ecologies

MDPI

Article

Beta-Diversity Enhancement by Archaeological Structures: Bacterial Communities of an Historical Tannery Area of the City of Jena (Germany) Reflect the Ancient Human Impact

Johann Michael Köhler [1,*], Linda Ehrhardt [1], Jialan Cao [1], Frances Möller [1], Tim Schüler [2] and Peter Mike Günther [1]

[1] Department of Physical Chemistry and Microreaction Technology, Institute for Micro- und Nanotechnologies/Institute for Chemistry and Biotechnology, Technische Universität Ilmenau, 98684 Ilmenau, Germany; jialan.cao@tu-ilmenau.de (J.C.); mike.guenther@tu-ilmenau.de (P.M.G.)
[2] Thuringian State Office for Archaeology and Monument Conservation, 99423 Weimar, Germany
* Correspondence: michael.koehler@tu-ilmenau.de

Abstract: Soil samples taken during archaeological investigations of a historical tannery area in the eastern suburb of the medieval city of Jena have been investigated by 16S r-RNA gene profiling. The analyses supplied a large spectrum of interesting bacteria, among them Patescibacteria, Methylomirabilota, Asgardarchaeota, Zixibacteria, Sideroxydans and Sulfurifustis. Samples taken from soil inside the residues of large vats show large differences in comparison to the environmental soil. The PCAs for different abundance classes clearly reflect the higher similarity between the bacterial communities of the outside-vat soils in comparison with three of the inside-vat soil communities. Two of the in-side vat soils are distinguishable from the other samples by separate use of each abundance class, but classes of lower abundance are better applicable than the highly abundant bacteria for distinguishing the sampling sites by PCA, in general. This effect could be interpreted by the assumption that less abundant types in the 16S r-RNA data tend to be more related to an earlier state of soil development than the more abundant and might be, therefore, better suited for conclusions on the state of the soils in an earlier local situation. In addition, the analyses allowed identification of specific features of each single sampling site. In one site specifically, DNA hints of animal residue-related bacteria were found. Obviously, the special situation in the in-site vat soils contributes to the diversity of the place, and enhances its Beta-diversity. Very high abundancies of several ammonia-metabolizing and of sulphur compound-oxidizing genera in the metagenomics data can be interpreted as an echo of the former tannery activities using urine and processing keratin-rich animal materials. In summary, it can be concluded that the 16S r-RNA analysis of such archaeological places can supply a lot of data related to ancient human impacts, representing a kind of "ecological memory of soil".

Keywords: soil; ngs sequencing; human impact; archaeology; tannery; memory effect; community evolution

Citation: Köhler, J.M.; Ehrhardt, L.; Cao, J.; Möller, F.; Schüler, T.; Günther, P.M. Beta-Diversity Enhancement by Archaeological Structures: Bacterial Communities of an Historical Tannery Area of the City of Jena (Germany) Reflect the Ancient Human Impact. *Ecologies* **2023**, *4*, 325–343. https://doi.org/10.3390/ecologies4020021

Academic Editor: Michael A. Herman

Received: 17 March 2023
Revised: 9 May 2023
Accepted: 17 May 2023
Published: 20 May 2023

1. Introduction

During the last decades, a lot of investigations have shown that agriculture, industry and mining have a strong impact not only on the macro flora and fauna but on the microorganism communities, as well [1–3]. It is not surprising that the release of heavy metal ions, pesticides, herbicides and other toxic substances have a strong influence on soil microbial communities because sensitive types are suppressed and only tolerant microorganisms can grow. In particular, the side effects of industrial mining, the large deposition of material and development of acid drains, cause strong damages in the related environment. Besides these strong effects of industrial activities, ancient settlements and production places can

have a significant effect on the local microorganism communities. Besides historical mining areas and melting places, this concerns living and celebration places as well [4].

Such effects of human impacts are not only restricted to places which still show the artificial changes of local environment on the surface. Soil samples from archaeological investigations have been included in microbiological and metagenomics investigations in recent years [5–7]. They make clear that, even at small sites and with intensive change, the microbial communities should not be levelled completely over time. Besides special high abundant species, special dormant types related to former human activity can contribute to the diversity of a place [8,9].

Here, the results of an analysis of 16S r-RNA NGS data from a historical suburb area of the city of Jena (Thüringen/Germany) are reported. This area is situated in the recent city centre, but was historically placed outside the eastern medieval city wall between a mill channel and the river Saale. The area was used as a tannery in the late middle age and early new age periods. During archaeological excavations on a recent construction site, residues of some large vats had been found and soil samples collected. Besides the facts that tannery activities were finished there centuries ago, the concerned soil is wet, the area was flooded several times by the river and was used in different ways during the last decades, there was the question of whether the bacterial DNA reflects the special history of the place. In particular, the analysis was motivated by the question of whether the former embossing of the bacterial communities by the ancient tannery activities could be detected by the analysis of recent bacterial compositions. Therefore, samples from soil inside some vats and samples from outside were taken. The analysis presented here is based on these soil sample set.

2. Experimental Procedures

2.1. Soil Samples

The investigations included 16 samples from 9 sampling sites from the archaeological excavation of a place with former tannery activities in a suburb area of the city of Jena. This archaeological area is situated in the river lowland of Saale and belonged to the eastern suburb of Jena with late middle age to early modern settlement and craft business. The samples were taken from inside of four objects identified as vats for tanning or dying activities and from 5 sites outside of the vats (Table 1). The vat with the feature number 61/V (HB35-1; HB35-2) was dated after 1846 by dendrochronology. Other wooden construction parts of the direct neighbourhood of the sampled vats were dated between 1732 and 1784 by the same method. The wood used for the vessels was determined as pine (*pinus sylvestris*).

Table 1. Samples, coordinates and soil pH values.

Feature/Area	Archaeological Situation	Sample	Coordinates	pH Values
58/V	Inside Vat	HB31	682091.3/5645202.8	7.41
58/Vref	Outside Vat	HB32-1, HB32-2	682091.3/5645202.8	8.09
60/V	Inside Vat	HB33	682088.5/5645201.9	7.71
60/Vref	Outside Vat	HB34-1, HB34-2	682088.5/5645201.9	8.37
61/V	Inside Vat	HB35-1, HB35-2	682087.5/5654201.3	8.1
61/Vref	Outside Vat	HB36-1, HB36-2	682087.5/5654201.3	8.29
70/Vref	Outside Vat	HB38-1, HB38-2	682088.4/5645197.8	8.6
57/V	Inside Vat	HB39-1, HB39-2	682088.4/5645197.8	7.65
57/Vref	Outside Vat	HB40-1, HB40-2	682092.0/5645192.7	8.18

2.2. Isolation of DNA and PCR Processes

DNA was obtained by extraction using DNeasy® PowerSoil® Pro Kits (Qiagen, Hilden (GE)). The kit was used following the instructions of supplier. For PCR, a lab thermocycler Edvocycler (Edvotek, Washington, DC, USA) was used. Gel electrophoresis was applied in order to check the quality of amplification products. Therefore, 1% agarose gels are used. This check was made for each single PCR process. Both products' first thermocycling

procedure as well as the DNA of completed pooled libraries were purified using the ProNex® Size-Selective Purification System (Promega, Madison, WI, USA)) following the Promega protocol.

Adapator primers were obtained from Eurofins Genomics (Ebersberg, Germany) and included primers Amplicon PCR A519F-Ad (5′ TCGTCGG-CAGCGTCAGATGTGTATAAG AGACAGCAGCMGCCGCGGTAA 3′) and Bact_805R-Ad (5′-GTCTCGTGGGCTCGGAG ATGTGTATAAGAGACAGGACTACHVGGGTATCTAATC 3′). They were applied at a concentration of 100 pmol/µL. The ready prepared PCR solutions (25 µL in total per reaction) consisted of: 0.5 µL of DNA isolation eluate, 2 mM $MgCl_2$, 200 µM dNTP mix, 0.65 Units GoTaq® G2 Hot Start DNA Polymerase, nuclease-free water (all reagents from Promega, Madison, WI, USA) and 1 µM of each primer. In the PCR process, several steps were included as follows: initial melting for 5 min at 94 °C (denaturation step), 30 temperature cycles for DNA amplification consisting on 30 s denaturation at 94 °C, 30 s primer annealing at 50 °C and 30 s extension of primers at 72 °C. At the end, a final primer extension step at 72 °C (5 min) was added in order to optimize the PCR yield.

The primers for index PCR (forward and reverse process) were obtained from Eurofins Genomics (Ebersberg, Germany). For index PCR, a primer concentration of 1.25 pmol/µL was applied. The PCR mixture (applied total volume: 25 µL per reaction) included: 2.5 µL of Amplicon PCR product, 2.5 mM $MgCl_2$, 300 µM dNTP mix, 0.5 Units GoTaq® Mdx Hot Start DNA Polymerase and nuclease-free water (reagent supplier: Promega, Madison, WI, USA).

The PCR program was slightly modified in case of index PCR: 3 min for first at 95 °C followed by 30 temperature cycles consisting on denaturation at 95 °C (30 s), annealing of primers at 55 °C (30 s) and elongation (primer extension) at 72 °C (30 s). After temperature cycling, an additional final extension step was made (5 min at 72 °C).

2.3. Conversion and Analysis of Sequence Data

The sequencing supplied two FastQ files, one for forward and one for reverse alignment. At first, the sequence data for 16S rRNA were converted to contig files represented as FastA files. In addition, quality files were generated (mothur (version 1.39.5)). Therefore, the open-source platform Galaxy was applied (https://usegalaxy.org/last accessed on 8 June 2022). The obtained files (datasets) were characterized by a median quality score. These values were high for all investigated samples.

For sequence alignment and type identification, the NCBI cloud was used. For the alignment procedure, the SILVAngs tool box was used (data analysis service: https://ngs.arb-silva.de/silvangs, accessed on 8 June 2022). This service supplies a detailed analysis of the input sequence files [10–12], in our case of the single soil bacteria communities. In all cases, the pre-set parameter configurations of the SILVAngs database version 138.1 were applied [12].

In most cases, the obtained sequences and alignment results give the possibility to assign taxonomical groups down to the genus level. For some types, the state of the database only allows the obtained sequences to be assigned with higher taxonomical levels. These are, typically, families, orders, or in some cases classes or phyla. The most specific identified level is described as "Operational Taxonomic Unit" (OTU). Thus, each distinguished bacterial type is classified as an "OTU", named by genera, families or a higher taxon. The composition of each soil bacterial communities is characterized by the contained OTUs, preferably on the level of genus. For the most part of the analysed data, the NGS values for each OTU of the taken sample pairs (HB32, HB34, HB35, HB36, HB38, HB39 and HB40) are similar. Thus, the values of sample pairs are summarized displayed in the Figures.

