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Evaluation of Biological Characteristics of Soil as Indicator for Sustainable Rehabilitation of a Post-Bauxite-Mining Land

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Abstract: This paper presents a study of the microbial abundance in post-bauxite-mining land soil from Zece Hotare, Bihor county, Romania. The soil samples were collected from 12 soil variants, in the year 2020, after 15 years of long-term restoration. Some chemical parameters and bacterial numbers of six groups of microorganisms were determined in the restored mining land, and these characteristics were compared with those of the soil from a beech forest situated in an adjacent area unaffected by bauxite exploitation. On the basis of the total number of microorganisms belonging to each group studied, the bacterial potential of the soil quality was assessed, calculating the bacterial soil quality index (BSQI), while the Shannon diversity index and the Jaccard distance were applied to show the level of bacterial diversity. The characteristics of the studied chemical and microbiological parameters determined in the beech adjacent area were very similar to those observed in the high-level plateau, low-level plateau, and Black locust areas, indicating similar soil conditions; therefore, the ecological reconstruction 15 years ago, had a very favorable impact on restoration in some affected areas.

Keywords: ecological reconstruction; bacteria diversity; sustainable rehabilitation

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1. Introduction

The main processes leading to soil degradation in former mining areas are as follows:

- Physical: structure destruction, compaction, crust formation, and heavy-metal pollution (Fe, Al, Mg, and Mn);
- Chemical: acidic vulnerability (levels 2–4) due to dissolution and large-scale circulation of contaminated soil and underground waters;
- Biological: reduction in microorganisms, mesofauna, and macrofauna;
- Removal through intense erosion by surface and underground waters, landslides, excavation, and covering with dumped sterile or waste material [1].

Mining activities lead to destructive changes in ground structure and biodiversity, triggering considerable environmental problems [2,3]. Bauxite mining involves serious threats to ecosystems. It causes alteration in species diversity, changes in soil composition, habitat loss, and various social threats. Most of the mining occurs around eco-sensitive areas due to climate change, nutrient imbalance, biodiversity loss, and interrupted ecosystem services [4]. It has been found that mining presents a serious challenge for physical, chemical, and biological restoration. Comprehensive knowledge of the ecology of the landscape structure and configuration, soil type, physical, chemical, and biological properties, dispersal mode, and identification and quantification/inventory of plant communities is critically important for planning restoration programs [5].

The soil degraded through social and economic activities is subjected to a complex system of measures (technical/mining, hydro-amelioration, and soil management) that

are meant to rehabilitate the degraded soil's fertility and create a land shaft proper for agriculture, forestry, and other socioeconomic activities [6–8].

The rehabilitation of post-mining land is required to repair damage to local environments. Various methods are employed to achieve this, such as landscape reclamation, planting ground cover crops, utilization of fast-growing plants, and remediation of water and soil contaminants [9,10]. The technology used for the recultivation of degraded soils constitutes two stages: the technical/mining stage to manage the exploitation material, as well as transportation and storage, and the measures needed in order to fight the negative phenomena (soil acidity and soil erosion) of the primitive technogenic soils capable of ensuring durable plant formation with self-tuning capabilities [11–14].

Ecological reconstruction of bauxite residue disposal areas is regarded as an effective approach to eliminating potential environmental risks. Soil microorganisms are very sensitive to environmental perturbation. Bauxite mining is a major threat to soil productivity by inducing perturbations to the soil microbiota that drive nutrient cycles [15]. The study of the abundance, diversity, and dynamics of some groups of bacteria involved in biogeochemical circuits is particularly important in the context of the scientific need to include the biological properties of soils in environmental impact assessment studies and soil quality monitoring programs. Thus, microbial communities and the total number of microorganisms in the soil can serve as a tool for assessing soil quality change. Some studies concluded that unmined soils consisted of higher numbers of microorganisms than rehabilitated sites [15]. The rehabilitation of mines requires an understanding of both soil chemistry and microbiological activity. Soil microorganisms play a key role in soil physicochemical properties, which permits the re-establishment of soil quality after mining [16].

