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Carbon Sequestration in Remediated Post-Mining Soils: A New Indicator for the Vertical Soil Organic Carbon Variability Evaluation in Remediated Post-Mining Soils

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Abstract: The present study experimentally investigated two different open-cast post-mining areas with different remediation methods for the vertical distribution of sequestered soil organic carbon (SOC). The study has been performed for two soil layers (0–15 cm, and 15–30 cm) for the four areas with different remediation advancement (up to 20 years) at both studied post-mining soils: the limestone post-mining soil remediated with embankment and lignite post-mining soil remediated with sewage sludge. The study revealed that SOC is more stable within soil depths for lignite post-mining soil remediated with sewage sludge in comparison to the limestone post-mining soil remediated with embankment. The lignite post-mining soil remediated with sewage sludge showed a better hydrophobicity, humidity, aromaticity, and C/N ratio according to the ¹³C NMR. Therefore, in that soil, an increased microbial community has been observed. The study observed a positive correlation between GRSP content with a fungi community within soil depths. For lignite post-mining soil remediated with sewage sludge, the activity of ureases and dehydrogenases was generally lower compared to the post-mining soil remediation with embankment. The investigation found good parameters of Ce and NCER which for both studied areas were negative which indicate for the privilege of the higher capturing of CO₂ over its release from the soil into the atmosphere. The study finds no relevant changes in SOC, POXC, TC, and LOI content within soil depth and remediation age. Due to the lack of a possible well-describing indicator of the vertical distribution of SOC stability in post-mining remediation soil, we proposed two different indicators for differentially managed post-mining soil remediations. The model of calculation of vertical SOC variability index can be universally used for different post-mining soils under remediation, however, both proposed calculated indexes are unique for studied soils. The proposed model of an index may be helpful for remediation management, C sequestration prediction, and lowering the carbon footprint of mining activity.

Keywords: post-mining soils; SOC sequestration; soil layers; vertical SOC variability index; indicator; GRSP; SOC; POXC; hydrophobicity; aromaticity; humification; C/N; climate change mitigation



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1. Introduction

Open-cast mining contributes to high soil degradation, and its restoration is very problematic due to the low organic matter content. The main consequences of open-cast mining are losses in soil biodiversity, water shortage, and soil erosion [1]. Therefore it causes deforestation and the removal of carbon-rich topsoil. Such effects are correlated with the high release of CO₂ into the soil [2]. While in mining high amounts of CO₂ are released into the soil [3], the post-mining soils are considered to be a powerful C sink which through carbon sequestration may capture CO₂ from the atmosphere [4]. The remediation

of these areas has been found to improve the soil potential for soil organic carbon (SOC) sequestration and storage [5]. It has been noticed that different land management types influence SOC sequestration [6]. Thus, the choice of remediation technique in the reclamation of post-mining soils is crucial for its capability for C sequestration. Since post-mining areas are characterized by a low organic matter content, the remediation with the initial application of organic soil additives is favorable for the restoration of the soil and promotes C sequestration [7]. The usage of organic soil additives such as sewage sludge is a common practice of external sources of organic matter application that is used for remediation technologies, especially in post-mining soils [8]. The application of sewage sludge or composts is widely studied on all continents, including Africa [9]. The first mentions of the use of sewage sludge in post-mining soils date back to the 1990s [10]. The legitimacy of using external substrates of organic matter in post-mining areas results from the fact that the top layer of soil is removed in the mining process, which results in the deprivation of soil organic matter. Sewage sludge applied at mine areas consists of the medium for growth and development of plants, as well as improving seed germination which in results accelerates remediation of these areas [11]. Therefore, reforestation using pioneer, and fast-growing plants are still considered the best remediation method for post-mining areas which brings benefits in economic and ecological aspects [12]. Such a method is widely used in post-mining soils, however, is contingent upon local abiotic and biotic factors [13]. Therefore, forest recultivation is sustainable over the long term [14].

It has been observed that reforestation accelerates soil organic carbon accumulation [15]. Besides the improvement of fertilization it increases soil enzymatic activity [16], promotes microbial growth and biodiversity [17], improves soil structure [18], and protects to erosion [19]. The production of glomalin-related soil proteins (GRSP) by arbuscular mycorrhizal fungi (AMF) tends to increase in remediated soils [20,21]. It has been reported that these glycoproteins influence soil aggregation, contribute to the recovery of SOC, and soil N, and stabilize sequestered SOC [22].

The higher microbial community in post-mining remediated soils positively influences plant growth [23]. It is caused by the increase in plant-growth-promoting rhizobacteria (PGPR) which have the potential to enhance plant growth [24]. The accelerated growth of soil microorganisms influences the soil enzymes and activates soil functioning [25]. The activation of soil enzymes directly influences soil's physical, chemical, and biological properties [26]. There is a direct influence of several soil enzymes such as urease and dehydrogenase on nutrient cycling, and SOC storage [27]. The enzymatic activation may be enhanced by the application of organic matter in remediation [28]. Urease is the main biocatalyst for decomposition, and thus, plays an important role in the mineralization of organic matter in the soil [29]. Soil ureases catalyze the reaction of hydrolysis of urea providing N fertilizer and other nitrogenous compounds to produce ammonia and release CO₂ [30]. Dehydrogenases usually are used as an index for soil microbial activity [31], as they occur only in living cells [32]. Dehydrogenase oxidizes soil organic matter, and its activity indicates the overall microbial respiratory activity [33]. Therefore, the activity of urease and dehydrogenases are closely linked with SOC availability [34].

The microbial availability of SOC usually results in elevated CO₂ release from the soil as a result of the increased microbial activity [35]. However, the formation of stable, and non-susceptible for microbial decomposition carbon forms alters the carbon budget in the soil [36]. The limitation of bioavailable organic matter in the soil results in a lower soil respiration rate which decreases the CO₂ release into the atmosphere [37]. On the other hand, elevated microbial activity allows for the formation of more stable organic matter for sequestration purposes [38]. Generally, soil remediation is considered to result in higher SOC, and total carbon (TC) content [39]. The SOC content is the most commonly used indicator for soil C sequestration. In turn, the permanganate oxidizable carbon (POXC) is more and more used in the assessment of soil health indicating the labile or active soil organic carbon as many studies reported that POXC is susceptible to soil management alterations and highly susceptible to microbial decomposition [40]. POXC represents

even higher sensitivity to the particular organic carbon (POC) which has been a standard indicator for labile C fraction [41]. The SOM persistence in the soil is mainly regulated by its microbial availability, the degree of its limitation, and carbon use efficiency [42]. The studies indicated the need to understand the dynamics of SOC indicating its high value as the biggest C pool on the Earth [43]. SOC storage in soil is highly connected with the conversion of each C functional group including alkyl C (derived from chitin, cutin, and lipids), O-alkyl C (derived from cellulose, semicellulose, and carbohydrates), aromatic C (associated with lignin degradation), and carbonyl C (originated from fatty acids, amino acids, and organic acids) [3,44]. The chemical heterogeneity of sequestered SOM in a young forest may be caused by the microbial metabolism of relatively simple C components in leaf litter [38]. The alkyl-C and lignin are considered as highly recalcitrant structures for microbial decomposition [45]. Since lignin is the second biggest composition of plant tissues [46] it is provided in forests at a high rate. After forming a big complex with humic substances it provides a high persistence in the soil enhancing C storage [47]. O-alkyl C is considered to highly influence the soil's physical conditions, cation exchange reaction, anion retention, and biological activities [48]. Therefore, O-alkyl C and carbonyl C represent the labile C fraction of SOC and show persistence for days to a year. In turn, the alkyl C and aromatic C represent the relatively stable SOC fraction with a half-life time of decades [49]. The stable C fraction mainly is deposited in soil aggregates [50]. It has been suggested that SOC decomposition susceptibility decreases in the soil profile [51]. Therefore, it has been observed an increased proportion of alkyl C, carbonyl, and aromatic C forms of SOC within the soil depth. Simultaneously indicated the reduction in O-alkyl C in deeper soil parts [52]. Several indicators for the characterization of SOC stability in the soil are used including hydrophobicity, aromaticity, humification, and C/N ratio. Hydrophobicity indicated the affinity of the soil particles to the water. It has been observed that hydrophobic soils may better stable storage of SOC compared to hydrophilic soils. Hydrophobicity refers to the share of hydrophobic C fractions (alkyl-C, and aromatic C) to hydrophilic C fractions (O-alkyl C and carbonyl C) [53]. Aromaticity refers to the share of aromatic C form in the sum of all C forms. The higher aromaticity suggests better stability of SOC [54]. Humification and C/N indicated the susceptibility of SOC to microbial breakdown [55]. All mentioned indicators are important tools for controlling and evaluating of C sequestration in the soil, as well as for assessing the SOC pool underground.

The previous studies indicated the need to understand the dynamic of SOC indicating its high value as the biggest C pool on the Earth [43]. However, the distribution of chemical heterogeneity of SOC has been well documented for most soil types, there is a lack of knowledge in the alternations and vertical distribution of chemical heterogeneity of SOC at highly remediated post-mining soils on which open-cast operations were carried out. In this study, we studied two open-cast post-mining soils under differentially managed remediation methods. We examined limestone post-mining soil remediated with embankment and lignite post-mining soil remediated with sewage sludge. The goal of this study is to provide an effective tool to evaluate the vertical stabilization of sequestered soil for differentially managed post-mining soils. The aim of the study was to describe the SOC distribution in two soil depths (0–15 cm and 15–30 cm) and access the vertical alternations in microbial community, enzyme activity, and GRSP content at different remediation advancements. In the study, we aim to evaluate a correlation between different soil remediation practices, biological soil properties, SOC stabilization, and CO₂ emission.

2. Materials and Methods

In this study we selected two open-cast post-mining areas under remediation located in central Poland: limestone post-mining area (S1) and lignite post-mining area (S2). The details of remediation technique, and characteristics of study areas are shown in Table 1. The area S1 has been recultivated with embankment collected in the mining industry. In turn, at area S2 remediation has been conducted with the initial application of sewage sludge originated from the company's wastewater treatment plant serving office buildings

of mine. The sewage sludge has been previously studied and approved for use on land for non-agricultural purposes according to the applicable legal regulations in accordance with the regulation of the Minister of the Environment on sewage sludge. Thus, applied sewage sludge has a suitability certificate for the reclamation of the land in question for non-agricultural purposes. The applied sewage sludge had been previously brought to the properties allowing it to meet the standards in force in Polish law. For the study areas, the Regulations of the Minister of the Environment in force at the start of reclamation were applied by the following: the Regulation of the Minister of the Environment of 6 February 2015 on municipal sewage sludge [56] for S2A, the Regulation of the Minister of the Environment of 13 July 2010 on municipal sewage sludge [57] for S2B, and the Regulation of the Minister of the Environment of 1 August 2002 on municipal sewage sludge [58] for S2C, and S2D. Following the above regulations regarding the use of municipal sewage sludge, *Salmonella* bacteria were not isolated, the sewage sludge did not exceed the standards for the content of the total number of live eggs of intestinal parasites *Ascaris* sp., *Trichuris* sp., *Toxocara* sp. d.m., the content of total nitrogen (including ammonium nitrogen), total phosphorus, calcium, and magnesium. Therefore, the sewage sludge used in the reclamation process of post-mining soils met the criteria regarding the content of heavy metals required for soil-applied sewage sludge for reclamation for non-agricultural purposes (Cd, Cu, Ni, Pb, Zn, Hg, and Cr).

Table 1. The characteristic of applied remediation technique and characteristic of studies post-mining areas.

