

Seasonal microbiological and enzymatic responses to selected soil properties in urbogenic soils beneath introduced ornamental plant species along the Southern Black Sea coast

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Abstract

The aim of this study was to evaluate the seasonal microbiological and enzymatic responses of urbogenic soils beneath introduced ornamental tree and shrub species in urban areas of Burgas and Sozopol along the Southern Black Sea coast of Bulgaria. Soil samples were collected under *Ligustrum lucidum* W.T.Aiton, *Magnolia liliiflora* 'Nigra', *Musa paradisiaca* L., *Nerium oleander* L., *Photinia × fraseri* 'Red Robin', *Pinus pinea* L., *Prunus serrulata* 'Kanzan' and *Trachycarpus fortunei* (Hook.) H.Wendl. from two soil layers during spring, summer and autumn. The abundance and composition of the main microbial groups, total microbial count, mineralisation coefficient, cellulase and catalase activities, soil pH, humus content, temperature and moisture were determined. The microbial status showed pronounced spatial differentiation and a general decrease in total microbial abundance with depth. The highest total microbial count was recorded under *N. oleander*, whereas the lowest values were found under *T. fortunei*. Non-spore-forming bacteria dominated the microbial community, followed by bacilli, while actinomycetes and filamentous fungi were less abundant. Seasonal dynamics of total microbial count and enzyme activities showed higher values in autumn at most sites, followed by spring, whereas summer maxima were observed in the surface soil layers at two sites under favourable soil moisture conditions. Two-way analysis of variance showed that season did not significantly affect the analysed parameters, whereas soil depth significantly affected total microbial count, cellulase and catalase activities. Cellulase activity was higher in the surface layer and slightly increased in autumn at most sites, whereas catalase activity followed a similar spatial pattern. The mineralisation coefficient varied considerably among sites and was not directly related to total microbial abundance. Spearman correlation analysis identified humus content as the factor most strongly associated with microbial abundance and enzymatic activity. Overall, the results confirm the value of combining microbiological, enzymatic and physicochemical indicators for assessing the biological quality of urbogenic soils.

Key words: catalase, cellulase, humus content, introduced vegetation, soil microbial activity, urban ecosystem.

Introduction

Soils in anthropogenic environments are exposed to numerous factors that influence the biological activity, structure, and composition of soil microbial communities, depending on local environmental conditions. Over the past two decades, the Bulgarian Black Sea coast, including the Southern Black Sea region, has experienced intensive development of tourism, residential, and transport infrastructure. Urbanisation and the expansion of tourist complexes have increased anthropogenic pressure on coastal ecosystems, resulting in changes in land use, fragmentation of natural habitats, and an increased risk of degradation of green areas and soil cover (Palazov and Stanchev 2007, Stanchev et al. 2015). In the Southern Black Sea region, these processes also affect the ecological condition of valuable coastal habitats (Prodanov et al. 2023), highlighting the need to assess the ecological status of urban soils. The concept of smart cities emphasises the integration of innovative approaches for more efficient urban management, including environmental monitoring and the sustainable management of green infrastructure (Trizlova 2017).

Soil microbiological and enzymatic activities are considered sensitive bioindicators of changes in soil quality and the functional status of urban ecosystems (Nannipieri et al. 2012, Igalavithana et al. 2017). As part of the efforts to develop urban green infrastructure, numerous landscaping initiatives have been implemented in the municipalities of Burgas and Sozopol during the same period, involving the introduction of ornamental trees and shrubs into residential green spaces, roundabouts, roadside plantings, central urban areas, and coastal parks. These introduced ornamental species create

distinct soil environments whose microbiological and enzymatic characteristics remain insufficiently investigated.

Urban soils are frequently exposed to the impacts of industrial activities, traffic, salinisation, construction, domestic and biological waste, as well as the accumulation of various anthropogenic pollutants, including heavy metals. These factors may alter the physical, chemical, and biological properties of soils and consequently affect soil microorganisms and the activities of microbial enzymes, depending on pollutant concentrations, soil characteristics, and the biological traits of individual microbial groups (Nustorova and Gencheva 1988, 1995; Nustorova and Plugchieva 1991; Deribeeva and Belichki 1995; Nustorova and Yorova 1995; Kandeler et al. 2000; Kenarova and Bogoev 2001; Lugauskas et al. 2005; Nustorova and Malcheva 2007, 2011; Malcheva 2008, 2019, 2020a,b; Atanasova 2015). Numerous studies have shown that bacteria constitute the major proportion of the total soil microbial community, whereas actinomycetes and micromycetes are generally less abundant. Soil microbial communities also exhibit pronounced seasonal dynamics, characterised by maxima in spring and autumn and minima in summer and winter with respect to abundance, community composition, and activity. These seasonal fluctuations are primarily driven by changes in soil temperature and moisture regimes, as well as by vegetation cover (Díaz-Raviña et al. 1995; Fierer et al. 2007; Fierer 2017; Delgado-Baquerizo et al. 2018; Malcheva et al. 2018, 2019; Malcheva 2020a, 2025; Naskova et al. 2016; Plamenov et al. 2016; Yankova et al. 2016; Schneckner et al. 2023). In contaminated and technogenically affected soils, representatives of the genus *Bacillus* are able to maintain their viability over

extended periods owing to their ability to form endospores, whereas non-spore-forming bacteria are generally more sensitive to adverse environmental conditions (Kandeler et al. 2000, Nicholson et al. 2000, Setlow 2014, Madigan et al. 2018, Pouyat et al. 2007, Fierer 2017). Owing to their mycelial organisation and their ability to degrade more recalcitrant organic compounds, actinomycetes and filamentous fungi play an important role in microbial succession and may exhibit greater resilience in stressed urban ecosystems, including soils affected by industrial activities and construction waste (Goodfellow and Williams 1983, Giller et al. 1998, Kandeler et al. 2000, Gadd 2010, Paul 2015).

Urban soils are characterised by high spatial heterogeneity resulting from anthropogenic disturbances, fill materials, and construction activities, leading to considerable variability in soil texture, humus content, pH, and other physicochemical properties (Lehmann and Stahr 2007). This heterogeneity is one of the major factors determining the structure and functional activity of soil microbial communities, which are strongly influenced by soil texture, soil organic matter content, soil reaction (pH), moisture, and anthropogenic disturbances (Nannipieri et al. 2012, Paul 2015, Fierer 2017).

Cellulase is a key enzyme in the carbon cycle, participating in cellulose degradation and the mineralisation of organic matter, whereas catalase is an oxidoreductase enzyme that catalyses the decomposition of hydrogen peroxide and reflects the intensity of aerobic microbial processes in soil. Because of their high sensitivity to changes in soil conditions, both enzymes are widely used as bio-indicators for assessing the biological status, functional activity, and quality of soils (Nannipieri et al. 2012, Torres et al.

