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Changes in Soil Microbial Parameters after Herbicide Application in Soils under Conventional Tillage and Non-Tillage

Marwa Douibi, María José Carpio, María Sonia Rodríguez-Cruz, María J. Sánchez-Martín and Jesús M. Marín-Benito *

Institute of Natural Resources and Agrobiology of Salamanca (IRNASA-CSIC), Cordel de Merinas 40-52, 37008 Salamanca, Spain; marwa.douibi@irnasa.csic.es (M.D.); mjose.carpio@ica.csic.es (M.J.C.); msonia.rodriguez@irnasa.csic.es (M.S.R.-C.); mjesus.sanchez@irnasa.csic.es (M.J.S.-M.)
* Correspondence: jesusm.marin@irnasa.csic.es; Tel.: +34-923-219-606

Abstract: This study evaluated the changes in microbial activity in the course of time following the joint application of the herbicides S-metolachlor, foramsulfuron, and thiencarbazone-methyl to two soils (S1 and S2) under conventional tillage (CT) and non-tillage (NT) management in field conditions. The biochemical parameters of soil respiration (RES), dehydrogenase activity (DHA), microbial biomass (BIO), and the phospholipid fatty acid (PLFA) profile were determined at 1, 34, and 153 days during herbicide dissipation. In the absence of herbicides, all microbial activity was higher under NT than CT conditions, with higher or similar mean values for S1 compared to S2. A continuous decrease was detected for RES, while DHA and BIO recovered over time. In the presence of herbicides, a greater decrease in all microbial activity was detected, although the changes followed a similar trend to the one recorded without herbicides. In general, a greater decrease was observed in S1 than in S2, possibly due to the higher adsorption and/or lower bioavailability of herbicides in this soil with a higher organic carbon content. The decrease was also greater under CT conditions than under NT conditions because the herbicides can be intercepted by the mulch, with less reaching the soil. These changes involved evolution of the structure of the microbial community.

Keywords: herbicide; conservation agriculture; soil respiration; dehydrogenase activity; microbial biomass; PLFAs

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1. Introduction

Conservation agricultural practices including non-tillage, mulch residues, and crop rotation have been widely applied in recent years to enhance the sustainability of agricultural systems and decrease the negative impacts of a more conventional approach [1,2]. The primary objectives of conservation agriculture are the improvement of soil moisture and the reduction of its degradation via erosion by maintaining crop stubble and residue on the surface after the harvest and to minimize the contamination of surface water by runoff or the movement of sorbed agrochemicals on eroded soil particles [3].

This alternative cultivation approach could change the soil's physicochemical, biological, and microclimate properties, thereby affecting its microbial population. Conservation tillage generally promotes higher microbial activity, as the accumulation of organic residues in the topsoil and minimal soil disturbance increase its carbon concentration, which correlates positively with microbial biomass and microbial communities. An increase in soil organic carbon (OC) under these conditions creates a stable bacterial network and greater microbial diversity, raising enzyme activity levels and increasing microbial metabolic activity under conservation agriculture compared to conventional management [4,5]. In addition, Panettieri et al. [6] have shown that non-tillage increases the quantity of soil fungi over bacteria, and Li et al. [7] have reported a significant increase in the microbial population, fungal biomass, and bacterial diversity with non-tillage compared to conventional tillage.

Soil microorganisms are key bioindicators of soil health, functionality, and eco- sustainability [8,9]. Soil microbiota has various functions in the soil, being responsible for mineralising and decomposing soil organic matter (OM) from residues; regulating nutrient cycling, including that of carbon and nitrogen; providing and supporting the flow of energy and biochemical cycles; and enhancing soil aggregation [10]. These functions amend soil health and boost nutrient availability for plant growth and increase crop yields [11]. They are also related to the diversity, biomass, composition, and size of the soil's microbial population, which, in turn, is influenced by agricultural management [12].

A disadvantage of conservation agriculture, mainly with reduced tillage, is the increase in weed pressure, which usually results in higher herbicide use [13,14]. These compounds could be further degraded by increasing microbial activity in conservation systems, although the two processes are not always correlated [15,16], as microbial activity may also be influenced by the herbicides applied. The usage of organic residues as soil amendments has been reported to reduce the persistence of a pesticide, whereas with others, it might increase or even have no impact on persistence [17–19]. The presence of organic residues may affect the pesticide adsorption–desorption process in soils and regulate their bioavailability in the soil solution, conditioning the possible interaction of pesticides and soil microbial communities or their potential impact on them [20,21]. The soil microbial community's function and activity could therefore be affected by the application of herbicides in conservation agricultural practices according to their behaviour in these systems. Studies on the response of soil microbial community to the joint application of pesticides and organic wastes have been conducted mostly under laboratory conditions [22], with field assays being less common [23,24].

The herbicides S-metolachlor (SMOC), foramsulfuron (FORAM), and thiencarbazone-methyl (TCM) are widely applied in conservation agriculture but scarcely studied in the literature, especially FORAM and TCM [25]. They belong to different chemical groups (chloroacetanilide, sulfonylurea, and sulfonylamino-carbonyl triazolinone, respectively) with different structures and physicochemical properties, being used to control grasses and some broadleaf weeds in a large variety of crops sown in different seasons of the year [26]. Previous studies by the authors of this research have found that straw from winter wheat mulch residues used as an organic soil amendment increases the adsorption of these herbicides depending on the amended soils' OC and/or dissolved organic carbon (DOC) content [27]. This adsorption process controls the dissipation of these herbicides in these soils amended with straw from winter wheat mulch residues and shows that their dissipation is due mainly to a biodegradation process [28]. Some studies have reported microbial degradation via co-metabolism for SMOC transformation in soil [29,30] or via catabolism by different degrading enzymes for sulfonylureas transformation as FORAM in soil [30] or the cleavage of sulfonic acid for TCM [31]. However, the effect these herbicides have on microbial activity during their dissipation in a conservation agricultural system is not well known. Some studies report an effect of SMOC on the enzymatic activities of maize-growing soil when it is applied on its own [32,33] or when applied to soils with a mixture of herbicides such as terbuthylazine and mesotrione [34] or with glyphosate [35]. These effects have also been studied for TCM when applied with a mixture of herbicides such as isoxaflutole + cyprosulfamide [36]. To the best of our knowledge, there are no studies in the literature on the impact that the joint application of the herbicides SMOC, FORAM, and TCM has on soil microbial activity in the case of conservation agricultural management.

Accordingly, the purpose of this research was to determine the changes in soil microbial communities in the course of time in field conditions after the joint application of the herbicides SMOC, FORAM, and TCM as commercial formulations compatible with each other and with the cropping systems (winter wheat–maize) studied in soil under conventional tillage and non-tillage management. The following biochemical parameters were determined: (1) microbial activity (respiration (RES) and dehydrogenase activity (DHA)) and abundance (microbial biomass (BIO) and the structure of soil microbial communities (phospholipid fatty acids—PLFAs)); and (2) the changes in these parameters at

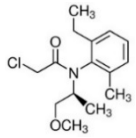
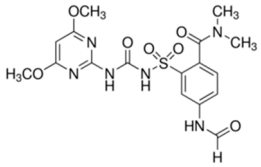
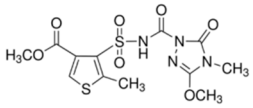
different sampling times during herbicide dissipation. This study's contribution involves considering the significance of microorganisms in soil health and its possible alteration by herbicides applied in a conservation agricultural system.

