Sulfur cycle microbiota in extremely contaminated Technosol with ongoing pedogenesis: culture-dependent and metagenomic approach.

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Abstract

Understanding the microbial communities involved in the global sulfur cycle is crucial for comprehending key biogeochemical processes on Earth. Most studies, however, are biased towards the marine ecosystems, as terrestrial sulfur cycle rarely investigated. In this study, we employed culture-dependent techniques and metagenomics to investigate functional, taxonomic and culturable of sulfur-cycling bacteria in extremely contaminated soils. Our findings reveal that elevated concentrations of xenobiotic pollutants, bulk sulfur content, and fluctuating redox conditions have shaped a unique microbial community involved in sulfur transformation. This community comprises non-canonical taxa carrying out typical reactions (e.g., sulfate-reducing acidobacteria), known taxa engaged in various oxidative, reductive, and organosulfur transformations (e.g., sulfur oxidizing alpha-, gamma-, and betaproteobacteria), as well as previously unidentified taxa that remain uncultured and may possess novel genes related to sulfur compound metabolism. Additionally, multiple taxa contribute to the replenishment of readily consumable compounds like sulfite and thiosulfate, facilitating cryptic biogeochemical cycling of molecules with high turnover rates but low absolute abundance. Our analysis also supports the idea that known sulfate-reducing bacteria behave differently in soils compared to aquatic habitats and belong to the soil rare biosphere, still greatly affecting their ecosystem.

Keywords: soil microbiome, sulfur cycle, sulfate-reducing bacteria, metagenome-assembled genomes, Technosols, pollution, rare biosphere.

Introduction

The study of microbiota involved in the sulfur cycle was initiated in the late 19th century through groundbreaking work by Winogradsky and Beijerinck (Lens et al., 2002). One of the most well-known experiments that paved the way to study sulfur cycle microbiota (SM, hereafter) was the establishment of the Winogradsky column. The column represents a model of submerged ecosystem with C and S sources. Over time, within the column exposed to light, the microbial community stratifies along oxygen and iron sulfide gradients (Zavarzin, 2006), giving rise to a diverse sulfur-transforming community. What thus initially began as the first observation of microbes directly linked to their ecological traits has now revealed an enigmatic picture of an immensely diverse group of microbes. These microbes are ubiquitous and possess the ability to mediate numerous traceable and cryptic biogeochemical transformations, making it increasingly challenging to assign them to specific processes, transformations, or ecosystems. Most of SM diversity concentrates within dissimilatory pathways, where inorganic sulfur compounds are metabolized and/or deposited outside the cell (Anantharaman et al., 2018). At least five compounds – elemental sulfur, polysulfides, thiosulfate, tetrathionate, and sulfite – spanning the most oxidized and reduced states of sulfur, can be oxidized, reduced or disproportionated by at least one distinct group of microorganisms, with many overlapping groups also capable of these processes. Recently, microbes liberating and reducing sulfite moiety from organosulfonates were also recognized in the context of dissimilatory sulfur metabolism (Hausmann et

al., 2018; Peck et al., 2019; Ye et al., 2023). The diversity of taxa oxidizing sulfide or reducing sulfate is even bigger. Two major sulfur-related operons, Sox and Dsr gene clusters, can act in a reverse way, thus widening our perspective on a possible genetic diversity and capabilities of the SM. Many sulfur oxidizing bacteria (SOB) demonstrate conspicuous morphology, from well-known sheathed filamentous *Beggiatoa* and *Thiothrix* to recently described giant *Thiomargarita* species (Volland et al., 2022; Ravin et al., 2022). On the other hand, sulfate-reducers (SRB) are unprecedentedly diversified taxonomically, with evaluated number of unique operational taxonomic units (OTU) exceeding tens of thousands (Vigneron et al., 2018). In some cases, SRB exhibits a unique natural behavior: they do not proliferate and maintain a low-density population, yet they remain transcriptomically and metabolically active (Hausmann et al., 2019). In that state, cells actively reduce sulfates and significantly influence their ecosystem, which led to the idea of SRB comprising rare biosphere. It is also has become evident that other microbial taxa, not affiliated with SM, can bear complete Dsr operons (Anantharaman et al., 2018). For example, a unique type of metabolism – degradation of complex plant biopolymers coupled to dissimilatory sulfate reduction – was recently discovered in Acidobacteriota members (Dyksma and Pester, 2023). Finally, a rare case of prokaryotic high-taxonomic level endemism has recently been observed in a relic Antarctic lakes. In this habitat, a novel class of bacteria within candidate Electryoneota phylum exists, possessing the complete genetic machinery necessary for both sulfate and sulfur reduction to sulfide (Vigneron, Vincent and Lovejoy, 2023).

SRB and SOB actively participate in elemental cycling beyond sulfur. SRB can degrade hydrocarbons, reduce and precipitate heavy metals from iron to plutonium (Merino et al., 2023), compete with methanogens for substrate (Hu et al, 2022), degrade end- or byproducts of others' bacteria metabolism; besides, they are also capable of direct extracellular electron transport (Lovley and Holmes, 2022). SOB members can fix CO2, oxidize heavy metals, and convert insoluble sulfide minerals. Some phototrophic members of SOB utilize near-infrared light, thus introducing new pathways for energy to enter biogeochemical systems (Beatty et al., 2005). In addition, some SRB and SOB can respire nitrites. Most of SM belongs to microbial dark matter or resists growing in the lab. Many naked-eye-observable SOB has yet to be isolated in axenic cultures, while the hundreds of SRB groups are known solely from metagenome binning data (Li et al., 2022; Vigneron, Vincent and Lovejoy, 2023). SM comprise functionally and taxonomically diverse groups of prokaryotes and are crucial for the global ecosystem.

