

Article

Permeable Organic Barriers as Effective Tools for Reducing Emissions of Nitrogen Compounds and PCBs from Manure to Groundwater

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Abstract

Agricultural pollution, such as contamination from manure storage or leaking livestock buildings, often spreads through the catchment, affecting groundwater and surface water. An effective solution is the construction of permeable organic barriers. This study evaluates the efficiency of an innovative bioactive barrier in removing nitrogen compounds (NO_3^- and NH_4^+) and polychlorinated biphenyls (PCBs). Two types of barriers were tested: a horizontal deposit under a manure storage point and a vertical deposit in the leachate flow path. The bioactivity of the barrier was confirmed by the presence of bacterial genes involved in nitrogen transformation and PCB degradation. Results showed a 70% reduction in NO_3^- ($368.4 \text{ mg} \cdot \text{L}^{-1}$) and 43% reduction in NH_4^+ ($42.4 \text{ mg} \cdot \text{L}^{-1}$). Genetic analysis identified bacteria capable of complete denitrification, resembling *Pseudomonas stutzeri*. The analysis also indicated that higher summer temperatures and pH levels fostered microbial communities capable of nitrogen transformation. Cluster analysis revealed that the vertical deposit zone was crucial for nitrogen removal. Additionally, the vertical barrier achieved a 53% reduction in PCBs, with *Pseudomonas aeruginosa*-like bacteria identified as PCB degraders.

Keywords: nitrogen compounds; PCB; nature-based solutions; organic barrier; protection of groundwater



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1. Introduction

Population growth and its associated greater demand for food and agricultural activity continue to place increasing pressure on the environment. The United Nations predicts the global population to exceed nine billion by the year 2050 [1]. To provide adequate access to food, its production needs to increase by about 60%. Unfortunately, the necessary growth in food production will accelerate ecosystem degradation and disrupt processes shaped over the course of evolution, such as the circulation of water and nutrients. In recent years, industrial agriculture has grown in prominence, which is focused on intensive exploitation

of the soil with extensive fertilization and chemical treatment. In the European Union (EU), as much as 71% of all EU agricultural land produces animal feed, including 63% of all arable land [2].

In recent years, meat consumption has been increasing, and with it the number of livestock. This contributes to an increasing amount of manure and slurry, and thus the need for their proper management. In many countries, including Poland, this manure is used as a natural fertilizer in spring and autumn. However, it is stored for several months in one place before use, often directly on the ground. Although these “hot spots” of pollution are easy to locate, they are often inadequately secured against leakage of manure-originated pollutants such as nitrogen compounds. Manure storage locations can be secured using a concrete surface plate with a leachate tank, but these measures are obligatory only in large farms in Poland: no such protection is present in the medium-sized or small farms which predominate in the country [3]. It is also worth emphasizing that some European countries are experiencing declining meat consumption and a reduction in livestock populations. For example, Germany has seen a decline in total meat consumption, with the largest relative decline in beef consumption, while poultry consumption has increased [4]. At the same time, Eurostat data indicate a general decline in pig and cattle populations in several EU countries between 2021 and 2023. These trends are the result of changes in consumer preferences (health, environment, animal welfare), economic factors, legal regulations, and market fluctuations [5]. Additionally, emerging livestock diseases (epizootics, panzootics) can cause short-term declines in production and herd size. Therefore, when assessing the impact of livestock farming or its concentration on the environment and human health in different countries, local and regional differences in meat consumption and production structure should be taken into account [6].

The manure storage locations associated with livestock production therefore represent significant sources of nitrogen pollution: in areas characterized by intensive use of manure and an absence of such low-permeable units in the ground (e.g., clay) it is common to see high levels of nitrate infiltration from shallow to deep aquifers [7]. Previous studies have confirmed that the density of pig and cattle livestock (heads·km²) positively correlates with nitrogen load exported from subcatchments to the Baltic Sea ($r = 0.600$ for pigs and $r = 0.567$ for cattle) [7]. More global studies have also demonstrated that the long-term storage of manure directly on the ground represents a strong point source of nutrients in agricultural catchments [8–10]. Such improper storage of manure also results in soil, water and food contamination. One of the most harmful groups of chemical pollutants is nitrates (III, V), causing anemia and methemoglobinemia in humans. They can be a source of carcinogenic nitrosamines [10]. Moreover, manure can be a source of micropollutants, such as polychlorinated biphenyls (PCBs) [11–15], that affect soil and groundwater quality and exert negative health effects. Therefore, it is important to ensure that such unsecured manure storage sites are not a source of water contamination.

One of the most effective methods of preventing the leakage of manure-originated compounds to water ecosystems appears to be the installation of special barriers limiting not only the direct outflow of the slurry from the manure landfill, but also enhancing the removal and biotransformation processes inside the organic deposit. The use of so-called permeable organic barriers, employing various carbon substrates for microorganisms, has been proposed for the removal of nitrogen compounds [10,16].

The barriers engage in natural denitrification and nitrification processes performed by microorganisms [17]. Denitrifying bacteria, participating in the reduction of NO_3^- to N_2 , use carbon compounds as an electron acceptor for growth. It is worth noting that in the case of cow's manure, ammonium (NH_4^+) predominates. The nitrifying bacteria inhabiting the soil profile oxidize NH_4^+ to NO_3^- , which can in turn, under appropriate

conditions, be reduced via denitrification to gaseous nitrogen (N_2). Thanks to this, the nitrogen cycle is closed without adverse effects on the environment. The present study evaluates the potential of using such barriers for the removal of various N compounds from farming wastewater (liquid manure) by the simultaneous activation of the denitrification and nitrification process. These results will facilitate future biotechnological solutions based on the Ecohydrology concept, leading to an improvement in groundwater quality in agricultural areas, and thus higher quality surface water [18,19].

Various reports have discussed the application of such permeable organic barriers in various regions differing in climate and pollution sources [10,20–25]. However, these studies concentrate mainly on non-point sources of pollutions and neglecting point sources. Additionally, so far, no studies have examined the efficacy of such barriers in removing other dangerous manure-originated compounds, such as PCBs.

Consequently, the aim of the present study tests the efficiency of an innovative permeable organic barrier in the protection of groundwater against point sources of pollution, i.e., stored cow manure, such as nitrogen and PCB compounds. It also examines the influence of the season on the operation of the studied barrier. The pollutant removal rates were calculated based on the concentrations of NO_3^- , NH_4^+ and PCBs above, inside and below the barrier. To confirm the ongoing denitrification, the area was tested for the presence of specific bacterial genes responsible for complete transformation of nitrogen compounds. A similar approach was used to confirm the presence of PCB-degrading bacteria. The phylogenetic characterization of the mentioned bacteria inhabiting the studied vertical deposit of permeable organic barrier was also described. Moreover, the physical parameters of groundwater (temp., pH, DO—dissolved oxygen, SEC—specific electrical conductivity, and water level) were monitored and compared with the concentration of nitrogen compounds.

2. Materials and Methods

2.1. Study Site and Construction of the Permeable Organic Barrier

The barrier was constructed in a cow farm in Jerwonice Village, 25 km to the west of Lodz, central Poland (location of the manure pile: $51^{\circ}46'44.78''$ N; $19^{\circ}07'03.34''$ E). It was originally constructed to protect groundwater against a point source of nitrogen compounds and PCB pollutants leaking from unprotected storage manure (Figure 1). The source had been located in the same place for 12 years. The manure was produced by about 20 cows.