2. Materials and Methods

2.1. Herbicides

The herbicides SMOC, FORAM, and TCM were applied in the field in the commercial formulations Efica 960EC® (SMOC 96% *w*/*v* ADAMA, Agriculture Spain, S.A., Madrid, Spain) and Monsoon® Active (TCM 1% *w*/*v*, and FORAM 3.15% *w*/*v*, Bayer Crop Science S.L., Valencia, Spain). PESTANAL™ analytical standards of the herbicides SMOC (≥99.1% purity), FORAM (≥98.5% purity), and TCM (≥99.8% purity) from Sigma-Aldrich were supplied by Merck Life Science S.L. (Madrid, Spain). The herbicides' main characteristics are specified in Table 1 [26].

Table 1. Chemical structure and physicochemical properties of the three herbicides.

Herbicide	Chemical Structure	WS [a] (mg L^{-1})	Log Kow [b]	Kf_{oc}/K_{oc} [c] (mL g^{-1})	DT_{50} [d] Field/Lab (Days)	GUS Index [e]
S-metolachlor (SMOC) [2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(1S)-2-methoxy-1-methylethyl] acetamide]		480	3.05	200.2	23.2/51.8	2.32
Foramsulfuron (FORAM) [2-[[[[(4,6-dimethoxy-2-pyrimidinyl) amino] carbonyl] amino] sulfony]-4-(formylamino)-N,N-dimethylbenzamide]		3293	−0.78	78.4	-/25.3	2.95
Thiencarbazone-methyl (TCM) [methyl 4-[[[(4,5-dihydro-3-methoxy-4-methyl-5-oxo-1H-1,2,4-triazol-1-yl) carbonyl] amino] sulfonyl]-5-methyl-3-thiophenecarboxylic acid]		436	−1.98	100	17/51.5	2.46

[a] Water solubility at 20 °C; [b] Octanol/water partition coefficient at pH 7 and 20 °C; [c] Adsorption coefficient normalized for soil organic carbon content; [d] Time needed for the concentration to decrease to 50% of the initial applied amount in the field/laboratory; [e] Groundwater Ubiquity Score index for leaching into groundwater [26].

2.2. Field Experiment

A field experiment was performed in experimental plots at the Muñovela farm in Spain (IRNASA-CSIC) (40°54′15″ N latitude and 5°46′26″ W longitude), for a two-year period (October 2019–December 2021). The selected soil was a Eutric–Chromic Cambisol [37] with a sandy loam texture (14.9% clay, 4.7% silt, and 80.4% sand). This soil has previously been used for growing cereal, with winter wheat being the cover crop in this experiment.

The layout involved 12 experimental plots of 81 m^2 (9 m × 9 m) involving the soils at two different sites, S1 and S2. Four treatments were applied in a random distribution resulting from the combination of two soil management systems (conventional tillage (CT) with a cultivator to a depth of 25–28 cm (S1 + CT and S2 + CT) and a non-tillage or conservation system (S1 + NT and S2 + NT)), each one of them with three repetitions. Two experimental winter wheat–maize cycles were cultivated over two successive years involving winter wheat that was sown as a cover crop in S1 + NT and S2 + NT, chemically destroyed, cut, and deposited on the NT surface plots as mulch (more than 85% of soil surface remained covered by the mulch layer) prior to sowing maize, while the soil was kept uncovered in S1 + CT and S2 + CT over the same period.

Commercial formulations of the herbicides SMOC, FORAM, and TCM were jointly applied with an automatic sprayer attached to a backpack during the pre-emergence of the maize. The herbicides were applied over two successive years, and this microbiological study was undertaken after the second application at a dose of 0.975, 0.840, and 0.267 kg a.i ha^{-1}, respectively, by which time the soil's NT management was well established (18 months after starting the field experiment) following more than 20 years of CT management. The herbicides rates were according to the agronomic dose for each herbicide applied, the percentage of active ingredient in the commercial formulations used and the agriculture practice assayed. The herbicides were applied to eight plots (two replicates per treatment), while one added replicate per treatment without herbicides was used as a control plot. The control plots were divided into two subplots of 45 m^2 for duplicating the microbiological studies. The application was made on uncovered soil in S1 + CT and S2 + CT, while the herbicides were partially intercepted by the wheat mulch in S1 + NT and S2 + NT. The plots were first irrigated 35 days after herbicide application, although precipitation was recorded after nine days.

Weather conditions were registered during the experiment (153 days) at an on-site meteorological station. Air temperature ranged from −2.6 °C to 36.5 °C (mean of 17.6 °C), and cumulative precipitation and additional irrigation during the study amounted to 215.5 mm and 234 mm (26 mm per week), respectively.

2.3. Experimental Procedure

Soil samples were taken from the top soil layer (0–10 cm) of all the experimental field plots on the first day (1 day) and at 34 and 153 days after herbicide application in the second experimental year.

Herbicide residues and the biochemical parameters RES, DHA, BIO, and PLFAs were determined for all the treatments over time. Five sub-samples were taken from each plot and homogeneously mixed to obtain representative average soil samples for each plot before they were transferred to polypropylene bottles. The samples were then taken to the laboratory in portable refrigerators.

The soil samples were divided into sub-samples and dried and sieved (<2 mm), whereupon standard analytical methods [38,39] (Table 2) were used to determine the physicochemical characteristics of the soils. The analytical methods described in Douibi et al. [27] were used for quantifying the residual concentration of herbicides. The residual amounts of SMOC, FORAM, and TCM were expressed as percentages of the initial amounts of herbicides applied (Table 2). The biochemical parameters RES and DHA were analysed immediately. Soil moisture was determined (in duplicate) in separate samples (5 g) by weight difference after drying each sample at 110 °C for 24 h. PLFAs were determined prior to extraction and analysis by lyophilising the samples at −80 °C.

Table 2. Physicochemical characteristics of soil (S1, S2) under conventional tillage (CT) and non-tillage (NT).

Treatment/Parameter	S1 + CT	S2 + CT	S1 + NT	S2 + NT
Sand (%)	80.4	76.7	80.4	76.7
Silt (%)	4.7	6.8	4.7	6.8
Clay (%)	14.9	16.5	14.9	16.5
pH	6.81	7.67	6.8	7.67
OC (%)	0.69	1.01	0.68	1.01

Soil DHA was evaluated by the Tabatabai method [40]. A detailed description of the used protocol is found in Carpio et al. [23]. The results were expressed as µg 1,3,5-triphenylformazan (TPF) g^{-1} dry soil.

Soil RES was determined by measuring the drop in pressure produced by the O_2 consumed by the microorganisms in 50 g of fresh soil over four days using OxiTop Control BM6 containers with an OxiTop Control OC 110 measurement system (WTW, Weilheim,

Germany). The CO_2 produced by the metabolism of soil microorganisms was captured in 10 mL of NaOH 1 M.

The microbial community in the soil samples was determined using PLFA analysis, as described in García-Delgado et al. [24]. PLFAs allow the profiling of soil microbial communities and provide an overall view of their composition, structure, and biomass [41]. The protocol followed for PLFA extraction and analysis is described by Carpio et al. [23]. PLFAs were identified using bacterial fatty acid standards and software from the Microbial Identification System (Microbial ID, Inc., Newark, DE, USA). Neodecanoic acid (19:0) was used as an internal standard for the quantitative determination of PLFAs. Certain specific fatty acids were used as biomarkers to quantify the relative abundance of Gram-positive (iso and anteiso saturated branched-chain fatty acids), Gram-negative (monounsaturated and 17:0 cyclopropyl fatty acids), Actinobacteria (10-methyl fatty acids), and saprophytic and arbuscular mycorrhizal fungi (18:2 ω6 cis and 16:1 ω5, respectively). Total BIO was estimated by the sum of PLFAs and expressed as nmol g^{-1}.

