agronomy

MDPI

Article

Soil Microbial Response to Cover Crop Termination Methods under Two Water Levels

Nelly Centurión [1], Kelly Ulcuango [2], Mariela Navas [3], Ignacio Mariscal-Sancho [1], Miguel A. Ibáñez [4], Ana Moliner [1] and Chiquinquirá Hontoria [1,*]

[1] Departamento de Producción Agraria, Universidad Politécnica de Madrid, Av. Puerta de Hierro 2, 28040 Madrid, Spain
[2] Instituto de Investigación de Biodiversidad Pachamamata Kamak, Universidad Intercultural de las Nacionalidades y Pueblos Indígenas Amawtay Wasi (UINPIAW), Juan León Mera 56, Quito 170143, Ecuador
[3] Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Finca El Encín, Autovía del Noreste A-2, Km. 38, 28805 Alcalá de Henares, Spain
[4] Departamento de Economía, Estadística y Gestión de Empresas, Universidad Politécnica de Madrid, Av. Puerta de Hierro 2, 28040 Madrid, Spain
* Correspondence: c.hontoria@upm.es

Abstract: Cover crops (CC) promote soil health, but the termination method can condition the benefits for soil microorganisms. In a greenhouse experiment, we evaluated the legacy effects of four common CC termination methods on mycorrhization, soil microbial abundance, structure, and activity, as well as other soil properties, and its interaction with water levels (well-watered and water deficit). Mowing and residue incorporation (INC), glyphosate (GLY), roller crimper (ROL) and glyphosate + roller crimper (RGL) were evaluated, together with no CC, at two sampling dates of a subsequent maize. The water level modulated the soil microbial response to CC termination methods, especially in the glyphosate methods. Legacy effects on soil microbial attributes were notable and evolved differently from maize, from pre-emergence to ~3 months later. At final sampling, INC showed the best microbial response at both water levels, enhancing most microbial attributes. ROL was the second most beneficial method, especially in well-watered soil, promoting fungi but nullifying the CC positive effect on bacteria. Regardless of water level, GLY and RGL showed a similar microbial response. In well-watered soil, GLY and RGL had a negative effect on the total fungi, which separated the RGL response from the ROL. Overall, the time since CC termination and water level modulated the soil microbial response to the termination methods. Further research is needed to investigate CC termination impacts under different environmental conditions, in order to better understand the processes involved and provide farm-level recommendations.

Keywords: glyphosate; roller crimper; incorporation; mycorrhizal fungi; qPCR

Citation: Centurión, N.; Ulcuango, K.; Navas, M.; Mariscal-Sancho, I.; Ibáñez, M.A.; Moliner, A.; Hontoria, C. Soil Microbial Response to Cover Crop Termination Methods under Two Water Levels. *Agronomy* **2022**, *12*, 3002. https://doi.org/10.3390/agronomy12123002

Academic Editors: Emanuele Radicetti, Roberto Mancinelli and Ghulam Haider

Received: 7 November 2022
Accepted: 25 November 2022
Published: 29 November 2022

1. Introduction

Replacing bare fallow with winter cover crops (CC) in annual rotations promotes agroecosystem sustainability by reducing environmental hazards such as soil degradation, nitrate leaching or climate change, as well as enhancing soil quality and nutrient cycling [1–5]. In particular, CC promotes soil health, thus enhancing the ecosystem services provided by soil microorganisms [6,7] with positive effects on microbial communities in semi-arid conditions [8]. In their meta-analysis, Kim et al. [9] indicate that CC improves abundance (27%), activity (22%) and, to a much lesser extent, diversity of soil microorganisms (2.5%) compared to the control without CC. However, the positive effect depends on the CC management and tillage system, as well as climate and soil type. In addition, Muhammad et al. [10] reported that CC improved abundance (more fungi than bacteria), C and N of the microbial biomass by a magnitude of 24, 40 and 51%, respectively, compared to no CC. With respect to arbuscular mycorrhizal fungi (AMF), replacement of bare fallow by CC (except Brassicaceae) provides

a host for these fungi, which are obligate symbionts [11]. Then, the inoculum is favored and the colonization of the subsequent crop is increased [12], promoting its nutrition, protection against pathogens and, in general, resistance to adverse conditions [13].

The effects of CC on soil microorganisms are conditioned by managing practices such as species choice, termination date, residue management, or termination method [14–18]. CC termination can involve physical means such as mowing, disking, rolling or frost; and chemical means such as herbicides, whereas residues can be incorporated or left on the surface as mulch. The way CC are terminated, together with the management of their residues, can modulate the expected CC benefits in rotations at a microbial level [7,9], with potential effects on the ecosystem services [19,20] and the subsequent main crop performance [21].

A traditional method to terminate CC has been mowing and incorporation into the soil by tillage, which can be effective when aiming to increase N in the soil using a legume CC [22]. However, soil disturbance is a factor that negatively affects soil microorganisms in the upper layers compared to no-tillage [23] with relevance to the total fungi and AMF [24,25]. In contrast, bacteria may be favored by tillage and the entry of organic debris into the soil [26]. When focusing on the residue, Muhammad et al. [10] found that incorporation of CC residues into the soil increased the total microbial biomass, total bacteria, AMF colonization and spore density compared to residues placed on the surface or removed from the soil.

In conservation agriculture, it is common to use herbicides such as glyphosate to terminate CC. The use of glyphosate is controversial due to its potential environmental and health effects [27], therefore a prohibition is envisaged. Although some recent reviews tend to minimize its effect on soil microbiota at commercial doses [28], the effects, direct or indirect, on microorganisms are often contradictory [25,29]. Whereas some studies reported no effects or irrelevant effects when used at recommended commercial doses [30], others indicated negative impacts for AMF [31] and certain N cycle genes [18], or even positive effects on some microbial groups such as culturable bacteria [32]. Compared to other termination methods, Kim et al. [9] found that the positive effects of cover cropping on microbial properties were reduced under chemical CC termination.

Among the techniques used to avoid or reduce tillage without using herbicides, the use of a roller crimper is spreading in both conservation and organic farming [33]. The roller crimper breaks the stems at different heights, forming a layer of residues on the surface [34]. Due to the reduced soil disturbance and the soil moisture preservation by mulching, a positive effect on soil microbiota, especially fungi, is to be expected [24]. Few studies have compared the effect of the roller with other methods on soil microbial properties, and results are divergent [14,18,35–37]. In the event that the roller crimper is combined with glyphosate, which occurs to ensure its effectiveness (as this may be diminished depending on the CC species and the phenological stage [38]), no studies on soil microbial properties were found. The effects could be expected to be intermediate between the two pure methods that are combined, glyphosate and roller.

Water availability influences root exudates [39] as well microbial community structure, lowering abundance and activity under water deficit conditions [40], which would tend to reduce the response to management practices [41]. Therefore, soil moisture can be expected to modulate the soil microbial response to the CC termination method; however, this interaction has hardly been studied. In the case of glyphosate, soil moisture affects its effectiveness as a herbicide [42] and its degradation [43], so it can be expected to also modulate the effects of glyphosate on soil microorganisms. To our knowledge, only Rhomdane et al. [18] analyzed the effect of different CC termination methods at two irrigation levels, finding a limited effect. In view of the few existing studies about the effects of different termination methods and their interaction with water levels on soil microbial attributes, more research is needed to shed light on the processes involved and the design of smart farming systems.

Therefore, the main objective of this study was to evaluate the legacy effects of different CC termination methods on soil microbial attributes, measured in the subsequent main crop and their interaction with two water levels, well-watered and deficit water. For this purpose, we set up a microcosm essay with maize as the main crop. On two sampling dates we measured variables related to microbial biomass, structure, and activity, as well as several soil physicochemical properties. We expected: (i) increased differences among the soil microbial responses to termination methods under well-watered conditions, (ii) a different soil microbial response according to the termination method, with bacteria being stimulated when CC residues were incorporated, while fungi and AMF stimulated when rolled; (iii) an intermediate response for the method combining roller crimper with glyphosate, between the methods of glyphosate and roller crimper separately.

2. Materials and Methods

The experiment was carried out with microcosms in a greenhouse located in central Spain. In short, a CC mixture was grown to evaluate the legacy effects of different CC termination methods under two water levels on selected variables at two sampling dates of a subsequent maize. We measured selected soil microbial variables shortly after the CC period, in pre-emergence, and both soil microbial and physicochemical variables were measured at the early growth stage of the maize.

2.1. Experimental Design and Setup of the Experiment

The experiment consisted of two study factors. The first was the CC termination method with five levels: mowing and incorporation (INC), glyphosate (GLY), roller crimper (ROL), the combination of glyphosate with roller crimper (RGL), and a control without CC (CON). The second factor was the water availability, with two irrigation levels: well-watered or high (H), near optimum conditions, and water deficit or low (L), with the low level being 75% of the high level. In total, ten treatments (5 × 2) were randomly distributed in five blocks, resulting in 50 microcosms. The greenhouse had a cooling and heating system with supplementary lighting allowing for semi-controlled conditions, which resulted in a mean temperature of 15 °C during the CC period and 19 °C during the main crop period.

The soil used in the microcosms was extracted from the surface horizon (0–20 cm) of a Haplic Calcisol at the experimental field station "La Chimenea", located in the Tajo River basin in Aranjuez (Madrid). The climate of this area is Bsk [44], with a mean annual temperature of 14.5 °C and a mean annual precipitation of 415 mm. The sampling site corresponded to an area devoted to irrigated maize cultivation under a traditional tillage system. The soil was sieved in the field at 1 cm and then homogenized. This initial soil showed alkaline pH (8.5), silt loam texture (27% sand, 52% silt, and 21% clay), relatively low organic carbon and nitrogen concentrations (1.01% and 0.11%, respectively), and relatively high calcium carbonate (20%). This soil is known to have a low structural stability and a tendency to form a crust [45]. In a previous microcosm test, the soil showed high compaction, so in order to facilitate drainage and infiltration, a substrate consisting of two volumes of soil with one volume of siliceous river sand (autoclaved at 121 °C for 2 h) was used to fill microcosms sized 30 × 12 × 10 cm.

Based on previous studies [21], the CC consisted of a mixture of barley (*Hordeum vulgare* L.), and vetch (*Vicia sativa* L.) at 1:1 proportion. The CC mixture was sown at a depth of 3 cm at a density of 16 plants per microcosm, and then thinned to leave 5 plants per species and microcosm. CC were grown for 14 weeks, and during that time they were irrigated 2 to 3 times per week according to crop demand. On average, CC received 47 and 36 mm per month at the high and low irrigation level, respectively. No fertilization was applied during the CC phase. Three months after CC sowing, glyphosate was applied at a commercial dose of 4 L ha^{-1} (isopropylamine salt of glyphosate at 36%) to terminate the CC in the GLY and RGL microcosms. For the glyphosate application, the microcosms were moved to another room to avoid cross contamination. A week later the remaining termination methods were applied. For INC, we simulated mowing by cutting the plant

shoots at ground level and then tearing with a grinder, and the incorporation was done with a hand cultivator at 5–6 cm depth. The termination by roller crimper was simulated with two passes of a mini roller crimper built ad hoc (publication number: ES1290154U) for the greenhouse experiment, to resemble that used in a field trial [21]. The mini roller crimper was designed to produce the effects of the roller crimper in microcosms, in such a way that the roller moved over the crop, breaking its stems at different heights, and forming a mulch on the ground. To do this, it was necessary to adjust the weight of the roller to produce the termination of the CC and to exert the same pressure on all replicates. In the ROL method, the mini roller was applied directly on the living plant, while in the combined method of glyphosate and roller crimper, it was applied on the plants treated with glyphosate a week before, following the usual procedure under field conditions [21].