A generalized comparison of the character of soil bacterial communities can be obtained by rank plots. In these diagrams, the frequency of the single OTUs N is shown

directly or by decade logarithms-related values r normalized to the total number of reads for each sample N_{sum}:

$$r = \log10(1 + 1000000 \times N/N_{sum}) \quad (1)$$

3. Results and Discussions

3.1. General Comparison of Soil Bacterial Communities at the Phylum Level

On the phylum level, the majority of samples are marked by similar qualitative composition, including significant amounts of *Proteobacteria*, *Planctomycetota*, *Patescibacteria*, *Firmicutes*, *Chloroflexi*, *Bacteroidota*, *Actinobacteria*, *Acidobacteria* and *Archaea* (Figure 1). *Proteobacteria* represent a very large and diverse group of bacteria. Their members are found in different environments, typically in soils. *Planctomycetota* are widely distributed, as well. They are found as well in aquatic and terrestrial environments, whereby many group members are known to be able to oxidize ammonia under anoxic conditions. *Patescibacteria* are probably mostly symbiotic microorganisms. *Myxococcota* are mostly aerobically living spore-forming bacteria. *Firmicutes* are a largely distributed phylum of gram-positive bacteria marked by low GC content in DNA that speaks to an evolutionary history related to low and moderate environment temperature. *Actinobacteria* are gram-positive, as well, but show a high GC content. *Desulfobacterota* are sulphate-reducing microorganism and could be regarded as an indicator for enhanced sulphur content in soils, for example due to former deposition of inorganic sulphate or decomposed animal wastes. The phylum *Chloroflexi* contains mostly thermophilic bacteria with photoautotroph or photoheterotroph metabolisms and the ability to switch to chemoorganotroph processes in the absence of light. *Acidobacteria* are typically adapted to lower soil pH. They were first isolated in acid mine drains, but have since been identified in many different soils.

In contrast to all other samples, the abundance of *Verrucomicrobiota*, *Patescibacteria* and *Archaea* is very low in sample HB31 (place 58/V). Complementary, HB31 has the highest abundance of *Proteobacteria*. In addition, HB31 is marked by a comparatively low abundance of Acidobacteria, but by a high abundance of Bacteroidia. A comparatively low abundance of *Firmicutes, Archaea* and *Planctomycetota* is observed in HB39. HB36 has a high abundance of *Patescibacteria*, but a low abundance of *Methylomirabilota* and *Verrucomicrobiota*.

Methylomirabilota (also called NC10) is a phylum of methanotrophic bacteria which are difficult to cultivate, but can be found enriched in urban parks [13]. Its low abundance in samples HB31 and HB36 could be interpreted as low available methane in the related sampling sites and, therefore, a higher availability of oxygen. Further, the very low abundance of *Archaea* in Sample HB31 seems to match conditions less suited for extremophiles.

The abundance of *Bacteroidota* might also be of interest for the local character of samples. This phylum is also widely distributed and abundant in soils. However, it is frequently related to gastrointestinal tracts of different organisms. Thus, it could be speculated that the abundance above 20% in sample HB31 might be related to a former high faeces content on the sampling site. Zixibacteria are a newly defined phylum recognised by genetic data. They were mainly observed in subsurface sediments, underground water and deep subsurface samples.

Besides the above-mentioned phyla, there are some other groups of bacteria distinguishing HB31 from the other samples. It is remarkable that some archaeal phyla, such as *Nanoarchaeota (Woesearchaeales)* and *Crenarchaeota (Nitrosophaeria)* as well as the bacteria phylum *Dadabacteria*, show significant abundances in the investigated samples with the exception of HB31.

The composition of bacterial communities in all single samples by phyla is displayed in Supplementary Materials Figure S1. This shows the similarity between the pairs of soil material taken at the same sampling site.

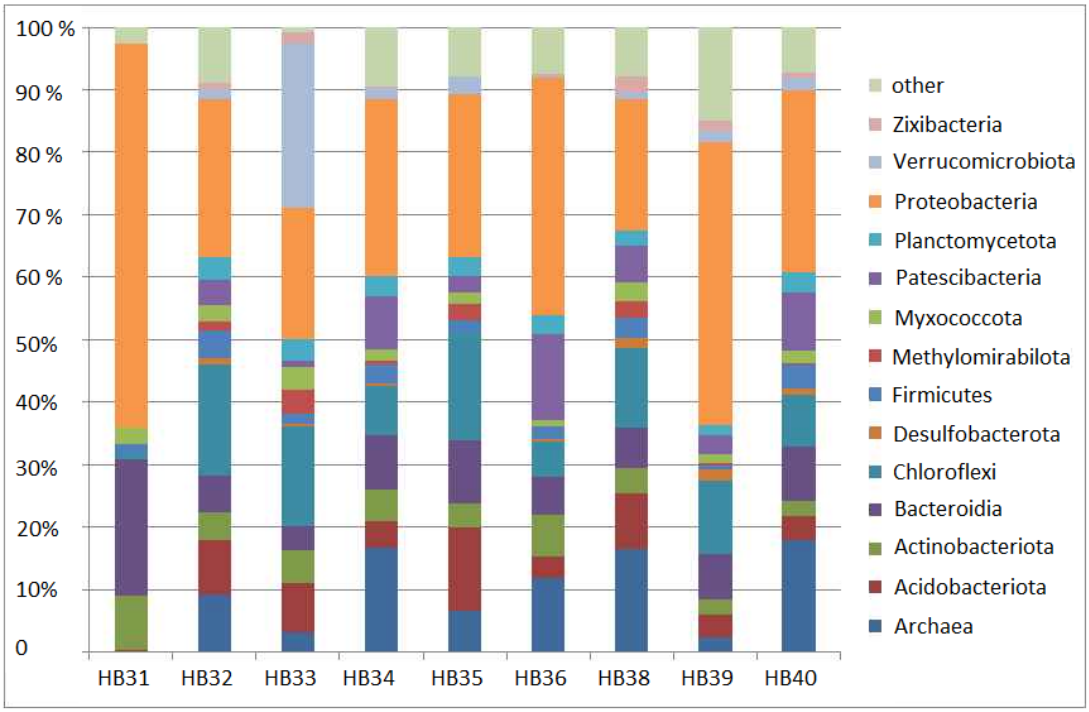

Figure 1. General composition of soil bacterial communities of sampling sites.

3.2. Comparison of Soil Sample Communities on the OTU Level

The majority of samples show similar rank functions. This concerns all outside-vat samples as well as the inside-vat samples HB35, HB33 and HB39. In contrast, the particular situation at site HB31 is reflected by the rank plots (Figure 2). The special shape of the rank function of sample HB31 is marked by an enhanced increase in the abundances over a small interval of ranks. Such a rank function could be interpreted by an echo of a formerly massive disturbance of environmental conditions or an important translocation of soil material [14]. Such an interpretation is based on the assumption that the rank function shows not only the recent coexistence of fast and slower growing components of soil microbial community but is significantly determined by a decrease of abundances of many types over time. This point of view agrees with the idea that a recent situation is determined by few active—and therefore highly abundant—populations, whereas the overwhelming majority of types have fallen into a more or less dormant state. Under this assumption, the low abundances in the rank function (left hand side in Figure 2) would be determined mostly by "older" dormant OTUs, while the right-hand side indicates younger dormant and active bacteria. Thus, it seems to be reasonable to assume that the rank order could reflect the history of bacterial communities of a sampling site to a certain extent, and less abundant OTUs might reflect ancient relations between different soils [9].

A principal component analysis (PCA) was applied in order to get a general picture of the similarities and differences in the soil bacteria communities from the single sampling sites. In general, PCA is used for transform a multi-dimensional data set—here the distribution of many bacterial OTUs in a sample set—into a data space organized by a hierarchical order of dimensions ("principal components") reflecting the most important dimension as "first principal component", the second most important as "second principal component" and so on. Here, we decided to regard not only the absolute abundance values for all OTUs in one analysis, but tried to distinguish the bacterial communities of sampling sites in dependence on the overall abundance of OTUs. From the picture of rank functions (Figure 2), it was concluded that the analysis of logarithmic values is more adequate for comparing the character of community composition because lower abundant OTUs are than better represented. The obtained result confirmed the power of the abundance class-

related approach, showing the best distinguishability of samples not in the overall analysis but at low to mediate total abundances (10–100 reads; see Figure 3 and text below).

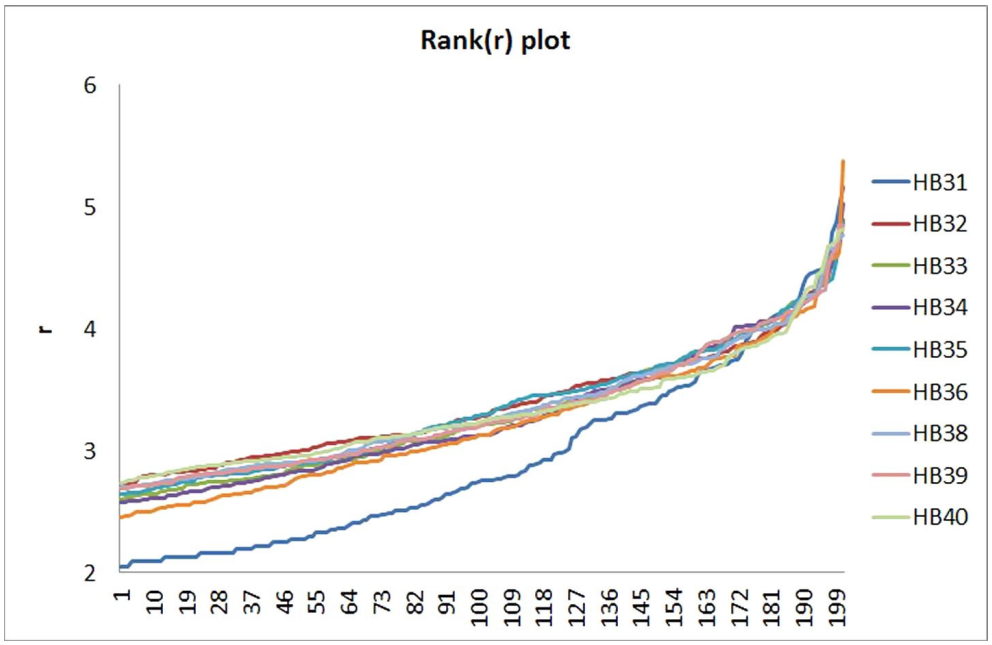

Figure 2. Rank diagrams for the most abundant OTUs for all investigated sampling sites based on r values (related to the decadic logarithms of abundances following Equation (1)). The diagram shows the r values for the 200 most abundant OTUs of each sampling site in the order of their abundance.

The principal component analysis (PCA) on the OTU level confirms the special character of the sampling sites HB31 and HB33, but shows that the bacterial communities of the vat-internal samples HB35 and HB39 are also significantly distinguished from the vat-external samples. Separate PCAs for different abundance classes (total of all samples) have been executed in order to relate the comparison between sampling sites to the corresponding rank orders. It is remarkable that the vat-external communities are found close together for the first and the second principal component (PC) for all abundance classes (Figure 3a–f). For higher abundance (1000–100,000 reads in total), the vat-internal sampling sites HB35 and HB39 are found in the same region as the vat-external samples in the PC1/PC2-plots (Figure 3b,c). In contrast, HB39 differs significantly in the PCA if only OTUs with low abundances (1–1000 reads) are considered. In the plots for abundances between 10 and 1000 (Figure 3d–f), HB35 tends to be separated from the vat-external samples, as well (Figure 3d,e). With the background of the interpretational concept of abundance functions in a temporal way (see above), it could be assumed that for high read numbers (recently or shortly before active types) a certain homogenization of bacterial community character took place for the vat-internal soils as well as for the soils inside vats of the sampling sites HB35 and HB39. HB31 and HB33 had not been included in this homogenization (Figure 3b). For lower read numbers (Figure 3e), all vat-internal samples are distinguished from the point of cloud of vat-external samples in the PC1/PC2 diagram.

