sustainability

MDPI

Article

Functional Diversity of Soil Microbial Communities in Response to the Application of Cefuroxime and/or Antibiotic-Resistant *Pseudomonas putida* Strain MC1

Kamila Orlewska [1], Anna Markowicz [2], Zofia Piotrowska-Seget [2], Joanna Smoleń-Dzirba [1] and Mariusz Cycoń [1,*]

[1] Department of Microbiology and Virology, School of Pharmacy with the Division of Laboratory Medicine, Medical University of Silesia, Jagiellońska 4, 41-200 Sosnowiec, Poland; kamila_orlewska@o2.pl (K.O.); jsmolen@sum.edu.pl (J.S.-D.)

[2] Department of Microbiology, Faculty of Biology and Environmental Protection, University of Silesia, Jagiellońska 28, 40-032 Katowice, Poland; anna.markowicz@us.edu.pl (A.M.); zofia.piotrowska-seget@us.edu.pl (Z.P.-S.)

* Correspondence: mcycon@sum.edu.pl; Tel.: +48-515-270-972

Received: 4 September 2018; Accepted: 30 September 2018; Published: 3 October 2018

Abstract: Cefuroxime (XM), the most commonly prescribed antibiotic from the cephalosporin group, may cause changes in the structure of the soil microbial community, and these changes may also be reflected in the alteration of its functionality. Therefore, due to the lack of studies on this topic, the scope of this study was to assess the functional diversity and catabolic activity of the microbial community in soil treated with XM (1 mg/kg and 10 mg/kg soil) using the community-level physiological profile (CLPP) approach during a 90-day experiment. In addition, the effect of antibiotic-resistant *Pseudomonas putida* strain MC1 (Ps) was also evaluated. The resistance/resilience concept and multifactorial analysis were used to interpret the data. The results showed that the introduction of XM and/or Ps into the soil caused changes in the catabolic activity and functional diversity of the microbial community. A decrease in the values of the CLPP indices (i.e., microbial activity expressed as the average well-color development (AWCD), substrate richness (R), the Shannon-Wiener (H) and evenness (E) indices and the AWCD values for the six carbon substrate groups) for the XM-treated soil was generally detected up to 30 days. In turn, at the same time, the activity measured in the Ps-inoculated soil was higher compared to the control soil. A stimulatory effect of XM at 10 mg/kg (XM10) and XM10+Ps on the utilization pattern of each substrate group was found at the following sampling times (days 60 and 90). The AWCD values for the utilization of amines, amino acids, carbohydrates, carboxylic acids, miscellaneous compounds and polymers for these treatments were found to be up to 2.3-, 3.1-, 2.3-, 13-, 3.4- and 3.3-fold higher compared to the values for the nontreated control, respectively. The resistance of the CLPP indices and the AWCD values for the carbon substrate groups were categorized as follows: E > H > R > AWCD and amino acids = carbohydrates > polymers > amines > miscellaneous > carboxylic acids, respectively. The results suggest a low initial resistance of the soil microbial community to XM and/or Ps, and despite the short-term negative effect, the balance of the soil ecosystem may be disturbed.

Keywords: cefuroxime; *Pseudomonas putida*; catabolic activity; CLPP; the resistance/resilience concept; soil microorganisms

1. Introduction

Antibiotics belong to a group of pharmaceuticals that have recently been excessively used, consequently leading to their accumulation in various parts of the environment, including the

soil. Studies have shown that the concentration of antibiotics in the soil can range from a few nanograms to even 50 mg/kg of soil [1–4]. Antibiotics, as organic compounds, can be subjected to various processes in the soil environment, the most important of which are sorption on soil components, as well as transformation and/or degradation [5–9]. Factoring in both various abiotic and biotic factors, which influence the behavior of the antibiotic in the soil, its degradation time (DT50 or half-life) may vary from less than one (e.g., amoxicillin) [10] up to 3466 (e.g., azithromycin) days [11]. In addition to antibiotics, bacterial strains resistant to these compounds may also enter the environment [12–15]. The consequences of such actions may be the development of antibiotic-resistant microorganisms [16–20]. It has been shown that even very low concentrations of antibiotics can cause changes in the genome of microorganisms and consequently lead to the transfer of resistance genes between individual members of an exposed population of microorganisms. In addition, microorganisms naturally present in the soil can be a source of resistance genes that can be transferred to other microorganisms such as human and animal pathogens [21–24]. In addition to this effect, antibiotics belonging to different classes may adversely affect the soil microflora, possibly manifesting themselves in the change of their structure [15,25–27] and functionality [6,9,28–30]. The consequence of these changes may be the disturbance of the soil ecosystem balance. Data on the effect of antibiotics on soil microorganisms are varied and include results related to the impact on individual species and whole populations of microorganisms obtained on the basis of biochemical and genetic indices analyses [9,30–33].

One of the most frequently used groups of antibiotics are the second-generation cephalosporins, particularly cefuroxime (XM) [34]. The mode of action of this antibiotic involves blocking the synthesis of the bacterial wall and is directed against a wide group of microorganisms [35–37]. For example, the consumption of cefuroxime in many European countries reaches as much as 50% of the total consumption of cephalosporins [34,38,39]. The consequence of such a high consumption of XM is its detection in wastewater and surface waters, with the highest concentration reaching 210 μg/L for wastewater from the pharmaceutical industry and hospitals around the world [40,41]. Considering that currently used wastewater treatment systems do not guarantee 100% removal of antibiotics, these compounds may enter the soil with the sewage sludge. However, there are no reports on concentrations of this antibiotic in the soil environment, and studies on its degradation have shown that in aerobic conditions, its elimination reached 42.8–80% in 64 days [42]. Additionally, data on the effect of XM on soil microorganisms are limited. In our previous studies [43], including the determination of the effect of XM and multidrug resistant *Pseudomonas putida* MC1 on the soil bacterial genetic structure, the antibiotic showed a negative effect at 10 mg/kg in soil on the bacterial population analyzed, resulting in a decrease in its biodiversity after 30, 60 and 90 days from the application of antibiotics. In turn, the effect of strain MC1 on the measured biodiversity indices was not demonstrated. Use of the resistance (RS)/resilience (RL) concept during the 90-day experiment demonstrated the progressively detrimental effect of XM on the genetic structure of the soil bacteria [43]. The changes observed in the diversity of microbial communities under the influence of XM may also be reflected in the altered functions of soil microorganisms, possibly translating into soil processes important for the ecosystem. Therefore, in this study, due to the lack of such studies, we assumed that the application of XM and/or strain MC1 to the soil may result in changes in the functional biodiversity of the soil microorganisms. To obtain this knowledge, the metabolic potential of the soil microbial community analyzed was assessed using the community-level physiological profile (CLPP) approach. In addition, the RS/RL concept and multivariate analysis were used to determine the ability of microorganisms to maintain their activity and functional diversity under the stress conditions caused by XM and/or strain MC1.

2. Materials and Methods

2.1. Experimental Design

The experiment was conducted using soil classified as a sandy loam [43], the parameters of which were determined using the ISO (International Organization for Standardization) standards [44]. The experiment had a random block system and consisted of three replicates for the control soil and soil contaminated with two concentrations of XM (1 mg/kg and 10 mg/kg soil) and/or inoculated with strain MC1. The concentrations of XM reflect the adverse scenarios associated with the entry of large quantities of antibiotics into the soil as a result of the uncontrolled disposal of unused drugs into municipal waste or depositing them in landfills. The experiment used MC1 (accession number in GenBank: MC327770), which was isolated from raw sewage in the presence of cefuroxime [43,45] and identified as *Pseudomonas putida* based on the analysis of the 16S RNA gene [45] and biochemical properties [46]. In addition, the strain MC1 was characterized by resistance to cefuroxime, clindamycin, erythromycin and vancomycin with MIC values above 256 µg/mL [43]. Strain MC1 was introduced into soil at 1.6×10^7 cells/g soil, and the preparation of its suspension was made with the use of a previously described method [45]. All the treatments were incubated in the dark at 22 ± 1 °C for 90 days. On specific days of the experiment (1, 15, 30, 60 and 90 days), samples were randomly removed to determine the biochemical potential of the soil microbial community. The design of the experiment and the list of analyses performed are shown in Figure 1.

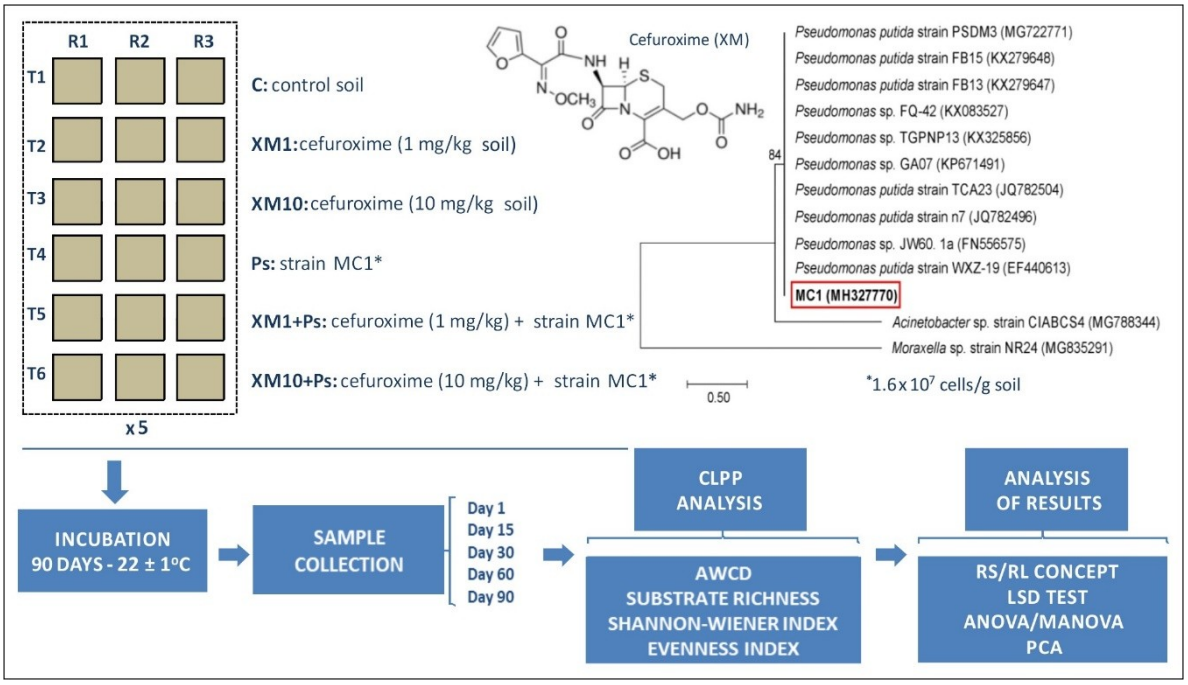

Figure 1. Experimental design and analyses performed. R1–3 = replicates. T1–6 = treatments. CLPP = community-level physiological profile. AWCD = average well-color development. RS = resistance. RL = resilience. LSD = least significant differences. ANOVA = analysis of variance. MANOVA = multivariate analysis of variance. PCA = principal component analysis.