In the present study, we investigate some chemical and microbiological characteristics of soils from a natural forest and post-mining sites, such as pH, humus content, mobile P, mobile K, and numbers of aerobic heterotrophic bacteria, fungi, ammonifiers, nitrifiers represented by nitrate bacteria and nitrite bacteria, and denitrifiers. The microorganism abundance was also used to evaluate the biological potential of the soil quality. The principal aim was to study how chemical and microbiological soil characteristics respond to restoration (tree planting and fertilization) by comparing post-mining sites with a natural forest site.

2. Materials and Methods

2.1. Study Site and Soil Sampling

This study took place in Bihor county, in Apuseni Mountains which belong to the Western Carpathians. The research area covered 10 ha and was located in Pădurea Craiului Mountains, in a restored bauxite mining land (Figure 1). The exploitation of bauxite ended in 1998, while extensive development works were carried out in 2004–2005: leveling, wattleworks on slopes, and planting of Black locust trees in the leveled area and Norway spruce in the higher zones. The Craiului Forest Mountains represent the area between the Crişul Repede River and the Crişul Negru River in the Apuseni Mountains. The Craiului Forest resembles a relatively suspended plateau, mainly formed from Mesozoic limestones with heights over 1000 m in some parts (1027 m in Hondringuşa) in the east; as we proceed westward, the heights decrease to 400–500 m close to Vârciorog and Bucuroaia (525 in Dealul Poiana and 442 m southwest of Vârciorog). These formations consist of Mesozoic limestones associated with the metamorphic schists, Permian conglomerates, sandstone, and rhyolites. Bauxite can be found in the Pădurea Craiului, Remeţi, and Pietrosul regions of the Apuseni Mountains.

The bauxite resources are located in the area enclosed by the Crişul Repede River, the Crişul Negru River, and the Roşia River. The bauxite exploitation was performed on the surface and in underground mines. The annual rainfall average is 615.1 mm: 585.2 mm in 2005, 872.0 mm in 2006, and 585.2 mm in 2007. The multiannual average temperature is 10.2 °C [19].

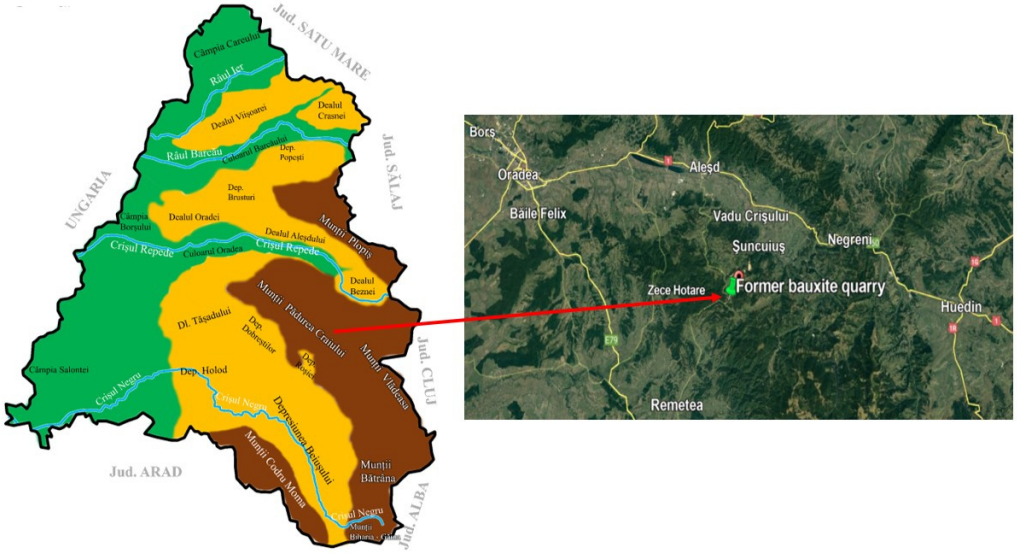

Figure 1. Location of the research area [17,18].