2016, Malcheva and Koleva 2025). Their activities are influenced by the combined effects of soil temperature and moisture, humus content, soil texture, pH, soil type, the abundance and activity of soil microorganisms, vegetation cover, and other environmental factors (Kanazawa and Miyashita 1987; Grozeva and Nustorova 1995; Uzun and Uyanöz 2011; Nannipieri et al. 2012; Paul 2015; Fierer 2017; Malcheva et al. 2019; Malcheva 2020a,b; Naskova et al. 2016; Datta 2024; Malcheva and Koleva 2025).

Despite numerous studies on urban soils, information on the seasonal dynamics of the main soil microbial groups and enzymatic activity under introduced ornamental plant species in the urban areas of the Southern Black Sea coast of Bulgaria remains limited. Different ornamental plant species may exert distinct effects on the physical, chemical, and biological properties of urban soils through differences in root architecture, the quantity and chemical composition of plant residues, and the release of root exudates, thereby promoting the development of distinct microbial communities and influencing of enzymatic activity (Bardgett and van der Putten 2014, Philippot et al. 2013, Fierer 2017). Therefore, the aim of the present study was to evaluate the seasonal responses of the main groups of soil microorganisms – non-spore-forming bacteria, bacilli, actinomycetes, and filamentous fungi – as well as cellulase and catalase activities in relation to selected soil properties (temperature, moisture, pH, and humus content) under different introduced ornamental plant species growing in urban soils. Based on the literature review and the objectives of the study, the following hypotheses were formulated: (1) seasonal changes would significantly affect the abundance of the main groups of soil microorganisms and

the activities of cellulase and catalase in urban soils under introduced ornamental plant species; (2) selected soil properties, including humus content, pH, temperature, and moisture, are key factors determining the microbiological and enzymatic activity of urban soils; and (3) different introduced ornamental plant species establish distinct microbiological and enzymatic profiles in urban soils, with their effects being modified by the heterogeneity of soil properties.

Materials and Methods

Research area

For the present study, soil samples were collected from anthropogenic urban soils

classified as Urbogenic soils according to the Bulgarian classification of anthropogenic soils (Gencheva, 1995) in urban and peri-urban areas of Burgas and Sozopol, Bulgaria. The investigated locations were designated as sampling sites (SS1–SS11) according to the corresponding ornamental plant species and urban location. The study area, showing the sampling sites and the associated vegetation, is presented in Figure 1.

The locations of the study sites and information on the introduction of the ornamental tree and shrub species into Bulgaria are presented in Table 1.

Experimental design

At each sampling site, soil samples were collected from two depth intervals

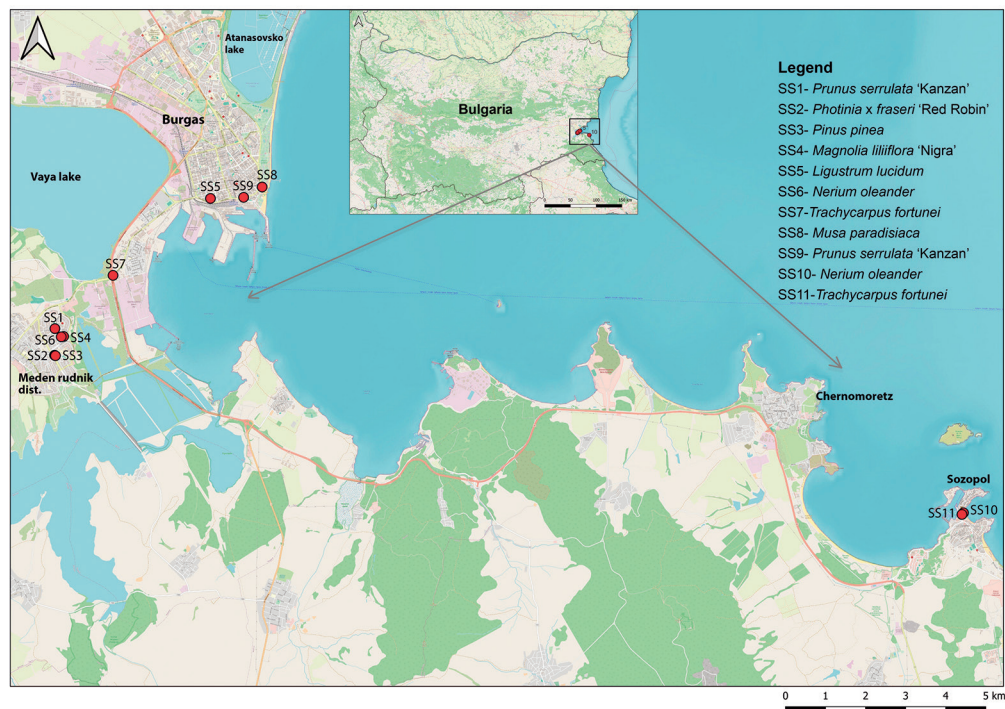


Fig. 1. Map of the sampling locations and vegetation.

Table 1. Location of the study sites and information on the introduction of ornamental tree and shrub species into Bulgaria.

No	Investigated species / Plant community	Location, GPS coordinates	Introduction source and Initiative	Year of introduction
SS1	<i>Prunus serrulata</i> 'Kanzan' / <i>Lolium perenne</i> L.; <i>Cynodon dactylon</i>	Meden Rudnik district, International Youth Centre, Burgas 42°27'43.7" N; 27°25'06.4" E	IYC	2024
SS2	<i>Photinia</i> × <i>fraseri</i> 'Red Robin' / <i>Cynodon dactylon</i> ; <i>Pinus nigra</i> Wulfen	Meden Rudnik district, Park Zone B, Burgas 42°27'22.3" N; 27°25'06.0" E	IPUDR	2015
SS3	<i>Pinus pinea</i> L. / <i>Trifolium repens</i> ; <i>Geranium sanguineum</i> ; <i>Photinia</i> × <i>fraseri</i> 'Red Robin'	Meden Rudnik district, Park Zone B, Burgas 42°27'21.9" N; 27°25'07.0" E	IPUDR	2015
SS4	<i>Magnolia liliiflora</i> 'Nigra' / <i>Capsella bursa-pastoris</i> (L.) Medik.; <i>Geranium sanguineum</i> L.	In the yard of 'Elin Pelin' Primary School, Burgas 42°27'37.4" N; 27°25'15.9" E	Earth Day – school initiative	2019
SS5	<i>Ligustrum lucidum</i> W.T.Aiton / <i>Ornithogalum umbellatum</i> L.	Iv. Shishman Str., Burgas, sidewalk plantation 42°29'28.5" N; 27°27'56.1" E	IPUDR	2015
SS6	<i>Nerium oleander</i> L. / <i>Nerium oleander</i> ; <i>Cynodon dactylon</i> (L.) Pers	In the yard of 'Elin Pelin' Primary School, Burgas 42°27'37.1" N; 27°25'13.3" E	Earth Day – school initiative	2019
SS7	<i>Trachycarpus fortunei</i> (Hook.) H.Wendl. / <i>Cynodon dactylon</i> ; <i>Trifolium repens</i>	Burgas–Sozopol road roundabout 42°28'26.5" N; 27°26'09.8" E	IPUDR	2013
SS8	<i>Musa paradisiaca</i> L. / <i>Lonicera nitida</i> E.H.Wilson	Seaside Park, Burgas 42°29'37.7" N; 27°28'52.5" E	Personal donation	2017
SS9	<i>Prunus serrulata</i> 'Kanzan' / <i>Plantago major</i> L.; <i>Cynodon dactylon</i>	Bulair Str., intersection with Tsaritsa Yoana Blvd., Burgas 42°29'29.4" N; 27°28'32.3" E	IPUDR	2015
SS10	<i>Nerium oleander</i> / <i>Trifolium repens</i> L.; <i>Cynodon dactylon</i>	Seaside Park, Sozopol (The Old Town) 42°25'15.7" N; 27°41'37.9" E	Municipal initiative	2024
SS11	<i>Trachycarpus fortunei</i> / <i>Hedera helix</i> L.	Dividing strip, Sozopol (The Old Town) 42°25'14.4" N; 27°41'36.2" E	Municipal initiative	2011

