

Article

Agricultural Reuse of Phosphogypsum in Contrasting Soils: Wheat Yield, Potentially Toxic Element Uptake, and Soil Microbial Community Responses

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Abstract

Phosphogypsum (PG) is a high-volume industrial by-product that is receiving increasing attention as an alternative soil amendment. This study presents a two-year pot experiment evaluating the effects of PG on spring wheat (*Triticum aestivum* L.) grown on two contrasting soils: an acidic, sulfur-deficient Arenosol and a sodium-affected Solonetz. Treatments comprised an untreated control and PG applications at 2 and 10 t ha^{−1}, applied once in year 1 and repeated at the same rates in year 2. In the Solonetz, PG increased grain yield by 48–73% in year 2 and 31–40% in year 1, with the lower dose (2 t ha^{−1}) providing sufficient agronomic benefits. In contrast, no yield response was observed in the Arenosol. In both soils, PG acted as a slow-release sulfur source, increasing straw sulfur concentrations in the second year. Even the repeated application of the highest PG dose (10 t ha^{−1}) did not increase Al, Cd, Cr, or Sr accumulation in wheat grain, indicating no treatment-related increase in trace-element accumulation. PG also increased soil microbial biomass and altered the microbial community structure in the Solonetz, including higher relative abundances of arbuscular mycorrhizal fungi and actinomycetes. Overall, these results demonstrate the dual agronomic function of PG in improving sodic soil productivity and supplying sulfur to nutrient-deficient sandy soils without increasing treatment-related trace-element accumulation, thus highlighting its potential as a soil amendment in sustainable nutrient management systems.



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Keywords: Phosphogypsum; spring wheat; Solonetz; Arenosol; soil amendment; potentially toxic elements; soil microbial community

1. Introduction

Soil degradation is a major global challenge that threatens food security and ecosystem stability. Worldwide, human-induced soil degradation affects nearly two billion hectares, and over 60% of soils in the European Union (EU) are already considered unhealthy [1–3]. In Hungary, low-lying areas such as the Great Hungarian Plain suffer from salinization and sodification due to challenging hydrological conditions, while improper agricultural practices accelerate these processes [4–6]. Effective soil amendments are therefore urgently needed [7].

Phosphogypsum (PG) is a calcium sulfate-rich by-product of wet-process phosphoric acid production that has accumulated worldwide in stockpiles exceeding 6 billion metric tons (t), with annual production of approximately 300 million t [8]. Its chemical composition, primarily $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, makes PG a potentially valuable soil amendment, particularly for reclaiming saline, sodic and acidic soils [9–13]. In sodic soils, Ca^{2+} released from PG replaces exchangeable Na^+ , thereby improving soil structure and permeability. In acidic soils, PG can alleviate Al^{3+} toxicity by increasing Ca^{2+} saturation [14–16].

A growing body of research has examined the agronomic benefits of PG [17–19]. A meta-analysis by Tiecher et al. [20], for instance, found that PG application increased mean maize yields by 16% and winter-cereals yields by 19%. Similarly, Outbakat et al. [14] reported that PG applied at a rate of 1.5–4.5 t ha^{−1} increased barley yield by up to 39% on both saline and acidic soils. Alcordo and Rechcigl [21] observed yield increases of 6–49% in tomatoes, potatoes, and watermelons following PG addition. Kim et al. [22] noted improved soil Ca and S status in onions, although excessive PG application (3.12 t ha^{−1}) was reported to reduce yield. Gao et al. [23] reported that PG use resulted in a 130% yield increase in buckwheat, attributed to improved soil moisture and enzymatic activity.

Despite these benefits, PG contains variable levels of potentially toxic trace elements and naturally occurring radionuclides, including Cd, Pb, Zn, Cr, Cu, Ni, As and Hg. The concentrations largely depend on the phosphate ore used during the production of wet-process phosphoric acid [8,24,25]. The EU's Fertilizing Products Regulation sets a Cd limit of 1.5 mg kg^{−1} for inorganic soil improvers [26] and PG can exceed this threshold, which limits its current use as a soil amendment in the EU. It is noteworthy that most studies indicate that at agronomically recommended rates, PG does not lead to significant accumulation of heavy metals in soil or crops [27–29].

This study evaluates the use of PG from Kutina, Croatia [30,31] as a soil amendment for spring wheat (*Triticum aestivum* L.) grown over two cropping seasons on two contrasting soils: a colloid-poor, acidic Arenosol (sandy, S-deficient) and a clay-rich, sodic Solonetz. While previous studies have demonstrated that PG can improve soil properties and crop performance, the novelty of the present work lies in its integrated plant–soil–microbiome assessment of PG effects within the same experimental framework. Specifically, we simultaneously evaluated the immediate, residual, and repeated effects of applying 2 and 10 t PG ha^{−1} on (i) soil chemical properties and nutrient availability, (ii) soil microbial biomass and communities, (iii) crop yield and nutrient uptake, and (iv) the accumulation and translocation of potentially toxic elements in the soil–plant system. By conducting this comprehensive assessment in two contrasting soil types over two consecutive growing seasons, the study goes beyond evaluating individual agronomic or soil-crop responses and

provides an understanding of how PG effects depend on soil characteristics, application history, and the persistence of these effects over time.

2. Materials and Methods

2.1. Soil and Pot Set-Up

A two-year pot experiment (2024–2025) was conducted in a controlled semi-field environment at the Institute of Agricultural Chemistry and Soil Science, Faculty of Agricultural, Food Sciences and Environmental Management at the University of Debrecen in Hungary, using two contrasting soils: an Arenosol (from Nyíregyháza, 47.95306° N 21.72713° E) and a Solonetz (from Karcag, 47°18'40" N 20°54'58" E) (Table 1). The Solonetz was heavy-textured, had a high clay content, and contained a deep solonetzic layer.

Table 1. Main physical and chemical parameters of the experimental soils.

	Arenosol	Solonetz
texture	sand	clay-rich/heavy-textured
pH (KCl)	4.92	6.17
CaCO ₃ (%)	<0.1	0.17
C _{org} (%)	0.71	1.57
Total water-soluble salts (<i>w/w</i> %)	0.02	0.04
EC _{se} (mS cm ^{−1})	0.282	1.75
CEC (cmol kg ^{−1})	10.4	29.0
ESP (%)	6.1	17.3
AL-P (mg kg ^{−1})	35.6	90.8
AL-K (mg kg ^{−1})	105.1	302.9
AL-Na (mg kg ^{−1})	58.6	190.1
KCl-SO ₄ ^{2−} (mg kg ^{−1})	6.43	23.7
Exchangeable K ⁺ (cmol kg ^{−1})	2.77	1.42
Exchangeable Ca ²⁺ (cmol kg ^{−1})	4.80	16.3
Exchangeable Mg ²⁺ (cmol kg ^{−1})	0.87	4.60

C_{org}: organic carbon content, EC_{se}: electrical conductivity of saturated extract, CEC: cation exchange capacity, ESP: exchangeable sodium percentage, AL-P: available phosphorus (ammonium lactate-extractable), AL-K: available potassium, AL-Mg: available magnesium, AL-Na: available sodium, KCl-SO₄^{2−}: available sulfur (potassium-chloride-extractable).

Plastic pots (diameter 20.5 cm, height 14.5 cm) were filled with 3 kg of air-dried, sieved (<10 mm) soil collected from the upper 0–30 cm layer of the soil profile. The pots were placed on movable carts, and each individual pot was considered an experimental unit.

Spring wheat (*Triticum aestivum* L., cv. IS Jariella) provided by Karintia Kft. (Vasvár, Hungary) was used as the indicator plant in the experiment. The carts were moved outdoors during daylight hours and returned under cover at night to prevent interference from rainfall. This setup ensured natural light and temperature conditions while allowing wheat to be grown under controlled semi-field conditions. Soil moisture in each pot was maintained at a constant level corresponding to 60% of field capacity throughout the experiment. Watering was performed using ion-exchanged water, with the required amount determined based on daily pot weighing. Water losses were replenished once daily and twice daily during warmer periods. The selected pot size provided sufficient rooting volume for spring wheat throughout the growing period. At harvest, intact soil cores were removed from the pots and visually inspected. The root systems uniformly occupied the available soil volume without exhibiting root-bound growth, indicating that root development was not restricted by the chosen pot size.

2.2. Phosphogypsum (PG)

The PG used in the experiment was sourced from Kutina, Croatia, approximately 2 km south of the Petrokemija fertilizer plant where it was produced. The plant is located approximately 90 km from the Hungarian border. The PG was originally produced from sedimentary Khouribga phosphate ore [32,33] imported from Morocco. The elemental composition of the PG was determined by inductively coupled plasma mass spectrometry (ICP–MS, Agilent 7900, Tokyo, Japan) after acid digestion ($\text{HNO}_3\text{--HClO}_4$, 4:1) and is presented in Table 2.

Table 2. The chemical composition of Kutina PG used in this experiment (mg kg^{-1}).