Characteristic	Limestone Post-Mining Area (S1)	Lignite Post-Mining Area (S2)
Assisted remediation technique	Remediation with embankment collected in the mining industry	Remediation with an initial application of sewage sludge mixed with seeds (<i>Trifolium repens</i> , <i>Trifolium pratense</i> , <i>Lolium multiflorum</i> , <i>Lolium perenne</i> , <i>Agrostis stolonifera</i>). Sewage sludge was produced in the municipal wastewater treatment plant serving the administrative buildings of the mining facility and contained >10% of solids. Dose of hydrated sewage sludge: 30 Mg ha ⁻¹
Type of remediation	Afforestation	Afforestation
Plantation	Scots pine (<i>Pinus silvestris</i>), silver birch (<i>Betula verrucosa</i>), and grey alder (<i>Alnus incana</i>)	Scots pine 45% (<i>Pinus silvestris</i>), black alder 30% (<i>Alnus glutinosa</i>), and pedunculate oak 25% (<i>Quercus robur</i>).
Topography	Lowland, locally changed by open-cast mining	Lowland, locally changed by open-cast mining
Average annual temperature	9.8 °C	9.1 °C vegetation period is above 220 days
Average annual rainfall	736 mm	707 mm
Vegetation period	above 220 days	between 210–217 days

In both post-mining areas, the four differentially advanced remediated areas were selected. This selection has been conducted assuming the approximate advancement of remediation in both post-mining areas. For the lignite post-mining area selected sites where remediation began in 2019 (6 ha), 2013 (10 ha), 2007 (5.5 ha), and 2002 (7 ha), while for limestone post-mining area selected sites with remediation beginning in 2019 (4 ha), 2014 (10 ha), 2008 (3.5 ha), and 2002 (6 ha).

The sampling was conducted in spring in April 2022. The soil samples were collected from soil depths of 0–15 cm, and 15–30 cm (Figure 1). For each study site, 12 separate soil samples were carefully collected taking into account the topography of the area (Figure 2). The sampling was conducted in the same weather conditions at both studied post-mining areas. Before the soil collection, litter and debris on the ground surface were removed. The sampling was carried out at a similar distance between samples with separate study

sites. The average volume of each soil sample was 1 dm³. All visible gravel and roots were removed from the soil samples, and a part of the soil sample was air-dried, grounded, and sieved with a diameter of <2 mm before to measuring its physicochemical properties. A part of a fresh soil sample was immediately used for biological analyses. The description of soil samples was shown in Table 2.



Figure 1. The sampling at different ranges of soil depth.

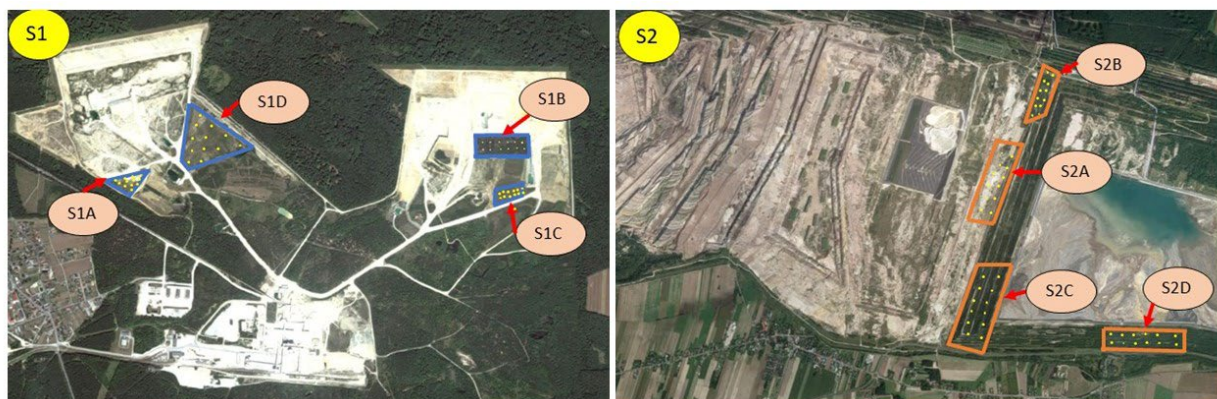


Figure 2. Localization of study sites and sampling scheme. S1—limestone open-cast mining remediated with embankment; S2—lignite open-cast mining remediated with sewage sludge. Subsequent letters A–D means study sites with different remediation advancement. Photos from satellite map. Source: Google Maps.

Table 2. The characteristic of soil samples.

Soil Sample	Study Area	Remediation Duration [Years]	Soil Depth Range of Sampling [cm]
S1A15	Limestone post-mining area	3	0–15
S1A30			15–30
S1B15		8	0–15
S1B30			15–30
S1C15		14	0–15
S1C30			15–30
S1D15		20	0–15
S1D30			15–30
S2A15	Lignite post-mining area	3	0–15
S2A30			15–30
S2B15		9	0–15
S2B30			15–30
S2C15		15	0–15
S2C30			15–30
S2D15		20	0–15
S2D30			15–30

2.1. *In Situ* Analyses

Simultaneously with the sampling, an *in situ* analysis of soil gas exchange analysis was performed using LCpro T[®] (ADC BioScientific, Hoddesdon, UK). In this analysis, an original adapter for the device dedicated to soil testing was used. The adapter consisted of a 1 dm³ soil chamber with a detachable collar with an area of 97.5 cm². The applied adapter ensures the maintenance of stable research conditions. The analysis was conducted in the same scheme as sampling. *In situ* study allowed us for determining Net Carbon Exchange Rate (NCER) with a soil respiration (Ce). In the study, the calculations of NCER and Ce has been performed according to the previous study [50,59].

2.2. Soil Physico-Chemical Properties

The pH value in soil samples was measured with pH meter (both in H₂O and 1 M KCl). The procedure of pH measurement applied in the study was by a standard procedure ISO 10390:2005. Soil samples were tested for calcium carbonate concentration with the use of the Scheibler apparatus according to the volumetric Scheibler method [60]. The total Kjeldahl nitrogen has been conducted under Polish standard PN-ISO 10694:2002. The measurement has been preceded by the mineralization using 95% H₂SO₄. The Kappen method has been used for calculation of sorption capacity [61].

2.3. TC, SOC, LOI, POXC

The total carbon (TC), SOC, LOI and POXC were measured according to the following methodologies:

- Total C—Polish standard PN-ISO 10694:2002 [62] (after dry combustion);
- Soil Organic Carbon—Tyurin method with K₂Cr₂O₇ as an oxidant for organic compounds from the soil [63];
- Loss of ignition (LOI)—Krogstad method [64];
- The active carbon was determined as a permanganate oxidizable organic carbon (POXC) with Weil et al. [65] method.

The soil samples were tested for the composition of carbon forms in the soil. It was determined with the method of ¹³C NMR on a high-field spectrometer Bruker Avance III 300.13 MHz equipped with a broadband CP-MAS 4 mm probe. The analysis was performed in 300 K with a sample rotation of 8 kHz. The ¹³C NMR spectra were referenced to the low field signal of adamantane (¹³C NMR: $\delta = 38.48$ ppm). The following four regions and C types were considered: alkyl-C (C_{AL}) (0–45 ppm), O-alkyl (C_{OAL}) (45–110 ppm), aromatic C (C_{AR}) (110–160 ppm), and carbonyl C (C_{CN}) (160–210 ppm), followed by the calculation of four the most important indicators for C stability: hydrophobicity (HB), aromaticity (AR), and humification (HF) according to the equations previously described [59].

2.4. Microbiology, Enzyme Activity and GRSP Analysis

The fresh soil samples were studied for the determination of the microbial community: bacteria (psychrophiles, mesophiles, thermophiles). and fungi. The microbial analyses were conducted with the cultivation method using Petri dishes. In the analyses, the dilution technique was applied. The microbial cultivation was preceded by the preparation of a soil extract using 1 g of fresh soil and 9 mL of PBS buffer with a shaking method (200 rpm per 2 h). Two different media were used for bacteria and fungi—Luria Broth and Sabouraud medium were used respectively. The analyses were conducted in sterile conditions preventing contamination. After the preparation of the soil extract, a series of dilutions were made from which inoculations were made and appropriately described a Petri dishes were placed in incubators, maintaining optimal temperatures for individual groups of microorganisms. The psychrophiles were cultivated for 24 h at 15 °C, mesophiles at 36 °C, thermophiles at 45 °C, and fungi at 25 °C.

Two enzyme activities were also studied in fresh soil samples. Using spectrometric methods dehydrogenases and ureases (EC 3.5.1.5) activity were determined. The dehydro-

genase activity was measured using TTC with a method of Casida et al. [66]. The method involves incubation of the soil with colorless water-soluble tetrazolium chloride (TTC) which is reduced to colorless water-insoluble triphenyl formazan (TPF). The urease activity was measured by the phenol hypochlorite assay method [67].

The easily extractable glomalin-related soil proteins (EE-GRSP), and total glomalin-related soil proteins (T-GRSP) content were determined according to the methods of Wright et al. [68]. For the determination of GRSP 1g of the air-dried, grounded and sieved (<2 mm) soil samples were used. The extraction of EE-GRSP was conducted with 8 mL of 20 mmol sodium citrate pH = 8.0 in the autoclave at 121 °C for 30 min. The extraction of T-GRSP was conducted by repeatedly extraction of proteins with 8 mL of 50 mmol sodium citrate pH = 9.0 in the autoclave at 121 °C for 60 min. Each extraction was followed by centrifugation at 4000 rpm/min for 15 min. The extraction of T-GRSP was repeated up to the disappearance of the red-brown color characteristic of glomalin. The GRSP was measured in the extracts with the Bradford method [69]. Bovine albumin was used as a standard.

2.5. Statistical Analysis

Each analysis were performed in triplicates. The results are shown as a mean value with standard deviation. The analysis of variance (ANOVA) with a Tukey's test to evaluate the significance of differences of the means at a significance level of $p \leq 0.05$. Statistical analyses were performed using Origin 8 software. The statistical analyses were performed individually between studied areas for different soil depths. For example, for the area S1, the statistic was performed together for the soils S1A15 and S1A30, and for the area S2 together for soils S1B15 and S1B30, and for the rest of studied soils identically scheme. Separate statistical analyzes are indicated by different letters of the alphabet as follow: a for S1A, b for S1B, c for S1C, d for S1D, e for S2A, f for S2B, g for S2C, and h for S2D. Statistically significant differences signed by the capitalization. The only exception is for analyses of Ce and NCER where the statistical analyses were performed for all studied soils around the study area (S1 or S2), due to the nature of the analyzes. In this case the different letters (small for S1, and large for S2) indicate separate statistical analyses.

3. Results

3.1. Physico-Chemical Soil Properties

The sorption capacity was not show statistically significant changes between soil depth, however, we noticed that for S1 at areas remediated, longer sorption capacity is higher in comparison to the shorter remediated areas (Table 3). In turn, for S2 sorption capacity showed a decreasing tendency with a length of remediation time. The pH in H₂O indicated statistically significant changes between soil depth only in the longest remediation for S1 (decrease in deeper soil), and for the shortest (decrease in deeper soil), and the longest (increase in deeper soil) remediation for S2. The pH in KCl showed statistically significant changes in the longest remediation for both studied soil (increase).

Table 3. Physical properties of soils collected from limestone open-cast mining (S1), and lignite open-cast mining (S2), from 4 differentially advanced in remediation areas (A–D) from the top layer of 0–15 cm (15) and sublayer of 15–30 cm (30). The letters next to the results indicate the statistical analysis, and the case of the letters determines the significance of the statistical differences within one remediation advancement of the soil ($p < 0.05$) according to Tukey's test. Different letters indicate separate statistical analyses. Results shown as a mean values \pm SD, $n = 3$.

Sample	Sorption Capacity [cmol(+)/kg]	pH	
		H ₂ O	KCl
S1A15	17.43 \pm 0.51 a	7.61 \pm 0.02 a	7.15 \pm 0.03 a
S1A30	16.23 \pm 0.41 a	7.51 \pm 0.01 a	7.11 \pm 0.02 a
S1B15	20.51 \pm 0.72 b	7.81 \pm 0.09 b	7.32 \pm 0.06 b
S1B30	21.17 \pm 1.12 b	7.72 \pm 0.02 b	7.32 \pm 0.04 b

Table 3. Cont.

