M., Andrades, M.S., Sánchez-Martín, M.J.,

630   Rodríguez-Cruz, M.S., 2018a. Application of green compost as amendment in an

631   agricultural soil: Effect on the behaviour of triasulfuron and prosulfocarb under

632   field conditions. J. Environ. Manage. 207, 180–191.

633   doi:10.1016/j.jenvman.2017.11.024

634   Marín-Benito, J.M., Barba, V., Ordax, J.M., Sánchez-Martín, M.J., Rodríguez-Cruz,

635   M.S. 2018b. Recycling organic residues in soils as amendments: Effect on the

636   mobility of two herbicides under different management practices. J. Environ.

637   Manage. (in press). doi:10.1016/j.jenvman.2018.07.045.

638   Marín-Benito, J.M., Brown, C.D., Herrero-Hernández, E., Arienzo, M., Sánchez-

639   Martín, M.J., Rodríguez-Cruz, M.S., 2013. Use of raw or incubated organic wastes

640   as amendments in reducing pesticide leaching through soil columns. Sci. Total

641   Environ. 463–464, 589–599. doi:10.1016/j.scitotenv.2013.06.051

642   Marín-Benito, J.M., Herrero-Hernández, E., Andrades, M.S., Sánchez-Martín, M.J.,

643   Rodríguez-Cruz, M.S., 2014. Effect of different organic amendments on the

644   dissipation of linuron, diazinon and myclobutanil in an agricultural soil incubated

645   for different time periods. Sci. Total Environ. 476–477, 611–21.

646   doi:10.1016/j.scitotenv.2014.01.052

647   Martin-Laurent, F., Piutti, S., Hallet, S., Wagschal, I., Philippot, L., Catroux, G., Soulas,

648   G., 2003. Monitoring of atrazine treatment on soil bacterial, fungal and atrazine-

649   degrading communities by quantitative competitive PCR. Pest Manag. Sci. 59,

650   259–268. doi:10.1002/ps.630

651   Nègre, M., Passarella, I., Boursier, C., Mozzetti, C., Gennari, M., 2006. Evaluation of

652   the bioavailability of the herbicide prosulfocarb through adsorption on soils and

653   model soil colloids, and through a simple bioassay. Pest Manag. Sci. 62, 957–964.

654   doi:10.1002/ps

655   Nyamwasa, I., Li, K., Rutikanga, A., Rukazambuga, D.N.T., Zhang, S., Yin, J., Ya-

656   zhong, C., Zhang, X.X., Sun, X., 2018. Soil insect crop pests and their integrated

657   management in East Africa: A review. Crop Prot. 106, 163–176.

658   doi:10.1016/j.cropro.2017.11.017

659   Pascual, J.A., Garcia, C., Hernandez, T., Moreno, J.L., Ros, M., 2000. Soil microbial

660   activity as a biomarker of degradation and remediation processes. Soil Biol.

661   Biochem. 32, 1877–1883. doi:10.1016/S0038-0717(00)00161-9

662   Pose-Juan, E., Igual, J.M., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S., 2017.

663   Influence of herbicide triasulfuron on soil microbial community in an unamended

664   soil and a soil amended with organic residues. Front. Microbiol. 8, 1–12.

665   doi:10.3389/fmicb.2017.00378

666   Pose-Juan, E., Sánchez-Martín, M.J., Andrades, M.S., Rodríguez-Cruz, M.S., Herrero-

667   Hernández, E., 2015. Pesticide residues in vineyard soils from Spain: Spatial and

668   temporal distributions. Sci. Total Environ. 514, 351–358.

669   doi:10.1016/j.scitotenv.2015.01.076

670   PPDB, 2018. Pesticide poperties data base. University of Hertfordshire. URL

671   https://sitem.herts.ac.uk/aeru/ppdb/en/index.htm (accessed 6.11.18).

672   Rouchaud, J., Neus, O., Callens, D., Bulcke, R., 1997. Enhanced soil biodegradation of

673   prosulfocarb herbicide in barley crop. Bull. Environ. Contam. Toxicol. 58, 752–

674   757. doi:10.1007/s001289900397

675   Said-Pullicino, D., Gigliotti, G., Vella, A.J., 2004. Environmental fate of triasulfuron in

676   soils amended with municipal waste compost. J. Environ. Qual. 33, 1743-1751.

677   doi:10.2134/jeq2004.1743

678   Santás-Miguel, V., Cutillas-Barreiro, L., Arias-Estévez, M., Fernández-Sanjurjo, M.J.,

679   Álvarez-Rodríguez, E., Núñez-Delgado, A., Fernández-Calviño, D., 2018. By-

680   products as an amendment of a mine soil: effects on microbial biomass determined

681   using phospholipid fatty acids. Spanish J. Soil Sci. 8, 1–11.

682   doi:10.3232/SJSS.2018.V8.N1.01

683   Sarmah, A.K., Kookana, R.S., Alston, A.M., 2000. Leaching and degradation of

684   triasulfuron, metsulfuron-methyl, and chlorsulfuron in alkaline soil profiles under

685   field conditions. Aust. J. Soil Res 38, 617–631. doi:10.1071/SR99114

686   Singh, S., Kumar, V., Chauhan, A., Datta, S., Wani, A.B., Singh, N., Singh, J., 2018.

687   Toxicity, degradation and analysis of the herbicide atrazine. Environ. Chem. Lett.

688   16, 211-237. doi: 10.1007/s10311-017-0665-8

689   Sofo, A., Scopa, A., Dumontet, S., Mazzatura, A., Pasquale, V., 2012. Toxic effects of

690   four sulphonylureas herbicides on soil microbial biomass. J. Environ. Sci. Heal. -

691   Part B Pestic. Food Contam. Agric. Wastes 47, 653–659.

692   doi:10.1080/03601234.2012.669205

693   Sparks, D.L., 1996. Methods of Soil Analysis. Part 3-Chemical Methods. Soil Science

694   Society of America, Inc., Madison, WI.

695   Stork, P.R., 1995. Field leaching and degradation of soil applied herbicides in a

696   gradationally textured alkaline soil: chlorsulfuron and triasulfuron. Aust. J. Agric.