Components	Ca	S	Al	Fe	Si	Mg	P	Sr	Cu	Cd	Cr	Zn	Pb	Mn	Ba	U	Th	As	V
mg kg^{-1}	250,565	188,947	1125	258.9	1564	92.9	2477	325.4	5.53	2.16	5.55	4.93	1.97	6.11	53.3	3.35	1.33	0.94	4.39

Notably, under the current EU regulations on inorganic soil improvers [26,34], the Cd concentration in the tested PG ($2.16 \text{ mg}\cdot\text{kg}^{-1}$) exceeded the EU limit of $1.5 \text{ mg}\cdot\text{kg}^{-1}$. However, the concentrations of lead were below their respective limit values, and the concentrations of the other reported trace elements were also relatively low [26].

2.3. Experimental Procedure

In the first year of the experiment (2024), three treatments (control, PG1, PG2) were established with six replicates per soil type, with PG1 and PG2 corresponding to application rates of 2 and 10 t ha^{-1} , respectively. In the second year (2025), each treatment group was divided into two subgroups with three replicates each. For one subgroup of each PG treatment, the residual effects of the previous year's PG application (RES-PG1, RES-PG2) were examined without additional PG application. For the other subgroup of each PG treatment, PG was reapplied at the same rates of 2 and 10 t ha^{-1} , to evaluate the cumulative effects of PG application over two years (REP-PG1, REP-PG2). Accordingly, in the first year the experiment consisted of three treatments (control, PG1, PG2), each with six replicates, and in the second year five treatments: control, residual PG1 (RES-PG1), residual PG2 (RES-PG2), repeated PG1 (REP-PG1), and repeated PG2 (REP-PG2), each with three replicates (Table 3).

Table 3. Overview of the PG treatments applied in the two-year experiment.

Treatments	Rates of PG (t ha^{-1})
First year ($n = 6$)	
control	-
PG1	2
PG2	10
Second year ($n = 3$)	
control	-
RES-PG1	-
RES-PG2	-
REP-PG1	2
REP-PG2	10

RES—residual, REP—repeated.

The experimental pots were arranged on carts according to a randomized complete block design (RCBD), with treatments and replicates randomly distributed within each block to minimize the effects of spatial variability. The experimental layout of the pot arrangement is presented in Figure 1.

First year Arenosol			Second year Arenosol		
240 cm	PG1	control	REP-PG2	control	
	PG2	PG2	control	REP-PG2	
	control	PG1	REP-PG1	RES-PG2	
	control	PG2	RES-PG1	REP-PG1	
	PG2	PG1	RES-PG2	RES-PG1	
	PG1	control	REP-PG1	-	
	PG2	PG1	RES-PG1	-	
	control	control	REP-PG2	-	
	PG1	PG2	control	-	
Solonetz			Solonetz		
240 cm	PG1	control	REP-PG2	control	
	PG2	PG2	control	REP-PG2	
	control	PG1	REP-PG1	RES-PG2	
	control	PG2	RES-PG1	REP-PG1	
	PG2	PG1	RES-PG2	RES-PG1	
	PG1	control	REP-PG1	-	
	PG2	PG1	RES-PG1	-	
	control	control	REP-PG2	-	
	PG1	PG2	control	-	
80 cm			80 cm		

Figure 1. Pot arrangement during the two experimental years.

2.4. Fertilization

To ensure that the nutrient requirements of spring wheat were met in both experimental years, nitrogen (N) and potassium (K) fertilizers were applied to all treatments, including the control. Nitrogen was supplied as ammonium nitrate (NH_4NO_3) and potassium as potassium chloride (KCl), with application rates adjusted to the soil mass in each pot to correspond to field-equivalent rates of 112 kg N ha^{-1} and 84 kg K ha^{-1} . Both fertilizers were thoroughly incorporated into the soil before sowing spring wheat. Phosphorus (P) fertilizer was not applied, as the experimental soils already had an adequate P supply, and additional phosphorus was provided through the PG treatments. The PG1 and PG2 doses supplied the equivalent of 4.95 and $24.75 \text{ kg P ha}^{-1}$, respectively. All application rates were adjusted to the soil mass of each experimental unit. In both experimental years, PG was thoroughly incorporated into the soil one week before sowing. Spring wheat was grown to maturity and harvested at full ripeness (BBCH 89). At harvest, plants were cut at soil level, and the aboveground biomass of each pot was measured. Grain yield and thousand-grain weight were determined. The harvest index (HI) was calculated as the ratio of grain yield to total aboveground biomass.

2.5. Plant and Soil Sample Analysis

For elemental analysis, wheat straw and grain samples were dried at 60°C to constant weight and finely ground to a powder. Aliquots of 0.5 g were digested with 1 mL of 65% nitric acid (HNO_3) using an Anton Paar Multiwave PRO microwave digestion system (Graz, Austria) under the following conditions: 45 min , 900 W , maximum pressure 60 bar , maximum temperature 260°C . After digestion, the samples were diluted to a final volume of 25 mL with deionized water. The concentrations of P, Ca, S, Fe, Al, Ba, Cd, Cr and Sr were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 2100 DV, PerkinElmer, Norwalk, CT, USA).

After the aboveground parts of the wheat were removed, soil samples were collected from the rhizosphere of each pot. A portion of each soil sample was air-dried and passed through a 2 mm sieve prior to chemical analysis, while another portion was lyophilized and stored at -20°C for microbial community analysis. Soil pH was measured in 1 M KCl solution at a soil-to-extractant ratio of $1:2.5$ using a Hanna Instruments HI-8525 pH meter (Woonsocket, RI, USA). For elemental analysis, soil samples were digested with nitric acid, and Al, Ba, Cd, Cr, and Sr concentrations were determined by ICP-OES.

Readily soluble P and Ca concentrations in soil were measured by the AL method, using a 1:20 soil-to-extractant ratio and a 2 h extraction time [35]. P concentrations in the AL extract were measured spectrophotometrically and Ca concentrations were determined by atomic absorption spectrometry (Varian SpectrAA-20, Varian Australia Pty Ltd., Mulgrave, Australia) [35].

Cation concentrations in the eluate were determined by atomic absorption spectrophotometry at 589.0 nm for Na⁺, 769.9 nm for K⁺, 422.7 nm for Ca²⁺, and 285.2 nm for Mg²⁺ [36]. Exchangeable sodium percentage (ESP) was calculated according to Bleam [37]. These parameters were determined only for selected treatments.

Soil microbial biomass and community composition were determined in the second experimental year using phospholipid fatty acid (PLFA) analysis following the method described by Ellis and Ritz [38]. Lipids were extracted from 2 g of freeze-dried soil using a methanol-chloroform-K₂HPO₄ mixture (1:0.5:0.4, v/v/v), followed by alkaline methanolysis, and analyzed by gas chromatography (Agilent 8890, Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a flame ionization detector. Phospholipid fatty acids were identified using standard fatty acid mixtures (PLFAD1 Calibration Standard—No. 1208) and Sherlock 6.5 software (Biolog, Microbial Identification Inc., Newark, DE, USA). Phospholipid fatty acid concentrations were quantified using a 19:0 internal standard and expressed as nmol PLFA g^{−1} dry soil. Microbial biomass was estimated from the total measured PLFA content, and microbial community structure was evaluated using specific PLFA biomarkers for fungi (18:2ω6), arbuscular mycorrhizal fungi (16:1ω5), Gram-positive bacteria (iso- and anteiso-saturated, branched PLFAs), Gram-negative bacteria (monounsaturated and cyclopropyl PLFAs), and actinomycetes (10-methyl fatty acids: 10Me16:0, 10Me17:0, 10Me18:0).

2.6. Statistical Analysis

Data from the different treatments were analyzed by one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$ using IBM SPSS Statistics (version 28). When significant differences were detected, post hoc pairwise comparisons were conducted using Tukey's Honestly Significant Difference (HSD) tests with the same software. Values in the tables are presented as mean ± standard deviation. Multivariate analyses and diversity calculations were performed in RStudio (version 2025.05.1+513). To test the significance of treatment effects on microbial community structure, a Bray–Curtis-based permutational multivariate analysis of variance (PERMANOVA) was applied using the 'vegan' package. Principal component analysis (PCA) was also used to assess the separation of microbial communities among the different treatments using the 'factoextra' and 'FactoMineR' packages. To assess microbial functional diversity, the Shannon diversity index was calculated based on the relative abundance of seven major microbial functional groups identified via PLFA data using the 'vegan', 'dplyr', and 'multcompView' packages. Spearman's rank correlation analysis was performed using the *stats* package in RStudio to evaluate the relationships between yield parameters, plant nutrient concentrations, soil properties, and microbial community groups based on the results obtained in the second year of the study. The statistical figures in RStudio were generated and customized using the 'ggplot2' and 'ggpubr' packages.

3. Results

3.1. Plant Parameters

3.1.1. Plant Yield

Spring wheat biomass production and yield parameters responded differently to PG treatments, depending on the soil type (Table 4). In the first year, no significant differences

among treatments were observed in the Arenosol for dry biomass, grain weight, thousand-grain weight, or harvest index. In the second year, most yield parameters showed no significant differences compared to the control following either residual or repeated PG applications.

Table 4. Yield parameters of spring wheat affected by PG application in Arenosol and Solonetz during the two-year experiment.

