Heliyon 9 (2023) e17208

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

The microbiome of buried soils demonstrates significant shifts in taxonomic structure and a general trend towards mineral horizons

A.A. Kichko [a,*], N.K. Sergaliev [b], E.A. Ivanova [a,c], T.I. Chernov [c], A.K. Kimeklis [a,d], O.V. Orlova [a], M.D. Kalmenov [b], K.M. Akhmedenov [e], A.G. Pinaev [a], N.A. Provorov [a], N.A. Shashkov [a,f], E.E. Andronov [a,c]

[a] *All-Russian Research Institute for Agricultural Microbiology, Saint-Petersburg, Russia*
[b] *West Kazakhstan Innovation and Technology University, Uralsk, Kazakhstan*
[c] *Dokuchaev Soil Science Institute, Moscow, Russia*
[d] *Department of Applied Ecology, St. Petersburg State University, Saint-Petersburg, Russia*
[e] *M. Utemisov West Kazakhstan University, Uralsk, Kazakhstan*
[f] *Federal State Budget-Financed Educational Institution of Higher Education The Bonch-Bruevich Saint Petersburg State University of Telecommunications, Saint-Petersburg, Russia*

ARTICLE INFO

Keywords:
Kurgan
Burial mound
Dark-chestnut soil
Microbiome
High throughput sequencing
Diagenesis
16s rRNA

ABSTRACT

Burial mounds represent a challenge for microbiologists. Could ancient buried soils preserve microbiomes as they do archaeological artifacts? To investigate this question, we studied the soil microbiome under a burial mound dating from 2500 years ago in Western Kazakhstan. Two soil profile cuts were established: one under the burial mound and another adjacent to the mound surface steppe soil. Both soils represented the same dark chestnut soil type and had the same horizontal stratification (A, B, C horizons) with slight alterations. DNA samples isolated from all horizons were studied with molecular techniques including qPCR and high throughput sequencing of amplicon libraries of the 16S rRNA gene fragment. The taxonomic structure of the microbiome of the buried horizons demonstrated a deep divergence from ones of the surface, comparable to the variation between different soil types (representatives of the soil types were included in the survey). The cause of this divergence could be attributed to diagenetic processes characterized by the reduction of organic matter content and changes in its structure. Corresponding trends in the microbiome structure are obvious from the beta-diversity pattern: the A and B horizons of the buried soils form one cluster with the C horizons of both buried and surface soil. This trend could generally be designated as 'mineralization'. Statistically significant changes between the buried and surface soils microbiomes were detected in the number of phylogenetic clusters, the biology of which is in the line of diagenesis. The trend of 'mineralization' was also supported by PICRUSt2 functional prediction, demonstrating a higher occurrence of the processes of degradation in the buried microbiome. Our results show a profound shift in the buried microbiome relatively the "surface" microbiome, indicating the deep difference between the original and buried microbiomes.

* Corresponding author.
E-mail address: 2014arki@gmail.com (A.A. Kichko).

https://doi.org/10.1016/j.heliyon.2023.e17208
Received 6 September 2022; Received in revised form 9 June 2023; Accepted 9 June 2023
Available online 12 June 2023

1. Introduction

Buried soils (or paleosols) are formed by a spontaneous or purposeful deposition of natural [1–3] or artificial material over surface soil. Soils buried under the material of natural origin are found on narrow slopes [3], river terraces [2,4], under peatlands [1] or in cryoturbated soils [5]. Paleosols are often used for paleo-archaeological reconstructions. Objects for reconstructing environmental conditions are found in soils buried under man-made structures, such as archaeological layers of ancient dwellings [6], earthworks [7, 8] and burial mounds (or kurgans) [9–13]. The comprehensive study of buried soils allows us to discover information about climate dynamics, vegetation, and other bioclimatic conditions [9,10,14,15] as well as to confirm the age of archaeological objects [16]. Such investigations often include a variety of methodological techniques, including the analysis of physical, chemical, and morphological properties such as pH, content of soluble salts, calcic and gypsum formations [9,10,17], common pool and available forms of carbon and nitrogen [5,18], elemental composition [19], humus content [9,10], mineral composition of clay fraction [20], and analysis of sporo-pollen and phytoliths [19].

The soil burial process is followed by a decrease in the effect of environmental factors such as fresh organic matter input and oxygen-enriched air. Along with shifts in thermic and water soil regimes, these changes significantly affect the abundance and structure of the microbiome [3,21,22] which is the most reactive and labile fraction of the soil system [23–26]. Investigations of buried soils often include assessment of the microbiome parameters using different methods of classic microbiology, which include: cultivation with selective media [1,21,27], microbiome stimulation with activity evaluation [2,9,12,17,27], estimation of the total microbial biomass [2,6,10,17,27] or the total abundance [28], estimation of trophic coefficients [5,10,12,16] and enzymatic activity [1, 5,6], accounting viable microorganisms with microscopy [27]) and molecular biology methods (analysis of membrane-associated DGDT [4], TRFLP [11,27] and DGGE analysis [2], quantification of marker gene copies [5,7,8,14] and high-throughput sequencing [5–7,18,29].

Microbial communities of paleosols are considered to be partially preserved, this provides some information about environmental conditions at the time of burial [2,9,22,27,30]. For example, according to radiocarbon analysis, the age of microbial biomass for the humic horizon of buried chestnut soil corresponds to the time of its burial [31]. Despite the decrease in microbial abundance and diversity [5,27] some total patterns such as profile stratification by morphology [9,21] and microbial abundance [8] or the trophic and taxonomic structure [3,7,9,32], are preserved in buried soils for a long time. On the other hand, the burial process is followed by diagenesis [33] with a loss in the quantity of organic matter [3,7,10,16] in the soil and its structural transformation [9]. Shifts in environmental conditions induce the accumulation of survival structures, such as spores and nanoforms [27], along with shifts in the trophic structure [1,5,10,12] and imbalance in soil microbial processes and pathways [21]. In addition, the high rates of soil respiration [3,9,21] and the size of active microbial biomass [10] indicate microbial activity under burial conditions. Diagenetic processes are highly intensive for the first 100–300 years of burial and then the decrease in soil organic matter slows down and the soil system becomes more stable [21,22].

There is no clear answer as to how the microbiome data of buried soils can help in the reconstruction of environmental conditions at the time of burial. Comparative analysis of buried soil and the adjacent surface soil of the same soil type (formed by similar soil-forming factors) is the classical approach in this case. There are previous researches on such soil pairs, including soil chronosequences, which used them to infer the environmental dynamics in a broader timespan [9,10,27,33].

The aim of our research was to assess and assign common and different features of the structures of prokaryotic communities of dark chestnut soil buried under a mound from 500 B.C. (the age was established by archaeological artifacts) and the adjacent dark chestnut surface soil. A comparative analysis of the prokaryotic communities of different soil types formed in distinct bioclimatic conditions was performed to better characterize the burial process and to scale observed divergence.

2. Materials and methods

2.1. Sampling location

Soil samples were collected during the archaeological survey of the Segizsay I (Lebedevka) burial complex, consisting of 20

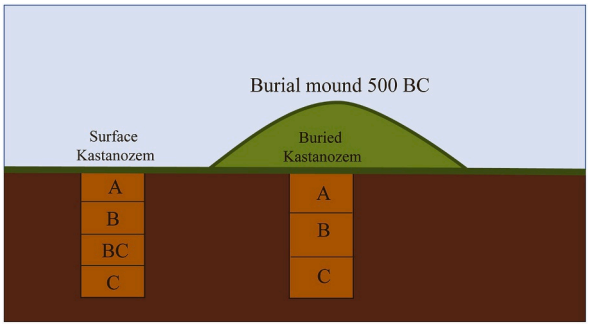

Fig. 1. Schematic picture of the burial mound, the buried and surface soil cuts.

kurgans, located at the flat surface of a watershed upland, 9 km south-west of Segizsay, Western Kazakhstan. Soil buried under Kurgan No 1 was excavated in 2018 close to the base of the upland and used in the current study. The burial place dates back to the end of the VI century - the middle of the V century BC, corresponding to the Scythian-Sarmatian period of the early Iron Age [34]. The buried soil presented in the current analysis was located at the side of a young woman's grave. Inside the burial place the following objects were found: a stone credence table, a tiny clay vessel, a bronze mirror, a glass flacon, a glass chaplet, a moulded clay crock, and sheep bones. Photographs of the kurgan and artifacts are attached in Figure S1. The climatic environment of Western Kazakhstan during that period was comparable to the current relatively humid conditions [35] with about 300–330 mm of average annual precipitation [9]. There are two contrary opinions about the climate continentality of that period. Some researchers assert a higher continentality rate, while others contend that the climate was milder [35].

Samples of paleosol were taken from under the burial place (embankment height 1.3 m); surface soil located on a mild slope was chosen as a modern analogue (Fig. 1). Both soils were classified as dark chestnut carbonate on Cretaceous rocks (Kastanozems [36]), with high carbonate content and increased humus content. The descriptions of the soil profiles and vegetation are given in the File S1.

