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DECREASE IN THE ACTIVITY OF SOIL FUNCTIONAL MICROBIOMES IN HEAVY METAL POLLUTED SOILS

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Abstract

The presence of heavy metals in urban soil is a consequence of anthropogenic activities and represents a continuous source of changes in microbial communities. The DEMSA model was used to analyse a series of decreases in the soil microbial functional profile, with a target on the groups and guilds that suffer a reduction due to the presence of heavy metals. The presence of heavy metals in the 5 sites analysed is visible in the reduction of the soil microbiome general activity. The Index of Narrowing is set in a very large interval, which indicates the absence of multiple functional groups from the altered community. The diversity indices show a large variability associated with the reduction of specialized functional activity. The sum of activities is a good indicator of functional contraction, with most of the functional guilds showing activities below 1.0 units, and a large variability between functional groups. Soil microbiomes show powerful changes due to the presence of heavy metals in soil, which is visible in the decrease of multiple groups functional activity.

Key words*: functional narrowing, index of contraction, indicator guild, microbial communities.*

INTRODUCTION

Historical soil pollution with heavy metals relates to the multitude of anthropogenic activities during the development of urban areas (Mehmood et al., 2020; Sharma et al., 2015). Green areas in cities possess multiple services necessary for human life, both from a physical and physiological point of view, and thus the presence of heavy metals in soil is important in the risk assessment procedures associate with human well-being (Luo et al., 2024; Poggio and Vrščaj, 2009).

For polluted soils within cities bioremediation approaches are effective methods for heavy metal removal, considering their beneficial environmental effects (Sánchez-Castro et al., 2023). An important aspect related to bioremediation effect is the continuous monitoring process, which enable the detection of the key factors that sustain its success (Liu et al., 2022; Vega et al., 2022; Zhang and Wang, 2020; Xiang et al., 2020). Phytoremediation as a bioremediation technique implies the use of plants that can remove heavy metals from soil and thus decrease their degree of pollution (Kumar et al., 2022; Tonelli et al., 2022). This process relies on microbial activity, especially in the rhizosphere of plants, which can increase the remediation effect (Yan et al., 2020).

An in-depth analysis of soil microorganism reveal that microbial components are severely affected by the presence of heavy metals (Adewumi and Ogundele, 2024; Tang et al., 2024). One of the most harmful effects is the reduction of populations and the variability of microbial activities (Horváth et al., 2021; Sager, 2020). In this context, the monitoring of microbial dynamics is a good instrument in the detection of remediation effect, including the diversity of microbial groups. Application of community-level physiological profiling to bioremediate soils can offer numerous indices on microbial functional activity and diversity, along with the assemblage of functional community (Martínez-Toledo et al., 2024).

The aim of this research was to analyse the structure of reduced functional guilds in soil microbiome after 1 year of bioremediation. Hypotheses related to the aim was formulated as research questions: i) are there still visible effects of historical pollution on functional diversity of microbiome? ii) an in-depth analysis of functional microbiome reveal a

reduced presence of specific functional guilds from the entire community? iii) is there a pattern of functional decrease in community associated with a site and a specific guild?

MATERIALS AND METHODS

The samples used in the research were collected from Baia Mare (Baia Mare County), from 5 sites (Craica - CR), Colonia Topitorilor - CT, Ferneziu - FR, Urbis - URB, Romplumb - ROMP) where phytoremediation was applied one year prior (Pop et al., 2024). The Biolog Ecoplates was used to analyse the functional microbiome, in a time interval of 96 hours. Each of the 3 replications from each site was 10^{-4} times diluted, and readings were performed with a spectrophotometer at 590 nm, every 24 hours. From all the samples, an average standard of activity was constructed, to which the standard deviation (+/-) was applied to set the local limits of the functional ecological niche.

The analysis of the results followed the DEMSA (Detailed Exploration of Microbial Sociological Assemblage) model (Stoian et al., 2022). All the readings from each site were compared with the standard and two matrices were built. The first matrix contained all the values that were between the average and the minimum activity of the standard, and this matrix was considered the Narrowing segment of the functional microbiome. The data that were below the minimum standard were considered as a part of the Contraction matrix. All substrates were considered as a functional group, while chemical similar substrates were considered as a functional guild. The 5 functional guilds followed in the research protocol were: Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM).