Treatments	Dry Biomass g pot ^{−1}		Grain Weight g pot ^{−1}		Thousand-Grain Weight g 1000 Grains ^{−1}		Harvest Index (HI)	
	Arenosol	Solonetz	Arenosol	Solonetz	Arenosol	Solonetz	Arenosol	Solonetz
First year (2024)								
control	22.2 ± 3.7 a	14.8 ± 3.7 a	7.11 ± 1.19 a	4.53 ± 1.61 a	24.5 ± 1.12 a	22.0 ± 3.63 a	0.32 ± 0.01 a	0.30 ± 0.04 a
PG1	24.1 ± 2.4 a	19.7 ± 2.6 b	7.78 ± 1.02 a	5.93 ± 1.08 ab	25.1 ± 2.51 a	25.0 ± 1.18 ab	0.32 ± 0.02 a	0.30 ± 0.05 a
PG2	24.0 ± 2.3 a	19.2 ± 2.3 b	8.13 ± 1.13 a	6.34 ± 0.73 b	24.6 ± 1.08 a	25.6 ± 1.33 b	0.34 ± 0.03 a	0.33 ± 0.03 a
Second year (2025)								
control	17.9 ± 0.7 a	12.9 ± 2.5 a	6.00 ± 0.09 ab	4.18 ± 0.66 a	24.7 ± 2.23 a	23.8 ± 1.12 a	0.34 ± 0.02 a	0.33 ± 0.01 a
RES-PG1	17.6 ± 0.6 a	17.5 ± 2.7 ab	6.54 ± 0.14 b	6.19 ± 1.23 b	22.7 ± 1.73 a	26.8 ± 2.10 a	0.37 ± 0.01 a	0.35 ± 0.02 ab
RES-PG2	17.4 ± 0.2 a	18.8 ± 1.9 b	5.85 ± 0.36 a	7.25 ± 0.57 b	23.6 ± 2.42 a	27.9 ± 1.60 a	0.34 ± 0.02 a	0.39 ± 0.02 b
REP-PG1	17.9 ± 0.6 a	18.6 ± 0.3 b	6.18 ± 0.29 ab	6.62 ± 0.30 b	22.9 ± 2.39 a	28.4 ± 3.21 a	0.35 ± 0.01 a	0.36 ± 0.01 ab
REP-PG2	17.1 ± 0.3 a	17.3 ± 0.6 ab	5.91 ± 0.13 a	6.34 ± 0.38 b	24.1 ± 1.57 a	26.9 ± 1.13 a	0.35 ± 0.00 a	0.37 ± 0.01 ab

Values marked with the same letter within a column are not significantly different at $p \leq 0.05$. Data are presented as mean ± SD ($n = 6$ in the first year; $n = 3$ in the second year).

In contrast, more pronounced effects were observed in the Solonetz. In the first year, PG application (PG1, PG2) significantly increased total aboveground biomass by 33.1% and 29.7%, respectively, compared to the control. Grain weight and thousand grain weight were also higher in the pots treated with PG1 and PG2, with PG2 showing the largest increase compared to the control. In the second year, both residual and repeated PG treatments (RES-PG1, RES-PG2, REP-PG1, and REP-PG2) generally increased most yield parameters (except thousand-grain weight) compared to the control, but there were no significant differences among the values of the different treatments.

3.1.2. Plant Nutrients

In the experiment, essential nutrients (P, Ca, S, and Fe) were measured in wheat straw and grain to evaluate the effects of the PG treatments (Tables 5 and 6). In the Arenosol, in the first year, PG had no measurable effect on straw P, Ca and Fe contents, while S concentration increased with increasing PG doses compared to the control. Grain nutrient concentrations remained unchanged in all treatments. In the second year, nutrient responses were more pronounced. Straw Ca and S were higher in the repeated PG treatments (REP-PG1, REP-PG2) than in the control, particularly in REP-PG2. Grain nutrient concentrations remained largely unchanged, with only a slight increase in S observed in REP-PG2. The residual PG treatments (RES-PG1, RES-PG2), particularly at the higher rate (RES-PG2), significantly increased straw S concentrations and, to a lesser extent, grain S concentrations.

In the first year, Solonetz straw Ca increased significantly under both PG1 and PG2 compared to the control, and straw S showed a dose-dependent response, with the highest values under PG2. In the second year, straw P showed a different pattern: the highest P concentration was observed in the control, while the lowest value was measured in pots that had received the higher PG dose (RES-PG2) in the previous year. Straw Ca also increased to significantly higher concentrations in RES-PG2 and both repeated treatments. Straw S likewise increased progressively from control to REP-PG2, with the highest S concentrations observed under repeated PG application. Grain Ca and S showed a significant response to PG application. In the pots treated with PG in the previous year (RES-PG1, RES-PG2), both Ca and S concentrations were higher than in the control. Additional PG applications (REP-

PG1, REP-PG2) in the second year did not further increase Ca concentrations; however, S concentrations increased, reaching their highest value at the higher PG dose (10 t ha⁻¹).

Table 5. Effects of PG application on P, Ca, S (%) and Fe (mg kg⁻¹) concentrations in wheat straw and grain over two growing seasons in the Arenosol.

Treatment	Wheat Straw				Wheat Grain			
	P (%)	Ca (%)	S (%)	Fe (mg kg ⁻¹)	P (%)	Ca (%)	S (%)	Fe (mg kg ⁻¹)
First year (2024)								
control	0.016 ± 0.004 a	0.362 ± 0.004 a	0.092 ± 0.006 a	203 ± 65 a	0.346 ± 0.058 a	0.046 ± 0.005 a	0.129 ± 0.017 a	35.7 ± 5.5 a
PG1	0.014 ± 0.002 a	0.357 ± 0.033 a	0.104 ± 0.020 ab	212 ± 60 a	0.347 ± 0.031 a	0.051 ± 0.012 a	0.135 ± 0.029 a	33.1 ± 7.2 a
PG2	0.014 ± 0.002 a	0.348 ± 0.044 a	0.135 ± 0.027 b	215 ± 67 a	0.350 ± 0.049 a	0.047 ± 0.004 a	0.130 ± 0.014 a	32.7 ± 4.5 a
Second year (2025)								
control	0.023 ± 0.004 a	0.361 ± 0.008 ab	0.043 ± 0.002 a	235 ± 61 ab	0.384 ± 0.030 a	0.048 ± 0.003 a	0.036 ± 0.002 a	30.0 ± 3.6 a
RES-PG1	0.019 ± 0.003 a	0.318 ± 0.021 a	0.041 ± 0.004 a	163 ± 40 a	0.398 ± 0.015 a	0.047 ± 0.002 a	0.037 ± 0.002 a	32.3 ± 0.6 a
RES-PG2	0.021 ± 0.002 a	0.339 ± 0.035 ab	0.078 ± 0.013 b	130 ± 26 a	0.397 ± 0.024 a	0.052 ± 0.007 a	0.040 ± 0.003 ab	29.0 ± 3.6 a
REP-PG1	0.023 ± 0.002 a	0.357 ± 0.011 ab	0.073 ± 0.010 b	298 ± 67 b	0.418 ± 0.036 a	0.052 ± 0.003 a	0.041 ± 0.002 ab	29.7 ± 4.0 a
REP-PG2	0.023 ± 0.004 a	0.392 ± 0.014 b	0.114 ± 0.004 c	230 ± 31 ab	0.398 ± 0.016 a	0.055 ± 0.003 a	0.043 ± 0.002 b	27.7 ± 0.6 a
Sufficient range								
Reference	0.05–0.15 [39]	0.15–0.3 [40]	0.08–0.15 [41]	100–250 [40]	0.25–0.45 [39]	0.02–0.06 [40]	0.12–0.2 [41]	40–60 [40]

Values marked with the same letter within a column are not significantly different at $p \leq 0.05$. Data are presented as mean ± SD ($n = 6$ in the first year; $n = 3$ in the second year).

Table 6. Effects of PG application on P, Ca, S (%) and Fe (mg kg⁻¹) concentrations in wheat straw and grain over two growing seasons in the Solonetz.

Treatment	Wheat Straw				Wheat Grain			
	P (%)	Ca (%)	S (%)	Fe (mg kg ⁻¹)	P (%)	Ca (%)	S (%)	Fe (mg kg ⁻¹)
First year (2024)								
control	0.040 ± 0.015 a	0.191 ± 0.015 a	0.125 ± 0.027 a	251 ± 96 a	0.398 ± 0.045 a	0.044 ± 0.007 a	0.129 ± 0.026 a	35.7 ± 7.7 a
PG1	0.048 ± 0.028 a	0.231 ± 0.037 b	0.202 ± 0.049 ab	258 ± 56 a	0.408 ± 0.065 a	0.049 ± 0.006 a	0.133 ± 0.024 a	31.1 ± 5.0 a
PG2	0.057 ± 0.029 a	0.268 ± 0.022 b	0.306 ± 0.052 b	238 ± 21 a	0.410 ± 0.061 a	0.046 ± 0.006 a	0.135 ± 0.012 a	31.9 ± 5.3 a
Second year (2025)								
control	0.073 ± 0.017 b	0.204 ± 0.019 a	0.066 ± 0.013 a	150 ± 41 a	0.448 ± 0.009 a	0.046 ± 0.003 a	0.044 ± 0.001 a	27.0 ± 2.0 a
RES-PG1	0.062 ± 0.015 ab	0.197 ± 0.007 a	0.060 ± 0.009 a	155 ± 23 a	0.460 ± 0.009 a	0.048 ± 0.001 ab	0.045 ± 0.001 ab	30.3 ± 5.0 a
RES-PG2	0.032 ± 0.008 a	0.258 ± 0.020 b	0.078 ± 0.002 ab	209 ± 81 a	0.444 ± 0.016 a	0.056 ± 0.003 c	0.047 ± 0.000 bc	30.3 ± 1.5 a
REP-PG1	0.049 ± 0.002 ab	0.279 ± 0.023 b	0.093 ± 0.012 bc	212 ± 36 a	0.461 ± 0.050 a	0.054 ± 0.003 bc	0.048 ± 0.001 c	32.3 ± 4.0 a
REP-PG2	0.045 ± 0.005 ab	0.287 ± 0.019 b	0.109 ± 0.011 c	161 ± 18 a	0.446 ± 0.031 a	0.058 ± 0.002 c	0.051 ± 0.001 d	31.7 ± 3.1 a

Values marked with the same letter within a column are not significantly different at $p \leq 0.05$. Data are presented as mean ± SD ($n = 6$ in the first year; $n = 3$ in the second year).