Note: IPUDR – Integrated Plan for Urban Development and Reconstruction of Burgas under the Operational Programme 'Regional Development 2007–2013'; IYC – Project No. BGLD-2.001-0002 'Establishment of the Burgas International Youth Centre' (2020–2024).

(0–10cm and 10–30 cm) during three seasons: spring, summer, and autumn of 2025. The following methods were applied:

- Soil pH, soil temperature, and soil moisture were measured using a combined probe, model PH328, SMART SENSOR. Soil moisture was also determined

under laboratory conditions using a DBS 60-3 moisture analyser for recalculating the microbiological results on an oven-dry soil weight basis.

- The serial dilution method followed by surface plating on solid nutrient media was used to determine the main groups of soil microorganisms: meat-peptone agar for non-spore-forming bacteria (NSB) and bacilli; Czapek-Dox agar for filamentous fungi (FF); and actinomycetes isolation agar for actinomycetes and bacteria utilising mineral nitrogen (Bacteria-N). Plates were inoculated with 0.05 mL from a 1:1000 soil dilution for the 0–10 cm soil layer and from a 1:100 soil dilution for the 10–30 cm soil layer. Cultivation was performed at 27 °C for 48 h for bacteria and for 7–10 days for the mycelial groups, namely actinomycetes and filamentous fungi (Gushterov et al. 1977). The total microbial count (TMC) was calculated as the sum of non-spore-forming bacteria, bacilli, actinomycetes, and filamentous fungi. The mineralisation coefficient (MC) was calculated as the ratio of bacteria utilising mineral nitrogen (Bacteria-N) to the sum of non-spore-forming bacteria (NSB) and bacilli (Malcheva and Naskova 2018). Each microbiological determination was based on triplicate surface plating for each microbial group. The results were expressed as mean values. Sampling for microbiological analyses was carried out aseptically using sterile instruments and sterile containers.

- Catalase activity was determined by the manganometric titration method of Khaziev (1976). Briefly, 2 g of air-dried soil were placed in conical flasks, and 5 mL of 1% H_2O_2 were added to the experimental samples, while 5 mL of 10% H_2SO_4 were added to the controls. After shaking for 15 min on a shuttle shaker, the reagents were exchanged: 5 mL of 10% H_2SO_4 were add-

ed to the experimental samples, and 5 mL of 1% H_2O_2 were added to the controls. Shaking was then continued for another 15 min. The resulting suspensions were filtered, and the residual hydrogen peroxide was titrated with 0.1 N KMnO_4 until a persistent pale pink colour appeared.

- Cellulase activity was determined by the dynamic filter-paper degradation method of Khaziev (1976). Sterile filter paper strips were placed in direct contact with the surface of the soil samples in Petri dishes. Soil moisture was maintained throughout the incubation period by periodic addition of sterile distilled water. Every 15 days, the degree of cellulose degradation was assessed as the percentage of degraded filter paper area using a grid standard. For each soil sample, three filter-paper strips (1 × 5 cm) were used, and cellulase activity was expressed as the mean value of the degradation of the three strips.

- Humus content was determined by the Turin method (Penkov et al. 1991), based on the complete oxidation of soil organic matter with potassium dichromate in an acidic medium (sulfuric acid), followed by titration of the residual potassium dichromate.

Statistical analyses were performed using jamovi (version 2.6.44). Descriptive statistics were calculated for all measured variables. For microbiological parameters and cellulase activity, mean values and coefficients of variation (CV) were calculated from the triplicate determinations. The normality of data distribution was assessed using the Shapiro–Wilk test, supported by histograms and Q–Q plots. As most variables did not meet the assumptions of normality, relationships among soil physicochemical properties, microbial groups, enzymatic activities, total microbial count (TMC), and the mineralisation coefficient

(MC) were evaluated using Spearman's rank correlation coefficient (ρ). The effects of season and soil depth on the analysed microbiological and enzymatic parameters were evaluated using two-way analysis of variance (ANOVA), with season and soil depth as fixed factors. The assumption of homogeneity of variances was evaluated using Levene's test. Statistical significance was accepted at $p < 0.05$.

Results and Discussion

Research results

Soil sampling was carried out at two depths (0–10 cm and 10–30 cm) to char-

acterise the vertical distribution of micro-organisms in the investigated urbogenic soils. The study included eleven urbogenic soils located beneath different introduced ornamental tree and shrub species in urban and suburban areas of Burgas and Sozopol. The sampling sites differed in location, intensity of anthropogenic influence, degree of urbanisation, age of the plantings, and characteristics of the vegetation cover. Some sites were situated in parks and schoolyards with relatively low anthropogenic pressure, whereas others were located adjacent to busy roads, roundabouts, and highly urbanised areas. These differences created diverse soil conditions that favoured the development of distinct soil microbial communities (Table 2).

Table 2. Abundance and composition of soil microorganisms, $\times 10^3$ CFU·g⁻¹ dry soil.