Sample	Sorption Capacity [cmol(+)/kg]	pH	
		H ₂ O	KCl
S1C15	20.31 ± 1.86 c	7.67 ± 0.06 c	7.49 ± 0.04 c
S1C30	22.16 ± 2.08 c	7.78 ± 0.03 C	7.61 ± 0.02 C
S1D15	18.74 ± 1.14 d	7.68 ± 0.03 d	7.52 ± 0.04 d
S1D30	18.94 ± 2.21 d	7.51 ± 0.03 D	7.62 ± 0.05 D
S2A15	16.45 ± 1.17 e	7.70 ± 0.01 e	7.33 ± 0.01 e
S2A30	17.51 ± 2.20 e	7.38 ± 0.02 E	7.38 ± 0.02 e
S2B15	13.01 ± 1.16 f	7.86 ± 0.04 f	7.86 ± 0.03 f
S2B30	12.98 ± 0.98 f	7.89 ± 0.05 f	7.89 ± 0.06 f
S2C15	4.67 ± 0.036 g	7.64 ± 0.02 g	7.51 ± 0.05 g
S2C30	4.82 ± 0.33 g	7.72 ± 0.01 g	7.71 ± 0.07 g
S2D15	5.78 ± 0.09 h	7.07 ± 0.04 h	6.96 ± 0.05 h
S2D30	6.02 ± 0.22 h	7.52 ± 0.05 H	7.55 ± 0.01 H

The CaCO₃ content was generally much higher in the S1 in comparison to the S2 (Figure 3). Therefore, the CaCO₃ content did not show statistically significant changes between soil depth for all studied areas at S1, however, we noticed significant changes for two the shortest remediation time (S2A, S2B). The CaCO₃ content was higher in deeper soil for S2A and S2B. For S2B the CaCO₃ content increased with a soil depth 4 times reaching almost 2%. The CaCO₃ content was generally lower in the longer remediated areas (for 20 years) in comparison to the areas remediated for 3 years (the shortest).

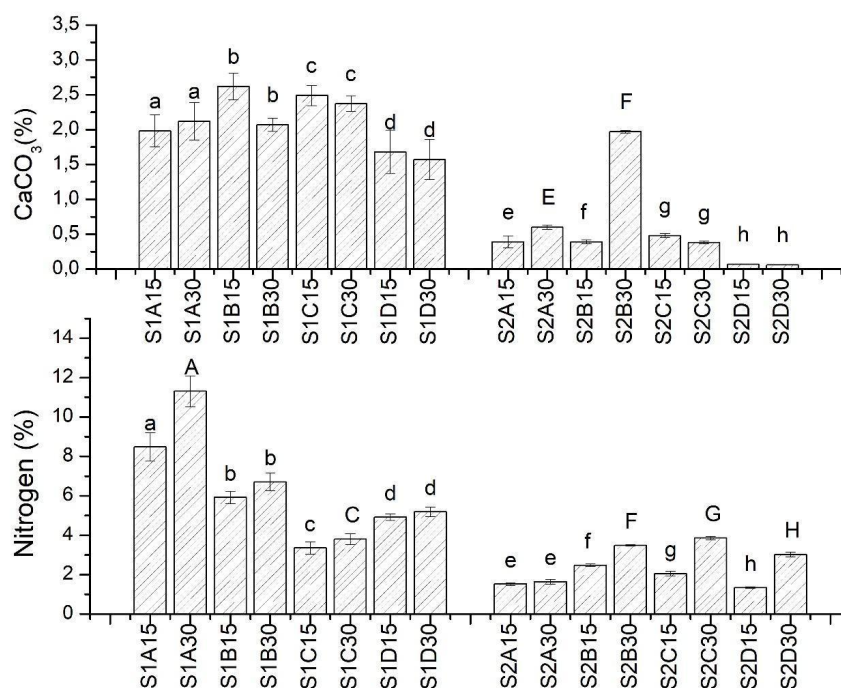


Figure 3. Calcium carbonate and nitrogen content in soils collected from limestone open-cast mining (S1), and lignite open-cast mining (S2), from 4 differentially advanced in remediation areas (A–D) from the top layer of 0–15 cm (15) and sublayer 15–30 cm (30). The letters next to the results indicate the statistical analysis, and the case of the letters determines the significance of the statistical differences within one remediation advancement of the soil ($p < 0.05$) according to Tukey's test. Different letters indicate separate statistical analyses for both sampling soils. Results shown as a mean values ± SD, $n = 3$.

Similarly to the CaCO_3 , the total nitrogen (TN) content was higher in the S1 compared to the S2. For S1 we observed a statistically significant change (increase) in TN content between soil depth for S1A, and S1C. Generally, the TN content for S1 showed a decreasing tendency with a remediation length. In turn, for S2, we noted statistically significant changes in TN content for S2B, S2C, and S2D. The TN content increased with soil depth in mentioned studies areas. The TN content for S2 tends to increase with remediation time, except the longest remediation time, where we observed a slight decrease in TN content. The areas S2A and S2D had a similar TN content for the soil depth of 0–15 cm, but they significantly differed in the soil depth of 15–30 cm where S2D showed a much higher TN content in comparison to S2A.

3.2. CO_2 Emissiones

The soil respiration (Ce) was generally negative for all studied soil either for S1 or for S2 (Figure 4). For S1 we observed statistically significant changes between S1A, and S1B, where S1B was lower in comparison to S1A. Therefore, for S1C and S1D the Ce value was slightly lower compared to the S1A. The Ce was generally much lower for S2 in comparison to S1. Therefore, we observed statistically significant changes in Ce for remediation time. The Ce was significantly lower for S2C and S2D in comparison to S2A and S2B.

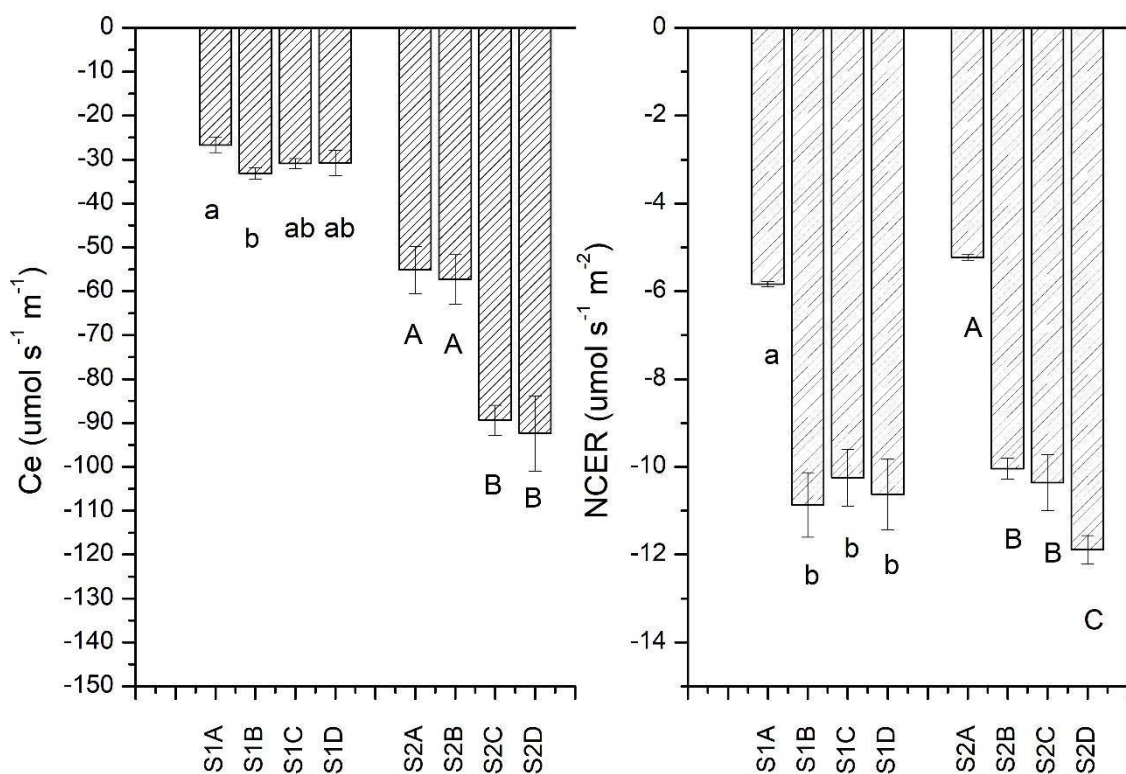


Figure 4. Soil respiration (Ce) and Net CO_2 emission rate (NCER) in soils collected from limestone open-cast mining (S1), and lignite open-cast mining (S2), from 4 differentially advanced in remediation areas (A–D). The letters next to the results indicate the statistical analysis, and the case of the letters determines the significance of the statistical differences within one remediation advancement of the soil ($p < 0.05$) according to Tukey's test. Different letters indicate separate statistical analyses. Results shown as a mean values \pm SD, $n = 3$.

The net CO_2 emission rate (NCER) decreased in soil with more advanced remediation in comparison to the soil remediated for 3 years for both studied soils. The statistically significant changes were observed for S1B, S1C, and S1D in comparison to S1A. For soil remediated for 14 and 20 years, the NCER for S1 did show any significant changes. For S2, the decrease in NCER was observed for S2B, S2C, and S2D compared to S2A. However,

S2D showed a lower NCER, and additionally indicated a statistically important decrease in comparison to S2B, and S2C. Generally, for both soils, the tendency of NCER was similar, showing a significant decline in soils remediated for 8 (for S1) or 9 (for S2) years of remediation.

3.3. Biological Properties

In the study, we noticed that in the microbial community, the mesophilic bacteria represented the most numerous groups (Figure 5). Generally, the microbial community was significantly higher in S2 in comparison to S1. For S1 did not observe colonies for each soil sample. The presence of psychrophilic microorganisms was observed only for S2B15, S2C15, S2C30, and S2D30. For S2 psychrophiles were observed in all studies of soil samples. Generally, in deeper soil (15–30 cm) the psychrophiles were more numerous in all studied areas for S2 in comparison to the soil depth of 0–15 cm. This difference was the highest for soil remediated for 15 and 20 years (S2C, and S2D respectively). For limestone post-mining soil (S1), the mesophilic microorganisms were generally most numerous in the soil depth of 15–30 cm with the exception of S1A. The soils with more advanced remediation showed a high significance in the increase of the number of mesophilic bacteria in a deeper part of the soil. Generally, the mesophilic bacteria's community tends to increase either with soil remediation advancement or with soil depth, however, this increase was more substantial for lignite post-mining soil (S2). The significant changes between soil depth were observed for S2A, S2B, and S2C, while, for S2D, the number of mesophiles was similar for both studied soil depth but remained much higher in comparison to the rest of studies soil samples from lignite post-mining soil. The thermophiles were more numerous for S2 in comparison to S1. We observed an increase in the number of thermophilic bacteria with a soil depth for S1C and S1D, while for S1A, and S1B this number was lower for a soil depth of 15–30 cm. The opposite tendency was noticed for lignite post-mining soil (S2). For S2, the number of thermophiles in the shorter remediated soil (S1) was much lower in both studies' soil depths in comparison to the rest of the studied area. For all study areas of S2, except S2A, the community of thermophiles was much higher in the soil depth of 15–30 cm (10.5 times higher for S2B). Moreover, we saw that with a remediation advancement, the number of thermophiles slightly decreased in both studies of soil depths. The fungi were the less numerous microbial group in the studied soils, and their number was much lower in S1 in comparison to S2. For S1 we noticed significant changes between soil depth only for S1D, where the number of fungi was 17 times higher for S1D30 compared to S1D15, and was higher for all studies of soil samples of S1. For lignite post-mining soil (S2) we noticed a significant increase in the community of fungi in soil depth of 15–30 cm compared to the depth of 0–15 cm. A higher increase was observed for S2C, where the number of fungi was about 37 times higher in the soil depth of 15–30 cm compared to 0–15 cm. Generally, the number of fungi tends to decrease in the top layer of the soil for S2 with the remediation advancement and was the lowest for the area remediated for 20 years. In that soil, we also observed the lowest differences between the number of fungi in samples collected from two studied soil depths.

