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Prokaryotic community structure of the soil matrices and the ferromanganese nodules in Luvisols from natural environment

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Abstract

Purpose This study aimed to investigate the structure and diversity of the bacterial community in soil matrices and Fe–Mn nodules from natural Luvisols and reveal the potential bacterial involvement in the transformation of different elements in nodules.

Materials and methods Soil matrices and Fe–Mn nodules (sized 2–4 mm) were collected from uncontaminated soil in the National Park "Land of the Leopard" (south of the Russian Far East) and studied using advanced analytical methods. The compositions of the soil matrix and nodule bacterial communities were analysed using high–throughput sequencing technology.

Results and discussion We identified 48 phyla, 122 classes, 238 orders, and 316 families of bacteria in the studied soil matrices and nodules. The bacterial diversity and abundance in the studied nodules were lower than those in the soil matrices. The taxonomic compositions of the bacterial communities in the soil matrices and nodules were similar; Proteobacteria, Actinobacteria, and Acidobacteria were the predominant phyla. Thirty–five dominant families were selected to identify the relationships between the main physicochemical parameters of the soil matrices and nodules and the relative bacterial abundances. The same dominant families were associated with different elements in the soil matrix and nodules. Mn–rich, organic compounds, and N–containing compounds were the main factors influencing bacterial diversity and abundance in the nodules. Compared to the soil matrix, the associations of bacteria with Fe–rich compounds in the nodules were not significant.

Conclusions The bacterial community identified in the nodules and soil matrix showed metabolic flexibility and adaptability, allowing them to play a significant role in the formation of nodules in the studied soils and in the biogeochemical cycles of Mn, SOC, TN, Fe and related elements in the soil matrix and nodules.

Keywords Fe–Mn nodules · Soil microbiome · Bacterial · Luvisols · Physicochemical parameters · Soil from natural environment

1 Introduction

Ferromanganese nodules (FMNs) are soil organomineral neoformations formed through pedogenesis in different types of soils (Sipos et al. 2011; Timofeeva et al. 2014; Hu et al. 2015; Ettler et al. 2017; Huang 2022). Nodules differ significantly from the soil matrix in terms of their chemical and mineralogical composition and several physicochemical parameters. FMNs are soil geochemical microbarriers that have a high ability to accumulation and sequestration of polyvalent cations of different elements (Tan et al. 2006; Liu et al. 2002; Gasparatos 2013; Timofeeva et al. 2021). Despite abundant information about FMNs morphology, composition and geochemistry and their influence on the element cycles in soil profiles, the mechanism of FMNs formation is still a topic under debate. One of the main conditions for FMNs formation in soils is seasonally contrasting alterations in the soil redox potential (Eh) (Cornu et al. 2009; Sipos et al. 2011; Timofeeva et al. 2014 Ali et al. 2025). The contrast cycles of reductive dissolution and oxidative precipitation of Fe and Mn (hydr)oxides resulted in the formation of microzones with relatively high

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concentrations of these elements in the soil matrix. These microzones promote proto–nodule formation. The main nodule–forming elements (Fe and Mn) and associated with their different elements precipitate onto the surface of the proto–nodules, which then grow (Dabard et al. 2020; Gasparatos et al. 2019).

Recent studies have shown that various bacterial phyla participate in the process of FMNs formation in soils from different parts of the world (He et al. 2008; Lysak et al. 2013, 2019; Zhang et al. 2014; Hu et al. 2015; Timofeeva et al. 2024; Sipos et al. 2025). The results of FMNs studies in soils from paddy fields and sugarcane fields in China indicate the presence of members of the phyla Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes, and Verrucomicrobia and members of the orders Burkholderiales, Rhodocyclales, Acidobacteriales, Desulfuromonales, and Clostridiales (He et al. 2008; Zhang et al. 2014; Hu et al. 2015). In FMNs from Albic Retisols, Stagnic Fluvisols and Gleyic Fluvisols in Russia, the following bacterial classes were identified: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Acidobacteria, and Planctomycetes (Lysak et al. 2019). Different bacterial taxa can be involved in FMNs formation and development by oxidizing and reducing Fe and Mn ions and producing nodule–specific minerals through biologically controlled (ions Fe and Mn are transformed inside the bacterial cell through intracellular biochemical enzymatic activity) and induced (deposition of minerals on the bacterial cell surface through interactions of bacterial metabolic products with metal ions) mineralization (Bazylinski et al. 2007; Dong et al. 2022; Qin et al. 2020; Ali et al. 2025). Several reports have shown that bacteria in soil nodules are characterized by increased physiological activity and the ability to nanotransform as a result of the stressful effects of the environment (Lysak et al. 2013). Nonetheless, the contribution of bacteria to the formation of FMNs, the transformation of the main nodule–forming elements and the diversity of the bacterial communities associated with FMNs are still limited. Additionally, most studies have examined the bacterial communities of FMNs from anthropogenically impacted soils (mainly urbanized landscapes and agricultural ecosystems), which precludes the ability to obtain reliable information on the natural functions of the bacterial communities of FMNs.

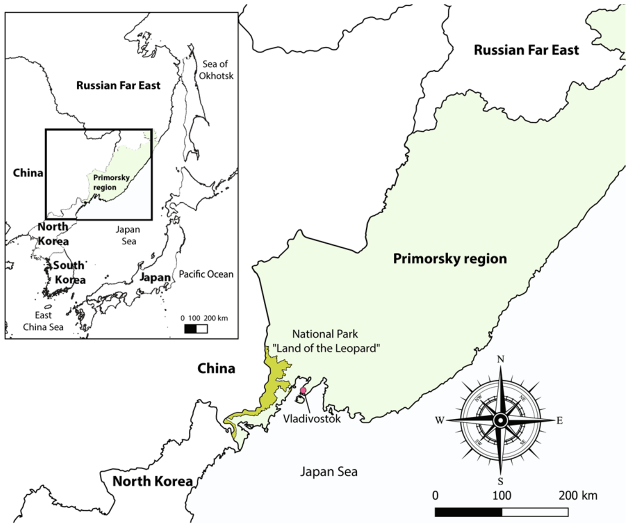

Fig. 1 Map showing sampling locations

We examined FMNs from the Gleyic Albic Luvisol (Manganiferric, Defferentic) formed in the territory of the National Park "Land of the Leopard" under the absence of direct anthropogenic impact (south of the Russian Far East). The soil–forming conditions in the south of the Russian Far East are known as favorable to the active formation and development of FMNs, which are characterized by well–defined sorption activities for trace elements and potentially toxic elements (Timofeeva 2008; Timofeeva et al. 2021, 2025). We previously found several genera of cultured bacteria, such as *Priestia*, *Bacillus*, *Rhodococcus*, *Lysinibacillus*, *Methylobacterium,* and *Pseudomonas*, in FMNs of the region under study (Timofeeva et al. 2024).

The objectives of this study are as follows: (i) to study the bacterial community of the soil matrices and FMNs from uncontaminated soil, and (ii) to identify the presumed influence of the bacterial community on the element composition of FMNs in view of bacterial involvement in the transformation of different elements in FMNs.

In this work, we describe the phylogenetic composition of the bacterial communities of FMNs and the host soil matrices from uncontaminated soil using next–generation sequencing (NGS) and show the metabolic flexibility and adaptability of the identified bacterial communities to various environments.

2 Materials and methods

2.1 Site description

The sampling site was located in the territory of the National Park "Land of the Leopard" in the south of the Russian Far East (Fig. 1). National Park covers an area of approximately 269 000 ha. The National Park was formed on the territory of a natural large massif of virgin coniferous broad–leaved forests, which are considered southern Ussuri taiga. The low–mountain (the eastern spurs of the Black Mountains and near the coast of the Yankovsky and Peschaniy peninsulas) and mid–mountain (the middle belt of the eastern spurs of the Black Mountains and the upper sections of the river basins) relief with small areas of flatlands (river valleys and coastal areas approaching Amur Bay) characterize the National Park territory. The hydrographic network of the National Park territory is quite defined and consists of twenty–one rivers and one lake. The climate of the studied territory is characterized as monsoon with northwestern continental winds, which bring clear, sunny weather and little precipitation in winter, and with southeastern and southern winds, which bring a large amount of precipitation (rain and fog) in summer. The mean winter and summer temperatures are approximately – 15.0 °C and + 20.1 °C, respectively. The mean annual precipitation is 826 mm, which mostly occurs from August to September (approximately 80%) when the typhoons bring in significant rains.

2.2 Soil characterization

Based on the whole–profile morphology, the studied soils are classified as a Gleyic Albic Luvisol (Manganiferric, Defferentic) according to the World Reference Base for Soil Resources (WRB) (2015). The Luvisol profiles were divided into the following depths: 0–18 cm (horizon A), 18–31 cm (E), 31–81 cm (Btg), and 81–153 cm (horizon BCg) (Fig. 2). The morphological characteristics of representative horizons of the Gleyic Albic Luvisol (Manganiferric, Defferentic) are given in Table 1.

2.3 Soil matrix and nodule sampling

Soil matrices and FMNs were collected from the A, E and Btg horizons of two soil profiles (43.26 N, 131.53 E; 43.22 N, 131.40 E). Two soil monoliths (3000–4000 g) were extracted from each horizon using sterile tools and packed into sterile material according to GOST 17.4.4.02–84. The soil monoliths were packed into cooled bags and transported to the laboratory immediately. The soil monoliths were wet–sifted through a nylon screen with a fine mesh size (0.05 mm). Nodules were hand–separated from the sediments and weighed. Samples of the soil matrix were hand–separated from the FMNs. Before DNA extraction, FMNs were sterilized by rinsing in sterile distilled water five times, immersion in 0.1% NaClO for 1 min, and then five rinses in sterile distilled water (Zhang et al. 2008; Hu et al. 2015). A portion of FMNs samples was dried at 105 °C for 12 h for chemical and mineralogical analyses and microscopy. The FMNs and soil matrices were ground to a powder using the planetary ball mill Pulverisette 5 (Fritsch GmbH) and passed through a sieve (0.06 mm). For scanning electron microscopy, 20 nodule samples were impregnated with an epoxide resin, cut in half and coated with Au by vacuum. Approximately 1100 nodule samples were analysed in this study.

2.4 DNA extraction, amplification and sequencing

DNA was extracted from the soil matrix and nodule samples using «Meta Soil» kit (Raissol™, Russia) according to the manufacturer's protocol. Purified DNA was used as the template for polymerase chain reaction (PCR). The V3–V4 regions of the 16S rRNA gene were amplified with the primer set 341 F (5′–CCTAYGGGRBGCASCAG–3′) and 806R (5′– GGACTACNNGGGTATCTAAT–3′) according to the recommendation of Caporaso et al. (2011). The PCR products of the proper length were confirmed by 2% agarose gel electrophoresis. The PCR products were purified using magnetic bead purification and then sequenced by Novogene Bioinformatics Technology (China) on the Illumina NovaSeq X Plus platform.

2.5 Physicochemical analyses

The pH was measured electrometrically in a 1:2.5 soil matrix or FMNs/deionized water solution after shaking 10 g of air–dried powder of the soil matrices and FMNs for 1 h.

Oxides of macro–element (Fe_2O_3, MnO, CaO, K_2O, and P_2O_5) and trace elements (Co, Cr, and Pb) contents of the soil matrices and FMNs were determined via energy–dispersive X–ray fluorescence spectrometry (EDX) using a Shimadzu EDX–800HS–P instrument as previously described by Timofeeva et al. (2021, 2024). Eight certified reference standard soil samples were used to obtain calibration curves and assess the analytical recovery and precision.

The organic carbon (OC) content of the dried (105 °C) soil matrices and FMNs was determined using a TOC–L analyser (Shimadzu) with a CSN configuration and a module for solid sample measurement, SSM–5000A. The total nitrogen (TN) content was analysed via a Flash 2000 elemental analyser (Thermo Scientific) with a CHNS configuration. Elemental data were analysed using the Eager Xperience software.

Elemental distribution maps were obtained from scanning electron microscope (MERLIN, Carl Zeiss with EDS INCA Energy 350 X–Max 150 (Oxford Instruments, Abingdon)).

2.6 X–ray diffraction analysis

The mineral compositions of whole FMNs were analysed using a Rigaku MiniFlex II X–ray diffractometer (Rigaku) from 2.5° to 60° 2Q. The International Centre for Diffraction Data 2010 database was used to determine the phases present in the FMNs based on the comparison of the diffraction patterns. A detailed description of the X–ray diffraction analysis used in this study was previously presented by Timofeeva et al. (2021).

