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Impact of Tree Species and Substrates on the Microbial and Physicochemical Properties of Reclaimed Mine Soil in the Novel Ecosystems

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Abstract: Evaluating how different tree species and substrates affect the microbial and physicochemical properties of technosols from combustion wastes and reclaimed mine soil (RMS) is vital in species selection to enhance restored ecosystem services. This research aimed to evaluate the impact of pioneer and N-fixing tree species and substrates on the post-mining soil microbial and physicochemical properties. Common birch (*Betula pendula* Roth) and Scots pine (*Pinus sylvestris* L.), as the commonly introduced species on reclaimed mine soils (RMS) in eastern and central Europe, were selected as pioneer species, whereas black alder (*Alnus glutinosa* (L) Gaernt.) and black locust (*Robinia pseudoacacia* L.) were selected as N-fixer species. Soil samples were collected from different RMS developed from three substrates (fly ashes, clay, and sand) and measured for the content of total nitrogen (N_t), organic carbon (C_{org}), exchangeable calcium (Ca^{2+}), exchangeable potassium (K^+), exchangeable magnesium (Mg^{2+}), C to N ratio (C:N), basal respiration rate (RESP), and microbial biomass carbon (C_{mic}). The research indicated that tested tree species influenced water holding capacity (WHC), N_t, C:N, and RESP value. The highest N_t accumulation in soil was observed under N-fixing, but it did not transfer into higher organic carbon content under N-fixers. The soil under pine had a greater C:N ratio than the soil under birch, alder, and locust. The RESP rate was highest under birch. In terms of substrate type, RMS developed on Miocene clays exhibited higher carbon and macronutrient contents followed by ashes, whereas sands exhibited the lowest values of both physicochemical and microbial properties. The study suggested that both tree species and substrates affect microbial activities and physicochemical properties of RMS; however, the substrate effect is stronger.

Keywords: reclamation; pioneer; N-fixing species; microbial biomass; soil carbon

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1. Introduction

The exploitation of minerals and other geological materials (such as sand and coal) is the primary driver of ecosystem service degradation [1,2]. The post-mining soil usually exhibits disturbed water–air–soil relationships, very low soil microbial biomass, deficiency of available water, variation in texture (from high clay content to dominant coarse fraction), highly acid or alkaline pH, and nutrient deficiency [3–6]. These features restrict the growth and development of trees in reclaimed mine soils (RMS) [7,8]. Additionally, the planted trees frequently face significant constraints of essential nutrient supply [9,10]. However, if appropriate reclamation and rehabilitation methods are implemented, post-mining sites have a high potential for ecosystem services [11–13]. Afforestation is likely one of the most promising methods of mitigating the effects of exploitation and land degradation by mining and industry [8,14]. It is vital for the accumulation of soil organic matter (SOM), which supports soil biota and releases essential nutrients to the soil [15–17], and thus for the redevelopment of sustainable ecosystems and their services in a changing climate [16].

The period of restoration and achievement of a sustainable novel ecosystem on a post-mining site is highly dependent on the species selected for afforestation [8]. Different tree species produce litter and root inputs with varying properties that significantly influence microbial activities and soil physicochemical properties in RMS [18–21]. As a result, the ability of different tree species to stabilize soils, increase soil organic carbon, and increase available nutrient content varies greatly [22]. Pioneer tree species, especially common birch (*Betula pendula* Roth) and Scots pine (*Pinus sylvestris* L.), and N-fixers such as black alder (*Alnus glutinosa* (L.) Gaertn.) and black locust (*Robinia pseudoacacia* L.) are commonly selected for restoration of post-mining sites in eastern and central Europe [23].

Afforestation of mining sites with pioneer species initiates the colonization process of soil biota and development of ecological functions [24,25]. N-fixing species accumulate more nitrogen in soils and soils under these species exhibit lower carbon to nitrogen (C:N) ratios and are often used for soil quality improvement [25–28]. However, their effect on biological properties of RMS is deviating. For example, Šourková et al. [29] observed higher microbial biomass in RMS under alder than under pine. On the other hand, Chodak and Niklińska [30] observed higher microbial biomass, basal respiration, and dehydrogenase activity in post-mining soils under birch than under alder.

Tree species growing in post-mining areas experience a variety of effects on their rhizosphere soil properties, which may influence their growth and survival directly or indirectly [9]. Soil enzymes released by microorganisms increase the rate of plant residues' decomposition and enhance release of plant available nutrients from organic resources [31]. Microorganisms in soil can increase soil porosity [32], aggregation [33], water retention [32], and organic matter turnover [34] and enhance soil fertility by improving nutrients [35], thereby increasing nutrient supply to trees planted on RMS. Therefore, tree species have considerable species-specific effects on soil properties by litters and root exudates. However, the effect of tree species on RMS properties may be modified by the quality of soil substrate [36]. Chodak and Niklińska [37] observed that the effect of substrate texture on microbiological properties of RMS was stronger than the effect of tree species.

