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The Effect of Environmental Stressors on Respiration Activity of Afforested Mine Soils**

Abstract: The functioning of soils strongly depends on the activity of the soil microbial communities and their ability to withstand different environmental stresses. The aim of this work is to compare the effect of two frequently occurring stressors (drought-rewet and freeze-thaw cycles) on the basal respiration rate of mine and natural soils. Soil samples (*n* = 18) were delivered from the Szczakowa open-cast sand quarry in Poland. The samples were measured for organic matter (OM) content, basal respiration, pH levels, and electric conductivity. The studied mine and natural soils had a similar texture and were classified as loamy sands. The natural soils contained significantly more OM than the mine soils but did not differ in terms of pH. There were no significant differences in the OM content, pH, and texture of the soils under the studied tree species (Pine, Birch, Larch). Mine soils exhibited significantly lower initial respiration rate (RESP value) than the natural soils (1.34 μg $C\text{-}CO_2$ g^{-1} 24 h^{-1} vs. 3.13 μg $C\text{-}CO_2$ g^{-1} 24 h^{-1}). Five freeze-thaw cycles reduced cumulative CO_2 evolution both in both the mine and the natural soils by 17.8% and 6.7%, respectively. Moreover, the reaction of the respiration rate to dry-rewet cycles differed distinctly between the mine and natural soils. In the natural soils, all dry-rewet cycles increased the respiration rate, wherein the increase was much more pronounced in the last two cycles. We conclude that periods of drought in the summer and freeze-thaw events in the autumn and spring may have a stronger negative effect on the functioning of forest ecosystems in the reclaimed lands than in natural stands.

Keywords: soil respiration rate, mine soils, natural forest soils, dry-wet cycles, freeze-thaw cycles

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1. Introduction

Reclamation of post-mining areas is often carried out for forestry. The ultimate goal of such a reclamation is the restoration of stable ecosystems able to withstand various environmental stresses and disturbances. The restoration of soils with properly functioning nutrient cycling is of crucial importance for reclamation success, as productive and fertile soils constitute the foundation of each terrestrial ecosystem [32].

The functioning of soils strongly depends on the activity of soil microbial communities, as microorganisms play a pivotal role in energy transfer and nutrient cycling [4, 25]. Mine soils are often built of infertile materials extracted from large depths or excavated during open-cast mining activities. These materials do not contain organic C nor N and often lack other important nutrients; hence, they are often characterized by extremely low microbial activity [3]. However, reclamation measures such as fertilization and the introduction of tree species improve these disadvantageous properties of initial mine soils relatively quickly and lead to a rapid increase in their biological activity [9, 15]. However, the young mine soils often contain less organic matter, are less biologically active, and have different structures of soil microbial communities than natural forest soils [5, 9, 15, 23].

The ability of soil microorganisms to withstand different environmental stresses depends on several factors, including the soil organic matter content, composition of vegetation cover, and occurrence frequency of the stressing factor. For instance, Hueso et al. reported that, in soils rich in organic matter, the effect of drought stress is lower than in OM-poor soils and underlined the role of vegetation diversity in the mitigating reaction of soil microorganisms to stressing moisture conditions [28]. Soil microbial communities regularly facing stressful conditions seem more resistant than those rarely exposed to stress [12, 14]. The resistance of soil microbial communities also depends on their taxonomic composition, as different groups of soil microbes developed various mechanisms to cope with different stressors [29, 33, 34].

When compared to natural forests, the low organic matter content and lower diversity of plant and soil microbial communities in reclaimed areas suggest that the microbial communities in mine soils could be more sensitive to environmental stress than those from natural soils [6]. As a consequence, the microbial processes in mine soils should be more affected than those in natural soils in the case of a stressing event. However, the young mine soils may be inhabited by specific microorganisms capable of living under harsh conditions. Furthermore, stressing conditions that occur frequently at the very beginning of the reclamation process may select stress-resistant microbial groups. However, little is known thus far about the resistance of mine soil microbial communities to various stressing factors.

