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Microbiological Nitrogen Transformations in Soil Treated with Pesticides and Their Impact on Soil Greenhouse Gas Emissions

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Abstract: Research was conducted in connection with the pressure exerted by man on the environment through the use of pesticides. The aim of the study was to assess the impact of pesticides on soil and to evaluate the effect of these changes on greenhouse gas emissions into the atmosphere. The research was carried out on soil sown with oilseed rape. The activity of protease and urease, ammonification, nitrification in soil, as well as CO_2 (carbon dioxide) and N_2O (nitrous oxide) gas emissions from soil were assessed. The analyses were carried out directly after harvest and 2 months after. Pesticides most frequently negatively affected the tested parameters, in particular enzymatic activities. Of the two herbicides used, Roundup had a stronger negative impact on microbial activity. The application of pesticides, especially the fungicide, resulted in an increase in gas emissions to the atmosphere over time. Pesticides disturbed soil environmental balance, probably interfering with qualitative and quantitative relationships of soil microorganism populations and their metabolic processes. This led to the accumulation of microbial activity products in the form of, among others, gases which contribute to the greenhouse effect by escaping from the soil into the atmosphere.

Keywords: pesticides; enzymatic activity; nitrification; ammonification; greenhouse effect; soil; bacteria; fungi; rape cultivation

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1. Introduction

Modern agriculture contributes significantly to the pollution of the natural environment, i.e., air, water and soil. Agricultural production can cause a number of negative changes in the natural environment, including movement of nitrates and pesticides to groundwater and greenhouse gas emissions (carbon dioxide, nitrous oxide and methane). Agriculture is a major anthropogenic source of these gases, emitting about 60% of CH_4, 15% of CO_2 and 61% of N_2O [1,2]. Emission sources of gases in agriculture are processes related to the transformation of mineral fertilizers, manure, decomposition of plant residues and enteric fermentation by ruminants, especially beef and dairy cattle [3–6]. The use of mineral fertilizers in the world contributed to the emission of 704,441.912 gigagrams of CO_2 and 2272.393 gigagrams of N_2O in 2017 [7]. On the one hand, the use of organic waste in agriculture leads to neutralization and improvement of soil quality, but on the other hand, it also contributes to atmospheric pollution by increasing greenhouse gas emissions from soil [8]. Microbial degradation of pesticides can lead to the formation of more toxic products than pesticides themselves [3]. Pesticides have become a crucial component of high-yielding agriculture. In the world, the use of pesticides amounted to 4,113,591.25 tons in 2017 [7]. An ideal chemical applied in agriculture should fulfill its role and quickly degrade to non-toxic products. The dynamics of changes related to pesticide residues in soil depends, among others, on the content of organic carbon (OC), dissolved organic carbon (DOC) and moisture [9]. Carpio et al. [9] found that OC reduced the mobility and availability of herbicides, while DOC and moisture increased the risk of soil and water contamination by them. Simultaneously, dissolved organic carbon (DOC) facilitated herbicide transport, which is favored by the initial soil moisture and rainfall shortly after the initial application of chemicals. Various indicators can be used to assess the risk of the presence of pesticide residues in soil. Astaykina et al. [10] developed a risk indicator for the negative effects of pesticides on soil and aquatic organisms based on the exposure to acute toxicity, long-term toxicity and bioconcentration. The use of pesticides is a source of environmental contamination that can affect the activity of soil fungi and bacteria associated with organic matter decomposition [11–13]. Tomkiel et al. [11] found a negative effect of the herbicide Boreal 58 WG on the development of various groups of bacteria (azotobacter, organotrophic bacteria, Actinomycetes) and fungi, as well as enzymatic activity. The latter authors also noted that this effect was dose- and time-dependent. Other studies found that the fungicide chlorothalonil exerted a stimulating effect on the growth of bacteria and actinomycetes, as well as on the activity of dehydrogenases, catalase and acid phosphatase, while fungal growth was inhibited [12]. Soil supplementation with materials such as compost, manure or sewage sludge may contribute to the stimulation of soil microorganisms with pesticide-decomposing abilities [13]. Changes in microbial activity can ultimately affect greenhouse gas emissions from the soil into the atmosphere [14]. Increasing CO_2 levels and global warming are driving interest in identifying sources and reducing greenhouse gas emissions from agriculture. Over 90% of CO_2 emitted from the soil is of microbial origin. Respiratory activity is considered an indirect indicator of the total number and activity of microorganisms in soil and can be an indicator of changes occurring in this environment [15]. Alterations in the intensity of respiratory processes may indicate ecological disturbances. They also indicate a high involvement of microorganisms in soil metabolism and global warming. Nitrous oxide is produced by microbial nitrogen transformations in soils and water. Nitrification and denitrification are two main processes recognized as contributing to gas emissions [16].