2.2. Determination of the Catabolic Activity of Soil Microorganisms

The biochemical potential of soil microorganisms referred as to CLPP were determined using the Biolog EcoPlate™ system (Biolog Inc., CA, USA). To extract the microbial community, soil suspensions (10 g dry weight of soil in 90 mL sterile 0.85% NaCl solution) were shaken for 1 h, and aliquots of 125 µL were inoculated onto plates and incubated at 22 °C in the dark. The readings were taken at 590 nm after inoculation and at 12 h intervals for 144 h using a BIOLOG™ microplate reader.

The absorbance measurements for individual substrates were corrected against the control well, which contained only water, and by subtracting the absorbance of the first reading to eliminate the background absorbance [47].

2.3. Analysis of the Data

The data obtained from the absorbance measurements were used to determine the CLPP indices, i.e., microbial activity expressed as the average well-color development (AWCD), substrate richness (R_S), and the Shannon-Wiener (H) and evenness (E) indices. The AWCD values were determined using Equation (1) [48].

$$AWCD = \sum OD_i/31 \quad (1)$$

where OD_i is the optical density value from each well.

The R_S and H values were calculated using Equations (2) and (3), respectively [48].

$$H = -\sum p_i \, (\ln p_i) \quad (2)$$

$$E = H/H_{max} = H/\ln R_S \quad (3)$$

where p_i is the ratio of the activity on each substrate (OD_i) to the sum of the activities on all of the substrates ($\sum OD_i$), and R_S is the number of substrates metabolized.

To assess the resistance (RS) of the catabolic activity of the soil microorganisms under the stress conditions caused by XM and/or strain MC1 at individual sampling days of the experiment and resilience (RL), indicating the recovery of balance, on day 90 after the application of the XM and/or strain MC1, indices proposed by Orwin and Wardle [49] were used. The RS index was calculated using Equation (4).

$$RS(t_0) = 1 - 2\,|D_0|\,/(C_0 + |D_0|) \quad (4)$$

where D_0 is the difference between the control (C_0) and the disturbed soil (P_0) at the end of the disturbance (t_0). The RL index was calculated using Equation (5).

$$RL(t_x) = 2\,|D_0|\,/(|D_0| + |D_x|) - 1 \quad (5)$$

where D_0 is as above and D_x is the difference between the nontreated control (C_x) and the exposed soil (P_x) at the time point (t_x) chosen to measure the resilience.

The data obtained for the CLPP indices (i.e., AWCD, R_S, H and E), and the AWCD values for the six carbon substrate groups (i.e., amines, amino acids, carbohydrates, carboxylic acids, miscellaneous and polymers) were analyzed using a three-way analysis of variance (ANOVA) to assess the level of variability (%) related to the factors tested, i.e., the concentration of XM, the presence of MC1 strain and the sampling time [50]. In the case of the data obtained for the RS and RL indices, two-way and one-way ANOVAs were applied to examine the differences among sampling time and differences among the different treatments, respectively. The statistical significance of the differences ($p < 0.05$) was assessed using the least significant differences (LSD) test. A principal component analysis (PCA) of the data for the CLPPs and the AWCD data for the carbon substrate groups was conducted for all the sampling days and separately for each sampling day. The principal component (PC) scores from the PCA were subjected to a three-way and two-way multivariate analysis of variance (MANOVA) for the first and second PCA sets, respectively. The Statistica 13.0 PL software package was used for all of the statistical analyses performed.

3. Results

3.1. The CLPP Indices

The results of this study showed that the introduction of XM and/or strain MC1 to the soil caused changes in the catabolic activity of the microbial community analyzed, and these changes were demonstrated by a significant decrease ($p < 0.05$) in the CLPP indices for the XM-treated soil generally up to 30 days. At the same time, the catabolic activity in the soil inoculated only with strain MC1 was significantly higher ($p < 0.05$) (Figure 2A–D). In general, statistical analysis of the results showed that the values of the CLPP indices measured were influenced by all of the factors analyzed, i.e., strain MC1, the concentration of XM and incubation time, and among them the time effect contributed to the most of the variability observed. In addition, the interactions between these factors had a significant impact (Table 1). The XM and XM+Ps treatments contributed to a significant decrease ($p < 0.05$) in the AWCD values, and this decrease accounted for 26–68%, 6–31% and 9–22% of the control values on days 1, 15 and 30 of the experiment, respectively (Figure 2A). In turn, in the second part of the experiment, a significant increase in the AWCD values was observed. The XM10 and XM10+Ps-treatments showed approximately three-fold higher AWCD values, when compared to the non-treated control on day 90 (Figure 2A). The study revealed that the substrate richness (R_S) value was influenced by the XM treatment, and its decrease was observed on days 1 and 15 compared to the control soil. In turn, an increase in the R_S index values for the XM10 and XM10 + Ps treatments within the range of 15–20% and 28–33% was noted on days 60 and 90, respectively, compared to the control soil (Figure 2B). A similar effect of XM and/or Ps was observed in the case of the H index. The XM and XM+Ps treatments contributed to a significant decrease ($p < 0.05$) in the H index values, and this decrease accounted for 85–95%, 76–94% and 74–88% of the control values on days 1, 15 and 30 of the experiment, respectively (Figure 2C). In turn, in the second part of the experiment, an increase in the H index values for the XM10 and XM10+Ps treatments within the range of 17–21% and 20–23% was noted on days 60 and 90, respectively, compared to the nontreated soil (Figure 2C).

Table 1. Results of the three-way ANOVA for the CLPP indices and carbon substrate groups.

SV	AWCD		R		H		E		Amines	
	VE	*p*	VE	*p*	VE	*p*	VE	*p*	VE	*p*
S	<1	0.004 **	<1	0.403	2	<0.001 ***	3	<0.001 ***	1	<0.001 ***
C	11	<0.001 ***	2	<0.001 ***	5	<0.001 ***	8	<0.001 ***	6	<0.001 ***
T	31	<0.001 ***	38	<0.001 ***	40	<0.001 ***	25	<0.001 ***	41	<0.001 ***
S × C	<1	0.019 *	<1	0.586	<1	0.063	<1	0.210	<1	<0.001 ***
S × T	<1	<0.001 ***	3	<0.001 ***	1	0.001 **	4	0.005 **	1	<0.001 ***
C × T	55	<0.001 ***	47	<0.001 ***	46	<0.001 ***	36	<0.001 ***	46	<0.001 ***
S × C × T	<1	0.866	2	0.029 *	2	0.002 **	9	<0.001	2	<0.001 ***
SV	**Amino acids**		**Carbohydrates**		**Carboxylic acids**		**Miscellaneous**		**Polymers**	
	VE	*p*	VE	*p*	VE	*p*	VE	*p*	VE	*p*
S	<1	0.084	<1	<0.001 ***	<1	<0.001 ***	<1	0.002 **	<1	0.013 *
C	8	<0.001 ***	9	<0.001 ***	6	<0.001 ***	18	<0.001 ***	11	<0.001 ***
T	41	<0.001 ***	53	<0.001 ***	44	<0.001 ***	9	<0.001 ***	26	<0.001 ***
S × C	<1	<0.001 ***	<1	0.077	<1	0.025 *	<1	<0.001 ***	<1	0.001 **
S × T	2	<0.001 ***	1	<0.001 ***	<1	<0.001 ***	<1	0.080	1	<0.001 ***
C × T	47	<0.001 ***	36	<0.001 ***	47	<0.001 ***	72	<0.001 ***	58	<0.001 ***
S × C × T	<1	<0.001 ***	<1	0.005 **	1	<0.001 ***	<1	<0.001 ***	1	0.002 **

SV: source of variation, S: strain, C: concentration, T: time, AWCD: average well-color development, R: substrate richness, H: Shannon-Wiener index, E: evenness index, VE: variance explained (%). Asterisks represent the significance level (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