Basal respiration is one of the fundamental soil properties and describes the general biological activity of the soil. Soil respiration is closely related to the decomposition of organic matter and nutrient cycling. Therefore, the measurement of basal respiration rate has been used often to study the development of soils in reclaimed areas [13].

This study was aimed at comparing the effect of two frequently occurring stressors (drought-rewet and freeze-thaw cycles) on the basal respiration rate of sandy mine soils. Since there are no threshold values for soil respiration, we used natural soils to assess the vulnerability of the basal respiration of mine soils to the stressors mentioned above.

2. Materials and Methods

2.1. Study Site

The study was carried out in Upper Silesia, Poland (19°26′E; 50°16′N) on the grounds of the Szczakowa open-cast sand quarry as well as its surroundings. The climate of the area is temperate, with mean annual precipitation of ca 700 mm and a mean annual temperature of 8°C. The soils of the study area (mainly Podzols) developed from sands. The dominant tree species in the forests surrounding the sand quarry is the Scots pine (*Pinus sylvestris*), which constitutes 72.9% of the forest stands, followed by common birch (*Betula pendula*) constituting 16.2% and the occasional stands of *Larix decidua* (data from the Chrzanów Forest District administration).

The Szczakowa open-cast quarry has been extracting sand since the early fifties of the Twentieth Century. Mining activity has occupied more than 2700 ha, and most of this area has been reclaimed for forestry. The reclamation procedures have changed over the years, recently including the forming and leveling off the surface, lupine (*Lupinus luteus L.*) cultivation for one or two years, and NPK fertilization (N: 50 kg·ha^{-1}, P_2O_5: 140 kg·ha^{-1}, K_2O: 120 kg·ha^{-1}). The fertilized areas have been planted with one-year old seedlings of Scots pine (*Pinus sylvestris*), common birch (*Betula pendula*), European larch (*Larix decidua*), common alder (*Alnus glutinosa*), and some other deciduous trees [26].

2.2. Soil Sampling

Soil samples were taken in November 2014 under pure Scots pine, common birch, and European larch forest stands in the reclaimed areas as well as in natural forests. The stand age was 30 years for the reclaimed areas and ca. 60 years for the natural stands. Each combination of forest stand (pine, birch, and larch) and soil type (mine and natural soil) was represented by three independent sites. At each site, a single mixed sample was taken. Each mixed sample consisted of five subsamples (area of each subsample = 0.16 m^2) located at the corners and in the middle of a 25 m × 25 m. After arrival at the laboratory, the samples were sieved (2 mm mesh) adjusted to 50% of their maximum water-holding capacity (WHC) and acclaimed at 22°C for 7 days prior to the experiments. The maximum water-holding capacity (WHC) was determined gravimetrically.

2.3. Freeze-thaw Experiment

Each soil sample was divided into two sub-samples (equivalent to 50 g of d.w.), one of which was then used as a stressed sample, and the other – a control sample. The subsamples were adjusted to 50% of their maximum water-holding capacity and placed in plastic cups, and their initial respiration rate was then measured. Subsequently, one subsample was frozen by placing it in a freezer at −17°C for 24 h. After freezing, the stressed subsamples were thawed at 22°C, and their respiration rate was measured 24 h after thawing. Simultaneously, the respiration rate of the control samples was measured. In total, there were 5 freeze-thaw cycles applied over 15 days. After the series of freeze-thaw cycles, the samples were measured for another three days at optimum conditions (22°C, 50% of maximum WHC).

2.4. Dry-rewet Experiment

Each soil sample was divided into two sub-samples (equivalent to 50 g of d.w.), one of which was then used as a stressed sample, and the other – a control sample. The subsamples were adjusted to 50% of their maximum water-holding capacity and placed in plastic cups without lids, and their initial respiration rate was then measured. Subsequently, the stressed sub-samples were placed under drought conditions for six days (temperature – 22°C, no water addition). After this time, the samples were rewetted to obtain a moisture level equal to 50–60% of their maximum WHC. Soil respiration rates were measured directly after the drought period in dried samples and 24 h after rewetting. In total, there were 5 dry-rewet cycles applied over the 45 days of the experiment. Simultaneously, the respiration rate was measured in the control sub-samples that were stored under optimum conditions (22°C, 50% of maximum WHC).