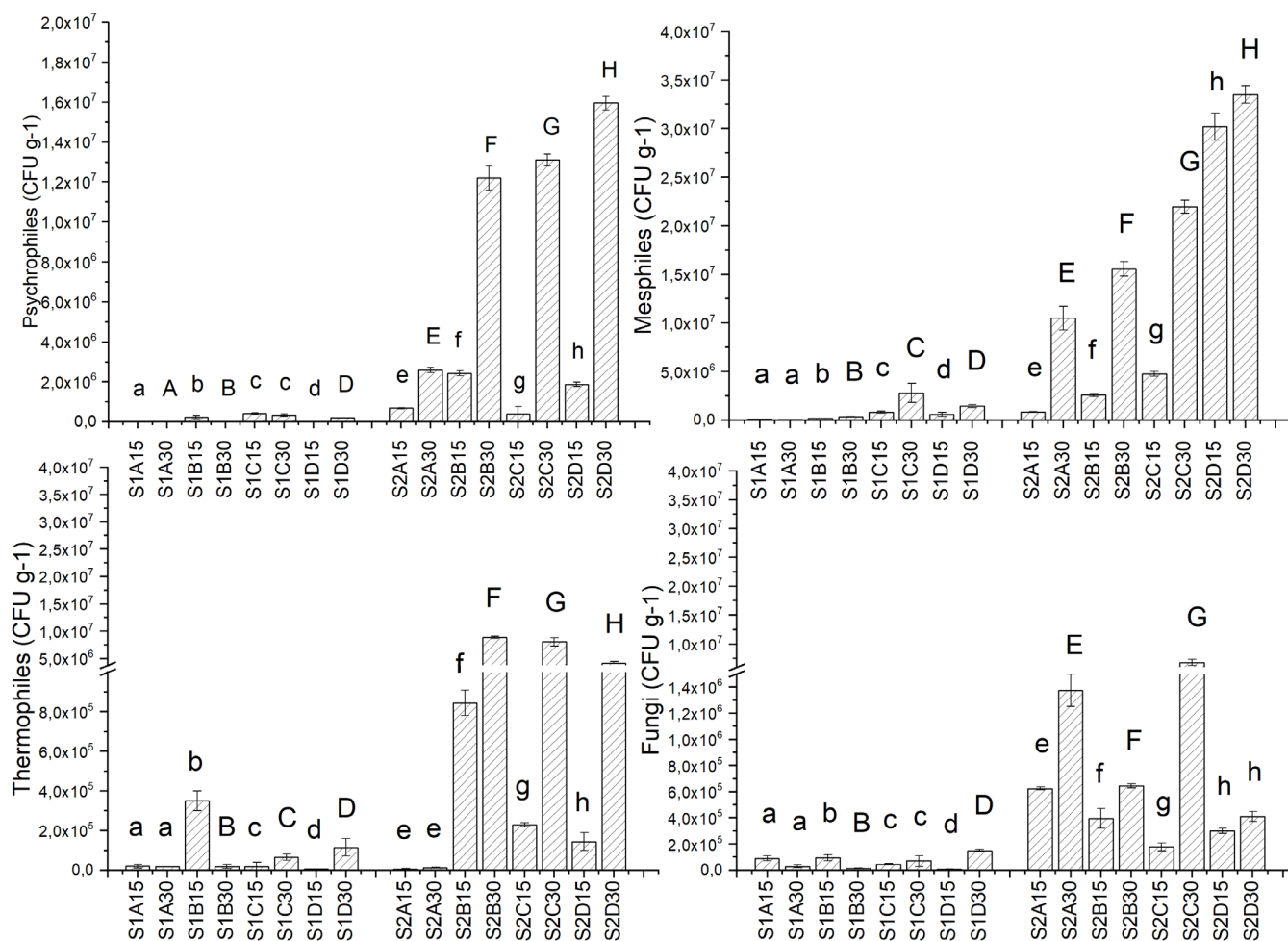


Figure 5. The community of selected microorganism groups in the soils collected from limestone open-cast mining (S1), and lignite open-cast mining (S2), from 4 differentially advanced in remediation areas (A–D) from the top layer of 0–15 cm (15) and sublayer of 15–30 cm (30). The letters next to the results indicate the statistical analysis, and the case of the letters determines the significance of the statistical differences within one remediation advancement of the soil ($p < 0.05$) according to Tukey’s test. Different letters indicate separate statistical analyses. Results shown as a mean values \pm SD, $n = 3$.

In the study, we noticed an interesting behavior of GRSP between soil depths in both studied soils (Figure 6). For S1, we observed statistically significant changes only for S2B and S2C, while for S1A, and S1D the GRSP remained statistically at the same level. In S2B we found a slight increase in GRSP while in S1C the GRSP were lower in soil depth of 15–30 cm in comparison to 0–15 cm. Generally, the GRSP tends to decrease with the soil remediation advancement in limestone post-mining soil (S1). For S2 we observed statistically significant changes between GRSP content in different soil depths for all studied areas. While for S2A and S2D, the total GRSP content was higher for deeper soil parts, for S2B, and S2C it was higher in a depth of 0–15 cm. Generally, except for S2A30, the total GRSP content was similar for all studied soil for the same soil depths.

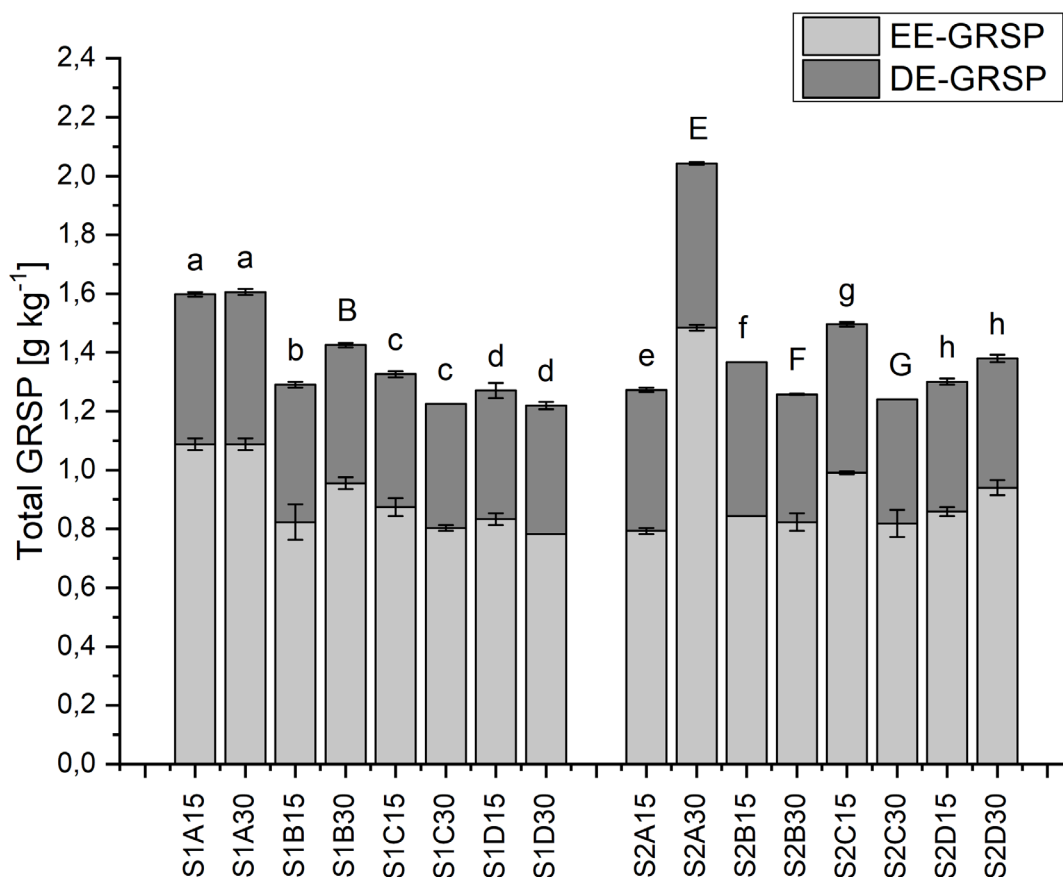


Figure 6. Total content of glomalin-related soil proteins (GRSP) in soils collected from limestone open-cast mining (S1), and lignite open-cast mining (S2), from 4 differentially advanced in remediation areas (A–D) from the top layer of 0–15 cm (15) and sublayer of 15–30 cm (30). The letters next to the results indicate the statistical analysis, and the case of the letters determines the significance of the statistical differences within one remediation advancement of the soil ($p < 0.05$) according to Tukey's test. Different letters indicate separate statistical analyses. Results shown as a mean values \pm SD, $n = 3$.

The activity of ureases was generally higher for S1 (Figure 7). We observed higher activity of urease in soil samples from deeper soil parts for S1A and S1B, however, for S2C and S2D this activity was much lower in the soil depth of 15–30 cm in comparison to 0–15 cm. For S2, the activity of ureases was higher for deeper soil parts in the areas of S2B and S2D. The areas S1 did show no significant changes in urease activity between soil depth. For S2C the urease activity was higher for the soil depth of 0–15cm.

The activity of dehydrogenases, similar to ureases, was higher for S1 in comparison to S2 (Figure 7). For limestone post-mining soil (S1) the activity of dehydrogenases tends to decrease with a soil depth (except S2D) as well as with a remediation advancement. For S2 we noticed an increasing dehydrogenase activity in the studied soil samples. While for the shortly remediated soil (S1A) we observed any changes in the activity of dehydrogenases, it altered in the rest of the studied areas and soil depths. For S2B and S2C the activity of dehydrogenases decreased with a soil depth, while for S2D it increased.

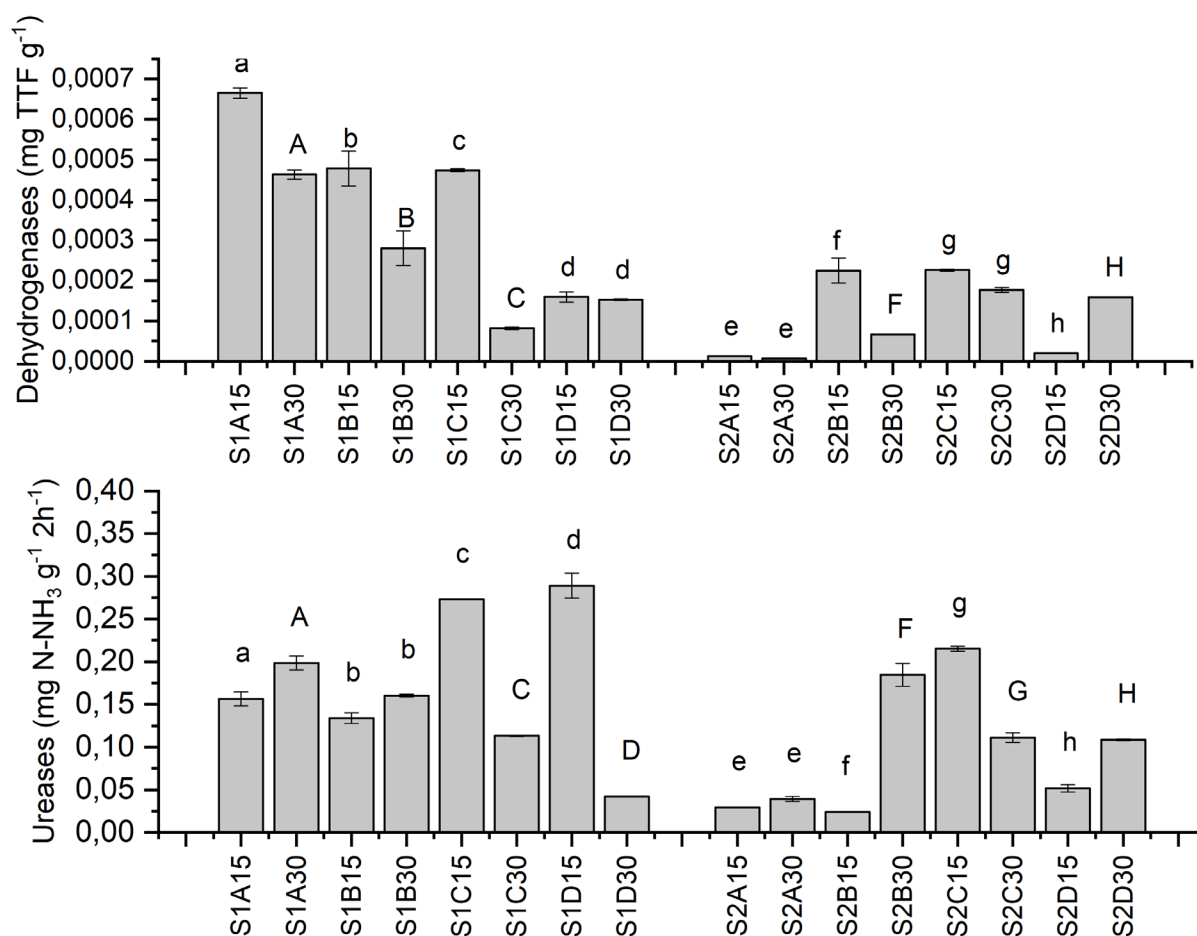


Figure 7. Activity of ureases and dehydrogenases in soils collected from limestone open-cast mining (S1), and lignite open-cast mining (S2), from 4 differentially advanced in remediation areas (A–D) from the top layer of 0–15 cm (15) and sublayer of 15–30 cm (30). The letters next to the results indicate the statistical analysis, and the case of the letters determines the significance of the statistical differences within one remediation advancement of the soil ($p < 0.05$) according to Tukey's test. Different letters indicate separate statistical analyses. Results shown as a mean values \pm SD, $n = 3$.

