



Article White Clover (*Trifolium repens* L.) Cultivation as a Means of Soil Regeneration and Pursuit of a Sustainable Food System Model

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Abstract: Background: Currently, in the face of constant climate change and the development of the mining industry, recovering soils degraded by industry for agricultural production and ensuring more food security for the world has become more difficult. Soil contamination is of particular concern as it affects not only human health but also vegetation growth and the biological environment. The aim: The aim of our research is to develop an appropriate cultivation technology in the area of former and present oil extraction areas and monitor their recovery for agricultural purposes and, thus, for food production. Methods: Experimental, descriptive, laboratory, and comparative methods were used. Results: A significantly decreased content of PAHs in the tested soil under the influence of the "Green technology" was observed just in the third year of the research. Eight years after the introduction of "green technology", the sum of PAHs in the soil degraded by the oil extraction industry was more than 2-fold reduced. Therefore, there is a need to develop a nature-friendly and cost-effective method of removing and minimizing the effects of soil contamination by oil and its products. Conclusions: T. repens turned out to be a species that significantly prevents the degradation of the agricultural environment and restores soil for agricultural use, consequently encouraging the production of food safe for humans. The immeasurable effect of the use of "Green technology" was to ensure the biodiversity of the grasslands and to return the sources of natural nitrogen bound by bacteria of the genus Rhizobium in coexistence with plants from the Fabaceae family.

Keywords: bioremediation; phytoremediation; phytoextraction; legumes; petroleum hydrocarbon; soil pollution; green technology; sustainable farming system

1. Introduction

White clover (*T. repens* L.) is one of the most important, perennial, small-seeded legumes cultivated in the world. *T. repens* is a natural, allotetraploid (2n = 4x = 32), easily interbreeding species and is characterized by an entomophily, xenogamous pollination pattern [1]. White clover is the most commonly sown legume species in temperate grasslands,



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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/). and it can grow in a fairly wide range of climatic conditions. This species can tolerate biting and trampling; therefore, it is suitable for sowing on pastures for farm animals, as it coexists well with grasses [2,3]. White clover has rhizobial bacteria in its roots, which "fix" nitrogen from the air, thanks to which it can provide 50-200 kg N ha⁻¹ per year. Increasing clover content in grasslands can result in increased production and quality with less nitrogen fertilization. White clover can increase the yield of dry biomass, improve the performance of animals (because it is a higher quality fodder), and can reduce the demand for chemical nitrogen (because it increases the efficiency of nitrogen use (NUE) on farms) [4,5]. This species is also distinguished by a high digestibility of organic matter and a high protein content. White clover benefits agriculture with its ability to fix nitrogen, its high nutritional value, its seasonal complementarity with grasses, and its ability to improve animal feed intake and utilization. It is also sown in various landscapes to protect the soil [1–3,6,7]. With proper management, *T. repens* can grow in a wide range of soil and climate conditions. Active growth from germinating seeds or existing plants begins with lower temperatures and increased humidity, and continues until freezing [3,8,9]. White clover is better adapted to difficult climatic and soil conditions than any other species of clover, and it also has the ability to absorb nitrogen from the air. It is also the most common cold-season companion species on vast, perennial grass pastures in Australia [10]. In recent years, white clover has gained importance as a species that fixes free nitrogen from the air as a result of symbiosis with nodular bacteria due to the preference for sustainable agriculture, and recently it has developed a strong reputation as a reclamation plant [1,3].

For several decades, the industrialized countries of the world have had serious problems with soil contamination by oil and its processed products. They get into the soil as a result of extraction, via the processing of crude oil, and as a result of failures during the storage of fuels. Excessive exploitation of oil deposits, failures during extraction, storage, and transport of this raw material, as well as military activities, have always been the main causes of soil contamination with oil derivatives [11–15]. In Central and Eastern Europe, the areas of former bases and post-Soviet military training grounds are heavily contaminated, but also areas after oil exploitation, adjacent to refineries, gas stations, car and railway rolling stock workshops, and airports [15–20]. Excessive amounts of petroleum hydrocarbons (PHC) contaminate agriculturally important soils and the ultimate source of drinking water, groundwater. The accidental release of PHCs such as gasoline, diesel, and heating oil are a common source of groundwater contamination. PHC concentrations in groundwater often exceed drinking water standards, and bioremediation measures should be taken. Due to their organic nature, PHCs are difficult to degrade as they are inaccessible to microbial action. For this reason, PHCs are the most widespread environmental pollutants. The synergistic association of plants and microbes for PHC remediation is versatile and an effective tool for rehabilitating the soil and the environment from these types of undesirable materials. In addition to promoting plant growth, microbes can efficiently degrade PHC [12–14,21].

A large area of soil, particularly in south-eastern Poland, is heavily polluted with petroleum substances due to past and present oil extraction. Such soils must be recultivated in accordance with the Environmental Protection Act [22,23]. These are activities aimed at removing or significantly reducing the number of substances that pose a real threat to human and animal health. They should also be constantly monitored and soil contamination with petroleum substances should be limited. There are two main strategies for soil reclamation: complete or partial cleaning combined with a temporary increase in the mobility of petroleum substances in the soil; or immobilizing these substances so that they can never migrate to other environments and become inaccessible to plants. Currently, there are many methods of soil remediation, including: physical, chemical, thermal, and biological. Methods related to extraction, rinsing, or thermal treatment are highly effective in cleaning the soil of petroleum substances, but they are very cost-intensive due to the need to remove contaminated soil and transport it. In addition, such methods destroy natural biological activity and generate a large amount of waste. Biological methods that are much

cheaper and more environmentally friendly are based on the use of soil microorganisms (bioremediation) and higher plants (phytoremediation) as remediators. Mechanisms of phytoremediation of oil contaminated soils include phytodegradation, rhizodegradation, phytostabilization, phytoextraction, and phytovolatility [13–15]. The phytoextraction technique seems to be the most beneficial, in which plants clean the soil of excess petroleum substances and prevent its further movement from the place of contamination, additionally preventing erosion of the cleaned surface. In turn, in phytoextraction methods, contaminated plant biomass, the selection of appropriate species, resistance to both the type and degree of contamination, as well as the long time needed to completely clean the soil all pose problems. When choosing the appropriate method to remediate soils contaminated with compounds of soil derivatives, one should also take into account not only the effectiveness of the method but also the degree of contamination, the location of the site, and its current and planned future use [13,14,24].

Phytoremediation is a method of cleaning soils contaminated with PAHs that uses plants to remove pollutants from the soil. This process is based on the ability of plants to take chemical pollutants from the soil and accumulate them in their tissues or transform them into harmless products. One of the methods of phytoremediation of soils contaminated with PAHs is the use of plants such as goldenrod, grasses, rapeseed, corn, and sunflower, which have the ability to accumulate heavy metals and other pollutants in the soil. These crops are grown in polluted areas and then harvested after a certain period of time, together with the pollutants accumulated in them. Phytoremediation has many advantages. It is an environmentally friendly method because it does not require the use of strong chemicals that can be harmful to the environment. It is also a method that is relatively easy to apply, and the cultivation of crops can also be used to recultivate degraded land. Phytoremediation is relatively inexpensive compared to other methods of treating contaminated soil. Plants used for phytoremediation have great potential for use as energy biomass, which further increases their economic value. However, this method also has its limitations, e.g., in some cases, the phytoremediation process can take a long time to effectively remove contaminants from the soil. Plants used for phytoremediation can be sensitive to extreme soil conditions such as salinity, low pH, or lack of nutrients [13,21]. Some contaminants can be very difficult to remove by phytoremediation because they are not easily soluble in water and are poorly taken up by plants. Overall, phytoremediation is a treatment method for contaminated soils that can be effective for some pollutants but may also require other treatment methods for heavily contaminated soil [21,24].

The resilience of global food security is a critical issue. Faced with limited access to land and potential disruption to food markets, alternative, scalable, and efficient production systems are needed as a supplemental buffer to maintain the integrity of human food and animal feed production [5]. Therefore, the purpose of our conducted research was to develop a safe cultivation technology in the oil post-exploitation areas and adapt these areas for agricultural use.

2. Materials and Methods

2.1. Experimental Design

The research was carried out in the years 2012–2019 in the Krosno poviat, in the area wherein oil extraction formerly took place in south-eastern Poland. The experiment was carried out using the method of randomized blocks in a split-plot design in four repetitions on grasslands of a pasture community. The factors of the first order were tillage treatments: A—control object (without overseeding), B—underseeding after heavy tine harrow, C—underseeding after disc harrow, D—underseeding after the rotary tiller. The second-order factor was clover seed sowing norms: (a) 25% of the norm; (b) 50% of the norm; (c) 75% of the sowing rate at full tillage. White clover seeds of the 'Tasman' cultivar were used for sowing. The same mineral phosphorus–potassium fertilization was applied annually in the amount of: 43.6 kg P and 124.4 kg K ha⁻¹. Phosphorus was used in the form of triphosphate superphosphate and potassium in the form of 60% potassium salt.