697   Res. 46, 1445–1458.

698   Sun, D., Li, K., Bi, Q., Zhu, J., Zhang, Q., Jin, C., Lu, L., Lin, X., 2017. Effects of

699   organic amendment on soil aggregation and microbial community composition

700   during drying-rewetting alternation. Sci. Total Environ. 574, 735–743.

701   doi:10.1016/j.scitotenv.2016.09.112

702   Tortella, G.R., Mella-Herrera, R.A., Sousa, D.Z., Rubilar, O., Acuña, J.J., Briceño, G.,

703   Diez, M.C., 2013. Atrazine dissipation and its impact on the microbial communities

704   and community level physiological profiles in a microcosm simulating the

705   biomixture of on-farm biopurification system. J. Hazard. Mater. 260, 459–467.

706   doi:10.1016/j.jhazmat.2013.05.059

707   Valle, A., Boschin, G., Negri, M., Abbruscato, P., Sorlini, C., D'Agostina, A.,

708   Zanardini, E., 2006. The microbial degradation of azimsulfuron and its effect on the

709   soil bacterial community. J. Appl. Microbiol. 101, 443–452. doi:10.1111/j.1365-

710   2672.2006.02937.x

711   Vischetti, C., Monaci, E., Cardinali, A., Casucci, C., Perucci, P., 2008. The effect of

712   initial concentration, co-application and repeated applications on pesticide

713   degradation in a biobed mixture. Chemosphere 72, 1739–1743.

714   doi:10.1016/j.chemosphere.2008.04.065

715   Wang, F., Zhu, L., Wang, X., Wang, J., Wang, J., 2015. Impact of repeated applications

716   of metalaxyl on its dissipation and microbial community in soil. Water. Air. Soil

717   Pollut. 226, 1–14. doi:10.1007/s11270-015-2686-x

718   Wu, X., Xu, J., Dong, F., Liu, X., Zheng, Y., 2014. Responses of soil microbial

719   community to different concentration of fomesafen. J. Hazard. Mater. 273, 155–

720   164. doi:10.1016/j.jhazmat.2014.03.041

721   Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the

722   characterisation of microbial communities in soil: a review. Biol. Fertil. Soils 29,

723   111-129. doi: 10.1007/s003740050533

724   Zhang, C., Xu, J., Liu, X., Dong, F., Kong, Z., Sheng, Y., Zheng, Y., 2010. Impact of

725   imazethapyr on the microbial community structure in agricultural soils.

726   Chemosphere 81, 800–806. doi:10.1016/j.chemosphere.2010.06.079

727

Figure captions:

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.

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.

