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Microbiome structure, function, and drivers across different soil groups in an agricultural region of Serbia

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Abstract. Soil is the largest reservoir of biodiversity, with distinct physical, chemical, and biological properties. Microorganisms play essential roles in soil formation and fertility. This study aimed to analyze the microbiomes of three selected soil groups in an important agricultural region of Vojvodina (Serbia) by 16S rRNA gene metabarcoding and explore their association with soil properties. Soil samples from a total of 26 field plots (in 5 replicates) were analyzed using Illumina MiSeq paired-end sequencing and processed through the QIIME2 pipeline. The obtained results indicate that the analyzed soils generally exhibit physicochemical properties typical for the respective soil groups. Alpha diversity indices revealed the highest microbiome diversity in Chernozem, consistent with its favourable physicochemical characteristics. Based on beta diversity, clear separation of soil groups according to their properties was determined. Proteobacteria, Acidobacteriota, and Actinobacteriota dominate the microbial community composition at the phylum level. Redundancy analysis revealed that soil properties account for 53.8% of the variation in community composition, with pH value, iron availability, and $CaCO_3$ content having the strongest influence, pH being particularly significant. The functional potential of microbial communities showed dominance of functions related to metabolism, with significant representation of functions belonging to the following groups: genetic information processing, environmental information processing, and cellular processes. The analysis of genes involved in nitrogen cycling using Kruskal-Wallis test showed no statistically significant differences in their abundances across different soil groups (p-value > 0.05). This study provides the first detailed analysis of soil microbial communities across Serbia and highlights factors shaping them. These findings underscore the importance of microbial diversity for ecosystem functioning and offer a framework for soil health monitoring, while providing insights relevant for sustainable agriculture.

1 Introduction

In the light of growing human population and declining resources, soil has increasingly been amended with various chemicals and fertilizers to increase food production. While these practices initially lead to higher yields, they result in soil fertility decline on a long-term basis. To combat the loss of fertile soil, it is crucial to reduce the application of chemical substances, opt for organic rather than mineral fertilizers, and practice frequent cover crop rotations (Kljajić et al., 2012). As a non-renewable resource, soil faces threats from contamination, erosion, floods, landslides, salinization, soil sealing, compaction, and biodiversity loss (Commission of the European communities, 2006).

Investigating soil health enables the monitoring of degradation processes including erosion, contamination, and the loss of organic matter, which are major global challenges. To that, soil preservation and investigation are critical for achieving the Sustainable Development Goals (SDGs), such as food security (SDG 2), climate action (SDG 13), and biodiversity conservation (SDG 15) (UN DESA, 2024). Initiatives like the European Soil Deal and the EU's LUCAS framework highlight the importance of understanding soil health and implementing effective conservation strategies (Orgiazzi et al., 2018; European Commission: Directorate-General for Research and Innovation, 2022). LUCAS is a European-wide program that collects standardized data on soil properties, land use, and land cover to track changes and inform policy decisions. However, Serbian soils remain outside the routine scope of the LUCAS framework. Serbia's unique agricultural landscapes and biodiversity, including fertile Chernozems, saline soils, and Vertisols, provide an important case study for understanding soil responses to management practices and contribute valuable information to these global monitoring and policy frameworks.

Building on global concerns about soil degradation, the loss of fertile soil in Serbia is additionally driven by soil acidification and drought (Vidojević et al., 2022). The loss of organic matter in soil also leads to its degradation and decreased fertility in Serbia (Vasin et al., 2021). Serbia's soils are particularly vulnerable to degradation due to steep relief, fragile geology, a variable climate, and human activities such as vegetation removal and unsustainable land use, which together promote erosion and other soil degradation processes (Malušević et al., 2025). Autonomous Province of Vojvodina, located in northern Serbia, is critical region for agricultural production, with 91% of its land being suitable for agriculture (Statistical Office of the Republic of Serbia, 2023), and soil groups that are classified among the most fertile in the world (Montanarella et al., 2021). The present study, applying methodology used in LUCAS monitoring system (Orgiazzi et al., 2018), contributes to a comprehensive understanding of soil health and land use in Europe, and promotes harmonized data collection across all European countries. Serbia's unique agricultural landscape and biodiversity could provide valuable insights into soil degradation patterns, climate change effects, and land use changes in Southeast Europe, facilitating comparisons with other European countries and supporting region-specific sustainable land management practices. To that, presented data will be a valuable asset for the stakeholders and legislative agencies in Serbia and would improve data-driven policymaking for the country's agricultural and environmental sectors, for enhancement of soil monitoring plans and preservation actions.

Soil physicochemical properties can provide insights into soil fertility (Milić et al., 2011), however, they often respond only after substantial environmental disturbances, making them less sensitive for detecting early soil degradation. This limitation highlights the need for additional, more responsive indicators, such as biological or biochemical soil properties (Filip, 2002). Biological indicators are among the most effective tools for assessing soil quality, given the complexity of soil as a matrix.

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Unlike physical or chemical properties, they integrate diverse interactions within the soil ecosystem, providing a dynamic and sensitive reflection of soil health and ecosystem functions. Meta-analyses and experimental studies show that microbial biomass and extracellular enzyme activities commonly respond within months to disturbances such as fire, herbicide application, or climate warming, while chemical properties (e.g., total organic carbon) typically change over years to decades (Holden and Treseder, 2013; Pertile et al., 2020; Fanin et al., 2022). Indicators such as microbial biomass and enzymatic activity, along with the analysis of the soil microbiome and environmental DNA (eDNA), allow for detailed insights into soil biodiversity and its functional roles. These tools not only detect early changes in soil quality but also highlight its capacity to support sustainable agriculture and environmental health (Stone et al., 2016; Bhaduri et al., 2022; Bonilla-Bedoya et al., 2023).

The soil microbiome, a dynamic and complex microbial community, interacts with its environment in ways that are essential for soil health, including nutrient cycling, microbiological degradation, symbiotic relationships, and responses to habitat changes (Fierer, 2017; Sergaki et al., 2018; Banerjee and van der Heijden, 2023). Understanding the structure and function of the soil microbiome is crucial to unravel ecosystem processes and functions, which leads to the promotion of more sustainable soil management practices. Soil health is believed to be closely linked to the health of animals, plants, and ecosystems; a concept known as “One Health” (Banerjee and van der Heijden, 2023). Linking soil health to the health of plants, animals, and ecosystems highlights the central role of microbial communities, which connect these components and drive the fitness, productivity, and resilience of ecosystems – emphasizing the importance of maintaining diverse and functional soil microbiomes for sustainable land management. Within this concept, soil is considered as a reservoir of pathogens, beneficial microorganisms, and microbial diversity, influencing the health of all interconnected components. There is no single typical soil microbiome, as the relative abundance of bacterial and archaeal taxa depends on various anthropogenic and environmental factors. Land-use perturbation has been identified as one of the main anthropogenic factors affecting microbial community composition (Tsiafouli et al., 2015). Additionally, shifts in climate and soil properties, such as pH (Fierer and Jackson, 2006), nitrogen availability (Frey et al., 2004), and texture (Girvan et al., 2003) have been identified as key factors leading to changes in bacterial communities. Plant community characteristics are also believed to explain a portion of the variation in soil microbial diversity (Delgado-Baquerizo et al., 2018). Importantly, the soil microbiome also shows marked variability across soil groups: Chernozem is dominated by Actinobacteriota, Verrucomicrobiota, and Proteobacteria (Semenov et al., 2018; Boyarshin et al., 2023), Solonchak soils harbor distinct halotolerant communities dominated by Actinobacteriota, with elevated Firmicutes (Pershina et al., 2018; Xu et al., 2021), while Vertisol is enriched in Proteobacteria, Acidobacteriota, and Bacteroidota (Knelman et al., 2015; Song et al., 2018). Such contrasts in microbial composition across soil groups highlight the importance of considering both edaphic context and land-use management when assessing soil microbial communities, a perspective central to the present study.

Despite the critical role of soil microbial communities in soil fertility and ecosystem functioning, comprehensive studies on soil microbiomes in Serbia are lacking. Prior work has mostly focused on soil physicochemical properties or microbial activity, without linking microbial composition to functional potential (Marinković et al., 2012, 2016; Milić et al., 2019;

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Zeremski et al., 2021). Our study addresses this gap by providing the first detailed survey of soil microbial communities across key soil groups in Vojvodina, using 16S rRNA gene metabarcoding to assess microbial diversity and its relationship with soil properties.

The objective of this study was to analyze the composition and diversity of soil microbial communities in a predominantly agricultural region of Vojvodina, focusing on three soil groups: Chernozem, Solonchak, and Vertisol. These soil groups differ in their physicochemical properties, fertility, and land-use suitability, making them ideal for examining how microbial communities respond to contrasting soil environments. Studying their microbiomes provides insights into how microbial diversity and function are linked to key soil properties. This study aimed to investigate the relationships between soil properties and the abundance of dominant bacterial taxa, as well as to assess the functional potential of soil microbial communities, particularly genes involved in the nitrogen cycle. We hypothesized that contrasting soil properties among Chernozem, Solonchak, and Vertisol would drive distinct microbial community structures and nitrogen-cycling gene abundances, reflecting the ecological adaptations of microbial taxa to specific soil environments. By conducting the first comprehensive soil microbiome survey in the Republic of Serbia based on 16S rRNA sequencing, this research seeks to align Serbia with global trends in soil microbiome analysis, contributing to European soil diversity monitoring programs and enhancing the understanding of microbial diversity and functions in key soil groups. This survey also provides baseline insights into microbial indicators of soil health, which could support sustainable land management, early detection of soil degradation, and data-driven agricultural and environmental policy development in Serbia.

2 Materials and methods

2.1 Study area and soil sampling

The study was conducted in northern Serbia, on the territory of the Autonomous Province of Vojvodina. The largest part of Vojvodina is a plain at an altitude of 70-130 m. Its relief features alluvial plains, loess terraces, loess plains, sandstones and low mountains – Fruška gora and Vršačke planine (Marković et al., 2014).

Northern Serbia is characterized by a continental climate. Summers are generally hot and often dry, while winters are relatively short (2-3 months) and moderately cold, with average January temperatures around –1°C. The average annual temperature is about 11°C. The prevailing wind is the Košava, a southeasterly wind that can occasionally be strong and gusty. Annual precipitation ranges between 540 and 720 mm, often insufficient for cultivated crops, with the highest amounts typically occurring in late spring and early summer, when water demand is greatest (Malinović-Milićević et al., 2018).

The most represented soil groups according to the international soil classification (IUSS Working Group WRB, 2022) are Chernozem, Vertisol, Gleysol, Phaeozem, Fluvisol and saline soils (Solonetz and Solonchaks).

2.1.1 Site selection

The investigation focused on Chernozem and Vertisol soil groups due to their widespread presence in the region, and additionally on Solonchak soils due to their specific characteristics as a result of higher salt concentration. Soil type identification was conducted in two steps. Initially, Vojvodina’s pedological map at the scale of 1:50.000 was consulted (Neugebauer et al., 1971). The second step involved field and laboratory observations for each location, followed by the determination of the soil group according to the WRB classification (IUSS Working Group WRB, 2022). Three locations were selected for sampling for each soil type. Control plots were selected for their similar characteristics and proximity to agricultural plots of the same soil group. These control plots, minimally affected by human activities over longer periods, included forests, meadows, and pastures.

2.1.2 Sampling design

At each location, five replicates of soil samples were collected from three plots, including two agricultural (different practices and crops) and one uncultivated control plot. Detailed description of sampling locations can be found in Kuzmanović et al. (2024). The agricultural plots were managed with conventional cropping practices, including tillage, fertilization, and periodic crop rotations, whereas control plots remained uncultivated and undisturbed. These differences in management likely influence soil properties such as organic matter content, nutrient availability, and microbial community composition, providing a basis for comparing the impacts of agricultural practices on soil health.