Nitrogen fertilization was not applied throughout the research period. Yield valuation was performed according to the pasture grazing schedule. Cows with calves grazed on the pasture. During harvest, in each pasture rotation, samples were taken for botanical and weight analyses of the sward. The experiment was conducted in accordance with the principles of good agricultural practice and integrated pest management [25].

2.2. Groundwater Measurement

In all growing seasons, the groundwater level was measured every 7 days in control wells located next to the experiments. Groundwater level studies were also carried out before the start of pasture experiments (autumn 2011) and after their completion (autumn 2019). The points indicated for monitoring were the existing test wells, drilled wells, exploited and not exploited, and in exceptional cases "hammered" wells. Similar location and technical requirements were adopted for these points as for the points of the State Environmental Monitoring. The points were selected in such a way that they captured one of the aquifers occurring here, which in a given region was the main usable aquifer, most often taken for water supply purposes or most exposed to contamination with petroleum substances.

2.3. Determination of Chemical and Physicochemical Properties of Soil

The physicochemical and chemical properties of the soil were determined in the laboratory of the Regional Chemical and Agricultural Station in Lublin (no. AB 1186) according to the following methods: Casagrande method modified by Prószyński according to the standard PN-EN ISO 10390:2022-09 [26]; soil reaction—pH in 1 M KCl—electrometrically according to ISO 10390:2005 [27]; Corg.—Tiurin method [28], content of Mg²⁺ and Cu, Mn and Zn—ASA method [29]; the amount of assimilable magnesium was determined by the Schachtschabel method [30]; the content of assimilable forms of phosphorus and potassium—by the Egner Riehm method [31,32].

Soil bulk density was used as a significant index of differences in the soil structure and moisture retentive measurements and was steadily measured from 50 mm diameter cores to a depth of 30 cm [33]. Soil cores were measured wet, desiccated in an oven at 105C for 48 h, and measured once more to determine the soil moisture content and bulk density. The total porosity was determined on the basis of the results of determinations of the density of the solid phase of the soil and the density of the soil, based on the formula: Total porosity = (1 - pbps), (1), where ρb is the bulk density and ρs is the average particle density (2.65 g cm⁻³) [33]. The density of the solid phase of the soil was determined by the pycnometric method. Soil density was determined in Kopecky cylinders with a volume of 100 cm³. Determination of soil water capacity was performed with a neutron moisture meter (NMM) [34].

2.4. Quantification of Polycyclic Aromatic Carbons

The determination of polycyclic aromatic hydrocarbons (PAHs) in soil was carried out on the basis of standard mixtures of these compounds in ampoules containing about 1 mL of solution, from which standard solutions were prepared. The dichloromethane-based solutions contained approximately 90 μ g mL⁻¹ of each component. A series of calibration samples (5) were prepared from these solutions. A total of 0, 100, 400, 600, 800 μ g of the stock solution were added to 10 mL volumetric flasks. For quantitative determinations, 100 μ g of an internal standard solution containing acenaphthene d10 and the addition of 100 μ g of a control standard (d10 phenanthrene) were added to dichloromethane solutions. This made it possible to control the extraction process and determine the recovery of the analyte. Chromatograms were recorded for standard solutions using an analytical program. In total, 1 μ g of standard solution was added to the chromatography column. The area under the peaks of individual compounds from the PAH group was measured, and then the ratios of these areas to the area of the control standard added in a constant amount to the calibration solutions were determined. Analyte peaks were determined on the basis of the calculated area ratio in comparison to the area of control standards. Then, plots were made for naphthalene, acenaphthylene, fluorene, and benzo[k]fluoranthene with the coordinate system: X-axis—ng of the analyte injected into the chromatograph; Y-axis—ratio of the analyte surface area to the internal standard area. The standards PN-ISO 10381-1, PN-ISO 10381-2 [35,36] were used to determine PAHs. Samples for testing were collected in sealed containers with a capacity of about 2 kg. Sampling sites in a potentially contaminated zone were designated in proportion to its size, with a minimum of six samples. A scheme of systematic sampling with a regular-shaped grid in a randomly selected area located in the vicinity of oil occurrence was applied. The samples were taken with a soil stick to a depth of 20 cm. The collected samples were transported to the laboratory, where the soil was then stored in a refrigerator at 4 °C until the analytical sample was prepared. Five soil samples were taken from each variant of the experiment. These areas were classified in accordance with the Regulation of the Minister of the Environment of 2002 (item 1359) as wasteland (group B) (ACT of 3 December 2010 on the implementation of some European Union regulations regarding equal treatment [37,38]).

2.5. Study of the PAH Extraction Process from the Soil

The determination of petroleum substances and PAHs in soils was carried out in accredited laboratories of the Provincial Sanitary and Epidemiological Station and the Laboratory of Analysis and Physicochemistry of Hydrocarbon Fuels in Kraków. A total of 15 PAHs approved for labeling in environmental samples by the US Environmental Protection Agency were analyzed, excluding the rarest and most volatile PAHs. In order to isolate polycyclic aromatic hydrocarbons from the sample matrix, an ultrasound-assisted solvent extraction process was used. It was carried out for 30 min then the obtained extract was separated from the soil sample in a laboratory centrifuge at 3000 rpm for a specified period of time. The extract was purified on SPE columns with a vacuum system. The adsorbed compounds were eluted into 2 mL vials with an appropriately selected solvent. The extract was concentrated under a stream of nitrogen, and in the last step, an internal standard solution (syringe standard) was added and sent for quantification. In the first stage, the extraction process on synthetic samples and the internal standard containing deuterated compounds of acenaphthene, chrysene, 1,4-dichlorobenzene, naphthalene, perylene, and phenanthrene were evaluated. The extraction was carried out in two cycles of 30 min each, with a new portion of the solvent (2-propanol). The centrifugation time was 10 min. The SPE column was used for purification with an octadecyl stationary phase conditioned with 3 mL of 2-propanol and 3 mL of a mixture of propanol and water. Compounds to be labeled on the column were eluted with $2.00-0.05 \mu g$ portions of dichloromethane. The extract was concentrated to a volume of about 100 μ L under a stream of nitrogen. To control the extraction, an addition of 200 μ g of the control standard was added to the sample before extraction [39–41].

2.6. Collection of Plant Samples and Determination of Their Air-Dry Mass

The tests were carried out in 4 repetitions on plots of $8 \times 5 = 40 \text{ m}^2$. The sample unit of the pasture sward was a square with an area of 0.5 m^2 . Two such units formed a single sample. Aggregate samples, on the other hand, were multiples of single samples and acted as sample size variants. They were created by combining, the weights of individual components in subsequent single samples and the weights of entire single samples.

The samples were taken before grazing, when the height of the main mass of the sward was approx. 18.5 cm, and its efficiency in terms of air-dry matter was 3.3 t ha^{-1} . This is because the accuracy of the assessment of the botanical composition by weight analysis is directly proportional to the number of sample units that the analyzed sample consists of. Then, the samples were weighed and dried at 60 °C in an oven (model SIM 500, Memmert, Schwabach, Germany) for 48 h until a constant weight was obtained [42].

2.7. Determination of Species Composition of the Sward

Cow grazing began around May 1 and lasted until November 1. The floristic diversity of plants on the pasture was determined on the basis of the share of species from distinguished botanical families in the total number of species [7,43]. The species composition of the sward in the 2012–2019 growing seasons was analyzed using the botanical-weight method in average samples from four repetitions annually, in the yield of all rotations. The grazing of cows in the pasture started around May 1st and lasted until November 1st. The floristic diversity of plants on the pasture was determined based on the share of species from distinguished botanical families in the total number of species [6,7,43]. The species composition of the sward in the 2012–2019 growing seasons was analyzed using the botanical-weight method in average samples from four repetitions taken before grazing animals during pasture yield valuation. Average samples of about 4 kg were averaged and a 1 kg sample was air dried (up to 18% dry weight). After drying, the entire sample was weighed and individual species fractions (grasses, sedges, legumes, herbs) were separated from it, which were also weighed and the weight percentage of these species in the sample was calculated. The weight percentage was multiplied by Nvu—numbers of value in use, according to Filipek [43]. To determine the value in use of the examined pasture vegetation, Nvu were used, according to Filipek's method [43]. The value in use scale covers the range from -3 to 10. In many cases, due to the significant share of taxa atypical for pastures, which have not been assigned numerical values of Nvu, this value was approximated and marked with the symbol "~". The value of the sward was classified in four categories: very good (Nvu 8.1-10.0), good (Nvu 6.1-8.0), mediocre (Nvu 3.1-6.0), and poor (Nvu < 3.0) [43].