The vegetation of the Craiului Forest includes pasture, a forest of *Picea abies*, and a forest of broad-leaved trees: *Fagus sylvatica*, *Carpinus betulus*, *Acer pseudoplatanus*, *Ulmus montana*, *Fraxinus excelsior*, *Prunus avium*, *Betula pendula*, *Sorbus aucuparia*, *Salix caprea*, and *Juglans regia*. Along the rivers, some specifical species can be encountered: *Salix alba*, *Salix purpurea*, *Salix triandra*, *Populus nigra*, *Alnus glutinosa*, *Carex* sp., *Juncus inflexus*, and *Iris pseudacorus*. There are different vegetative species associated with different areas of the mountain [20–23].

The wattle-work on the hillside determined a better growth rhythm of *Pinus sylvestris*. The following spontaneous species were installed in the post-bauxite-mining land: *Tussilago farfara*, *Cirsium arvense*, *Poa pratensis*, *Rubus caesius*, *Hypericum perforatum*, *Equisetum arvense*, *Polygonum persicaria*, and *Juncus inflexus* [24].

The soil samples were collected in March 2020 from 12 experimental sites. A soil profile was placed in each experimental field. Sampling was conducted using the square method (100 m^2 surface in each area), and the depth of sample collection was 0–20 cm. Chemical and microbiological parameters were determined on the soil samples taken in a natural layout from all 12 profiles using cylinders of 100 cm^3 each in five replicates.

The pH soil values were determined using the potentiometric method, whereas soil organic matter was calculated using the method of wet oxidation and titrimetric dosing (Walkey–Black method). The content of macroelements (mobile phosphorus and potassium) was determined through extraction with ammonium acetate lactate and dosing using a spectrophotometer.

2.2. Microbiological Analysis

The foreign materials (plant debris, calcareous concretions, and other impurities) were removed, and the soil was passed through a sieve with a diameter of <2 mm. The analytical sample (10 g) was extracted from the sifted soil by quartering (this method of tillage was applied for each variant of soil separately, starting with 10 g of soil in each case).

In the studied area, six eco-physiological groups of microorganisms were determined: aerobic heterotrophic bacteria, fungi, ammonifying bacteria, nitrifying bacteria, and denitrifiers.

The microbial abundance of the six groups of microorganisms was determined in 12 experimental fields with different characteristics in terms of fertilization factor, slope influence, and natural vegetation installation:

1. Black locust without fertilization factor (N_0P_0) (BlF0);
2. Black locust with fertilization factor ($N_{60}P_{60}$) (BlF60);
3. Black locust with fertilization factor ($N_{120}P_{120}$) (BlF120);

4. Slope (10%) without wattle-work and fertilization (N_0P_0), planted with Norway spruce (Ns10%F0);
5. Slope (10%) without wattle-work, with fertilization factor ($N_{60}P_{60}$), planted with Norway spruce (Ns10%F60);
6. Slope (10%) without wattle-work, with fertilization factor ($N_{120}P_{120}$), planted with Norway spruce (Ns10%F120);
7. Slope (40%) without fertilization (N_0P_0), planted with Norway spruce (Ns40%F0);
8. Slope (40%) with fertilization factor ($N_{60}P_{60}$), planted with Norway spruce (Ns40%F60);
9. Slope (40%) with fertilization factor ($N_{120}P_{120}$), planted with Norway spruce (Ns40%F120);
10. High-level plateau planted with Black locust (hlpBl);
11. Low-level plateau planted with Black locust (llpBl);
12. Beech tree adjacent area (unaffected by the bauxite exploitation) (btaa).

For the microbiological determinations, six successive decimal dilutions (10^{-1}–10^{-6}) of the soil samples (10 g) were conducted. The first dilution of the soil samples (10^{-1}) was homogenized using a magnetic stirrer at 110 rpm for 5 min.

The total number of aerobic heterotrophic bacteria was determined using the plate culture method [25] (three repetitions) with a solid nutrient medium containing meat extract (incubation: 7 days at 30 °C). Yeasts and molds were grown on *Sabouraud* agar (incubation: 4–5 days at 25 °C). After the plate incubation period, a colony count was performed using a colony counter. Total microflora was expressed as CFU (colony-forming units)/g soil (dry weight).