Soil material was sampled from each horizon in 5 replicates, placed in envelopes, delivered to a laboratory within 24 h, and then stored at −20 °C until further analysis.

2.2. Carbon and nitrogen content

Total carbon content was determined titrimetrically by the Tyrin wet oxidation method (bichromatic) with Nikitin modifications [37]. The water-soluble carbon content was measured in an aqueous extract with soil:water proportion equal to 1:5 [37]. The concentration of organic carbon in extracts was measured by wet oxidation with a sulphochromic mixture (0.04 n) and further spectrophotometric analysis on an 'Ultraspec' device («LKB», Switzerland; $\lambda = 340$ nm) [38].

Total nitrogen was measured on an automatic nitrogen analyser (Boshu, Switzerland) with the Kjeldahl method [39].

All measurements were performed only for the A and B horizons of the surface and buried soil.

2.3. pH and electroconductivity

The pH was measured in soil suspension in the proportion soil:distilled water 1:2.5. The suspension was shaken for 4 h, after which the pH was measured with METTLER TOLEDO Seven Compact pH-meter and electroconductivity was measured in the same suspension with STARTER 300C device.

2.4. DNA extraction and purification

DNA extraction and purification were performed with a NucleoSpin Soil kit (MACHEREY-NAGEL, Germany) in accordance with the manufacturer's guidance. For DNA extraction SL1 lysis buffer and 150 mkl of enhancer were added to 0.25 g of soil. For homogenization, a Precellys 24 machine (Bertin technologies, France) was used (6000 rpm: 2 × 30″).

2.5. Quantitative analysis of bacterial DNA

For the quantitative analysis of bacterial DNA, Real-Time PCR was performed. Universal primers to the prokaryotic 16s rRNA gene were EUB338 – ACTCCTACGGGAGGCAGCAG/EUB518 – ATTACCGCGGCTGCTGG [40]. A range of ten-fold dilutions of 16S rRNA *E. coli* was used as a standard. PCR was performed in three technical replicates using the qPCR mix-HS SYBR kit (Evrogen, Russia) mixture, in accordance with the manufacturer's guidance on a CFX96 Real-Time PCR Detection System (BIO-RAD, USA) device using the protocol: 95 °C - 3′; 95 °C - 20″, 50 °C - 20″, 72 °C - 20" (40 cycles); final melt curve generation.

2.6. High throughput sequencing and further data analysis

The library preparation for sequencing included amplification of the target fragment of the variable region V4 of the 16S rRNA gene using universal primers (515F - GTGCCAGCMGCCGCGGTAA/806R - GGACTACVSGGGTATCTAAT) [41] together with linkers and unique barcodes. PCR was performed on a T100 Thermal Cycler (BIO-RAD Laboratories, USA) in 15 μL of a reaction mixture containing 0.5 units of Q5® High-Fidelity DNA Polymerase (New England BioLabs, USA), 1X Q5 Reaction Buffer, 5 pM of each primer, 3.5 mM dNTP (Evrogen, Russia) and 1–10 ng of DNA template. The PCR program included the stage of denaturation at 94 °C - 1′, amplification of the product during 35 cycles (94 °C - 30″, 50 °C - 30″, 72 °C - 30″), and final elongation at 72 °C - 3′. Further sample preparation and sequencing were carried out in accordance with Illumina protocol ("'16S Metagenomic Sequencing Library Preparation") on an Illumina MiSeq device (Illumina Inc., USA) using a MiSeq Reagent Kit v3 (600 cycles) with pair-end reading (2 × 300 b) (Illumina Inc., USA).

The pre-processing of the obtained data included primer sequences removal using the cutadapt program [42] with further denoising, joining paired reads, and chimera removal using the dada2 software package [43] implemented in the R software environment (at the stage of quality filtering truncation length of forward and reverse reads was 200 b). A naiive Bayesian classifier, implemented in the dada2 package and trained using the SILVA 132 database [44], was used for taxonomic classification of the obtained ASV (amplicon sequence variant) sequences. Further processing was carried out within the QIIME2 package [45] and the plugins implemented in it. This included the construction of a phylogenetic tree using the SEPP algorithm [46] (fragment insertion on

the SILVA database reference tree with further truncation of branches not presented in the set of our ASVs), calculation of alpha and beta diversity, and prediction of the functional properties of the community in the PICRUSt2 program [47]. To assess alpha diversity, we used the indices of diversity reflecting phylogenetic diversity (Faith PD), the degree of evenness (Shannon), and dominance (inverted Simpson). The ggviolin and stat_compare_means functions from package ggpubr [48] were used to compare alpha-diversity indices and plot violinplots (comparison was performed with Wilcox test with Benjamini-Hochberg p-value adjustment).

The weighted UniFrac metric [49] was used for the estimation of beta diversity. Microbiomes of different horizons of the surface and buried soils were compared.

To scale the differences between buried and control chestnut soils, microbiome data for some contrasting soil types, each represented by A horizon (Table 1: chernozem [50], sod-podbur, grey-humus soil [51]), were included in the beta-diversity analysis. The soils for this comparison were taken from different projects in our laboratory and for this reason, are represented in different numbers of replicates. The geographic location of these soils is presented at Fig. 2.

PAST 2.7 program was used for cluster analysis of horizon group centroids for the buried and surface soils.

The adonis2 test (PERMANOVA, R environment) on the weighted UniFrac-based matrices was used to study the stability of the horizon structure during the burial period. The adonis2 test was applied to the set of horizons of each soil sample separately in order to assess differentiation through the whole profile. The differentiation of the microbiome structure between different horizons of both surface and buried soils was assessed. Retention of differentiation was accepted if the adonis2 test returns a significant effect for factor "horizon".

The phyloseq [52] and DESeq2 [53] packages in the R environment were used to obtain more details for the burial-dependent differentiation of "homologous" horizons of the surface and buried soils. DESeq2 performs a Wald test to test the null hypothesis that the LFC (log2-fold change) between groups is 0 and provides a p-value and a corrected p-value (Bejamini-Hochberg adjustment) for the resulting statistics. Using this approach we identified a group of phylotypes (level of ASV) with a significant shift in abundance (p-value after Benjamini-Hochberg adjustment $\leq$0.1) and constructed corresponding phylogenetic trees consisting of these phylotypes (ggtree package [54] was used). To construct these trees, we pruned a SEPP-generated tree in phyloseq with function prune_taxa.

To assess functional shifting during the burial we used the PICRUSt2 pathway abundance prediction. Only pathways with a significant shift in abundance were used for further interpretation (Kruskal-Wallis Rank Sum Test, p-value after Benjamini-Hochberg adjustment $\leq$0.1, R environment).

Raw sequences are available in the SRA under PRJNA649708 accession number ("SRA: PRJNA649708").

3. Results

Sequencing resulted in 21,500 to 111,319 raw reads per sample (mean 46,736). The mean reads count per sample after chimera removal was 28,542. The minimal number of reads per sample after data pre-processing was 10,987. This number was used as a threshold value in further data rarefaction, which is the default normalization strategy for alpha and beta-diversity estimation in the QIIME2.

3.1. Beta-diversity analysis

Some representatives of other soil types in the comparative analysis (chernozem, grey-humus soil, sod-podbur) were included to assess the differentiation and scale of the difference between the buried and surface soils. An analysis of the A horizons of different soil types based on weighted UniFrac shows how much the buried soil diverges from the surface soil (Fig. 3). The difference between the A horizons of the buried and surface soils is comparable to the difference between the A horizons of the different soil types.

Table 1
Description of the samples included in the analysis.

Soil	Horizons	C total, %	N total, %	WRB 2014 classification	Location	Short description
Buried dark-chestnut	A	0.47 ± 0.011	0.079 ± 0.002	Kastanozem	50°07′43.4″ N, 54°01′39.9″ E	soil under the burial mound
	B	0.43 ± 0.007	0.073 ± 0.001			
	C	–	–			
Surface dark-chestnut	A	0.9 ± 0.014	0.144 ± 0.002	Kastanozem	50°07′43.4″ N, 54°01′39.9″ E	soil near the burial mound
	B	0.52 ± 0.007	0.095 ± 0.006			
	BC	–	–			
	C	–	–			
Chernozem	A	–	–	Chernozem	51°01′41.6″ N, 40°43′39.3″ E	edge of a field, 50 years fallow
Sod-podbur	A	–	–	Umbrisol	59°39′25.1″ N, 31°22′23.3″ E	cone forest
Grey-humus	A	–	–	Arenosol	53°29′43.80″ N, 49°20′56.44″ E	cone forest