2.4. Data Analysis

The data obtained from the dissipation studies were statistically analysed using IBM SPSS Statistics v.29.0.0.0 software (IBM, Armonk, NY, USA). Standard deviation (SD) was used to indicate variability among replicates in the soil RES, DHA, BIO, and PLFAs. One-way and two-way ANOVA were performed, and the Tukey or Games–Howell post hoc test (according to Levene's test for homogeneity of variance) at $p < 0.05$ was used to determine significant differences between these parameters and evaluate the effects of the different soil management practices at the same and different sampling times within the same treatment. Simple linear regression models were used to relate the residual amounts of herbicides and/or soil characteristics ($p < 0.05$).

3. Results and Discussion

3.1. Herbicide Residues in Soils under Tillage and Non-Tillage Management

The residual herbicide concentrations determined in S1 and S2 ranged between 0.898 and 0.000 (SMOC), 0.675 and 0.009 (FORAM), and 0.102 and 0.001 (TCM) μg g^{-1} dry soil the day after their application in the experimental plots under CT and NT management (Table 3). These concentrations were high in both cases and decreased significantly over time ($p < 0.05$), indicating a continuous dissipation of the three herbicides in all the samples. A one-way ANOVA revealed the significant influence of time ($F_{2,3} > 20.07$, $p < 0.05$) and treatment ($F_{3,4} > 14.02$, $p < 0.05$) on the residual amounts of each herbicide (Table 3). The residual concentrations of the three herbicides were generally higher in S2 than in S1 and under CT than under NT (Table 3). Higher adsorption of herbicides by S2 than by S1 has been described by Douibi et al. [27] and could explain the higher residual amounts of herbicides in this soil. By contrast, the lower amount of herbicide residues in soils under NT may have been because the soil surface covered by mulch affected the amounts of herbicides reaching the soil surface, as reported previously [42–44].

Table 3. Remaining amounts of S-metolachlor, foramsulfuron, and thiencarbazone-methyl in soils (S1, S2) under conventional tillage (CT) and non-tillage (NT) at different sampling times after the application of the herbicides.

Herbicides/Soil	Residual Herbicide (μg Herbicide g^{-1} Dry Soil) ± SD [a]		
	1 Day	34 Days	153 Days
S-metolachlor			
S1 + CT	0.898 ± 0.09 aA	0.421 ± 0.08 aB	0.024 ± 0.00 -C
S1 + NT	0.013 ± 0.02 cA	0.007 ± 0.00 bB	0.000 ± 0.00 -C
S2 + CT	0.659 ± 0.04 bA	0.423 ± 0.14 aB	0.036 ± 0.05 -C
S2 + NT	0.000 ± 0.00 c-	0.050 ± 0.05 b-	0.014 ± 0.02 --

Table 3. *Cont.*

Herbicides/Soil	Residual Herbicide (μg Herbicide g^{-1} Dry Soil) ± SD [a]		
	1 Day	34 Days	153 Days
Foramsulfuron			
S1 + CT	0.555 ± 0.28 ab-	0.005 ± 0.00 c-	0.002 ± 0.00 --
S1 + NT	0.022 ± 0.02 b-	0.011± 0.00 bc-	0.041 ± 0.00 -B
S2 + CT	0.675 ± 0.72 aA	0.023 ± 0.00 aB	0.041 ± 0.00 -B
S2 + NT	0.009 ± 0.10 bB	0.018 ± 0.00 abA	0.002 ± 0.00 -C
Thiencarbazone-methyl			
S1 + CT	0.102 ± 0.02 aA	0.031 ± 0.00 -B	0.003 ± 0.00 -B
S1 + NT	0.003 ± 0.01 bB	0.012 ± 0.00 -A	0.001 ± 0.00 -B
S2 + CT	0.097 ± 0.00 aA	0.041 ± 0.01 -B	0.004 ± 0.00 -C
S2 + NT	0.001 ± 0.00 b-	0.021 ± 0.00 --	0.003 ± 0.00 --

[a] Standard deviation of the mean. Values marked with lowercase letters in the same column indicate significant differences ($p < 0.05$) between different treatments at each time, according to the Tukey post hoc test. Values marked with uppercase letters in the same line indicate significant differences ($p < 0.05$) between different sampling times for each treatment according to the Tukey post hoc test. The lack of letters (-) indicates non-significant differences.

3.2. Soil Microbial Respiration

Figure 1 shows the soil RES rates for conventional tillage (S1 + CT, S2 + CT) and non-tillage (S1 + NT, S2 + NT) with or without herbicides (H). The mean values of soil microbial respiration with no herbicides for the three times ranged from 267.99 (S1 + CT) to 411.52 (S1 + NT) mg O_2 kg^{-1} dry soil in S1 and from 177.39 (S2 + CT) to 272.88 (S2 + NT) mg O_2 kg^{-1} dry soil in S2. These values were significantly higher in S1 than in S2 ($F_{7,24}$ = 79.18, p = 0.001), and in both soils, they were higher under NT than under CT. Minimal soil disturbance under NT is known to enhance the physical quality of soil by conserving its structural stability, withholding moisture, and enhancing OM content [45]. Furthermore, the presence of an organic layer of crop residues on the soil surface provides a consistent OC supply that stimulates the microbial community and its activity by means of an extended bacterial and fungi population, thereby increasing the rate of soil RES [45,46]. However, soil RES rates significantly decreased over time in soils under NT compared to those under CT, which remained constant (Figure 1), indicating NT's negative impact on microbial activity over time. Although NT initially has a positive effect on soil RES, a mulch layer on the soil surface could be a hindrance. Khan et al. [47] have reported similar findings by observing a decline in soil RES over time under CT practices with NT and straw retention, reporting that a thick mulch layer could act as a physical barrier, reducing oxygen availability in NT fields and providing a slow release of carbon.

By contrast, RES values decreased under all conditions over time following herbicide application. Mean values determined at the three times evaluated ranged from 179.25 (S1 + CT + H) to 208.07 (S1 + NT + H) and from 112.65 (S2 + CT + H) to 158.53 (S2 + NT + H) mg O_2 kg^{-1} dry soil (Figure 1). A two-way ANOVA including all data and time and treatments as factors showed that this interaction is statistically significant ($F_{14,24}$ = 9.61, p = 0.001) for soil RES under different management practices (Figure 1). A significant decrease in soil RES was recorded under CT and NT in both soils in the presence of herbicides, with S1 generally recording higher RES rates than S2, probably due to higher herbicide adsorption by S2 because of its higher OC content [27]. This could decrease the bioavailability of the herbicide adsorbed by microbial communities, although a non-significant correlation (r = −0.13, p = 0.66) was observed between soil RES and herbicide residues when all the results were considered jointly. The results indicate that the herbicide could have a toxic effect on the soil microbial rate, which is consistent with the progressive decrease in RES over time and its non-recovery at the last sampling date. This is consistent with the results reported by Zhou et al. [48] who have found that SMOC stimulates soil RES at the beginning of incubation, while it has an inhibitory effect later on in the experiment. No results have been found in the literature for the other herbicides studied.

