Metagenomic analysis of bacterial communities from nitrification-denitrification treatment of landfill leachates by Ion PGM System

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ABBREVIATIONS: **PGM**, personal genome machine, **COD**, chemical oxygen demand, **BOD**, biological oxygen demand, **VFA**, volatile fatty acids.

KEYWORDS

Bacterial community; nitrification-denitrification; PGM sequencing; wastewater treatment; metagenomic.

ABTRACT

The efficiency of the biological removal of carbon and nitrogen from leachates is determined by the activity of microbial populations present in biological reactors. In this work, a complete characterization of bacterial communities revealed by PGM sequencing has been carried out from different point of a nitrification-denitrification process operated in an urban landfill sited in the North of Spain. The leachate fed to the treatment was a mixture of young leachate, old leachate and effluent from an anaerobic digestion process, in a ratio of 1/0.9/0.12 (v/v), respectively. The anoxic and oxic reactors were followed by an ultrafiltration step. Samples were taken from different points of the process and PGM sequencing was used to characterize microbial communities. Results revealed the microbial diversity of samples, which included detection of minority populations that are difficult to be explored by other methods. Bacteria belonging to *Bacteroidetes* and *Proteobacteria* were dominant in all the samples analyzed. This last phylum represented more than 50% of the total population in all cases. Samples taken after the biological treatment showed a significant reduction in the relative abundance of *Firmicutes*, *Tenericutes* and *Lentisphaerae* phyla in comparation with the initial leachate. The relative abundance of the classes was studied being *Betaproteobacteria* and *Flavobacteria* the most abundant in the samples taken throughout the biological treatment.

1. INTRODUCTION

Landfill leachate is the liquid that results from water percolating through waste deposits. The specific composition of leachates depends on the type of wastes, landfill age, climate conditions and hydrogeology of the landfill site [1]. These effluents are usually characterized by high concentrations of organic matter, ammonium as well as heavy metals and chlorinated salts [2]. Young leachates are commonly characterized by high biochemical oxygen demand (BOD) and chemical oxygen demand (COD), as consequence of a rapid anaerobic fermentation that generates volatile fatty acids (VFA) as main products [3]. In matures leachates, the methanogenic phase occurs and the VFA are converted to biogas. Therefore, the organic fraction of the leachate becomes dominated by recalcitrant or bio-refractory compounds [4].

Pollutants present in the leachate can contaminate groundwaters, rivers and soils, causing high environmental impact. Therefore, its collection and treatment is one of the main problems in urban waste landfills. Biological processes have been reported as the most effective for the treatment of these wastewaters [3, 5]. These processes take advantage of the abilities of microbes to degrade organic matter, remove nutrients and transform toxic compounds into harmless products [6]. During biological treatment, the nitrogen of landfill leachate is removed through nitrification and denitrification processes, which are carried out by ammonium-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), and denitrifying bacteria [7]. These bacterial communities are highly sensitive to environmental factors, such as pH, salinity, temperature or dissolved oxygen [8]. To go in depth these biological transformations, it is essential to characterize the microbiota at each stage of the process, which depends on the substrate characteristics and the operational conditions [9].

Several molecular techniques, such as terminal restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE), single-strand conformation polymorphism (SSCP) and Sanger sequencing of clone libraries have been employed in the last decades to describe microbial communities in wastewater processes [10]. However, the information obtained from these techniques was limited because only a few hypervariable regions are considered. In recent years, the application of more advanced techniques, i.e. the next generation of sequencing (NGS) based on 16S rRNA gene sequencing, has provided a cheaper and higher throughput alternative to sequencing DNA [11]. This technology allows the generation of millions of short sequencing reads for massive studies of genes, giving higher taxonomic resolution. It offers a great opportunity and new insights to rapidly examine the composition as well as the interaction of the great diversity of microorganisms involved in wastewater treatments [12].

However, despite of the evident interest, as far as we know, PGM sequencing has not yet been employed for the study in depth of microbial ecology in nitrification-denitrification processes of landfill leachates. This technique is used in this work to carry out a microbial characterization throughout a real biological treatment of wastewater mainly composed by a mixture of young and old leachate.

In particular, the aims of this work were: i) To characterize the bacterial population in the raw leachate and in the nitrification-denitrification reactors. ii) To determine the effect of operational parameters on the distribution of bacterial communities and its repercussions in the effectiveness.

2. MATERIAL AND METHODS

2.1. Plant operation parameters

The samples used in this study were taken from the biological leachate treatment plant sited in COGERSA, the wastes treatment center of Asturias (Spain). This center has a non-hazardous-wastes landfill with a capacity of 16 million of m^3, a hazardous-waste landfill with a capacity of 600 m^3 and an anaerobic digestion plant, which can treat 30000 t/years of sludges from urban wastewater treatment plants and the organic fraction of municipal solid waste.

The treated process was fed with a mixture of young leachate, old leachate and an effluent from the anaerobic digestion process, in an approximate ratio of 1/0.9/0.12 (v/v), respectively.

Approximately 700 m^3/day of leachates were treated by the biological treatment, which consisted of one denitrification reactor (anoxic), one mixed reactor which operated as denitrifying or nitrifying depending on the conditions of the plant, and four nitrification reactors (oxic). At the time when the samples were taken, the mixed reactor was operating as a nitrifying reactor. The nitrification-denitrification process occurred under pressure (2.5 bar) at mesophilic temperatures (37-40ºC). The volume of each reactor was 175 m^3 with a total hydraulic retention time of 7 h. During the process pH maintained between 6.5 and 7, and 3 m^3/day of methanol were supplied as carbon source. Oxygen was supplied by air compressors through bottom ejectors to the nitrification reactors in order to assure an oxygen concentration of 2.5 ppm. The injection pumps circulated the air-mud mixture, favoring the dissolution of oxygen and the homogenization of the sludge inside the reactors. After the treatment process, a recirculation from the last nitrification tank (OXIC-4) to the initial denitrification tank (ANOXIC-1) was carried out in a ratio of 80.5%. The rest of treated water was separated from the biological sludge by ultrafiltration process formed by 5 units of ultrafiltration with a total membrane surface of 280 m^2 with a pore size of 0.02 μm. After the process, for

the final sample, the efficiency of nitrogen removal higher than 80% compared with the fed sample to the treatment plant. A flow diagram of the treatment plant is shown in Figure 1.

FIGURE 1

2.2. Sampling

Five different samples were collected throughout the biological treatment to be analyzed microbiologically. Sample 1 (S1) corresponded to the raw leachate incoming the biological treatment. This sample was taken before mixing with the recirculate permeate from the ultrafiltration process. Sample 2 (S2) was taken from the effluent of the denitrification reactor, sample 3 (S3) corresponds to the effluent of nitrification reactor OXIC-3 and sample 4 (S4) corresponds to the recirculated effluent to the head of the process coming from nitrification reactor OXIC-4. Finally, sample 5 (S5) was taken from the sludge of the ultrafiltration process. Detailed information from each point of sampling is shown in Figure 1 and Table 1.

TABLE 1

2.3. Sample processing and DNA extraction

Sample processing was preformed according to [13]. A volume of 160 ml of each sample was centrifuged for 20 minutes at 13000g. The supernatant was discarded, and the solid fraction was preserved at -20ºC for DNA extraction. With this aim, Power Biofilm DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA), specific for leachate samples, was employed and 0.25 g of the solid fraction were weighted and treated according to the manufacturer's instructions. Due to the excessive colour of samples, 200 µl of solution BF3 were added (recommended in the kit protocol). The extracted DNA was concentrated using the Concentrator Plus Vacufuge (Eppendorf, Hamburg, Germany) and a BioPhotometer Plus (Eppendorf, Hamburg, Germany) was used to ensure that the amount of DNA was high enough to continue the process.

2.4. DNA amplification and purification

An Ion 16S Metagenomics Kit (Ion Torrent, Life Technologies) was employed for DNA amplification. This kit allows the simultaneous examination of 7 of the 9 hypervariable regions in the bacterial 16S rRNA gene, using one primer for the V2-4-8 regions and another primer for V3-6 and V7-9 regions. The DNA samples were amplified by PCR reaction, which was performed in several steps: i) heating at 95 °C for 10 minutes, ii)25 cycles of denaturation at 95 °C for 30 seconds, iii)alignment at 58 °C for 30 seconds, iv) extension at 72 °C for 30 seconds, v)elongation at 72 ° C for 7 minutes and vi) preservation at 4 °C for 20 minutes. The resulting products were purified using the Agencourt AMPure XP Kit (Beckman Coulter,

Atlanta, GA, USA) and the 16S rRNA amplicons were quantified with a Qubit 2.0 Fluorometer using dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA).