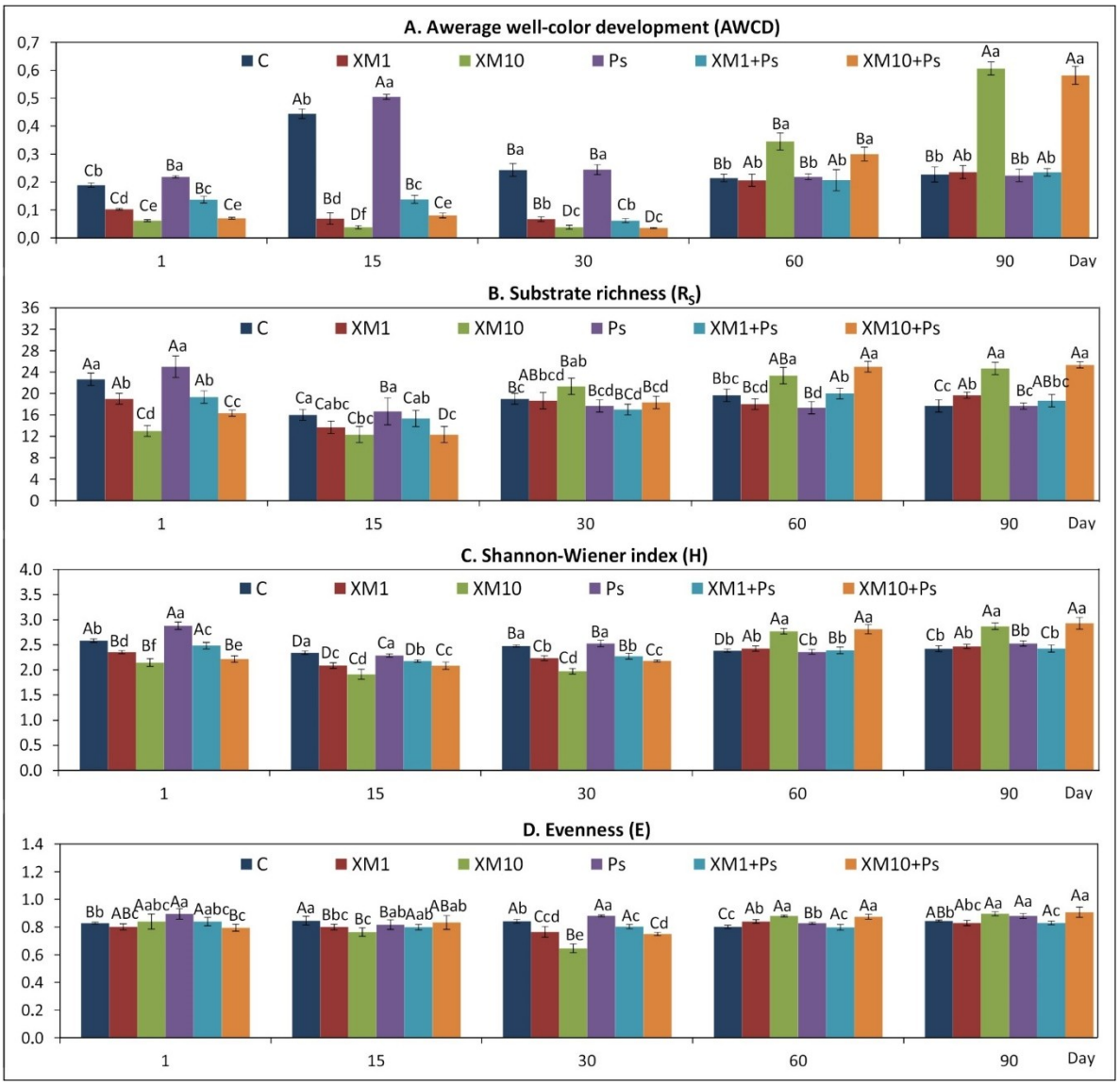

Figure 2. Effect of XM and/or strain MC1 on the values of the CLPP indices, i.e., AWCD (**A**), Rs (**B**), H (**C**) and E (**D**). The data presented are the means of three replicates with standard deviations. Significant differences (LSD test, $p < 0.05$) between treatments at the same sampling time and between sampling times within the same treatments are denoted with different lower and uppercase letters, respectively. The treatment abbreviations are explained in Figure 1.

3.2. Carbon Substrate Utilization Pattern

An analysis of the AWCD values showed that XM and/or strain MC1 changed the pattern of carbon substrate group utilization during the experiment (Figure 3). The XM and XM+Ps treatments contributed to a significant decrease ($p < 0.05$) in the AWCD values for the utilization of all of the carbon substrate groups up to 30 days of the experimental period. At the same time, the catabolic activity in the soil inoculated only with strain MC1 was significantly higher ($p < 0.05$) (Figure 3A–F). At the following sampling times (days 60 and 90), a stimulatory effect of XM10 and XM10+Ps on the utilization pattern of each substrate group was observed (Figure 3). The AWCD values for the utilization of amines, amino acids, carbohydrates, carboxylic acids, miscellaneous and polymers for these treatments were found to be up to 2.3-, 3.1-, 2.3-, 13-, 3.4- and 3.3-fold higher compared to the value for the nontreated control, respectively (Figure 3). In general, statistical analysis of the results showed that the AWCD values of all the substrate groups were affected by all of the factors

analyzed, i.e., strain MC1, the concentration of XM and incubation time, and among them the time effect contributed to the most of the variability observed. In addition, the interactions between these factors had a significant impact (Table 1).

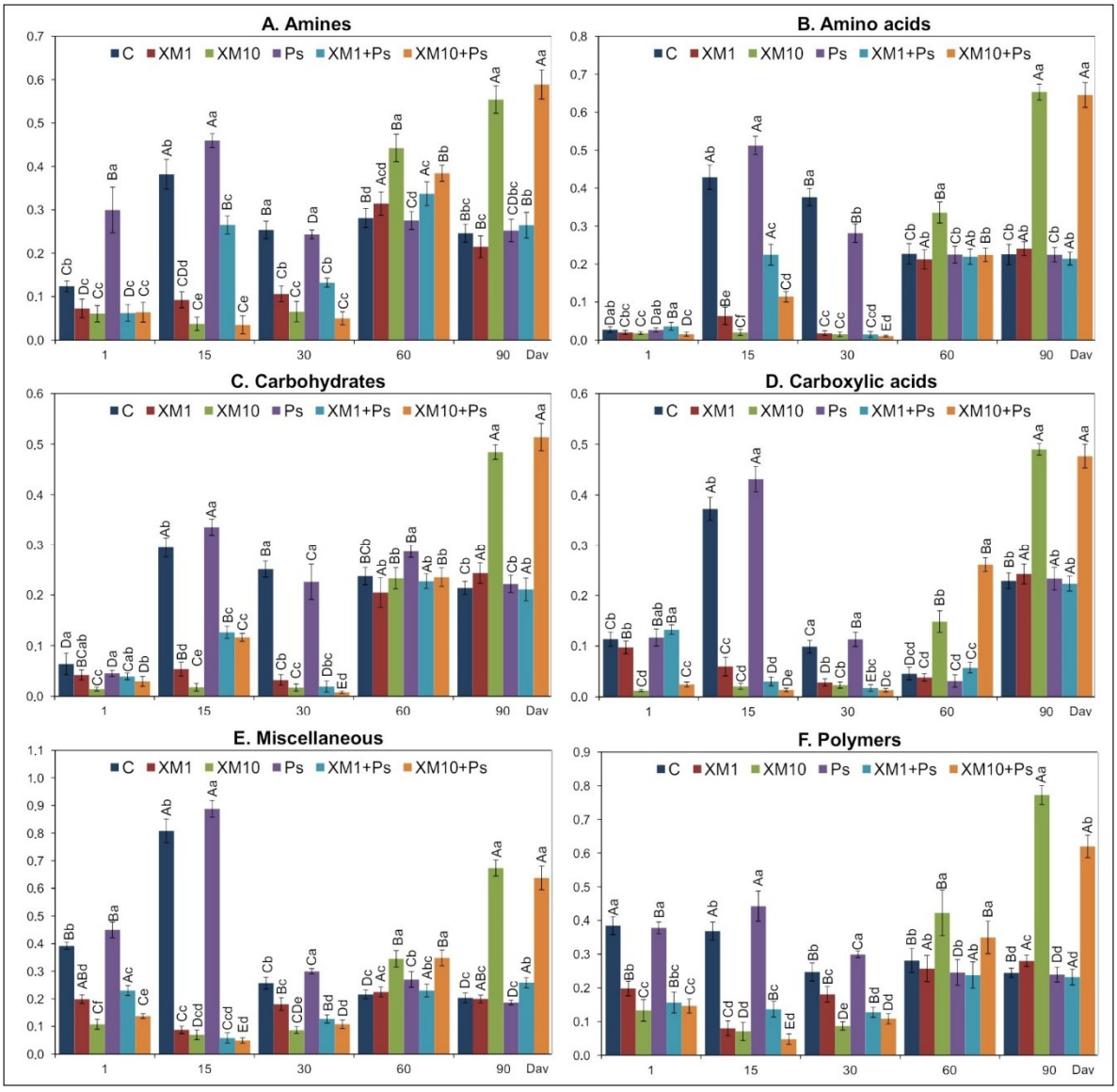

Figure 3. Effect of XM and/or strain MC1 on the AWCD values of carbon substrate groups, i.e., amines (**A**), amino acids (**B**), carbohydrates (**C**), carboxylic acids (**D**), miscellaneous (**E**) and polymers (**F**). The data presented are the means of three replicates with standard deviations. Significant differences (LSD test, $p < 0.05$) between treatments at the same sampling time and between sampling times within the same treatments are denoted with different lower and uppercase letters, respectively. The treatment abbreviations are explained in Figure 1.

3.3. Principal Component Analysis

The PCA plots obtained for the CLPP indices (Figure 4A) and the carbon substrate groups (Figure 4B) revealed a pattern of variability dependent on all of the factors tested (i.e., bacterial strain, XM dosage and time). The PCA plot generated for all of the sampling days showed that the samples were primarily scattered along the PC 1 axis, and this scattering contributed to 71.1% and 85.0% of the total variability for the CLPP indices and the carbon substrate groups, respectively (Figure 4). The first PCA axis was characterized by a decreasing gradient of all of the CLPP indices (Figure 4A) and carbon

substrate groups (Figure 4B). Generally, a significant impact of the XM dosage and/or Ps was evident during the whole experimental period. It was clearly observed that the CLPP profiles and carbon substrate utilization patterns for the soil samples treated with XM and/or Ps were separated from those obtained for the control soil (Figure 4A,B). This observation was also confirmed by analysis of the scores from PC 1 and PC 2 using a three-way MANOVA. However, among the three factors tested, the primary contribution in the observed variability was obtained for time (Table 2).

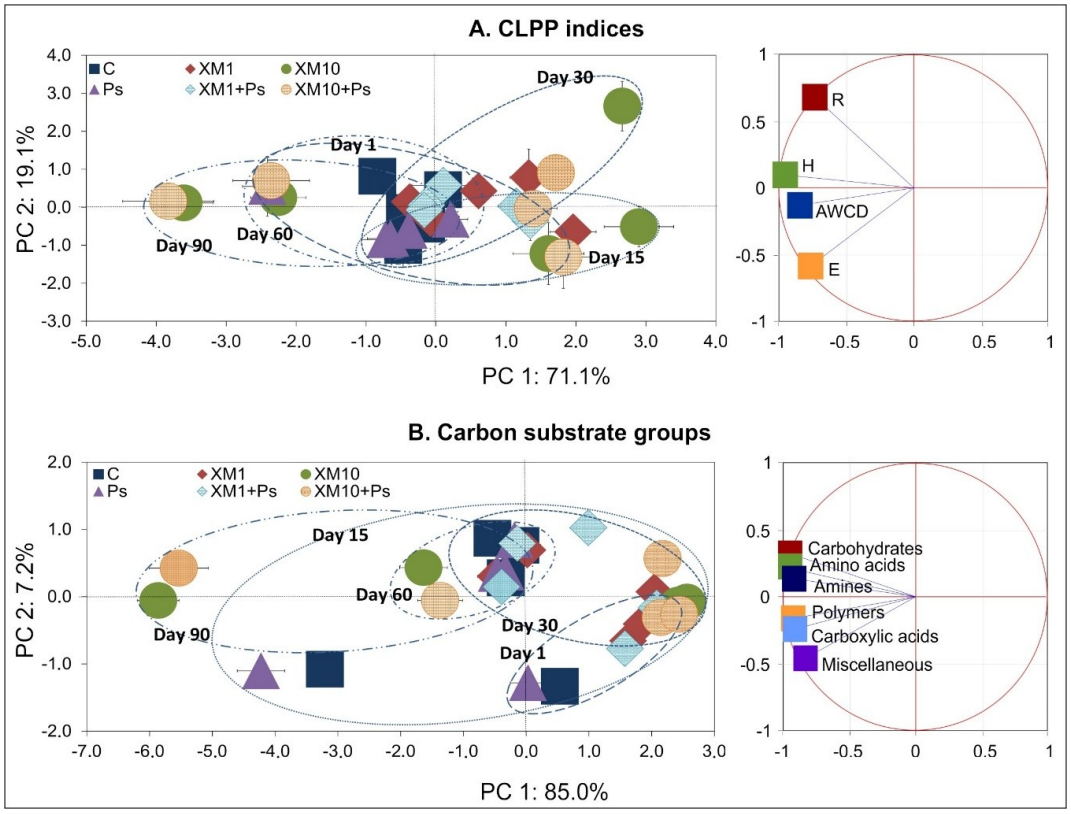

Figure 4. The PCA plots for the CLPP indices (**A**) and carbon substrate groups (**B**) generated for all the days of the experiment. The treatment abbreviations are explained in Figure 1.