2.5. Analytical Methods

The organic matter content in the samples was estimated using the loss on ignition method by placing the samples at 550°C for 24 h. The texture of the samples was determined hydrometrically [24].

To measure basal respiration (RESP), the soil samples were incubated at 22°C in gas-tight jars for 24 h. The jars contained small beakers with 5 ml 0.2 M NaOH to trap the evolved CO_2. After the jars were opened, 2 ml of 0.5 M $BaCl_2$ was added to the NaOH, and the excess hydroxide was titrated with 0.1 M HCl in the presence of phenolphthalein as an indicator [7].

The soil pH was measured potentiometrically in KCl at 5:1 (*V:m* ratio) [24].

2.6. Statistical Analyses

The effect of stressors on soil respiration was expressed as the reaction indicator (RI) calculated according to the following formula: $RI = -100(C0\text{-}S0/C0)$, where C0 is the respiration rate of the control sample and S0 is the respiration rate of the stressed sample [16]. The RI values were calculated for soil respiration rates after each stressing cycle (freeze-thaw and dry-rewet) and for total CO_2 evolution during the whole experiment with each particular stressor (five cycles of freeze-thaw and five cycles of dry-rewet).

A two-way ANOVA was used to test the effect of the soil type (natural vs. mine soil) and tree species (Scots pine, silver birch, European larch) on the OM content, pH, and texture as well as the initial soil respiration rate and RI values in the studied soils. Tukey's Honestly Significant Difference test (HSD) for multiple comparisons was run to see if significant differences were found ($p < 0.05$). Prior to the analysis, the data was log-transformed as necessary to fulfill the assumption of normality.

3. Results

The studied mine and natural soils had a similar texture and were classified as loamy sands (Tab. 1). The natural soils contained significantly more OM than the mine soils but did not differ in terms of pH. There were no significant differences in the OM content, pH, nor texture of soils under the studied tree species (Tab. 1).

Table 1. Effects of soil type and tree species on soil texture (sand, silt, and clay), organic matter (OM) content, and pH by two-way ANOVA. Mean values ±standard error; asterisks indicate statistically significant differences ($p < 0.05$, HSD Tukey test); N.S. – not significant)

Factor	Level	Sand	Silt	Clay	OM	pH
		%				
Soil (So)		N.S.	N.S.	N.S.	N.S.	N.S.
	Mine soil	83 ±5	14 ±4	3 ±1	7.6 ±1.2	5.4 ±0.3
	Natural soil	81 ±2	15 ±2	4 ±1	2.9 ±0.5	5.5 ±0.2
Tree (Tr)		N.S.	N.S.	N.S.	N.S.	N.S.
	Pine	79 ±7	17 ±6	4 ±1	5.1 ±1.1	5.5 ±0.5
	Birch	87 ±2	11 ±2	2 ±1	5.0 ±1.2	5.5 ±1.1
	Larch	81 ±2	15 ±3	4 ±0	5.6 ±2.2	5.3 ±2.0
Interaction	So×Tr	N.S.	N.S.	N.S.	N.S.	N.S.

There was a visible correlation between the content of organic matter (OM) and initial RESP value (Fig. 1). The correlation coefficient was 0.76, with *R*-squared 58.39%.

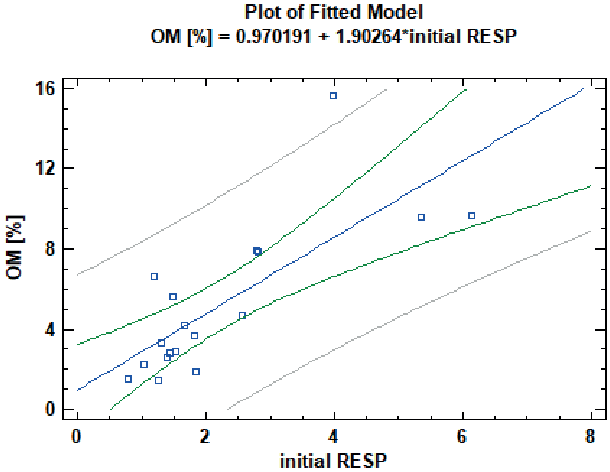

Fig. 1. Correlation OM (%) and initial RESP value (*P*-value < 0.05)