3.1.3. Potentially Toxic Elements in Wheat

In the Arenosol, the concentrations of Al, Ba, Cd, Cr, and Sr in wheat straw were higher than those in wheat grain in both experimental years (Table 7). In the first year, grain Cr concentration remained below the limit of quantification (LOQ) in all treatments. Al, Cd, Cr, and Sr concentrations in straw and grain were not significantly affected by PG1 or PG2. In contrast, Ba concentrations in both straw and grain were significantly influenced by PG application. The PG2 treatment resulted in a significant reduction in Ba concentration compared to the control.

Table 7. Effect of PG application on Al, Ba, Cd, Cr and Sr (mg kg^{−1}) concentrations in wheat straw and grain over two growing seasons in the Arenosol.

Treatment	Wheat Straw (mg kg ^{−1})					Wheat Grain (mg kg ^{−1})				
	Al	Ba	Cd	Cr	Sr	Al	Ba	Cd	Cr	Sr
First year (2024)										
control	203 ± 67 a	28.6 ± 7.3 b	0.30 ± 0.04 a	1.81 ± 0.40 a	21.5 ± 1.7 a	2.4 ± 0.8 a	5.6 ± 1.2 b	0.22 ± 0.03 a	<0.25 *	4.9 ± 1.2 a
PG1	245 ± 78 a	20.3 ± 7.1 ab	0.29 ± 0.04 a	1.97 ± 0.28 a	19.9 ± 3.5 a	2.3 ± 0.7 a	5.0 ± 1.3 ab	0.28 ± 0.04 a	<0.25 *	5.0 ± 1.0 a
PG2	233 ± 42 a	15.0 ± 2.1 a	0.33 ± 0.05 a	1.97 ± 0.30 a	18.2 ± 2.7 a	2.1 ± 0.7 a	3.8 ± 0.7 a	0.27 ± 0.09 a	<0.25 *	4.4 ± 0.9 a
Second year (2025)										
control	317 ± 58 ab	24.7 ± 0.6 c	<0.05 *	<0.25 *	23.6 ± 0.6 a	6.6 ± 1.2 a	4.3 ± 0.6 b	<0.05 *	<0.25 *	4.7 ± 0.6 a
RES-PG1	225 ± 60 a	22.7 ± 1.5 bc	<0.05 *	<0.25 *	21.3 ± 2.1 a	5.7 ± 0.6 a	5.0 ± 1.0 b	<0.05 *	<0.25 *	5.0 ± 0.0 a
RES-PG2	232 ± 70 a	16.3 ± 0.6 b	<0.05 *	<0.25 *	21.7 ± 1.5 a	5.0 ± 1.0 a	4.0 ± 1.0 b	<0.05 *	<0.25 *	5.0 ± 1.0 a
REP-PG1	435 ± 77 b	18.3 ± 3.5 ab	<0.05 *	<0.25 *	22.0 ± 1.2 a	5.3 ± 2.3 a	3.3 ± 0.6 ab	<0.05 *	<0.25 *	5.0 ± 0.0 a
REP-PG2	337 ± 55 ab	10.0 ± 1.0 a	<0.05 *	<0.25 *	22.7 ± 0.6 a	6.7 ± 1.5 a	1.7 ± 0.6 a	<0.05 *	<0.25 *	5.0 ± 0.0 a

* Below LOQ. Values marked with the same letter within a column are not significantly different at $p \leq 0.05$. Data are presented as mean ± SD ($n = 6$ in the first year; $n = 3$ in the second year).

In the second year, Cd and Cr concentrations in both grain and straw were below the quantification limit. Al concentrations in straw were slightly lower in the residual treatments (RES-PG1 and RES-PG2) compared to the control. However, repeated PG applications (REP-PG1 and REP-PG2) resulted in higher Al concentrations in straw than in pots that had received PG only in the previous year, especially at the lower PG dose (REP-PG1). Ba concentrations in wheat also remained lower in PG-treated pots during the second year. In wheat straw, a reduction was observed in the pots with RES-PG treatments compared to the control. Repeated PG applications (REP-PG1, REP-PG2) caused a further decrease in Ba concentrations.

In the Solonetz, concentrations of Al, Ba, Cd, Cr, and Sr were also higher in straw than in grain (Table 8). In the first year, there were no changes in straw Al, Cd or Cr concentrations in PG-treated crops compared to the control, while Ba and Sr showed some treatment-related differences. In the pots treated with PG1 and PG2, Ba concentrations were lower than in the control. The PG1 treatment slightly decreased the Sr concentration, and the PG2 treatment resulted in a further decrease. PG had no significant effects on the measured element concentrations in wheat grain and the Cr concentration remained below the limit of quantification (LOQ) in all treatments.

Table 8. Effect of PG application on Al, Ba, Cd, Cr and Sr (mg kg^{−1}) concentrations in wheat straw and grain over two growing seasons in the Solonetz.

Treatment	Wheat Straw (mg kg ^{−1})					Wheat Grain (mg kg ^{−1})				
	Al	Ba	Cd	Cr	Sr	Al	Ba	Cd	Cr	Sr
First year (2024)										
control	310 ± 79 a	22.1 ± 4.4 b	0.15 ± 0.01 a	2.49 ± 0.80 a	22.5 ± 1.5 a	2.5 ± 1.1 a	2.3 ± 0.7 a	0.18 ± 0.04 a	<0.25 *	6.1 ± 1.0 a
PG1	332 ± 88 a	16.6 ± 3.8 a	0.20 ± 0.06 a	2.32 ± 0.37 a	25.8 ± 4.0 ab	2.4 ± 0.8 a	1.7 ± 0.6 a	0.18 ± 0.04 a	<0.25 *	6.6 ± 1.0 a
PG2	288 ± 32 a	13.9 ± 2.6 a	0.29 ± 0.06 a	2.16 ± 0.15 a	28.9 ± 1.5 b	3.1 ± 1.4 a	1.4 ± 0.4 a	0.17 ± 0.07 a	<0.25 *	6.2 ± 0.7 a
Second year (2025)										
control	225 ± 72 a	15.3 ± 1.5 b	<0.05 *	<0.25 *	28.3 ± 3.2 a	5.0 ± 2.6 a	3.0 ± 0.0 c	<0.05 *	<0.25 *	8.3 ± 0.6 a
RES-PG1	225 ± 38 a	13.3 ± 1.5 b	<0.05 *	<0.25 *	26.7 ± 1.5 a	4.3 ± 1.2 a	3.0 ± 0.0 c	<0.05 *	<0.25 *	8.0 ± 1.0 a
RES-PG2	270 ± 91 a	9.0 ± 1.0 a	<0.05 *	<0.25 *	31.7 ± 9.2 a	7.3 ± 2.1 a	2.0 ± 0.0 b	<0.05 *	<0.25 *	9.0 ± 1.0 a
REP-PG1	317 ± 52 a	8.7 ± 2.1 a	<0.05 *	<0.25 *	33.0 ± 2.0 a	4.3 ± 1.5 a	1.0 ± 0.0 a	<0.05 *	<0.25 *	8.3 ± 0.6 a
REP-PG2	238 ± 31 a	5.7 ± 0.6 a	<0.05 *	<0.25 *	32.0 ± 1.7 a	6.3 ± 0.6 a	1.0 ± 0.0 a	<0.05 *	<0.25 *	8.3 ± 0.6 a

* Below LOQ. Values marked with the same letter within a column are not significantly different at $p \leq 0.05$. Data are presented as mean ± SD ($n = 6$ in the first year; $n = 3$ in the second year).

In the second year, Cd and Cr concentrations were below the quantification limit in both plant parts. In pots that had received PG1 and PG2 doses in the first year (RES-

PG1 and RES-PG2), Ba concentrations were generally lower than in the control, whereas repeated PG application (REP-PG1, REP-PG2) led to a further decrease in Ba.

3.2. Soil Parameters

3.2.1. Soil pH and Plant Nutrients

After the crop harvest, soil pH and residual soil nutrients were measured. Soil pH decreased by the end of the second year in both soil types and across all treatments, including the control. PG application had different effects on soil pH, as well as on ammonium lactate (AL)-soluble P and Ca, in Arenosol and Solonetz soils over the two experimental years (Table 9). In the Arenosol, soil pH remained unchanged across the different treatments in both experimental years. In the first year, AL-P was significantly lower in the PG-treated pots (PG1, PG2) than in the control, likely due to increased plant uptake of P under PG application. In contrast, AL-Ca increased with increasing PG doses, reaching its highest value in the PG2 treatment. Similar changes in AL-soluble P, Ca, and pH (KCl) were detected in the Solonetz following PG application.

