EFFECT OF MOISTURE CONTENT ON RESPIRATION RATE OF FOREST SOILS WITH THE ADDITION OF POLYMERIC SUPERABSORBENTS

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Abstract

The present study examined the role of moisture on the soil respiration rate in forest soils supplemented with two different types of polymeric superabsorbents. Soil samples were collected in the Tyniecki Forest near Krakow, Poland. The physicochemical characteristics of the soils were studied, as well as the basal soil respiration rate under standard substrate moisture conditions, which is 60% of the maximum soil water capacity. An experiment was conducted in which two different types of polymeric superabsorbents (SAP) were added to the soils: SAP 1, a classic representative of synthetic acrylic polymers, and SAP 2, a copolymer based on oxidized starch and acrylic acid. To accomplish the research objective, moisture conditions of the studied samples were determined - simulated variants of constant optimal moisture, normal sediment followed by dry conditions, and over-gravel sediment followed by dry conditions. The experiment had 2 dry-rain cycles. During the experiment, changes in the soil respiration rate were measured. The study concluded that soil moisture and the role of SAP are of paramount importance - the maximum soil respiration rate was achieved under conditions of constant soil moisture at 60% WHC and under conditions of over-gravel precipitation and subsequent drying - in these variants, the soil had sufficient moisture due to hydrogels. SAP 2 performed better than SAP 1 - this is due to the chemical structure of the hydrogel, where oxidized starch played the role of an additional easily digestible carbon substrate for soil microorganisms.

Keywords: *superabsorbents, soil respiration, soil moisture, microbial activity*

1. INTRODUCTION

Soil respiration is one of the most important processes of the carbon cycle in ecosystems, reflecting the total metabolic activity of soil microbiota and plant roots. This process directly depends on factors such as temperature, organic matter content, and, to a large extent, soil moisture. In forest ecosystems, especially in the context of climate change, the resistance of soil microbiota to moisture fluctuations is becoming increasingly important.

Soil water has a complex effect on the metabolic activity of microorganisms. With reduced moisture, substrate mobility decreases, cellular activity is disrupted, and microbial communities are suppressed. On the other hand, excessive moisture can cause anaerobic conditions that inhibit aerobic respiration. Thus, there are optimal moisture ranges in which the greatest intensity of soil respiration is observed.

In the last decade, much attention has been paid to the use of polymer superabsorbents (SAP) - materials that can repeatedly absorb and retain water, and then gradually release it. Originally developed for agriculture, SAPs have come to be regarded as promising additives for improving the water regime in various soil types [1]. It is assumed that they can increase the availability of moisture for soil microflora, especially during drought periods, thereby supporting the activity of microbial communities and soil respiration. SAPs are currently widely used as soil additives and, as numerous studies have shown, they affect the structure of the soil and improve its water regime. SAPs can also have a positive effect on the microbiological activity of the soil [14]. Despite the growing interest in the use of SAPs, the effect of these materials on microbiological processes in forest soils, in particular on the rate of soil respiration at different moisture levels, remains poorly understood. different from agroecosystems, which requires a separate approach to research. The main objective of the current work is to study the effect of moisture content on the respiration rate of forest soils with the addition of polymer superabsorbents.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1 Soil collection and description

Soil samples for the study were collected in the Tyniec Forest (coordinates: 50°00'18.1 ‘N, 19°49'56.2 ’E), located near the city of Kraków (Małopolskie Voivodeship, Poland). The Tynieckie Forest is part of Bielańsko-Tynieckiego Landscape Park. Samples were collected in October 2023 on a square of terrain about half a kilometre apart. The study site is a square with four sampling sites placed in the corners of the square and a fifth site in the centre. Five sub-samples were collected at each site. Samples were collected from the mineral soil layer at a depth of 0 - 5cm beneath identical stands of trees (Fágus sylvática) up to 20 years old.

After transporting the samples to the laboratory, each sample was homogenised to obtain a total sample of each site. Afterwards, the samples were sieved through a sieve with a mesh diameter of 2 mm. They were divided into two parts: one part was used for analyses of soil physico-chemical characteristics after reaching air-dry condition, and the second, fresh part of the sample was stored at 4 °C until the samples were incubated before microbiological analyses. For this purpose, the samples were incubated at 22 °C for six days, maintaining the moisture level at 60% of the maximum water-holding capacity (WHC) to avoid a spike in microbial activity following changes in temperature and moisture level.

