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Influence of phytoremediation on soil microbial biomass and activity in degraded soils

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Abstract. Phytoremediation of roadside soils modifies the landscape and could be used for extraction of potentially toxic elements from the soil, but little is known about how it affects the physiological activity of the soil microbial communities. The present study aimed to evaluate the reliability of three perennial species i.e. *Lolium perenne* (L.), *Agropyron cristatum* (L.), and *Festuca arundinacea* (Schreb), and one leguminous grass species (*Lotus corniculatus* L.) for sustainable phytoremediation of roadside soils by assessing their impact on the microbiological indicators for soil fertility, including microbial biomass soil basal respiration and nitrification potential of the communities. The experimental design tested the individual effects of the species as well as their impact when sown in polyculture (1:1:1:1). The results showed that the formed vegetation cover did not significantly affect the inherent chemical properties of the soils. There was no significant correlation between organic matter, total nitrogen, and phosphorus concentrations with microbial biomass, dehydrogenase activity and soil basal respiration instead only with a C: N ratio suggesting no C limitation in the studied soils. Data show that, when grown in monoculture the studied plant species does not have the desired positive effect on microbiological indicators of soil health. Only the polyculture (1:1:1:1) leads to a significant positive effect on the microbial physiological activity, making it a suitable candidate for future research on its applicability for sustainable phytoremediation.

Key words: phytoremediation, microbial biomass, dehydrogenase activity, soil basal respiration

Introduction

According to the EU Roadmap for Resource Efficiency in Europe (Ferreira et al., 2022), soil is a finite and non-renewable resource responsible for multiple ecosystem services and should be considered natural capital. Large urban centers are continuously developing and expanding territorially, often at the expense of natural green spaces (Renella, 2020). Their importance is generally overlooked, despite their involvement in urban pollutant capture and removal processes, while providing a range of ancillary benefits, e.g., ecosystem processes mediated by microorganisms that improve air and water quality, in addition to associations with plant and tree rhizospheres (Joyner et al., 2019). Currently, 55% of the global population is living in urban areas, and Global estimates suggest that by 2050, over 68% of the population will inhabit large cities (Chen et al., 2021). Urbanization leads to signifycant pressures on urban soils through the input of various pollutants, including heavy metals (HM) (Chen et al., 2005, 2015) and petroleum hydrocarbons (Liu et al., 2019; Wang et al., 2015).

Urban soil contains microorganisms associated with key functions for urban biota, and disturbances in this complex and dynamic biogeochemical structure have direct implications for human health. The effects of various contaminants on microbial communities have been extensively studied in soils of agricultural lands (Ren et al., 2022), urban parks (Zhang et al., 2021), open areas (Singh et al., 2019) and even mining sites (Kavehei et al., 2022), while little is known about the response of microbial communities associated with contaminants in roadside soils. They act as sinks for many pollutants including heavy metals, hydrocarbons, etc. due to motor vehicles (Joshi et al., 2010). It is the pollution of roadside soils that is one of the most significant unaddressed harms of urbanization (Kumar & Fulekar, 2021).

The diversity and activity of soil bacterial communities are diminishing due to environmental stress triggered by heavy metals. Soil contamination has the potential to decrease overall microbial biomass (Romero-Freire et al., 2016) and bring about changes in metabolic capabilities (Abd et al., 2013; Wang et al., 2010). This can impact numerous microbial activities in the soil, disrupting nutrient cycling and the ability to carry out essential ecological functions, such as the breakdown of organic compounds and the production of organic substances (Moreno et al., 2009). Due to their nature, contaminants are metabolized slowly or accumulate at different levels of the food chain (Kumar & Fulekar, 2019; Singh & Hiranmai, 2021). This severely limits roadside soils in terms of their capacity to provide the services that people will need in the coming years and poses a serious problem for the safe use of soils (Igwe et al., 2005; Tanu & Hoque, 2013). This growing threat to ecological resources and public health represents a driving force for communities to adopt sustainable practices that improve and maintain urban environmental health.

Several strategies exist to contain and remove soil contaminants, but they rely primarily on costly physical and chemical remediation methods. These strategies are labor-intensive, alter soil properties and disrupt the natural soil microbiome (Raffa et al., 2021). One solution is to redesign the urban landscape through the use of plants and associated soil microorganisms to enhance 'green' infrastructure (Greipsson, 2011), in a process known as phytoremediation. Unlike conventional methods, phytoremediation is recognized as cost-effective, non-invasive and environmentally friendly (Beans, 2017; Bernardino et al., 2020). Despite numerous breakthroughs, the process of effective phytoremediation is undergoing continuous development (Ashraf et al., 2019) and is difficult to find shield-scale applications (Garbisu et al., 2020). The focus has mainly been on contaminant removal by phytoextraction (Yan et al., 2020; Petrova et al., 2022; Yu et al., 2022). There has been relatively limited information available on the response of soil microorganisms, even though over 90% of the energy flow in the soil ecosystem is mediated by microbes (Nannipieri & Badalucco, 2004), making them probably one of the most suitable groups to study soil degradation (Nannipieri et al., 2003; Romero-Freire et al., 2016). Because of their quick response to environmental changes, high sensitivity, ecological relevance and capacity to provide information that integrates many environmental factors characteristics, it has been proposed that these elements should be included in ecological risk assessments as crucial indicators to monitor toxicity over time (Frey et al., 2006; Gómez-Sagasti et al., 2012; Mijangos et al., 2010).