2.5. Library construction and sequence analysis

The DNA obtained in the purification phase was fragmented in order to obtain smaller fragments of up to 150 base pairs (bp) by using an Ion Plus Fragment Library Kit (AB Library Builder). For the library construction, each fragment of the obtained DNA was coupled to a marker and two adapters. Each library corresponds to a different collection of DNA fragments to be sequenced and is unique to each sample. Construction of the library was conducted using the PGM Hi-Q OT2 Kit. Subsequently, the samples were sequenced using the PGM Hi-Q Sequencing Ion Kit and the Ion 318 Chip Kit v2, which has a minimum capacity of 4 million readings.

The results obtained were analyzed by using Life Technologies Ion Reporter Software, that uses both the Premium Curated MicroSEQ ID 16S rRNA reference database and the Curated Greengenes Database. The restriction criteria applied was as follows: i) read length filter: 150 bp, ii) minimum alignment coverage: 90%, iii) read abundance filter: 10, iv) genus cut off: 97%, and v) species cut off: 99%. These criteria were selected according to previous works about microbial identification that used databases employed in this study [13, 14]

2.6. Nucleotide sequence accession numbers

The sequences obtained in this study are available in the National Center for Biotechnology Information (NCBI) under accession numbers SAMN09765719 to SAMN09765723. The SRA database accession number is SRP156554.

3. RESULTS AND DISCUSSION

The PGM sequencing and the amplification of hypervariable regions of 16S rRNA allowed us to obtain a detailed taxonomic bacterial classification throughout the nitrification-denitrification treatment. A total of 21 phyla, 250 families, 128 genera and 77 species were identified in the five samples analyzed. The classification of microorganisms up to specie level is shown in the Supplementary Material (Fig.S1 to Fig.S5). After the analysis with Ion Reporter Software, a total of 1056150 effective sequences were obtained. In general, hypervariable V3 and V6-7 regions presented a greater number of mapped reads, followed by V4 and V8 regions. This information highlights the importance of sequencing all hypervariable regions to obtain a more accurate identification of microorganisms. The Simpson index, which represents

the probability that two individuals within a habitat and selected at random belong to the same species, was employed to determine the species diversity in each sample [15].

As can be observed, the Simpson index was lower for S1 indicating that the diversity was higher in the initial leachate (S1) than in the rest of samples taken from the different points of the biological treatment. This fact was expected because the initial sample was a mixture of effluents from different sources, with different microbial environments, whereas the conditions of the nitrification and denitrification reactors inhibits the activity of some microorganisms and favors the development of others.

3.1. Raw leachate (S1)

The raw leachate is a mixture of young leachate, mature leachate and an effluent from an anaerobic digestion process as indicated in Material and Methods section. This sample, as shown in Table 1, was characterized by high concentrations of ammonium (> 2000 mg/L) and COD_t (> 4000 mg/L) with moderate biodegradability (BOD_5/COD~3). As shown in Figure 2A, in the initial leachate (S1), *Proteobacteria* and *Firmicutes* phyla were the most abundant, achieving 51% and 18% of total relative abundance, respectively. Previous studies highlighted the dominance of these phyla in landfill leachates and wastewater treatments, followed by other groups such as *Bacteroidetes* and *Tenericutes* also found in this sample, but with relative abundances lower than 8% [6].

FIGURE 2

The relative abundance of classes within *Proteobacteria* phylum is shown in Figure 3A, where *proteobacteria* class, which accounted for 80% of the total bacteria, was the most abundant. Within this class, the genus *Arcobacter* was detected in S1. The presence of this genus has been reported as typical in urban wastewater and some microorganisms within it as *Arcobacter butzleri* has been described as potential pathogens and fecal pollution indicator [16]. Lu et al. [17] reported the efficiency of activated sludge in full-scale water treatment systems for the elimination of this specie. In this study, *Arcobacter skirrowii* and *Arcobacter venerupis* were detected in S1, S2 and S3. Nevertheless, its relative abundance was significantly reduced throughout process and it has not been detected in S4 and S5. This fact indicates that the nitrification-denitrification process here considered is effective for the elimination of this pathogenic bacterium.

FIGURE 3

The second class in order of relative abundance within *Proteobacteria* phylum was *γ-proteobacteria* class, which accounted for approximately 15%. Genus as *Pseudomonas*, *Teredinibacter*, *Idiomarina* and

Marinospirillum were the most abundant. Previous studies reported *Pseudomonas* genus as bacteria with capacity to biodegrade organic substances and to reduce the biotoxicity caused by xenobiotic organic chemicals. Besides, it is known that these bacteria use primarily nitrate as an electron acceptor and play an important role in the conversion of nitrite to molecular nitrogen [18]. Du et al. [19] applied bacteria of this genus as a bioaugmented system to treat complex and high concentrated wastewater with great contents of nitrate and nitrite.

This class also includes important nitrifiers and denitrifiers microorganisms which have an important role during the biological process. Genus such as *Nitrosomonas*, *Nitrosospira*, *Nitrosococcus*, *Tissierella*, *Pseudomonas*, *Clostridium* and *Paracoccus* were detected in S1, all of them have been associated with fermentative metabolism of macromolecular organic compounds [20].

Köchling et al. [21], who analyzed microbial communities in raw leachates of different ages, reported that *Proteobacteria*, mainly *Pseudomonadales* order, were more abundant in rainy seasons, whereas microorganisms belonging to *Firmicutes*, mainly *Clostridiales* order, were predominant in dry seasons and they increased their proportion with the landfill age. *Firmicutes* was related to the secretion of extracellular enzymes as cellulases, lipases and proteases. So, their main function in landfills consists in degrading complex polysaccharides, such as starch and cellulose [22]. In our case, the landfill is located in a high rainfall zone and the proportion of old leachate was lower than the proportion of young leachate, which is in agreement with the fact that the relative abundance of *Firmicutes* phylum was quite lower. *Clostridia* class was the most representative within *Firmicutes* phylum, with more than 50% of relative abundance. The next microorganisms belonging to this class were identified: *Cellulosibacter alkalithermophilus*, *Clostridium sp.*, *Tissierella creatinini*, *Syntrophomonas byantii*, *Syntrophomonas sapovorans*, and *Proteiniborus ethanoligenes*.

As shown in Figure 2, the *Bacteroidetes* phylum accounted for 8% of total microorganisms in S1. Microorganisms within this phylum have been described as expert bacteria for the degradation of high molecular weight organic matter to acetic and propionic acid [20a]. Their presence has been reported in anaerobic digestion processes fed with vegetal biomass, sludge or mixed organic residues [13, 23]. Within *Bacteroidetes* phylum, *Bacteroidia* class was the most abundant representing around 70% of the phylum. This class plays an important role in hydrolyzing and fermenting organic materials, producing organic acids, CO_2 and H_2 during the anaerobic digestion process that takes place in landfills [24]. Within this class, species of the order *Bacteroidales*, i.e. *Petrimonas sp.*, were detected. *Flavobacteria* and *Sphingobacterii*

classes were also detected with relative abundances of 12% and 16%, respectively. These classes have been described as typical populations in leachates [2][22].

3.2. Denitrification reactor output (S2)

During the denitrification step (anoxic tank), organic matter is consumed by heterotrophic bacteria responsible for the transformation of nitrate into molecular nitrogen. In this reactor, methanol was added as carbon source for increase the biodegradable organic matter available for denitrifying bacteria. For this reason, COD values reported in Table 1 for S2 was higher than values reported for S1. As can be estimated from data shown in Table 1, nitrate recirculated to the anoxic tank is removed in this step with efficiencies higher than 80%.

The relative abundances of majority phyla found in S2 are shown in Figure 4. With respect to S1, the relative abundance of Bacteroidetes increased, accounting in this sample 27% of total. This phylum together with *Proteobacteria* has been described as dominant in denitrification processes [25].

FIGURE 4

The relative abundance of the phylum *Proteobacteria* suffered an increase of 6% with respect to the raw leachate. This fact was expected since it was reported that the relative abundance of *Proteobacteria* phylum was higher when the ammonium concentration was reduced [2] and the ammonium concentration is S2 was five times lower than in S1 due to the recirculations (see Table 1 and Figure 1). Potential denitrifying genera within this phylum, i.e. *Thauera*, *Comamonas* and *Azoarcus*, were detected.

