

Soil Organic Matter and Its Fractions at Different Stages of Soil Salinization, as well as Their Resistance to Decomposition in Relation to Soil Bioactivity

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Abstract. *The aim of the study was to conduct a comparative assessment of key biological activity indicators in soils with varying degrees of salinity. The research objectives included determining the decomposition rates of plant OM and cellulose, the intensity of soil respiration, and analyzing the total number of microorganisms and bacterial populations in the upper humus horizon of the saline soils. The stabilization process is closely linked to high soil moisture, elevated silt and clay content, and rising nitrogen levels. While rapid changes occur in the surface soil layers (0–10 cm), deeper layers respond much more slowly, retaining specific carbon structures. Ultimately, ecological restoration promotes effective soil carbon sequestration, although this effect is unevenly distributed and concentrated primarily in the surface layer. Based on the results obtained, it was established that the molecular structures of carbon are positively correlated with soil depth, and the dynamics of recovery change in the opposite direction. In both models developed, the processing of the topsoil is clearly separated from the processing of the deeper layers, indicating a noticeable vertical stratification: the topsoil responds more quickly to revegetation than the deeper soil.*

Keywords: *decomposition, microorganisms, soil bioactivity, polysaccharides, phyto-organic nutrient, ecological restoration*

Introduction

Chemical composition and the degree of salinity are key factors influencing the functional activity of soils, determining their natural fertility, productivity, biodiversity, and ecosystem stability. The basis for the biodiagnostic assessment of lowland soils with varying degrees of salinity is biological activity defined as the aggregate of biological processes characterizing the scale and direction of matter and energy transformation in terrestrial ecosystems, as well as the intensity of organic matter (OM) decomposition and synthesis. The biodiagnostics of soil condition using biological activity indicators represents an important area of scientific research. Soil respiration, measured as O₂ consumption or CO₂ production, is an important and informative indicator of OM recycling and soil activity (Acevedo & Govan, 2026; Swaminathan et al., 2021). The biodiagnostics of soil condition using biological activity indicators represents an important area of scientific research. Soil respiration, measured as O₂ consumption or CO₂ production, is an important and informative indicator of OM recycling and soil activity (Acevedo & Govan, 2026; Swaminathan et al., 2021).

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The decomposition of cellulose and plant material in general allows for an assessment of decomposition intensity a process considered a natural ecosystem attribute that is sensitive to changes in ecosystem functioning and involves the breakdown of residues and the transfer of OM and nutrients within the soil (Sadigov et al., 2025a; Mirzazadeh et al., 2025). Determining microbial counts by inoculating soil suspensions onto solid culture media enables the identification of biological activity patterns. Soil respiration and microbial counts serve as indicators of potential soil biological activity, measured under optimal conditions for a specific biological process. In contrast, the breakdown of cellulose and plant material indicates actual soil biological activity, reflecting the activity occurring under field conditions (Hasanova et al., 2026a; Nasirova et al., 2026).

Materials and Methods

Four sites with varying degrees of salinity in the Mil-Mugan Plain of Azerbaijan, located in close proximity to one another under similar climatic conditions, were selected as experimental sites. The research was conducted from September 2023 to September 2024. The degree and chemical composition of soil salinity were determined using the classical method of water extract analysis. Aqueous extract analysis method: slightly saline (salt content 0.23%), sulfate type ($\text{Cl}:\text{SO}_4 \leq 0.3$); moderately saline (0.6%), chloride type ($\text{Cl}:\text{SO}_4 \geq 2.5$); highly saline (2.18%), sulfate type ($\text{Cl}:\text{SO}_4 \leq 0.3$); very highly saline (4.95%), sulfate-chloride type ($\text{Cl}:\text{SO}_4=2.5-1.0$). According to soil maps, the experimental plots are situated on meadow and saline-meadow soils-including areas with saline marsh patches as well as on clay and heavy-clay soils (Hasanova & Mammadova, 2023; Hasanova et al., 2026a). Key physicochemical soil parameters were determined using standard methods: chemical composition and salinity were assessed via water extraction, OM content was determined by calcining soil samples, and soil acidity was measured using a pH meter directly on the experimental plots. The soils of the experimental plots are similar in terms of OM content and total water-holding capacity. To assess the decomposition rate, we employed soil exposure methods using filter paper samples and hay bags techniques frequently used in studies evaluating soil activity and metabolism (Bashir et al., 2021; Hasanova et al., 2026b).