It has been suggested that the development of the root system of the plants through exudate release and provision of an ecological niche leads to an increase of the soil microbial biomass and microbiological activities, which in turn enhances soil health. To test the hypothesis that microbial activity depends on plant development, a three-year field experiment was conducted within the limits of the city of Plovdiv, Bulgaria, where roadside soils were planted with different perennial grasses. The objective was to evaluate the effect of newly created roadside lawns on the microbiological indicators for soil fertility, including microbial biomass soil basal respiration and nitrification potential of the communities during the process of phytoremediation.

Materials and methods

Study area and soil sampling

Six sites were selected for this study within the limits of the city of Plovdiv, Bulgaria (N 42.1354079, E 24.7452904), which is the second largest city in Bulgaria with a population of nearly 450 000 inhabitants (NSI, 2022). They were located in permanent grass areas near major roads in each of the six administrative areas (one per area – East, North, South, West, Central, Trakiya) according to the General Development Plan (Plovdiv Municipality, 2022). Each site was organized in five experimental adjacent plots measuring 1 × 1 m following road direction. Monocultures of three perennial and one leguminous grass species namely perennial ryegrass (*Lolium perenne* L.), variety IFC Harmoniya; crested wheatgrass (*Agropyron cristatum* L.), variety Svejina; tall fescue (*Festuca arundinacea* Schreb), variety Albena; and bird's foot trefoil (*Lotus corniculatus* L.), variety Leo were established in experimental plots one to four. Additionally, the fifth plot was planted with a combination of the grass species (1:1:1:1) as described by Petrova et al. (2022). The newly formed lawns were maintained by the municipality alongside the adjacent green areas in which they were situated.

The sites were sampled 5 times over the three-year study period between April 2019 and April 2021. First samples were taken before sowing and used for estimation of the base levels of microbial activity at the beginning of the experiment (control), followed by additional sampling every six months (autumn and spring of each year). Samples of the 0-20 cm surface soil layer from a 25 × 25 cm area were collected in sterile containers and transported in the dark under refrigerated conditions. The debris and plant materials were removed before analysis and all soil samples were sieved to 2 mm. Microbial analyses were performed no later than one week after sampling.

Determination of soil physicochemical properties

Soil pH and electrical conductivity were determined in 1:1 (v/w) water: bulk soil suspension using pHotoFlex Set, 2512000, WTW-Germany (ISO 10390) and Multiset, F340, WTW- Germany (ISO 11265). Water content was estimated using the thermogravimetric method (Carter & Gregorich, 2008). Content of C org (by Turin), total N (Kjeldahl Method), P and S (spectrophotometrically) has been analyzed in the Laboratory of the Department of Agroecology and Evironmental Protection and Department of Soil Science and Agrochemistry, Agricultural University - Plovdiv.

Soil microbial biomass

Soil microbial biomass was determined using the chloroform fumigation - K_2SO_4 extraction method (Burton et al., 2010). For each soil sample, three moist subsamples of 25 g (70% WHC) were fumigated with ethanol-free chloroform for 24 h in vacuum desiccators. Another three were not fumigated. Both fumigated and non-fumigated subsamples were suspended in 100 mL of 0.5 M K_2SO_4, shaken at 200 rpm for 30 min at 25 °C, and filtered through folded filter paper. The carbon concentration in the filtrates was determined by the potassium dichromate method (Vance et al., 1987). Soil microbial biomass was calculated as the difference in the concentration of carbon between the fumigated and non-fumigated samples with a conversion factor of 0.38 (Vance et al., 1987).

Basal respiration rate (BAS)

Basal respiration was measured by the static incubation method (Anderson, 1982). In brief, 40 g of soil with a water-holding capacity of 50-70% is enclosed in 1 L air-tight jars along with a vial containing 2 ml 1M NaOH and preincubated for 10 days at 22°C. At the start of BAS measurement, the scintillation vial was replaced with 2 ml 0.1 M NaOH and the vessels were incubated for an additional 24 h. CO_2 released was determined by the back titration of the remaining OH^- against 0.01 M HCl, after $BaCl_2$ carbonate precipitation. Basal respiration rate in units μg CO_2–C/ g DW/ h, was calculated from the formula:

$$\boldsymbol{BAS = \frac{Mc \times V \times 0.01}{Sdw \times t \times 2} \times 10^3}$$

where Mc is the molar weight of carbon; V is the volume of 0.01M HCl used for back titration of remaining OH^-, expressed as the difference between the volume of 0.01M HCl used for blanks and volume of 0.01M HCl used for samples; Sdw is gram dry weight of the sample; t is the incubation time in hours. The qCO_2 is calculated from basal respiration (CO_2-C·h^{-1}) per unit microbial biomass carbon (Insam & Haselwander, 1989).