Data analysis was performed in RStudio (R Core Team, 2024), with basic statistics extracted from "psych" package (Revelle, 2024) and the differences between sites were assessed with ANOVA and LSD test from "agricolae" package (de Mendiburu, 2023). For each of the matrices used, diversity indices and NMDS ordination ("vegan" package, Oksanen et al., 2022) were extracted to analyse the stability of site-specific functional microbiomes and their potential reduction pattern. Additionally, the correlations between parameters were analysed with "Hmisc" package (Harrell, 2025).

RESULTS AND DISCUSSIONS

The narrowing matrix showed a variety of activities and diversity but not present in all sites (Table 1). This phenomenon was caused by site-specific soil conditions, which favoured specific activities. CH guild is absent in CT sites, but present extremely high values in ROMP and URB. Both sites present a potential reduction of CH functional activity, which is associated with ten different functional groups. For this guild, CT sites present conditions that enable the survival of CH groups, and near to this condition FR is set to almost zero. For P functional guild, CT and FR sites offers the conditions for microbiome survival, both sites being absent from the constraining matrix. In terms of CX guild, the same two sites present a limiting activity near to zero, while for ROMP and URB the narrowing is set to more than 4.5 units. An interesting case was observed for CR site, where CX guild have a restrictive potential of 1.5, while CX of only near to 1 unit. The same site offers the conditions for the survival of AA and AM guilds, with less than 1 unit narrowing of activities. On the other hand, both ROMP and URB act to reduce the AA activity in a similar manner, while for AM was observed a 20% difference.

In terms of the total potential sum of narrowing, ROMP is the most unfavourable site, with 16 units, and followed by URB (Table 1). Both sites act to drastically reduce the potential functional activities. Compared to these conditions, for CT and FR the reducing of activities is set to less than 1 unit, which correspond to less restrictive conditions of both sites.

By analysing the index of confining, a different point of view is set toward the population of the communities that reduce their activities (Table 1). ROMP is responsible for the almost complete change of microbial community. This high value of narrowing index can be explained by the lack of pattern in the reduction of functional activity at microbiome scale. Also, this indicate that several functional groups are more sensitive to soil conditions and reduce

their activities at a larger scale. URB site produce a narrowing index of more than 58%, which explain a change in the initial functional pattern of more than half of community. For both CT and FR sites the values of narrowing are less than 2%, which means a negligible effect for the reduction of a large share of community.

In terms of diversity, the analysis of site-specific microbiomes showed high differences in terms of both community groups and the effect of soil conditions (Table 1). Shannon diversity was set to more than 3 (URB and ROMP), a value which indicate a strong effect on the functional microbiome and that numerous functional groups are reducing their activities. An interesting case was recorded for CT and CR sites, where this index values were lower, which indicates the reduction of activity for very specific functional groups.

Table 1. Microbial functional guilds and diversity in Narrowing range of activities

Sites	CH	P	CX	AA	AM
CR	1.51±0.54bc	0.89±0.24b	1.03±0.47b	0.84±0.34b	0.76±0.14a
CT			0.04±0.04b	0.34±0.34b	
FR	0.35±0.09c		0.29±0.14b	0.10±0.10b	0.02±0.02b
ROMP	4.96±0.40a	1.89±0.35a	5.09±0.66a	3.32±0.56a	0.77±0.38a
URB	3.10±1.00b	1.18±0.22b	4.58±0.48a	3.19±0.35a	0.97±0.11a
Sites	Sum	Index of	Shannon	Simpson	Pielou
CR	5.06±1.08b	12.6±3.45b	2.54±0.16b	0.91±0.01a	0.94±0.00a
CT	0.39±0.32c	0.73±0.60b	0.27±0.19d	0.51±0.26b	0.39±0.28b
FR	0.78±0.15bc	1.51±0.29b	1.25±0.12c	0.68±0.04ab	0.92±0.01a
ROMP	16.0±1.94a	98.8±24.5a	3.31±0.05a	0.96±0.00a	0.97±0.00a
URB	13.0±2.10a	58.1±15.6a	3.15±0.13a	0.95±0.00a	0.94±0.01a

Note: values ± s.e. followed by different letters present significant differences according to LSD ($p<0.05$). Legend: sites - Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR), Urbis (URB), Romplumb (ROMP); guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM); Index of - Index of Narrowing.

Simpson index values indicate a medium heterogeneity for these two sites, and less groups that are reducing their activity (Table 1). In contrast, all the other three sites act for the reduction of activities for larger functional groups. These trends are sustained by Pielou`s index, where almost balanced share of communities is reducing their activities, apart from CT site where the narrowing affects differently microbial groups.