According to the Intergovernmental Panel on Climate Change (IPCC), it is necessary to reduce global gas emissions by 70–80% [1]. Agriculture must contribute to this and it is important to find solutions that will help farmers and land users make better decisions regarding crop management and environmental protection [16]. The nitrogen cycle consists of a series of steps, and the processes associated with these changes in the nitrogen oxidation state are an important aspect in understanding environmental microbiology [17]. Therefore, research was conducted to examine the impact of various chemicals applied in the cultivation of winter oilseed rape on the activity of microorganisms associated with this biogen circulation, i.e., ammonification, nitrification, protease and urease activity, and emission of their activity products—N_2O and CO_2.

The results of previous studies concerning the impact of individual pesticides on the activity of soil microorganisms are ambiguous. Bruckner et al. [18] explained this by the fact that many of these studies were performed under laboratory conditions, sometimes without the involvement of plants, and thus they did not take into account the interactions between all biotic factors. Therefore, the presented research provides valuable information to the current state of knowledge, because it was conducted in field conditions, taking into account the influence of all biotic factors. We hypothesized that individual types of pesticides and preparations, by shaping the activity of soil microorganisms, affected greenhouse gas emissions with varying intensity.

2. Materials and Methods

2.1. Field Experiment

The soil was taken from winter oilseed rape cultivation fields located at the Experimental Station for Variety Testing in Glebokie (Kujawsko-Pomorskie Province, Poland, 52°38′41″ N, 18°26′18″ E). This region has an average annual temperature of +8 °C (−2 °C in January and +18 °C in July), and an average annual precipitation ranges from 600 to 650 mm.

The soil was a black soil (Mollic Gleysols) characterized as light silty clay [19]. Soil grain size composition was as follows: 65% sand fraction (0.05–2 mm), 23% silt fraction

(0.002–0.05 mm), 12% clay (<0.002 mm). The chemical characteristics of the soil are given in Table 1.

Table 1. Chemical characteristics of the soil used in the experiment.

Parameter	Unit	Value
pH	pH_{KCl}	6.1
Corg. Total nitrogen	g/kgd.m.	9.8 1.3
C:N		7.5
Total phosphorus K	g/kg d.m.	0.7 0.1
Zn Cd Cu Pb Ni Cr Hg	mg/kg d.m.	34.0 0.15 10.8 9.6 7.5 14.4 0.1
HA	cmol/kg d.m.	1.38

pH—hydrogen ions concentration, Corg—organic carbon, HA—hydrolytic acidity, d.m.—dry matter. Ph—potentiometric method, Corg-IR spectrometry method, Ntot-Kjeldahl method, content of other elements—Mercury analyzer, gravimetric method, determination by atomic absorption spectrometry with excitation in acetylene-air flame.

The experiment was established using a split-block design. The plots were sown with a winter oilseed rape. The experiment involved basic agriculture procedures applied in winter oilseed rape cultivation. Oilseed rape was sown on August 20. This date is optimal because oilseed rape enters the winter period with a rosette of 6–8 shaped leaves and a thick root collar, which is favorable for its wintering. Mineral fertilization was used according to nutritional requirements for winter oilseed rape: nitrogen—140 kg/ha, phosphorus—18.33 kg/ha, potassium—58 kg/ha, sulfur—36 kg/ha.

One of the listed pesticides was used at the highest recommended dose in individual treatments: the herbicide Roundup 360 SL (4 dm^3/ha), herbicide Reglone 200 SL (2 dm^3/ha), fungicide Caramba 60 SL (1 dm^3/ha) and adjuvant Spodnam 555 SC (1.2 dm^3/ha). Soil without pesticides served as control. The area of each plot, including control, was 12 m^2.

2.2. Characteristics of Pesticides

Roundup 360 SL was used 7 days before, while Reglone 200 SL and Spodnam 555 SC 5 days before oilseed rape harvest and Caramba in the spring. All agents were applied using a knapsack sprayer. The chemical characteristics of individual preparations are listed in Table 2.

