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Fungal and Prokaryotic Communities in Soil Samples of the Aral Sea Dry Bottom in Uzbekistan

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Abstract: Due to the falling water level in the Aral Sea and Muynak Lake, the content of salts dissolved in the water has gradually increased, and toxic elements have been deposited at the lake's bottom and subsequently washed into the Aral region by the river. Bacteria, archaea and fungi are crucial for the cycling of several important inorganic nutrients in soils. From 15 genera and 31 species of recovered microscopic filamentous fungi, a big group was melanized, of which most of them were also phytopathogenic. The second group consisted of keratinophilic species. Isolated bacteria mainly included members of the genera *Arthrobacter*, *Bacillus*, *Massilia*, *Rhodococcus* and *Nocardiopsis*. High-throughput sequencing analysis permitted a better view of the mycobiome and prokaryotic communities (comprising archaea). The cultivation and sequencing approaches were shown to be complementary. The aim of the work was to identify soil microorganisms, including the order Halobacteriales, and to discover the differences in species diversity depending on soil salinity and the presence of PTEs in soil.

Keywords: archaea; bacteria; microscopic filamentous fungi; slight halophiles environment

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1. Introduction

The Aral Sea (a fresh water lake) is situated in the Central Asian deserts, on the border between Kazakhstan and Uzbekistan (in the autonomous Republic of Karakalpakstan). Up until the third quarter of the 20th century, the Aral Sea was the fourth largest lake in the world, with an area of 68,000 km^2, and the largest inland body of salty reservoirs in the world. The Aral Sea has been steadily shrinking since the 1960s after the rivers that fed it were diverted by Soviet irrigation projects. In the past, the Aral Sea was supplied with water from the rivers Amu Darya (from the south) and Syr Darya (from the northeast). Those rivers were diverted in the 1960s for the irrigation of the desert region surrounding the Sea in order to favor agriculture over supplying the Aral Sea basin. Much of the Amu Darya and Syr Darya rivers feeding the lake were diverted to irrigate cotton fields and rice paddies. Irrigation works during that period turned many desolate areas into flourishing oases and provided water and tillable soil. However, in the last fifty years, more than 85 percent of the Aral Sea has disappeared.

From 1973 to 2020, there has been a profound reduction in the overall area of the Aral Sea and an increase in land area as the basin. Due to the shortage of water in the Aral Sea region, the watering of the natural lakes in the Amu Darya delta has sharply decreased. Currently, there are only about 10 lakes formed as a result of waste and collector-drainage waters, and the share of natural lakes is only about 5 thousand hectares [1].

The remnants of the Aral Sea water and exposed lake bottom provide extreme environmental conditions for organisms. A secondary problem of this area is the strong winds that pick up and deposit bed soil. The crops from agricultural land have been appreciably affected due to heavily salt-laden particles falling on arable land in and around the former Aral Sea. The intense scattering of saline soil by wind also contributes to a significant reduction in breathable air quality and causes human health problems [2].

Soil salinization is the source of many of environmental problems, such as land degradation, reduced crop yields, contaminated freshwater, biodiversity reduction, etc. Anthropogenic salinization of soils is a global threat. This process has been recorded, e.g., in the Euphrates Basin in Syria [3], the Yellow River Basin in China [4] and also in other regions of the world. Together with salinity, the presence of heavy metals or other types of potential toxic elements (PTEs) is to be considered as another limiting factor to the reduced biodiversity. In fact, recent studies have shown that over the years, various quantities of toxic material coming from different types of industrial manufactures [5] have been deposited in the lake sediment. The results of these works highlighted the high presence of Al, As, Cd, Pb, U and PTEs, but unfortunately, they are related only to regions belonging to Kazakhstan. According to information found in the literature, data on the conditions of the Aral Sea in Uzbekistan are scarce and relate to the Amu Darya River Basin or to Dautkul Lake, situated on the right bank of the Amu Darya River, 47 km north of the city of Nukus [6]. On the other hand, it is known that the contaminated sediment of the former seabed (in the Karakalpakstan region, in the vicinity of the Aral Sea) has been disseminated over the surrounding area by strong winds, and this deterioration of the ecosystem has created a hazardous situation for the health of the people of this region [7].

The desiccated Aral Sea Basin, rich in salt and toxins, offers a unique natural setting for the examination of microbial community structures and their ecological functions. Many microorganisms are able to adapt to extreme environmental conditions of natural origin [8]. However, the extreme conditions of anthropogenic origin which man has caused by his careless and inappropriate interference with nature are much worse. In addition to soil salinity, these are, for example, contaminated environment by radiation from 3 to 5 orders higher than the background radioactivity, as at the Chernobyl Nuclear power Plant [9], contamination by heavy metals and toxic elements after mining activities [10], and many others. The soil salinity reduces microbial activity and changes the microbial community structure [11]. Salinity reduces microbial biomass mainly because the osmotic stress results in the drying and lysis of cells [12]. On the other hand, bacteria, archaea and fungi are crucial for the cycling of several important inorganic nutrients in soils [13].

The combination of salinity and heavy metals contributes to the presence of halophilic and halotolerant bacterial and archaeal species capable of resisting and metabolizing these dangerous elements [14]. In addition to the ecological value brought by the identification of these microbial species, it is also very interesting to learn about them for their possible use in various biotechnological fields. For example, some groups of archaea can be used in agriculture so that plants can tolerate soil salinity [15]. Bacteria can be exploited as plant promoters and also to remediate potential agricultural soil from heavy metals [16].

Microscopic fungi represent an extensive group of organisms that occur under extreme conditions, such as a halophilic environment [17,18], a hypersaline environment [19] or an alkaline environment [20]. The adaptation mechanisms of microfungi are different. In a hypersaline environment, some important mechanisms are the high osmolarity glycerol signaling pathway for sensing and responding to increased salt concentration [8], the accumulation of inorganic ions intracellularly to balance the salt concentrations in their environment [21], and adaptation to low water activity and high concentrations of toxic

ions [22]. In other extreme environments, they are able to translocate an array of naturally occurring waste, such as man-made radionuclides in the mycelium, and have also been shown to adapt to stress conditions caused by melanin pigments, which are among the most stable and resistant of biochemical materials [9]. Despite the difficulties in both sample collection and cultivation of microscopic fungi from extreme environments, this environment can also be a promising source of novel species [23]. They are considered to be potential sources for the discovery of bioactive compounds and compatible solutes, including novel and/or extraordinary enzymes. In addition, some extremophilic microorganisms are capable of producing novel bioactive secondary metabolites [24], as well as massive amounts of compatible solutes that are useful as stabilizers for biomolecules and in various fields in biotechnology [25], as remediation, for biosorption of heavy metals [26] and for the application of indigenous species as biorefineries [17].

Because macroscopic species have all but disappeared with the reduction in the Aral Sea water area and the extreme climatic conditions of the desert region have limited the biodiversity, we focused our study on the soil microbiota. We compared the species diversity of bacteria, archaea and fungi in localities situated at different distances from the Muynak (Moynaq; Uzbekistan) water body, which is among the remnants of the Aral Sea in the southern part of the original water reservoir. The aim of the work was to identify soil microorganisms and to discover the differences in species diversity depending on soil salinity and the presence of PTEs in soil.