The "most likely number" (MPN) method was used to determine the total number of ammonifying, nitrifying (nitrite bacteria and nitrate bacteria), and denitrifying microorganisms. A liquid culture medium with peptone water was used. The culture medium used for the determination of nitrite bacteria and nitrate bacteria contained Winogradski's saline solution, diluted at 1:20 [26]. Denitrifying bacteria were determined on "de Barjac" liquid culture medium. For each decimal dilution of the soil samples, five test tubes were used. Each test tube was inoculated with 1 mL of the respective dilution. After an incubation period of 3 weeks at 28 °C, the typical reaction product was analyzed in each test tube as follows:

- Cultures of ammonifying bacteria were analyzed using the Nessler reagent to highlight the ammonia produced by these bacteria. To 1 mL of culture medium was added 1 mL of Nessler reagent. The appearance of a yellow-orange coloration in the inoculated medium indicated the formation of ammonia and, therefore, the presence of ammonifiers;
- In the case of nitrite bacteria, the presence of nitrites was tested with diphenylamine sulfuric reagent, and, in the case of nitrate bacteria, nitrates were detected in the presence of urea in the sulfuric medium (the appearance of a blue coloration was observed);
- The cultures of the denitrifying bacteria were analyzed to highlight the nitrites produced by these bacteria following the reduction of nitrates, using diphenylamine sulfur reagent.

The most probable number of ammonifying bacteria, nitritbacteria, nitrate bacteria, and denitrifiers was determined using McCrady's statistical table. The number of microorganisms was determined by counting the number of tubes giving positive reactions (color change) and comparing the pattern of positive results (the number of tubes showing growth at each dilution) with standard statistical tables [26].

On the basis of the total number of microorganisms belonging to each group studied, the bacterial potential of the soil quality was assessed, calculating the bacterial soil quality index (BSQI) [27] according to the following formula:

$$BSQI = 1/n \times \sum SQI = N, \quad (1)$$

where BSQI is the bacterial index of soil quality, n is the number of bacterial groups, and N is the number of bacteria belonging to each group studied.

2.3. Shannon Diversity Index and Statistical Analysis

For analyses of the diversity of microbial groups identified in the 12 experimental variants, we used the Shannon diversity index and the Jaccard distances, both computed using the vegan package [28] in the R environment.

$$H' = -\sum^{S}_{i=1} pi \ln pi, \quad (2)$$

where H′ is the Shannon diversity index, pi is the relative abundance of species i, S is the total number of species present, and ln is the natural logarithm [29].

The statistical analysis of the collected data, including Pearson correlation, ANOVA, and Kruskal–Wallis test, was performed in the R environment [30].

3. Results

Chemical and Microbiological Analysis

The soil from btaa is a skeletic calcic luvosol, while that in the former bauxite quarry (from the other 11 variants) is a leptosol (according to WRB).

The ANOVA test, which was performed among the 12 sites for the investigated chemical and microbiological characteristics of soils (pH, humus content, mobile P, and mobile K), revealed no significant differences between sites or between repetitions regarding the chemical characteristics of soils.

The microbiological analysis was computed as a function of the microbial abundance. In addition, the results for the microbial abundance were interpreted as a logarithmic expression of the number of microorganisms belonging to each microbial group studied (Table 1).

Table 1. Logarithmic expression of the number of microorganisms belonging to each microbial group studied (log cells/g dry soil).

Experimental Variants	Heterotrophic Bacteria	Fungi	Ammonifying Bacteria	Nitribacteria	Nitratbacteria	Denitrifying Bacteria
BlF0	7.09	6.57	2.56	3.44	2.96	2
BlF60	5.73	5.63	2.96	3.89	1.87	2.07
BlF120	7.25	6.30	2.30	2.38	1.56	2.74
Ns10%F0	5.23	6.47	2.74	-	-	2.83
Ns10%F60	5.91	6.60	3.30	-	-	2.65
Ns10%F120	5.82	6.16	3	-	-	2.96
Ns40%F0	6.54	5.82	2.78	-	-	2.30
Ns40%F60	6.50	6.75	2.86	-	-	2.25
Ns40%F120	7.26	6.92	3.07	-	-	2.91
hlpBl	7.27	6.44	3.30	5.11	1.60	3.04
llpBl	6.90	6.43	2.91	5.36	1.78	2.78
btaa	5.79	5.96	2.96	3.32	2.89	2.55