Ten days after glyphosate application and 3 days after applying the other termination methods, the maize (*Zea mays* L. cycle 700), was sown at a density of 3 seeds per microcosm. During the growth of the main crop, the maize was irrigated according to the two irrigation levels, receiving on average 61 mm per month at the high level and 46 mm at the low one. All treatments received fertilization equivalent to 37 units of N, 35 units of P_2O_5 and 60 units of K_2O (kg/ha) under a low-input approach, mainly for N.

2.2. Sampling and Measurements

During the experiment, several non-destructive soil and plant measurements were carried out, of which soil penetration resistance (PR), soil moisture and soil temperature are reported here for the final sampling. Penetration resistance at the topsoil (0.5 mm) was measured with a ST 315 pocket penetrometer, 6.35 mm diameter (Farnell, Lainate, Italy). Soil temperature and soil moisture in the top 5 cm were recorded with an ECH2O 5TM sensor (Decagon Devices, Inc., Washington, DC, USA), previously calibrated in the study soil.

Soil sampling was conducted twice, at maize pre-emergence 4 days after sowing (DAS), which was 14 days after glyphosate application, and at the end of the experiment, at 57 DAS maize. In maize pre-emergence, soil samples were taken with a cylindrical sampler (3 cm Ø and 8 cm depth); the resulting holes were filled with the original mixture of soil and autoclaved sand (2:1) and marked to avoid being sampled again. Selected microbial variables were determined in this sampling according to the methods described below. In the final sampling, undisturbed soil samples were extracted with a metal cylinder (5 cm Ø and 5 cm height) to obtain the soil bulk density. The 3 maize plants of each microcosm were taken, together with its soil. The soil was carefully separated from the roots, and then the shoots from the roots. Secondary roots were separated from the primary ones and stored at 4 °C in a 50% ethanol solution for subsequent AMF root colonization. To do this, the fine maize roots were emptied using KOH, stained with ink and vinegar [46], and the AMF structures were counted using the magnified intersections method [47].

The soil samples from each microcosm were pooled into one and then subdivided into 3 parts for air drying, refrigeration at 4 °C, and storage at −20 °C. Samples were sieved at 2 mm, and for certain chemical properties the air-dried samples were milled with a ball mill. Soil pH and electrical conductivity at 25 °C (EC) were measured in a 1:2.5 (*w*/*v*) aqueous extract. Total organic carbon (TOC) concentration was measured by the Walkley-Black method [48] and total nitrogen (TN) concentration by the Kjeldahl method [49]. Dissolved organic carbon (DOC) was obtained by the dichromate method adapted to low concentrations [50] after extraction with potassium sulphate 0.5 M [51]. Available soil P was determined by ICP-OES (iCAP 6500-Duo, Thermo Scientific, Horsham, England) after extraction with Mehlich III solution (1:10, *w*:*v*).

Microbial variables were determined in the cold preserved samples. When frozen samples were used, they were incubated for 7 days at 22 °C and 60% of water holding capacity before analysis. Soil basal respiration (BR) was measured by CO_2 quantification in an alkaline trap after incubation for 24 h at 22 °C [52]. Substrate-induced soil respiration (SIR) was obtained by the same procedure after adding glucose (3:1 ratio talc to glucose)

and incubation for 4 h at 22 °C [52,53]. Microbial biomass C (MBC) was measured by the fumigation-extraction method [51]; then, the metabolic quotient (qCO_2, basal respiration to MBC ratio) and the microbial quotient (qMIC, MBC to TOC ratio) were obtained [54,55]. The length of the extra-radical hyphae was determined from an aqueous extraction, as described by García-Gonzalez et al. [56] based on the membrane filter technique [57] and the grid-line-intersect method [58].

Deoxyribonucleic acid (DNA) was extracted from each soil sample with the PowerSoil® DNA isolation kit (Mo-Bio laboratories, Carlsbad, CA, USA) to estimate the abundances of total bacteria, total fungi, and total archaea by quantitative polymerase chain reaction (qPCR). The 16S ribosomal ribonucleic acid (rRNA) genes were used to estimate total bacteria and total archaea, whereas the abundance of the total fungal community was studied using the ITS region. The primers used to amplify the genes are detailed in Table S1. The DNA extracts were evaluated with a series of dilutions to identify possible inhibitions and to determine the dilution that produced the highest copy number. The base DNA of the standard curve and the control without DNA were amplified in duplicate on the same plate as the samples. All qPCR amplifications were carried out in a final volume of 20 µL containing 10 µL of the KAPA SYBR® FAST qPCR Master Mix Kit (2×) (Kapa Biosystems, Washington, MA, USA), 4.2 µL of nuclease-free water, 0.4 µL of each primer (10 µM), and 5 µL of pre-diluted template DNA using a real-time system (LightCycler® 480-Roche, Rotkreuz, Switzerland). Standard curves were generated using dilutions of linearized cloned plasmids. The genes were cloned into P-GEM T-easy (Promega, Madison, WI, USA) and the inserts were sequenced to confirm their correct length and identity. The standard curves generated in each reaction were linear (serial dilutions of plasmids from 10^2 to 10^7 gene copies) with R^2 values greater than 0.98. The amplification efficiencies of all quantification reactions were 80–100%. The copy number of bacteria, archaea and total fungi were expressed as Log10 gen g^{-1} dry soil. The ratio fungi/bacteria (F/B) were obtained by dividing the respective Log10 of the fungal and bacterial copy numbers. Overall, MBC was taken as proxy for soil microbial abundance; total bacteria, total fungi, and total archaea for microbial structure; AMF colonization and hyphal length for mycorrhizal variables; and BR, SIR, qCO_2 and qMIC for microbial activity.

2.3. Statistical Analysis

An analysis of variance was applied with a mixed linear model for a factorial randomized block design. The termination method, the water level and their interaction were considered fixed effects, while the block was considered a random effect. The normality and homoscedasticity of the data were verified with 95% confidence. A Box-Cox transformation was applied to the variables that required transformations, so that a logarithm transformation was used for the microbial variables (AMF colonization, hyphal length, qCO_2) and for physicochemical variables (PR, DOC/TOC, C/N, C/P, soil moisture). Differences between means were evaluated with the Tukey test for a p-value < 0.05. Given the strong interaction between the termination method and the water level, for microbial variables, ANOVA was also performed separately for high and low irrigation doses. The Pearson's product-moment and their significance levels were calculated to assess relationships among the soil properties and the microbial variables in the maize soil. To relate microbial variables (response variables) to treatments and soil physicochemical variables (explanatory variables), a redundancy analysis (RDA) was performed. Treatments were the combinations of the 5 levels of the termination factor and the two levels of irrigation. Both response and explanatory variables were transformed as indicated above to ensure linearity and symmetry, and standardized. The RDA was performed firstly considering only treatments, secondly only soil variables and, finally, both groups together. For multicollinearity reasons, microbial variables were reduced to 9 variables and soil variables to 10 in a first approximation. A Monte Carlo permutation test was conducted using 999 permutations for a forward, backward, and stepwise forward procedure to select the soil variables ($p < 0.05$). All three procedures resulted in the same soil variables. The soil variables resulting from

the selection procedure were used in the final RDA together with the treatments. Separate ellipses on the biplot show a well-defined region in the biplot plane. A variance partitioning analysis was carried out following a Monte Carlo permutation test to explore how much of the microbial response was explained by the treatments, and how much by the soil variables. In the next analysis, conditional effects were tested using treatments as a covariate. The significance level was set at $p \leq 0.05$. Analyses were performed with the software R version 4.2. [59]. The RDA analysis was performed with the function *rda* of the vegan package [60] and the RDA plot with the *ggplot2* package [61].

3. Results

3.1. Soil Microbial Variables at Maize Pre-Emergence and 57 Days after Sowing Maize

Most soil microbial variables were measured at maize pre-emergence, and 57 DAS (Tables 1 and 2) were affected by the termination factor. A significant proportion of them showed interaction between the termination method and the water level (Figures 1 and 2). At maize pre-emergence (Table 1), hyphal length was affected by both factors but not by its interaction: INC increased hyphal length by more than 50% compared to the control without CC, and a similar increase was shown by the high compared to the low water level. Bacterial abundance, fungal abundance, and F/B ratio showed a strong interaction between factors (Table 1 and Figure 1). A high water level triggered differences between termination methods for bacteria and F/B. At a high water level (Table S2), GLY decreased bacteria by 13% (in terms of log10) compared to CON, while INC showed the highest bacterial abundance (8% more than CON). At a low water level, all CC treatments showed more bacteria than CON, but GLY remained below the others. All CC treatments, and especially GLY showed more fungi than CON, regardless of water level. At a high water level, GLY showed the highest F/B (25% higher than CON, INC and ROL) followed by RGL (15% higher), whereas at a low water level, the peak moved to RGL (12% higher than CON). Archaea responded to the termination method with the lowest value for CON and the highest for the treatments with the roller crimper (Table 1).

Table 1. Soil microbial variables as affected by termination method (TM) and water level (WL) at maize pre-emergence.

Factors	Levels	Hyphal Length cm g^{-1}	Total Bacteria Log10 Copies g^{-1}	Total Fungi Log10 Copies g^{-1}	Fungi/Bacteria	Total Archaea Log10 Copies g^{-1}
TM	CON	16.78 ^{a}	8.60	6.31	0.73	6.77 ^{a}
	INC	26.75 ^{b}	9.37	7.07	0.75	7.54 ^{b}
	GLY	23.87 ^{ab}	8.35	7.04	0.84	7.60 ^{bc}
	RGL	19.70 ^{ab}	9.07	7.53	0.83	7.74 ^{c}
	ROL	22.75 ^{ab}	9.22	6.86	0.74	7.76 ^{c}
WL	High	26.70 ^{b}	8.89	6.91	0.78	7.49 ^{a}
	Low	17.87 ^{a}	8.95	7.01	0.78	7.47 ^{a}
TM		**	***	***	***	***
WL		***	*	***	ns	ns
TM x WL		ns	***	***	***	ns

CON: control, INC: mowing + incorporation, GLY: glyphosate, RGL: glyphosate + roller, ROL: roller crimper. Different letters indicate significant differences between means according to Tukey's HSD test (p-value < 0.05). *** p-value < 0.001, ** p-value < 0.01, * p-value < 0.05, ns: p-value > 0.05.