2. Materials and Methods

2.1. Study Area and Soil Sampling

Soil samples were taken from the dry bottom of the southern part of the Aral Sea in Uzbekistan (Figure 1), from three sites near Lake Muynak between which the distance is approximately 8–9 km (Figure 2). This is a freshwater lake fed by water from the Amu Darya River. The water supply in the lake from the river is currently limited. River water is used to irrigate cotton fields in Uzbekistan. Much of the water evaporates before it reaches the fields and the original river delta, and only a small volume of water reaches Muynak Lake. As a result of increased evaporation, water is declining in all surface water bodies that have been supported by river water in the past. The Amu Darya delta supported about 2600 lakes in the 1960s; in 1985 it supported only 400 [27], and the current number is even lower. The samples were taken from this territory because the concentrations of salts and minerals began to rise in the shrinking body of water (Figure 3). This process was the reason for changes in the lake's ecology, causing an extreme reduction in biodiversity. The level of salinity rose from approximately 10 g/L to often more than 100 g/L in the remaining Southern Aral [28]. The selection criterion of the sites was the different distances from the water body of different parts of the Aral Sea. The mutual distance between the research sites (A1, A2, A3) was 25 km. Samples from site A3 are situated closest to the former water body of the Aral Sea, with brackish water, and farthest from the Muynak Lake. Site A2 is between A1 and A3. Soil samples were taken at two soil depths, up to 5 cm (samples marked a) and from a depth of 50 cm (samples marked b). Soil samples from each localityA1a, A1b, A2a, A2b, A3a and A3b—were taken from five sampling points. In the laboratory, soil samples from each sampling point were homogenized by quartering and sieved through a 2 mm sieve to obtain a fine soil. The fine soils were stored at 4 °C in darkness for approximately 10 days, until the soil samples were processed. The resulting fine soils were used for all chemical and microbiological analyses.

2.2. Chemical Analyses

Basic chemical parameters, such as pH_{H2O}, the amount of TC, TN, humus and $CaCO_3$, and the amount of available nutrients and salts (as Ca^{2+}, Na^{2+}, Mg^{2+}, K^{+}, Al^{3+}, Mn^{2+}, Fe^{2+} and H^{+}), were analyzed in the certified laboratory of the National Forest Centre, Zvolen, Slovakia. All soil samples were also analyzed for their metal content (Ag, Al, Be, Bi, Cd, Co, Cr, Cu, Fe, Hf, Ga, In, La, Mg, Mo, Nb, Ni, Pb, Re, Sc, Sn, Ta, Ti, Tl, V, W, Y, Zn, Zr),

lanthanide content (Ce, Dy, Eu, Gd, Nd, Pr, Sm, Tb), alkali metal content (Cs, Na, K, Li, Rb), alkali earth metals content (Ba, Ca, Sr), semi-metal content (As, Sb and Te), non-metals (P, S, Se) and actinides content (U and Th) in the Certified Laboratories of Bureau Veritas Commodities Canada, Ltd., Vancouver, BC, Canada (Tables as Supplementary Material). Analyses of all elements are included in the Supplementary Material.

Figure 1. Localization of Aral Sea (origin area) in the Central Asian deserts. (https://mapcruzin.com/free-uzbekistan-maps.htm, accessed on 10 January 2024).

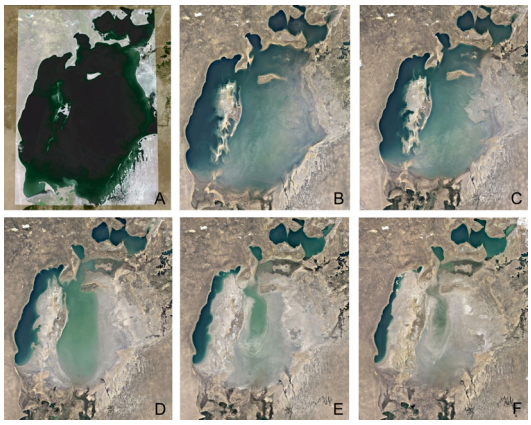

Figure 2. Reduction in the water surface of the Aral Sea in the period 1973–2020 ((© GoolgeEarth, Landsat/Copernicus, accessed on 10 January 2024). Legend: (**A**) 1973; (**B**) 1984; (**C**) 1993; (**D**) 2003; (**E**) 2013; (**F**) 2020).

Figure 3. Localization of research sites (the distance between sites is approximately 8–9 km) in the south Aral Sea—places of soil samples near by Muynak lake (© GoolgeEarth, Landsat/Copernicus, accessed on 10 January 2024).

2.3. Mycological Analyses

The soil mycobiota was isolated by the plating method up to a dilution of 10^4 CFU (Colony Forming Units) per 1 g of dry sample plates of SAB (Sabouraud Dextrose Agar), CDY (Czapek Dox Yeast Extract), MEA (Malt Extract Agar Base w/Mycological Peptone) and DMB (Dichloran Medium Base w/Rose Bengal; all media from HiMedia, Mumbai, India). An adequate concentration of NaCl in % (NaCl p. a., Merck, Darmstadt, Germany) was always added to the agar media used, depending on the sampling point A1, A2 and A3. Cultivation was carried out in the dark at 25 °C for 7–10 days. Each soil sample was always processed in three replications. All morphologically distinctive colonies were selected from the resulting mixed culture and purified. The isolates were then maintained on the original isolation agar media. Pure cultures were identified according to the phenotype using mycological diagnostic keys [29] and according to their Internal Transcribed Spacer (ITS) sequences of the amplicons produced by the primers ITS1–ITS4 [30]. The obtained sequences were deposited to the GenBank database under the accession numbers: PP085461–PP085492.

Valid names of microscopic filamentous fungi were modified according to Hubka et al. [31] and Visagie et al. [32]. All figures of microscopic filamentous fungi were observed under an Axio Scope A 1 Carl Zeiss Jena light (Carl Zeiss Microscopy GmbH, Jena, Germany) microscope in a drop of lactic acid enriched with cotton blue stain (0.01%).

2.4. Bacterial Analyses

Ten grams (wet weight) of each soil sample (A1a, A1b, A2a, A2b, A3a and A3b) was combined in sterile 250 mL Erlenmeyer flasks with 90 mL of a 0.9% (w/v) NaCl solution. The mixture was then incubated at 28 °C in a shaker incubator at 90 rpm for 2 h. After incubation, the resulting suspensions were filtered through Whatman 1 filter paper (Merck, Darmstadt, Germany). Subsequently, these filtered soil suspensions were decimally diluted, and the dilutions were inoculated onto two distinct agar media for the isolation of bacteria: tenfold-diluted Luria–Bertani (LB10 agar; peptone 1 g L^{-1}, yeast extract 0.5 g L^{-1}, NaCl 1 g L^{-1}, agar 15 g L^{-1}; [33] and Reasoner's 2A (R2A; Himedia, Mumbai, India)) supplemented with cycloheximide (80 mg L^{-1}; Sigma-Aldrich, Seelze, Germany). The incubation lasted a minimum of two weeks at room temperature (24–26 °C). Following this incubation period, isolated bacterial colonies were selected on the basis of their macro-morphological characteristics and maintained on R2A plates.

Bacterial representatives were identified by the sequencing of their 16S rRNA genes using the primers 27F and 685R [30]. The PCR amplicons of bacterial isolates were subjected to purification using ExoSAP-IT (Affymetrix, Cleveland, OH, USA) and subsequently sent for sequencing at a commercial facility, GATC-Biotech, in Konstanz, Germany. The resulting sequences were directly compared with those available in GenBank using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 3 January 2024). The sequences were then deposited in GenBank, with accession numbers PP078790–PP078818.

The similarity of soil micromycocoenoses and bacteriocenoses based on identified fungi and bacteria was determined according to Sőrensen SS = 2a/(2a + b + c) and Jaccard SJ = a/(a + b + c), where a is the number of species in sample a; b is the number of species in sample b; and c is the number of species in common in both (a and b) samples [34].