The contraction potential of microbial communities is visible in only 3 sites (Table 2), and only for CX guild. The rest of the sites show a variable contraction potential, with a maximum in ROMP for CX guild. All the rest of the guilds show a contraction potential of less than 1 unit of their activities, which indicates that even their metabolic activities are reduced due to the soil conditions there is no possible disappearance from the community.

In terms of contraction index, the highest value was recorded in ROMP site, where the community that contract its activities is 26% different from the average pattern (Table 2). Both CR and URB sites showed less than 6% contraction index values.

Shannon present index a powerful effect of contraction in ROMP community contraction, compared to CR site where the effect was considered negligible (Table 2). This effect is visible in the value of Simpson index, with a heterogeneous community that contract their activities due to soil conditions. At the opposite, for both CR and URB sites, the contraction is visible in a moderate homogeneous community. Pielou`s index present specific functional shares of the microbiomes that reduce drastically their activities in CR site, while the contraction is visible in almost the entire community for the ROMP and URB sites.

Table 2. Microbial functional guilds and diversity in Contraction range of activities

Sites	CH	P	CX	AA	AM
CR			0.03±0.03b		
ROMP	0.81±0.26a	0.37±0.16a	1.90±0.38a	0.82±0.35a	0.22±0.11
URB	0.18±0.10b	0.02±0.02b	0.45±0.24b	0.47±0.25a	0.02±0.02
Sites	Sum	Index of	Shannon	Simpson	Pielou
CR	0.04±0.04b	0.12±0.12b	0.19±0.19c	0.75±0.24a	0.13±0.13b
ROMP	4.14±0.88a	26.1±8.24a	2.77±0.18a	0.92±0.01a	0.90±0.00a
URB	1.15±0.63b	5.63±3.36b	1.59±0.54b	0.68±0.17a	0.85±0.05a

Note: values ± s.e. followed by different letters present significant differences according to LSD ($p<0.05$). Legend: sites - Craica (CR), Urbis (URB), Romplumb (ROMP); guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM); Index of - Index of Contraction.

The projection on NMDS ordination for the limiting segment of functional microbiomes show a different stability of communities (Figure 1). Both CT and FR sites are placed in the same area of the ordination and the plots are near one to each other, indicating stable communities and a similar reduction pattern. On the other hand, CR site show a medium dispersion of plot-specific communities, and a position in the -+ quadrat, near to the CT and Cr sites. Both ROMP and URB are placed in different quadrats and are defined by a great dispersion of their communities.

An interesting aspect is that each of these microbiomes are associated with specific

functional guilds, that are responsible for major changes inside the assemblage of the community (Figure 1). CX and AA guilds are very significant in the changes of URB community, and additionally the AM variability can produce distinct changes. For ROMP, a part of the communities is associated with the variability and changes in CH and P guilds, but not completely correlated one with each other. The other two communities are associated with the sum of potential restriction and the index of narrowing, that will modify both the community consortium and the loss of a part of activities.

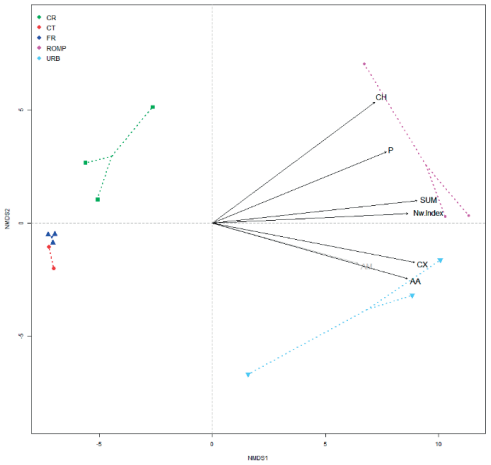

Figure 1. NMDS projection of microbial communities in Narrowed niche. Legend: sites - Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR), Urbis (URB), Romplumb (ROMP); Nw.Index - Index of narrowing; H - Shannon index; S - Simpson index; guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM)

In term of contraction potential, CR site present an almost identical community pattern to one URB site (Figure 2). This similarity of contractions indicates a similar microbial assemblage pattern that overlap in both sites. Both ROMP and URB show a high dispersion in on the ordination graph. URB dispersion is oriented parallel with axis 1 of ordination and is not associated with a specific functional guild activity.