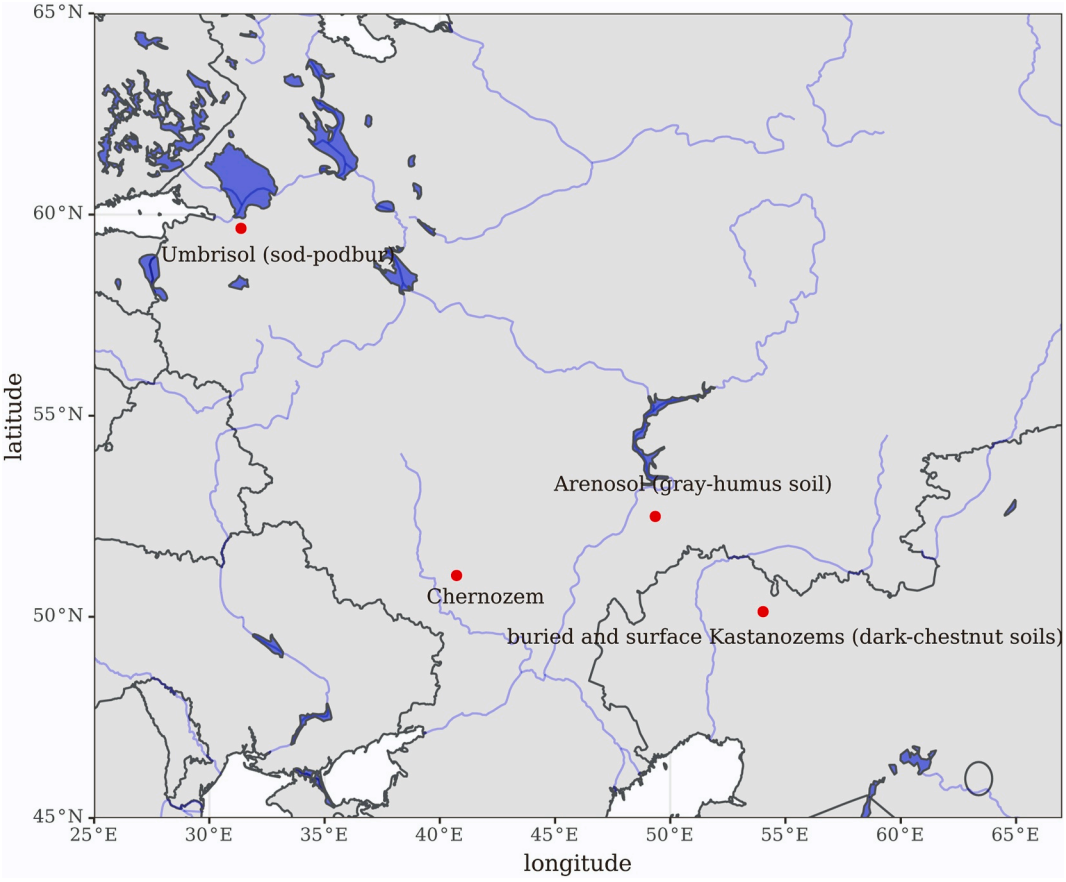

Fig. 2. The geographic location of soils included in the comparative analysis with different soil types.

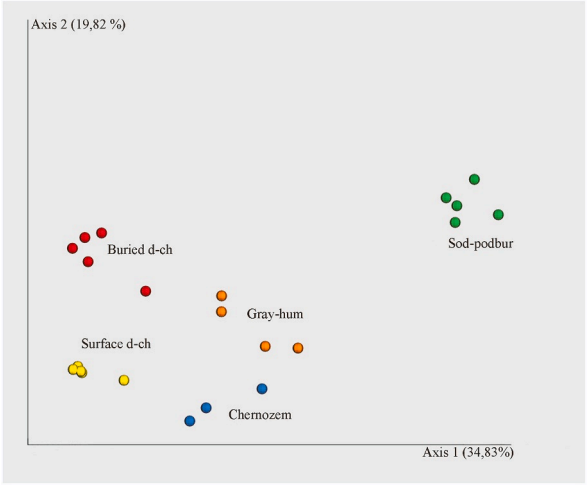

Fig. 3. Difference between the A horizons of the buried and surface dark-chestnut soils in the context of differences between different soil types. Buried d-ch stands for buried dark-chestnut soil, surface d-ch - surface dark-chestnut soil, grey-hym – grey-humus soil.

A more detailed further analysis compares only the buried and surface soils. Prior to performing an analysis of microbiomes, total carbon and nitrogen analyses were performed. Data from this analysis is available in Table 1. An almost twofold decrease in total carbon and nitrogen in the buried A horizon was detected and there was almost no decrease in the B horizon Besides total C and total N the EC, pH, C/N, C water-soluble and fraction of C water-soluble in C total were measured (see S2 Table). Data for electroconductivity

(EC) shows the B horizon has traces of salinity (1.294 mS/cm) and pH 7.5 (minimal value relative to the rest) (S2 Table). The highest pH was characteristic for the buried A horizon - 8.3. C/N values were approximately equal for the A and B horizons of both soils.

The profile structure of the buried soil has similar morphological properties to dark chestnut soils in terms of horizontal stratification with distinct A, B, and C horizons. Notably surface soil has a transitional horizon BC which is absent in the buried soil. For details on the soil profile descriptions, please refer to S1 File.

A microbiome analysis shows that while the horizon structure is stable, the microbiome structure had undergone significant changes. Beta-diversity between horizons of the buried and surface soils based on the weighted UinFrac metric is shown in Fig. 4. It shows the "sequential transition" of surface soil horizons (A, B, BC) through buried A and B horizons to the mineral C horizons (of both soils). The Adonis2 (PERMANOVA) tests were performed separately for each soil, they show profile differentiation by microbiome structure (measured by weighted UniFrac) with R^2 of 0.63 and 0.65 for the surface and buried soils, respectively, with a p-value of 0.001.

3.2. Quantitative analysis of bacterial 16s rRNA

Quantitative analysis of bacterial 16s rRNA shows a substantial decrease in bacterial abundance in all buried horizons compared with corresponding horizons of the surface soil (Fig. 5). The A horizon demonstrates a 1.8-fold decrease, the B horizon a 15.7-fold decrease (6.5-fold in comparison to the BC horizon of the surface soil) and the C horizon shows a 2.7-fold decrease.

A minimal decrease was seen in the A horizon (1.8-fold) and a maximal one in the B horizon (15.7 and 6.5-fold compared with the B and BC horizons of the surface soil) (Fig. 5). Both soil profiles demonstrated a gradual decrease in bacterial abundance with an increase of depth.

3.3. Alpha-diversity analysis

Comparison of alpha diversity indices of pairs of homologous horizons (buried and surface) shows significant shifts only for B horizons (Fig. 6). The buried B horizon is characterized by a decrease in evenness (Shannon index and inverted Simpson indices). The plot of comparison between all horizons is attached in the supplement (S2 Figure).

3.4. Taxonomic structure of the microbiomes

The microbiomes of the surface and buried soils were dominated by eight phyla which represent at least 1% of the community in any of the given horizons: *Actinobacteria*, *Proteobacteria*, *Firmicutes*, *Thaumarchaeota* (*Archaea*), *Chloroflexi*, *Acidobacteria*, *Bacteroidetes*, *Verrucomicrobia* and *Planctomycetes* (Fig. 7). Actinobacteria is one of the main phyla for both the surface and buried soils, it represented 35%–68% of the total community and included 1677 identified phylotypes (ASVs). The difference between the surface and buried soil microbiomes is also noticeable within the class of *Actinobacteria* (S3 Fig).

The surface soil has a higher abundance of *Thaumarchaeota.* The buried soil stands out for its presence of *Verrucomicrobia* and a higher proportion of *Bacteroidetes* in the A horizon. It also has a higher proportion of *Proteobacteria*, *Firmicutes*, *Acidobacteria,* and *Chloroflexi* in the A and B horizons. Remarkably the C horizons of both soils demonstrate an increase in the proportion of *Proteobacteria* and *Firmicutes*.

A comparative analysis of the surface and buried soil microbiomes shows a significant difference in the taxonomic structure even at the phylum level (the most shifts were determined for *Proteobacteria*, *Verrucomicrobia*, *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Bacteroidetes*, and *Thaumarchaeota*). Structural changes in the microbiome show complex patterns. While actinobacteria keep a dominant position in the buried microbiome; the structure of this group is reorganized at a more subtle taxonomic level. Moreover, the

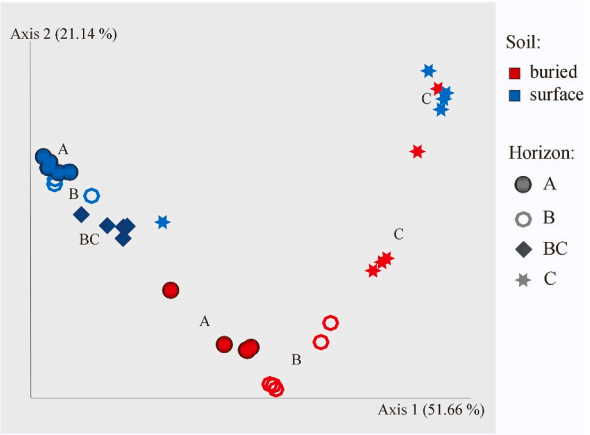

Fig. 4. Beta-diversity (PCoA based on weighted UniFrac metric), differentiation between the buried and surface soil horizons.