2.1.2 Superabsorbents

Polymeric superabsorbent materials (SAPs), which are hydrogels, have the ability to retain water and biological fluids in volumes hundreds of times their own mass. According to a study [3], under simulated soil moisture deficit conditions, the addition of SAPs increased the water content of soil by 30% compared to the control sample without SAPs, especially during alternating dry and wet periods. A decrease in soil bulk density was also observed. Synthetic hydrogels have been the subject of active research over the last forty years and this field is still very relevant [1].

Two different types of superabsorbents were used in this experiment, a synthetic one represented by SAP 1, a synthetic acrylic polymer, and a natural one represented by SAP 2, a copolymer based on starch and acrylic acid. SAP 1 is a traditional synthetic superabsorbent produced by polymerisation of vinyl monomers. SAP2 is synthesised from acrylic acid and oxidised starch. It is suggested that due to the content of starch, which is an available carbon source, SAP2 may have a favourable effect on soil microbiota by stimulating microbial degradation processes [9]. These superabsorbents have different water-holding capacity: for SAP 1 it is about 240 grams of water per gram of dry matter, and for SAP 2 it is about 200 grams of water per gram.

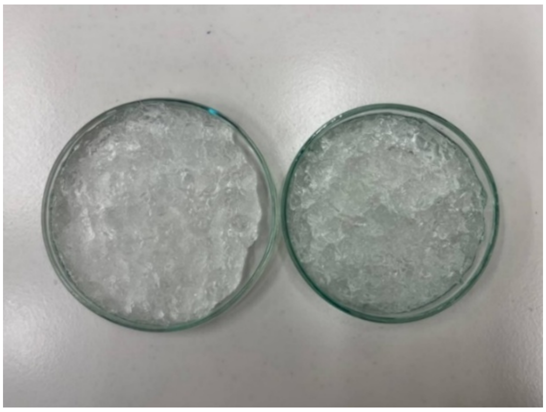

Fig. 1. SAPs used in the assays – SAP1 (left side) and SAP2 (right side) – product after absorption of water. Source: our elaboration

2.2. Methods

The study includes study of physicochemical characteristics of soil samples: analysis of granulometric composition, determination of pH, determination of WHC, amount of total carbon, as well as measurement of basic soil respiration under conditions of constant moisture and without addition of SAP. The study of soil respiration under different variants of water regimes and addition of two different types of SAP was carried out in accordance with Figure 2. The moisture regimes tested during the study are shown in Table 1.

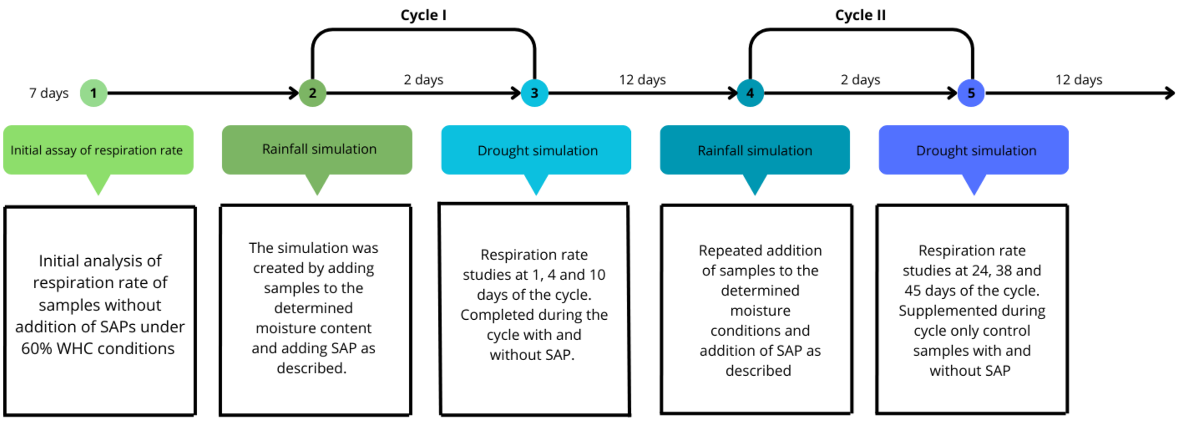

Fig. 2. Schematic diagram of the main study with a description of each stage

Source: own development

After sample preparation, analyses of soil physicochemical characteristics and respiration were carried out without the addition of SAP (step 1). The experimental conditions took into account different variations in soil moisture content as well as the addition of SAP. The stages of the study are shown in Figure 2.