2.8. Test Conditions

The research was conducted out in the area of the first oil route in Europe, in the Jasielsko-Krośnieńska Valley and Foothills, which are the oldest oil extraction regions in Europe. These areas have been subjected to significant depletion and a decrease in their oil content over time [44]. As a result, the former oil extraction areas need to be restored for agricultural use to help ensure food security.

2.8.1. Climatic and Meteorological Conditions

Krosno County is located at 49°41′19″ N; 21°46′14″ E and has an altitude above sea level ranging from 278–340 m. The area of the Krosno poviat is situated in the agricultural and climatic region of Podkarpacie, which is a transitional zone between the mountains and foothill valleys. The climate in this region is transitional, with features of both oceanic and continental climates. The average annual temperature ranges from 6.0–8.5 °C, and the vegetation period of plants lasts up to 230 days, while the thermal winter lasts for 80–150 days [44].

The mountainous climatic region of Krosno County is characterized by a stratified climate with an average temperature decrease of 0.5 °C per 100 m of altitude and an increase in precipitation of about 60 mm per 100 m of altitude. The average rainfall in the western part of the county is 750–800 mm, while in the eastern part, it is 800–850 mm. In the mountain valleys, there is significant climatic variation caused by the local microclimate [44,45].

The description of the vegetation period was based on Vinczeffy's method [46], which uses the climatic precipitation index as an indicator of plant growth and development. Over the 8-year research period, the climatic precipitation index exceeded 0.13 only in 2015 and 2017, which can be considered average years. Years such as 1999 should be considered dry. In contrast, 1999 was a dry year, while the years 2012–2014, 2016, and 2018 were classified as too dry (Figure 1).



Figure 1. Rainfalls and air temperature during vegetation period in the years 2012–2019 acc. to meteorological station in Krosno.

The groundwater level was higher than the average level in all years of the study, as shown in Figure 2. It reached its highest point in early spring, following the start of vegetation, and gradually decreased until August before increasing again.

Given the current groundwater level, meadow vegetation experiences a shortage of moisture in the root layer during the summer. According to Hohendorf after Denis et al. [44], the optimal groundwater level on muck-peat soils should range from -50 to -60 cm. Szajda and Łabędzki [46] consider -60 cm the optimal level of groundwater in the conditions of peat-muck soils. In the opinion of Okruszko [47], a groundwater level above -70 cm ensures a good supply of water to plants, reduces the drying of the top layers of muck, and protects the positive properties of peat-muck soils. As stated by the authors, if the water table drops below this threshold, it can result in the drying out of the upper layers of soil and adverse modifications in the underlying turf layer. It can be assumed that a periodically maintained groundwater level below -70 cm in the experimental objects could have contributed to the deepening of the root and sub-root layers as a result. These changes could have affected the productivity and species composition of grass communities in the studied complex, as indicated by the results of Baryła and Kulik [48].

The groundwater level characteristics presented in this study have been determined based on the daily averages for several decades (as shown in Figure 2), using the method of measuring on grasslands. This information has proven to be useful for the ongoing management of groundwater resources in wet, dry, and subsoil dry environments. It has allowed for the determination of optimal groundwater levels, which can be adjusted based on the actual evapotranspiration rates of grasslands. According to Szajda and Łabędzki [46], maintaining an optimal groundwater level on drained grasslands is essential for maximizing the yield of green pastureland and effectively reducing the mineralization of organic matter.



Ground water level in the years 2012-2019 Mean ground water level in the years 1990-2019

Figure 2. Grassland groundwater in 2012–2019, compared to the long-term average (1990–2019).

2.8.2. Soil Conditions

The soils in the Krosno poviat exhibit typological variability and geological structure, as well as land morphology, water conditions, and the nature of vegetation. The most common types of soils found in this region are brown, acidic, leached, clay-loam, medium-deep, and deep soils [49]. In mountainous regions, they are more homogeneous but shallow, acidic, brown, and podzolic. Soils ranging from class IIIb to V are dominant in this region. Soil degradation is primarily caused by erosion, soil acidification, contamination with chemicals, and the exploitation of raw materials, particularly crude oil [50,51]. The mountain soils in the Krosno Basin are deluvial and form due to erosion [50,52].

2.9. Statistical Analysis

Statistical calculations were based on the analysis of variance with the assumed significance level of p = 0.05. The effect of variance on the main effects of the studied factors and their interactions was analyzed. Detailed analysis focused on the main effects and two-way interactions. The results of the study were also analyzed using correlation and polynomial regression. A gradual, progressive construction of the regression model was used. The significance of the sources of variability was checked with the Fischer–Snedecor F test, and the significance of differences between the compared means was assessed using Tukey confidence intervals. The parameters of the function were determined by the least squares method, and the significance was verified by Student's *t*-test. The obtained values were used to determine the optimal conditions for obtaining the most expected soil quality indicators, as well as to apply support in the face of changing edaphic conditions and to eliminate the negative effects of weather. In addition, descriptive statistics of the studied features were carried out using the SPSS Statistics 26 program [53–55].

3. Results

3.1. Soil Condition

The experiment involving the reseeding of white clover was conducted on muck-peat soil (Mt IIc) that was previously marshy. The soil was composed of sedge peat on top of reed peat, and its profile is presented in Table 1. The chemical composition of the soil was analyzed before and after the end of the experiment, and the results are shown in Tables 2 and 3, respectively.

Table 1. Soil profile before establishment of experiment with reseeding of *T. repens*.

Depth of Deposition (cm)	Genetic Horizon	Description of Soil Profiles
0–5	M_1	humus-turf, overgrown with roots;
6–23	M ₁	peat with a lot of roots, strongly decomposed with pockets of a gritty structure;
24–55	T ₁	sedge-rush peat with a fibrous structure, poor distribution (R1);
56–230	T2	rush peat with amorphous structure, strong decomposition (R3).

Table 2. Chemical characteristics of soils before establishment of experiment (2012).

		0		Avail	able Compon	ents Content in	Soil			
Cepth (cm)	рн (KCl)	Matter (%)		mg 100 g^{-1}			${ m mg}{ m kg}^{-1}$			
			P_2O_5	K ₂ O	Mg	Cu	Zn	Mn		
0–10	4.5	78	50.0	19.5	2.7	1.7	9.6	34.0		
10-15	4.2	74	17.5	14.0	7.0	1.6	1.3	48.0		
40-50	4.8	84	12.5	12.5	9.5	>30.0	0.5	_		
70-80	5.0	54	125.0	12.0	13.6	-	1.3	41.0		

Table 3. Chemical characteristics of soils after finishing experiment with reseeding of *T. repens.*

		Available Components Content in Soil							
(cm)	рн (KCl)		mg.100 g^{-1}						
		P_2O_5	K ₂ O	Mg					
0–10	5.4	33.5	15.0	5.5					
10-15	5.2	18.5	7.0	12.5					
40-50	6.0	43.0	19.5	2.8					
70-80	5.0	26.0	9.0	13.7					

The soils that were tested in the experiment were classified as Terric Histosols, and according to the classification of hydrogenic soils [49], they were identified as peat-muck, moderately decomposed (MtII). Their ash content was $0.225-0.227 \text{ g.g}^{-1}$. The soils on which the experiment was set up differed in the degree of decay after drainage [46]. This process affected the top layer of soil with a thickness of approximately 15–22 cm, which was characterized by the structure of decomposing peat.

During the first year of the experiment, the pH of the topsoil was found to be acidic (pH 4.5 in KCl). Upon analyzing the soil profile, the pH value in 1 mole of KCl was found to be in the range of 4.2–5.0. The average content of organic matter content in the top layer of soil was 78%, while in the analyzed profile, it ranged from 54% at the depth of 70–80 cm to 84% at the depth of 40–50 cm (Table 2).

In the first year of the experiment, the soil used in the study had a low abundance of available phosphorus in layers 0–10 (50.0 mg 100 g⁻¹) and even lower levels (12.5–17.5 mg 100 g⁻¹) in deeper layers. The content of assimilable potassium in the soil profile ranged from 12.5 to 19.5 mg 100 g⁻¹, indicating a low and very low abundance. Similarly, the content of assimilable magnesium was very low, ranging from 2.7–13.6 mg 100 g⁻¹.

The soil was characterized by a high abundance of available phosphorus in the layers of 0–10 and 70–80 cm, but low in the layers of 10–15 and 40–50 cm, and a low content of available potassium and magnesium [46,49] (Table 2). Compared to the surface layer, lower levels of almost all ash components were observed in the deeper levels of the soil profile, except for phosphorus in the 70–80 cm layer and copper in the 40–50 cm layer. The average content of organic matter in the top layer of soil was 78%, and in the examined profile, it ranged from 54% in the 70–80 cm layer to 84% in the depth of 40–50 cm, as shown in Table 2.