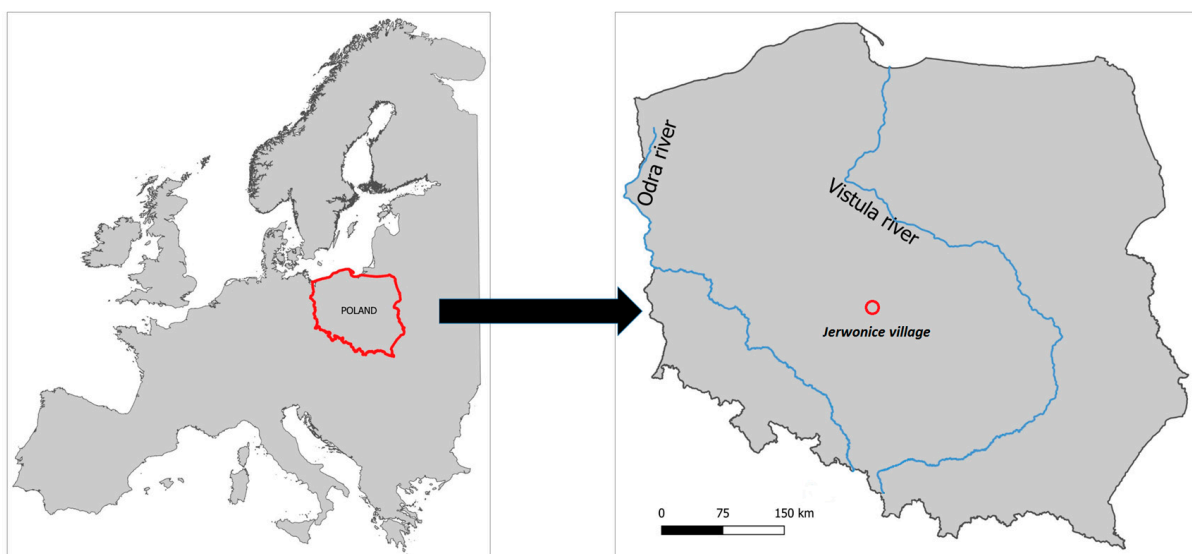


Figure 1. Location of the constructed organic barrier.

The average rainfall for the analyzed region over the 50-year period is 582 mm (515 to 648 mm) [26]. The average groundwater level at the location of the manure piles, based on wepon tests, was 0.91 m. It was dependent on the season ($SD = 0.40$ m). The vertical deposit of the permeable organic barrier was constructed first, in 2010. For this, a ditch 1.3–1.5 m deep and 1 m wide was dug, situated perpendicularly to the slope/direction of groundwater flow (Figure 2A–C). Lignite and charcoal lime were used as sources of organic carbon for the bacteria processing the studied pollutants. The carbon material constituted 30% of the deposit volume, with each component added equally (15% of the total volume each) (Figure 2B). In autumn 2011, when all manure was removed and used as a fertilizer on the field, a permeable horizontal deposit, with the same organic carbon source, was prepared under the storage site (Figure 3). Nine pipe wells were constructed in the permeable organic barrier (PCV pipes 50 mm diameter, 2.0 m long): three in the transect upslope from the horizontal deposit (above vertical deposit), three directly in the vertical deposit (inside deposit) and three downslope from the barrier (below vertical deposit) (Figure 2A). The pipe wells were 1.3–2 m deep.



Figure 2. Constructed vertical deposit of permeable organic barrier in the leachate flow path from unprotected storage manure in a cow farm (photo S. Szklarek, J. Wójcik). (A)—vertical deposit of the permeable organic barrier with pipe wells for monitoring ground water, (B)—lignite and charcoal lime were used as sources of organic carbon, (C)—ditch 1.3–1.5 m deep and 1 m wide situated perpendicular to the slope/direction of groundwater flow.

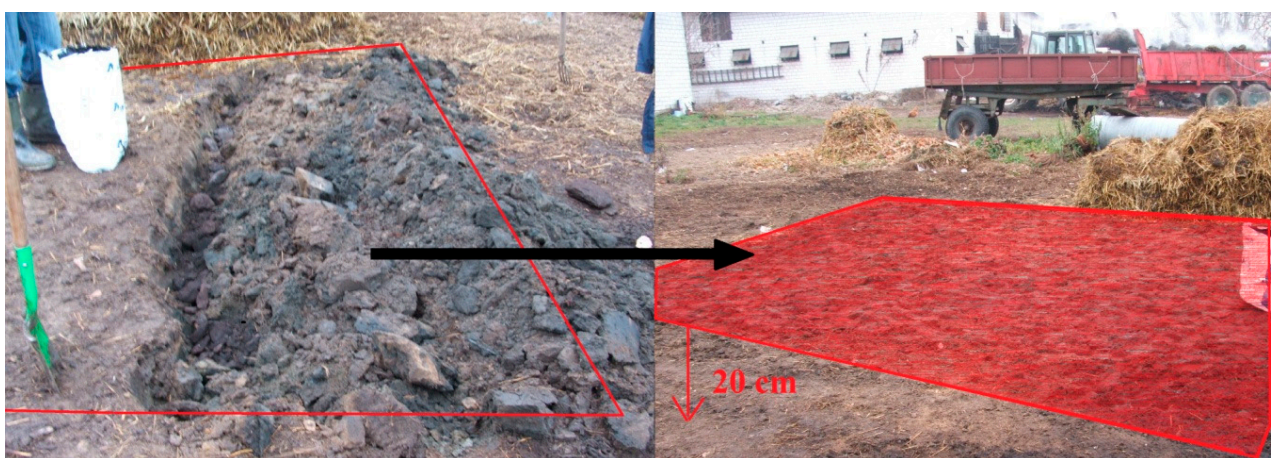


Figure 3. Horizontal deposit of the permeable organic barrier for protecting groundwater against toxic leachates containing nitrogen compounds and PCBs, the red color indicates the area with the constructed biologically active plate (photo M. Urbaniak).

2.2. Measurements of Physical Parameters

All groundwater samples were analyzed in situ for pH, conductivity, dissolved oxygen and temperature using a YSI Professional Plus[®] Multiparameter instrument (Yellow Springs, OH, USA).

2.3. Chemical Analysis

2.3.1. Nitrogen Compounds

Samples of groundwater were taken from nine pipe wells (see Section 2.1) once per month from November 2012 to November 2013 (119 samples in total). Samples were collected in 100 mL containers and transported at a temperature of 4 °C. The water was analyzed on the same day. The concentration of selected ions (NO_3^- and NH_4^+) was analyzed using a Dionex[®] ion chromatograph with a cation column (CG18, IonPac CS18, CSRS-ULTRA II) and an anion column (AG22, IonPac AS22, ASRS—ULTRA II). For ion identification, combined standards were used (Dionex Corporation, Sunnyvale, CA, USA). The analytical procedure is described in detail elsewhere [17].

2.3.2. PCBs

Samples of groundwater were taken for PCB analysis from nine pipe wells (see Section 2.1). Three samples were taken in 2013, i.e., spring, summer and autumn (12 samples in total). Water samples for PCBs were collected in 50 mL tubes and frozen to prevent PCB degradation. The PCB contents in the leachate samples were analyzed using PCB Rapid Assay kit (Tigret Ltd., Warsaw, Poland) as described by Wyrwicka et al. [27] and Urbaniak et al. [28]. The PCB Rapid Assay kit uses ELISA principles to determine the PCB concentration. In brief, the leachate sample was added, along with an enzyme conjugate, to a disposable test tube, followed by paramagnetic particles with antibodies specific to PCBs. Both the PCBs and the enzyme conjugate compete for the antibody binding sites on the magnetic particles. The presence of PCBs was detected by adding the enzyme substrate (hydrogen peroxide) and chromogen (3,3',5,5'-tetramethylbenzidine). The reaction was stopped by adding a stopping solution containing 2 M sulfuric acid. The absorbance of each vial was measured at 450 nm using an SDI Differential Spectrophotometer (Massachusetts, CA, USA).

