

Response of catalase and cellulase to microbial biomass carbon and some soil indicators in forest, agricultural and urban soils of Sofia Municipality with and without influence of local water sources

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Abstract

Microbial biomass carbon (MBC) and the enzymatic activities of catalase and cellulase were investigated in soils within Sofia Municipality in an agricultural ecosystem (Vrazhdebna district), forest ecosystems (Vitosha Nature Park, Zheleznitsa, and Dolni Pasarel areas), and urban soil (green area, Dolni Pasarel village), in a seasonal context, with and without the influence of local water sources. MBC and the activities of catalase and cellulase were higher in the agricultural soils than in the forest soils, which is also attributable to the fact that they are tilled and irrigated, with trends of higher microbiological activity in spring compared to summer. In most soils of Vitosha, the inflow of water from local sources creates anaerobic conditions that reduce the accumulation of microbially derived carbon and the enzymatic activity of microorganisms. Soils in Vitosha, Zheleznitsa and Dolni Pasarel are acidic, while those in Vrazhdebna are neutral, suggesting mainly bacterial biomass in the latter and more mould fungi in acidic soils; anaerobic conditions in waterlogged sites further limit mycelial microorganisms. Correlation analysis showed a strong negative relationship between MBC and soil moisture ($r \approx -0.73$; -0.69), a weak positive relationship with pH ($r \approx 0.08$ – 0.15), a moderate positive relationship with temperature ($r \approx 0.30$ – 0.38) and catalase ($r \approx 0.50$ – 0.60) and a strong positive relationship with cellulase ($r \approx 0.61$ – 0.66). Catalase activity was moderately negatively correlated with moisture ($r \approx -0.53$; -0.45) and moderately positively with pH ($r \approx 0.49$ – 0.52); cellulase showed a weak to moderate negative association with moisture ($r \approx -0.33$; -0.27), a weak to moderate positive association with temperature ($r \approx 0.28$ – 0.31) and a moderate negative association with pH ($r \approx -0.21$; -0.26). Regression analysis confirmed the role of pH as a stable predictor – positive for catalase and negative for cellulase in both seasons, and showed that in spring catalase was the strongest positive predictor of cellulase, whereas in summer cellulase was significantly influenced by temperature, MBC, depth and catalase.

Key words: agricultural soils, enzymatic activities, forest soils, microbial carbon, urban ecosystem.

Introduction

The accumulation of microbial biomass carbon in soils from forest and agroecosystems in urban environments, is of essential importance for the content of organic carbon in them and the course of the carbon cycle in nature. Organic carbon of microbial origin indicates the activity of soil microorganisms, which in turn are sensitive indicators of the processes taking place in soils. They participate in the cycle of substances, provide nutrition for plants and self-cleaning of soils from pollutants. The activity of soil microorganisms is also indicative through the study of the enzymes produced by them. Cellulase is a primary enzyme involved in the carbon cycle in the decomposition of cellulose, a main component in plant residues. Catalase is a respiratory enzyme that participates in the decomposition of toxic hydrogen peroxide into water and oxygen. All three indicators – microbial biomass carbon, cellulase and catalase activity, are used to study microbiological soil activity. However, results are often contradictory due to the complex of environmental factors affecting the quantity, composition, and activity of soil microflora.

Soil microbial biomass is the living mass of organic matter, half of which is located in the soil top layer (15 cm) (Das et al. 2023) and represents a labile pool of nutrients, including bacteria, actinomycetes, fungi, algae, protozoa, and other soil microfauna (Henrot and Robertson 1994), playing a major role in the decomposition and build-up of the soil carbon stock (Das et al. 2023). According to a study (Das et al. 2023) covering publications on microbial biomass carbon from 1989 to 2022, microbial organic carbon stocks in soils of different forest ecosystems worldwide ranged from 55 mg·kg⁻¹ (decid-

uous forests) to 2214.4 mg·kg⁻¹ in montane mixed forests. Total organic carbon stocks ranged from 800 mg·kg⁻¹ (boreal forests) to 350,800 mg·kg⁻¹ (mixed natural forests). Microbial carbon was positively correlated with organic carbon stocks and soil basal respiration and influenced by temperature, moisture, seasonal factors, and other environmental conditions. Soil microbial biomass includes microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), microbial biomass phosphorus, and microbial biomass sulphur, with MBC and MBN accounting for 1–5 % and 2–6 % of the total soil C and N content, respectively, and tending to be relatively more stable (Babur and Dindaroglu 2019). Approximately 1.4 % of the global soil organic C content is of microbial origin, but it contributes significantly to the global C cycle (Wardle 1992). The contribution of carbon from microbial biomass to total organic carbon in agricultural soils has been reported to be 1.13–3.37 %, and microclimatic variations as well as different agricultural practices have been found to influence changes in microbial biomass (Bhuyan et al. 2013).

The accumulation of microbial biomass and its decomposition with the help of produced enzymes depend on both the amount and activity of the soil microflora and on environmental factors: climate, mechanical composition and structure of the soil, nutrient availability, soil temperature and moisture, type of vegetation, also on the depth of sampling and other factors (Gulde et al. 2008, Russell et al. 2015, Hassink 2016, Kara et al. 2016, Magnússon et al. 2016, Babur 2019, Malcheva 2020a, Qu et al. 2023, Zhan 2024). The ratio of microbial biomass carbon to total organic carbon can be indicated as an indicator of the dynamics of carbon in the soil (Insam et al. 1989). Soil microbial diversi-

ty, microbial biomass, microbial respiration and soil enzymes should be monitored as more indicative of any changes in soil conditions to provide more accurate and rapid information on soil health and quality and to measure ecosystem health (Rice et al. 1986, Smith 1993, Sparling 1997, Nielsen and Winding 2002, Alvarez et al. 2009, Schnecker et al. 2023, Zhang 2024).

Cellulase is a key enzyme in the carbon cycle and, together with catalase, is a sensitive indicator that determines the biochemical characteristics of soils (Torres et al. 2016, Malcheva et al. 2023). Soil biogenicity and cellulase activity are higher in *Fagus sylvatica* L. beds than in *Pinus sylvestris* L., decreasing with depth in the soil profile (Malcheva et al. 2024). Cellulase activity depends on soil temperature and moisture, organic matter content, amount and composition of microorganisms (bacteria dominate), sampling depth, soil type, mechanical composition, structure and pH of the soil, type of plant residues and other factors (Kanazawa and Miyashita 1987, Malcheva et al. 2015a, 2015b, 2019, 2024, Malcheva 2020b, 2020c, Naskova et al. 2016, Datta 2024). A very strong positive correlation has been found between catalase activity and microbial biomass in carbon, more pronounced in broadleaved than in coniferous forest vegetation. (Malcheva et al. 2023). Both MBC and catalase activity decrease sharply from dead forest litter to A soil horizon, followed by a weaker and smoother decrease with increasing soil depth. Catalase activity correlates with iron content, hygroscopic moisture, humus, the amount and activity of soil microorganisms, soil temperature and pH, soil type, clay content, sampling depth, vegetation type, presence of inhibitors, research methods, and agrotechnical practices (Grozeva and Nustorova 1995; Uzun and Uyanöz 2011; Malcheva

et al. 2015a,b, 2019; Naskova et al. 2016; Malcheva 2020b,c).

The aim of the present study was to analyse the seasonal (spring vs. summer) dynamics of sensitive microbiological and enzymatic indicators – MBC, catalase, and cellulase activities – in relation to selected soil parameters in agricultural, forest, and urban soils of Sofia Municipality, including irrigated soils and over-waterlogged soils near local water sources. We hypothesised that: (i) MBC, catalase, and cellulase activities would be significantly higher in agricultural soils compared to forest and urban soils due to cultivation and irrigation; (ii) over-moistening and partial anaerobiosis in soils near local water sources (springs, boreholes, fountains) would lead to lower MBC and enzyme activities; (iii) soil temperature and pH would emerge as significant predictors of MBC and enzymatic activities; and (iv) MBC and enzyme activities would be interdependent, showing significant positive associations in the regression models.

Materials and Methods

The analysed soils were collected from four regions within Sofia Municipality during two seasons – spring and summer: the villages of Zheleznitsa and Dolni Pasarel, the Vrazhdebna district, and Vitosha Nature Park. Sampling included soils influenced by local water sources (fountains, springs, or irrigation) as well as nearby soils without such influence. Soil samples were collected at two depths: 0–10 cm and 10–25 cm. According to the World Reference Base for Soil Resources (WRB 2022), the soils in Vitosha Nature Park and the Zheleznitsa area are predominantly brown forest soils (Cambisols), with exceptions at SS17 and SS18 (Regosols)

and SS21 (Fluvisols). The soils in Dolni Pasarel area are cinnamon forest soils, also classified as Cambisols. At locations directly influenced by water from fountains and springs, Cambisols with Stagnic properties (SS1, SS5, SS13, SS15, SS19), Regosols with Stagnic properties (SS17), and Cambisols with Gleyic properties (SS3) were identified. The soils in Vrazhdebna Training and Experimental

Field are alluvial-meadow soils (Fluvisols). Previous studies report that Cambisols are the prevailing soil type in Vitosha Nature Park (Karatoteva 2016, Hristov et al. 2025), while other authors indicate that the soils of the University of Forestry's Vrazhdebna Training and Experimental Field are Fluvisols (Kercheva et al. 2021, Dimitrov 2024). The sampling sites (SS) are listed and described in Table 1.

Table 1. Sampling sites and soil description.