However, the modern picture of SM biology and ecology is biased towards the processes in aquatic environments (Li et al., 2022) (oceans, lakes, groundwater) and controlled biotechnological systems (bioleaching tanks, corroded metal structures). Among terrestrial habitats, rice paddy fields, peats and volcanic muds are well represented in current studies, while a wide spectrum of soils, phyllosphere, arid environments, caves and host-associated consortia are largely overlooked. Furthermore, for almost a century, we have been witnessing the emergence of entirely new types of environments because of human activities and the interactions between technology and nature. These include highly contaminated Technosols, landfills, urban areas, as well as metal and/or concrete infrastructure. All these niches are readily colonized by SRB and SOB.

Because of the development and active implementation of functional metagenomic approaches, it has become easier to characterize complex microbial consortia. The binning and subsequent annotation of metagenome-assembled genomes (MAGs) already allows scientists to bring new microbes into a laboratory condition and comprehensively study them. However, metagenomic methods alone are unable to resolve complexity of an environmental microbial consortia; it is important to combine culture-dependent and molecular techniques. In the present work, we describe SM microbiota of a unique ecosystem: soil with severe polymetallic, bulk sulfur and PAHs contamination, formed on a site of a dried-up lake. The main hypothesis is that a unique and diverse sulfur-transforming part of the soil microbial community has evolved and

stabilized as a result of the adaptation to a rare set of conditions: high concentrations of bulk sulfur, extreme technogenic contamination and regular flooding events.

Materials and methods

Design of the study

The study aims to characterize the sulfur cycle microbiota in the polluted soils of the former Atamanskoe Lake. The first step involved analysis amplicon sequencing data covering the entire lake territory and adjacent soils. In total, 27 samples were taken, with 22 belonging to the lake territory and 5 to the adjacent areas. These samples were used for DNA extraction and amplification of the 16S rRNA gene. Based on the analysis of that data, 9 samples were selected for determining the physicochemical properties of the soils, extracting and sequencing total DNA to characterize the community's taxonomic profile and reconstruct genomes of individual species, as well as for enumeration of sulfate-reducing and sulfur-oxidizing bacteria.

Object description

The soils of the dried-up Atamanskoe Lake, located at the South of Russia, served as the object of study. As a result of the usage of the lake's territory as a sludge collector from the 1960s to the mid-1990s, a large part of industrial waste from chemical production accumulated in its bottom sediments. Over the past two decades, a Technosol has formed on the bottom sediments of Atamanskoe Lake, characterized by unprecedented technogenic pollution, such as the content of HM and PAH, (Linnik et al., 2022). The amount of technogenic sludge in the lake is about 400,000-420,000 m^3, containing up to 98,000 mg kg^{-1} of zinc, 1,050 mg kg^{-1} of lead, 13.6 mg kg^{-1} of mercury, 52 mg kg^{-1} of arsenic, and 303 mg kg^{-1} of copper (Minkina et al., 2019). Compared to average lithospheric values, much higher concentrations of Zn, Cd, Pb, Cu, Ni, and Cr are observed, hundreds of times higher for Zn, ten times higher for Cd and Pb, and several times higher for Cu, Ni, and Cr (Bauer et al., 2018). The total concentration of PAH ranges from 499 to 7,178 mg kg^{-1} (Sushkova et al., 2020). According to the World Reference Base for Soil Resources (WRB), the soils are classified as Spolic Technosols. As a control, soil from a floodplain site near Lake Atamanskoe with background values of PAH and TM indicators, classified as Fluvisol, was taken. In addition, the Atamanskoe soil is characterized by a unique mineralogical composition, where more than half of the minerals are sulfate minerals, and a sulfide geochemical barrier operates in the deep soil layers (Bauer et al., 2018).

Sample collection

Soils were collected by taking an average sample from five points, packaged in sterile plastic bags, and stored at t=4°C until subsequent analysis (ISO 10381-1, 2002). For the screening amplicon study, 27 samples were selected, of which 22 represented the lake area and 5 were background soil samples. For the determination of physicochemical properties, microbiological cultures, and shotgun metagenomic analysis, 9 samples were chosen from the 27. Soil cores were separately excavated to a depth of 50 cm for these samples, from which sub-samples were taken at depths of 15, 30, and 45 cm. The map of soil sample collection is presented in Figure 1.

Figure 1. Soil sample collection map: the green zone indicates a former island, currently covered with abundant vegetation, and the gray zone represents the area of the Technosol (former lake).

Soil physicochemical analysis

The data on pH, concentration of PAH and HM mentioned in the study were obtained using the methods specified in (Gorovtsov et al., 2021). In the study, the data were used to analyze their correlation with the content of bacteria mediating the sulfur cycle. The table containing the soil chemical data is provided in the Supplementary material table 1.