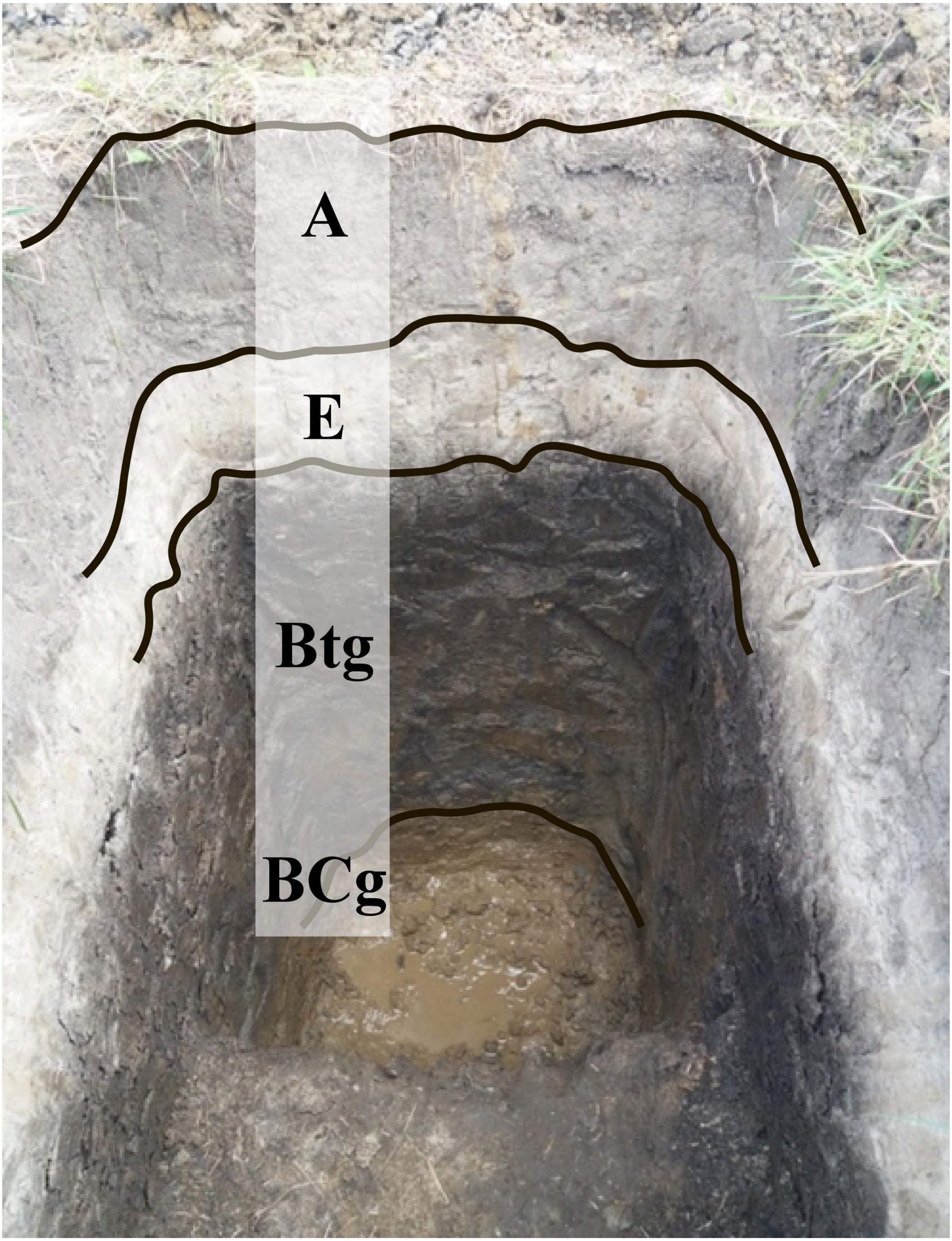

Fig. 2 View of the Gleyic Albic Luvisol (Manganiferric, Defferentic) profile

Table 1 Morphological characteristics of representative horizons of the Gleyic Albic Luvisol (Manganiferric, Defferentic)

Horizon	Color moist	Description
A	Gray 5YR 5/1	Homogenously colored; loamy sand; medium–strong moderate granular structure; hard; friable; found brown nodules; wavy boundary to
E	Light gray 5YR 7/1	Unevenly colored with a numerous fine distinct brown and reddish mottles; loamy; medium blocky subangular structure; slightly hard; very friable; numerous brown nodules; clear wavy boundary to
Btg	Dark reddish brown 5YR 3/2	Unevenly colored with a numerous oximorphic mottles; clay loamy; blocky subangular and angular structure; sticky; plastic; few medium gravel with cutans on pedfaces; numerous brown nodules; transitional boundary to
BCg	Reddish brown 5YR 5/4	Unevenly colored with a numerous oximorphic mottles and abundant fine soft iron–manganese accumulations in the upper part; clay; dense; sticky; plastic; medium weathered fragments of rocks

2.7 Data treatment and bioinformatics analysis

The raw reads were preprocessed using FLASH (V1.2.11) and QIIME (Version 1.9.1) (Magoč and Salzberg 2011). The chimeric sequences were removed with the vsearch package (V2.16.0) (Edgar et al. 2011). The obtained sequences clustered into OTUs with more than 97% sequence identity. Representative OTUs were identified using Mothur method and the SILVA database (Edgar 2013).

The CCA was conducted using the Canoco (Version 4.5). Alpha diversity is applied to analyse the complexity of species diversity for a sample through 6 indices, including the observed species, Chao1, Shannon, Simpson, ACE and Good coverage indices. All these indices in our samples were calculated with QIIME (Version 1.9.1) and displayed with R software (Version 4.3.2). Histograms of relative abundance distributions (10 dominant taxa from each sample) were constructed through the SVG function. A heatmap and unweighted pair group based on the weighted UniFrac distance matrix were created to display beta diversity, which was calculated via QIIME software (Version 1.9.1). R (Version 4.3.2) was used to construct a heatmap showing 35 dominant families, and correlation analyses were performed between the relative abundance of identified families and the physicochemical parameters of the soil matrices and FMNs. FAPROTAX software was used to predict metabolic bacterial function.

Each chemical analysis was performed with three parallel probes to identify an adequate iteration number. The data were processed statistically (median value and regression analyses) using Microsoft Excel and SPSS software (SPSS Inc., Version 13, 2018).

3 Results

3.1 Nodule abundance, morphology and mineralogy

In the studied Luvisols, FMNs existed as hard compact spherical or ellipsoidal segregations distinctly separated from the host soil matrices, varying in size from 1 to 6 mm (Fig. 3a). In this study, we considered only FMNs with sizes of 2–4 mm, as they account for 88% of all FMNs. The vertical distribution of FMNs within the soil profiles was characterized by a consecutive increase from the upper to the lower soil horizons (Table 2). Although gley features clearly occur in the Btg horizon, the abundance of FMNs indicates that differences in redox potential levels during dry/wet periods are sufficient for the formation of FMNs.

The examined FMNs had uneven surface brown and reddish–brown colors. Scanning electron microscopy with elemental mapping demonstrated that the FMNs had an outer Fe–rich zone and an internal Mn–rich zone (Fig. 3b). The complex and irregular layers and antiphase effects between the distributions of Fe and Mn were identified in the internal structure of the FMNs. The distributions of C, Ca, K, and P were homogeneous, lacking visual signs of large enrichment areas within the FMNs (Fig. 3b). The FMNs also contain a few angular aluminosilicate grains.

In the internal zone of the FMNs, similar to bacterial cells, spherical microformations were also observed (Fig. 3c). These microformations have smooth surfaces with small cracks. The diameters of the microformations varied from 7 to 29 µm. Spherical microformations are located either separately or combined in "colonies" (Fig. 3d).

The results of the XRD analysis indicated the presence of a complex of primary (quartz, albite, and tephroite) and

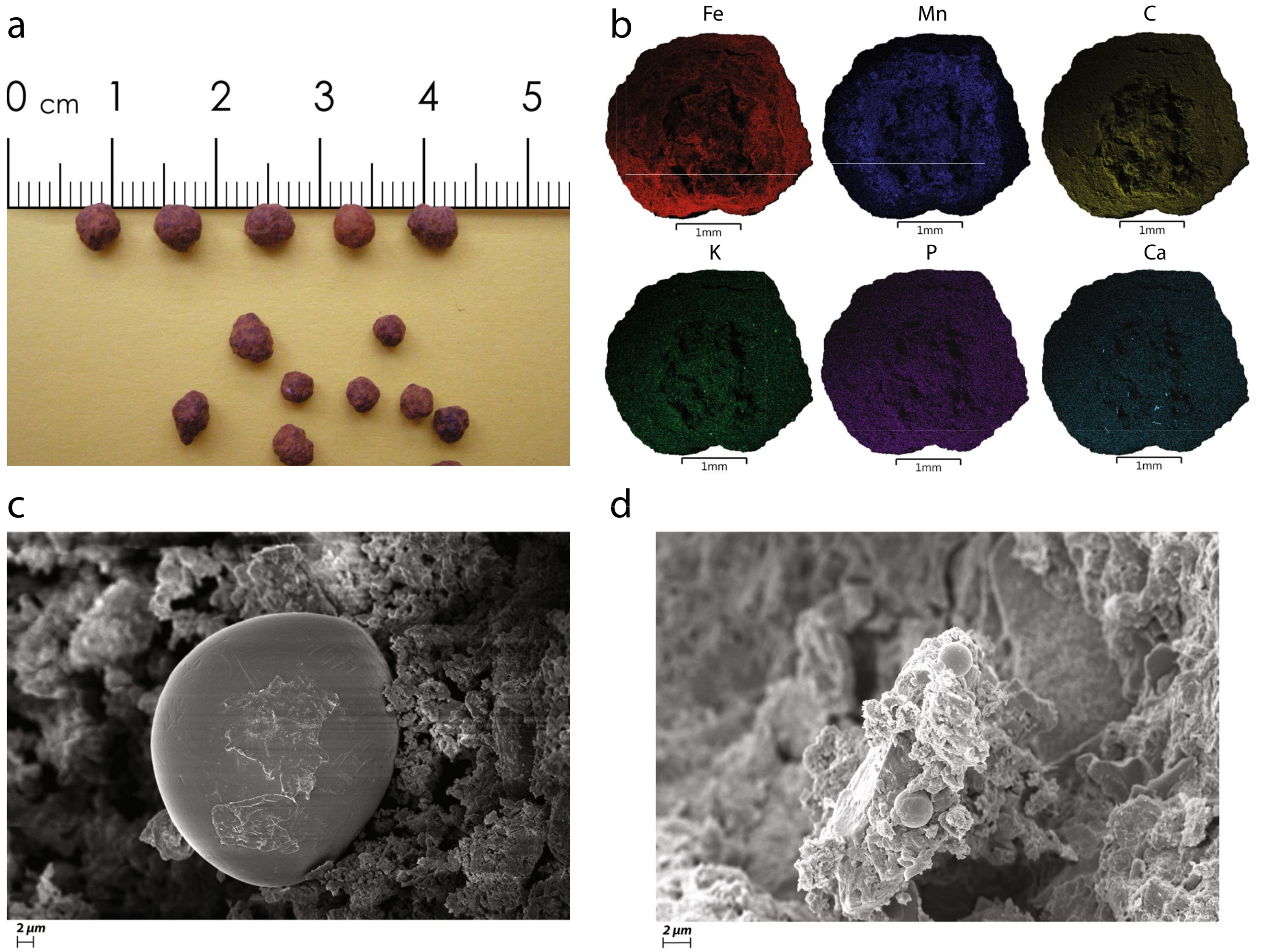

Fig. 3 Morphologies and element composition of typical nodules: a – photo of the representative nodules, b – elemental distribution maps of within the opened nodule, c, d – spherical microformations similar to bacterial cells within the nodules

Table 2 Physicochemical parameters of nodules and soil matrices and the abundance of nodules in different horizons of the Gleyic Albic Luvisol (Manganiferric. Defferentic)

Parameter	Horizon					
	A		E		Btg	
	Soil matrix	Nodules	Soil matrix	Nodules	Soil matrix	Nodules
pH (H_2O)	5.93±0.15*	5.38±0.11	6.03±0.18	5.62±0.13	5.21±0.10	4.54±0.11
SOC (%)	2.78±0.08	2.51±0.07	0.11±0.002	0.78±0.004	0.17±0.001	0.29±0.001
TN (%)	0.51±0.006	0.36±0.002	0.014±0.001	0.013±0.001	0.012±0.001	0.005±0.0001
CaO (%)	0.87±0.03	0.65±0.02	0.67±0.02	0.51±0.01	0.86±0.03	0.73±0.03
K_2O (%)	2.13±0.07	1.77±0.06	2.00±0.06	1.69±0.05	2.14±0.08	2.03±0.06
P_2O_5 (%)	0.12±0.006	0.08±0.002	0.13±0.007	0.11±0.005	0.13±0.006	0.14±0.006
Fe_2O_3 (%)	3.82±0.15	24.17±0.73	2.97±0.12	25.56±0.78	5.31±0.22	15.06±0.41
MnO (%)	0.10±0.003	0.81±0.026	0.03±0.001	0.79±0.022	0.09±0.003	3.90±0.092
MnO/Fe_2O_3	0.03±0.001	0.03±0.001	0.01±0.001	0.03±0.001	0.02±0.001	0.24±0.003
Pb (mg kg^{-1})	21.36±0.65	106.36±5.28	22.43±0.61	143.97±6.27	28.39±0.74	171.43±8.27
Co (mg kg^{-1})	30.52±0.61	55.79±1.54	32.83±0.72	61.03±1.12	18.05±0.43	25.99±0.50
Cr (mg kg^{-1})	61.25±1.53	118.62±5.07	63.42±1.39	146.97±5.89	78.32±2.62	121.14±4.28
Nodule abundance (g kg^{-1})	24.83±0.83		128.39±5.99		204.56±11.40	

[*] Mean values±standard deviations, n within horizon=12

clay (kaolinite and muscovite) minerals and iron (goethite and feroxyhyte) and manganese (birnessite) minerals in the studied FMNs (Fig. S1).