With respect to the relative abundance of classes within this phylum (Fig 3A), in comparation with S1 it was observed, a significant decrease in the *ε-protebacteria* and an increase in *α-proteobacteria* and *β-proteobacteria*, which are related with nitrification-denitrification processes. The ammonia-oxidizing bacteria (AOB) are phylogenetically restricted to *β-proteobacteria*, including the genera *Nitrosomonas*, *Nitrosospira*, *Nitrosovibrio* and *Nitrosolobus* and to *γ-proteobacteria*, including the genus *Nitrosococcus* [26]. *Nitrosomonas* and *Nitrosococcus* were detected in S2, whereas *Nitrosospira*, *Nitrosovibrio* and *Nitrosolobus* were not identified in this study.

The relative abundance of *Firmicutes* in S2 was very low whereas in S1 was the second in order of abundance. This phylum has been described as one of the most abundant in anaerobic processes [27]. Again, the high recirculation from the oxic reactors seems to be the reason for the decrease in the relative abundance of this phylum.

The second dominant phylum, *Bacteroidetes*, has been related with the degradation of particulate organic matter, especially high-molecular-weight compounds [28]. Regarding the relative abundance within *Bacteroidetes* phylum (Fig 3B), *Cytophagia*, *Flavobacteria* and *Sphingobacterii* classes were dominant. Guo et al. [29] described these classes, in especial, *Flavobacteria* and *Sphingobacterii* as dominant in activate sludge treatments plants. Gabarró et al. [30] that investigated microbial communities in the treatment of madure landfill leachates reported that these classes are key in nitrification processes. Microorganisms belonging to these classes utilize complex organic substrates as cellulose, which might suggest that they can promote the degradation of recalcitrant compounds [31]. With respect to *Bacteroidia* class, it suffered a sharp decline till values lower than 7% in all the samples taken throughout the treatment process, whereas in S1 was the most abundant class. This fact was expected since most of these microorganisms are known to be obligate anaerobes. The subsequent nitrification step was carried out under aerobic conditions, inhibiting bacteria belonging to *Bacteroidia* class and decreasing its relative abundance in all the samples, except for S1. Hu et al. [32] reported that microorganisms within *Proteobacteria* phylum were most abundant in aerobic conditions whereas *Bacteroidetes* phylum, to which *Bacteroidia* class belongs, was most abundant in anaerobic bioreactors.

Other phyla as *Verrucomicrobia*, *Actinobacteria*, *Chloroflexi*, *Firmicutes* and *Planctomycetes* were detected with relative abundances lower than 5%.

3.3. Nitrification reactors output (S3 and S4) and ultrafiltration sludge (S5)

Nitrification processes are typically conducted by autotrophic bacteria. Consequently, as is shown in Table 1, the concentration of COD and BOD were similar along S2, S3 and S4. However, due to the activity of nitrifying bacteria, more than 80% of the ammonium contained in S2 was removed. Results obtained for the samples S3 and S4 from the nitrification process are shown in Figure 5A and Figure 5B, respectively.

FIGURE 5

Therefore, *Proteobacteria* and *Bacteroidetes* phyla were again the most abundant, representing around 90% of total relative abundance in these samples. Most of the microorganisms responsible for carrying out nitrification processes, (AOB and NOB) are found within these phyla [33].

It is striking the higher relative abundance of *Proteobacteria* in sample S3. Heterotrophic nitrifiers from genera belonging to this phylum, such as *Comamonas*, *Thauera*, *Paracoccus* and *Azoarcus* were detected in S3 reaching the classification of the microorganisms up to specie level, i.e., *Comamomas denitrificans*, *Thauera amoniaromatica*, *Thauera phenylacetica* and *Paracoccus solventivorans*. These genera have been

reported in activated sludge reactors treating ammonium-rich, high-organic tannery and coking wastewater [34].

Guo et al. [29] studied the microbial structure and diversity of activated sludge in a full-scale simultaneous nitrogen and phosphorus removal plant. They described *Proteobacteria*, *Nitrospirae*, *Bacteroidetes*, *Actinobacteria* and *Firmicutes* as dominant phyla. In addition to *Proteobacteria* and *Bacteroidetes* phyla, *Actinobacteria*, *Firmicutes* and *Nitrospirae* were also detected in S3 and S4, although with low relative abundance. Other phyla as *Chloroflexi*, *Verrucomicrobia* and *Planctomycetes* were detected in these samples with relative abundances lower than 5%.

The ultrafiltration sludge (S5) is basically a concentrated mixture of sludges coming out from the last oxic reactor. So, as expected, the microbiota found in this sample was similar to S3 and S4 microbiota with again *Bacteroidetes* and *Proteobacteria* as the dominant phyla (See Figure 5C).

The relative abundance of classes within these phyla (Fig 3) remained almost constant in all the samples taken through the process (S2 to S5), and only slight variations could be observed.

CONCLUDING REMARKS

Results here obtained proved that Ion Torrent methodology, based on PGM sequencing and the amplification of all variable regions of 16S rRNA gene, makes possible to obtain an exhaustive taxonomic classification of bacterial populations in complex samples taken from biological treatments, such as the nitrifying-denitrifying process here analyzed. The predominant phylum throughout the leachate treatment was *Proteobacteria* with more than 50% of total relative abundance in all the samples analyzed. This predominance was expected because most of microorganisms involved in nitrification-denitrification processes are included within this phylum, mainly in *β-proteobacteria* and *γ-proteobacteria* classes.

In the initial leachate (S1), the relative abundance of *Firmicutes* was higher than in samples taken at the outlet of biological reactors (S2 to S5). On the contrary, *Bacteroidetes* abundances were higher throughout the biological process, reaching values between 20% and 30%. This phylum together with *Proteobacteria* represented more than 90% in samples from S2 to S5.

In relation to class level, *γ-proteobacteria* was the most abundant in the initial leachate. However, throughout the biological process *α-proteobacteria* and *β-proteobacteria* became also dominant classes, according with others works that analyzed leachate treatments.

In relation with the phyla troughtout biological treatment, significative differences in relative abundances have been detected between oxic and anoxic reactors, with higher percentages of *Proteobacteria* in the

samples taken from the oxic reactors. Despite the different environments in anoxic an oxic reactors, the high recirculation contributes to achive a high degree of mixturealthought several differences could be detected with respect to minority populations.

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TABLES

Table 1. Characteristics of the samples analyzed. The values correspond to the averages (± standard deviations) of four samples taken along 2016.

	Parameters							
	pH (ud.)	**COD_T (mg/L)**	**COD_S (mg/L)**	**BOD_5 (mg/L)**	**NH_4^+ (mg/L)**	**NO_3^- (mg/L)**	**NO_2^- (mg/L)**	**TS (mg/L)**
S1	8.56 ± 0.10	5155 ± 1159	4988 ± 966	1788 ± 798	2330 ± 171	< 60	2330± 171	10565± 1416
S2	7.53 ± 0.41	23738 ± 2375	7988 ± 491	2500 ± 616	412 ± 83	< 60	412 ± 83	28250 ± 2371
S3	6.73 ± 0.23	25775 ± 2207	7890 ± 616	2275 ± 618	78 ± 40	352 ± 138	78 ± 40	20009 ± 1825
S4	6.68 ± 0.21	21475 ± 6974	7788 ± 709	1950 ± 656	41 ± 34	368 ± 136	41 ± 34	29168 ± 2593
S5	6.81 ± 0.16	25150 ± 2362	9142 ± 2847	2125 ± 591	80 ± 43	316 ± 154	80 ± 43	29008 ± 2643

FIGURE CAPTIONS

Fig 1 Process flow diagram of biological treatment plant.

Fig 2 Relative abundance for the phyla detected in the raw leachate (S1)

Fig 3 Relative abundance for the classes detected in the *Proteobacteria* (A) and *Bacterioidetes* (B) phyla.

Fig 4 Relative abundance for denitrification reactor output (B) sample (S2).

Fig 5 Relative abundance for the phyla detected in the nitrification reactors output (A and B) and ultrafiltration sludge (C) samples (S3, S4 and S5, respectively).