2.4. Molecular Analysis

2.4.1. Denitrifying Bacteria

The abundance and phylogenetic characterization of bacteria with the potential to complete denitrification were determined in samples collected from pipe wells (see Section 2.1) in autumn 2013. For tracking the complete process of denitrification, the following genetic markers were amplified: *nirK* (NO_2^- reductase), *cnorB* (NO reductase) and *nosZ* (N_2O reductase). The DNA was isolated using a FastDNA[®] SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA). The mentioned functional reductase genes were studied based on the following primers: *nirK*1F (5'-GG(A/C)ATGGT(G/T)CC(C/G)TGGCA-3') and *nirK*5R (5'-GCCTCGATCAG(A/G)TT(A/G)TGG-3') for *nirK* (514 bp) [29], *cnorB*2F (5'-GACAAGNNNTACTGGTGGT-3') and *cnorB*6R (5'-GAANCCCCANACNCCNGC-3') for *cnorB* (389 bp) [30], and *nosZ*-F-1181 (5'-CGCTGTTCITCGACAGYCAG-3') and *nosZ*-R-1880 (5'-ATGTGCAKIGCRTGGCAGAA-3') for *nosZ* (700 bp) [31]. A nucleotide BLAST analysis (Basic Local Alignment Search Tool, ver. 2.2.29+) was performed to identify sequence similarities between the *nosZ* gene fragment and other published strains.

2.4.2. PCB-Degrading Bacteria

The presence of PCB-degrading bacteria was determined in samples according to Section 2.4.1. The DNA was isolated using the GeneMATRIX Soil DNA Purification Kit EurX® (Gdansk, Poland) and the samples were examined for the occurrence of functional PCB-degradative gene (*bphA*, 826 bp) using the following primers: 352f (5'-TTCACCTGCASCTAYCACGGC-3') and 1178r (5'-ACCCAGTTYTCDCCTCGTCCTGC-3') [32,33]. PCR was performed using Taq QIAGEN Polymerase (QIAGEN, Hilden, Germany) according to the manufacturer's instructions but with minor modifications of annealing temperature.

To verify the homology with other strains, the *bphA* gene fragment was additionally amplified by PCR using thermostable Pfu DNA polymerase (ThermoScientific, Carlsbad, CA, USA). The amplified gene fragment was purified using a QIAGEX II Gel Extraction Kit (Qiagen, Hilden, Germany) and subjected to sequencing. Homology searches were performed using the National Center for Biotechnology Information microbial and nucleotide BLAST network service [34,35] and Vector NTI Advance™ 9 software (Invitrogen, Vector NTI Advance™ 9, Waltham, MA, USA).

2.5. Statistical Analysis

Principal component analysis (PCA) was used to evaluate the spatial distance between the seven sampling periods in 2013: (i) one representing winter (11 February), spring (25 April), and autumn (1 October), (ii) and four representing summer (19 June, 12 July, and 9 and 30 August), with the environmental parameters (oxygen concentration, pH, temperature, conductivity, water level and the concentration of nitrate and ammonium). For each period, three samples were collected according to the barrier's vertical profile: (i) above, (ii) inside, and (iii) below zones, obtaining a total of 21 samples. A higher number of samples were taken during summer as it was speculated that this season would be subject to greater microbial metabolic activity due to the higher registered temperatures.

To avoid skewedness, the data were transformed by subtracting the mean and dividing with the standard deviation: $(x - \text{mean}) / \text{Sd}$. The samples were grouped according to the season of collection, and significance of the differences between them was tested with the Kruskal–Wallis and Mann–Whitney pairwise tests ($p < 0.05$) according to the scores obtained for the PC1. This statistical analysis was used to condensate the multivariate database, thus highlighting the most important factors that explain the greatest variances within samples [36].

Furthermore, cluster analysis with heat map visualization was utilized to observe differentiation between the zones of the vertical barrier. Clustering was performed with paired grouping (UPGMA) and the Gower's distance index (d), which allows the differences between samples to be estimated. The PCA, cluster and heat map were prepared with the PAST 4.03 package [37].

3. Results and Discussion

3.1. Physical Parameters of Groundwater in Permeable Organic Barrier

The temperatures monitored in the permeable organic barrier, including the point above the vertical deposit, the sample taken from the vertical deposit itself, and the point below the permeable organic barrier, were within the range of 1.9–20.8 °C. The mean temperature inside the deposit was 14.08 °C (Table 1). According to Tomaszek [38], 8 to 20 °C is the optimal temperature range for activation of denitrification process. Nevertheless, it is important to note that temperature affects the final products of the denitrification process: temperatures between 10–35 °C led to the production of N₂, while lower temperatures, i.e., between 5 and 15 °C, can generate NO and N₂O as the end product [39].

Consequently, in the present case, all three forms of gaseous nitrogen compounds could be produced, viz. NO, N₂O and N₂.

Table 1. The physical parameters of groundwater sampled above the vertical permeable organic barrier, inside it and below it during 2012–2013.

Parameters	Above	Inside	Below
Water level [m]—Average $\pm$ SD (Minimum—Maximum)	1.10 $\pm$ 0.27 (0.23–1.23)	1.09 $\pm$ 0.24 (0.60–1.76)	1.10 $\pm$ 0.20 (0.62–1.50)
Temperature [°C]—Average $\pm$ SD (Minimum—Maximum)	15.16 $\pm$ 4.02 (6.00–20.80)	14.08 $\pm$ 4.78 (1.90–20.60)	12.73 $\pm$ 4.21 (4.30–18.70)
pH—Average $\pm$ SD (Minimum—Maximum)	7.81 $\pm$ 0.63 (6.35–9.01)	7.76 $\pm$ 0.80 (4.50–9.31)	7.97 $\pm$ 0.64 (6.65–9.15)
Dissolved oxygen (DO) [mg·L ^{−1}]—Average $\pm$ SD (Minimum—Maximum)	1.38 $\pm$ 1.12 (0.29–5.00)	1.54 $\pm$ 1.23 (0.21–4.60)	2.10 $\pm$ 2.10 (0.17–9.39)
Specific electrical conductivity (SEC) [μS cm ^{−1}]—Average $\pm$ SD (Minimum—Maximum)	3925 $\pm$ 2586 (133–10,666)	4365 $\pm$ 1804 (700–8408)	2928 $\pm$ 1614 (680–7574)

The pH values ranged from 4.5 to 9.3 (Table 1), but the mean pH inside the vertical deposit was 7.76. Hence, the pH was within the optimal ranges of pH 7–8 for the denitrification process [38,40]. Additionally, studies from other countries indicates that a pH above 7.3 contributes to the formation of N₂ as the final product, which is safer for the environment [16,40,41]. Therefore, the observed mean pH value inside the vertical deposit (pH 7.76) was optimal for closing the nitrogen cycle and facilitating a lasting improvement in groundwater quality (Table 1).

Another factor influencing the rate of denitrification is the water level. The amount of water in the soil determines denitrification processes because it affects the concentration of O₂ in the soil profile and stabilizes the activity of bacteria. Although denitrification is essentially an anaerobic process, the presence of oxygen in water does not preclude this process [38]. In the present study, the mean oxygen concentration inside the vertical deposit was 1.54 mg·L^{−1}, ranging periodically from 0.21 to 4.60 mg·L^{−1} (Table 1). It could be speculated that the relatively high oxygen content of the tested barrier, in parallel with microhabitats, intensified the process of ammonia nitrification from outflow and mineralization of organic matter, which increased the availability of nitrates for denitrifiers. The mean levels of oxygenation of groundwater varied between locations, viz. 1.38 mg·L^{−1} above the barrier, 1.54 mg·L^{−1} inside it and 2.10 mg·L^{−1} below it (Table 1). This should provide stable conditions for the parallel development of both nitrification and denitrification bacteria, which was the goal of the proposed nature-based solution.