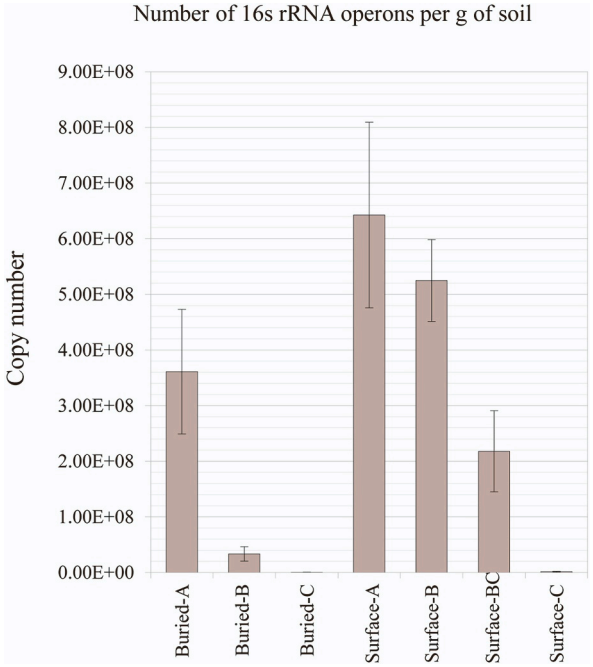

Fig. 5. Quantity of 16s rRNA operons per g of soil for the different horizons of the buried and surface soils.

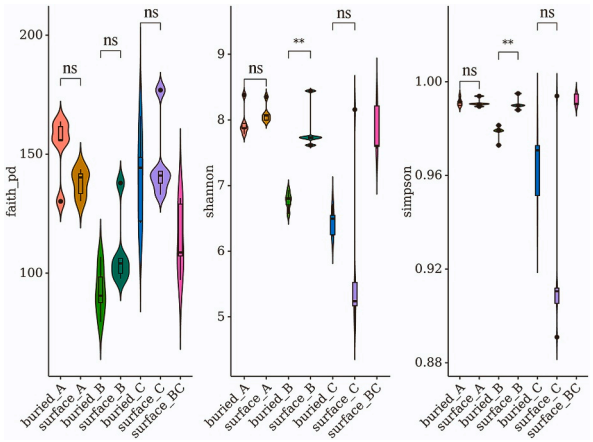

Fig. 6. Violin plots of the alpha-diversity indices (Faith phylogenetic diversity, Shannon, Simpson). Buried – stands for buried dark-chestnut soil, surface-surface dark-chestnut soil. A, B, BC and C – designation of soil horizons. Coding the significance of difference: 'ns' – p-value >0.5, ** - p-value 0.009–0.001.

taxonomic structure of dominants in the microbiome differs significantly at the level of individual phylotypes for horizons A. *Nitrososphaera* was dominant in both the soils but was represented in the buried and surface soils by different subgroups (Fig. 8).

A more detailed analysis of changes in taxonomic structure was performed at the level of separate phylotypes with the DESeq2 package.

3.5. Phylogenetic structure of differentially abundant parts of the microbiome

Burial also clearly affects minor taxa and the general picture is quite complex. To cover all the phylotypes with significant shifts in abundance, we performed the differential abundance test DESeq2 and constructed a phylogenetic tree with those phylotypes. A distinctive feature of the burial process was the formation of separate clades with negative or positive dynamics in burial conditions (S1 Fig 4, Fig 5). Those distinctive clades were considered as 'markers' of the burial process (at least for dark-chestnut soil).

Microbial markers of the burial process in the A horizon can be considered to be: 1) a decreased abundance of representatives of *Gaiella, Rubrobacterales (Actinobacteria)* and Candidatus Nitrososphaera (*Archaea*), 2) specific shifts in the composition of some

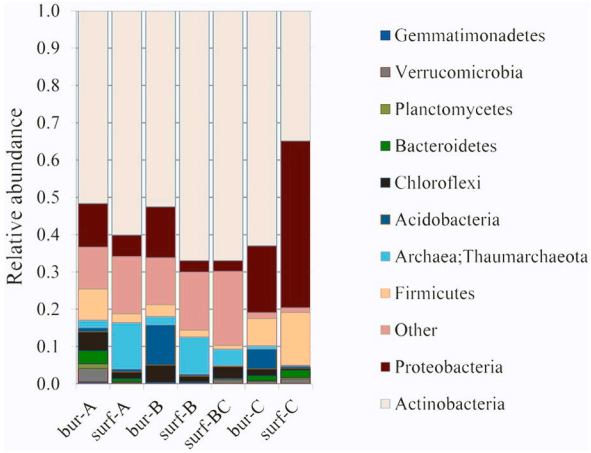

Fig. 7. Microbiome taxonomic structure at phylum level for different horizons of the surface and buried dark-chestnut soil. 'Other' stands for representatives of low-abundant phyla and unclassified prokaryotes.

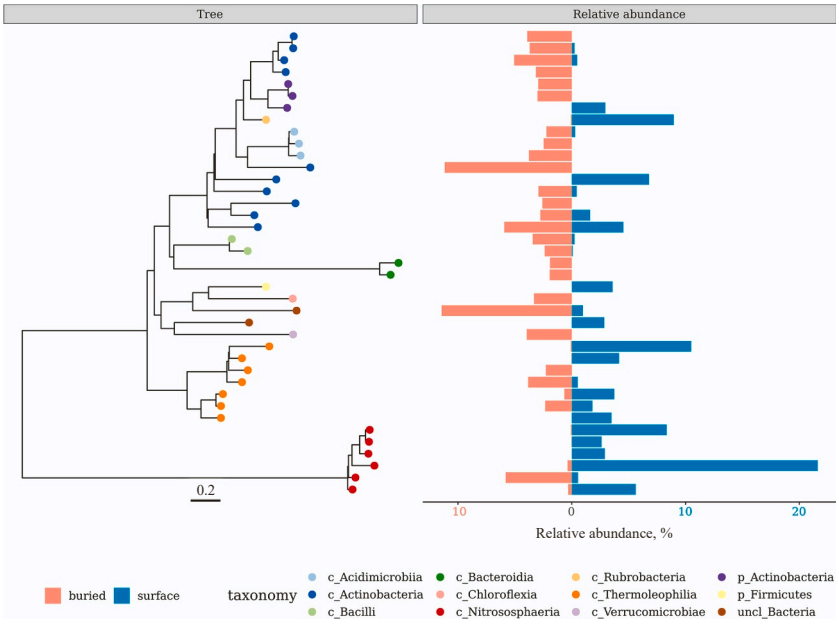

Fig. 8. Taxonomic classification of dominant phylotypes and their relative abundances in the A horizons of the buried and surface dark-chestnut soils. Phylogenetic tree is constructed using SEPP algorithm and includes representatives of dominant phylotypes marked according to taxonomical affiliation at the class (or phylum in some cases) level. Bar plots (both left and right sides) show the relative abundance of these phylotypes in buried (coral, left) and surface (cyan, right) dark-chestnut soils.

unclassified bacteria, 3) a higher proportion of proteobacteria, and 4) an increased abundance of *Microtrichales* (*Acidimicrobiia*), *Bacillales (Firmicutes), Thermomicrobiales, Caldilineales (Chloroflexi), Sphingobacteriales (Bacteroidetes)*, and *Verrucomicrobia* (S4 Fig).

The burial process in the B horizons was accompanied by a decrease in the abundance of representatives of *Gaiella* and Candidatus Nitrososphaera, with the addition of *Solirubrobacteriales* and *Frankiales*. An increase in diversity and abundance of some actinobacteria (*Acidimicrobiia, Propionibacteriales, Micromonosporales, Euzebyales*), *Firmicutes (Bacilli), Chloroflexi (*mostly *Thermomicrobiales), Acidobacteria* (Subgroup 6), *Proteobacteria* (particularly *Tistrellales*). Shifts in the phylogenetic structure of some unclassified actinobacteria as well as some unclassified bacteria were also observed (S5 Fig).

3.6. Difference in the 'functional structure' (PICRUSt2 analysis)

Since features of the diagenetic process were more pronounced for the A horizon (decrease in total carbon and nitrogen), we tried to infer the functional structure of the A horizon with the PICRUSt2 package and trace corresponding features in the functional profile

differences. Differential abundance analysis (DESeq2) on the PICRUSt2 data shows significant shifts in abundance for 55 inferred pathways, with 18 pathways related to organic degradation (S1 Table). 14 of them are more abundant in the buried soil, and 4 – in the surface soil. More detailed information on the PICRUSt2 results is attached in the supplement (table of pathways with significant shifts in abundance for the A horizons).

Notably, a cluster analysis of centroids (weighted UniFrac) shows a grouping of microbiomes of the A and B horizons of the buried soil with the C horizons (the horizon of the soil-forming rock). This is in accordance with the PICRUSt2 data, as mineralization is the consequence of degradation (Fig. 9).