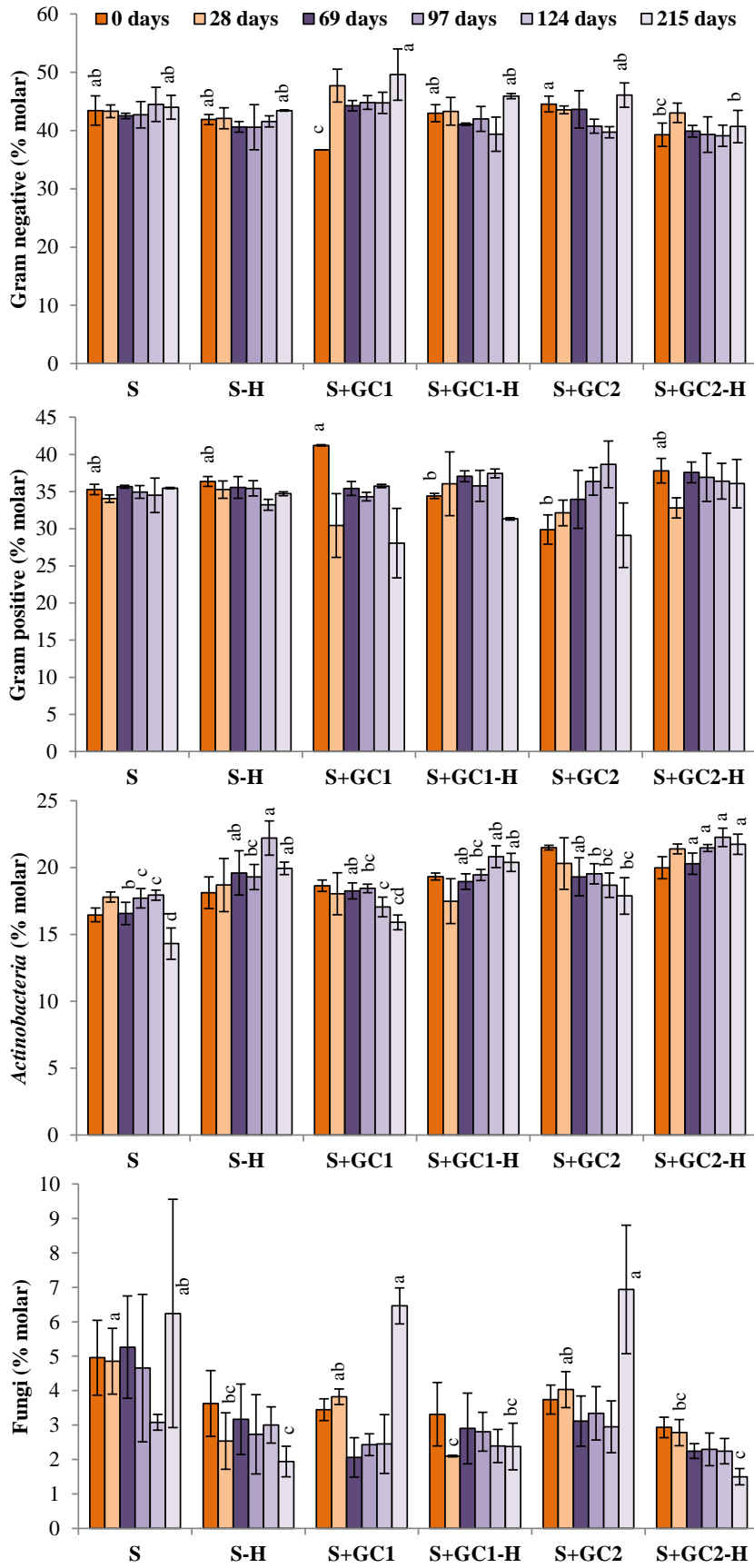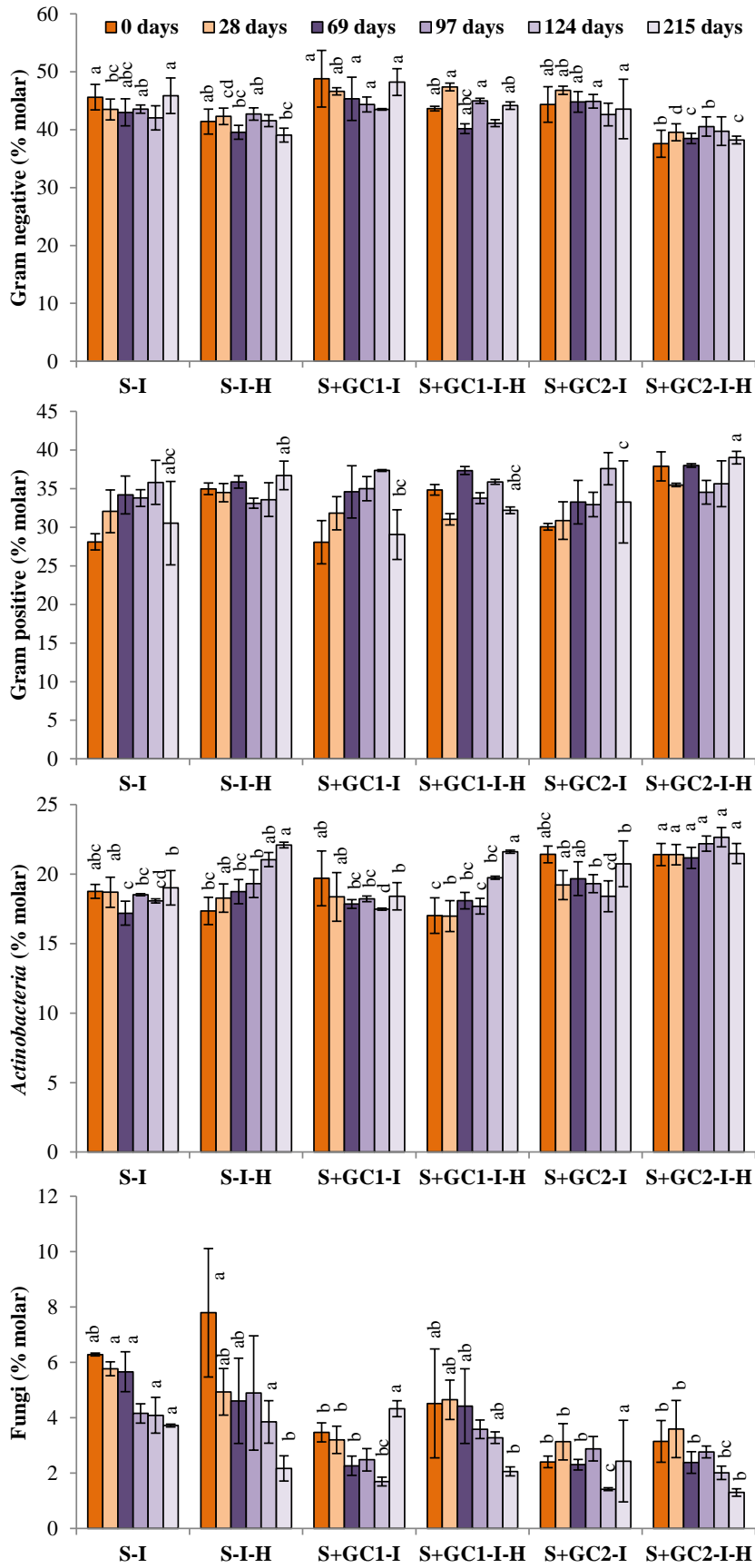**Figure 1**
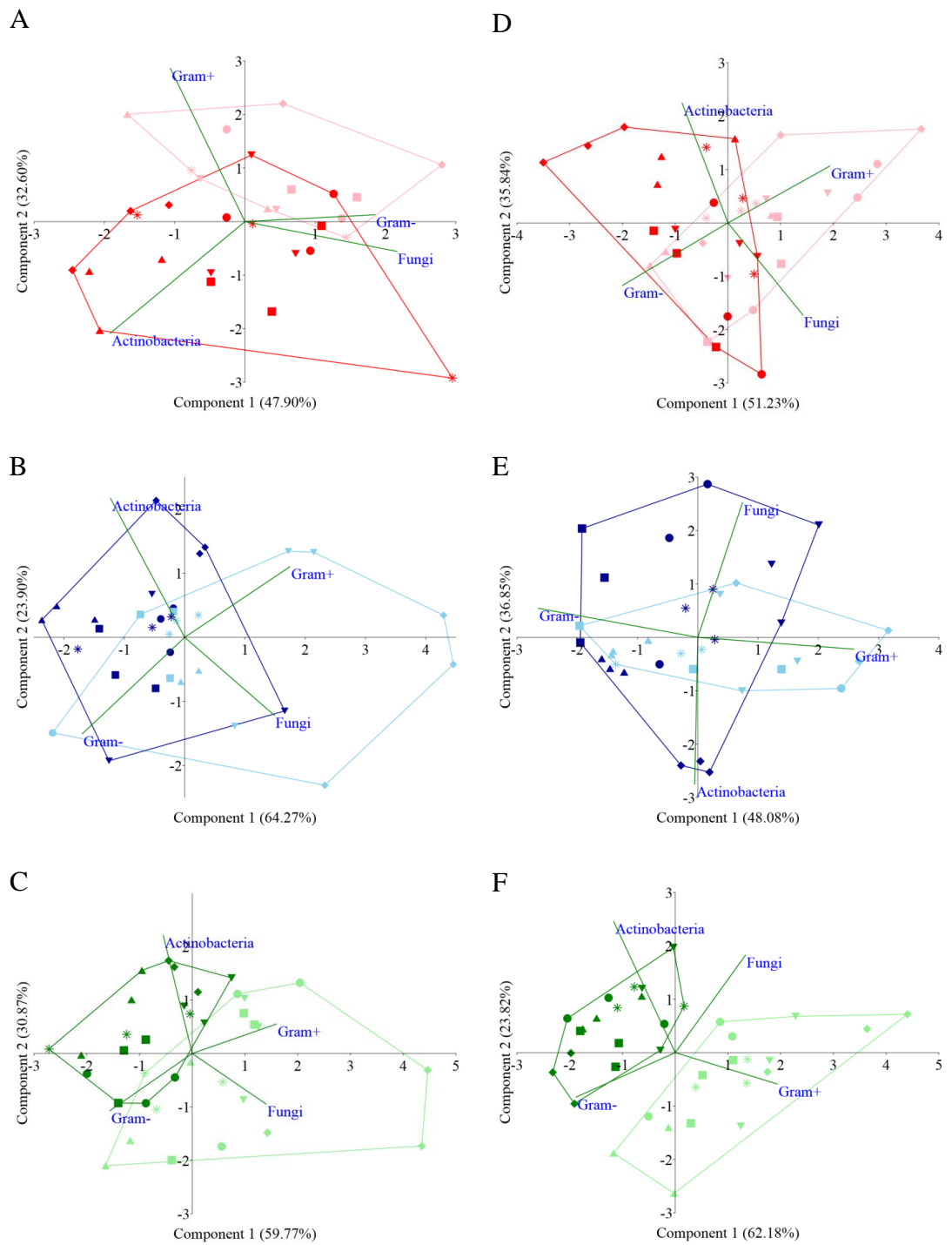
Figure 2