The mine soils exhibited a significantly lower initial RESP value (1.34 μg C-CO_2 g^{-1} 24 h^{-1}) than the natural soils (3.13 μg C-CO_2 g^{-1} 24 h^{-1}), and their respiration activity under optimum conditions remained lower than the activity of the natural soils over the whole experiment, resulting in significantly lower cumulative CO_2 evolution after 15 and 45 days (Tab. 2). Five freeze-thaw cycles reduced cumulative CO_2 evolution in both the mine and natural soils by 17.8% and 6.7%, respectively (Tab. 2). However, the difference in the reaction to the freeze-thaw stress between the mine and natural soils was not statistically significant. As a consequence, cumulative CO_2 evolution after the freeze-thaw cycles was lower in the mine soils than in the natural soils. The reaction of the respiration rate to five consecutive freeze-thaw cycles differed between the mine and natural soils. The first freeze-thaw cycle increased the respiration rate of the mine soils, while this was not the case for the natural soils (Fig. 2). Conversely, after the fourth and fifth freeze-thaw cycles, the respiration rate in the mine soils was reduced further than in the natural soils. Similarly, the respiration rate measured after the whole experiment was reduced further in the mine soils than in the natural ones (Fig. 2).

The reaction of respiration rates to dry-rewet cycles differed distinctly between the mine and natural soils. In the natural soils, all dry-rewet cycles increased respiration rates, wherein this increase was much more pronounced in the last two cycles (Fig. 3). In the mine soils, the respiration rate was not stimulated after rewetting, returning only to the initial values. Considering that the respiration rate was reduced to 0 upon drying, the five dry-rewet cycles resulted in a reduction of cumulative CO_2 evolution in the mine soils, while cumulative CO_2 evolution increased in the natural soils (Tab. 2).

Table 2. Effects of soil type and tree species on initial basal respiration (RESP), cumulative CO_2 evolution after 15 ($Cumul_{15}$) and 45 ($Cumul_{45}$) days in non-stressed soils, and soils affected by 5 cycles of freeze-thaw (Cumulfreeze) and dry-rewet (Cumuldry) stress as well as on reaction of respiration rate to applied stressors (reaction indices – RI) by two-way ANOVA. Mean values ±standard error; asterisks indicate statistically significant differences ($p < 0.05$, HSD Tukey test); N.S. – not significant

Factor	Level	RESP	$Cumul_{15}$	$Cumul_{freeze}$	$Cumul_{45}$	$Cumul_{dry}$	$RICumul_{freeze}$	$RICumul_{dry}$
		μg C-CO_2 g^{-1} 24 h^{-1}	μg C-CO_2 g^{-1}				%	
Soil (So)		*	*	*	*	*	N.S.	*
	Mine soil	1.34 ±0.11	16.05 ±1.16	13.42 ±1.69	50.81 ±4.02	34.35 ±2.35	−17.8 ±6.7	−30.3 ±5.4
	Natural soil	3.13 ±0.56	33.87 ±7.13	31.09 ±6.44	111.05 ±27.70	123.79 ±27.39	−6.7 ±5.6	18.2 ±10.2
Tree (Tr)		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Pine	2.12 ±0.68	23.55 ±6.56	21.99 ±7.45	84.36 ±33.97	82.47 ±32.12	−13.0 ±5.91	−3.9 ±12.75
	Birch	2.61 ±0.72	29.35 ±9.96	27.00 ±8.18	90.85 ±32.22	90.23 ±39.29	−3.5 ±8.80	−7.2 ±18.02
	Larch	1.99 ±0.47	21.97 ±4.94	17.78 ±4.71	67.57 ±13.18	65.50 ±19.16	−20.4 ±7.67	−7.1 ±13.56
Interaction	So×Tr	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