Sampling site	Depth, cm	TMC	NSB	Bacilli	Actino-mycetes	FF	Bacteria-N
Spring							
SS1	0–10	2224	1424	672	112	16	2400
	10–30	416.5	183.6	188.7	25.5	18.7	715.7
SS2	0–10	3728.8	1975	1580	126.4	47.4	2354.2
	10–30	771.04	549.84	180.12	20.54	20.54	630.42
SS3	0–10	2635	1649	442	425	119	6766
	10–30	788.8	596.7	86.7	93.5	11.9	676.6
SS4	0–10	6350.4	5040	1041.6	184.8	84	1780.8
	10–30	790.16	605.9	134.46	33.2	16.6	597.6
SS5	0–10	2997	2089.8	777.6	64.8	64.8	4860
	10–30	852.8	632	158.4	35.2	27.2	400
SS6	0–10	2181.2	1164.4	623.2	377.2	16.4	2246.8
	10–30	358.4	284.8	49.6	22.4	1.6	524.8
SS7	0–10	1968	1104	496	272	96	2656
	10–30	468	316.68	31.2	106.08	14.04	310.44
SS8	0–10	2780.8	1943.4	458.2	221.2	158	1580
	10–30	304.94	219.62	47.4	25.28	12.64	150.1
SS9	0–10	5848	4896	306	578	68	3417
	10–30	510.72	431.76	45.36	25.2	8.4	339.36
SS10	0–10	2125	1530	340	187	68	3400
	10–30	251.6	161.5	44.2	39.1	6.8	664.7
SS11	0–10	5743.6	4448.8	381.8	846.6	66.4	1411
	10–30	426.06	324	25.92	64.8	11.34	369.36

Sampling site	Depth, cm	TMC	NSB	Bacilli	Actino-mycetes	FF	Bacteria-N
Summer							
SS1	0–10	2108.1	1737.76	284.88	56.98	28.49	1780.49
	10–30	290.94	213.9	55.62	17.11	4.28	256.71
SS2	0–10	3704.11	3001.91	280.88	315.99	105.33	3282.79
	10–30	355.6	280	36.4	33.6	5.6	298.2
SS3	0–10	2548	2080	273	156	39	2600
	10–30	644.3	557.6	57.8	15.3	13.6	586.5
SS4	0–10	6115.2	5275.2	453.6	285.6	100.8	5779.2
	10–30	730.4	664	36.52	26.56	3.32	630.8
SS5	0–10	2870	2254	266	322	28	2814
	10–30	669.2	557.2	36.4	70	5.6	420
SS6	0–10	3038	2254	504	140	140	2660
	10–30	350	271.6	44.8	14	19.6	280
SS7	0–10	2632	1610	420	280	322	3010
	10–30	333.2	254.8	30.8	44.8	2.8	281.4
SS8	0–10	2729.3	2189.28	233.52	218.93	87.57	2977.42
	10–30	301.78	213.3	37.92	25.28	25.28	282.82
SS9	0–10	5610	5134	374	51	51	6817
	10–30	460.6	392	42	9.8	16.8	476
SS10	0–10	2093	1599	390	91	13	2197
	10–30	244.4	213.2	22.1	7.8	1.3	302.9
SS11	0–10	4909.6	4066.4	612	176.8	54.4	4052.8
	10–30	421.6	310.08	59.84	50.32	1.36	380.8
Autumn							
SS1	0–10	2320	1456	704	128	32	2416
	10–30	406.3	185.3	178.5	23.8	18.7	720.8
SS2	0–10	3760.4	2006.6	1564.2	142.2	47.4	2385.8
	10–30	772.62	553	178.54	22.12	18.96	630.42
SS3	0–10	2771	1700	476	459	136	6766
	10–30	785.4	596.7	85	90.1	13.6	681.7
SS4	0–10	6384	5023.2	1058.4	201.6	100.8	1831.2
	10–30	790.16	604.24	137.78	33.2	14.94	595.94
SS5	0–10	3045.6	2122.2	761.4	97.2	64.8	4876.2
	10–30	851.2	632	156.8	36.8	25.6	408
SS6	0–10	2279.6	1213.6	623.2	410	32.8	2279.6
	10–30	366.4	284.8	52.8	24	4.8	526.4
SS7	0–10	2048	1120	528	288	112	2704
	10–30	468	315.12	34.32	104.52	14.04	312
SS8	0–10	2844	1975	474	237	158	1627.4
	10–30	311.26	221.2	50.56	25.28	14.22	153.26
SS9	0–10	5865	4879	340	561	85	3451
	10–30	517.44	433.44	47.04	26.88	10.08	342.72

Sampling site	Depth, cm	TMC	NSB	Bacilli	Actinomycetes	FF	Bacteria-N
SS10	0–10	2227	1564	374	204	85	3417
	10–30	258.4	163.2	45.9	40.8	8.5	663
SS11	0–10	5743.6	4448.8	398.4	830	66.4	1510.6
	10–30	430.92	327.24	27.54	63.18	12.96	372.6

Note: TMC is total microbial count; NSB is non-spore-forming bacteria; FF is filamentous fungi; CV ≤ 10 % for all microbiological parameters, indicating low variability.

At all investigated sites, the total microbial count was higher in the surface soil layer (0–10 cm); however, the magnitude of the differences between the two soil layers varied considerably. The strongest vertical stratification was observed at SS2 (*Photinia* $\times$ *fraseri* 'Red Robin'), SS4 (*Magnolia liliiflora* 'Nigra'), SS9 (*Prunus serrulata* 'Kanzan'), and SS11 (*Trachycarpus fortunei*), where the total microbial count in the 0–10 cm layer was several-fold higher than in the 10–30 cm layer throughout the study period. The vertical differences were most evident for non-spore-forming bacteria and bacteria utilising mineral nitrogen. In contrast, at SS3 (*Pinus pinea*), SS5 (*Ligustrum lucidum*), SS6 (*Nerium oleander*), and SS7 (*Trachycarpus fortunei*), relatively high microbial abundance was maintained in the 10–30 cm soil layer, indicating that active microbiological processes also occurred in the deeper soil horizon. This is likely associated with root system development, the penetration of root exudates, and the more stable temperature and moisture regime at greater soil depth. Actinomycetes and filamentous fungi exhibited less pronounced vertical differentiation, reflecting their greater adaptation to the environmental conditions prevailing in deeper soil horizons.

The seasonal dynamics of the total microbial count were not uniform across all sampling sites. At most sites, the highest values were recorded in autumn, whereas

at SS6 (*Nerium oleander*) and SS7 (*Trachycarpus fortunei*), the maximum total microbial count in the surface soil layer (0–10 cm) was observed in summer. This suggests that microbial development is influenced not only by seasonal temperature changes but also by the combined effects of soil moisture, vegetation cover, and local habitat conditions.