Table 2. Soil microbial variables as affected by the termination method (TM) and water level (WL) at 57 DAS maize.

Factors	Levels	AMF Col. %	Hyphal Length cm g^{-1}	Basal Resp. mg C-CO_2 $kg^{-1}h^{-1}$	SIR mg C-CO_2 $kg^{-1}h^{-1}$	MBC mg kg^{-1}	qCO_2 mg C-CO_2 mg MBC h^{-1}	qMIC mg MBC g^{-1} C	Total Bacteria Log10 Copies g^{-1}	Total Fungi Log10 Copies g^{-1}	Fungi/ Bacteria	Total Archaea Log10 Copies g^{-1}
TM	CON	10.8 ^{a}	18.13 ^{a}	0.64 ^{a}	1.52 ^{a}	42.61 ^{a}	0.019 ^{b}	8.78 ^{a}	9.87 ^{a}	6.32 ^{a}	0.64 ^{a}	7.32 ^{a}
	INC	26.6 ^{b}	30.51 ^{b}	0.76 ^{a}	0.93 ^{a}	68.8 ^{ab}	0.011 ^{ab}	13.46 ^{a}	10.41 ^{c}	7.14 ^{c}	0.68 ^{c}	8.53 ^{e}
	GLY	16.0 ^{ab}	19.14 ^{a}	0.71 ^{a}	1.67 ^{a}	65.0 ^{ab}	0.011 ^{ab}	12.98 ^{a}	10.15 ^{b}	6.86 ^{b}	0.67 ^{bc}	8.21 ^{c}
	RGL	16.6 ^{ab}	16.86 ^{a}	0.55 ^{a}	1.32 ^{a}	67.4 ^{ab}	0.008 ^{a}	13.61 ^{a}	10.18 ^{b}	6.73 ^{b}	0.66 ^{b}	8.01 ^{b}
	ROL	22.1 ^{b}	24.27^{ab}	0.76 ^{a}	1.53 ^{a}	72.8 ^{b}	0.012 ^{ab}	14.83 ^{a}	9.98 ^{a}	7.10 ^{c}	0.70 ^{d}	8.40 ^{d}
WL	High	21.5 ^{b}	24.01 ^{a}	0.69 ^{a}	1.43 ^{a}	66.1 ^{a}	0.012 ^{a}	13.13 ^{a}	10.26 ^{b}	6.73 ^{a}	0.65 ^{a}	8.15 ^{b}
	Low	15.3 ^{a}	19.76 ^{a}	0.68 ^{a}	1.36 ^{a}	60.5 ^{a}	0.012 ^{a}	12.34 ^{a}	10.00 ^{a}	6.91 ^{b}	0.69 ^{b}	8.04 ^{a}
TM		***	**	ns	ns	*	*	ns	***	***	***	***
WL		*	ns	ns	ns	ns	ns	ns	***	***	***	***
TM x WL		ns	ns	ns	ns	*	ns	*	***	***	***	***

CON: control, INC: mowing + incorporation, GLY: glyphosate, RGL: glyphosate + roller, ROL: roller crimper, AMF Col.: AMF colonization, Basal resp: basal respiration, SIR: substrate induced respiration, MBC: microbial biomass carbon, qCO_2: metabolic quotient, qMIC: microbial quotient. Different letters indicate significant differences between means according to Tukey's HSD test (p-value < 0.05). *** p-value < 0.001, ** p-value < 0.01, * p-value < 0.05, ns: p-value > 0.05.

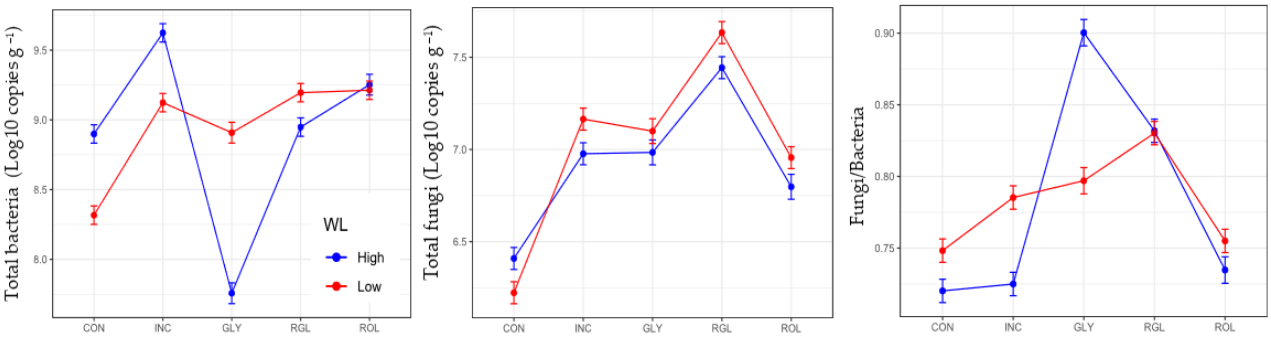

Figure 1. Total bacteria, total fungi, and ratio Fungi/Bacteria at maize pre-emergence for the termination methods: control (CON), mowing + incorporation (INC), glyphosate (GLY), glyphosate + roller (RGL), and roller crimper (ROL), under two water levels. Bars represent 95% confidence intervals according to Tukey's HSD method.

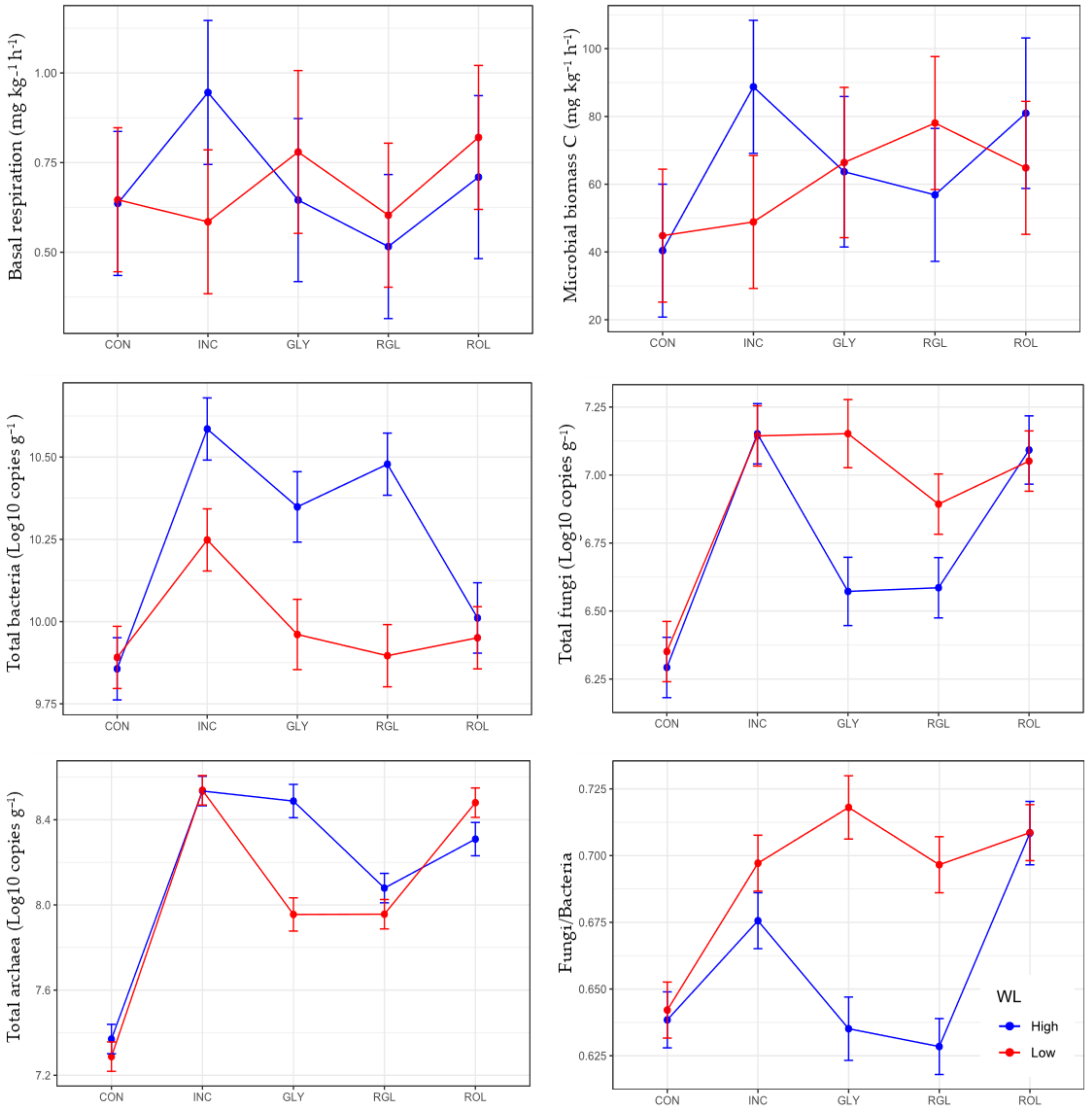

Figure 2. Basal respiration, microbial biomass carbon, total bacteria, total fungi, and F/B ratio at 57 DAS maize for the termination methods: control (CON), mowing + incorporation (INC), glyphosate (GLY), glyphosate + roller (RGL), and roller crimper (ROL), under two water levels. Bars represent 95% confidence intervals according to Tukey's HSD method.

At 57 DAS maize (Tables 2 and S3), all microbial variables, except BR and SIR, were sensitivity to the termination factor, and a large part showed interaction between the factors (Figure 2). INC followed by ROL stimulated the mycorrhizal variables the most. INC increased mycorrhizal colonization by 2.5-fold and hyphal length by 1.7-fold compared to CON. ROL showed the highest values of MBC and qMIC ($p < 0.1$) compared to CON. For INC, a high water level stimulated MBC to a greater extent than a low water level, with basal respiration showing the same trend ($p < 0.1$). Water level had no effect on the other termination methods. For the major groups of microorganisms, we found a strong interaction, and in general a high water level tended to widen the differences between termination methods. For bacteria, INC showed the highest abundance at both water levels, whereas CON and ROL showed the lowest values overall with no effect on irrigation dose. Compared to low, a high water level significantly increased bacterial abundance for INC, GLY and RGL, the latter showing the greatest difference between irrigation levels (6% more at the high dose). For fungi, the water level only influenced GLY and RGL, with greater differences between levels for GLY. The F/B ratio behaved similarly. GLY was also the most sensitive to water level for archaea, with higher abundance at the high irrigation dose.

Regarding the evolution of microbial variables from pre-emergence to 57 DAS (Table S2), i.e., already under the influence of the main crop, the termination methods tended to reduce their differences over time, except for archaeal abundance. The overall hyphal length increased, as did bacteria and archaea abundances, while F/B tended to decrease. Regarding bacteria, the strong negative effect of GLY under high irrigation disappeared and became positive. Over time, the positive effect of ROL on fungi improved from the lowest values (except CON) to the highest. In contrast, RGL went from the highest values at pre-emergence to the lowest at 57 DAS (considering both water levels together). In GLY, the water level went from having no effect to having a notable effect at 57 DAS. F/B also underwent marked changes over time, especially because of the sharp drop in the two methods with glyphosate. The passing of time favored the positive effect of INC on archaea over ROL, while that of RGL declined.