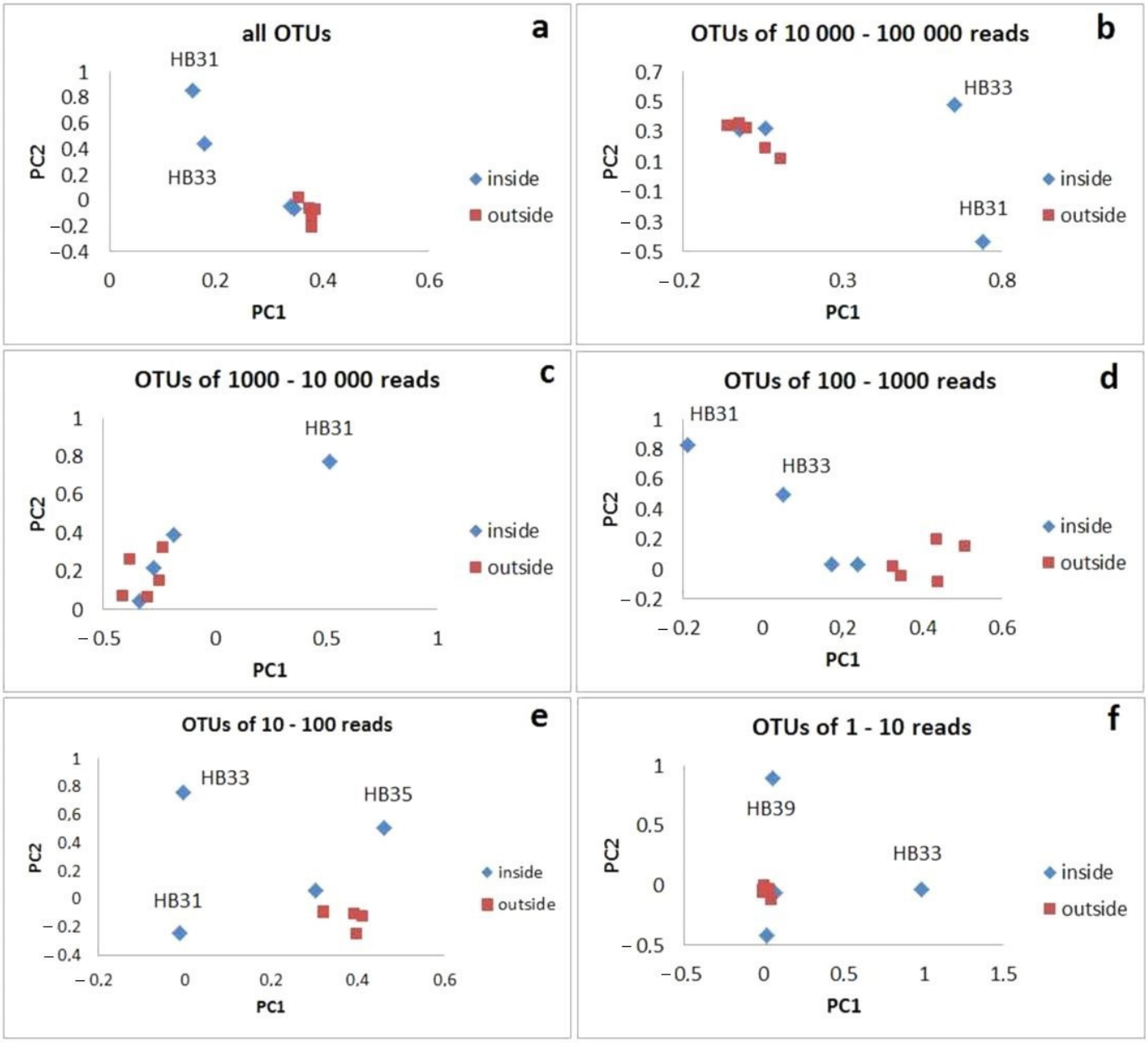

Figure 3. Abundance classes-related principal component analyses of OTUs shown by correlation diagrams of first (PC1) and second principal component (PC2): (**a**) reconsidering all OTUs, (**b**) OTUs with more than 10,000 reads in total, (**c**) OTUs with 100–10,000 reads in total, (**d**) OTUs with 100–1000 reads in total, (**e**) OTUs with 10–100 reads in total, (**f**) OTUs with 10 or less reads in total.

The historical interpretation of rank functions suggests that the pattern of this PCA correlation plot is due to the different ancient ecological situations on the different sampling sites. It is astonishing that the differences between inside- and outside-vat situations are also still reflected for the PCA reconsidering exclusively OTUs with very low read numbers (10 or less in total over all samples). This observation speaks for the possibility to take even OTU groups with very low read numbers of the NGS analyses into comparisons between different soil samples [6]. Taking this into account, the low-abundant types might be particularly suited for searching for ancient relations between the ecological situations of different sampling points and for learning something about similarities of human impacts on ancient living and working places.

3.3. Comparison by Dominating OTUs

Specific differences between samples can be seen in many cases from comparisons on the genera (or OTI-) level. Two examples of groups of genera which seem to be typical for one sample, but not for others, are displayed in Figure 4. HB31 is clearly distinguished from other samples by their dominating OTUs. There are six types, which are highly present in this sample, but mostly missing in most other samples (Figure 4a). *Methylophaga* is the most abundant genus. It is known to include halophilic methylotrophic species. The genus is highly abundant in upper layers of oceans. C_1-compounds, such as methanol, methylamine and halomethanes, can be metabolized [15]. *Caenimonas* was first isolated from activated sludge. It is a strict aerobic organism [16]. *Limnohabitans* was first found in a freshwater lake. It is also an aerobic active chemoorganotrophic organism, but is described as a facultative anaerobic [17]. An *Algoriphagus* strain was first isolated from Antarctic sea ice. It is strictly aerobic and cold-adapted [18]. *Streptomyces* is a typical soil-associated genus and includes a large number of species. These species are aerobic and many of them are famous for production of different secondary metabolites as antibiotics. *Ramlibacter* strains have been found in subdesert soil in Tunesia. They have been identified to be strictly aerobic and chemoorganotrophic [19]. In summary, it can be concluded that these six most abundant OTUs of HB31 indicate a dominating bacterial community associated with availability of oxygen and chemoorganotrophic metabolisms.

The most abundant OTUs of HB35 are TRA3-20 (*Burkholderiales*), uncultivated *Anaerolineaceae* and *Limnobacter*. These and further highly abundant OTUs in HB35-1 and HB35-2 were also frequently found in the outside-vat samples. This observation indicates that the bacterial soil community of the vat/sampling site 61/V (HB35-1 and 2) was much more similar to the outside-vat situation than the other three inside-vat sampling sites.

The bacterial community in samples of vat 57/V (HB39) is dominated by *Sulfurifustis* and *Sideroxydans* (Figure 4b). *Sulfurifustis* was also frequently found in most other samples, whereas *Sideroxydans* was much less abundant there. *Sulfurifustis* is a chemolithoautotrophic organism, able to oxidize sulphur, sulphides, thiosulphate and other sulphur compounds to sulphate. It was first isolated from lake sediment in Japan [20]. *Sideroxydans* is a Fe(II)-oxidizing organism, first isolated from marine iron-oxidizing microbial mats [21]. Both highly abundant OTUs indicate the availability of oxygen in the environment, but at the same time the used substrates (Fe(II), reduced chemical sulphur species) speak for a transition region with high content of organic material.

With about 2% each, *Thiobacillus*, *Bauldia* and *Gallionella* are three other OTUs which are comparatively abundant in HB39. In addition, SAR324 clade Marine Group B is present in about same concentration. *Thiobacillus* is known as a genus to be able to oxidize sulphur, sulphides, thiosulphate and other sulphur compounds to sulphate and is frequently found in transition milieus between aerobic and anoxic conditions. *Bauldia* is a heterotrophic aerobic growing genus [22]. *Gallionella* is a chemolithotrophic organism and famous for oxidizing Fe(II) to Fe(III). It is an indicator for reduced oxygen partial pressure or for a transition milieu to anoxic conditions in the soil water. Obviously, the highly abundant OTUs of HB39 represent a chemolithotrophic bacterial community typical for an environment with reduced oxygen content and high availability of reduced sulphur compounds and Fe(II).

The identification of groups of bacteria which are present in a part of samples but less abundant or absent in other samples could support the interpretation of data and the search of former ecological specificities. Such a group of types was filtered out for comparing inside-vat and outside-vat samples, for example (Figure 5). Besides TRA3-20 (*Burkholderiales*), uncultivated *Anaerolineaceae* and *Limnobacter,* ammonia-oxidizing OTUs are highly abundant in the outside-vat samples, among them *Nitrosoarchaeum*, *Nitrosopumilaceae* and *Nitrosophaeraceae*. They belong to the phylum *Crenarcheota* which includes a lot of extremophilic archaea [23]. It is interesting that these organisms are lower or not present by reads from samples HB31 and HB39 (both inside-vat sampling sites). The high abundance of ammonia-oxidizing bacteria in the outside-vat samples could be due to a high input

of urine and faeces in historical times over the whole area between the vats. It must be kept in mind that urine was traditionally used in tanning, because its decomposition under formation of ammonia was useful for treating the skin of animals in the leather production. The high abundance of DNA indicating ammonia-oxidizing bacteria could be interpreted as an echo of the former application of human or animal urine in the well-known historical tannery activity of the investigated place.