Figure 1

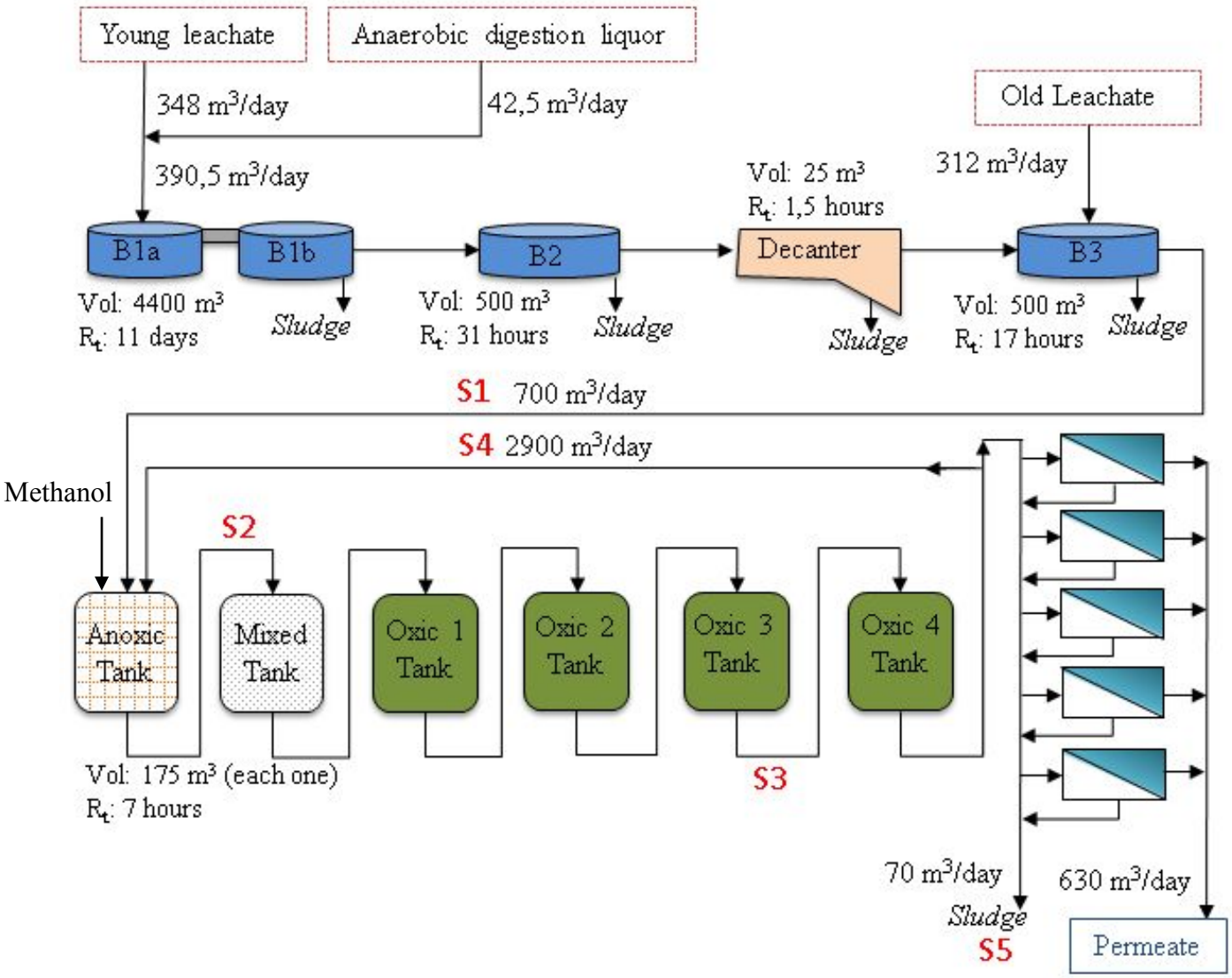

Young leachate
Anaerobic digestion liquor
348 m³/day
42,5 m³/day
390,5 m³/day
Old Leachate
312 m³/day
Vol: 25 m³
Rt: 1,5 hours
B1a
B1b
B2
Decanter
B3
Vol: 4400 m³
Rt: 11 days
Sludge
Vol: 500 m³
Rt: 31 hours
Sludge
Sludge
Vol: 500 m³
Rt: 17 hours
Sludge
S1 700 m³/day
S4 2900 m³/day
Methanol
S2
Anoxic Tank
Mixed Tank
Oxic 1 Tank
Oxic 2 Tank
Oxic 3 Tank
Oxic 4 Tank
Vol: 175 m³ (each one)
Rt: 7 hours
S3
70 m³/day
630 m³/day
Sludge
S5
Permeate

Figure 2

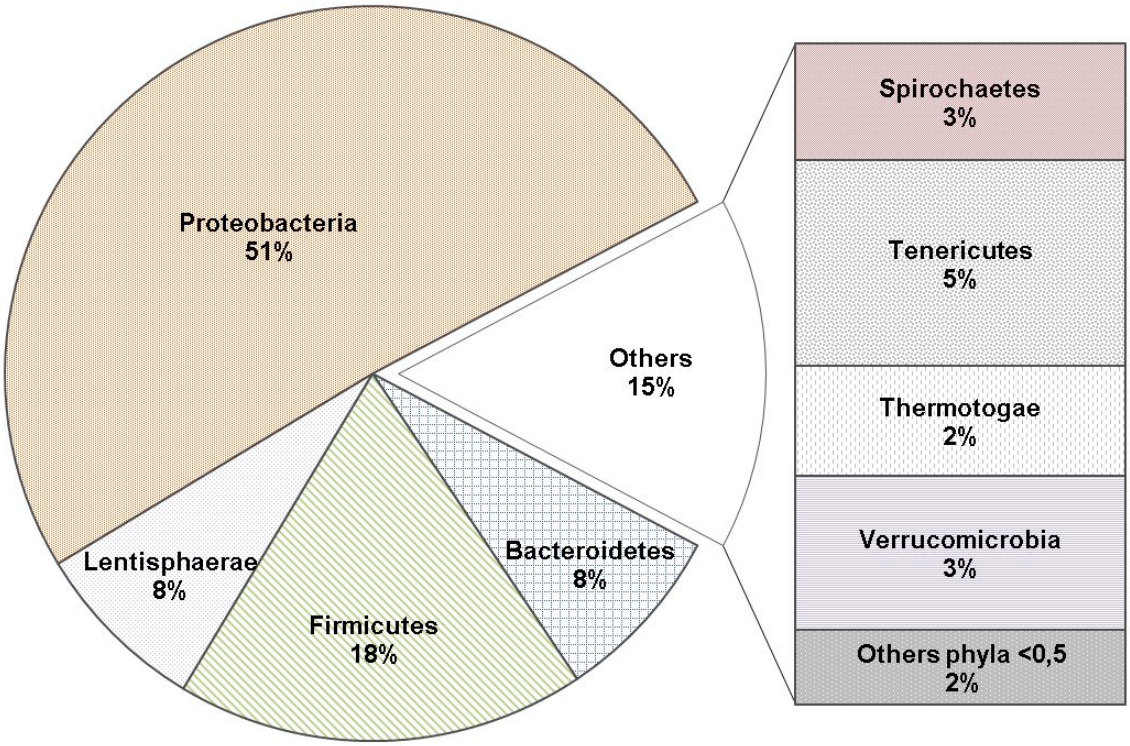
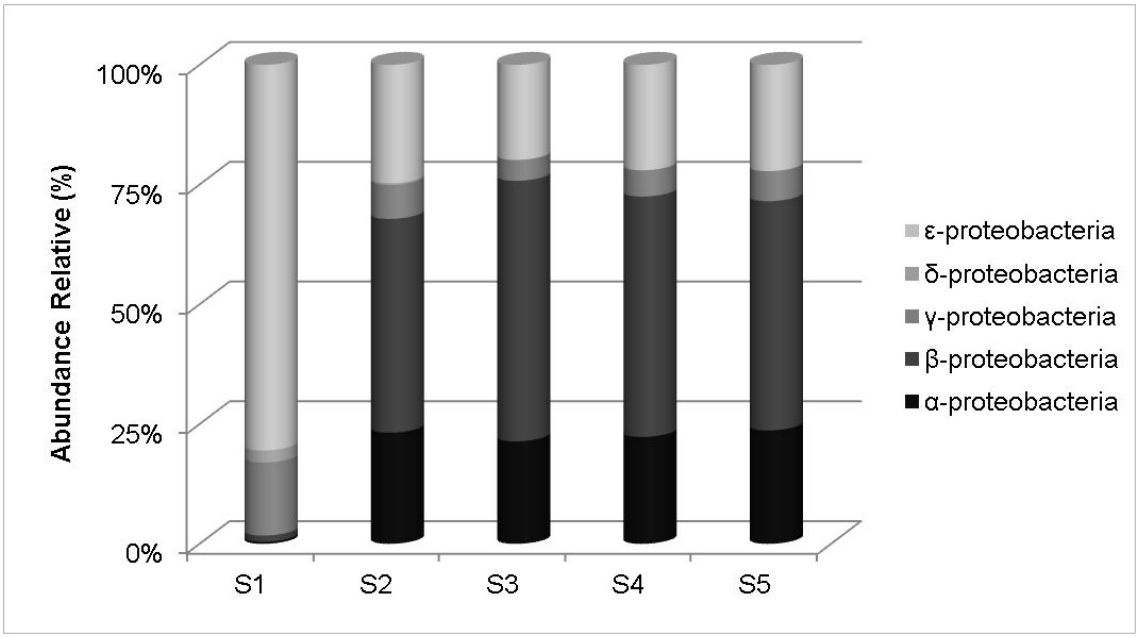