A

D

B

E

C

F

Figure 3

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	10.85-10.4
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.

Table 2: 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	-0.021	1								
Gram- (%)	**-0.288***	**-0.510*****	**-0.505*****	**-0.422****	**-0.435****	**0.970*****	0.168	1							
Gram + (%)	**0.535*****	**0.733*****	**0.600*****	**0.301***	**0.634*****	**-0.789*****	0.020	**-0.765*****	1						
Actinobacteria (%)	**-0.318****	**-0.344***	-0.087	**-0.504*****	**-0.331***	-0.243	**-0.390****	-0.187	-0.256	1					
Fungi (%)	0.172	0.145	0.041	**0.669*****	0.158	0.067	**-0.952*****	-0.053	0.02	**-0.401****	1				
[TSF]	**0.652*****	**0.651*****	**0.609*****	**0.478*****	**0.659*****	-0.261	-0.247	0.266	**0.412****	**-0.330***	**0.283***	1			
[PSC]	**0.602*****	**0.597*****	**0.573*****	**0.368****	**0.605****	-0.220	-0.223	-0.212	**0.341***	-0.207	**0.301***	**0.811*****	1		
%TSF	**0.614*****	**0.624*****	**0.570*****	**0.535*****	**0.639*****	-0.196	**-0.362***	-0.217	**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 3: 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	**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

* $p < 0.050$; ** $p < 0.010$; *** $p < 0.001$

INFLUENCE OF DIFFERENT AGRICULTURAL MANAGEMENT PRACTICES ON SOIL MICROBIAL COMMUNITY OVER DISSIPATION TIME OF TWO HERBICIDES

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Number of Figures: 3

Soil sampling and sample processing

Surface soil samples from 0 to 10 cm were collected on the first day after herbicide application (0 days), and at different times up to 215 days after treatment to determine herbicide dissipation and at 0, 28, 69, 97, 124 and 215 days to determine soil phospholipid fatty acids (PLFAs) in all the treatments. Five sub-samples were taken in each plot, mixing them before they were transferred to polypropylene bottles. All the samples were transported to the laboratory in portable refrigerators. The soil water content of the bulk sample for each 10-cm soil layer was gravimetrically determined by weight difference, measuring the soil sample mass before and after drying at 110ºC for 24 h. Soil samples, previously air-dried overnight if necessary, were then sieved (< 2 mm) and their characteristics determined by standard methods (Sparks, 1996). The pH of the soils was determined in soil/water suspensions (1:2.5). The organic carbon (OC) content was determined by the modified Walkley-Black method. The dissolved organic carbon (DOC) content was determined in a suspension of soil in deionized water (1:2) after shaking (24 h), centrifugation (20 min at 10000 rpm), and filtering using a Shimadzu 5050 organic carbon analyser (Shimadzu, Columbia, MD, USA). Total N content was determined by the Kjeldahl method. Particle size distribution was determined using the pipette method. Soil samples to determine herbicide dissipation were analyzed immediately. To determine PLFAs, the samples were frozen at -80ºC and lyophilized prior to extraction and analysis.