3.2. Soil Physicochemical Variables at 57 Days after Sowing Maize

The soil physicochemical variables responded to the factors in a weaker way than the microbial ones, with only EC and penetration resistance standing out (Tables 3 and S4). There was hardly any interaction between factors, and only a few variables (C/N, N/P, and soil moisture) showed a weak interaction. As for the microbial variables, a high water level tended to widen the differences between the termination methods. ROL showed the lowest value of EC with a decrease of ≈40% compared to CON, which showed the highest value. C/P ratio was higher in GLY than in CON, regardless of water level. The rest of C variables showed no response to these factors. At a high water level, GLY increased N/P relative to CON. Soil moisture did not vary with the termination methods at a low water level, while at a high level RGL showed higher values than INC and CON. Soil moisture differences between water levels were higher for RGL followed by GLY, whereas there were no differences for INC. Temperature tended to be lower for RGL and ROL. INC increased penetration resistance by ≈50% compared to the two methods with glyphosate, but with no difference to CON. The soil variables that showed the highest correlations with the microbial variables were pH and soil moisture, especially with bacteria (r = 0.39 ** and 0.47 ***, respectively), and to a lesser extent, DOC and electrical conductivity (Table S5).

3.3. Redundancy Analysis to Explain Soil Microbial Variables at 57 Days after Sowing Maize from Treatments and Soil Physicochemical Variables

The 10 treatments resulting from the combination of the five termination methods and the two water levels explained almost 61% of the variability of the 9 microbial variables (SIR, MBC, qCO2, AMF colonization, Hyphal length, Bacteria, Fungi, Fungi/Bacteria, Archaea). The first two canonical axes were significant and jointly explained 83% of the 61%, with an adjusted R^2 of 51.6%. Figure 3 shows the biplot relating the microbial variables (vectors

in black) with the treatments (ellipses grouping the microcosms of each treatment, blue at high water level and red at low level). The two treatments without CC, at high and low water level, were projected together in the left part of the biplot and showed a microbial response that clearly differentiated from that of the treatments with CC, especially INC. The control treatments were associated with lower values of microbial variables except for the metabolic quotient.

Table 3. Soil physicochemical variables as affected by termination method (TM) and water level (WL) at 57 DAS maize.

Factors	Levels	$pH_{1:2.5}$	$EC_{1:2.5}$ µS cm^{-1}	TOC g kg^{-1}	DOC mg kg^{-1}	%DOC/TOC	C/N	C/P	N/P	Moisture %v	T °C	PR kg cm^{-2}	Db g cm^{-3}
TM	CON	8.35 ^{a}	244 ^{c}	4.90 ^{a}	58.1 ^{a}	1.19 ^{a}	8.14 ^{a}	0.16 ^{a}	0.02 ^{a}	5.12 ^{ab}	24.7 ^{a}	1.82 ^{ab}	1.30 ^{a}
	INC	8.45 ^{a}	188 ^{ab}	5.09 ^{a}	56.3 ^{a}	1.11 ^{a}	9.24 ^{a}	0.18 ^{ab}	0.03 ^{a}	4.67 ^{a}	24.2 ^{a}	2.13 ^{b}	1.32 ^{a}
	GLY	8.48 ^{a}	191 ^{abc}	5.08 ^{a}	54.6 ^{a}	1.08 ^{a}	7.28 ^{a}	0.21 ^{b}	0.02 ^{a}	6.70 ^{ab}	24.7 ^{a}	1.39 ^{a}	1.37 ^{a}
	RGL	8.41 ^{a}	217 ^{bc}	5.05 ^{a}	54.6 ^{a}	1.10 ^{a}	8.04 ^{a}	0.17 ^{ab}	0.02 ^{a}	7.70 ^{b}	23.6 ^{a}	1.37 ^{a}	1.33 ^{a}
	ROL	8.43 ^{a}	143 ^{a}	4.99 ^{a}	54.0 ^{a}	1.10 ^{a}	6.73 ^{a}	0.18 ^{ab}	0.02 ^{a}	5.57 ^{ab}	23.8 ^{a}	1.75 ^{ab}	1.35 ^{a}
WL	High	8.46 ^{a}	190 ^{a}	5.07 ^{a}	55.8 ^{a}	1.10 ^{a}	8.00 ^{a}	0.19 ^{b}	0.02 ^{a}	8.48 ^{b}	24.0 ^{a}	1.60 ^{a}	1.35 ^{a}
	Low	8.39 ^{a}	204 ^{a}	4.98 ^{a}	55.3 ^{a}	1.12 ^{a}	7.80 ^{a}	0.16 ^{a}	0.02 ^{a}	3.06 ^{a}	24.5 ^{a}	1.78 ^{b}	1.32 ^{a}
	TM	ns	***	ns	ns	ns	ns	*	ns	*	ns	***	ns
	WL	ns	ns	ns	ns	ns	ns	**	ns	***	ns	*	ns
	TM x WL	ns	ns	ns	ns	ns	*	ns	*	*	ns	ns	ns

CON: control, INC: mowing + incorporation, GLY: glyphosate, RGL: glyphosate + roller, ROL: roller crimper; EC: electrical conductivity at 25 °C, TOC: total organic carbon, DOC: dissolved organic carbon, C/N: carbon/nitrogen ratio, C/P: carbon/phosphorus ratio, T: soil temperature, PR: penetration resistance, Db: bulk density. Different letters indicate significant differences between means according to Tukey's HSD test (p-value < 0.05). *** p-value < 0.001, ** p-value < 0.01, * p-value < 0.05, ns: p-value > 0.05.

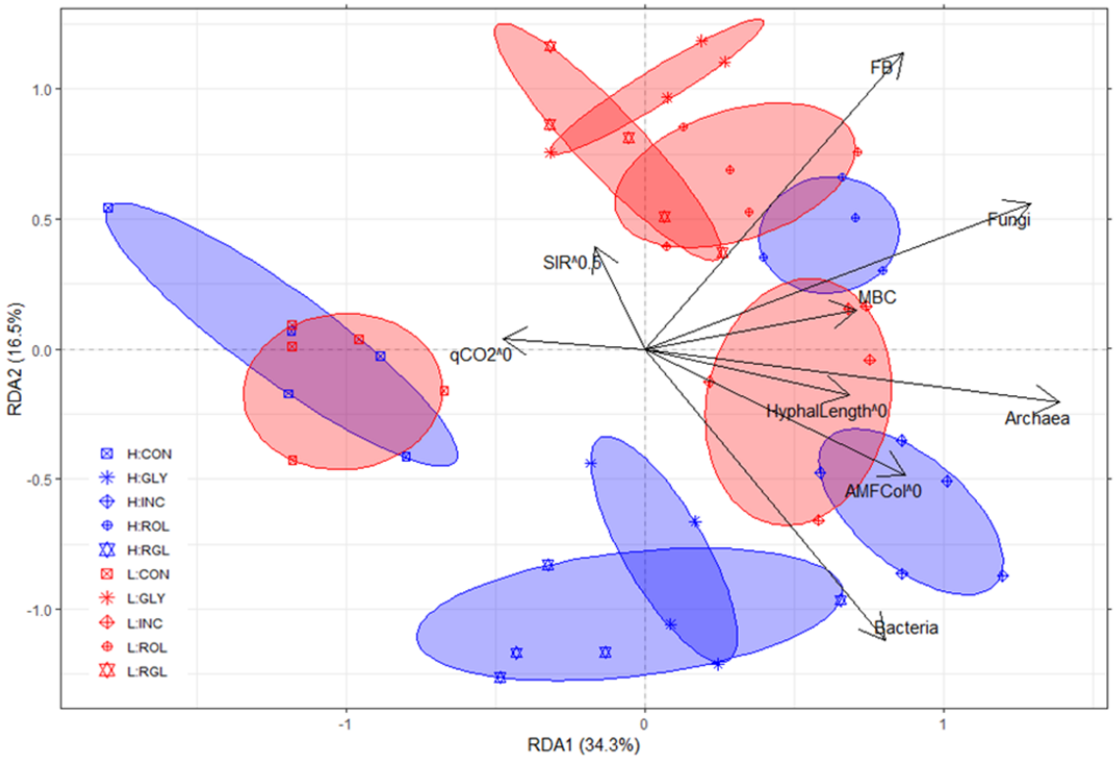

Figure 3. Redundancy analysis (RDA) results at 57 DAS maize for soil microbial variables and ten combined treatments of termination methods and water levels. CON: control, INC: mowing + incorporating, GLY: glyphosate, RGL: glyphosate + roller, ROL: roller crimper; H: high water level, L: low water level; qCO_2: metabolic quotient, SIR: substrate induced respiration, Col: AMF colonization, MBC: microbial biomass carbon.

Regarding the CC treatments, and in view of how microcosms are grouped into the ellipses (Figure 3), the high water level increased the differences between termination methods to a greater extent than the low water level. INC and ROL, located to the right of the biplot, together with most of the vectors of the microbial variables, showed higher values of these variables. ROL was associated with higher fungal abundance and high F/B, while INC stood out for its level of archaea, bacteria, and AMF colonization. Water level had little influence on both methods (overlapping ellipses of high and low water levels), with higher abundance and activity of microorganisms at higher water level (ellipses shifted to the right).

The two methods applying glyphosate (GLY and RGL) showed a similar microbial response regardless of the water level, but this response was strongly influenced by the irrigation dose. Thus, at a high water level (blue), the ellipses of these two treatments overlapped at the top of the biplot, associated with high bacteria abundance, low fungi abundance and low F/B. However, at a low water level, their behavior was opposite to the high level (lower part of the biplot), associated with less bacteria, more fungi, higher F/B, and lower abundance of archaea. At this level, the ellipses of GLY, RGL and ROL were close, with the microbial response of RGL being intermediate between GLY and ROL. It should be noted that GLY showed a different response to the other methods (except for RGL) at both low and high water levels.

In summary, the CON response differed from the CC treatments, especially INC, and in general, CON showed lower values for the microbial variables except for the metabolic ratio. INC stimulated mycorrhizal variables as well as the major groups of microorganisms and F/B ratio. ROL was also noted for its positive effect on microbial variables, especially for fungi, to the detriment of bacteria and a high MBC value. GLY and RGL were more sensitive to water level than the others, for bacteria, fungi, and F/B, and in general had a moderate positive effect, even neutral on mycorrhizal variables.