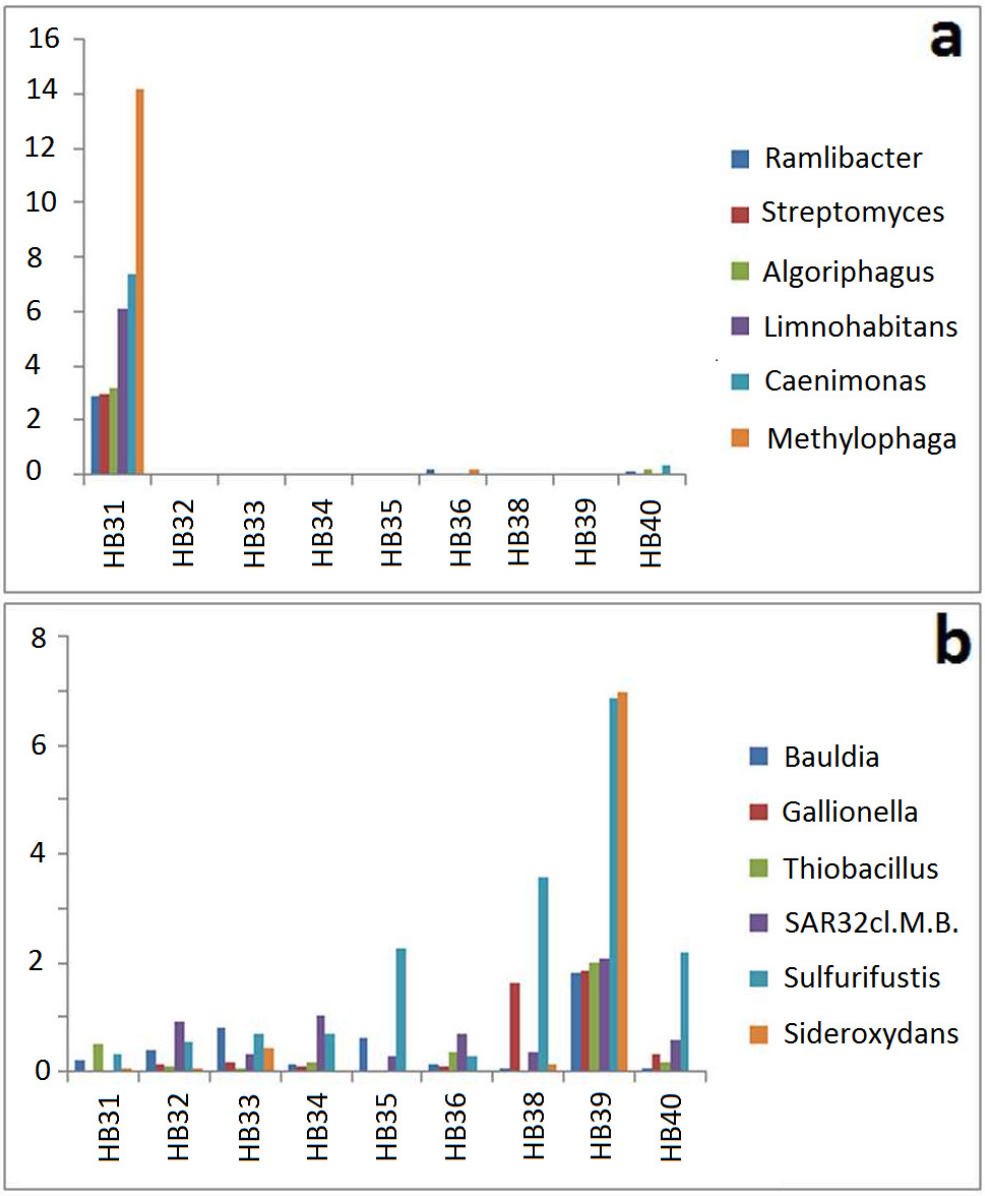

Figure 4. Groups of dominating OTUs shown by percentage of reads per sampling site: (**a**) Dominant OTUs in 58/V (HB31), (**b**) dominant OTUs in 57/V (HB39).

Possibly, the presence of sulphur species-oxidizing bacteria such as *Sulfurifustis* can be interpreted in an analogous way. It can be assumed that the processing of cadavers, animal skin and horn led to a permanent input and accumulation of keratin and its decomposition products in the local soil. This input of the sulphur-rich protein keratin was probably connected with the formation of a large spectrum of sulphur-containing chemical compounds which could have served as substrate in sulphate-forming bacterial metabolism. Up to now, the alkaline hydrolysis of keratin has been used in the technical production of the sulphur-containing amino acid cysteine.

The composition of single samples by lower taxonomical levels (OTUs of bacteria in Supplementary Materials Figure S2 in the Supplementary part, *Archaea* in Supplementary Materials Figure S3) confirms the similarity inside pairs of samples, mostly, but the differences inside sample pairs HB32 and HB35 appear for bacteria larger than in the comparison at the phylum level and for *Archaea*.

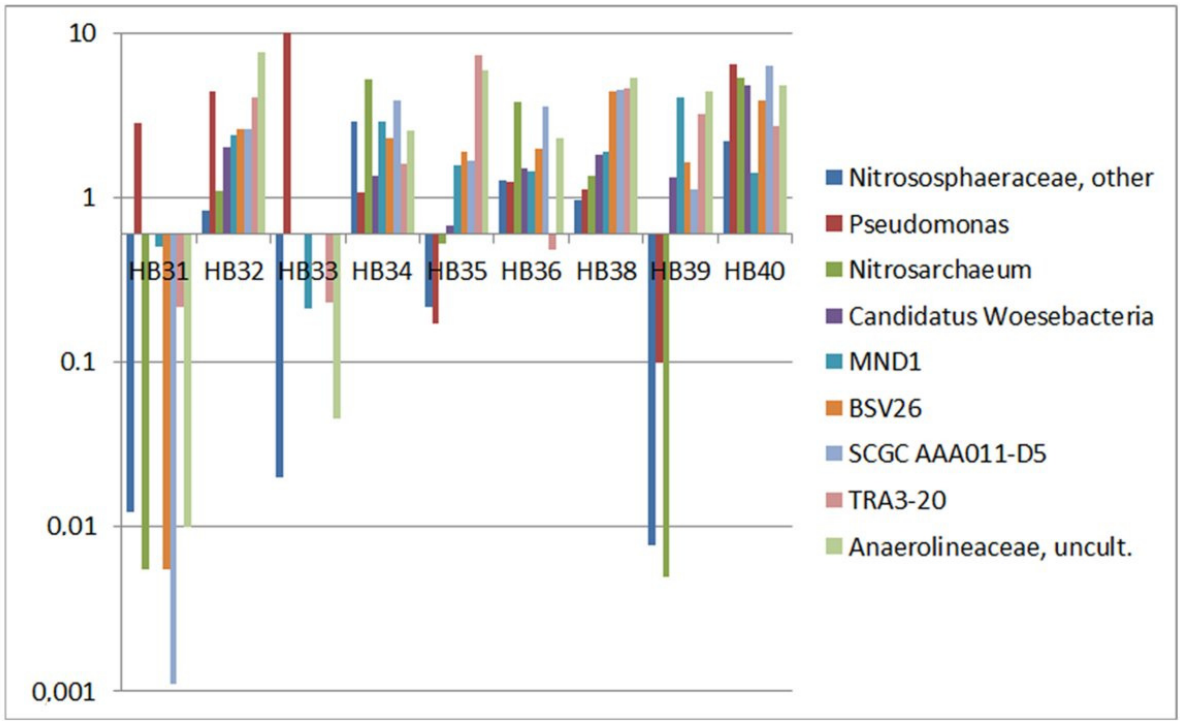

Figure 5. Highly abundant OTUs (percentages of reads) from outside-vat sampling sites illustrating their similarity as well as the moderate strangeness of the inside-vat samples HB39 (57/V) and HB35 (61/V) and the strong strangeness of HB31 (58/V) and HB33 (60/V).

3.4. Specificity of Sampling Sites

The inclusion of less abundant OTUs into the community picture allows a more specific picture of the single sampling sites to be obtained. There are several OTUs which possess certain specificity because they are found in only one or a few samples. Some examples are given in Figure 6a.

Thus, HB31 is specifically marked by *Thioalkalimicrobium* and *Thiomicrospira*. *Thioalkalimicrobium* strains have been first isolated from a highly alkaline soda lakes in Siberia. These bacteria are chemolithoautotrophic and oxidize sulphur or sulphur compounds to sulphate [24]. *Thiomicrospira* are sulphur-oxidizing microorganisms, as well. They have been isolated from marine mud flats [25]. The specific abundance of both these types in vat 58/V (HB31) speaks to the fact that there was a reducing milieu, but with a high content on sulphur-containing chemical components. Obviously, this is in contrast to the environment where ammonia seems to play a more important role than sulphur compounds. A possible conclusion could be that the purpose of this vat was related to stronger sulphur- or sulphur compound-containing materials, for example horn, skin and leather or related raw material, tannery side products or waste.

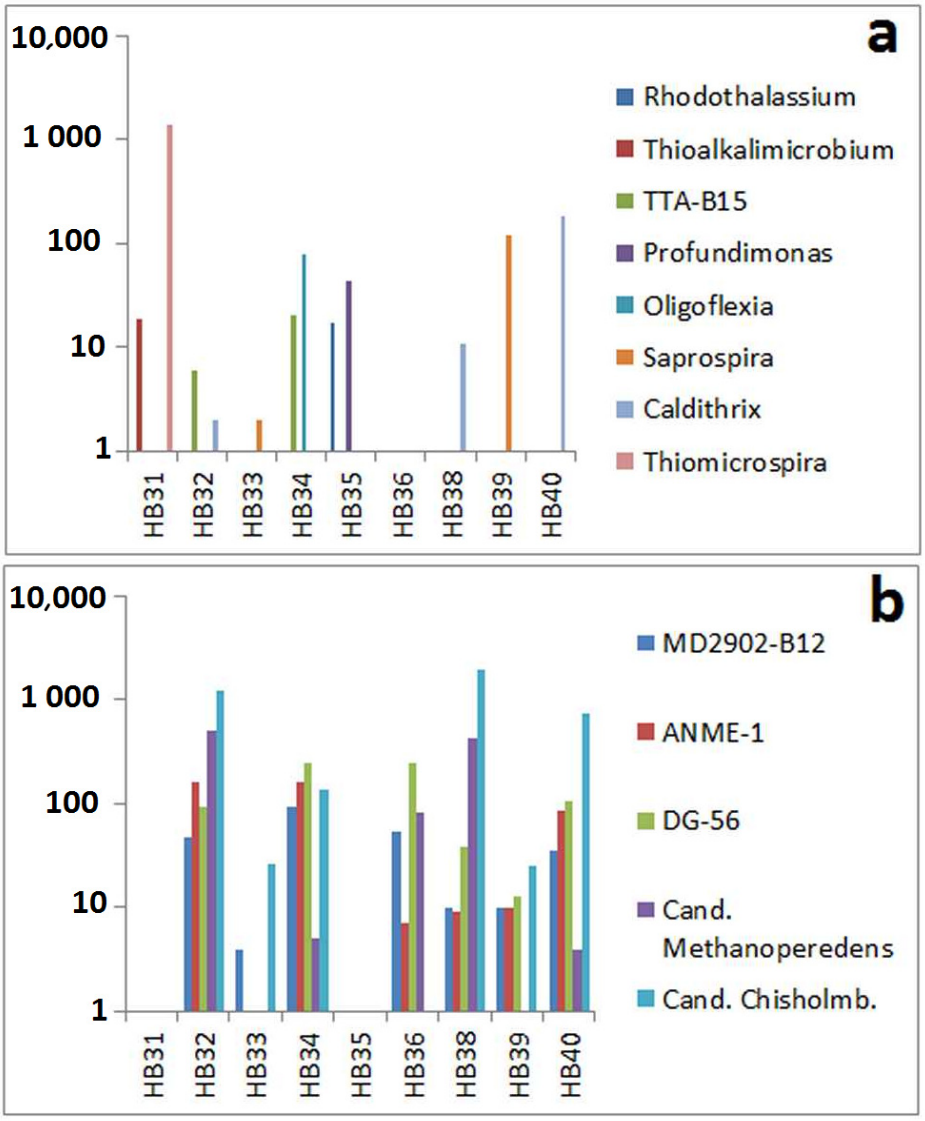

Figure 6. Stepwise enhanced similarity reflected by groups of OTUs (read numbers): (**a**) Examples of OTUs present in one or a few samples only, (**b**) group of OTUs present in most outside-vat samples, but mostly absent in HB31, HB33 and HB35.