During the test period, the water level in the permeable organic barrier fluctuated seasonally from 0.60 to 1.76 m (Table 1). However, the monitored vertical deposit never dried out, which would limit the efficiency of its work. Since the permeable organic barrier was built on a site where manure had been stored directly on the soil surface in previous years, the noted specific electrical conductivity (SEC) values were high and ranged from 133 to 8408 μS cm^{−1} (Table 1). This indicated strong contamination of the entire environment and the need for a longer self-purification period.

3.2. Chemical Parameters of Groundwater in Permeable Organic Barrier

Changes in the Concentrations of Nitrogen Compounds

The concentrations of NO_3^- ranged widely from 0.02 to 3184.9 $\text{mg}\cdot\text{L}^{-1}$ (Figure 4). Over the study period, the mean concentrations of NO_3^- fell by 70% from the sample point above the vertical deposit (368.4 $\text{mg}\cdot\text{L}^{-1}$) to the point below the horizontal and vertical deposits (110.7 $\text{mg}\cdot\text{L}^{-1}$) (Figure 4). Most other studies, mainly focused on diffused sources of pollution, showed a 30–80% reduction on NO_3^- [10,16,17]. The observed differences in efficiency could result from the time elapsed from construction, the material used and the environmental conditions. Similar studies based on barriers built to reduce the nitrogen load from area sources also confirm that increasing the concentrations of nitrates flowing into permeable organic barriers, regardless of the source of pollutants, results in higher denitrification efficiency [20,24].

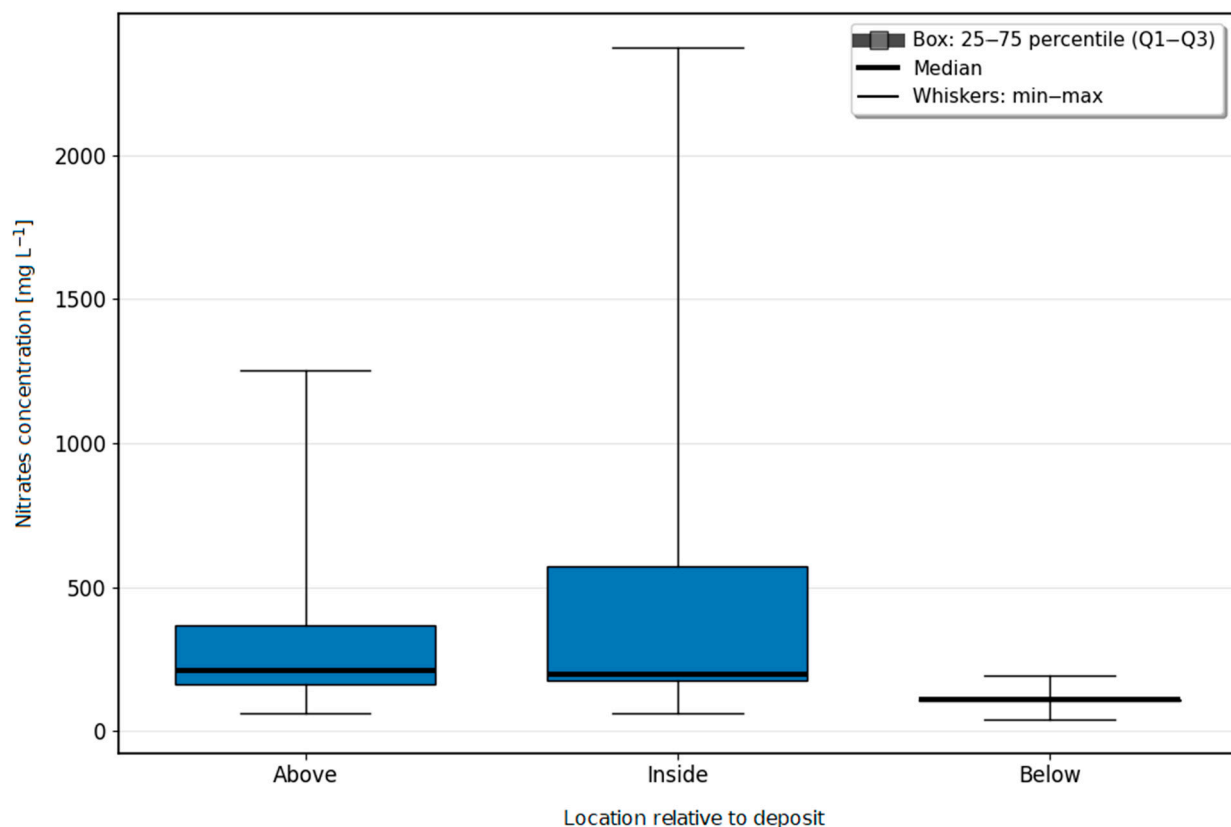


Figure 4. Mean nitrate concentrations (and $\pm$ DS and Min/Max value) in groundwater above the vertical deposit, inside the vertical deposit and below the permeable organic barrier.

It is important to note that European regulations—the Nitrate Directive [42] and the Groundwater Directive [43]—specify the maximum nitrate level in groundwater to be 50 $\text{mg}\cdot\text{L}^{-1}$. Although the proposed permeable organic barrier reduced the nitrate concentration by 70%, the final concentration still exceeded the allowable limit according to the Nitrate Directive by more than two-fold. This may have been due to the long storage period of manure in the same place, and also the naturally occurring ammonium ion nitrification processes taking place in the immediate vicinity.

Most studies evaluating the efficiency of permeable organic barriers or denitrifying walls have typically examined only the reduction of NO_3^- [16]. However, such assessments should also include NH_4^+ level when evaluating the application of the barrier for protecting point sources of pollution. This form of nitrogen was found to be present at 0.01–263.34 $\text{mg}\cdot\text{L}^{-1}$ in the studied barrier (Figure 5). High concentrations of ammonium

ions are typically observed in permeable organic barriers in the case of cow manure pollution, as an effect of ammonification [16]. In the present study, the mean concentrations of NH_4^+ were 42.4 mg L^{-1} (maximum 250 mg L^{-1}) in the groundwater above the vertical deposit, and 24.4 mg L^{-1} below the barrier, i.e., a 43% reduction across the barrier (Figure 5). This clearly demonstrated the possibility of reducing ammonium ions from point source pollution directly in the environment.

