



Article Impact of Cypermethrin (Arpon G) on Soil Health and Zea mays Growth: A Microbiological and Enzymatic Study

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Abstract: In defining the research objective, consideration was given to the expanding range of applications of third-generation pyrethroids, including cypermethrin-the active substance in Arpon G preparation. The interest in cypermethrin is due to its high thermostability and photostability. This study verified the effect of Arpon G on both the soil condition and the growth and development of Zea mays. To this end, the alpha and beta diversity of bacterial and fungal communities were characterized using the NGS (Next Generation Sequencing) method, as was the response of soil enzymes. The positive response of Z. mays to the soil application of cypermethrin corresponded to higher soil microbial and biochemical activity. Sowing the soil with Z. mays moderated changes in the biodiversity of alpha- and beta-bacterial communities to a greater extent than cypermethrin. The influence of both parameters was less significant for fungi. Although bacteria belonging to the Actinobacteria phylum and fungi from the Ascomycota phylum dominated in the soil, the use of Arpon G reduced the abundance of unique nucleotide sequences in the mycobiome to a greater extent than in the bacteriobiome. The inhibitory potential of Arpon G was only evident for acid phosphatase (by 81.49%) and arylsulfatase (by 16.66%) in the soil sown with Z. mays. The activity of catalase, dehydrogenases, β -glucosidase, arylsulfatase, and alkaline phosphatase was most strongly associated with the abundance of bacteria, while dehydrogenases were correlated with the abundance of fungi at the genus level. Arpon G can, thus, be considered a safe insecticide for soil conditions and, consequently, for its productive function.

Keywords: insecticides; fungal community; bacterial community; biochemical properties

1. Introduction

Increasing urbanization and industrialization, together with economic development, have led to a higher flow of food across countries and continents. Additionally, population migration from rural to urban areas results in an increased demand for food [1,2]. To meet the growing global food demand, farmers are increasing agricultural production. However, intensification of agricultural production requires an increased use of plant protection measures against diseases, pests, and weeds [3,4]. The current technological advancement contributes to enhanced accessibility of new types of plant protection agents that are both more effective and safer to use [5,6]. They are also more efficient, allowing for their dosages to be reduced. This is in line with the principles of sustainable agriculture and a balanced approach that considers environmental protection and public health. According to FAO-STAT [7,8], overall global pesticide consumption continues to increase. Between 1990 and 2021, 416,668.05 tons of pesticides were used in the United States of America, 295,226.06 tons were used in Brazil, 271,092.31 tons were used in China, 203,108.67 tons were used in Indonesia, and 135,669.63 tons were used in Argentina. Considering the agricultural use of pesticides in these five countries, the total pesticide consumption in the years 2020–2021 ranged from 457,385.42 tons (United States of America) to 241,407.08 tons (Argentina) [7]. According to



Citation: Borowik, A.; Wyszkowska, J.; Zaborowska, M.; Kucharski, J. Impact of Cypermethrin (Arpon G) on Soil Health and *Zea mays* Growth: A Microbiological and Enzymatic Study. *Agriculture* 2023, *13*, 2261. https:// doi.org/10.3390/agriculture13122261

Academic Editor: Cristina Abbate

Received: 31 October 2023 Revised: 8 December 2023 Accepted: 9 December 2023 Published: 11 December 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). data from the Food and Agriculture Organization of the United Nations (UN) [9,10], the highest agricultural use of insecticides in the years 2020–2021 was recorded in Indonesia, amounting to 144,362.49 tons, followed by Brazil with 114,116.76 tons and the United States of America with 73,771.80 tons. Russia used 13,385.60 tons of insecticides during that time, while France used 6607.92 tons, Canada used 3143 tons, and Poland used 632 tons. The increased use of insecticides can become a significant environmental pollutant, leading to the development of pest resistance to pesticides and a loss of biodiversity [8,11]. The need to replace these chemical insecticidal agents with alternative methods of pest control that prioritize environmental protection is highly justified. In this area, pest pathogens such as bacteria, viruses, or fungi, represent an interesting alternative to the production of microbial-based insecticides [6,12].

Pyrethroids are used to control various plant and animal pests, as well as for public health purposes to control insect-borne disease. Cypermethrin is an organic chemical compound belonging to the pyrethroid group (type II), which includes synthetic analogs of natural plant-based insecticides [2,13,14]. It belongs to the third generation of pyrethroids, which are known for their increased thermal stability, photostability, and selectivity compared with previous generations of insecticides [15]. According to the United States Environmental Protection Agency (EPA), cypermethrin is primarily used in the cultivation of pecans, peanuts, broccoli, and sweet corn and the treatment of livestock. The European Food Safety Authority also highlights its use in the cultivation of lettuce, sugar beets, wheat, leguminous, and oilseed crops, including cotton [11]. Cypermethrin is also widely used in veterinary medicine as well. It is effective in eliminating external parasites of domestic and farm animals [16,17]. The vast majority of applications for insecticides containing cypermethrin as the active substance occur in non-agricultural settings, including in commerce, industry, residential areas, and households. It is also used in smaller enclaves like residential areas to control ants, cockroaches, or fleas [8,10]. Additionally, it is an ingredient in preparations for pest control in indoor spaces, clothing, and bedding [18].

The Pyrethroid Working Group (PWG) recognizes cypermethrin as one of nine hazardous substances and classifies it for re-registration with the US EPA (EPA-HQ-OPP-2005-0293-0036, EPA-HQ-OPP-2012-0167). This is driven by the increasing body of evidence supporting the belief that there is absorption through the skin or gastrointestinal tract upon contact with this pyrethroid. Importantly, the greater the efficacy of pyrethroids in combating pests, the stronger their adverse impact on other living organisms inhabiting the soil. Within this group of pests, particular attention should be given to those with biting and sucking mouthparts, such as insects transmitting yellow fever or malaria [19–21]. According to Chen et al. [22]; Cycoń and Piotrowska-Seget [23] and Bhatt et al. [24], the half-life of cypermethrin ranges from 14 to 77 days depending on the physicochemical properties of the soil. Therefore, research into the plant rhizosphere, the area encompassing the root surface involved in the degradation of toxic chemicals, is also an important research topic.

Z. mays is a highly adaptable crop that is cultivated worldwide under various conditions. Unfortunately, the production of this plant is frequently constrained, mainly reduced, by environmental and biotic pressures from pathogens and insect pests both during cultivation and storage. This is evidenced by the studies by Carrière et al. [25] and Jiménez-Galindo et al. [26], which investigated corn yield losses due to pest attacks in North America and Europe in 2012, estimated at approximately USD 1.6 billion.