On the other hand, the 10 soil variables (pH, EC, DOC, DOC/TOC, C/N, C/P, N/P, penetration resistance, soil temperature, and soil moisture) explained 34.4% of the variability of the microbial variables. The first two axes explained 72.4% of the 34.4%, with an adjusted R^2 of 16.1%. After applying different variable selection processes, they resulted in a model with the variables pH, EC, DOC and DOC/TOC, which were all significant. This reduced model obtained an adjusted R^2 of 17.6%. When both the treatments and the four selected soil variables were included together in the model, 67.7% of the variability was explained. The first two canonical axes were significant and explained 77% of this variability, i.e., 52% (Figure S1). The third axis was also significant and explained an additional 10%. The four soil variables were significant and the adjusted R^2 of the model was 55%. Regarding partition, if we discount what is explained by the soil variables, the treatments explained 37.3% of the variability of microbial variables. In contrast, if we discount what is explained by the treatments, the soil variables explained only 3.3% of the variability, with pH being the only significant variable. The two sets of variables had in common 14.3% of the variability explained.

4. Discussion

As expected, the replacement of bare soil by CC modified the microbial structure and enhanced variables of microbial abundance and activity compared to no CC. However, the CC benefits on microbial attributes in the subsequent maize were sometimes reduced or even cancelled out depending on how the CCs were terminated, with the effects evolving from maize pre-emergence till ~3 months after sowing. Water level differently modulated the microbial response to the termination methods, with differences between methods amplified under the high irrigation dose.

The bare soil treatment showed a microbial response in the subsequent maize (57 DAS) globally different from that of the CC treatments (Figure 3, Table 2), with lower values of AMF colonization, MBC, qMIC ($p < 0.1$), F/B, and abundances of total bacteria, fungi and archaea, but a higher metabolic quotient, suggesting a larger level of stress [62]. In contrast,

the presence of CC living roots and their exudates [63], the ability to harbor AMF [12], the higher functional diversity of plants provided by the CC mixture (grass and legume) [64], as well as the contribution of its residues (shoot and root) [65], modified the microbial structure and reinforced microbial abundance and activity relative to no-CC. These effects were favored by the coarse texture and low level of organic matter of the study soil [66].

4.1. Effects of CC Incorporation and Rolling on Soil Microbial Attributes at 57 DAS Maize

Termination by INC, followed by ROL, showed the most positive microbial response overall compared to no CC. INC stood out for high levels of mycorrhizal variables, abundances of total bacteria, fungi and archaea, and high F/B. Being a soil poor in OM and mixed with sand, the incorporation of fresh and fragmented shoot debris into the soil, together with root breakage, provided an important C source which, together with the oxygen influx, stimulated microorganisms [15]. The positive effect on bacteria is well known and results from bringing a C source closer to this group of decomposers with limited mobility [26]. For the same reason, archaea responded very well to CC residue incorporation, although they showed less sensitivity to the termination method, perhaps because of their greater hardiness and adaptability [67]. With respect to fungi, INC did not have a negative effect as might be expected from soil disturbance [23]. Being a single vertical, shallow tillage, the positive effect of the input of organic residues in a poor soil predominated over the negative effect on mycelium due to soil disturbance. Moreover, the negative effect of INC on soil penetration resistance did not affect the improvements mentioned above.

Termination by ROL, especially at a high water level, also enhanced most microbial variables. The positive role of ROL can be attributed to the lack of soil disturbance, moisture protection and higher temperature stability [68], although ROL partly failed to preserve moisture due to ineffective killing (data not shown), so its best performance occurred at a high water level. Muhammad et al. [10] reported the CC positive effect on soil microbial attributes both when residues were incorporated and when residues were left on the surface, with a slight advantage for INC in bacteria and AMF colonization. The relative similarity between both methods was also reported by several studies [35–37] for a variety of soil biochemical and biological parameters. In contrast to INC, leaving CC residues on the surface promoted fungi [26], especially mycelium-formers judging by the high MBC, to the detriment of bacteria. Limited access to shoot residues, together with lower killing effectiveness [38], which reduces the release of labile C forms from dying roots, can explain the low performance of ROL to such an extent that cancelled out the positive effect of CC on bacteria, triggering the F/B ratio. Elfstrand et al. [69] found a higher level of bacteria over time when red clover residues were incorporated than when left on the surface, while the levels of total fungi and AMF tended to equalize in line with our results. That is, INC enhanced both prokaryotes and fungi, while ROL was notable for its negative effect on bacteria.

The slightly worse performance of ROL versus INC is partly in line with the results of the meta-analysis by Kim et al. [9], who found that conventional management with incorporation of CC residues had an overall more positive effect at the microbial level than conservative management without soil disturbance. In our case, INC performance is certainly noticeable compared to when residues are left on the surface after glyphosate (INC > GLY, RGL), but it is less important when the roller crimper is applied alone (INC≈ROL). Then, the differences seem to be more related to glyphosate, which is common in conservative management, than to the non-incorporation of residues. Therefore, in future meta-analyses it would be important to differentiate within the residues left on the surface, whether glyphosate was applied or not.

Soil moisture is a key factor in the abundance and activity of microorganisms [70], so that lack of water has a negative effect with and without tillage [71]. In that line was the microbial response of INC and ROL with the worse performance at a low water level, but this effect was slight given the overlapping ellipses at high and low water levels in

both methods (Figure 3). Bacteria were particularly sensitive to water level, as shown by their positive correlation with soil moisture (r = 0.47 ***). Thus, under INC, bacteria were the most sensitive group to water level [40], so that water lack reduced the positive effect of CC by almost half, while other termination methods without incorporation even cancelled out the positive effect of CC on bacteria. The combination of dry conditions with the consequent restriction in solute diffusion [40], together with the lack of fresh residue input to the soil, may have been especially limiting for bacteria in such a nutrient-poor soil. Lower competition from bacteria in dry conditions may have benefited fungi and archaea, reducing the impact of water stress on these groups, and enhancing F/B. In ROL, archaea were somewhat more sensitive to water level, with slightly higher abundance in water deficit than in well-watered conditions. Archaea, which are slower growing than bacteria, may have benefited from reduced bacterial growth due to both reduced water and lack of residue incorporation [16].

4.2. Effects of Glyphosate with and without Rolling on Soil Microbial Attributes at 57 DAS Maize

The legacy of RGL and GLY on soil microbial attributes of the subsequent maize was quite similar regardless of water level. GLY and RGL share the application of glyphosate, but while CC are left intact in GLY, in RGL a roller is subsequently rolled over forming a mulch of residues on the surface. This mulch can be expected to protect against evaporation resulting in higher soil moisture and lower temperature [72], which are important variables regulating the activity of microorganisms in the soil [70]. Although the numerical value of moisture is somewhat higher and that of temperature somewhat lower in RGL than in GLY (7.70% and 23.6 °C vs. 6.70% and 24.7 °C, respectively), the differences were not significant, suggesting a limited effect of mulch that may have brought the microbial responses of GLY and RGL closer.

Both GLY and RGL showed an overall less positive response relative to CON than INC and ROL. These results agree with Kim et al. [9], who found that benefits of cover cropping on certain microbial variables, such as microbial abundance, phosphatase activity and Shannon's diversity index, were reduced under chemical compared to mechanical termination. Other studies found a worse performance of glyphosate only versus mowing (closer to our ROL because it is mechanical and the residue remains on the surface) as Liang et al. [14] for microbial biomass and nitrification potential, or Adetunji et al. [7] for beta-glucosidase. In contrast, Romdahne et al. [18] found no differences between glyphosate and other termination methods (roller crimper, frost) in bacterial abundance, but they did in certain N cycling parameters.

It is known that besides plants, microbes including fungi and bacteria contain the molecular target of glyphosate [28]. Different groups of microorganisms display variations in this target's sensitivity which, besides some differences in the glyphosate degradation capacity or in coping with the oxidative damage caused by this herbicide, can generate important shifts in the microbial community composition in the soil [27]. In this study, the legacy effects of glyphosate on the major groups of microorganisms were strong and modulated by water level. At a high water level, we found a positive effect of GLY/RGL on bacteria, which was lower than that of INC in the case of GLY, but much higher than the ROL performance. Apart from the CC effect itself, the positive effect may be related to that found by other studies with commercial doses [32,73,74]. This is attributed to the glyphosate nature as a source of nutrients that bacteria can extensively exploit [28,75], being favored by the scarcity of colloids that could provide protection against biodegradation. Furthermore, the effectiveness of killing by glyphosate and rapid release of labile C forms after root decay [32] provides an additional stimulus for bacteria. Compared to INC, GLY, with erect and intact shoot, can hardly compete with the advantage of intimate in-soil contact of previously fragmented shoot residues with bacteria. In dry conditions, it seems that water lack, together with the lack of residue incorporation, cancelled out the expected benefit of CC on bacteria, except for INC as mentioned above. Sheng et al. [73] reported

that the stimulatory effect of glyphosate did not occur under dry conditions for the more drought-sensitive Gram-bacteria, in line with our work.

Termination by GLY also favored archaeal abundance in well-watered conditions. As with bacteria, our results are in line with Means et al. [76], who reported a positive effect of glyphosate when coupled with adequate soil water conditions. However, under a low water level, glyphosate reduced the positive effect of CC compared to INC and ROL, suggesting an adverse effect. Some studies reported a negative effect of glyphosate on total archaea [74] or on nitrifying archaea [77], although other studies indicate neutral effects on nitrifying archaea [78].

Regarding fungi, fewer studies analyzed the effect of glyphosate compared to bacteria. In general, none or minor effects were reported [28,79–81]; in some cases, sensitivity depended on fungal taxa [82], and in others, stimulation of pathogenic fungi were inherited by the main crop [29]. Compared to INC and ROL, and for a high water level, the CC positive effect on fungi was reduced by more than half by glyphosate, indicating an adverse effect on this group in agreement with the findings of Vazquez et al. [83]. Sheng et al. [73] suggested a higher enzyme sensitivity to glyphosate in fungi than in bacteria, which could explain why few fungal groups are able to biodegrade it [74]. In our case, with a potentially more sensitive, coarse-textured soil [84], higher soil water availability may have enhanced the effectiveness of glyphosate [42] and altered the composition of exudates [39], being affected by those fungal groups with higher susceptibility [82].

Taken together, the responses of the major groups of microorganisms confirm that well-watered conditions would tend to widen the differences between CC termination methods, mainly because under water restriction microbes become resource limited [85], equaling microbial responses, or because the effect of glyphosate is reduced [42]. It is worth noting that at a high water level, RGL clearly differed from ROL due to the reinforcement of the glyphosate effect under optimum water conditions, while in a low water level, RGL maintained similarities with both GLY and ROL in a kind of intermediate behavior.

Regarding AMF, our results suggest that the use of glyphosate to terminate CC, without subsequent incorporation, tends to cancel out the expected positive effect of CC on the studied mycorrhizal variables regardless of the irrigation level. If CON is ignored to compare only methods with CC, glyphosate treatments showed lower hyphal length and AMF colonization ($p < 0.1$) than INC, suggesting a negative effect of glyphosate in line with studies in greenhouse [86,87] and field conditions [31,88]. However, being the means for GLY and RGL lower than for ROL in numerical terms, the differences were not significant at 95%, and we cannot conclude a negative effect of glyphosate on mycorrhizal variables. This is in line with other studies in greenhouse [80] and field conditions [73].