3.4. Soil Carbon Pool

The soil organic carbon (SOC) for S1 we noticed statistically significant differences in all studies areas except S1A (Figure 8). The SOC in the shortly remediated areas (S1A) was the highest in comparison to other studied areas with different advancements of remediation. While for S1B. and S1C the SOC content in the soil depth of 15–30 cm was lower in comparison to the layer of 0–15 cm, for S1D we observed an increase of SOC content with a soil depth. In the case of lignite post-mining soil (S2), we found statistically significant changes for each studied area. However, the highest content of SOC was observed for S1A with the shortest remediation. Only in this area (S2A) the SOC content was significantly higher in deeper soil parts. For the areas S2B, S2C, and S2D the SOC content was lower in the soil layer of 15–30 cm compared to the soil layer of 0–15 cm. Generally, the SOC content tends to decrease with the remediation advancement for both studied post-mining soils.

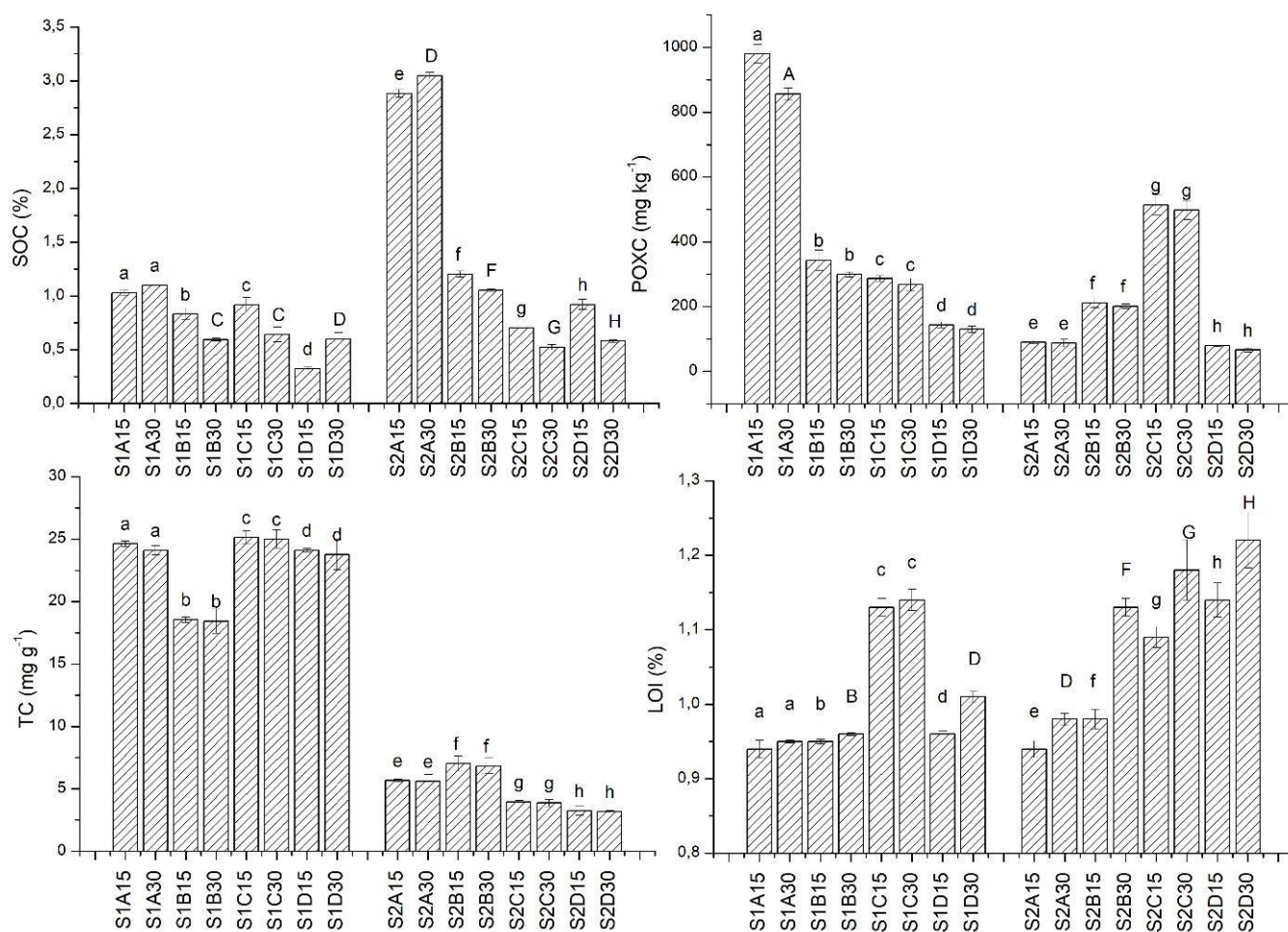


Figure 8. Soil organic carbon (SOC), permanganate oxidizable organic carbon (POXC), total carbon (TC) and loss of ignition (LOI) in soils collected from limestone open-cast mining (S1), and lignite open-cast mining (S2), from 4 differentially advanced in remediation areas (A–D) from the top layer of 0–15 cm (15) and sublayer of 15–30 cm (30). The letters next to the results indicate the statistical analysis, and the case of the letters determines the significance of the statistical differences within one remediation advancement of the soil ($p < 0.05$) according to Tukey's test. Different letters indicate separate statistical analyses. Results shown as a mean values \pm SD, $n = 3$.

The POXC, considered as an active carbon in the soil, was the highest for S1A at limestone post-mining soil at both studied soil depths (Figure 8). For S1 we noted a statistically significant decrease in POXC with a soil depth only for S1A. For other studied areas at limestone post-mining soils (S1), we observe any alteration. The POXC tends to decrease with a remediation advancement for S1, and for the soil remediated for 20 years (S1D) was the lowest. For lignite post-mining soil (S2) any statistically significant changes in POXC content between soil depth were noticed. The POXC increased with a remediation advancement in comparison to S2A, however, it decreased for the longest remediation areas (S2D) to the level of S2A.

Total carbon (TC) was generally higher (about 5 times) for limestone post-mining soil (S1) compared to the lignite post-mining soil (S2) (Figure 8). We observed any statistically significant changes between soil depth for all studied areas and both post-mining soils. The TC content for S1 was the lowest for the area remediated for 8 years (S1B), while in the rest of the studied areas, it remained at a similar level of about 25 mg g^{-1} . In contrast to S1, at lignite post-mining soil the TC concentration was the highest for the area remediated for 9 years (S2B). The TC content tends to decrease with a remediation stage and was the lowest for the area remediated for 20 years (S2D).

The loss of ignition (LOI) was generally very low for both studied post-mining soils (Figure 8). For S1 we found statistically significant changes in LOI content between soil depth only for the longest remediation area (S1D). The LOI content was the highest for S1C, while for the rest of the studied soil with different remediation advancements, it was at a similar level. For S2 the LOI content differed in all studied areas between soil depth and was higher in the layer of 15–30 cm compared to 0–15 cm. The LOI content tends to increase with a soil remediation advancement and was the highest for S2D, and the lowest for S2A.

3.5. Heterogeneity of SOC Composition

The carbon form content differed in both studied soils. The alkyl-C is considered a stable C form in the soil. For S1 the alkyl-C content was higher in the soil layer of 0–15 cm compared to 15–30 cm (Figure 9). For S2 the differences in alkyl-C content between soil depth were lower compared to S1. For S2A the alkyl-C was lower in the deeper soil part (S2A30) in comparison to the soil layer of 0–15 cm (S2A15). For S2B the alkyl-C content was the same at both studied soil depths, while for S2C it was lower for S2C30 compared to S2C15. In contrast for the longer remediated areas (S2D), the alkyl-C content was slightly higher for S2D30 compared to S2D15. Generally alkyl-C content for S2 tends to increase with a length of remediation.

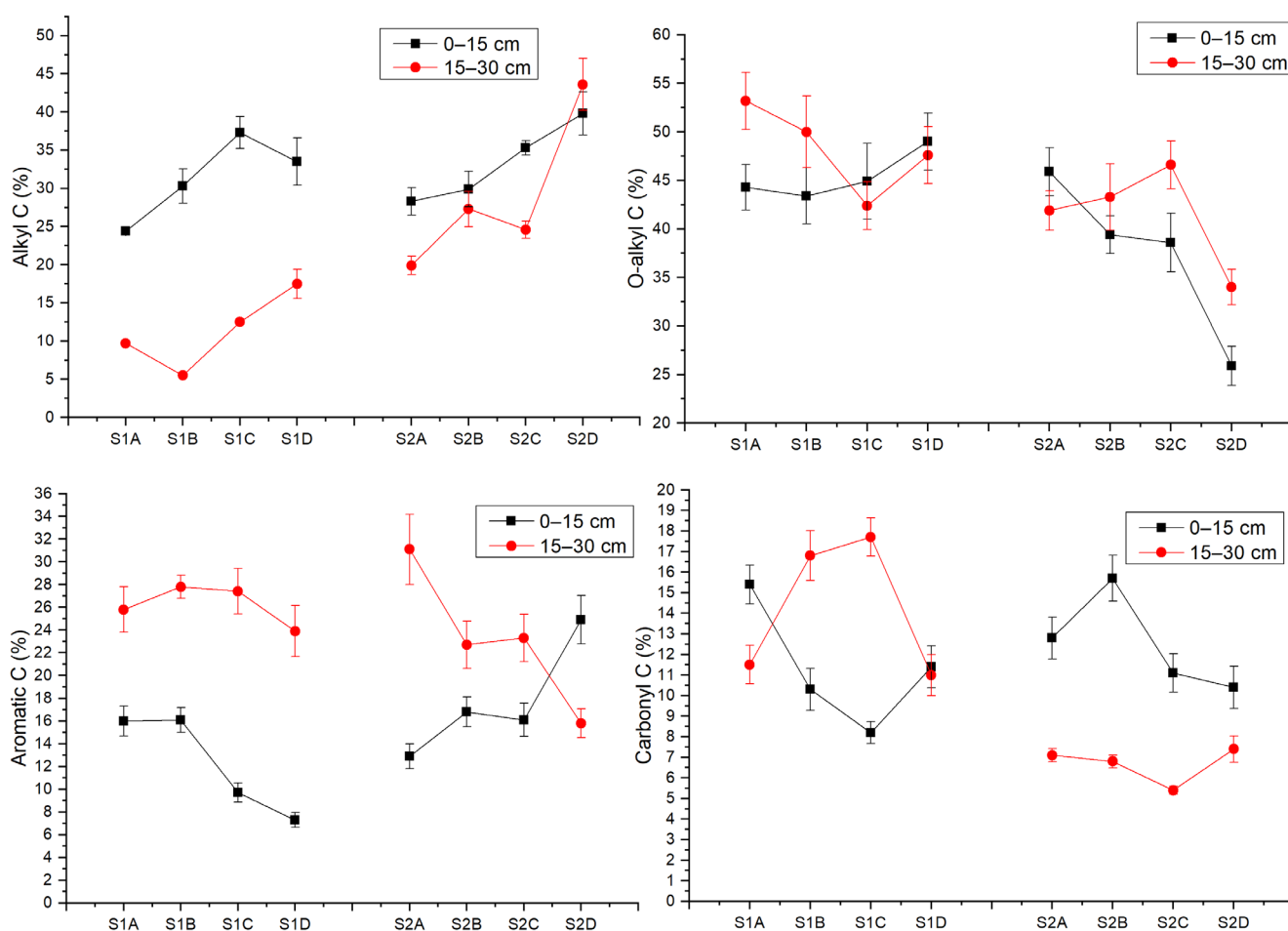


Figure 9. A percentage content of carbon forms in soils collected from limestone open-cast mining (S1), and lignite open-cast mining (S2), from 4 differentially advanced in remediation areas (A–D). The toplayer of 0–15 cm has been presented by black color, and the sublayer of 15–30 cm by red color. Results shown as a mean values \pm SD, $n = 3$.