DNA extraction and sequencing

Metabarcode sequencing. According to the manufacturer's instructions, total DNA was extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals, UK) for sequencing the 16S rRNA gene. Metagenomic libraries were prepared following the "Preparation of 16S rRNA Gene Amplicons for the Illumina MiSeq System" protocol. The amplification of the V3-V4 region of the 16S rRNA gene was performed using the following prokaryotic primers: forward - TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG; reverse - GTCTCGTGGGCTCGGAGATGTGTATAAGATACAGGTATCACCHG. Sequencing was carried out on the MiSeq platform (Illumina) with v3 reagents (600 cycles) at the Interdisciplinary Shared Facility of Kazan Federal University (Amplicon et al., 2013).

Shotgun metagenomics. Total soil DNA was extracted using the FastDNA™ Spin Kit for Soil. For library preparation, the NEBNext Ultra II DNA Library Prep Kit (NEB) was used according to the manufacturer's instructions. Genomic DNA was fragmented using the Covaris S220. The quality of the prepared libraries was assessed using the 2100 Bioanalyzer (Agilent) with the DNA High Sensitivity Kit (Agilent). The DNA concentration in the samples was quantified using the Qubit 2.0 instrument. Sequencing was performed on the MGI platform (DNBSEQ).

Bioinformatics and statistical analysis

***Analysis of 16S rRNA gene pool sequencing data*:**

The filtration of raw sequencing data, quality control, removal of chimeras, and merging of paired reads were performed using Trimmomatic, Pandaseq, and VSEARCH programs. Subsequently, the filtered reads were fed into the Kraken2 program (Wood, Lu & Langmead, 2019) and classified using the SILVA database. The results from Kraken2 were further processed using the Bracken software.

Analysis of shotgun sequencing data:

Quality control: Reads corresponding to human DNA were removed using the BMTagger program. Read duplicates were removed using clumpify from the bbmap package. The FastQC program was used for quality control. Adapter sequences, low-quality reads (Q < 10), and short reads (<40 bp) were removed using Trimmomatic ver. 0.38.

Assembling and binning: Quality-controlled reads were assembled into contigs using metaSpades. The contigs were then binned using three independent programs: Metabat2, CONCOCT, and Maxbin2. The bins obtained from these programs were merged into consensus bins using the DAS-Tool program (Kang et al., 2019; Alneberg et al., 2013; Wu, Simmons & Singer, 2016; Sieber et al., 2018). Further processing of the bins, including contig removal based on tetranucleotide frequency, GC content, and presence/absence of clade-specific marker genes, was performed using the MAGpurify program (Nayfach et al., 2019). Quality control and assembly statistics of the bins were assessed using the CheckM program (Parks et al., 2015). The bins that passed all quality control steps, had an assembly completeness >60%, and contamination level <15% were considered MAGs. Annotation was performed using the Ghost Koala and EggNOG. Based on the annotation outputs, conclusions about the functions of the reconstructed taxa in the community were made. All quality-filtered MAGs were taxonomically classified using TypeMat and GTDB datasets available at MiGA online

resource (Rodriguez-R et al., 2018). In case when taxonomic assignments varied between the two datasets, GTDB inference were used.

Analysis of metagenomic taxonomic profiles: Quality-filtered reads from the quality control step were subjected to taxonomic identification using the Kraken2 program with the PlusPFP dataset. Re-estimation of taxon proportions in the community based on the reads counts was additionally performed using the Braken program. Further analysis and visualization were conducted in the R 4.3.2 programming environment.

Quantitative analysis of sulfate-reducing bacteria

The determination of sulfate reducers was carried out using the most probable number (MPN) method as follows: Bacteria were separated from soil particles in a sterile mortar using a rubber pestle (soil-to-water ratio of 1:10), and then shaken on a rotary shaker in flasks for 40 minutes. A 1:10 cell suspension, after separation from soil particles, was serially diluted up to 10^{-7}. Each dilution, in 4 replicates, was then used to inoculate a tube containing 20 milliliters of modified Postgate B medium with pH of 7.5, the composition of which is specified in (Kushkevych, 2020). The tubes were sealed with rubber stoppers and incubated at t=30°C for three weeks, after which the number of replicates showing iron sulfide precipitation was recorded for each dilution. The recorded data were used to calculate the most probable number using with the MPN package in R 4.3.2., which utilizes a modified version of the classical MPN calculation (Ferguson & John, 2019).

Quantitative analysis of sulfur-oxidizing microorganisms

Soil dilutions were prepared as described above. Then, 1 milliliter of each dilution was used to inoculate 10 mL of Waksman medium with elemental sulfur (pH = 7.5), poured in 50 mL plastic containers 50 mL (each dilution in triplicate). Incubation was carried out for two months, t=25°C. The presence of growth was determined based on three indicators: a decrease in pH to values <5, visible solubilization of elemental sulfur, and visible microbial growth. A positive result was considered when the medium exhibited an acidic reaction after the incubation period, and microscopy of solubilized sulfur demonstrated the presence of cells. The medium's reaction was tested using acidity indicator solutions (methyl red, bromocresol green). MPN was calculated as described above.

Quantitative analysis of thiosulfate-oxidizing microorganisms

Soil dilutions were prepared as described. Then, 100 microliters of each dilution were used to inoculate Beijerinck medium with thiosulfate (pH = 7.5) and pH indicator bromocresol green (0.04%), distributed at 600 µL per deep-well polystyrene plate with a well volume of 2.5 mL (each dilution in quadruplicate). Incubation was carried out for one month, t=25°C. The presence of growth was determined by a color change of the pH indicator from violet-blue to yellow and the formation of a yellow cell precipitate at the bottom of the well. MPN was calculated as described above.