2.1.3 Soil sampling and handling

Soil sampling following the circular reference sample method (Bogdanović et al., 2014) is explained in detail in Kuzmanović et al. (2024) including a specific protocol for sampling for environmental DNA (eDNA). Briefly, at each sampling location, five composite samples were collected as replicates. Each composite was formed by pooling 20-25 individual soil samples taken from a depth of 30 cm using a sterilized agrochemical probe. Sampling equipment was thoroughly cleaned and sterilized to minimize contamination, and samples were transported at 4°C and processed within 24 h. For subsequent microbial analyses, separate subsamples of fresh soil were retained at –80°C for DNA extraction, whereas the subsamples for physical and chemical properties analyses were kept at 4°C until further processing.

2.2 Soil physical and chemical properties analyses

Soil samples were air-dried at room temperature (~20-25°C) to an air-dry state (moisture content of approximately 3-5%) and subsequently sieved to <2 mm (ISO 11464:2006). The moisture correction factor (MCF) was determined by oven-drying 5 g of soil at 105 °C to constant weight (van Reuwijk, 2002), and was subsequently used to express results on an absolutely dry soil mass. Particle size distribution was determined in the <2 mm fraction by the pipette method (van Reeuwijk, 2002). The size fractions were defined as clay (<2 µm), silt (2-20 µm), fine sand (20-200 µm) and coarse sand (200-2000 µm). Soil samples were then classified into textural classes based on their mechanical composition (IUSS Working Group WRB, 2022). The pH value in 1:5 (volume fraction) soil suspension with water (active acidity) and 1 mol/l KCl (exchangeable acidity) was determined by potentiometric method (ISO 10390:2021). Total CaCO3 content was determined by the volumetric method (ISO 10693:1995). Organic matter content was measured using the Tyurin method (Shamrikova et al., 2022). Total carbon (TC), total organic carbon (TOC) and total nitrogen (N) were determined by elementary analysis (CHNSO VarioEL III) after dry combustion in accordance with the ISO 10694:1995 and AOAC Official Method 972.43:2006, respectively. Readily available phosphorus (AL-P) was determined by extraction with ammonium lactate (Egner et al., 1960) and detected spectrophotometrically at a wavelength of 830 nm (UV/VIS spectrophotometer) using the phospho-molybdate-blue-method (Murphy and Riley, 1962). Readily available potassium AL-K) in the soil was determined by extraction of ammonium lactate (Egner et al., 1960) using a flame photometer. The content of plant-available micronutrients (Fe, Mn, Zn, and Cu) was determined after soil extraction with a buffered diethylenetriaminepentaacetic acid (DTPA) solution, following ISO 14870:2001. Plant-available boron (B) was extracted using hot water. The concentration of micronutrients (Fe, Mn, Zn, Cu, and B) was determined by Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP-OES) (Vista Pro - Axial, Varian) in accordance with US EPA method 200.7:2001. ICP-OES operation conditions were: RF power 1.10 kW; plasma and auxiliary gas flow rate 15.0 and 1.50 L min-1, respectively; nebulizer flow 1.20 L min-1; number of replicates 3. The determination of cation exchange capacity (CEC) was carried out by soil extraction in an ammonium acetate solution (van Reuwijk, 2002). All soil physical and chemical analyses were conducted at the Laboratory for Soil and Agroecology, Institute of Field and Vegetable Crops, accredited under ISO/IEC 17025:2017. Quality assurance and quality control (QA/QC) were ensured using Laboratory Control Samples (LCS) for all analyses. The LCS were verified with certified reference materials and through participation in inter-laboratory proficiency testing using standardized methods. For subsequent microbial analyses, separate subsamples of fresh soil were retained at –80°C for DNA extraction.

2.3 DNA extraction and sequencing

A single extraction of environmental DNA was performed per soil sample from 250 mg of material using a DNeasy PowerSoil Pro kit (Qiagen, Germany) following the manufacturer's instructions with several modifications. After the addition of the C1 solution, a 10-minute incubation step at 70°C (Dry Block Heater 2, IKA, Germany) was included to improve cell disruption and DNA recovery from recalcitrant soil matrices characteristic of Chernozem, Solonchak, and Vertisol soils. For sample homogenization in solution C1 after incubation, BeadBug Microtube Homogenizer (Benchmark Scientific, USA) was used. Homogenization involved three steps: mixing at 3200 rpm for 20 seconds, pausing for 20 seconds, and mixing again at 3200 rpm for 20 seconds. The quality and yield of the extracted DNA were assessed using a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, USA) and a QFX fluorometer (DeNovix, USA). DNA samples were stored at -80°C. DNA sequencing was conducted using the Illumina MiSeq paired-end sequencer (Illumina, USA). Primers used for the hypervariable V4 region of the 16S rRNA gene were 515-F (Parada) (5'-TGYCAGCMGCCGCGGTAA-3') and 806-R (Apprill) (5'-GGACTACNVGGGTWTCTAAT-3') (Thompson et al., 2017).

Sequences were deposited in the NCBI database under the accession number PRJNA1116093 (http://www.ncbi.nlm.nih.gov/bioproject/1116093).

2.4 Quantitative real-time PCR

The abundances of nitrogen cycling genes, including *nifH* (nitrogen fixation), *nrfA* (dissimilatory nitrate reduction to ammonium), archaeal and bacterial *amoA* (nitrification), and *nirK*/*nirS* (denitrification), were determined using quantitative real-time PCR (qPCR) on a Mastercycler ep Gradient S (Eppendorf, Germany). Each 10 µl reaction consisted of 5 µl Luna Universal qPCR Master Mix (New England Biolabs, USA), 0.5 µl forward and reverse primer mix (10 µM), 1 µl of DNA template diluted 1:1000, and 3.5 µl DNase-, RNase-, Protease-Free H2O. qPCR reactions were performed in duplicate for each sample, and a no-template control containing all reaction components except DNA was included in each run to monitor potential contamination. Primers for this study were designed in PCR Primer-BLAST Tool (Ye et al., 2012), and their sequences, along with the annealing temperatures, are detailed in Table 1. Standard curves for each target gene were generated using ten-fold serial dilutions of synthetic ultramers (4 nmol) as qPCR standards. The calculated PCR efficiencies ranged from 95% to 110%. The qPCR amplification protocol involved an initial denaturation step at 95°C for 10 minutes, followed by 45 cycles of amplification with the following parameters: denaturation at 95°C for 10 seconds, annealing at the gene-specific temperature for 30 seconds, and elongation at 72°C for 30 seconds. A final extension step at 72°C for 10 minutes was included to ensure complete amplification. Melting curve analysis was performed to verify amplicon specificity and to exclude the presence of primer-dimers.

Table 1 Quantitative real-time PCR primer sequences (Tm – melting temperature)

Gene	Forward and reverse primers (5’→3’)	Product length	Tm	GC%
nirK	f: ACGGACTCAAGGACCACAAG	129 nucleotides	59,61	55,00
	r: AGGCTTCACCAGCACTTTCG		60,89	55,00
nirS	f: ACCGATGTCACCAAGATTCCG	164 nucleotides	60,41	52,38
	r: AGCGTACGCACTACCTTCCA		61,25	55,00
nrfA	f: TGCCATACCCAGGACAAAGC	109 nucleotides	60,32	55,00
	r: CGAAGTGAGCGTGAACCAG		58,85	57,89
nifH	f: GTCCACCACTTCGCAGAACA	78 nucleotides	60,53	55,00
	r: TTCGGATCGCACCCTACAATC		60,20	52,38
bacterial *amoA*	f: TGCTGGATTTCACCCTGTACC	120 nucleotides	60,00	52,38
	r: GCAGATGAGTCGGACCAAAGA		60,07	52,38
archaeal *amoA*	f: TGCTCACTGTAGGTGCGTG	167 nucleotides	60,01	57,89
	r: TGCTGATGGTAGCCAAACTGG		60,61	52,38

2.5 Bioinformatic and statistical data analysis

Sequence reads of 16S rRNA gene were analyzed using the Quantitative Insights Into Microbial Ecology 2 pipeline (QIIME2 version 2022.2) (Bolyen et al., 2019; Estaki et al., 2020). Prior to downstream analyses, sequence data were quality filtered to remove low-quality reads. Chloroplast and mitochondrial sequences were removed, and the remaining sequences were rarefied to 15,000 reads per sample to standardize sequencing depth across samples. Primers were removed from the sequences using the *cutadapt* algorithm (Martin, 2011). The DADA2 algorithm was employed for denoising (Callahan et al., 2016). Only forward reads were used for sequence analysis, with the DADA2 *trunc-len* parameter set at 220, due to the lower quality of the reverse reads. Taxonomic classification of 16S rRNA reads was performed using a classifier specific for the primer pair 515-F (Parada)/806-R (Apprill), trained on SILVA 138 database (Quast et al., 2013; Bokulich et al., 2018). The classifier uses a probabilistic naive Bayes algorithm to assign taxonomy to each sequence based on k-mer composition. Visualization of bacterial community structure and diversity analyses were conducted in R version 4.2.2 (R Core Team, 2022). Data from the QIIME2 analysis were imported in R using the *phyloseq* package version 1.34.0 (McMurdie and Holmes, 2013). Taxa abundances at different taxonomic levels were visualized using the *ggplot2* version 3.4.0 (Wickham, 2016) and *ampvis2* version 2.5.7 (Andersen et al., 2018) packages. Alpha diversity was visualized with the *phyloseq* package's *plot_richness* function, and statistically significant differences in diversity indices were calculated using the Pairwise Wilcox test. Beta diversity was assessed through Principal Coordinates Analysis (PCoA) based on Bray-Curtis and Jaccard indices. The proportion of variance explained by PCoA axes was calculated to assess the adequacy of the representation. Group differences were evaluated statistically using PERMANOVA (Adonis function, permutation number 999). To include the taxonomy information, Weighted UniFrac analysis was also conducted, followed by PERMANOVA. The *microeco* package version 0.6.5 in R (Liu et al., 2021) was used to visualize statistically significant differences in the abundances of the dominant taxa across different soil groups and to determine correlations between taxa and soil properties. This package was also used for Redundancy Analysis (RDA) to identify the factors with the highest influence on microbial community composition. Constrained analysis of principal coordinates (CAP) was performed using the *capscale* function in *vegan* version 2.6.4 (Oksanen et al., 2022) to assess the influence of individual soil properties and all measured soil factors on bacterial community composition, with statistical significance tested using PERMANOVA (999 permutations). Functional prediction was conducted using the FAPROTAX database (Louca et al., 2016) and the *Tax4Fun* package version 0.3.1 (Aßhauer et al., 2015). Linear Discriminant Analysis (LDA) with the LEfSe method (LDA Effective Size) was performed to identify the most significant functions differentially abundant in the analyzed soil types. Gene copy numbers obtained from qPCR analysis were visualized using *ggplot2*. To test for statistically significant differences in soil properties and gene copy numbers between different soil groups, the Kruskal-Wallis test with post-hoc Dunn test was conducted in R. For statistical analyses involving multiple comparisons, appropriate corrections were applied to control for false positives. Specifically, pairwise comparisons using Dunn's test were adjusted with the Bonferroni method. These steps ensure that reported differences are robust and reduce the likelihood of type I errors.

3 Results

3.1 Soil physicochemical properties

The analysis of soil properties highlighted the differences between Chernozem, Solonchak, and Vertisol (Table 1). A part of the results (pH in KCl and H2O, CaCO3, organic matter, total N, organic C, P2O5, and K2O) was previously published in Kuzmanović et al. (2024). Obtained results are in agreement with the expected values for each soil group. Chernozem showed intermediate pH values (6.78 in KCl and 7.64 in H_2O), Solonchak the highest (7.95 and 8.98), and Vertisol the lowest (5.30 and 6.39). $CaCO_3$ content was significantly higher in Solonchak (20.16%) than in Chernozem (3.49%) and Vertisol (0.32%). Organic C, total N, and P2O5 did not differ significantly among the soils. K_2O differed significantly between Solonchak (17.44 mg/100g) and Vertisol (35.06 mg/100g), with Chernozem (20.72 mg/100g) not significantly different from either.

Table 2 Means and standard deviations of soil physicochemical properties for Chernozem, Solonchak, and Vertisol. Statistically significant differences were assessed using Kruskal–Wallis test followed by post hoc Dunn's test; different letters indicate significant differences.