O-alkyl C was slightly higher (for S1A, S1B) or similar (S1C, S1D) for the layer of 15–30 cm compared to 0–15 cm (Figure 9). For S2 we observed that O-alkyl C content was the highest for the shortly remediated area (S2A), and the lowest for the longer remediated area (S2D). The O-alkyl C was lower in S2A30 compared to S2A15. For S2B we noticed no differences between O-alkyl C content in both studied depths. For S2C the content of O-alkyl C was significantly higher in S2C30 in comparison to S2C15. The highest difference between the content of O-alkyl C was noticed for the area with the highest advancement of remediation (S2D) where O-alkyl C content was higher for S2D30 compared to S2D15.

The aromatic C was higher in all samples of S1 in the layer of 15–30 cm compared to 0–15 cm (Figure 9). The aromatic C content increased for S1 with the remediation years for soil layer 15–30 cm, while it decreased with a remediation advancement in the layer 0–15 cm. For S2 we noticed an increasing content of aromatic C for each area with the remediation years. The aromatic C was higher in deeper soil parts for all studied areas except S2D where it was lower in S2D30 compared to S2D15.

The percentage share of carbonyl C in SOC was similar for both studied post-mining soils. For S1 the carbonyl C was slightly higher in S1A15 compared to S1A30 (Figure 9). For the area S1B and S1C the carbonyl C was higher in the layer of 15–30 cm (S2B30, S2C30) compared to the layer of 0–15 cm (S2B15, S2C15). For the longest remediated area at S1, we observed any differences in the content of carbonyl C between studied soil depths. For S2 we found lower carbonyl C in the soil layer of 15–30 cm compared to the layer of 0–15 cm for all studied areas. The highest content of carbonyl C was for the S2B15 ($15.7 \pm 1.12\%$). For the areas S2B we observed the biggest differences in the content of carbonyl C between both studied soil depths.

For the area S1, and S2 (except S2D15, and S2D30) in all studied areas in both soil layers the O-alkyl C was a dominant form of C.

3.6. SOC Stability

In the study, four important indicators for soil carbon sequestration were calculated. For hydrophobicity for S1, we noticed statistically significant differences between studied soil layers for S1A, S1B, and S1C (Figure 10). In all mentioned areas, the hydrophobicity was lower in the deeper soil layer of 15–30 cm compared to the layer of 0–15 cm. For the area S1D, the hydrophobicity was similar for both studied soil depths. The hydrophobicity in S1 did not alter between areas with different soil remediation advancements. For S2, we noticed statistically significant differences in hydrophobicity between soil depths, and it was higher in a deeper soil layer for S1A, and S1B, and lower for S1C, and S1D. The hydrophobicity tends to increase with remediation years for lignite post-mining soil (S2) and was the highest for S2D (above 2 times higher for S1D15 compared to S1A15).

We observed statistically significant differences in aromaticity for all studied soils (Figure 10). For S1 the aromaticity was much higher in a deeper soil (15–30 cm) for all studied areas. The aromaticity tends to decrease with remediation years for S1 in a soil depth of 0–15 cm, while for a depth of 15–30 cm, it was similar for all studied remediation advancements. For S2 The aromaticity was higher in a deeper soil for all studies areas except S2D. The highest differences in aromaticity between soil depth were observed for the areas with the shortest remediation years (S2A) where aromaticity was 2 times higher for S2A30 compared to S2A15. The aromaticity tends to increase in soil layers of 0–15 cm with a remediation advancement. In contrast, in soil layers of 15–30 cm, the automaticity decreased with remediation years.

The humification was lower for a deeper soil layer of 15–30 cm compared to the layer of 0–15 cm for all studied post-mining areas (Figure 10). For S2 the humification tends to increase (except S1D) with remediation years in a soil layer of 0–15 cm. For S1D15 the humification decreased with remediation years compared to S1C15 reaching a level similar to S1B15. For the soil depth of 15–30 cm the humification at S1 tends to increase (except S1B). The humification for S2 was higher in a soil depth of 0–15 cm compared to 15–30 cm for all studied areas. For S2 we observed a gradual increase of humification with a remediation

advancement for both studied soil depths (except S2C30). The highest humification was noticed for S2D for both soil depths.

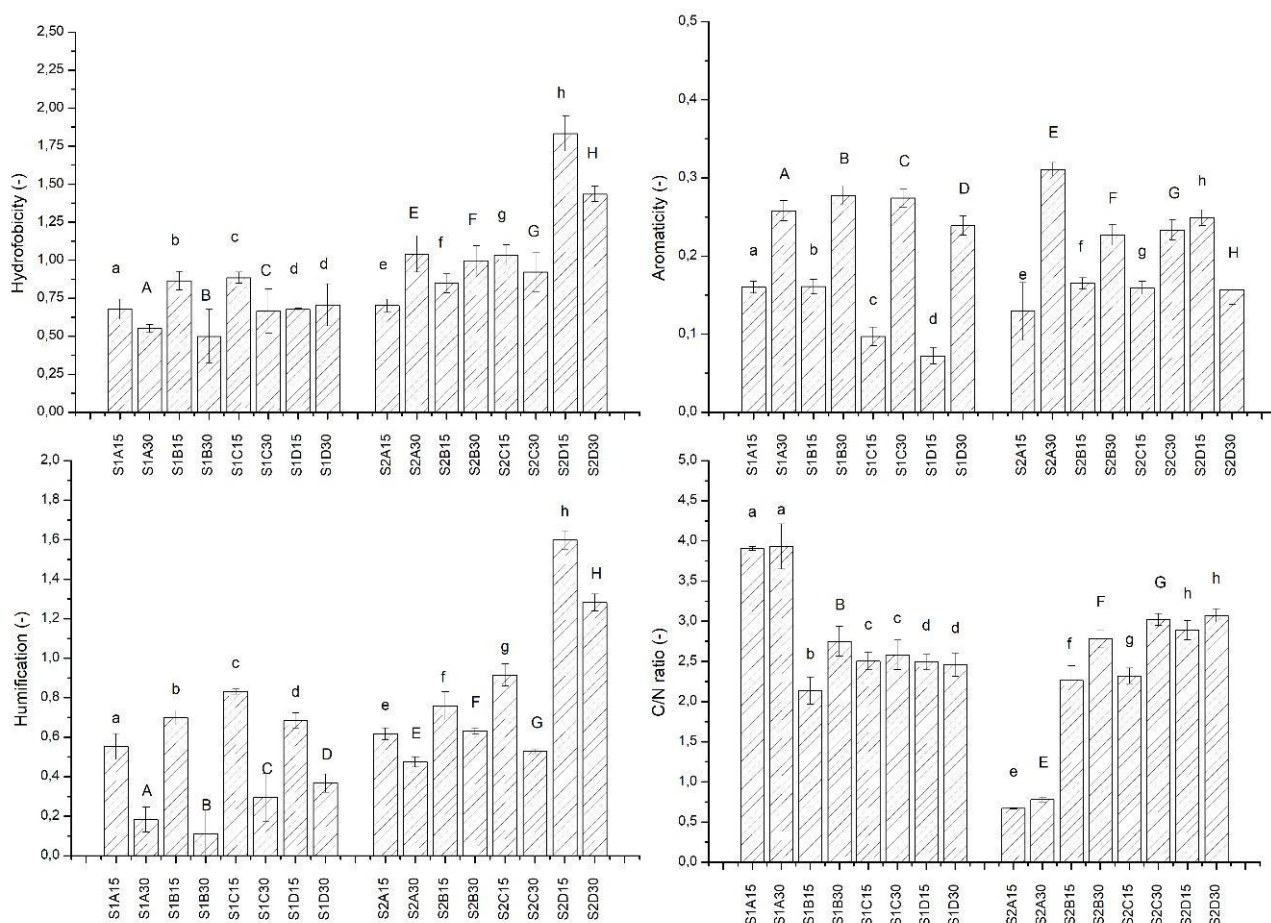


Figure 10. Hydrophobicity, aromaticity, humification and C/N ratio for soils collected from limestone open-cast mining (S1), and lignite open-cast mining (S2), from 4 differentially advanced in remediation areas (A–D) from the top layer of 0–15 cm (15) and sublayer of 15–30 cm (30). The letters next to the results indicate the statistical analysis, and the case of the letters determines the significance of the statistical differences within one remediation advancement of the soil ($p < 0.05$) according to Tukey's test. Different letters indicate separate statistical analyses. Results shown as a mean values \pm SD, $n = 3$.

The C/N ratio for S1 showed statistically significant differences between soil depths only for the area S1B, where the C/N ratio was higher in deeper soil 15–30 cm compared to 0–15 cm (Figure 9). The C/N ratio was the highest for area S1A, and decreased with soil remediation years in S1B, S1C, and S1D compared to S1A, however, after this decline after 8 years of remediation it remained stable for S1C and S1D. For S2 we noted statistically significant differences for S2A, S2B, and S2C between soil depths. The C/N ratio was higher in deeper soils (15–30 cm) compared to 0–15 cm for all mentioned areas. In contrast to S1, for S2 we observed that the C/N ratio was the lowest for S2A, and tends to increase for S2B followed by a stabilization for the rest of the studied remediation advancements.

3.7. Model for Estimation of a Vertical Variability of SOC in Post-Mining Soils

In the study, we found that according to the calculation of hydrophobicity which corresponds to the product of stable and labile forms of coal in the soil it is possible to calculate the vertical degree of carbon stabilization in the soil. After calculating the

hydrophobicity value in individual soil layers, we calculated the product of their values according to the formula:

$$HF_{\Delta depth} = \frac{HF_{15-30\text{ cm}}}{HF_{0-15\text{ cm}}}$$

where “ $HF_{15-30\text{ cm}}$ ” means the hydrophobicity for the soil layer of 15–30 cm, and “ $HF_{0-15\text{ cm}}$ ” means hydrophobicity for a soil layer of 0–15 cm.

Next, we included the remediation time according to the formula:

$$VSOC_{index} = HF_{\Delta depth} \times t$$

where “ t ” means the time of remediation [years].

Proceeding in this way, we obtained the regularities for both studied post-mining soils: for S1 we noticed an exponential function of VSOC time variability index based on hydrophobicity (VSOC index), while for S2 we observed a logarithmic dependence of VSOC index on reclamation time (Figure 11).