Table 2. Preparation characteristics.

Preparation	Type	Active Substance	Formula	Producer
Roundup 360 SL	herbicide	glyphosate	$C_3H_8NO_5P$	Monsanto Europe S.A Antwerp, Belgium
Reglone 200 SL	herbicide	diquat dibromide	$C_{12}H_{12}N_2Br_2$	Syngenta Crop Protection AG Basel, Switzerland
Caramba 60 SL	fungicide	metconazole	$C_{17}H_{22}ClN_3O$	BASF Agricultural Solution, Ludwigshafen am Rhein, Germany
Spodnam 555 SC	adjuvant	di-1-P-menten	$(C_{10}H_{18})_2$	Mandops Limited Eastleigh, United Kingdom

2.3. Soil Sampling

Soil samples were collected twice, i.e., directly after harvest (August—1st time point) and two months after harvest (October—2nd time point). Soil samples were collected from the topsoil (0–20 cm) into sterile plastic bags. Samples were collected from 10 randomly selected sites from each plot treated with a given pesticide and from the control plot. The bulk sample from each treatment (three plots) consisted of soil cores 4 cm in diameter each. Laboratory analyses were performed in three replicates of an averaged sample from a given treatment. Soil samples were sieved through a 2-mm mesh sieve and stored in a refrigerator at +4 °C.

2.4. Enzymatic Analyses

Protease activity was determined in 2-g soil samples incubated in 0.2 M Tris-HCl buffer (pH 8.0) for 1 h at 50 °C using casein as a substrate (5 mL sodium caseinate solution) [20]. The concentration of released tyrosine was measured spectrophotometrically at 578 nm. Urease activity was determined in 10-g soil samples using urea solution as a substrate (10 mL) and incubation for 18 h at 37 °C [21]. The concentration of ammonium ions was measured spectrophotometrically at a wavelength of 410.

2.5. Biochemical Analyses

Intensification of the ammonification process was determined in 25-g soil samples containing 0.1% asparagine. After 3 days of incubation, ammonium ions were extracted using 2 M KCl (mixing for 20 min) and their content was determined using the Nessler method [22]. Intensification of the nitrification process was determined in 25-g soil samples containing 0.1% monoammonium phosphate. After 7 days of incubation, nitrate ions were extracted using 2 M KCl (mixing for 20 min) and their level was measured by the brucine method [22].

2.6. Gas Emission

In order to evaluate gas emission a mixture of 2 g of soil with 2 mL of distilled water were incubated in 25 °C in tightly closed vessels with rubber stoppers with a membrane enabling gas sampling. Air samples (0.025 mL) were collected from above the incubated soil. CO_2 and N_2O emissions were determined after 7 days of above-mentioned soil incubation according to Włodarczyk [23] and Horn et al. [24]. Gas samples were analyzed for CO_2 and N_2O content using a gas chromatograph (GC-14, Shimadzu, Kyoto, Japan) equipped with TCD and ECD.

2.7. Statistical Analysis

All analyses were carried out in triplicate. The results were statistically analyzed using the Statistica 13 software (StatSoft Inc., Tulsa, OK, USA) with ANOVA (analysis of variance) models and multiple Tukey’s t-tests at the significance level of $\alpha = 0.05$. To verify whether ANOVA assumptions were met, including dataset normality and homogeneity of the variance, the Shapiro-Wilk and Levene tests were used, respectively, and they showed that indeed those criteria were met. The results are shown in graphs with standard deviation indicated. The graphs depict mean values of three replicates obtained for a given sample. The relationships between the analyzed microbial activities and gas emissions in each object was determined using principal component analysis (PCA method).

3. Results

3.1. Enzymatic Activity

The results regarding the impact of pesticides on soil enzymatic activity are presented in Figure 1A,B. Chemicals applied, i.e., herbicides (Roundup, Reglone), fungicide (Caramba) and adjuvant (Spodnam) caused a statistically significant decrease in protease activity in soil compared to the control treatment (no pesticides), where the activity was 13.65 mg in the first sampling and 7.79 mg in the second sampling (Figure 1A). Inhibition was maintained both in the first and the second sampling point. The lowest proteolytic activity was observed in the treatment with the herbicide Roundup (1.20 mg) and fungicide Caramba (1.24 mg). The weakest negative effect was caused by the adjuvant Spodnam (7.43–8.65 mg).