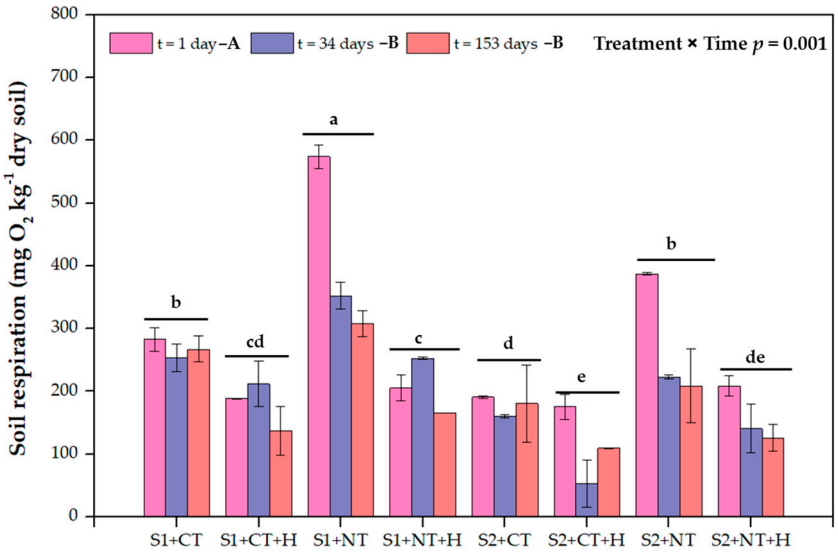


Figure 1. Microbial respiration rate for soils under conventional tillage (S1 + CT, S2 + CT) and non-tillage (S1 + NT, S2 + NT) in the absence or presence of herbicides (H). Data present the mean ± standard deviation of two replicated plots. Different capital letters inside the graph indicate significant differences between treatments at each sampling time according to the Tukey post hoc test ($p \leq 0.05$). Different letters above bars indicate significant differences between sampling times at each treatment (Tukey post hoc test; $p \leq 0.05$).

3.3. Soil Dehydrogenase Activity

Figure 2 shows the DHA for soils under CT and NT with and without herbicides. The mean DHA values in the absence of herbicides ranged from 136.72 (S1 + CT) to 185.03 (S1 + NT) µg TPF g^{-1} dry soil and from 122.60 (S2 + CT) to 165.74 (S2 + NT) µg TPF g^{-1} dry soil. These values are significantly higher in S1 than in S2 ($F_{7,24} = 18.85$, $p = 0.001$), and they are higher for both soils under NT than under CT. As indicated for RES, the increase in DHA under NT compared to CT could be attributed to the minimal disturbance of the soil under NT treatment that creates a favourable microclimate for microbial growth, resulting in greater enzyme activity [8,49,50]. Moreover, soil OM content generally increases in soils under NT, and this improves soil structure and its stability, which, in turn, results in an enhanced and stable bacterial network and higher microbial diversity [51], although such an increase in soil OM content was not observed here (Table 2). The accumulation of crop residues at the soil surface under NT regulates its microclimate by moderating soil temperature (up to 13 °C in this study) and reducing water evaporation, which maintains a suitable moisture content for a long period [8,52]. Repetitive tillage practices seriously disturb the soil, affecting its structure and its capacity to retain moisture and decreasing OM, thereby reducing soil microbial abundance and activity.

DHA decreased significantly ($p < 0.05$) over a period of 34 days in soils + CT and soils + NT. However, DHA recovered at the final sampling time (Figure 2), which contrasted with the trend observed for RES (Figure 1). This discrepancy could be attributed to a shift in the composition of the soil microbial community, where some microbial groups may have become less active in terms of RES, while others became more dominant, increasing DHA [53]. Such changes in the structure and function of the microbial community are not uncommon [7]. Moreover, NT could increase carbon sequestration in the soil, and therefore lead to a build-up of OC [54]. This might stimulate DHA because microorganisms play a crucial role in OC stabilisation and transformation.

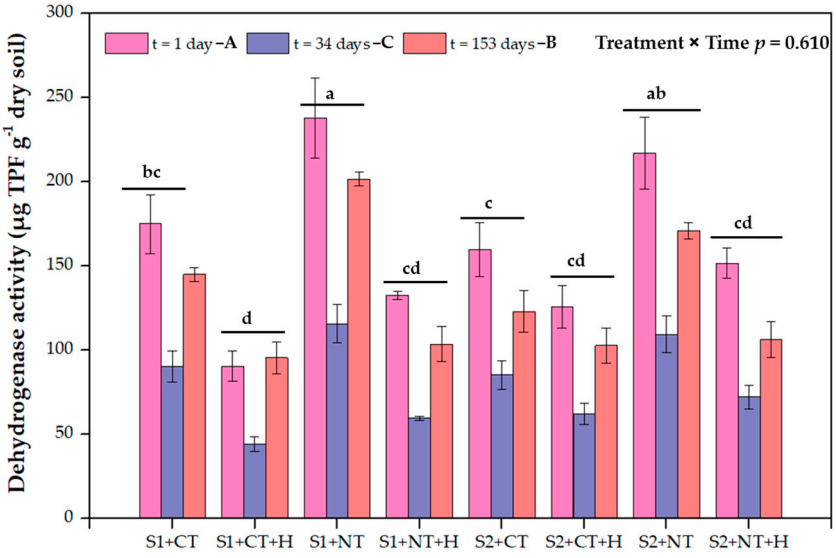


Figure 2. Dehydrogenase activity for soils under conventional tillage (S1 + CT, S2 + CT) and non-tillage (S1 + NT, S2 + NT) in the absence or presence of herbicides (H). Data present the mean ± standard deviation of two replicated plots. Different capital letters inside the graph indicate significant differences between treatments at each sampling time according to the Tukey post hoc test ($p \leq 0.05$). Different letters above bars indicate significant differences between sampling times at each treatment (Tukey post hoc test; $p \leq 0.05$).

The application of herbicides significantly decreased ($p < 0.05$) DHA values in all the soils at the three times evaluated, ranging from 76.62 (S1 + CT + H) to 98.48 (S1 + NT + H) µg TPF g^{-1} dry soil and from 96.78 (S2 + CT + H) to 109.94 (S2 + NT + H) µg TPF g^{-1} dry soil. This may be attributed to the inhibition of DHA caused by the toxic effect of the herbicides. Under NT, a significant decrease in DHA was observed, with similar values in both S2 and S1, while under CT higher DHA values were recorded in S2, which contrasted with the higher RES rates for S1 under NT and CT. A significant decrease in DHA values was observed at t = 34 days after herbicide application, as also observed without herbicides. However, the inhibition of DHA did not persist over time, and recovery occurred at the last sampling date (153 days) in both CT and NT with and without herbicides, being the opposite to RES. According to the two-way ANOVA conducted to examine the effect that management and time had on soil, DHA was recorded to have a non-significant interaction ($F_{14,24} = 0.86$, $p = 0.610$) between management and time under different conditions.

A non-significant relationship was also detected between herbicide residues and DHA values in soils under CT and NT at the three times, probably due to the combination of used herbicides with different performance regarding adsorption, dissipation, and bioavailability rates. The persistence of SMOC may have greater toxic influence on soil microbial biomass and activity compared to the sole application of one of other two herbicides. Lipșa et al. [55] have observed that soils treated with SMOC record a significant reduction of 10–30% in DHA levels compared to untreated soils. The same trend has been reported by Borowik et al. [34], who have found an 83% decrease in DHA in soils treated with a herbicide mixture containing terbuthylazine and SMOC. Filimon et al. [56] support these findings by reporting that high doses of SMOC consistently and significantly reduce soil DHA levels throughout the soil incubation period.

3.4. Soil Microbial Biomass and Structure

Figure 3 shows results for the BIO determined for soils under CT and NT with or without herbicides, and Figure 4 includes the results corresponding to the soil microbial structure determined by the relative abundance of PLFAs that diagnose Gram-negative and Gram-positive bacteria, Actinobacteria, and fungi.