3.2 Nodule and soil matrix chemistry

The physicochemical parameters of the FMNs and host soil matrices are presented in Table 2. These parameters are selected on the basis of their relationship with the diversity and composition of soil bacterial communities, which have been reported in different studies (Yang et al. 2023; Cahyani et al. 2007; Hua et al. 2024). Additionally, trace elements (Pb, Co, and Cr) in FMNs are commonly associated with Fe– and Mn–rich compounds and most likely may influence the bacterial communities of FMNs.

The acidity of the soil matrices varied from slightly acidic (horizons A and E) to acidic (horizon Btg). The levels of most studied elements in the soil matrices varied with depth. The quantitative distributions of the SOC and TN contents in the soil matrix were characterized by an abrupt decrease from the surface horizon to the underlying layers. The contents of Ca and Mn oxides were the highest in the soil matrix of the upper horizons; however, a pronounced secondary peak of these elements was found in the soil matrix of the Btg horizons. The contents of K and Fe oxides in the soil matrix also had two concentration peaks (horizons A and Btg), but the maximum contents were found in the lower horizon. The distribution of P oxide in the soil matrices throughout the soil profiles was homogeneous. Other trends in the vertical element distributions were found for trace elements (Pb, Cr, and Co). The contents of Pb and Cr gradually increased in the soil matrix with depth. The soil matrix of the studied soils had a relatively distinct Co content peak in the E horizon.

Compared with the host soil matrices, the FMNs have a lower pH. Nodules from all horizons were significantly enriched in Fe_2O_3, MnO, Pb, Co, and Cr compared with the host soil matrices. The contents of these elements in FMNs formed in different soil horizons were different. The maximum Fe_2O_3, Co and Cr concentrations were observed in FMNs formed in the E horizon. The contents of MnO and Pb were highest in FMNs formed in the underlying horizon Btg. According to the mentioned Fe– and Mn–rich compound distributions, in the FMNs from the Btg horizon, the Mn/Fe ratio was maximal and reached 0.24. Nodules from the Btg horizon were also characterized by enrichment by P_2O_5. Nodules with the highest levels of SOC were located within the upper A horizon. The content of SOC in the FMNs decreased with increasing soil depth; however, the FMNs formed in the E and Btg horizons accumulated SOC. Compared with those in the soil matrix, the FMNs from all the studied horizons were depleted of Ca and K oxides and TN.

3.3 Bacterial diversity and community structure in soil matrices and FMNs

The V3–V4 regions of the 16S rRNA gene were sequenced by next–generation sequencing (NGS). Sequencing of the soil matrix and nodule samples from three soil horizons yielded a total of 604,891 16S paired–end reads. After quality filtering and chimera sequence removal, a total of 591,380 effective sequences were obtained from all the studied samples. The filtered reads were clustered into operational taxonomic units (OTUs), which were used for further analysis of alpha and beta bacterial diversity.

The analysis of alpha biodiversity indices showed that the predicted bacterial community (ACE) diversity of the soil matrix exceeded the ACE value of the FMNs in all studied soil horizons (Table 3). According to the OTUs, the number of directly observed bacterial species suggested this pattern. The variation in the alpha diversity indices (ACE, Shannon, Chao1, and Simpson) and the OTU values indicated a decrease in bacterial diversity in both the soil matrix and FMNs with increasing soil depth (Table 3).

On the basis of the taxonomic analysis, a total of 48 phyla, 122 classes, 238 orders, and 316 families were identified in all the studied samples. The top 10 taxa from each sample at each taxonomic rank (type, class) were selected for further analysis of the relative abundance distribution of the taxa (Fig. 4). Proteobacteria, Actinobacteria, and Acidobacteria were the predominant phyla in the most studied soil matrices and FMNs. The relative abundance of Proteobacteria (from 11 to 91%), Actinobacteria (from 2 to 23%), and Acidobacteria (from 1 to 22%) varied within the soil profile. The vertical distribution of the relative abundance

Table 3 Alpha diversity indices of bacterial communities from the soil matrices and nodules at the OTU level

Horizon	Object	Alpha diversity indices				
		ACE index	Observed_species	Shannon index	Chao1 index	Simpson index
A	Soil matrix	1999.749	1785	8.158	2002.191	0.982
	Nodules	1559.847	1373	7.673	1581.509	0.982
E	Soil matrix	1230.474	1203	6.734	1239.513	0.878
	Nodules	555.828	531	7.205	570.6	0.986
Btg	Soil matrix	86.003	72	1.949	99.5	0.525
	Nodules	73.407	63	1.94	68	0.574

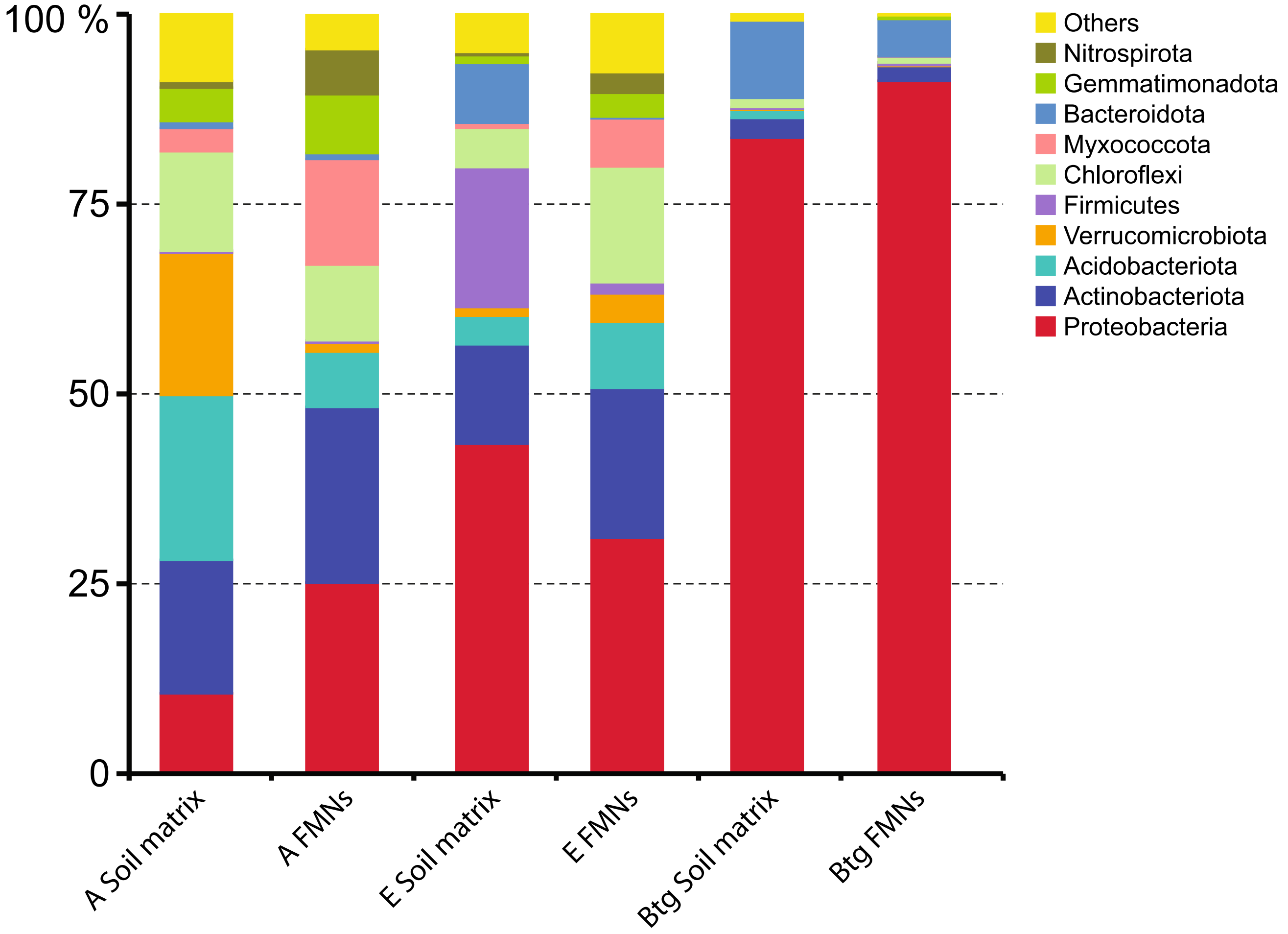

Fig. 4 Relative abundance of the dominant 10 phyla in the soil matrix and nodule samples

of Actinobacteria and Acidobacteria decreased with increasing soil depth, whereas the relative abundance of Proteobacteria increased significantly with increasing soil depth. In addition, a high abundance of Verrucomicrobiota (19%) was detected in the soil matrix from the upper part of the profile. In the soil matrix from the middle horizon, a high relative abundance of Firmicutes (18%) was identified. The soil matrix and nodule samples from the upper and middle horizons contained a prominent relative abundance of Chloroflexi (5–13% and 10–15%, respectively), Myxococcota (1–3% and 6–14%, respectively), and Gemmatimonadota (1–4% and 3–8%, respectively). The soil matrices and FMNs from the lower part of the soil profiles characterized increase relative abundance of Bacteroidota (10% and 5%, respectively).

The most abundant classes in the soil matrix and FMNs were Gammaproteobacteria (5–89%), Alphaproteobacteria (2–18%), and Thermoleophilia (1–14%) (Fig. 5). The relative abundance of Gammaproteobacteria increased with soil profile depth, and the highest abundance was found in the soil matrix and FMNs from the Btg horizon. The relative abundance of Alphaproteobacteria and Thermoleophilia in the FMNs was significantly higher than those in the host soil matrix. The highest Alphaproteobacteria and Thermoleophilia abundance was identified in the FMNs formed in the upper and middle parts of the soil profile. Additionally, the FMNs formed in the upper and middle parts of the soil profile were characterized by higher relative abundance of Myxococcia (6–13%), Ktedonobacteria (5–8%), and Gemmatimonadetes (3–8%) than those in the host soil matrix. At the same time, the relative abundance of Verrucomicrobiae (1–19%), Bacteroidia (1–10%), and Acidobacteriae (1–8%) was higher in the soil matrix samples. The Verrucomicrobiae and Acidobacteriae were relatively concentrated in the soil matrix from the upper horizons, while the abundance of Bacteroidia increased significantly with soil depth (Fig. 5**)**.