In the first year of the experiment, the soil was characterized by a low abundance of available phosphorus in layers 0–10 (50.0 mg 100 g⁻¹) and very low abundance in deeper layers (12.5–17.5 mg 100 g⁻¹). The content of assimilable potassium in the profile of the tested soil was in the range of 12.5–19.5 mg 100 g⁻¹, indicating low and very low abundance. Similarly, the content of assimilable magnesium was very low (2.7–13.6 mg 100 g⁻¹) (Table 2).

Soil acidity tests carried out after the end of the experiments did not show any unfavorable changes in reaction compared to the condition of the soil before the experiment was established, but they did not show significant improvement either. After 8 years of the experiment, the pH value in the entire profile increased and ranged from pH 5.2 to 6.0, although the soil was still acidic. Despite the systematic use of phosphorus–potassium fertilization, the content of assimilable forms of phosphorus and potassium decreased over time, as shown in Table 3.

After 8 years of pasture use, the abundance of assimilable phosphorus in the top layer of the soil decreased significantly indicating a very low abundance. A similar trend could be observed in the remaining layers, except for the 40–50 cm layer, where the abundance was determined as low. The content of assimilable potassium ranged from 7.0 to 19.5 mg 100 g^{-1} . The content of magnesium, similar to before the start of the experiment, remained low, ranging from 2.8–13.7 mg 100 g^{-1} .

The density of the soil tested varied depending on the depth of the layer. It was found to be twice as high in the root layer compared to the other layers. The porosity of the soil had a significant impact on its permeability and drainage. The total porosity was found to be between 68.59% in the root layer and 86.0% in the remaining layers.

The field water capacity in the root layer was 64.1 vol.%, while the full water capacity was 68.5 vol.%. The other layers had higher field water capacity ranging from 78.7 to 79.8% and full water capacity ranging from 82.1 to 86.0%. Please refer to Table 4 for more information.

	Specific	Weight of Soi	il (% vol.)			Water Capacity						
Depth	Depth Real	Temporary at Water Capacity		Specific Gravity	Total Porosity	Field Wate	er Capacity	Full Water Capacity				
(cm)	Weight Field Full Water Water Capacity Capacity		(Kg.m ⁻³)	(% vol.)	(% DM)	(% vol.)	(% DM)	(% vol.)				
5-15	0.468	1.109	1.154	1.49	68.59	138.1	64.1	147.6	68.5			
35-45	0.203	1.001	1.063	1.45	86.00	393.1	79.8	423.9	86.0			
70-80	0.214	1.001	1.035	1.20	82.17	370.1	78.7	387.2	82.1			

Table 4. Physical characteristics of soil prior to the establishment experiment with reseeding *T. repens.*

3.2. PAH Soil Contamination

The results of the PAH contamination analysis of the tested soil can be found in Table 5. The sum of PAH was found to be the highest in the first year of the study. However, a significant decrease in their content was observed only in the third year of the study. By the last year of the study, a more than two-fold decrease in their number was found in comparison to Rison in the first year. No significant year-to-year differences were identified

Years	Sum of PAH	Naphtalene	Fenantren	Anthracene	Fluoranthene	Chrysene	Benzo(a)anthracene	Benzo(a)pyrene	Benzo (a) Fluoranthene	Benzo(ghi)perylene	Fluorene	Pyrene	Benzo(b)fluoranthene	Benzo(bk)fluoranthene	Diabezn(ah)anthracene	Inden(1,2,3-cd)pyrene
2012	328.5	39.0	26.0	11.0	49.3	12.0	10.0	21.0	20.2	33.0	28.0	23.0	5.0	10.0	21.0	20.0
2013	311.7	37.8	23.8	14.0	42.0	10.6	10.6	24.0	16.7	28.0	29.0	25.0	4.0	9.0	20.2	17.0
2014	290.3	36.0	30.8	24.6	23.8	16.7	14.1	-	22.0	18.5	26.4	24.6	3.5	9.7	19.4	20.2
2015	278.0	30.0	21.0	24.6	23.8	16.7	14.1	-	25.5	18.5	26.4	24.6	3.5	9.7	19.4	20.2
2016	240.5	23.0	28.2	9.7	36.1	9.7	7.9	10.6	23.0	13.2	22.0	22.0	-	4.4	19.4	11.4
2017	210.8	19.4	25.0	12.3	20.2	15.0	8.8	-	19.0	20.2	15.0	11.0	2.6	7.9	17.6	16.7
2018	182.0	22.0	23.8	20.2	18.5	12.0	7.9	0.9	18.5	12.3	10.6	8.0	4.4	7.0	7.9	8.0
2019	161.9	16.7	15.0	19.4	15.8	13.2	9.7	-	17.6	23.8	7.9	6.0	1.0	8.8	7.0	-
Mean	250.5	28.0	24.2	17.0	28.7	13.2	10.4	7.1	20.3	20.9	20.7	18.0	3.0	8.3	16.5	14.2
LSDp ₀	.0515.5	1.7	1.5	1.1	1.8	ns *	ns	ns	1.3	1.3	1.3	1.1	ns	ns	1.0	0.9

in the content of the following aromatic hydrocarbons: chrysene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, and benzo(bk)fluoranthene.

Table 5. PAH content in the soil of the field trials between the years 2012 and 2019 (expressed in $\mu g kg^{-1}$).

* not significant at p_{0.05}.

The content of other aromatic hydrocarbons showed a significant dependence on the years of research, as presented in Table 5. The highest content of naphthalene, fluoranthene, and diabezno(ah)anthracene, as well as indeno(1,2,3-cd)pyrene, was found in 2012. The content of individual polycyclic aromatic hydrocarbons ranged from 0.0 (in the case of indene(1,2,3-cd)pyrene) to 9 times higher (in the case of benzo(bk)fluoroantenna) than the permissible values of PAH concentrations.

The lowest total PAH contamination was found in 2019, and on average, it was 2.03 times lower than in 2012. Nevertheless, it was still significantly higher than the PAH contamination standard for class B soils.

The descriptive statistics of PAHs are presented in Table 6. Among the analyzed aromatic hydrocarbons, diabezno(ah)anthracene showed the greatest variability, while fluorene was found to be the most stable. Kurtosis, as a relative measure of distribution concentration and flattening, determines the distribution and concentration of values (sets) close to the mean.

The higher the kurtosis value, the more the population clusters around the mean value. A low kurtosis value has the opposite effect, i.e., a greater dispersion of values consequently flattens the abundance curve. Skewness is a measure of the asymmetry of the analyzed results. Skewness above 'zero' indicates a right-sided asymmetry of the distribution, while scores below 'zero' for fluorene, pyrene, benzo(b)fluoranthene, benzo(bk)fluoranthene, diabezno(ah)anthracene, and indeno(1,2,3-cd) pyrene indicate a left-sided asymmetry of the distribution (as presented in Table 6).

Table 6. Descriptive statistics of polycyclic aromatic hydrocarbons (PAH) content in the soil before the start of the research ($\mu g k g^{-1}$).

Specification	Sum of PAH	Naphtalene	Fluoranthene	Chrysene	Benzo(a)anthracene	Benzo (a)pyrene	Benzo(a)fluoranthene	Benzo(ghi)perylene	Fluorene	Pyrene	Benzo(b)fluoranthene	Benzo bk)fluoranthene	Diabezno(ah)anthracene	Indeno(1,2,3-cd)pyrene
Mean	250.5	28.0	28.7	13.2	10.4	7.1	20.3	20.9	20.7	18.0	3.0	8.3	16.5	14.2
Median	259.3	26.5	23.8	12.6	9.8	0.4	19.6	19.4	24.2	22.5	3.5	8.9	19.4	16.9
Standard dev.	36.5	8.9	1.2	2.7	2.5	1.0	3.0	0.7	0.8	0.8	0.2	1.9	5.6	0.7
Kurtosis	1.5	2.0	0.9	1.4	0.7	0.8	-0.5	-0.4	1.5	1.8	0.2	2.2	0.1	0.8
Skewness	0.2	0.1	0.8	0.2	0.9	1.1	0.6	0.5	-0.6	-0.7	-0.9	-1.5	-1.3	-1.2
Coefficient of variation (%)	14.6	31.6	4.3	20.2	23.8	14.5	14.7	3.4	4.0	4.5	6.6	22.4	34.2	5.1

3.3. Yields of Air-Dry Mass of White Clover

Yields of air-dry matter obtained from the use of white clover pasture in conditions devoid of nitrogen fertilization doses were found to be high. The objects sown with white clover yielded significantly more than the control object, as shown in Figure 3.



Figure 3. The influence of tillage equipment's and reseeding norms of *T. repens* on the yield of ADM (Mean of pasture rotations). Tillage treatments: A—control object (without overseeding), B—underseeding after heavy tine harrow, C—underseeding after disc harrow, D—underseeding after the rotary tiller.