DHA assay (dehydrogenase activity)

DHA was determined using a 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride (INT) assay (von Mersi & Schinner, 1991). The determinations were carried out in triplicate. For each sample, 1 g soil was mixed with 2.5 ml 0.3% INT and incubated in the dark at 25°C for 1 hour. During the incubation, the tubes were inverted every 15 min to resuspend the solids. After incubation, 10 ml N, N-dimethylformamide–ethanol mixture (1:1) was added followed by additional 1h incubation for the extraction of the generated water-insoluble iodonitrotetrazolium formazan (INTF). The INTF concentration was determined by spectrophotometry at 484 nm. DHA was calculated using the formula:

$$DHA\ (\mu g\ INTF/g/\ h) = \frac{([INTFs] - [INTFc]) \times 12.5}{\text{ECT}}$$

where $INTF_s$ concertation of INTF ion the sample; $INTF_c$ concentration of INTF in the sterile control; ECT – equivalent dry weight for 1 g soil, 12.5 – the final volume of the reaction mixture.

Nitrification potential

The potential nitrification activity (PNA) method is used to determine the maximum nitrifying activity under optimal conditions (Belser, 1979; Hart et al., 1994). Briefly, 10 g fresh soil was added to 100 mL of 1 mM phosphate buffer (pH 8) and 5 mM $(NH_4)_2SO_4$, and the mixture was incubated at 25°C and 200 rpm for 26 h. NO_2^- content was determined with colorimetry of N-(1-Naphthyl) ethylenediamine dihydrochloride at 540 nm. NO_3^- content was determined with colorimetry of sulfanilamide at 430 nm. The final concentrations were calculated as follows:

$$NO_2^-\ or\ NO_3^-\ \ conc\ (\mu g\ N/g\ DW) = NO_2^-\ or\ NO_3^-\ \ conc\ (\mu M) \times 14 \times \frac{\left(\frac{100 + (10 - DW)}{1000}\right)}{DW}$$

where: DW = dry weight of the oven-dry soil (g); 14 = conversion factor (1 M nitrogen = 14 g nitrogen).

Statistical analyses

Analyses of enzyme activities were performed with Primer 6 (E-Primer); Statistica v.10 (StatSoft) and Microsoft Excell 2016 with XLSTAT statistical package (Addinsoft). The normality of the data and the number of linear correlations of the dataset were assessed before statistical analyses were performed. Principal coordinate analysis (PCA) of the normalized data was applied for the characterization of the relation between soil physiological parameters and the type of vegetation used in the experimental plots. Permutational multivariate analysis of variance (perMANOVA) and analysis of similarities (ANOSIM) were used to test the differences between the five experimental design plots as well as between the spatially distant identical plots (Anderson & Walsh, 2013).

Results

Physical and chemical characteristics

The three-year evaluation of the studied parameters showed that planting with perennial grasses did not significantly affect the inherent chemical properties of the soils (Table 1). Analysis of the physico-chemical properties of roadside soils studied found that the soils were generally neutral to slightly acidic ranging from 6.65 to 7.03, while electro conductivity (EC) varied from 143.8 μs/ cm to as high as 188.5 μs/ cm with no notable data dispersion as indicated by standard deviations. The results illustrate that roadside soils in Plovdiv are characterized by low salinity, with no significant differences between districts ($p>0.05$). Soil moisture content was in the range of 62.3% to 66.7%. Data characterizing other attributes of soils, namely organic matter, N, P, and S contents exhibited similar patterns. For example, the organic matter content varied from 2.32% to 3.99% with an overall average of 3.21% mimicking the pattern exhibited by the soil EC. The carbon-to-nitrogen ratio, which is an essential indicator of the decomposition rate of soil organic matter, was low in all substrates.

Table. 1. Physical and **c**hemical characterization of studied soils at 0–20 cm layer.

Parameter	Central C	North (N)	West (W)	South (S)	East (E)	Trakia (T)
OM %	3.135±0.179	3.993±0.781	3.975±0.501	2.321±0.045	3.162±0.147	2.69±0.173
TN %	0.17 ±0.025	0.268±0.039	0.216±0.006	0.143±0.007	0.143±0.009	0.193±0.039
C/N	10.91±1.04	8.64±0.37	10.67±1.05	9.44±0.28	12.86±0.24	8.32±1.14
P %	0.198±0.113	0.236±0.099	0.164±0.131	0.199±0.148	0.237±0.135	0.163±0.107
S %	0.039±0.001	0.057±0.014	0.115±0.017	0.029±0.001	0.112±0.072	0.072±0.049
pH	6.81±0.1	6.65±0.21	6.84±0.08	6.81±0.07	7.03±0.11	6.85±0.05
WC %	61.9±2.4	62.6±4.8	64.68±3.3	62.5±6.2	65.9±4.1	66.3±2.9
EC, μS/ cm	187.4±0.7	167.8±23.4	156.9±4.9	143.8±4.3	188.5±23.6	177.9±8.5

Microbial biomass carbon of the five plots

We found a very low level of variability in the microbial biomass (BMC) values for the 3-year study period across the six urban districts of the city of Plovdiv, taking into account their relatively small spatial dispersion. The control biomass-C (MBC) measurements although subjected to inherently spatial variability of the individual roadside soils, showed no significant differences ($p>0.05$) in the initially supported microbial biomass. The MBC varied between 291.6 µg C/g DW in the soils from the Trakia district to 684 µg C/ g DW in the North district soils. These relative differences between district soils persisted throughout the experiment, mainly due to seasonal rather than spatial variations (Fig. 1a) with an average MBC of 471.7 µg C/g DW.