The relative abundance of the top 35 families in the studied samples is represented in the heatmap (Fig. 6). The dominant bacterial families were different in the soil matrix and nodule samples throughout the studied soil profiles. Chthoniobacteraceae and Solibacteraceae were predominant in the soil matrix from the upper horizon (A), while

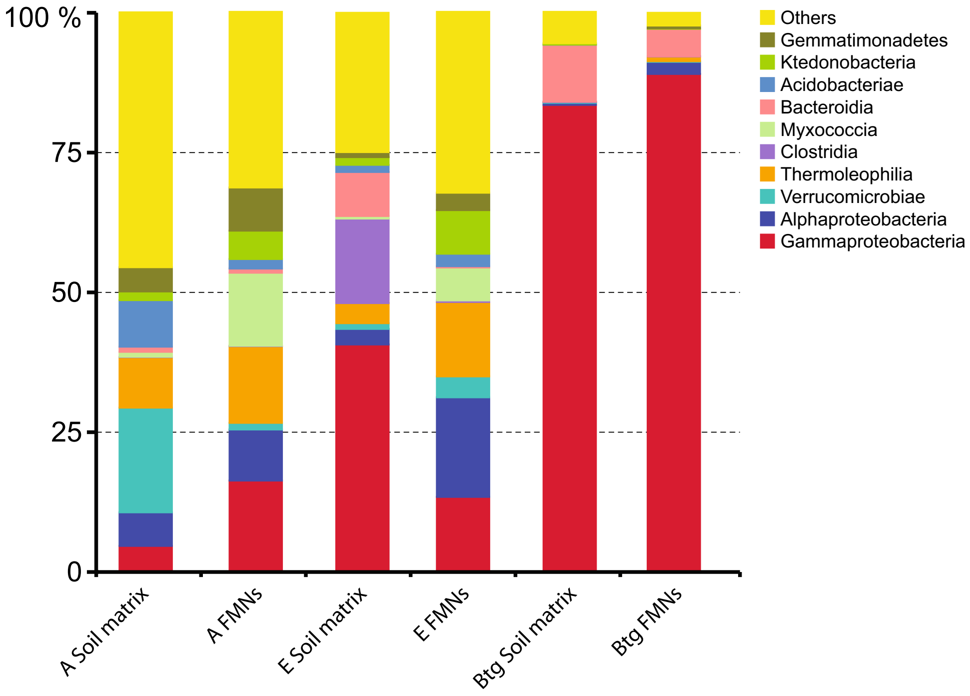

Fig. 5 Relative abundance of the dominant 10 classes in the soil matrix and nodule samples

in the FMNs from this horizon, Oxalobacteraceae, Geodermatophilaceae, and Burkholderiales (B1−7BS) were predominant. In the soil matrix of the middle soil horizon (E), Nocardiaceae, Sporichthyaceae, Muribaculaceae, Enterobacteriaceae, Lachnospiraceae, and Lactobacillaceae were predominant, whereas the FMNs formed in this horizon were characterized by the predominance of Nitrospiraceae, Reyranellaceae, Hyphomicrobiaceae, and Rhizobiales (Amb−16S−1323). In the lower part of the soil profile, the compositions of the dominant families identified in the soil matrix and FMNs were similar and included Comamonadaceae, Burkholderiaceae, and Weeksellaceae. The relative abundance of the dominant families decreased in the soil matrices and FMNs with increasing soil depth.

The results of the weighted UniFrac and unweighted UniFrac analyses also suggested significant differences in bacterial diversity between the soil matrix and FMNs from the upper and middle parts of the soil profile (Fig. 7). In the lower part of the soil profile, the difference in bacterial diversity between the soil matrix and FMNs was relatively small.

Additionally, the results of the cluster analysis integrated with the relative abundance (UPGMA) of the top 10 dominant bacterial phyla that the bacterial community compositions of the soil matrix and FMNs from the lower part of the soil profile formed a distinct clade (Fig. 8). Nodule samples formed in the upper and middle parts of the soil were united into a single cluster. The samples of FMNs and the soil matrix from the upper horizons did not unite into a common clade, but significant differences in the composition of the bacterial community between FMNs and the soil matrix were not observed.

3.4 Correlations between physicochemical parameters of soil matrices and FMNs and bacterial relative abundance

Canonical correspondence analysis of the physicochemical characteristics of the soil matrices and FMNs and the bacterial community OTU abundance in the different horizons is shown in Fig. 9a, b. The results showed that the first and second ordination axes of the CCA explained 94.9% and 72.7%, respectively, of the bacterial community changes in the soil matrix (Fig. 9a). These values were lower in FMNs; the first axis of the CCA diagram explained 91.9% of the data, and the second axis accounted for 27.5% (Fig. 9b). Most of the selected parameters significantly affected the bacterial community distribution. However, the main characteristics affecting the bacterial community distribution varied among the different soil horizons. The bacterial community distribution in the soil matrix from the upper soil horizon (A) was obviously positively correlated with the contents of SOC, TN, K_2O, MnO, and the Mn/Fe ratio. In the soil matrix of the middle horizon (E), none of the selected factors had a significant influence on the bacterial community distribution. The bacterial community distribution in the soil matrix of the lower horizon (Btg) was related to the contents of Fe_2O_3, Cr, and Pb. The acute angles of arrow rays between SOC and TN, and between Fe_2O_3 and Cr indicate the combined influence of pair parameters on

Fig. 6 Heatmap of the relative abundance of the dominant 35 families in the soil matrix and nodule samples

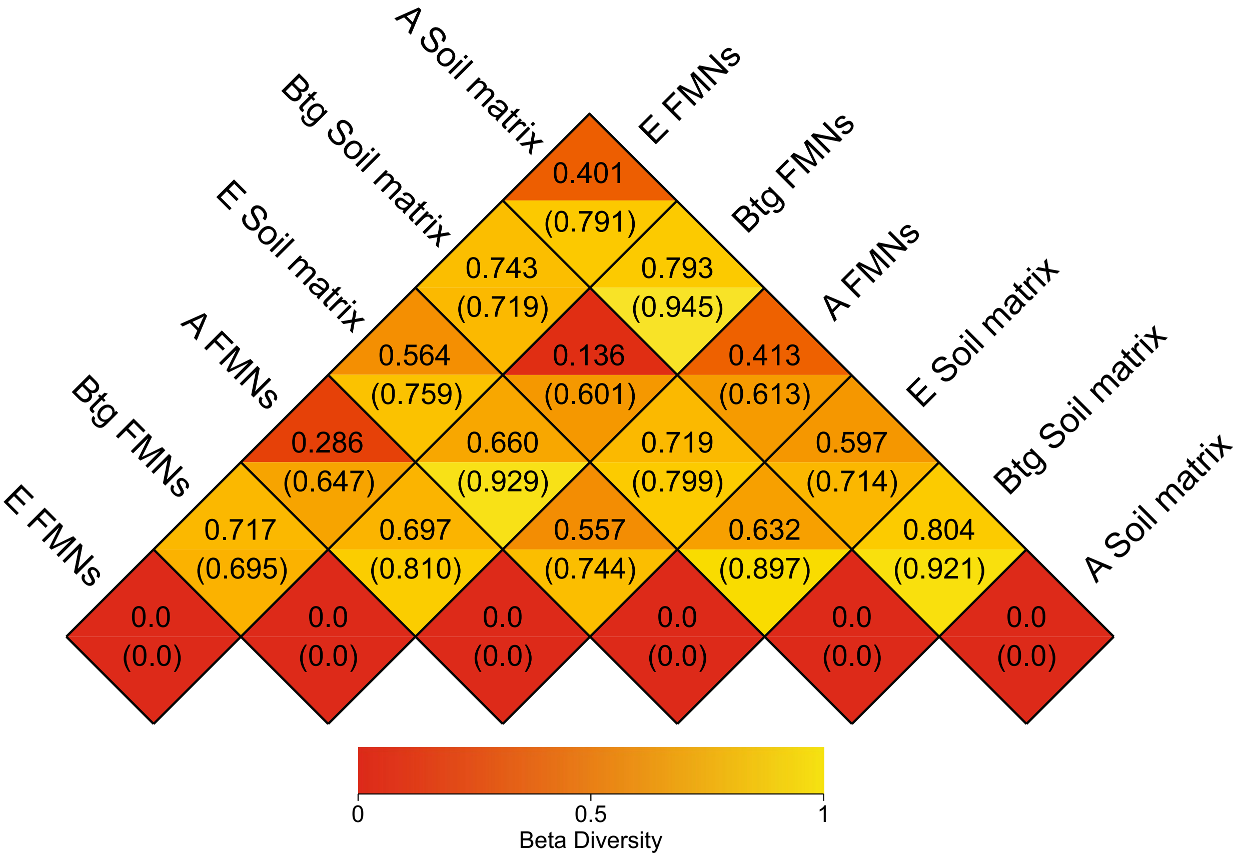

Fig. 7 Beta diversity of soil matrices and nodules bacterial community (weighted and unweighted UniFrac)

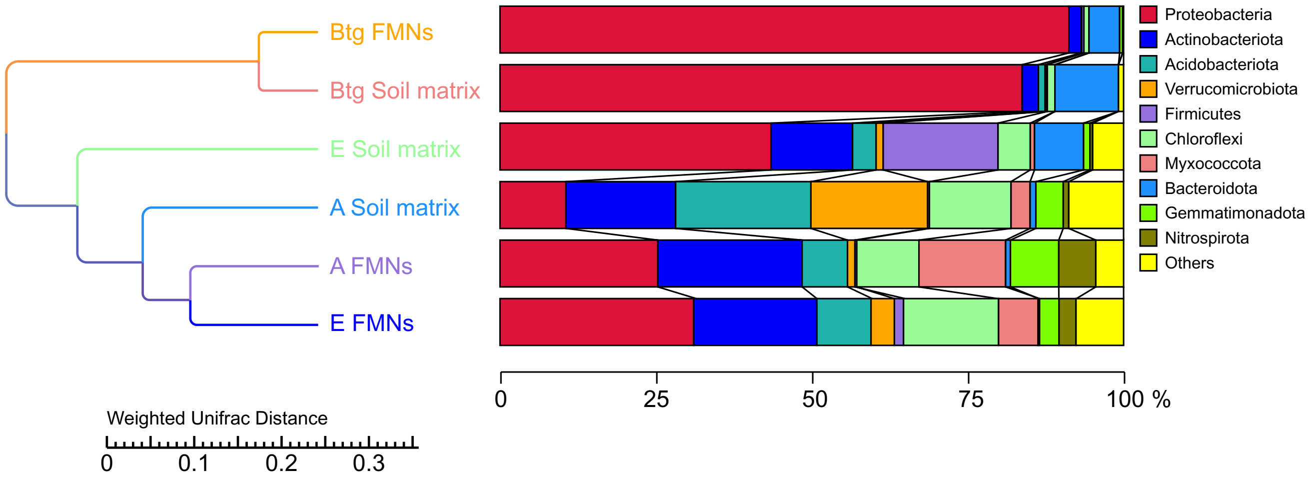

Fig. 8 UPGMA clustering tree based on weighted UniFrac distance and maps of phyla relative abundance

the distribution of the bacterial community. The bacterial community distribution of FMNs is controlled by a lesser amount of studied parameters compared with the soil matrix. The main parameters affecting the distribution of the bacterial community in FMNs from the A horizon are SOC and TN. In FMNs from the Btg horizon, the bacterial community is a multicollinear complex consisting of MnO, Pb, and the Mn/Fe ratio. Our results demonstrated that, as well as in the soil matrix, the distribution of the bacterial community in FMNs formed in the E horizon was not significantly associated with the studied parameters.

According to the results of the correlation analysis between the relative abundance of bacterial families and the physicochemical characteristics of the

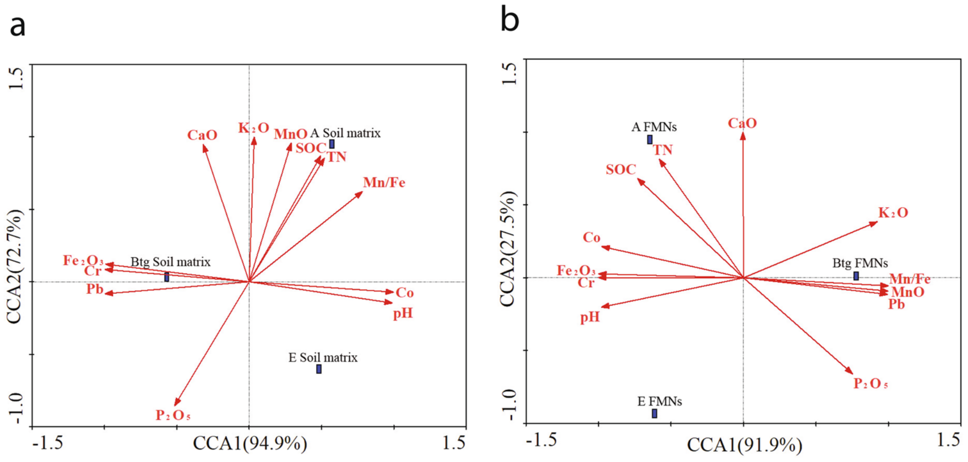

Fig. 9 Canonical correspondence analysis (CCA) scatter plot representing the influence of physicochemical parameters on bacterial community: a – soil matrices, b – nodules