Proteobacteria
51%
Lentisphaerae
8%
Firmicutes
18%
Bacteroidetes
8%
Others
15%
Spirochaetes
3%
Tenericutes
5%
Thermotogae
2%
Verrucomicrobia
3%
Others phyla <0,5
2%

Figure 3

A)


100%
75%
50%
25%
0%
Abundance Relative (%)
S1
S2
S3
S4
S5
ε-proteobacteria
δ-proteobacteria
γ-proteobacteria
β-proteobacteria
α-proteobacteria

B)

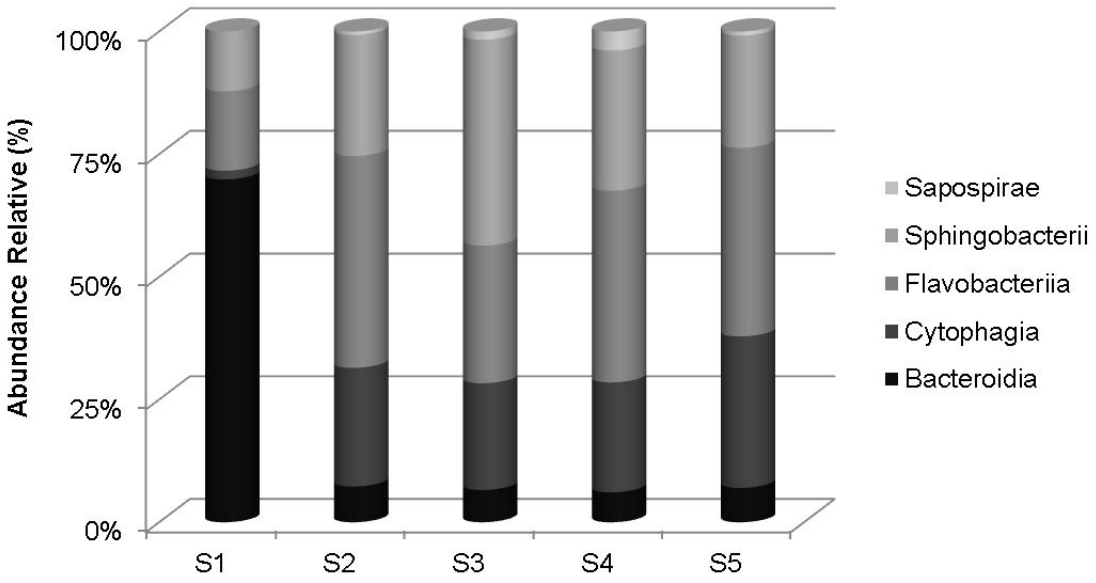

100%
75%
50%
25%
0%
Abundance Relative (%)
S1
S2
S3
S4
S5
Sapospirae
Sphingobacterii
Flavobacteriia
Cytophagia
Bacteroidia

Figure 4

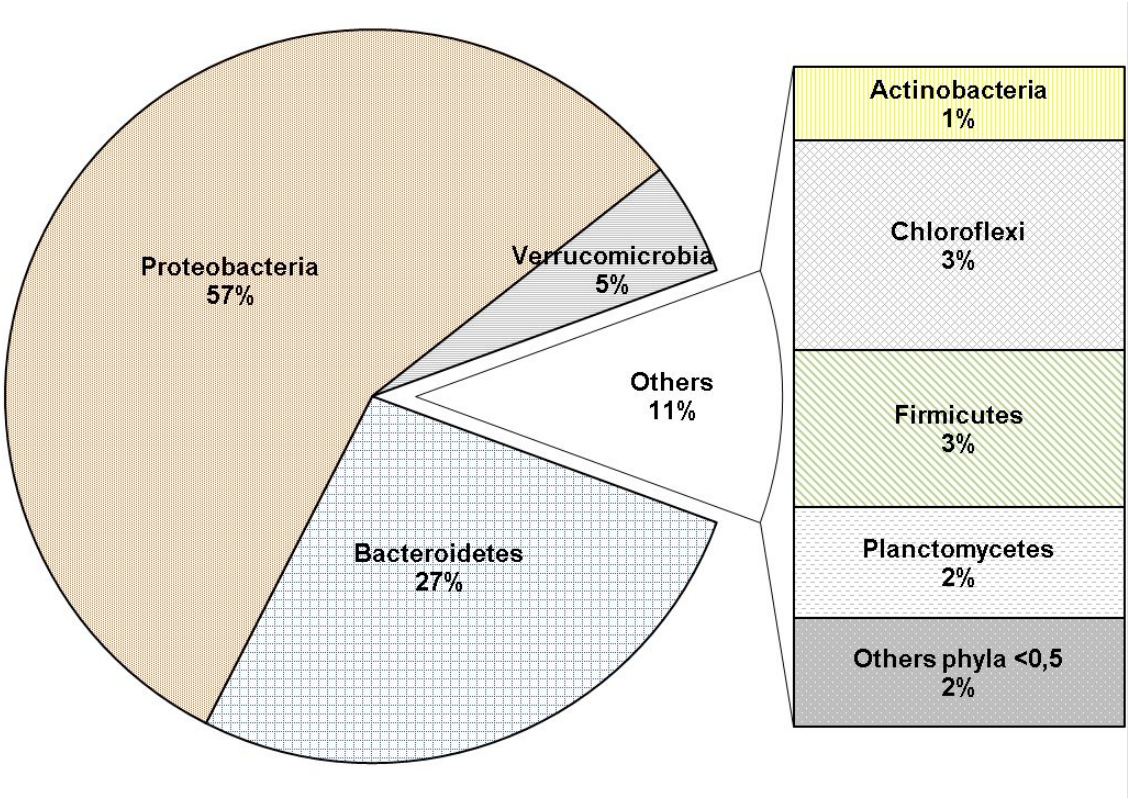
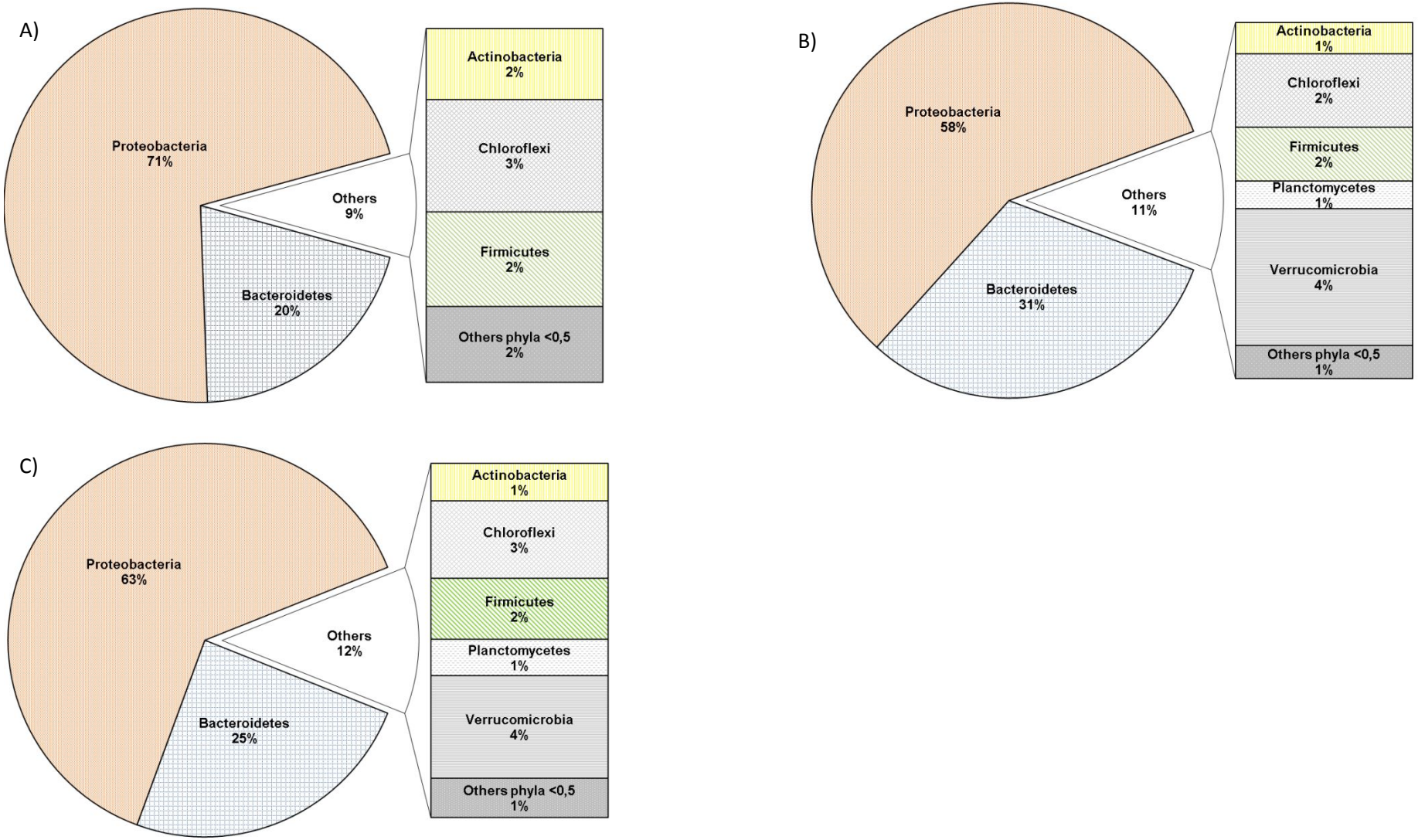