Bags containing a specific amount of plant material or filter paper are placed in the soil for a set period. The decomposition rate is calculated as the daily mass loss per gram of OM ($\text{mg} \cdot \text{g}^{-1} \cdot 24 \cdot \text{h}^{-1}$). The decomposition of standardized, homogeneous material enables a comparison of the decomposition capacity of plant material across different soil types with specific compositions and structures. The method of exposing filter paper samples to the soil is used to assess the decomposition rate of pure cellulose and serves as an indicator of the cellulolytic activity of microorganisms, whereas the method of exposing bags allows for the assessment of the decomposition rate of plant OM as a whole and reflects the degradative activity. Samples of filter paper and vegetables material were in the soils of the experimental plots in the post-vegetation period (September – November 2023) and growing season (June – September 2024) periods. Since the regime of CO_2 in soil air is more dynamic than O_2 , which is related to several sources of its entry into the gas phase and high solubility in the water environment, we estimated soil respiration as the amount of $\mu\text{lO}_2 \cdot \text{h}^{-1} \cdot \text{g}^{-1}$. One of the study's objectives was to demonstrate the respiratory potential of the different soil types rather than to measure O_2 consumption under actual natural conditions. Laboratory measurements were conducted using a volumetric respirometer with 25 ml flasks, following the standard method, at three temperatures (8, 18, and 25 °C) and a moisture content of 60% of the soil's total water capacity a level commonly used in such studies. Microorganisms in the soils of the experimental plots were quantified using 1:10,000 dilutions of soil suspensions plated on solid culture media (Swaminathan et al., 2021; Li et al., 2023). Meat-peptone agar was used to isolate and quantify the total bacterial count, while *Azotobacter* numbers were assessed by culturing on nitrogen-free media. Statistical analysis of all obtained results was performed using STATISTICA version 10 (Ismayilova et al., 2025a; Verdiyeva et al., 2026).

Results and Discussion

The obtained data indicate that the decomposition rates of plant OM in the soils of each of the four experimental plots varied significantly (t-test, $p < 0.05$) during both the growing season and the autumn (Tunlid et al., 2022). In all experimental plot soils, the decomposition rate increased markedly under conditions favorable for active microbiological activity and biochemical processes, as well as under the temperature and moisture conditions characteristic of the growing season. During this period, the decomposition rate of hay and filter paper more than doubled in moderately and highly saline soils. It has been established that the rate of phyto-organic decomposition is inversely proportional to salinity (Ismayilova et al., 2025b; Mammadova et al., 2026a; Sadigov et al., 2025b).

The highest values were observed in the soil of the slightly saline site during the growing season, whereas the lowest decomposition rates during this period were observed in the soil of the highly saline site. A similar inverse relationship regarding decomposition rates along the salinity gradient was evident in autumn: the most intense decomposition of phyto-organic matter occurred in the slightly saline site, while the lowest rate was observed in the highly saline soil. Across all experimental sites, the rate of cellulose decomposition also decreased along the soil salinity gradient during both the growing and post-growing seasons (Shukurov et al., 2025; Mammadova et al., 2026c). The dry conditions typical of an arid climate characterized by high air temperatures and low summer precipitation during the sample exposure period were similar across the adjacent experimental plots. All soils in the experimental plots were similar in terms of total water-holding capacity and OM content (Table 1).

This enabled us to track the influence of salinity on the decomposition rate of phyto-organic nutrients while keeping the effects of climatic conditions and other soil physicochemical parameters constant. The data confirmed that the higher the soil salinity level, the lower the rate of phyto-organic nutrient decomposition (Mammadova et al., 2025; Mammadova et al., 2026b). Soil respirometer measurements were obtained at temperatures of 25 °C, 18 °C, and 8 °C. A temperature of 25 °C proved optimal for soil biological activity across all experimental plots and closely approximates the natural growing season in the study area. A temperature of 18 °C proved critical for moderately, strongly, and very strongly saline soils, with no O₂ consumption observed. For soils in the slightly saline plot, no O₂ consumption was observed at a temperature of 8 °C. The respiration rate of the studied soils at 25 °C varied significantly along the salinity gradient ($p < 0.05$, t-test). The maximum rate was observed in the moderately saline soil ($3.586 \pm 0.173 \mu\text{O}_2\text{h}^{-1} \cdot \text{g}^{-1}$ dry mass), a value slightly higher than that of the slightly saline soil. Respiration rates were virtually identical in the highly and very highly saline soils ($p < 0.05$, t-test). At 18 °C, O₂ consumption was observed only in samples from the slightly saline soil site ($2.045 \mu\text{O}_2\text{h}^{-1} \cdot \text{g}^{-1}$ dry mass); the respiration rate was lower than at 25 °C ($p < 0.05$, t-test).