The study on the influence of N-fixing and pioneer tree species and substrates on the physicochemical and microbial parameters of post-mining soils is limited. Thus, it is vital to know how specific tree species selected to afforest post-mining sites affect the properties of the soils. We hypothesized that differences in microbial and physicochemical properties in RMS are primarily caused by differences in tree species and substrates. Therefore, the objective of this study was to compare the effect of pioneer (Scots pine and silver birch) and N-fixing (black locust and black alder) tree species on the microbial and physicochemical properties in RMS developed from various substrates (nutrient poor Quaternary sands, acid and alkaline Miocene clays, and ashes after combustion of lignite).

2. Materials and Methods

2.1. Study Sites

The study was carried out in Poland at five rehabilitated post-mining sites with three different substrates. The sites included Szczakowa sand pit, Bełchatów open-pit lignite mine, Lubień combustion waste disposal site of Bełchatów lignite power plant, Turów open-pit lignite mine, and Piaseczno open-pit sulphur mine. Soil samples were collected from pure stands of Scots pine, common birch, black alder, and black locust grown on sand substrates in Szczakowa and Bełchatów, clay substrates in Turów and Piaseczno, and combustion waste ash substrates in Lubień (near Bełchatów lignite mine). Table 1 describes the study sites in detail.

Table 1. Basic characteristics of the experimental sites.

Study Site	Mean Annual Temperature (°C)	Mean Annual Precipitation (mm Year^{-1})	Age of Forest Stands (Years)	Substrate Type	Reclamation Treatments
Szczakowa sand pit 50°16′ N; 19°26′ E	8.1	700	30–35	Quaternary sand	Cultivation of lupine (*Lupinus polyphyllus* Lindl.) as a green manure for 1 year, mineral fertilization with NPK (70 kg N ha^{-1}, 120 kg P ha^{-1}, 120 kg K ha^{-1}), and tree planting.
Bełchatów external spoil heap 51°13′ N; 19° 25′ E	7.6	580	30–35	Quaternary sand	Surface forming and leveling, mineral fertilization with NPK (60 kg N ha^{-1}, 70 kg P ha^{-1}, 60 kg K ha^{-1}), cultivation of leguminous plants and grasses for 1 year, and planting of trees.
Lubień lignite combustion waste disposal site 51° 27′ N, 19° 27′ E	7.6	580	25–30	Ashes after lignite combustion	Mineral fertilization with NPK (60 kg N ha^{-1}, 36 kg P ha^{-1}, 36 kg K ha^{-1}), hydro-seeding of a mixture of grasses (*Dactylis glomerata* L., *Lolium multiflorum* Lam.) and a sewage sludge and tree planting.
Turów external spoil heap 50°52′ N; 14°52′ E	8.3	706	38–44	Acid Miocene clays	Mineral fertilization with NPK (50 kg N ha^{-1}, 28 kg P ha^{-1}, 16 kg K ha^{-1}), cultivation of grasses (*Festuca rubra* L., *Phleum pratense* L.) and legumes (e.g., *Lupinus polyphyllus* Lindl, *Melilotus albus* Desr), and tree planting.
Piaseczno external spoil heap 50°35′ N; 21°47′ E	7.0	650	38–44	Alkaline Miocene clays	Mineral fertilization with NPK (80 kg N ha^{-1}, 50 kg P ha^{-1} and 60 kg K ha^{-1}), cultivation of *Melilotus albus* L. and grasses, and tree planting.

Sources for climatic data: [38–40].

2.2. Soil Sampling and Measurements

A total of seventy-two (6 replications × 3 RMS substrates × 4 tree species) 10 × 10 m plots were established randomly. Soil samples were collected in August and September 2019. Tree stands on plots ranged in age from 18 to 44 years (Table 1). At each plot, one composite sample was collected from five locations (from four corners and at the center) at the depth of 0–5 cm with 5 cm diameter auger after the removal of the litter layer. Because of the presence of large waste rock fragments below 5 cm, the soil sample was only taken from the top layer. The samples were sieved with 2 mm mesh prior to laboratory analysis. Samples for physicochemical analyses were air-dried, whereas samples for microbial analyses were stored field-moist at 4 °C.