Although seasonal changes are considered one of the main drivers of soil microbial dynamics, in the present study season did not significantly affect the total microbial count and enzymatic activity. These findings suggest that, under coastal urban conditions, stable site-specific factors may have a stronger influence than short-term seasonal fluctuations. Seasonal variations in microbial activity have been reported for anthropogenic soils of Sofia Municipality, with higher values during spring and autumn and lower activity during summer and winter periods (Malcheva 2020a). Other studies have also demonstrated that seasonal patterns of microbial communities and their activity vary depending on vegetation type, organic matter availability, soil properties, and environmental conditions (Díaz-Raviña et al. 1995, Zhu and Carreiro 2004, Schneckner et al. 2023). These differences confirm that seasonal effects are strongly dependent on vegetation type, soil properties, moisture regime, regional climatic conditions, and local environmental characteristics. The relatively mild

coastal climate of the Southern Black Sea region, characterised by reduced temperature amplitudes, higher air humidity, and prolonged favourable conditions for biological activity, may also contribute to reducing sharp seasonal fluctuations in microbial processes.

Among the investigated sites, SS4 (*Magnolia liliiflora* 'Nigra') consistently exhibited one of the highest total microbial counts, whereas the lowest values were found at SS10 (*Nerium oleander*). A comparison between the two *Trachycarpus fortunei* sites (SS7 and SS11) revealed substantial differences in microbial abundance despite representing the same ornamental species. Although both sites were associated with the same ornamental species, SS11 consistently exhibited considerably higher microbial abundance than SS7. This difference is probably related to more favourable local habitat conditions, including older established vegetation, additional plant cover, greater accumulation of organic residues and humus, whereas the roadside location of SS7 may contribute to stronger anthropogenic influence. Similar differences were observed between the two *Nerium oleander* sites (SS6 and SS10), probably reflecting differences in planting age, location, vegetation cover and local soil conditions. These findings indicate that plant species contribute to the development of specific rhizosphere environments; however, their effects are strongly modified by habitat characteristics, humus accumulation and anthropogenic influence. Urban soils are highly heterogeneous ecosystems affected by soil disturbance, compaction, vegetation management and organic matter input (Zhu and Carreiro 2004; Pouyat et al. 2010).

The structure of the soil microbial communities was clearly dominated by

bacteria. On average, non-spore-forming bacteria accounted for 72.8 % of the total microbial count, followed by bacilli (16.2 %), filamentous fungi (8.4 %), and actinomycetes (2.6 %). Thus, bacteria (non-spore-forming bacteria and bacilli) represented nearly 89 % of the total microbial count, confirming their dominant role in microbial processes in urban soils, in agreement with previous reports for mineral soils (Fierer et al. 2007; Paul 2015; Fierer 2017; Malcheva et al. 2018, 2019; Malcheva 2020a,b, 2025; Naskova et al. 2016; Plamenov et al. 2016; Yankova et al. 2016).

The relative abundance of the individual microbial groups varied among the sampling sites, reflecting the high spatial heterogeneity of urban soils. Non-spore-forming bacteria exhibited the greatest variability, probably due to their sensitivity to readily available organic substrates, soil moisture, and local environmental conditions. In contrast, the relative abundance of bacilli increased at some sites, which may be attributed to their ability to form endospores and withstand adverse conditions, including periodic drought and anthropogenic disturbance (Nicholson et al. 2000, Setlow 2014).

Filamentous fungi were more abundant than actinomycetes, probably owing to the favourable temperature and moisture regime of the coastal urban soils and the continuous input of leaf litter and other plant residues. Although less abundant than bacteria, these filamentous microorganisms play an important role in the decomposition of cellulose and other recalcitrant organic compounds. Overall, the structure of the soil microbial communities was shaped by the combined effects of plant species, soil properties, the temperature–moisture regime, and anthropogenic influence.

The relative abundance of the individual microbial groups exhibited clear seasonal dynamics. During summer, the proportion of non-spore-forming bacteria increased to $80.0 \pm 7.0\%$, whereas the relative abundance of bacilli and filamentous fungi decreased to $11.0 \pm 4.0\%$ and $6.4 \pm 3.6\%$, respectively. This probably reflects more favourable conditions for the development of non-spore-forming bacteria during the summer period. In contrast, the relative abundance of actinomycetes remained relatively stable ($2.5\text{--}2.8\%$), indicating lower sensitivity to seasonal environmental changes.

The mineralisation coefficient exhibited considerable spatial variability among the investigated sites, reflecting differences in the functional organisation of the soil microbial communities. Its mean value for all samples was 1.15, with similar values recorded in spring (1.204 ± 0.812) and autumn (1.195 ± 0.787), whereas a slight decrease was observed in summer (1.039 ± 0.169). Only minor differences were found

between the two soil layers (1.168 ± 0.680 at 0–10 cm and 1.124 ± 0.638 at 10–30 cm), indicating that mineralisation processes were active in both soil horizons (Fig. 2).

The highest mean mineralisation coefficients were recorded at SS10 (*Nerium oleander*) and SS3 (*Pinus pinea*), whereas the lowest values were found at SS4 (*Magnolia liliiflora* 'Nigra'), SS11 (*T. fortunei*), and SS8 (*Musa paradisiaca*). A comparison between the total microbial count and the mineralisation coefficient revealed that high microbial abundance was not necessarily associated with greater mineralisation activity. For example, SS4 exhibited one of the highest total microbial counts but the lowest mineralisation coefficient, whereas the opposite trend was observed at SS10. Furthermore, the marked differences between the two *T. fortunei* sites, as well as between the two *Nerium oleander* sites, indicate that mineralisation processes are influenced not only by plant processes but also by differ-

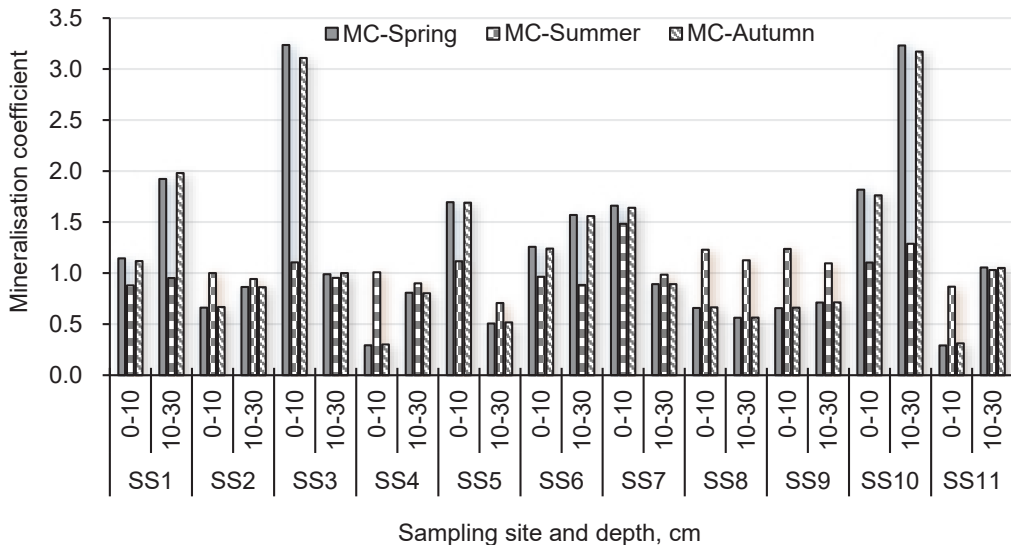


Fig. 2. Mineralisation coefficient.

ences in microbial community structure, organic matter availability, and site-specific soil conditions. These findings indicate that the mineralisation coefficient complements total microbial count and enzyme activity, providing additional information on the functional organisation of soil microbial communities.