Variant number	Humidity conditions (WHC), [%]	Type of simulation	Added superabsorbent
1	60%	Control, constant soil moisture	no
2	60%	Optimum humidity conditions (60% WHC)	Acrylic polymer - SAP 1
3	>100%	Extreme rain - drought	
4	100%	Normal rain - drought	
5	60%	Optimum humidity conditions (60% WHC)	Starch-acrylic copolymer - SAP 2
6	>100%	Extreme rain - drought	
7	100%	Normal rain - drought	

Table 1. Description of options, presenting the conditions for conducting the study

Source: our elaboration

2.2.1. Investigation of basic characteristics of soil without addition of SAP

In accordance with Figure 2, at the first stage of the study, analyses of physicochemical characteristics of all 5 soil samples were carried out. The measurement of maximum soil water capacity (WHC) was carried out according to [24]. The pH was determined according to PN-ISO 10390:2021 in H_2O and KCl. Total carbon was determined on a CS500-Eltra elemental analyser. The presence of carbonates in the soil was checked by field method. Areometric analysis of soil particle size distribution was analysed according to the Casagrande method [20]. Detailed physico-chemical characteristics of the studied soil samples are presented in Table 2.

2.2.2 Soil respiration

To measure the basal rate of soil respiration, 50-gram samples were incubated for 24 h at 22°C in gas-tight transparent jars. Beakers with 5 ml of 0.2 M NaOH were kept in them to capture the CO_2 released during soil respiration. At the end of incubation, 2 ml of 0.9 M $BaCl_2$ was added to the beaker with NaOH. Excess hydroxide was titrated using 0.1 M HCl in the presence of phenolphthalein as indicator. Microbial carbon (C_{mic}) was calculated from the rate of substrate-induced respiration according to the equation [2] $C_{mic}[mg\times g^{-1}] = 40.04\ y + 0.37$, where "y" is mL $CO_2\times h^{-1}\times g^{-1}$.

The prepared fresh soil was incubated for 6 days at 22°C under optimal moisture conditions of 60% WHC to avoid microbiological stress and activity spikes before the start of the experiment. Soil respiration measurements for each of the samples without the addition of SAP were also carried out after preliminary preparation. The experiment simulated 2 rain-dry cycles by changing the soil moisture regime according to Figure 1. Before the start of the first cycle, soils were flooded to a certain level of their HWC, as well as SAPs were added at the rate of 0.1% of soil weight according to Table 1. Measurements of the respiration rate were taken two days after flooding and were performed on days 1,4 and 10 (step 2-3). After the measurements were completed, the samples were not submerged for 12 days, simulating dryness. After this time, the rain-dry cycle was repeated and measurements were taken on days 24, 38 and 45 from the start of the experiment. During the whole experiment, only samples that were kept at 60% of their maximum water capacity were refilled according to Table 1.

3. RESULTS

The studied physicochemical characteristics of the soil, as well as the basic respiration rate of each sample, are presented in Table 2.

Area number	***Parameter examined***							
	Granulometric group			**pH (H_2O)**	**pH (KCl)**	**Carbonates [%]**	**C total [%]**	**RESP [µM CO2/g/24h]**
	Sand [%]	**Dust [%]**	**Clay [%]**					
1	Sandy loam			4,63	3,28	0,0	3,1123	0,60
	58	34	8					
2	Sandy loam			4,51	3,46	0,0	3,6905	0,78
	57	36	7					
3	Fine sand			4,48	3,31	0,0	1,2960	0,32
	86	12	2					
4	Sand			4,73	3,79	0,0	0,4151	0,36
	94	4	2					
5	Sand			4,53	3,19	0,0	0,8048	0,34
	94	4	2					

Table 2. Physicochemical and starter respiration tempo of the studied soil samples

Source: our elaboration

3.1 Soil respiration

Soil respiration was measured in three stages: starter respiration, first cycle after adding water to a certain percentage of WHC, according to the test scheme (Figure 2) and adding two different types of superabsorbents to the soil, second cycle - refilling the soil with the same amount of water.