Proteobacteria
57%
Verrucomicrobia
5%
Others
11%
Bacteroidetes
27%
Actinobacteria
1%
Chloroflexi
3%
Firmicutes
3%
Planctomycetes
2%
Others phyla <0,5
2%

Figure 5


A)
Proteobacteria
71%
Others
9%
Bacteroidetes
20%
Actinobacteria
2%
Chloroflexi
3%
Firmicutes
2%
Others phyla <0,5
2%
B)
Proteobacteria
58%
Others
11%
Bacteroidetes
31%
Actinobacteria
1%
Chloroflexi
2%
Firmicutes
2%
Planctomycetes
1%
Verrucomicrobia
4%
Others phyla <0,5
1%
C)
Proteobacteria
63%
Others
12%
Bacteroidetes
25%
Actinobacteria
1%
Chloroflexi
3%
Firmicutes
2%
Planctomycetes
1%
Verrucomicrobia
4%
Others phyla <0,5
1%

Supplementary Material to

"Metagenomic analysis of bacterial communities from nitrification-denitrification treatment of landfill leachates by Ion PGM System"

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Fig.S1

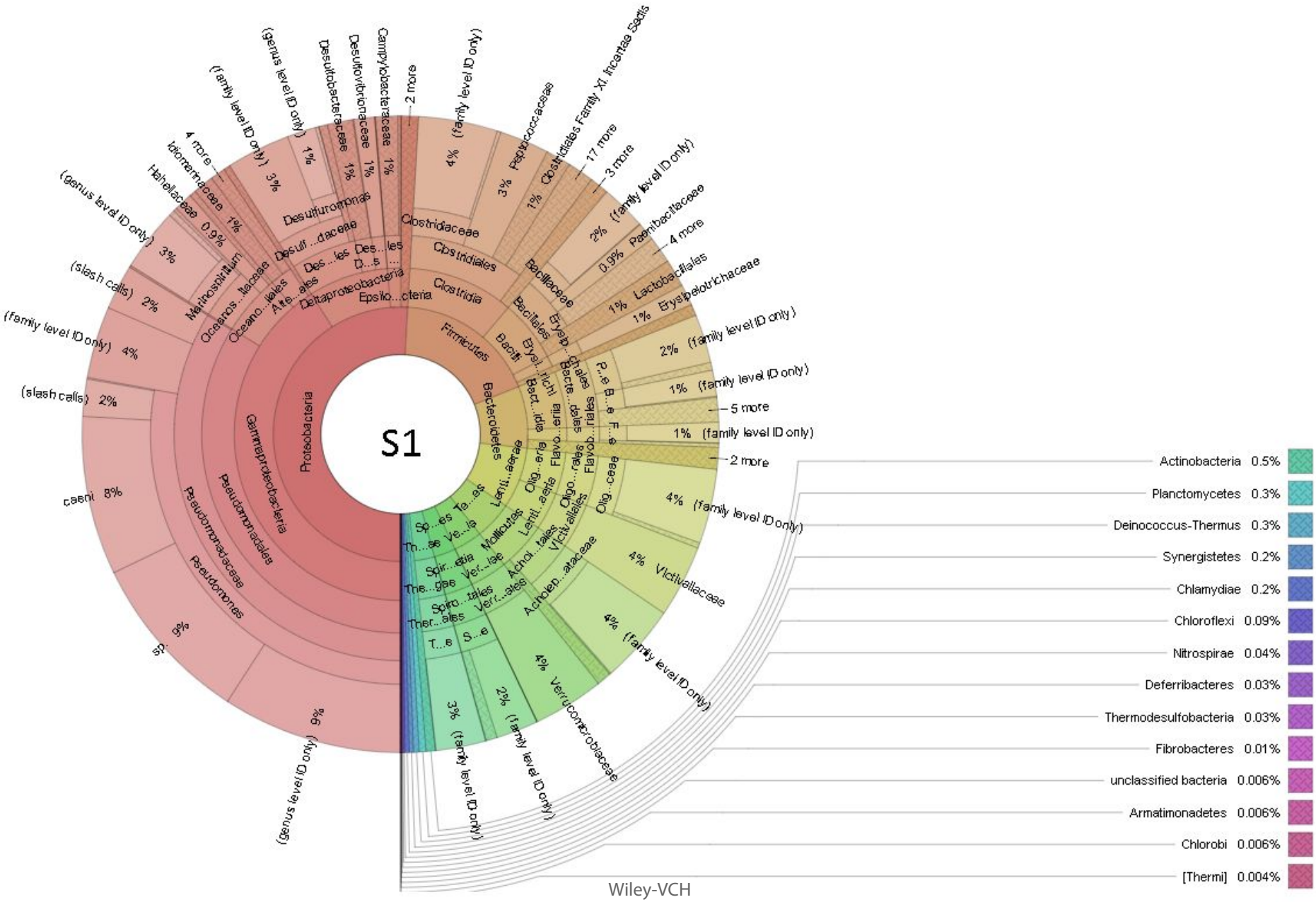

S1
Actinobacteria 0.5%
Planctomycetes 0.3%
Deinococcus-Thermus 0.3%
Synergistetes 0.2%
Chlamydiae 0.2%
Chloroflexi 0.09%
Nitrospirae 0.04%
Deferribacteres 0.03%
Thermodesulfobacteria 0.03%
Fibrobacteres 0.01%
unclassified bacteria 0.006%
Armatimonadetes 0.006%
Chlorobi 0.006%
[Thermi] 0.004%