With respect to MBC and metabolic variables, termination methods and specifically glyphosate had a rather neutral effect, with little sensitivity to water level. In a meta-analysis, Nguyen et al. [89] also found little effect of glyphosate on MBC and basal respiration at the commercial field dose. In contrast, in a greenhouse trial with glyphosate-resistant soybean varieties, Alan et al. [90] reported adverse changes in MBC and metabolic parameters such as basal respiration, qCO_2 and qMIC resulting from glyphosate use, which were amplified under water deficit conditions. Apart from water level, other studies reported a short-term increase in soil respiration and SIR [74,80,89] that is explained by both the use of glyphosate as a C-source and the increased availability of readily available C after plant death. In our study, GLY showed a trend towards higher SIR values than INC, which was not statistically significant. It is more in line with Dennis et al. [30], who reported limited effects of glyphosate at the metabolic level.

4.3. Physicochemical Soil Properties and Microbial Attributes as Affected by CC Termination Methods

The physicochemical properties explained the soil microbial response to a limited extent (almost 18%), and did so mainly due to the treatments themselves as they shared around 14% of variability. This may be due to the weak effect that the termination methods

had on soil properties (Table 3), possibly because it was a short-term experiment. We attribute the lower EC values in ROL (which suggest a lower nutrient content) to its lower killing effectiveness [38], which may: (i) reduce nutrient inputs from root decomposition, and (ii) lead to higher nutrient extraction by the higher CC regrowth (data not shown). The last would explain the relatively low soil moisture in ROL, whereas RGL, more effective for killing due to glyphosate and at the same time generating a protective mulch, had the highest soil moisture at high water level. Soil disturbance and the absence of mulch would explain the lower soil moisture in INC [72]. Due to the inverse relationship between PR and soil moisture found in the original soil [45], RGL and GLY with higher soil moisture, showed the lowest resistance to penetration, whereas INC with lower moisture showed the highest.

In general, a high water level tended to increase the differences between termination methods relative to soil properties (soil moisture, N/P; and to a lesser extent pH, EC, DOC and DOC/TOC) (Table 1). In contrast to Romdhane et al. [18], with significant field spatial variability and high explanatory capacity of soil properties, the low variability explained by soil variables excluding the termination effect (3%) is consistent with a microcosm experiment where the soil was homogenized. The fact that pH showed correlations with a number of microbial variables (Table S5), and was the only significant variable in the final RDA analysis, confirms the importance of pH as a driver of microbial populations, especially for bacteria [91]. The different inputs and rates of residue decomposition (shoot and root), as well as glyphosate and its effect on nutrient availability, may explain the relative importance of variables such as EC, DOC, and DOC/TOC, which also showed remarkable correlations with the microbial variables (Table S5).

4.4. Legacy Effects of CC Termination Methods over Time

Once the CCs were terminated and as the maize grew, the microbial populations were progressively shaped by maize exudates [63], so that the differences due to the different termination methods were reduced (Table S2). With the progressive decomposition of dead roots over time, labile C sources were released. This stimulated the growth of different groups of organisms, especially bacteria and archaea which are more dependent on substrate location within the soil [26], whereas fungi populations tended to be stabilized or decreased. INC was the method least affected by the passing of time, with a sustained positive effect for up to 3 months due to the decomposition of both roots and shoots within the soil. By contrast, fungi were growing in ROL, as bacteria slowed down due to reducing substrate availability, as explained above. Several studies indicate transitory glyphosate effects on soil microbial variables [89,92]. In our study, we found an adverse effect on bacteria two weeks after glyphosate application, in agreement with Zobiole et al. [93]. However, this vanished and changed to a neutral or even positive effect three months later, as explained above. The rapid evolution of the response over time may be due to changes in exudates [39] and biodegradation of glyphosate [43], and is also affected by water availability. These complex interactions between glyphosate, soil moisture, plant, soil type, and the passing of time may explain the disparity of glyphosate effects on soil microbiota found in the literature.

5. Conclusions

Most soil microbial variables measured in the subsequent maize were affected by the CC termination method and its interaction with the water level. From the formulated hypotheses, it was confirmed that a high water level amplified the differences between treatments. Overall, mowing and incorporation of CC residues was the most beneficial method for the studied microbial attributes, enhancing mycorrhizal variables and abundances of total bacterial, fungi and archaea, with less affectation by water level and the passing of time. Termination by rolling crimper was the second best method, mainly when well-watered. This was also associated with a higher fungal abundance, F/B, and MBC. Fungal stimulation was similar in both INC and ROL, whereas bacterial abundance

dropped under ROL, but INC did not specifically boost bacteria and ROL fungi, as was hypothesized. Both glyphosate application methods (GLY and RGL) showed a similar soil microbial response regardless of the irrigation rate, but was greatly influenced by it, with notable changes between maize preemergence and ~3 months later. Only under a low water level did RGL resemble both GLY and ROL, so our third hypothesis was not fully met. Changes in glyphosate effectiveness as herbicide, exudates, and biodegradation may explain the complexity of the microbial response under chemical termination. The adverse legacy effects of glyphosate found after three months on fungi and archaea under certain water conditions should be further studied. The way in which CC are terminated, and its interaction with water availability may cancel out the expected benefits of CC on certain microbial attributes. Further research under different environments and field conditions is needed to better understand the mechanisms involved and to improve recommendations for more sustainable agriculture.

6. Patents

Mini-roller crimper https://www.upm.es/recursosidi/offers-resources/patentes/rodillo-agricola-desgarrador/ (publication number: ES1290154U).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12123002/s1. Table S1: Set of primers selected from previous studies for the quantification of the major groups of microorganisms in the soil [94–96]; Table S2: Soil microbial variables at maize pre-emergence and 57 days after sowing maize; Table S3: AMF colonization, microbial biomass and variables of microbial activity at 57 days after sowing maize; Table S4: Soil physicochemical variables at 57 days after sowing maize; Table S5: Pearson correlation matrix of soil physicochemical and soil microbial variables; Figure S1: Redundancy Analysis (RDA) results for soil microbial variables, soil properties and ten combined treatments of termination methods and water levels. Refs. [94–96] are cited in the Supplementary Materials file.

Author Contributions: Conceptualization, I.M.-S. and C.H.; Data curation, N.C. and C.H.; Formal analysis, N.C., M.A.I. and C.H.; Funding acquisition, C.H.; Investigation, N.C., K.U., M.N., A.M., I.M.-S. and C.H.; Methodology, M.N., M.A.I., A.M., I.M.-S. and C.H.; Supervision, C.H.; Validation, C.H.; Visualization, N.C. and C.H.; Writing—original draft, N.C. and C.H.; Writing—review & editing, N.C., K.U., M.N., M.A.I., A.M., I.M.-S. and C.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research has been funded by the Spanish Ministry of Science and Innovation through the project AGL2017-83283-C2-1-R, by the Community of Madrid and the European Social Fund through the project AGRISOST-CM S2018/BAA-4330, and by the "Programa de becas de postgrado en el exterior Carlos Antonio Lopez (BECAL)" of Paraguay.

Data Availability Statement: The data sets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments: We are grateful to the Ministry of Science and Innovation of Spain for the financial support (AGL2017-83283-C2-1-R), to the Comunidad de Madrid (Spain) and Structural Funds 2014-2020 (ERDF and ESF) for the financial support (project AGRISOST-CM S2018/BAA-4330). Nelly Centurión is the recipient of a grant from the "Programa de becas de postgrado en el exterior Carlos Antonio Lopez (BECAL), Paraguay". In addition, we thank Javier González-Canales for his laboratory work.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Schipanski, M.E.; Barbercheck, M.; Douglas, M.R.; Finney, D.M.; Haider, K.; Kaye, J.P.; Kemanian, A.R.; Mortensen, D.A.; Ryan, M.R.; Tooker, J.; et al. A framework for evaluating ecosystem services provided by cover crops in agroecosystems. *Agric. Syst.* **2014**, *125*, 12–22. [CrossRef]
2. Kaye, J.P.; Quemada, M. Using cover crops to mitigate and adapt to climate change. A review. *Agron. Sustain. Dev.* **2017**, *37*, 4. [CrossRef]
3. Abdalla, M.; Hastings, A.; Cheng, K.; Yue, Q.; Chadwick, D.; Espenberg, M.; Truu, J.; Rees, R.M.; Smith, P. A critical review of the impacts of cover crops on nitrogen leaching, net greenhouse gas balance and crop productivity. *Glob. Change Biol.* **2019**, *25*, 2530–2543. [CrossRef]
4. Jian, S.; Li, J.; Wang, G.; Kluber, L.A.; Schadt, C.W.; Liang, J.; Mayes, M.A. Multi-year incubation experiments boost confidence in model projections of long-term soil carbon dynamics. *Nat. Commun.* **2020**, *11*, 5864. [CrossRef]
5. Garba, I.I.; Bell, L.W.; Williams, A. Cover crop legacy impacts on soil water and nitrogen dynamics, and on subsequent crop yields in drylands: A meta-analysis. *Agron. Sustain. Dev.* **2022**, *42*, 34. [CrossRef]
6. Vukicevich, E.; Lowery, T.; Bowen, P.; Úrbez-Torres, J.R.; Hart, M. Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. *Agron. Sustain. Dev.* **2016**, *36*, 48. [CrossRef]
7. Adetunji, A.T.; Ncube, B.; Mulidzi, R.; Lewu, F.B. Management impact and benefit of cover crops on soil quality: A review. *Soil Tillage Res.* **2020**, *204*, 104717. [CrossRef]
8. Thapa, V.R.; Ghimire, R.; Acosta-Martínez, V.; Marsalis, M.A.; Schipanski, M.E. Cover crop biomass and species composition affect soil microbial community structure and enzyme activities in semiarid cropping systems. *Appl. Soil Ecol.* **2021**, *157*, 103735. [CrossRef]
9. Kim, N.; Zabaloy, M.C.; Guan, K.; Villamil, M.B. Do cover crops benefit soil microbiome? A meta-analysis of current research. *Soil Biol. Biochem.* **2020**, *142*, 107701. [CrossRef]
10. Muhammad, I.; Wang, J.; Sainju, U.M.; Zhang, S.; Zhao, F.; Khan, A. Cover cropping enhances soil microbial biomass and affects microbial community structure: A meta-analysis. *Geoderma* **2021**, *381*, 114696. [CrossRef]
11. Bowles, T.M.; Jackson, L.E.; Loeher, M.; Cavagnaro, T.R. Ecological intensification and arbuscular mycorrhizas: A meta-analysis of tillage and cover crop effects. *J. Appl. Ecol.* **2017**, *54*, 1785–1793. [CrossRef]
12. García-González, I.; Hontoria, C.; Gabriel, J.L.; Alonso-Ayuso, M.; Quemada, M. Cover crops to mitigate soil degradation and enhance soil functionality in irrigated land. *Geoderma* **2018**, *322*, 81–88. [CrossRef]
13. Smith, S.E.; Read, D.J. *Mycorrhizal Symbiosis*, 3rd ed.; Elsevier Ltd.: NewYork, NY, USA, 2008.
14. Liang, S.; Grossman, J.; Shi, W. Soil microbial responses to winter legume cover crop management during organic transition. *Eur. J. Soil Biol.* **2014**, *65*, 15–22. [CrossRef]
15. Nevins, C.J.; Nakatsu, C.; Armstrong, S. Characterization of microbial community response to cover crop residue decomposition. *Soil Biol. Biochem.* **2018**, *127*, 39–49. [CrossRef]
16. 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] [PubMed]
17. Hontoria, C.; García-González, I.; Quemada, M.; Roldán, A.; Alguacil, M.M. The cover crop determines the AMF community composition in soil and in roots of maize after a ten-year continuous crop rotation. *Sci. Total Environ.* **2019**, *660*, 913–922. [CrossRef] [PubMed]
18. Romdhane, S.; Spor, A.; Busset, H.; Falchetto, L.; Martin, J.; Bizouard, F.; Bru, D.; Breuil, M.; Philippot, L.; Cordeau, S. Cover crop management practices rather than composition of cover crop mixtures affect bacterial communities in no-till agroecosystems. *Front. Microbiol.* **2019**, *10*, 1618. [CrossRef]
19. Jani, A.D.; Grossman, J.M.; Smyth, T.J.; Hu, S. Influence of soil inorganic nitrogen and root diameter size on legume cover crop root decomposition and nitrogen release. *Plant Soil* **2015**, *393*, 57–68. [CrossRef]
20. Daryanto, S.; Fu, B.; Wang, L.; Jacinthe, P.; Zhao, W. Quantitative synthesis on the ecosystem services of cover crops. *Earth-Sci. Rev.* **2018**, *185*, 357–373. [CrossRef]
21. Alonso-Ayuso, M.; Gabriel, J.L.; Hontoria, C.; Ibáñez, M.Á.; Quemada, M. The cover crop termination choice to designing sustainable cropping systems. *Eur. J. Agron.* **2020**, *114*, 126000. [CrossRef]
22. Turmel, M.; Speratti, A.; Baudron, F.; Verhulst, N.; Govaerts, B. Crop residue management and soil health: A systems analysis. *Agric. Syst.* **2015**, *134*, 6–16. [CrossRef]
23. Mbuthia, L.W.; Acosta-Martínez, V.; DeBruyn, J.; Schaeffer, S.; Tyler, D.; Odoi, E.; Mpheshea, M.; Walker, F.; Eash, N. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. *Soil Biol. Biochem.* **2015**, *89*, 24–34. [CrossRef]
24. Chen, H.; Dai, Z.; Veach, A.M.; Zheng, J.; Xu, J.; Schadt, C.W. Global meta-analyses show that conservation tillage practices promote soil fungal and bacterial biomass. *Agric. Ecosyst. Environ.* **2020**, *293*, 106841. [CrossRef]
25. Hage-Ahmed, K.; Rosner, K.; Steinkellner, S. Arbuscular mycorrhizal fungi and their response to pesticides. *Pest Manag. Sci.* **2019**, *75*, 583–590. [CrossRef] [PubMed]
26. Six, J.; Frey, S.D.; Thiet, R.K.; Batten, K.M. Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Sci. Soc. Am. J.* **2006**, *70*, 555–569. [CrossRef]