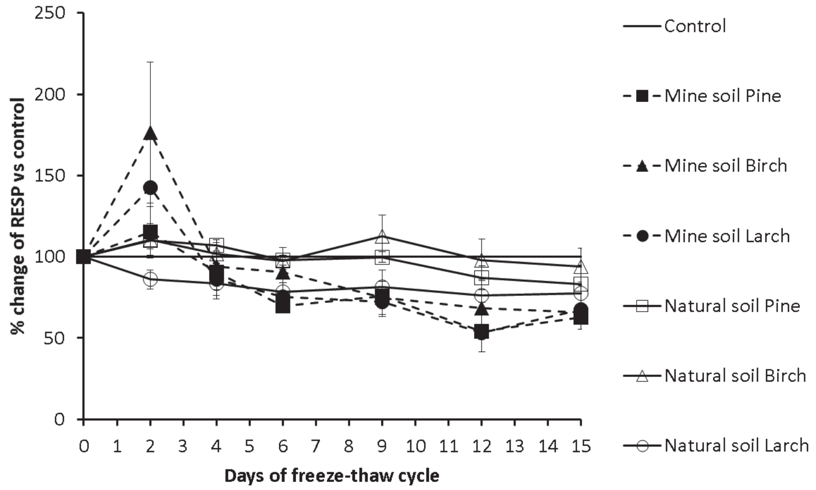

Fig. 2. Respiration in freeze-thaw cycle

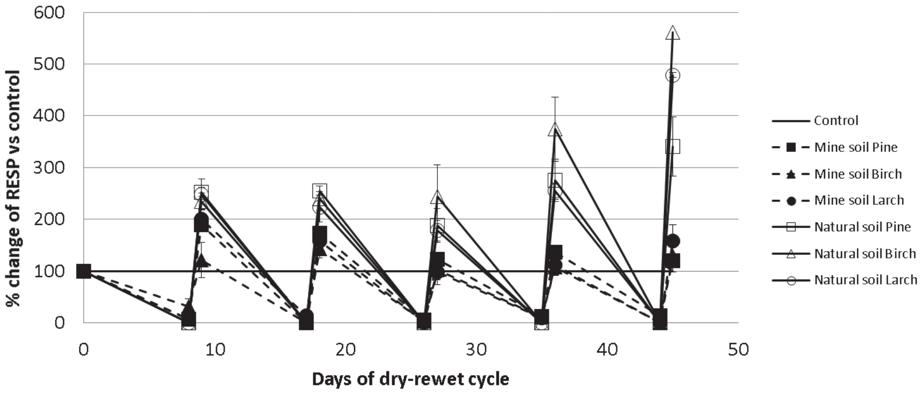

Fig. 3. Respiration in dry-rewet cycle

There were no significant differences in RESP, CO_2 evolution (over 20 or 50 days under optimum or stressing conditions), nor in the reaction to freeze-thaw or dry-rewet related to the tree species (Tab. 2).

4. Discussion

The mine soils exhibited much-lower values of initial respiration rates and cumulative CO_2 evolution over the 15- or 45-day experiments than the natural forest soils. The lower respiration activity of the mine soils was apparently related to their lower OM content. It is well known that a soil's microbial activity is positively related to its OM content, as organic compounds are an energy source for microbes [20]. The studied mine soils developed from excavated sands that did not contain organic matter. As a consequence, they contained only the organic matter that accumulated over 30 years of forest stand development at the time of sampling. The adjacent natural forest stands were older (60 years old) and grew on soils that likely contained some pool of organic matter before planting the last generation of trees. Consequently, they were more active biologically than the studied mine soils.