The MBC in soil plots seeded with perennial grass monocultures experienced no significant changes ($p<0.05$) compared to the control samples (387 µg C/g DW) with levels ranging from 344 µg C/g DW in the perennial ryegrass (*Lolium perenne* L.) plots to 414 µg C/g DW in the tall fescue plots (*Festuca arundinacea* Schreb), while seeding with bird's foot trefoil (*Lotus corniculatus* L.), lead to a significant reduction ($p=0.0488$) to an average of 299 µg C/g DW. Interestingly the seeding of plot 5 with a combination of the 4 grass species (1:1:1:1) resulted in the elevation of MBC content ($p=0.00001$) in the constructed roadside lawns (Fig. 1b).

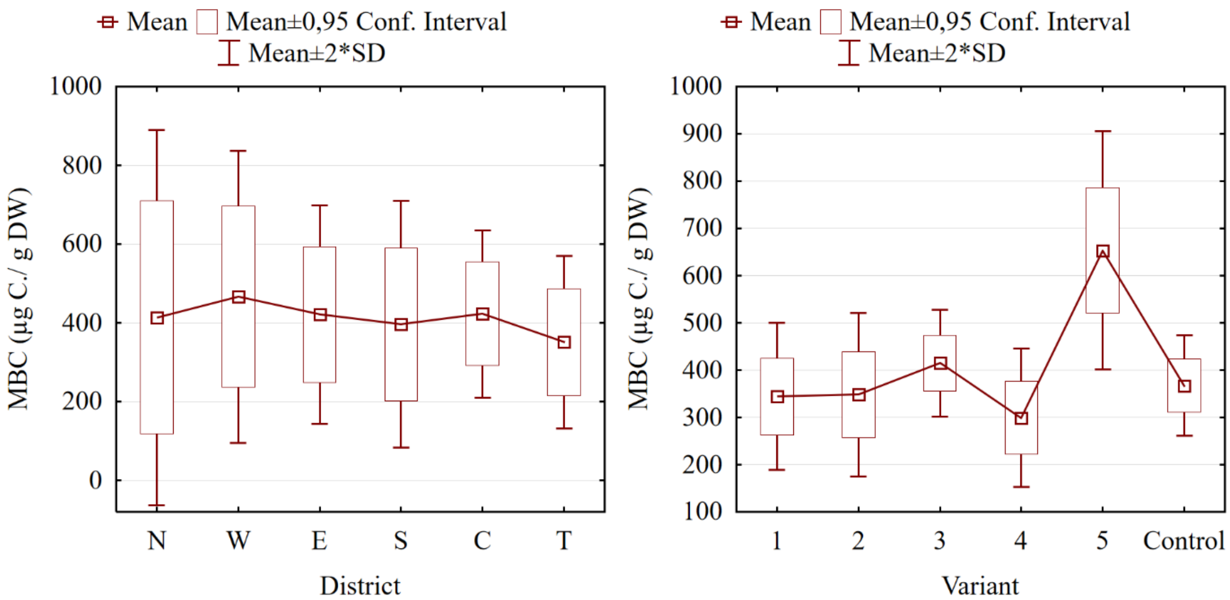

Fig. 1. Average soil microbial biomass carbon concentration in: a) six roadside soil sites selected for this study within the limits of the city of Plovdiv; b) five experimental plots planted with different grass species.

Soil basal respiration, dehydrogenase activity and potential nitrification rates

The basal respiration (BAS) of the roadside soils selected (Fig. 2a) displayed a high degree of similarity, and this pattern was strongly influenced by the vegetation chosen for each individual soil plot (Fig. 2b). The soil plots with mixed grass cultures (1:1:1:1) exhibited the highest basal respiration, reaching up to 41.7 µg CO_2-C/g/day, representing a significant 61.4% increase compared to the control samples. In contrast, the monoculture soil plots showed a substantial decrease in BAS ($p = 0.0002$), ranging from 22.1 to 30.1 µg CO_2-C/g/day. Despite these variations, the metabolic quotient (qCO_2) did not follow the same pattern as basal respiration among the five experimental plots (Fig. 1c), possibly influenced by differences in microbial biomass carbon (MBC). Data analysis grouped the plots into two categories, with mixed vegetation and perennial ryegrass (*Lolium perenne* L.) plots exhibiting lower soil qCO_2 compared to the crested wheatgrass (*Agropyron cristatum* L.), tall fescue (*Festuca arundinacea* Schreb), and bird's foot trefoil (*Lotus corniculatus* L.) monoculture plots.