Sampling sites	Location, GPS coordinates (WGS84)	Altitude, m	Type of influence on soil (water source / plant cover)
SS1	Zheleznitsa 42°32'25.476" N, 23°23'5.604" E	900	Influenced by mineral water from a borehole. <i>Plantago major</i> L., <i>Athyrium filix-femina</i> (L.) Roth., <i>Urtica dioica</i> L.
SS2	Zheleznitsa 42°32'25.476" N, 23°23'5.648" E	900	Near SS1, not influenced by mineral water from a borehole. <i>Plantago lanceolata</i> L., <i>Urtica dioica</i> .
SS3	Zheleznitsa 42°32'25.542" N, 23°23'5.868" E	905	Influenced by a natural spring water. Without vegetation.
SS4	Zheleznitsa 42°32'25.575" N, 23°23'5.802" E	905	Near SS3, not influenced by natural spring water <i>Urtica dioica</i> , <i>Lolium perenne</i> L., <i>Poa pratensis</i> L.
SS5	Dolni Pasarel 42°32'23.266" N, 23°29'40.776" E	710	Influenced by roadside fountain water. <i>Hedera helix</i> L., <i>Geranium robertianum</i> L.
SS6	Dolni Pasarel 42°32'23.266" N, 23°29'40.732" E	710	Near SS5, not influenced by roadside fountain water. <i>Geranium robertianum</i> , <i>Lolium perenne</i> .
SS7	Dolni Pasarel 42°32'23.435" N, 23°29'43.498" E	708	Next to the Colourful fountain, not influenced by the water from it. <i>Geranium robertianum</i> , <i>Taraxacum officinale</i> F.H.Wigg., <i>Sambucus nigra</i> L.
SS8	Vrazhdebna 42°42'26.001" N, 23°26'11.804" E	538	Irrigated by an irrigation basin. <i>Brassica oleracea</i> L. var. <i>capitata</i> L.
SS9	Vrazhdebna 42°42'26.065" N, 23°26'11.805" E	538	Irrigated by a borehole 1. <i>Brassica oleracea</i> var. <i>capitate</i> .
SS10	Vrazhdebna 42°42'25.968" N, 23°26'11.848" E	538	Irrigated by a borehole 2. <i>Capsicum annuum</i> L.

Sampling sites	Location, GPS coordinates (WGS84)	Altitude, m	Type of influence on soil (water source / plant cover)
SS11	Vrazhdebna 42°42'25.968" N, 23°26'11.936" E	538	Irrigated by an outdoor fountain. <i>Capsicum annuum</i> .
SS12	Vrazhdebna 42°42'25.871" N, 23°26'11.892" E	538	Unirrigated soil. Without vegetation.
SS13	Vitosha Nature Park, Vitosha tulip area 42°35'10.590" N, 23°18'26.257" E	1665	Influenced by fountain water. <i>Euphrasia officinalis</i> L., <i>Luzula multiflora</i> (Ehrh.) Lej.
SS14	Vitosha Nature Park, Vitosha tulip area 42°35'10.558" N, 23°18'26.345" E	1665	Near SS13, not influenced by fountain water. <i>Pinus sylvestris</i> L., <i>Luzula luzuloides</i> (Lam.) Dandy & Wilmott, <i>Euphrasia officinalis</i> .
SS15	Vitosha Nature Park 42°36'29.890" N, 23°17'51.280" E	1270	Influenced by roadside fountain water. <i>Fagus sylvatica</i> L., <i>Geranium robertianum</i> .
SS16	Vitosha Nature Park 42°36'30.071" N, 23°17'51.302" E	1270	Near SS15, not influenced by roadside fountain water. <i>Fagus sylvatica</i> , <i>Geranium robertianum</i> .
SS17	Vitosha Nature Park 42°38'41.280" N, 23°14'25.692" E	910	Influenced by Stone fountain water. <i>Plantago major</i> , <i>Deschampsia cespitosa</i> (L.) P.Beauv.
SS18	Vitosha Nature Park 42°38'41.313" N, 23°14'25.736" E	910	Near SS17, not influenced by Stone fountain water. <i>Carpinus betulus</i> L.
SS19	Vitosha Nature Park 42°36'59.148" N, 23°14'18.624" E	1401	Influenced by Pavlova fountain water. <i>Fagus sylvatica</i> , <i>Athyrium filix-femina</i> , <i>Geranium robertianum</i> .
SS20	Vitosha Nature Park 42°36'59.181" N, 23°14'18.580" E	1401	Near SS19, not influenced by Pavlova fountain water. <i>Fagus sylvatica</i> , <i>Athyrium filix-femina</i> (L.) Roth., <i>Geranium robertianum</i> .
SS21	Vitosha Nature Park, Golden bridges area 42°36'36.020" N, 23°14'19.987" E	1382	Influenced by Vladayska River water. Without vegetation.
SS22	Vitosha Nature Park, Golden bridges area 42°36'36.013" N, 23°14'19.987" E	1382	Near SS21, not influenced by Vladayska River water. <i>Angelica sylvestris</i> L., <i>Plantago lanceolata</i> , <i>Lolium perenne</i> .

All sampling sites were located within the administratively urbanised area of Sofia Municipality (including protected natural areas, rural settlements, and an urban

experimental field). For the purposes of this study, soils were grouped both by land use and by genesis: (i) forest soils from protected natural areas (Vitosha Nature

Park) and rural settlements (Zheleznitsa and Dolni Pasarel); (ii) agricultural soils from the University of Forestry's Vrazhdebna Training and Experimental Field (cultivated and irrigated Fluvisols); and (iii) urban soils from green areas within settlements (SS7 – Dolni Pasarel, near the Colourful fountain). This dual classification highlights both the fact that all sites are located within Sofia Municipality (an urbanised territory) and the differences in soil origin and land use.

The sampling site location map was prepared using Google Earth Pro (version 7.3.6; Google LLC) (Fig. 1).

Sampling was performed under sterile conditions, as part of the samples were also analysed for major groups of micro-organisms. The samples were transported in a cooler at 4 °C, and analyses were initiated immediately upon delivery to the laboratory on the day of sampling. MBC was determined using the fumigation method (Cai et al. 2011). Soil extracts were prepared with potassium sulphate,

followed by digestion with a dichromate–sulphuric acid solution. The samples were heated at 140 °C for 30 min, and absorbance was measured spectrophotometrically at 350 nm. Carbon concentration was calculated using a glucose calibration curve.

Catalase activity was determined using the manganometric titration method (Khaziev 1976). In the experimental samples, hydrogen peroxide was added to the soil, while sulphuric acid was used for the controls. After 15 min of shaking on a shuttle apparatus, the reagents were exchanged, and shaking was continued for a further 15 min. The suspensions were then filtered and titrated with potassium permanganate until a pink colour appeared. Catalase activity was calculated as the difference between the volume of oxygen required for titration of the control and that of the experimental sample.

Cellulase activity was determined dynamically by placing sterile filter strips on the soils, periodically moistening and

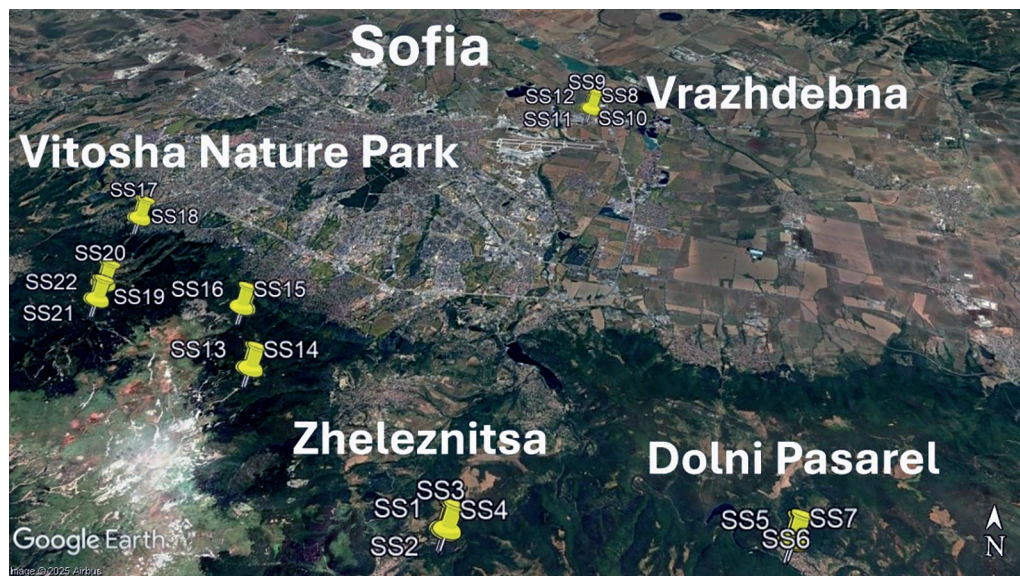


Fig. 1. Sampling site location map.

counting the percentage of degraded area every 15 days with a grid standard (Khaziev 1976).

Soil temperature was monitored in the field using a temperature probe (model Sv218) at the time of soil sampling for microbiological and enzymatic analyses, once per season (spring and summer) at two depths: 0–10 cm and 10–25 cm.