2.5. Next-Generation Sequencing Analysis

2.5.1. DNA Amplification of Bacterial, Archaeal and Fungal Communities

DNA extraction from the six soil samples was carried out using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Subsequently, the elution process was performed multiple times, concluding with a final volume of 2 × 30 µL of TE buffer.

The total DNA of bacterial, archaeal and fungal communities was amplified using the sets of primers oriented to bacterial (27f/685r) and archaeal (Arc344f-mod/Arch958r-mod) 16S rRNA [35] and to fungal ITS (ITS1/ITS4; [36]).

2.5.2. High-Throughput Sequencing and Data Analysis

PCR amplicons (amplified by primer sets for 16S rRNA gene for bacteria and archaea, ITS for fungi) were transposon-tagmented using the Nextera XT DNA Library Preparation Kit (Illumina Inc., San Diego, CA, USA) and consequently indexed and amplified with low-cycle pcr. The DNA profile of the sequencing libraries was verified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and a High-Sensitivity DNA Kit (Agilent Technologies, Santa Clara, CA, USA) and quantified using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The DNA libraries were analyzed using the Illumina MiSeq platform via 200 bp paired-end reads.

The quality of sequencing reads was verified using the FastQC tool (Version 0.12.0). The paired reads were trimmed for sequence quality and length and taxonomically classified with kraken2 using [37] and the Standard RefSeq database, including archaea and bacteria (v:2022-06-07). The produced sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/, accessed on 17 January 2024) Bioproject PRJNA1065900.

Alpha-diversity of bacterial, fungal and archaeal communities was performed using Simpson's diversity index. The statistical test used was Mann–Whitney U.

3. Results and Discussion

Due to the loss of water in the Aral Sea, the conditions for living organisms have changed in the studied area from the 1960s to the present. A fundamental change occurred in the hydrological regime and subsequently in the chemical and physical parameters of the soils. Increasing soil salinity, circulation change and oxygenation have had a direct negative impact on biodiversity [13]. Plants and animals that got stuck in the gradually drying lake and subsequently died became a source of organic matter for microorganisms. Soil microscopic filamentous fungi have gradually adapted to the new extreme conditions.

3.1. Soil Characteristic

According to the pH range [38], the soil reaction of all the study soil samples from the depth of 5 cm (assigned as "a") is medium alkali, and of all the study soil samples from the depth of 50 cm (assigned as "b") are highly alkali. The amount of organic matter is also very low. The calcium content in the soils is variable and significantly influenced by the parent rock and especially by precipitation (Table 1). Therefore, it accumulates in areas with limited precipitation, which is evident in samples from a depth of 50 cm (A3b, A2b and A1b). The salinity content of the analyzed soil samples is shown in Table 2. In the studied soil samples, a total of 59 elements were detected, including metals, lanthanides, alkali metals, alkali earth metals, semi-metals, non-metals and actinides. From this analysis, there is an evident increase in the values of metals in both soil samples from locality A2 (Cu, Pb, Zn, Ag, Ni, Co, Fe, V, La, Cr, Mg, Al, W Zr, Sn, Sc, Y, Nb and Ga). The same situation also occurred in the analysis of lanthanides (Ce, Pr, Nd, Dy, Er). From localities A2 and A3, increased values of Sr were detected. From the soil samples of locality A3, the highest amount of U was detected compared to the other soil samples (Table in Supplementary Material).

Table 1. Basic chemical characteristic of analyzed soil samples.

Samples	pH_{H20}	%				
		TC	TN	C Org.	Humus	$CaCO_3$
A1a	8.4	1.6	0.03	0.1	0.17	14
A1b	8.6	1.0	0.01	0.1	0.17	8.5
A2a	8.1	2.1	0.04	0.2	0.34	16.5
A2b	8.5	2.6	0.04	0.2	0.34	19.3
A3a	8.2	1.6	0.02	0.5	0.86	8.6
A3b	8.6	3.2	0.04	0.6	1.03	22.3

Table 2. Salt content in analyzed soil samples.

Samples	Ca^{2+}	Na^{2+}	Mg^{2+}	K^{+}	Al^{3+}	Mn^{2+}	Fe^{2+}	H^{+}	Salinity
	g/kg								%
A1a	1.1	0.06	0.4	0.1	0.02	0.005	0.56	0.002	0.22
A1b	3.1	0.9	1.3	0.1	0.02	0.005	0.56	0.002	0.60
A2a	3.3	1.3	1.6	0.4	0.02	0.005	0.56	0.002	0.72
A2b	2.1	4.8	3.3	0.5	0.02	0.005	0.56	0.002	1.13
A3a	26.0	5.6	3.6	0.3	0.02	0.005	0.56	0.002	3.61
A3b	2.5	5.9	0.7	0.5	0.02	0.005	0.56	0.002	1.02

3.2. Cultivable Soil Microscopic Filamentous Fungi

From the analyzed soil samples, 15 genera and 31 species of soil microscopic filamentous fungi were recovered (Table 3). The species *Rhizopus microsporus* (Figure 4A) and *Saksena vasiformis*, from the strain Zygomycota, occurred only in samples A1a, A2a and A2b. All other species belonging to the strain Ascomycota were much more abundant. The most species (seven) were recorded in the genera *Aspergillus*, as *A. flavus*, *A. fumigatus*, *A. pseudoglaucus*, *A. jensenii*, *A. niger* (Figure 4B), *A. oryzae* and *A. proliferans* (Figure 4C). In the genus *Cladosporium*, five species were identified, as *C. cladosporioides*, *C. floccosum*, *C. herbarum*, *C. iridis* (Figure 4D) and *C. sphaerospermum.* Also, in the genus *Penicillium,* five species were identified, as *P. chrysogenum*, *P. corraligenum* (Figure 4E–H), *P. echinulatum*, *P. expansum* and *P. rugulosum*. Four species were identified in the genus *Alternaria*, as *A. alternata*, *A. atra* (Figure 5A), *A. japonica* and *A. tenuissima.*

Table 3. Soil microscopic filamentous fungi isolated from study soil samples.

Genera/Species/Sequence Similarity	Study Soil Samples					
	A1a	A1b	A2a	A2b	A3a	A3b
KF747365 *Alternaria alternata* 100%	+	–	–	–	–	–
MG780401 *Alternaria atra* 100%	+	–	+	–	–	–
MF462308 *Alternaria japonica* 100%	+	–	–	–	–	–
MH345962 *Alternaria tenuissima* 100%	+	–	–	–	–	–
MT645322 *Aspergillus flavus* 100%	–	–	–	+	+	–
MT487775 *Aspergillus fumigatus* 100%	+	–	–	+	–	+
KX258805 *Aspergillus pseudoglaucus* 100%	–	–	–	+	–	–
LN898704 *Aspergillus jensenii* 100%	–	–	–	–	+	–
KJ701548 *Aspergillus niger* 100%	–	–	–	+	+	+
MW058064 *Aspergillus oryzae* 99%	–	–	–	+	–	–
KX696377 *Aspergillus proliferans* 100%	–	–	–	+	–	–
MH857026 *Auxarthron umbrinum* 100%	–	–	–	+	–	–
MT529803 *Chaetomium globosum* 100%	–	–	+	–	–	–
MT131338 *Cladosporium cladosporioides* 100%	–	–	–	–	+	+
MF472979 *Cladosporium floccosum* 100%	–	–	+	–	–	–
OR243878 *Cladosporium herbarum* 100%	–	–	+	–	–	–
OQ608649 *Cladosporium iridis* 100%	–	–	–	+	–	+
MG787259 *Cladosporium sphaerospermum* 100%	+	–	–	–	–	
ON229430 *Epicoccum nigrum* 100%	–	–	–	–	–	+
MH191137 *Isaria farinosa* 100%	–	–	–	–	–	+
MK719910 *Neomicrosphaeropsis italica* 99%	–	–	+	–	–	–
MH861337 *Myriodontium keratinophilum* 100%	–	–	–	+	–	–
MN947607 *Parengyodontium album* 100%	–	+	–	–	–	
MT103060 *Penicillium chrysogenum* 100%	–	+	–	–	+	+
KP016836 *Penicillium coralligerum* 99%	–	–	–	+	–	–
KP411588 *Penicillium echinulatum* 100%	–	–	–	–	+	–
MN643064 *Penicillium expansum* 99%	–	+	–	–	–	–
GU566230 *Penicillium rugulosum* 100%	–	–	–	+	–	–
KC206538 *Rhizopus microsporus* 100%	+	–	+	–	–	–
FR687323 *Saksenaea vasiformis* 100%	–	–	–	+	–	–
NR_111027 *Simplicillium sympodiophorum* 100%	–	–	+	–	–	–
MK802874 *Stachybotrys chlorohalonata* 100%	–	+	–	–	–	–
∑ 15 genera/32 species	7	4	7	12	6	7