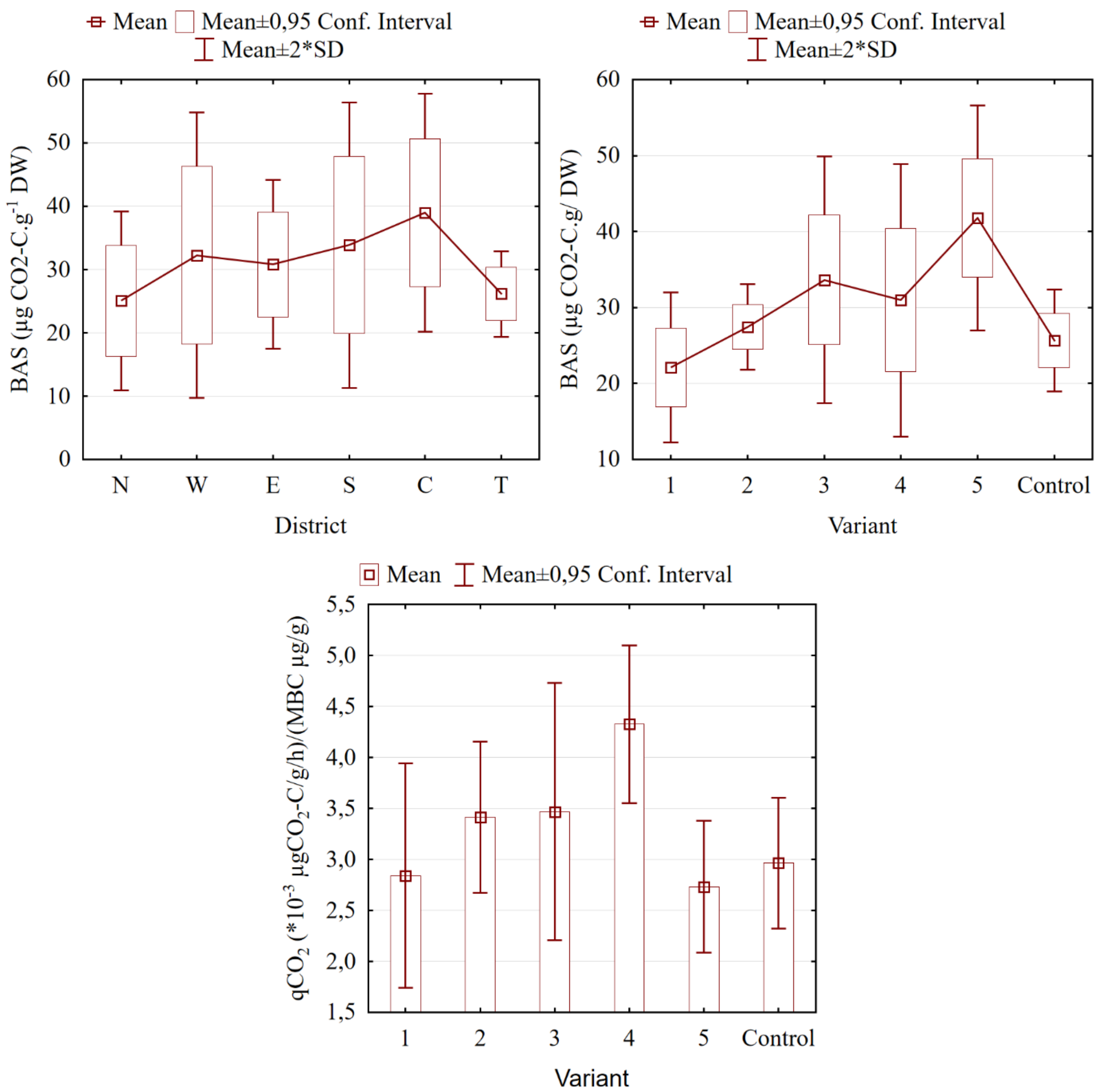

Fig. 2. Average values for soil basal respiration in: a) six roadside soil sites selected for this study within the limits of the city of Plovdiv; b) five experimental plots planted with different grass species; c) metabolic quotient reflecting the relationship between BAS and MBC

There were no significant effects of the district location on the soil dehydrogenase activity (p=0.2113) (Fig. 3a), but DHA showed wide plot-dependent variations, ranging from 3.9 to 5.5 µg INTF/g/h for the monoculture lawns and from 7.66 to 8.31 µg INTF/g/h for the polyculture plot (Fig. 3b). In the polyculture lawns the DHA was 88, 52, 24 and 34% higher than those in perennial ryegrass (*Lolium perenne* L.), crested wheatgrass (*Agropyron cristatum* L.), tall fescue (*Festuca arundinacea* Schreb), and bird's foot trefoil (*Lotus corniculatus* L.), respectively. Nitrification potential (NP) rates were influenced by site location and by the experimental plot design, (Fig. 4a and Fig. 4b). Overall, the potential nitrification rate, i.e., the transformation of NH_4^+ to NO_2^-, was about 7 times higher than the control samples (p=0.0048). In contrast to the other indicators studied, the variant with plant polyculture showed comparable values to the monoculture plots (p=0.9232).