Herbicide extraction and analysis

Duplicate subsamples of moist soil (6 g) from each plot were transferred to a glass tube, and extracted with methanol (12 mL). The samples were sonicated for 1 h, shaken at 20ºC for 24 h, and then centrifuged at 5045 g for 15 min. A volume of 8 mL was transferred to a clean glass tube and evaporated until dryness at 25ºC under a nitrogen

stream using an EVA-EC2-L evaporator (VLM GmbH, Bielefeld, Germany). The residue was dissolved in 0.5 mL of methanol+formic acid (1%), filtered to remove particles > 0.45 μm in a GHP Acrodisc filter (Waters Corporation), and transferred to a HPLC glass vial for analysis (Marín-Benito et al., 2018).

The analysis of triasulfuron and prosulfocarb was performed by HPLC. The apparatus used was a Waters chromatograph (Waters Assoc., Milford, MA, USA), equipped with a model e2695 multisolvent delivery and autosampler system attached to a ZQ mass spectrometer detector (MS), with Empower software as the data acquisition and processing system. A Luna® 3 μm PFP(2) 100 Å (150 × 4.6 mm) column by Phenomenex (Torrance, CA, USA) was used at ambient temperature, and the mobile phase was acetonitrile:water+1% formic acid (70:30). The flow rate of the mobile phase was 0.4 mL min^{-1}, and the sample injection volume was 10 μL. Detection involved monitoring the positive molecular ion [m/z] 402.8 $[M+H]^+$ (triasulfuron) and 252.4 $[M+H]^+$ (prosulfocarb) after applying an optimized cone voltage of 20 V, and the retention times were 6.1 min and 14.1 min, respectively (Marín-Benito et al., 2018).

The matrix-matched calibration standards were between 0.1 and 2.5 μg mL^{-1} for both herbicides, and the limit of detection (LOD) and limit of quantification (LOQ) were in the ranges 0.018-0.026 μg mL^{-1} or 0.059-0.088 μg mL^{-1} for triasulfuron, and 0.005-0.008 μg mL^{-1} or 0.017-0.027 μg mL^{-1} for prosulfocarb in the unamended and amended soils, respectively. The method's recoveries were determined by spiking three unamended and amended soil samples with triasulfuron (76.9 μg kg^{-1}) or prosulfocarb (3.46 mg kg^{-1}) and performing the extraction procedure as described above. The mean recoveries of triasulfuron and prosulfocarb were >80% for the unamended and GC-amended soils. The amounts of triasulfuron and prosulfocarb extracted from soils were not corrected for

recovery values because matrix-matched calibration method was used (Marín-Benito et al., 2018).

Dissipation kinetics

The dissipation kinetics for both herbicides was fitted to a single first-order (SFO) kinetic model ($C = C_0\, e^{-kt}$) or first order multicompartment (FOMC) model ($C = C_0/(t/\beta) + 1)^{\alpha}$), known also as the Gustafson and Holden model. FOCUS work group guidance recommendations were followed (FOCUS, 2006) for selecting the kinetic model that best describes the dissipation results. The coefficient of determination (R^2) and the chi-square (χ^2) test were calculated as indicators of the goodness of fit. The χ^2 test considers the deviations between observed and calculated values relative to the uncertainty of the measurements for a specific fit, and was used to compare the goodness of fit of the two models tested. The error value at which the χ^2 test is fulfilled at a given degree of freedom should be below 15% (at a 5% significance level). Values for the time to 50% dissipation (DT_{50}) 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).

References

FOCUS, 2006. Guidance document on estimating persistence and degradation kinetics from environmental fate studies on pesticide in EU registration. Report of the FOCUS Work Group on Degradaton Kinetics, EC Document Reference Sanco/10058/2005 version 2.0.

Marín-Benito, J.M., Barba, V., Ordax, J.M., Andrades, M.S., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S., 2018. Application of green compost as amendment in an

agricultural soil: Effect on the behaviour of triasulfuron and prosulfocarb under field

conditions. J. Environ. Manage. 207, 180–191.

Sparks, D.L., 1996. Methods of Soil Analysis. Part 3-Chemical Methods. Soil Science

Society of America, Inc., Madison, WI.

Table S1. Results of PERMANOVA of the effect of herbicides application (H), sampling time (T), soil treatment (ST) and their interaction on the relative abundance (%mol) of PLFAs specific for Gram positive and negative bacteria, *Actinobacteria* and fungi in unamended and amended soils.