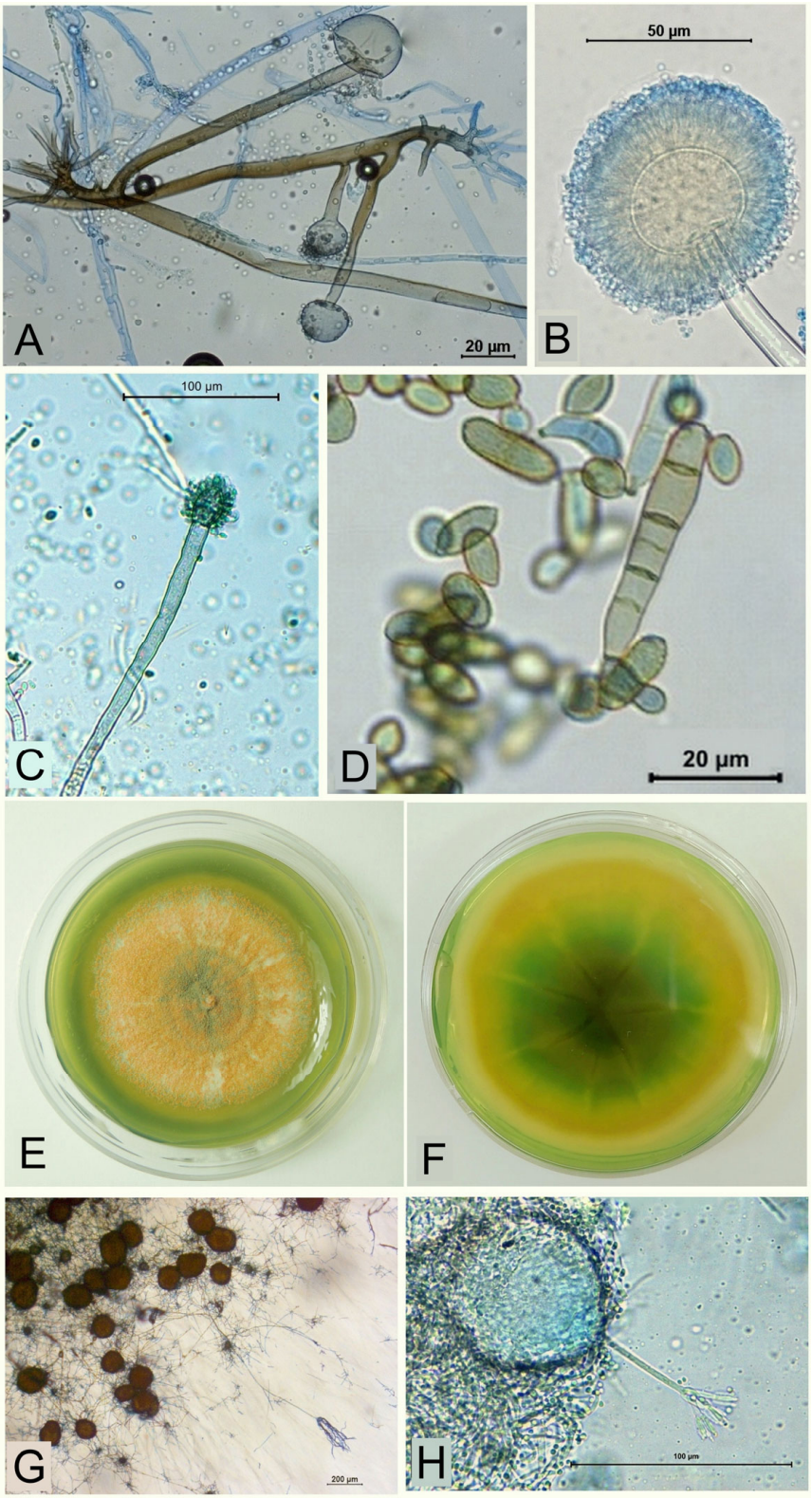

Figure 4. Microscopic filamentous fungi isolated from study soil samples. *Rhizopus microsporus* ((**A**) sporangiophores with collumela and rhizoides); *Aspergillus niger* ((**B**) conidial head); *Aspergillus proliferans* ((**C**) conidial head); *Cladosporium iridis* ((**D**) conidia of various shapes and magnifications); *Penicillium coralligerum* ((**E**) rough structure of aerial mycelium; (**F**) revers of Petri dish with dark green pigment; (**G**) cleistothecia as sexually reproducing forms; (**H**) detail of cleistothecium with asexually form *Penicillium coralligerum*).