Statistical analysis

Standard statistical tests, such as the Shapiro-Wilk test for normality of distribution, the pairwise Wilcoxon test, and Spearman correlation analysis, were carried out using base packages in the R 4.3.0. If it was necessary to compare groups of unequal sample sizes, the required statistical test was performed based on 100 random subgroups of a bigger group, with a size of such subgroup equal to the overall size of a smaller group). Ecological analysis (calculation of biodiversity indexes and ecological distances) was also performed in R using the vegan package (Oksanen, 2013).

Results and discussiuon

Metabarcode screening shows predominance of spore-forming SRB and acidophilic chemolithoautotrophic SOB in Technosols

In addition to typical members of the reductive and oxidative stages of the sulfur cycle, such as *Desulfovibrio* and *Thiobacillus*, many other microorganisms are involved in sulfur cycling. Therefore, for comprehensive screening of the obtained metagenomic data, keyword searches, systematic reviews and articles containing information about species and genera with documented metabolism of inorganic sulfur were used. Information on the oxidative stage was obtained from studies (Ghosh & Dam, 2009; Kumar et al., 2018), on the reductive stage from studies (Rabus, Hansen & Widdel, 2006; Anantharaman et al., 2018), and on sulfur-disproportionating taxa from the review (Slobodkin & Slobodkina, 2019). Only taxa represented by at least two reads of the ribosomal gene were taken into consideration. Additionally, sampling sites were divided into three groups: background (control) soils, Atamanskoe soils with neutral pH, and Atamanskoe soils with low pH. The screening results are presented in Figures 2 and 3. A total of 35 genera capable of dissimilatory metabolism of sulfur compounds were identified in the dataset. The majority of taxa were represented by a small number of the reads. The most abundant were the genera *Desulfosporosinus* (1927 reads), *Desulfallas* (700 reads), *Desulfofarcimen* (593 reads), and *Desulfitomicrobium* (468 reads). Among them, *Desulfosporosinus* surpassed the rest both in terms of read count and its proportion, reaching up to 3-4% of the entire metagenome in some samples (d2, 13, 23, 30). The greatest taxonomic diversity was observed in samples 43 and d3, while the lowest was observed in samples t6, 1, and 24, with no sulfate reducers detected in sample 16 at all. Some lake samples had similar read counts to the background soils, specifically samples 28 and t6, located in an area with abundant tree cover, as well as sample 36.

Notably, there is a predominance of spore-forming genera belonging to the Bacillota phylum: *Desulfosporosinus*, *Desulfofarcimen*, *Desulfitomicrobium*, *Desulfurispora*, *Thermincola*, and *Pelotomaculum*. This may reflect the variable conditions of Atamanskoe soils, particularly the fluctuating redox potential due to periodic flooding. Such conditions inevitably create selective pressure in favor of spore-forming species.

Figure 2. Diversity and abundance of SRB genera based on amplicon data; SR - sulfate reducers, SR+SD – disproportionating microorganisms.

Among the sulfur bacteria, 24 genera were identified, of which 7 are phototrophs (Figure 3).

Figure 3. Diversity and abundance of SOB genera based on total soil DNA sequencing; ASO – autotrophic sulfur oxidizers; GSB - green sulfur bacteria; PSB - purple sulfur bacteria; PNSB - purple non-sulfur bacteria.

No significant diversity differences or dominance within or between samples were observed for the PNSB group. A small number of reads were recorded for the purple sulfur bacteria *Thiovirga*, and only two samples contained low amounts of GSB *Oscillochloris* detected. Conversely, many colorless sulfur bacteria were recorded. *Thiobacillus*, *Sulfobacillus*, *Sulfurifustis Acidiphilium*, and *Sulfuriferula* were the predominant genera. The most diverse sites were 43, 35, d1, d4, d3, and 14. Some genera reached exceptional proportions of reads in samples d4, wk, tr1, and 35, accounting for 5.5%, 5.4%, 7%, and 10% of all sample reads, respectively. The purple non-sulfur bacteria were distributed most evenly among the groups, although overall diversity was higher in Atamanskoe. In the control soils adjacent to the lake area, only a small number of sulfur-oxidizing taxa were detected, like that of the Atamanskoe samples t6 and 28. The spatial distribution of the sulfur microbiome across the Atamanskoe territory is also of a great interest (Figure 4).

Figure 4. Spatial distribution of SM genera across the Atamanskoe territory.

The main abundance of both sulfur bacteria and sulfate reducers is concentrated in the former lake channels, while sulfur bacteria are only found in large quantities in isolated samples in the border territories and on the island.

Based on the above data, it can be concluded that there is high heterogeneity in the sulfur microbiome of the soils formed in the lake territory. It is worth noting that for such non-systematic and diverse groups of bacteria like sulfate reducers, the use of ribosomal genes may not be informative for community classification. False-positive signals and an inflated number of unique genera can be expected. However, all of this indicates a greater diversity of 16S rRNA gene variants. Additionally, PCR errors and variations in primer annealing can significantly distort the community profile. For a more in-depth study of the microbial diversity in the sulfur cycle, samples were selected from each soil subgroup: tr1, 13, and d2 from acidic soils in Atamanskoe, d4, d3, 42, t6, and 28 from neutral soils, and sample 3 as background soil.