Table 9. Effect of PG application on plant-available P and Ca as well as soil pH in the Arenosol and Solonetz.

Treatment	pH (KCl)		AL-P (mg kg ⁻¹)		AL-Ca (mg kg ⁻¹)	
	Arenosol	Solonetz	Arenosol	Solonetz	Arenosol	Solonetz
First year (2024)						
control	5.01 ± 0.07 a	6.05 ± 0.13 a	31.0 ± 1.62 b	100.5 ± 2.76 b	1041 ± 220 a	2926 ± 187 a
PG1	4.99 ± 0.03 a	6.05 ± 0.03 a	28.0 ± 0.68 a	94.2 ± 1.79 a	1102 ± 180 ab	3181 ± 490 ab
PG2	4.98 ± 0.07 a	5.95 ± 0.03 a	28.5 ± 1.00 a	94.0 ± 2.24 a	1620 ± 256 b	4009 ± 398 b
Second year (2025)						
control	4.89 ± 0.04 a	5.83 ± 0.02 b	20.4 ± 0.3 ab	102.8 ± 5.3 c	767 ± 36 a	2811 ± 34 a
RES-PG1	4.83 ± 0.10 a	5.81 ± 0.04 b	19.5 ± 0.2 a	84.4 ± 0.4 b	847 ± 68 a	2794 ± 41 a
RES-PG2	4.82 ± 0.06 a	5.83 ± 0.02 b	20.0 ± 1.9 ab	77.2 ± 0.6 a	1180 ± 271 a	3558 ± 145 b
REP-PG1	4.87 ± 0.02 a	5.77 ± 0.02 ab	22.0 ± 0.9 ab	80.2 ± 1.1 ab	989 ± 14 a	3326 ± 12 ab
REP-PG2	4.91 ± 0.07 a	5.75 ± 0.02 a	22.5 ± 0.9 b	83.1 ± 1.5 ab	1700 ± 283 b	4829 ± 416 c

Values marked with the same letter within a column are not significantly different at $p \leq 0.05$. Data are presented as mean ± SD ($n = 6$ in the first year; $n = 3$ in the second year).

In the second year, AL-P values in the Arenosol showed no statistically significant differences among treatments. Similarly, no differences in AL-Ca were observed in pots previously treated with PG (RES-PG1, RES-PG2) or in those receiving the lower repeated dose (REP-PG1) compared with the control. A significant increase in AL-Ca was observed only under the higher repeated PG application (REP-PG2). Soil pH showed no significant differences between the treatments and the control.

Solonetz soil pH decreased in the second year in response to the higher PG doses (REP-PG2) compared with the control, and AL-P was significantly lower under all PG treatments and there was a difference in AL-P between RES-PG1 and RES-PG2. AL-Ca further increased in response to the PG application. As in the Arenosol, Ca responded most strongly to the higher PG doses, with both RES-PG2 and REP-PG2 showing higher values than the control, and the highest concentration was measured under the higher repeated PG application (REP-PG2).

3.2.2. Potentially Toxic Elements in Soil

In the Arenosol (Table 10), at the end of the first year, the total concentrations of Al, Ba, Cr, and Sr were not affected by either the lower (PG1) or higher (PG2) doses, whereas

Cd was significantly lower under PG1 than in the control. By the end of the second year, the total concentrations of Al, Ba, Cd, Cr, and Sr in the PG-treated pots did not differ from the control, indicating that neither residual nor repeated PG applications affected their soil levels.

Table 10. Changes in total concentrations of Al, Ba, Cd, Cr, and Sr in the Arenosol (mg kg^{-1}).

Treatment	Al (mg kg^{-1})	Ba (mg kg^{-1})	Cd (mg kg^{-1})	Cr (mg kg^{-1})	Sr (mg kg^{-1})
First year (2024)					
control	11,210 $\pm$ 1234 a	61 $\pm$ 5.3 a	2.77 $\pm$ 0.00 b	16.1 $\pm$ 0.7 a	11.6 $\pm$ 0.5 a
PG1	9687 $\pm$ 537 a	54 $\pm$ 3.7 a	2.63 $\pm$ 0.02 a	15.1 $\pm$ 0.8 a	10.3 $\pm$ 0.8 a
PG2	10,131 $\pm$ 513 a	59 $\pm$ 3.4 a	2.72 $\pm$ 0.08 ab	15.3 $\pm$ 0.6 a	11.7 $\pm$ 0.4 a
Second year (2025)					
control	9716 $\pm$ 84 b	51 $\pm$ 1.00 a	2.00 $\pm$ 0.07 a	16.5 $\pm$ 0.73 a	8.46 $\pm$ 0.42 ab
RES-PG1	9658 $\pm$ 874 b	48 $\pm$ 3.14 a	1.96 $\pm$ 0.25 a	14.7 $\pm$ 1.51 a	9.29 $\pm$ 1.06 b
RES-PG2	8739 $\pm$ 478 ab	46 $\pm$ 2.12 a	1.79 $\pm$ 0.07 a	15.8 $\pm$ 1.17 a	8.36 $\pm$ 0.17 ab
REP-PG1	7760 $\pm$ 660 a	44 $\pm$ 3.21 a	1.71 $\pm$ 0.35 a	15.2 $\pm$ 0.45 a	7.56 $\pm$ 0.50 a
REP-PG2	8531 $\pm$ 245 ab	47 $\pm$ 2.20 a	1.83 $\pm$ 0.17 a	15.2 $\pm$ 0.31 a	7.92 $\pm$ 0.13 ab

Values marked with the same letter within a column are not significantly different at $p \leq 0.05$. Data are presented as mean $\pm$ SD ($n = 6$ in the first year; $n = 3$ in the second year).

In the Solonetz (Table 11), in the first year, the total concentrations of Al, Ba, Cd, Cr, and Sr were significantly lower in PG-treated soils than in the control, with no significant difference between the two PG rates. In the second year, however, total concentrations of Al, Ba, Cd, Cr, and Sr did not differ significantly among treatments, indicating that neither residual nor repeated PG applications resulted in detectable changes compared to the control. The observed changes, particularly in total Al, likely reflect analytical and/or spatial soil variability rather than an effect of PG application.

Table 11. Changes in total concentrations of Al, Ba, Cd, Cr, and Sr in the Solonetz (mg kg^{-1}).

Treatments	Al (mg kg^{-1})	Ba (mg kg^{-1})	Cd (mg kg^{-1})	Cr (mg kg^{-1})	Sr (mg kg^{-1})
First year (2024)					
control	47,491 $\pm$ 3084 b	194 $\pm$ 36 b	6.86 $\pm$ 0.31 b	58.0 $\pm$ 4.7 b	53.7 $\pm$ 6.0 b
PG1	35,885 $\pm$ 1440 a	144 $\pm$ 14 a	6.49 $\pm$ 0.13 a	50.0 $\pm$ 2.0 a	47.1 $\pm$ 2.0 a
PG2	34,229 $\pm$ 811 a	134 $\pm$ 4 a	6.48 $\pm$ 0.15 a	49.2 $\pm$ 0.0 a	48.3 $\pm$ 3.3 a
Second year (2025)					
control	25,600 $\pm$ 111 a	122 $\pm$ 0.86 a	6.15 $\pm$ 0.09 a	43.3 $\pm$ 0.2 a	40.7 $\pm$ 1.00 a
RES-PG1	28,667 $\pm$ 2861 a	135 $\pm$ 17.8 a	6.50 $\pm$ 0.09 a	48.1 $\pm$ 3.5 a	43.4 $\pm$ 1.92 a
RES-PG2	28,007 $\pm$ 2414 a	126 $\pm$ 5.51 a	6.57 $\pm$ 0.32 a	51.0 $\pm$ 8.0 a	45.9 $\pm$ 4.81 a
REP-PG1	26,640 $\pm$ 3320 a	125 $\pm$ 11.5 a	6.35 $\pm$ 0.14 a	45.2 $\pm$ 2.8 a	41.1 $\pm$ 1.47 a
REP-PG2	24,973 $\pm$ 575 a	118 $\pm$ 5.15 a	6.14 $\pm$ 0.07 a	42.5 $\pm$ 0.5 a	42.0 $\pm$ 3.16 a

Values marked with the same letter within a column are not significantly different at $p \leq 0.05$. Data are presented as mean $\pm$ SD ($n = 6$ in the first year; $n = 3$ in the second year).

3.2.3. Microbial Biomass and Community Structure

Soil microbial biomass is considered a biological indicator of soil health due to the high sensitivity of microorganisms to soil management, pollution, and environmental changes. The total microbial biomass (as total PLFA) ranged from 157.0 to 231.1 nmol g^{-1} dry soil in the Arenosol and was significantly influenced by the application of PG (Figure 2). Both residual PG effects and repeated PG applications resulted in higher microbial biomass than

the control, with the highest values observed under the repeated, high-dose PG treatment (REP-PG2).