The growth and development of crop plants are mainly correlated with the soil condition designated for their cultivation, and a reliable indicator of changes in the soil is its microbiome [27,28]. Microorganisms inhabiting the soil constitute a diverse group of organisms that is also involved in nutrient conversion and the detoxification of organic and inorganic pollutants [29–31]. They therefore contribute to maintaining the proper functioning and health of the soil [32].

The extent of the toxicological effects of cypermethrin that disrupt the soil balance can also be diagnosed by analyzing the interactions between these chemical compounds and soil enzymes [33]. Considering that enzymes are produced by living organisms and play an important role in the soil microbial environment of soil and plants, they are valuable potential quality indicators for sustainable management [34].

Considering the above facts, research hypotheses were formulated assuming that Z. mays is resistant to the effects of cypermethrin. It was also assumed that the insecticide does not cause drastic changes in the soil microbiome. The research hypotheses were verified based on defined objectives, which included the determination of the microbiological diversity and enzyme activity in the soil of the northern part of Poland after the application of Arpon G preparation. The specific objectives were: (i) to assess the bacterial and fungal diversity in soil under cypermethrin pressure; (ii) to determine the response of soil enzymes to cypermethrin application; (iii) to establish the relationship between the abundance of bacteria and fungi in the soil, (iv) to determine the relationships between bacteria and fungi and enzyme activity under insecticidal stress conditions, and (v) to define the response of Z. mays to the application of cypermethrin.

2. Materials and Methods

2.1. Soil Characterization

This study was conducted on soil from the Mazury Plain in northeastern Poland, in the Olsztyn Lakeland region (NE Poland, 53.72° N, 20.42° E). This soil, according to the grain size classification of the International Union of Soil Sciences (IUSS), Polish Soil Classification 2019, and the U.S. Department of Agriculture, is classified as typical brown soil with a granulometric composition of clayey sand. Soil samples were collected from the 0–20 cm layer, and then, in the greenhouse of the University of Warmia and Mazury in Olsztyn (Poland), the soil was homogenized and sieved through a 5 mm mesh, clearing it of any potential plant roots. The prepared material was mixed, and then 3 kg portions were weighed into 3.5 dm³ polyethylene pots. A detailed soil characterization is presented in the publications [31,35,36].

2.2. Experimental Design

The factors tested were: (I) soil contamination with the insecticide Arpon G: (1) uncontaminated soil and (2) soil contaminated with 40 mg of cypermethrin in the form of Arpon G preparation, and (II) soil use method: (1) unsown soil and (2) sown soil. *Z. mays* was used for soil sowing. The experiment was conducted in 4 replicates. The soil in the pots was packed after prior mixing with NPKMg fertilizers and in the appropriate objects with the insecticide Arpon G. The exact dimensions of the pot are as follows: upper base diameter: 18.5 cm; lower base diameter: 14 cm; and height: 15 cm.

This study utilized fertilization with urea, heptahydrate magnesium sulfate, dipotassium hydrogen phosphate, and potassium chloride. The doses of individual fertilizers in pure form in mg per 1 kg of soil dry matter were: N—150, P—50, K—150, and Mg—20. In the corresponding sets, 8 seeds of *Z. mays* of the LG 32.52 variety were sown in the soil and planted in the pots. After their germination, the plants were thinned out, leaving 4 plants in each pot.

The experiment was conducted for 60 days. Throughout the study period, the soil moisture was maintained at 60% of field capacity. Soil moisture was checked three times a day, and any water losses were replenished with demineralized water. The leaf greenness index SPAD was determined at BBCH (Biologische Bundesanstalt, Bundessortenamt, and Chemical Scale) stage 14 and BBCH stage 19. At BBCH stage 51, the above-ground and root yields of maize were determined. The measurement of the leaf greenness index was performed with a SPAD 502 Chlorophyll Meter 2900P (Konica Minolta, Inc., Chiyoda, Tokyo, Japan). After the plants were harvested, soil samples were collected for microbiological and enzymatic analyses. Analyses were conducted using fresh soil material, following prior sieving through a 2 mm mesh. Meteorological data, a more detailed description of the soil, the methodology, and the laboratory equipment for the physicochemical and chemical analyses of the soil were described in our previous research [36,37].

2.3. Characteristics of the Insecticide Arpon G

The Arpon G emulsion for dilution (ZOTAL Laboratorios, Camas, Seville, Spain) is an insecticide, with its active substance being cypermethrin $[(\pm)-\alpha$ -cyano-(3-phenoxyphenyl)methyl (\pm) -cis/trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] (C₂₂H₁₉C₁₂NO₃). The concentration of cypermethrin in Arpon G is 100 g per 1 dm³. It is an insecticidal agent used in agriculture [34,38] and beyond. The half-life of cypermethrin depends on the physicochemical conditions of the soil in a given environment and typically ranges from 14 to 77 days [22,23].

2.4. DNA Isolation and Identification of Bacteria and Fungi Using the NGS Method

The isolation of DNA from soil samples, amplification of 16S rRNA and ITS gene fragments, and sequencing were conducted as previously described [31,35]. The genetic material was sequenced for the V3-V4 region using an Illumina MiSeq sequencer (Genomed S.A., Warsaw, Poland). Bacterial and fungal sequences were deposited in GenBank NCBI under the accession numbers Prokaryotic 16S rRNA (OP914644:OP916021) and (OP897054:OP897145), accessed: 2–4 December 2022, and Eukaryotic nuclear rRNA/ITS (OP978693:OP979103), accessed: 14 December 2022.

2.5. Enzymatic Analyses of Soils

Immediately after delivering the soil to the laboratory, the activity of two enzymes of the oxidoreductase class: dehydrogenases (Deh) (μ mol TFF kg⁻¹ d.m. of soil h⁻¹) and catalase (Cat) (mol O₂ kg⁻¹ d.m. of soil h⁻¹), and five from the hydrolase class: urease (Ure) (mmol N-NH₄ kg⁻¹ d.m. of soil h⁻¹), alkaline phosphatase (AlP), acid phosphatase (AcP), arylsulfatase (Aryl), and β -glucosidase (Glu) (mmol 4-Nitrophenol (PN) kg⁻¹ d.m. of soil h⁻¹) were determined. The analyses were conducted in triplicate using the methods described by Öhlinger (1996), Johnson and Temple (1964), and Alef and Nannipieri (1998). A Perkin-Elmer Lambda 25 spectrophotometer (Waltham, MA, USA) was used to determine the absorbance of enzymatic reaction products, excluding catalase. A detailed description of the methods and laboratory equipment used for biochemical analyses was presented in our previous work [37].