Soil moisture was determined gravimetrically in the laboratory using a moisture balance (model DBS 60-3) by drying the samples at 105 °C until constant weight. Measurements were taken once per season (spring and summer) at two depths (0–10 cm and 10–25 cm) immediately after soil collection for microbiological and enzymatic analyses. Soil moisture was also monitored in the field using a moisture probe (model Sv218); results from both methods were comparable and averaged. Values of microbial biomass

carbon and enzymatic activities were subsequently expressed on an oven-dry soil basis using the determined moisture coefficients.

The active reaction (pH) of the soil was determined potentiometrically in a water extract according to ISO 10390:2005.

Data preprocessing and descriptive statistics were performed using Microsoft Excel 2010, while stepwise multiple regression analysis was conducted with IBM SPSS Statistics, version 25.0.

Results and Discussion

The accumulation of organic carbon of microbial origin was studied in two seasons – spring and summer at two depths (0–10 cm, 10–25 cm) and is presented in figures 2–5.

The values of MBC are higher in ag-

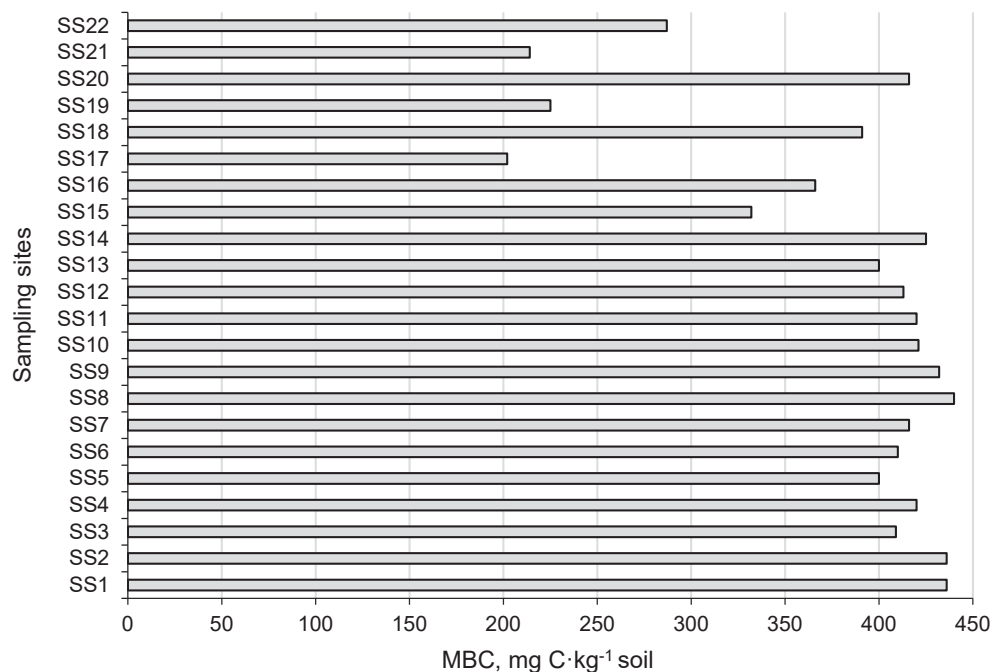


Fig. 2. Microbial biomass carbon in spring (0–10 cm).

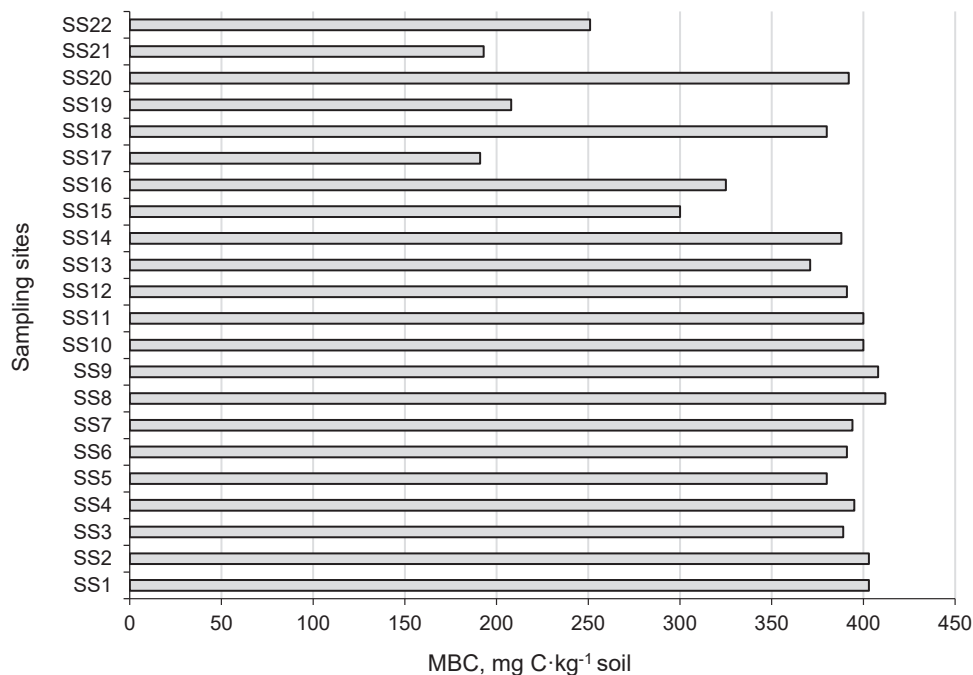


Fig. 3. Microbial biomass carbon in spring (10–25 cm).

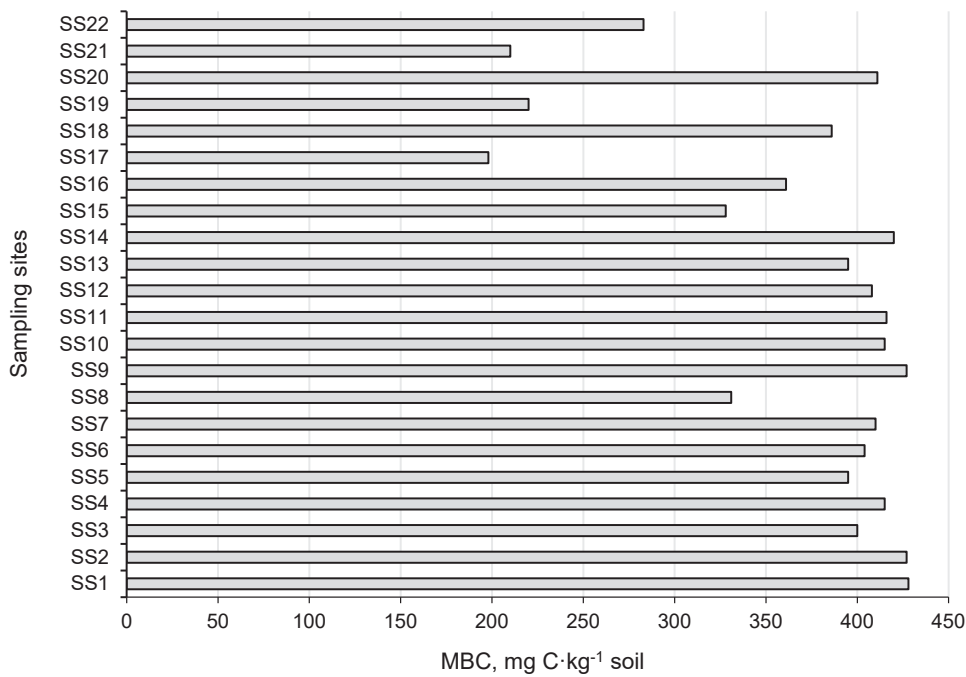


Fig. 4. Microbial biomass carbon in summer (0–10 cm).

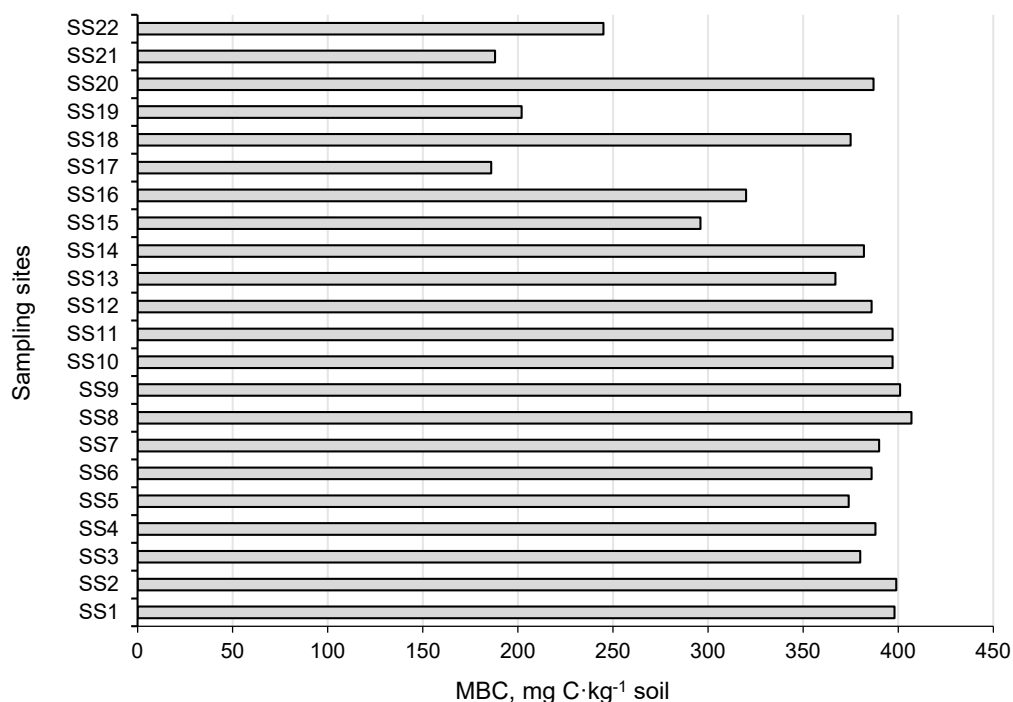


Fig. 5. Microbial biomass carbon in summer (10–25 cm).