Analysis of total soil DNA shows predominance of SM, but contradicts taxonomy distribution inferred from amplicon data

Before assembling reads into contigs, the workflow for sequencing total soil DNA was not different from that for amplicons. The same taxa were selected for analysis as in the amplicon metagenomes. A minimum threshold of 1,000 reads was set to include a taxon in the analysis. Figure 5 presents the profile of sulfate-reducing bacteria in 9 soil samples. The abundance and diversity differ significantly from the amplicon profile. *Desulfosporosinus*, *Desulfitobacterium*, and *Desulfofarcimen* are no longer dominant. The most abundant were *Desulfuromonas*, *Pseudodesulfovibrio*, *Desulfovibrio*, and *Desulfosarcina*. The background soil did not differ much from the lake soils in terms of absolute abundance of sulfate reducers. *Desulfuromonas*, a typical molecular sulfur reducer, was found in a significant amount.

Figure 5. Diversity and abundance of SRB genera based on total soil DNA sequencing; SR - sulfate reducers, SR+SD – disproportionating microorganisms.

Sulfur-oxidizing taxa identified by shotgun sequencing are presented in Figure 6. *Sulfuriferula*, *Sulfurifustis*, *Sulfuricaulis*, and *Thiobacillus* are present in high numbers in the tr1 sample. This sample also has the highest MPN values for sulfur and thiosulfate oxidizing microorganisms. The same pattern is observed for the other samples and groups: higher abundance and diversity in Atamanskoe soils. For example, the tr1, 13, and d2 samples have a significant presence of PNSB bacteria, such as *Rhodoferax* and *Rhodopseudomonas*, as well as a greater variety of GSB, such as *Chlorobium* and *Pelodictyon*, although in lower counts. Although in small counts, almost all screened taxa were present in background soil sample, indicating that sulfur-oxidizing bacteria can be found in typical soils too, as a rare part of the community.

Figure 6. Diversity and abundance of SOB genera based on total soil DNA sequencing; ASO – autotrophic sulfur oxidizers; GSB - green sulfur bacteria; PSB - purple sulfur bacteria; PNSB - purple non-sulfur bacteria.

Diversity of SM is higher in acidic soil samples

To understand the differences in SM diversity estimates of biodiversity were calculated (Figure 7). All results were calculated based solely on abundance of SM genera, excluding other taxa and taxonomic categories.

Figure 7. Analysis of alpha- and beta-diversity of the studied soils based on the content of sulfur cycle microorganisms; Shannon index estimates two metagenomic datasets (top); the ordination of samples based on the bray-curtis dissimilarities of their sulfur microbiome (bottom).

Shannon index, calculated based on the read counts of all the genera mentioned above, was used to indicate alpha-diversity. To determine the relationships between samples (beta-diversity), an NMDS analysis was conducted using bray-

curtis dissimilarities. As seen from the figures, the distribution of the sulfur microbial community is influenced by both spatial factors and soil acidity. Based on amplicon data, the diversity of the sulfur cycle microbiota is significantly higher in acidic soils compared to neutral soils in Atamanskoe, as well as the background soil (Wilcox test, $p < 0.01$ and $p < 0.05$, respectively). Neutral soils do not significantly differ from the background in terms of alpha-diversity. In addition to the H index, the three groups significantly differ from each other in terms of the composition and structure of the sulfur microbial community. Beta-diversity analysis shows three clusters - acidic soils, neutral soils, and background soils (with three neutral Atamanskoesoil samples showing similarity to background soils, the same that previously demonstrated the lowest abundance of sulfur microbiome). All three groups significantly differ from each other ($p < 0.001$, PERMANOVA) in terms of sulfur microbiome composition. Based on amount of shotgun metagenomes, it is hard to resolve community dissimilarities between groups; still, t6 and 28 samples cluster with background soil, while other samples demonstrate high dissimilarity.

Pollutants content is associated with SM abundance

In addition to acidity level, the content of PAHs and certain HMs was also significantly associated with both the abundance and diversity of the sulfur cycle microbiota. Table 1 presents the results of the Spearman correlation analysis between these indicators and the concentrations of Cu, Pb, Zn, Cd, and the sum of 16 types of PAHs.

Table 1. Spearman correlation coefficients between pollutant concentrations and statistics of sulfur microbiome. *'Ns'* – non-significant, '*' – *p* value < 0.05, '**' – *p* value < 0.01.

Parameter	Zn	Pb	Cd	Cu
SRB abundance	ns	ns	0,5 **	ns
SOB abundance	ns	0,41 *	0,54 **	ns
SM abundance	ns	0,39 *	0,55 **	ns
Shannon diversity	ns	ns	0,43 *	ns

The diversity of total SM abundance was significantly correlated with bulk Cd content, as both SRB and SOB demonstrated moderate correlation with this indicator. Pb concentration and total PAH content were significantly correlated only with SOB. It is worth noting that Zn is the main pollutant in Atamanskoesoils, followed by Cu. Zn concentration averages 7% in Atamanskoesoils, and the maximum recorded concentration was up to 15% (unpublished data). However, no significant correlations were found with Zn or Cu.