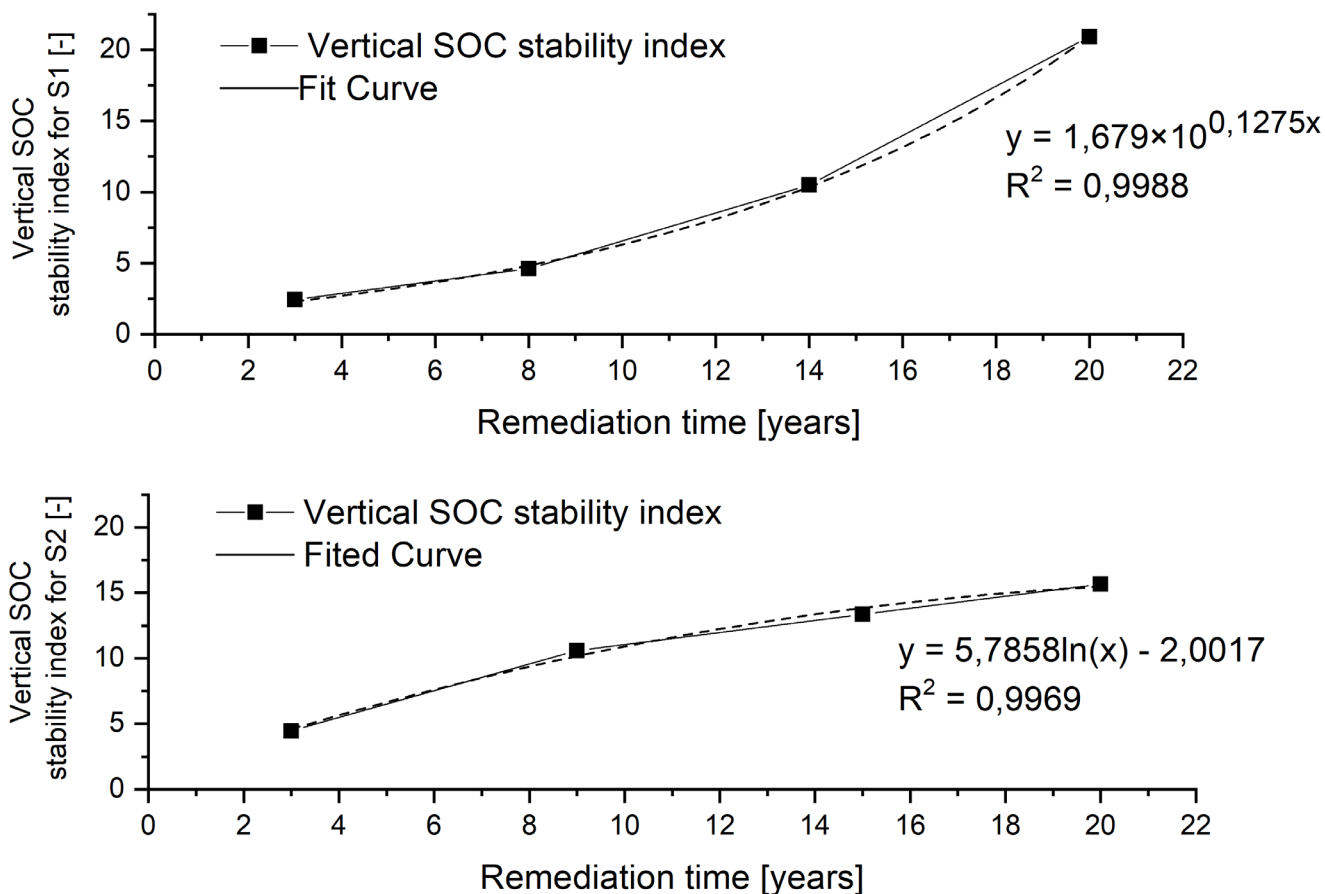


Figure 11. The dependence of VSOC variability index based on hydrophobicity for studied soils. S1—limestone open-cast mining remediated with embankment; S2—lignite open-cast mining remediated with sewage sludge.

3.8. Mathematical Validation of Models

Observations of the predicted resilient VSOC variability index vs. experimental VSOC variability index for functions are exhibited in Figure 12. The VSOC variability index provides accurate assessments of the destination values. On the other hand, VSOC variability index provides a good estimation and provides R^2 value for training and testing as 0.9971 (for S1), and 0.99628 (for S2) respectively.

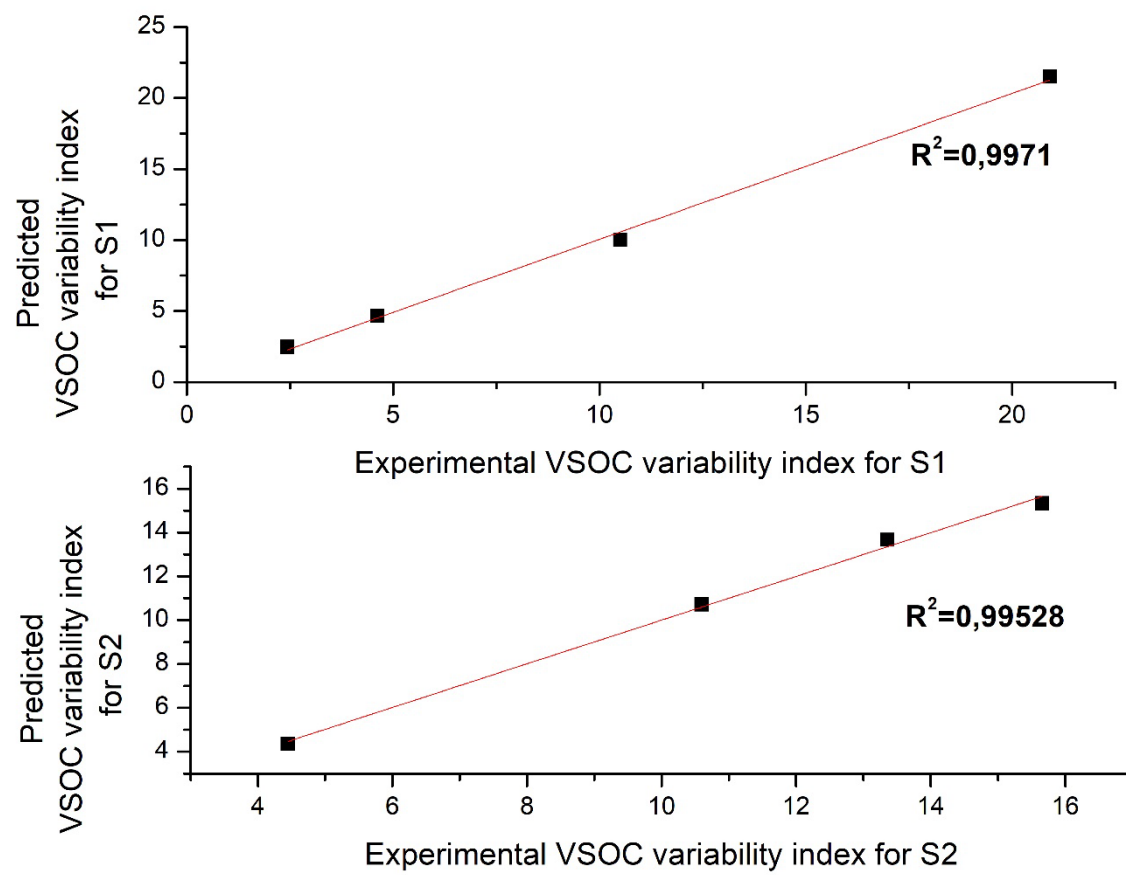


Figure 12. Regression plot of predicted VSOC variability index against experimental VSOC variability index for both studied soils. S1—limestone open-cast mining remediated with embankment; S2—lignite open-cast mining remediated with sewage sludge.

An extensive analysis of the errors has been carried out for both models for the stabilization of carbon in post-mining soils. We calculated error (E_t), percentage error (PE_t), mean error (ME), mean absolute error (MAE), mean squared error (MSE), and root mean squared error ($RMSE$) according to the equations shown in Table 4.

Table 4. Equations of modeling errors used in the study (y_t —the value of the target variable y_t^P —variable forecast value).

Modeling Error	Equation
Error (E_t)	$E_t = y_t - y_t^P$
Percentage error (PE_t)	$PE_t = \frac{y_t - y_t^P}{y_t} \cdot 100\%$
Mean error (ME)	$ME = \frac{1}{n} \sum_{t=1}^n (y_t - y_t^P)$ $t = 1, 2, 3, \dots, n$
Mean absolute error (MAE)	$MAE = \frac{1}{n} \sum_{t=1}^n y_t - y_t^P $ $t = 1, 2, 3, \dots, n$
Mean squared error (MSE)	$MSE = \frac{1}{n} \sum_{t=1}^n (y_t - y_t^P)^2$ $t = 1, 2, 3, \dots, n$
Root mean squared error ($RMSE$)	$RMSE = \sqrt{MSE}$

The values of calculated errors for the proposed models are presented in Table 5. All modeling errors calculated for the proposed index were generally very low indicating good suitability, and correctness of forecasting.

Table 5. Calculated errors for the proposed models.

Model		E_t	PE_t	ME	MAE	MSE	$RMSE$
VSOC variability index for S1	S1A	−0.02545	−1.0447	−0.03929	2.359535	0.147499	0.384055
	S1B	−0.04074	−0.88277				
	S1C	0.494683	4.7109				
	S1D	−0.58564	−2.79977				
VSOC variability index for S2	S2A	0.094081	2.114781	−0.00007	1.888384	0.055306	0.235173
	S2B	−0.11335	−1.06961				
	S2C	−0.30622	−2.29203				
	S2D	0.325198	2.07712				

4. Discussion

4.1. Physicochemical Fluctuation in Top Layer and Sublayer of Post-Mining Remediated Soil with Remediation Age

In the study, we observed that there are no changes in sorption capacity between soil depths at limestone post-mining soil remediated with embankment (S1), as well as at lignite post-mining soil remediated with the application of sewage sludge (S2). The initial increase of sorption capacity for longer remediated areas at limestone post-mining soil was followed by the decrease in the longest remediated area. It indicated the lack of stability in sorption capacity changes at post-mining soil remediated with embankment. However, the higher sorption capacity at post-mining soil remediated with embankment would decrease the ecotoxicity of the potentially present contaminants of the soil [70], lowering soil toxicity for soil microorganisms and plants [71] which promote reforestation. Therefore, the application of sewage sludge for remediation of lignite post-mining soils resulted in the decrease of sorption capacity with the remediation advancement, which is a very interesting phenomenon. Sorption capacity in the remediation with organic amendments such as sewage sludge generally tends to increase with a remediation advancement [72]. In our study, the remediation of post-mining soil with sewage sludge resulted in the decrease of sorption capacity at longer remediated areas.

The much higher content of CaCO_3 in limestone post-mining soil compared to lignite post-mining soil was most likely related to the type of host rock and the raw material extracted. The soil depth as well as remediation advancement did not influence CaCO_3 content in limestone post-mining soil. The persistent content of CaCO_3 in the soil even after 20 years of reclamation may indicate a slight transformation of this compound over the years. Thus it may indicate a small contribution of CaCO_3 to the carbon cycle at limestone post-mining soil remediated with embankment. Therefore, the fluctuations of CaCO_3 content in deeper soil parts (15–30 cm) noticed for lignite post-mining soil most likely suggest a large CaCO_3 transformation over the years. That may have been driven by organic matter in the applied sewage sludge, which may have activated soil microorganisms [73].

The decrease in TN noticed for limestone post-mining soil over the remediation years, and between soil depth for all studied areas may be connected with an alkaline nature of the soil (pH = 7.5–7.8). Such an alkaline environment would promote the activity of nitrifying bacteria [74]. Therefore, such a decrease may have the origin in the volatilization of ammonium in the soil with an alkaline nature and high CaCO_3 [75]. Very low content of nitrogen (2–4%) in lignite post-mining soil indicates soil impoverishment over the years. While the total amount of nitrogen in the sludge-amended soil tends to be higher after plant

cultivation [74] in our study the TN content was not changed over the remediation. Our finding, unlike other studies, indicated the higher TN content in the soil layer of 15–30 cm compared to 0–15 cm for sludge-amended soil [75]. The tendency of TN to increase with soil depth suggests a positive influence of soil remediation on soil quality.