gricultural soils compared to forest soils, which is also due to the fact that they are cultivated and irrigated. An increased accumulation of organic carbon is found in spring compared to summer. This trend depends on the quantity, composition and activity of soil microorganisms. Malcheva et al. (2025) found for the same variants that the total quantity of microorganisms is also higher in agricultural soils, with the main share in the composition of the soil microflora occupied by non-spore-forming bacteria, followed by bacilli, weaker and even absent development of mycelial groups of microorganisms – actinomycetes and mould fungi in waterlogged soils, due to the anaerobic conditions created. The same authors point out that the mineralisation rate of decomposition of organic substances in soils, however, does not

depend solely on the quantity of microorganisms. MBC depends on a complex of factors (CF), including the quantity, composition and activity of the soil microflora, soil temperature, moisture and pH, soil depth, soil type and mechanical composition, nutrient availability, the presence and type of vegetation, altitude and other site-specific conditions (Gulde et al. 2008, Russell et al. 2015, Hassink 2016, Kara et al. 2016, Magnússon et al. 2016, Babur 2019, Qu et al. 2023, Zhan 2024).

In spring, the MBC values in both soil layers are highest in agricultural soils from Vrazhdebna (SS8, SS9) and from Zheleznitsa, soils influenced by a mineral spring and in its immediate vicinity (SS1, SS2). These results correlate with a higher abundance of microorganisms and higher mineralisation activity in these soils

(Malcheva et al. 2025). The agricultural soils in Vrazhdebna are arable and regularly irrigated, while the mineral spring in Zheleznitsa is rich in dissolved organic matter, both contributing to enhanced microbial biomass. In summer, microbial organic carbon again reached its maximum values in the same soil samples, except for SS8, where MBC decreased more sharply than the total microbial abundance and mineralisation activity. The lowest MBC values were recorded in SS17, SS21, SS19 and SS22, corresponding mainly to Vitosha Nature Park sites, where high soil moisture and overmoistening from adjacent water bodies lead to leaching of the upper soil horizon and a reduction in organic matter and microbial abundance. Seasonal variations in temperature, precipitation, plant growth and the accumulation of organic matter from different types of plant residues also have a major impact on soil microbial biomass (Maithani et al. 1996, Chen et al. 2005, Tonon et al. 2005, Devi and Yadava 2006, Schneckner et al. 2023, Qu et al. 2023). Maximum microbial biomass has been reported either in spring (Diaz-Raviña et al. 1995, Zhu and Carreiro 2004), in summer (Gallardo and Schlesinger 1994, Zhong and Makechin 2006), or without pronounced seasonal trends (Bauhus and Barthel 1995). The studied sites are part of an urban environment, with soils variably affected by transport traffic emissions (mainly in Vrazhdebna) and tourism or mineral extraction (mainly in Vitosha Natural Park and Zheleznitsa). In soils under greater anthropogenic influence, organic matter undergoes more intense mineralisation and carbon loss to the atmosphere, limiting humus accumulation, whereas in urban park areas, elevated soil organic carbon values are often observed (Nikolov et al. 2020). In our study, however, the park

sites were strongly affected by topsoil erosion due to inflow from nearby water bodies. Disturbance of soil texture, increase bulk density and reduced soil moisture are main factors responsible for the depletion of microbial biomass in urban soils (Rai et al. 2018).

The studied enzymes – catalase and cellulase – reached their maximum activities in spring and showed higher values in agricultural soils. Catalase activity correlated with microbial biomass accumulation: the highest enzyme levels were recorded in soils from Vrazhdebna (SS8 and SS9) and in the site influenced by the mineral spring in Zheleznitsa (SS1). The lowest catalase activities were recorded in soils near Vladayska River – Golden Bridges (SS21 and SS22) and at Pavlova fountain (SS19), while comparatively low values were also observed in soils from the other sites located within Vitosha Nature Park. It should be noted that catalase, in addition to its microbial origin, may also have a plant origin. Regarding cellulase activity, complete cellulose degradation was detected at one of Vrazhdebna sites (SS8, both depths, spring) and in the soil influenced by the mineral spring in Zheleznitsa (SS1, both depths, spring). Catalase activity is shown in figures 6–9.

Similarly, catalase activity is influenced by the same CF, with additional effects related to humus, iron content, clay fraction, and the presence of inhibitors, research methods, agrotechnical measures (Grozeva and Nustorova 1995; Uzun and Uyanöz 2011; Malcheva et al. 2015b, 2019, 2023; Naskova et al. 2016; Malcheva 2020b,c; Wang et al. 2024). The absence of vegetation at sites directly affected by water sources, together with washout by surface flow, also negatively impacts microbial biomass accumulation and enzyme activities.

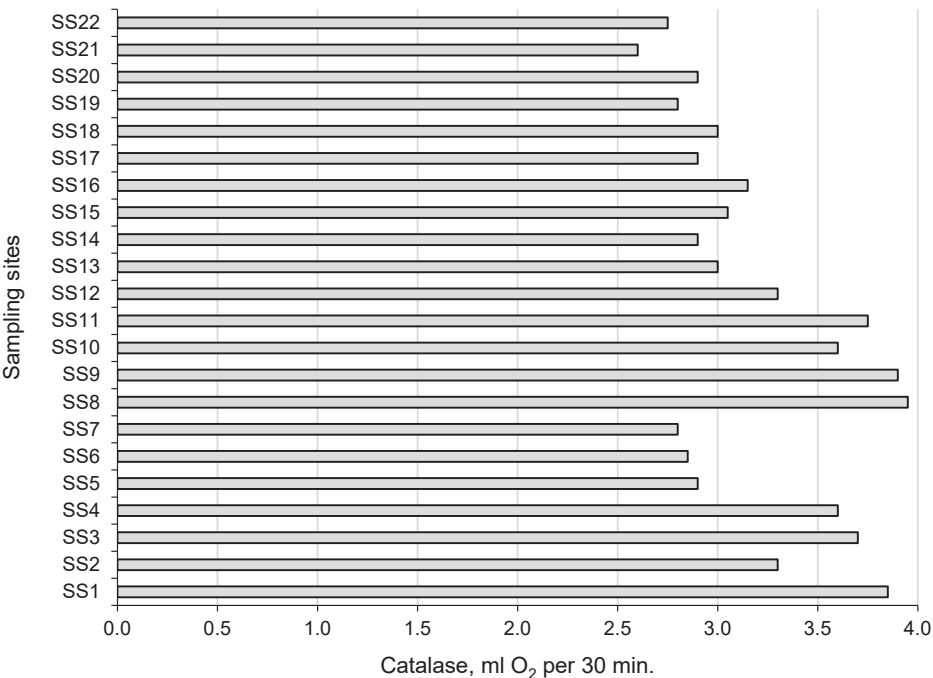


Fig. 6. Catalase activity of soil microorganisms in spring (0–10 cm).

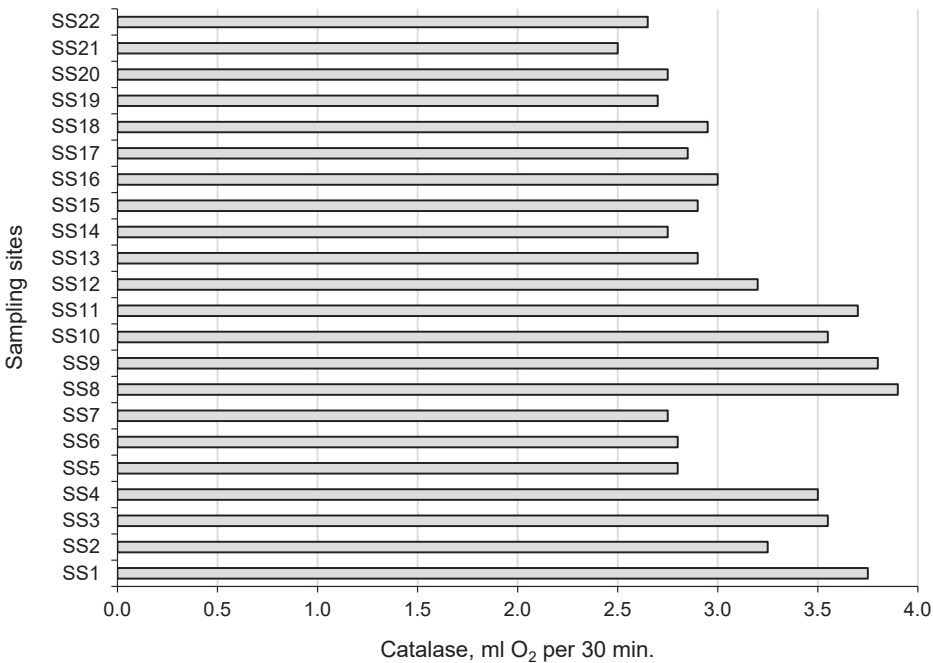


Fig. 7. Catalase activity of soil microorganisms in spring (10–25 cm).