Area 1 (Figure 3):

The initial soil respiration at 60% WHC without additives was 0.51 µM CO_2/g/24h. In cycle I of the control sample, the peak (0.85 µM CO_2/g/24h) occurred on day 4, after which activity decreased. After a 12-day dry period, cycle II started at 0.67 µM CO_2/g/24h, decreased to 0.26 µM CO_2/g/24h on day 4, and then increased to 0.56 µM CO_2/g/24h on day 10. In SAP1 samples, activity initially increased, then decreased. In cycle II, a sharp increase (1.73 and 1.0 µM CO_2/g/24h) was followed by a decrease to 0.56 and 0.01 µM CO_2/g/24h. SAP 1 showed higher activity than SAP 2 and the control.

With SAP 2 addition, the trend was similar, with the highest activity observed under heavy rainfall. In cycle II, activity on day 1 was higher than in cycle I, while the control remained stable.

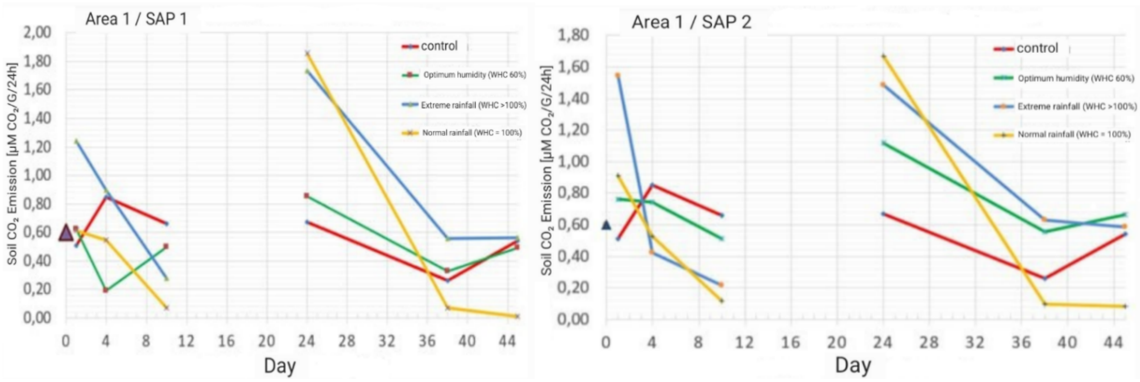

Fig. 3. Soil respiration activity graph - start, cycle I and cycle II for area 1 with addition: SAP1, SAP2

Source: own elaboration

Area 2 (Figure 4):

The initial respiration rate at this location was 0.55 µM CO_2/g/24h. The control sample showed a consistent pattern—peak activity on day 4, followed by a gradual decline. At 60% WHC, optimal for microorganisms, respiration peaked on day 4 in both cycles I and II, after which activity decreased. SAP 1 addition under these conditions increased the maximum respiration rate to 1.71 µM CO_2/g/24h, 1.7 times the peak value of cycle 1.

Excessive rainfall led to an increase in respiration from a minimum of 0.37 µM CO_2/g/24h to a maximum on day 10 of cycle I. In cycle II, activity started lower (0.42 µM CO_2/g/24h), peaked on day 14, and then decreased. At 100% WHC, simulating normal rainfall, respiration increased to 1.53 µM CO_2/g/24h (day 4, cycle I). In cycle II, activity started at twice the value of cycle I end and decreased by day 45.

SAP 2 addition caused a more pronounced increase than SAP 1. Both 60% and 100% WHC conditions showed similar trends—peak activity on day 4 of cycle I and day 1 of cycle II, with maximum values of 4.49 and 4.51 µM CO_2/g/24h (cycle I) and 3.61 and 3.63 µM CO_2/g/24h (cycle II). The difference in maximum values for optimal moisture conditions was 4.53 µM CO_2/g/24h.