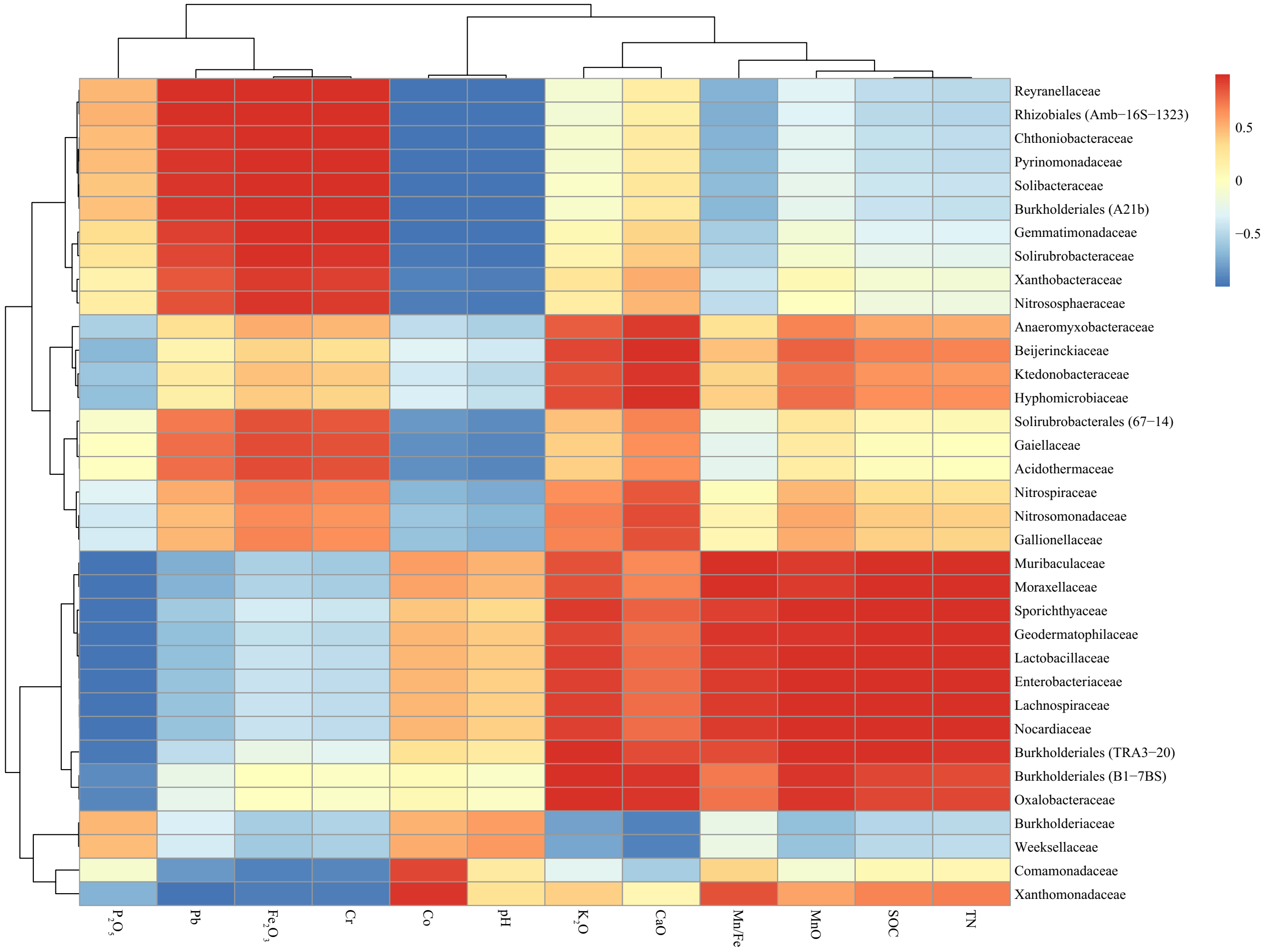

Fig. 10 The correlation heatmap between relative abundances of the dominant bacterial families and physicochemical parameters of the soil matrices

soil matrices and FMNs, the bacterial community was divided into four groups in the soil matrix and four groups in the FMNs (Fig. 10; Fig. 11). The first bacterial group in the soil matrix exhibited extremely significant correlations with the contents of MnO, SOC, TN, CaO, K$_2$O, and with the Mn/Fe ratio (Fig. 10). This group consists of Muribaculaceae, Moraxellaceae, Sporichthyaceae, Geodermatophilaceae, Lactobacillaceae, Enterobacteriaceae, Lachnospiraceae, Nocardiaceae, Burkholderiales (TRA3−20), Burkholderiales

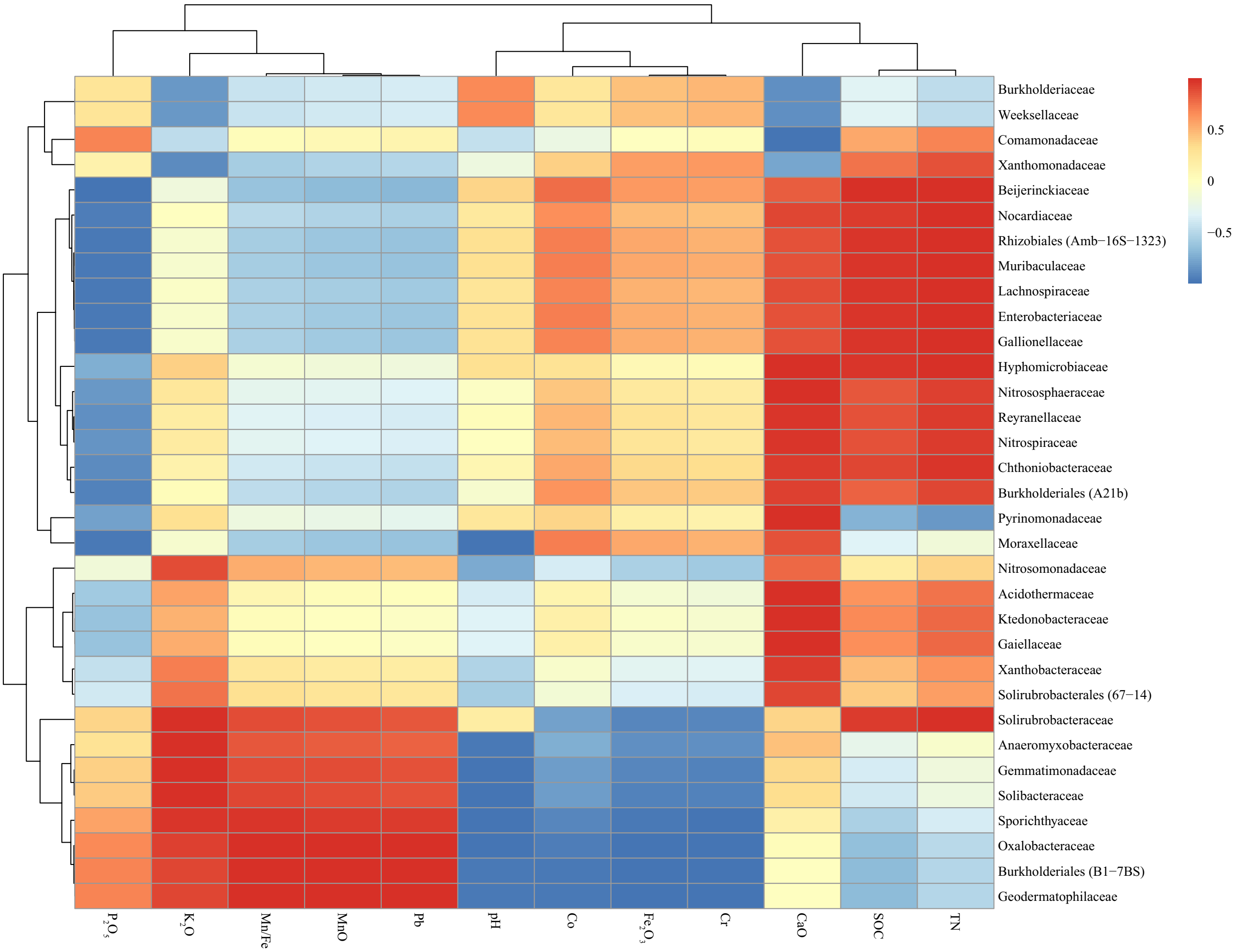

Fig. 11 The correlation heatmap between relative abundances of the dominant bacterial families and physicochemical parameters of the nodules

(B1 − 7BS), Oxalobacteraceae, Anaeromyxobacteraceae, Beijerinckiaceae, Ktedonobacteraceae, and Hyphomicrobiaceae families. Notably, the abundances of the Anaeromyxobacteraceae, Beijerinckiaceae, Ktedonobacteraceae, and Hyphomicrobiaceae families were not significantly correlated with the Mn/Fe ratio. The significant parameters for the relative abundance of the second bacterial group presented by the families Reyranellaceae, Rhizobiales (Amb − 16S − 1323), Chthoniobacteraceae, Pyrinomonadaceae, Solibacteraceae, Burkholderiales (A21b), Gemmatimonadaceae, Solirubrobacteraceae, Xanthobacteraceae, Nitrososphaeraceae, Solirubrobacterales (67 − 14), Gaiellaceae, and Acidothermaceae were the Fe_2O_3, Cr, and Pb contents. The abundance of bacterial community in the third group (Nitrospiraceae, Nitrosomonadaceae, and Gallionellaceae families) was significantly correlated with the CaO content and positively correlated with the K_2O, Fe_2O_3, and Cr contents. The main peculiarity of the fourth bacterial group (Comamonadaceae and Xanthomonadaceae) was the increased influence of Co concentration on bacterial abundance.

The group differentiation of the bacterial community in the soil matrices and FMNs differed widely. In FMNs, the first bacterial group consists of the Solirubrobacteraceae, Anaeromyxobacteraceae, Gemmatimonadaceae, Solibacteraceae, Sporichthyaceae, Oxalobacteraceae, Burkholderiales (B1 − 7BS), and Geodermatophilaceae families (Fig. 11). The relative abundance of bacterial families in this group was significantly related to the contents of MnO, Pb, the Mn/Fe ratio, and K_2O content. The relative abundance of the bacterial families of this group was strongly negatively correlated with Fe_2O_3, Cr, and Co. The second bacterial group in FMNs included the Beijerinckiaceae, Nocardiaceae, Rhizobiales (Amb − 16S − 1323), Muribaculaceae, Lachnospiraceae, Enterobacteriaceae, and Gallionellaceae families. Correlation analysis has demonstrated that the abundance of the bacterial community from the second group was significantly controlled by the contents of SOC, TN, and CaO and was less affected by the Fe_2O_3, Cr, and Co contents. The third bacterial group showed that the contents of SOC, TN, and CaO in FMNs were significantly positively correlated with the relative abundance of the Acidothermaceae, Ktedonobacteraceae, Gaiellaceae, Hyphomicrobiaceae, Nitrososphaeraceae, Reyranellaceae, Nitrospiraceae, Chthoniobacteraceae, and Burkholderiales (A21b) families. The fourth bacterial group in FMNs was related mainly to the CaO and K_2O contents and consisted of three families: Xanthobacteraceae, Solirubrobacterales (67 − 14), and Nitrosomonadaceae.