Soil property	Soil group		
	Chernozem	Solonchak	Vertisol
Chemical properties			
CEC (cmol$_c$/kg)	19.28 ± 6.32 **a**	28.43 ± 4.23 **ab**	33.57 ± 14.48 **b**
Ca (ppm)	3774.97 ± 1980.93 **a**	6561.64 ± 1701.76 **b**	4725.18 ± 2886.01 **ab**
K (ppm)	187.03 ± 94.94 **ab**	145.77 ± 33.87 **a**	312.09 ± 129.42 **b**
Mg (ppm)	349.11 ± 112.86 **a**	742.72 ± 438.99 **b**	900.73 ± 514.73 **b**
Na (ppm)	25.73 ± 7.34 **a**	1285.34 ± 2014.01 **b**	77.29 ± 52.01 **ab**
Cu (mg/kg)	2.78 ± 1.22 **a**	1.56 ± 0.57 **a**	21.53 ± 22.65 **b**
Fe (mg/kg)	20.84 ± 24.20 **a**	18.76 ± 12.64 **a**	157.83 ± 78.15 **b**
Mn (mg/kg)	20.03 ± 19.38 **ab**	9.49 ± 2.53 **a**	18.48 ± 10.85 **b**
Zn (mg/kg)	2.19 ± 1.58 **a**	0.69 ± 0.19 **b**	3.42 ± 1.23 **a**
B (mg/kg)	0.68 ± 0.21 **a**	1.61 ± 0.73 **b**	0.71 ± 0.29 **a**
Physical properties			
coarse sand (%)	3.64 ± 5.48 **a**	1.23 ± 0.47 **a**	5.49 ± 8.11 **a**
fine sand (%)	51.32 ± 11.13 **a**	47.17 ± 4.72 **a**	24.42 ± 9.21 **b**
silt (%)	23.99 ± 7.30 **a**	24.94 ± 3.18 **a**	26.72 ± 5.30 **a**
clay (%)	21.15 ± 8.40 **a**	26.92 ± 3.35 **a**	44.12 ± 10.43 **b**

Statistically significant differences were found between Chernozem and Solonchak in the content of Ca (3774.97 and 6561.64 ppm), Mg (349.11 and 742.72 ppm), Na (25.73 and 1285.34 ppm), Zn (2.19 and 0.69 mg/kg), and B (0.68 and 1.61 mg/kg). Chernozem and Vertisol differed significantly in cation exchange capacity (19.28 and 33.57 cmol$_c$/kg) and in Mg

(349.11 and 900.73 ppm), Cu (2.78 and 21.53 mg/kg), and Fe (20.84 and 157.83 mg/kg). Statistically significant differences between Solonchak and Vertisol were observed in several properties, including K (145.77 and 312.09 ppm), Cu (1.56 and 21.53 mg/kg), Fe (18.76 and 157.83 mg/kg), Mn (9.49 and 18.48 mg/kg), Zn (0.69 and. 3.42 mg/kg), and B (1.61 and. 0.71 mg/kg). Additionally, the analysis of soil physical properties revealed statistically significant differences in fine sand (24.42% in Vertisol vs. 51.32% in Chernozem and 47.17% in Solonchak) and clay content (44.12% in Vertisol vs. 21.15% in Chernozem and 26.92% in Solonchak).

The observed differences in soil pH and nutrient contents among Chernozem, Solonchak, and Vertisol have potential agronomic implications. For example, the high pH and $CaCO_3$ in Solonchak may limit nutrient availability, Chernozem's balanced pH and nutrient levels support favorable microbial activity and crop growth, and Vertisol's elevated P_2O_5 and K_2O, combined with its high clay content, indicate high nutrient retention and potential fertility, although the dense texture and lower pH may limit aeration and require management such as liming or tillage adjustments.

3.2 Alpha and beta diversity of microbial communities

The alpha diversity of the bacterial community, expressed by Shannon and Simpson indices, showed heterogeneity among different soil groups, with Solonchak samples displaying overall lower variability compared to Chernozem and Vertisol (Fig. 1). The richness values, expressed by the Chao1 index, varied significantly among all tested soil groups, showing the highest values in Chernozem, and the lowest in Solonchak. Similarly, Observed diversity was highest in Chernozem and lowest in Solonchak. These differences suggest that Chernozem harbors a more diverse and potentially more stable microbial community, which may enhance ecosystem resilience and nutrient cycling, whereas lower richness in Solonchak could indicate a more vulnerable or specialized microbial ecosystem.

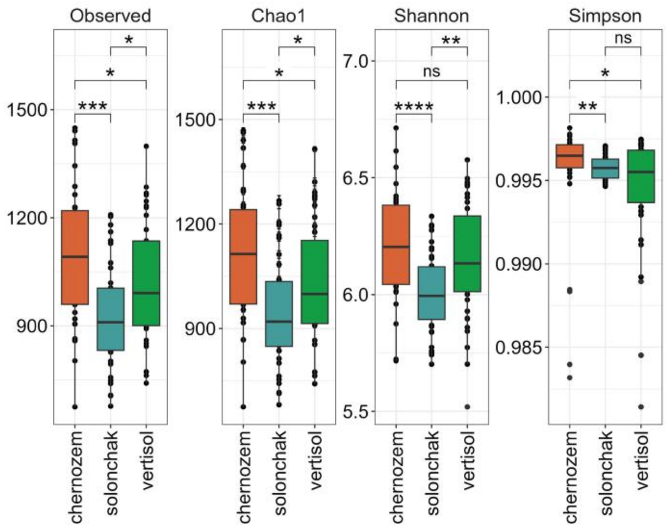

Figure 1 Alpha diversity indices (Observed, Chao1, Shannon, Simpson) across different soil groups in Vojvodina. Data represent 5 replicate samples from each of 8 field plots on Chernozem and 9 on Solonchak and Vertisol. Statistically significant differences between soil groups are indicated by stars: $p \leq 0.05$ *, $p \leq 0.01$ **, $p \leq 0.001$ ***, $p \leq 0.0001$ ****, ns - not significant. Overall differences were tested with Kruskal-Wallis (Observed p = 0.00028, Chao1 p = 0.00048, Shannon p = 0.00039, Simpson p = 0.021).

PCoA analysis of bacterial beta diversity, based on Bray-Curtis and Jaccard distances, revealed distinct differences between the microbial communities of various soil groups (Fig. 2). Chernozem and Vertisol were separated along the PCoA1 axis, which accounted for 23.3% of the variation between samples, while the PCoA2 axis, explaining 10.1% of the variation, separated Solonchak from Chernozem and Vertisol, based on Bray-Curtis. Based on Jaccard index, PCoA1 and PCoA2 axes explained 14.5 and 6.4% of the variation, respectively. PCoA analysis indicated significant differences between soil groups, as confirmed by PERMANOVA (Bray-Curtis: $R^2 = 0.26$, p = 0.001***; Jaccard: $R^2 = 0.17$, p = 0.001***) statistical test. Weighted UniFrac analysis also confirmed that microbial community composition was significantly structured by soil group (PERMANOVA, $R^2 = 0.17$, p = 0.001***), with PCoA1 axis accounting for 50.3%, and PCoA2 9.2% of the variation between samples.

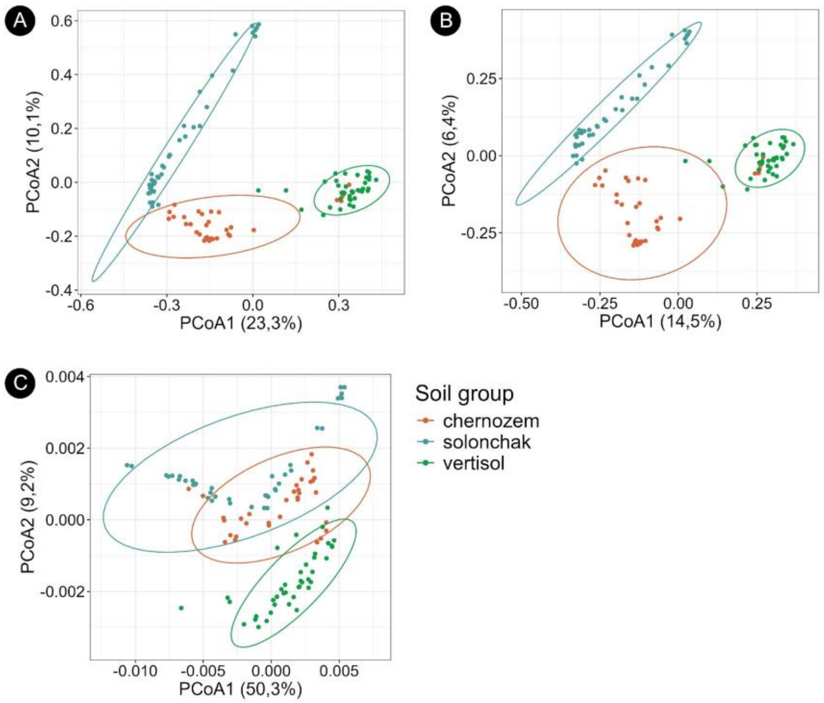

Figure 2 Beta diversity of soil bacterial communities in different soil groups in Vojvodina was assessed by Principal Coordinates Analysis (PCoA) using three distance metrics: (A) Bray-Curtis, (B) Jaccard, and (C) weighted UniFrac. Each point represents a single sample, with 5 replicate samples from each of 8 field plots on Chernozem and 9 on Solonchak and Vertisol. Bray-Curtis PCoA1 and PCoA2 axes explained 23.3% and 10.1% of the variation, respectively, while Jaccard PCoA1 and PCoA2 axes explained 14.5% and 6.4%. Weighted UniFrac PCoA1 and PCoA2 axes explained 50.3% and 9.2% of the variation. PCoA revealed distinct clustering of microbial communities

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according to soil group, confirmed by PERMANOVA (Bray-Curtis: $R^2 = 0.26$, $p = 0.001$***; Jaccard: $R^2 = 0.17$, $p = 0.001$***; weighted UniFrac: $R^2 = 0.17$, $p = 0.001$***).

3.3 Bacterial community composition

The most dominant phyla across the analyzed field plots were Proteobacteria, Acidobacteriota, Actinobacteriota, and Verrucomicrobiota, with similar abundances in field plots of the same soil group (Fig. 3A). In several Chernozem plots, phyla Bacteroidota and Crenarchaeota were also abundant. A higher abundance of Chloroflexi and Firmicutes was observed in Solonchak. Chernozem and Vertisol showed more similarity in phyla abundance, whereas Solonchak exhibited a different phyla distribution pattern (Fig. 3B, Fig. 3C). Specifically, Proteobacteria, Acidobacteriota, and Verrucomicrobiota were less abundant, while phylum Actinobacteriota was more abundant in Solonchak. Statistically significant differences in abundance were observed in 8 out of 10 most abundant phyla (Fig. 5A). Acidobacteriota, Verrucomicrobiota, Chloroflexi, Bacteroidota, Gemmatimonadota, and Crenarchaeota showed significant differences in abundance across all soil groups. Proteobacteria was significantly less abundant, while Actinobacteriota was significantly more abundant in Solonchak compared to Chernozem and Vertisol. These phylum-level differences may be driven by soil properties; for instance, the higher salinity and $CaCO_3$ content measured in Solonchak could favor Actinobacteriota and Chloroflexi, while more neutral pH and balanced nutrients in Chernozem and Vertisol support higher abundances of Proteobacteria, Acidobacteriota, and Verrucomicrobiota.