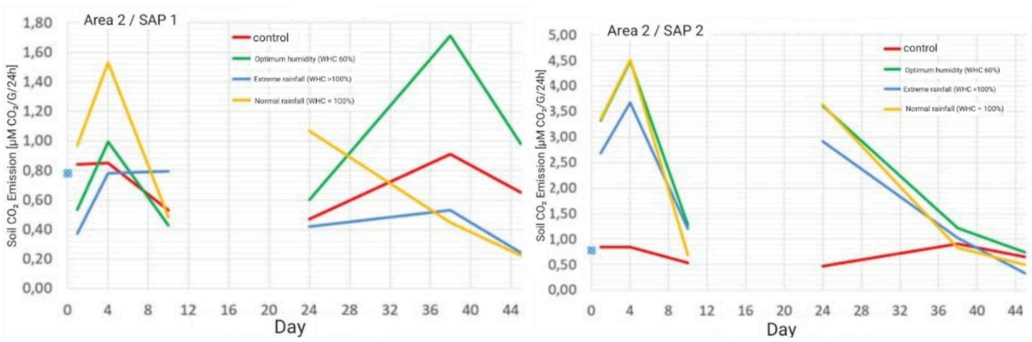

Fig. 4. Soil respiration activity graph - start, cycle I and cycle II for area 2 with addition: SAP1, SAP2

Source: own elaboration

Area 3 (Figure 5):

The initial respiration rate was 0.21 µM CO_2/g/24h. In the control sample, peak respiratory activity occurred on day 4 of each cycle, with cycle 2 starting at the same level despite a 12-day drought. In all variants, maximum activity was recorded on days 4 and 14 of subsequent cycles.

SAP 1 showed the highest activity under excessive rainfall in cycle I (0.64 µM CO_2/g/24h) and at optimal moisture (60% WHC) in cycle II (0.60 µM CO_2/g/24h). SAP 2 had higher peak values: 0.74 µM CO_2/g/24h under excessive rainfall (cycle I) and 0.63 µM CO_2/g/24h at 60% WHC (cycle II).

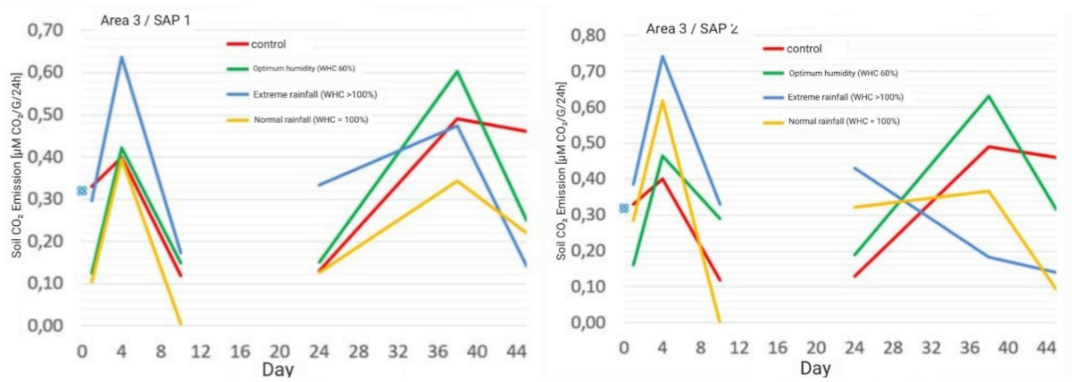

Fig. 5. Soil respiration activity graph - start, cycle I and cycle II for area 3 with addition: SAP1, SAP2

Source: own elaboration

Area 4 (Figure 6):

The initial respiration rate was 0.31 µM CO_2/g/24h. In control samples, peak activity (0.66 µM CO_2/g/24h) was recorded on day 4 of cycle I. In cycle II, activity increased from 0.36 to 0.46 µM CO_2/g/24h, then dropped to 0.09 µM CO_2/g/24h (day 4), before doubling by the end of the study.

Samples with SAP 1 followed a similar trend to the control but differed in CO_2 release levels. The highest respiratory activity occurred under excessive rainfall, while the lowest was at 100% WHC (normal rainfall).

SAP 2 caused a sharp increase in respiration in cycle I, aligning with SAP 1 levels in cycle II. The peak (1.66 µM CO_2/g/24h) occurred under excessive rainfall, while the minimum (0.26 µM CO_2/g/24h) was at 100% WHC. In cycle II, normal rainfall conditions were more stable, whereas under excessive rainfall, respiration dropped from 0.88 to 0.06 µM CO_2/g/24h on day 14.