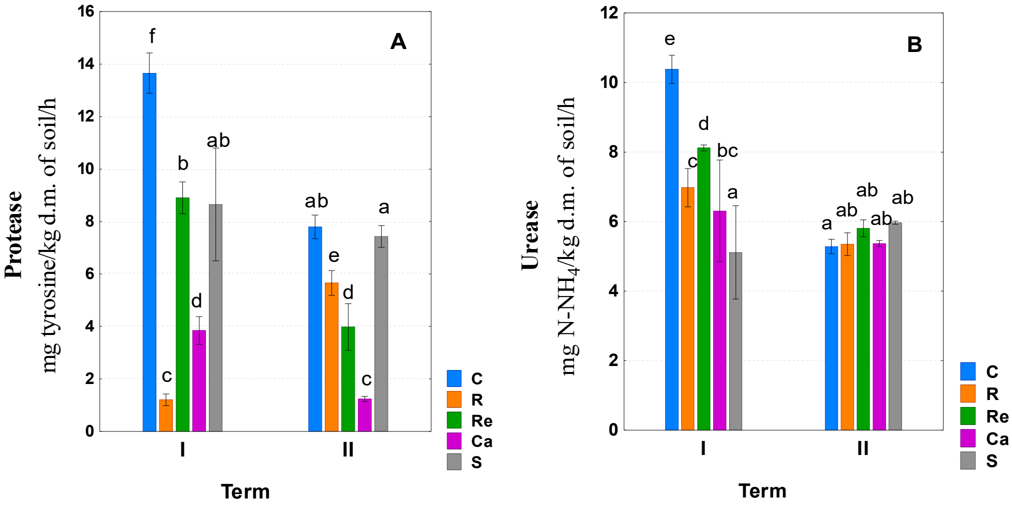

Figure 1. Enzymatic activity in the soil. Legend: 🟦 C-control (without pesticides), 🟧 R-herbicide Roundup, 🟩 Re-herbicide Reglone, 🟪 Ca-fungicide Caramba, ■ S-adjuvant Spodnam. A—Protease activity, B—Urease activity.

Urease activity was also significantly inhibited by the applied pesticides (Figure 1B). The negative impact was visible only directly after harvesting the plants, i.e., at the first time point. The adjuvant Spodnam showed the strongest negative effect. Urease activity was the lowest (5.11 mg) in the treatment with this pesticide compared to that in the control soil (10.38 mg). The herbicide Reglone caused the lowest inhibition. Urease activity in soil treated with this agent was the highest among all treatments with pesticides and amounted to 8.12 mg. Of the two herbicides used, as in the case of protease (Figure 1A), Reglone caused lower urease inhibition than Roundup (6.98). It is noteworthy that the negative impact of pesticides declined over time. At the second time point, urease activity in Roundup (5.35 mg) and Caramba (5.37 mg) treatments was at a similar level to the control soil (5.28 mg). The herbicide Reglone and adjuvant Spodnam even caused a small but significant increase in urease activity (5.81 mg and 5.97 mg, respectively).

3.2. *Biochemical Activity*

Data on the effects on biochemical processes, i.e., ammonification and nitrification are presented in Figure 2A,B. Herbicides (Reglone, Roundup) as well as the fungicide (Caramba) and adjuvant (Spodnam) had a significant influence on soil ammonification (Figure 2A). Inhibition of this process was recorded in treatments with the use of pesticides. This phenomenon occurred shortly after oilseed rape harvest, i.e., on the first date. The most adverse effect was noted for the adjuvant Spodnam, which caused the greatest decrease in ammonification (18.08 mg) compared to the control soil (91.19 mg). Of the herbicides applied, Reglone (57.49 mg) caused less inhibition of ammonification than Roundup (80.32 mg). It is worth emphasizing that no negative impact of pesticides on the ammonification process was observed in the second date. This parameter was at the control level (300.68–379.73 mg) in treatments with both herbicides, i.e., Roundup and Reglone, and the fungicide Caramba. In the treatment with adjuvant Spodnam, it was even significantly higher and amounted to 401.12 mg. Similar to the first time point, Reglone proved to be less beneficial for this process than Roundup. The intensity of ammonification in the treatment with Reglone was weaker and amounted to 331.94 mg compared to 379.73 mg in the Roundup treatment.