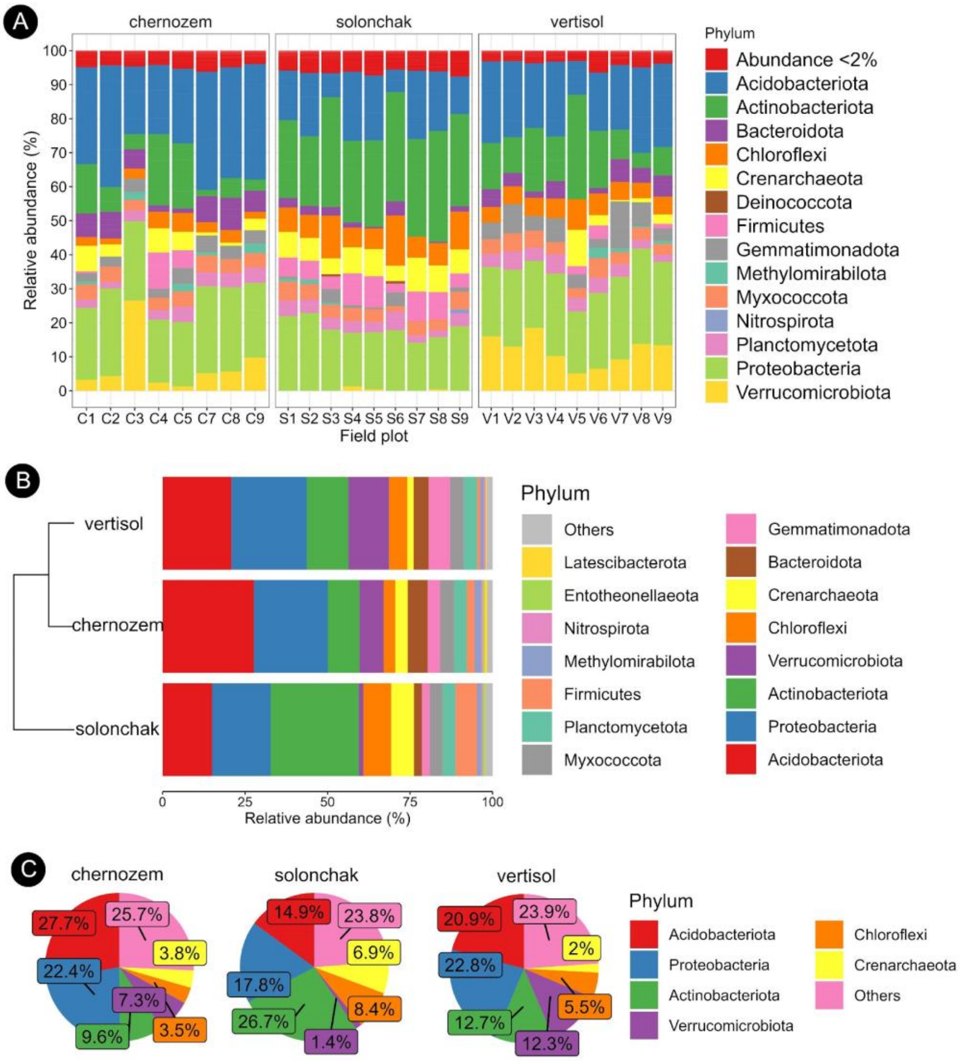

Figure 3 Relative abundance of bacterial phyla on field plot (A) and soil group (B, C) levels. Low-abundance phyla (< 2%) are grouped as "Abundance < 2%". Colors corresponding to individual phyla as indicated in legends. Relative abundances are expressed as percentages. Sample sizes: 8 field plots on Chernozem and 9 on Solonchak and Vertisol, 5 samples per plot.

The most dominant bacterial families in Chernozem and Solonchak were Vicinamibacteraceae and an unidentified family belonging to phylum Acidobacteriota, order Vicinamibacterales (Fig. 4). Unidentified taxa are a common challenge in soil microbiome studies due to the large proportion of uncultivated and unclassified soil bacteria. This taxonomic gap reflects the limitations of current 16S rRNA databases and highlights the presence of unexplored microbial diversity in soils. In Vertisol, in addition to the above-mentioned unidentified family, Chthoniobacteraceae was the most abundant. Besides mentioned, family Nitrososphaeraceae showed higher abundance in Solonchak, and Gemmatimonadaceae in Vertisol. Statistically significant differences were observed in the abundance of all 10 most dominant families across different soil groups (Fig. 5B). The abundances of Chthoniobacteraceae, Bacillaceae, SC-I-84, Nitrososphaeraceae, Gemmatimonadaceae, Nitrosomonadaceae, and Xanthobacteraceae differed significantly among the soil groups. Vicinamibacteraceae was significantly more abundant in Chernozem, whereas family 67-14 was more abundant in Solonchak. The abundance of family bacteriap_25 was significantly lower in Vertisol compared to Chernozem and Solonchak.

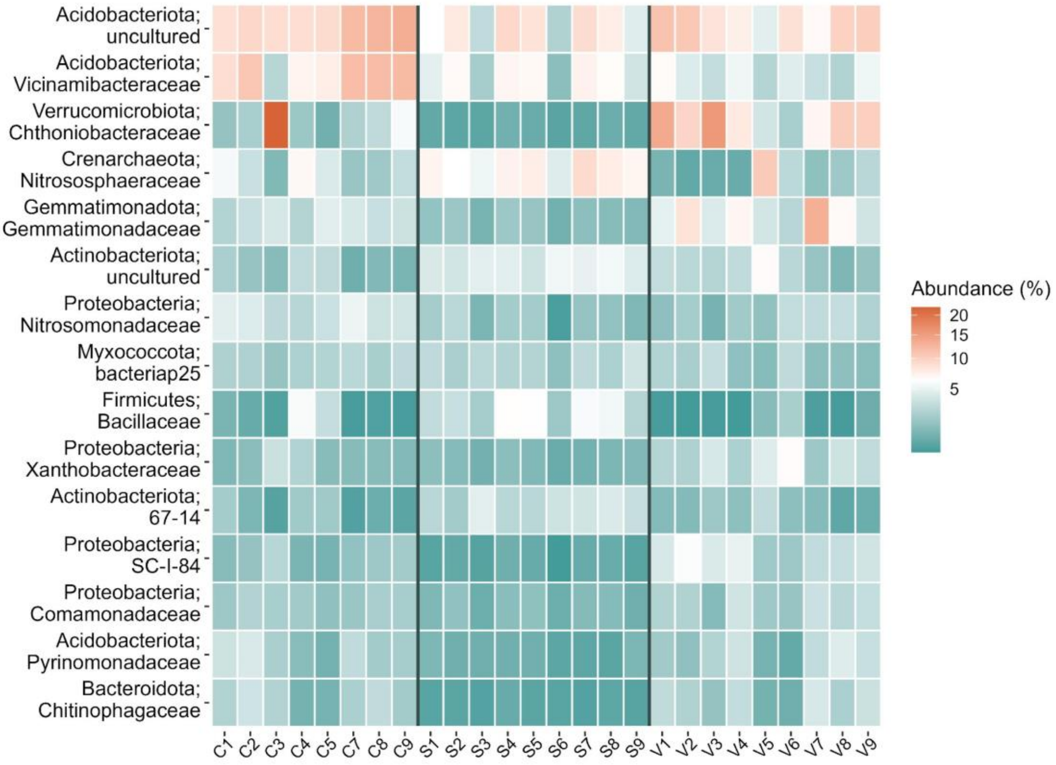

Figure 4 Relative abundance of 15 prevalent bacterial families in field plots of different soil groups (C1-C9: Chernozem, S1-S9: Solonchak, V1-V9: Vertisol). The color gradient indicates relative abundance values, ranging from dark blue (0%) to dark red (highest relative abundance observed). Rows correspond to bacterial families and columns to individual field plots. Differences in color intensity illustrate variation in community composition among soil groups.

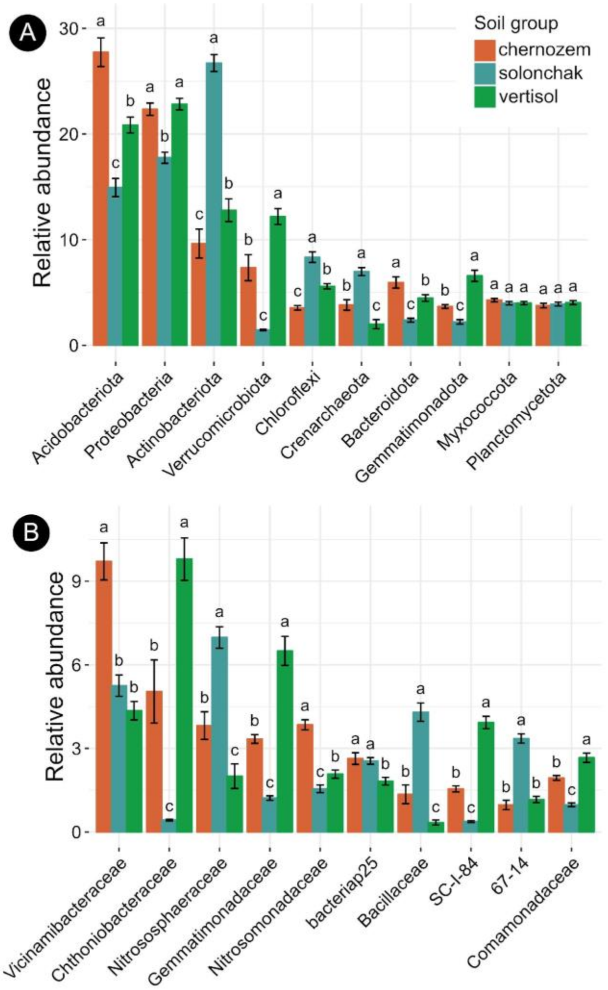

Figure 5 Statistically significant differences in the abundance of 10 most dominant phyla (A) and families (B) in different soil groups (Chernozem, Solonchak, Vertisol). Differences were assessed using the Kruskal–Wallis test followed by post hoc Dunn's test. Soil groups not sharing the same letter differ significantly ($p \leq 0.05$) in the abundance of certain taxa.

3.4 The relationship between soil properties and microbiome

Soil physical and chemical properties were found to have a statistically significant influence on the 15 most abundant phyla in the soil (Fig. 6A). Various bacterial phyla showed the strongest correlations with soil pH and $CaCO_3$ content. The heatmap revealed interesting correlation patterns. Namely, phyla positively correlated with pH value were also positively correlated with $CaCO_3$ content, cation exchange capacity, exchangeable Ca, and B content. Phyla positively correlated with sand content tended to be negatively correlated with clay content. Additionally, available micronutrients (Cu, Fe, Mn, and Zn) were all either positively or negatively correlated with the same taxa. Similar correlation patterns were observed at the taxonomic level of family (Fig. 6B). The phyla identified as most abundant across all three soil types – Actinobacteriota, Acidobacteriota, and Proteobacteria, showed strong statistically significant correlations with the soil properties identified as

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the most influential for microbial community composition, namely pH, $CaCO_3$ content, and, to a moderate extent, sand and clay fractions. In the case of Actinobacteriota, correlations with pH and $CaCO_3$ were positive, while the correlation with coarse sand was negative. By contrast, Acidobacteriota and Proteobacteria exhibited negative correlations with pH, $CaCO_3$, and clay content, and positive correlations with sand content.