2.6. Data Analysis and Statistical Processing

The experiment was designed as randomized sub-blocks with four replications. The results were processed using Statistica 13.1 software [39]. The data obtained were verified with a normality test (Kruskal–Wallis) and Tukey's post-hoc test. An analysis of variance was performed using the ANOVA test. A Venn diagram of unique and shared sequences was graphically presented from the total number of reads for 4 soil samples, separately for bacteria and fungi [40]. Community diversity indices were calculated from all sequences, separately for bacteria and fungi, i.e., the Shannon, Simpson, Shaneven, Margalefa, Richness, Pielou J, and Brillouin indices. The functions in Microsoft Excel (version 2311) were used to process the above indicators. Dominant types and genera of bacteria and fungi (OTU \geq 1%) were statistically compared using the G-test (with Yates' correction) and Fisher's test with the application of STAMP 2.1.3 software [41]. Pearson's simple correlation coefficients between bacteria and fungi and soil enzyme activities at the genus level were presented as a heat map using R software (4.2.2) [42–44].

3. Results

3.1. The Effect of Cypermethrin on Z. mays Biomass and Greenness Index

The cypermethrin soil application did not significantly affect the development of the above-ground parts and root system of *Z. mays* (Figure 1). It also had no negative effect on the photosynthesis process, as evidenced by the Soil and Plant Analysis Development (SPAD) leaf greenness index values.



Figure 1. The effect of cypermethrin on the biomass of aerial parts (g d.m. pot^{-1}) (**a**), root biomass (g d.m. pot^{-1}) (**b**), and the *Z*. *Mays* greenness index (SPAD) measured in the 4th leaf phase (**c**) and the 6th leaf phase (**d**). CS—soil sown with *Z*. *mays* without cypermethrin, CypS—soil sown with *Z*. *mays* with cypermethrin, SPAD—greenness index. Homogeneous groups denoted with letters (a, b) were calculated separately for the tested property.

3.2. *The Reaction of Bacteria and Fungi to Cypermethrin and Z. mays* 3.2.1. Distribution Characteristics of the Soil Bacterial Community

A higher number of bacterial readings were observed in the soil sown with *Z. mays* after the application of cypermethrin than in the soil sown without cypermethrin (117,562 and 115,918 OTUs, respectively). A lower number was found in the unsown soils, specifically, in the unsown soil after cypermethrin application (109,929 OTUs) and in the unsown soil without cypermethrin (109,929 OTUs) (Figure 2). Each of the tested soil samples was characterized by unique sequences, which can be arranged from the highest to the lowest abundance in the following order: CS > CypS > CN > CypN. The total number of reads from the obtained sequences was 64,373 OTUs.



Figure 2. Venn diagram illustrating unique and shared bacterial sequences in a soil sample. CS soil sown with *Z. mays* without cypermethrin, CypS—soil sown with *Z. mays* with cypermethrin, CN—unsown soil without cypermethrin, CypN—unsown soil with cypermethrin.

Based on the taxonomic classification of all sequence reads, 27 phyla, 89 classes, 127 orders, 167 families, 205 genera, and 47 species were identified. *Actinobacteria* were the dominant phylum in the soils, except in the soil without cypermethrin, where *Proteobacteria* exhibited dominance. *Actinobacteria* ranged from 38.4% in CypS to 47.6% in CS, while *Proteobacteria* ranged from 28.5% in CS to 40.3% in CypN.

The obtained indices describing the alpha diversity of bacteria were highest in the soil samples sown with *Z. mays* (CS, CypS) and lowest in the unsown soil without cypermethrin

(CypN), indicating that both the plant and the application of cypermethrin stimulate the abundance of these microorganisms in the soil environment (Table 1). The Shannon index ranged from 3.834 for unsown soil to 4.446 for soil sown after cypermethrin application. The Simpson index ranged from 0.929 for CN to 0.964. The Shannon index ranged from 0.639 to 0.719, the Margalef index ranged from 34.727 to 41.423, the Pielou J index ranged from 0.639 to 0.719, the Brillouin index ranged from 3.825 to 4.434, and the bacterial richness ranged from 404 to 484.

Object	Shannon	Simpson	Shaneven	Margalefa	Richness	Pielou J	Brillouin
CS	4.313 ^b	0.960 ^a	0.698 ^b	41.423 ^a	484 ^a	0.698 ^b	4.302 ^b
CypS	4.446 ^a	0.964 ^a	0.719 ^a	41.404 ^a	484 ^a	0.719 ^a	4.434 ^a
ĊN	3.834 ^d	0.929 ^c	0.639 ^c	34.724 ^b	404 ^b	0.639 ^c	3.825 ^d
CypN	4.074 ^c	0.951 ^b	0.678 ^b	34.800 ^b	407 ^b	0.678 ^b	4.065 ^c

Table 1. Alpha diversity of bacterial communities in soil samples.

CS—soil sown with *Z. mays* without cypermethrin, CypS—soil sown with *Z. mays* with cypermethrin, CN unsown soil without cypermethrin, CypN—unsown soil with cypermethrin. Homogeneous groups denoted with letters (^{a-d}) were calculated separately for the each diversity indicator in soil samples.

After extracting sequences larger than 1%, it was found that cypermethrin in unplanted soil (CypN) compared with unplanted soil without cypermethrin (CN) most significantly reduced the relative abundance of *Actinobacteria* (by 4.8%) and increased *Gemmatimonadetes* (by 3.1%) (Figure 3). Sowing *Z. mays* in soil exposed to cypermethrin (CypS) reduced the relative abundance of *Actinobacteria* by 9.2% and increased *Proteobacteria* by 8.4% compared with soil sown with *Z. mays* but without cypermethrin (CS). Analyzing the effect of cypermethrin in soil sown with *Z. mays* (CypS) compared with unsown soil (CypN), it was observed that cypermethrin only reduced the abundance of *Actinobacteria* by 3.4%. Sowing of uncontaminated soils increased the relative abundance of *Actinobacteria* by 3.8% and increased the relative abundance of *Proteobacteria* by 10.8%.



Figure 3. The relative abundance of dominant bacterial types in soils, calculated using STAMP statistical analysis software, $OTU \ge 1\%$. CS—soil sown with *Z. mays* without cypermethrin, CypS—soil sown with *Z. mays* with cypermethrin, CN—unsown soil without cypermethrin, CypN—unsown soil with cypermethrin. *—statistical significance.