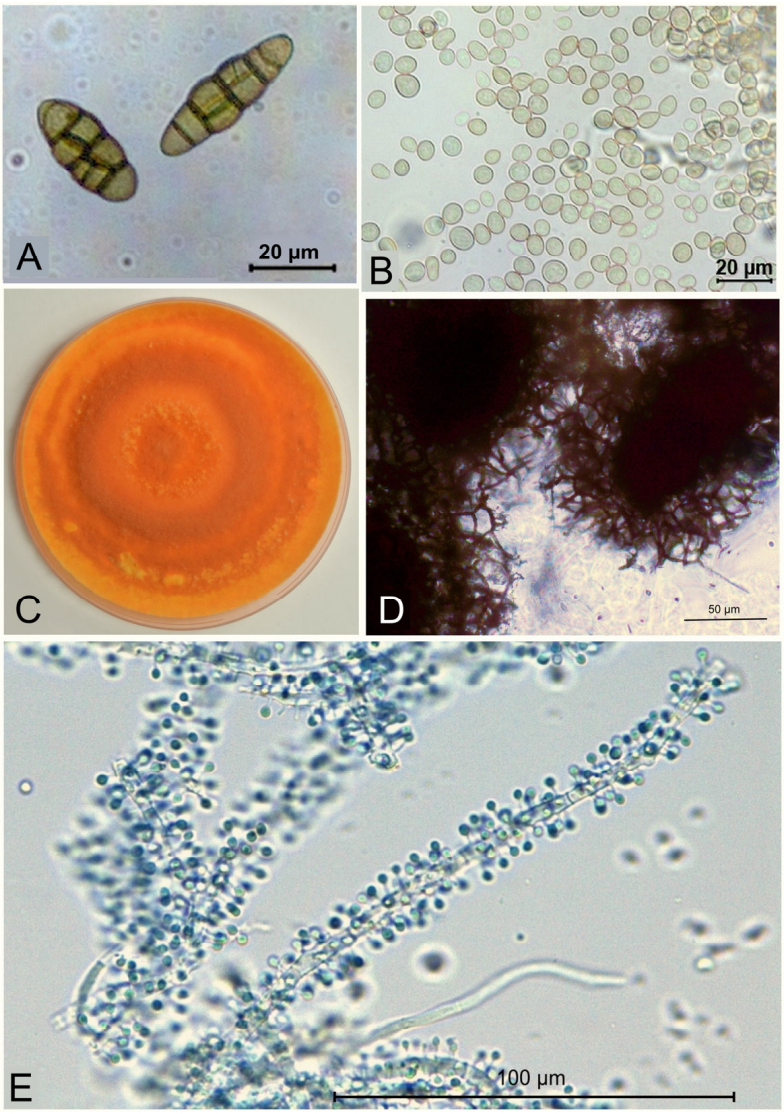

Figure 5. Microscopic filamentous fungi isolated from study soil samples. *Alternaria atra* ((**A**) conidia), *Neomicrosphaeropsis italica* ((**B**) conidia), *Auxarthron umbrinum* ((**C**) growth of aerial mycelium in orange colours; (**D**) rough-walled reticuloperidium terminated by spines), *Myriodontium keratinophilum* ((**E**) fertile hyphae and conidia).

All of the identified species of the genus *Alternaria* were isolated from soil sample A1a (the depth of 5 cm). *Alternaria* is one of the most ubiquitous fungal genera, inhabiting nearly every environmental substrate, but most species have been recorded on an extremely wide range of plants. Among the less common species is *Alternaria japonica*, occurring on seeds, which causes black spot on turnips, and head rot and leaf spots on Brassicaceae [39]. Also, *Cladosporium sphaerospermum* belongs to the group of melanized fungi isolated from the same sample. *Aspergillus fumigatus* and *Rhizopus microsporus* also occurred in soil sample A1a. The identification of *Rhizopus microsporus* is very interesting, because of its pathogenicity on plants such as maize, sunflower or rice. It has also been isolated from pristine soils from sites in Antarctica. According to Durán et al. [40], the extreme conditions that coexist in Antarctica produce a strong selective pressure that could lead to the evolution of novel mechanisms for stress tolerance by indigenous microorganisms. The lowest number of species (*Paraengyodontium album*, *Penicillium expansum*, *P. chrysogenum* and melanized *Stachybotrys chartarum*) was isolated from soil sample A1b (the depth of 50 cm). All of these species are known as cosmopolitan, occurring in variable soils [41,42]. According to Jaouani et al. [43], the keratinophilic species *Paraengyodontium album* is halotolerant and

able to grow in solid and liquid media with a salt concentration from 10% to 15% NaCl. This strain is even able to tolerate 20% NaCl and alkaline tress at pH 10. It was identified from locality A1, together with 11 species of soil microscopic filamentous fungi (Table 3).

From a mycological point of view, locality A2 is very interesting. From the depth of 5 cm (sample A2a), only six species were isolated (*Chaetomium globosum*, *Cladosporium floccosum*, *C. herbarum*, *Neomicrosphaeropsis italica* (Figure 5B), *Rhizopus microsporus* and *Simplicillium sympodiophorum*). All these species occur in soil but all of them are also plant pathogens [44–46]. Of all the studied soil samples, the most microfungal species (11) were identified from sample A2b (from the depth of 50 cm). A total of six cosmopolitan aspergili were identified. According to Hubka et al. [31], *Aspergillus proliferans* is an economically important strain, and it seems to be relatively common. It has been isolated from variable soils in Tibet or China, from moldy wood, cave sediment, inside books from a library, from air in a living room and from unknown sources [47], as well as from onychomycoses [48]. The species *Auxarthron umbrinum* (Figure 5C,D), in contrast, was until now isolated only from onychomycoses [49]. The phytopathogenic species *Cladosporium iridis* has been isolated from leaf spot and blotch of *Iris* sp. from many countries, such as Africa and Cyprus and also from Uzbekistan, Turkmenistan, Kazakhstan, Kyrgyzstan and others [50]. The species *Myriodontium keratinophilum* (Figure 5E) is widespread in the environment and able to colonize keratinous surfaces of human body [51]. It has been isolated from soil samples in India [52] and also from the funeral clothes of Cardinal Peter Pázmany [53]. *Penicillium* species are among the most widespread fungal organisms on the Earth, but marine environments and marine subaqueous soils have been poorly studied. *Penicillium coralligerum* (Figure 4E–H) is a marine species sometimes referred to as a deep-sea fungus and in some languages named the equivalent of a "deep-sea mold", isolated from subaqueous soil in the Sakhalin shelf [54]. According to the study of Takahashi et al. [55], among 91 deep-sea fungal strains, *Penicillium coralligerum* could produce notable anti-*Saprolegnia parasitica* activity. *Saksena vasiformis* is a species able to cause severe human infections. It has also been isolated from soils, driftwood or grains [56].

Locality A3 is closest to the southern part of the Aral Sea, where there is still water. From the soil depth of 5 cm (sample A3a), three aspergili were isolated, from which *Aspergillus jensenii* was described as a new species in 2012. This species belongs to the section Versicolores, and according to Jurjevic et al. [57], variable propagules of *A. versicolor* have been recovered from the highly saline Dead Sea, showing an ability to survive conditions of salinity or drying. High tolerance to salinity may extend to other species in the section Versicolores. *Aspergillus jensenii* was also isolated from an old manuscript from Indonesia [58] and from the soil of potted plants [59]. All other microscopic fungi isolated from this soil sample belong to ubiquitous species, including in the case of the sample from a depth of 50 cm (A3b). Among the species isolated are the phytopathogenic *Cladosporium cladosporioides*, *C. iridis* and *Epicoccum nigrum* and the entomopathogenic *Isaria farinosa* [60].

Based on the calculation according to Sörensen and Jaccard [34]. (Table 4), the similarity of mycocoenoses is the highest between samples A1a and A1b (0.64). The similarity values of 0.57 (between samples A2b and A3b) and 0.54 (between samples A1a and A3a) are on the border of similarity and difference of mycocoenoses.

Table 4. Similarity of mycocoenosis according to Sörensen and Jaccard (S/J) [34].

	A1a	**A1b**	**A2a**	**A2b**	**A3a**	**A3b**
A1a	-	S/J = 0.64	S/J = 0.47	S/J = 0.35	S/J = 0.54	S/J = 0.47
A1b	S/J = 0.64	-	S/J = 0.36	S/J = 0.25	S/J = 0.36	S/J = 0.33
A2a	S/J = 0.36	S/J = 0.36	-	S/J = 0.37	S/J = 0.54	S/J = 0.5
A2b	S/J = 0.35	S/J = 0.25	S/J = 0.37	-	S/J = 0.5	S/J = 0.57
A3a	S/J = 0.47	S/J = 0.36	S/J = 0.54	S/J = 0.63	-	S/J = 0.37
A3b	S/J = 0.47	S/J = 0.33	S/J = 0.5	S/J = 0.57	S/J = 0.37	-

The species composition of microscopic filamentous fungi of the arid and slightly halophile environment of the Aral Sea dry bottom is characterized by a big group of melanized species of the genera *Alternaria*, *Cladosporium*, *Epicoccum* and *Stachybotrys*, from which most of them are also phytopathogenic. The second group consists of keratinophilic species, such as *Auxarthron umbrinum*, *Isaria farinosa*, *Myriodontium keratinophilum*, *Paraengyodontium album* and *Saksena vasiformis*, which are widespread in nature but found most abundantly in keratin-rich environments, such as insects, feathers, animal fur, nails and hair. Fungal species belonging to the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Chaetomium* and *Penicillium* have been isolated from saline or hypersaline environments by many other authors [17,19,22], and for this reason they have been assigned as halophiles. Halophiles are defined as organisms requiring > 3% NaCl for growth [24]. Fungal species which can tolerate salt concentrations of 2 to 5% *w*/*v* are known as slight halophiles [17]. However, the long-term adaptation of microscopic fungi to salinity requires cellular and metabolic responses that differ from short-term osmotic stress signaling [18].