| Non-Irrigation	F-value	*p*-value	Irrigation	F-value	*p*-value
Soil			**Soil**		
Herbicide	8.811	**0.0001**	Herbicide	7.235	**0.0017**
Time	0.9423	0.5235	Time	1.192	0.3059
H * T	-0.1369	0.4663	H * T	2.247	**0.0340**
GC1			**GC1**		
Herbicide	5.772	**0.0127**	Herbicide	3.071	0.0664
Time	3.710	**0.0057**	Time	4.741	**0.0004**
H * T	0.5160	**0.0306**	H * T	1.627	**0.0009**
GC2			**GC2**		
Herbicide	6.520	**0.0082**	Herbicide	32.87	**0.0001**
Time	3.103	**0.0092**	Time	0.6189	0.7198
H * T	1.977	0.0811	H * T	4.175	**0.0044**
Global			**Global**		
Herbicide	15.37	**0.0001**	Herbicide	26.69	**0.0001**
Time	5.067	**0.0001**	Time	2.819	**0.0061**
ST	3.409	0.0110	ST	6.398	**0.0003**
H * T	1.899	**0.0060**	H * T	3.124	**0.0001**
H * ST	-0.8854	0.2920	H * ST	3.123	**0.0021**
T * ST	0.2592	0.2448	T * ST	-0.7416	0.7282
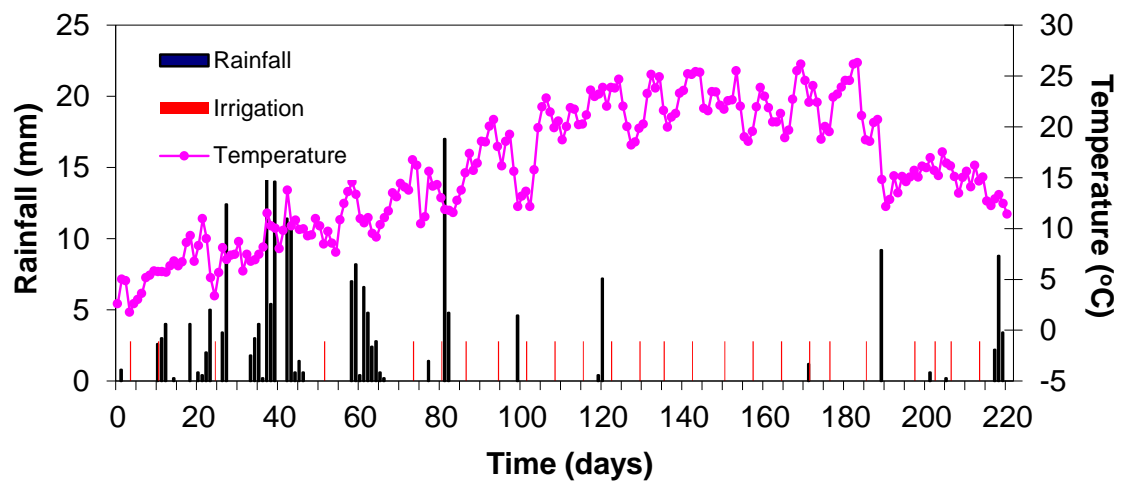
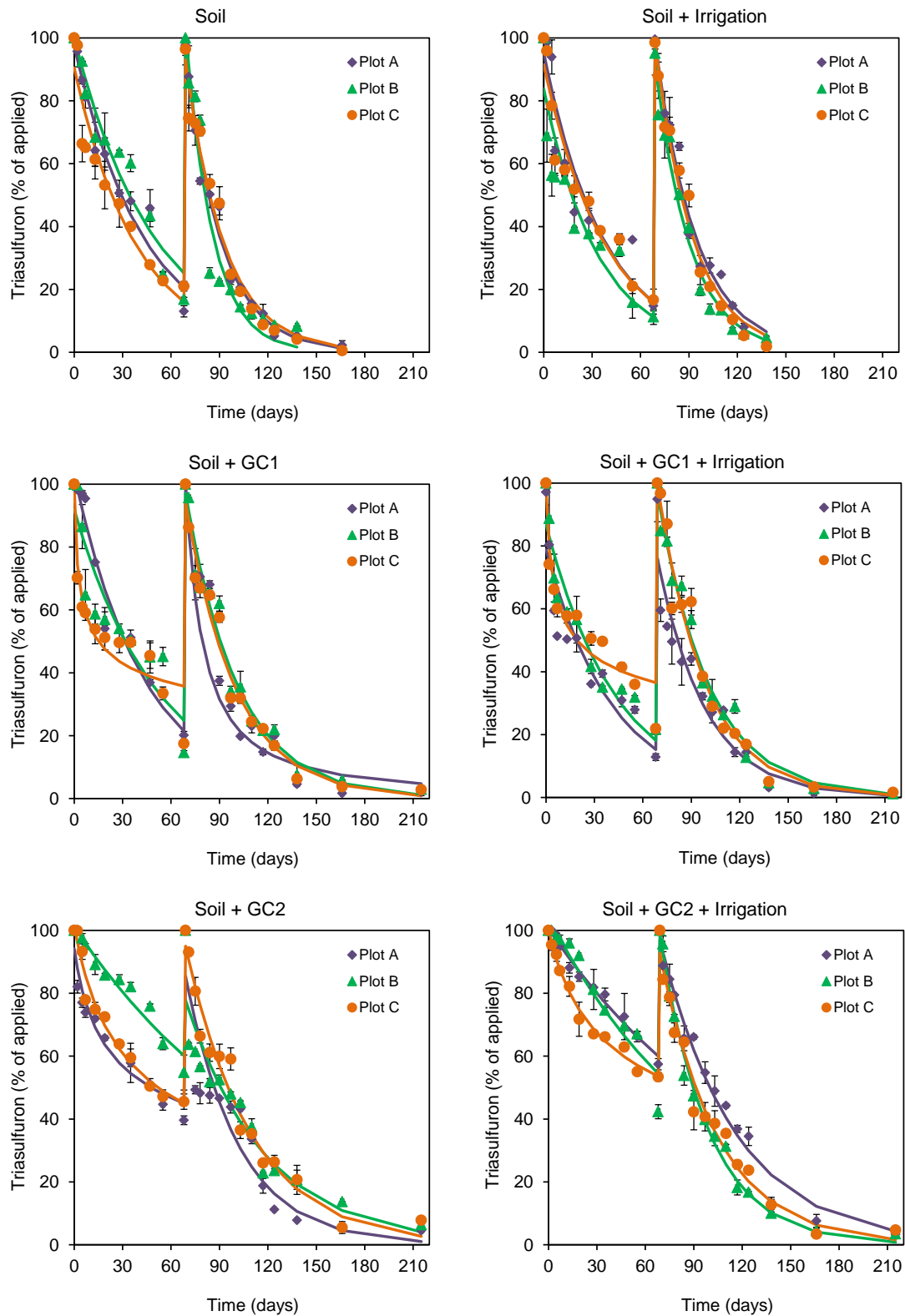**Fig. S1**. Rainfall, additional irrigation and temperature evolution over time of experiment.