Out of the 205 sequences classified to the genus level, 14 were dominant, with a relative abundance of >1% (Figure 4). The dominant genus in all soil samples was *Cellulosimicrobium* (39.06–46.60%). In sown soils, both with and without cypermethrin, the second dominant genus was *Kaistobacter*, comprising 16.54 to 12.80% of the community, respectively. In unsown soils, both with and without the application of cypermethrin (CypN and CN), the second dominant genus was Sphingomonas, constituting 13.94 to 15.95% of the community, respectively. In the CypN soil, cypermethrin had the most significant effect, decreasing the relative abundance of *Cellulosimicrobium* (by 4.1%) and *Sphingomonas* (by 2.5%) while increasing Kaistobacter (by 2.9%). In the CypS soil, cypermethrin had the most significant effect, decreasing the relative abundance of *Kaistobacter* bacteria (by 4.2%) and Terracoccus and Arthrobacter (by 3.6%) while increasing Pseudomonas by 9%. Considering the effect of soil use, it was found that sowing uncontaminated soil with Z. mays increased the abundance of Kaistobacter bacteria by 9.4%, Arthrobacter by 8.2%, Terracoccus by 6.3%, Rhodoplanes by 3.3%, Nocardioides by 2.6%, Phycicoccus by 2.3%, and Luteolibacter by 1.3%. It also reduced Sphingomonas and Cellulosimicrobium by 14.3% and 7.5%, respectively, as well as bacteria of the genera Thermomonas (by 4.3%), Devosia (by 4.3%), and Bacillus and Rhodanobacter (by 2.1% and 1.6%). In the CypN treatments, cypermethrin reduced the relative abundance of *Pseudomonas* (by 9%), Arthrobacter (by 5.2%), and bacteria from the genera Kaistobacter, Terracoccus, Luteolibacter, Nocardioides, and Phycicoccus (from 3.1% to 1.1%). In the CypS treatments, cypermethrin increased the relative abundance of bacteria from the genus Sphingomonas by 12.5% and Devosia, Bacillus, Cellulosimicrobium, Thermomonas, and Rhodanobacter by 3.9%, 3.1%, 2.6%, 2.2%, and 1.6%, respectively. Z. mays had the most positive effect on bacteria of the genus Kaistobacter, Arthrobacter, and Terracoccus, increasing their relative abundance by 9.4%, 8.2%, and 6.3%, respectively. To a lesser extent, it stimulated the growth of bacteria belonging to the genera *Rhodoplanes*, *Nocardioides*, *Phycicoccus*, and Luteolibacter. Sowing uncontaminated soils with Z. mays significantly reduced the relative abundance of bacteria of the genera Sphingomonas and Cellulosimicrobium by 14.3% and 7.5%, respectively. It also decreased the abundance of bacteria from the genera Thermomonas (by 4.3%), Devosia (by 4.3%), and Bacillus and Rhodanobacter (by 2.1% and 1.6%, respectively). In the unsown soil after the application of cypermethrin (CypN), the relative abundances of Pseudomonas and Arthrobacter decreased by 9% and 5.2%, respectively.

Bacteria from the genera *Kaistobacter*, *Terracoccus*, *Luteolibacter*, *Nocardioides*, and *Phycicoccus* also decreased from 3.1% to 1.1% (Figure 4). Sowing soils where cypermethrin was applied had an increased relative abundance of bacteria from the *Sphingomonas* genus of 12.5%. In addition, *Devosia*, *Bacillus*, *Cellulosimicrobium*, *Thermomonas*, and *Rhodanobacter* increased by 3.9%, 3.1%, 2.6%, 2.2%, and 1.6%, respectively. *Z. mays* had the most positive effect on bacteria from the genera *Kaistobacter*, *Arthrobacter*, and *Terracoccus*, increasing their relative abundance by 9.4%, 8.2%, and 6.3%, respectively. To a lesser extent, it also had a positive impact on bacteria from the genera *Rhodoplanes*, *Nocardioides*, *Phycicoccus*, and *Luteolibacter*.

The spatial differentiation of the soil bacteriobiome at the genus level as a function of soil use and cypermethrin contamination is well-illustrated by the PCA plot (Figure 5). Soil use had a greater effect on the sequences of individual genera than contamination with the tested pyrethroid, as evidenced by the vector arrangement in the *Z. mays*-sown soil, regardless of cypermethrin application, and the vector arrangement in the unsown soil. In all soils, the highest number of sequences was obtained for *Cellusimicrobium*, *Sphingomonas*, and *Kaistobacter*, with *Kaistobacter* sequences showing the strongest correlation with CypS and CS, while *Sphingomonas* was correlated with CN and CypN.



Figure 4. The relative abundance of the dominant bacterial genera in soils, calculated using STAMP statistical analysis software, $OTU \ge 1\%$. CS—sown soil with *Z. mays* without cypermethrin, CypS—sown soil with *Z. mays* with cypermethrin, CN—unsown soil without cypermethrin, CypN—unsown soil with cypermethrin. *—statistical significance.



Figure 5. Relative abundance of dominant bacterial genera in soils, presented as a principal component analysis (PCA). CS—sown soil with *Z. mays* without cypermethrin, CypS—sown soil with *Z. mays* with cypermethrin, CN—unsown soil without cypermethrin, CypN—unsown soil with cypermethrin.

3.2.2. Distribution Characteristics of Soil Fungal Community

More fungal sequences were observed in the CS and CN soils than in CypS and CypN (Figure 6). In CS, 156,863 OTUs were recorded; in CN, 67,296 OTUs were recorded; in CypS, 41,268 OTUs were recorded; and in CypN, 29,266 OTUs were recorded. Each of the investigated soil samples was characterized by unique sequences, which can be arranged in the following order from the largest to the smallest amount: CS > CN > CypS > CypN. The total number of sequence reads obtained was 6207 OTUs (Figure 6). Based on the cumulative number of fungal sequences, 14 types, 35 classes, 81 orders, 130 families, and 196 genera were identified. The dominant type of fungi in the soils was *Ascomycota*.



Figure 6. A Venn diagram illustrating the unique and shared fungal sequences in soil samples. CS—sown soil with *Z. mays* without cypermethrin, CypS—sown soil with *Z. mays* with cypermethrin, CN—unsown soil without cypermethrin, CypN—unsown soil with cypermethrin.

Similar to the 16S sequencing, all analyzed indices for all ITS fungal sequences were higher in the CypS samples and lower in the CypN samples. The Shannon index ranged from 2.249 for cultivated soil to 3.048 for cultivated soil treated with cypermethrin. The Simpson index ranged from 0.643 for CS to 0.855 for CypS. The Shannon index ranged from 0.395 to 0.594, the Margalef index ranged from 10.974 to 24.910, the Pielou J index ranged

from 0.395 to 0.594, the Brillouin index ranged from 2.244 to 3.037, and the fungal richness ranged from 116 to 299 (Table 2).

Table 2. Alpha diversity of fungal communities in soil samples.