The applied stressors induced distinct reactions of the respiration rate in the studied soils. This was probably due to the different nature of these two stressors. The reaction of soil microbes to the stressing factors may be variable due to the different effects of the stressors on the soil's chemical and physical properties [16] and the diverse mechanisms that the microbes developed to cope with them [2]. Freeze-thaw cycles may disrupt a large proportion (up to 50%) of soil microbial biomass [31]. Dry-rewet cycles pose an osmotic stress on soil microbes, both upon drying and later rewetting. A period of drought affects soil microorganisms through starvation, osmotic stress, and resource competition, while rewetting causes stress for soil microorganisms (as they must rapidly dispose their osmolytes in order to counteract the rapid flow of water into their cells). As the microbial osmotolerance mechanisms are energetically expensive, drought and rewetting are among the most-potent environmental stressors and may lead to substantial changes in the structure of soil microbial communities [19, 31].

The effect of the freeze-thaw cycles on respiration was relatively weak. The calculated reaction indices for cumulative CO_2 evolution did not differ between the mine and natural soils. In both of the studied soil types, the series of freeze-thaw cycles reduced cumulative CO_2 evolution; however, the decrease was relatively small (by 6.7–17.8%). This was probably because the initial freeze-thaw cycles tended to stimulate soil respiration. Indeed, increased soil respiration with thawing has been reported for different soils [18] and attributed to the decomposition of organic compounds released from the cells of microorganisms killed by soil freezing. However, similar to our results, Schimel and Clein [31] reported that pulses of CO_2 decreased with successive freeze-thaw cycles. These authors argued that the decrease of CO_2 pulses in consecutive freeze-thaw cycles was related to soil OM availability and the size of the soil microbial biomass. According to their argumentation, soils with readily available OM and an active microbial biomass would have a low CO_2 pulse upon the first freeze-thaw cycle, but cumulative CO_2 evolution over longer periods of freeze-thaw cycles would be less affected than in soils with a lower pool of available OM. In our study, the differences in the reaction of soil respiration to freeze-thaw stress between the mine and natural soils were not statistically significant due to the large variability of soil respiration. However, the mine soils tended to have higher CO_2 evolution after the first freeze-thaw cycle, but their cumulative CO_2 evolution over five cycles decreased more than in the natural soils. This suggests that mine soils are more sensitive to environmental stressors than natural soils.

The reaction of soil respiration to dry-rewet cycles was stronger than for freeze-thaw cycles and differed between the mine and natural soils. In the mine soils, cumulative CO_2 evolution over five dry-rewet cycles was strongly reduced (by ca. 30%) as compared to the non-stressed control, while this was increased significantly in the natural soils (by ca. 18%). An evident reason for the stronger cumulative respiration of the natural soils was their much-higher CO_2 pulses after consecutive rewetting cycles (Fig. 2). These pulses in the natural soils were so large that they overcompensated the reduced respiration during drought periods. For the mine soils, the CO_2 pulses did not compensate the reduced respiration over dry periods, as their magnitude was much lower. Increased CO_2 evolution upon the rewetting of dried soils is known as the Birch effect and may be caused by the rapid mineralization of C released from dead cells killed by drought [22], release of previously unavailable organic compounds [1], and resuscitation of soil microorganisms that survived the drought period in a dormant state [27]. We presume that the lower CO_2 pulses after consecutive dry-rewet cycles in mine soils and the consequential decrease in cumulative CO_2 release resulted mainly from their lower content of organic matter (and, as a consequence, lower microbial biomass). Indeed, Fierer and Schimel [11] reported that several dry-rewet cycles increased cumulative respiration in the OM-rich forest soils while decreasing it in the OM-poor grassland soil. They concluded that CO_2 pulses would be higher in soils with a larger OM pool and microbial biomass, owing to the larger organic C pool available for the microbes that survived the drought period.

After consecutive dry-rewet cycles, the CO_2 pulses were lower in the mine soils, while these pulses were higher in the natural soils after the last two cycles. Kemmitt et al. [21] suggested that the mineralization of soil organic matter is not regulated by the size, activity, nor composition of the soil microbial biomass but is governed by abiotic processes that convert non-bioavailable soil OM into bioavailable soil OM. We presume that repeated dry-rewet cycles finally cause a breakdown of the organic matter, causing a release of readily available organic compounds and, as a consequence, stronger CO_2 pulses in the natural soils. In the mine soils, the OM pool was much lower; thus, the bioavailable OM and their CO_2 pulses after dry-rewet cycles were also lower.