Other samples are marked by special types as *Oligoflexia* (HB34), *Profundimonas* (HB35) and *Saprospira* (HB39). The outside-vat sampling sites HB38 and HB40 show a common special triple of OTUs: *Caldithrix* and the groups Milano-WF1B-03 and GWA2-50-13 (Figure 6a). *Caldithrix* was described first as a nitrate-reducing, thermophilic, anaerobic bacterium which had been isolated from an oceanic hydrothermal vent [26]. It could indicate particularly high nitrate availability in the related part of the excavation area in the past.

A group of five OTUs connects all outside-vat sampling sites (HB32, HB34, HB36, HB38 and HB40). These types are obviously typical for the environment of vats, but less abundant than the above-mentioned more abundant outside-vat-typical OTUs (Figure 6b). It is remarkable that these five OTUs have no reads in all samples taken inside the vats 61/V (HB35) and 58/V (HB31) and only one is present in 60/V (HB33). Two of these groups (ANME-1b and *Candidatus Methanoperedens*) belong to the archaeal phylum *Halobacterota*. This branch of Archaea is famous for its extreme halophilic character. This might give a hint that the general environment of vats was affected by application of larger concentrations of salt, whereas the three mentioned vats had not been confronted with this high salt content and, therefore, ANME-1b and *Candidatus Methanoperedens* had not been verifiable in the

obtained NGS data of these sites. Besides HB35 and among the above-mentioned three inside-vat samples, 57/V (HB39) has the highest similarity to the outside-vat samples.

Finally, HB31 is the strangest in comparison with all other samples. Thus, a rank order of strangeness of inside-vat samples from the environment can be formulated as follows: 58/V (HB31) > 60/V (HB33) > 57/V (HB39) ≈ 61/V (HB35). This corresponds well to the results of PCA (Figure 3).

Besides the above discussed strangeness and similarity between samples, there are identified groups of OTUs which are mainly or exclusively found on one sampling site. Figure 7 gives examples for OTU groups reflected by reads from one site only. Figure 8 shows groups of OTUs which are found preferable on one site only, characterized by the percentage of reads for each OTU in relation to the total number of reads for this OTU summarized over all samples. It has to be considered that the read numbers of exclusively-found OTUs are partially low and might by meaningless if single OTUs are regarded. However, the sight on the composition of exclusively-found groups can support the evaluation of the character of the related samples.

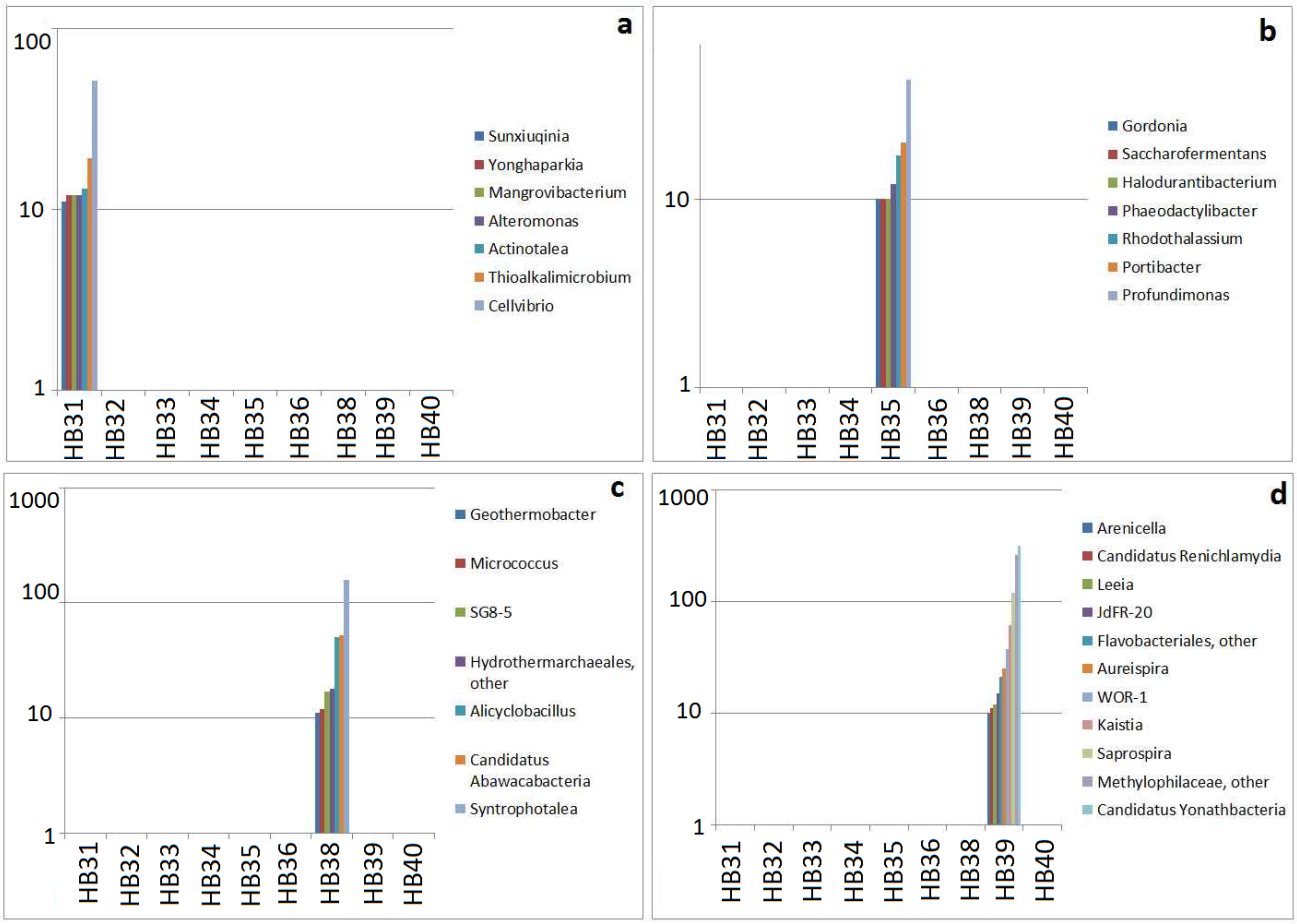

Figure 7. OTUs found exclusively on one sampling site (number of reads): (**a**) 58/V (HB31), (**b**) 61/V (HB35), (**c**) 70/Vref (HB38), (**d**) 57/V (HB39).

Thus, for HB31, *Sunxiuqinia*, *Yonghaparkia*, *Mangrovibacterium*, *Alteromonas* and *Actinotalea* were found there in addition to *Thioalkalimicrobium* and *Cellvibrio* (Figure 7a). These exclusively-found OTUs are mostly described in marine environments, which hints to a tolerance in the microbial community to enhanced salt content of the soil. In addition, a certain alkalinity could be supposed as suggested by the reads for *Thioalkalimicrobium* and *Yonghaparkia*. *Sunxiuqinia* was isolated from the sediment of a sea cucumber farm [27]. *Yonghaparkia* was first found in an alkaline soil in Korea [28]. *Mangrovibacterium* has been

described as a nitrogen-fixing bacterium from mangrove sediment [29]. *Alteromonas* was isolated from a marine environment, as well [30]. *Actinotalea* was found in a tidal flat sample [31].

The composition of the group of preferably in HB31-found OTUs (Figure 8a) supports the impression of a salt-tolerant bacterial community. Thus, *Labedella* was isolated from beach sand of Korean coast [32], and *Zexanthinibacter* was found in a marine environment [33].

The genus *Treponema* belongs to the *Spirochaetes* and includes several human- and animal-pathogenic species. With 1272 reads, it is astonishingly highly abundant in HB31. *Mucilaginibacter* is known to be able to metabolize different polysaccharides [34]. *Meniscus* was first found in the digester of a municipal waste treatment plant [35]. *Leptothrix* belongs to iron bacteria and forms characteristic micro capillaries formed by Fe(III) hydroxide precipitates. Besides the concluded salt tolerance, the preferentially abundant bacteria of HB31 hint at a high level of organic components, possibly partially related to animals.

Despite similarities in the bacterial composition with the outside-vat samples, the samples from vat 61/V (HB35) include some interesting reads for exclusively-found OTUs (Figure 7b). *Saccharofermentans* is an anaerobic bacterium which was found in the waste water sludge of a brewery [36]. Bacteria of the genus *Halodurantibacterium* were isolated from production in a Chinese oilfield. They were found to be highly salt tolerant [37].

Some of the exclusively-found OTUs of HB39 (Figure 7d) speak for mostly aerobic living conditions and a certain salt stress. For example, *Arenicella* was isolated from a marine sandy sediment [38]. *Leeia* is a strictly aerobic bacterium and was isolated from a rice field [39]. Aureispira was found in a marine environment [40]. The preferentially-found OTUs in HB39 support and extend this picture. For example, *Desulfatitalea* was found in tidal flat sediment [41].

The exclusively- or strongly preferentially-found OTUs suggest also a certain specificity of the outside-vat sampling sites. Despite the general similarity of these samples, in particular concerning the most dominant OTUs, groups of OTUs were also found at such sampling sites which had not been proved or were less abundant at all other sites. Among others, the sampling site 58/V (HB32) showed an exclusive or preferential existence of several OTUs in comparison with all other samples. This includes halophilic and thermophilic OTUs as well as OTUs with special metabolic features, among them genera such as *Sulfuriflexus*, *Dehalogenimonas*, *Ketobacter*, *Salinispira*, *Thiohalophilus*, *Metallibacterium*, *Methanocella* and *Alterococcus*.

Sulfuriflexus is a genus of Sulphur oxidizing bacteria which was isolated from brackish lake sediment [42]. *Dehalogenimonas* is a genus including bacterial members able to dehalogenate halogenoalkane compounds by a reductive mechanism. Strains are described as strictly anaerobic and mesophilic [43]. *Ketobacter* was isolated from surface seawater. It is salt tolerant, mesophilic and remarkable for its ability to degrade hydrocarbons [44]. *Salinispira* was isolated from a hypersaline environment [45]. *Thiohalophilus* was isolated from a hypersaline environment, as well. It is a halophilic microorganism growing under microoxic or anaerobic conditions and is able to oxidize reduced sulphur species by reduction of nitrate [46]. The acidophilic *Metallibacterium* was first isolated from an acid biofilm of a pyrite mine [47]. *Methanocella* was isolated from rice paddy soil and is described as a hydrogenotrophic methane producing bacterium [48]. Five strains of *Alterococcus* had been isolated from two hot springs. The bacteria are facultative anaerobic, halophilic, thermophilic and can decompose agar [49]. This sample-specific group of bacterial community seems to reflect a more microoxic milieu of enhanced salt content and content of hydrocarbons and other lipid-similar organic compounds.