Object	Shannon	Simpson	Shaneven	Margalefa	Richness	Pielou J	Brillouin
CS	2.249 ^c	0.643 ^c	0.395 ^c	24.910 ^a	299 ^a	0.395 ^c	2.244 ^c
CypS	3.048 ^a	0.855 ^a	0.594 ^a	15.808 ^b	169 ^b	0.594 ^a	3.037 ^a
ĊŇ	2.603 ^b	0.848 ^b	0.541 ^b	10.974 ^d	123 ^c	0.541 ^b	2.597 ^b
CypN	2.588 ^b	0.829 ^b	0.544 ^b	11.182 ^c	116 ^d	0.544 ^b	2.578 ^b

CS—soil sown with *Z. mays* without cypermethrin, CypS—soil sown with *Z. mays* with cypermethrin, CN unsown soil without cypermethrin, CypN—unsown soil with cypermethrin. Homogeneous groups denoted with letters (^{a-d}) were calculated separately for the each diversity indicator in soil samples.

At the phylum level, the fungal community in all soils, regardless of land use and cypermethrin application, was dominated by *Ascomycota*, ranging from 78.19% in CypS soil to 93.90% in CS. Considering sequences constituting more than 1% apart from *Ascomycota*, the phyla *Basidiomycota*, *Mortierellomycota*, *Rozellomycota*, and *Chytridiomycota* were also identified. Both soil contamination with cypermethrin and sowing with *Z. mays*, similar to bacterial sequences, differentiated the proportions of sequences among the soil samples studied (Figure 7). Cypermethrin in unplanted soil (CypN) primarily decreased the relative abundance of *Ascomycota* (by 10.3%) and increased *Rozellomycota* (by 7.8%). Comparing CypS and CS objects, cypermethrin reduced the relative abundance of *Ascomycota* by 7.5%. When comparing CypS and CypN objects, the pyrethroid under discussion decreased the abundance of *Rozellomycota* by 9.8% and increased *Basidiomycota* by 7.3%. Sowing in uncontaminated soils increased the relative abundance of *Ascomycota* by 1.8% and *Rozellomycota* by 3.5%.



Figure 7. The relative abundance of the dominant fungal types in soils, calculated using STAMP statistical analysis software, $OTU \ge 1\%$. CS—soil sown with *Z. mays* without cypermethrin, CypS—soil sown with *Z. mays* with cypermethrin, CN—unsown soil without cypermethrin, CypN—unsown soil with cypermethrin. *—statistical significance.

Out of 196 sequences classified to the genus level, 18 had a relative abundance of \geq 1% (Figure 8). In the CS and CypS soils, *Chaetomium* dominated at 72.21% and 44.52%,

respectively, whereas in the CypN and CN treatments, *Penicillium* dominated at 53.88% and 41.02%, respectively. Analyzing the data presented in Figure 6, it was observed that cypermethrin moderated fungal sequences at the genus level in a somewhat different manner. Specifically, in the CypN objects, cypermethrin most significantly reduced the relative abundance of fungi from the genera *Humicola* and *Penicillium* (by 15.5% and 12.9%, respectively) while increasing *Botryotrichum* (by 35.2%). In the CypS objects, cypermethrin most significantly reduced the relative abundance of *Chaetomium* fungi (by 27.7%) and increased *Penicillium* (by 21.5%). The mycobiome of the soil was also influenced by the soil's use. Sowing of uncontaminated soil with *Z. mays* most significantly reduced the relative abundance of *Penicillium* and *Humicola* fungi by 52.4% and 14.9%, respectively, and increased *Chaetomium* by 36.8%. In the unsown soil, cypermethrin reduced the relative abundance of *Chaetomium* by 38.5% and *Solicoccozyma* and *Mortierella* by 5.7% and 4.5%, respectively, whereas it increased *Botryotrichum* by 34.5% and *Penicillium* by 18.1% compared with the *Z. mays*-sown soil.



Figure 8. The relative abundance of the dominant fungal genera in soils, calculated using STAMP statistical analysis software, $OTU \ge 1\%$. CS—soil sown with *Z. mays* without cypermethrin, CypS—soil sown with *Z. mays* with cypermethrin, CN—unsown soil without cypermethrin, CypN—unsown soil with cypermethrin. *—statistical significance.

The direction of vectors representing independent variables in soil (Figure 9) was positive, while those describing bacteria (Figure 4) were negative. Similar to the bacterial sequences, the fungal sequences depended more on soil usage than on contamination with cypermethrin. The vectors CypS and CS, as well as CypN and CN, were positively correlated. In CypN and CN, *Penicillium* and *Botryotrichum* dominated, whereas in CypS and CS, *Chaetomium* was the dominant species. The remaining fungal sequences formed a homogeneous group and were less abundant.



Figure 9. Relative abundance of dominant fungal genera in soils, presented as a principal component analysis (PCA). CS—soil sown with *Z. mays* without cypermethrin, CypS—soil sown with *Z. mays* with cypermethrin, CN—unsown soil without cypermethrin, CypN—unsown soil with cypermethrin.

3.3. Response of Soil Enzymes to Cypermethrin and Z. mays

The activity of soil enzymes, similar to the abundance of bacterial and fungal sequences, was more influenced by soil management than cypermethrin dose (Table 3). The activities of Deh, AlP, Aryl, and Glu were significantly higher in soils sown with *Z. mays*, regardless of cypermethrin application, whereas Cat activity was only higher in unsown soil. In the unsown soil, cypermethrin significantly reduced the activities of Deh, Cat, and AcP and stimulated Ure activity. In contrast, in *Z. mays*-sown soil, cypermethrin reduced AcP and Aryl activities but stimulated AlP activity.

Table 3. Tl	he effect of ind	lependent va	riables on enz	yme activity per	1 of soil \times h ⁻¹ .
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Object	Deh µmol TFF	Cat mol O _{2.5}	Ure	AcP	AlP Aryl			
			mmol N-NH ₄					
CS	$4.41~^{a}\pm0.28$	$0.17\ ^{\mathrm{c}}\pm0.01$	$0.06^{\text{ b}}\pm0.00$	$3.89~^{a}\pm0.10$	$0.63^{\text{ b}} \pm 0.01$	$0.12~^{a}\pm0.01$	$0.34~^a\pm0.01$	
CypS	$4.61\ ^{a}\pm0.31$	$0.15~^{\rm c}\pm0.00$	$0.06\ ^{ m b}\pm 0.00$	0.73 $^{\rm c}\pm 0.01$	$0.74~^{\rm a}\pm0.01$	$0.10^{\ \mathrm{b}} \pm 0.00$	$0.33~^{\mathrm{ab}}\pm0.01$	
ĊN	$1.39^{\text{ b}} \pm 0.05$	$3.98~^{a}\pm 0.03$	$0.05~^{\rm c}\pm0.03$	$3.03 \ ^{ m b} \pm 0.05$	$0.59~^{\rm c}\pm0.03$	$0.09~^{ m b}\pm 0.01$	$0.29~^{\rm c}\pm0.02$	
CypN	0.75 $^{\rm c}\pm 0.06$	$3.88 \text{ b} \pm 0.03$	0.07 $^{a}\pm0.03$	$0.53~^{\rm d}\pm0.01$	$0.50~^{d}\pm0.02$	$0.09~^{\mathrm{b}}\pm0.00$	$0.30 \text{ bc} \pm 0.00$	