3.3. Bacterial Isolates

According to the isolated bacteria, the soil that had the richest bacterial diversity was from sample A1a. From this sample, 11 different species were isolated, mainly members of the genera *Arthrobacter*, *Bacillus*, *Massilia* and *Rhodococcus*. Soils from A3a and A3b displayed the poorest bacterial diversity, with four and three species isolated, respectively. Half of these species belong to the *Bacillus* genus (Table 5). *Bacillus* species were the only isolates spread in all the samples. Members of this genus have already been isolated from Aral soils, and some of them were able to mineralize [61] and to protect plants against pathogenic fungi [62]. Bacteria isolated exclusively from soils at A1a were *Massilia* strains; these bacteria have also been isolated in other arid soils from Uzbekistan [63] and Morocco and show interesting hydrolytic properties [64].

Table 5. Soil bacteria isolated from study soil samples.

Genera/Species/Sequence Similarity	Study Soil Samples					
	A1a	**A1b**	**A2a**	**A2b**	**A3a**	**A3b**
KF747044 *Massilia* sp. 99%	+	–	–	–	–	–
MF077219 *Massilia varians* 99%	+	–	–	–	–	–
JQ977399 *Arthrobacter* sp. 100%	+	–	–	+	–	–
OR539574 *Arthrobacter humicola* 100%	+	+	–	–	–	–
KY753214 *Arthrobacter crystallopoietes* 100%	+	–	–	–	–	–
MN931288 *Bacillus coreaensis* 100%	+	–	–	–	–	–
MG705966 *Bacillus flexus* 100%	+	–	+	+	–	–
LN995471 *Bacillus firmus* 100%	–	–	–	–	+	+
MH819519 *Bacillus* sp. 100%	+	+	+	+	+	+
MH806388 *Bacillus cereus* 100%	–	+	+	+	–	–
MF620082 *Bacillus paralicheniformis* 100%	–	–	–	+	–	–
MT804101 *Pontibacter* sp. 98%	–	–	+	–	–	–
KC354466 *Agrococcus citreus* 100%	+	–	–	–	–	–
KU204869 *Pseudarthrobacter siccitolerans* 99%	+	–	–	–	–	–
NR_113620 *Oxalicibacterium horti* 100%	+	–	–	–	–	–
MG254794 *Kocuria* sp. 99%	+	–	–	–	–	–
KT922050 *Rhodococcus erythropolis* 100%	+	+	–	–	–	–
KF040418 *Rhodococcus* sp. 99%	+	+	–	–	–	–
KT003514 *Pseudomonas* sp. 100%	+	+	–	+	–	–
MW089200 *Microbacterium* sp. 100%	–	+	–	–	–	–
LC565814 *Paraliobacillus sp.* 100%	–	+	–	–	–	–
KF876899 *Nocardiopsis flavescens* 100%	–	–	+	+	–	–
KC493983 *Nocardiopsis aegyptia* 100%	–	–	–	+	–	–
JQ885684 *Nocardiopsis* sp. 99%	–	–	–	+	–	–
KC336307 *Streptomyces* sp. 100%	–	–	+	–	–	–
KY952739 *Halomonas* sp. 99%	–	–	+	–	–	–
MH392690 *Sphingomonas* sp. 99%	–	–	–	–	+	–
MH813363 *Rathayibacter* sp. 99%	–	–	–	–	+	–
FR727710 *Paenisporosarcina* sp. 99%	–	–	–	–	–	+
∑ 18 genera/28 species	15	8	7	9	4	3

Other soil-specific isolates were members of the genera *Rhodococcus* and *Nocardiopsis*, which occurred in the soils at A1 and A2, respectively. The *Rhodococcus* species was previously isolated in Aral Sea areas in association with halophytes [62] and detected by metagenomics analysis in water samples [65]. However, *Rhodococcus* strains are able to adapt to different soil conditions, including high concentrations of salts and pollutants. These characteristics permit them to be versatile bacteria for bioremediation applications [66]. Several *Nocardiopsis* species have also been isolated from halophytes of the west Aral Sea basin, and they display strong proteolytic and cellulolytic activities [62]. They can be considered as free-living entities occurring in a variety of soils and other saline or hypersaline habitats, and they are producers of diverse bioactive compounds and extracellular enzymes [67].

Typical strains of the soils from A3 were as follows: *Sphingomonas* sp., *Rathayibacter* sp. and *Paenisporosarcina* sp. Wicaksono et al. [68] found that *Sphingomonas* were part of the bacterial community in the rhizosphere of the plant *Suaeda acuminata*, rather than being present in Aral soil samples, and Osman et al. [63] frequently found *Sphingomonas* in soil samples from the Kyzyl-Kum desert in Uzbekistan. *Rathayibacter* sp. and *Paenisporosarcina* sp. were not previously identified in Aral-related samples. However, *Rathayibacter* species are known mainly as opportunistic phytopathogenic bacteria that frequently occur in arid areas [69].

The highest similarity of bacteria is between samples A1a and A3b (0.76), A1a and A3b (0.75), A2b and A3b (0.69), A1a and A3b (0.67), A1a and A2a (0.65) and A2a and A3b (0.64). Much less samples show dissimilarity of bacteriocenosis (Table 6).

Table 6. Similarity of bacteriocenosis according to Sörensen and Jaccard (S/J) [34].

	A1a	A1b	A2a	A2b	A3a	A3b
A1a	-	S/J = 0.54	S/J = 0.65	S/J = 0.62	S/J = 0.75	S/J = 0.79
A1b	S/J = 0.54	-	S/J = 0.47	S/J = 0.40	S/J = 0.61	S/J = 0.67
A2a	S/J = 0.65	S/J = 0.47	-	S/J = 0.35	S/J = 0.58	S/J = 0.64
A2b	S/J = 0.62	S/J = 0.40	S/J = 0.35	-	-	S/J = 0.69
A3a	S/J = 0.75	S/J = 0.61	S/J = 0.58	S/J = 0.58	-	S/J = 0.44
A3b	S/J = 0.79	S/J = 0.67	S/J = 0.64	S/J = 0.69	S/J = 0.44	-

3.4. High-Throughput Sequencing Analysis

To see the differences in alpha-diversity between bacterial fungal and archaeal communities, we performed the Mann–Whitney U test, which did not show significant differences among communities. However, the highest Simpson's index was found for bacterial communities and the lowest for archaea (Figure 6).

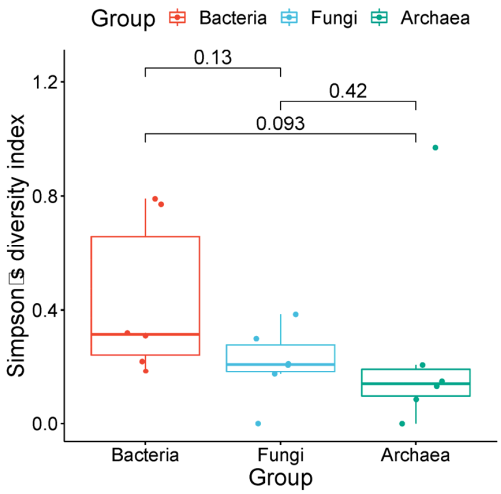

Figure 6. Simpson's diversity index for bacterial fungal and archaeal communities.