The absence of a direct relationship between total microbial abundance and mineralisation intensity confirms that microbial quantity does not always correspond to microbial activity. Similar conclusions have been reported for soil biological indicators, where microbial abundance, enzyme activities, and functional processes may respond differently de-

pending on microbial composition and environmental factors (Nielsen and Winding 2002, Schnecker et al. 2023). Therefore, the combination of microbial abundance, mineralisation coefficient, and enzyme activities provides a more complete assessment of soil biological status.

All investigated urbogenic soils were characterised by a moderate humus content (2–4 %) and a soil reaction ranging from slightly acidic to slightly alkaline. Approximately 87 % of all analysed samples were slightly acidic to neutral, around 10 % were alkaline, and only 3 % were strongly acidic, indicating generally favourable conditions for the development of diverse soil microbial communities (Table 3).

In the surface soil layer, soil reaction may be partially buffered by external factors such as dust deposition, irrigation water, and the more intensive decomposition of organic matter, which releases calcium and magnesium (Rao et al. 2002). Therefore, the observed decrease in pH with depth is likely the result of the combined effects of vegetation cover, rhizosphere processes, water regime, and local soil conditions.

Humus content was consistently higher in the surface soil layer (0–10 cm) and decreased with soil depth at all sampling sites, reflecting the accumulation of leaf litter and other plant residues in the upper horizon. Similarly, pH tended to decrease slightly with increasing depth, probably due to reduced buffering capacity and the leaching of base cations. At some sites, these vertical dif-

Table 3. Soil pH and humus content of the investigated urbogenic soils.

Sampling site	Depth, cm	Spring	Summer	Autumn	Humus, %
SS1	0–10	5.70	6.40	7.00	3.54
	10–30	5.10	6.55	8.10	2.18
SS2	0–10	5.64	7.80	6.46	3.60
	10–30	6.02	6.94	6.25	2.30
SS3	0–10	5.92	7.18	6.18	3.49
	10–30	6.59	6.90	6.58	2.33
SS4	0–10	5.44	7.24	6.11	3.88
	10–30	5.55	6.66	5.87	2.45
SS5	0–10	6.54	6.77	6.93	3.55
	10–30	5.87	5.90	6.36	2.54
SS6	0–10	6.64	7.61	5.95	3.43
	10–30	6.25	6.39	7.25	2.15
SS7	0–10	7.90	7.90	7.24	3.34
	10–30	6.45	6.45	6.30	2.22
SS8	0–10	8.46	8.46	6.21	3.54
	10–30	5.70	5.70	5.65	2.05
SS9	0–10	8.11	8.11	7.44	3.67
	10–30	6.30	6.30	5.91	2.26
SS10	0–10	7.00	7.00	7.40	3.38
	10–30	6.64	6.64	7.60	2.00
SS11	0–10	6.15	6.15	7.40	3.62
	10–30	5.80	5.80	6.72	2.20

ferences were less pronounced, likely because of the well-developed root systems of the ornamental trees and shrubs and the downward transport of organic matter.

Organic matter and soil reaction are among the main factors regulating the structure and activity of soil microbial communities (Nannipieri et al. 2012, Paul 2015, Fierer 2017). The present results indicate that neither higher humus content nor soil pH alone determines microbial abundance or activity. Instead, their effects are expressed in combination with soil temperature, moisture, vegetation cover, and local habitat conditions, emphasising the complex interactions controlling the biological status of urban soils.

Among the studied soil parameters, humus content showed the strongest relationship with microbial abundance and enzyme activities, confirming the central role of soil organic matter as a source of carbon, nutrients, and energy for microorganisms. Organic matter accumulation

stimulates the development of microbial communities and provides substrates for extracellular enzyme production. Similar relationships between organic matter availability, microbial parameters, and enzyme activities have been reported, highlighting the importance of organic substrates in maintaining soil biological functioning (Bastida et al. 2008). The strong influence of humus in the present study indicates that organic matter availability is a more stable regulator of microbial communities in urban soils than temporary variations in temperature and moisture.

Soil temperature and moisture are among the main factors determining the development, structure, and functional activity of soil microbial communities. The results demonstrated clear seasonal dynamics for both parameters (Fig. 3).

The mean temperature in the 0–10 cm layer was approximately 23 °C in spring, 28 °C in summer, and 21.5 °C in autumn, whereas in the 10–30 cm layer it was

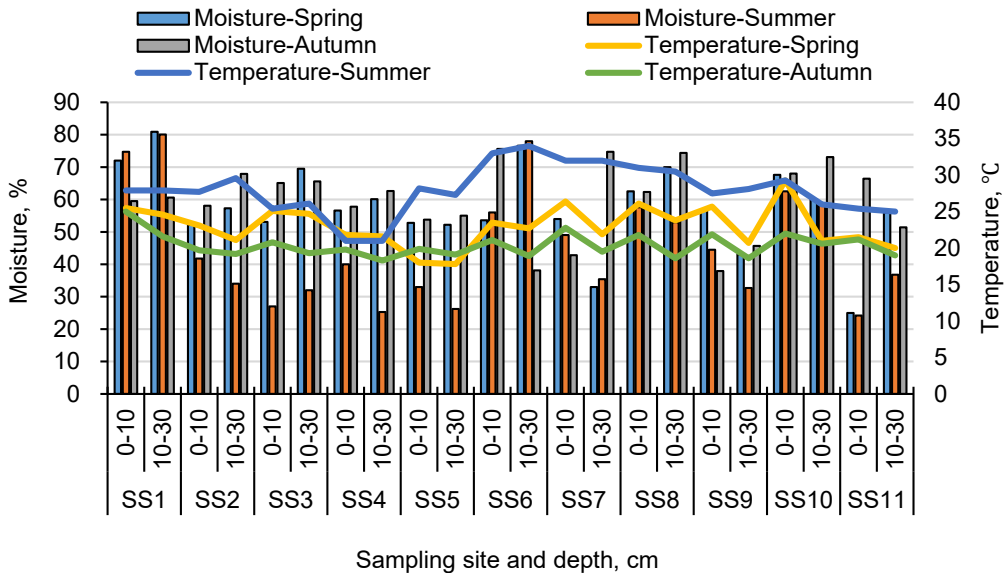


Fig. 3. Seasonal dynamics of soil temperature and moisture.

about 20 °C, 27 °C, and 19 °C, respectively. Soil temperature decreased with increasing depth, with the most pronounced seasonal fluctuations observed in the surface soil layer.