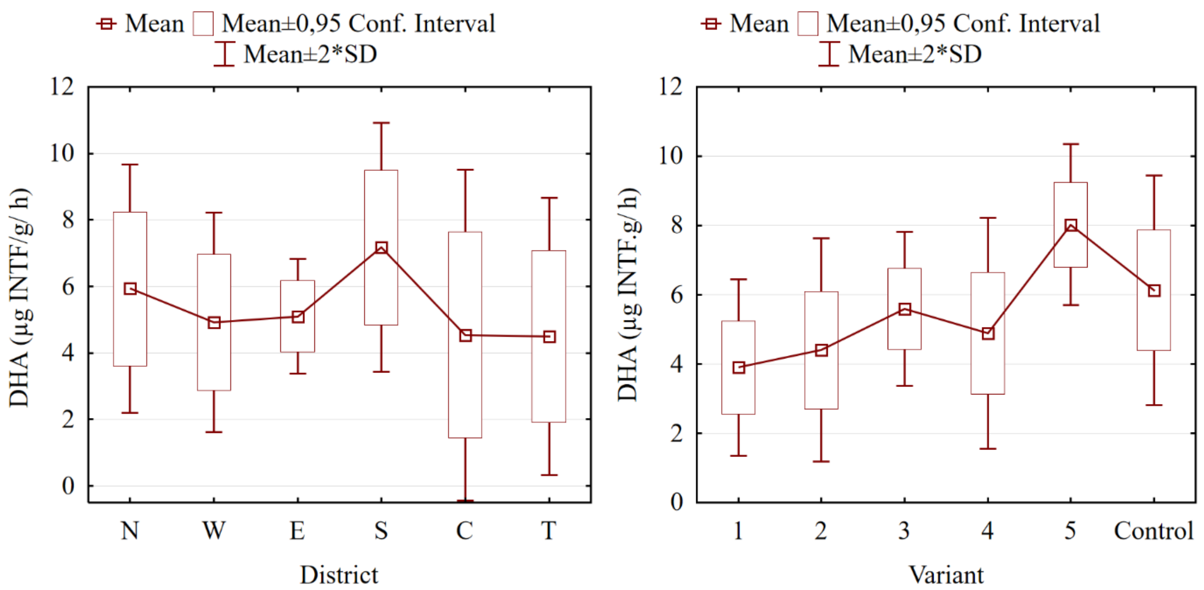

Fig. 3. Average soil microbial dehydrogenase activity in: a) six roadside soil sites selected for this study within the limits of the city of Plovdiv; b) five experimental plots planted with different grass species.

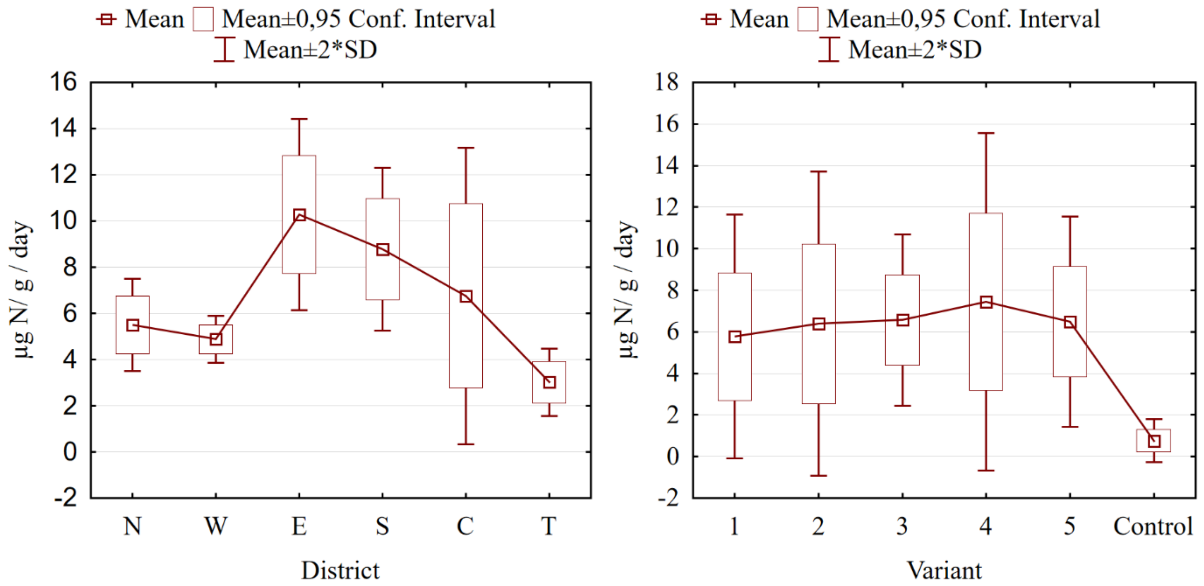

Fig. 4. Average soil nitrification potential in: a) six roadside soil sites selected for this study within the limits of the city of Plovdiv; b) five experimental plots planted with different grass species.

Multivariate analysis of soil physical, chemical and microbial properties

A Pearson correlation matrix was employed to investigate the impact of soil chemical properties on the physiological activity of the soil microbial community in the experimental plots. The analysis indicated significant correlations among most of the examined soil parameters (refer to Table 2). Notably, the total nitrogen content exhibited significant correlations with soil organic carbon, as well as total phosphorus and sulfur concentrations. Interestingly, the findings imply that changes in soil pH do not significantly influence the studied chemical parameters. Additionally, water content (WC) did not play a significant role, as it remained relatively stable over the studied period due to regular irrigation by the Plovdiv municipality.