Table 2. Results of the three-way MANOVA for the PC 1 and PC 2 based on the CLPP indices and carbon substrate groups.

SV	CLPP Indices				Carbon Substrate Groups			
	PC 1		PC 2		PC 1		PC 2	
	VE	*p*	VE	*p*	VE	*p*	VE	*p*
S	1	<0.001 ***	1	0.072	<1	0.003 **	<1	<0.001 ***
C	8	<0.001 ***	4	0.005 **	11	<0.001 ***	2	<0.001 ***
T	35	<0.001 ***	25	<0.001 ***	34	<0.001 ***	46	<0.001 ***
S × C	<1	0.267	1	0.456	<1	0.045 *	1	<0.001 ***
S × T	1	0.003 **	7	0.001 **	1	<0.001 ***	4	<0.001 ***
C × T	50	<0.001 ***	32	<0.001 ***	54	<0.001 ***	41	<0.001 ***
S × C × T	1	0.005 **	10	0.002 **	<1	0.003 **	4	<0.001 ***

SV: source of variation, S: strain, C: concentration, T: time, VE: variance explained (%). Asterisks represent the significance level (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

The PCA plots generated for each sampling day also showed that the samples were primarily scattered along the PC 1 axis, and this scattering contributed to 68.2–89.0% and 63.9–98.4%% of the total variability for the CLPP indices (Figure 5A) and the carbon substrate groups (Figure 5B), respectively.

This observation was also confirmed by analysis of the scores from PC 1 and PC 2 using a two-way MANOVA. However, among the two factors tested, the primary contribution for PC 1 (up to 93% and 99% for the CLPP indices and the carbon substrate groups, respectively) in the observed variability was obtained for the dosage of XM (Table 3). The first PCA axis was characterized by an increasing and decreasing gradient of all of the CLPP indices on days 1 and 15 and on days 30, 60 and 90, respectively (Figure 5A). In the case of the carbon substrate groups, the gradient generally decreased, and a significant impact of the XM dosage and/or Ps was evident for each sampling day (Figure 5B).

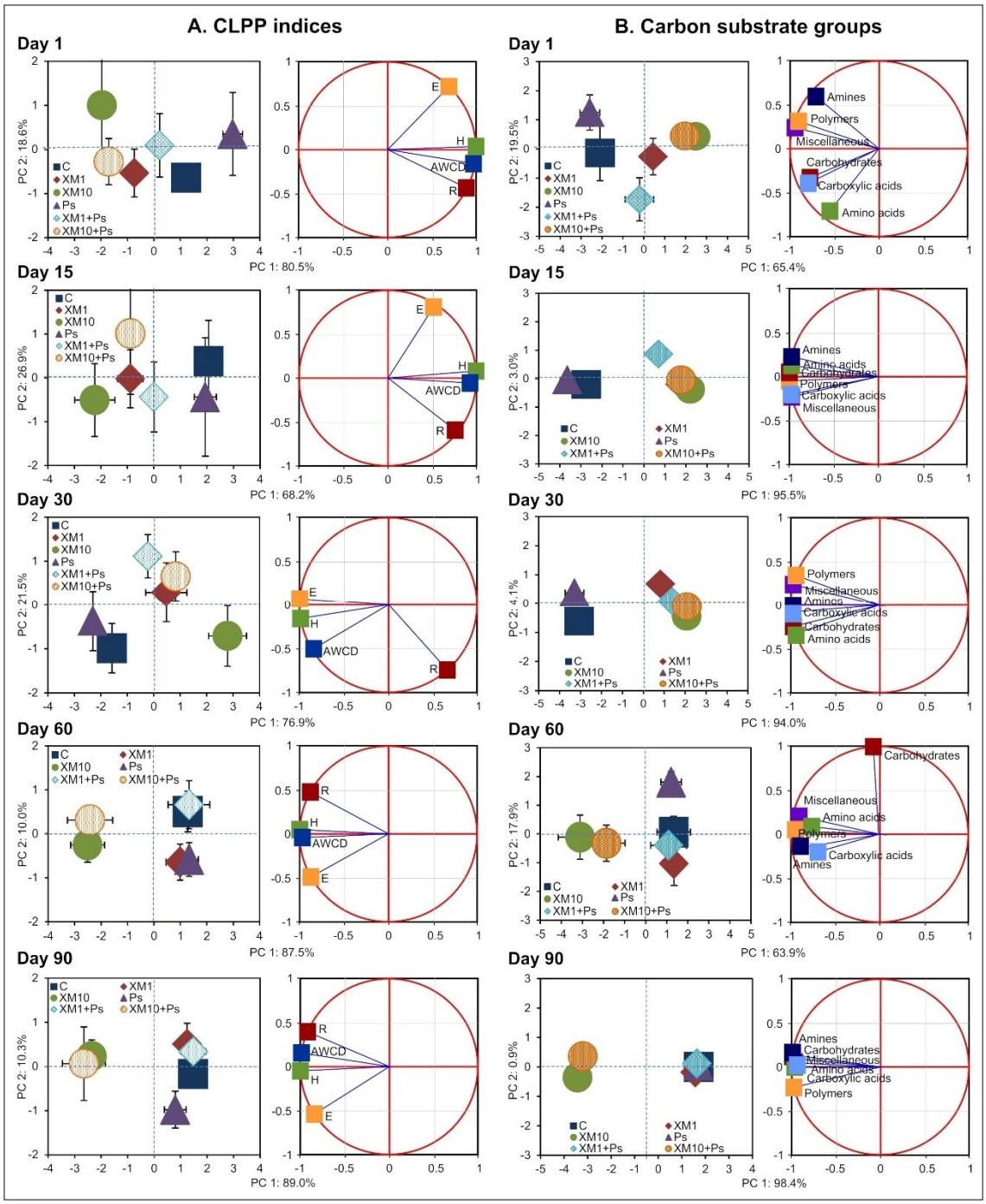

Figure 5. The PCA plots for the CLPP indices (**A**) and carbon substrate groups (**B**) generated for each sampling day of the experiment. The treatment abbreviations are explained in Figure 1.

Table 3. Results of the two-way MANOVA for the PC 1 and PC 2 based on the CLPP indices and carbon substrate groups.

Day	SV	CLPP Indices				Carbon Substrate Groups			
		PC 1		PC 2		PC 1		PC 2	
		VE	*p*	VE	*p*	VE	*p*	VE	*p*
1	S	8	<0.001 ***	<1	0.758	2	0.032 *	<1	0.942
	C	86	<0.001 ***	10	0.379	94	<0.001 ***	45	0.002 **
	S × C	3	0.014 *	35	0.051	<1	0.955	31	0.007 **
15	S	5	0.009 **	<1	0.849	3	<0.001 ***	40	<0.001 ***
	C	86	<0.001 ***	4	0.725	96	<0.001 ***	33	<0.001 ***
	S × C	4	0.057	26	0.154	<1	0.079	22	<0.001 ***
30	S	11	<0.001 ***	27	0.007 **	<1	0.500	7	0.033 *
	C	81	<0.001 ***	39	0.007 **	98	<0.001 ***	33	<0.001 ***
	S × C	3	0.066	3	0.569	1	0.194	47	<0.001 ***
60	S	<1	0.592	4	0.201	1	0.438	13	0.027 *
	C	92	<0.001 ***	<1	0.959	85	<0.001 ***	47	0.002 **
	S × C	<1	0.907	66	<0.001 ***	3	0.214	16	0.051
90	S	<1	0.332	9	0.114	<1	0.514	59	<0.001 ***
	C	93	<0.001 ***	46	0.009 **	99	<0.001 ***	1	0.210
	S × C	1	0.334	6	0.435	<1	0.780	36	<0.001 ***

SV: source of variation, S: strain, C: concentration, VE: variance explained (%). Asterisks represent the significance level (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

3.4. The Resistance (RS)/Resilience (RL) Indices

The changes observed in the catabolic activity of the microbial community were reflected in its resistance to stress factors, i.e., XM and/or Ps (Table 4). In general, the ANOVA revealed that the treatment, time and interaction between the factors tested had a significant impact on the resistance of the CLPP indices and the metabolic activity expressed as the AWCD for the carbon substrate groups (Table 5). The decrease observed in the RS index values for the soil treated with XM or XM+Ps at the beginning of the experiment corresponded to the inhibitory effect, while in the second part of the study (days 60 and 90), they were generally associated with the stimulatory effect of the parameters measured (Table 4). Factoring in the mean values of the RS index for all of the sampling days, the resistance of the CLPP indices and the AWCD values for the carbon substrate groups was categorized as follows: E (0.868) > H (0.830) > R (0.748) > AWCD (0.417) and amino acids = carbohydrates (0.441) > polymers (0.422) > amines (0.398) > miscellaneous (0.360) > carboxylic acids (0.325), respectively. An analysis of the RL index for measured parameters demonstrated differences in its value for individual treatments at the end of the experiment (day 90) (Table 6). In general, the positive values of the RL index were obtained for the XM1-, Ps- and XM1+Ps treated soils in relation to all of the parameters tested. In contrast, the RL index was generally found to be negative in the case of the XM10- and XM10+Ps treated soils for all of the CLPP indices and the AWCD values for carbon substrate groups (Table 6).

Table 4. The resistance (RS) index for the CLPP indices and carbon substrate groups.