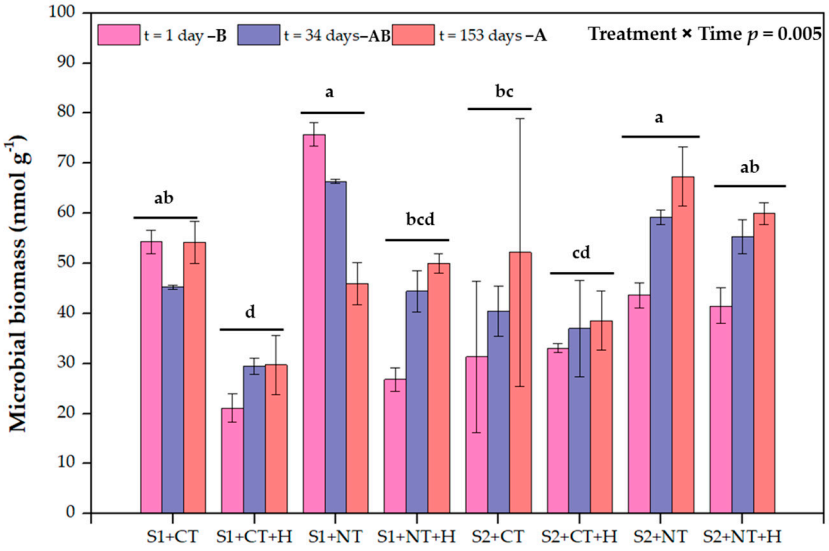


Figure 3. Total microbial biomass for soils under conventional tillage (S1 + CT, S2 + CT) and non-tillage (S1 + NT, S2 + NT) in the absence or presence of herbicides (H). Data present the mean ± standard deviation of two replicated plots. Different capital letters inside the graph indicate significant differences between treatments at each sampling time according to the Tukey post hoc test ($p \leq 0.05$). Different letters above bars indicate significant differences between sampling times at each treatment (Tukey post hoc test; $p \leq 0.05$).

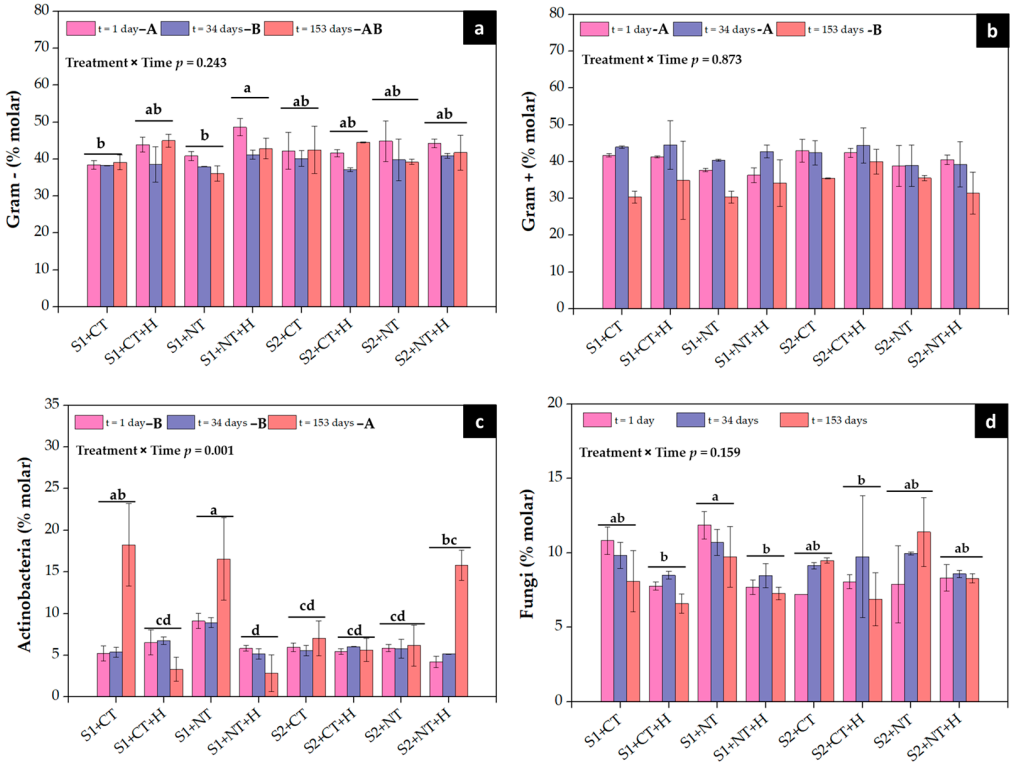


Figure 4. Relative abundance (% mol) of PLFAs specifically diagnostic of Gram-negative (**a**) and Gram-positive bacteria (**b**), Actinobacteria (**c**), and fungi (**d**) in soils under conventional tillage (S1 + CT, S2 + CT) and non-tillage (S1 + NT, S2 + NT) in the absence or presence of herbicides (H). Data present the mean ± standard deviation of two replicated plots. Different capital letters inside the graph indicate significant differences between treatments at each sampling time according to the Tukey post hoc test ($p \leq 0.05$). Different letters above bars indicate significant differences between sampling times at each treatment (Tukey post hoc test; $p \leq 0.05$).

The mean concentration of BIO in the absence of herbicides determined at the three times evaluated ranged from 51.27 (S1 + CT) to 62.69 (S1 + NT) nmol g^{-1} and from 41.30 (S2 + CT) to 56.74 (S2 + NT) nmol g^{-1}. Higher values of BIO ($F_{7,24}$ = 15.93, p = 0.001) were recorded under NT than under CT, with higher BIO concentrations in S1 than in S2, as indicated for the other microbial activities. A higher BIO concentration under NT has previously been reported [7,50,57]. Crop residues left on the surface under NT decrease the fluctuation in soil temperature and increase soil water content, thereby creating a suitable microhabitat for microbial growth [7,58,59]. Furthermore, the minimal disturbance of the soil under NT slows the rate of OM decomposition, resulting in more efficient carbon use and creating an appropriate environment for microbial growth. This is in contrast to CT practices that destroy the soil structure, prompting faster OM mineralisation, as previously indicated. BIO increased significantly over time under CT and NT, and, in general, it was observed in both soils (Figure 3).

The application of herbicides significantly decreased ($p < 0.05$) BIO under CT and NT for all the sampling times, which is consistent with the results reported for DHA (Figure 2) and RES (Figure 1) due to the initial effect of herbicides applied to suppress microbial activity. The mean concentration of BIO in the presence of herbicides at the three times ranged from 26.75 (S1 + CT + H) to 40.39 (S1 + NT) nmol g^{-1} and from 36.21 (S2 + CT + H) to 52.28 (S2 + NT + H). However, a significant increase in BIO in soils + CT + H and soils + NT + H was observed after 34 days through to 153 days, reflecting the recovery of the microbial population after an initial inhibition. This trend is probably due to microbial adaptation to the herbicides consistent with the recovery of DHA values observed at the last sampling time, indicating the microbial populations restored enzymatic activity. On the other hand, the higher BIO in soils + NT + H compared to soils + CT + H might be due to the lower amount of herbicides reaching the soils under NT when intercepted by the mulch compared to CT [42–44].

The results on the microbial community structure in Figure 4 indicate that the relative abundance of Gram-negative bacteria was lower than that of Gram-positive bacteria in all the treatments (Figure 4a,b). Non-significant interaction was detected between treatment × time for the relative abundance of Gram-negative ($F_{14,24}$ = 3.35, p = 0.243) and Gram-positive ($F_{14,24}$ = 0.55, p = 0.873) bacteria in all the treatments without herbicides at the three sampling times. However, a significant decrease in Gram-positive ($p < 0.05$) bacteria was detected at the last sampling time (153 days) for all the treatments.

Non-significant differences were recorded in Actinobacteria and fungi for the same soil under NT and CT (Figure 4c,d). However, an increase in Actinobacteria was observed at the last sampling time in S1 in both treatments. The two-way ANOVA revealed a significant interaction between treatment × time ($F_{14,24}$ = 11.80, p = 0.001) solely for Actinobacteria. For S1, Actinobacteria were higher in S1 + NT than S1 + CT. Fungi decreased in S1 + CT and S1 + NT over time. Meanwhile, a progressive increase in fungi was recorded over time for S2 + CT and S2 + NT, indicating that the type of tillage did not influence the relative abundance of fungi because it depends on soil characteristics.