27. Van Bruggen, A.H.; He, M.M.; Shin, K.; Mai, V.; Jeong, K.C.; Finckh, M.R.; Morris, J.G., Jr. Environmental and health effects of the herbicide glyphosate. *Sci. Total Environ.* **2018**, *616*, 255–268. [CrossRef]
28. Zabaloy, M.C.; Allegrini, M.; Hernandez Guijarro, K.; Behrends Kraemer, F.; Morrás, H.; Erijman, L. Microbiomes and glyphosate biodegradation in edaphic and aquatic environments: Recent issues and trends. *World J. Microbiol. Biotechnol.* **2022**, *38*, 98. [CrossRef]
29. Van Bruggen, A.H.; Finckh, M.R.; He, M.; Ritsema, C.J.; Harkes, P.; Knuth, D.; Geissen, V. Indirect effects of the herbicide glyphosate on plant, animal and human health through its effects on microbial communities. *Front. Environ. Sci.* **2021**, *9*, 464. [CrossRef]
30. Dennis, P.G.; Kukulies, T.; Forstner, C.; Orton, T.G.; Pattison, A.B. The effects of glyphosate, glufosinate, paraquat and paraquat-diquat on soil microbial activity and bacterial, archaeal and nematode diversity. *Sci. Rep.* **2018**, *8*, 2119. [CrossRef]
31. Druille, M.; Omacini, M.; Golluscio, R.A.; Cabello, M.N. Arbuscular mycorrhizal fungi are directly and indirectly affected by glyphosate application. *Appl. Soil Ecol.* **2013**, *72*, 143–149. [CrossRef]
32. Imparato, V.; Santos, S.S.; Johansen, A.; Geisen, S.; Winding, A. Stimulation of bacteria and protists in rhizosphere of glyphosate-treated barley. *Appl. Soil Ecol.* **2016**, *98*, 47–55. [CrossRef]
33. Navarro-Miró, D.; Blanco-Moreno, J.M.; Ciaccia, C.; Testani, E.; Iocola, I.; Depalo, L.; Burgio, G.; Kristensen, H.L.; Hefner, M.; Tamm, K. The concurrent assessment of agronomic, ecological and environmental variables enables better choice of agroecological service crop termination management. *J. Appl. Ecol.* **2022**, *59*, 1026–1037. [CrossRef]
34. Mirsky, S.B.; Curran, W.S.; Mortensen, D.A.; Ryan, M.R.; Shumway, D.L. Control of cereal rye with a roller/crimper as influenced by cover crop phenology. *Agron. J.* **2009**, *101*, 1589–1596. [CrossRef]
35. Manici, L.M.; Caputo, F.; Nicoletti, F.; Leteo, F.; Campanelli, G. The impact of legume and cereal cover crops on rhizosphere microbial communities of subsequent vegetable crops for contrasting crop decline. *Biol. Control* **2018**, *120*, 17–25. [CrossRef]
36. Navarro-Miró, D.; Blanco-Moreno, J.M.; Ciaccia, C.; Chamorro, L.; Testani, E.; Kristensen, H.L.; Hefner, M.; Tamm, K.; Bender, I.; Jakop, M. Agroecological service crops managed with roller crimper reduce weed density and weed species richness in organic vegetable systems across Europe. *Agron. Sustain. Dev.* **2019**, *39*, 55. [CrossRef]
37. Bloszies, S.A.; Reberg-Horton, S.C.; Heitman, J.L.; Woodley, A.L.; Grossman, J.M.; Hu, S. Legume cover crop type and termination method effects on labile soil carbon and nitrogen and aggregation. *Agron. J.* **2022**, *114*, 1817–1832. [CrossRef]
38. Kornecki, T.S.; Kichler, C.M. Effectiveness of Cover Crop Termination Methods on No-Till Cantaloupe. *Agriculture* **2022**, *12*, 66. [CrossRef]
39. Williams, A.; de Vries, F.T. Plant root exudation under drought: Implications for ecosystem functioning. *New Phytol.* **2020**, *225*, 1899–1905. [CrossRef]
40. Manzoni, S.; Schimel, J.P.; Porporato, A. Responses of soil microbial communities to water stress: Results from a meta-analysis. *Ecology* **2012**, *93*, 930–938. [CrossRef] [PubMed]
41. Schimel, J.P. Life in dry soils: Effects of drought on soil microbial communities and processes. *Annu. Rev. Ecol. Evol. Syst.* **2018**, *49*, 409–432. [CrossRef]
42. Mollaee, M.; Matloob, A.; Mobli, A.; Thompson, M.; Chauhan, B.S. Response of glyphosate-resistant and susceptible biotypes of Echinochloa colona to low doses of glyphosate in different soil moisture conditions. *PLoS ONE* **2020**, *15*, e0233428. [CrossRef]
43. Bento, C.P.M.; Yang, X.; Gort, G.; Xue, S.; van Dam, R.; Zomer, P.; Mol, H.G.J.; Ritsema, C.J.; Geissen, V. Persistence of glyphosate and aminomethylphosphonic acid in loess soil under different combinations of temperature, soil moisture and light/darkness. *Sci. Total Environ.* **2016**, *572*, 301–311. [CrossRef]
44. Köppen, W.; Geiger, R. *Handbuch Der Klimatologie*; Gebrüder Borntraeger: Berlin, Germany, 1930.
45. Gabriel, J.L.; García-González, I.; Quemada, M.; Martin-Lammerding, D.; Alonso-Ayuso, M.; Hontoria, C. Cover crops reduce soil resistance to penetration by preserving soil surface water content. *Geoderma* **2021**, *386*, 114911. [CrossRef]
46. Vierheilig, H.; Coughlan, A.P.; Wyss, U.; Piché, Y. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Appl. Environ. Microbiol.* **1998**, *64*, 5004–5007. [CrossRef]
47. McGonigle, T.P.; Miller, M.H.; Evans, D.G.; Fairchild, G.L.; Swan, J.A. A new method which gives an objective measure of colonization of roots by vesicular—Arbuscular mycorrhizal fungi. *New Phytol.* **1990**, *115*, 495–501. [CrossRef] [PubMed]
48. Nelson, D.W.; Sommers, L.E. Total carbon, organic carbon, and organic matter. In *Methods of Soil Analysis: Part 3 Chemical Methods*; Soil Science Society of America and American Society of Agronomy: Madison, WI, USA, 1996; Volume 5, pp. 961–1010. [CrossRef]
49. Bremner, J.M.; Mulvaney, C.S. Total nitrogen. In *Methods of Soil Analysis*; Page, A.L., Miller, R.H., Keeney, D.R., Eds.; Soil Science Society of America and American Society of Agronomy: Madison, WI, USA, 1982; Part 2; pp. 595–624.
50. Yakovchenko, V.P.; Sikora, L.J. Modified dichromate method for determining low concentrations of extractable organic carbon in soil. *Commun. Soil Sci. Plant Anal.* **1998**, *29*, 421–433. [CrossRef]
51. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **1987**, *19*, 703–707. [CrossRef]
52. Alef, K.; Nannipieri, P. *Methods in Applied Soil Microbiology and Biochemistry*; Academic Press: Cambridge, MA, USA, 1995.
53. Anderson, J.P.; Domsch, K.H. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biol. Biochem.* **1978**, *10*, 215–221. [CrossRef]
54. Anderson, T.; Domsch, A.K. The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. *Soil Biol. Biochem.* **1993**, *25*, 393–395. [CrossRef]