Indirect indicators of retention of biogenic nutrient cycles were observed during the burial. For the nitrogen cycle as an example, we observed the presence of potential nitrogen-fixers (*Bacillus, Frankiales* - surface*; Bacillus, Clostridia* - buried), nitrifiers (*Nitrospirae, Candidatus Nitrososphaeria* - surface*; Nitrolancea, Nitrospirae, Candidatus Nitrososphaeria* - buried), and denitrifiers (*Gaiella* - surface; *Candidatus Alisiosphaera, Gaiella* - buried) in the microbiomes of both the surface and buried soil.

4. Discussion

The object of this investigation is soil buried under a kurgan 2500 years ago. This research aimed to compare the microbiomes of nearby surface soil and its "buried" version to understand the changes in microbial community that have occurred over a sufficiently long time. The burial causes a radical change in providing soil horizons with water and nutritional substances. So, although buried soil preserved horizontal stratification comparable to the surface soil, the "buried microbiome" changed significantly, which is described by diagenesis.

Changes in the properties of buried soils associated with diagenesis (decreased thickness and increased density of the A1 horizon due to reduction of fresh organic matter input and the pressure of the burial rock, decreased content of biophilic elements (primarily, C, N), etc.) [8,55,56] substantially transform the ecological conditions of existence and functioning of the soil microbial complex [27,57, 58]; and, generally, diagenetic processes affect mostly the upper, humus, A1 horizon. Our study shows that, even though the profile structure characteristic of a typical chestnut soil (S1 File) is preserved, the genetic horizons of the buried soil under study showed significant shifts in the composition, structure, and functioning of the microbiomes. Thus, changes in the trophic, air, and water regimes led to a decrease in the number of bacteria in the buried soil horizons, with the maximum decrease in the mineral horizons of the buried soil (the number of copies of ribosomal operons of the 16 S rRNA gene decreased by more than an order of magnitude in the B horizon of the buried soil as compared to that in the surface soil (Fig. 3). The decrease in microbial biomass in soils during burial has been reported in many studies as a consequence of decreased input of fresh organic matter combined with its mineralization and increased oligotrophic regime in buried soil horizons compared to surface soil [8,27,57].

The amount of organic matter in soils during their burial sharply and significantly decreases in the first 200 years, subsequently stabilizing at 30–40% since burial [56,59]. The preservation of trace amounts of humus in the upper horizon of the buried soil may contribute to the relatively greater buffering of its physicochemical properties compared to the underlying horizons, which may explain the less sharp decrease in the abundance of prokaryotes in the A1 horizon of the buried soil (Fig. 3). These factors may also explain the observed less dramatic decrease in the A1 horizon of the buried chestnut soil in total diversity, as determined by comparable values of alpha diversity indices (Fig. 6).

No correlation in the scale of the decrease in the A and B horizons of the bacterial abundance (5.1 and 15.6-fold, respectively (Fig. 3)) and C and N content (by a factor of 2 in both horizons (Table 1)) may be partially explained by the presence of most microorganisms in the mineral horizons mainly in the form of dormant, non-functioning spores, as well as by the influence of other environmental factors and physical and chemical conditions in the mineral horizons on prokaryote activity, which could remain

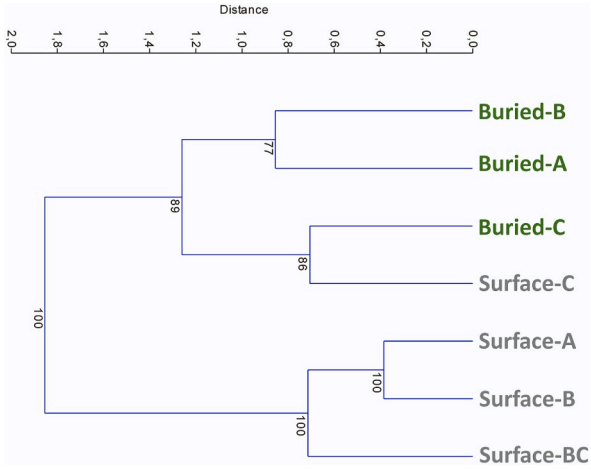

Fig. 9. Cluster analysis of centroids for microbiomes of the different horizons of the surface and buried dark-chestnut soil. Centroids are average weighted UniFrac for each of the microbiomes.

beyond the scope of the current investigation.

The higher values of the biophilic element content in the A horizon of the buried soil may explain, in connection with its high biological potential (in comparison with the mineral horizons), the absence of differences in the values of diversity indices in it and the upper horizon of the surface soil. Meanwhile, the differences in the values of the diversity indices of the B-horizon were more pronounced, with a significant decrease in the B horizon of the buried soil (Fig. 6). A sharp decrease in the biomass, including its functionally active part, was diagnosed for paleosoils of southern Russia confined to the range of chestnut and solonetz soils [60]; this difference was significantly increased in higher-aged buried soils (4000 and 2000 years). Since these soils are dated at approximately 2500 years, this may explain significant degradation transformations in the structure of both the genetic mineral horizons of the buried soil and its microbial community.

Despite the generally close values in the total values of biodiversity, the observed decreases in the total amount of nutrients, in particular, C and N, as well as the changes in the aeration regime occurring during burial, contributed to shifts in the composition of the buried soil microbiomes as well, which was expressed in predominant clustering of the buried soil microbiomes, including the A horizon, with the mineral horizons of surface soils. The general trend of "mineralization" was also a characteristic of the functional structure of the buried soil microbiome overall: the reconstruction of the potential functional community structure demonstrated the predominance of degradation processes in the buried soil compared to the surface soil (S1 Table). This, along with the negative dynamics of carbon and nitrogen in the buried soil (Table 1) and the results of cluster analysis (Fig. 9), is generally consistent with the concept of soil diagenesis. The latter assumes a gradual mineralization of soil organic matter and demonstrates a gradual shift of the "buried" communities towards the microbiome of mineral horizons. It is worth noting that not only the age, but also the embankment/mound height, can influence the intensity of transformation of the ecological environment of buried soils. Thus, in Chernysheva et al. (2013) [60] it was shown that the soil buried under the 90 cm mound showed a sharper decrease in the value of microbial biomass (both total and substrate-induced) compared to the surface soil, as well as the soil buried under the 44 cm mound. The 130 m mound height in our study could be a significant factor influencing highly the physicochemical parameters of the buried chestnut soil, determining the specificity of the taxonomic and functional structure of its microbiome.

4.1. Comparative analysis of taxonomic structure (differentially abundant part) of the prokaryotic community of buried and surface chestnut soil

The observed changes in the microbiome structure are characteristic of buried soils [1,21] and are associated with an increase in the number of microorganisms resistant to adverse environmental conditions. Many of these bacteria have an oligotrophic nutritional type, high hydrolytic activity, the ability to nanoform, spore formation, and anaerobic respiration. Members of *Acidobacteria* which are abundant in the 'buried' B horizon are oligotrophs [61–64], able to live in soils with low salinity [65]. *Bacilli* detected throughout the buried profile are known as spore-forming bacteria, capable of surviving periods of drought [66] or salting [67], adapting to frequent changes in redox conditions [68], hydrolyzing hard-to-access substances in anaerobic conditions [5,32], and fixing nitrogen. *Chloroflexi* are known as anaerobic hydrolytic bacteria [5], capable of surviving in nutrient scarcity [61] and using CO_2 as an energy substrate [69]. The representatives of the genus *Nitrolancea* (*Chloroflexi*) are nitrifiers [69]. *Proteobacteria* are well-known as active hydrolytics [1]. Representatives of Candidatus Alisiosphaera (*Alphaproteobacteria*) are abundant in the buried B horizon and can consume nitrites and nitrates in the anaerobic environment [70].

The phylum *Actinobacteria* is widely represented in both soils. Its representatives are well-known 'universals' with variable metabolic pathways [71] that can consume complex polymers [1,71]. They are resistant to low temperatures [5] and moisture fluctuations [72].

Some actinobacteria were associated more with the surface soil. For example, representatives of the genus *Rubrobacteriaceae* which are thermophiles and resist radiation and drought [73,74] were abundant in the A horizon of the surface soil. Members of *Frankiales* are nitrogen-fixing [75] amphimicrobion [73], and its presence in the B horizon can be linked to spore-formation [73], which impacts long-term survival in deeper soil layers. *Gaiella* and *Solirubrobacteraceae* are more typical for the surface soil and are known as non-spore-forming bacteria. Representatives of the genus *Gaiella* are sensitive to pH variations and are denitrifiers, reducing nitrate to nitrite [73]. Representatives of *Micromonosporaceae* are more typical for the B horizon of the buried soil. These bacteria can hydrolyze complex carbohydrates (lignin, chitin, cellulose, pectin) and are known as utilisers of plant residues. The fact that *Micromonosporaceae* was discovered in cryogenic soils [73] is evidence of its ability to survive in a poor-of-labile-nutrient, low-competitive environment.