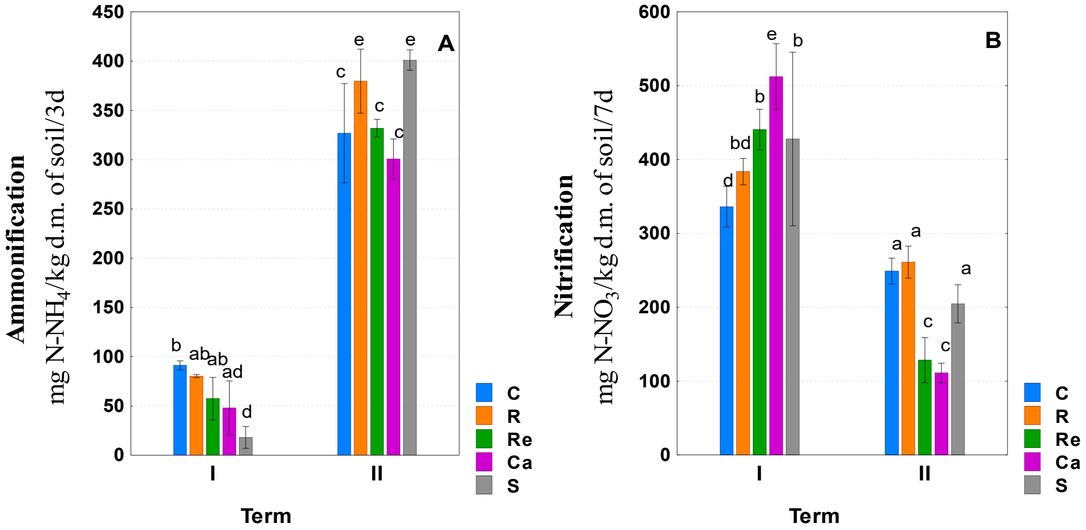

Figure 2. Biochemical activity in the soil. Legend: 🟦 C-control (without pesticides), 🟧 R-herbicide Roundup, 🟩 Re-herbicide Reglone, 🟪 Ca-fungicide Caramba, ⬛ S-adjuvant Spodnam. A—Ammonification process, B—Nitrification process.

The nitrification process varied at individual time points (Figure 2B). Shortly after plant harvest (1st time point), pesticides significantly increased nitrification compared to the control treatment, i.e., without pesticides (336.16 mg). The highest NO_3 ion concentration was found in the soil treated with the fungicide Caramba (512.42 mg), and the lowest under the influence of the herbicide Roundup (383.74 mg). Over time, the beneficial effects of pesticides on the process under study were declining. However, a significant nitrification inhibition was recorded under the influence of the herbicide Reglone (128.48 mg) and fungicide Caramba (111.07 mg) in the second time point in comparison to the control soil (248.91 mg).

3.3. Gas Emission

The data in Figure 3A show that the intensity of N_2O emissions was at a significantly different level in individual treatments in relation to the control. They also varied at individual time points. Data obtained after harvesting the plants, i.e., on the first date, indicated that the application of the herbicide Reglone, fungicide Caramba and adjuvant Spodnam resulted in a decrease in N_2O emissions to the atmosphere compared to emissions from the control soil (0.88 mg). The level of this chemical parameter in the treatment with Reglone was 0.12 mg, and no emissions of this gas were observed with Caramba and Spodnam. In the second sampling, a significant increase in the emission of this gas from soil to the atmosphere was noted in almost all pesticide treatments. The highest amount of N_2O was released from the soil treated with the fungicide Caramba and adjuvant Spodnam (2.47 mg and 2.62 mg, respectively) compared to control (0.38 mg). This gas emission was similar to the first time point only in the treatment with the herbicide Roundup, i.e., at the control level, and amounted to 0.51 mg.