The Pearson correlation among the six groups of microorganisms showed that the presence of some bacterial species was related to the presence of another species (Figure 2). The strongest positive correlations ($r = 0.77$) were identified between ammonifying bacteria and nitrite bacteria, indicating that an increase in the number of ammonifying bacteria would also increase the number of nitrite bacteria.

The BISQ values shown in Figure 3 indicate a high presence of microorganisms in hlpBl, llpBl, and BlF0, with the exception of soil samples collected from the following experimental areas: Ns10%F120, Ns40%F0, and Ns10%F0, where the BISQ values indicate a moderate abundance of microbial groups. The Kruskal–Wallis test revealed no significant differences among the 12 experimental variants regarding BISQ values ($p = 0.443$), but with a slight superiority of hlpBl and llpBl, which recorded a higher number of bacterial species.

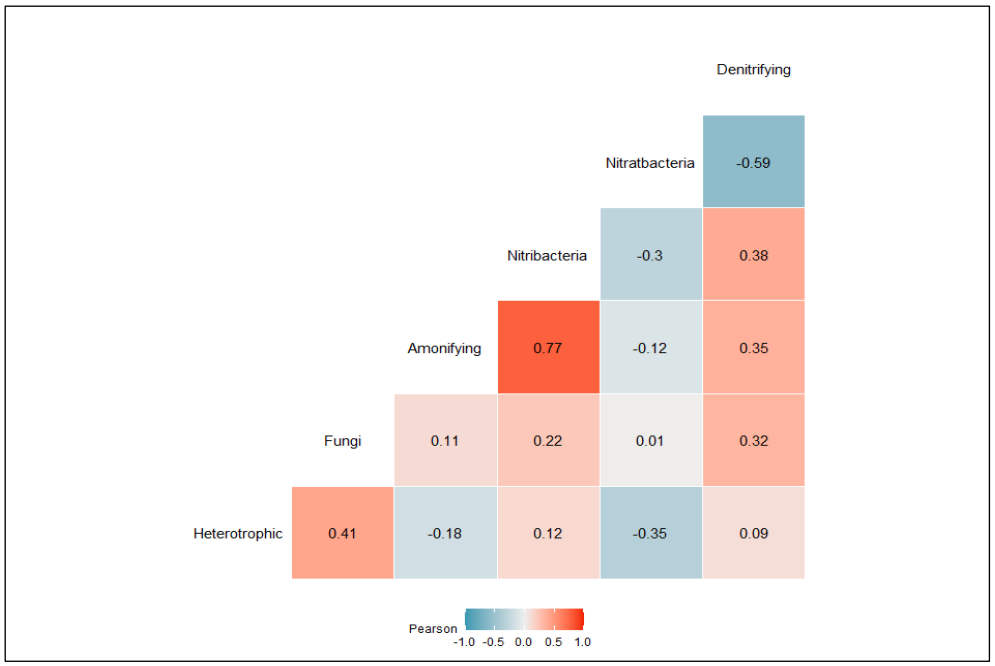

Figure 2. The Pearson correlation computed for the six microbial groups identified in the 12 experimental variants.