Studies have reported changes in the microbial community structures with the implementation of NT [11,60,61]. Other studies have found a non-significant effect on these structures [62,63]. Li et al. [7] have conducted a thorough meta-analysis and revealed a varied response of microbial community structures and population size in soils under NT compared to CT. They suggest that NT has a differential impact on microbes throughout all the populations within microbial communities. This, in turn, could imply a potential microbial resistance to alterations under this soil management practice [64]. In addition, the changes in microbial community structure could be related mainly to the period of adaptation when transitioning from CT to NT, as the microbial communities may take some time to adapt to the new soil management [65].

After herbicide application, a non-significant effect was observed for Gram-negative and Gram-positive bacteria for all the treatments, except a significant increase in Gram-negative bacteria in S1 + NT + H compared to the control without herbicide (S1 + NT). A

significant decrease was observed for Actinobacteria in S1 + CT + H and S1 + NT + H after herbicide application, with significant increases in Actinobacteria in S2 + NT + H at the last sampling time compared to the controls. Herbicides also decreased fungi in all the treatments, albeit only significantly in S1 + NT + H. These results indicate that herbicides application had a contrasting effect on the structure of the microbial communities in soils under CT and NT treatments. In general, herbicide application had a significant impact on Actinobacteria and fungi, highlighting the sensitivity these microorganisms have to these chemical compounds. In contrast, minor changes were detected in the structure of Gram-positive and Gram-negative bacteria. Such variations in the reaction of microorganisms to herbicide exposure can be attributed to the varying levels of resistance shown by these microorganisms [58].

4. Conclusions

The implementation of conservation practices such as non-tillage in agricultural soils caused significant changes in microbial activities compared to the conventional management. RES, DHA, and BIO increased in NT compared to CT practices in the absence of herbicides. All microbial activities decreased initially, but DHA and BIO subsequently recovered over time. Herbicides application decreased microbial activities in CT and NT, although the changes followed a similar trend to the one observed in the absence of herbicides. In general, microbial activity was favoured in the soil with lower OC as herbicides bioavailability increases due to lower adsorption and under NT because herbicides are intercepted by the mulch compared to CT. Changes were found in Gram-positive bacteria, Actinobacteria, and fungi over time, although they did not depend upon CT or NT, as the influence of the type of soil was more significant, especially for Actinobacteria and fungi. The non-significant changes in microbial structure under NT are due mainly to the short-term transition (two years) to this type of soil management. The results highlight the interest in considering the impact herbicides have on microbial activities when these compounds are applied in a conservation agricultural system, the need for longer-term experiments, as well as for optimizing the amount of mulch covering the soil surface that control the amount of herbicide reaching the soil surface and, consequently, its degree of impact on soil microbial communities.

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References

1. Boselli, R.; Fiorini, A.; Santelli, S.; Ardenti, F.; Capra, F.; Maris, S.C.; Tabaglio, V. Cover crops during transition to no-till maintain yield and enhance soil fertility in intensive agro-ecosystems. *Field Crops Res.* **2020**, *255*, 107871. [CrossRef]
2. Kader, M.A.; Senge, M.; Mojid, M.A.; Ito, K. Recent advances in mulching materials and methods for modifying soil environment. *Soil Tillage Res.* **2017**, *168*, 155–166. [CrossRef]
3. Jacobs, A.A.; Evans, R.S.; Allison, J.K.; Garner, E.R.; Kingery, W.L.; McCulley, R.L. Cover crops and no-tillage reduce crop production costs and soil loss, compensating for lack of short-term soil quality improvement in a maize and soybean production system. *Soil Tillage Res.* **2022**, *218*, 105310. [CrossRef]
4. Wang, G.; Jia, H.; Zhuang, J.; Glatzel, S.; Bennett, J.M.; Zhu, Y. Growing-season soil microbial respiration response to long-term no tillage and spring ridge tillage. *Int. J. Agric. Biol. Eng.* **2020**, *13*, 143–150. [CrossRef]
5. Zheng, F.; Wu, X.; Zhang, M.; Liu, X.; Song, X.; Lu, J.; Wang, B.; van Groenigen, K.J.; Li, S. Linking soil microbial community traits and organic carbon accumulation rate under long-term conservation tillage practices. *Soil Tillage Res.* **2022**, *220*, 105360. [CrossRef]
6. Panettieri, M.; de Sosa, L.L.; Domínguez, M.T.; Madejón, E. Long-term impacts of conservation tillage on Mediterranean agricultural soils: Shifts in microbial communities despite limited effects on chemical properties. *Agric. Ecosyst. Environ.* **2020**, *304*, 107144. [CrossRef]
7. Li, Y.; Zhang, Q.; Cai, Y.; Yang, Q.; Chang, S.X. Minimum tillage and residue retention increase soil microbial population size and diversity: Implications for conservation tillage. *Sci. Total Environ.* **2020**, *716*, 137164. [CrossRef]
8. Baghel, J.K.; Das, T.K.; Raj, R.; Paul, S.; Mukherjee, I.; Bisht, M. Effect of conservation agriculture and weed management on weeds, soil microbial activity and wheat (*Triticum aestivum*) productivity under a rice (*Oryza sativa*)-wheat cropping system. *Indian J. Agric. Sci.* **2018**, *88*, 1709–1716. [CrossRef]
9. Belmonte, S.A.; Celi, L.; Stahel, R.J.; Bonifacio, E.; Novello, V.; Zanini, E.; Steenwerth, K.L. Effect of Long-Term Soil Management on the Mutual Interaction Among Soil Organic Matter, Microbial Activity and Aggregate Stability in a Vineyard. *Pedosphere* **2018**, *28*, 288–298. [CrossRef]
10. Arantes, A.C.C.; Cotta, S.R.; da Conceição, P.M.; Meneghin, S.P.; Martinelli, R.; Próspero, A.G.; Boaretto, R.M.; Andreote, F.D.; Mattos, D., Jr.; de Azevedo, F.A. Implication of *Urochloa* spp. Intercropping and Conservation Agriculture on Soil Microbiological Quality and Yield of Tahiti Acid Lime in Long Term Orchard Experiment. *Agriculture* **2020**, *10*, 491. [CrossRef]
11. Jayaraman, S.; Dang, Y.P.; Naorem, A.; Page, K.L.; Dalal, R.C. Conservation Agriculture as a System to Enhance Ecosystem Services. *Agriculture* **2021**, *11*, 718. [CrossRef]
12. Legrand, F.; Picot, A.; Cobo-Díaz, J.F.; Carof, M.; Chen, W.; Le Floch, G. Effect of tillage and static abiotic soil properties on microbial diversity. *Appl. Soil Ecol.* **2018**, *132*, 135–145. [CrossRef]
13. Alletto, L.; Coquet, Y.; Benoit, P.; Heddadj, D.; Barriuso, E. Tillage management effects on pesticide fate in soils. A review. *Agron. Sustain. Dev.* **2010**, *30*, 367–400. [CrossRef]
14. Malone, M.; Foster, E. A mixed-methods approach to determine how conservation management programs and techniques have affected herbicide use and distribution in the environment over time. *Sci. Total Environ.* **2019**, *660*, 145–157. [CrossRef] [PubMed]
15. Mahía, J.; Martín, A.; Carballas, T.; Díaz-Raviña, M. Atrazine degradation and enzyme activities in an agricultural soil under two tillage systems. *Sci. Total Environ.* **2007**, *378*, 187–194. [CrossRef]
16. Okada, E.; Costa, J.L.; Bedmar, F. Glyphosate Dissipation in Different Soils Under No-Till and Conventional Tillage. *Pedosphere* **2019**, *29*, 773–783. [CrossRef]
17. Hussain, S.; Arshad, M.; Springael, D.; Sørensen, S.R.; Bending, G.D.; Devers-Lamrani, M.; Maqbool, Z.; Martin-Laurent, F. Abiotic and Biotic Processes Governing the Fate of Phenylurea Herbicides in Soils: A Review. *Crit. Rev. Environ. Sci. Technol.* **2015**, *45*, 1947–1998. [CrossRef]
18. Álvarez-Martín, A.; Hilton, S.L.; Bending, G.D.; Rodríguez-Cruz, M.S.; Sánchez-Martín, M.J. Changes in activity and structure of the soil microbial community after application of azoxystrobin or pirimicarb and an organic amendment to an agricultural soil. *Appl. Soil Ecol.* **2016**, *106*, 47–57. [CrossRef]
19. Marín-Benito, J.M.; Herrero-Hernández, E.; Andrades, M.S.; Sánchez-Martín, M.J.; Rodríguez-Cruz, M.S. Effect of different organic amendments on the dissipation of linuron, diazinon and myclobutanil in an agricultural soil incubated for different time periods. *Sci. Total Environ.* **2014**, *476–477*, 611–621. [CrossRef]
20. Hussain, S.; Siddique, T.; Arshad, M.; Saleem, M. Bioremediation and Phytoremediation of Pesticides: Recent Advances. *Crit. Rev. Environ. Sci. Technol.* **2009**, *39*, 843–907. [CrossRef]
21. Pose-Juan, E.; Igual, J.M.; Curto, N.; Sánchez-Martín, M.J.; Rodríguez-Cruz, M.S. Mesotrione dissipation and response of soil microbial communities in a soil amended with organic residues. *Span. J. Soil Sci.* **2015**, *5*, 12–25. [CrossRef]
22. Imfeld, G.; Vuilleumier, S. Measuring the effects of pesticides on bacterial communities in soil: A critical review. *Eur. J. Soil Biol.* **2012**, *49*, 22–30. [CrossRef]
23. Jose Carpio, M.; Garcia-Delgado, C.; Maria Marin-Benito, J.; Jesus Sanchez-Martin, M.; Sonia Rodriguez-Cruz, M. Soil Microbial Community Changes in a Field Treatment with Chlorotoluron, Flufenacet and Diflufenican and Two Organic Amendments. *Agronomy* **2020**, *10*, 1166. [CrossRef]