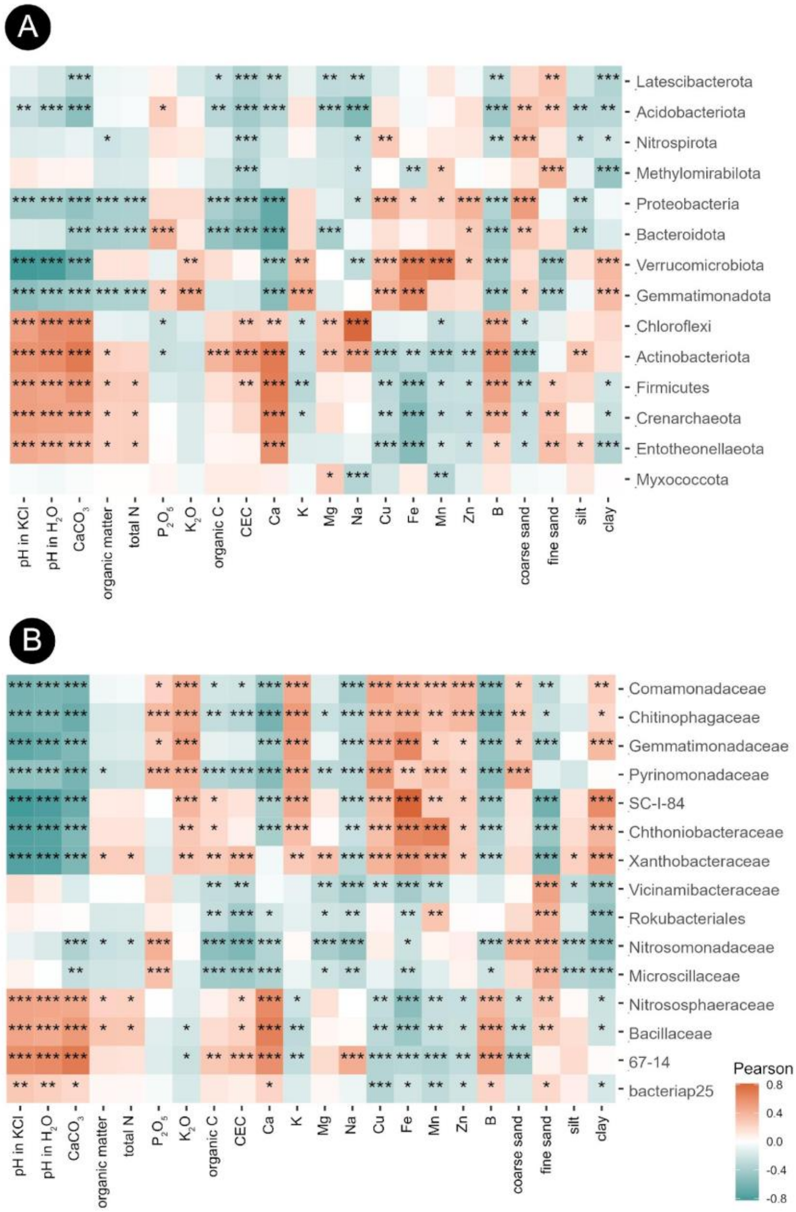

Figure 6 Correlation between dominant bacterial phyla (A) or families (B) and analyzed soil physicochemical properties. Pearson's correlation coefficients are represented by a color scale (blue - negative correlation, white - no correlation, red - positive correlation). Statistically significant correlations after are marked with * ($p \le 0.05$), ** ($p \le 0.01$), and *** ($p \le 0.001$).

Redundancy analysis confirmed the strong influence of soil properties on the microbial community (Fig. 7). The first two axes accounted for 53.8% of the total variation in community composition at the level of detected ASV (Amplicon Sequence Variants). Based on arrow lengths and statistical analysis, pH, CaCO3, and Fe content were identified as properties having the strongest positive statistically significant influence on microbial community. A Mantel test based on Pearson’s correlation coefficient indicated that all soil properties, except P2O5, coarse sand and silt content, significantly impacted bacterial community composition (Table B.1). The lack of significant correlations for P_2O_5, coarse sand, and silt may be due to their relatively low variability across the studied sites (significant differences between soil groups were not detected) or weaker direct effects on microbial habitats compared to other chemical properties such as pH and $CaCO_3$.

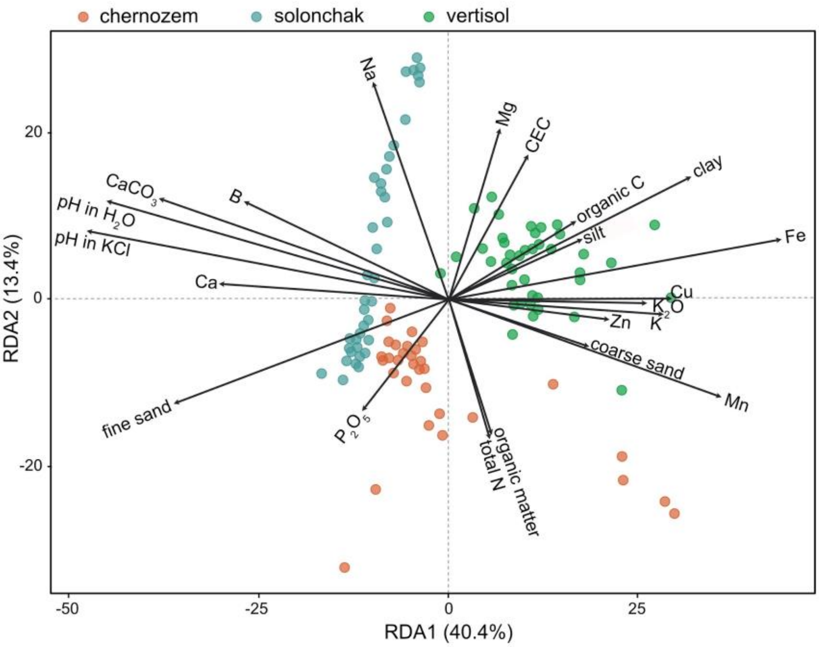

Figure 7 The influence of soil physicochemical properties on microbial community composition in different soil groups at the level of detected ASV based on redundancy analysis. Each point represents a soil sample, color-coded by soil group. Arrows indicate soil physicochemical variables significantly associated with microbial community composition, with arrow length proportional to the strength of the correlation. The percentages on the axes represent the proportion of variance in community composition explained by the corresponding canonical axis.

Constrained analysis of principal coordinates (CAP) indicated that soil properties significantly influenced bacterial community composition (Table 3). Among individual factors, pH (in H_2O and KCl) was the strongest driver, explaining ~16–17% of the variation, followed by $CaCO_3$ (~13%), Fe content (~16%), and clay content (~15%) (all $p < 0.001$). The full

CAP model including all measured soil properties explained 59.7% of the variation in bacterial community composition (F = 6.49, p = 0.001), demonstrating that combined soil characteristics collectively shape microbial community structure.

Table 3 Results of the Mantel test based on Pearson's correlation coefficient for the effect of physicochemical properties on the community composition at the ASV level for all three soil groups together and individually (r – correlation coefficient; ns – not statistically significant)

Soil property	All 3 soil groups		Chernozem		Solonchak		Vertisol	
	r	*p*-value	r	*p*-value	R	*p*-value	r	*p*-value
pH in KCl	0.749	0.001***	0.597	0.004**	0.750	0.002**	0.349	0.001***
pH in H_2O	0.746	0.001***	0.617	0.004**	0.863	0.002**	0.258	0.001***
Fe	0.430	0.001***	0.542	0.004**	0.711	0.002**	0.196	0.013*
$CaCO_3$	0.425	0.001***	0.152	0.114ns	0.243	0.016*	0.216	0.001***
Na	0.388	0.001***	0.129	0.092ns	0.803	0.002**	0.331	0.001***
fine sand	0.332	0.001***	0.193	0.011*	0.226	0.003**	0.475	0.001***
clay	0.328	0.001***	0.191	0.011*	0.323	0.002**	0.404	0.001***
Ca	0.211	0.001***	0.212	0.011*	0.695	0.002**	0.547	0.001***
B	0.194	0.001***	-0.052	0.698ns	0.226	0.011*	0.334	0.001***
CEC	0.172	0.001***	0.283	0.004**	0.282	0.003**	0.587	0.001***
Mn	0.164	0.001***	0.508	0.004**	0.375	0.002**	0.270	0.001***
Cu	0.163	0.001***	0.056	0.277ns	0.590	0.002**	0.290	0.001***
total N	0.162	0.001***	0.208	0.033*	0.502	0.002**	0.461	0.001***
organic matter	0.161	0.001***	0.237	0.011*	0.519	0.002**	0.470	0.001***
K	0.136	0.001***	0.097	0.199ns	-0.057	0.792ns	0.135	0.013*
Mg	0.129	0.001***	0.217	0.014*	0.109	0.150ns	0.571	0.001***
K_2O	0.120	0.002**	0.197	0.020*	-0.092	0.900ns	0.193	0.005**
organic C	0.117	0.001***	0.361	0.004**	0.296	0.003**	0.479	0.001***
Zn	0.083	0.031**	0.186	0.060ns	-0.072	0.882ns	0.237	0.013*
coarse sand	0.060	0.074ns	-0.113	0.884ns	0.229	0.036*	0.340	0.001***
P_2O_5	0.035	0.174ns	0.123	0.170ns	-0.063	0.792ns	0.303	0.002**
silt	0.034	0.139ns	0.156	0.027*	-0.072	0.904ns	0.525	0.001***

3.5 Functional potential of soil microbial communities

The putative role of bacterial community was determined using FAPROTAX database. The most abundant KO (KEGG Orthology) categories were metabolism, environmental information processing, genetic information processing, cellular processes, human diseases, and organismal systems, with metabolism being dominant across all three analyzed levels (Fig. 8A). At level 2, it was found that 75% of the detected functions across all soil types belonged to 10 gene groups (Fig. 8B).

Of these, 6 were related to metabolism (carbohydrate metabolism, amino acid metabolism, energy metabolism, metabolism of cofactors and vitamins, nucleotide metabolism, and xenobiotics biodegradation and metabolism), 2 to environmental information processing (membrane transport and signal transduction), and 2 to genetic information processing (replication and repair and translation). At level 3, 27 categories had an abundance above 1% (Fig. 8C). On this level, 17 categories were related to metabolism, 4 to genetic information processing, 3 to environmental information processing, and 3 to cellular processes.

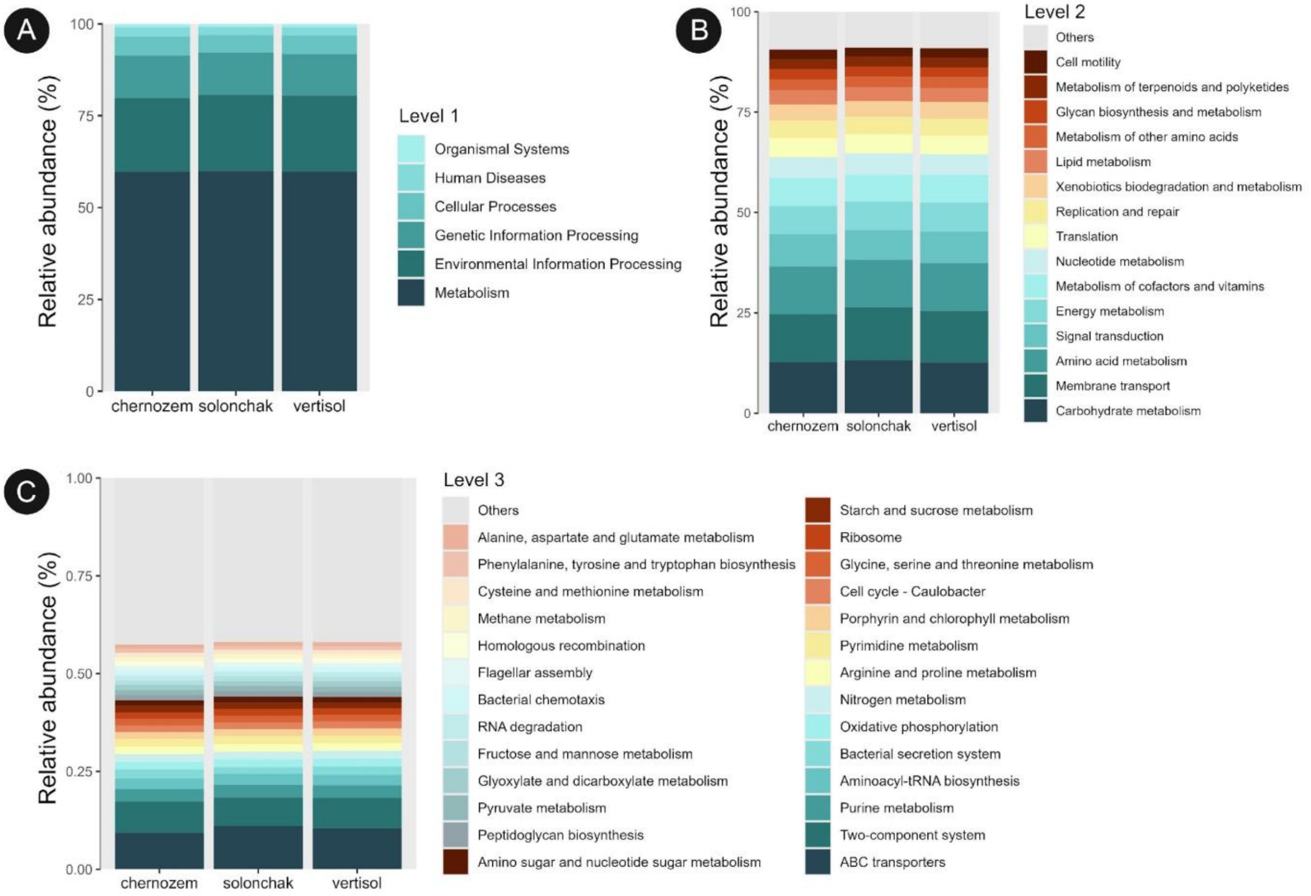

Figure 8 Functional prediction of bacterial communities in different soil groups on level 1 (A), level 2 (B), and level 3 (C).