Parameter	Day	Treatment					$\bar{x}$
		XM1	XM10	Ps	XM1+Ps	XM10+Ps	
AWCD	1	0.374 ^{Bc}	0.196 ^{Ad}	0.731 ^{Ba}	0.571 ^{Bb}	0.229 ^{Bd}	0.420 ^{A}
	15	0.085 ^{Dc}	0.045 ^{Bc}	0.759 ^{Ba}	0.184 ^{Cb}	0.100 ^{Cc}	0.235 ^{B}
	30	0.161 ^{Cb}	0.086 ^{Bbc}	0.969 ^{Aa}	0.146 ^{Cb}	0.080 ^{Cc}	0.288 ^{B}
	60	0.927 ^{Aa}	0.244 ^{Ad}	0.956 ^{Aa}	0.842 ^{Ab}	0.427 ^{Ac}	0.679 ^{A}
	90	0.924 ^{Aab}	−0.252 ^{Cc}	0.966 ^{Aa}	0.899 ^{Ab}	−0.221 ^{Dc}	0.463 ^{A}
Substrate richness (R)	1	0.721 ^{Db}	0.402 ^{Cd}	0.816 ^{BCa}	0.743 ^{Db}	0.564 ^{Bc}	0.649 ^{C}
	15	0.745C ^{Db}	0.626 ^{Bc}	0.862 ^{Ba}	0.919 ^{ABa}	0.626 ^{Bc}	0.755 ^{ABD}
	30	0.952 ^{Aa}	0.783 ^{Ac}	0.868 ^{Bb}	0.809 ^{Cbc}	0.932 ^{Aa}	0.869 ^{A}
	60	0.844 ^{Bb}	0.686 ^{Bc}	0.787 ^{Cb}	0.966 ^{Aa}	0.573 ^{Bd}	0.771 ^{AB}
	90	0.795 ^{BCc}	0.432 ^{Cd}	0.958 ^{Aa}	0.893 ^{Bb}	0.394 ^{Cd}	0.694 ^{CB}
Shannon-Wiener index (H)	1	0.838 ^{Cb}	0.711 ^{Ae}	0.796 ^{Cc}	0.925 ^{Ba}	0.752 ^{Ad}	0.804 ^{A}
	15	0.804 ^{Cc}	0.691 ^{ABd}	0.955 ^{ABa}	0.868 ^{Cb}	0.801 ^{Ac}	0.824 ^{A}
	30	0.822 ^{Cbc}	0.662 ^{Bd}	0.960 ^{Aa}	0.848 ^{Cb}	0.786 ^{Ac}	0.816 ^{A}
	60	0.925 ^{Bab}	0.723 ^{Ac}	0.978 ^{Aa}	0.979 ^{Aa}	0.697 ^{Bc}	0.860 ^{A}
	90	0.964 ^{Aa}	0.689 ^{ABc}	0.918 ^{Bb}	0.991 ^{Aa}	0.656 ^{Cc}	0.844 ^{A}
Evenness (E)	1	0.936 ^{Aa}	0.920 ^{Aa}	0.564 ^{Bb}	0.955 ^{Aa}	0.922 ^{Aa}	0.859 ^{A}
	15	0.897 ^{Aa}	0.823 ^{ABa}	0.934 ^{Aa}	0.895 ^{Aa}	0.961 ^{Aa}	0.902 ^{A}
	30	0.834 ^{Aab}	0.623 ^{BCb}	0.911 ^{Aa}	0.912 ^{Aa}	0.804 ^{Aab}	0.817 ^{A}
	60	0.909 ^{Aa}	0.551 ^{Cb}	0.937 ^{Aa}	0.981 ^{Aa}	0.834 ^{Aa}	0.842 ^{A}
	90	0.963 ^{Aa}	0.886 ^{Aa}	0.920 ^{Aa}	0.964 ^{Aa}	0.866 ^{Aa}	0.920 ^{A}
AWCD—amines	1	0.414 ^{Ba}	0.323 ^{Ab}	−0.167 ^{Cc}	0.335 ^{Dab}	0.351 ^{Bab}	0.251 ^{B}
	15	0.137 ^{Cc}	0.051 ^{Ccd}	0.660 ^{Ba}	0.532 ^{Cb}	0.047 ^{Cd}	0.285 ^{B}
	30	0.264 ^{Cb}	0.147 ^{Bc}	0.927 ^{Aa}	0.353 ^{Db}	0.108 ^{Cc}	0.360 ^{B}
	60	0.789 ^{Ab}	0.270 ^{Ae}	0.961 ^{Aa}	0.668 ^{Bc}	0.461 ^{Ad}	0.630 ^{A}
	90	0.774 ^{Ab}	−0.113 ^{Dc}	0.952 ^{Aa}	0.863 ^{Aab}	−0.166 ^{Dc}	0.462 ^{AB}
AWCD—amino acids	1	0.582 ^{Bb}	0.519 ^{Ab}	0.892 ^{Ba}	0.521 ^{Bb}	0.382 ^{Bc}	0.579 ^{AB}
	15	0.079 ^{Cd}	0.024 ^{Cd}	0.673 ^{Ca}	0.354 ^{Cb}	0.153 ^{Cc}	0.257 ^{CD}
	30	0.024 ^{Cb}	0.020 ^{Cb}	0.597 ^{Da}	0.020 ^{Db}	0.014 ^{Db}	0.135 ^{D}
	60	0.879 ^{Aa}	0.350 ^{Bb}	0.968 ^{Aa}	0.942 ^{Aa}	0.939 ^{Aa}	0.816 ^{A}
	90	0.868 ^{Aa}	−0.311 ^{Db}	0.953 ^{ABa}	0.914 ^{Aa}	−0.303 ^{Eb}	0.424 ^{BC}
AWCD—carbohydrates	1	0.514 ^{Bab}	0.126 ^{Bd}	0.611 ^{Ca}	0.477 ^{Bb}	0.299 ^{Bc}	0.406 ^{BC}
	15	0.099 ^{Cc}	0.030 ^{Bc}	0.755 ^{Ba}	0.272 ^{Cb}	0.246 ^{Bb}	0.280 ^{BC}
	30	0.067 ^{Cb}	0.035 ^{Bb}	0.818 ^{Ba}	0.039 ^{Db}	0.015 ^{Cb}	0.195 ^{C}
	60	0.757 ^{Ab}	0.963 ^{Aa}	0.655 ^{Cb}	0.920 ^{Aa}	0.980 ^{Aa}	0.855 ^{A}
	90	0.762 ^{Ab}	−0.115 ^{Cc}	0.932 ^{Aa}	0.933 ^{Aa}	−0.166 ^{Dc}	0.469 ^{B}
AWCD—carboxylic acids	1	0.748 ^{Bb}	0.057 ^{Abc}	0.947 ^{Aa}	0.709 ^{Bb}	0.119 ^{Ac}	0.516 ^{A}
	15	0.086 ^{Cb}	0.028 ^{Bb}	0.728 ^{Ba}	0.042 ^{Db}	0.018 ^{BCb}	0.180 ^{B}
	30	0.165 ^{Cb}	0.130 ^{Abc}	0.747 ^{Ba}	0.094 ^{Dbc}	0.069 ^{Abc}	0.241 ^{AB}
	60	0.733 ^{Ba}	−0.396 ^{Dc}	0.500 ^{Cb}	0.575 ^{Cb}	−0.653 ^{Dd}	0.152 ^{B}
	90	0.891 ^{Aa}	−0.067 ^{Cb}	0.950 ^{Aa}	0.952 ^{Aa}	−0.040 ^{Cb}	0.537 ^{A}
AWCD—miscellaneous	1	0.339 $^{Ba\,bc}$	0.160 ^{Abc}	0.436 ^{Ca}	0.415 $^{BC\,ab}$	0.212 $^{AB\,bc}$	0.312 ^{AB}
	15	0.057 ^{Cb}	0.045 ^{Bb}	0.819 ^{Aa}	0.037 ^{Db}	0.031 ^{Bb}	0.198 ^{B}
	30	0.541 ^{Ba}	0.203 ^{ABb}	0.715 ^{ABa}	0.329 ^{Cb}	0.266 ^{Ab}	0.411 ^{AB}
	60	0.773 ^{Aab}	0.255 ^{Ac}	0.606 ^{BCb}	0.884 ^{Aa}	0.244 ^{Ac}	0.552 ^{A}
	90	0.964 ^{Aa}	−0.396 ^{Cc}	0.851 ^{Aa}	0.576 ^{Bb}	−0.362 ^{Cc}	0.326 ^{AB}
AWCD—polymers	1	0.346 ^{Db}	0.208 ^{Bc}	0.959 ^{Aa}	0.254 ^{Dc}	0.233 ^{Bc}	0.400 ^{B}
	15	0.121 ^{Ec}	0.105 ^{Cc}	0.670 ^{Ca}	0.226 ^{Db}	0.068 ^{Cc}	0.238 ^{B}
	30	0.576 ^{Cb}	0.213 ^{Be}	0.648 ^{Ca}	0.347 ^{Ccd}	0.280 ^{Bde}	0.413 ^{B}
	60	0.842 ^{Aa}	0.337 ^{Ad}	0.774 ^{Bab}	0.731 ^{Bb}	0.609 ^{Ac}	0.659 ^{A}
	90	0.748 ^{Bb}	−0.367 ^{Dd}	0.939 ^{Ba}	0.901 ^{Aa}	−0.210 ^{Dc}	0.402 ^{B}

The data presented are the means of three replicates. Significant differences (LSD test, $p < 0.05$) between treatments at the same sampling time and between sampling times within the same treatments are marked with different lower and uppercase letters, respectively. The treatment abbreviations are explained in Figure 1.

Table 5. Results of the two-way ANOVA for the resistance (RS) indices.

SV/Parameter	AWCD		R		H		*E*		Amines	
	VE	*p*	VE	*p*	VE	*p*	VE	*p*	VE	*p*
Tr	58	<0.01 **	50	<0.01 **	73	<0.001 ***	13	0.019 *	37	<0.01 **
T	16	<0.01 **	19	<0.01 **	3	<0.001 ***	5	0.306	15	<0.01 **
Tr × T	26	<0.01 **	28	<0.01 **	21	<0.001 ***	30	0.060	46	<0.01 **
SV/Parameter	**Amino acids**		**Carbohydrates**		**Carboxylic acids**		**Miscellaneous**		**Polymers**	
	VE	*p*	VE	*p*	VE	*p*	VE	*p*	VE	*p*
Tr	36	<0.01 **	26	<0.01 **	60	<0.01 **	47	<0.001 ***	51	<0.01 **
T	34	<0.01 **	35	<0.01 **	14	<0.01 **	10	<0.001 ***	15	<0.01 **
Tr × T	30	<0.01 **	37	<0.01 **	24	<0.01 **	36	<0.001 ***	33	<0.01 **

SV: source of variation, Tr: treatment, T: time, AWCD: average well-color development, R: substrate richness, H: Shannon-Wiener index, E: evenness index, VE: variance explained (%). Asterisks represent the significance level (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

Table 6. Values of the resilience (RL) index for measured parameters obtained at the end of the experiment.