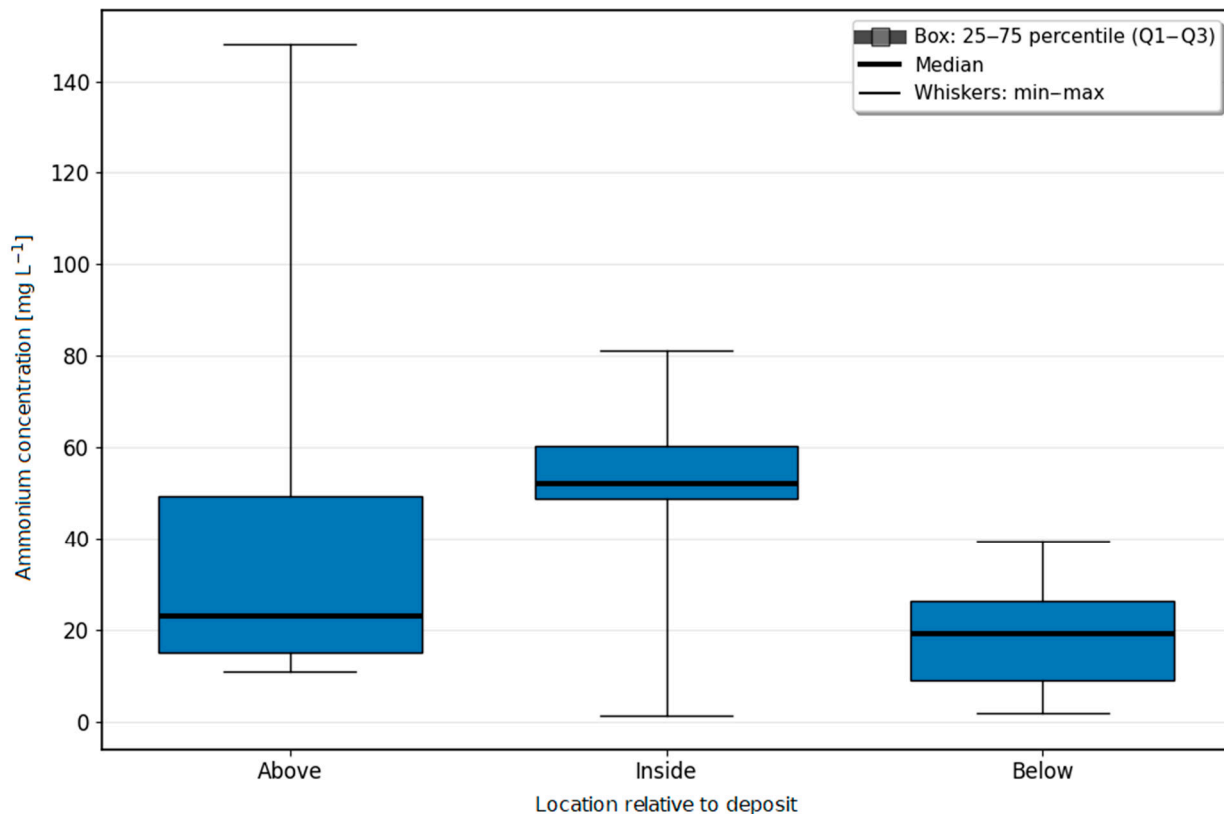


Figure 5. Mean ammonium concentration (and $\pm$ DS and Min/Max value) in groundwater above the vertical deposit, inside the vertical deposit and below the permeable organic barrier.

3.3. Bacterial Transformation of Nitrite to Gaseous Nitrogen

Complete denitrification involves the cascade $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$. Our findings confirm that bacteria capable of performing the entire process were present in the studied vertical deposit. The PCR confirmed the presence of markers for the denitrification genes *nirK*, *cnorB* and *nosZ* (Figure 6). These genes were also detected before the vertical deposit, which may suggest an intensification of the denitrification process by groundwater runoff from the horizontal deposit. However, the genes *nirK*, *cnorB* and *nosZ* were not amplified behind the permeable organic barrier, including the horizontal and vertical deposits (Figure 6). These findings suggest that the carbon substrate used, lignite and charcoal lime, provided a good habitat for bacteria involved in nitrogen transformation, and that the denitrifiers prefer this constructed barrier.

The Nucleotide BLAST analysis showed that the identified *nosZ* gene fragment was closely related to an uncultured bacterial isolate DGGE band 1-1 (96% identity, accession number KJ541495), which interestingly has also been detected in cow manure compost. Furthermore, the *nosZ* gene fragment shared approximately 90% of its identity with the *Pseudomonas stutzeri* strains TR2, DSM 1070, and RCH2 (accession numbers AB764137, CP003725, and CP003071, respectively), these being the closest identified bacteria, which have also been described to perform complete denitrification [44,45].

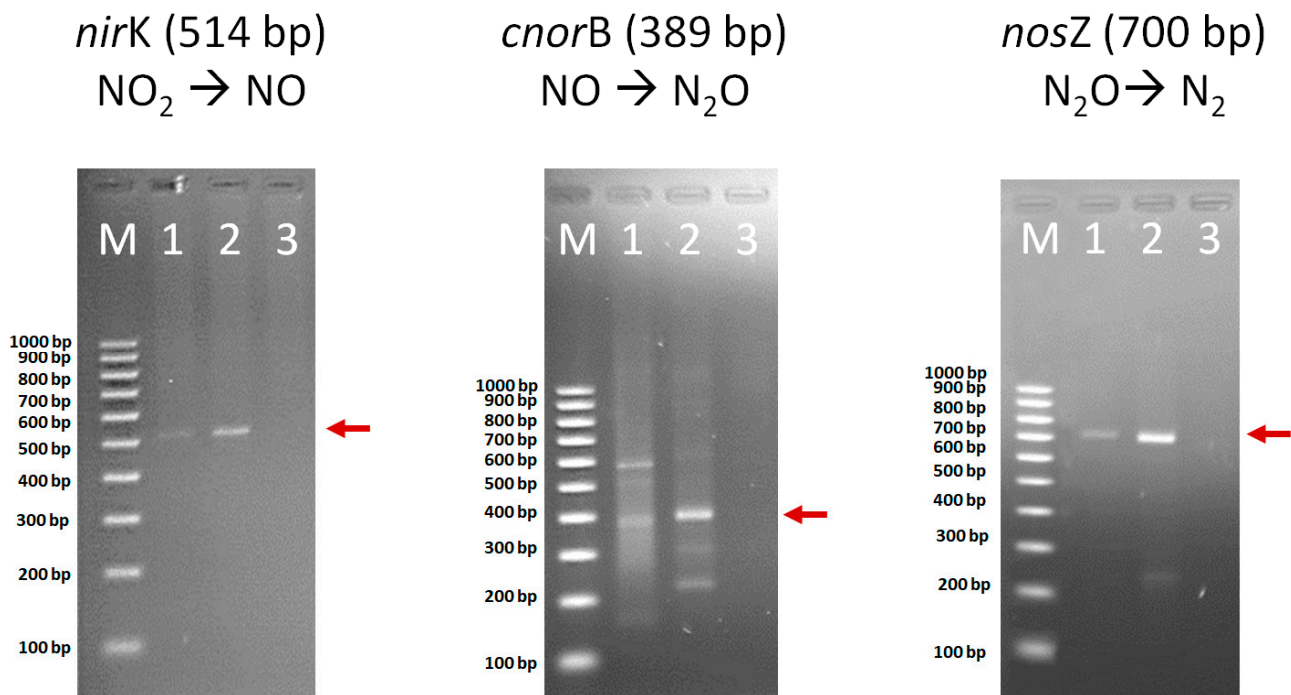


Figure 6. Presence of bacterial genes (*nirK*, *cnorB* and *nosZ*) involved in denitrification process: 1—a sample taken above the vertical deposit of permeable organic barrier, 2—a sample taken from the vertical deposit, 3—a sample taken below the permeable organic barrier, M—marker 100–1000 bp.

3.4. PCBs in the Groundwater of the Permeable Organic Barrier

3.4.1. Changes in PCB Concentrations

PCBs are considered as persistent and bioaccumulative compounds which may exert toxic effects on living organisms, and consequently contribute to extensive environmental degradation. Our findings indicate the presence of PCB concentrations several times higher than the method detection limit ($0.20 \mu\text{g L}^{-1}$), suggesting reduced soil and groundwater quality. The relatively high concentration of PCBs observed in the groundwater of areas of agricultural use suggest that commonly used organic fertilizers, such as manure [11], may also be sources of PCBs [12,13]. Additionally, Elsknes et al. [15] confirm that commonly used fertilizers can be contaminated with dioxins and PCBs. Dumortier et al. [14] and Elsknes et al. [15] report that 49% of the annual national input of dioxin/PCB in Belgium is supplied by manure, 22% by sludge, 12% by compost, 12% by chemical fertilizers and 4% by liming materials. The authors emphasize that despite its relatively low dioxin and PCB content ($0.6 \text{ ng TEQ kg d.w.}$), manure is the most commonly used source that contributes about half of the total dioxin and PCB load. As a result, manure seems to be one of the most significant sources of PCBs in agricultural soils.