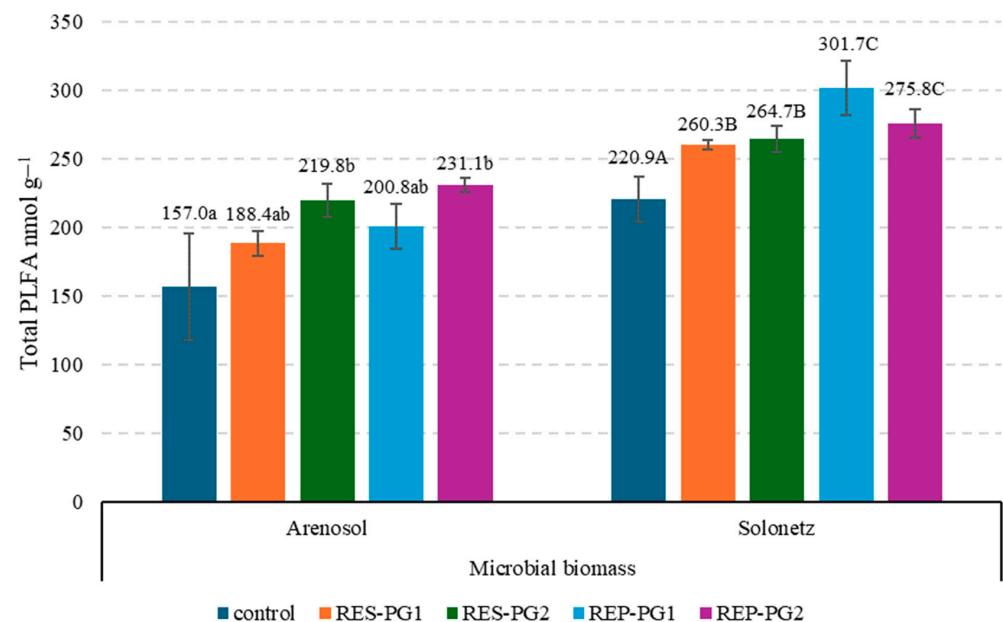


Figure 2. Effects of PG application on microbial biomass in the Arenosol and Solonetz ($n = 3$). Lowercase letters indicate significant differences in Arenosol and uppercase letters indicate significant differences in Solonetz.

In the Solonetz, total PLFA ranged from 220.9 to 301.7 nmol g⁻¹ dry soil and increased in response to PG application. The residual effect of PG application (RES-PG1; RES-PG2) resulted in higher microbial biomass than the control, while repeated PG application (REP-PG1; REP-PG2) further increased the values. However, a slight decrease was observed under the repeated high-dose treatment (REP-PG2) compared to the lower dose (REP-PG1).

In the Solonetz, higher biomass values were observed for most measured microbial groups when comparing results to the Arenosol. The exception was Gram-negative bacteria, which were more abundant in Arenosol (Table 12). PLFA analysis showed that microbial communities responded differently to PG application depending on the soil type. In the Arenosol, PG treatments produced smaller changes in the biomass of the measured microbial groups. Even at the higher PG2 dose, changes in AM fungi, other fungi, Gram-positive and Gram-negative bacteria, and actinomycetes were limited. In contrast, the Solonetz showed more pronounced responses to PG than the Arenosol, especially regarding the abundance of AM fungi and actinomycetes. All PG treatments (both residual and repeated treatments) significantly increased AM fungi abundance compared to the control, but responses did not differ significantly among the PG treatments. In pots receiving the lower PG dose, actinomycetes abundance exceeded that of the control under repeated low-dose treatment. However, after repeated application of the higher dose (REP-PG2), a slight but nonsignificant decrease in actinomycetes abundance was recorded. The biomass of Gram-negative and Gram-positive bacteria, as well as fungi, remained unchanged in both soil types, with no detectable differences among the PG treatments.

Table 12. Effect of PG application on microbial communities in the Arenosol and Solonetz.

	AM fungi (nmol g ⁻¹)		Fungi (nmol g ⁻¹)		GN Bacteria (nmol g ⁻¹)		GP Bacteria (nmol g ⁻¹)		Actinomycetes (nmol g ⁻¹)	
	Arenosol	Solonetz	Arenosol	Solonetz	Arenosol	Solonetz	Arenosol	Solonetz	Arenosol	Solonetz
control	7.2 ± 1.5 a	10.7 ± 6.0 a	4.29 ± 0.6 a	5.62 ± 1.3 a	73.4 ± 27.1 a	43.2 ± 7.5 a	38.3 ± 3.8 a	93.7 ± 10.5 a	6.3 ± 2.4 a	11.2 ± 0.7 a
RES-PG1	8.5 ± 0.5 a	37.3 ± 3.4 b	5.61 ± 0.8 a	5.49 ± 0.4 a	81.7 ± 8.3 a	46.3 ± 2.0 a	41.0 ± 8.3 a	90.3 ± 2.1 a	5.0 ± 1.5 a	11.8 ± 0.5 a
RES-PG2	10.3 ± 1.4 a	49.0 ± 9.6 b	5.21 ± 0.2 a	5.70 ± 0.1 a	97.0 ± 8.8 a	61.5 ± 21.2 a	44.2 ± 2.3 a	99.4 ± 9.9 a	7.1 ± 0.3 a	14.8 ± 1.6 b
REP-PG1	8.4 ± 2.9 a	47.3 ± 10.1 b	4.77 ± 1.4 a	6.07 ± 0.5 a	95.5 ± 16.4 a	57.6 ± 2.0 a	36.2 ± 3.4 a	103.2 ± 5.5 a	5.3 ± 1.8 a	14.6 ± 0.6 b
REP-PG2	10.8 ± 2.1 a	32.7 ± 2.8 b	6.04 ± 0.3 a	5.56 ± 0.1 a	99.0 ± 1.6 a	56.1 ± 8.0 a	47.4 ± 3.9 a	92.3 ± 5.3 a	4.3 ± 1.5 a	13.0 ± 0.8 ab

AM fungi: arbuscular mycorrhizal fungi; GP bacteria: Gram-positive bacteria; GN bacteria: Gram-negative bacteria. Values marked with the same letter within a column are not significantly different at $p \leq 0.05$. Data are presented as mean $\pm$ SD ($n = 6$ in the first year; $n = 3$ in the second year).

3.2.4. Relative Abundance of Microbial Groups

The Solonetz was characterized by a greater relative abundance of Gram-positive (GP) bacteria, which made up a significantly larger proportion of the community than in the Arenosol, where Gram-negative (GN) bacteria were more abundant (Figure 3). The Solonetz exhibited a significant shift, with AM fungi increasing from approximately 6% in the control group to about 15–20% in the PG-treated pots. In contrast, the Arenosol maintained a more uniform distribution of microbial groups across all treatments. These patterns indicate that the microbial community composition in Solonetz soil was considerably more responsive to PG applications than that in Arenosol.

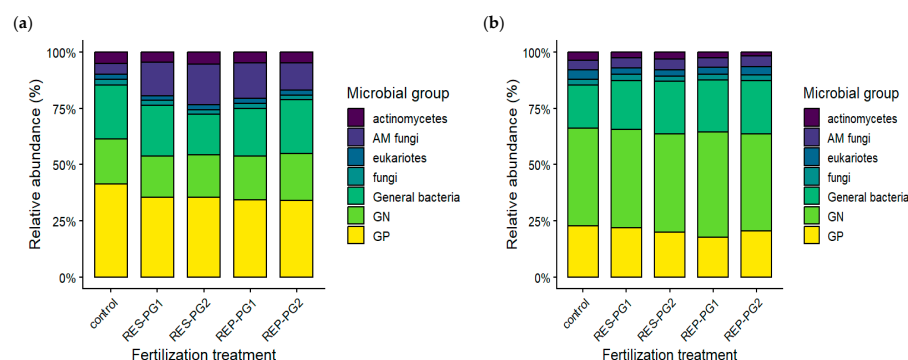


Figure 3. Relative abundance of soil microbial groups under different fertilization treatments in (a) Solonetz and (b) Arenosol soils.

3.2.5. Shannon Index

The trends in the Shannon index shown in Figure 4 are consistent with the relative-abundance results for both soil types. In Solonetz soil, functional diversity indices increased significantly across all PG treatments (RES and REP) compared to the control. In contrast, no statistically significant differences were observed among the groups in Arenosol.

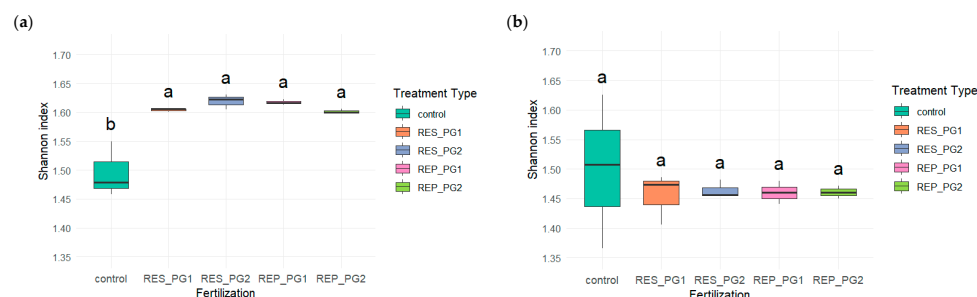


Figure 4. Shannon index across different PG treatments in Solonetz (a) and Arenosol (b) soils. Boxplots marked with the same lowercase letter are not significantly different at $p \leq 0.05$.