3.4.1. Fungi

The detected fungi belonged mainly to the phylum Ascomycota, namely the classes Saccharomycetes, Eurotiomycetes, Schizosaccharomycetes, Leotiomycetes and Sordariomycetes, while only two classes, Tremellomycetes and Malasseziomycetes, represented Basidiomycota (A3b 0.5%–A3a 5.1%). The PCR of sample A2b did not produce any amplicon; therefore, no data are available.

Nonetheless, 19 fungal genera were identified (Figure 7), of which *Aspergillus*, represented by *Aspergillus oryzae* and *Aspergillus fumigatus*, *Colletotrichum higginsianum* and *Botrytis cinerea*, comprised over 50% of all detected taxa (A1a—57%; A1b—73%; A2a—71%; A3b—82%). While sample A3a was uniquely dominated by *Saccharomyces cerevisiae* (59%), followed by *Schizosaccharomyces pombe* (16%), the latter was also detected in samples A1a (20%) and A1b (9%). Other species were also detected, such as *Neurospora crassa* (not found in A1a) and *Malassezia restricta* (not found in A3b), which belonged to the widely spread fungi.

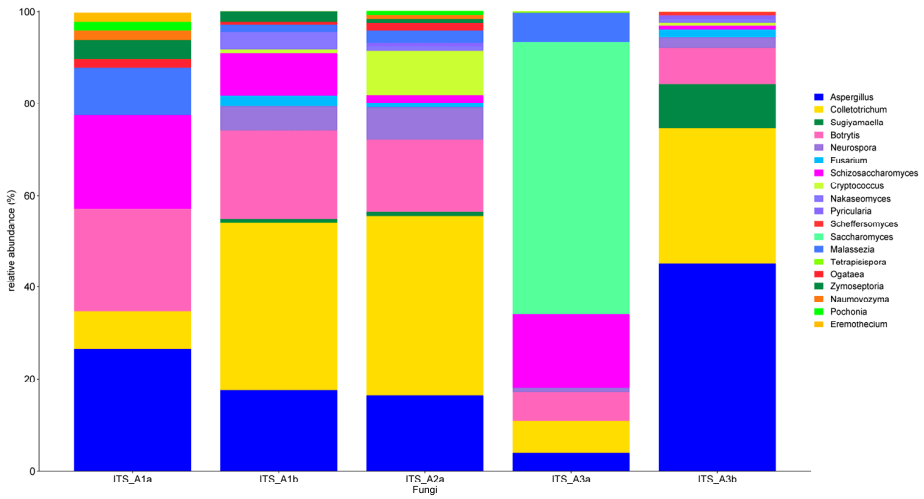

Figure 7. Fungi: Graphical visualization of the main representatives of fungi. All detected fungal genera were included.

Practically speaking, only a few links occurred between the cultivation and sequencing analysis. The correlation regarded only the high presence of *Aspergillus* species, and some members belonged to the order Sordariales (*Neurospora*/*Chaetomium*).

Considering the most abundant genera detected by high-throughput sequencing, new taxa were added to the isolated ones. In fact, the well-known phytopathogens *Colletotrichum* and *Botrytis* [70] were revealed. Members of the genera *Saccharomyces* and *Schizosaccharomyces* are not usually considered as halophilic microorganisms, and perhaps their detection is more associated with their ability to grow in the presence of toxic contaminants [71].

3.4.2. Bacteria

A total of 30 different bacterial phyla were identified (Figure 8), of which Pseudomonadota (A1b—49%, A3a—89%, A3b—28%), Bacteroidota (A2a—88%) and Actinomycetota (A2b—53%) were the most abundant, followed by Cyanobacteria (A1a—34%) and Bacillota (2–8%). The highest bacterial diversity at the phylum level was detected in samples A2b (26 phyla) and A3b (23 phyla).

The most abundant bacterial family of the A1a sample, Hymenobacteraceae (8%), was also identified in A2a (11%), while Chitinophagaceae, Rubrobacteraceae, Cytophagaceae and Oscillatoriaceae (from 7% to 5%) were unique. For each sample, a different profile of the most abundant bacterial families was typical: A1b (Marinobacteraceae 45%, Iamiaceae 9%), A2a (Flavobacteriaceae 57%, Hymenobacteraceae 11%, Fulvivirgaceae 9%), A2b (Iamiaceae

23%, Egicoccaceae 17.5%, Borreliaceae 5%), A3a (Comamonadaceae 33%, Burkholderiaceae 27%, Pseudomonadaceae 14%) and A3b (Flavobacteriaceae 17.5%, Egicoccaceae 11.3%, Iamiaceae 7.5%).

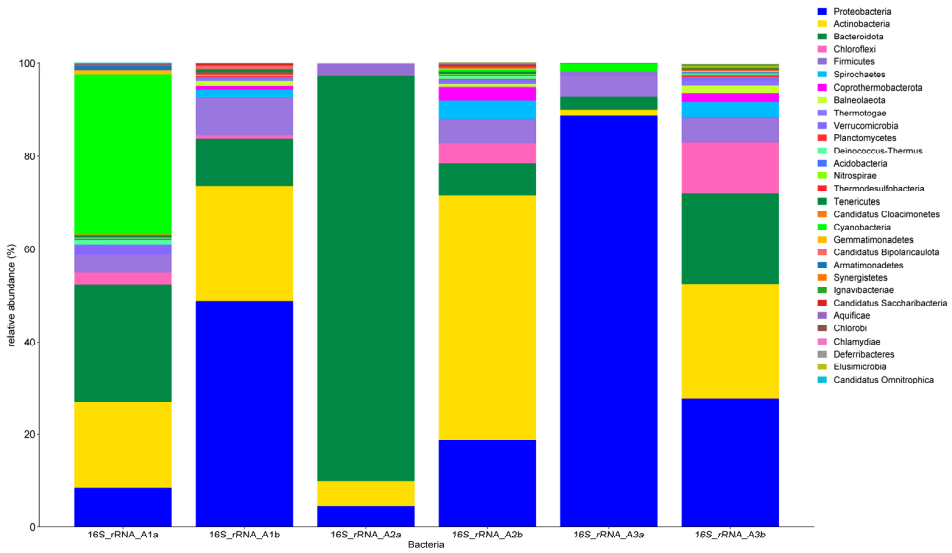

Figure 8. Bacteria: Graphical visualization of identified bacterial phyla. All detected bacterial phyla were included.