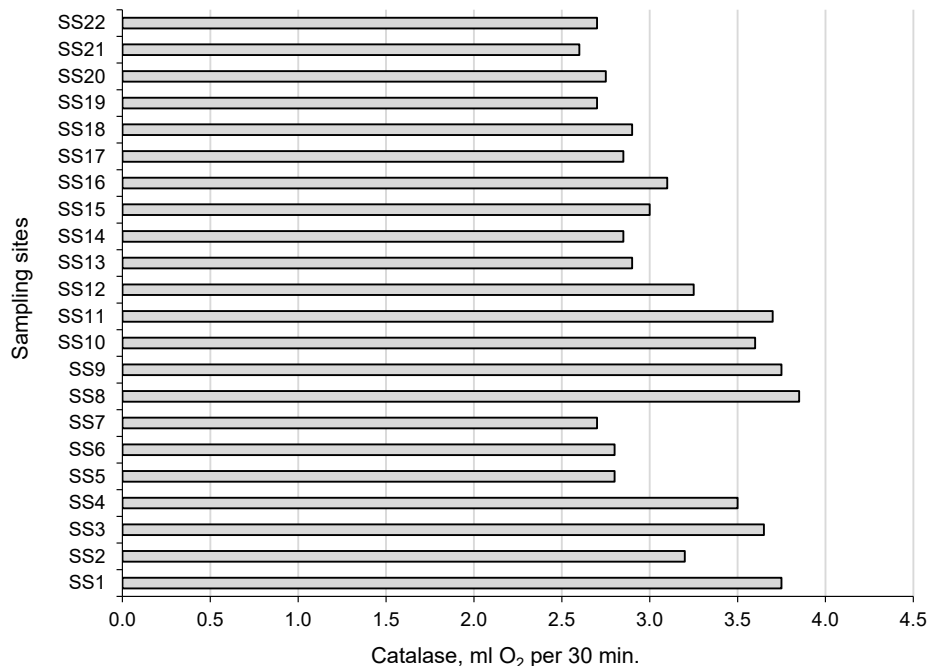


Fig. 8. Catalase activity of soil microorganisms in summer (0–10 cm).

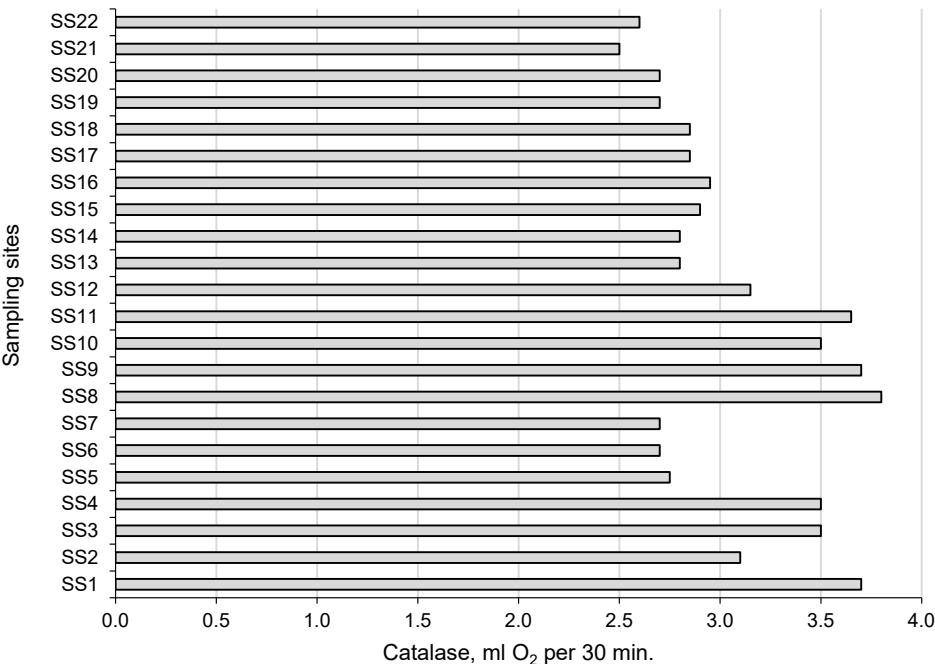


Fig. 9. Catalase activity of soil microorganisms in summer (10–25 cm).

Cellulase activity is significantly lower at SS17 (soil influenced by the water flow of Stone fountain in Vitosha Nature Park) – 4 times in spring and 3 times in summer lower compared to the highest values. This trend is maintained in both seasons (figs 10–13).

Cellulase activity is also determined by the same CF that influence microbial biomass and catalase activity, with the abundance and composition of microorganisms – particularly bacteria – playing a decisive role in the decomposition of plant residues and the overall cellulolytic activity (Kanazawa and Miyashita 1987, Malcheva et al. 2015a, 2015b, 2019, 2024, Naskova et al. 2016, Malcheva 2020b, 2020c, Datta 2024).

In summer, the accumulation of microbial biomass carbon and the production of enzymes is by mesophilic groups of microorganisms, while in spring, psy-

chrophiles also participate, especially in forest areas with higher altitudes. In most of the waterlogged soils of Vitosha Nature Park, due to spillage of water from local water sources to them, the abundance of aerobic microorganisms decreases at the expense of anaerobes (Malcheva et al. 2025), the accumulation of microbial biomass decreases and, accordingly, the enzymatic activity of microorganisms decreases. The values of soil temperature and moisture in spring and summer are presented in figures 14 and 15.

The dynamics in the temperature regime of the soils is more significant in spring – higher temperatures in more open areas (Vrazhdebna, Zheleznitsa and Dolni Pasarel) and lower in the sites of Vitosha Nature Park. For soil moisture, the trends are opposite – higher values in the soils of Vitosha Nature Park and the soil

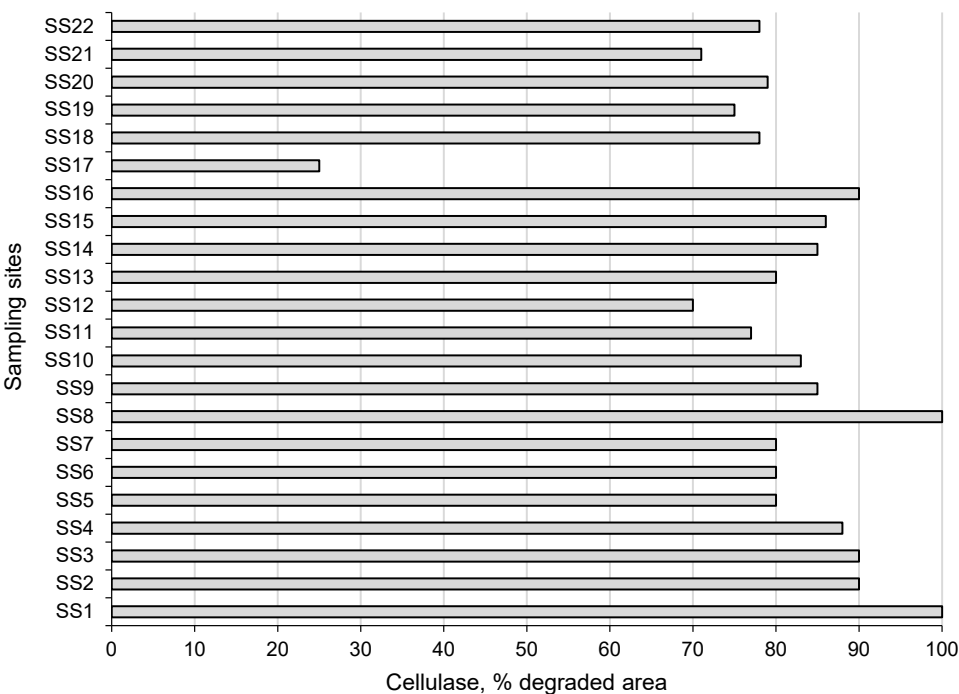


Fig. 10. Cellulase activity of soil microorganisms in spring (0–10 cm).

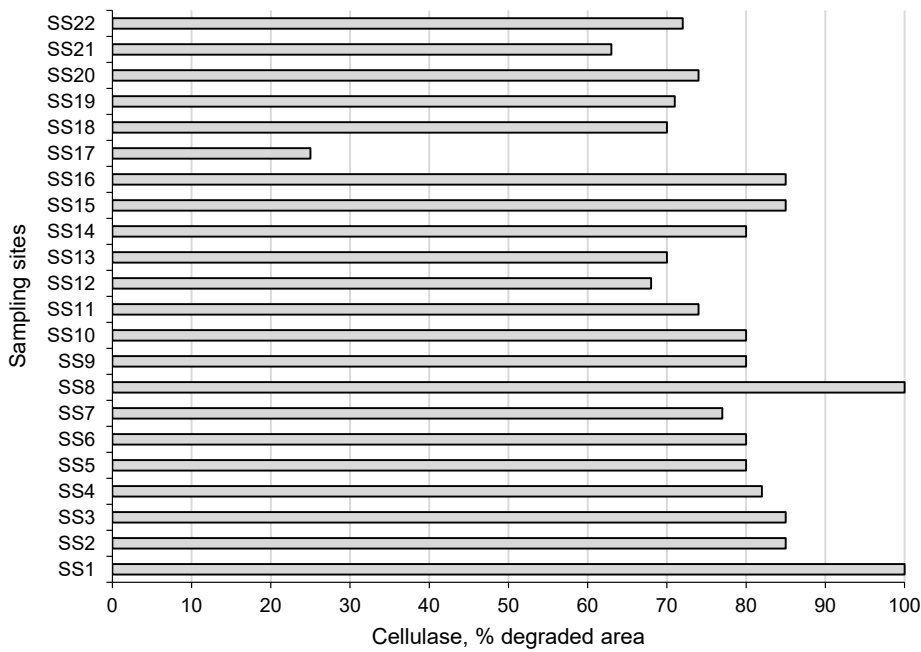


Fig. 11. Cellulase activity of soil microorganisms in spring (10–25 cm).