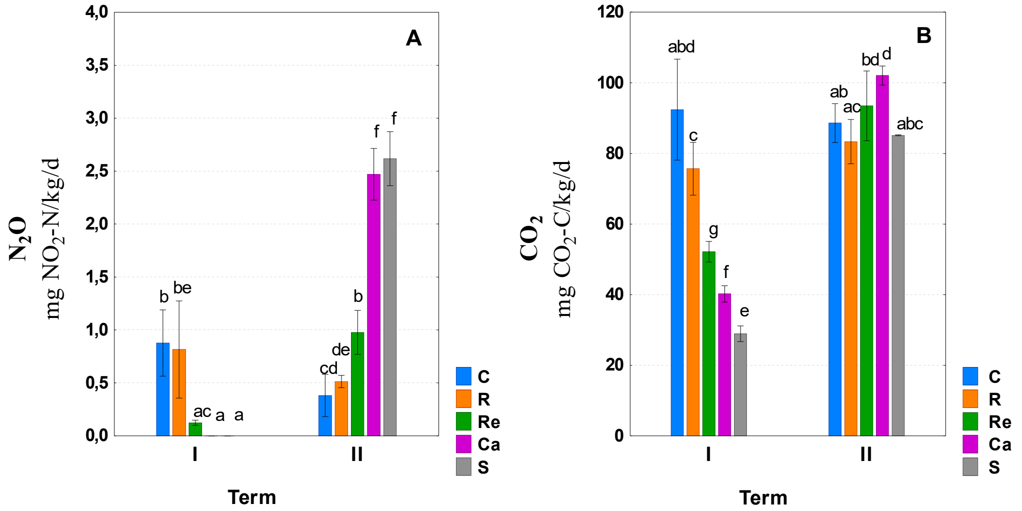

Figure 3. Greenhouse gases emissions from soil. Legend: 🟦 C-control (without pesticides), 🟧 R-herbicide Roundup, 🟩 Re-herbicide Reglone, 🟪 Ca-fungicide Caramba, ■ S-adjuvant Spodnam. A—nitrous oxide emissions, B—carbon dioxide emission.

CO_2 emissions from soil to the atmosphere changed significantly in individual treatments at the first time point (Figure 3B). Soil treated with the herbicide Reglone, fungicide Caramba and adjuvant Spodnam released significantly less of this gas than the control soil (92.40 mg). The lowest concentration of this gas was recorded in the case of Spodnam (28.95 mg). Of the two herbicides (Roundup, Reglone), Roundup caused more intense CO_2 emissions, at a level of 75.70 mg, compared to 52.18 mg for Reglone. The differences in CO_2 emissions between treatments disappeared over time. In the second time point, a significantly higher level of gas emission (102.07 mg) (88.62 mg) was recorded only in the treatment with the fungicide Caramba compared to the control treatment. The level of this chemical parameter in other treatments with chemical agents remained the same as in the control soil (83.34–93.48 mg).

3.4. Relationships between Enzymatic and Biochemical Activities and Gas Emissions in Individual Objects (PCA Method)

Principal component analysis (PCA) showed the distribution of treatments on the score plot in four areas: I—control, II—treatment with the herbicide Reglone, III—treatment with the fungicide Caramba and IV—treatment with the herbicide Roundup and treatment with the adjuvant Spodnam (Figure 4A). This distribution suggested that the soil from all pesticide treatments clearly differed in the tested parameters from the control soil. Factor 1 (PC1) was responsible for 57% of the differences between individual treatments, and factor 2 (PC2) was responsible for 30% of the variance.

The loading plot (Figure 4B) showed a correlation between the analyzed enzymatic, biochemical and chemical parameters. Strong positive correlations were noted between ammonification and CO_2 and N_2O emissions. In addition, these parameters strongly correlated with nitrification. A positive relationship was recorded between protease and urease.

The analysis of Figure 4A,B demonstrates that the overlapping position of the object with ammonification and gas emissions at the fungicide Caramba application site indicated the intensification of these processes in this treatment.