Deh—dehydrogenase, Cat—catalase, Ure—urease, AcP—acid phosphatase, AlP—alkaline phosphatase, Glu— β -glucosidase, Aryl—arylsulfatase, TFF—1,3,5-triphenyl formazan, PNP—4-nitrophenol, CS—sown soil with *Z. mays* without cypermethrin, CypS—sown soil with *Z. mays* with cypermethrin, CN—unsown soil without cypermethrin, CypN—unsown soil with cypermethrin. Homogeneous groups denoted with letters (^{a-d}) were calculated separately for each enzyme.

The PCA (Figure 10) indicates that the vectors describing the independent variables CypS and CS, as well as CypN and CN, were positively correlated. In the CypN and CN objects, the highest catalase activity was observed, while in the CypS and CS objects, dehydrogenase activity was the highest. On the other hand, acid phosphatase activity was highest in the CN and CS objects. The activities of AcP, Glu, Ure, and Aryl formed a homogeneous group.



Figure 10. Soil enzyme activity presented as a principal component analysis (PCA). CS—sown soil with *Z. mays* without cypermethrin, CS—sown soil with *Z. mays* without cypermethrin, CypS—sown soil with *Z. mays* with cypermethrin, CN—unsown soil without cypermethrin, CypN—unsown soil with cypermethrin; Deh—dehydrogenase, Cat—catalase, Ure—urease, AcP—acid phosphatase, AlP—alkaline phosphatase, Glu— β -glucosidase, Aryl—arylsulfatase.

3.4. Interdependence between Bacteria and Fungi and Soil Enzyme Activity at the Genus Level3.4.1. Correlation between Bacteria and Fungi

The changes occurring in the microbial communities in soil exposed to cypermethrin are well-described by correlation coefficients between the number of bacterial genus sequence counts and the number of fungal genus sequence counts (Figure 11). Particularly noteworthy are the positive correlations between the abundance of the Kaistobacter, Rhodoplanes, Phycicoccus, Terracoccus, Arthrobacter, Nocardioides and Pseudogymnoascus, Trichoderma, Gibberella, Acremonium, Chaetomium, Fusarium, Botryotrichum, Mortierella, Naganishia, Solicoccozyma, Paracremonium and Devosia, Sphingomonas, Rhodanobacter, Thermomonas, Cellulosimicrobium, Stenotrophomonas and Meyerozyma, Penicillium, Iodophanus, Humicola, Dichotomopilus sequences. Significant negative correlations were observed between the sequences of Kaistobacter, Rhodoplanes, Phycicoccus, Terracoccus, Arthrobacter, Nocardioides and Meyerozyma, and Penicillium and between Devosia, Rhodanobacter, Thermomonas, Cellulosimicrobium and Pseudogymnoascus, Trichoderma, Chaetomium, Fusarium, Mortierella, Naganishia, Solicoccozyma, Paracremonium, and Ascobolus. Additionally, considering the cluster analysis, two homogeneous subsets were identified for bacteria (1-Devosia, Sphingomonas, Rhodanobacter, Thermomonas, Cellulosimicrobium, Bacillus, and Stenotrophomonas and 2-Pseudomonas, Luteolibacter, Kaistobacter, Rhodoplanes, Phycicoccus, Terracoccus, Arthrobacter, and Nocardioides) and three for fungi (1—Pseudogymnoascus, Trichoderma, Gibberella, Acremonium, Chaetomium, Fusarium, and Botryotrichum; 2-Mortierella, Naganishia, Solicoccozyma,



Paracremonium, and *Ascobolus* and 3—*Dichotomopilus*, *Humicola*, *Iodophanus*, *Penicillium*, *Meyerozyma*, and *Pseudeurotium*).

Figure 11. Pearson's correlation coefficients between the abundance of dominant bacterial genera and fungi are significant at p = 0.05, n = 12. *—statistical significance.

3.4.2. Correlation between Bacteria and Soil Enzymes

The strongest connections between soil enzyme activity and the number of bacterial genus-level sequences were observed for catalase, dehydrogenases, β -glucosidase, arylsulfatase, and alkaline phosphatase. No significant correlation was found between urease and acid phosphatase activity (Figure 12). However, even the significant correlation between enzyme activity and individual bacterial genera was specific and had both negative and positive values. The data analysis allows us to distinguish a group of genera (*Kaistobacter*, *Arthrobacter*, *Terracoccus*, *Rhodoplanes*, *Nocardioides*, and *Phycicoccus*) that were positively correlated with Deh, Aryl, and Glu activity and a group that was negatively correlated mot only with these enzymes but also with AIP (*Devosia*, *Sphingomonas*, *Bacillus*, and *Rhodanobacter*). Interestingly, in the case of a positive correlation between dehydrogenases and individual bacterial genera, there was a negative correlation with catalase, and, conversely, a positive correlation between genera and catalase resulted in a negative correlation with dehydrogenases.



Figure 12. Pearson's correlation coefficients between enzyme activity and the abundance of dominant bacterial genera. * significant at p = 0.05, n = 12, Deh—dehydrogenase, Cat—catalase, Ure—urease, AcP—acid phosphatase, AlP—alkaline phosphatase, Glu— β -glucosidase, Aryl—arylsulfatase. *— statistical significance.

3.4.3. Correlation between Fungi and Soil Enzymes

Similar to the correlation between bacteria and oxidoreductases, the correlations between fungal genera and dehydrogenases and catalase were arranged in an opposite manner (Figure 13). Fungi that were significantly positively correlated with dehydrogenase activity were negatively correlated with catalase activity. Those that were least correlated with soil enzyme activity were the genera *Humicola* and *Dichotomopilus*, and the most correlated were *Chaetomium*, *Fusarium*, and *Acremonium* because these genera were positively correlated with the activity of four enzymes (Deh, AcP, Aryl, and Glu). *Mortierella* and *Naganishia* were positively correlated with Deh, AlP, Aryl, and Glu. *Pseudogymnoascus* and *Gibberella* were significantly positively correlated with three enzymes (AcP, Aryl, Glu), as well as *Paracremonium* (Deh, AlP, and Glu). The strongest negative correlation between soil enzyme activity and the number of sequences occurred in the *Meyerozyma* genus (Deh, Ure, Aryl, and Glu) and the *Iodophanus* genus (Ure, Aryl, and Glu).