LDA analysis identified 273 KEGG metabolic pathways, with 248 showing significant differences among different soil types. The number of the most significant detected pathways with the LDA score above 2 was 93. Out of those 93 pathways, 36 were in chernozem, 31 in solonchak, and 26 in vertisol. The most pathways differentially abundant across the different soil types belonged to metabolism group (Fig. 9).

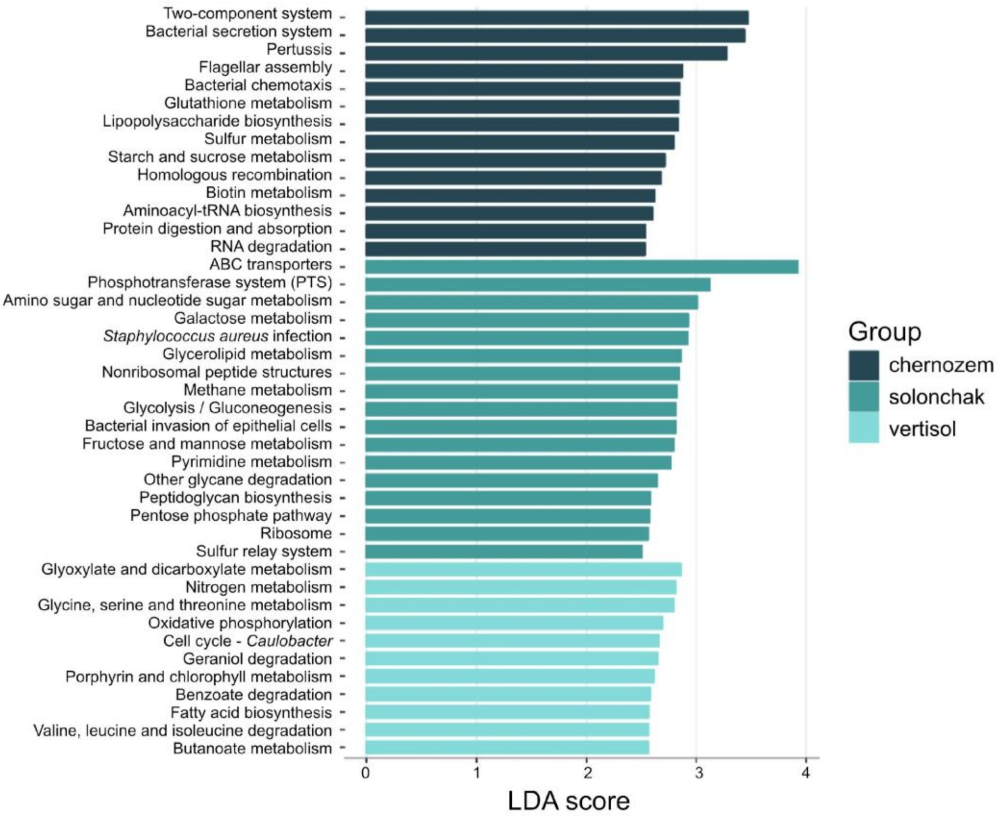

Figure 9 Differentially abundant bacterial functions (level 3) in different soil groups – Chernozem, Solonchak, and Vertisol.

3.6 Quantification of genes encoding key enzymes involved in nitrogen cycling

No statistically significant differences in nitrogen cycling gene copy numbers across different soil groups were detected (Table 4). However, at the field plot level, distinct patterns in gene abundances were observed (Fig. 10). Based on the quantification of archaeal and bacterial *amoA* gene copies, ammonia-oxidizing bacteria (AOB) exhibited consistently higher abundances compared to ammonia-oxidizing archaea (AOA) across all field plots. In the case of nitrite reductase genes, we observed a difference between *nirK* and *nirS* gene abundances, with *nirS* being prevalent in the majority of field plots. Among the genes associated with nitrate-reducing enzymes (*nirK*, *nirS*, and *nrfA*), we observed that *nrfA* consistently showed the lowest copy numbers in all field plots. These plot-level variations may reflect localized micro-environmental conditions, such as soil moisture, nutrient availability, or differences in management practices, which could influence the distribution of nitrogen-cycling microorganisms. Understanding the distribution of nitrogen-cycling microorganisms is critical, as they mediate essential soil processes that regulate nitrogen availability for plants and influence the production of nitrogenous gases, with implications for both soil fertility and ecosystem functioning.

Table 4 Means and standard deviations of selected nitrogen cycling gene copy numbers for Chernozem, Solonchak, and Vertisol. Statistically significant differences were assessed using Kruskal–Wallis test followed by post hoc Dunn's test; different letters indicate significant differences. In this case, no significant differences were observed.

Gene	Soil group		
	Chernozem	Solonchak	Vertisol
nifH ($\cdot 10^4$ copies/g dry soil)	10.33 ± 7.17 **a**	8.45 ± 2.10 **a**	9.49 ± 6.06 **a**
AOB *amoA* ($\cdot 10^8$ copies/g dry soil)	6.70 ± 3.79 **a**	6.62 ± 3.32 **a**	7.92 ± 1.12 **a**
AOA *amoA* ($\cdot 10^5$ copies/g dry soil)	2.06 ± 1.32 **a**	2.50 ± 0.51 **a**	2.41 ± 0.44 **a**
nirK ($\cdot 10^7$ copies/g dry soil)	10.17 ± 5.41 **a**	13.92 ± 3.49 **a**	16.43 ± 10.51 **a**
nirS ($\cdot 10^7$ copies/g dry soil)	12.63 ± 4.76 **a**	17.33 ± 6.53 **a**	16.44 ± 9.75 **a**
nrfA ($\cdot 10^4$ copies/g dry soil)	6.86 ± 5.93 **a**	9.79 ± 4.60 **a**	8.47 ± 3.13 **a**

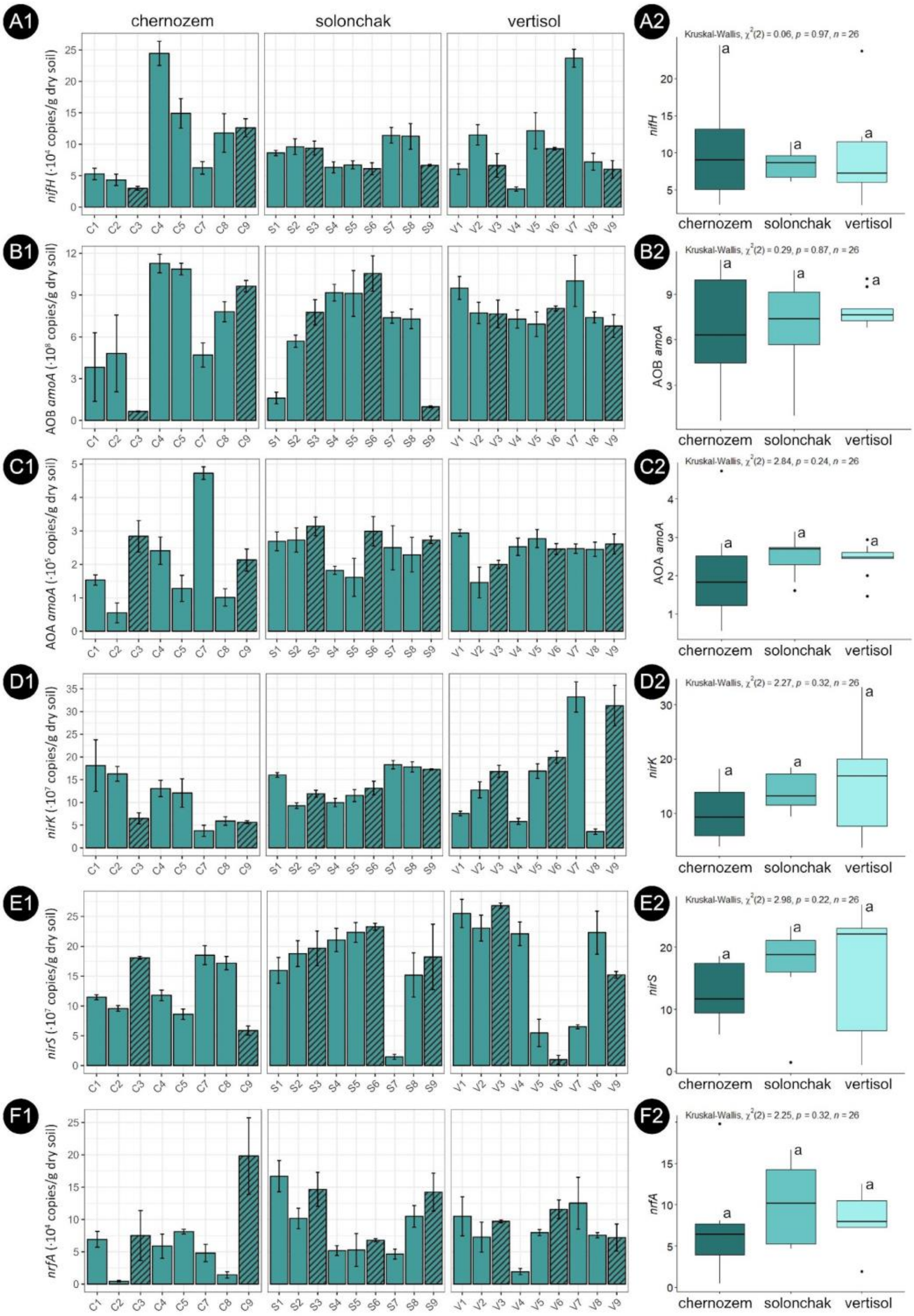

Figure 10 The number of copies of microbial functional genes involved in the nitrogen cycle in different soil groups. Figures A1–F1 show gene abundances across field plots for: A1) *nifH*, B1) bacterial *amoA*, C1) archaeal *amoA*, D1) *nirK*, E1) *nirS*, and F1) *nrfA*; ▨ – control

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plots. Figures A2–F2 show the same genes, with bars representing mean values and whiskers indicating the range (min–max) of samples within each soil group. Statistical significance was tested using the Kruskal–Wallis test followed by post hoc Dunn's test; differences among soil groups were indicated with different letters (no significant differences were observed).

4 Discussion

4.1 Alpha and beta diversity of soil microbial communities

The current study aimed to assess the variations in microbial communities across Chernozem, Solonchak, and Vertisol soil groups. These differences are evident based on alpha diversity indices, with Chernozem exhibiting the highest diversity, while Solonchak shows the lowest. The observed diversity in previous studies in Chernozem was found to be between 501.5 and 1519.8 (Zverev et al., 2020), while the Shannon index varied from 5 to 7.5 (Semenov et al., 2018; Zverev et al., 2020), which is consistent with our results. In Vertisol, the Shannon index typically ranges from 6.25 to 6.6, while the observed diversity is reported to be between 1250 and 1700 (Steinberger et al., 2024). The Shannon index is known to decrease as soil salinity increases (Rath et al., 2019), with typical values around 6 or 7 (Shi et al., 2019). Consequently, saline soils generally exhibit the lowest Shannon index (Xu et al., 2021), a finding consistent with our results. The reduced diversity under stress conditions, typical of saline soils, is often associated with diminished functional redundancy, leading to lower resilience and potential shifts in key soil processes such as nutrient cycling and decomposition. Conversely, the richer microbial communities in Chernozem may provide a broader functional capacity, thereby supporting ecosystem stability under environmental fluctuations (Griffiths and Philippot, 2013; Delgado-Baquerizo et al., 2016).