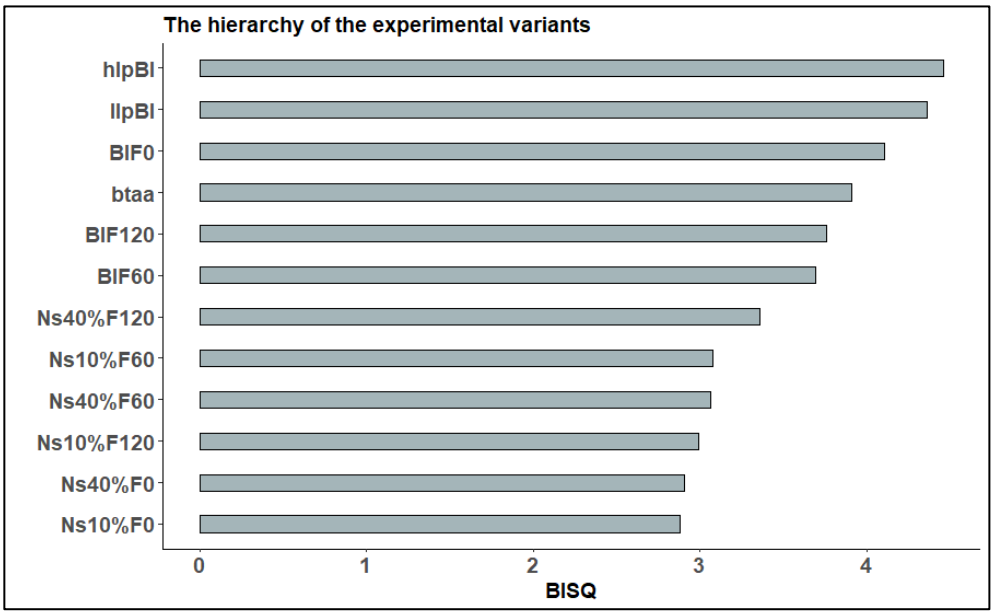

Figure 3. The hierarchy of the experimental variants according to the values of the bacterial indicator of soil quality.

The 12 experimental sites were arranged into two groups as a function of the Shannon diversity index, and the differences among sites were small and insignificant (Figure 4). Higher values for this diversity index were recorded in the btaa, suggesting that this site contained the highest number of bacterial species. The lowest value for this diversity index was identified in Ns40%F60, which seemingly contained the lowest number of bacterial species.

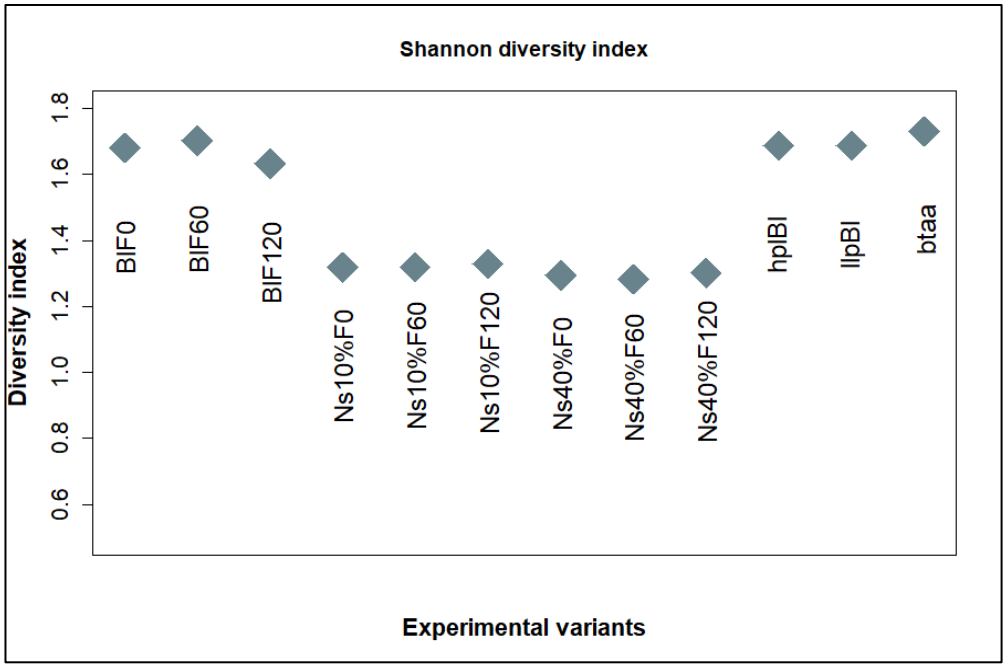

Figure 4. Values of Shannon diversity computed for the 12 experimental variants.