LECO TruMac CNS analyzer was used to analyze the content of organic carbon (C_{org}) and total nitrogen (N_t). Soil texture was measured with a Fritsch GmbH Laser Particle Sizer ANALYSETTE 22 (Idar-Oberstein, Germany). Basic exchangeable cations (Ca^{2+}, Mg^{2+}, and K^+) were measured with an ICP OES ICAP 6000 series spectrophotometer after extraction in 1 M NH4Ac. The pH of the samples was measured in H_2O (pH_{H_2O}) and 1 M KCl solution (pH_{KCl}) (soil/liquid ratio 1:5, w/v) with a digital pH meter (CPC-411, ELMETRON) at 20 °C.

Prior to microbial analyses, the samples were adjusted to 50% of maximum water holding capacity (WHC) and pre-incubated at 22 °C for 6 days; WHC was determined gravimetrically according to Schlichting and Blume [41]. For microbial biomass carbon (C_{mic}) and basal respiration rate (RESP) measurement, samples (50 g d.w.) unamended for RESP measurements and amended with 8 mg glucose for C_{mic} measurements were incubated at 22 °C in gas-tight jars. The incubation time was 24 h for the determination of RESP and 4 h for C_{mic}. The jars contained beakers with 5 mL 0.2 M NaOH to trap the evolved CO_2. After the jars were opened, 2 mL 0.9 M $BaCl_2$ was added to the NaOH; the excess of hydroxide was titrated with 0.1 M HCl in the presence of phenolphthalein as an indicator. C_{mic} was calculated from the substrate-induced respiration rate according to the equation given by Anderson and Domsch (1978): C_{mic} [mg g^{-1}] = 40.04 y + 0.37, where "y" is mL $CO_2 \times h^{-1} \times g^{-1}$.

2.3. Statistical Analysis

Data were analyzed using Statistica 12.0 Software (StatSoft, Inc., (Tulsa, OK, USA), 2014). Prior to analysis, the data were tested for normality (Shapiro–Wilk test, $p < 0.05$). Two-way analysis of variance (ANOVA) was used to compare the effect of tree species and substrates on the measured properties. HSD test was run if significant ($p < 0.05$) effects were found. A linear correlation was used to assess the relationships between macronutrients and microbial activities. All correlation coefficients significant at $p < 0.05$ are shown.

3. Results

3.1. Soil Texture and Water Holding Capacity

The soil under the pioneer and N-fixing tree species did not show any significant difference in soil texture on the same substrate (Table 2). The lowest value of sand fraction was observed under alder on clays (22%) and the highest under pine on sands (89%). The lowest silt fraction was under pine on sands (5%) and the highest under black locust on ashes (30%) and for the clay fraction the lowest value was observed under birch on ashes and pine on sands (6%) and the highest under alder on clays (64%). The soils under birch on ashes revealed the highest WHC (104.7%), whereas soils under alder on sands revealed the lowest (29.8%; Table 2).

Table 2. Texture and water holding capacity (WHC) in studied technosols under different tree species.

Species-Substrate	Sand (%)	Silt (%)	Clay (%)	WHC (%)
A-As	71 ± 12^{ab}	21 ± 10^{abc}	8 ± 3^{c}	97.4 ± 11.9^{ab}
A-Cl	22 ± 8^{c}	14 ± 5^{bcd}	64 ± 11^{a}	75.9 ± 22.3^{bcd}
A-Sa	82 ± 7^{ab}	8 ± 5^{cd}	10 ± 3^{c}	29.8 ± 6.4^{g}
B-As	74 ± 9^{ab}	20 ± 10^{abc}	6 ± 2^{c}	104.7 ± 7^{a}
B-Cl	27 ± 8^{c}	19 ± 5^{abc}	54 ± 11^{ab}	68.6 ± 7.7^{cde}
B-Sa	78 ± 6^{ab}	13 ± 5^{bcd}	9 ± 2^{c}	41.4 ± 10.3^{fg}
Bl-As	63 ± 12^{b}	30 ± 10^{a}	7 ± 2^{c}	93.1 ± 12.3^{abc}
Bl-Cl	28 ± 18^{c}	14 ± 3^{bcd}	58 ± 18^{ab}	69.8 ± 17.4^{cde}
Bl-Sa	78 ± 8^{ab}	12 ± 7^{bcd}	10 ± 2^{c}	47.3 ± 14.8^{efg}
P-As	70 ± 5^{b}	23 ± 5^{ab}	7 ± 2^{c}	86.7 ± 8.3^{abcd}
P-Cl	37 ± 9^{c}	16 ± 2^{bcd}	47 ± 11^{b}	62.1 ± 15^{def}
P-Sa	89 ± 2^{a}	5 ± 2^{d}	6 ± 2^{c}	30.7 ± 11.8^{g}

Explanations: 71 ± 12—mean ± SD; P = pine; B = birch; A = alder; Bl = black locust; As = ashes; Sa = sands; Cl = clays; WHC = water holding capacity. Means in each column followed by different letters are significant at $p < 0.05$ level.