4 Discussions

Our data indicated that the differences in the composition of the bacterial communities were obvious both for the soil matrices and for FMNs formed in different parts of the profile of the same soil. Proteobacteria, Actinobacteria and Acidobacteria were the predominant bacterial phyla in the studied soil matrix and nodule samples. The same dominant bacterial phyla have been reported in soils and FMNs from North China to East China (Cahyani et al. 2007; Zhang et al. 2014). Additionally, Proteobacteria and Acidobacteria were the dominant bacterial phyla in FMNs and soil samples from Central China (He et al. 2008). The widespread occurrence of Proteobacteria, Actinobacteria, and Acidobacteria in soils and soil neoformation indicates the metabolic versatility of these taxa. The increase in the relative abundance of Actinobacteria in the soil matrix and FMNs from the upper horizons is consistent with the involvement of this phylum in the decomposition of complex polymeric organic substrates (chitin and lignin) and in the process of organic soil compounds mineralization (Chernitsyna et al. 2016; Lewin et al. 2016). In our study, this finding is in agreement with the dominance of the Thermoleophilia class belonged to the phylum Actinobacteria, whose ability to decompose both soil dissolved organic compounds and stable organo–mineral complexes and mineralize soil organic matter was shown earlier (Yang et al. 2023). Our results demonstrate that Thermoleophilia can decompose and mineralize organic–rich compounds in FMNs. The soil matrix and FMNs of the upper horizons contain high content of nutrients available for these bacteria, resulting in active bacterial growth (Timofeeva and Purtova 2024). Most Acidobacteria are oligotrophic bacteria and occur in soils with low nutrient content (Conradie and Jacobs 2020). However, some representatives of this phylum may participate in the degradation of soil organic matter (Catão et al. 2014; Vélez-Martínez et al. 2024). The dominance of the Acidobacteriae class belonged to the phylum Acidobacteria in the soil matrix and FMNs from upper horizons confirmed their participation in the transformation of organic matter and the decomposition and mineralization of organic residues. The Proteobacteria phylum is known to dominate in soils with high organic matter content (Yang et al. 2022; Yaghoubi Khanghahi et al. 2024). However, our results demonstrate an inverse trend. The relative abundance of Proteobacteria in the soil matrices and FMNs increased as the SOC content decreased. The dominance of different classes belonged to the phylum Proteobacteria in the studied soil matrices and FMNs is attributed more to the fractional composition of organic matter, as was also suggested by Yang et al. (Yang et al. 2023). A high relative abundance of Gammaproteobacteria was identified in the samples from the lower horizons. Our previous studies demonstrated the predominant fulvic acid fractions in the composition of organic matter in the soil matrix and FMNs from the lower part of the soils with similar main soil–forming factors and processes (Timofeeva and Purtova 2024). Most likely, in the studied soil matrix and nodule samples, organic compounds with low molecular weights (fulvic substance) are more readily available for transformation by Gammaproteobacteria, while more stable organic compounds presented mainly as humic substance and humin are more essential factors affecting the relative abundance of Alphaproteobacteria dominating the upper and middle parts of the soil profiles. The maximum abundance level of Alphaproteobacteria was found in the FMNs formed in the middle horizons, which may be due to the higher content of SOC in the FMNs than in the soil matrix and the highest levels of SOC accumulation in the FMNs of these horizons. The classes Gammaproteobacteria and Alphaproteobacteria include bacteria that are capable of Mn oxidization (Cahyani et al. 2007; Tebo et al. 2004). The profile distribution of the relative abundance of Gammaproteobacteria and Alphaproteobacteria and the contents of Mn in the soil matrices and FMNs indicate greater involvement of these bacterial classes in the Mn biogeochemical cycles in the soil matrix samples (Table 2; Fig. 10; Fig. 11). Most likely, in the lower soil horizons with a longer reducing regime period, Gammaproteobacteria oxidizes Mn–containing compounds and precipitates Mn (hydr)oxides on the cell surface, promoting the formation of microzones with oxidation–reduction potential differing from that of the host soil mass, which may contribute to the formation of proto–FMNs.

The relative abundance of Verrucomicrobia, Firmicutes, Chloroflexi, Myxococcota, Gemmatimonadota and Bacteroidota was also significant in the different soil matrix and nodule samples. In general, the Verrucomicrobiota phylum is often identified in bacterial communities of different soil types (Borsodi et al. 2024). However, the factors influencing their diversity and abundance in the soil environment are currently unclear. Some representatives of the Verrucomicrobiota phylum prefer acidic soil environments (Willms et al. 2021). This confirms the presence of Verrucomicrobiae belonged to the phylum Verrucomicrobiota, in the studied slightly acidic and acidic samples of the soil matrix and FMNs. The high relative abundance of the classes Ktedonobacteria of the Chloroflexi phylum and Myxococcia of the Myxococcota phylum in the soil matrices and FMNs from the upper and middle parts of the soil profile are most likely related to their ability to grow both in nutrient–rich and nutrient–poor soil environments and survive by generating energy through solar radiation (Yabe et al. 2017; Lan et al. 2022). Additionally, organic–poor microenvironments in the middle and lower parts of soil profiles are favourable for the growth and reproduction of bacteria that are capable of oligotrophy or r–strategy, such as the Firmicutes and Bacteroidota phyla (Liu et al. 2023a, b; Borsodi et al. 2024). On the basis of the vertical distribution of bacterial abundance, the lower horizons constitute the optimal zone for the growth and development of the Bacteroidia class (belonged to the phylum Bacteroidota) in the soil matrix and FMNs. Soil–specific studies have demonstrated that an increase in the relative abundance of the Gemmatimonadota phylum is found in soil microenvironments with relatively low SOC content (Hua et al. 2024). Our results did not support this finding because the maximum relative abundance of Gemmatimonadetes belonged to the phylum Gemmatimonadota, was located in the soil matrix and nodule samples from the upper horizons containing the highest level of SOC (Table 2).

The alpha– and beta–diversity of the bacterial communities also significantly differed between the samples from the different horizons of the studied soils. The decrease in bacterial diversity and abundance of the most identified phyla with soil depth is explained mainly by the decreased content of organic matter and nutrients, increased acidity and accumulation of the clay fraction, and more long–term waterlogging periods in the lower soil horizons of the studied soil. This finding was also supported by the results of UniFrac (weighted) and UPGMA analyses. Based on this and the vertical distribution of the relative abundance of the predominant phyla in the studied soil matrices and FMNs, we may suggest that the Proteobacteria phylum is more stable to variations in conditions in the soil environment. Beta diversity analysis showed differences between the bacterial community of FMNs and the host soil matrix from the same horizon. According to the UniFrac (weighted) analysis, the highest difference was identified in the upper and middle soil horizons, whereas in the lower horizons, the difference between the bacterial community of the FMNs and the host soil matrix was significantly lower. This might be explained both by the vertical variation in organic matter and nutrient contents in the soil matrix and FMNs and by the differences in the level of redox potential between the soil matrix and the surface of FMNs. Compared with the host soil matrix, FMNs are localized zones with a higher redox potential (Kostenkov 1987; Gasparatos et al. 2019). More contrasting values of redox potential between the soil matrix and the surface of FMNs were mentioned in the upper and middle horizons of the soil, with similar main soil–forming processes. (Kostenkov 1987). A longer period with reducing conditions in the lower horizon resulted in a decrease in the difference in redox potential between the host soil matrix and the surface of FMNs and the formation of a bacterial community of similar composition.

Our data showed that differences in the physicochemical characteristics of the soil matrices and FMNs can affect the adaptation of bacterial communities to different microenvironmental conditions. Correlation analyses revealed the capability of the same bacterial families to transform different complex compounds in the soil matrix and FMNs.

The results of our studies have shown that the abundance of thirteen families was associated mainly with the contents of Fe_2O_3, Cr, and Pb in the soil matrix (Fig. 10). This relationship may be explained by the presence of iron ions in many enzymes involved in basic biological processes (respiration, central metabolism, and DNA reparation) (Andreini et al. 2008; Frawley and Fang 2014). The formation of Fe–Cr complexes in the soil matrix influences the involvement of Cr in the process of microbiological transformation, which was also suggested by the significant contribution of Fe–reducing bacteria to the reduction of Cr ions in the different soils (Xiao et al. 2014). In general, the relationship between the abundance of the identified families and the Pb content was based on the different mechanisms of Pb biosorption (on the cell surface, on the extracellular polymers, and substances), bioaccumulation (by metallothioneins), and precipitation (Tiquia-Arashiro 2018). However, on the basis of the mentioned group differentiation of the bacterial community, most likely, in the studied soil matrices, the predominant process is the microbiological chelation of Pb by siderophores, which effectively bind Pb^{2+} and reduce Pb mobility in the soil (Tiquia-Arashiro 2018). The nodule parameters controlling the abundance of the thirteen mentioned families significantly changed. The variation in the influencing parameters indicates the metabolic universality of the mentioned families. The abundance of Reyranellaceae, Rhizobiales (Amb−16S−1323), Chthoniobacteraceae, Nitrososphaeraceae, Acidothermaceae, Gaiellaceae, and Burkholderiales (A21b) was associated mainly with the contents of CaO, SOC, and TN, which is likely related to the participation of such families in the process of organic matter transformation and carbon stabilization in FMNs. Based on the composition of relative parameters, the abundance of these families in FMNs might be explained by the association of bacteria with Ca–bound humic and fulvic acids, which were identified in FMNs of the region under study (Timofeeva and Purtova 2024). The abundance of Solibacteraceae, Gemmatimonadaceae and Solirubrobacteraceae was affected by the MnO, K_2O and Pb contents, and the Mn/Fe ratio in FMNs. This may be attributed to the increase in the requirement of these bacteria for Mn^{2+} under oxidative stress (Vandieken et al. 2012; Bosma et al. 2021). Compared with the host soil matrices, FMNs are more oxidized microzones with a predominance of reduction processes in soil (Gasparatos et al. 2019; Huang 2022; Huang et al 2023). The contents of Pb and K are mainly governed by Mn–rich compounds in the studied FMNs (Fig. 11). Most likely, Solibacteraceae, Gemmatimonadaceae, and Solirubrobacteraceae used Pb and K in the composition of complex compounds with Mn. The abundance of the Solirubrobacteraceae family in FMNs was related to the larger number of studied parameters (MnO, Mn/Fe, K_2O, Pb, SOC, and TN), which may be due to the more high metabolic versatility. The relative abundance of the Pyrinomonadaceae, Xanthobacteraceae, and Solirubrobacterales (67−14) families was positively correlated with the content of CaO in FMNs. This may explain the role of Ca ions and Ca–binding proteins in the physiological adaptations of bacteria in response to changes in the environment and the volume and composition of available nutrients (Domínguez et al. 2015).

For some bacterial families in the correlation group associated with Mn in the soil matrix, changes in parameters controlling their abundance in FMNs were found. Previous studies have shown that Mn cations are involved in essential bacterial physiological processes such as metabolism, photosynthesis, antioxidation, and protection from environmental insults (Tebo et al. 2005; Hansel 2017; Bosma et al. 2021). Among the fifteen Mn–transforming bacterial families in the soil matrix, only five families (Anaeromyxobacteraceae, Oxalobacteraceae, Burkholderiales (B1−7BS), Geodermatophilaceae, and Sporichthyaceae) participate in the Mn transformation process in FMNs. Most likely, these families may consider manganese–centric bacteria that use Mn as a cofactor for the formation of diverse enzymes. However, analysis of literature data has shown that the five mentioned bacterial families have not been previously attributed to manganese–centric bacteria. The abundance of four other bacterial families (Enterobacteriaceae, Lachnospiraceae, Nocardiaceae, and Beijerinckiaceae), related to Mn–transforming processes in the soil matrices, in FMNs was associated mainly with C–, N– and Ca–containing compounds and less with Fe–rich compounds and Fe–bounded Cr and Co ions. These results might indicate that Mn–rich nodule compounds are not suitable for inclusion in intracellular processes by these bacterial families. Although manganese compounds are energetically much more favourable electron acceptors than iron compounds are for bacteria, most likely in FMNs, these four bacterial families use Fe ions for the synthesis of diverse enzymes involved in central carbon metabolism. Additionally, the presence of Co in the composition of Fe–rich complex compounds in FMNs is a competitive advantage for bacterial growth due to the possibility of remobilizing Co from complex compounds and subsequent Co involvement in the process of producing enzyme cofactors (cobamides) in cobalamin (vitamin B12). The abovementioned four bacterial families refer to taxonomic bacterial classes known as cobamide producers (Zhang et al. 2009; Shelton et al. 2019; Dulay et al. 2020). The main parameters controlling the abundance of Ktedonobacteraceae, Hyphomicrobiaceae, Muribaculaceae, and Moraxellaceae in FMNs were C–, N–, and Ca–containing compounds. The involvement of such families in the process of transformation of organic materials, both in the soil matrices and in FMNs, may indicate that the Mn concentration in the environment does not influence the growth and reproduction of these bacteria.

The peculiarities of two bacterial families (Nitrospiraceae and Gallionellaceae) grouped on the basis of their relationships with the K and Ca oxide contents in the soil matrices were their involvement in the process of transforming organic materials in FMNs. In general, K and Ca ions play regulatory role in the physiology of prokaryotes (Epstein 2003; Domínguez et al. 2015). These results might indicate that the involvement of Nitrospiraceae and Gallionellaceae in the process of organic matter transformation is based primarily on the presence of Ca in the organic complexes within the FMNs. The abundance of the Nitrosomonadaceae family was controlled by the K and Ca oxide contents both in the soil matrices and in FMNs, which indicates their resistance to changes in external microenvironmental factors.

The Comamonadaceae and Xanthomonadaceae family abundance showed similar variations under changes in the environment. The main activity of these bacterial families in FMNs is associated with organic compounds transformation, while in the soil matrices, the main parameter influencing the abundance of the two families is the content of Co, and for Xanthomonadaceae abundance, an additional parameter is the Mn/Fe ratio. These results demonstrate that Fe–Co complex formation in FMNs limits the participation of the Comamonadaceae and Xanthomonadaceae families in Co transformation.