Interestingly *Verrucomicrobia,* associated with soil carbon flux [3], are abundant in the surface B and buried A horizons. This data contradicts some metagenomic-based research [7,8,21,33] where a decrease of *Verrucomicrobia* was determined during burial. Since buried soils can often maintain relatively high humidity [76], and taking into account the greater confinement of this group of bacteria to less arid conditions [77], we assume their presence can be explained by comparatively more humid conditions of the buried A and surface B horizons.

In our research, there were indicated markers of the burial process (at least for chestnut soils). The A horizon was characteristic of 1) a decrease in abundance of representatives of *Gaiella, Rubrobacterales (Actinobacteria)* and Candidatus Nitrososphaera (*Archaea*), 2) specific shifts in the composition of some unclassified bacteria, 3) a higher proportion of proteobacteria, and 4) an increased abundance of *Microtrichales* (*Acidimicrobiia*), *Bacillales (Firmicutes), Thermomicrobiales, Caldilineales (Chloroflexi), Sphingobacteriales (Bacteroidetes)*, and *Verrucomicrobia* (S4 Fig).

The B horizon demonstrated a decrease in the abundance of representatives of *Gaiella* and Candidatus Nitrososphaera, with the addition of *Solirubrobacteriales* and *Frankiales.* An increase in diversity and abundance of some actinobacteria (*Acidimicrobiia, Propionibacteriales, Micromonosporales, Euzebyales*), *Firmicutes (Bacilli), Chloroflexi (*mostly *Thermomicrobiales), Acidobacteria* (Subgroup

6), *Proteobacteria* (particularly *Tistrellales*). Shifts in the phylogenetic structure of some unclassified actinobacteria as well as some unclassified bacteria were also observed (S5 Fig).

Probably, the analysis of certain groups of microorganisms, or the processes carried out by them, associated with a specific function of the soil microbial community - the transformation of organic matter, plant nutrition, etc., rather than the analysis of general microbiome parameters would be more informative for the tasks of paleoecological reconstructions.

It is worth noting that, of course, our study has several significant limitations. They are associated with the peculiarities of the methods of analysis we used. First of all, this limitation is related to the analysis of total soil DNA. For this reason, we cannot judge the physiological status of the microorganisms identified. Are they living forms, dormant, dead, or is it just DNA left over from these microorganisms (eDNA)? Soil RNA analysis is needed to address this question. The second question is, to what extent this analysis can reconstruct the historical microbiome? Given the pronounced influence of diagenetic processes and the trend to mineralization, which may have significantly altered the original microbiome, we consider it unlikely that conclusions can be drawn about the historical microbiome based on metagenomic data for the buried soil. Although it has been showed that even in 100-year-old archival soils stored in dry collections, important components of the historical microbiome can be identified, especially for soils with high humus content [14]. A very interesting continuation of this study would be an attempt to isolate the cultured microbiome with subsequent analysis of changes in the genome of corresponding microorganisms relative to their modern relatives. This would make it possible to understand adaptational processes not only at the level of taxonomical structures of full microbiomes but also through comparative evolutional genomics.

5. Conclusions

The interpretation of the 'buried' microbiome data is challenging. On the one hand, buried soil keeps evolving in the same soil environment such as climate conditions and parent rock material, and preserves a lot of features of the natural dark-chestnut soils. On the other hand, the burial process significantly changes the air, thermal, water, and nutritional soil regimes. The dominance of microbial decomposition of soil organic matter (soil organic matter diagenesis) is observed in the buried soil. Additionally, these processes occur over an extended period, and we cannot make conclusions about their dynamic, only detect their consequences. This means that the microbiome data is a 'one-time stamp' of 2500-year of the burial dynamics and shows a detailed but hard-to-formalize snapshot of that process with shifts at the various phylogenetic levels in the different microbial groups. In summary, we conclude that the burial process causes changes in the soil environment and affects the upper A and B horizons, rather than the parent rock material. Microbial communities of the buried soil gradually mineralize the soil organic matter and undergo a complex, presumably long-term and sequential, structural shift and a decrease in the total biomass.

Considering this we do not believe that microbiomes of soils buried under kurgans can be used as a source of information for paleo archaeological reconstructions. Rather the identification of the specific soil and microbiome features of the buried soils can describe the degree and course of microbiome shifts during the long-term burial, as well as assess the functional shifts linked to the diagenetic processes.

Author contribution statement

A.A. Kichko; E.A. Ivanova; T.I. Chernov: Analyzed and interpreted the data; Wrote the paper.
N.K. Sergaliev; M.D. Kalmenov; K.M. Akhmedenov: Conceived and designed the experiments; Performed the experiments.
A.K. Kimeklis; O.V. Orlova; N.A. Shashkov: Analyzed and interpreted the data.
A.G. Pinaev: Contributed reagents, materials, analysis tools or data.
N.A. Provorov: Conceived and designed the experiments.
E.E. Andronov: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Data availability statement

Data associated with this study has been deposited at SRA under the accession number PRJNA649708.

Funding statement

The article was made with the support of the Ministry of Science and Higher Education of the Russian Federation in accordance with the agreement No 075-15-2020-920, dated November 16, 2020, on providing a grant in the form of subsidies from the Federal budget of the Russian Federation. The grant was provided for state support for the creation and development of a world-class scientific center, "Agrotechnologies for the Future".

Declaration of competing interest

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.

Acknowledgements

The research was done using equipment of the Core Centrum 'Genomic Technologies, Proteomics, and Cell Biology' in ARRIAM.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e17208.