Significant differences in soil texture and WHC were observed between the substrates. Ashes and sands contained significantly more sand fraction (ranged 63%–74% in ashes and 78%–89% in sands) than clays (ranged 22%–37%). Ashes and clays contained more silt fraction than sands. Clays contained significantly more clay fraction (47%–64%) than both ashes (6%–8%) and sands (6%–10%) (Table 2). The lowest WHC values were observed in sands. Ashes exhibited the highest values of WHC (86.7%–104.7%) followed by clays (62.1%–75.9%).

3.2. Soil Chemical Properties and Microbial Activities

Tree species (TS) had only effect on N_t, C:N, and RESP, while substrate (SU) significantly affected all studied chemical and microbial activities. No interaction effect between tree species and soil substrate (TS × SU) was found (Table 3).

Table 3. Two-way ANOVA results for tree species (TS) and substrate (SU) effect on soil chemical properties and microbial activities: N.S.—not significant, C_{org}—organic carbon, N_t—total nitrogen, Ca^{2+}—calcium, K^+—exchangeable potassium, Mg^{2+}—exchangeable magnesium, C:N—C to N ratio, RESP—basal respiration rate, and C_{mic}—microbial biomass carbon.

		Df	SS	MS	F	P
pH_{H_2O}	TS	3	N.S.	N.S.	N.S.	N.S.
	SU	2	68.51	34.26	38.44	0.000
	TS × SU	6	N.S.	N.S.	N.S.	N.S.
pH_{KCl}	TS	3	N.S.	N.S.	N.S.	N.S.
	SU	2	98.04	49.02	32.30	0.000
	TS × SU	6	N.S.	N.S.	N.S.	N.S.
C_{org} (g kg^{-1})	TS	3	N.S.	N.S.	N.S.	N.S.
	SU	2	35,072.52	17,536.26	62.30	0.01
	TS × SU	6	N.S.	N.S.	N.S.	N.S.
N_t (g kg^{-1})	TS	3	15.59	5.20	7.10	0.000
	SU	2	40.98	20.49	28.01	0.000
	TS × SU	6	N.S.	N.S.	N.S.	N.S.
Ca^{2+} (g kg^{-1})	TS	3	N.S.	N.S.	N.S.	N.S.
	SU	2	142.51	71.26	36.80	0.000
	N.S.	6	N.S.	N.S.	N.S.	N.S.
K^+ (g kg^{-1})	TS	3	N.S.	N.S.	N.S.	N.S.
	SU	2	0.34	0.17	26.08	0.01
	TS × SU	6	N.S.	N.S.	N.S.	N.S.
Mg^{2+} (g kg^{-1})	TS	3	N.S.	N.S.	N.S.	N.S.
	SU	2	0.08	0.04	13.78	0.000
	TS × SU	6	N.S.	N.S.	N.S.	N.S.
C:N	TS	3	1069.38	356.46	9.45	0.000
	SU	2	1531.99	766	20.30	0.000
	TS × SU	6	N.S.	N.S.	N.S.	N.S.
RESP (µM CO_2 g^{-1} 24 h^{-1})	TS	3	9.51	3.17	4.18	0.010
	SU	2	7.02	3.51	4.62	0.014
	TS × SU	6	N.S.	N.S.	N.S.	N.S.
C_{mic} (µg g^{-1})	TS	3	N.S.	N.S.	N.S.	N.S.
	SU	2	1,405,123.85	702,561.93	13.34	0.000
	TS × SU	6	N.S.	N.S.	N.S.	N.S.

The N_t content was higher under N-fixing species (alder and locust) than under pine. Soils under pine exhibited significantly higher C:N ratio than under other tree species. Soils under birch exhibited significantly higher RESP compared to soils under pine and black locust (Table 4).

Table 4. Tree species effect on soil chemical properties and microbial activities: C_{org}—organic carbon, N_t—total nitrogen, Ca^{2+}—calcium, K^+—exchangeable potassium, Mg^{2+}—exchangeable magnesium, C:N—C to N ratio, RESP—basal respiration rate, and C_{mic}—microbial biomass carbon. Means in each row followed by different letters are significant at $p < 0.05$ level.