Mean soil moisture was approximately 56 % in spring, 45 % in summer, and 60 % in autumn, with higher values generally recorded in the 10–30 cm layer due to lower evaporation and greater water retention capacity. At some sampling sites, moisture remained relatively high even during summer, probably due to shading by tree vegetation and specific microclimatic conditions. At these sites, no substantial decrease in the total microbial count was observed, indicating that local conditions may mitigate the influence of seasonal climatic factors. These findings are consistent with previous studies showing that soil temperature and moisture are among the key factors controlling the seasonal

dynamics, structure, and activity of soil microbial communities (Nannipieri et al. 2012, Fierer 2017, Frindte et al. 2019).

Enzyme activity exhibited pronounced spatial variability but comparatively weaker seasonal variation. Cellulase activity was higher in the surface soil layer (0–10 cm) at most sampling sites, which can be attributed to the accumulation of leaf litter and other plant residues (Fig. 4). The values presented correspond to the final assessment (90th day) and are shown by season.

The highest cellulase activity was recorded at SS4 (*Magnolia liliiflora* 'Nigra'), followed by SS9 (*Prunus serrulata* 'Kanzan') and SS11 (*Trachycarpus fortunei*), whereas the lowest activity was observed at SS10 (*Nerium oleander*). A slight increase in cellulase activity was observed during autumn at most sampling sites, probably due to the input of fresh plant

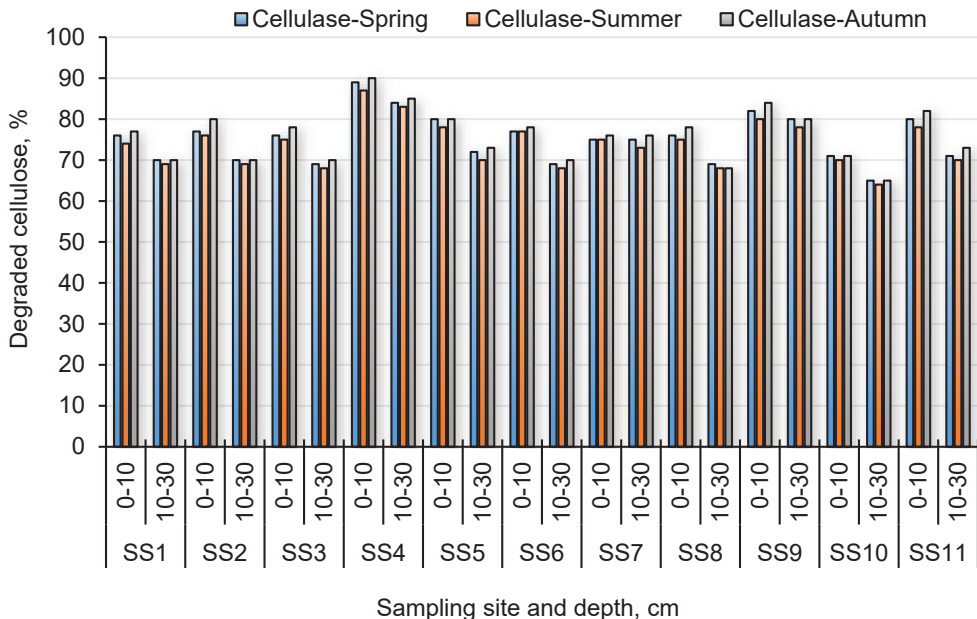


Fig. 4. Cellulase activity of soil microorganisms.

Note: CV ≤10 % for cellulase activity in all samples, indicating low variability.

residues and the increased availability of cellulose as a substrate for cellulolytic microorganisms. Cellulase activity was assessed using a method that monitors cellulose degradation over time. During the 15-day monitoring intervals, the most intensive cellulose degradation was also observed at site SS4.

Catalase activity followed a similar spatial pattern. The highest values were again recorded at SS4, whereas the lowest were observed at SS10, indicating that habitat conditions have a substantial influence on the intensity of aerobic microbial processes (Fig. 5).

Higher catalase activity generally coincided with sites characterised by greater total microbial abundance and more favourable conditions for microbial development. However, this relationship was not absolute, suggesting that catalase activity reflects not only microbial abundance but also the physiological status of the

microbial community and the intensity of aerobic metabolic processes. The higher enzyme activities in the surface soil layer can be explained by the greater accumulation of organic residues, root-derived substrates, and more intensive microbial activity. These findings confirm that cellulase and catalase are sensitive bioindicators of the functional status of urban soils. Cellulase activity reflects the intensity of decomposition of cellulose-containing plant residues and carbon cycling, whereas catalase activity is associated with overall microbial activity and oxidative metabolism (Dick 1994, Bastida et al. 2008, Nannipieri et al. 2012, Paul 2015, Torres et al. 2016, Malcheva 2020a, Malcheva and Koleva 2025).

Statistical analyses

Two-way analysis of variance (ANOVA) showed that soil depth had a statistical-

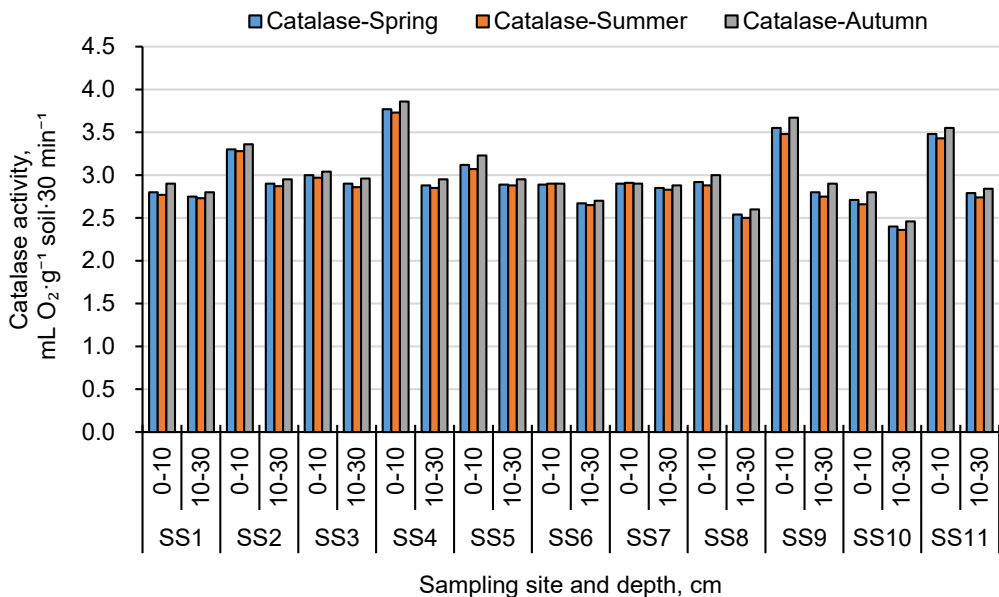


Fig. 5. Catalase activity of soil microorganisms.