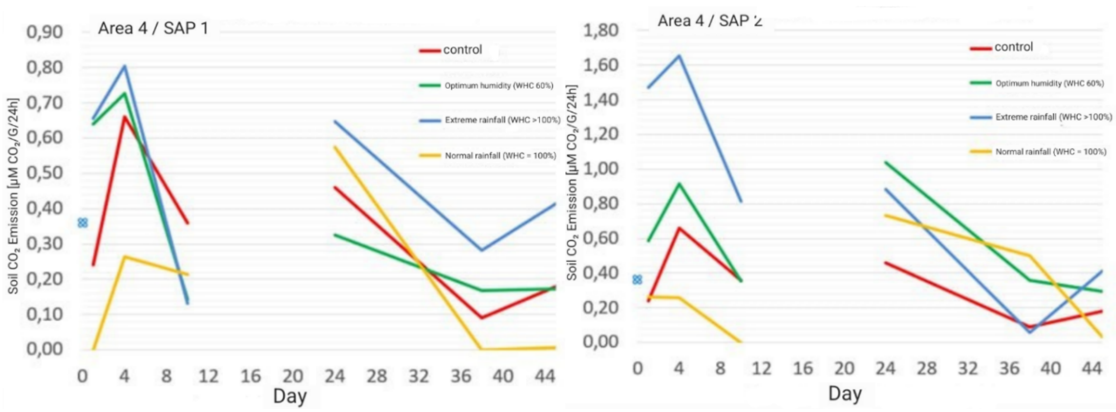

Fig. 6. Soil respiration activity graph - start, cycle I and cycle II for area 4 with addition: SAP1, SAP2

Source: own elaboration

Area 5 (Figure 7):

The initial soil respiration rate was 0.24 µM CO_2/g/24h, with peak activity recorded on day 4 in all samples. In the control sample, CO_2 release dropped from 0.30 µM CO_2/g/24h at the start of cycle I to 0.09 µM CO_2/g/24h at the beginning of cycle 2, matching the value recorded on day 10 of the first cycle.

Samples with SAP 1 showed the highest activity under excessive rainfall in cycle I (0.57 µM CO_2/g/24h) and under normal and optimal conditions in cycle II (0.46 µM CO_2/g/24h). The addition of SAP 2 resulted in higher respiratory activity, reaching 0.78 µM CO_2/g/24h in cycle I (>100% WHC) and 0.69 µM CO_2/g/24h in cycle II (optimal conditions).

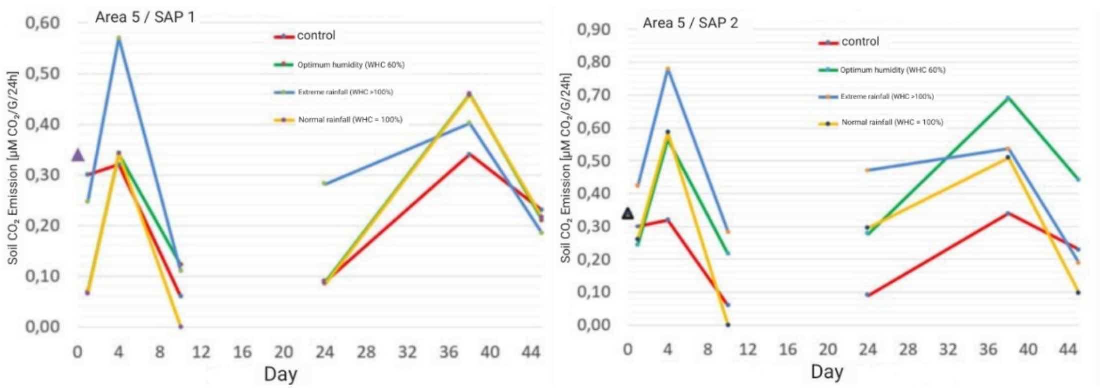

Fig. 7. Soil respiration activity graph - start, cycle I and cycle II for area 5 with addition: SAP1, SAP2