The analysis of beta diversity conducted through PCoA based on Bray-Curtis and Jaccard indices, as well as Weighted UniFrac, revealed clear distinctions between samples, with grouping occurring according to soil group. These spatial patterns suggest that variations in microbial community structure correspond to environmental gradients and combinations of soil properties within each soil group. Other research has also demonstrated that soil group significantly influences bacterial communities in soil, resulting in similar microbial communities in soils with similar properties (Xue et al., 2024). Sample distribution and separation pattern on PCoA plot has also been attributed to a single soil property, such as pH. For instance, soil samples with low pH are significantly different from those with higher pH (Xia et al., 2020). This trend was observed in our study as well, where plot C3, characterized by atypically low pH for Chernozem, clustered with slightly acidic Vertisol field plots. Furthermore, RDA and CAP analyses conducted on our data confirmed that pH, $CaCO_3$, Fe, fine sand, and clay content are the primary drivers of microbial composition, supporting the observed clustering patterns by soil group and highlighting the combined influence of soil chemical and physical properties on microbial beta diversity.

4.2 Soil bacterial community composition

Phyla Actinobacteriota (up to 55.5%), Proteobacteria (up to 35%), and Verrucomicrobiota (up to 55%) have previously been identified as dominant in Chernozem (Semenov et al., 2018). In our study, Actinobacteriota and Proteobacteria were identified as the most prevalent phyla, with the addition of Acidobacteriota, while Verrucomicrobiota was observed to be less abundant, comprising less than 10% in most field plots. The dominance of Proteobacteria and Acidobacteriota in Chernozem likely reflects the neutral pH and high nutrient availability in this soil type, which favor metabolically versatile taxa capable of rapidly utilizing available carbon and nitrogen sources (Rousk et al., 2010.; Fierer et al., 2012; Delgado-Baquerizo et al., 2018). Consistent with previous findings (Boyarshin et al., 2023), bacterial communities in Chernozem appear similar across agricultural and non-agricultural plots in Vojvodina. Saline soils, on the other hand, are typically dominated by Actinobacteriota (Pershina et al., 2018), while Acidobacteriota and Nitrospirota are less abundant, and Firmicutes are more abundant compared to non-saline soils (Xu et al., 2021), which is consistent with our results. This pattern can be explained by the high salinity and osmotic stress in Solonchak, which select for halotolerant and stress-adapted taxa, such as Actinobacteriota and certain Firmicutes, that possess genes providing osmoprotection, drought tolerance, and nutrient acquisition under low-fertility conditions (Rath et al., 2019; Pershina et al., 2018; Zhang et al., 2023). In Vertisol, the dominant phyla include Proteobacteria, Acidobacteriota, Bacteroidota, and Verrucomicrobiota (Knelman et al., 2015), with Proteobacteria being the most prevalent, comprising approximately 20% (Song et al., 2018). Vertisol under vineyard cultivation demonstrate increased abundances of Bacteroidota, Planctomycetota, and Proteobacteria, compared to other land use types (Steinberger et al., 2024). These trends were observed in our samples as well, particularly in field plot V7 under vineyard and corresponding field plot under conventional agriculture and uncultured soil (V8 and V9). The prevalence of these taxa in Vertisol can be linked to its clayey texture, which affects water retention, oxygen diffusion, and microhabitat heterogeneity, favoring taxa adapted to variable moisture and nutrient conditions. Differences in phyla abundance between soil groups may reflect variations in the distribution of lower taxonomic categories that are adapted to specific environmental conditions (Naylor et al., 2022). Bacteria belonging to families Sphingomonadaceae, Xanthobacteraceae, Gemmatimonadaceae, Chitinophagaceae, and Rubrobacteriaceae are commonly found in Chernozem (Boyarshin et al., 2023). Meta-analysis of saline soils have revealed increased relative abundance of distinct families within phyla such as Proteobacteria (Xanthomonadaceae, Halomonadaceae, Nitrospiraceae, and Sphingomonadaceae), Acidobacteriota (Holophagaceae), Actinobacteriota (Micrococcaceae, Propionobacteraceae, Streptomycetaceae, Rubrobacteriaceae, and Solirubrobacteraceae), Bacteroidetes (Sphingobacteriaceae, and Flavobacteriaceae), Chloroflexi (Anaerolineaceae and Caldilineaceae), and Firmicutes (Bacillaceae and Clostridiaceae) (Ma and Gong, 2013). In our study, the most abundant families in Solonchak were an uncultured family belonging to Acidobacteriota, Vicinamibacteraceae, and Nitrososphaeraceae. In addition, Halomonadaceae, Micrococcaceae, Propionobacteraceae, Streptomycetaceae, Rubrobacteriaceae, Bacillaceae, Clostridiaceae, and Caldilineaceae were more abundant in Solonchak compared to Chernozem and Vertisol. The prevalence of these families in Solonchak may be linked to salinity tolerance, osmotic stress adaptation, and nutrient acquisition strategies, which enable them to thrive under high-salt or low-nutrient conditions (Rath et al., 2019). Our results show partial similarity with results of stated meta-analysis; however, the three most abundant families in our research were not reported as prevalent in previous study. This discrepancy may be attributed to methodological differences, including DNA extraction method, primer choice and sequencing platform (Fouhy et al., 2016), regional environmental variations such climate and land use (Drenovsky et al., 2010), as well as differences in sampling strategy, including depth and location (Hao et al., 2021). Vertisol is characterized by higher abundances of Chthoniobacteraceae, Nitrososphaeraceae, Gemmatimonadaceae, Bacillaceae, Nocardioidaceae, Bradyrhizobiaceae, Syntrophobacteraceae, Pseudomonadaceae, and Micrococcaceae (Rincon-Florez et al., 2020). Vicinamibacteraceae in our study was significantly more abundant in Chernozem compared to Solonchak and Vertisol, likely reflecting its preference for neutral pH soils and nutrient-rich conditions that support its metabolic activities in organic matter decomposition and nitrogen cycling (Huber and Overmann, 2018). The composition of bacterial communities across different taxonomic levels in this research largely corroborates previous studies. However, we identified variations in the presence and abundance of certain taxa, which may result from the complex interactions of climatic, biotic, and edaphic factors that influence the structure of soil microbial communities (Waldrop et al., 2017; Labouyrie et al., 2023; Xue et al., 2024). Variations in soil physical and chemical properties could explain some portion of the biogeographic patterns observed in soil community composition, as well (Yu et al., 2022). Overall, the observed dominance of Proteobacteria and Acidobacteriota in Chernozem and Vertisol, and Actinobacteriota in Solonchak, can be attributed to the interplay of soil pH, salinity, nutrient availability, and texture, which favor taxa with specific metabolic capabilities and stress tolerances.

This study provides novel insights into the distribution of bacterial families across Chernozem, Solonchak, and Vertisol soils in our region, highlighting the previously unreported high abundance of Vicinamibacteraceae, a relatively recently described bacterial family (Huber and Overmann, 2018) that is integral to soil health due to its roles in organic matter decomposition, nitrogen cycling, and adaptability to varying environmental conditions, in Chernozem. Additionally, the distinct community composition observed under different management practices (e.g. vineyards and conventional agriculture in Vertisol) further emphasizes how soil type and land use interact to shape microbial communities through changes in soil chemistry, moisture, and nutrient distribution. These findings demonstrate that specific soil properties and agricultural practices strongly influence microbial community structure, offering new understanding of the environmental and management factors that drive microbial diversity and potential ecosystem functions in these soils.

4.3 The relationship between soil properties and microbiome

Regarding soil properties, our findings suggest that pH has the most significant influence on bacterial community composition, which is consistent with previous findings (Bartram et al., 2014; Zhang et al., 2017). This effect can be explained by the fact that pH regulates nutrient solubility, metal availability, and enzyme activity, thereby directly shaping microbial metabolism and competitiveness (Lauber et al., 2009; Rousk et al., 2010) Some phyla, such as Proteobacteria, exhibit varying correlations with pH across different studies, displaying either positive or negative relationships (Ko et al., 2017; Yan et al., 2019). These discrepancies may reflect the differing abundances of lower taxonomic groups within the phylum, which have distinct physiological pH optimum (Shen et al., 2013; Yun et al., 2016). Moreover, the complex interactions between soil properties, as well as different crops, agricultural practices, and field rotation, can increase heterogeneity, modulating the influence of individual factors like pH on various taxa (Karimi et al., 2018). In saline soils, pH value along with soil salinity represents the most influential factor shaping microbial communities (Rath et al., 2019). In our

Solonchak soils, elevated $CaCO_3$ and salinity likely reduce phosphorus availability and impose osmotic stress, creating conditions that favor stress-tolerant bacterial groups. Soil texture is another factor with a significant influence on bacterial communities. Sandy soils, characterized by lower aggregate stability and higher oxygen concentration, differ from clayey soils, which are susceptible to aggregate compaction and bonding, creating a less favorable environment for microorganisms (Shah et al., 2017). This was evident in our Vertisol soils, where high clay content likely limited aeration and diffusion, favoring microbial groups adapted to lower oxygen availability, while Chernozem soils with higher sand content supported more aerated and nutrient-cycling communities. Accordingly, we observed that phyla in our study demonstrated positive correlation with sand, and negative with clay, and vice versa. In European soils, pH, CaCO3, and the carbon-to-nitrogen ratio have been identified as key factors affecting bacterial diversity (Labouyrie et al., 2023). In our study, nearly all of the dominant phyla showed significant positive or negative correlations with pH and CaCO3. Other soil properties, such as phosphorus, iron, manganese, nitrogen, copper, and organic matter, have also been recognized as critical factors influencing several prevalent taxa (Liu et al., 2013; Song et al., 2018). Additionally, interactive effects among soil properties and management practices, such as crop rotations, fertilization, and tillage regimes, can further influence microbial community composition by altering nutrient availability, soil structure, and microhabitat conditions (Zhang et al., 2017; Zong et al., 2024). These interactions may modulate how individual factors, like pH, $CaCO_3$, and texture, shape the abundance and distribution of specific taxa, contributing to the heterogeneity observed across field plots. The relationship between bacterial families and soil properties exhibits considerable variation across studies (Wang et al., 2019; Xia et al., 2020; Yang et al., 2022), likely due to differences in other soil characteristics and the abundance of lower taxonomic ranks. Redundancy analysis identified organic matter, pH, nitrogen, potassium, and phosphorus as significant drivers of microbial populations (Niu et al., 2021). Unlike previous studies, however, phosphorus was not found to be a significant factor in our research, while CaCO3 and iron were observed to have important roles. This suggests that in our study area, where phosphorus levels might be stabilized by fertilizer use, other factors, such as CaCO3 and iron availability emerge as stronger drivers of community structure. Phosphorus did not exhibit a significant effect on microbial community composition in our study likely because fertilizer application across sites has led to relatively uniform levels of available P_2O_5, reducing variability and masking its influence. Consistent with previous findings (Tian et al., 2017), we observed a negative correlation between pH and total nitrogen, potassium, phosphorus, and organic carbon. Among physical soil properties, clay content is known to significantly affect microbial communities (Xu et al., 2020). This was also observed in our research, where the clay content exhibited the strongest correlation with microbial communities in Vertisol, soil group characterized by clayey texture. These results support the idea that the microstructural environment created by clay-rich soils imposes constraints on microbial colonization and activity, explaining the distinct community profiles in Vertisol. Samples in our study were clearly separated on RDA plot according to soil group. Given that soil properties that consequently determine soil group significantly influence bacterial populations (Yeoh et al., 2017), we conclude that differences in microbial communities observed in our study can largely be attributed to variations in soil groups. In addition, it is important to note that soil temperature was not measured in this study and therefore was not included as an explanatory variable. Soil temperature significantly influences microbial community composition and activity. Elevated temperatures can reduce microbial diversity and alter the abundance of major soil microbial phyla, such as Firmicutes, Acidobacteriota, and Proteobacteria (Zhao et al., 2024). Additionally, warming can increase microbial activity, potentially leading to higher soil carbon losses and exacerbating climate change (Metze et al., 2024). Considering that Vojvodina frequently experiences summer temperatures exceeding 35°C, future studies incorporating soil temperature measurements would provide valuable mechanistic insights into seasonal shifts in microbial diversity and function.