Fig. S2. Dissipation kinetics of triasulfuron in unamended soil and soil amended with green compost (GC1 and GC2) after the first application (0 - 68 days) and after the second application (69 - 215 days). Error bars represent the standard deviation of the mean values of plots treated with the herbicide (n=3).
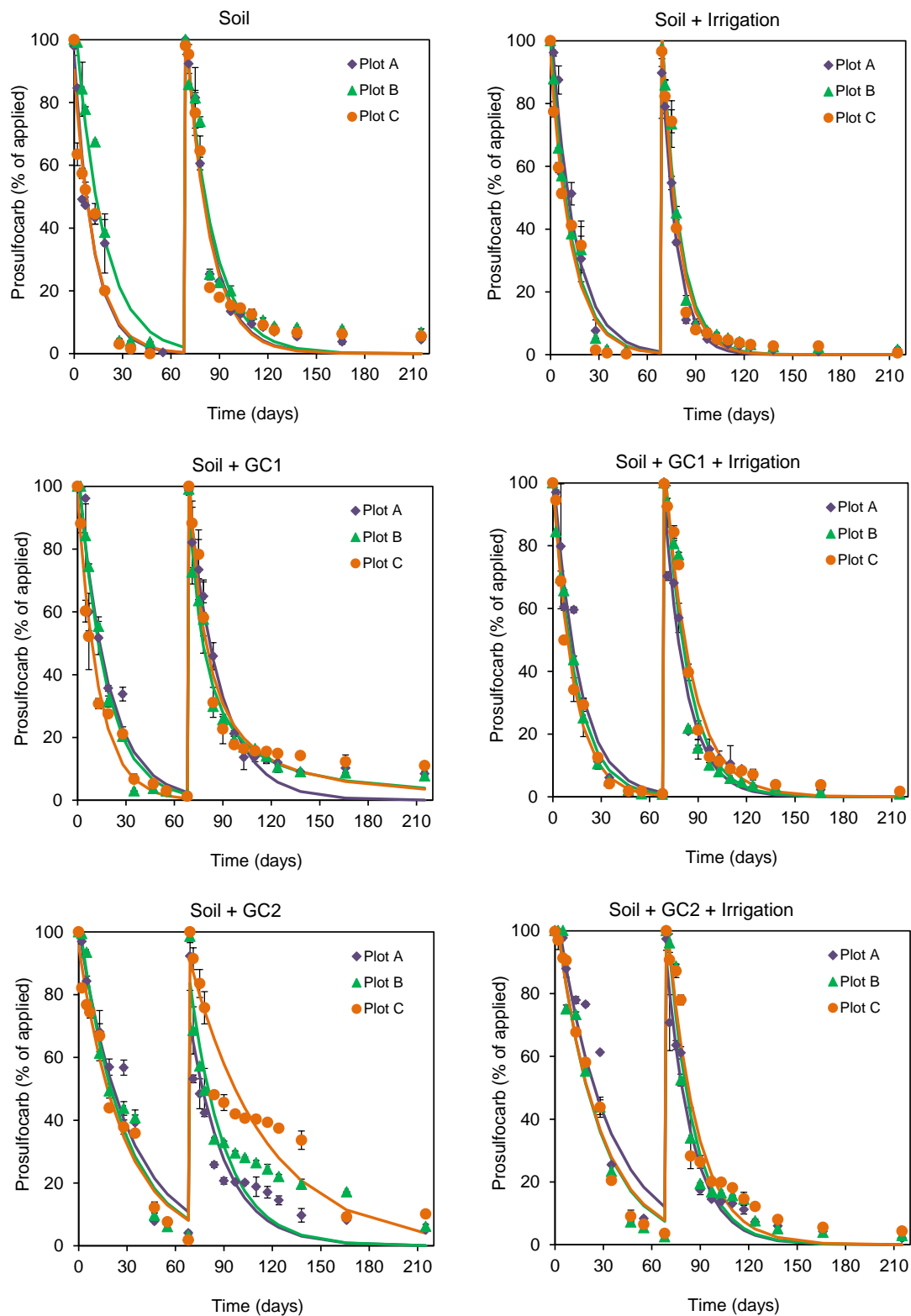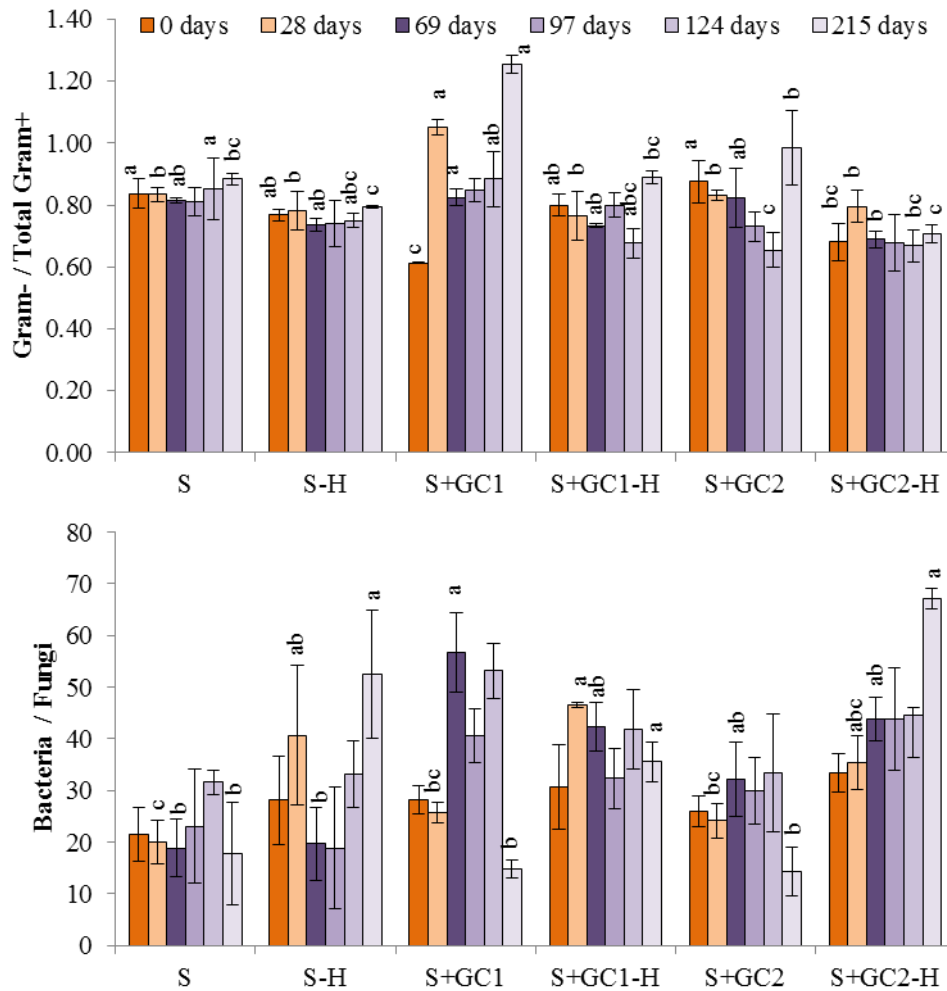
Fig S3. Dissipation kinetics of prosulfocarb in unamended soil and soil amended with green compost (GC1 and GC2) after the first application (0 - 68 days) and after the second application (69 - 215 days). Error bars represent the standard deviation of the mean values of plots treated with the herbicide (n=3).