24. García-Delgado, C.; Barba, V.; Marín-Benito, J.M.; Igual, J.M.; Sánchez-Martín, M.J.; Rodríguez-Cruz, M.S. Simultaneous application of two herbicides and green compost in a field experiment: Implications on soil microbial community. *Appl. Soil Ecol.* **2018**, *127*, 30–40. [CrossRef]
25. Wendt, M.J.; Kenter, C.; Ladewig, E.; Wegener, M.; Märländer, B. Duration of Soil Activity of Foramsulfuron Plus Thiencarbazone-methyl Applied to Weed Species Typical of Sugar Beet Cultivation. *Weed Technol.* **2017**, *31*, 291–300. [CrossRef]
26. Lewis, K.A.; Tzilivakis, J.; Warner, D.J.; Green, A. An international database for pesticide risk assessments and management. *Hum. Ecol. Risk Assess. Int. J.* **2016**, *22*, 1050–1064. [CrossRef]
27. Douibi, M.; Krishtammagari, A.; Sánchez-Martín, M.J.; Rodríguez-Cruz, M.S.; Marín-Benito, J.M. Mulching vs. organic soil amendment: Effects on adsorption-desorption of herbicides. *Sci. Total Environ.* **2023**, *892*, 164749. [CrossRef] [PubMed]
28. Douibi, M.; Sánchez-Martín, M.J.; Rodríguez-Cruz, M.S.; Marín-Benito, J.M. Impacto de la Temperatura y de Prácticas Agrícolas Sostenibles en la Degradación de Herbicidas. *Rev. Ciências Agrárias* **2022**, *45*, 583–586.
29. Cassigneul, A.; Benoit, P.; Nobile, C.; Bergheaud, V.; Dumeny, V.; Etiévant, V.; Maylin, A.; Justes, E.; Alletto, L. Behaviour of S-metolachlor and its oxanilic and ethanesulfonic acids metabolites under fresh vs. partially decomposed cover crop mulches: A laboratory study. *Sci. Total Environ.* **2018**, *631–632*, 1515–1524.
30. Huang, X.; He, J.; Yan, X.; Hong, Q.; Chen, K.; He, Q.; Zhang, L.; Liu, X.; Chuang, S.; Li, S.; et al. Microbial catabolism of chemical herbicides: Microbial resources, metabolic pathways and catabolic genes. *Pestic. Biochem. Physiol.* **2017**, *143*, 272–297. [CrossRef]
31. Ahmad, K.S.; Hafeez, N.; Gul, M.M.; Ali, D.; Shaheen, A.; Aslam, B. Xenobiotic thiencarbazone-methyl biotransformation investigation by bacteria *Streptococcus pneumoniae*, *Escherichia coli* and *Streptococcus pyogenes*. *Int. J. Env. Sci. Technol.* **2021**, *18*, 1753–1760. [CrossRef]
32. Wołejko, E.; Kaczyński, P.; Łozowicka, B.; Wydro, U.; Borusiewicz, A.; Hrynko, I.; Konecki, R.; Snarska, K.; Dec, D.; Malinowski, P. Dissipation of S-metolachlor in plant and soil and effect on enzymatic activities. *Env. Monit Assess* **2017**, *189*, 355. [CrossRef] [PubMed]
33. Xu, N.; Qu, Q.; Zhang, Z.; Yuan, W.; Cui, H.; Shen, Y.; Lin, W.; Lu, T.; Qian, H. Effects of residual S-metolachlor in soil on the phyllosphere microbial communities of wheat (*Triticum aestivum* L.). *Sci. Total Environ.* **2020**, *748*, 141342. [CrossRef] [PubMed]
34. Borowik, A.; Wyszkowska, J.; Kucharski, J.; Baćmaga, M.; Tomkiel, M. Response of microorganisms and enzymes to soil contamination with a mixture of terbuthylazine, mesotrione, and S-metolachlor. *Environ. Sci. Pollut. Res.* **2017**, *24*, 1910–1925. [CrossRef] [PubMed]
35. Rabelo, J.S.; Dos Santos, E.A.; de Melo, E.I.; Vaz, M.G.M.V.; de Oliveira Mendes, G. Tolerance of microorganisms to residual herbicides found in eucalyptus plantations. *Chemosphere* **2023**, *329*, 138630. [CrossRef] [PubMed]
36. Koçak, B.; Cenkseven, S. Effects of Three Commonly Used Herbicides in Maize on Short-Term Soil Organic Carbon Mineralization. *Water Air Soil Pollut.* **2021**, *232*, 386. [CrossRef]
37. World Reference Base; Schád, P.; van Huyssteen, C.; Micheli, E. World Reference Base for Soil Resources 2014, Update 2015. Available online: https://www.fao.org/3/i3794en/I3794en.pdf (accessed on 30 March 2023).
38. Marín-Benito, J.M.; Barba, V.; Ordax, J.M.; Sánchez-Martín, M.J.; Rodríguez-Cruz, M.S. Recycling organic residues in soils as amendments: Effect on the mobility of two herbicides under different management practices. *J. Environ. Manag.* **2018**, *224*, 172–181. [CrossRef] [PubMed]
39. Sparks, D.L. *Methods of Soil Analysis. Part 3: Chemical Methods*; SSSA Series; Wiley: Hoboken, NJ, USA, 1996.
40. Tabatabai, M.A. Soil Enzymes. In *Methods of Soil Analysis, Part 2-Microbiological and Biochemical Properties*; Weaver, R.W., Angl, J.S., Bottomley, P.S., Eds.; Soil Science Society of America (SSSA): Madison, WI, USA, 1994; pp. 903–947.
41. Frostegård, Å.; Bååth, E.; Tunlio, A. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biol. Biochem.* **1993**, *25*, 723–730. [CrossRef]
42. Alletto, L.; Benoit, P.; Bolognsi, B.; Couffignal, M.; Bergheaud, V.; Dumny, V.; Longueval, C.; Barriuso, E. Sorption and mineralisation of S-metolachlor in soils from fields cultivated with different conservation tillage systems. *Soil Tillage Res.* **2013**, *128*, 97–103. [CrossRef]
43. Schmitz, G.L.; Witt, W.W.; Mueller, T.C. The Effect of Wheat (*Triticum aestivum*) Straw Levels on Chlorimuron, Imazaquin, and Imazethapyr Dissipation and Interception. *Weed Technol.* **2001**, *15*, 129–136. [CrossRef]
44. Sperry, B.P.; Ferguson, J.C.; Bond, J.A.; Kruger, G.R.; Johnson, A.B.; Reynolds, D.B. Effect of differential levels of simulated overhead irrigation on residual herbicides applied to wheat straw–covered soil for barnyardgrass control. *Weed Technol.* **2022**, *36*, 648–654. [CrossRef]
45. Sapkota, T.B.; Mazzoncini, M.; Bàrberi, P.; Antichi, D.; Silvestri, N. Fifteen years of no till increase soil organic matter, microbial biomass and arthropod diversity in cover crop-based arable cropping systems. *Agron. Sustain. Dev.* **2012**, *32*, 853–863. [CrossRef]
46. Mirzavand, J.; Asadi-Rahmani, H.; Moradi-Talebbeigi, R. Biological indicators of soil quality under conventional, reduced, and no-tillage systems. *Arch. Agron. Soil Sci.* **2022**, *68*, 311–324. [CrossRef]
47. Khan, M.H.; Liu, H.; Zhu, A.; Khan, M.H.; Hussain, S.; Cao, H. Conservation tillage practices affect soil microbial diversity and composition in experimental fields. *Front. Microbiol.* **2023**, *14*, 1227–1297. [CrossRef] [PubMed]
48. Zhou, Y.; Liu, W.; Ye, H. [Effects of pesticides metolachlor and S-metolachlor on soil microorganisms in aquisols. II. Soil Respiration]. *Ying Yong Sheng Tai Xue Bao* **2006**, *17*, 1305–1309. [PubMed]