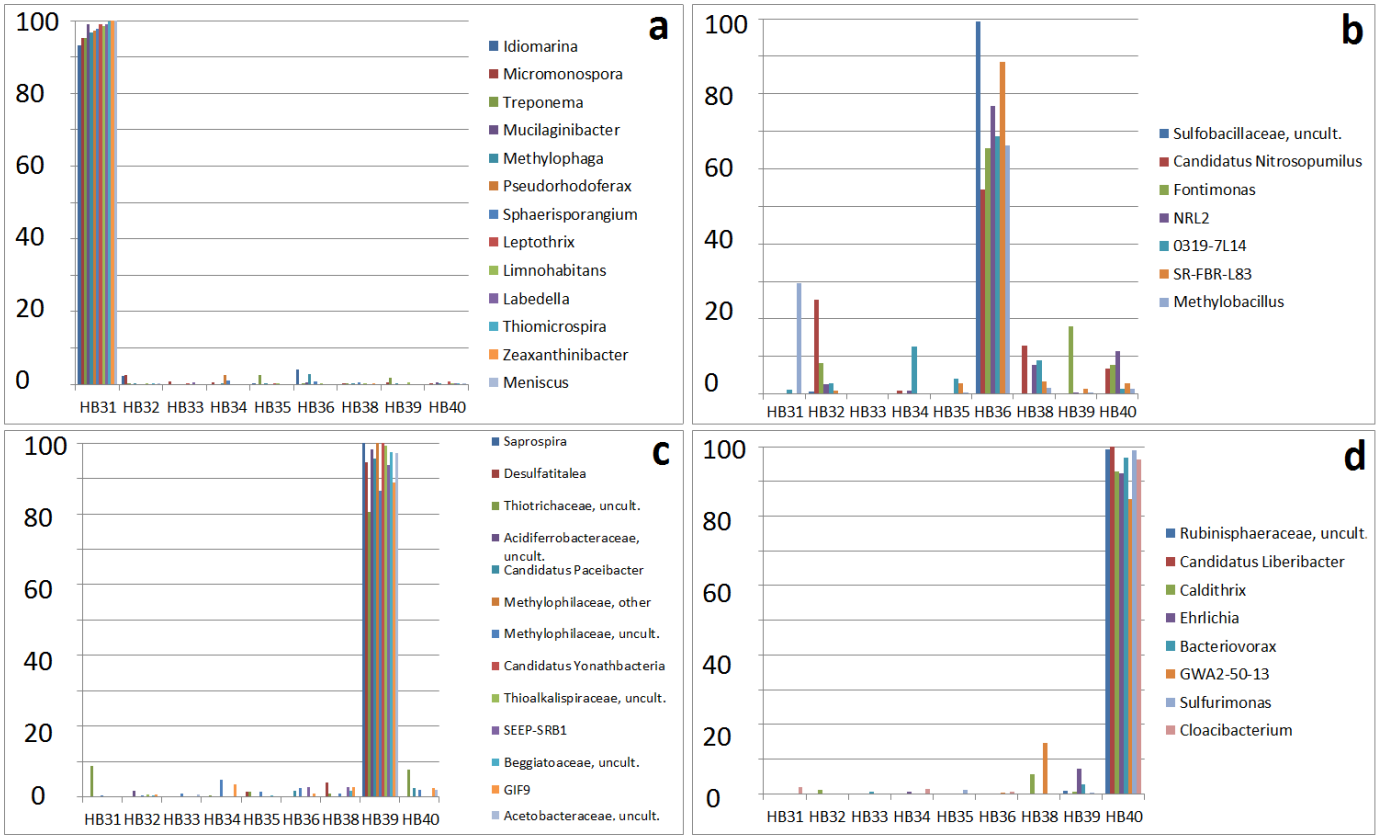

Figure 8. Preferentially-found OTUs on one sampling site (percentage of the total over all samples): (**a**) 58/V (HB31), (**b**) HB36, (**c**) 57/V (HB39), (**d**) 57/Vref (HB40).

In sample HB 34, a certain number of specific OTUs could also be identified, among them *Effusibacillus*, *Pseudoclostridium* and *Rugosimonospora*. In general, the bacterial communities of sample HB34 seem to be less specific than the samples from other sites. Effusibacillus was found in a Japanese fresh water lake. It is thermophilic and facultatively anaerobic [50]. *Rugosimonospora* was described by two acidophilic strains of this genus [51].

Fontimonas, *Methylobacillus* and 11 other OTUs are preferentially found in HB36 (Figure 8b). *Fontimonas* is an aerobic thermophilic bacterium isolated from a freshwater hot spring [52]. *Methylobacillus* is a strong methylotrophic genus [53].

Geothermobacter, Micrococcus and *Alicyclobacillus* showed NGS reads exclusively or mostly in samples from 70/V (HB38; Figure 7c). *Geothermobacter* was found in a pacific hydrothermal vent with a high accumulation of polysaccharides on the sediment surface [54]. It grows on a mixture of Fe(III) and malate. *Alicyclobacillus* is a thermophilic aerobically-growing bacterium [55]. Micrococcus is mesophilic and aerobic. The three types seem to reflect a phase of warm aerobic growth conditions.

Lapillicoccus, Caryophanon, Filimonas, Anaerosalibacter, Tardiphaga, Klebsiella, Lacibacter, Caldithrix, Ehrlichia, Bacteriovorax, Sulfurimonas, Cloacibacterium, Schlesneria and Nitrosomonas were exclusively or preferentially found in sample HB40 (Figure 8d). Anaerosalibacter is a strict anaerobic halotolerant and thermotolerant organism [56]. Tardiphaga robiniae was described as a Robinium root-associated bacterium [57]. Lacibacter was isolated from the sediment of a eutrophic lake, but is described as growing aerobically [58]. Caldithrix was isolated from an Atlantic hydrothermal vent. It is a thermophilic anaerobically growing organism [26]. Sulfurimonas is a mesophilic bacterium able to oxidizing sulphur, thiosulphate, sulphide and other sulphur compounds. It was isolated from deep ocean sediments [59]. Cloacibacterium is a facultative anaerobic bacterium which was first isolated from raw sewage [60]. Schlesneria was described as a moderate acidophilic mesophilic organism [61]. Nitrosomonas is a genus of ammonia-oxidizing bacteria [62] and

its presence demonstrates obviously ammonia oxidation activity in the related soil sample. This confirms roughly the situation reflected by the dominating OTUs. The observed differences in bacterial communities from the different sampling sites and, in particular, the differences between inside-vat and outside-vat samples, confirm the hypothesis that former human activities affect strongly the local bacterial composition in soils [4,6]. It supports the concept that the local bacteria communities represent a kind of local ecological memory which is related to the former use of place [5,63].

The outside-vat samples HB34, HB36 and HB40 show a considerable abundance of *Archaea* above 10% of OTUs, in total [63]. In general, the *Archaea* abundance of vat-internal samples is lower than that of the outside samples. It is known that recent tannery activities cause a significant shift in composition of soil microbial communities [64–66]. For the vat-internal samples, it can be concluded in general that a higher specificity of communities is reflected by the less abundant OTUs than by the dominant OTUs, whereas the outside-vat samples are less marked by such a general difference between highly abundant and sample site-specific less abundant types. These difference could support the hypothesis, that the dominant types more strongly represent the recent state or younger history of the local soil, whereas the specific, less abundant types more strongly represent the dominant OTUs of the original character and the former use of the related vats. This corresponds to the well-known fact that soil bacterial communities are marked by a small group of highly abundant—mostly active—bacteria and a large group of low abundant—mostly less active or dormant—types. This large group of bacteria is mainly responsible for the microbial diversity inside soils and is very important for the possibility of a soil microbiome to react to changing environmental conditions [8]. On the other hand, the composition of dormant bacteria reflect the ecological past of a soil more than the recent state, containing information about former environmental conditions which can be used for analysing special situations of a place and to distinguish local differences in former conditions in the soil.

4. Conclusions

The investigations on soil samples from different sampling sites of the historical suburban tannery area of Jena show, on the one hand, that the compositions of DNA of soil bacterial communities match well the idea that the former use of the area—dating back for several centuries—is reflected by the 16S r-RNA data from NGS sequencing. The echo of former use seems to be confirmed, in particular, by high fractions of ammonia metabolizing organisms and bacteria adapted to enhanced soil salinity and—partially—alkaline soil character. In addition, the bacterial composition is marked by types able to oxidize sulphur-containing chemical species, which might be caused by the former deposition of keratin-rich material from animal skins and other remains.

On the other hand, it can be seen that the different sampling sites show differences in their interrelations depending on the abundance of regarded groups of soil bacteria. It is assumed that the majority of related special bacterial strains were already deposited in the local soils during the use of the regarded area for tannery and related activities. Obviously, a part of the less abundant types in particular reflect the specific character of the single sampling sites in former times. This impression supports the idea that former chemical impact on soil by human activities left traces in the signature of soil bacteria composition and developed abundance peaks of special groups of bacteria and their DNA that are decaying gradually, but can carry some information about the history of the soil and ancient human impacts after centuries. This effect is regarded as the "ecological memory of soil". Here, such specificity can be concluded for three vat-internal soils, at least. Their specific soil character enlarges the spectrum of soil bacteria of the investigated area, confirming that ancient local impact and archaeological structures tend to enhance the Beta-diversity of a place.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ecologies4020021/s1. Figure S1. Taxonomical composition of all samples by phyla. Figure S2. Taxonomical composition of all samples by OTUs. Figure S3. Taxonomical composition of Archaea in all samples.

Author Contributions: Conceptualization: J.M.K. and J.C.; formal analysis: P.M.G.; investigation: L.E., F.M., J.M.K. and T.S.; writing—original draft preparation: J.M.K.; writing—review and editing: J.C., L.E. and P.M.G.; supervision: J.M.K. All authors have read and agreed to the published version of the manuscript.