Parameter	Treatment					$\bar{x}$
	XM1	XM10	Ps	XM1 + Ps	XM10 + Ps	
AWCD	0.750 ^{a}	−0.206 ^{b}	0.321 ^{ab}	0.690 ^{a}	−0.226 ^{b}	0.266
Substrate richness (R_S)	0.302b ^{c}	0.160 ^{cd}	0.711 ^{a}	0.538 ^{ab}	−0.095 ^{d}	0.323
Shannon−Wiener index (H)	0.678 ^{a}	−0.011 ^{b}	0.478 ^{c}	0.793 ^{a}	−0.158 ^{b}	0.356
Evenness (E)	0.344 ^{ab}	−0.246 ^{b}	0.476 ^{a}	0.053 ^{ab}	−0.249 ^{b}	0.076
AWCD—amines	0.242 ^{c}	−0.660 ^{c}	0.938 ^{a}	0.537 ^{b}	−0.705 ^{c}	0.070
AWCD—amino acids	−0.278 ^{a}	−0.959 ^{b}	−0.354 ^{ab}	0.078 ^{a}	−0.945 ^{b}	−0.491
AWCD—carbohydrates	−0.189 ^{b}	−0.693 ^{c}	0.283 ^{ab}	0.456 ^{a}	−0.797 ^{c}	−0.188
AWCD—carboxylic acids	0.115 ^{b}	−0.442 ^{c}	−0.354 ^{c}	0.521 ^{a}	−0.471 ^{c}	−0.126
AWCD—miscellaneous	0.960 ^{a}	−0.247 ^{d}	0.699 ^{b}	0.495 ^{c}	−0.260 ^{d}	0.329
AWCD—polymers	0.682 ^{a}	−0.354 ^{b}	0.021 ^{b}	0.898 ^{a}	−0.222 ^{b}	0.205

The data presented are the means of three replicates. Significant differences (LSD test, $p < 0.05$) between the values of each parameter are marked with different letters. The treatment abbreviations are explained in Figure 1.

4. Discussion

Due to the significant share of microorganisms in maintaining the balance of the soil ecosystem, it is important to determine the impact of various factors on their catabolic potential. This potential may be measured by the Biolog method and EcoPlates™ that allow for insight into the functional activity of the soil microbial community. However, some authors stated that the effects of contamination can be better evaluated by measuring the activity of some soil enzymes rather than use of Biolog EcoPlates™ [51]. This conclusion may be due to the fact that the Biolog technique does not take into account the activity of catabolically inactive microorganisms that exist in a dormant state or non-culturable microorganisms. Moreover, mainly fast growing microorganisms are involved in this analysis. Despite some limitations, as other studies have shown, this method can be useful to assess the functional biodiversity of soils contaminated with antibiotics [32,52–54]. There are no reports on the effects of XM and/or antibiotic-resistant *Pseudomonas putida* on soil microbial activity. However, the results of other studies indicate that antibiotics may negatively affect the microbial activity of the soil [9,25,52,55].

The presented study showed that the introduction of XM and/or strain MC1 to the soil caused changes in the catabolic activity of the microbial community analyzed. At both doses of the antibiotic, a decrease in the values of all the CLPP indices measured was generally observed up to 30 days. In turn, at the beginning of the experiment, the catabolic activity in the soil inoculated with the MC1 strain was higher compared to the control soil. In contrast to this effect, at the following

measurement times (days 60 and 90), the values of the CLPP indices increased, particularly for the XM-treated soil (10 mg/kg) in combination with strain MC1. A short-term negative effect on the catabolic activity of soil microorganisms has also been noted for other antibiotics. For example, Fang et al. [56] found that AWCD and functional diversity indices decreased significantly up to 35 days after chlortetracycline application. A slight reduction in the value of the H index was observed for soil treated with a gradient of oxytetracycline concentration (1–300 mg/kg in soil). In contrast, a marked decline in functional diversity and AWCD values with increasing concentrations of oxytetracycline was reported by Kong et al. [52]. Antibiotics from the sulfonamide group, such as sulfamethoxazole and sulfamethazine, can also alter the activity of microbial populations; however, they only had short-term detrimental effects [54,57]. Furthermore, sulfadimethoxine or chlortetracycline did not affect the CLPP indices including AWCD, whereas other antibiotics such as monensin contributed to an increase in the value of the H index [53].

An analysis of the AWCD values showed that XM and/or strain MC1 changed the pattern of carbon substrate group utilization during the experiment. The introduction of XM at both dosages contributed to a decrease in the AWCD values for the utilization of all of the carbon substrate groups up to 30 days. In contrast, for soil inoculated only with strain MC1, the AWCD values increased up to 30 days for all the carbon substrate groups. On the following measurement days, i.e., on day 60 and 90, a significant increase in the catabolic activity of the microbial community analyzed for all of the substrate groups was observed in the XM+MC1 treatments. In many studies, changes in the preferential degradation by microorganisms of some of the substrate groups were observed over the course of an experimental period. For example, Xu et al. [27] revealed that sulfadiazine at a higher concentration contributed to a decrease in the utilization rates of four categories of substrates (carboxylic acids, amino acids, carbohydrates, and aromatic acids). Also, Liu et al. [58] observed a short-term decrease in the usage of carbohydrates and miscellaneous by the analyzed microbial community in soil treated with sulfamethoxazole. In contrast, the application of doxycycline generally contributed to a stimulation of the substrate utilization [59].

In this study, it was noted that regardless of whether only XM was used alone or in combination with strain MC1, the antibiotic contributed to environmental stress conditions resulting in changes in the catabolic activity and functional diversity of the microbial community analyzed. The loss of the capability of the soil microbial community to utilize selected carbon substrates at the beginning of the experiment could be associated with the negative effect of XM on specific enzymes produced by microorganisms. Since XM is active against both Gram-positive and Gram-negative bacteria, some microorganisms could have been killed or their metabolic activity inhibited. As a consequence of this phenomenon, catabolic activity and functional diversity significantly decreased at the beginning of the experiment. However, the negative effect of the antibiotic in combination with the MC1 strain observed at the beginning of the experiment was slightly smaller compared to that observed for the soil contaminated with only XM at both dosages. This suggests that part of the negative antibiotic effect was abolished by inoculation with the MC1 strain. This is also confirmed by the results obtained for the soil only inoculated with the MC1 strain. Higher values of the parameters measured suggest that the bacterial strain introduced had the ability to survive in soil and increased the catabolic potential of the microbial community. Since the MC1 strain is resistant to many antibiotics, including cefuroxime, its activity in the soil has not been disturbed by XM and therefore a higher catabolic activity could have been observed for XM+Ps treatments in comparison with XM treatments. In addition, a higher catabolic activity for the Ps treatment could be the result of a larger starting number of microorganisms as compared to the non-inoculated control. Many studies on the degradation of organic contaminants in the soil environment showed synergy between inoculated strains and natural soil microflora, resulting in the accelerated degradation of pollutants [60–62]. However, the lack of any effect after the introduction of bacterial strains into the soil was also observed. For example, a study by Cycoń et al. [9] showed that the antibiotic-resistant strain of *Citrobacter freundii* did not affect the catabolic activity and functional diversity of the soil microorganisms whether it was introduced alone or in combination

with vancomycin. This phenomenon may be related to the survivability of the inoculants in the soil environment, which is often a foreign environment for them [46]. Soil is a very complex ecosystem and many biotic and abiotic factors may determine the survival of the inoculants. In addition, there may be competition between the natural soil microflora and the strains of microorganisms introduced. The phenomenon of soil microorganisms producing various inhibitory substances that limit the activity of inoculants is also of great importance [60,63].

After the initial inhibition caused by XM, the values of the CLPP indices, i.e., AWCD, R, H, E and the AWCD values for the six carbon substrate groups increased over the next few sampling days. This effect could be related to the development of the ability of selected microorganisms to degrade the XM introduced into the soil and use it as a source of carbon and energy. This could result in an increase in the number of degrading microorganisms and therefore, an increase in enzyme production. Alternatively, the negative effect of XM could have been masked by the increased activity of other microorganisms capable of surviving in the presence of an antibiotic and/or using compounds released from the cells of killed microorganisms [9,64,65]. The effect observed could also be related to the XM degradation in the soil. Available studies on degradation, however very scarce, have shown that XM belongs to a group of compounds with relatively low stability under soil conditions, as evidenced by the DT50 value at the level of several dozen days [42]. In addition, the loss of the antibacterial properties of XM during the degradation in the soil could also have been of great importance.

The observed changes in the catabolic activity of the microbial community analyzed were reflected in its resistance to stress factors. Evaluation of the resistance of the CLPP indices and the metabolic activity expressed as the AWCD for the carbon substrate groups showed that the RS index was affected by XM and strain MC1. According to the interpretation proposed by Orwin and Wardle [50], the RS and RL indices may have values between −1 and +1. A value of +1 for the RS index shows maximal resistance and that the exposure had no influence, while lower values show stronger effects (less resistance) related to either stimulation or inhibition. In the case of the RL index, a value of +1 at the sampling time shows maximal resilience, while lower values show a slower recovery rate. The results obtained generally suggest a low initial resistance of microorganisms to the introduction of XM and/or strain MC1, but they were resilient in the long term. A similar phenomenon was noted by Cycoń et al. [9] and Baćmaga et al. [66] who studied the effect of another antibiotic, vancomycin, and the pesticide azoxystrobin on the soil metabolic activity, respectively. Our results suggest that the properties of the microbial community are a key factor responsible for maintaining the soil balance. Despite the initial perturbations caused by stress factors, the initial balance may be restored [67–69]. However, alternatively, there may be changes in the structure of microorganisms, but their activity related to the specific microbial process may be similar to that obtained from soil not subjected to stress factors [37,69,70].

5. Conclusions

In this study, it was noted that regardless of whether XM was used alone or in combination with *Pseudomonas putida* strain MC1, the antibiotic contributed to environmental stress conditions at the beginning of the study, resulting in changes in the catabolic activity and functional diversity of the soil microbial community. However, it was shown that strain MC1 introduced into the soil was characterized by some properties to compensate for the negative effect caused by the antibiotic at the beginning of the experiment. Higher values of measured parameters obtained for the soil inoculated with solely the bacterial strain suggest that it had the ability to survive in the soil and increased the catabolic potential of the soil microorganisms. The loss of the ability of a microbial community to degrade selected substrates at the beginning of the experiment could be associated with the negative effect of XM on selected microorganisms responsible for the production of specific enzymes. However, after the initial inhibition, the increase in the catabolic activity of soil microorganisms was noted during the following sampling days. This effect could be related to the increase in the number of microorganisms degrading XM and/or masking the effect of XM through the increased activity of

other microorganisms using compounds released from the cells of killed microorganisms. In addition, the degradation of XM and its loss of antibacterial properties could also have great significance. In general, the results obtained suggest a low level of resistance of soil microorganisms to XM and/or strain MC1 at the beginning of the exposure time, but the microbial community had the ability to recover its initial catabolic activity over the experimental period.