According to previous studies on saline soils in the lowlands of the Republic of Azerbaijan, low concentrations of readily soluble salts up to 1 mol eq. per 100 g of soil have a positive effect on the functioning of microbial communities; the higher the concentration, the greater the vitality of the microorganisms. This is clearly reflected in the respiration rate of moderately saline soils, where peak O₂ consumption values are observed, whereas the salinity levels found in the third and fourth study sites prove detrimental to microbial activity. Analysis of the number of microorganisms isolated by plating diluted soil suspensions showed that the total number of microbial cells, as well as the number of nitrifying bacteria per gram of soil in each of the four experimental plots, decreased along the salinity gradient.

It was found that the number of colony-forming units for both the total microbial population and nitrogen-fixing bacteria was more than five times higher in soil samples from the slightly saline plot than in soils with moderate or high salinity. Low salt concentrations have no negative effect on the functioning and total biomass of soil microorganisms, while high salt concentrations can cause osmotic stress, limiting the growth and activity of microbial communities (Mammadova et al., 2025).

The decomposition rate of plant OM and cellulose in the soils of experimental plots under natural conditions serves as an indicator of their actual biological activity. It was found that, given identical natural hydrothermal conditions and similar OM content and soil water-holding capacity, the decomposition rate of plant OM and cellulose decreases along the salinity gradient. Data obtained via respirometer and microbial counts indicate the potential biological activity of the soils, as measurements were taken under artificially optimized conditions. O₂ consumption potential was found to be quite high in soils with low salinity levels. The total number of microorganisms including both the total count and specific nitrogen-fixing bacteria was more than five times higher in slightly saline soils than in moderately saline soils. Evidently, the salt concentration in moderately saline soils does not critically inhibit microbial activity, allowing the microbes to exhibit high activity potential despite lower population numbers.

This is corroborated by data showing maximum respiration rates in moderately saline soils with low bacterial populations. High and very high salinity levels proved detrimental to the soil's biological activity potential, affecting both respiration rates and total microbial counts. To evaluate the relationship between soil organic matter (SOM) composition, thermal stability, and environmental factors (EF) at different restoration stages and soil depths, redundancy analysis (RDA) was conducted (Figure 1). In Figure 1a, the first two axes explain 28.48% of the total variance, with RDA1 dominating at 26.47% and RDA2 accounting for 2.01%. Ordination revealed a clear spatial division of soil samples according to RDA1, based on the chronological sequence of restoration.

Early recovery samples (5 years, pink/red) clustered on the positive side of RDA1, showing strong positive correlations with labile OM, soil depth, and pH. Conversely, late recovery samples (30 years, in green) clustered on the negative side of RDA1, which was largely driven by recovery age (years) and soil water content (SWC). The refractory OM and recalcitrant OM vectors indicated longer recovery stages, suggesting accumulation of stable organic components over time accompanied by an increase in silt + clay content and NH₄⁺-N.

In Figure 1b, the RDA models the shifts in specific carbon functional traits (aliphatic and aromatic carbon, polysaccharides, and the decomposition resistance index) in response to environmental drivers. The first two canonical axes captured 25.96% of the total structural variance (RDA1: 25.62%, RDA2: 0.34%).

Mirroring the trends in the fraction analysis, the polysaccharides and the recalcitrant index vectors pointed in the direction of the late-stage restoration plots (30 years), aligning closely with the long-term increase in years, SWC, and nitrogen availability NH₄⁺-N and NO₃⁺-N. On the other hand, aliphatic C and aromatic C molecular structures were positively aligned with soil depth, shifting opposite to the restoration timeline. Notably, in both models, the 0–10 cm surface soil layer (demarcated by the dashed ellipses) clustered distinctly away from the deeper core layers, highlighting a profound vertical stratification where topsoil's exhibit an accelerated response to vegetation restoration compared to subsoils.