Regardless of the cultivation tools used, a significant increase in the air-dry mass of crops was demonstrated in plots where white clover was sown. However, the differences between the applied seed sowing standards and cultivation tools were found to be insignificant, as shown in Figure 4.

A clear and significant difference in ADM annual yields was observed between the different years of the study. The highest yields of ADM were obtained during the first three years after sowing (as shown in Figure 4). However, over time, the yields decreased due to an increased presence of weeds and low-quality grasses in the yield structure, as well as a decrease in the proportion of white clover, which limits its beneficial effects on soil fertility.



Figure 4. The influence of tillage equipment's, reseeding norms and years of *T. repens* on the yield of ADM (Mean of pasture rotations). Tillage treatments: A—control object (without overseeding), B—underseeding after heavy tine harrow, C—underseeding after disc harrow, D—underseeding after the rotary tiller.

The lowest yields were obtained during the seventh and eighth year of the study, with no significant difference between them. Regardless of the cultivation tools and sowing standards used, the statistically lowest yields were obtained in 2012 (an extremely dry) and in 2019 (a dry year). These years were characterized by relatively high temperatures and low rainfall, and the groundwater table from May to the end vegetation period was below the level that the pasture vegetation roots could reach.

The second and third pasture rotations produced higher yields, while the lowest yields were obtained during the fourth rotation (as shown in Figure 5). Such a pattern in the yield can be attributed to weather conditions and the proportion of white clover in the ADM yield.

Regardless of the experiment factors and years, clover had the largest contribution to the ADM yield in the 4th pasture rotation, while the lowest participation of this species was recorded in the yield from the 3rd pasture rotation (as shown in Figure 6). The relatively high proportion of clover from the 2nd rotation can be associated with better thermal conditions that favored the development of *T. repens* during this growing season.

The share of *T. repens* in the ADM yield during the first rotation followed a seconddegree parabolic curve with the coefficient of determination D = 76.7%. In the second and fourth rotations, it followed a third-degree equation (D = 89.7% and 65.8%, respectively), while in the third pasture rotation, it followed a fifth-degree curve with a coefficient of determinations of D = 93.4%. The high value of this coefficient indicates the high reliability of the regression equations.

The relationship between the proportion of *T. repens* in the pasture sward and the experimental factors (cultivation equipment and seed sowing norms) was demonstrated over the years of the study and pasture rotations (as shown in Figures 7 and 8). The figures present the results for the first and last pasture rotations, which are considered the most important for the research.



Figure 5. The influence of tillage equipment's and norms of reseeding of *T. repens* in pasture rotation on the yield of ADM. Tillage treatments: A—control object (without overseeding), B—underseeding after heavy tine harrow, C—underseeding after disc harrow, D—underseeding after the rotary tiller.



Figure 6. The influence of tillage equipment and reseeding norms on the share of *T. repens* in the pasture rotations. Tillage treatments: A—control object (without overseeding), B—underseeding after heavy tine harrow, C—underseeding after disc harrow, D—underseeding after the rotary tiller.



Figure 7. The share of *T. repens* in ADM of 1st pasture rotation (year 2012–2019). Tillage treatments: A—control object (without overseeding), B—underseeding after heavy tine harrow, C—underseeding after disc harrow, D—underseeding after the rotary tiller.



Figure 8. The share of *T. repens* in ADM of 4th pasture rotation (2012–2019). Tillage treatments: A—control object (without overseeding), B—underseeding after heavy tine harrow, C—underseeding after disc harrow, D—underseeding after the rotary tiller.

During the first rotation of pasture use, the proportion of white clover in the ADM yield was relatively low in the first year after sowing but increased systematically in the fol-

lowing. The contribution of *T. repens* to the ADM yield was modeled using a fourth-degree regression equation, which was dependent on the techniques and norms of reseeding, with the coefficient of determination D = 86.3%, which attests to its high reliability.

In 2014, during the fourth year of the study, the share of white clover in ADM's total yield was the highest. The proportion was influenced by the sowing methods and standards, and it followed a fourth-degree equation with the coefficient of determination of D = 95.5%, indicating the high reliability of the equation model. In contrast, the lowest proportion of white clover in ADM's yield was observed in 2019, which was the last year of the study. Depending on the sowing methods and norms, this correlation was represented by a 3-degree curve, with a coefficient of determination of D = 48.0%. However, this did not fully satisfy the reliability conditions of the regression equation [54] (Figure 7).

In the fourth pasture rotation, the share of *T. repens* in ADM's yield was modified in different ways depending on the sowing technique and norms, as well as the seed sowing and the conditions during the study years. In the first year, the proportion of white clover was the lowest and followed a third-degree equation model, with a coefficient of determination D = 71.0%. In the third year of the study, the proportion of clover in the ADM's yield was the highest. However, depending on the experiment factors, it was represented by a 5-degree regression equation with a coefficient of determination of 57.7%, indicating credibility.

In the final year of the study, the share of clover in the pasture sward was at its lowest. Depending on the sowing technique and seed sowing norms, it followed a third-degree regression curve, with a regression coefficient of 52.2%. However, according to Marwick and Krishnamoorthy [54], this coefficient does not provide credibility to the regression model.

3.4. Dependence of ADM Yield on Experimental Factors and Random Factors

The relationship between ADM's and white clover's sowing standards, pasture rotation, and years were observed. The yield of air-dry matter in all rotations was shaped by curvilinear regression equations, with high coefficients of determination ranging from D = 65.7 to 93.4%, meeting the reliability condition (Figure 9).



Figure 9. The mean share of plant groups in % of ADM in 1st pasture rotation (Years 2012–2019). Tillage treatments: A—control object (without overseeding), B—underseeding after heavy tine harrow, C—underseeding after disc harrow, D—underseeding after the rotary tiller.

During the first pasture rotation, the ADM's yield followed a second-degree equation. For the second and fourth rotation it followed a third-degree equation, and for the 3rd pasture rotation it followed a fifth degree equation. The highest ADM yields were observed in the third year of the study, while the lowest yield was in the first year due to the poor rooting of the undersown white clover (Figure 9).

During the first year of the experiment, the control plot was dominated by herbs and weeds (approximately 46%) and tall grasses (approximately 42%). The proportion of low grasses was small (approximately 9%), as well as sedges and rushes (approximately 3%) (Figure 9).

Over the years, the proportion of tall grasses in the yield decreased from 49.5 to 5.8%, while the proportion of short grasses increased from 35.0 to 77.7%. Humidity conditions played an important role in the proportion of these plants groups. During wetter years such as 2013 and 2016, changes in the proportions between overgrowth and undergrowth grasses were slowed down and, in some cases, even reversed. The absence of fertilization in the control plot resulted in the complete disappearance of tall grasses from the sward.

In the final year of the study, *Festuca rubra* (71.6%) dominated species in the plot, and weed infestation decreased from 45.8% to 17.5%. In wet years, a big problem in the object without fertilization was the high share of *Deschampsia caespitosa* (43.0% in the most unfavorable year). However, its proportion decreased to 0.8% after subsequent dry years.

In the plot that received only PK fertilization, the dominance of tall grasses persisted throughout the study period, while the proportion of low grasses decreased over time (Figure 9). This was likely due to differences in the depth of grass rooting. However, weed infestation increased over time in these plots, rising from 15.3 to 47.6%. Economically valuable grasses constituted for 78% of the sward and were represented by nine species (*Agrostis alba, Alopecurus pratensis, Dactylis glomerata, Festuca arundinacea, Festuca rubra, Festuca pratensis, Phalaris arundinacea, Phleum pratense, Poa pratensis*). There were only three species of legumes (*T. repens, T. pratense, Lathyrus sativus*), five species of herbs and weeds, and two collective species of sedges and rushes.

The usable value of the sward, assessed on the Filipek scale [43], was deemed mediocre in the control plot (without any fertilization) and good in the remaining plots fertilized only with phosphorus–potassium fertilizers. There was a tendency to decrease the use value of the sward over the years of research from 8.8 points (good) to 6.2–6.8 points, and a decrease in the value of very good sward from 9.5 to 7.6 points.

4. Discussion

4.1. The Impact of Land Use on the Formation of Plant Biodiversity and Soil Physicochemical Properties

An essential prerequisite for land management is the study of the effects of changes in land use and biodiversity of vegetation and topography. The conducted research shows that the change in land use and the vegetation used for land development had a significant impact on the physicochemical properties of the soil in the study area. The bulk density, soil organic carbon, and porosity are higher in the studied, and agricultural use of land reclaimed from the oil mining industry shows a downward trend due to difficulties in their maintenance. Converted agricultural land had a higher bulk density than other land uses and showed a positive correlation with soil organic carbon and nitrogen. Cultivated areas in the study area were characterized by low rhizobia, as well as a small amount of available potassium and phosphorus. This means that soil characteristics are more susceptible to changes in land management and land use processes. In addition, there is a loss of essential nutrients in the study area, which may lead to a reduction in the productivity of agricultural land. Therefore, soil management practices that increase soil organic carbon, nitrogen, and rhizobia, and improve soil pH towards neutral or alkaline on agricultural land must be prioritized [2,11].