Funding: A scholarship from the state of Thuringia for Linda Ehrhardt is gratefully acknowledged.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We thank Carl-Eric Wegner (Jena) for critical discussion and very valuable hints. The lab support by Steffen Schneider is gratefully acknowledged. L.E. thanks the state of Thuringia for the scholarship enabling this research.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hedrich, S.; Schippers, A. Metallgewinnung mittels Geobiotechnologie. *Chem. Ing. Tech.* **2017**, *89*, 29–39. [CrossRef]
2. Thavamani, P.; Samkumar, R.A.; Sathees, V.; Subashchandrabose, S.R.; Ramadass, K.; Naidu, R.; Venkateswarlu, K.; Megharaj, M. Microbes from mined sites: Harnessing their potential for reclamation of derelicted mine sites. *Environ. Pollut.* **2017**, *230*, 495–505. [CrossRef] [PubMed]
3. Barns, S.M.; Cain, E.C.; Sommerville, L.; Kuske, C.R. Acidobacteria phylum sequences in uranium-contaminated subsurface sediments greatly expand the known diversity within the phylum. *Appl. Environ. Microbiol.* **2007**, *73*, 3113–3116. [CrossRef] [PubMed]
4. Margesin, R.; Siles, J.A.; Cajthaml, T.; Ohlinger, B.; Kistler, E. Microbiology meets archaeology: Soil microbial communities reveal different human activities at archaic Monte Iato (Sixth century BC). *Microbial Ecol.* **2017**, *73*, 925–938. [CrossRef] [PubMed]
5. Köhler, J.M.; Kalensee, F.; Günther, P.M.; Schüler, T.; Cao, J. The local ecological memory of soil: Majority and minority components of bacterial communities in prehistoric urns from Schöps (Germany). *Int. J. Environ. Res.* **2018**, *12*, 575–684. [CrossRef]
6. Köhler, J.M.; Beetz, N.; Günther, P.M.; Möller, F.; Schüler, T.; Cao, J. Microbial community types and signature-like soil bacterial patterns from fortified prehistoric hills of Thuringia (Germany). *Community Ecol.* **2020**, *21*, 107–120. [CrossRef]
7. Chernysheva, E.; Korobov, D.; Borisov, A. Thermophilic microorganisms in arable land around medieval archaeological sites in Northern Caucasus, Russia: Novel evidence of past manuring practices. *Geoarchaeology Int. J.* **2017**, *32*, 494–501. [CrossRef]
8. Jones, S.E.; Lennon, J.T. Dormancy contributes to the maintenance of microbial diversity. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 5881–5886. [CrossRef]
9. Köhler, J.M.; Kalensee, F.; Cao, J.; Günther, P.M. Hadesarchaea and other extremophile bacteria from ancient mining areas of the East Harz region (Germany) suggest an ecological long-term memory of soil. *SN Appl. Sci.* **2019**, *1*, 839. [CrossRef]
10. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glockner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **2013**, *41*, D590–D596. [CrossRef]
11. Klindworth, A.; Pruesse, E.; Schwee, T.; Peplies, J.; Quast, C.; Horn, M.; Glockner, F.O. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequenc-ing-based diversity studies. *Nucleic Acids Res.* **2013**, *41*, e1. [CrossRef]
12. Yilmaz, P.; Parfrey, L.-W.; Yarza, P.; Gerken, J.; Pruesse, E.; Quast, C.; Schweer, T.; Peplies, J.; Ludwig, W.; Glockner, F.O. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acid Res.* **2014**, *42*, D643–D648. [CrossRef]
13. Zhang, W.W.; Han, J.G.; Wu, H.B.; Zhong, Q.C.; Liu, W.; He, S.W.; Zhang, L. Diversity patterns and drivers of soil microbial communities in urban and suburban park soils of Shanghai, China. *PeerJ* **2021**, *9*, 11231. [CrossRef]
14. Ehrhardt, L.; Köhler, J.M. Three soil bacterial communities from an archaeological excavation site of an ancient coal mine near Bennstedt (Germany) characterized by 16S r-RNA sequencing. *Environments* **2022**, *9*, 115. [CrossRef]
15. Janvier, M.; Frehel, C.; Grimont, F.; Gasser, F. *Methylophaga marina* gen. nov., sp. nov. and *Methylophaga thalassica* sp. nov., Marine Methylotrophs. *Int. J. Syst. Bacteriol.* **1985**, *35*, 131–139. [CrossRef]
16. Ryu, S.H.; Lee, D.S.; Park, M.; Wang, Q.; Jang, H.H.; Park, W.; Jeon, C.O. *Caenimonas koreensis* gen. nov., sp. nov., isolated from activated sludge. *Int. J. Syst. Evol. Microbiol.* **2008**, *58*, 1064–1068. [CrossRef]
17. Hahn, M.W.; Kasalický, V.; Jezbera, J.; Brandt, U.; Jezberová, J.; Šimek, K. *Limnohabitans curvus* gen. nov., sp. nov., a planktonic bacterium isolated from a freshwater lake. *Int. J. Syst. Evol. Microbiol.* **2010**, *60*, 1358–1365.

18. Bowman, J.P.; Nichols, C.M.; Gibson, J.A.E. Algoriphagus ratkowskyi gen. nov., sp. nov., *Brumimicrobium glaciale* gen. nov., sp. nov., *Cryomorpha ignava* gen. nov., sp. nov. and *Crocinitomix catalasitica* gen. nov., sp. nov., novel flavobacteria isolated from various polar habitats. *Int. J. Syst. Evol. Microbiol.* **2003**, *53*, 1343–1355. [CrossRef]
19. Heulin, T.; Barakat, M.; Christen, R.; Lesourd, M.; Sutra, L.; De Luca, G.; Achouak, W. *Ramlibacter tataouinensis* gen. nov., sp. nov., and *Ramlibacter henchirensis* sp. nov., cyst-producing bacteria isolated from subdesert soil in Tunisia. *Int. J. Syst. Evol. Microbiol.* **2003**, *53*, 589–594. [CrossRef]
20. Kojima, H.; Shinohara, A.; Fukui, M. *Sulfurifustis variabilis* gen. nov., sp. nov., a sulfur oxidizer isolated from a lake, and proposal of Acidiferrobacteraceae fam. nov. and Acidiferrobacterales ord. nov. *Int. J. Syst. Evol. Microbiol.* **2015**, *65*, 3709–3713.
21. Emerson, D.; Rentz, J.A.; Lilburn, T.G.; Davis, R.E.; Aldrich, H.; Chan, C.; Moyer, C.L. A Novel Lineage of Proteobacteria Involved in Formation of Marine Fe-Oxidizing Microbial Mat Communities. *PLoS ONE* **2007**, *2*, e667. [CrossRef] [PubMed]
22. Yee, B.; Oertli, G.E.; Fuerst, J.A.; Staley, J.E. Reclassification of the polyphyletic genus *Prosthecomicrobium* to form two novel genera, *Vasilyevaea* gen. nov. and Bauldia gen. nov. with four new combinations: *Vasilyevaea enhydra* comb. nov., *Vasilyevaea mishustinii* comb. nov., *Bauldia consociata* comb. nov. and *Bauldia litoralis* comb. nov. *Int. J. Syst. Evol. Microbiol.* **2010**, *60*, 2960–2966. [PubMed]
23. Jung, M.-Y.; Islam, M.A.; Gwak, J.-H.; Kim, J.G.; Rhee, S.K. *Nitrosarchaeum koreense* gen. nov., sp. nov., an aerobic and mesophilic, ammonia-oxidizing archaeon member of the phylum *Thaumarchaeota* isolated from agricultural soil. *Int. J. Syst. Evol. Microbiol.* **2018**, *68*, 3084–3095. [CrossRef] [PubMed]
24. Sorokin, T.Y.; Lysenko, A.M.; Mityushina, L.L.; Tourova, T.P.; Jones, B.E.; Rainey, F.A.; Robertson, L.A.; Kuenen, G.J. *Thioalkalimicrobium aerophilum* gen. nov., sp. nov. and *Thioalkalimicrobium sibericum* sp. nov., and *Thioalkalivibrio versutus* gen. nov., sp. nov., *Thioalkalivibrio nitratis* sp.nov., novel and *Thioalkalivibrio denitrificancs* sp. nov., novel obligately alkaliphilic and obligately chemolithoautotrophic sulfur-oxidizing bacteria from soda lakes. *Int. J. Syst. Evol. Microbiol.* **2001**, *51*, 565–580.
25. Kuenen, J.G.; Veldkamp, H. *Thiomicrospira pelophila*, gen. n., sp. n., a new obligately chemolithotrophic colourless sulfur bacterium. *Antonie Van Leeuwenhoek* **1972**, *38*, 241–256. [CrossRef]
26. Miroshnichenko, M.L.; Kostrikina, N.A.; Chernyh, N.A.; Pimenov, N.V.; Tourova, T.P.; Antipov, A.N.; Spring, S.; Stackebrandt, E.; Bonch-Osmolovskaya, E.A. *Caldithrix abyssi* gen. nov., sp. nov., a nitrate-reducing, thermophilic, anaerobic bacterium isolated from a Mid-Atlantic Ridge hydrothermal vent, represents a novel bacterial lineage. *Int. J. Syst. Evol. Microbiol.* **2003**, *53*, 323–329. [CrossRef]
27. Qu, L.; Zhu, F.; Hong, X.; Gao, W.; Chen, J.; Sun, X. *Sunxiuqinia elliptica* gen. nov., sp. nov., a member of the phylum Bacteroidetes isolated from sediment in a sea cucumber farm. *Int. J. Syst. Evol. Microbiol.* **2011**, *61*, 2885–2889. [CrossRef]
28. Yoon, J.-H.; Kang, S.-Y.; Schumann, P.; Oh, T.K. *Yonghaparkia alkaliphila* gen. nov., sp. nov., a novel member of the family Microbacteriaceae isolated from an alkaline soil. *Int. J. Syst. Evol. Microbiol.* **2006**, *56*, 2415–2420.
29. Huang, X.-F.; Liu, Y.L.; Dong, J.D.; Qu, L.Y.; Zhang, Y.Y.; Wang, F.-Z.; Tian, X.P.; Zhang, S. *Mangrovibacterium diazotrophicum* gen. nov., sp. nov., a nitrogen-fixing bacterium isolated from a mangrove sediment, and proposal of *Prolixibacteraceae* fam. nov. *Int. J. Syst. Evol. Microbiol.* **2014**, *64*, 875–881. [CrossRef]
30. Vandecandelaere, I.; Nercessian, O.; Segaert, E.; Achouak, W.; Mollica, A.; Faimali, M.; De Vos, P.; Vandamme, P. *Alteromonas genovensis* sp. nov., isolated from a marine electroactive biofilm and emended description of *Alteromonas macleodii*. Baumann et al. 1972 (Approved Lists 1980). *Int. J. Syst. Evol. Microbiol.* **1972**, *58*, 2589–2596. [CrossRef]
31. Yi, H.; Schumann, P.; Chun, J. *Demequina aestuarii* gen. nov., sp. nov., a novel actinomycete of the suborder Micrococcineae, and reclassification of *Cellulomonas fermentans* Bagnara et al. 1985 as *Actinotalea fermentans* gen. nov., comb. Nov. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 151–156. [CrossRef]
32. Lee, S.D. *Labedella gwakjiensis* gen. nov., sp. nov., a novel actinomycete of the family *Microbacteriaceae*. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 2498–2502. [CrossRef]
33. Asker, D.; Beppu, T.; Ueda, K. *Zeaxanthinibacter enoshimensis* gen. nov., sp. nov., a novel zeaxanthin-producing marine bacterium of the family *Flavobacteriaceae*, isolated from seawater off Enoshima Island. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 837–843. [CrossRef]
34. Pankratov, T.A.; Tindall, B.J.; Liesack, W.; Dedysh, S.N. *Mucilaginibacter paludis* gen. nov., sp. nov. and *Mucilaginibacter gracilis* sp. nov., pectin-, xylan- and laminarin-degrading members of the family *Sphingobacteriaceae* from acidic *Sphagnum* peat bog. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 2349–2354. [CrossRef]
35. Irgens, R.L. Meniscus, a New Genus of Aerotolerant, Gas-Vacuolated Bacteria. *Int. J. Syst. Evol. Microbiol.* **1977**, *27*, 62829204. [CrossRef]
36. Chen, S.; Niu, L.; Zhang, Y. *Saccharofermentans acetigenes* gen. nov., sp. nov., an anaerobic bacterium isolated from sludge treating brewery wastewater. *Int. J. Syst. Evol. Microbiol.* **2010**, *60*, 2735–2738. [CrossRef]
37. Lv, X.-L.; Xie, B.S.; Cai, M.; Tang, Y.-Q.; Wang, Y.-N.; Cui, H.-L.; Liu, X.-Y.; Tan, Y.; Wu, X.-L. Halodurantibacterium flavum gen. nov., sp. nov., a non-phototrophic bacterium isolated from an oil production mixture. *Curr. Microbiol.* **2015**, *70*, 141–148. [CrossRef]
38. Romanenko, L.A.; Tanaka, N.; Frolova, G.M.; Mikhailov, V.V. *Arenicella xantha* gen. nov., sp. nov., a gammaproteobacterium isolated from a marine sandy sediment. *Int. J. Syst. Evol. Microbiol.* **2010**, *60*, 1832–1836.
39. Lim, J.-M.; Jeon, C.O.; Lee, G.S.; Park, D.-J.; Kang, U.-G.; Park, C.-Y.; Kim, C.J. *Leeia oryzae* gen. nov., sp. nov., isolated from a rice field in Korea. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 1204–1208.