This problem is amplified in manure storage sites, where high levels of PCBs and nutrients can enter the deeper layers of soil and facilitate groundwater pollution. Therefore, permeable organic barriers may be promising solutions for reducing PCB content in such areas. However, no studies appear to have evaluated the efficiency of such barriers in PCB removal. Our present results show that the permeable organic barrier reduced the average concentration of PCBs in groundwater of about 39% (from 2.49 above to $1.53 \mu\text{g L}^{-1}$ below the barrier). The lowest mean value ($1.18 \mu\text{g L}^{-1}$), however, was noted inside of the vertical deposit: a reduction of 53% in comparison to the concentration observed above the deposit (Figure 7). This low concentration is probably related to the fact that that organic matter content was highest in this part of the barrier. This favors stronger sorption of PCBs inside

the barrier and its consequent decrease in groundwater. In addition, the PCB concentration inside the deposit could be biodegraded, as noted below.

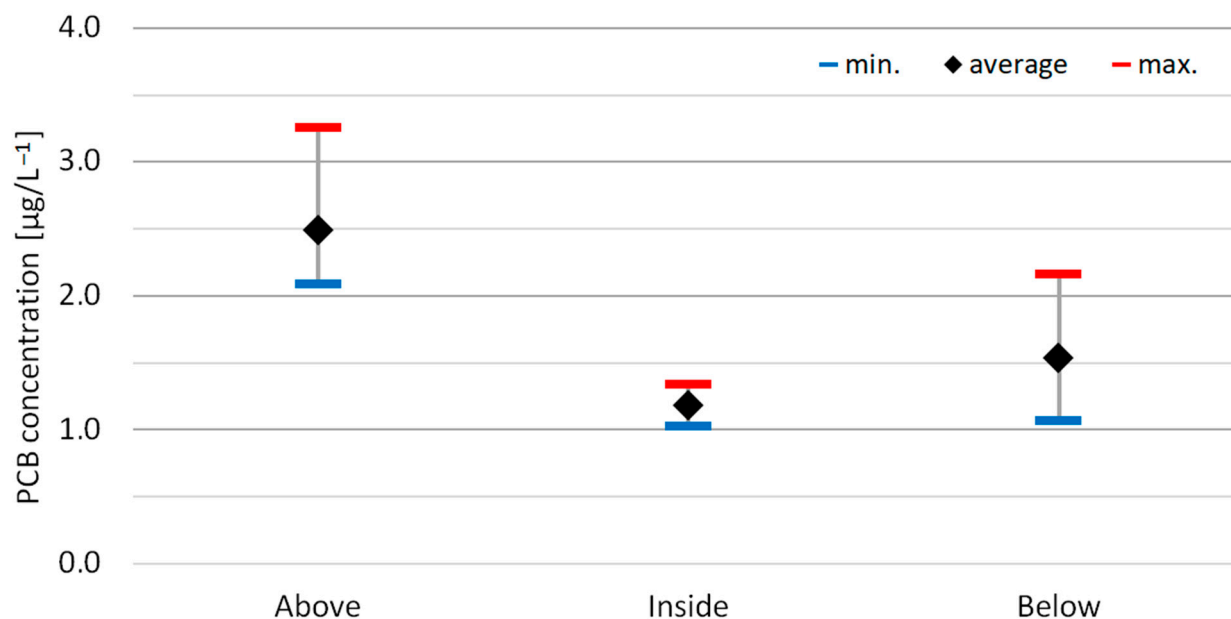


Figure 7. Mean concentrations of PCBs (and $\pm$ DS and Min/Max value) in groundwater above the vertical deposit, inside the vertical and below the permeable organic barrier ($n = 4$).

3.4.2. Bacterial Degradation of PCBs

The carbon source used for barrier construction can also enhance microbial activity inside the barrier [46]. This has been attributed to aerobic bacteria co-metabolizing the PCBs through oxidative pathways [47,48]. The potential of the bacteria inhabiting the present barrier to degrade PCBs was confirmed in the molecular analyses. Our results confirm the presence of a gene coding for PCB-degrading biphenyl dioxygenase (*bphA*), suggesting the occurrence of oxidative PCB biodegradation processes (Figure 8). It is important to note that oxygen was dissolved inside the barrier (1.54 mg L^{-1} , Table 1) and that this was needed for the oxidative removal of PCBs [46,49].

Aerobic PCB degradation is performed in part by oxygenases acting via the biphenyl (bph) catabolic pathway, with *bphA* (dioxygenase) acting as the first step in the degradation process. Bacteria utilizing the bph pathway co-metabolize a variety of PCB congeners depending on the strain. Various species of aerobic bacteria have been described to oxidatively degrade PCBs in soil. Most belong to the Gammaproteobacteria and Betaproteobacteria, including species from the genera *Pseudomonas*, *Alcaligenes* and *Bacillus*, and are mostly involved in the first step of aerobic biphenyl ring cleavage (*bphA*) [48]. In the present study, the sequencing analysis revealed the presence of bacteria homologous in 86.44% to *Pseudomonas aeruginosa* strain DVT412 (accession number CP050333.1) inside the vertical deposit of the permeable organic barrier. These bacteria are known to be effective degraders of PCBs in environmental matrices [50]. The obtained results confirm that due to its high organic carbon content, the permeable organic barrier stimulated the biodegradation of highly toxic and environmentally persistent PCBs and contributes to reducing the risk of groundwater contamination.