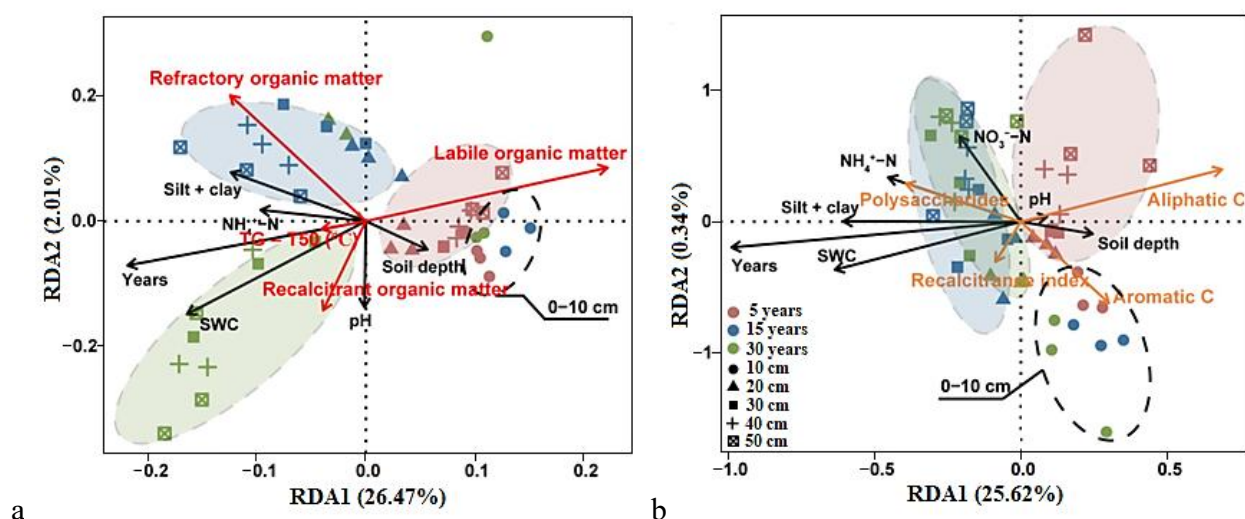


Figure 1

RDA analysis of SOM composition and EF at various stages of restoration: (a) OM fractions in relation to EF and the thermal stability index; (b) aliphatic and aromatic carbon, polysaccharides, and the decomposition resistance index in relation to EF

Table 1

Physicochemical properties, rates of decomposition of plant OM and cellulose, and soil respiration rates in the soils of experimental plots

The degree of soil salinity in experimental sites	OM, %	Maximum capillary water capacity, ml/g	pH	Soil texture
Slightly saline	7.74	0.38	7.1	middle to heavy loamy soils
Medium saline	5	0.35	6.2	middle to heavy loamy soils
Highly saline	7.48	0.39	4.6	loamy and heavy loamy soils
Very saline	7.44	0.4	6.0	loamy and heavy loamy soils
Samples of hay				
September–November 2023		June–September 2024		
The degree of soil salinity in experimental sites	Number of samples	Average decomposition rate $\bar{X} \pm S_x$	Number of samples	Average decomposition rate $\bar{X} \pm S_x$
Slightly saline	14	4.25 ± 0.27	24	5.51 ± 0.12
Medium saline	19	2.67 ± 0.26	21	5.31 ± 0.17
Highly saline	20	1.28 ± 0.13	15	4.49 ± 0.16
Very saline	19	0.86 ± 0.07	8	2.63 ± 0.44
Samples of filter paper				
September–November 2023		June–September 2024		
The degree of soil salinity in experimental sites	Number of samples	Average decomposition rate $\bar{X} \pm S_x$	Number of samples	Average decomposition rate $\bar{X} \pm S_x$
Slightly saline	29	11.12 ± 0.22	21	10.07 ± 0.02
Medium saline	23	0.22 ± 0.03	20	3.13 ± 0.12
Highly saline	21	0.16 ± 0.03	13	3.84 ± 0.15
Very saline	22	0.10 ± 0.01	23	1.50 ± 0.2
Temperature regime				
25 °C		18 °C		

The degree of soil salinity in experimental sites	Number of samples	O ₂ uptake $\bar{X} \pm Sx$	Coefficient of variation, %	Number of samples	O ₂ uptake $\bar{X} \pm Sx$	Coefficient of variation, %
Slightly saline	30	3.025 ± 0.126	18.6	23	2.045 ± 0.086	18.4
Medium saline	30	3.586 ± 0.173	21.5	24	0	–
Highly saline	33	2.168 ± 0.131	30.7	12	0	–
Very saline	31	1.886 ± 0.096	22.7	10	0	–

Conclusion

Slightly saline soils have the highest potential activity. They hold more than five times the number of microbes and nitrogen-fixing bacteria than moderately saline soils. Moderately saline soils have fewer microbes, but those microbes are highly efficient. They can still achieve maximum respiration rates because the salt level is not yet critical. High and very high saline soils are toxic. The extreme salt levels severely damage both the microbe populations and their ability to breathe. Overall, while low-to-moderate salt levels change how microbes behave, high salt levels destroy the soil's biological life.

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Conflict of Interest

The authors declare no competing interests that could be perceived as influencing the research or its publication. T.H. is an Editor of the journal, while G.M. is a member of the Editorial Board. Neither T.H. nor G.M. was involved in the editorial evaluation or publication decision concerning this manuscript.

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