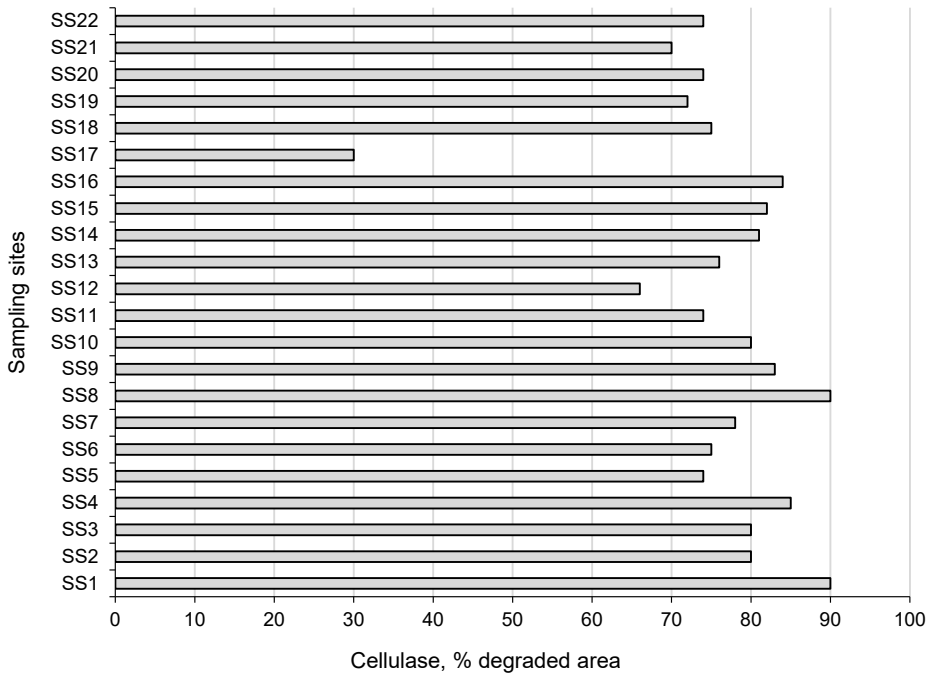


Fig. 12. Cellulase activity of soil microorganisms in summer (0–10 cm).

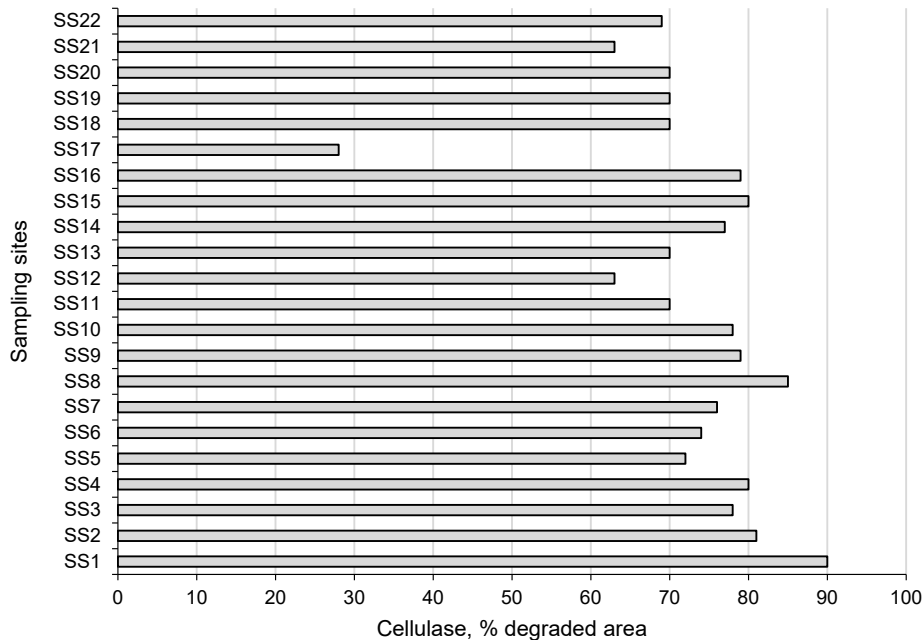


Fig. 13. Cellulase activity of soil microorganisms in summer (10–25 cm).

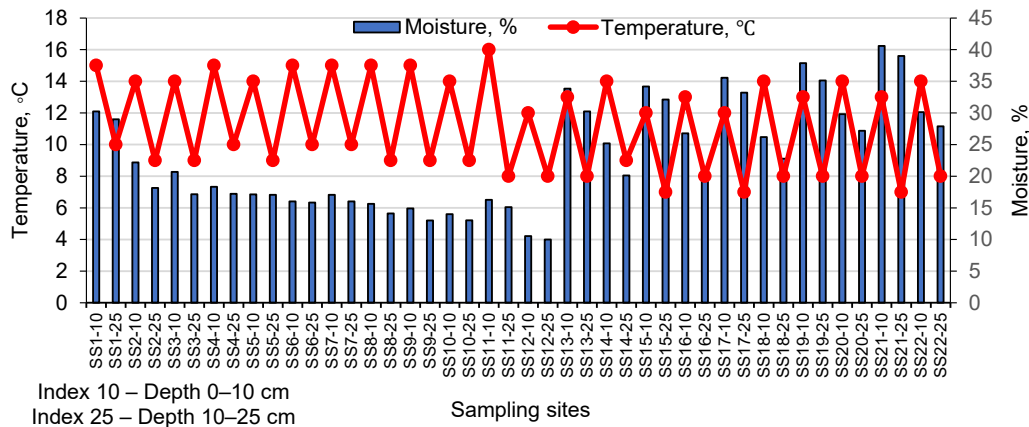


Fig. 14. Soil temperature and moisture in spring.

directly influenced by the mineral spring in Zheleznitza and lower values in the arable soils and the remaining sites of Zheleznitza and Dolni Pasarel.

In the sites of Vitosha Nature Park, the villages of Zheleznitza and Dolni Pasarel,

the soils have an acidic reaction, while the agricultural soils of Vrazhdebna district have a neutral reaction, which suggests an accumulation of biomass mainly of bacterial origin in the agricultural soils, and in those with an acidic reaction an in-

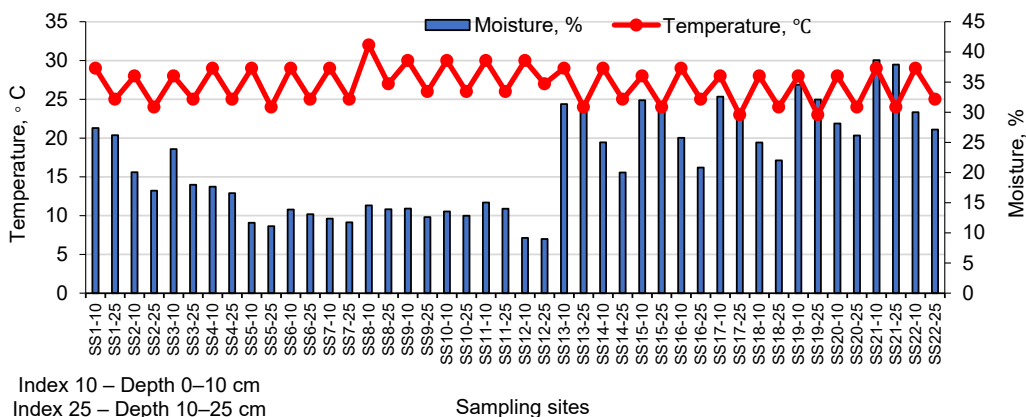


Fig. 15. Soil temperature and moisture in summer.

crease in the amount and activity of mould fungi, but on the other hand, anaerobic conditions in overmoistened soils limit the development of mycelial groups of micro-organisms (Fig. 16).

Microbial biomass carbon and enzyme activities depend moderately, positively, on soil temperature. Catalase activity depends significantly, and cellulase activity highly, positively, on the accumulated microbial biomass. A negative correlation is established between moisture and microbial biomass, as well as between moisture and enzyme activities, respectively significant negative between moisture and cellulase, moderate negative between

moisture and catalase and high negative between moisture and microbial biomass. A significant positive correlation is established between pH and catalase, and weak between pH and microbial biomass, while between cellulase and pH the correlation is moderate, negative (Table 2).

Coefficients with $p < 0.05$ are considered reliable predictors; the sign indicates direction (Table 3).