Figure 13. Simple correlation coefficients between enzyme activity and the abundance of dominant fungal genera. * significant at p = 0.05, n = 12. Deh—dehydrogenase, Cat—catalase, Ure—urease, AcP—acid phosphatase, AlP—alkaline phosphatase, Glu— β -glucosidase, Aryl—arylsulfatase. *— statistical significance.

4. Discussion

4.1. The Effect of Arpon-G on the Growth and Development of Z. mays

Our results demonstrate that soil contamination with Arpon G preparation at a rate of 40 mg of cypermethrin per kg⁻¹ of soil did not disrupt the growth and development of Z. mays or the plant's greenness index. Based on the results of a previous study [35], in which it was demonstrated that cypermethrin at a dose of 80 mg per kg⁻¹ soil dry mass disrupts the yield of Z. mays, an equally negative response of the cultivated plant was anticipated in our research. This is justified by the induction of increased lipid peroxidation, which indicates the degradation of the cell membrane system. Consequently, pyrethroid insecticides enhance the production of reactive oxygen species (ROS), thereby disrupting fundamental physiological processes in plants [45]. Proteins and chlorophyll pigments are also decomposed, which translates into a reduction in photosynthetic efficiency [46]. On the other hand, the milder response of Z. mays to the applied substance undoubtedly resulted from the half dose of cypermethrin. This response likely originated from the activation of plant xenobiotic detoxification mechanisms, including plant protection agents. These mechanisms primarily involve glutathione conjugation and the expression of three genes in Z. mays, namely, ZmGT1, ZmMRP1, and ZmGST27. These genes encode glutathione transporters, ATP-binding cassette (ABC) transporter, and glutathione S-transferase, respectively [47].

4.2. Response of Bacteria and Fungi to Cypermethrin

In our research, special attention was paid to the bacteria *Cellosimicrobium*, *Sphingomonas*, and *Kaistobacter*, as well as the fungi *Penicillium*, *Botryotrichum*, and *Chaetomium*, which were the most abundant in all soils, regardless of land use and cypermethrin application. Considering the abundance of sequences from the genera *Pseudomonas*, *Thermomonas*, *Kaistobacter*, *Bacillus*, *Luteolibacter*, and *Cellulosimicrobium* among the bacteria, as well as *Penicillium*, *Paracremonium*, *Meyerozyma*, *Ascobolus*, *Pseudeurotium*, and *Iodophanus* among the fungi in

the soil samples to which the Arpon G preparation was applied, it can be inferred that cypermethrin, the active ingredient of the tested insecticide formulation, served as a valuable carbon and energy source and acted as a proton and electron donor for these microorganisms. Huang et al. [48] and Deng et al. [49] emphasized that the primary mechanisms of pesticide degradation are mineralization and cometabolism, whereby microorganisms can utilize certain compounds present in pesticides as nutritional components.

The degradation process of cypermethrin depends on various factors, such as soil type, the presence of microorganisms, and environmental conditions [23]. Bhatt et al. [24] also emphasized the soil's organic matter content, microbial communities, and the type of vegetation cover.

Our research indicates that the application of the insecticide Arpon G contributed to a reduction in the number of unique nucleotide sequences in both bacteria and fungi. Greater perturbations occurred in the mycobiome than in the bacteriobiome. Larger changes in the mycobiome may be due to the increased sensitivity of certain fungal genera that inhabit soil extensively. This primarily concerns fungi from the *Chaetomium* genus, the abundance of which decreased by 27.7% in *Z. mays*-sown soil under the influence of cypermethrin and by 38.5% in unsown soil. These results align with those obtained by Zhang et al. [50], who demonstrated that an increase in soil bacterial populations following cypermethrin addition resulted from a reduction in fungal populations, leading to decreased competition for nutrients among the remaining microbial populations.

Insecticidal formulations containing cypermethrin as the active substance are currently of considerable interest and are widely used in agriculture [23,24,51,52]. However, despite the fact that the half-life of cypermethrin in the soil ranges from 14 to 77 days [22–24], due to its frequent use and its lability to enter the environment, it can accumulate in the soil in quantities that pose a threat to living organisms under certain conditions [53].

Intermediate transformation products of cypermethrin, such as 3-phenoxybenzoic acid (3-PBA) [24,49], may be particularly hazardous, potentially leading to secondary contamination of agricultural products [48]. Additionally, it should be noted that this acid is highly persistent in the environment, and microorganisms capable of converting cypermethrin into 3-PBA demonstrate relatively low efficiency in its further degradation. Moreover, these microorganisms are not well-understood [51]. Hence, metagenomic studies are of paramount importance as they allow for the analysis of soil microbiomes [54,55]. Current next-generation sequencing methods, targeting 16S rRNA and ITS, which were used in this study, provide new insights that deepen our understanding of the interactions between microbial communities and insecticides [24,56,57]. In the literature [15,32,48,56,58], it is evident that there is a wide range of bacteria, including *Acinetobacter*, *Brevibacillus*, *Brevibacterium*, *Sphingomonas*, *Bacillus*, *Brevibacillus*, *Pseudomonas*, *Serratia*, *Klebsiella*, *Rhodococcus*, *Alcaligenes*, *Micrococcus*, *Rhodococcus*, *Comamonas*, and *Lysinibacillus* and fungi such as *Fusarium*, *Aspergillus*, *Trichoderma*, *Monilochaetes*, and *Candida*, which possess the capability to degrade cypermethrin.

Both our research and the literature [34] indicate that the bacterial and fungal genera that respond with a reduction in the number of nucleotide sequences in the soil due to a stressor, undoubtedly represented by the application of the insecticide Arpon G, can be useful in determining soil diversity.

Furthermore, considering that the majority of previous studies regarding the effect of cypermethrin on soil health have focused primarily on bacterial degradation [56], the authors of this study decided to incorporate nucleotide sequences of both bacteria and fungi to determine correlations between the abundance of these microorganisms. It has been demonstrated that the bacteria *Kaistobacter*, *Rhodoplanes*, *Phycicoccus*, *Terracoccus*, *Arthrobacter*, and *Nocardioides* are positively correlated with *Pseudogymnoascus*, *Trichoderma*, *Gibberella*, *Acremonium*, *Chaetomium*, *Fusarium*, *Botryotrichum*, *Mortierella*, *Naganishia*, *Solicoccozyma*, and *Paracremonium* and the bacteria *Devosia*, *Sphingomonas*, *Rhodanobacter*, *Thermomonas*, *Cellulosimicrobium*, and *Stenotrophomonas* are correlated with fungi *Meyerozyma*, *Penicillium*, *Iodophanus*, *Humicola*, and *Dichotomopilus*. Determining these interdependencies between the bacteriome and mycobiome may be crucial for analyzing the potential biodegradation of cypermethrin, especially since among these microorganisms, there are also bacteria (*Arthrobacter, Bacillus*, and *Sphingomonas*) and fungi (*Trichoderma*) from the plant growth-promoting rhizobacteria group. Therefore, they may also induce systemic plant resistance [59,60].