Table 2. Correlation coefficients (Pearson correlation) between soil physical-chemical and physiological parameters**.**

	TN	C/N	OM	P	S	pH	WC	EC	NP	DHA	MBC	BAS
TN	**1.000**											
C/N	0.540	**1.000**										
OM	**0.982**	0.655	**1.000**									
P	**0.999**	0.563	**0.985**	**1.000**								
S	**0.999**	0.543	**0.981**	**0.999**	**1.000**							
pH	0.135	-0.589	-0.047	0.112	0.138	**1.000**						
WC	0.049	-0.635	-0.132	0.025	0.052	**0.995**	**1.000**					
EC	-0.479	0.401	-0.324	-0.458	-0.478	-0.812	-0.772	**1.000**				
NP	**0.910**	0.789	**0.593**	**0.528**	0.514	**-0.926**	-0.557	0.244	**1.000**			
DHA	0.805	0.731	0.856	0.817	0.803	-0.280	-0.350	-0.161	0.542	**1.000**		
MBC	-0.045	**0.553**	0.135	-0.025	-0.049	**-0.961**	-0.968	0.683	0.508	0.346	**1.000**	
BAS	0.543	0.646	0.622	0.558	0.537	**-0.567**	-0.632	0.112	0.671	**0.742**	**0.568**	**1.000**

*Correlations (in bold) are significant at α=0.05 (2-tailed)

In terms of biological parameters, there was a negative correlation observed between soil pH and NP, MBC, and basal respiration. For the remaining chemical variables, only a weak positive correlation was identified between total nitrogen (TN) and the nitrification potential of soil communities, as well as between the C/N ratio and the carbon content of soil microbial biomass. Electrical conductivity values vary within a narrow range and do not appear to be a factor influencing the variation in biological parameters. In general, the various biological activities were inter-correlated, except for the nitrification potential in the soil plots under examination. The absence of a noteworthy positive correlation among all measures of biological activity and the majority of chemical parameters implies that the primary factor influencing physiological diversity among experimental soil plots is the planted vegetation rather than the soil characteristics. This underscores the reliability of physiological activities as indicators of changes in soil quality during phytoremediation practices. Principal Coordinate Analysis (PCoA), based on a correlation matrix of total biological activity, highlighted significant variations that clearly distinguished between the control and Var 5 zones, while no notable differences were observed in the other variants (see Fig. 5). In the PCoA, the first axis, which accounted for almost 45% of the overall dataset variance, was composed of MBC, DHA, and BAS in the Principal Coordinate Analysis.

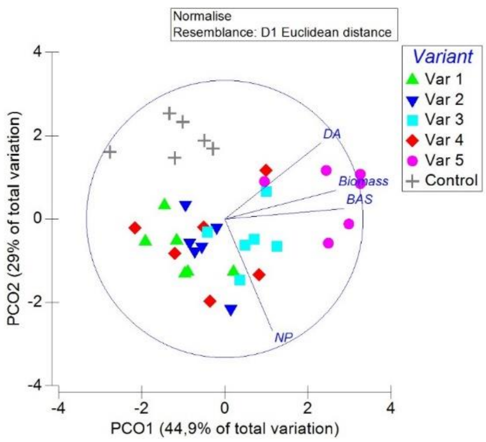

Fig. 5. Results of principal coordinate analysis (PCoA) of measures of microbial biomass carbon, basal respiration, dehydrogenase activity and soil nitrification potential across the 5 studied soil plots in the urban districts in the city of Plovdiv.

The permutational MANOVA design test and ANOSIM R values, based on the vegetation type, and district location, substantiated this result and showed significant differences in the physiological profiles of the soil microbial communities between the control samples and soils planted with *Lolium perenne* (L.), *Agropyron cristatum* (L.), *Festuca arundinacea* (Schreb), and *Lotus corniculatus* (L.) in polyculture (R = 0.894; p=0.002). At each site, the polyculture plot was consistently found to be significantly distinct from the four monoculture variants (R=0.956; p=0.002). In contrast, there was no distinction between the four monoculture plots (ANOSIM, R=0.135; p=0.147) as well as between identical plots, situated in different city districts (ANOSIM, R=0.108; p=0.476).

Discussion

The present study aimed to determine soil microbial responses to phytoremediation of roadside soils, based on the ability of perennial and leguminous grasses to remove toxic substances from the environment and to improve soil structure (Paz-Alberto & Sigua, 2013; Fatima et al., 2018; Petrova et al., 2022; Sladkovska et al., 2022). Our hypothesis that microbiological indicators for soil fertility will increase over time under the influence of plant root development during the process of phytoremediation was only partially supported by the results. The current findings showed that biological parameters were statistically the same among the roadside soil plots sown with the tested grass species in monoculture. Moreover, the analyzed physiological parameters maintained their levels from the control measurements before planting the vegetation cover. When grown in polyculture the perennial and leguminous grasses (Variant 5) resulted in a drastic increase in the microbial biomass, dehydrogenase activity and soil basal respiration. This result is consistent with previous studies on the soil health changes during the process of ecosystem restoration (Zhang et al., 2018; Feng et al., 2019; Wang et al., 2021).

Microbial biomass carbon functions as an indicator of the dynamic organic elements in soil, revealing the richness and diversity of microbial populations. Typically, irrigated agricultural soils that receive consistent carbon inputs, foster a thriving microbial community. In contrast, urban roadside soil microbial communities appear to experience considerable strain due to inadequate energy, substrates, and water, compounded by potential exposure to harmful pollutants. The MBC in the plotted variants in the six districts of the city of Plovdiv was inter-correlated with DHA and BAS and varied between 299 and 414 μg C/g DW for monoculture plots. The lowest values were found for variant 4 (leguminous grass), reaching an average of 653 μg C/g DW in the variant 5 soils. The results are higher than those reported for other urban soils (De Silva et al., 2021; Renella, 2020; Wang et al., 2011) and are comparable with agricultural soils (Kumar et al., 2015), but are still one to two orders of magnitude lower than those of the forest soils (Das et al., 2023). Usually, lower MBC in the urban soils serves as an indicator for slower biogeochemical cycling of C, N, and P, limiting the soil's ability to sustain perennial plant population. Carbon (C), nitrogen (N), phosphorus (P) and sulphur (S) are probably the most important macroelements comprising the biomass of living organisms and play crucial roles in the Earth's biogeochemical cycles (Luo et al., 2022; Zhang et al., 2021). Their concertation may depend on ongoing land management methods (Nugent & Allison, 2021; Thompson & Kao-Kniffin, 2019). The lack of significant correlation between OM, TN, P and S concentration with MBC and other biological parameters instead only with C: N ratio suggests no C limitation. This is confirmed by the established average C: N: P ratio of 106:12:2 which is close to the optimal Redfield ratio (Luo et al., 2022), which reveals neither N nor P are limiting factors for net primary production and C storage in the present study.