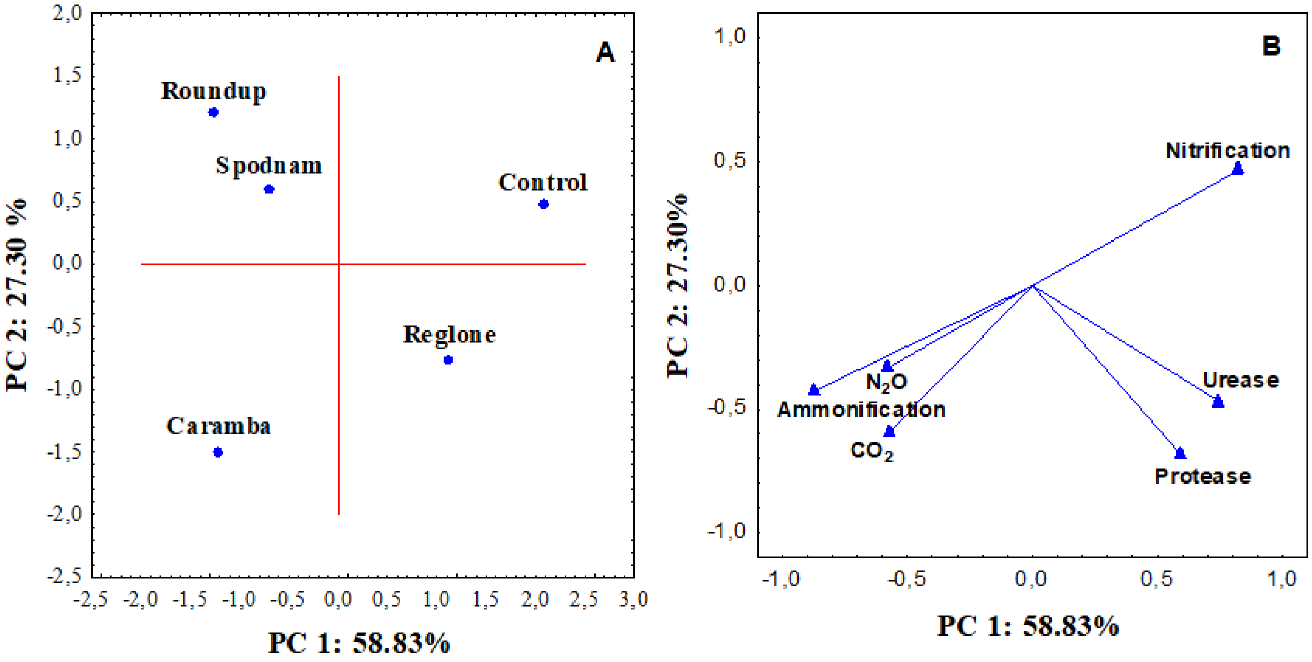

Figure 4. PCA score plot (**A**) and loading plot (**B**) showing the results of the analyzed soil parameters.

4. Discussion

The presented data demonstrated that pesticides significantly affected the activity of soil microorganisms, as well as the formation of CO_2 and N_2O gases, i.e., the products of their biochemical and enzymatic activities. Muñoz-Leoz et al. [25] argued that microorganisms participating in nitrogen transformations were the most sensitive to plant protection products. The available literature shows that the impact of pesticides on soil microorganisms is not homogeneous. Inhibition of microorganism activity caused by various pesticides has also been noted by other authors [26–29]. According to Riah et al. [30], conclusions derived from research on pesticides should not be generalized, because their impact on soil microorganisms depends on many factors, including soil type, pesticide availability, dose and type.

In the present study, it should be noted that ammonification inhibition and urease activity declined with time. Probably, there was a selection of microorganisms resistant to harmful agents. Other authors explained changes occurring over time in a similar manner. Other authors also came to similar conclusions with respect to the gradual disappearance of the negative impact of human pressure on soil microbial activity [28,31]. This phenomenon was the effect of death of sensitive and proliferation of resistant microorganisms, which resulted in changes in their abundance and biodiversity [32–34].

According to other authors, stimulation of ammonification in soil with pesticides could result from the mineralization of organic compounds present in pesticides or dead biomass of microorganisms that proved sensitive to these chemicals [35]. In addition to the negative effect caused by their binding to the active center of enzymes, thereby reducing their catalytic activity, pesticides can also be an additional source of nutrients for microorganisms, e.g., C and N [3,30,36]. As a result, the activity of bacteria and fungi may increase [37]. In the current study, an increase in nitrification and a slight increase in urease activity were recorded in individual treatments. PCA distribution showed that the nitrification process was negatively correlated with ammonification. Therefore, the intensification of nitrification could have been the result of the continuous utilization of ammonification products by nitrifiers. Joniec et al. [31] also reported a close relationship between these two processes related to nitrogen transformations in soil. Muñoz-Leoz et al. [25] observed stimulation of nitrification in their study on the effects of pesticides as well. In contrast, Lang and Cai [26] reported that nitrification under the influence of pesticides was inhibited.

The analysis of CO_2 emissions showed that the reduction in enzymatic activity and ammonification (especially in the first time point) was accompanied by a decrease in this chemical parameter. This should be connected to the fact that biochemical activities/processes carried out by microorganisms are accompanied by decarboxylation, and hence CO_2 release, therefore the decrease in their activity was reflected in a lower amount of CO_2. Relationships between the amount of CO_2 produced and enzymatic and biochemical activity in soil were also found by other authors [15,38]. In the present study, PCA distribution showed a strong relationship between ammonification and CO_2 emission, suggesting that ammonifiers contributed to the production of this gas under the conditions of this experiment. It is well known that ammonification is accompanied by CO_2 release. This is due to the fact that ammonification is a process related to the decomposition of nitrogen and carbon organic matter in the soil.