4.3. Response of Soil Enzymes to Cypermethrin

Biochemical activity plays a crucial role in maintaining the functional stability of soil ecosystems [27,61]. It serves as a good indicator of soil health [62–65]. To date, most studies have also described the phytoremediation potential of plants for cypermethrin-contaminated soils [35,66–69]. Some studies also considered accumulation in plants [66,67] as well as the potential of bacteria in the biodegradation of these pollutants [51,70,71].

In our research, it was observed that the effect of cypermethrin was dependent on the land use of the soil. Specifically, in the unsown soil, cypermethrin significantly reduced the activity of Deh, Cat, and Pac while stimulating the activity of Ure. Conversely, in the *Z. mays*-sown soil, cypermethrin only decreased Pac and Aryl activity while stimulating Pal. The differential impact of cypermethrin on soil endoenzymes and exoenzymes in unsown and *Z. mays*-sown soil is likely due to the secretion of organic compounds by the root system [72,73]. The different response of dehydrogenases to Arpon G in unsown and *Z. mays*-sown soil is probably also the result of greater adsorption of cypermethrin by colloids in the sown soil, which resulted in its lower content in the soil solution and, therefore, decreased its availability for microorganisms [34].

Therefore, in our research, determining the correlation between the abundance of bacteria and fungi most densely inhabiting the soil and the activity of seven important soil enzymes appears to be highly significant. It was demonstrated that bacteria (*Arthrobacter, Nocardioides, Phycicoccus, Terracoccus, Rhodoplanes, Kaistobacter, Luteolibacter,* and *Pseudomonas*) and fungi (*Trichoderma, Fusarium, Chaetomium, Acremonium, Mortierella, Nagasishia, Solicoccozyma, Paracremonium,* and *Ascobolus*), which were significantly positively correlated with dehydrogenase activity, were negatively correlated with catalase activity. Furthermore, it was observed that there is a group of bacteria, including *Kaistobacter, Arthrobacter, Terracoccus, Rhodoplanes, Nocardioides, Phycicoccus,* and fungi, including *Chaetomium, Fusarium,* and *Acremonium,* which is positively correlated with Deh, Aryl, and Glu activities. The determination of these dependencies provided crucial insights into the microbial responses and plant productivity under soil pollution stress induced by the Arpon G pesticide.

Frequent insecticide application leads to the accumulation of cypermethrin metabolites in the soil [74–77], which can significantly affect soil microbiological and enzymatic activity [78,79]. Insecticide use often affects enzymes involved in the carbon and nitrogen cycles, such as cellulase and urease, as well as phosphatases and arylsulfatases, which affect phosphorus and sulfur cycling [32,80–85]. However, there is a limited amount of research on the impact of cypermethrin on soil enzyme activity [34]. Therefore, in our studies, to assess the influence of cypermethrin on soil environmental quality, we used enzymes belonging to the classes of oxidoreductases and hydrolases. According to Tejada et al. [34], Wu et al. [86], Borowik et al. [87], and Caglayan et al. [88], elevated concentrations of pyrethroids in the soil can significantly affect both the microbiological and enzymatic activity of the soil. Therefore, we believe that the variability in the results obtained under the influence of cypermethrin on the soil bacterial and fungal populations and soil enzymatic activity is a function not only of cypermethrin itself but also of the interactions between cypermethrin and microorganisms, bacteria and fungi, bacteria and soil enzymes, and fungi and soil enzymes.

Based on the conducted research, it can be concluded that establishing correlations between the abundance of nucleotide sequences of bacteria and the activity of key enzymes involved in the C, N, P, and S cycles, as well as between the abundance of fungi and enzyme activity, can be highly valuable in designing strategies for the bioremediation of agricultural soils contaminated not only with the insecticide Arpon G but also with other pesticides.

5. Conclusions

The insecticide Arpon G does not disrupt the productive function of the soil, as measured with the quantity of Z. mays biomass obtained. The lower number of unique nucleotide sequences of bacteria and fungi in soil samples containing Arpon G compared with samples without this insecticide demonstrates the succession of microorganisms involved in the transformation of cypermethrin. The alpha diversity of bacteria is more dependent on soil management practices than the alpha diversity of fungi. However, the beta diversity of these microorganisms is determined more by soil management practices than by the effect of cypermethrin and is, therefore, more influenced by the presence of Z. mays than by the action of the Arpon G. In the soils studied, Actinobacteria bacteria and Ascomycota fungi are dominant. The mutual relationships are specific to individual types of microorganisms. The number of nucleotide sequences of bacterial genera can be positively or negatively correlated with the number of sequences of fungal genera. Within both bacterial and fungal genera, genera that are positively and negatively correlated with the activity of specific enzymes can be distinguished. Z. mays stimulates soil enzyme activity, whereas Arpon G modifies it only slightly. Among the seven enzymes examined, it reduces the activity of only two enzymes (acid phosphatase and arylsulfatase). This conducted research indicates that the insecticide Arpon G does not pose a significant threat to the soil environment, provided that good agricultural practices are followed during its application.

Author Contributions: Conceptualization, experimental design, and methodology, J.W., A.B., M.Z. and J.K.; investigation, J.W. and A.B.; statistical analyses, J.W., A.B. and M.Z.; writing—review and editing, J.W., A.B. and M.Z.; supervision, J.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the University of Warmia and Mazury in Olsztyn, Faculty of Agriculture and Forestry, Department of Soil Science and Microbiology (grant No. 30.610.006-110) and project financially supported by the Minister of Education and Science under the program entitled "Regional Initiative of Excellence" for the years 2019–2023, Project No. 010/RID/2018/19, and the amount of funding 12.000.000 PLN.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript: CS—soil sown with *Z. mays* without cypermethrin, CypS—soil sown with *Z. mays* with cypermethrin, CN—unsown soil without cypermethrin, CypN—unsown soil with cypermethrin. SPAD—greenness index; Deh—dehydrogenase; Cat—catalase; Ure—urease; AcP—acid phosphatase; AlP—alkaline phosphatase; Aryl—arylsulfatase, Glu— β -glucosidase.

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