Much less variation in diversity is traceable in shotgun data. Taxonomic analysis using total DNA has a much higher resolution than the amplicon approach, resulting in greater sequence diversity within a sample. The absolute number of reads corresponding to sulfur microbiome taxa is small, but they are distributed more evenly across background soil. In technosols, on the other hand, there is a clear dominance of certain taxa (especially *Thiobacillus* and *Sulfuriferula*).

Overall (gamma) diversity of SM is higher in Technosols

As mentioned earlier, individual sites in Atamanskoeseem to be highly heterogeneous in terms of microbiome composition. Therefore, to characterize their biodiversity, data on taxon abundance from all sampling sites were used to calculate gamma diversity (Table 2).

Table 2. Descriptive statistics of the SM abundance and diversity, calculated from the data of two metagenomes.

Group	Gamma-diversity	Number of unique genera	Mean group abundance in a sample, %	Mean group read counts in a sample
Amplicon data				
Technosol, neutral soils	3,2	51	5,0 ± 0,7	1997 ± 274
Technosol, acidic soils	4	40	8,2 ± 0,5	4186 ± 249
Background soils	2,5	15	0,8 ± 0,3	168 ± 52
Shotgun data				
Technosol, neutral soils	4,8	72	3,2 ± 0,1	469359 ± 22270
Technosol, acidic soils	4	66	3,6 ± 0,2	610738 ± 48683
Background soil	3,5	51	1,7	222125

A more accurate assessment of the biodiversity of the former lake's sulfur microbiome was achieved. The overall diversity of the sulfur microbiome is higher when considering the composition of different sampling sites. The largest number of sulfur microbiome taxa on a genus level is observed in neutral soils (72 unique genera considering shotgun data, 51 unique genera considering amplicons). Estimates of overall diversity vary because different metagenomes provide different pictures of community evenness: amplicon data show a stronger predominance of the minority in neutral soils, while shotgun sequencing data show it in acidic soils. In addition, dominant species information was extracted from shotgun metagenomes (Figure 8).

The most abundant species of SM reflect extreme technogenic pollution of the studied area

Figure 8. Most abundant species of SM according to shotgun metagenome data.

Figure 8 shows SOB species represented by >=20000 reads, and the SRB species represented by >=2000 reads. As expected, the dominant species were found to be of the genera *Sulfuriferula*, *Thiobacillus*, *Sulfurifustis*, *Desulfuromonas*, and *Desulfovibrio*. Only two samples contained SRB taxa comprising enough reads to surpass the threshold, once again confirming the hypothesis of a small population size of sulfate reducers in soils, even under conditions most favorable for their growth (i.e., high sulfate concentrations and low redox potential). Additionally, it is interesting to note that among the dominant species, there were those associated with metallic pollution (*Sulfuriferula plumbiphila*) and polycyclic aromatic hydrocarbon contamination (*Desulfosarcina alkanivorans*). This supports the connections between pollutants and the sulfur cycle microbiota, as discussed in the introduction and confirmed by the correlation analysis (Table 1).

Enumeration of culturable SRB and SOB demonstrates differences in the ecology of the two groups

Quantitative assessment of sulfate reducers was conducted during the spring and autumn seasons over a period of two years, from 2021 to 2023 (Figure 9).

Figure 9. Quantitative assessment of sulfate reducers over the course of four seasons.

Estimates ranged from hundreds of cells per gram of dry soil (c gds^{-1}) to hundreds of thousands in different seasons and years between samples. The highest value was recorded in sample d3 during spring 2022, reaching over 6×10^5 c gds^{-1}., while the lowest value was observed in sample 28 during autumn 2022, with less than a hundred c gds^{-1}. Seasonal dynamics revealed both oscillations of SRB quantity in studied soils and its predominance in Technosol samples. SRB content-soil depth relationships were completely different from what one could expect for anaerobic microorganisms: no significant correlation between MPN and depth was observed (figure 10).

Figure 10. Quantitative assessment of sulfate-reducers depending on the depth of soil sampling.

The absolute numbers of SRB cells are actually similar to that of typical numbers observed in certain groups of soil microorganisms. For instance, soil yeasts typically range between thousands and hundreds of thousands c gds^{-1}, while culturable actinomycetes can reach counts in the millions c gds^{-1}. Estimated SRB content by culture-dependent methods demonstrates only a tendency rather than strict correlation: samples with high quantity of SRB-affiliated reads were shown to have highest MPN values. Laboratory cultivation conditions are almost non-existent in soil (such as permanent anoxia, excess sulfate concentrations and readily available nutrients), which may "smooth out" the true representation of the active portion of soil SRB population. Moreover, a typical approach is to express SRB community in cells or Dsr gene copies per volume or a mass of specific material. It might be justified in the case of water environment, since oxygen gradient is practically vertical and sulfate content is high, which leads to more or less linear relationship between SRB content and depth. Soils, though, are highly heterogeneous. Redox changes, particle-size distribution and availability of multiple, often solid-state electron acceptors should dramatically influence distribution of mostly anaerobic group of bacteria. Obtained MPN values indicate that SRB in soils indeed can be a part of rare biosphere and exhibit different behavior from that of aquatic habitats.

The seasonal variation in the abundance of sulfur-oxidizing microorganisms was not considered. The bar diagrams of MPN of thiosulfate oxidizers and elemental sulfur oxidizers are shown in Figure 11.

Figure 11. Quantitative assessment of sulfate oxidizers in Atamanskoe soils.