Environmental factors had a significant impact on the monitoring and optimization of phytoremediation processes. These typically include: temperature, soil type, pH, organic matter content, water and oxygen availability, sunlight and nutrients. Some factors directly affect degradation processes, while others affect phytoremediation by changing the bioavailability of pollutants [56]. The type of soil, which includes the structure, texture, and content of organic matter, may significantly limit the bioavailability of oil-derived pollutants and affect the quality and quantity of root exudates, which may affect the efficacy of phytoremediation. Soil microorganisms require a low clay or silt content for good activity. Some of the hydrocarbon impurities in crude oil may already be strongly adsorbed on organic matter in the soil system [57]. In some cases, petroleum hydrocarbons are not readily desorbed. Therefore, they are not available for phytoremediation. In addition, increasing the concentration of petroleum hydrocarbons in the soil will not only have a negative impact on plant growth but will worsen human and animal health through the consumption of water, soil, and food produced on contaminated soils. In addition, high concentrations of pollutants will even lead to the death of plants [58]. Soils heavily contaminated with PAHs tend to have a poor physical condition that is not suitable for the vigorous growth of vegetation and rhizosphere bacteria. Therefore, it is crucial to apply amendments to improve the quality of the soil before sowing. Typical limitations are poor moisture holding capacity, insufficient aeration, low permeability, and nutrient deficiencies. Organic changes such as manure, sewage sludge, compost, straw, or mulch can be used to increase the water holding capacity of contaminated soil. Soil pH can be increased and decreased by adding lime and sulfur, respectively [58,59]. Contaminated soils tend to be deficient in the macro- and micronutrients necessary to create healthy, vigorous plants and to stimulate the degradation of microbial contaminants. In addition, contamination with hydrocarbon-based compounds affects the carbon to nitrogen ratio (C:N) in the soil and can lead to nitrogen immobilization [60]. The mineral nutrients that are most commonly reported to reduce the breakdown of petroleum hydrocarbons in the soil are nitrogen and phosphorus [46].

It should be assumed that slow changes in the soil's physicochemical properties did not have a major impact on water management and the species composition of the plant community. However, Warda [7], Cwintal, and Bartoszek [8] claim that the vegetation cover significantly affects the thermal conditions of the top layer of soil, limiting its extreme temperatures. The low heat capacity and low thermal conductivity of these plants mean that heat is not transferred deep into the soil. As a result, night radiation occurs and the risk of frost increases. However, in the subsequent years of our research (2015–2019), significant changes were observed in the species composition of the sward, which was expressed in a successive decrease in the share of tall grasses and an increase in the share of short grasses, of which *Poa pratensis* was the dominant species. The share of dicotyledonous plants, mainly xerophytic weeds, also increased. Similar observations regarding species composition changes in pasture sward were noted by the following authors: Mikołajczak and Mikołajczak [61] Warda and Sawicki [62], Rysiak et al. [1], Pepeta et al. [63]. In the post-bog habitat, on peat-muck soil, the most durable grasses were loose-tufted stolon grasses (Poa pratensis, Festuca rubra, Alopecurus pratensis). This is also confirmed by the results of Baryła and Kulik [48]. According to Rysiak et al. [1], changes to the conditions in hydrogenic habitats, leading to the drying of soil in the turf layer, cause the succession of species such as *Festuca rubra*, Poa pratensis, Arabis arenosa, Polygonum bisorta, Filipendula ullmaria, Rumex acetosa, sward receding: Phleum pratense, Dactylis glomerata, Festuca pratensis, and legumes.

4.2. The Role of White Clover in the Reclamation of Soils Contaminated by the Industry

White clover (*T. repens*) is one of the few species from the Fabaceae family that is suitable for the reclamation of soils contaminated with PAHs, which is confirmed by our own research. According to Issoufi et al. [64], Merkl et al. [56], Huang et al. [65], Smith et al. [59], Aisien et al. [11], Norton et al. [2], this species is useful in Phytostabilization, phytodegradation, and rhizodegradation, and can deactivate BTEX, TPH, PAHs, TPR of diesel fuel. Key mechanisms controlled by soil microbial biomass are involved in soil transformation and nutrient cycling, soil organic preservation, and macro-aggregation for optimal aeration and nutrient supply [7]. It is an important pool of variable nutrients in the soil, accounting for 1–5% of organic carbon and over 5% of total nitrogen [9,10]. The number of microorganisms present in the soil also affects the nutritional status and

microbiological changes of the soil [2]. They are important for the decomposition of plant and animal residues and the release of nutrients [11], and their activities are very susceptible to management methods, including fertilization, cultivation systems, irrigation, plant protection and others [59]. As a result, the amount of soil microorganisms is a key determinant of soil health [11]. The conversion of land devastated by the oil extraction industry into agricultural land to cope with the global economy and its recovery for agricultural purposes and thus food production affects soil organic matter dynamics, biodiversity and ecosystem changes in general [5,19]. In this, it also strongly affects soil functions, in particular, the activity of microorganisms, nitrogen, organic carbon in the soil and other physical properties of the soil [24]. Overexploitation of oil deposits and changes in land use are important factors of soil degradation that modify the quality of soil and vegetation and cause disturbances in the functioning of agricultural ecosystems, and even inhibit their natural regeneration [11,57,65].

In recent years, the conversion of land use, from natural ecosystems or those devastated by the mining industry to farmland, is a common process worldwide, especially in the temperate zone. In addition, climate change and its effects are extreme conditions in terrestrial ecosystems that have a large impact on its productivity, nutrient cycling and microbial biomass, and soil physicochemical properties [2]. In view of the recent shrinking of land intended for securing food for the growing world population, areas devastated by the oil extraction industry have recently been transformed into agricultural land due to the increased demand for animal feed and food base [4,5].

Ecologists have recently focused their attention on soil SOC, microbial characteristics, and microbial activity due to the effect of changing land use, from being devastated by the mining industry to land that is suitable for agricultural production [66]. Overall soil quality, including its physicochemical and microbiological properties, have been identified as factors affecting soil organic matter. Therefore, the physicochemical properties of soil are inextricably linked to the organic matter of the soil [67]. The microbial properties of soil more readily respond to soil disturbances in any ecosystem than the chemical or physical properties of the soil [66,67]. Therefore, any change in microbial properties can be used as a sensitive indicator of soil disturbance. Land use change has been shown to have a significant impact on the soil microbial community, especially in temperate regions.

A proper management approach for sustainable agricultural production requires, above all, reliable and up-to-date soil information; however, the source of spatial knowledge on soil microbiological and physicochemical parameters at the level of small farms is seriously limited [8]. Geospatial technologies offer hope for new and better ways to enrich soil information by providing an accelerated, repeatable, spatial-temporal perspective. GIS and remote sensing, in particular, are useful methods for assessing geographic problems and can enable spatial analysis; therefore, there is an opportunity to increase the accuracy of soil research and the use of GIS and remote sensing technologies.

The main objective of this study was to assess the impact of land use change and to analyze the variability of soil's physicochemical and microbiological properties in practical land use in the former oil mining area of the Low Beskids.

4.3. Influence of Soil Conditions on PAH Soil Contamination

Oil spill cleanup and recovery techniques are difficult and usually involve complex mechanical, chemical, and biological methods. Typically, the mechanical removal of free oil is used as an effective cleaning strategy for aquatic and terrestrial environments. However, these environments are expensive and require specialized personnel and equipment. Another commonly used method is the use of chemical materials such as dispersants, cleaners, demulsifiers, biosurfactants, and soil oxidants. Nevertheless, these reagents may have a potentially harmful impact on the environment, which may effectively limit their use. Alternatively, bioremediation may cause less environmental risk; however, limitations of soil microbial activity may make this option unsuitable [11,57,68].