References

[1] T.G. Dobrovol'skaya, D.G. Zvyagintsev, I.Y. Chernov, A.V. Golovchenko, G.M. Zenova, L.V. Lysak, et al., The role of microorganisms in the ecological functions of soils, Eurasian Soil Sci. 48 (9) (2015 Sep 10) 959–967.
[2] G. Subrahmanyam, G. Archana, L.S. Chamyal, Microbial activity and diversity in the late Pleistocene palaeosols of alluvial Mahi River basin, Gujarat, western India, Curr. Sci. 101 (2011).
[3] M.V. Semenov, T.I. Chernov, A.D. Zhelezova, D.A. Nikitin, A.K. Tkhakakhova, E.A. Ivanova, et al., Microbial communities of interglacial and interstadial paleosols of the late pleistocene, Eurasian Soil Sci. [Internet 53 (6) (2020 Jun 30) 772–779. http://link.springer.com/10.1134/S1064229320060101.
[4] G. Jia, Z. Rao, J. Zhang, Z. Li, F. Chen, Tetraether biomarker records from a loess-paleosol sequence in the western Chinese Loess Plateau, Front Microbiol. [Internet] 4 (2013) 199. https://www.frontiersin.org/article/10.3389/fmicb.2013.00199.
[5] A. Gittel, J. Bárta, I. Kohoutová, R. Mikutta, S. Owens, J. Gilbert, et al., Distinct microbial communities associated with buried soils in the Siberian tundra, ISME J. 8 (4) (2014) 841–853.
[6] R. Margesin, J.A. Siles, T. Cajthaml, B. Öhlinger, E. Kistler, Microbiology meets archaeology: soil microbial communities reveal different human activities at Archaic Monte Iato (sixth century BC), Microb. Ecol. 73 (4) (2017 May 1) 925–938.
[7] T.I. Chernov, A.D. Zhelezova, O.V. Kutovaya, A.O. Makeev, A.K. Tkhakakhova, N.A. Bgazhba, et al., Comparative analysis of the structure of buried and surface soils by analysis of microbial DNA, Microbiol. (Russian Fed.) 87 (6) (2018 Nov 1) 833–841.
[8] T. Chernov, F. Kurbanova, O. Kutovaya, A. Zhelezova, Microbial DNA analyses of soils buried under earthworks, Sofia, Bulgaria, in: 18th International Multidisciplinary Scientific GeoConference SGEM 2018, 2018, pp. 641–648.
[9] V.A. Demkin, A.S. Skripkin, M.V. Yeltsov, B.N. Zolotareva, T.S. Demkina, T.E. Khomutova, et al., in: S.V. Gubin (Ed.), Natural Environment of the Volga-Ural Steppes During Savromatian-Sarmatian Epoch (VI c. BC – AD IVc.) [In Russian], Institute of Physicochemical and Biological Problems in Soil Science, Russian Academy of Sciences, Puschino, Russia, 2012, p. 215.
[10] T.E. Khomutova, T.S. Demkina, V.A. Demkin, The state of microbial communities in buried paleosols in relation to prevailing climates in steppes of the Lower Volga region, Quat. Int. 324 (2014 Mar 4) 115–123.
[11] T.E. Khomutova, A.V. Borisov, Estimation of microbial diversity in the desert steppe surface soil and buried palaeosol (IV mil. BC) using the TRFLP method, J. Arid Environ. (2019 Dec 1) 171.
[12] E.V. Blagodatskaya, M.V. Semenov, A.V. Yakushev, in: V.N. Kudeyarov (Ed.), Activity and Biomass of Soil Microorganisms under Changing Environmental Conditions [In Russian] [Internet], KMK Scientific Press, Moskow, 2016, p. 243. https://istina.msu.ru/publications/book/36560269/.
[13] T.E. Khomutova, T.S. Demkina, N.N. Kashirskaya, V.A. Demkin, The state of microbial communities in paleosoils of the solonetz assemblage on the Northern Yergeni upland as indicator of climate humidity within the middle sarmatuan time-window (I c. AD) (Состояние Микробных сообществ палеопочв солонцового комплекса Се, Volga River Reg. Archaeol. [Internet] 3 (5) (2013) 79–90. http://archaeologie.pro/en/archive/5/81/.
[14] E.A. Ivanova, I.O. Korvigo, B.F. Aparin, E.L. Chirak, E.V. Pershina, N.S. Romaschenko, et al., The preservation of microbial DNA in archived soils of various genetic types, PLoS One 12 (3) (2017) 1–14.
[15] A. Rusakov, A. Makeev, O. Khokhlova, P. Kust, M. Lebedeva, T. Chernov, et al., Paleoenvironmental reconstruction based on soils buried under Scythian fortification in the southern forest-steppe area of the East European Plain, Quat. Int. [Internet] 502 (May 2018) (2019) 197–217, https://doi.org/10.1016/j.quaint.2018.05.016.
[16] V.A. Demkin, V.M. Klepikov, S.N. Udaltsov, T.S. Demkina, M.V. Eltsov, T.E. Khomutova, New aspects of natural science studies of archaeological burial monuments (kurgans) in the southern Russian steppes, J. Archaeol. Sci. 42 (1) (2014) 241–249.
[17] T.E. Khomutova, T.S. Demkina, A.V. Borisov, Shishlina II. State of microbial communities in paleosols buried under kurgans of the desert-steppe zone in the Middle Bronze Age (27th–26th centuries BC) in relation to the dynamics of climate humidity, Eurasian Soil Sci. 50 (2) (2017 Feb 1) 229–238.
[18] R. Sheng, K. Li, W. Zhang, H. Wang, H. Liu, X. Zhu, et al., Differentiations of determinants for the community compositions of bacteria, fungi, and nitrogen fixers in various steppes, Ecol. Evol. 9 (6) (2019 Mar 1) 3239–3250.
[19] T. Puzanova, E. Aseeva, M. Lebedeva, F. Kurbanova, T. Chernov, The methods of research of buried soils under archaelogical sites, in: 18th International Multidisciplinary Scientific GeoConference SGEM 2018, STEF92 Technology OOD, Sofia, Bulgaria, 2018, pp. 611–618.
[20] N.P. Chizhikova, I.V. Kovda, A.V. Borisov, N.I. Shishlina, The solonetzic process in surface soils and buried paleosols and its reflection in the mineralogical soil memory, Eurasian Soil Sci. 42 (10) (2009) 1179–1189.
[21] T.I. Chernov, A.D. Zhelezova, The dynamics of soil microbial communities on different timescales: a review, Eurasian Soil Sci. [Internet] 53 (5) (2020) 643–652, https://doi.org/10.1134/S106422932005004X.
[22] A.V. Mitusov, O.E. Mitusova, K. Pustovoytov, C.C.M. Lubos, S. Dreibrodt, H.R. Bork, Palaeoclimatic indicators in soils buried under archaeological monuments in the Eurasian steppe: a review, Holocene 19 (8) (2009 Dec) 1153–1160.
[23] A.B. Chase, C. Weihe, J.B.H. Martiny, Adaptive differentiation and rapid evolution of a soil bacterium along a climate gradient, Proc. Natl. Acad. Sci. India 118 (18) (2021 May 4), e2101254118. http://www.pnas.org/content/118/18/e2101254118.abstract.
[24] S. Jiao, W. Chen, G. Wei, Resilience and assemblage of soil microbiome in response to chemical contamination combined with plant growth, Appl. Environ. Microbiol. [Internet] 85 (6) (2019 Mar 6), e02523-18. https://pubmed.ncbi.nlm.nih.gov/30658982.
[25] J.D. Rocca, M. Simonin, J.R. Blaszczak, J.G. Ernakovich, S.M. Gibbons, F.S. Midani, et al., The microbiome stress project: toward a global meta-analysis of environmental stressors and their effects on microbial communities, Front Microbiol. [Internet] 9 (JAN) (2019 Jan 10). https://www.frontiersin.org/article/10.3389/fmicb.2018.03272/full.
[26] J.W. Sorensen, A. Shade, Dormancy dynamics and dispersal contribute to soil microbiome resilience, Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 375 (1798) (2020 May), 20190255, https://doi.org/10.1098/rstb.2019.0255.
[27] T.S. Demkina, T.E. Khomutova, N.N. Kashirskaya, I.V. Stretovich, V.A. Demkin, Microbiological investigations of paleosols of archeological monuments in the steppe zone, Eurasian Soil Sci. 43 (2) (2010 Feb) 194–201.
[28] L.M. Polyanskaya, V.E. Prikhod'ko, D.G. Lomakin, I.Y. Chernov, The number and biomass of microorganisms in ancient buried and recent chernozems under different land uses, Eurasian Soil Sci. [Internet] 49 (10) (2016) 1122–1135, https://doi.org/10.1134/S1064229316100100.
[29] N. Sergaliev, M. Kakishev, A. Zhiengaliev, M. Volodin, E. Andronov, A. Pinaev, Investigation of soil microbiota in Western Kazakhstan region using high-throughput sequencing, in: R. Kizlkaya, C. Gülser (Eds.), Abstract Book 9th International Soil Science Congress on "The Soul of Soil and Civilization", Erol Ofset, Samsun, Turkey, 2014, p. 49.

[30] K. Frindte, E. Lehndorff, S. Vlaminck, K. Werner, M. Kehl, F. Khormali, et al., Evidence for signatures of ancient microbial life in paleosols, Sci. Rep. 10 (1) (2020 Dec 1).

[31] T.S. Demkina, T.E. Khomutova, N.N. Kashirskaya, E.V. Demkina, I.V. Stretovich, G.I. El-Registan, et al., Age and activation of microbial communities in soils under burial mounds and in recent surface soils of steppe zone, Eurasian Soil Sci. 41 (13) (2008) 1439–1447.

[32] J.M. Young, T. Skvortsov, B.P. Kelleher, W.C. Mahaney, P. Somelar, C.C.R. Allen, Effect of soil horizon stratigraphy on the microbial ecology of alpine paleosols, Sci. Total Environ. [Internet] 657 (November 2018) (2019) 1183–1193, https://doi.org/10.1016/j.scitotenv.2018.11.442.

[33] F. Kurbanova, A. Makeev, T. Puzanova, P. Kust, T. Chernov, Soil indicators of late Holocene forest-steppe landscape evolution on the Russian plain, in: 18th International Multidisciplinary Scientific GeoConference SGEM 2018 [Internet]. 51 Alexander Malinov Blvd, Sofia, 1712, STEF92 Technology, Bulgaria, 2018, pp. 487–494 (International Multidisciplinary Scientific GeoConference-SGEM; vol. 18), https://www.sgem.org/index.php/jresearch-article?citekey=Kurbanova201813487494.

[34] M.L. Oparin, O.S. Oparina, Changes of the natural steppe complexes on the left bank of the Volga river in connection with the climate dynamics and anthropogenic effect [In Russian], Povolzhskiy J. Ecol. [Internet] 1 (2003) 31–40. http://www.sevin.ru/volecomag/issues/2003_1/PEJ_2003_1_31-40.pdf.

[35] A.D. Tairov, in: L.L. Gaiduchenko (Ed.), Changes in the Climate of the Steppes and Forest-Steppes of Central Eurasia in the II-I Millennium BC. Materials for Historical Reconstruction. [In Russian], Chelyabinsk, Russia, 2003, p. 68.

[36] IUSS Working Group WRB, World Reference Base for Soil Resources. International soil classification system for naming soils and creating legends for soil maps, in: World Soil Resources Reports No. 106. FAO, Food and Agriculture Organization of the United Nations, Rome, 2014, pp. 1–191. http://www.fao.org/3/i3794en/I3794en.pdf.

[37] A.V. Sokolov (Ed.), Agrochemical Methods of Soil Research [In Russian], Nauka, Moscow, USSR, 1975, p. 656.

[38] Panikov NS, Gorbenko AY, Svetlov SV. Method of determining total water-soluble-organic substances content of soil [In Russian]. USSR: USSR; SU 1318909 A1.

[39] J. Kjeldahl, A new method for the determination of nitrogen in organic matter, Z. für Anal. Chem. 22 (1883) 366–382.

[40] J.D. Lane, 16S/23S rRNA sequencing, Nucleic Acid. Tech. Bact. Syst. [Internet] (1991) 125–175. http://ci.nii.ac.jp/naid/10008470323/en/.

[41] S.T. Bates, D. Berg-Lyons, J.G. Caporaso, W.A. Walters, R. Knight, N. Fierer, Examining the global distribution of dominant archaeal populations in soil, ISME J. [Internet] 5 (5) (2011) 908–917, https://doi.org/10.1038/ismej.2010.171.