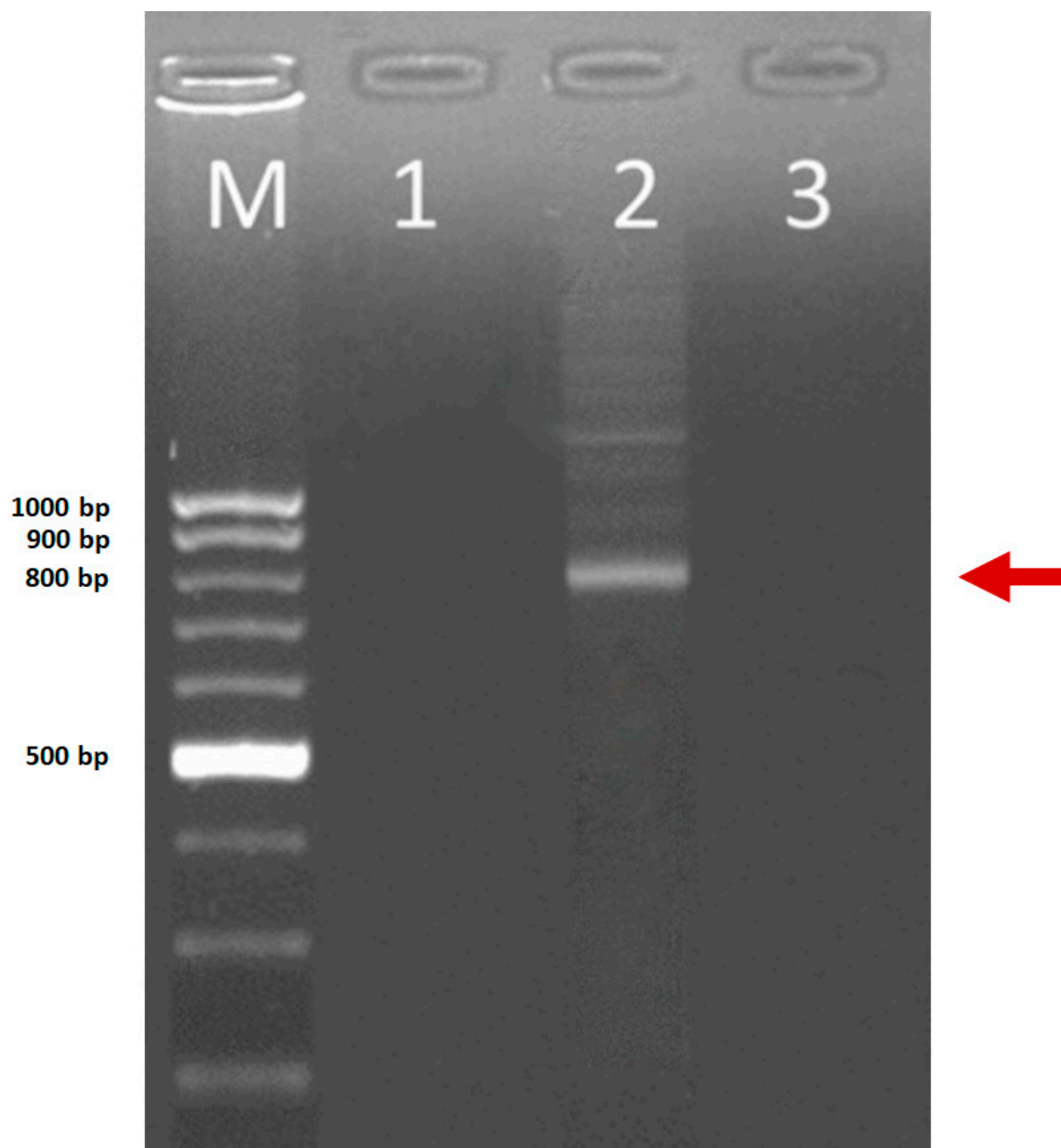


Figure 8. Presence of bacterial *bphA* gene (826 bp) involved in degradation of PCB: 1—sample taken above the vertical deposit of permeable organic barrier, 2—sample taken from the vertical deposit, 3—sample taken below the permeable organic barrier, M—marker 1000–500 bp.

3.5. Seasonal Influence on the Operation of the Permeable Organic Barrier

The influence of the season of collection on the environmental parameters is presented in Figure 9. PC1 and PC2 represented up to 63.7% of the total variance within samples (42.0% and 21.7%, respectively), and the scores and loadings are described in Tables S1 and S2. The PCA indicated sample agglomeration according to the season of collection. However, only the group containing the majority of the summer samples was significantly different from the other seasonal groups (Kruskal–Wallis $H = 14.51$, $p = 2.28 \times 10^{-3}$, Mann–Whitney pairwise test $p < 0.05$, see Table S3). Nevertheless, the autumn and spring samples were also agglomerated and differentiated (Figure 9). Only the winter season was not clearly grouped, due to strong differences between its samples (Figure 9).

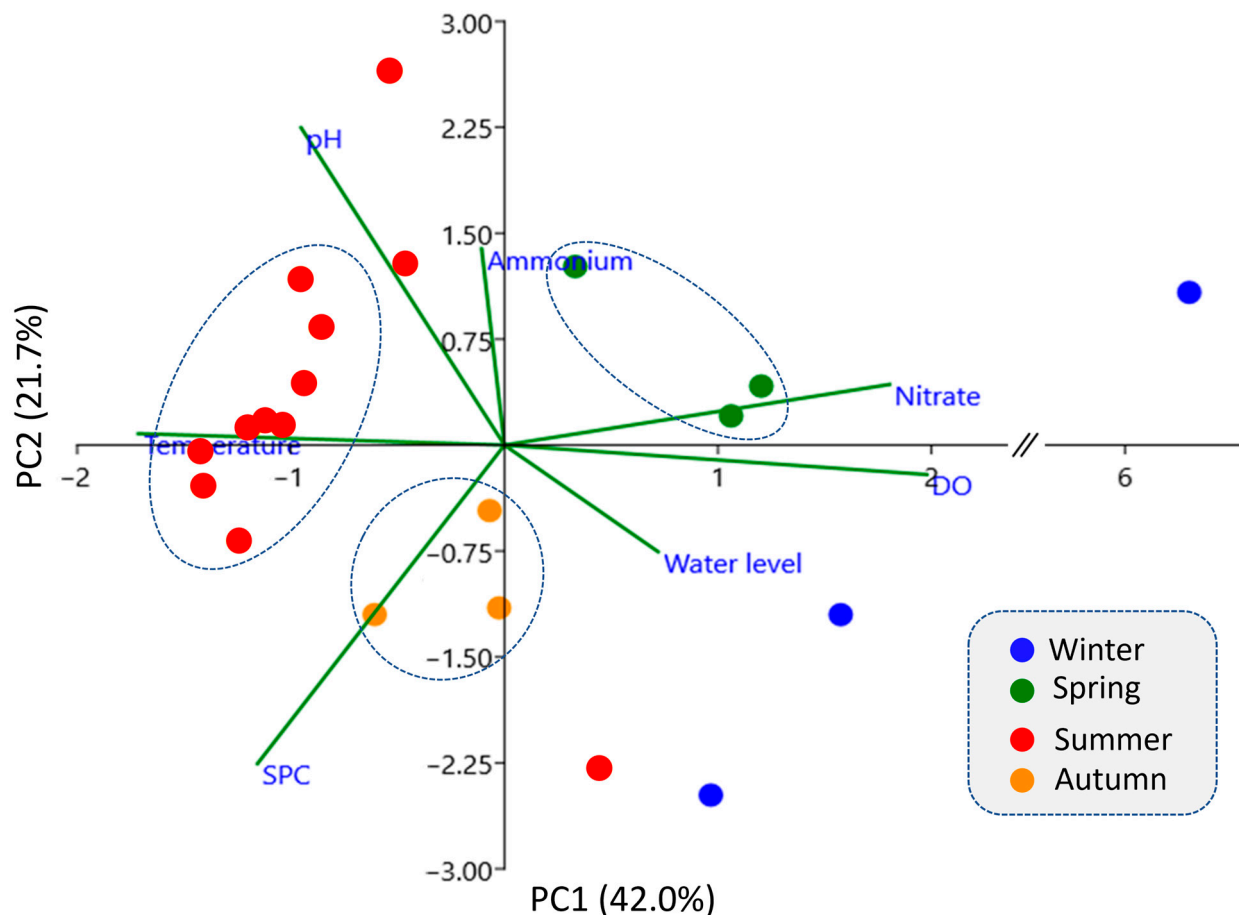


Figure 9. Principal component analysis (PCA) explaining the relationship between the samples according to the season of collection. The environmental parameters were used to explain the data variation. The samples were grouped according to the season of collection.

Strong correlations were noted between temperature and PC1 ($r = -0.82$), and pH with PC2 ($r = 0.77$) (Table S2). These were the most important physical and chemical parameters that differentiated the summer samples from the others (Figure 9). Autumn was differentiated by an increase in conductivity, spring with an increase in ammonium concentration, and the winter samples were independently segregated by an increase in water level, nitrate and oxygen concentration (Figure 9).

The physical and chemical differentiation of the barrier's vertical profile is described in more detail in Figure 10. These findings also indicate a similar seasonal agglomeration to that demonstrated by the PCA (Figure 9). A notable difference was that the cluster analysis showed closer similarities between summer and autumn ($d = 0.22$), when compared to the other seasons ($d = 0.34$) (Figure 10). The greatest distances were observed for the barrier during winter, especially the inside zone ($d = 0.52$) when the highest concentrations of oxygen and nitrates, and the lowest conductivity, were recorded (Figure 10). Significantly lower nitrate concentrations were found in the other two zones, suggesting that the nutrient was most likely stored within the barrier during winter (Figure 10). The presence of low temperatures could have hindered microbial metabolic activity during winter, with the barrier accumulating nitrate rather than transforming and removing it.