Fig S4. Ratio Gram- / total Gram+ bacteria (Gram+ bacteria and *Actinobacteria*) and bacteria / fungi in 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.
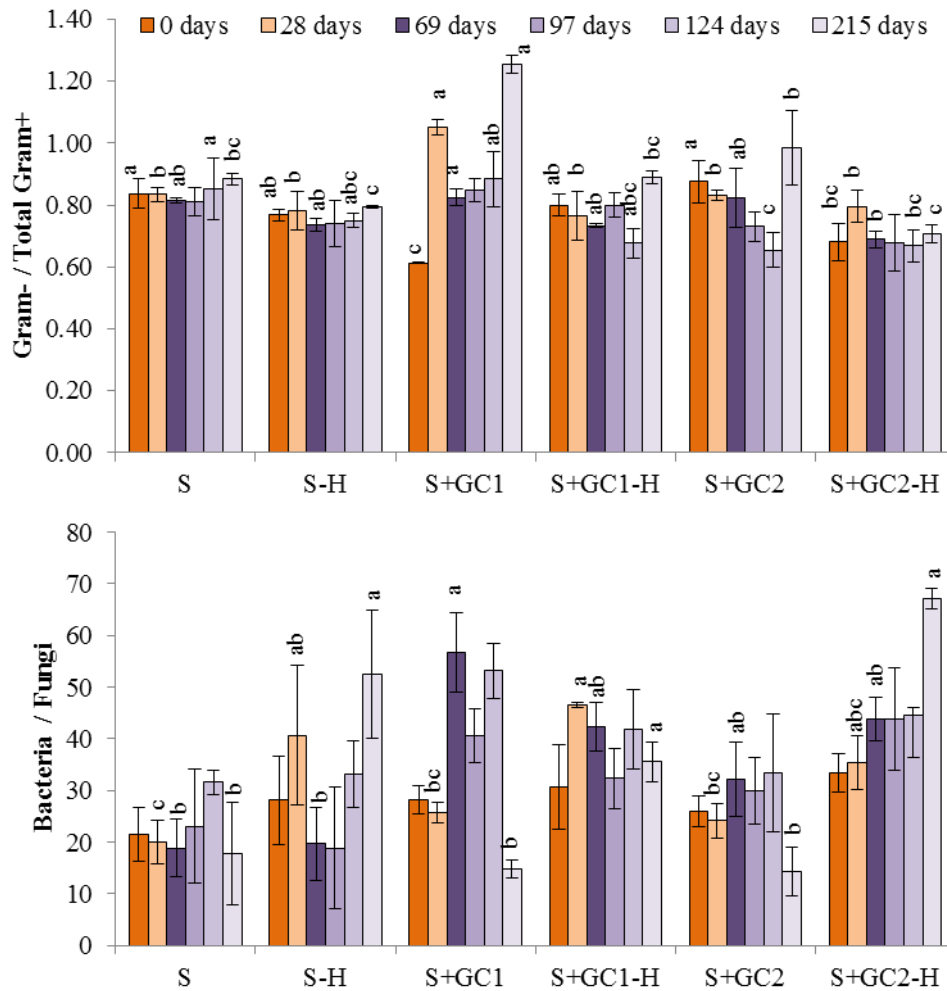
Fig S5. Ratio Gram- / total Gram+ bacteria (Gram+ bacteria and *Actinobacteria*) and bacteria / fungi in irrigated soils. Vertical bars represent the standard deviation of three replicates. Different letters indicate significant differences among treatments at the same sampling time (Tukey post hoc test, $p < 0.05$). 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.

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