[42] M. Martin, CUTADAPT removes adapter sequences from high-throughput sequencing reads, EMBnet J. (2011 Aug 2) 17.

[43] B.J. Callahan, P.J. McMurdie, M.J. Rosen, A.W. Han, A.J.A. Johnson, S.P. Holmes, DADA2: high-resolution sample inference from Illumina amplicon data, Nat. Methods [Internet] 13 (7) (2016) 581–583, https://doi.org/10.1038/nmeth.3869.

[44] C. Quast, E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, et al., The SILVA ribosomal RNA gene database project: improved data processing and web-based tools, Nucleic Acids Res. [Internet] 41 (D1) (2013 Jan 1) D590–D596, https://doi.org/10.1093/nar/gks1219.

[45] E. Bolyen, J.R. Rideout, M.R. Dillon, N.A. Bokulich, C.C. Abnet, G.A. Al-Ghalith, et al., Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2, Nat. Biotechnol. [Internet] 37 (8) (2019) 852–857, https://doi.org/10.1038/s41587-019-0209-9.

[46] J. Stefan, M. Daniel, G. Antonio, A. N-MJ, J. Lingjing, X.Z. Zech, et al., Phylogenetic placement of exact amplicon sequences improves associations with clinical information, mSystems [Internet] 3 (3) (2021 Jun 22), e00021-18, https://doi.org/10.1128/mSystems.00021-18.

[47] G. Douglas, V. Maffei, J. Zaneveld, S. Yurgel, J. Brown, C. Taylor, et al., PICRUSt2: an Improved and Extensible Approach for Metagenome Inference, 2019.

[48] Alboukadel Kassambara, Ggpubr: "Ggplot2" Based Publication Ready Plots, 2020. https://rpkgs.datanovia.com/ggpubr/.

[49] C. Lozupone, R. Knight, UniFrac: a new phylogenetic method for comparing microbial communities, Appl. Environ. Microbiol. [Internet] 71 (12) (2005 Dec) 8228–8235. https://pubmed.ncbi.nlm.nih.gov/16332807.

[50] A.O. Zverev, E.V. Pershina, V.M. Shapkin, A.K. Kichko, O.P. Mitrofanova, V.D. Kobylyanskii, et al., Molecular analysis of the rhizosphere microbial communities from gramineous plants grown on contrasting soils, Microbiol. [Internet] 89 (2) (2020) 231–241, https://doi.org/10.1134/S002626172001018X.

[51] G.V. Gladkov, E.Y. Chebykina, E.V. Evdokimova, E.A. Ivanova, A.K. Kimeklis, A.O. Zverev, et al., Restoration of soil microbiome in various soil horizons after crown and surface wildfires, Ecol. Genet. 18 (3) (2020 Oct 9) 343–356.

[52] P.J. McMurdie, S. Holmes, Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data, PLoS One [Internet] 8 (4) (2013 Apr 22), e61217, https://doi.org/10.1371/journal.pone.0061217.

[53] M.I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, Genome Biol. [Internet] 15 (12) (2014) 550, https://doi.org/10.1186/s13059-014-0550-8.

[54] G. Yu, Using ggtree to visualize data on tree-like structures, Curr. Protoc. Bioinforma. 69 (1) (2020 Mar) e96.

[55] J. Catt, Field recognition, description and spatial relationships of paleosols, Quat. Int. [Internet] 6 (1990) 2–95. https://www.sciencedirect.com/science/article/pii/104061829090003M.

[56] T.V. Alekseeva, Soils in devonian and carboniferous, Curr. State Knowledge Russia: Rev. 53 (10) (2020) 1343–1353.

[57] N.N. Kashirskaya, T.E. Khomutova, T.S. Demkina, V.A. Demkin, The microbial biomass in paleosols buried under kurgans and in recent soils in the steppe zone of the Lower Volga region, Eurasian Soil Sci. [Internet] 42 (5) (2009) 536–542, https://doi.org/10.1134/S1064229309050093.

[58] E.V. Chernysheva, N.N. Kashirskaya, A.V. Borisov, A.N. Zhuravlev, V.A. Demkin, Biomass and microbial activity in modern and buried chestnut soils of lower Volga region [In Russian], Vestn TGU 18 (3) (2013) 842–846.

[59] B.N. Zolotareva, V.A. Demkin, Humus in paleosols of archaeological monuments in the dry steppes of the Volga-Don interfluve, Eurasian Soil Sci. [Internet] 46 (3) (2013) 262–272, https://doi.org/10.1134/S1064229313030149.

[60] T.E. Khomutova, T.S. Demkina, V.A. Demkin, Estimation of the total and active microbial biomasses in buried subkurgan paleosoils of different age, Microbiol. [Internet] 73 (2) (2004) 196–201, https://doi.org/10.1023/B:MICI.0000023989.04745.7b.

[61] J. Yang, Y. Wang, X. Cui, K. Xue, Y. Zhang, Z. Yu, Habitat filtering shapes the differential structure of microbial communities in the Xilingol grassland, Sci. Rep. 9 (1) (2019) 1–12, https://doi.org/10.1038/s41598-019-55940-y.

[62] J.L. Wood, C. Tang, A.E. Franks, Competitive traits are more important than stress-tolerance traits in a cadmium-contaminated rhizosphere: a role for trait theory in microbial ecology, Front. Microbiol. 9 (FEB) (2018) 1–12.

[63] K. Etingoff, Agricultural Resource Use and Management, first ed., Apple Academic Press, New York, USA, 2014, p. 348.

[64] N. Fierer, M.A. Bradford, R.B. Jackson, Toward an ecological classification of soil bacteria, Ecology 88 (2007).

[65] X. Li, A. Wang, W. Wan, X. Luo, L. Zheng, G. He, et al., High salinity inhibits soil bacterial community mediating nitrogen cycling, Microb. Ecol. 87 (21) (2021), e01366-21.

[66] J. Liu, X. Cui, Z. Liu, Z. Guo, Z. Yu, Q. Yao, et al., The diversity and geographic distribution of cultivable bacillus-like bacteria across black soils of northeast China, Front. Microbiol. 10 (JUN) (2019) 1–11.

[67] D. Godoy-flores, A.M. Díaz-rodríguez, F.I. Parra-cota, S.D.L. Santos-villalobos, Salt-tolerant Bacillus Species as a Promising Strategy to Mitigate the Salinity Stress in Wheat (Triticum Turgidum Subsp. Durum), 2021, p. 186.

[68] K.M. DeAngelis, W.L. Silver, A.W. Thompson, M.K. Firestone, Microbial communities acclimate to recurring changes in soil redox potential status, Environ. Microbiol. 12 (12) (2010 Dec) 3137–3149.

[69] T.M. Schmidt, in: T.M. Schmidt (Ed.), Encyclopedia of Microbiology, fourth ed., Elsevier Science, Amsterdam, Netherlands, 2019, p. 3496.

[70] C. Kragelund, Y. Kong, J. van der Waarde, K. Thelen, D. Eikelboom, V. Tandoi, et al., Ecophysiology of different filamentous Alphaproteobacteria in industrial wastewater treatment plants, Microbiology 152 (10) (2006 Oct) 3003–3012.

[71] A. Wolinska, D. Górniak, U. Zielenkiewicz, A. Kuzniar, D. Izak, A. Banach, et al., Actinobacteria structure in autogenic, hydrogenic and lithogenic cultivated and non-cultivated soils: a culture-independent approach, Agronomy 9 (10) (2019 Sep 29).

[72] F.N. Lisetskii, A.V. Zemlyakova, A.D. Kirichenko, Variability of microbiota under diverse conditions of soil moistening, Biol. Bull. 45 (4) (2018 Jul 1) 337–344.

[73] E. Rosenberg, E.F. DeLong, S. Lory, E. Stackebrandt, F. Thompson, The Prokaryotes: Actinobacteria, fourth ed., 2014, pp. 1–1061.

[74] A. Novara, V. Catania, M. Tolone, L. Gristina, V.A. Laudicina, P. Quatrini, Cover crop impact on soil organic carbon, nitrogen dynamics and microbial diversity in a mediterranean semiarid vineyard, Sustain. Times 12 (8) (2020).

[75] A. Sellstedt, K.H. Richau, Aspects of nitrogen-fixing actinobacteria, in particular free-living and symbiotic frankia, FEMS Microbiol. Lett. 342 (2) (2013) 179–186.
[76] T. Arkhangelskaya, M. Prokhorov, M. Mazirov, Annual temperature dynamics of arable soils of Vladimir Opolye paleocryogenic complexes, Earth Cryosph. 3 (2008) 80–86.
[77] J.W. Neilson, K. Califf, C. Cardona, A. Copeland, W. Van Treuren, K.L. Josephson, et al., Significant impacts of increasing aridity on the arid soil microbiome, mSystems [Internet] 2 (3) (2017 May 1). http://www.scopus.com/inward/record.url?scp=85032301887&partnerID=8YFLogxK.