Fig.S2

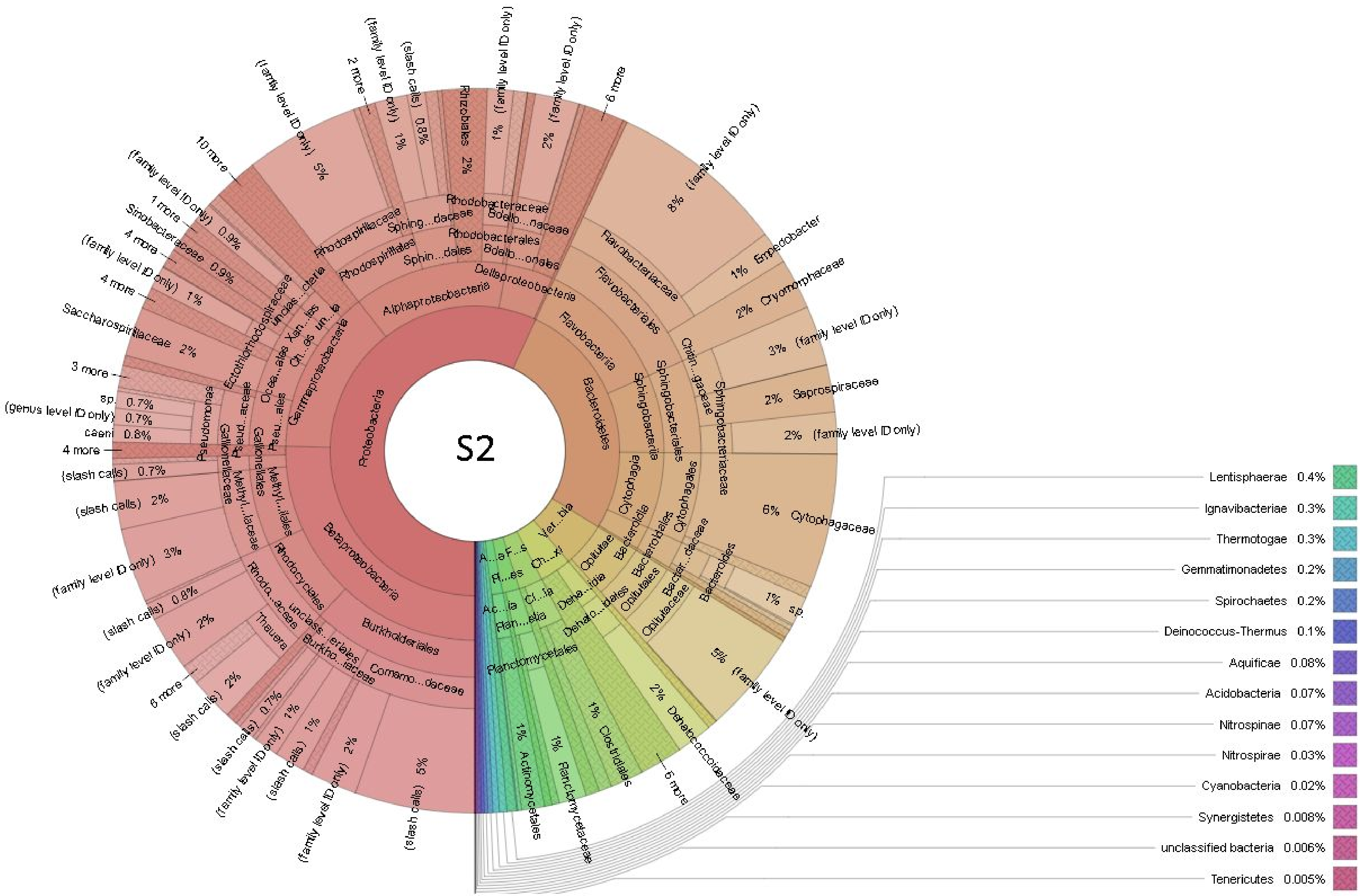

S2
Proteobacteria
Alphaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Deltaproteobacteria
Bacteroidetes
Flavobacteriia
Sphingobacteriia
Cytophagia
Flavobacteriales
Flavobacteriaceae
Sphingobacteriales
Sphingobacteriaceae
Cytophagales
Cytophagaceae
Burkholderiales
Rhodocyclales
Rhodospirillales
Rhodospirillaceae
Rhodobacterales
Rhodobacteraceae
Rhizobiales
Planctomycetales
Planctomycetaceae
Actinomycetales
Clostridiales
Dehalococcoidaceae
Saccharospirillaceae
Sinobacteraceae
Ectothiorhodospiraceae
Pseudomonas
Gallionellaceae
Thauera
Empedobacter
Cryomorphaceae
Saprospiraceae
Opitutaceae
Lentisphaerae 0.4%
Ignavibacteriae 0.3%
Thermotogae 0.3%
Gemmatimonadetes 0.2%
Spirochaetes 0.2%
Deinococcus-Thermus 0.1%
Aquificae 0.08%
Acidobacteria 0.07%
Nitrospinae 0.07%
Nitrospirae 0.03%
Cyanobacteria 0.02%
Synergistetes 0.008%
unclassified bacteria 0.006%
Tenericutes 0.005%

Fig.S3

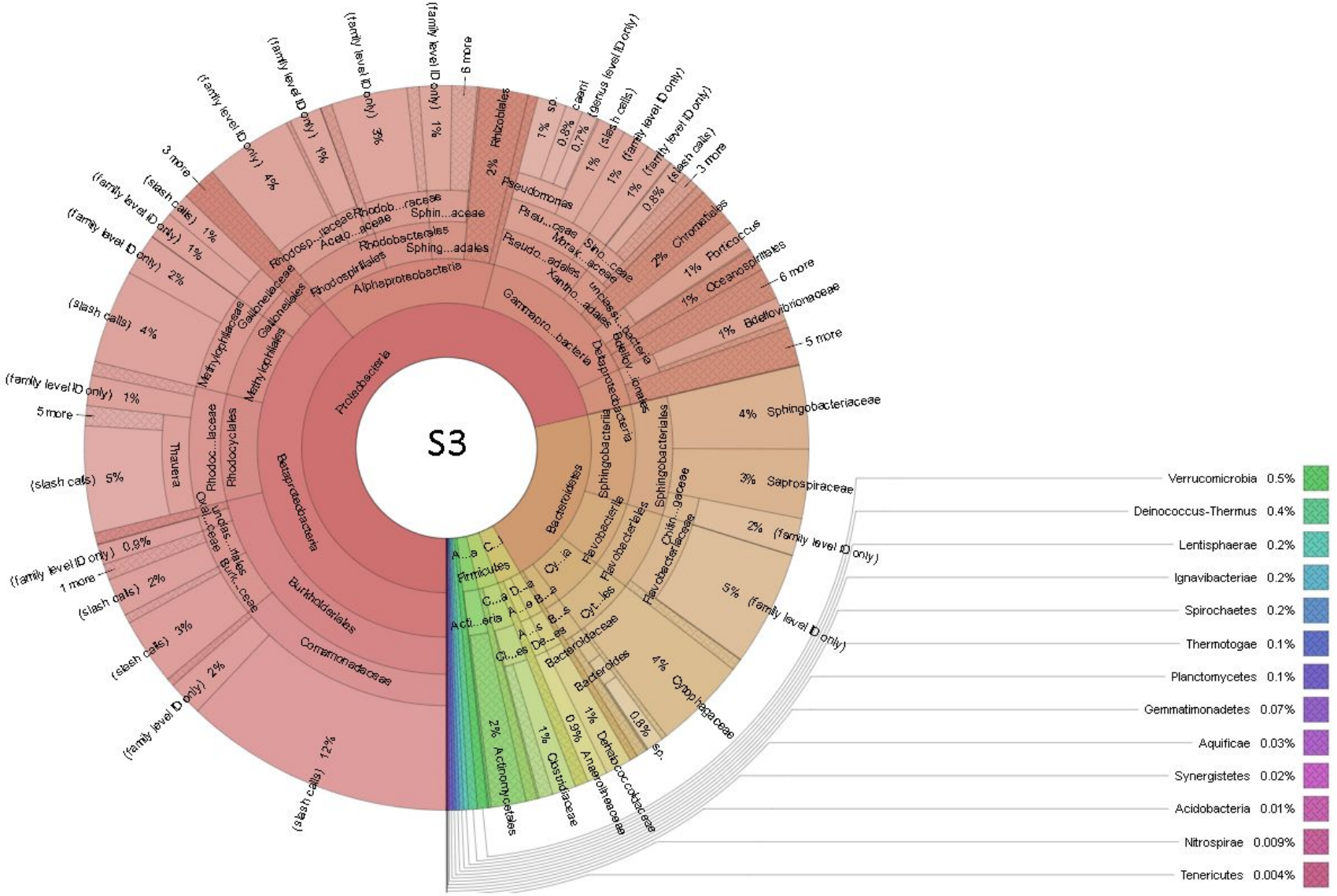

S3
Verrucomicrobia 0.5%
Deinococcus-Thermus 0.4%
Lentisphaerae 0.2%
Ignavibacteriae 0.2%
Spirochaetes 0.2%
Thermotogae 0.1%
Planctomycetes 0.1%
Gemmatimonadetes 0.07%
Aquificae 0.03%
Synergistetes 0.02%
Acidobacteria 0.01%
Nitrospirae 0.009%
Tenericutes 0.004%