The ROMP site-specific communities are present in three quadrats of the ordination, associated with the contraction of different groups activities (Figure 2). The upper point of this site is placed in -+ quadrat, and AA guild vector is oriented toward it. The middle point is placed in the -- quadrat and is more related to the sum of activities that are contracting within the community. Between these two points are placed the P activity guild, which indicate a different contraction scenario based on the contraction of this guild. This scenario is the most related to the index of contraction and that can completely modify the structure of the community. The last point is placed in the +- quadrat but is not related to any of the guild's activities. Between this and the second point, the presence of CX vector indicate another contraction scenario that is related to the drastic reduction of this guild activity.

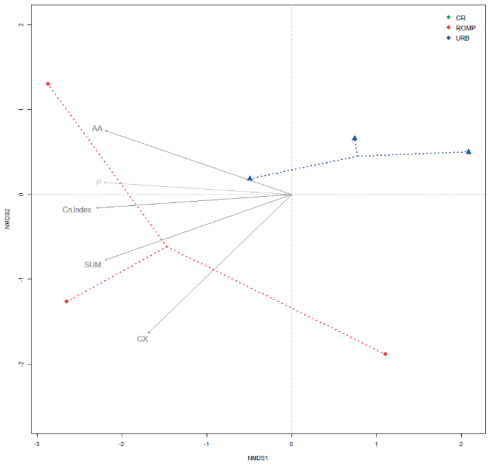

Figure 2. NMDS projection of microbial communities in Expansion niche. Legend: sites - Craica (CR), Urbis (URB), Romplumb (ROMP);Cn.Index - Index of contraction; H - Shannon index; S - Simpson index; guilds - Carboxylic & acetic acids (CX), Amino acids (AA)

The NMDS projections for narrowing and contraction of microbiome showed a great impact of specific functional guilds. The in-depth exploration of functional group relation with community projections shows a gradual reduction from restriction to contraction in niche (Table 3). Most of the CH and CX groups are present in the selective community, which indicate a sensitivity to pollution of these microbiome components. Their impact in the stability of communities range from very to distinct (***/**). The same phenomenon was observed for P and AA groups, indicating that a complex segment of microbiome is narrowing due to the soil conditions. AM2 group show a

significant presence in the reduction of microbial activity, being the only group from AM guild that show sensitivity to soil conditions.

The contraction observed in microbial communities relies on a reduced number of functional groups, indicating a high sensitivity to soil pollution (Table 3). For the three sites that show this type of extreme activity reduction, most of the functional groups belong to CX substrate category. Their impact in the community ranges from significant to distinct (CX7), with a total of 6 functional groups in this category. Both CH and P are rare in the contracted community, with an overall significant impact in the assemblage. On the other hand, the most present set of groups belong to AA guild, with only AA2 that present a significant impact in the contraction status. An interesting case is observed for AM1, which have a significant share in the contracted community.

Table 3. Microbial functional groups associated with Intensification and Expansion in the range of activities

Niche segement	Group	Sig	Group	Sig	Group	Sig
Narrowing	CH1	***	CX1	**	P1	***
	CH2	**	CX2	***	P2	**
	CH3	**	CX3	****	P3	**
	CH4	**	CX4	***	P4	**
	CH5	***	CX5	**	AA1	***
	CH7	**	CX6	***	AA2	***
	CH8	**	CX7	**	AA3	**
	CH9	***	CX8	***	AA4	**
	CH10	***	CX9	**	AA5	***
					AA6	***
					AM2	*
Contraction	CH4	*	CX2	*	P1	*
	CH9	*	CX4	*	AA1	*
			CX6	*	AA2	**
			CX7	**	AA3	*
			CX8	*	AA6	*
			CX9	*	AM1	*

Note: groups by * significant impact in NMDS ordination (*p<0.05*; p<0.01**; p<0.001* ***). Legend: functional guilds - CH1 - Pyruvic acid methyl ester, CH2 - d-Cellobiose, CH3 - α-d-Lactose, CH4 - β-Methyl-d-glucoside, CH5 - d-Xylose, CH7 - d-Mannitol, CH8 - N-Acetyl-d-glucosamine, CH9 - Glucose-1-phosphate, CH10 - d,l-α-Glycerol phosphate, CX1 - d-Glucosaminic acid, CX2 - d-Galactonic acid γ-lactone, CX3 - d-Galacturonic acid, CX4 - 2-Hydroxy benzoic acid, CX5 - 4-Hydroxy benzoic acid, CX6 - γ-Hydroxy butyric acid, CX7 - Itaconic acid, CX8 - α-Keto butyric acid, CX9 - d-Malic acid, P1 - Tween 40, P2 - Tween 80, P3 - α-Cyclodextrin, P4 - Glycogen, AA1 - l-Arginine, AA2 - l-Asparagine, AA3 - l-Phenylalanine, AA4 - l-Serine, AA5 - l-Threonine, AA6 - Glycyl-l-glutamic acid, AM1 - Phenylethylamine, AM2 - Putrescine.