The Jaccard similarity also grouped the 12 experimental sites into two groups (Figure 5). Moreover, in the case of each group, some differences were identified. In the case of group A, the experimental sites were arranged into two groups; the sites Ns10%F0, Ns10%F60, and Ns10%F120 were seemingly homogeneous, as they were characterized by a 10% slope without wattle-work, but differences were observed when considering the fertilization factor.

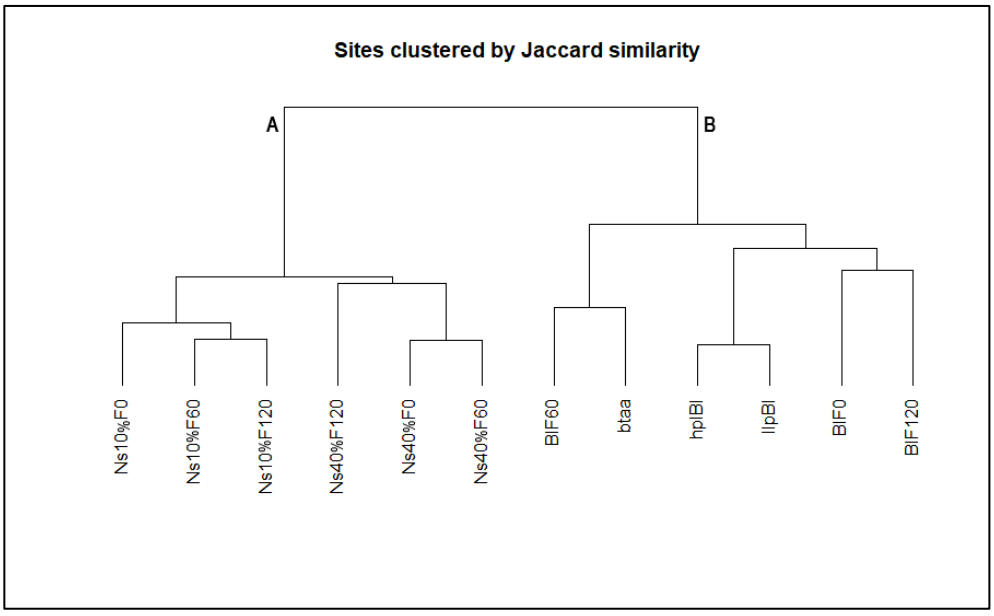

Figure 5. The Jaccard similarity computed for the results obtained by the Shannon diversity index.

Site Ns10%F0 belonged to one group, while sites Ns10%F60 and Ns10%F120 belonged to another. Identical results were recorded in the case of sites Ns40%F0, Ns40%F60, and Ns40%F120, which were similar in terms of slope (40%) but different in terms of fertilization factor. Group B was arranged into three subgroups. In the first subgroup, btaa was similar to BlF60 in terms of bacterial diversity. In the second subgroup, hlpBl and llpBl were also similar in terms of bacteria diversity and slope (0%) but differed in terms of altitude. The

third subgroup (BlF0 and BlF120) was similar with respect to the presence of Black locust, which seemingly influenced the diversity of bacterial species.

4. Discussion

The luvisol from the research area is widespread in Romania, accounting for 23% of the total forest soils [31]; it is well supplied with carbon [32–34], water [35,36], and nutritive elements [37–39]. Increasing knowledge about the chemical and bacterial numbers of the rehabilitated sites affected by long-term bauxite exploitation is crucial to assess the soil quality. In this research, we specifically studied how chemical and microbiological soil characteristics respond to restoration by comparing rehabilitated sites with an un-mined site.

Some authors found that restoration was associated with the recovery of the activity and diversity of soil bacterial communities, reaching similar levels to those observed in the conserved forest. Within a few years, restoration allowed recovering crucial physical, chemical, and microbiological soil attributes, reaching levels comparable to those found in conserved forests [40]. Some chemical parameters and bacterial numbers of six groups of microorganisms were determined in the restored mining land. These characteristics were compared with those of the soil from a beech forest situated in an adjacent area unaffected by bauxite exploitation.