Focusing on the genus level, *Rubrobacter tropicus*, *Oscillatoria nigro-viridis*, *Rhodocytophaga rosea* and mainly *Hymenobacter oligotrophus* (4–6%) dominated from the 139 bacterial genera in sample A1a, while *Marinobacter* (46%; *M.* sp. Arc7-DN-1, *M. salarius*, *M.* sp. LPB0319), the oversized *Actinomarinicola tropica* (6.5%) and another 75 bacterial genera dominated in the A1b sample. *Antarcticibacterium* (32%; *A. flavum*, *A.* sp. 1MA-6-2, *A. arcticum*) dominated over *Fulvivirga* (11%; *F.* sp. W9P-11, *F.* sp. SS9-22) and another 80 genera with an abundance of less than 10% in the A3a sample, while of the 214 bacterial genera observed in the A2b sample, *Egicoccus halophilus*, *Actinomarinicola tropica* and *Aquihabitans* sp. Kera 3 altogether represented over 40% of all bacteria. Sample A3a was very scarce in terms of bacterial diversity, since "only" 47 bacterial genera were detected, mainly representatives of the phylum Pseudomonadota (*Acidovorax* sp. *YS12*, *Lautropia mirabilis*, *Rubrivivax gelatinosus*, *Schlegelella thermodepolymerans* and *Pseudomonas* spp.), which formed almost 70% of all classified sequences. In contrast, the environment represented by the A3b sample was dominated by *Egicoccus halophilus* and *Aquihabitans* sp. Kera 3 (14% and 6%; Actinomycetota), *Antarcticibacterium* sp. 1MA-6-2 (7%; Bacteroidota) and the Candidatus *Promineofilum breve* (7%, Chloroflexota), followed by another 174 bacterial genera.

Comparing the culture-dependent analysis with the high-throughput sequencing makes it clear that the two approaches are complementary. There is no link between the genera isolated by cultivation and those detected by Illumina sequencing. An example is given by *Bacillus* species which were isolated from each sample but which were not among the most abundant bacteria according to sequencing. Some small similarity between the two strategies was seen only at the phylum level regarding Pseudomonadota and Actinomycetota.

It is difficult to compare our results with the previous studies related to Aral Sea samples. Other authors oriented their taxonomical analysis at the phylum or maximally at the class level [65,72]. Correlations with earlier investigations only regarded the genera *Rubrobacter*, *Hymenobacter*, *Marinobacter* and *Pseudomonas* [63,68].

However, considering the most abundant detected genera, *Antarcticibacterium*, *Egicoccus*, *Actinomarinicola* and *Aquihabitans*, they are typical of marine environments, and they can tolerate a high concentration of salt [73].

3.4.3. Archaea

Almost all of the analyzed samples were found to be positive for Archaea, mostly belonging to the phylum Euryarchaeota (Figure 9). While the A1a sample was exclusively inhabited by *Thaumarchaeota*, especially with *Candidatus Nitrososocosmicus franklandus* (class Nitrososphaeria), the A1b sample was also represented by *Halocatena* (11%) and *Haladaptatus* (29%), in addition to *Candidatus Nitrososocosmicus* (32%). The latter, together with *Halalkalicoccus* and *Halorussus*, was also found to be the main representative of the A2a and A2b environments. At the species level, A2a and A2b were found to be dominated by *Halalkalicoccus jeotgali* (A2a 35.2%; A2b 22%), followed by *Halorussus halophilus* (A2a 10%; A2b 6%), while *Natronomonas salina* was present both in A2b (8%) and A3b (12%). The highest diversity could be observed in the A3b sample, which was represented by several abundant genera belonging to the order Halobacteriales (*Haladaptatus*, *Halobacterium*, *Haloarcula*, *Halapricum*, *Halorhabdus*, *Natronomonas* and *Halorussus*), as well as a representative of Nitrosopumilaceae—*Nitrosopumilus*. We were not able to detect any archaea in the A3a sample, because the PCR did not work with this sample.

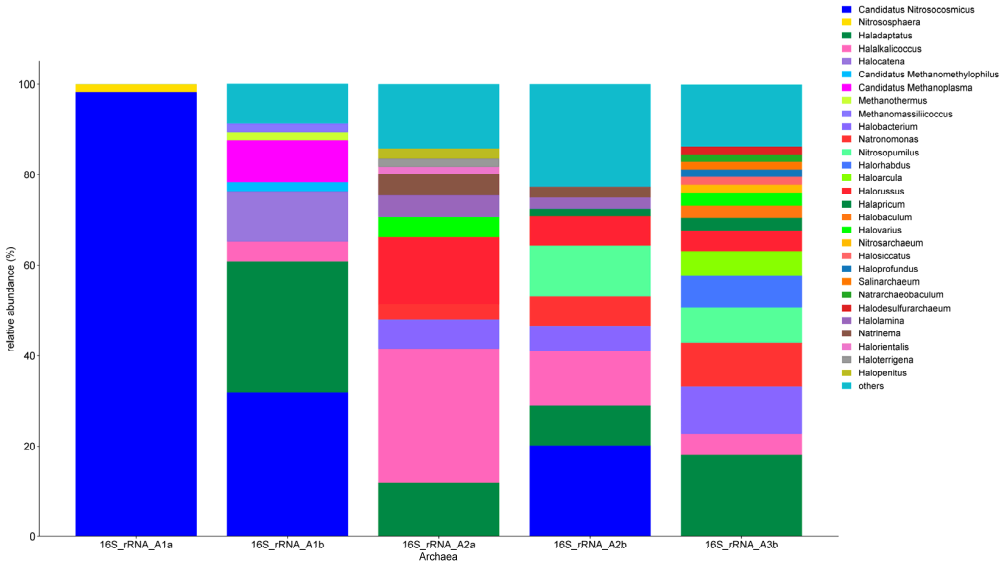

Figure 9. Archaea. Graphical visualization of the main representatives of *Archaea*. Representatives were selected according to their relative abundance with a minimum of 1.5% of all archaeal OTUs at genus level.

It is evident that the archaeal community in all the analyzed samples is composed mainly of halophilic genera. Members of the order Halobacteriales are the most widespread; they were previously detected in samples of water [74] and soil [68] from the Aral Sea. These archaea in this habitat are involved in denitrification processes [68].

Archaea of the class Nitrososphaeria have also previously been detected in this kind of environment [72]. Members of this class harbored genes encoding a methane/ammonia monooxygenase and are involved in denitrification, dissimilatory nitrate reduction to ammonium and ammonia oxidation [68].

4. Conclusions

The environmental changes in the Aral Sea and also Muynak Lake have led to considerable transformation of all ecosystem elements. From the analyzed soil samples, 15 genera and 31 species of soil microscopic filamentous fungi were identified. The microfungal composition is characterized by a big group of melanized species of the genera *Alternaria*, *Cladosporium*, *Epicoccum* and *Stachybotrys*, from which most are also phytopathogenic. The isolated bacteria were typical of an arid environment, and several of them can be used for biotechnological applications exploiting their hydrolytic properties (*Bacillus* and

Massilia), bioremediation activities (*Rhodococcus*) and their ability to produce bioactive compounds (*Nocardiopsis*).

The high-throughput sequencing approach displayed different microorganisms with respect to the cultivation strategy. The most abundant taxa detected by this method were *Colletotrichum*, *Botrytis*, *Saccharomyces*, *Schizosaccharomyces*, *Antarcticibacterium*, *Egicoccus*, *Actinomarinicola* and *Aquihabitans*. Moreover, the archaeal community mainly evidenced its halophilic character including the genera *Haladaptatus*, *Halobacterium*, *Haloarcula*, *Halapricum*, *Halorhabdus*, *Halorussus* and *Halalkalicoccus*.

The ecological crisis of the Aral Sea bottom caused by human activities has created the conditions for a change in biodiversity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/soilsystems8020058/s1.

Author Contributions: A.Š. and S.N.—processing and analyses of soil samples and isolation, cultivation of soil microscopic fungi and their photo documentation. E.P. and M.O.—soil collection, data processing and cartographic images. N.K.—bacterial identification; F.M.—fungal identification; L.K.—sequencing analysis; D.P.—coordination of molecular analyses of isolated microfungi, data processing; K.Š.—processing and evaluation of prokaryotic communities. J.P.—bioinformatic data analysis. All authors have read and agreed to the published version of the manuscript.

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