The reduction of functional activity is observed as the interaction between the different functional guilds (Table 4), that shape the structure of communities. The narrowing at community level implies a significant positive correlation between all functional guilds, indicating large coordinate process. The highest correlation was observed between CX and AA guilds, both presenting numerous groups in the narrowed niche. The AM is the less associated with the rest of functional guilds, indicating a more independent activity of this microbiome in terms of the other ones. Based on their coupled activity, CX and AA guilds present an important share in the narrowing of the total sum of activities. These values indicate that any restriction of activities in these two groups will impact the total activity almost completely. Both P and CH guilds are identically correlated with the sum of activities, which indicate that any of these guilds can be used to trace the narrowing of the activity. Related to the index of alteration, the sum of activities has the most important role, the high value of correlation indicating a potential change in the pattern of microbiome groups that reduce their presence and functionality. All guilds present correlation values with Shannon diversity between 0.75-0.85, sustaining that a complex microbiome, from different guilds, narrow their activities, but at different levels. The shift between heterogenous and homogeneous of the selective communities is associated with all the guilds at values under 0.60, an aspect that sustain that a great change within a community will be produced only when numerous guilds will reduce at once their activities.

Table 4. Interrelations between microbial guilds and indices in Narrowing and Contraction niche

									Narrowing
	P	CH	CX	AA	AM	Sum	In	H	S
P		0.86	0.90	0.87	0.84	0.94	0.92	0.85	0.58
CH	0.71		0.88	0.87	0.64	0.94	0.90	0.84	0.56
CX	0.87	0.74		0.97	0.78	0.98	0.93	0.85	0.55
AA	0.87	0.74	0.84		0.80	0.97	0.92	0.83	0.52
AM	0.96	0.56	0.90	0.84		0.81	0.74	0.81	0.57
Sum	0.93	0.84	0.97	0.93	0.90		0.96	0.88	0.58
In	0.97	0.82	0.93	0.93	0.93	0.99		0.75	*0.48*
H	0.74	0.83	0.91	0.88	0.72	0.93	0.87		0.69
S	*0.45*	0.55	0.60	0.59	*0.45*	0.61	0.56	0.68	
Contraction									

Note: values in *italic* did not present significant correlations (*p<0.05*). Legend In - Index of alteration; H - Shannon index; S - Simpson index; guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM).

The contraction correlations present lower values and a lower association potential between guilds and parameters (Table 4). In terms of this niche segment, AM is significant associated with P and CX, all three of them being highly correlated with the sum of

activities that are extremely reduced. An interesting case was observed for AA, which have a lower association potential with the other guilds but present a high importance in the total sum of activities. This makes AA a good candidate for the detection of contraction phenomena, even when small changes are visible within this guild. The index of alteration is almost completely determined by the total activity, indicating that contraction process will produce high imbalances in the share that each group have in the community. The changes in P activities are the most important in the assemblage of the contracted communities, while CX, AA and AM changes will act in a similar way. The CX guild is the most important type of microbiome that affect the diversity in contracted community, affecting at the same time the homogeneity.

CONCLUSIONS

The narrowing and contraction potential in functional communities is visible in the low scores recorded for all the guilds analysed, resulting in potential losses of high shares of the total activities.

The index of narrowing recorded almost 99% for Romplumb site, and an extra alteration of 26.1% in the contraction segment of ecological niche.

For both the narrowing and contraction segments of functional niche, the diversity indices present fluctuating values and indicate a high impact of pollution.

The narrowing phenomenon is visible in two different scenarios, with the maintenance of community stability and response (Colonia Topitorilor and Ferneziu), or with a plot-specific response and reduced community stability (Romplumb and Urbis).

Contraction phenomena lead to the high reduction of multiple functional groups activities, the lack of community stability and multiple scenarios of future functional assemblages.

In terms of narrowing, most of the groups can influence the development of different functional assemblages based on one-to-one positive interactions.

The contraction phenomenon is accelerated by the separate interaction between Amines/amides with Polymers and Carboxylic & acetic acids guilds.

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