The moisture of peat, and especially its surface layer, is very important due to the activity of soil microflora. According to Waraczewska [12], high humidity in the top layer of peat reduces the number of some groups of soil microflora and lowers the rate of cellulose decomposition. Moisture of peat which is in the range of 60–70% is the most favorable for nitrogen mineralization processes. Increasing the humidity above 75–80% limits this process. Thus, changes in the moisture content of peat soils during the growing season may affect the rate of microbiological processes occurring in the top layer of peat. The soil suction pressure in the root zone of the unsaturated profile of hydrogenic soils, according to Szajda and Łabedzki [46], has a significant impact on the amount of evapotranspiration and yields, which in peat-muck soils is obtained at the optimal suction pressure (corresponding to pF 2.0–2.7) in the root zone of the soil profile. At a pressure higher than optimal, evapotranspiration and yield are reduced, and the amount of this reduction depends on the soil moisture level. Changes in the physical, chemical, and water properties of the soil, especially the structure of the soil, its compaction, and retention capacity in the post-bog habitat, occurred as a result of lowering the groundwater level, which is confirmed by the studies of Baryła and Kulik [48]. The beneficial reduction of RDC content in the 0–5 cm layer is very important for increasing the stable structure of the soil and very important for protecting the soil environment against erosion [66]. Therefore, the top layer of the soil is most exposed to crusting, which causes poor aeration and plant emergence, especially during drought, and in periods of excessive moisture, it can increase surface runoff and intensify soil erosion processes.

Limitations for research on PAH soil. Research on soil contamination with polycyclic aromatic hydrocarbons (PAHs) in former oil production areas may encounter several limitations, including:

- Difficulty in obtaining representative soil samples from different parts of the site, as soil composition can vary greatly from site to site. As a result, surveys may not provide an accurate picture of soil contamination throughout the site. PAH soil contaminants are difficult to identify and locate due to their chemical instability and tendency to migrate in the soil. As a result, testing can be complex and require advanced technologies to identify and locate contaminants [41,59].
- Frequently changing regulations, for example, in the EU, regarding the maximum levels of PAHs in traditionally smoked meat and meat products, and in traditionally smoked fish and fishery products, as well as on the establishment of maximum levels of PAHs in foods of plant-based origin [38].
- In addition, the high cost of soil testing, which requires the use of complex technologies to determine the type and level of soil contamination. As a result, studies may require significant financial outlays, which in turn limits the number of studies conducted [57,59].

There are also no clear, international soil quality standards that would determine the level of soil contamination with PAHs. As a result, it is difficult to determine when soil is heavily contaminated and when it is not, which can make research difficult [56,59]. The time required to remove PAHs from the soil is also long, as these compounds are difficult to break down and remove from the site. As a result, the removal of pollutants may require a long-term strategy and high costs.

4.4. Influence of Habitat Conditions on Resistance of Pasture Plants to Drought

Meteorological conditions in the years of the study clearly affected the yield and survival of white clover. The species has so far had a reputation for being drought-sensitive, while alfalfa has been considered quite drought-tolerant, although this tolerance is primarily due to its deep root system. Norton et al. [2], after conducting a strict greenhouse experiment on the drought stress of these two species, proved that white clover is significantly less sensitive to drought than hybrid alfalfa, which was previously considered a species very resistant to drought stress. This experiment of limiting the deep rooting of alfalfa compared the dehydration tolerance of alfalfa and white clover. The authors compared the dehydration tolerance and plant survival of 11 varieties of white clover (*T. repens* L.) and 12 varieties of alfalfa (*Medicago sativa* L.) in a drying cycle experiment. Although white clover consumed more soil water, when drying the plants to find out the final gravimetric water content in the soil (θ g), they obtained a content of 4.7%, while the final gravimetric water content in the soil for alfalfa was almost twice as much (83%). This experiment highlighted the anatomical and physiological aspects of the main differences that exist between white clover and alfalfa in terms of their ability to withstand drought. Norton et al. [2] identified important traits that may be used in breeding or agronomic solutions for managing the cultivation of *T. repens* to overcome soil constraints and in seeking to improve the adaptation of white clover to drought conditions [1,2].

The groundwater level in all years of the research was higher than the average level. In general, the level of groundwater in the study area, in all years of research, was higher than the multi-year average. It was the highest in early spring, after the start of vegetation, and gradually decreased until August, after which the groundwater level rose again. With the lowered groundwater level, the pasture vegetation suffered from a lack of moisture in the root layer, mainly in the summer months. According to Denis et al. [44], after Hohendorf, the optimum level of groundwater on muck-peat soils should be from -50to -60 cm. Szajda and Łabędzki [46] consider the level of -60 cm to be optimal in the conditions of peat-muck soils. On the other hand, Okruszko [47] believes that a groundwater level above -70 cm ensures a good water supply for plants, limits the drying of the top layers of muck, and protects the properties of peat-muck soils. In the opinion of many authors [44,46–48], lowering the groundwater table below this limit causes drying of the top layers of the soil and unfavorable changes in the layer under the sod. It can be assumed that a periodically maintained groundwater level below -70 cm in the experimental objects could have contributed to the increase in the putrefaction process and the deterioration of the physical and water properties of the root and sub-root layers. This fact could have influenced the yield and species composition of grass communities in the study area, which is confirmed by the results of Baryła and Sawicki [6], Baryła and Kulik [48], and Szajda's and Łabędzki's [46]. The drying of grasslands results in the irreversible mineralization of organic matter, leading to the transformation of shallow mucky soils into muck [44,47] until their complete degradation, greenhouse gas emissions and related climate changes, unfavorable changes in retention, and conductive properties hydrogenic soils result in an increase in their dryness [46,48]. From the lysimetric studies of maximum evapotranspiration and yielding of grasslands carried out in the Lublin region on the medium-transformed peat-muck soil MtIIbb [44,46] and parallel studies of actual evapotranspiration and yielding of meadows in conditions with varied drainage depth, it makes sense that in order to ensure the maximum yielding of grasslands, it is necessary to maintain an optimal level of groundwater, under which capillary infiltration from the saturated zone fully balances outlays for evapotranspiration and ensures adequate moisture in the root layer (pF = 1, 9; 1.7; 2.1), respectively, in the periods: April–May; June–July, August–September. Such humidity limits unfavorable soil changes caused by soil drought, reduces the mineralization of organic matter, and ensures maximum yields in conditions of economical water consumption [46].

4.5. Influence of Pratotechnical Treatments on Yielding of White Clover

The presented results allow us to conclude that the differences between the yields of individual pasture rotations in subsequent years were much smaller than between the years of the research and gradually blurred. At the same time, they indicated a large share of yields of the first pasture rotation in annual yields (up to 70% in years with very unfavorable weather conditions). This is confirmed by the studies of Sawicki and Warda [62], Komárek and Kohoutek [69], and Norton et al. [2]. The increase in floristic biodiversity is accompanied by a decrease in the yield of meadow and pasture communities. From the farmer's point of view, this will be a disadvantageous process. Therefore, activities in the field of grasslands management should create such pratotechnical solutions that will enable the maintenance of appropriate biomass yield, while at the same time enrich

the botanical composition of the sward. One of the elements of such a procedure should be to introduce and maintain plants from the Fabaceae family. These plants have a huge potential on grasslands and should be used to a greater extent than before. Their main advantage is the ability to obtain atmospheric nitrogen through symbiosis with the soil bacteria *Rhizobium*. It is estimated that for every 1% share of legumes in the sward in the conditions of Central and Eastern Europe, 2–4 kg of N ha⁻¹ may be bound [67]. If there are about 20% of legumes on the pasture, about 40–80 kg of biological N ha⁻¹ is obtained. The impact of legumes is also manifested in the improved growth of plants accompanying them in the community. The nitrogen fixed by these species can be used by grasses and other dicotyledonous plants.

Warda [7], Cwintal et al. [70], Opitz von Boberfeld [71], Norton et al. [2] all prove that the best results on undersown pastures are obtained from *Lolium perenne* grasses and from plants of the Fabaceae family (T. repens). Although, Norton et al. [2] also draw attention to alfalfa (Medicago sativa). White clover is a thermophilic plant. Every year, an increase in its share in the yield biomass has been observed, along with the ongoing vegetation period. On average, in the long-term study period, regardless of the cultivation tools used for sowing and seed sowing standards, its highest share was proved in the yield of the fourth pasture rotation (20.6%) and its lowest in the yield of the first rotation (16.2%). The largest, most significant share of white clover in the yield weight was observed in the first three years of the study, and in the variant of the experiment with white clover sowing after tillage with a rotary tiller, also in the fourth year of the study. This proves a clear, positive correlation between the share of white clover in the yield and its size. The weeds were dominated by species characteristic of periodically dry habitats, such as Taraxacum officinale and Ranunculus repens—a common species on acidic soils. Urtica dioica also appeared in the pasture sward, which may indicate the ongoing mineralization of the soil. From the group of grasses, Poa pratensis and Alopecurus pratensis were the most common. Over the years, there was a slow decrease in the share of white clover in the pasture sward, and the share of grasses and weed infestation increased. In the opinion of Goliński and Kozłowski [72], as well as Baryła and Kulik [48], the use of phenologically diverse T. repens varietal mixtures ensure increased efficiency of overseeding the pasture due to better stability of the species composition of the sward. In their opinion, the polyploid and phenological diversity of varieties is particularly valuable in varietal mixtures, which determines the uniformity of feed supply during the growing season.