Microorganisms from soils affected by degradation processes [41–45] due to human activities represent the form of life that adapts the fastest to new environmental conditions [46]. In other studies on bauxite residue, the bacterial communities were observed to be similar to those from normal soil only after the restoration process [47], as proven in this study. On the other hand, some studies showed that a larger concentration of bauxite residues affected only the activity of the microbial community and not its structure [14]. A smaller number of bacterial species was statistically established in areas with a high slope in this study, as also observed by other authors [48–52].

Vachova et al. [51] investigated the relationship between vegetation and selected soil characteristics as part of landscape restoration. Microorganisms are most important in the phytoremediation process of mining sites because they can contribute to the mobilization or immobilization of metals and metalloids in soil, thus facilitating vegetation development [53]. Our findings are in accordance because we observed that experimental sites with a high abundance of microorganisms (BlF60, hlpBl, and llpBl) were quite similar to sites unaffected by bauxite exploitation (btaa). Moreover, we found no significant differences between the 12 sites or between repetitions regarding the chemical characteristics of soils.

Wu et al. [54] found that the establishment of microbial communities and associated functions may improve the physical and chemical properties of soil, as well as stimulate its formation in bauxite residue. The authors highlighted that the microbiota was significantly developed after long-term natural restoration, as also observed in our research. The long-term restoration created microbial community diversity in bauxite residue.

Bauxite mining activities have the potential to impact the environment, including changes in soil fertility, low soil pH, reduced ability of the soil to hold water, inadequate supply of nutrients for plants, erosion, and exposure to rocks containing sulfides, resulting in the potential for acid mine drainage and disruption of the ecosystem. The authors found significant differences in the chemical properties of soil according to the age of reclamation [55]. The results obtained were not the same as the present study, where the ANOVA test revealed no significant differences in the chemical properties of the soil across the 12 sites, thus indicating their similarity to soil from the natural forest site (btaa).

5. Conclusions

The results of this study emphasize the importance of microorganisms as biological indicators of changes taking place in reclaimed soils. Little is known about the microbial abundance and chemical properties, as well as their role in the characterization process of soil formation in bauxite residue.

After 15 years of long-term restoration, we found that bacterial numbers were similar to those in unexploited soil. In addition, the Pearson correlation among the six groups of microorganisms showed relationships between the presence of some bacterial species.

As bacterial indicators of soil quality, we observed that a high presence of microorganisms was recorded in hlpBl and llpBl, as well as in black locust areas (BlF0, BlF60, and BlF120), indicating their high potential to sustain the life of the microorganisms. In the remaining sites, a moderate presence of microorganisms was recorded, indicating a low potential for microorganisms to adapt and live in these conditions.

The Shannon diversity index showed that experimental sites btaa, hlpBl, and llpBl, as well as black locust areas (BlF0, BlF60, and BlF120), recorded a higher diversity than in the other areas, thus sustaining that these experimental sites were able to support the life of microorganisms and, hence, the growth of the tree species.

Another aspect that needs to be mentioned is that the studied chemical and microbiological parameters determined in btaa were very similar to those observed on the plateau (hlpBl and llpBl) and at experimental sites, BlF0, BlF60, and BlF120, indicating the similarity of the soil conditions in these areas. Therefore, the ecological reconstruction 15 years ago had a very favorable impact on restoring some affected areas.

The Jaccard similarity showed that the factors underlying the differences among the 12 experimental sites regarding the diversity index were the slope, fertilization factor, and Black locust species installed in some areas, which had a positive influence on the land rehabilitation affected by bauxite mining. Additionally, this analysis revealed that BlF60 was the site most similar to the natural experimental site (btaa), thus representing one of the best-reclaimed sites regarding the level of bacterial diversity.

The results obtained in this study offer a biogeochemical perspective describing soil formation in bauxite residue. We found that soil conditions were similar across all 12 experimental sites, enabling us to conclude that the ecological reconstruction 15 years ago had a very favorable impact on the restoration of areas affected by long-term bauxite exploitation.

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