Author Contributions: Conceptualization, K.O. and M.C.; Methodology, K.O., A.M. and M.C.; Software, M.C.; Validation, K.O., A.M. and M.C.; Formal Analysis, K.O., A.M., J.S.-D. and M.C.; Investigation, K.O., A.M., J.S.-D. and M.C.; Resources, M.C.; Data Curation, K.O., A.M. and M.C.; Writing—Original Draft Preparation, K.O., Z.P.-S. and M.C.; Writing—Review and Editing, K.O., Z.P.-S. and M.C.; Visualisation, K.O. and M.C.; Supervision, M.C.; Project Administration, M.C.; and Funding Acquisition, M.C.

Funding: This research was funded by the National Science Center, Poland (No. DEC-2014/15B/NZ9/04414).

Conflicts of Interest: The authors declare no conflict of interest and the funder 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. Zhou, L.-J.; Ying, G.-G.; Liu, S.; Zhao, J.-L.; Yang, B.; Chen, Z.-F.; Lai, H.-J. Occurrence and fate of eleven classes of antibiotics in two typical wastewater treatment plants in South China. *Sci. Total Environ.* **2013**, *452–453*, 365–376. [CrossRef] [PubMed]
2. Hou, J.; Wan, W.; Mao, D.; Wang, C.; Mu, Q.; Qin, S.; Luo, Y. Occurrence and distribution of sulfonamides, tetracyclines, quinolones, macrolides, and nitrofurans in livestock manure and amended soils of Northern China. *Environ. Sci. Pollut. Res.* **2015**, *22*, 4545–4554. [CrossRef] [PubMed]
3. Yang, Y.; Owino, A.A.; Gao, Y.; Yan, X.; Xu, C.; Wang, J. Occurrence, composition and risk assessment of antibiotics in soils from Kenya, Africa. *Ecotoxicology* **2016**, *25*, 1194–1201. [CrossRef] [PubMed]
4. Pan, M.; Chu, L.M. Leaching behavior of veterinary antibiotics in animal manure-applied soils. *Sci. Total Environ.* **2017**, *579*, 466–473. [CrossRef] [PubMed]
5. Kong, W.; Li, C.; Dolhi, J.M.; Li, S.; He, J.; Qiao, M. Characteristics of oxytetracycline sorption and potential bioavailability in soils with various physical-chemical properties. *Chemosphere* **2012**, *87*, 542–548. [CrossRef] [PubMed]
6. Cui, H.; Wang, S.-P.; Fu, J.; Zhou, Z.-Q.; Zhang, N.; Guo, L. Influence of ciprofloxacin on microbial community structure and function in soils. *Biol. Fertil. Soils* **2014**, *50*, 939–947. [CrossRef]
7. Pan, M.; Chu, L.M. Adsorption and degradation of five selected antibiotics in agricultural soil. *Sci. Total Environ.* **2016**, *545–546*, 48–56. [CrossRef] [PubMed]
8. Duan, M.; Li, H.; Gu, J.; Tuo, X.; Sun, W.; Qian, X.; Wang, X. Effects of biochar on reducing the abundance of oxytetracycline, antibiotic resistance genes, and human pathogenic bacteria in soil and lettuce. *Environ. Pollut.* **2017**, *224*, 787–795. [CrossRef] [PubMed]
9. Cycoń, M.; Orlewska, K.; Markowicz, A.; Zmijowska, A.; Smoleń-Dzirba, J.; Bratosiewicz-Wasik, J.; Wasik, T.J.; Piotrowska-Seget, Z. Vancomycin and/or multidrug-resistant *Citrobacter freundii* altered the metabolic pattern of soil microbial community. *Front. Microbiol.* **2018**, *9*, 1047. [CrossRef] [PubMed]
10. Braschi, I.; Blasioli, S.; Fellet, C.; Lorenzini, R.; Garelli, A.; Pori, M.; Giacomini, D. Persistence and degradation of new β-lactam antibiotics in the soil and water environment. *Chemosphere* **2013**, *93*, 152–159. [CrossRef] [PubMed]
11. Walters, E.; McClellan, K.; Halden, R.U. Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids-soil mixtures in outdoor mesocosms. *Water Res.* **2010**, *44*, 6011–6020. [CrossRef] [PubMed]
12. Kay, P.; Blackwell, P.A.; Boxall, A.B.A. Fate of veterinary antibiotics in a macroporous tile drained clay soil. *Environ. Toxicol. Chem.* **2004**, *23*, 1136–1143. [CrossRef] [PubMed]
13. Popowska, M.; Rzeczycka, M.; Miernik, A.; Krawczyk-Balska, A.; Walsh, F.; Duffy, B. Influence of soil use on prevalence of tetracycline, streptomycin, and erythromycin resistance and associated resistance genes. *Antimicrob. Agents Chemother.* **2012**, *56*, 1434–1443. [CrossRef] [PubMed]
14. DeVries, S.L.; Zhang, P. Antibiotics and the Terrestrial Nitrogen Cycle: A Review. *Curr. Pollut. Rep.* **2016**, *2*, 51–67. [CrossRef]

15. Orlewska, K.; Piotrowska-Seget, Z.; Bratosiewicz-Wąsik, J.; Cycoń, M. Characterization of bacterial diversity in soil contaminated with the macrolide antibiotic erythromycin and/or inoculated with a multidrug-resistant *Raoultella* sp. strain using the PCR-DGGE approach. *Appl. Soil Ecol.* **2018**, *126*, 57–64. [CrossRef]
16. Binh, C.T.T.; Heuer, H.; Gomes, N.C.M.; Kotzerke, A.; Fulle, M.; Wilke, B.M.; Schloter, M.; Smalla, K. Short-term effects of amoxicillin on bacterial communities in manured soil. *FEMS Microbiol. Ecol.* **2007**, *62*, 290–302. [CrossRef] [PubMed]
17. Ghosh, S.; LaPara, T.M. The effects of subtherapeutic antibiotic use in farm animals on the proliferation and persistence of antibiotic resistance among soil bacteria. *ISME J.* **2007**, *1*, 191–203. [CrossRef] [PubMed]
18. Kyselková, M.; Jirout, J.; Chroňáková, A.; Vrchotová, N.; Bradley, R.; Schmitt, H.; Elhottová, D. Cow excrements enhance the occurrence of tetracycline resistance genes in soil regardless of their oxytetracycline content. *Chemosphere* **2013**, *93*, 2413–2418. [CrossRef] [PubMed]
19. Ma, J.; Lin, H.; Sun, W.; Wang, Q.; Yu, Q.; Zhao, Y.; Fu, J. Soil microbial systems respond differentially to tetracycline, sulfamonomethoxine, and ciprofloxacin entering soil under pot experimental conditions alone and in combination. *Environ. Sci. Pollut. Res.* **2014**, *21*, 7436–7448. [CrossRef] [PubMed]
20. Wepking, C.; Avera, B.; Badgley, B.; Barrett, J.E.; Franklin, J.; Knowlton, K.F.; Ray, P.P.; Smitherman, C.; Strickland, M.S. Exposure to dairy manure leads to greater antibiotic resistance and increased mass-specific respiration in soil microbial communities. *Proc. R. Soc. B Biol. Sci.* **2017**, *284*, 20162233. [CrossRef] [PubMed]
21. Du, L.; Liu, W. Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. *Agron. Sustain. Dev.* **2012**, *32*, 309–327. [CrossRef]
22. Keen, P.L.; Patrick, D.M. Tracking change: A look at the ecological footprint of antibiotics and antimicrobial resistance. *Antibiotics* **2013**, *2*, 191–205. [CrossRef] [PubMed]
23. Tang, X.; Lou, C.; Wang, S.; Lu, Y.; Liu, M.; Hashmi, M.Z.; Liang, X.; Li, Z.; Liao, Y.; Qin, W.; et al. Effects of long-term manure applications on the occurrence of antibiotics and antibiotic resistance genes (ARGs) in paddy soils: Evidence from four field experiments in south of China. *Soil Biol. Biochem.* **2015**, *90*, 179–187. [CrossRef]
24. Grenni, P.; Ancona, V.; Barra Caracciolo, A. Ecological effects of antibiotics on natural ecosystems: A review. *Microchem. J.* **2018**, *136*, 25–39. [CrossRef]
25. Pinna, M.V.; Castaldi, P.; Deiana, P.; Pusino, A.; Garau, G. Sorption behavior of sulfamethazine on unamended and manure-amended soils and short-term impact on soil microbial community. *Ecotoxicol. Environ. Saf.* **2012**, *84*, 234–242. [CrossRef] [PubMed]
26. Akimenko, Y.V.; Kazeev, K.S.; Kolesnikov, S.I. Impact assessment of soil contamination with antibiotics (For example, an ordinary chernozem). *Am. J. Appl. Sci.* **2015**, *12*, 80–88. [CrossRef]
27. Xu, Y.; Yu, W.; Ma, Q.; Wang, J.; Zhou, H.; Jiang, C. The combined effect of sulfadiazine and copper on soil microbial activity and community structure. *Ecotoxicol. Environ. Saf.* **2016**, *134*, 43–52. [CrossRef] [PubMed]
28. Schmitt, H.; Haapakangas, H.; Van Beelen, P. Effects of antibiotics on soil microorganisms: Time and nutrients influence pollution-induced community tolerance. *Soil Biol. Biochem.* **2005**, *37*, 1882–1892. [CrossRef]
29. Brandt, K.K.; Sjøholm, O.R.; Krogh, K.A.; Halling-Sørensen, B.; Nybroe, O. Increased pollution-induced bacterial community tolerance to sulfadiazine in soil hotspots amended with artificial root exudates. *Environ. Sci. Technol.* **2009**, *43*, 2963–2968. [CrossRef] [PubMed]
30. Liu, B.; Li, Y.; Zhang, X.; Wang, J.; Gao, M. Effects of chlortetracycline on soil microbial communities: Comparisons of enzyme activities to the functional diversity via Biolog EcoPlates^{TM}. *Eur. J. Soil Biol.* **2015**, *68*, 69–76. [CrossRef]
31. Chen, W.; Liu, W.; Pan, N.; Jiao, W.; Wang, M. Oxytetracycline on functions and structure of soil microbial community. *J. Soil Sci. Plant Nutr.* **2013**, *13*, 967–975. [CrossRef]
32. Ma, T.; Pan, X.; Chen, L.; Liu, W.; Christie, P.; Luo, Y.; Wu, L. Effects of different concentrations and application frequencies of oxytetracycline on soil enzyme activities and microbial community diversity. *Eur. J. Soil Biol.* **2016**, *76*, 53–60. [CrossRef]
33. Reichel, R.; Michelini, L.; Ghisi, R.; Thiele-Bruhn, S. Soil bacterial community response to sulfadiazine in the soil-root zone. *J. Plant Nutr. Soil Sci.* **2015**, *178*, 499–506. [CrossRef]
34. Versporten, A.; Coenen, S.; Adriaenssens, N.; Muller, A.; Minalu, G.; Faes, C.; Vankerckhoven, V.; Aerts, M.; Hens, N.; Molenberghs, G.; et al. European Surveillance of Antimicrobial Consumption (ESAC): Outpatient cephalosporin use in Europe (1997–2009). *J. Antimicrob. Chemother.* **2011**, *66*, 25–35. [CrossRef]