3.2.6. PERMANOVA and PCA

The non-overlapping confidence ellipses in the PCA plot (Figure 5) show clear separation of the microbial communities of the two soil types. A distinct separation between the two soil types was observed along the first principal component (PCA1), which explains 55.7% of the total variance. The PERMANOVA results also confirmed the PCA findings ($R^2 = 0.773$, $p = 0.001$).

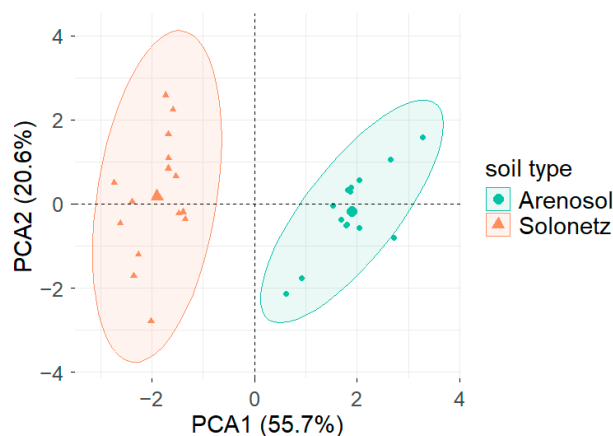


Figure 5. Principal component analysis (PCA) of microbial community structure: comparison between the Solonetz and the Arenosol.

PERMANOVA showed that treatments significantly affected microbial community composition in both Solonetz and Arenosol soils (Figure 6). In Solonetz soil, the treatment explained a large proportion of the variation ($R^2 = 0.725$, $p < 0.001$), while in Arenosol, the effect was also evident, though less pronounced ($R^2 = 0.485$, $p = 0.049$). In the Solonetz PCA plot (Figure 6a), the REP-PG1 group (solid blue circles) showed the most pronounced separation from the control group, primarily along the PCA1 axis. This distinct rightward shift accounts for 59.5% of the total variance. In Arenosol, the REP-PG2 treatment (solid red triangles) shows the most distinct separation, shifting furthest to the right along the PCA1 axis, which explains 46.8% of the total variance.

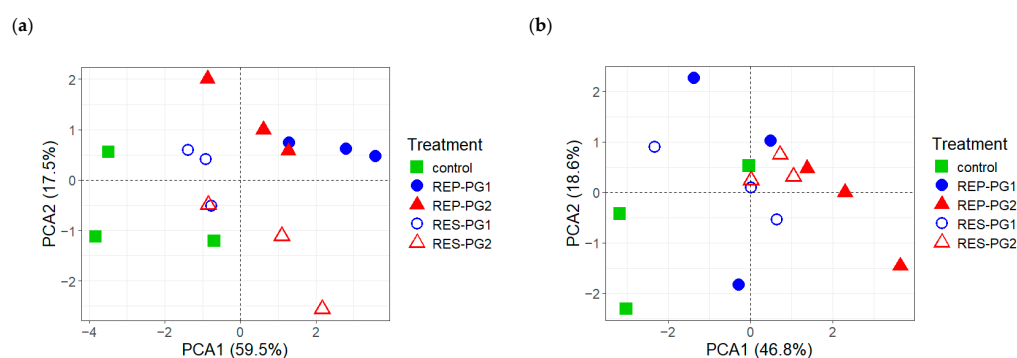


Figure 6. PCA score plots of microbial community composition across different treatment groups in the Solonetz (a) and Arenosol (b).

3.2.7. Correlation Analysis

The second-year Spearman correlation analysis (Figure 7) revealed strong positive associations among soil AL-Ca concentrations, straw and grain Ca and S concentrations, and soil microbial biomass in both soil types. These relationships indicate strong covariation among these variables, which primarily reflects the cumulative effect of increasing PG doses. The yield components showed no significant correlations with the measured plant

and soil properties in either soil type. This lack of correlation may be attributed to a saturation effect: in the Solonetz soil, PG application did not result in further increases in yield above 2 t ha^{-1} , whereas no significant yield response was observed in the sandy soil.

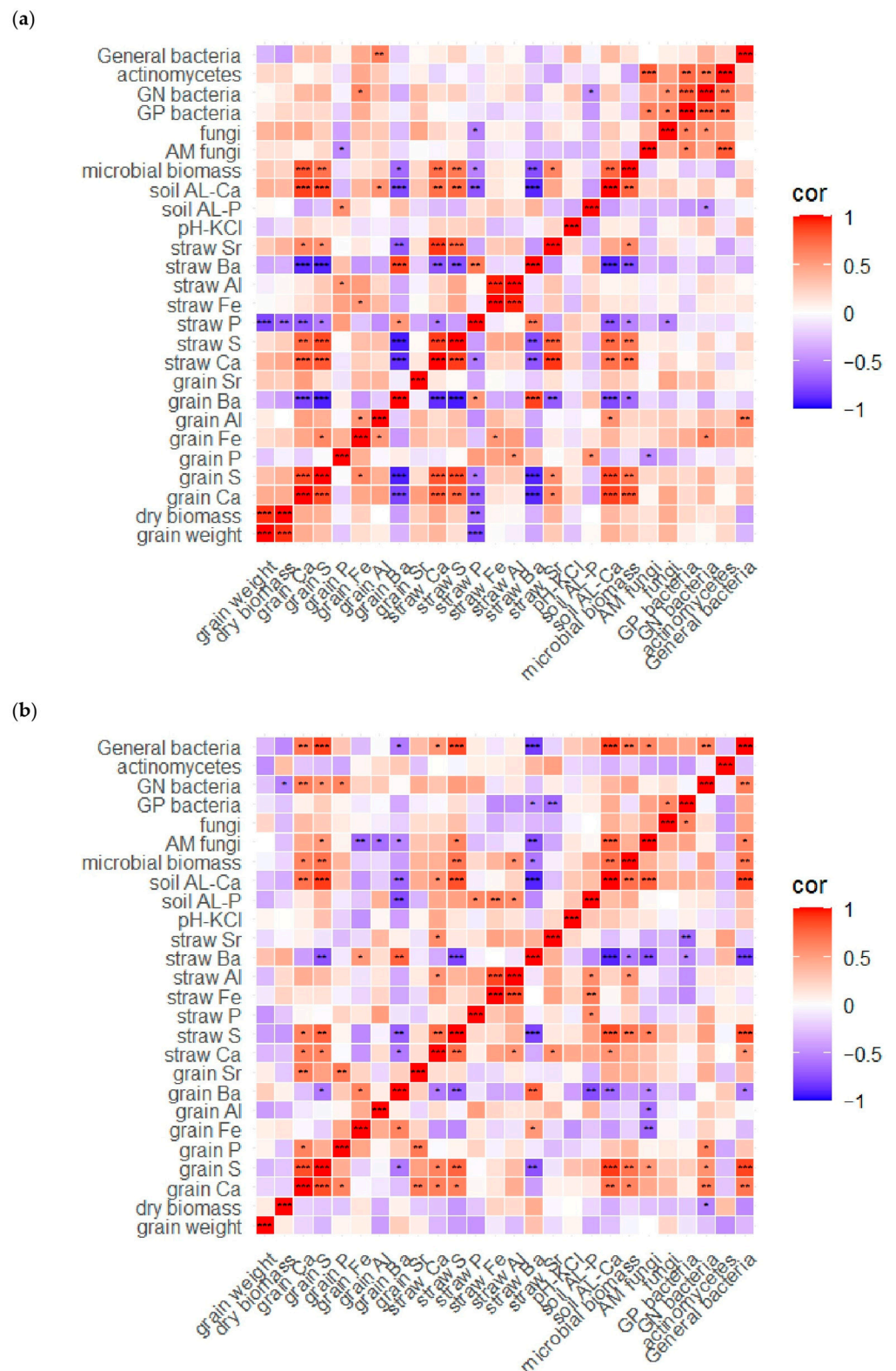


Figure 7. Correlation heatmaps of crop yield parameters, plant nutrient concentrations, soil properties and microbial communities in (a) Solonetz and (b) Arenosol soils (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

The two soil types showed distinct correlation patterns between individual microbial groups and AL-Ca concentrations. In the Solonetz soil (Figure 7a), AL-Ca showed no significant correlation with any individual microbial group, although some microbial groups correlated strongly with each other. In contrast, in the Arenosol (Figure 7b), AL-Ca was strongly and positively correlated with AM fungi and general bacteria, which also showed a strong positive correlation with each other.

Highly significant negative correlations were found between plant Ca and Ba concentrations in both soils, as well as between plant Ca and P concentrations in the Solonetz soil.

4. Discussion

4.1. Soil-Specific Agronomic Performance of PG

PG can serve as a highly effective soil conditioner and a source of plant-available S and Ca. The two-year pot experiment presented here demonstrated soil-dependent agronomic responses to PG application. In the Solonetz, PG significantly increased spring wheat yield. Conversely, no yield response was recorded in the acidic Arenosol. Similar positive crop yield responses following PG application on degraded soils have been reported in numerous studies [42–46]. Notably, the lower application rate (PG1: 2 t ha^{−1}) was sufficient if compared to the higher rate (PG2: 10 t ha^{−1}). These findings have important practical implications for agricultural management. They indicate that moderate application rates are sufficient to achieve favorable crop responses under the conditions investigated. Such an approach would minimize unnecessary costs and avoid potential environmental risks associated with over-application of PG.