	Pine	Birch	Alder	Black Locust
pH_{H_2O}	6.61 ± 1.38 ^{a}	6.78 ± 1.3 ^{a}	6.68 ± 1.35 ^{a}	6.74 ± 1.4 ^{a}
pH_{KCl}	5.94 ± 1.79 ^{a}	6.2 ± 1.66 ^{a}	6.07 ± 1.63 ^{a}	6.09 ± 1.65 ^{a}
C_{org} (g kg^{-1})	39.36 ± 32.02 ^{a}	46.08 ± 24.64 ^{a}	48.79 ± 28.85 ^{a}	49.68 ± 27.81 ^{a}
N_t (g kg^{-1})	1.38 ± 1.04 ^{b}	2.01 ± 0.95 ^{ab}	2.24 ± 1.23 ^{a}	2.67 ± 1.35 ^{a}
Ca^{2+} (g kg^{-1})	2.37 ± 1.97 ^{a}	2.16 ± 2.02 ^{a}	2.47 ± 2.22 ^{a}	2.62 ± 1.92 ^{a}
K^+ (g kg^{-1})	0.14 ± 0.13 ^{a}	0.13 ± 0.07 ^{a}	0.13 ± 0.12 ^{a}	0.17 ± 0.12 ^{a}
Mg^{2+} (g kg^{-1})	0.1 ± 0.07 ^{a}	0.11 ± 0.07 ^{a}	0.1 ± 0.06 ^{a}	0.1 ± 0.06 ^{a}
C:N	28.87 ± 10.16 ^{a}	22.69 ± 5.40 ^{b}	20.34 ± 8.25 ^{b}	18.73 ± 5.94 ^{b}
RESP (µM CO_2 g^{-1} 24 h^{-1})	1.76 ± 0.9 ^{b}	2.70 ± 0.9 ^{a}	2.08 ± 1.04 ^{ab}	1.87 ± 0.89 ^{b}
C_{mic} (µg g^{-1})	377.67 ± 329.24 ^{a}	503.32 ± 243.80 ^{a}	362.67 ± 201.16 ^{a}	376.46 ± 284.77 ^{a}

The highest pH_{H_2O} and pH_{KCl} values were observed in ashes (8.02 and 7.64, respectively), while the lowest values were in sands (5.68 and 4.84, respectively). Clays exhibited the highest values of C_{org} (72.86 g kg^{-1}) and N_t (3.06 g kg^{-1}), whereas sands exhibited the lowest values. Ca^{2+} was significantly higher in ashes (4.21 g kg^{-1}) and lower in sands (0.77 g kg^{-1}). Sands also exhibited significantly lower values of K^+, $Mg^{2+}$and C:N (0.04 g kg^{-1}, 0.06 g kg^{-1} and 16.14, respectively). The higher RESP value was observed in ashes (2.40 μM CO_2 g^{-1} 24 h^{-1}) and the lower RESP value was observed in sands (1.67 μM CO_2 g^{-1} 24 h^{-1}). The highest value of C_{mic} was measured in clays substrate (600.79 μg g^{-1}) than ashes and sands (Figure 1).

3.3. Relationships between Macronutrients and Microbial Activities

Basal respiration rate and microbial biomass carbon were positively correlated with C_{org}, N_t, Ca^{2+}, Mg^{2+}, and K^+. C_{org} was positively correlated with N_t, Ca^{2+}, Mg^{2+}, and K^+. N_t was also positively correlated with C_{org}, Ca^{2+}, Mg^{2+}, and K^+ (Table 5).

Table 5. Correlation coefficients (*n* = 72) between macronutrients content and microbial biomass and respiration. Coefficients significant at *p* = 0.05 are marked by *.

	C_{org}	N_t	Ca^{2+}	K^+	Mg^{2+}	RESP	C_{mic}
C_{org}	1						
N_t	0.81 *	1					
Ca^{2+}	0.41 *	0.49 *	1				
K^+	0.62 *	0.64 *	0.77 *	1			
Mg^{2+}	0.75 *	0.6 *	0.54 *	0.69 *	1		
RESP	0.48 *	0.58 *	0.62 *	0.46 *	0.54 *	1	
C_{mic}	0.54 *	0.67 *	0.31 *	0.42 *	0.53 *	0.70 *	1