The research showed that T. repens is very useful for reseeding in ecologically endangered habitats. Undersown white clover remained in significant amounts in the pasture sward twice as long as initially assumed. The highest share of white clover in the last, eighth year of the research was ensured by sowing after a rototiller with the highest seed sowing rate. The obtained pioneering research results indicate the need to re-see the white clover after four years of use. In conditions almost devoid of nitrogen fertilization doses, this is economically justifiable. The immeasurable effect of the ecological method of overseeding ensures the biodiversity of the grasslands and the return to the sources of natural nitrogen bound by *Rhizobium* bacteria in coexistence with plants of the Fabaceae family. According to Kasperczyk et al. [73], the introduction of white clover into the pasture sward by means of undersowing is expedient, and one should strive to quickly increase its share in the grass community. Otherwise, achieving this goal is only possible after 3-4 years, under the conditions of lower inputs, thanks to regular fertilization with phosphorus and potassium. An additional advantage supporting the usefulness of overseeding white clovers in protected and ecologically endangered habitats is the fact that the agri-environmental payment can be granted, in the first place, for biological, agrotechnical, breeding methods or the cultivation of white clovers. This species is also included in the list of plant species covered by the agri-environmental payment under Package 2. 'Organic farming' [23].

Based on the research, it was found that, regardless of the cultivation tools used and white clover sowing standards, a significant increase in yields was found in undersown objects. Similar results were obtained by Carlton et al. [74] and Radkowski and Kuboń [75],

who found that, regardless of the method of sward renovation, the yields of dry matter, NEL energy, and total protein were at a similar level. In the opinion of Radkowski and Kuboń [75] and Norton et al. [2], the renovation of a grassland sward via reseeding is much cheaper than nitrogen fertilization. Therefore, it is worth recommending for agricultural practice.

Yield fidelity is mainly a species trait, although, to a large extent, it is also varietal. Therefore, the yielding of grassland depends on the botanical composition of the sward, and is also modified by the habitat and weather conditions, as well as by cultivation, fertilization, and use [76]. During the 8-year pasture use, there were very clear changes in the floristic composition of the tested mixtures. Already, in the first year, the share of grass species in the pasture sward changed. These differences resulted from the competitiveness between the species used, the speed of development of individual components, and the reaction to unfavorable weather conditions (periods of drought). This is confirmed by multiple studies by many authors [6,7,62,72,76]. Research on long-term persistence (durability) in the sward of individual species of grasses and legumes in various habitat conditions is still insufficient, most often due to an insufficiently long a period of research. Therefore, it is necessary to undertake further long-term research on the selection of components for pasture mixtures [76].

The course of weather conditions during the research period was very diverse, generally being unfavorable for the growth and development of grasses and legumes in the pasture sward. This was reflected in the changes in the botanical composition of the pasture, and, in particular, in the sward species from the Fabaceae family, such as *T. repens*, and in the yields of ADM in subsequent years, which is consistent with the data from the literature [2,6–8,73,76–78]. The level of the groundwater table depended on rainfall, and during the research period, it showed large fluctuations from 20 to 103 cm. Especially in the years 2012–2019, when water during the growing season was often beyond the reach of plant roots, which had a very adverse effect on their growth and development. This means that, in all those years, the yield and its quality, apart from proto-technical factors, were determined by thermal and humidity conditions, limiting the production of proto-ecosystems. Similar observations were made by Borawska-Jarmułowicz [77] and Bryant et al. [78], who compared the productivity of meadow mixtures in a 12-year period of use, where the groundwater level ranged from 10 to 150 cm.

Stability of Pasture Communities

Analyzing the species composition of the pasture sward during the research period (2012–2019), attention is paid to its stability for several years after development. The basic mass of the plants consisted of grasses with appropriate proportions of tall to short grasses. The following factors may have contributed to the loss of legume species, especially white clover, from the sward: inadequate soil pH, intensive mineral fertilization with PK, and late spring and early autumn frosts. Their level of harmfulness, according to Czarnecki et al. [43], has been attributed to the fact that organic soils require twice the amount of heat than clay soils, and three times more than sandy soils. The ability to conduct heat through the dry mass of these soils is almost twice lower than in mineral soil; therefore, they heat up more slowly and conduct heat more slowly. A factor limiting the development of this group of plants may also be the high temperature of the surface layer of organic soils. According to Baryła and Kulik [48], the process of peat decay proceeds at a slow pace and covers only the shallow root layer of the soil. Research by Rysiak et al. [1] showed that extensive grazing induces quantitative and qualitative changes in the flora and was a key determinant of the dynamics of the flora. These authors believe that the first two years of use are crucial for the dynamics of the flora. At this time, a significant increase in species richness and the formation of ecological groups are observed. In the following years, the stabilization of species characteristics is noted. Significant differences were noted in the species richness (SR) and the number of meadow species (M), grassland (G), segetal (S), ruderal (R), aquatic (W), and trees and shrubs (T) in favor of managed areas, compared with abandoned lands.

4.6. Towards the Future

PAH-contaminated soils in areas where oil is extracted or explored are found all over the world. In the past, many methods of cleaning oil-contaminated soils were used, but phytoremediation turned out to be one of the most friendly, efficient, and cost-effective technologies for humans and the environment. Many plant species, especially legumes and microorganisms, can participate in phytoremediation processes. Currently, research is underway on other potential plants and microorganisms that can better and faster remediate. The potential prospect of using legumes for this purpose is encouraging, and in the not-too-distant future, environmental problems related to oil-contaminated soils around the world will become a thing of the past, especially thanks to enhanced phytoremediation. White clover is most often cultivated in the world as a component of mixtures used for pasture and less often in pure sowing and mowing [2,8,48]. In the conditions of cultivation for seeds, it is possible to obtain additionally valuable fodder as a side crop. In recent years, the demand for white clover seeds has been increasing, which results from both the high fodder value of this species and the possibility of using the seeds for other purposes, e.g., for the renovation of permanent grasslands, for undersown crops, for reclamation purposes, and in organic farming [1,3,8,33,48,78]. Such a situation may result in an increase in white clover seed crop, because the national breeding potential of this species is significant and valuable, as evidenced by the registration of new varieties distinguished by favorable biological and fodder properties. Studies determining the possibility of obtaining an additional yield of green or dry matter as a by-product, apart from seed production, seem to be fully justified. This experiment is a confirmation of the high potential of this species as a component of pasture mixtures but also as a key species for phytoremediation of soils contaminated with PAHs. Future research should focus on breeding varieties resistant to environmental pollution with nitrates, aflatoxins, heavy metals, and PAHs. Satisfying food needs should therefore be in line with the model of a sustainable food system.

5. Conclusions

An essential prerequisite for land management is the study of the effects of changes in land use and the mapping of landform diversity. Research has shown that changes in land use and land use systems have a significant impact on the physicochemical properties of soils in the study area. The soil on grasslands was characterized by a high bulk density and showed a positive correlation with organic carbon in the soil.

The area that previously had oil wells in the current study area was characterized by low levels of rhizobia, a small amount of available potassium, and phosphorus in the soil. This indicates that the soil characteristics are more susceptible to changes in land management and land use. Therefore, it is crucial to prioritize best practices in soil management, such as improving soil organic carbon, nitrogen, and rhizobia, as well as enhancing soil pH on agricultural land.

Considering both economic and environmental aspects, overseeding white clover on peat-muck soils has led to an improved quantity and quality of fodder for animals by introducing new pratotechnical techniques.

T. repens has proven to be a species that effectively counters the degradation of the agricultural environment, regenerates the soil, and allows for its recovery to produce feed that is safe for animals, which ultimately leads to safe food for humans. Meeting food demands should not come at the expense of the environment but should align with the model of a sustainable food system.

The method used in the study also has its limitations, including the fact that in some cases, the phytoremediation process may require a long time to effectively remove contaminants from the soil. Additionally, plants used for phytoremediation can be sensitive to extreme soil conditions, such as salinity, low pH, or nutrient deficiencies.

The groundbreaking results of many years of research indicate the need to reseed white clover after four years of use. This is economically justified, especially under conditions that have underwent a complete reduction in nitrogen fertilization doses. The immeasurable

effect of this ecological method is ensuring the biodiversity of grasslands and the return to the natural sources of nitrogen, which is bound by bacteria of the genus *Rhizobium* in coexistence with plants of the *Fabaceae* family.

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Abbreviation List

air dry mass
benzene, toluene, ethylbenzene, o,m.p-xylene
Carbon to Nitrogen ratio
Nitrogen
Ammonium-Nitrogen
Nitrate
Nitrogen use efficiency
numbers of value in use
polycyclic aromatic hydrocarbons
phosphorus-potassium fertilization
kerosene, diesel, jet fuel, bunker C
Total Physical Response

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