Source: own elaboration

4. DISCUSSION

4.1 Analysis of the results obtained for starter soil samples (without SAP) at constant soil moisture (60% WHC)

4.1.1 Correlation between the percentage of total C in soil samples and their respiration rate

Respiration rate, or soil respiration, is an integral indicator of biological activity and soil quality. The respiration process involves the decomposition of soil organic matter by microorganisms, which release carbon dioxide, thereby releasing plant-available matter. Organic matter accounts for about 50% of carbon (C) [28]. Inorganic carbon is absent in the study areas, so only the organic form is shown under the phrase "total carbon". All soil samples from sites 1-5 have low carbon levels. Soils with low organic carbon content, as in the present study, are characterized by low fertility and poor microbial activity [19]. In the present study, the relationship between the respiration rate and the percentage of total carbon was positively correlated (determination coefficient R^2=0.8916).

These observations confirm the findings of Lai [16], who suggests that increasing the content of labile carbon in the soil in the form of organic amendments increases the soil respiration rate. In addition, Gao's research [13] shows that increasing soil carbon content by more than 50% increases microbial carbon, stimulates microbial growth, and enhances microbial activity by stimulating the synthesis of extracellular enzymes with active decomposition of soil organic matter.

4.1.2 Relation of respiration rate to types of microorganisms

Soil respiration in the studied samples is not stable and typical for any control samples maintained at a constant substrate moisture of 60% WHC. The study did not include the study of microbial community representatives in the samples. Several hypotheses can explain the atypical pattern of soil respiration in the control samples. Rapid bursts of respiration can be observed in the middle of the cycle (during drying), which can be associated with specific soil microbial communities. The fungal community among soil microorganisms is most adapted to low moisture conditions than bacteria, representing a higher biomass and percentage of activity among all microorganisms in moisture-deficient soils [14,34]. Fungi are able to survive in harsh conditions of high soil acidity with unavailable substrate, exerting a strong influence on soil activity [15]. Most likely, fungi dominate in the studied samples. This assumption comes from the study [6], in which microbial activity was analysed under drought conditions - the respiration rate correlated with fungal biomass.

Whitaker J. [30] also suggested a similar hypothesis that increased respiration rates are associated with the abundance of fungi. Fungi have a greater impact on soil respiration than other microbial communities [4]. Water stress affects microbial activity in soils. It is believed that 60% WHC is considered optimal conditions [33]. However, in this study, maintaining constant moisture at 60% WHC in the control samples may be insufficient and provide unfavourable conditions for microbial activity, which should also be taken into account.

4.2 Analysis of the results obtained with the addition of SAP from varying substrate humidity

4.2.1 Soil respiration rate tests

Soil respiration after the addition of superabsorbents can cause various changes. Many studies [6,7] conclude that improving soil structure and increasing its water-holding capacity by adding SAP to soils increases soil microbial activity. The use of SAP as a soil additive significantly affects soil quality. Long-term studies [32] describe the effect of superabsorbents as positive because of the correlation between SAP and increased rate of water and nutrient utilisation in soil by improving soil structure and increasing organic carbon content and microbial activity.

In the present study, it is also found that SAP creates more favourable conditions for microbiota activity, especially under conditions of high water content of hydrogels.

- Variable moisture conditions

The use of constant soil moisture of 60% of the maximum soil water capacity for both superabsorbents had a relatively stable effect on soil moisture retention - both hydrogels had the effect of maintaining respiration activity at a level not lower than the initial respiration rate. Based on the literature data [33], it was assumed that the use of moisture conditions equal to 60% of the relative soil moisture is considered optimal for the development of microcommunities in the soil. A different trend was observed in the present study. The conditions of the control samples for these groups are stable, not providing a rapid increase in respiration, as was observed under other soil moisture conditions. Conditions above 100% soil moisture are more favourable than conditions at 100% soil moisture with respect to soil respiration activity. This level of moisture assumes the presence of free water, which ensures the solubility and availability of substrates [29]. Under conditions of excess water, anaerobic bacteria thrive, capable of decomposing organic matter in the absence of oxygen, maintaining the respiration rate [18]. While 100% WHC conditions form microaerobic communities and the activity is gradually or abruptly suppressed after the available nutrients are depleted.