Abundance of oxidizers varied much less compared to SRB, reaching several tens of thousands per gram dry weight for both groups in all samples, except for tr1, where the minimum and maximum values were 4.5×10^4 c gds^{-1} for thiosulfate oxidizers and 1.1×10^6 c gds^{-1} for sulfur oxidizers, respectively. In contrast to the oxidation of elemental sulfur which was observed in all samples, no thiosulfate oxidation was observed during the monitoring period in samples 3, d3, t6, and 28. It is worth noting that media composition and laboratory conditions in SOB enumeration studies typically promote the growth of *Thiobacillus* species (Sattley & Madigan, 2006), however, in this study, no typical *Thiobacillus* cells were observed in any samples with elemental sulfur. Instead, visible filamentous microorganisms with complex morphology were observed throughout. Any occurrence of such microorganisms was accompanied by decrease in pH value by >2 points and visible sulfur solubilization. Interestingly, both amplicon and shotgun results identified *Sulfurifustis* among dominant SOB genera, which is filamentous and can oxidize a variety of sulfur compounds. Universal media for SOB do not exist, and each set of conditions will favor a specific group or taxon over the others. Moreover, we did not estimate cell numbers of photosynthetic SOB, so reported values should be considered underestimations. However, these results agree well with metagenomic results: SOB are large and impactful part of Atamanskoe' soil microbial communities.

Metagenome-assembled genomes

After quality control, shotgun metagenome reads were assembled into contigs, which were grouped into bins using three independent binning programs. A total of 288 MAGs were obtained with assembly completeness above 60% and contamination below 15%. Among them, 146 genomes achieved assembly completeness of >90%. Data on contig numbers, quantity of essential genes, closest relative in terms of average aminoacid identity (AAI), contamination and other statistics were obtained using both MiGA and CheckM software. All the genomes were then annotated using KEGG GhostCoala tool and EggNOG web resource. Raw data produced by EggNOG were searched against a manually compiled list of sulfur cycle genes (104 genes in total, supplementary material table 2). A total of 50 MAGs were selected based on the presence of individual genes or gene clusters associated with sulfur cycle metabolic pathways. Taxonomy and assembly statistics for selected genomes are presented in table 4. Among 50 MAGs, only two were classified at the species level: *Pseudomonas yamanorum* (MAG 42_83) and *Sulfuriferula multivorans* (MAG d4_19). Most of the other MAGs reached only phylum,

class, order or family level of identification. MAGs corresponded to the following phyla: Pseudomonadota (32), Acidobacteriota (8), Binatota (7), Gemmatimonadota (1), Nitrospirota (1), Chloroflexota (1).

Table 4. Taxonomy, assembly statistics and potential contribution to sulfur cycle of studied MAGs.

MAG	Taxonomy	Contigs	Completeness, %	Contamination, %	Potential contribution to S cycle
42_87	Nitrosomonadales	25	90.6	0.9	Oxidation of reduced sulfur compounds by Sox and oxidative Dsr complex
42_6	Gammaproteobacteria	27	100	1.9	Oxidation of reduced sulfur compounds by Sox and oxidative Dsr complex
42_98	Betaproteobacteria	29	99.1	1.9	Oxidation of reduced sulfur compounds by Sox and oxidative Dsr complex, Liberation of sulfite from organosulfonates
d2_10	Betaproteobacteria	46	99.1	6.6	Oxidation of reduced sulfur compounds by Sox and oxidative Dsr complex, Liberation of sulfite from organosulfonates
42_7	Alphaproteobacteria	56	88.7	0.9	Sulfate reduction to sulfite, Liberation of sulfite from organosulfonates
13_5	Binatota	58	96.2	6.6	Liberation of sulfite from organosulfonates, production of sulfide from methanethiol
13_92	Binatota	73	96.2	0.9	Liberation of sulfite from organosulfonates, production of sulfide from methanethiol, Dissimilatory sulfate reduction by Asr complex
13_24	Binatota	80	95.3	1.9	Liberation of sulfite from organosulfonates, production of H2S from methanethiol
tr1_59	Sulfuricellaceae	95	87.7	5.7	Oxidation of reduced sulfur compounds by Sox complex, sulfate reduction to sulfite, oxidation of H2S to elemental sulfur
13_56	Terriglobia	96	98.1	4.7	Dissimilatory sulfate reduction by Dsr complex, tetrathionate reduction
tr1_73	Nitrosomonadales	101	78.3	0.9	Oxidation of reduced sulfur compounds by Sox complex
13_90	Nitrobacteraceae	105	100	1.9	Oxidation of reduced sulfur compounds by Sox complex,