4.2. *The Impact of the Use of Sewage Sludge and Its Non-Use in Remediation on CO₂ Emission*

The lower soil respiration with a remediation year for both remediated post-mining soils indicates the lower emission of CO₂ into the atmosphere. Therefore, the observed higher decrease in soil respiration with a remediation year for lignite post-mining area (S2) (up to 37.19 $\mu\text{mol s}^{-1} \text{m}^{-1}$) compared to limestone post-mining area (S1) (up to 6.66 $\mu\text{mol s}^{-1} \text{m}^{-1}$) suggest that remediation with organic additives promote soil C sequestration and influence on the higher limitation of emission of CO₂. However, the NCER was negative and similar for all studied soil at each remediation stage. It suggests that remediation of post-mining soils allows to limit soil contribution to climate change through the lower emission of CO₂. The remediation with sewage sludge resulted in a slightly higher decrease in NCER for the soil remediated for 20 years (the longest) compared to the remediation with embankment. Such negative NCER with a negative Ce indicates the capturing of CO₂ from the atmosphere which probably is built to the plant biomass which is favorable for soil C sequestration [76]. Since the CO₂ emissions are flexible with a C input, the differences in Ce and NCER observed between studied remediation techniques may be connected with the application of sewage sludge into soil [77]. The initial organic C input into soil most likely has an effect on the lower Ce and NCER in lignite post-mining soil (S2) remediated with sewage sludge compared to the limestone post-mining soil remediated with embankment (S1). Although other studies indicated a decrease in CO₂ emissions with the reduction of organic matter inputs such as litter [78], our study reported a decrease in soil remediation with an application of organic matter in sewage sludge. The initial application of organic C most likely stimulated soil processes (microbial activity, plant growth) which improved the soil functioning [79] and resulted in higher CO₂ capturing. Our findings are in good agreement with other studies which indicated that post-mining soils may act as an offset of C, released by the extraction of raw material [80]. The remediation year exerted greater control over the CO₂ emissions in the soil, showing some stabilization of carbon transformations in the soil. Khelifa et al. [81] also reported such an impact of soil remediation age. Other studies indicated increased CO₂ emission with vegetation restoration [82]. It is in contrast to our study, but we assign it to the very low quality of the initial, degraded soils.

4.3. *Effect of Initial Application of Sewage Sludge and Its Non-Application on Biochemical Soil Features*

The delivery of C inputs at the beginning of remediation of opencast post-mining soil affects the high increase in the microbial community in deeper soil parts, while remediation with embankment has no such influence on the soil microorganisms. Such an effect was most likely connected with the provision of substrates for microorganisms, which in more favorable conditions have increased their community. A similar finding was reported by Börjesson et al. [83]. Soil microbial community structure affected by 53 years of nitrogen fertilization and different organic amendments. The high increase in deeper soil at soil fertilized with organic matter for all studied remediation stages may be result of the gradual release of primary and micronutrients to the soil which has been suggested by Lewu et al. [84]. The controlled release of nutrients into soil could support the nutritional balance in the soil allowing also for proper plant growth [84]. The small microbial community in the soil and the significant difference between S1 and S2 soils may suggest that the application of bio-waste significantly increases the microbial community in depleted and degraded soil. It also suggests that the use of biowaste, as a technique to support the remediation of degraded post-mining soils, besides the delivery of exogenic organic carbon, is also a source of valuable microorganisms whose presence in the soil affects, among others, the soil structure [85]. While in our study we noted an exact influence of the initial application of

organic matter into soil remediation on the microbial community, other studies suggested that the remediation age [86], and age of the stand [87] influence soil microorganisms. Therefore, the higher number of microorganisms at longer remediated areas may result from the higher amount of litter provided by the higher biomass of stand. Liu et al. [88] reported that increased litter was favorable for microbial communities and enzymatic activities. In our study, we reported a higher activity of ureases and dehydrogenases activity for soil remediated with embankment which most likely is connected with the high content of active organic carbon (POXC) in those soils. The other study also indicated the highest urease and dehydrogenase activity in calciferous soils which positively corresponds with our finding [89]. The dehydrogenase activity was parallel with POXC content. In the soil with the highest POXC content, the dehydrogenase activity was the highest. However, the microbial community was negatively correlated with the activity of ureases and dehydrogenases. Therefore, we found that the remediation age has a different influence on soil enzymatic activity in different soil layers. For soil remediated with embankment, we observed a positive correlation with remediation age for the layer of 15–30 cm, while for the layer of 0–15 cm the soil enzymatic activity showed a negative influence of remediation age. However, the ureases and dehydrogenase activity in a subsurface level of soil remediated with sewage sludge did not show a clear tendency, which is in accordance with other study [90]. Moreover, in our study, the activity of ureases was parallel with the total nitrogen content. Lower activity of ureases resulted in lower total nitrogen content. Other studies indicated the increase of urease activity with higher nitrogen content [91]. Therefore, the lowering activity of ureases and dehydrogenases was negatively correlated with the indicator for soil C sequestration. The lower soil enzymatic activity resulted in a higher stability of C in the soil visible in a higher hydrophobicity, aromaticity C/N ratio, and humification at post-mining soil remediated with sewage sludge. Thus, the decreased enzymatic activity would be probably affected by the stabilization of C in the soil, and a higher share of stable, non-susceptible for degradation C forms. In contrast, Zhang et al. [28] showed that fertilization with biowastes increased urease activity in the soil and they suggest that such alternation improved SOC sequestration. The activity of soil enzymes was negatively correlated with the total glomalin-related soil proteins (GRSP) content, while the fungi community was parallel with GRSP content. Similar studies also indicated a positive correlation between fungi biomass and GRSP content [92]. While GRSP are considered to stabilize organic carbon in the soil [93], and may reduce the organic matter. Our finding indicated that in the soils with a higher GRSP content, the organic matter had better stabilization features. Other studies confirm such dependence [94,95]. The increase of GRSP content within the remediation age in post-mining soil remediated with sewage sludge suggest was negatively influenced in opposition to the previous finding [96].

4.4. Effects of Remediation Type on Ecosystem Carbon Pool in the Top Layer and Sublayer

The application of organic additives into soil such as sewage sludge generally causes an increase in organic matter content compared to the initial value [97]. The remediation of soil usually results in a higher SOC content in the top layer in comparison to the sublayer [98]. However, in our findings the SOC decreased with the soil depth for S1, it slightly decreased for the sublayer compared to the topsoil for S2. Therefore, we observed that the application of soil additives has any influence on SOC within remediation years at post-mining soils. In contrast, in many studies, the increased SOC content has been indicated with the application of sewage sludge [99,100]. Wu et al. [101] indicated also the improvement of soil fertilization with an application of sewage sludge in mine land soil which is contrary to our research. This could be caused by very poor soil quality before the reclamation process and confirms that post-mining soils are difficult to recultivation [102]. Our findings are in good agreement with another study, where remediation effected a higher SOC in the top layer, but not in the soil profile within 17 years [53]. While SOC showed a tendency to decrease with soil depth, POXC was similar in both soil depths. The increase in the remediation age for soil fertilized with sewage sludge may result from the

easily degrading organic matter delivered from the wood residues as reported by Danish et al. [103]. Therefore, while the remediation of opencast mining soil was reported to increase the total C content with the remediation age [104], our study did not indicate the relevant changes in TC content within soil depth and remediation age. It may suggest that the vertical transport of soil carbon and SOC stability in the layer 0–30 cm cannot be clearly described by SOC, POXC, TC, and LOI for the early years of soil recovery (20 years).

4.5. Stability of Sequestered SOC

We found that the remediation with sewage sludge causes an increase in the share of stable C (alkyl-C and aromatic C) forms in the deeper layers of the soil (15–30 cm), while its decreases in the upper layers. This is concomitant with a decrease in carbonyl C and an increase in O-alkyl C, representing labile forms of carbon liable to a quick degradation. Thus, the stronger stabilization of sequestered C in the deeper soil layer was assessed under remediation with sewage sludge. Such proportion of stable and labile C forms in a deeper soil layer is considered to promote C sequestration in a long-term perspective which has been confirmed by del Mar Montiel-Rozas et al. [105]. Therefore, as Wan et al. [106] reported, forming of the structure of Ca^{2+} ions with SOC (Ca-OC) potentially may be a stable SOC pool, we suggest that the increase in non-labile C fraction in lignite post-mining soil can be connected with a high content of Ca^{2+} ions in the soil. Therefore, Dick et al. [107] suggested that a high proportion of O-alkyl C and Carbonyl C in the soil may result from the coal contamination of the native site which in our study is unlikely due to the similar values for both studied post-mining soils. An improved hydrophobicity within soil depth for post-mining soils suggests the better stabilization of SOC and limits its susceptibility to microbial decomposition [108]. Therefore, such improvement in the top layer and the sublayer of the soil indicates better C storage in the soil [109] which is promoting climate change lowering. Very few studies in that issue, confirm our findings that the aromaticity was higher after soil remediation [110]. Such observations have been confirmed by Singh et al. [111] for post-mining soils. Other studies indicated an increase in alkyl-C, aromatic C, carbonyl C, and O/N-Alkyl C for remediation soil [112]. An et al. [49] suggested that forest land use improves soil organic carbon quality. Our study confirmed that reforestation has a high positive influence on SOC quality as reported previously [113,114]. Such a positive influence is also noticeable in soil depths. However, we observed a different distribution of SOC and its stabilization with an implemented remediation method, and higher improvement has been noticed for the remediation with sewage sludge. Similar to our study, Guo et al. [115] noted that different afforestation practices result in different SOC accumulation pathways among soil depths.

4.6. Estimation of Vertical Stability of SOC during Post-Mining Soil Remediation

While several indicators for SOC stability in the soil samples provide a good view of the formation of stable SOC fractions, there is a lack of indicator for the fluent and easy determination of the vertical variability of sequestered SOC. In our study, for the first time, we provided an indicators for the description of penetration of stable C forms in the soil into deeper soil layers. Such an indicator as a function of remediation year allows us to predict the vertical distribution of stable SOC. Therefore, thanks to the proposed indicators the remediation of post-mining soils remediated with sewage sludge or without may be effectively managed, which in an important value especially for soil C sequestration and mitigation of climate change. Since the different nature of the remediation method, such an indicator cannot be universal, and thus we proposed individual indicators for both studied post-mining soils. The advantages of proposed VSOC models include the ability to predict and simulate the vertical stability of sequestered carbon for post-mining soils. Therefore, the models are relatively easy to use, have a clear tendency, low modeling errors calculated during the validation which provides good predictions. The models are characterized by a high accuracy and may be effectively used already at the early remediation stages to predict vertical stabilization of sequestered carbon. Required input parameters can

be easily measured in laboratory analyses, and the calculations are not laborious and difficult to perform and process. The VSOC models might be effectively used either for the description of vertical variability in the past or in the future within a 20-years remediation process. The models help greatly in the design and management of soil remediation at post-mining areas which allow for quick corrections of the reclamation process to manage the carbon sequestration process as efficiently as possible. Open-pit mines can use the proposed models to prepare documentation on mitigating the impact of mines on climate change, as well as to describe the effectiveness of reclamation of post-mining soils. In addition, the practical use of the VSOC models proposed is also their implementation in the documentation proving the mine's efforts to reduce its carbon footprint. Therefore, the proposed models may be an indicator of the pursuit of climate neutrality in line with the Green Deal. Most likely those indexes may have only application for the studied post-mining soils, however, the procedure of its preparation, and calculations of functions are universal for post-mining soils.

5. Conclusions

In the study, we evaluated the distribution of important parameters for soil quality, SOC sequestration, and SOC stability. We observed better SOC stability within soil depths for lignite post-mining soil remediated with sewage sludge in comparison to the limestone post-mining soil remediated with embankment. In the post-mining soil remediated with sewage sludge, we noticed an increased microbial community which has been assigned directly to the application of biowastes which may be also a source of microorganisms, and their substrate. Therefore, a positive correlation between GRSP content with a fungi community within soil depths has been noticed. At lignite post-mining soil remediated with sewage sludge, the activity of ureases and dehydrogenases were generally lower compared to the post-mining soil remediation with embankment. We observed well parameters of Ce and NCER which for both studied areas were negative which indicate for the privilege of the higher capturing of CO₂ over its release from the soil into the atmosphere. Such phenomenon proves that post-mining soils are a good carbon sink and may be helpful in the mitigation of climate change. Moreover, in the study, we did not observe relevant changes in SOC, POXC, TC, and LOI content within soil depth and remediation age. It suggests that these parameters cannot describe the distribution of stable C in the soil layers. While these indicators clearly show the variability of sequestered SOC in the soil, there was a lack of the easily used and well-working indicator for vertical stabilization in post-mining soils under remediation. In our study, we proposed a vertical SOC variability index. It is impossible to provide a universal indicator for vertical SOC variability based on hydrophobicity for both studied post-mining soils due to the different nature of remediation. The proposed individual indicator allows for the prediction of vertical SOC variability based on hydrophobicity and may be useful for remediation management and decision-making. Therefore, due to the high importance of stabilization of SOC into deeper soil layers for C sequestration, this indicator can be helpful for decision-making in the aspects of climate change mitigation, lowering the carbon footprint of mines, and also for soil remediation efficiency.

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