Soil pH was the only studied chemical parameter directly correlated to the MBC and BAS. The results are in agreement with previous studies indicating pH as a strong driver of microbial composition and activity due to the narrow optimal pH range for many taxa (Glassman et al., 2017; Rousk et al., 2010; Wang et al., 2021). The established close to neutral pH values in the present study differ from the typically alkaline nature of the urban soils (Zhang & Yang, 2015), and are a prerequisite for good physiological activity (Liu et al., 2014). However, the role of pH of roadside urban soils in the metabolic activity of the communities is largely unknown (Wang et al., 2021). The role of water content as a major factor shaping the activity and function of urban soil microbial communities (Nkongolo et al., 2022), was not confirmed in the present findings. This is probably due to the regular irrigation, conducted by the municipality, which substantially supports the vegetation.

Irrigation additionally enhances nitrogen availability for microbes (Stark & Firestone, 1995), potentially leading to the leaching of NO_3^- from urban soils (Hall et al., 2008; Kaye et al., 2004). Our study identified a similar process, where the overall potential nitrification rate was more than seven times higher than in control samples ($p=0.0048$). Insufficient soil moisture levels on the other side can impede microbial activity by diminishing intracellular water potential, thereby decreasing hydration (Stark & Firestone, 1995). Hence, regulating irrigation could be crucial for effecttively managing urban green areas while reducing nitrogen loss (Bijoor et al., 2008).

Generally, all monoculture plots (variants 1 to 4) showed comparable steady-state levels of basal respiration, comparable to the average BAS found in the roadside soils before sowing of the perennial as well as leguminous grasses. In this case, the microbial response to basal respiration is similar to that for other legumes (Wobeng et al., 2020). The only exception again were the polyculture plots (variant 5), where phytoremediation improved not only microbial biomass but also community function. This indicates that the part of the microbial community governing basal respiration is induced by the site-specific conditions formed by the combined rhizosphere of the four plant species.

Correlation analysis indicated that C, N, and P did not directly affect soil basal respiration levels. BAS is used to estimate total microbial activity, reflecting the quantitative and qualitative composition of carbon sources (Cheng et al., 1996). The higher soil respiration in variant 5 supports the claim that soils sown with perennial and leguminous grasses in polyculture have higher soil microbial activity. This implies rapid decomposition of organic residues associated with making nutrients available for subsequent stimulation of heterotrophic microorganisms (Cheng et al., 2013). A claim that is supported by the lower soil microbial metabolic coefficient (qCO_2). The metabolic coefficient (qCO_2) is often perceived as a useful indicator reflecting the organic carbon assimilation efficiency of the microbial community. Its values are inversely proportional to BAS and increase when the soil microbial community is exposed to stress (Gonzalez-Quinones et al., 2011; Feketeová et al., 2021). The trend observed for experimental variants (1-4) sown in monoculture. In variant 5, the lowest qCO_2 levels were found, indicating less stress for microorganisms, higher C use efficiency, lower energy requirements and better soil quality sown in polyculture. These findings indicate that the microbial community structure and diversity in the rhizosphere are influenced by the particular plant species throughout phytoremediation (Grayston et al., 1998; Jaeger et al., 1999).

Conclusions

The negative effects of urban development and population growth in large cities on the quality of the natural environment are evident and therefore it is necessary to develop environmentally sound strategies for roadside soil rehabilitation through the construction of green buffer zones. In the present study, soil chemistry and soil microbial biomass and activity in phytoremediated roadside soils were characterized. Data show that planting the perennial grass species *Lolium perenne* (L.), variety IFC Harmoniya, *Agropyron cristatum* (L.), variety Svejina and *Festuca arundinacea* (Schreb), variety Albena, as well as leguminous grass *Lotus corniculatus* (L.), variety Leo, with good bioaccumulation potential in monoculture can significantly alter the soil environment, but does not always have the desired positive effect on microbiological indicators of soil health. This calls into question the long-term effectiveness of the phytoremediation process. Only loans formed by the combined sowing of the four species (1:1:1:1) had a significant positive effect on the microbial physiological activity, in terms of basal respiration, dehydrogenase activity and increased microbial biomass. This indicates that the microbial community in the studied soils is induced by the site-specific conditions formed by only the combined rhizosphere of the species.

Additional studies are needed to determine the specific rhizosphere-microbial community interactions arising during the phyto-remediation process in the roadside soils in conditions of specific microclimate characteristics for the city of Plovdiv.

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