The integration of microbial community data with detailed soil property analyses provides a comprehensive understanding of the factors shaping soil biodiversity and ecosystem function. By linking taxonomic composition and edaphic variables such as pH, $CaCO_3$ content, texture, and nutrient availability, this approach allows for the identification of soil management strategies that support beneficial microbial communities. These insights can guide targeted interventions, such as crop rotation planning, salinity mitigation, or organic amendment application, to enhance soil fertility, maintain microbial diversity, and improve resilience against environmental stressors. Ultimately, incorporating microbial indicators into land management and soil restoration practices can foster sustainable agricultural productivity while preserving soil ecosystem health.

4.4 Functional potential of soil microbial communities

We evaluated the functional potential of microbial communities by analyzing enriched metabolic pathways. Chen et al. (2020) identified the same functional groups to those observed in our study – metabolism, environmental information processing, genetic information processing, and cellular processes – with a combined abundance exceeding 90%. However, their study reported a higher abundance of the genetic information processing group compared to environmental information processing, which contrasts with our findings. Previous research has also confirmed the dominance of metabolic functional group across the soil types analyzed in our study (Steinberger et al., 2024; Tang et al., 2022). Further analysis of differentially abundant metabolic pathways in chernozem, solonchak, and vertisol revealed that metabolic pathways were dominant in all soil types. We observed that ABC transporters were more abundant in solonchak. Sulphur transport system, a subgroup of ABC transporters, plays a significant role in bacterial adaptation to salinity stress. These transporters modulate molecular transport into cells and regulate osmotic pressure in microbial cells inhabiting saline soils (Wang et al., 2023). The functional composition of microbial communities reflects interaction, regulation and adaptability of soil microbiome, which are critical for soil and plant health. Microbiome is a key driver of numerous important plant functions and a regulator of soil nutrient cycling. Therefore, enhancing the genomic and metabolic capacities of microbiomes may improve plant resistance to biological stress and increase nutrient uptake (Cordovez et al., 2019). Essential functional genes contribute to amino acid absorption (Rahman et al., 2014), nitrogen fixation, and the transport of metabolites and ions across cell membrane (Z. Wu et al., 2016), supporting bacterial survival and reproduction, nutrient cycling, and plant growth and development. The higher abundance of specific metabolic pathways in different soil types may be attributed to variations in microbial community composition. For example, Acidobacteriota is known for its high metabolic and genetic diversity (Lee et al., 2008), so its increased or decreased abundance in certain soils could result in the differential abundance of corresponding functional groups. Functions related to plant growth and disease suppression, such as sulfur metabolism, lipopolysaccharide synthesis, and siderophore production, are crucial for soil health (Lemanceau et al., 1992; Zaccardelli et al., 2013). Lipopolysaccharide synthesis, which promotes systematic resistance to plant pathogens (van Loon et al., 1998), was enriched in chernozem in our study and is likely linked to the higher abundance of plant disease-suppressing bacteria such as *Bacillus*, *Burkholderia*, *Pseudomonas*, *Serratia*, *Streptomyces*, and *Stenotrophomonas* (Dube et al., 2019). Thus, selective enrichment of beneficial bacterial taxa and functions could significantly enhance soil and plant health, thereby improving agricultural productivity and increasing crop yields.

4.5 Nitrogen cycling genes in soil

Additionally, this study aimed to investigate genes involved in nitrogen cycling, particularly focusing on nitrogenase gene abundance and nitrifying and denitrifying communities. Our results showed that nitrogenase coding gene copy numbers, specifically the *nifH* gene, were lower compared to those reported in previous studies (Wang et al., 2017). It is observed that the abundance of *nifH* gene is higher in soils without fertilizer application (Chen et al., 2021). Given the extensive use of fertilizers in Vojvodina, the lower abundance of the *nifH* gene observed in our study is consistent with the expected impact of fertilizer application on nitrogen-fixing microbial communities. With regard to the nitrifier community, certain studies have often reported that AOA were more abundant relative to AOB (Zhang et al., 2013; Sun et al., 2015; Wang et al., 2017; Zhu et al., 2018). In contrast, our findings are in agreement with other studies that have demonstrated the opposite trend - a higher abundance of AOB (Banning et al., 2015; Ke et al., 2015; Zhao et al., 2015; H. Wu et al., 2016; Xue et al., 2016). This discrepancy may be explained by higher oxygen availability at the soil surface, leading to increased bacterial *amoA* gene count (Ke et al., 2015). Additionally, AOB population is much more prominently increased (326% increase) in response to the application of nitrogen fertilizers compared to AOA (27% increase) (Carey et al., 2016). In addition to fertilizer application, other environmental and methodological factors may contribute to the observed lower *nifH* gene abundance and the contrasting dominance of AOB over AOA. Soil moisture, depth, pH, and organic matter have been shown to strongly influence *nifH* abundance (Wang et al., 2017). For ammonia oxidizers, shifts in pH and oxygen availability are critical drivers of the relative balance between AOA and AOB populations (Prosser and Nicol, 2012; Xue et al., 2016). In addition, primer bias and methodological variation may also explain divergent results across studies, especially in the detection of AOA vs. AOB *amoA* genes (Dechesne et al., 2016). Together, these factors, along with intensive fertilization in the Vojvodina region, provide a broader ecological and technical context for interpreting our results. Our study also found that the abundance of *nirS* gene was consistently higher than that of *nirK* across most of the analyzed field plots (Wang et al., 2017). Previous research indicated that the addition of organic fertilizer could lead to increase in *nir* gene copy numbers (Dong et al., 2015; Tao et al., 2018). However, we observed the increase only in *nirS* gene abundance in field plot under organic agriculture. Contrary, *nirK* gene abundance was lower under organic agriculture compared to conventional and control plots, which is consistent with the findings of Wang et al. (2020), who reported similar trends following the addition of biochar. Furthermore, the observed discrepancies in the abundance of *nrfA* gene between our study and literature (Tatti et al., 2014) could be attributed to competition for nitrates between denitrifiers and bacteria involved in disimilatory nitrite reduction to ammonia (DNRA). The addition of nitrate fertilizers usually reduces *nrfA:nir* ratio, suggesting that denitrifying community becomes more competitive under these conditions (Putz et al., 2018). Given that the abundance of nir genes was significantly higher than that of *nrfA* in our study, we can conclude that denitrifiers have a competitive advantage in fertilized soils of Vojvodina. The observed dominance of denitrifiers in fertilized soils suggests important functional consequences for nitrogen cycling. Higher denitrifier abundance may accelerate nitrogen loss through denitrification, potentially reducing nitrogen availability for crops. At the same time, this process can increase emissions of N_2O, a potent greenhouse gas, highlighting an environmental trade-off. These results highlight the need to consider microbial dynamics in soil and fertilization management to optimize nutrient use and reduce environmental impacts.

Our study revealed distinct patterns in nitrogen cycling gene abundances across different soil types, which correspond to variations in microbial community composition. In Chernozem and Vertisol soils, the higher observed abundance of Proteobacteria can be associated with increased *nifH* gene copy numbers, suggesting that these soils support a more active nitrogen-fixing community. This observation aligns with findings by Sepp et al. (2023), who reported a positive correlation between Proteobacteria abundance and *nifH* gene presence across various soil types. Similarly, the elevated presence of Proteobacteria in these soils corresponded with higher *amoA* gene copy numbers, indicating a more active ammonia-oxidizing bacterial community. This is consistent with research by Adair et al. (2013). In contrast, Solonchak soils exhibited a higher abundance of Crenarchaeota, which was linked to increased archaeal *amoA* gene copy numbers (Leininger et al., 2006). Proteobacteria were particularly abundant in Chernozem and Vertisol soils, whereas Actinobacteriota dominated in Solonchak. Given that *nirK* and *nirS* gene abundances were similar across all three soil types, these results suggest that denitrification may be mediated by different members of these two phyla depending on the soil group. This is in agreement with Pessi et al. (2022), who identified these phyla as dominant in denitrifying communities across various ecosystems. These connections between microbial community composition and nitrogen cycling gene abundances underscore the influence of soil type and microbial diversity on nitrogen cycling processes. Understanding these relationships is crucial for developing sustainable agricultural practices that optimize nitrogen use and minimize environmental impacts.

While this study provided valuable insights into microbial diversity, future research should investigate fungal community composition and its relationship with soil properties to achieve a more comprehensive understanding of soil health. Prioritizing the exploration of microbial activity through advanced methods, such as metatranscriptomics, will further deepen our knowledge. Additionally, expanding the analysis to encompass a broader range of soil groups, thereby including greater pedodiversity, will contribute to the achievements in examining the overall ecosystem services of soil as a vital resource.

5 Conclusion

Our work presented the first detailed systematic study of soil bacterial diversity using the metabarcoding approach in Serbia, covering the three key soil groups of an important agricultural region (Chernozem, Solonchak, and Vertisol). Obtained in-depth look at the bacterial community profiles revealed important connections with physical and chemical soil properties and land management practices. The study highlighted the differences in alpha diversity across analyzed soil groups, with Chernozem having the most diverse (Shannon index mean = 6.20) and Solonchak the least diverse bacterial community (Shannon index mean = 6.01). Both beta diversity and RDA analysis indicated clear statistically significant separation of samples based on soil group and soil properties. These findings support our hypothesis that contrasting soil properties among Chernozem, Solonchak, and Vertisol drive distinct microbial community structures, reflecting the ecological adaptations of taxa to specific edaphic conditions. We demonstrated that each soil group possesses a distinct bacterial community, with the dominance of selected taxa based on optimal soil properties for each taxon. Additionally, the strong influence of soil properties on microbial communities was observed, with pH value, iron availability, and $CaCO_3$ content having the highest statistically significant ($p\text{-value} \leq 0.05$) impact.

The present study marks a first step towards soil monitoring with the use of modern methods harmonized with European monitoring programs in Serbia. Our findings provide an essential baseline for continual monitoring and preservation of soil health, enabling at the same time the enhancement of agricultural production. This baseline can guide practical soil management practices, such as targeted pH adjustments, nutrient management, or organic amendments, to support beneficial microbial communities and improve crop productivity. Moreover, it can inform agricultural policy and monitoring programs by identifying priority areas for soil conservation, supporting sustainable land-use planning, and integrating microbial diversity metrics into soil health assessments.

In future research, it would be valuable to explore fungal community dynamics, the functional activity of microbes and fungi, and their role in ecosystem services; future investigations employing advanced methods, such as metatranscriptomics, will further elucidate these processes while expanding the analysis across diverse soil groups to enhance our understanding of soil ecosystem services as a vital resource.

Code and data availability

DNA sequences generated for this study were deposited in the NCBI – Sequence Read Archive (SRA), under the accession number PRJNA1116093 (http://www.ncbi.nlm.nih.gov/bioproject/1116093). Any additional data used or analyzed in this study is available from the corresponding author upon request.

Author contributions

RD, SM, MD, DT: conceptualization. SM, AK: data collection. SM, DT, AK: investigation. SM, DT, RD, MD, AK: methodology. MD: project administration, funding acquisition, resources. RD, JV, JN, DT, SM, MD, AK: data analysis and interpretation. RD, SM, MD, DT: supervision. RD, SM, DT, MD, JV, JN: validation, writing – review and editing. AK: visualization, writing – original draft. All authors contributed to the article and approved the submitted version.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Disclaimer

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