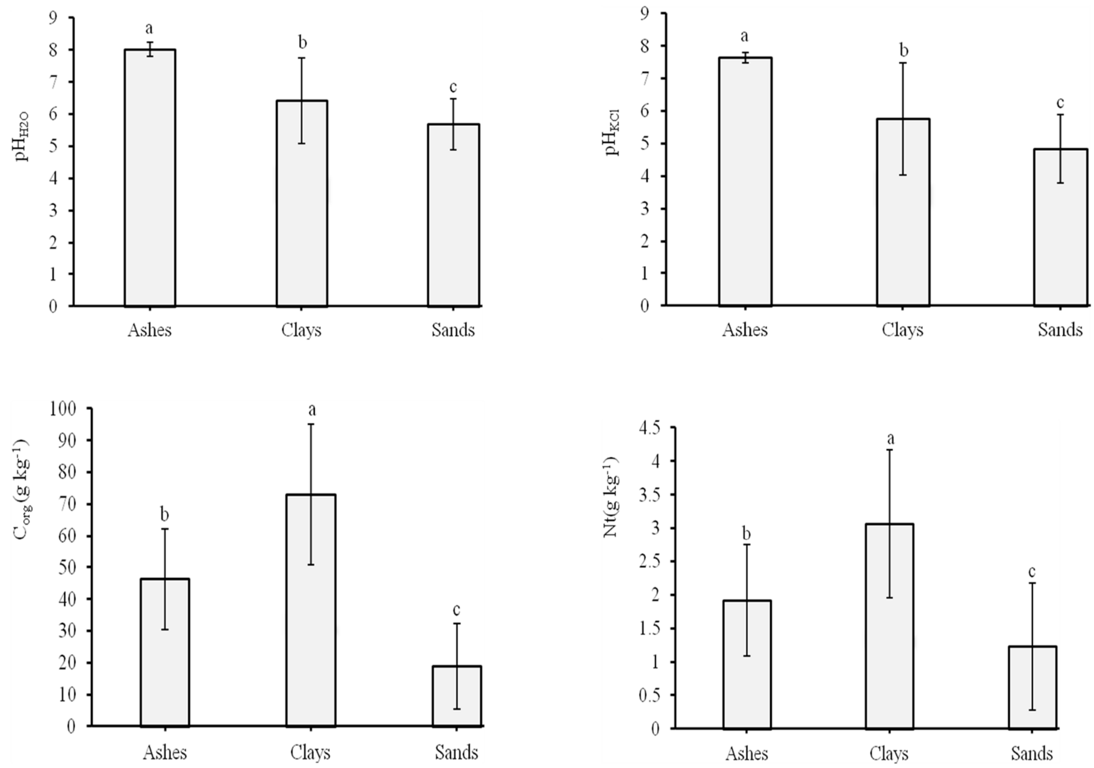

Figure 1. *Cont.*

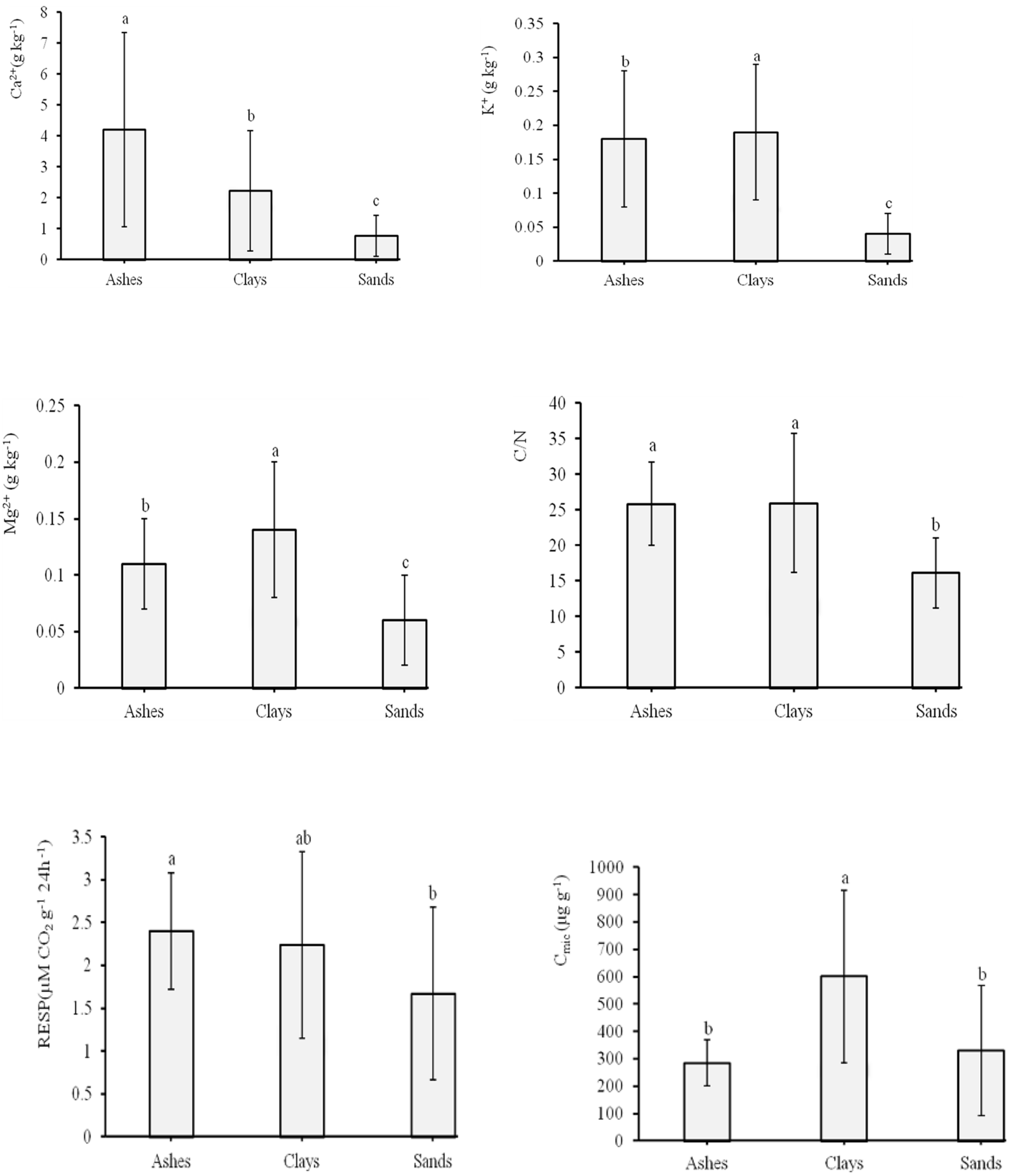

Figure 1. Substrate effect on soil chemical properties and microbial activities: C_{org}—organic carbon, N_t—total nitrogen, Ca^{2+}—calcium, K^+—exchangeable potassium, Mg^{2+}—exchangeable magnesium, C:N—C to N ratio, RESP—basal respiration rate, and C_{mic}—microbial biomass carbon. Means in each bar chart followed by different letters are significant at $p < 0.05$ level.

4. Discussion

4.1. Tree Species Effect on Soil Properties and Microbial Activities

The impact of tree species on soil WHC differed significantly between birch and pine. Cejpek et al. [42] also measured the lowest soil moisture in the reclaimed pine sites, whereas the highest soil moisture was in the reclaimed alder sites which may be related to soil development. The influence of soil substrate on studied chemical and biological properties was greater than that of tree species at the early stage of novel ecosystem development. Similarly, spoil heap after lignite mining in Poland had a greater impact on the content of C, N, P, C:N ratio, and microbial properties (microbial biomass carbon and respiration, enzyme activity) than tree species [30]. In contrast to our findings, Šnajdr et al. [43] found that tree species is the most important determinant of the chemical and biological properties of soils in the Sokolov brown-coal mining district's post-mining sites (Czech Republic). This phenomenon, however, could be attributed to the low diversity of substrates on the spoil heap in the Sokolov brown-coal mining district, which was formed of Miocene clays of the Cypris formation [43]. In contrast to Józefowska et al. [44], our study found no interaction effect between tree species and soil substrate on soil microbial biomass and respiration. According to these authors, the specific mix of tree species and the substrate has a large impact on faunal activity and microbial community composition.