In general, our results suggest flexible metabolism and high adaptive potential of the identified bacterial families, suggesting that many soil bacterial communities use multiple energy sources simultaneously or alternately (Liu et al. 2023a, b; Chen et al. 2021). Among the studied FMNs, most of the studied bacterial families were associated with either the process of organic matter transformation (sixteen families) or the transformation of Mn–rich compounds (eight families) (Fig. 11). We observed a negative correlation between the content of Mn oxides and the SOC and TN contents in FMNs (Fig. 11). This result might indicate the specialization of the nodules bacterial community in the transformation of organic and inorganic compounds. The FAPROTAX–based functional predictions showed that ten bacterial families associated with the SOC and TN contents in FMNs were chemoheterotrophs. According to the FAPROTAX functional assemblages, degradation of the primary complex organic compounds trapped from the soil matrix within FMNs might be promoted by the Xanthomonadaceae, Beijerinckiaceae, and Nocardiaceae families. Products of organic matter degradation reactions could be substrates for the growth of fermentative bacteria (Comamonadaceae, Lachnospiraceae, and Enterobacteriaceae families) capable of converting more simple organic compounds into organic acids, alcohols, and gases. Aerobic chemoheterotrophs (Hyphomicrobiaceae, Reyranellaceae, Chthoniobacteraceae, and Solirubrobacteraceae families) can be involved in the further decomposition of organic acids and alcohols. The function of the Nitrososphaeraceae family predicted with FAPROTAX was related to aerobic ammonia oxidation and nitrification in FMNs. For five bacterial families associated with organic compounds content in FMNs (Muribaculaceae, Gallionellaceae, Nitrospiraceae, Rhizobiales (Amb−16S−1323), and Burkholderiales (A21b)), functional groups were not identified via FAPROTAX analysis.

The manganese oxidation function was annotated only for the Geodermatophilaceae family associated with Mn–rich compounds in FMNs. Among the other bacterial families associated with mainly Mn–rich compounds in FMNs, the FAPROTAX analysis predicted that the function of the Oxalobacteraceae family was relevant to nitrogen fixation and ureolysis. The six bacterial families (Solirubrobacteraceae, Anaeromyxobacteraceae, Gemmatimonadaceae, Solibacteraceae, Sporichthyaceae, and Burkholderiales (B1−7BS)) associated with Mn–rich compounds in FMNs were not associated with any functional groups according to the FAPROTAX analysis results. According to the Mn distribution within the FMNs, we suggest that the optimal zones for the location and function of the Mn–transforming bacterial families were the internal zone of the FMNs, which preferentially occurring dehydration of the Mn–rich oxyhydroxides, and the microareas of the FMNs in the outer zone, which had high concentrations of newly precipitated Mn compounds. On the basis of the strong association of bacterial abundance with Mn–rich compounds, the identified bacterial families might influence the biologically controlled and biologically induced biomineralization of amorphous or weakly crystallized Mn–rich compounds of FMNs, as demonstrated by Ali et al. (2025). The probable result of Mn–rich compounds biomineralization could be the formation of lamellar pseudocrystalline structures presented by birnessite in the internal zone of FMNs (Fig. S1). Ali et al. (2025) reported that Mn–oxidizing bacteria promote the formation and growth of Mn–rich minerals by catalyzing the oxidation of Mn^{2+} to Mn^{4+} by bacterial enzymes in FMNs. In addition to the direct involvement of eight mentioned bacterial families in Mn transformation, these bacteria are involved in the processes of Pb transformation, which could influence the accumulation of Pb in FMNs and Pb cycling in soils.

The non–significant positive relationship between the abundance of seven bacterial families (Beijerinckiaceae, Nocardiaceae, Rhizobiales (Amb−16S−1323), Muribaculaceae, Lachnospiraceae, Enterobacteriaceae, and Gallionellaceae) and the content of Fe–rich compounds indicates an indirect influence of bacteria on Fe transformation in FMNs. On the basis of the specialization of these bacterial families in FMNs, bacteria most likely use iron ions that are released due to the degradation of Fe–organic complex compounds. Our previous studies identified the presence of Fe–organic compounds in FMNs (Timofeeva and Purtova 2024). The identified Mn–transforming bacterial families may also have an indirect impact on Fe–rich compounds in FMNs. Biogenically formed Mn–rich compounds in the internal zone of FMNs serve as ideal sites for the precipitation of Fe due to an increase in the oxidation rate of Fe^{2+}(Luo et al. 2018; Gasparatos et al. 2019). Additionally, this confirmed the presence of unstable slightly crystallized mineral (feroxyhyte) in the studied FMNs (Fig. S1). Feroxyhyte mainly form in FMNs during the combined biogenic deposition of oxidized Fe and Mn forms (Vodyanitskii and Sivtsov 2004).

5 Conclusions

The predominant bacterial classes in the studied soil matrix and nodule samples were Alphaproteobacteria and Gammaproteobacteria belonged to the phylum Proteobacteria; Thermoleophilia belonged to the phylum Actinobacteria; and Acidobacteriae belonged to the phylum Acidobacteria. The vertical differentiation of bacterial relative abundance related that Thermoleophilia and Acidobacteriae classes were mainly localized in the soil matrix and nodule samples from the upper horizons and the Alphaproteobacteria class in the samples from the upper and middle horizons, whereas the Gammaproteobacteria class significantly increased with increasing soil depth. Additionally, the soil matrices and FMNs from the upper and middle parts of the soil profiles were characterized by high relative abundance of phyla such as Verrucomicrobia (class Verrucomicrobiae), Firmicutes, Chloroflexi (class Ktedonobacteria), Myxococcota (class Myxococcia), and Gemmatimonadota (class Gemmatimonadetes). Despite the differences in the physicochemical characteristics and composition of the soil matrices and FMNs, the bacterial taxa specific to FMNs were not identified.

Our results showed that the biodiversity of the bacterial community in the FMNs was lower than the biodiversity of the soil matrices. The difference in bacterial communities between FMNs and the soil matrices was higher in the upper and middle parts of the soil profile and significantly decreased in the lower part of the soil profile. This finding provides evidence of the adaptation of bacterial communities to the unique microenvironment in FMNs.

The distribution of the bacterial community in FMNs was mainly controlled by the content of organic matter and Mn–rich compounds. The identified bacterial families associated with SOC–, TN–, and Ca–containing compounds were involved in different stages of organic matter transformation in FMNs (primary organic compound destructors (Xanthomonadaceae, Beijerinckiaceae, and Nocardiaceae), fermentative bacteria (Comamonadaceae, Lachnospiraceae, and Enterobacteriaceae), aerobic chemoheterotrophs (Hyphomicrobiaceae, Reyranellaceae, Chthoniobacteraceae, and Solirubrobacteraceae), aerobic ammonia oxidizers, and nitrifiers (Nitrososphaeraceae)). This process leads to the formation of stable organo–mineral complexes and significant accumulation of SOC in FMNs. According to the FAPROTAX functional assemblages, only one bacterial family (Gemmatimonadaceae) identified in the studied FMNs was related to the transformation of Mn. Our results showed that the Mn–transforming process and related compounds containing ions K and Pb in FMNs occurred with the direct participation of the Solibacteraceae, Anaeromyxobacteraceae, Gemmatimonadaceae, Solibacteraceae, Sporichthyaceae, Oxalobacteraceae, Burkholderiales (B1—7BS), and Geodermatophilaceae families. These bacterial families most likely promote the formation of FMNs. This finding indicated the capability of bacterial community associated with FMNs to change potential metabolic functions.

Despite the occurrence of a broad group of Fe–transforming bacterial families (Reyranellaceae, Rhizobiales (Amb − 16S − 1323), Chthoniobacteraceae, Pyrinomonadaceae, Solibacteraceae, Burkholderiales (A21b), Gemmatimonadaceae, Solirubrobacteraceae, Xanthobacteraceae, Nitrososphaeraceae, Solirubrobacterales (67 − 14), Gaiellaceae, and Acidothermaceae) in the soil matrices, the direct influence of the identified bacterial families on Fe transformation in FMNs was not observed. In the studied FMNs, the transformation of Fe and related elements (Co and Cr) by bacteria most likely proceeded predominantly through the degradation of Fe–organic complex compounds. This study revealed significant contributions of identified Mn–transforming bacterial families to the biogenic aspect of nodules formation in the studied soils.

The mentioned changes in the relationships between the different parameters in the soil matrices and FMNs and abundance of bacterial families suggest that the same bacterial families are able to participate in the biogeochemical cycles of different elements due to the metabolic versatility and adaptation of microorganisms to different environmental conditions.

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Declarations

Competing interests The authors declare no competing interests.

References

Ali D, Koner S, Hussain A, Hsu B (2025) Molecular mechanisms and biomineralization processes of ferromanganese nodule formation: insights its effect on nutrient imbalance and heavy metal immobilization in native soil profiles. Earth-Sci Rev 261:105029. https://doi.org/10.1016/j.earscirev.2024.105029

Andreini C, Bertini I, Cavallaro G, Holliday GL, Thornton JM (2008) Metal ions in biological catalysis: from enzyme databases to general principles. J Biol Inorg Chem 13(8):1205–1218. https://doi.org/10.1007/s00775-008-0404-5

Bazylinski DA, Frankel RB, Konhauser KO (2007) Modes of biomineralization of magnetite by microbes. Geomicrobiol J 24(6):465–475. https://doi.org/10.1080/01490450701572259

Borsodi AK, Megyes M, Zsigmond T, Horel Á (2024) Soil bacterial communities affected by land-use types in a small catchment area of the Balaton Uplands (Hungary). Biologia Future 75(3):313–325. https://doi.org/10.1007/s42977-024-00233-3

Bosma EF, Rau MH, van Gijtenbeek LA, Siedler S (2021) Regulation and distinct physiological roles of manganese in bacteria. FEMS Microbiol Rev 45(6):fuab028. https://doi.org/10.1093/femsre/fuab028

Cahyani VR, Murase J, Ishibashi E, Asakawa S, Kimura M (2007) Bacterial communities in manganese nodules in rice field subsoils: estimation using PCR-DGGE and sequencing analyses. Soil Sci Plant Nutr 53(5):575–584. https://doi.org/10.1111/j.1747-0765.2007.00176.x

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci U S A 108(Suppl 1):4516–4522. https://doi.org/10.1073/pnas.1000080107

Catão EC, Lopes FA, Araújo JF, de Castro AP, Barreto CC, Bustamante MM, Quirino BF, Krüger RH (2014) Soil Acidobacterial 16S rRNA Gene Sequences Reveal Subgroup Level Differences between Savanna-Like Cerrado and Atlantic Forest Brazilian Biomes. Int J Microbiol 2014(1):156341. https://doi.org/10.1155/2014/156341

Chen YJ, Leung PM, Wood JL, Bay SK, Hugenholtz P, Kessler AJ, Shelley G, Waite DW, Franks AE, Cook PLM, Greening C (2021) Metabolic flexibility allows bacterial habitat generalists to become dominant in a frequently disturbed ecosystem. ISME J 15(10):2986–3004. https://doi.org/10.1038/s41396-021-00988-w

Chernitsyna SM, Mamaeva EV, Lomakina AV, Pogodaeva TV, Galach'yants YuP, Bukin SV, Pimenov NV, Khlystov OM, Zemskaya TI (2016) Phylogenetic diversity of microbial communities of the Posolsk Bank bottom sediments, Lake Baikal. Microbiology 85(6):672–680. https://doi.org/10.1134/S0026261716060060

Conradie T, Jacobs K (2020) Seasonal and agricultural response of *Acidobacteria* present in two fynbos rhizosphere soils. Diversity. https://doi.org/10.3390/d12070277

Cornu S, Cattle JA, Samouelian A, Laveuf C, Guilherme LRG, Alberic P (2009) Impact of redox cyclers on manganese, iron, cobalt, and lead in nodules. Soil Sci Soc Am J 73:1231–1241. https://doi.org/10.2136/sssaj2008.0024

Dabard M, Loi A (2012) Environmental control on concretion-forming processes: Examples from Paleozoic terrigenous sediments of the North Gondwana margin, Armorican Massif (Middle Ordovician and Middle Devonian) and SW Sardinia (Late Ordovician). Sediment Geol 267–268:93–103

Domínguez DC, Guragain M, Patrauchan M (2015) Calcium binding proteins and calcium signaling in prokaryotes. Cell Calcium 57(3):151–165. https://doi.org/10.1016/j.ceca.2014.12.006

Dong H, Huang L, Zhao L, Zeng Q, Liu X, Sheng Y, Shi L, Wu G, Jiang H, Li F, Zhang L, Guo D, Li G, Hou W, Chen H (2022) A critical review of mineral-microbe interaction and co-evolution: mechanisms and applications. Natl Sci Rev 9(10):nwac128. https://doi.org/10.1093/nsr/nwac128

Dulay H, Tabares M, Kashefi K, Reguera G (2020) Cobalt resistance via detoxification and mineralization in the iron-reducing bacterium *Geobacter sulfurreducens*. Front Microbiol 11:600463. https://doi.org/10.3389/fmicb.2020.600463