40. Hosoya, S.; Arunpairojana, V.; Suwannachart, C.; Kanjana-Opas, A.; Yokota, A. *Aureispira marina* gen. nov., sp. nov., a gliding, arachidonic acid-containing bacterium isolated from the southern coastline of Thailand. *Int. J. Syst. Evol. Microbiol.* **2006**, *56*, 2931–2935.
41. Higasjioka, Y.; Kojima, H.; Watanabe, M.; Fukui, M. *Desulfatitalea tepidiphila* gen. nov., sp. nov., a sulfate-reducing bacterium isolated from tidal flat sediment. *Int. J. Syst. Evol. Microbiol.* **2013**, *63*, 761–765. [CrossRef] [PubMed]
42. Kojima, H.; Fukui, M. *Sulfuriflexus mobilis* gen. nov., sp. nov., a sulfur-oxidizing bacterium isolated from a brackish lake sediment. *Int. J. Syst. Evol. Microbiol.* **2016**, *66*, 3515–3518. [CrossRef] [PubMed]
43. Moe, W.M.; Yan, J.; Nobre, M.F.; DaCosta, M.S.; Rainey, F.A. *Dehalogenimonas lykanthroporepellens* gen. nov., sp. nov., a reductively dehalogenating bacterium isolated from chlorinated solvent-contaminated groundwater. *Int. J. Syst. Evol. Microbiol.* **2009**, *59*, 2692–2697. [CrossRef] [PubMed]
44. Kim, S.-H.; Kim, J.-G.; Jung, M.-Y.; Kim, S.J.; Gwak, J.-H.; Yu, W.-J.; Roh, S.W.; Kim, Y.H.; Rhee, S.-K. *Ketobacter alkanivorans* gen. nov., sp. nov., an n-alkane-degrading bacterium isolated from seawater. *Int. J. Syst. Evol. Microbiol.* **2018**, *68*, 2258–2264. [CrossRef]
45. Hania, W.B.; Joseph, M.; Schumann, P.; Bunk, B.; Fiebig, A.; Spröer, C.; Klenk, H.P.; Fardeau, M.-L.; Spring, S. Complete genome sequence and description of *Salinispira pacifica* gen. nov., sp. nov., a novel spirochaete isolated form a hypersaline microbial mat. *Stand. Genom. Sci.* **2015**, *10*, 7. [CrossRef]
46. Sorokin, D.Y.; Tourova, T.P.; Bezsoudnova, E.Y.; Pol, A.; Muyzer, G. Denitrification in a binary culture and thiocyanate metabolism in *Thiohalophilus thiocyanoxidans* gen. nov. sp. nov.—A moderately halophilic chemolithoautotrophic sulfur-oxidizing Gammaproteobacterium from hypersaline lakes. *Arch. Microbiol.* **2007**, *187*, 441–450. [CrossRef]
47. Ziegler, S.; Waidner, B.; Itoh, T.; Schumann, P.; Spring, S.; Gescher, J. Metallibacterium scheffleri gen. nov., sp. nov., an alkalinizing gammaproteobacterium isolated from an acidic biofilm. *Int. J. Syst. Evol. Microbiol.* **2013**, *63*, 1499–1504. [CrossRef]
48. Sakai, S.; Imachi, H.; Handa, S.; Ohashi, A.; Harada, H.; Kamagata, Y. *Methanocella paludicola* gen. nov., sp. nov., a methane-producing archaeon, the first isolate of the lineage 'Rice Cluster I', and proposal of the new archaeal order *Methanocellales* ord. nov. *Int. J. Syst. Evol. Microbiol.* **2008**, *58*, 929–936. [CrossRef]
49. Shieh, W.Y.; Jean, W.D. *Alterococcus agarolyticus*, gen.nov., sp.nov., a halophilic thermophilic bacterium capable of agar degradation. *Can. J. Microbiol.* **1998**, *44*, 637–645. [CrossRef]
50. Watanabe, M.; Kojima, H.; Fukui. M. Proposal of *Effusibacillus lacus* gen. nov., sp. nov., and reclassification of *Alicyclobacillus pohliae* as *Effusibacillus pohliae* comb. nov. and *Alicyclobacillus consociatus* as *Effusibacillus consociatus* comb. nov. *Int. J. Syst. Evol. Microbiol.* **2014**, *64*, 2770–2774. [CrossRef]
51. Monciardini, P.; Cavaletti, L.; Rhangetti, A.; Schumann, P.; Rohde, M.; Bamonte, R.; Sosio, M.; Mezzelani, A.; Donadio, S. Novel members of the family *Micromonosporaceae*, *Rugosimonospora acidiphila* gen. nov., sp. nov. and *Rugosimonospora africana* sp. nov. *Int. J. Syst. Evol. Microbiol.* **2009**, *59*, 2752–2758. [CrossRef]
52. Losey, N.A.; Stevenson, B.S.; Verbarg, S.; Rudd, S.; Moore, E.R.B.; Lawson, P.A. *Fontimonas thermophila* gen. nov., sp. nov., a moderately thermophilic bacterium isolated from a freshwater hot spring, and proposal of *Solimonadaceae* fam. nov. to replace Sinobacteraceae Zhou et al. 2008. *Int. J. Syst. Evol. Microbiol.* **2013**, *63*, 254–269. [CrossRef]
53. Yordy, J.R.; Weaver, T.L. Methylobacillus: A new Genus of Obligately Methylotrophic Bacteria. *Int. J. Syst. Evol. Microbiol.* **1977**, *27*, 247–255. [CrossRef]
54. Kashefi, K.; Holmes, D.E.; Baross, J.A.; Lovely, D.R. Thermophily in the Geobacteraceae: *Geothermobacter ehrlichii* gen. nov., sp. nov., a novel thermophilic member of the Geobacteraceae from the "Bag City" hydrothermal vent. *Appl. Environ. Microbiol.* **2003**, *69*, 2985–2993. [CrossRef]
55. Kusube, M.; Sugihara, A.; Moriwaki, Y.; Ueoka, T.; Shimane, Y.; Minegishi, H. *Alicyclobacillus cellulosilyticus* sp. nov., a thermophilic, cellulolytic bacterium isolated from steamed Japanese cedar chips from a lumbermill. *Int. J. Syst. Evol. Microbiol.* **2014**, *64*, 2257–2263. [CrossRef]
56. Maaroufi, A.; Fardeau, M.-L.; BenAliGam, Z.; Cayol, J.-L.; Hamed, S.-B.; Labat, M. *Anaerosalibacter bizertensis* gen. nov., sp. nov., a halotolerant bacterium isolated from sludge. *Int. J. Syst. Evol. Microbiol.* **2012**, *62*, 2469–2474.
57. DeMeyer, S.E.; Coorevits, A.; Willems, A. *Tardiphaga robiniae* gen. nov., sp. nov., a new genus in the family Bradyrhizobiaceae isolated from *Robinia pseudoacacia* in Flanders (Belgium). *Syst. Appl. Microbiol.* **2012**, *35*, 205–214. [CrossRef]
58. Qu, J.-H.; Yuan, H.-L.; Yang, J.-S.; Li, H.F.; Chen, N. *Lacibacter cauensis* gen. nov., sp. nov., a novel member of the phylum Bacteroidetes isolated from sediment of a eutrophic lake. *Int. J. Syst. Evol. Microbiol.* **2009**, *59*, 1153–1157. [CrossRef]
59. Inagaki, F.; Hideki, K.T.; Kobayashi, H.; Nealson, K.H.; Horikoshi, K. *Sulfurimonas autotrophica* gen. nov., sp. nov., a novel sulfur-oxidizing epsilon-proteobacterium isolated from hydrothermal sediments in the Mid-Okinawa Trough. *Int. J. Syst. Evol. Microbiol.* **2003**, *53*, 1801–1805. [CrossRef]
60. Allen, T.D.; Lawson, P.A.; Collins, M.D.; Falsen, E.; Tanner, R.S. *Cloacibacterium normanense* gen. nov., sp. nov., a novel bacterium in the family *Flavobacteriaceae* isolated from municipal wastewater. *Int. J. Syst. Evol. Microbiol.* **2006**, *56*, 1311–1316. [CrossRef]
61. Kulichevskaya, I.S.; Ivanova, A.O.; Belova, S.E.; Baulina, O.I.; Baudelier, P.L.E.; Ripstra, W.I.C.; Damsté, J.S.S.; Zavarsin, G.A.; Dedysh, S.N. *Schlesneria paludicola* gen. nov., sp. nov., the first acidophilic member of the order *Planctomycetales*, from Sphagnum-dominated boreal wetland. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 2680–2687. [CrossRef] [PubMed]

62. Koops, H.-P.; Böttcher, B.; Möller, U.C.; Pommerening-Röser, A.; Stehr, G. Classification of eight new species of ammonia-oxidizing bacteria: *Nitrosomonas communis* sp. nov., *Nitrosomonas ureae* sp. nov., *Nitrosomonas aestuarii* sp. nov., *Nitrosomonas marina* sp. nov., *Nitrosomonas nitrosa* sp. nov., *Nitrosomonas eutropha* sp. nov., *Nitrosomonas oligotropha* sp. nov. and *Nitrosomonas halophila* sp. nov. *J. General Microbiol.* **1991**, *137*, 1689–1699.
63. Köhler, J.M.; Ehrhardt, L.; Günther, P.M. Archaeal and Extremophilic Bacteria from Different Archaeological Excavation Sites. *Int. J. Mol. Sci.* **2023**, *24*, 5519. [CrossRef] [PubMed]
64. Araujo, A.S.F.; DePereira, A.P.D.; Antunes, J.E.L.; Oliveira, L.M.D.; DeMelo, W.J.; Rocha, S.M.B.; DoAmorim, M.R.; Ataujo, F.F.; Melo, V.M.M.; Mendes, L.W. Dynamics of bacterial and archaeal communities along composting of tannery sludge. *Environ. Sci. Pollut. Res.* **2021**, *28*, 64295–64306. [CrossRef]
65. Miranda, A.R.L.; Mendes, L.W.; Lemons, L.N.; Antunes, J.E.L.; Amorim, M.R.; Melo, V.M.M.; DeMelo, W.J.; VandenBrink, P.J.; Araujo, A.S. dynamics of archaeal community in soil with application of composted tannery sludge. *Sci. Rep.* **2019**, *9*, 7347. [CrossRef]
66. Araujo, A.S.F.; Lima, L.M.; Santos, V.M.; Schmidt, R. Repeated application of composted tannery sludge affects differently soil microbial biomass, enzymes activity, and ammonia-oxidizing organisms. *Environ. Sci. Poll. Res.* **2016**, *23*, 19193–19200. [CrossRef]