Welch's t-test showed significant seasonal differences in temperature ($p < 0.001$), while cellulase activity did not differ significantly between spring and summer ($p > 0.05$). Soil moisture was also higher in spring, but the difference was

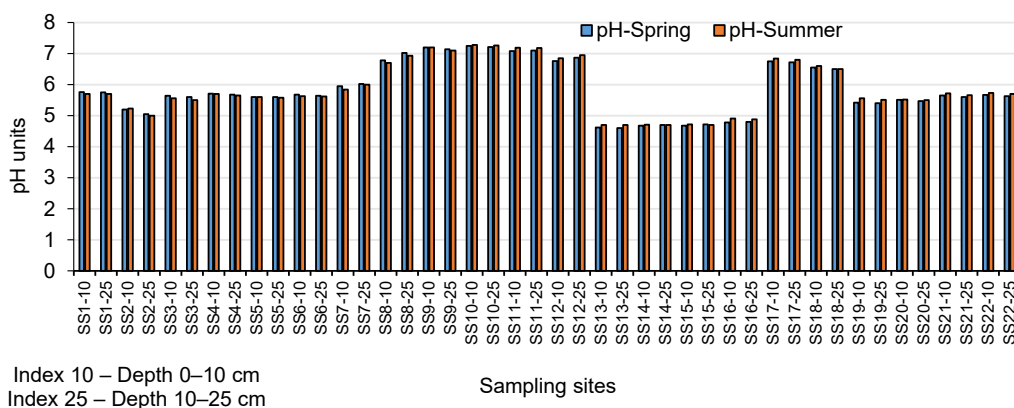


Fig. 16. Soil pH values.

Table 2. Correlation coefficients between biological and physicochemical soil parameters in spring and summer.

Variable	Depth	MBC	Catalase	Cellulase	Moisture	Temperature	pH
Spring							
Depth	1						
Microbial biomass C	-0.167	1					
Catalase	-0.110	0.5960	1				
Cellulase	-0.132	0.6563	0.4864	1			
Moisture	-0.130	-0.7296	-0.5277	-0.3279	1		
Temperature	-0.935	0.3786	0.2603	0.3127	-0.0724	1	
pH	-0.003	0.1483	0.5157	-0.2076	-0.4914	0.0728	1
Summer							
Depth	1						
Microbial biomass C	-0.136	1					
Catalase	-0.083	0.5074	1				
Cellulase	-0.137	0.6055	0.4355	1			
Moisture	-0.120	-0.6861	-0.4474	-0.2720	1		
Temperature	-0.900	0.2962	0.3374	0.2823	-0.1652	1	
pH	-0.010	0.0797	0.4905	-0.2561	-0.4508	0.2362	1

Table 3. *P*-values.

Predictor	Catalase (Spring)	Cellulase (Spring)	Catalase (Summer)	Cellulase (Summer)
Depth	0.649	0.069	0.188	0.024
MBC	0.065	0.163	0.055	0.026
pH	<0.001	<0.001	<0.001	<0.001
Temperature	0.547	0.052	0.227	0.010
Moisture	0.271	0.999	0.111	0.514
Cellulase	0.002		0.022	
Catalase		0.002		0.022

not statistically significant ($p > 0.05$). Catalase, microbial biomass carbon, and pH showed no significant seasonal variation (Table 4).

Multicollinearity diagnostics indicated acceptable levels for all predictors, with VIF values below 5 in most cases. Higher Variance Inflation Factor (VIF) values (>10) were observed for depth and temperature, reflecting their natural correlation structure, but tolerance values remained above 0.05. Slightly elevated collinearity

between temperature and moisture was noted, yet not at a level compromising model stability (Table 5)

Correlation analysis offers an initial indication of the direction and strength of relationships. However, in some cases variables that show significant correlations with enzyme activity may drop out of the regression model. The reasons include: (1) multicollinearity (strong association with another predictor), (2) lack of independent contribution once other factors are

Table 4. T-test results (spring vs summer).

Variable	Mean spring	Mean summer	t (Welch)	df (approx)	Sig. (2-tailed)
Temperature	11.23	26.91	-27.86	82.47	<0.001
Cellulase	78.55	74.43	1.45	82.68	0.152
Moisture	23.13	21.56	0.87	85.99	0.387
Catalase	3.16	3.10	0.69	85.95	0.490
MBC	365.36	357.70	0.48	85.98	0.631
pH	5.86	5.88	-0.11	86.00	0.912
Depth	17.50	17.50	0.00	86.00	1.000

Table 5. Multicollinearity diagnostics (VIF and tolerance).

Predictor	Catalase (Spring)	Cellulase (Spring)	Catalase (Summer)	Cellulase (Summer)
Depth	13.76 (Tol=0.073)	12.65 (Tol=0.079)	12.34 (Tol=0.081)	11.25 (Tol=0.089)
MBC	4.49 (Tol=0.222)	4.68 (Tol=0.214)	3.43 (Tol=0.291)	3.32 (Tol=0.301)
pH	1.81 (Tol=0.553)	2.28 (Tol=0.439)	2.22 (Tol=0.451)	2.09 (Tol=0.479)
Temperature	15.16 (Tol=0.066)	13.80 (Tol=0.072)	13.59 (Tol=0.074)	11.80 (Tol=0.085)
Moisture	3.96 (Tol=0.253)	4.09 (Tol=0.244)	3.63 (Tol=0.275)	3.85 (Tol=0.260)
Cellulase	2.34 (Tol=0.427)	—	2.57 (Tol=0.389)	—
Catalase	—	2.29 (Tol=0.437)	—	2.31 (Tol=0.434)

considered, or (3) the automatic stepwise selection procedure, which removes variables with $p > 0.05$. Soil moisture showed a negative and statistically significant correlation with catalase. Nevertheless, in the regression model this factor was excluded due to multicollinearity with temperature and pH. This indicates that the independent contribution of moisture is limited once other predictors are taken into account. In the correlation analysis, the relationship between pH and catalase is weak and non-significant. However, in the multiple regression model pH emerges as a significant positive predictor. This represents a classic example of a 'hidden effect': once the influences of temperature and mois-

ture are controlled for, the independent contribution of pH becomes statistically significant. Thus, pH is a key factor that is not revealed in a bivariate analysis but is crucial in a multivariate context. Stepwise multiple linear regression was applied to evaluate the factors influencing enzyme activities. This method is suitable when several potential predictors are available and their relative importance is not known in advance. The correlation analysis did not reveal extremely high intercorrelations ($r > 0.7$), but a moderate positive association was observed between microbial biomass and cellulase, with $r \approx 0.66$ in spring and $r \approx 0.61$ in summer. This indicates a certain degree of interdependence among

some variables. The stepwise procedure sequentially adds or removes predictors based on their statistical significance and independent contribution, thereby avoiding model oversaturation and reducing the potential impact of multicollinearity. Thus, an optimal balance is achieved between explanatory power and model simplicity (Table A1 in Appendix A).

The regression model for catalase in spring is given by equation (1):

$$\begin{aligned} \text{Catalase} = & -0.5359 - 0.0091 \cdot \text{Depth} + \\ & + 0.0022 \cdot \text{MBC} + 0.3497 \cdot \text{pH} - \\ & - 0.0331 \cdot \text{Temperature} + 0.0106 \cdot \text{Moisture} + \\ & + 0.0145 \cdot \text{Cellulase}, \end{aligned} \quad (1)$$

where: pH was identified as a significant positive predictor ($B \approx 0.35$, $p < 0.001$), while cellulase activity also contributed significantly and positively. Microbial biomass carbon (MBC) had a positive but marginal effect ($p \approx 0.065$), and depth, temperature, and moisture were not significant. Although MBC correlated positively with catalase in the correlation analysis, this relationship lost significance in the regression model, likely due to overlapping influence among predictors (multicollinearity).

The corresponding regression for cellulase activity in spring is presented in equation (2):

$$\begin{aligned} \text{Cellulase} = & 1.5432 + 1.2005 \cdot \text{Depth} + \\ & + 0.0562 \cdot \text{MBC} - 9.6367 \cdot \text{pH} + \\ & + 3.5300 \cdot \text{Temperature} - 0.0005 \cdot \text{Moisture} + \\ & + 16.5418 \cdot \text{Catalase}, \end{aligned} \quad (2)$$

where: depth, temperature, and catalase activity emerged as strong positive predictors, whereas pH had a strong negative effect ($B \approx -9.64$, $p < 0.001$). MBC and soil moisture were not significant contributors. Despite the moderate positive correlation between MBC and cellulase ($r \approx 0.66$), this effect disappeared in the multivariate model due to stronger contributions from

other predictors, reflecting a shared or mediated relationship rather than a direct one.

During summer, the model for catalase activity took the form shown in equation (3):

$$\begin{aligned} \text{Catalase} = & -3.3941 + 0.0262 \cdot \text{Depth} + \\ & + 0.0021 \cdot \text{MBC} + 0.2998 \cdot \text{pH} + \\ & + 0.0812 \cdot \text{Temperature} + 0.0156 \cdot \text{Moisture} + \\ & + 0.0135 \cdot \text{Cellulase}, \end{aligned} \quad (3)$$

where: pH ($B \approx 0.30$, $p < 0.001$) and cellulase activity were significant positive predictors. Depth, MBC, temperature, and soil moisture showed positive but statistically non-significant effects. The positive yet non-significant temperature coefficient ($B \approx 0.0812$, $p > 0.05$) indicates that the observed correlation between temperature and catalase is explained largely by covariance with other predictors – particularly pH and cellulase – rather than by an independent effect.

Finally, cellulase activity in summer was described by equation (4):

$$\begin{aligned} \text{Cellulase} = & -74.4463 + 1.1870 \cdot \text{Depth} + \\ & + 0.0650 \cdot \text{MBC} - 8.6619 \cdot \text{pH} + \\ & + 4.5112 \cdot \text{Temperature} + 0.1750 \cdot \text{Moisture} + \\ & + 9.8751 \cdot \text{Catalase}, \end{aligned} \quad (4)$$

where: temperature, MBC, and catalase activity acted as significant positive predictors, while pH remained a significant negative predictor, consistent with the spring model. Depth and soil moisture were not significant. Notably, depth contributed significantly in spring but not in summer, suggesting a seasonal pattern in which the influence of depth diminishes under warmer and drier summer conditions. The non-significance of soil moisture may again be attributed to its strong correlation with temperature.