The reaction of soil respiration did not depend on the dominating tree species. Silver birch, Scots pine, and European Larch are known to produce litter of different quality and support the development of diverse microbial communities [8, 10, 30]. However, the effect of the tree species on soil OM quality and soil microbial community structure is evident mainly in the soil O horizon and disappears quickly with soil depth [17]. In our study, we used soil samples taken from depths of 0–25 cm, containing mainly humified organic matter. Thus, the quality of soil OM might have been similar under all studied tree species; as a consequence, the OM content controlled the reaction of soil respiration to the applied stressors. Since the soil OM contents did not differ between the tree species, there was no difference in the reaction of soil respiration between the soils under the studied tree species.

5. Conclusions

The reaction of soil respiration to two common environmental stressors (freeze-thaw and dry-rewet cycles) differed between the mine and natural soils. The stressors decreased the soil respiration of the mine soils, while this was not always the case for the natural soils. The observed difference could be mainly attributed to the differences in organic matter content in the two studied soils types, as the mine soils contained less organic matter and were less-biologically-active than the natural forest soils. Since soil respiration is related to nutrient cycling, we conclude that periods of drought in the summer and freeze-thaw events in the autumn and spring may have a stronger negative effect on the functioning of forest ecosystems in reclaimed lands than in natural stands.

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Wpływ stresogennych czynników środowiskowych na tempo respiracji zalesionych gruntów kopalnianych

Streszczenie: Funkcje gleb silnie zależą od aktywności mikroorganizmów i ich zdolności do przeciwstawiania się różnym stresom środowiskowym. Celem pracy było porównanie wpływu dwóch często występujących czynników stresowych – cykli susza-wilgoć i mrożenie-rozmrażanie – na respirację gleb industrioziemnych i gleb naturalnych. Próbki gleby (n = 18) zostały pobrane na zrekultywowanych terenach kopalni piasku w Szczakowej oraz w lasach naturalnych w jej sąsiedztwie. W próbkach oznaczono zawartość materii organicznej, tempo respiracji, pH i przewodność elektrolityczną. Badane próbki z gleb industroziemnych i naturalnych miały podobne uziarnienie i zostały sklasyfikowane jako piaski gliniaste. Gleby naturalne zawierały znacznie więcej OM [%] niż gleby industrioziemne, natomiast obie rozpatrywane kategorie gleb nie różniły się znacząco pod względem pH. Ponadto nie zaobserwowano istotnych różnic w zawartości materii organicznej, pH i uziarnienia gleb w próbkach pobranych pod różnymi drzewostanami (sosna, brzoza, modrzew). Gleby industroziemne wykazywały znacznie niższe początkowe wartości respiracji (1,34 μg C-CO_2 g^{-1} 24 h^{-1}) niż gleby naturalne (3,13 μg C-CO_2 g^{-1} 24 h^{-1}). Pięciocyklowy proces mrożenia-rozmrażania zredukował skumulowane wartości wydzielania CO_2 zarówno w industrozimenych, jak i naturalnych glebach o odpowiednio 17,8% i 6,7%. Ponadto reakcja na wskaźnik respiracji w cyklu susza-wilgoć różniła się znacząco w przypadku gleb industroziemnych i naturalnych: w glebach naturalnych wszystkie cykle suszenia-nawadniania zwiększały wartość respiracji, przy czym wzrost ten był bardziej widoczny w ostatnich dwóch cyklach. Podsumowując, można stwierdzić, że okresy suszy w lecie i mrożenia-rozmrażania jesienią oraz wiosną mogą wywierać bardziej negatywny wpływ na leśne ekosystemy na ternach zrekultywowanych niż na obszarach występowania naturalnych drzewostanów.

Słowa kluczowe: wskaźnik respiracji gleby, gleby industroziemne, naturalne gleby leśne, cykle susza-wilgoć, cykle mrożenia-rozmrażania