55. Sparling, G.P. Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. *Soil Res.* **1992**, *30*, 195–207. [CrossRef]
56. García-González, I.; Quemada, M.; Gabriel, J.L.; Hontoria, C. Arbuscular mycorrhizal fungal activity responses to winter cover crops in a sunflower and maize cropping system. *Appl. Soil Ecol.* **2016**, *102*, 10–18. [CrossRef]
57. Jakobsen, I.; Abbott, L.K.; Robson, A.D. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. *New Phytol.* **1992**, *120*, 371–380. [CrossRef]
58. Tennant, D. A test of a modified line intersect method of estimating root length. *J. Ecol.* **1975**, *63*, 995–1001. [CrossRef]
59. RStudio Team. *RStudio: Integrated Development Environment for R*; RStudio, PBC: Boston, MA, USA, 2022. Available online: http://www.rstudio.com/ (accessed on 10 May 2022).
60. Oksanen, J.; Blanchet, F.G.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; Minchin, P.R.; O'Hara, R.B.; Simpson, G.L.; Solymos, P. *Vegan: Community Ecology Package*, R Package Version 2.5; Viena, Austria, 2020. Available online: https://www.researchgate.net/publication/346579465_vegan_community_ecology_package_version_25-7_November_2020 (accessed on 15 July 2022).
61. Wickham, H. *Data Analysis, in Anonymous Ggplot2*; Springer: Berlin/Heidelberg, Germany, 2016; pp. 189–201.
62. Dilly, O. Microbial energetics in soils. In *Anonymous Microorganisms in Soils: Roles in Genesis and Functions*; Springer: Berlin/Heidelberg, Germany, 2005; pp. 123–138.
63. Sasse, J.; Martinoia, E.; Northen, T. Feed your friends: Do plant exudates shape the root microbiome? *Trends Plant Sci.* **2018**, *23*, 25–41. [CrossRef]
64. Chen, L.; Yuan, P.; Pozsgai, G.; Chen, P.; Zhu, H.; You, M. The impact of cover crops on the predatory mite Anystis baccarum (Acari, Anystidae) and the leafhopper pest Empoasca onukii (Hemiptera, Cicadellidae) in a tea plantation. *Pest Manag. Sci.* **2019**, *75*, 3371–3380. [CrossRef] [PubMed]
65. Drost, S.M.; Rutgers, M.; Wouterse, M.; De Boer, W.; Bodelier, P.L. Decomposition of mixtures of cover crop residues increases microbial functional diversity. *Geoderma* **2020**, *361*, 114060. [CrossRef]
66. Cordeiro, C.F.D.S.; Rodrigues, D.R.; Rocha, C.H.; Araujo, F.F.; Echer, F.R. Glomalin and microbial activity affected by cover crops and nitrogen management in sandy soil with cotton cultivation. *Appl. Soil Ecol.* **2021**, *167*, 104026. [CrossRef]
67. Valentine, D.L. Adaptations to energy stress dictate the ecology and evolution of the Archaea. *Nat. Rev. Microbiol.* **2007**, *5*, 316–323. [CrossRef] [PubMed]
68. Vincent-Caboud, L.; Casagrande, M.; David, C.; Ryan, M.R.; Silva, E.M.; Peigne, J. Using mulch from cover crops to facilitate organic no-till soybean and maize production. A review. *Agron. Sustain. Dev.* **2019**, *39*, 45. [CrossRef]
69. Elfstrand, S. *Impact of Green Manure on Soil Organisms*; Swedish University of Agricultural Sciences: Upsala, Sweden, 2007.
70. Davidson, E.A.; Belk, E.; Boone, R.D. Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. *Glob. Change Biol.* **1998**, *4*, 217–227. [CrossRef]
71. Kaurin, A.; Mihelič, R.; Kastelec, D.; Grčman, H.; Bru, D.; Philippot, L.; Suhadolc, M. Resilience of bacteria, archaea, fungi and N-cycling microbial guilds under plough and conservation tillage, to agricultural drought. *Soil Biol. Biochem.* **2018**, *120*, 233–245. [CrossRef]
72. Teasdale, J.R.; Mohler, C.L. Light transmittance, soil temperature, and soil moisture under residue of hairy vetch and rye. *Agron. J.* **1993**, *85*, 673–680. [CrossRef]
73. Sheng, M.; Hamel, C.; Fernandez, M.R. Cropping practices modulate the impact of glyphosate on arbuscular mycorrhizal fungi and rhizosphere bacteria in agroecosystems of the semiarid prairie. *Can. J. Microbiol.* **2012**, *58*, 990–1001. [CrossRef]
74. Zhelezova, A.D.; Manucharova, N.A.; Gorlenko, M.V. Structural and functional characteristics of the prokaryotic community of soddy-podzolic soil influenced by the herbicide glyphosate. *Mosc. Univ. Soil Sci. Bull.* **2018**, *73*, 89–94. [CrossRef]
75. Chen, Y.; Chen, W.; Huang, Y.; Li, J.; Zhong, J.; Zhang, W.; Zou, Y.; Mishra, S.; Bhatt, P.; Chen, S. Insights into the microbial degradation and resistance mechanisms of glyphosate. *Environ. Res.* **2022**, *215*, 114153. [CrossRef]
76. Means, N.E.; Kremer, R.J. Influence of soil moisture on root colonization of glyphosate-treated soybean by Fusarium species. *Commun. Soil Sci. Plant Anal.* **2007**, *38*, 1713–1720. [CrossRef]
77. Jenkins, M.; Locke, M.; Reddy, K.; McChesney, D.S.; Steinriede, R. Glyphosate applications, glyphosate resistant corn, and tillage on nitrification rates and distribution of nitrifying microbial communities. *Soil Sci. Soc. Am. J.* **2017**, *81*, 1371–1380. [CrossRef]
78. Zabaloy, M.C.; Carné, I.; Viassolo, R.; Gómez, M.A.; Gomez, E. Soil ecotoxicity assessment of glyphosate use under field conditions: Microbial activity and community structure of Eubacteria and ammonia-oxidising bacteria. *Pest Manag. Sci.* **2016**, *72*, 684–691. [CrossRef] [PubMed]
79. Ratcliff, A.W.; Busse, M.D.; Shestak, C.J. Changes in microbial community structure following herbicide (glyphosate) additions to forest soils. *Appl. Soil Ecol.* **2006**, *34*, 114–124. [CrossRef]
80. Bruckner, A.; Schmerbauch, A.; Ruess, L.; Heigl, F.; Zaller, J. Foliar Roundup application has minor effects on the compositional and functional diversity of soil microorganisms in a short-term greenhouse experiment. *Ecotoxicol. Environ. Saf.* **2019**, *174*, 506–513. [CrossRef] [PubMed]
81. Kepler, R.M.; Epp Schmidt, D.J.; Yarwood, S.A.; Cavigelli, M.A.; Reddy, K.N.; Duke, S.O.; Bradley, C.A.; Williams, M.M., Jr.; Buyer, J.S.; Maul, J.E. Soil microbial communities in diverse agroecosystems exposed to the herbicide glyphosate. *Appl. Environ. Microbiol.* **2020**, *86*, 1744. [CrossRef]
82. Schlatter, D.C.; Yin, C.; Burke, I.; Hulbert, S.; Paulitz, T. Location, root proximity, and glyphosate-use history modulate the effects of glyphosate on fungal community networks of wheat. *Microb. Ecol.* **2018**, *76*, 240–257. [CrossRef]

83. Vázquez, M.B.; Moreno, M.V.; Amodeo, M.R.; Bianchinotti, M.V. Efecto del glifosato en las comunidades fúngicas del suelo: Estudio a campo. *Rev. Argent. Microbiol.* **2021**, *53*, 11–20.
84. Nguyen, D.B.; Rose, M.T.; Rose, T.J.; Van Zwieten, L. Effect of glyphosate and a commercial formulation on soil functionality assessed by substrate induced respiration and enzyme activity. *Eur. J. Soil Biol.* **2018**, *85*, 64–72. [CrossRef]
85. Schimel, J.P.; Bennett, J. Nitrogen mineralization: Challenges of a changing paradigm. *Ecology* **2004**, *85*, 591–602. [CrossRef]
86. Zaller, J.G.; Heigl, F.; Ruess, L.; Grabmaier, A. Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem. *Sci. Rep.* **2014**, *4*, 5634. [CrossRef] [PubMed]
87. Wilkes, T.I.; Warner, D.J.; Davies, K.G.; Edmonds-Brown, V. Tillage, glyphosate and beneficial arbuscular mycorrhizal fungi: Optimising crop management for plant–fungal symbiosis. *Agriculture* **2020**, *10*, 520. [CrossRef]
88. Helander, M.; Pauna, A.; Saikkonen, K.; Saloniemi, I. Glyphosate residues in soil affect crop plant germination and growth. *Sci. Rep.* **2019**, *9*, 19653. [CrossRef] [PubMed]
89. Nguyen, D.B.; Rose, M.T.; Rose, T.J.; Morris, S.G.; Van Zwieten, L. Impact of glyphosate on soil microbial biomass and respiration: A meta-analysis. *Soil Biol. Biochem.* **2016**, *92*, 50–57. [CrossRef]
90. Alan, M.Z.; Fabiano, A.P.; Jlio, C.A.N.; Leandro, P.P.; Francisco, A.N.; Fabrcio, R.A. Microbiological attributes in a Latosol in glyphosate application under water deficit conditions. *Afr. J. Agric. Res.* **2014**, *9*, 2495–2505. [CrossRef]
91. Buyer, J.S.; Teasdale, J.R.; Roberts, D.P.; Zasada, I.A.; Maul, J.E. Factors affecting soil microbial community structure in tomato cropping systems. *Soil Biol. Biochem.* **2010**, *42*, 831–841. [CrossRef]
92. Wolmarans, K.; Swart, W.J. Influence of glyphosate, other herbicides and genetically modified herbicide resistant crops on soil microbiota: A review. *S. Afr. J. Plant Soil* **2014**, *31*, 177–186. Available online: https://hdl.handle.net/10520/EJC163053 (accessed on 15 July 2022). [CrossRef]
93. Zobiole, L.; Kremer, R.J.; Oliveira, R.S., Jr.; Constantin, J. Glyphosate affects micro-organisms in rhizospheres of glyphosate-resistant soybeans. *J. Appl. Microbiol.* **2011**, *110*, 118–127. [CrossRef] [PubMed]
94. White, T.; Bruns, T.; Lee, S.; Taylor, J. *Amplification and Direct Sequencing of Fungal Ribosomal RNA Gnes for Phylogenetics*; Elsevier: Amsterdam, The Netherlands, 1990; pp. 315–322.
95. López-Gutiérrez, J.; Henry, S.; Hallet, S.; Martin-Laurent, F.; Catroux, G.; Philippot, L. Quantification of a novel group of nitrate-reducing bacteria in the environment by real-time PCR. *J. Microbiol. Methods* **2004**, *57*, 399–407. [CrossRef] [PubMed]
96. Ochsenreiter, T.; Selezi, D.; Quaiser, A.; Bonch-Osmolovskaya, L.; Schleper, C. Diversity and abundance of Crenarchaeota in terrestrial habitats studied by 16S RNA surveys and real time PCR. *Environ. Microbiol.* **2003**, *5*, 787–797. [CrossRef] [PubMed]