35. Ishibiki, K.; Inoue, S.; Suzuki, F.; Okumura, K.; Takeda, K.; Toshimitsu, Y. Investigation of adsorption, metabolism and excretion of cefuroxime axetil in volunteers of gastrectomized patients. *Jpn. J. Antibiot.* **1990**, *43*, 337–344. [CrossRef] [PubMed]
36. Cheng, G.; Hu, Y.; Yin, Y.; Yang, X.; Xiang, C.; Wang, B.; Chen, Y.; Yang, F.; Lei, F.; Wu, N.; et al. Functional screening of antibiotic resistance genes from human gut microbiota reveals a novel gene fusion. *FEMS Microbiol. Lett.* **2012**, *336*, 11–16. [CrossRef] [PubMed]
37. Bhattacharya, D.; Dey, S.; Kadam, S.; Kalal, S.; Jali, S.; Koley, H.; Sinha, R.; Nag, D.; Kholkute, S.D.; Roy, S. Isolation of NDM-1-producing multidrug-resistant *Pseudomonas putida* from a paediatric case of acute gastroenteritis, India. *New Microbes New Infect.* **2015**, *5*, 5–9. [CrossRef] [PubMed]
38. Coenen, S.; Ferech, M.; Dvorakova, K.; Hendrickx, E.; Suetens, C.; Goossens, H. European Surveillance of Antimicrobial Consumption (ESAC): Outpatient cephalosporin use in Europe. *J. Antimicrob. Chemother.* **2006**, *58*, 413–417. [CrossRef] [PubMed]
39. Iatrou, E.I.; Stasinakis, A.S.; Thomaidis, N.S. Consumption-based approach for predicting environmental risk in Greece due to the presence of antimicrobials in domestic wastewater. *Environ. Sci. Pollut. Res.* **2014**, *21*, 12941–12950. [CrossRef] [PubMed]
40. Oğuz, M.; Mihçiokur, H. Environmental risk assessment of selected pharmaceuticals in Turkey. *Environ. Toxicol. Pharmacol.* **2014**, *38*, 79–83. [CrossRef] [PubMed]
41. Yu, X.; Tang, X.; Zuo, J.; Zhang, M.; Chen, L.; Li, Z. Distribution and persistence of cephalosporins in cephalosporin producing wastewater using SPE and UPLC–MS/MS method. *Sci. Total Environ.* **2016**, *569–570*, 23–30. [CrossRef] [PubMed]
42. Material Safety Data Sheet. Available online: http://www.lupinpharmaceuticals.com/pdf/07-08/MSDS%20Cefuroxime%20Axetil%20Tablets.pdf (accessed on 25 September 2018).
43. Orlewska, K.; Piotrowska-Seget, Z.; Cycon, M. Use of the PCR-DGGE method for the analysis of the bacterial community structure in soil treated with the cephalosporin antibiotic cefuroxime and/or inoculated with a multidrug-resistant *Pseudomonas putida* strain MC1. *Front. Microbiol.* **2018**, *9*, 1387. [CrossRef] [PubMed]
44. Cycoń, M.; Piotrowska-Seget, Z.; Kozdrój, J. Linuron effects on microbiological characteristics of sandy soils as determined in a pot study. *Ann. Microbiol.* **2010**, *60*, 439–449. [CrossRef]
45. Cycoń, M.; Borymski, S.; Orlewska, K.; Wąsik, T.J.; Piotrowska-Seget, Z. An analysis of the effects of vancomycin and/or vancomycin-resistant *Citrobacter freundii* exposure on the microbial community structure in soil. *Front. Microbiol.* **2016**, *7*, 1015. [CrossRef] [PubMed]
46. Cycoń, M.; Zmijowska, A.; Piotrowska-Seget, Z. Biodegradation kinetics of 2,4-D by bacterial strains isolated from soil. *Cent. Eur. J. Biol.* **2011**, *6*, 188–198. [CrossRef]
47. Insam, H. A new set of substrates proposed for community characterization in environmental samples. In *Microbial Communities: Functional Versus Structural Approaches*; Insam, H., Rangger, A., Eds.; Springer: Berlin, Germany, 1997; pp. 259–260.
48. Garland, J.L. Analysis and interpretation of community-level physiological profiles in microbial ecology. *FEMS Microbiol. Ecol.* **1997**, *24*, 289–300. [CrossRef]
49. Orwin, K.H.; Wardle, D.A. New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances. *Soil Biol. Biochem.* **2004**, *36*, 1907–1912. [CrossRef]
50. Cycoń, M.; Markowicz, A.; Piotrowska-Seget, Z. Structural and functional diversity of bacterial community in soil treated with the herbicide napropamide estimated by the DGGE, CLPP and r/K-strategy approaches. *Appl. Soil Ecol.* **2013**, *72*, 242–250. [CrossRef]
51. Floch, C.; Chevremont, A.-C.; Joanico, K.; Capowiez, Y.; Criquet, S. Indicators of pesticide contamination: Soil enzyme compared to functional diversity of bacterial communities via Biolog® Ecoplates. *Eur. J. Soil Biol.* **2011**, *47*, 256–263. [CrossRef]
52. Kong, W.-D.; Zhu, Y.-G.; Fu, B.-J.; Marschner, P.; He, J.-Z. The veterinary antibiotic oxytetracycline and Cu influence functional diversity of the soil microbial community. *Environ. Pollut.* **2006**, *143*, 129–137. [CrossRef] [PubMed]
53. Toth, J.D.; Feng, Y.; Dou, Z. Veterinary antibiotics at environmentally relevant concentrations inhibit soil iron reduction and nitrification. *Soil Biol. Biochem.* **2011**, *43*, 2470–2472. [CrossRef]
54. Pino-Otín, M.R.; Muñiz, S.; Val, J.; Navarro, E. Effects of 18 pharmaceuticals on the physiological diversity of edaphic microorganisms. *Sci. Total Environ.* **2017**, *595*, 441–450. [CrossRef] [PubMed]

55. Liu, B.; Li, Y.; Zhang, X.; Wang, J.; Gao, M. Combined effects of chlortetracycline and dissolved organic matter extracted from pig manure on the functional diversity of soil microbial community. *Soil Biol. Biochem.* **2014**, *74*, 148–155. [CrossRef]
56. Fang, H.; Han, L.; Cui, Y.; Xue, Y.; Cai, L.; Yu, Y. Changes in soil microbial community structure and function associated with degradation and resistance of carbendazim and chlortetracycline during repeated treatments. *Sci. Total Environ.* **2016**, *572*, 1203–1212. [CrossRef] [PubMed]
57. Demoling, L.A.; Bååth, E.; Greve, G.; Wouterse, M.; Schmitt, H. Effects of sulfamethoxazole on soil microbial communities after adding substrate. *Soil Biol. Biochem.* **2009**, *41*, 840–848. [CrossRef]
58. Liu, F.; Wu, J.; Ying, G.-G.; Luo, Z.; Feng, H. Changes in functional diversity of soil microbial community with addition of antibiotics sulfamethoxazole and chlortetracycline. *Appl. Microbiol. Biotechnol.* **2012**, *95*, 1615–1623. [CrossRef] [PubMed]
59. Wang, J.; Lin, H.; Sun, W.; Xia, Y.; Ma, J.; Fu, J.; Zhang, Z.; Wu, H.; Qian, M. Variations in the fate and biological effects of sulfamethoxazole, norfloxacin and doxycycline in different vegetable-soil systems following manure application. *J. Hazard. Mater.* **2016**, *304*, 49–57. [CrossRef] [PubMed]
60. Cycoń, M.; Piotrowska-Seget, Z. Pyrethroid-degrading microorganisms and their potential for the bioremediation of contaminated soils: A review. *Front. Microbiol.* **2016**, *7*, 1463. [CrossRef] [PubMed]
61. Hirth, N.; Topp, E.; Dörfler, U.; Stupperich, E.; Munch, J.C.; Schroll, R. An effective bioremediation approach for enhanced microbial degradation of the veterinary antibiotic sulfamethazine in an agricultural soil. *Chem. Biol. Technol. Agric.* **2016**, *3*, 29. [CrossRef]
62. Zhang, H.; Zhou, Y.; Huang, Y.; Wu, L.; Liu, X.; Luo, Y. Residues and risks of veterinary antibiotics in protected vegetable soils following application of different manures. *Chemosphere* **2016**, *152*, 229–237. [CrossRef] [PubMed]
63. Cycoń, M.; Mrozik, A.; Piotrowska-Seget, Z. Bioaugmentation as a strategy for the remediation of pesticide-polluted soil: A review. *Chemosphere* **2017**, *172*, 52–71. [CrossRef] [PubMed]
64. Ding, G.C.; Radl, V.; Schloter-Hai, B.; Jechalke, S.; Heuer, H.; Smalla, K.; Schloter, M. Dynamics of soil bacterial communities in response to repeated application of manure containing sulfadiazine. *PLoS ONE* **2014**, *9*, e92958. [CrossRef] [PubMed]
65. Chessa, L.; Pusino, A.; Garau, G.; Mangia, N.P.; Pinna, M.V. Soil microbial response to tetracycline in two different soils amended with cow manure. *Environ. Sci. Pollut. Res.* **2016**, *23*, 5807–5817. [CrossRef] [PubMed]
66. Baćmaga, M.; Kucharski, J.; Wyszkowska, J. Microbial and enzymatic activity of soil contaminated with azoxystrobin. *Environ. Monit. Assess.* **2015**, *187*, 615. [CrossRef] [PubMed]
67. Allison, S.D.; Martiny, J.B.H. Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 11512–11519. [CrossRef] [PubMed]
68. Song, H.-S.; Renslow, R.S.; Fredrickson, J.K.; Lindemann, S.R. Integrating ecological and engineering concepts of resilience in microbial communities. *Front. Microbiol.* **2015**, *6*, 1298. [CrossRef] [PubMed]
69. Schaeffer, A.; Amelung, W.; Hollert, H.; Kaestner, M.; Kandeler, E.; Kruse, J.; Miltner, A.; Ottermanns, R.; Pagel, H.; Peth, S.; et al. The impact of chemical pollution on the resilience of soils under multiple stresses: A conceptual framework for future research. *Sci. Total Environ.* **2016**, *568*, 1076–1085. [CrossRef] [PubMed]
70. Ludwig, M.; Wilmes, P.; Schrader, S. Measuring soil sustainability via soil resilience. *Sci. Total Environ.* **2018**, *626*, 1484–1493. [CrossRef] [PubMed]