The N_t content was significantly higher under N-fixing species (alder and locust) than under pine (Table 4). The higher N_t value observed in soils under alder and black locust indicates the effectiveness of N-fixing tree species in enriching this deficient element in RMS. Similarly, Wang et al. [45] revealed that N-fixing forests had 20%–50% higher N_t in the 0–5 cm soil layer than non-N-fixing forests. The lack of differences in N_t content between birch and N-fixers could be attributed to the stimulation of non-symbiotic N-fixing bacteria growth [46,47]. The non-significant differences in C_{org} content observed among tree species are opposite to the findings of previous studies. The soils under N-fixing species usually contain more carbon than soils under non-N-fixers [48]. For example, Ussiri et al. [49] observed a 42% increase in soil carbon under black locust in 10 years afforestation on reclaimed mine soils, while only 11% increase under pine, and Chodak and Niklińska [30] observed higher organic carbon accumulation under alder than under pine. Increased soil organic matter input and reduced decomposition of older soil carbon are associated with higher C_{org} content in N-fixing tree species [48]. However, this phenomenon was not observed in our soils.

Soils under pine exhibited significantly higher C:N ratio than under other tree species. The lowest C:N values were observed under alder and black locust. This may be due to the lower carbon to nitrogen ratio in the litter of nitrogen fixing species [27,50,51]. Similarly, Józefowska et al. [44] observed lowest (14) C:N under alder compared to non-N-fixing species growing on different substrates in RMS.