Our data do not correlate with studies [54, 13] that show that 100% WHC soil is optimal for organic matter decomposition and supply of essential nutrients to plants. At the same time, the results of [30] indicate that excess moisture conditions create unfavourable conditions for soil microbial activity and organic matter decomposition. Testing with SAP substrate at different moisture conditions requires an individual approach to each soil and its unique microbial composition.

In his work, Xi Li [17] argues that SAPs can be useful for the formation of larger soil aggregates (< 0.25 mm), which improve water infiltration and aeration in the soil. SAPs are directly adsorbed onto soil particles, which helps to prevent dispersion [21]. Such soil aggregates are able to maintain more stable moisture levels over long testing periods [11].

- Effect of SAP on soil respiration rate

A higher soil respiration activity index throughout the experiment was recorded with SAP 2. This is due to the fact that this superabsorbent consists of more than 50% oxidized starch, which can be a source of additional readily available carbon for microorganisms [1]. The addition of SAP 2 for conditions of 60% WHC and excess water (more than 100% WHC) resulted in a higher increase in respiration activity compared to SAP 1, indicating that this SAP is able to significantly increase the microbial activity of rather poor soils. The presence of additional substrate significantly correlates with an increase in soil biomass, which is confirmed by studies [25].

Due to the pre-adopted study design, it is impossible to follow this phenomenon in more detail. We assume that this is due to the rapid growth of biomass and assimilation of the available carbon substrate under comfortable conditions for microbiota [8]. SAP 1 can improve soil moisture conditions, affect soil quality and enhance soil microbial activity, but not to the same extent as SAP 2.

SAP 1 probably acts purely as a soil conditioner [1], helping the microbiota obtain water during periods of drought. The amount of stored water in SAP 1 particles is higher compared to SAP 2 due to its higher water absorption compared to SAP 2, which may contribute to local soil drying (soil water is absorbed by the hydrogel). Soil water retention was also influenced by soil texture, which differed between the study areas: where the percentage of silt and clay was higher, moisture was retained better than in sandy soils. They did not dry out as quickly during the simulated drought period to create destructive conditions for the soil microflora, and thus maintained favourable conditions for microbial activity longer [10]. Under simulated excess rainfall, both types of SAP are likely to absorb excess water, leaving the soil moist enough for comfortable microbial activity. When drought occurs, both types of superabsorbents release water and are likely to maintain the most optimal conditions. Therefore, it can be said that SAP 2 under high humidity conditions (above 100% WHC) are able to act most effectively in maintaining optimal soil respiration conditions. Studies [12] show that acrylic acid-based SAPs are able to enhance soil microbial activity under water stress conditions. Barakat et al. [5] confirm that SAPs can have a significant effect on water retention in the root zone of plants in sandy soils by preventing water loss through drainage. There are also studies [27] in which polyacrylamide-based SAPs are described as not recommended for agricultural soils due to the lack of obvious effect on sandy soils.

- Response of soil microorganisms to water stress

Under all moisture conditions tested, sharp spikes in respiration activity were observed in the middle of each rain-dry cycle and after flooding the samples with water as part of a rainfall simulation. Samples after re-wetting may show ‘Birch effect’ - microbial stress. [33] argues that under drought conditions, microbial activity is highly dependent on moisture levels, increasing the soil respiration response immediately after the addition of water. The so-called ‘respiration pulse’ or ‘Birch effect’ described in [26, 31] occurs. Sharp spikes in respiration rates can be described as the effect of stress and a small increase in biomass during it [23].

The drought-induced decrease in soil respiration rates may be the result of reduced microbial activity and soil biomass, probably due to reduced substrate availability under drought conditions in the organic layer and upper mineral horizon [22].

5. CONCLUSIONS

The conducted experiment allowed us to draw several important conclusions:

- SAP as a soil additive is capable of increasing the microbiological activity in the studied soil samples due to its ability to retain water and, in the case of SAP 2, to provide an additional carbon substrate due to its chemical composition;
- Soil moisture is a key parameter influencing the microbiological activity of the soil, namely the rate of soil respiration. The moisture conditions under which the highest activity is observed for both SAPs were 60% of the maximum water capacity of the soils, as well as conditions simulating heavy rain (over 100% WHC);
- Soil respiration studies should be carried out on an ongoing basis to demonstrate the effect of the SAP additive on the microbial activity of soils during wet and dry periods.

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