Edgar RC (2013) Uparse: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10(10):996–998. https://doi.org/10.1038/nmeth.2604

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) Uchime improves sensitivity and speed of chimera detection. Bioinformatics 27(16):2194–2200. https://doi.org/10.1093/bioinformatics/btr381

Epstein W (2003) The roles and regulation of potassium in bacteria. Prog Nucleic Acid Res Mol Biol 75:293–320. https://doi.org/10.1016/s0079-6603(03)75008-9

Ettler V, Chren M, Mihaljevič M, Drahota P, Kříbek B, Veselovský F, Sracek O, Vaněk A, Penížek V, Komárek M, Mapani B, Kamona F (2017) Characterization of Fe-Mn concentric nodules from Luvisol irrigated by mine water in a semi-arid agricultural area. Geoderma 299:32–42. https://doi.org/10.1016/j.geoderma.2017.03.022

Frawley ER, Fang FC (2014) The ins and outs of bacterial iron metabolism. Mol Microbiol 93(4):609–616. https://doi.org/10.1111/mmi.12709

Gasparatos D (2013) Sequestration of heavy metals from soil with Fe-Mn concretions and nodules. Environ Chem Lett 11:1–9. https://doi.org/10.1007/s10311-012-0386-y

Gasparatos D, Massas I, Godelitsas A (2019) Fe-Mn concretions and nodules formation in redoximorphic soils and their role on soil phosphorus dynamics: current knowledge and gaps. CATENA 182:104106. https://doi.org/10.1016/j.catena.2019.104106

Hansel CM (2017) Manganese in marine microbiology. Adv Microb Physiol 70:37–83. https://doi.org/10.1016/bs.ampbs.2017.01.005

He J, Zhang L, Jin S, Zhu Y, Liu F (2008) Bacterial communities inside and surrounding soil iron-manganese nodules. Geomicrobiol J 25(1):14–24. https://doi.org/10.1080/01490450701829014

Hu M, Li F, Lei J, Fang Y, Tong H, Wu W, Liu C (2015) Pyrosequencing revealed highly microbial phylogenetic diversity in ferromanganese nodules from farmland. Environ Sci Process Impacts 17:213–224. https://doi.org/10.1039/c4em00407h

Hua H, Sui X, Liu Y, Liu X, Chang Q, Xu R, Li M, Mu L (2024) Effects of land use type transformation on the structure and diversity of soil bacterial communities. Life 14(2):252. https://doi.org/10.3390/life14020252

Huang L (2022) Pedogenic ferromanganese nodules and their impacts on nutrient cycles and heavy metal sequestration. Earth-Sci Rev 232:104147. https://doi.org/10.1016/j.earscirev.2022.104147

Huang L, Wang Y, Shao M (2023) Micromorphology, mineralogy, and geochemistry of ferromanganese nodules in tropical soils and their impacts on heavy metal sequestration. J Clean Prod 383:135498. https://doi.org/10.1016/j.jclepro.2022.135498

Kostenkov NM (1987) Redox regimes in soils of periodic waterlogging. Nauka Publ, Moscow (**(In Russ.)**)

Lan J, Wang S, Wang J, Qi X, Long Q, Huang M (2022) The shift of soil bacterial community after afforestation influence soil organic carbon and aggregate stability in Karst region. Front Microbiol 13:901126. https://doi.org/10.3389/fmicb.2022.901126

Lewin GR, Carlos C, Chevrette MG, Horn HA, McDonald BR, Stankey RJ, Fox BG, Currie CR (2016) Evolution and ecology of Actinobacteria and their bioenergy applications. Annu Rev Microbiol 70:235–254. https://doi.org/10.1146/annurev-micro-102215-095748

Liu F, Colombo C, Adamo P, He JZ, Violante A (2002) Trace elements in manganese-iron nodules from a Chinese Alfisol. Soil Sci Soc Am J 66:661–670. https://doi.org/10.2136/sssaj2002.6610

Liu G, Shan Y, Liu R, Sun C (2023a) Insights into the bacterial and archaeal population dynamics in the deep-sea hydrothermal sediments exposed to lights with different wavelengths. Deep-Sea Res I Oceanogr Res Pap 195:103996. https://doi.org/10.1016/j.dsr.2023.103996

Liu L, Chen Y, Shen J, Pan Y, Lin W (2023b) Metabolic versatility of soil microbial communities below the rocks of the hyperarid Dalangtan Playa. Appl Environ Microbiol 89(11):e0107223. https://doi.org/10.1128/aem.01072-23

Luo Y, Ding J, Shen Y, Tan W, Qiu G, Liu F (2018) Symbiosis mechanism of iron and manganese oxides in oxic aqueous systems. Chem Geol 488:162–170. https://doi.org/10.1016/j.chemgeo.2018.04.030

Lysak LV, Kadulin MS, Konova IA, Lapygina EV, Ivanov AV, Zvyagintsev DG (2013) Population number, viability, and taxonomic composition of the bacterial nanoforms in iron-manganic concretions. Euras Soil Sci 46:668–675. https://doi.org/10.1134/S1064229313060069

Lysak LV, Konova IA, Lapygina EV, Soina VS, Chekin MR (2019) Filtered forms of prokaryotes and bacteriophages in soil concretions. IOP Conf Ser Earth Environ Sci 11:368. https://doi.org/10.1088/1755-1315/368/1/012030

Magoč T, Salzberg SL (2011) Flash: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27(21):2957–2963. https://doi.org/10.1093/bioinformatics/btr507

Qin W, Wang C-y, Ma Y-x, Shen M-j, Li J, Jiao K, Tay FR, Niu L-n (2020) Microbe-mediated extracellular and intracellular mineralization: environmental, industrial, and biotechnological applications. Adv Mater 32:1907833. https://doi.org/10.1002/adma.201907833

Shelton AN, Seth EC, Mok KC, Han AW, Jackson SN, Haft DR, Taga ME (2019) Uneven distribution of cobamide biosynthesis and dependence in bacteria predicted by comparative genomics. ISME J 13(3):789–804. https://doi.org/10.1038/s41396-018-0304-9

Sipos P, Kovács I, Tóth A, Németh P, Demény A (2025) Paragenetic relationship between ferromanganese and calcareous nodules in a hydromorphic toposequence. Geoderma 454:117179. https://doi.org/10.1016/j.geoderma.2025.117179

Sipos P, Nemeth T, May Z, Szalai Z (2011) Accumulation of trace elements in Fe-rich nodules in neutral-slightly alkaline floodplain soil. Carp J Earth Environ Sci 6(1):13–22

Tan WF, Liu F, Li YH, Hu HQ, Huang QY (2006) Elemental composition and geochemical characteristics of iron-manganese nodules in main soils of China. Pedosphere 16:72–81. https://doi.org/10.1016/S1002-0160(06)60028-3

Tebo BM, Bargar JR, Clement BG, Dick GJ, Murray KJ, Parker D, Verity R, Webb SM (2004) Biogenic manganese oxides: properties and mechanisms of formation. Annu Rev Earth Planet Sci 32:287–328. https://doi.org/10.1146/annurev.earth.32.101802.120213

Tebo BM, Johnson HA, McCarthy JK, Templeton AS (2005) Geomicrobiology of manganese(II) oxidation. Trends Microbiol 13(9):421–428. https://doi.org/10.1016/j.tim.2005.07.009

Timofeeva YO (2008) Accumulation and fractionation of trace elements in soil ferromanganese nodules of different size. Geochem Int 46:260–267. https://doi.org/10.1134/S0016702908030038

Timofeeva YO, Karabtsov AA, Burdukovskii ML, Purtova LN, Martynenko ES (2025) Iron-manganese nodules in soils of natural landscapes in the south of the Russian Far East. Eurasian Soil Sci 58:13. https://doi.org/10.1134/S1064229324603202

Timofeeva YO, Martynenko ES, Sidorenko ML, Kim AV, Kazarin VM (2024) Taxonomic composition of cultured Fe- and Mn-oxidizing bacteria and microbial abundance in Fe–Mn nodules of different sizes. Microbiology 93:293–304. https://doi.org/10.1134/S0026261723603676

Timofeeva YO, Purtova LN (2024) Influence of organic compounds on Ni, Co, Cu, Cr, and Pb accumulation by nodules in Agro-Dark-Humus Podbels (Planosols) in the south of Primorskii Region. Eurasian J Soil Sci 57(4):562–576. https://doi.org/10.1134/S106422932360330X

Timofeeva YO, Karabtsov A, Ushkova M, Burdukovskii ML, VA, Semal' (2021) Variation of trace element accumulation by iron-manganese nodules from Dystric Cambisols with and without contamination. J Soils Sediments 21:1064–1078

Timofeeva YO, Karabtsov AA, VA, Semal', ML, Burdukovskii, NV, Bondarchuk (2014) Iron-manganese nodules in udepts: the dependence of the accumulation of trace elements on nodule size. Soil Sci Soc Am J 78:767–778. https://doi.org/10.2136/sssaj2013.10.0444

Tiquia-Arashiro SM (2018) Lead absorption mechanisms in bacteria as strategies for lead bioremediation. Appl Microbiol Biotechnol 102(13):5437–5444. https://doi.org/10.1007/s00253-018-8969-6

Vandieken V, Pester M, Finke N, Hyun J, Friedrich MW, Loy A, Thamdrup B (2012) Three manganese oxide-rich marine sediments harbor similar communities of acetate-oxidizing manganese-reducing bacteria. ISME J 6(11):2078–2090. https://doi.org/10.1038/ismej.2012.41

Vélez-Martínez GA, Reyes-Ardila WL, Duque-Zapata JD, Rugeles-Silva PA, Muñoz Flórez JE, López-Álvarez D (2024) Soil bacteria and fungi communities are shaped by elevation influences in Colombian forest and páramo natural ecosystems. Int J Microbiol 27(2):377–391. https://doi.org/10.1007/s10123-023-00392-8

Vodyanitskii YN, Sivtsov AV (2004) Formation of ferrihydrite, feroxyhyte, and vernadite in soil. Eurasian Soil Sci 37(8):863–875

Willms IM, Bolz SH, Yuan J, Krafft L, Schneider D, Schöning I, Schrumpf M, Nacke H (2021) The ubiquitous soil verrucomicrobial clade '*Candidatus Udaeobacter*' shows preferences for acidic pH. Environ Microbiol Rep 13(6):878–883. https://doi.org/10.1111/1758-2229.13006

IUSS Working Group WRB (2015) World Reference Base for Soil Resources 2014. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. World Soil Resources Reports. FAO, Rome.

Xiao W, Yang X, He Z, Li T (2014) Chromium-resistant bacteria promote the reduction of hexavalent chromium in soils. J Environ Qual 43(2):507–516. https://doi.org/10.2134/jeq2013.07.0267

Yabe S, Sakai Y, Abe K, Yokota A (2017) Diversity of ktedonobacteria with actinomycetes-like morphology in terrestrial environments. Microbes Environ 32(1):61–70. https://doi.org/10.1264/jsme2.ME16144

Yaghoubi Khanghahi M, Curci M, Cazzato E, Lasorella C, Traversa A, Crecchio C, Spagnuolo M (2024) Shifts in soil bacterial communities under three-year fertilization management and multiple cropping systems. Soil Syst 8:5. https://doi.org/10.3390/soilsystems8010005

Yang W, Cai X, Wang Y, Diao L, Xia L, An S, Luo Y, Cheng X (2022) Increased soil bacterial abundance but decreased bacterial diversity and shifted bacterial community composition following secondary succession of old-field. Forests 13:1628. https://doi.org/10.3390/f13101628

Yang Y, Cheng S, Fang H, Guo Y, Li Y, Zhou Y, Shi F, Vancampenhout K (2023) Linkages between the molecular composition of dissolved organic matter and soil microbial community in a boreal forest during freeze-thaw cycles. Front Microbiol 13:1012512. https://doi.org/10.3389/fmicb.2022.1012512

Zhang GY, He JZ, Liu F, Zhang LM (2014) Iron-manganese nodules harbor lower bacterial diversity and greater proportions of *Proteobacteria* compared to bulk soils in four locations spanning from north to south China. Geomicrobiol J 31(7):562–577. https://doi.org/10.1080/01490451.2013.854428

Zhang LM, Liu F, Tan WF, Feng XH, Zhu Y, He J (2008) Microbial DNA extraction and analyses of soil iron– manganese nodules. Soil Biol Biochem 40(6):1364–1369. https://doi.org/10.1016/j.soilbio.2007.01.004

Zhang Y, Rodionov DA, Gelfand MS, Gladyshev VN (2009) Comparative genomic analyses of nickel, cobalt and vitamin B12 utilization. BMC Genomics 10:78. https://doi.org/10.1186/1471-2164-10-78