Across the four models, pH consistently emerges as a key factor – positive for catalase and negative for cellulase. Al-

though the simple correlation between pH and catalase is weak and non-significant, in regression analysis pH becomes a significant predictor, representing a classic example of a 'hidden effect'. Temperature strongly stimulates cellulase and shows a stable positive role, while for catalase its influence disappears once other factors are considered. Microbial biomass carbon supports both enzymes, particularly in summer, with a more pronounced contribution to cellulase activity. The inclusion of one enzyme as predictor for the other highlights the interdependence of enzymatic activities and the complex nature of soil biochemical processes. Overall, step-wise multiple regression enabled the identification of the most relevant independent factors, while avoiding model oversaturation and reducing the risk of multicollinearity, thus providing a more reliable and transparent interpretation of the role of individual predictors.

The relationships established in the present study confirm that enzyme activities are closely linked to the microbiological and chemical properties of the soil, which corresponds to the results of Wang et al. (2023), who reported that enzyme activities are mainly determined by soil nutrient status and the content of microbial biomass carbon and nitrogen, with biomass nitrogen being particularly important for cellulase. In both studied seasons, pH emerged as a key predictor of catalase and cellulase activity, although with opposite directions of influence. Although higher temperatures generally enhance enzymatic reactions, pH may restrict catalytic efficiency (Souza and Billings 2021), while in our study it limited cellulase activity. Under more stressful or waterlogged conditions, the reduced effect of pH in our data resembles the trends described by Chander and Joergensen (2001), who

noted that pH often loses significance when stronger stressors such as toxic metals or oxygen deficiency prevail. The inclusion of depth, pH, temperature and microbial biomass among the consistent predictors in our models corresponds to the synthesis by Daunoras et al. (2024), who emphasised that these parameters jointly determine microbial community activity and enzymatic potential.

Conclusions

The obtained results showed that microbial and enzymatic activities respond sensitively to land use, soil depth, and environmental conditions. In spring, microbial biomass carbon (MBC) reached approximately $360 \text{ mg C} \cdot \text{kg}^{-1}$ and was about 2–3 times higher in the agricultural soils of Vrazhdebna and in the soil near the mineral spring at Zhelezmitsa compared with the forest soils of Vitosha, reflecting the effects of cultivation and irrigation. The activities of catalase and cellulase were also highest in spring and greater in agricultural soils, decreasing with depth and under waterlogged conditions. Catalase activity reached up to $3.7 \text{ L O}_2 \cdot \text{g}^{-1} \cdot 30 \text{ min}^{-1}$, while complete cellulose degradation (100%) was recorded in the most biologically active soils.

Soil moisture exerted a strong negative effect on MBC ($r \approx -0.73$) and enzyme activities, whereas temperature and pH acted in opposite directions: temperature promoted ($r \approx 0.28$ – 0.38), while pH limited cellulase activity ($r \approx -0.21$ to -0.26) but enhanced catalase ($r \approx 0.49$ – 0.52). Regression analysis confirmed the role of pH as a stable predictor—positive for catalase ($B \approx +0.30$ – 0.35 ; $p < 0.001$) and negative for cellulase ($B \approx -8.7$; $p < 0.001$) in both seasons. In the spring

model for catalase, pH and cellulase activity emerged as significant positive predictors, whereas in summer pH and cellulase again remained significant, while depth, temperature, moisture, and MBC were positive but non-significant. For cellulase in spring, catalase activity was the strongest positive predictor ($B \approx +16.5$; $p = 0.002$), pH had a strong negative effect ($B \approx -9.6$; $p < 0.001$), and temperature and depth had positive effects at the margin of significance. In summer, cellulase was significantly stimulated by temperature ($B \approx +4.5$; $p = 0.010$), MBC ($B \approx +0.065$; $p = 0.026$), depth ($B \approx +1.19$; $p = 0.024$), and catalase ($B \approx +9.88$; $p = 0.022$), with pH remaining a negative predictor.

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Appendix A

Table A1. Regression analysis.

Model summary – Catalase (spring)									
R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson	N				
0.817	0.668	0.614	0.266	0.797	44				
ANOVA									
Source	Sum of Squares	df	Mean Square	F	Sig.				
Regression	5.2650	6	0.8775	12.40	<i>p</i> <0.001				
Residual	2.6179	37	0.0708						
Total	7.8829	43							
Coefficients									
Term	B	Std. Error	Standardised Beta	t	Sig.	95% CI Lower	95% CI Upper	Tolerance	VIF
Intercept	-0.5359	1.1164		-0.48	0.634	-2.7979	1.7261		
Q(Depth)	-0.0091	0.0198	-0.162	-0.46	0.649	-0.0493	0.0311	0.073	13.76
Q(MBC)	0.0022	0.0011	0.381	1.90	0.065	-0.0001	0.0045	0.222	4.494
Q(pH)	0.3497	0.0654	0.681	5.35	<0.001	0.2172	0.4822	0.553	1.808
Q(Temperature)	-0.0331	0.0544	-0.224	-0.61	0.547	-0.1435	0.0772	0.066	15.16
Q(Moisture)	0.0106	0.0095	0.210	1.12	0.271	-0.0086	0.0298	0.253	3.957
Q(Cellulase)	0.0145	0.0042	0.495	3.42	0.002	0.0059	0.0231	0.427	2.342
Model summary – Cellulase (spring)									
R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson	N				
0.822	0.675	0.623	8.984	1.170	44				
ANOVA									
Source	Sum of Squares	df	Mean Square	F	Sig.				
Regression	6216.5	6	1036.0905	12.84	<i>p</i> <0.001				
Residual	2986.4	37	80.7126						
Total	9202.9	43							
Coefficients									
Term	B	Std. Error	Standardised Beta	t	Sig.	95% CI Lower	95% CI Upper	Tolerance	VIF
Intercept	1.5432	37.8229		0.04	0.968	-75.09	78.18		
Q(Depth)	1.2005	0.6421	0.623	1.87	0.069	-0.101	2.5016	0.079	12.65
Q(MBC)	0.0562	0.0395	0.288	1.42	0.163	-0.024	0.1363	0.214	4.676
Q(pH)	-9.6367	2.4779	-0.549	-3.89	<0.001	-14.66	-4.6160	0.439	2.276
Q(Temperature)	3.5300	1.7547	0.700	2.01	0.052	-0.025	7.0854	0.072	13.80
Q(Moisture)	-0.0005	0.3251	-0.000	-0.00	0.999	-0.659	0.6583	0.244	4.091
Q(Catalase)	16.542	4.8411	0.484	3.42	0.002	6.7329	26.351	0.437	2.289

Model summary – Catalase (summer)

R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson	N
0.790	0.624	0.563	0.276	0.797	44

ANOVA

Source	Sum of Squares	df	Mean Square	F	Sig.
Regression	4.6757	6	0.7793	10.23	$p<0.001$
Residual	2.8190	37	0.0762		
Total	7.4948	43			

Coefficients

Term	B	Std. Error	Standardised Beta	t	Sig.	95% CI Lower	95% CI Upper	Tolerance	VIF
Intercept	-3.3941	2.0065		-1.69	0.099	-7.4596	0.6715		
Q(Depth)	0.0262	0.0195	0.475	1.34	0.188	-0.0133	0.0656	0.081	12.34
Q(MBC)	0.0021	0.0011	0.371	1.98	0.055	-0.0000	0.0042	0.291	3.433
Q(pH)	0.2998	0.0753	0.598	3.98	<0.001	0.1472	0.4524	0.451	2.220
Q(Temperature)	0.0812	0.0660	0.457	1.23	0.227	-0.0526	0.2149	0.074	13.59
Q(Moisture)	0.0156	0.0095	0.314	1.63	0.111	-0.0038	0.0349	0.275	3.631
Q(Cellulase)	0.0135	0.0057	0.385	2.38	0.022	0.0020	0.0249	0.389	2.572

Model summary - Cellulase (summer)

R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson	N
0.814	0.663	0.608	7.472	0.943	44

ANOVA

Source	Sum of Squares	df	Mean Square	F	Sig.
Regression	4063.0	6	677.1697	12.13	$p<0.001$
Residual	2065.8	37	55.8318		
Total	6128.8	43			

Coefficients

Term	B	Std. Error	Standardised Beta	t	Sig.	95% CI Lower	95% CI Upper	Tolerance	VIF
Intercept	-74.446	55.0326		-1.35	0.184	-185.95	37.060		
Q(Depth)	1.1870	0.5039	0.754	2.36	0.024	0.1661	2.2079	0.089	11.254
Q(MBC)	0.0650	0.0281	0.403	2.32	0.026	0.0081	0.1219	0.301	3.318
Q(pH)	-8.6619	1.9774	-0.604	-4.38	<0.001	-12.668	-4.6554	0.479	2.087
Q(Temperature)	4.5112	1.6650	0.888	2.71	0.010	1.1375	7.8849	0.085	11.800
Q(Moisture)	0.1750	0.2656	0.123	0.66	0.514	-0.3633	0.7132	0.260	3.848
Q(Catalase)	9.8751	4.1436	0.345	2.38	0.022	1.4793	18.271	0.434	2.305