There was no difference observed for microbial biomass among tree species. However, the highest RESP was exhibited under birch (Table 4). The previous studies by Chodak and Niklińska [37] and Józefowska et al. [44] also revealed higher respiration rate and microbial biomass under birch than pine on post-mining sites. Differences in microbial biomass and basal respiration rate between tree species and substrates may have implications for tree nutrient availability. High microbial biomass and basal respiration rate frequently result in high nutrient availability to trees [52,53], by increasing both microbial biomass turnover and non-microbial organic material degradation. This result showed that a strong and positive correlation exists between soil microbial biomass and basal respiration rate with N_t content (Table 5). This confirms the findings of Józefowska et al. [44], who indicated that N_t content was positively and strongly correlated with soil microbial activities in mine soil. Frouz et al. [27] also reported a positive correlation between soil microbial biomass and N_t content. Soil microorganisms play a vital role in the biological transformations and develop most of the stable N and C nutrients and other vital nutrients [54].

4.2. Substrate Effect on Soil Properties and Microbial Activities

All of the investigated physicochemical and microbial properties were significantly influenced by the substrates. The lowest WHC values were observed in sands. Ashes exhibited the highest values of WHC (86.7%–104.7%) followed by clays (62.1%–75.9%). The storage of water on spoil heaps is hampered by the soil's incomplete development [55]. The pH values varied significantly across all substrates. According to Soil Science Division Staff [56], sands were strongly acidic to very strongly acidic, clays were moderately acidic to slightly acidic, and ashes were moderately alkaline to slightly alkaline, which is a characteristic of combustion wastes [40,57].

C_{org} content varied significantly across substrates, with clays having significantly higher C_{org} content than ashes and sands. In line with this, Chodak and Niklińska [37] revealed that substrate texture had a greater effect on C_{org} of RMS than tree species. Woś and Pietrzykowski [39] also observed the highest organic carbon stock in soils developed from Neogene (Miocene) clays, which is linked to the element's in-situ accumulation and geogenic (fossil) carbon content. The differences found in our study could also be attributed to geogenic carbon content [58]. In contrast to sands, Miocene clays contain some geogenic carbon [59]. The higher values found in clay-based soils revealed that soils with more silt and clay content have more organic carbon [39]. This discrepancy is caused by clay particles binding SOC in the form of organic-mineral complexes. These complexes are resistant to leaching and microorganism breakdown [60,61]. Because of the incomplete combustion of lignite in the power plant, combustion wastes contain some unburned carbon [62,63]. In the case of ashes from the Bełchatów power plant, the amount of unburned material was estimated to be 2% [64]. Despite this, the carbon content of ashes-derived soils was lower than that of clay and comparable to that of sands. Clays, like C_{org}, had a higher N_t value than ashes and sands. C_{org} accumulation in soils is generally highly correlated with N_t accumulation [65,66]. Similarly, Treschevskaya et al. [67]'s study on soil development processes on post-mining sites found that clay substrates had higher N_t content than chalk and marl, sand, and sandy loam.

Significantly higher Ca^{2+} was observed in ashes than in sands and clays and it was mainly due to the combustion of lignite coal [68]. Ashes and clays, on the other hand, had significantly higher K^{+} and Mg^{2+} values than sands. Except for Ca^{2+}, the observed higher macronutrient content in clay may be due to its high capacity for holding macronutrients [69]. Mining waste sites with sandy substrates are characterized by unfavorable soil structure and nutrient deficiencies [70].

The study revealed that substrates have a greater influence on microbial activities than tree species. Clays, in particular, had greater microbial biomass than ashes and sands. In line with this, Chodak and Nikliska [37] found that soil texture, rather than vegetation properties, influenced microbial activity on reforested mine spoils in Poland. The type of soil is a major factor in determining microbial activity [71,72]. Carletti et al. [73] found that geologic parent material had a significant impact on the microbial community in northern Italian montane spruce forests. According to Józefowska et al. [25] and Reich et al. [74], the substrate influences soil biological properties in both natural and post-mining soils.

5. Conclusions

The substrate influences physicochemical and microbial properties more than tree species. Except for N_t, there is no significant difference in physicochemical and microbial properties between pioneer and N-fixing tree species. The highest carbon and macronutrient content was found in Miocene clays, followed by combustion wastes, and the lowest physicochemical and microbial properties were found in sands. The study found that tree species influenced WHC, N_t, C:N, and RESP value. The highest N_t in soils was observed under N-fixers, but it did not transfer into higher organic carbon content under N-fixers. The C:N ratio of pine soils was higher than that of birch, alder, and locust soils. Birch had the highest basal respiration rate. As a result of the stronger effect of substrate on RMS than tree species, reclamation of the substrate improves physicochemical and microbial properties and facilitates ecosystem restoration in mine sites.

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