The results indicated that the pesticides (except for Roundup) contributed to increased N_2O emissions to the atmosphere. The results of the studies published in this area are ambiguous. A literature review showed that some pesticides reduced CO_2 and N_2O emissions from the soil to the atmosphere, others contributed to their intensification or had no effect [39–41]. Changes in greenhouse gas emissions over time can be affected by temperature, humidity, oxygen availability and agricultural treatments [6,40,42]. The variability of annual respiration in studies concerning respiration in grassland ecosystems was closely related to the annual gross primary production and soil water content during the growing season [40]. Shi et al. [14] showed a significant direct relationship between respiration, soil moisture and soil-absorbed CH_4, which can be a carbon source for microorganisms.

The emission of N_20 under the conditions of the experiment was a direct result of the activity of denitrifiers, which reduced the products of the activity of the nitrifiers. Bacteria growing under anaerobic conditions can utilize nitrates or nitrites generated in the nitrification process as electron acceptors in respiratory processes. It is a process of denitrification and N_2O is one of the products of this process [17]. Considering the above observations, it should be stated that there was an adverse disruption of the nitrogen transformation cycle in the second sampling point.

The fungicide Caramba turned out to be the most unfavorable for the analyzed microbiological activities and contributed to the increase of greenhouse gas emissions. Riah et al. [30] classified fungicides to the group of most hazardous pesticides to soil microorganisms. The literature shows that fungicides disrupt the balance in soil microorganism populations, affect the proportions between fungi and bacteria, as well as relationships between them and their activity [11,12,28,29,43]. Fungi, together with bacteria, participate in the mineralization of organic matter and release of nutrients. Therefore, disturbing their number and function results in changes in soil conditions [30]. The overlap of fungicide treatment with soil greenhouse gas emissions into the atmosphere in this study suggested that CO_2 and N_2O were mainly the products of bacteria, not fungi.

The comparison of the interaction of the two herbicides, Roundup and Reglone, demonstrated that Roundup caused a stronger inhibition of enzymatic activities and nitrification. Roundup has been shown to affect the structure of soil microbial populations, which may result in soil activity changes [18,44]. Other authors also reported negative effects of glyphosate-based pesticides [28,45]. However, it should be noted that despite the negative impact on the studied activities, Roundup did not contribute to the increase of greenhouse gas emission to the atmosphere in the conditions of this experiment. Research indicated a role of pesticides applied in agriculture in the emission of greenhouse gases from soil. The present results imply the possibility of reducing the role of agriculture in the greenhouse effect through the selection of appropriate pesticides. This fact was also noted by other authors [14]. The results provide new information on the contribution of not only individual groups of pesticides but also single preparations in the emissions of greenhouse gases from the soil during the cultivation of winter oilseed rape. This is particularly important due to the large-scale cultivation of this plant in the world. Choosing the right pesticides can be one way to reduce greenhouse gas flux from agricultural practice.

5. Conclusions

The pesticides caused significant changes in the activity of soil microorganisms and the amount of gases emitted to the atmosphere as a result of this activity. The intensity and direction of these changes alternated over time.

The observed effect of pesticides was usually negative and most apparent in the case of enzymatic activities. A positive effect was observed in the form of an increase in nitrification intensification in the initial period, particularly pronounced after fungicide application. This effect appeared to be caused by reduced competition between fungi and bacteria in favor of the latter. Of the chemicals applied, the fungicide and adjuvant showed the strongest inhibitory effect. A comparison of the impact of both herbicides on the activity of soil microorganisms indicated that Roundup was an agent with a stronger negative effect compared to Reglone.

The observed increase in N_2O and CO_2 gas emissions over time indicated that these pesticides interfered with microbiological processes occurring in the soil, thereby contributing to homeostasis disturbance of this environment. This in turn led to the accumulation of gaseous products of microbial activity. The results demonstrated that the fungicide Caramba probably reduced the number of fungi and contributed to the disruption of relationships within the population of soil microorganisms. This was reflected in changes in soil microbial activity. Considering the increasing greenhouse gas emissions from soil over time, long-term monitoring of pesticide application effects is advisable not only due to the condition of the soil environment, but also the atmosphere.

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