49. García-Orenes, F.; Guerrero, C.; Roldán, A.; Mataix-Solera, J.; Cerdà, A.; Campoy, M.; Zornoza, R.; Bárcenas, G.; Caravaca, F. Soil microbial biomass and activity under different agricultural management systems in a semiarid Mediterranean agroecosystem. *Soil Tillage Res.* **2010**, *109*, 110–115. [CrossRef]
50. Zuber, S.M.; Villamil, M.B. Meta-analysis approach to assess effect of tillage on microbial biomass and enzyme activities. *Soil Biol. Biochem.* **2016**, *97*, 176–187. [CrossRef]
51. Dang, Y.P.; Page, K.L.; Dalal, R.C.; Menzies, N.W. No-till Farming Systems for Sustainable Agriculture: An Overview. In *No-till Farming Systems for Sustainable Agriculture*; Dang, Y.P., Dalal, R.C., Menzies, N.W., Eds.; Springer International Publushing: Cham, Switzerland, 2020; pp. 3–20. Available online: http://link.springer.com/10.1007/978-3-030-46409-7_1. (accessed on 30 January 2023).
52. Ravichandran, M.; Samiappan, S.C.; Pandiyan, R.; Velu, R.K. Improvement of crop and soil management practices through mulching for enhancement of soil fertility and environmental sustainability: A review. *J. Exp. Biol. Agric. Sci.* **2022**, *10*, 697–712. [CrossRef]
53. Mathew, R.P.; Feng, Y.; Githinji, L.; Ankumah, R.; Balkcom, K.S. Impact of No-Tillage and Conventional Tillage Systems on Soil Microbial Communities. *Appl. Environ. Soil Sci.* **2012**, *2012*, e548620. [CrossRef]
54. Six, J.; Frey, S.D.; Thiet, R.K.; Batten, K.M. Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems. *Soil Sci. Soc. Amer. J* **2006**, *70*, 555–569. [CrossRef]
55. Lipșa, F.D.; Ulea, E.; Chiriac, I.P.; Coroi, I.G. *Effect of Herbicide S-Metolachlor on Soil Microorganisms*; Seria Agronomie; Lucrări Științifice, Universitatea de Stiinte Agricole si Medicină Veterinară 'Ion Ionescu de la Brad': Iași, Romania, 2010; Volume 53, pp. 110–113.
56. Filimon, M.N.; Roman, D.L.; Caraba, I.V.; Isvoran, A. Assessment of the Effect of Application of the Herbicide S-Metolachlor on the Activity of Some Enzymes Found in Soil. *Agriculture* **2021**, *11*, 469. [CrossRef]
57. Kaschuk, G.; Alberton, O.; Hungria, M. Three decades of soil microbial biomass studies in Brazilian ecosystems: Lessons learned about soil quality and indications for improving sustainability. *Soil Biol. Biochem.* **2010**, *42*, 1–13. [CrossRef]
58. Kaur, P.; Jain, D.; Singh Bhullar, M. Effect of Conventional and Conservation Agriculture Practices on Dissipation of Metribuzin and Clodinafop-Propargyl, Soil Enzymatic and Microbial Activities in Wheat in a Rice-Wheat System. *Soil Sediment Contam. Int. J.* **2023**, 1–21. [CrossRef]
59. Zhang, B.; Li, Y.; Ren, T.; Tian, Z.; Wang, G.; He, X.; Tian, C. Short-term effect of tillage and crop rotation on microbial community structure and enzyme activities of a clay loam soil. *Biol. Fertil Soils* **2014**, *50*, 1077–1085. [CrossRef]
60. Kumar, S.; Rana, S.S.; Kumar, R.; Sharma, N. Effects of Conservation Tillage and Weed Management On Soil Microbial Community and Enzymatic Activity. *Bangladesh J. Bot.* **2022**, *51*, 425–431. [CrossRef]
61. Schmidt, R.; Gravuer, K.; Bossange, A.V.; Mitchell, J.; Scow, K. Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. *PLoS ONE* **2018**, *13*, e0192953. [CrossRef]
62. Helgason, B.L.; Walley, F.L.; Germida, J.J. Long-term no-till management affects microbial biomass but not community composition in Canadian prairie agroecosytems. *Soil Biol. Biochem.* **2010**, *42*, 2192–2202. [CrossRef]
63. Lupwayi, N.Z.; May, W.E.; Kanashiro, D.A.; Petri, R.M. Soil bacterial community responses to black medic cover crop and fertilizer N under no-till. *Appl. Soil Ecol.* **2018**, *124*, 95–103. [CrossRef]
64. Allison, S.D.; Martiny, J.B.H. Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci. USA* **2008**, *105* (Suppl. 1), 11512–11519. [CrossRef]
65. Khmelevtsova, L.E.; Sazykin, I.S.; Azhogina, T.N.; Sazykina, M.A. Influence of Agricultural Practices on Bacterial Community of Cultivated Soils. *Agriculture* **2022**, *12*, 371. [CrossRef]