Fig.S4

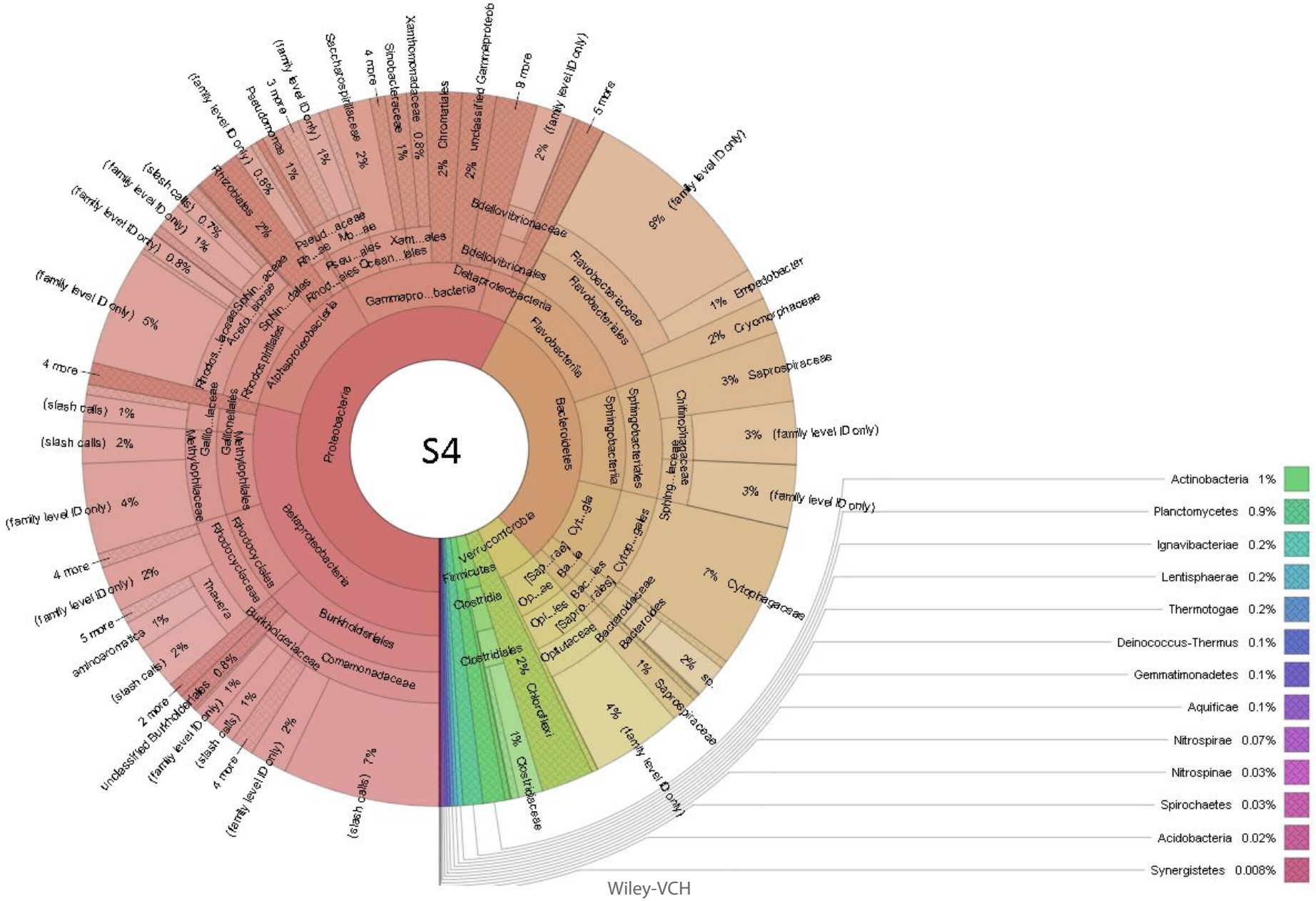
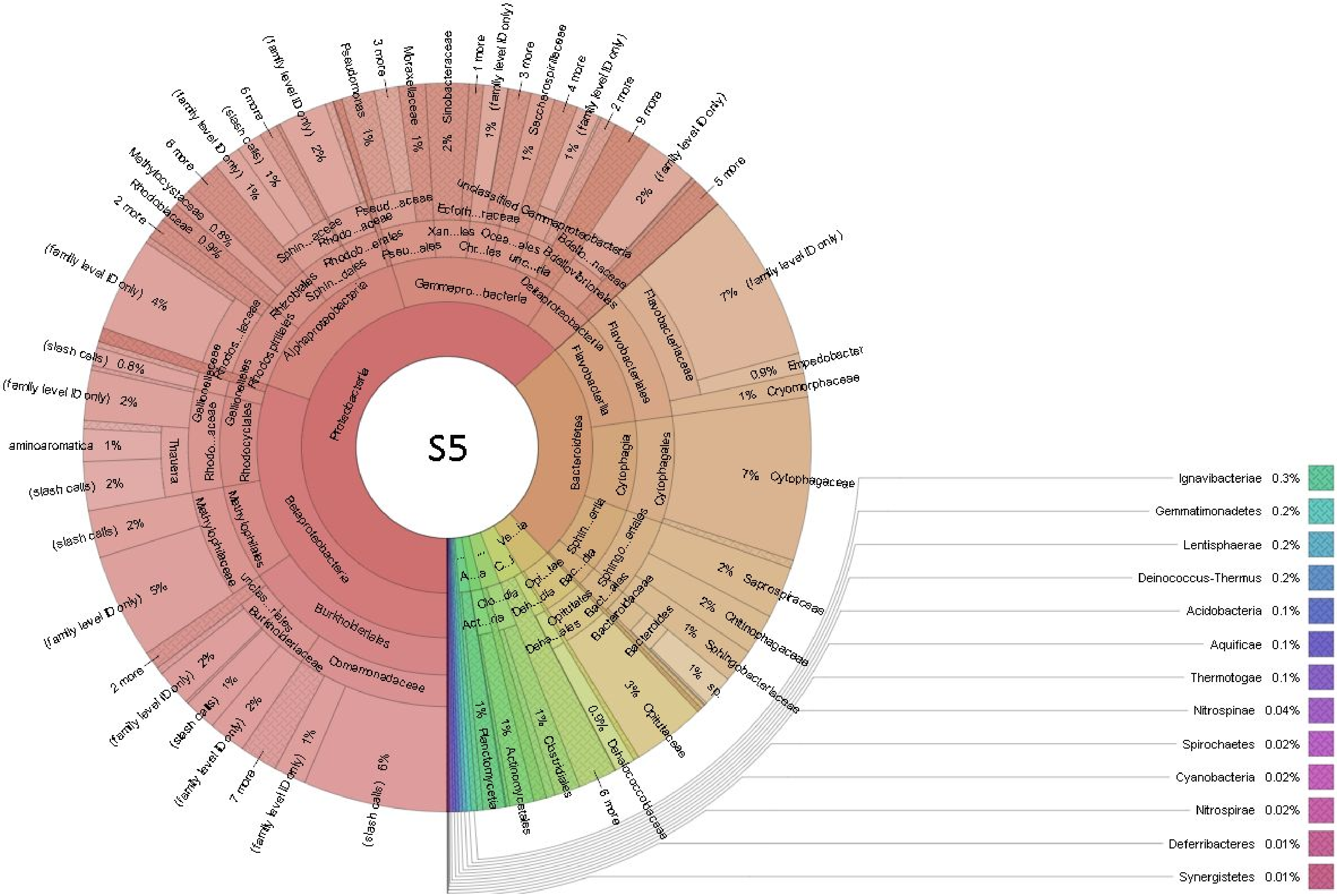

S4
Actinobacteria 1%
Planctomycetes 0.9%
Ignavibacteriae 0.2%
Lentisphaerae 0.2%
Thermotogae 0.2%
Deinococcus-Thermus 0.1%
Gemmatimonadetes 0.1%
Aquificae 0.1%
Nitrospirae 0.07%
Nitrospinae 0.03%
Spirochaetes 0.03%
Acidobacteria 0.02%
Synergistetes 0.008%

Fig.S5


S5
Proteobacteria
Bacteroidetes
Gammapro...bacteria
Alphaproteobacteria
Betaproteobacteria
Deltaproteobacteria
Flavobacteriia
Cytophagia
Flavobacteriales
Flavobacteriaceae
Cytophagales
7% Cytophagaceae
7% (family level ID only)
0.9% Empedobacter
1% Cryomorphaceae
2% Saprospiraceae
2% Chitinophagaceae
1% Sphingobacteriaceae
3% Opitutaceae
0.9% Dehalococcoidaceae
1% Clostridiales
1% Actinomycetales
1% Planctomycetia
Burkholderiales
Comamonadaceae
Burkholderiaceae
Methylophilales
Methylophilaceae
Rhodocyclales
Gallionellales
Gallionellaceae
Thauera
aminoaromatica 1%
Rhodospirillales
Rhizobiales
Sphingomonadales
Methylocystaceae 0.8%
Rhodobacteraceae 0.9%
Moraxellaceae 1%
Pseudomonas 1%
Sinobacteraceae 2%
Saccharospirillaceae 1%
unclassified Gammaproteobacteria
Bdellovibrionales
Ignavibacteriae 0.3%
Gemmatimonadetes 0.2%
Lentisphaerae 0.2%
Deinococcus-Thermus 0.2%
Acidobacteria 0.1%
Aquificae 0.1%
Thermotogae 0.1%
Nitrospinae 0.04%
Spirochaetes 0.02%
Cyanobacteria 0.02%
Nitrospirae 0.02%
Deferribacteres 0.01%
Synergistetes 0.01%