In the Solonetz soil, the initial exchangeable sodium percentage (ESP = 17%) exceeded the critical threshold of 15%, while the base saturation was already very high (99.1%). Consequently, repeated PG application did not result in a substantial change in this parameter. In contrast, the proportion of exchangeable Ca²⁺ increased from 60.4% in the control to 64.6% at 2 t ha^{−1} PG and to 72.5% at 10 t ha^{−1} PG. At the same time, the proportion of exchangeable Na⁺ decreased from 17.2% in the control to 16.5% and 8.5% at 2 and 10 t ha^{−1} PG, respectively. These changes in the relative proportions of exchangeable Ca²⁺ and Na⁺ had a marked effect on the main soil characteristics, indicating considerable chemical amelioration of sodicity [47–49]. The improvement in soil chemical conditions may explain the observed yield response. Furthermore, the Solonetz soil had higher clay and organic matter contents, allowing it to retain more moisture and nutrients than the free-draining Arenosol [50]. Beyond supplying Ca and improving soil structure, PG also provided plant-available S, which further enhanced wheat yield.

The response of the Solonetz to increasing PG doses showed that higher application rates do not necessarily provide greater agronomic benefits. At the same time, the increase in Ca²⁺ concentrations was accompanied by a reduction in plant-available P (AL-P) and lower straw P concentrations in the second year. This reduction may have resulted from the formation of insoluble Ca-P precipitates [51]. This complex nutritional interplay is further supported by the second-year Spearman correlation analysis, in which a highly significant negative correlation was found between plant Ca and P concentrations (Table 6, Figure 7a). Thus, our results suggest that excessive PG application can alter nutrient availability without providing a corresponding increase in crop yield.

In the Arenosol soil, initial EC_{se} and ESP values were within the normal range (EC_{se} < 4, ESP < 15%). Repeated PG application at 2 t ha^{−1} increased base saturation from 73.1% to 93.1%, with no further substantial increase at 10 t ha^{−1}. Similarly, the exchangeable Ca²⁺ proportion increased from 53.4% to 65.0% at the 2 t ha^{−1} PG dose and reached 66.8% when 10 t ha^{−1} PG was applied.

The Arenosol soil was initially characterized by low KCl-soluble SO_4^{2-} (6.43 mg kg^{-1}), which is below the critical threshold of 10 mg kg^{-1} [38]. This deficiency was further confirmed during the second year of the experiment in the control treatment, where S concentrations in straw (0.043%) and grain (0.036%) were below the respective critical values of 0.08 and 0.12% [41].

Due to the low solubility of CaSO_4 ($\approx 2 \text{ g L}^{-1}$ at 25°C), PG acted primarily as a slow-release S source in the Arenosol. The S-supplying effect persisted into the second year, as reflected by increased S concentrations in straw and, to a lesser extent, grain. Costa and Crusciol [52] reported similar findings, observing increased S absorption in sorghum and soybean following PG application at 2.1 t ha^{-1} . Repeated high-rate PG application (REP-PG2: 10 t ha^{-1}) further increased S and Ca levels in the vegetative tissues but had a limited effect on grain nutrient concentrations. This aligns with our previous findings [53], which showed that while S fertilization increases both total and organic S concentrations in the vegetative parts of spring wheat, grain S concentrations remain relatively constant even under varying N:S ratios. This limited response of grain S to increased S supply is primarily driven by genetic regulation rather than changes in soil nutrient availability [54,55]. Ultimately, the lack of yield response despite successful S uptake indicates that other co-limiting nutrients or soil constraints in the Arenosol were the primary limitations to crop growth.

4.2. Potentially Toxic Element Accumulation and Crop Safety

The assessment of potentially toxic elements indicated no additional short-term risk attributable to PG under the investigated conditions. The total concentrations of potentially toxic elements were consistently higher in the straw than in the grain. These findings indicate limited translocation to edible plant parts, thereby reducing potential food safety risks. This finding aligns with observations by Al-Oudat et al. [56] and Papastefanou et al. [57], who reported that physiological barriers in wheat limit the accumulation of potentially toxic elements in grain. Across both soil types, PG applications had no significant impact on Al, Cd, Cr and Sr concentrations in grain. This observation is further supported by Al-Oudat et al. [58] and Enamorado et al. [59], who reported that PG application does not promote heavy metal accumulation in soil–plant systems. In contrast, Ba concentrations in both straw and grain decreased significantly, particularly at 10 t ha^{-1} (Tables 7 and 8, Figure 7). This decrease likely resulted from precipitation of insoluble BaSO_4 caused by the high sulfate input from PG. Among the analyzed elements, Cd is the only metal with an established EU regulatory threshold for wheat grain (0.10 mg kg^{-1} , Commission Regulation (EU) 2023/915). In the first year, grain Cd concentrations exceeded this threshold in all treatments, including the untreated control, this elevation likely reflects baseline soil Cd levels rather than the PG treatments. Increasing PG application rates and repeated treatments did not enhance Cd accumulation. In fact, Cd concentrations were below the limit of quantification in all treatments in the second experimental year. This result confirms that PG did not enhance Cd mobility or bioavailability in the short term and is consistent with the finding of Outbakat et al. [60], who observed no increase in Cd uptake in barley following PG applications of 15 to 45 t ha^{-1} .

However, it is critical to separate these short-term experimental observations from long-term environmental safety implications. Evidence from long-term field applications further highlights the potential for Cd accumulation. Repeated application of PG at high rates (20 and 25 t ha^{-1} every 2–3 years between 1978 and 2006) was reported, for instance, to increase Cd concentrations in tomato fruits [61]. Therefore, while no immediate risk was observed in this short-term pot experiment, longer-term field experiments are necessary to determine the environmental risks of continuous PG application.

4.3. Responses of Microbial Biomass and Community Structure to PG

PG application increased total microbial biomass in both experimental soils. However, its impacts on microbial community composition and functional diversity were substantially more pronounced in the Solonetz than in the Arenosol. When interpreting these shifts, it is important to emphasize that the Shannon diversity index derived from PLFA profiling primarily reflects the functional balance and relative abundance among major microbial groups (such as AM fungi, saprotrophic fungi, Gram-positive and Gram-negative bacteria, and actinomycetes) rather than taxonomic species richness [62,63].

In the Solonetz, the increase in microbial biomass under PG application was likely associated with the improved soil chemical and structural conditions [9,64]. The improved Ca^{2+} - Na^+ balance created more favorable conditions for soil aggregate stability, potentially providing a more suitable habitat for microbial communities. These structural improvements were accompanied by greater abundances of specific microbial groups, particularly AM fungi and actinomycetes, greater functional diversity, and an increase in the Shannon index from 1.48 to 1.60. Specifically, AM fungal biomass increased from 10.7 nmol g^{-1} in the control, to $32.7\text{--}49.0 \text{ nmol g}^{-1}$ across PG treatments, expanding their community contribution from ~5% to 12–19%. Actinomycetes biomass also increased significantly, reaching 14.8 nmol g^{-1} under RES-PG2 and 14.6 nmol g^{-1} under REP-PG1. Accordingly, these microbial community responses may serve as sensitive indicators of ecological recovery in the Solonetz [65]. While repeated application of the lower PG dose (REP-PG1) resulted in the greatest increase in microbial biomass, the higher repeated dose (REP-PG2) was associated with a slight decline in both total microbial biomass and actinomycete biomass. This dose-dependent response may indicate that excessive PG application induces transient salinity stress, potentially constraining the abundance of sensitive microbial groups [66].

The Arenosol also showed an increase in microbial biomass, but the changes in community composition were less pronounced than those observed in the Solonetz. The low clay and low organic matter contents of the Arenosol likely limited the formation and stabilization of microaggregates. Consequently, although PG supplied Ca^{2+} , its application was insufficient to induce microaggregate formation, resulting in limited changes in microbial community structure and functional diversity.

5. Conclusions

This two-year pot experiment demonstrated the beneficial agronomic effects of PG application, as evidenced by increased wheat yield and improved soil properties, although these effects were strongly soil dependent. In the slightly sodic Solonetz ($\text{EC} = 1.750 \text{ mS cm}^{-1}$, $\text{ESP} = 17.3\%$), PG improved soil fertility and crop yield by supplying Ca and S and replacing exchangeable Na^+ with Ca^{2+} . It also stimulated microbial biomass and functional diversity, with a marked increase in AM fungi and a smaller increase in actinomycetes. In the sulfur-deficient Arenosol, PG acted primarily as a slow-release S source, correcting S deficiency and maintaining S availability over time. However, these improvements did not result in a significant yield response. The PG application rate of 2 t ha^{-1} provided the most favorable overall agronomic and biological response, while the higher rate of 10 t ha^{-1} offered limited additional benefits and reduced P availability in the Solonetz. In this work, PG application did not increase the accumulation of the measured potentially toxic elements, including Cd, in wheat grain, indicating no additional short-term risk attributable to PG under the tested conditions. However, the tested PG contained Cd above the current EU threshold; given the relatively short two-year duration of the experiment, longer-term field studies are strongly recommended to evaluate its sustained effectiveness and potential cumulative environmental risks.

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