ly significant effect on the total microbial count, cellulase activity, and catalase activity ($p < 0.001$), whereas season had no significant effect on any of the analysed parameters ($p > 0.05$). No significant interaction between season and soil depth was detected. These results indicate that the vertical distribution of microbial communities and enzyme activities is a more stable characteristic of urban soils than seasonal variation, highlighting the dominant role of local soil conditions (Table 4).

Spearman correlation analysis showed that humus content was the soil property most strongly associated with the microbiological parameters. Very strong positive correlations were found between humus content and total microbial count ($\rho = 0.978$), non-spore-forming bacteria ($\rho = 0.973$), bacilli ($\rho = 0.799$), filamen-

tous fungi ($\rho = 0.756$), actinomycetes ($\rho = 0.739$), bacteria utilising mineral nitrogen ($\rho = 0.789$), as well as cellulase ($\rho = 0.756$) and catalase activity ($\rho = 0.850$) ($p < 0.001$), confirming the key role of organic matter in the development and functional activity of soil microbial communities.

In contrast, soil temperature and moisture showed weak and generally non-significant correlations with most microbiological parameters. Soil moisture was weakly negatively correlated with non-spore-forming bacteria, cellulase activity, and catalase activity, whereas soil temperature was not significantly correlated with any of the analysed microbiological and enzymatic parameters.

The Spearman correlation matrix (Table 5) showed that soil pH exhibited moderate positive correlations with several

Table 4. Results of the two-way ANOVA.

Effect	Sum of squares	df	Mean square	F	p	Partial η^2
TMC						
Season	1.02e+11	2	5.10e+10	0.04033	0.961	0.001
Depth	1.50e+14	1	1.50e+14	118.61824	<.001	0.664
Season $\times$ Soil depth	2.05e+10	2	1.03e+10	0.00812	0.992	0
Residuals	7.59e+13	60	1.27e+12			
MC						
Season	0.3771	2	0.1886	0.4126	0.664	0.014
Depth	0.0318	1	0.0318	0.0695	0.793	0.001
Season $\times$ Soil depth	0.0295	2	0.0148	0.0323	0.968	0.001
Residuals	27.4174	60	0.457			
Cellulase						
Season	54.94	2	27.47	1.0675	0.35	0.034
Depth	630.55	1	630.55	24.5031	<.001	0.29
Season $\times$ Soil depth	2.45	2	1.23	0.0477	0.953	0.002
Residuals	1544	60	25.73			
Catalase						
Season	0.104	2	0.0519	0.70032	0.5	0.023
Depth	2.311	1	2.3109	31.2041	<.001	0.342
Season $\times$ Soil depth	7.30e-4	2	3.65e-4	0.00493	0.995	0
Residuals	4.444	60	0.0741			

Note: TMC is total microbial count; MC is mineralisation coefficient; df is degrees of freedom; F is the ANOVA F statistic; p is the probability value; Partial η^2 is partial eta squared (effect size).

Table 5. Spearman correlation matrix.

Indicators	TMC	NSB	Bacilli	FF	Actino- mycetes	Bacte- ria-N	MC	Mois- ture	Tepera- ture	pH	Humus	Celulase
TMC	-											
NSB	0.982	-										
Bacilli	0.822	0.760	-									
FF	0.779	0.752	0.646	-								
Actinomycetes	0.801	0.767	0.758	0.716	-							
Bacteria-N	0.813	0.799	0.795	0.723	0.759	-						
MC	-0.224	-0.222	0.025	0.008	-0.020	0.334	-					
Moisture	-0.207	-0.253	0.069	-0.138	-0.027	-0.109	0.105	-				
Temperature	0.007	0.076	0.007	0.098	0.130	0.126	0.187	-0.107	-			
pH	0.225	0.278	0.126	0.297	0.284	0.426	0.319	-0.143	0.296	-		
Humus	0.978	0.973	0.799	0.756	0.739	0.789	-0.237	-0.238	0.076	0.221	-	
Celulase	0.769	0.747	0.547	0.544	0.515	0.520	-0.298	-0.290	-0.224	0.019	0.756	-
Catalase	0.876	0.833	0.648	0.650	0.662	0.626	-0.340	-0.306	-0.186	0.129	0.850	0.752

Note: TMC is total microbial count; NSB is non-spore-forming bacteria; FF is filamentous fungi; Bacteria-N is bacteria utilising mineral nitrogen; MC is mineralisation coefficient.

microbial groups and the mineralisation coefficient, with the strongest relationship observed for bacteria utilising mineral nitrogen ($p = 0.426$). The mineralisation coefficient was negatively correlated with cellulase and catalase activities but showed no significant relationship with the total microbial count, indicating that it reflects a different aspect of the functional status of soil microbial communities.

The findings of the present study confirm that urban soils represent complex and heterogeneous ecosystems, where microbial communities are controlled by interactions between vegetation, soil properties, and anthropogenic pressure. Consistent with previous studies, biological indicators respond sensitively to changes in soil conditions and provide valuable information about soil functioning (Dick 1994, Nielsen and Winding 2002, Bastida et al. 2008). These findings demonstrate that the combined application of microbiological, enzymatic, and physicochemical indicators can serve as a reliable approach for assessing the ecological quality and functional status of urban green areas.

Conclusions

The present study represents one of the first comprehensive investigations of soil microbial communities, enzyme activities, and physicochemical properties of urbogenic soils beneath introduced ornamental tree and shrub species along the Southern Black Sea coast of Bulgaria. The results demonstrated that spatial heterogeneity, soil depth, and local soil conditions exerted a stronger influence on microbial communities and enzyme activities than seasonal variation, while season had no statistically significant effect on the analysed biological parameters. Humus content emerged as the principal factor governing both microbial abundance and the functional activity of these soils, whereas cellulase activity, catalase activity, and the mineralisation coefficient proved to be reliable bioindicators of soil biological status. The integrated application of microbiological, enzymatic and physicochemical indicators represents a reliable approach for monitoring the biological quality of urbogenic soils and supports the sustainable management of urban green infrastructure.

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