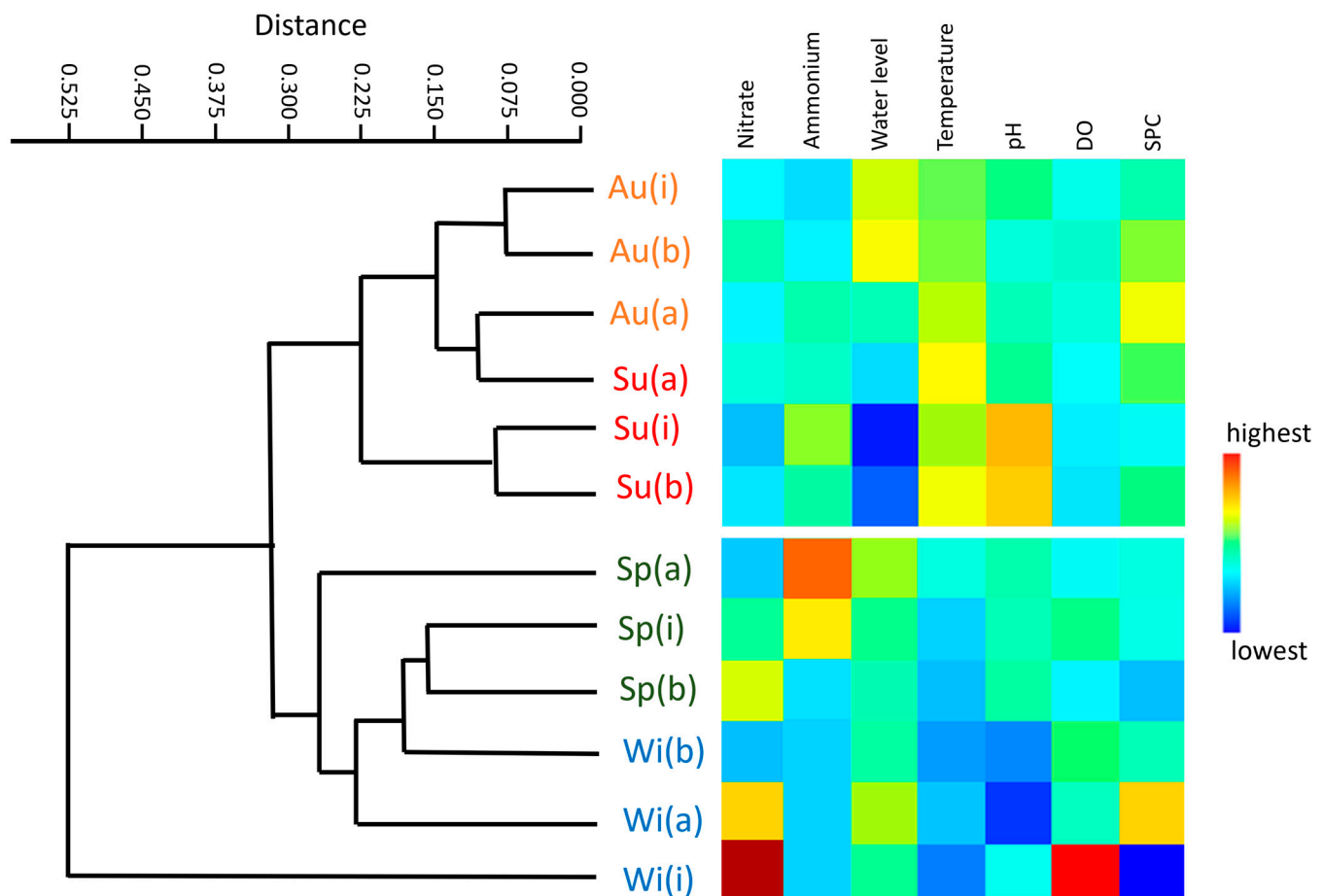


Figure 10. Cluster and heat map analysis indicating the physico-chemical differentiation of the barrier's vertical profile. Summer samples were averaged for each vertical zone. The Gower index was used to estimate the distance between samples, where $d = 1$ represents totally different samples and $d = 0$ indicates no difference between samples. Wi: winter, Sp: spring, Su: summer, Au: autumn, a: above, i: inside, b: below.

In spring, a significant load of ammonium entered the barrier and was considerably removed towards the lower zone (Figure 10). The increase in temperature from winter, and relatively high oxygen concentrations in the inside zone, were regarded as important factors triggering microbial transformation and ammonium removal (Figure 10). We speculated that microbial nitrification was an important process occurring in spring, since nitrate, the common product of nitrification, was subsequently detected in the lower zone (Figure 10).

In summer, microbial metabolic activity was believed to be the highest, particularly in the inside zone, due to the considerable increase in temperature and pH (Figure 10). In contrast to spring, microbial denitrification was believed to be the most important process occurring in summer, especially because nitrate, an important substrate for denitrification, dropped considerably when compared to winter and spring (Figure 10). The environmental conditions found inside the barrier, in the vertical deposit, i.e., a basic pH and limiting oxygen concentrations (Figure 10), were suitable for supporting the microbial communities involved in denitrification. Similar conditions have also been observed in sediments and biofilms from urban river treatment solutions known as sequential sedimentation-biofiltration systems (SSBSs) [51], where nitrification was found to be the dominant process occurring in spring and denitrification in summer. The present study suggests that similar microbial dynamics could occur during the seasonal operation of such underground vertical barriers.

In autumn, despite the relatively high temperature, microbial metabolic activity was believed to be reduced due to a strong decrease in pH (Figure 10). These observations indicate that the barrier operation was reduced towards autumn. This may also be associated with the low load of nitrogen compounds received during that season, as manure was removed from the storage location (Figure 10).

4. Conclusions

In the case of Poland, national legislation and action programs do not cover all sources of pollution by nitrogen compounds. The Law on fertilizers and fertilization [52] only obliges large farms to have structures for the storage of solid and liquid manure: smaller farms with a small number of animals are ignored. This also appears to be the case in other countries. When these installations are not present, nitrogen leaches through the soil and penetrates the water resources [53]. The spatial variation in NO_3^- concentration observed in the groundwater of the Baltic Sea Basin [54,55] suggests that in agricultural areas, the implementation of denitrification programs drawing on the concepts of Ecohydrology and Biotechnology could provide efficient low-cost nature-based solutions for protecting groundwater and surface water quality, restraining eutrophication and preventing harmful algal blooms.

A key aim of the present study was to demonstrate the value of permeable organic barriers for both the transformation of nitrogen (NO_3^- and NH_4^+) and elimination of persistent organic pollutants (PCBs) from the leachate generated by the stored manure. During the course of the study, a 70% reduction was observed for NO_3^- , 43% for NH_4^+ and 44% for PCBs based on results from the zone below the vertical deposit. Such high efficiency confirms that the permeable organic barrier is an effective solution for reducing point pollution in agriculture. Such barriers, i.e., nature-based solutions aimed at protecting groundwater against manure leachate, are especially recommended in areas lacking properly prepared storage sites.

The conditions created for microorganisms in the constructed permeable barrier, including lignite and charcoal lime as a carbon substrate, provided an environment for the enrichment of bacteria with the potential to perform complete nitrogen transformation. More specifically, a particularly important role in the transformation of nitrogen compounds was played by the inside zone, where nitrification was most likely the dominant process occurring during spring, and denitrification in summer. These findings indicate that the inside zone within the vertical deposit is a potential area for further optimization of barriers for nutrient removal, i.e., inoculation with microbial activators [17]. Our data also provide the first such demonstration that the proposed barrier is highly effective at removing PCBs: our genetic analysis confirmed the ubiquitous enrichment of the carbon deposit with *Pseudomonas aeruginosa*, a known PCB-degrader.

5. Patents

The research was part of a larger project based on which Patent No. P.439257 was obtained: “Biogeochemical reef for reducing area pollution” [Biogeochemiczna rafa do redukcji zanieczyszczeń obszarowych] (in Polish), granted by the Patent Office of the Republic of Poland.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/nitrogen6040105/s1>, Table S1. Description of PCA scores for the samples collected in 2013; Table S2. PCA loadings describing the correlation between environmental parameters and the collection of samples in 2013; Table S3. Statistical Kruskal–Wallis (a) and Mann–Whitney pairwise test (b) to determine significant differences between seasonal groups in the PCA.

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