					Liberation of sulfite from organosulfonates
d2_14	Steroidobacterales	107	74.5	0	Oxidation of reduced sulfur compounds by Sox complex
tr1_38	Binatota	120	96.2	0.9	Liberation of sulfite from organosulfonates, production of H2S from methanethiol, dissimilatory sulfate reduction by Asr complex
13_37	Gammaproteobacteria	123	95.3	0.9	Oxidation of reduced sulfur compounds by Sox complex, Liberation of sulfite from organosulfonates
tr1_93	Hyphomicrobiales	129	99.1	0.9	Oxidation of reduced sulfur compounds by Sox complex, Liberation of sulfite from organosulfonates, Sulfate reduction to sulfite
d4_78	Acetobacteraceae	136	93.4	0.9	Oxidation of reduced sulfur compounds by Sox complex, Liberation of sulfite from organosulfonates
42_11	Acidiferrobacterales	137	87.7	0.9	Oxidation of reduced sulfur compounds by Sox complex, sulfite reduction to H2S, oxidation of H2S to elemental sulfur
d2_36	Terriglobia	139	96.2	0.9	Dissimilatory sulfate reduction by Dsr complex, tetrathionate reduction
d2_22	Nitrosomonadales	143	85.8	0.9	Oxidation of reduced sulfur compounds by Sox complex, Liberation of sulfite from organosulfonates
d2_130_2	Acidoferrales	161	94.3	1.9	Disproportionation of thiosulfate and sulfite, Liberation of sulfite from organosulfonates
42_54	Gammaproteobacteria	169	93.4	0.9	Oxidation of H2S to elemental sulfur
13_32	Binatota	174	94.3	1.9	Liberation of sulfite from organosulfonates, production of H2S from methanethiol, dissimilatory sulfate reduction by Asr complex, polysulfide disproportionation to sulfide
t6_1	Pyrinomonadales	182	96.2	5.7	Dissimilatory sulfate reduction by Asr complex
d2_57	Alphaproteobacteria	185	91.5	0	Oxidation of reduced sulfur

					compounds by Sox complex, Liberation of sulfite from organosulfonates
d4_9	Acidiferrobacterales	186	89.6	1.9	Sulfite reduction to H2S, oxidation of H2S to elemental sulfur
d3_50	Gammaproteobacteria	190	96.2	2.8	Oxidation of reduced sulfur compounds by Sox and oxidative Dsr complex
d2_114	Binatota	192	86.8	2.8	Liberation of sulfite from organosulfonates, production of H2S from methanethiol
d2_76	Alphaproteobacteria	210	90.6	0.9	Oxidation of reduced sulfur compounds by Sox complex, Liberation of sulfite from organosulfonates
d3_36_2	Gemmatimonadales	223	84	1.9	Oxidation of reduced sulfur compounds by Sox complex
tr1_72	Gammaproteobacteria	226	88.7	0.9	Oxidation of reduced sulfur compounds by Sox and oxidative Dsr complex
13_32_2	Nitrosomonadales	237	91.5	1.9	Oxidation of reduced sulfur compounds by Sox and oxidative Dsr complex complex, Liberation of sulfite from organosulfonates
d4_42	Gammaproteobacteria	252	95.3	4.7	Oxidation of reduced sulfur compounds by Sox and oxidative Dsr complex complex
d4_45	Alphaproteobacteria	279	95.3	1.9	Oxidation of reduced sulfur compounds by Sox complex, Liberation of sulfite from organosulfonates, Oxidation of sulfitfe to sulfate
d3_99	Betaproteobacteria	299	90.6	11.3	Oxidation of reduced sulfur compounds by Sox complex, Liberation of sulfite from organosulfonates, Oxidation of sulfitfe to sulfate
d4_12	Pseudomonadota	349	96.2	6.6	Sulfite reduction to H2S, Oxidation of reduced sulfur compounds by Sox complex, DMSO metabolism
d2_64	Terriglobia	406	77.4	5.7	Dissimilatory sulfate reduction by Dsr complex
42_42	Gammaproteobacteria	420	65.1	5.7	Oxidation of reduced sulfur compounds by Sox and oxidative Dsr complex

					complex
d3_108	Burkholderiales	431	95.3	0.9	Oxidation of reduced sulfur compounds by Sox complex, DMSO metabolism, Liberation of sulfite from organosulfonates
d4_19	*Sulfuriferula multivorans*	464	67.9	3.8	Oxidation of reduced sulfur compounds by Sox complex, Alternative oxidation of thiosulfate by Dox and glpE genes
d2_57_2	Casimicrobiaceae	494	67.9	0	Oxidation of reduced sulfur compounds by Sox complex, Liberation of sulfite from organosulfonates
tr1_27	Binatota	552	72.6	1.9	Production of H2S from methanethiol
42_23	Nitrosomonadales	642	65.1	4.7	Oxidation of reduced sulfur compounds by Sox and oxidative Dsr complex complex
d3_36	Nitrospirales	656	95.3	5.7	DMSO metabolism, sulfite oxidation
42_94	Gammaproteobacteria	673	84	9.4	Oxidation of reduced sulfur compounds by Sox and oxidative Dsr complex complex, Oxidation of H2S to elemental sulfur
13_10	Terriglobia	678	92.5	7.5	Dissimilatory sulfate reduction by Dsr complex, Liberation of sulfite from organosulfonates
42_83	*Pseudomonas yamanorum*	732	88.7	0.9	Oxidation of reduced sulfur compounds by Sox complex, Liberation of sulfite from organosulfonates, sulfur disproportionation
42_52	Terriglobia	910	83	6.6	Liberation of sulfite from organosulfonates
d_009	Pyrinomonadales	412	93.4	9.4	Thiosulfate oxidation, Liberation of sulfite from organosulfonates
d_019	Limnocylindria	1172	80.2	7.5	Alternative oxidation of thiosulfate by Dox, Sulfur disproportionation

There are no typical sulfate-reducers among obtained MAGs

The first striking observation was that no MAGs belonging to a typical member of SRB (such as *Desulfosporosinus*, *Desulfovibrio* or *Desulfuromonas*) were assembled. We hypothesized that most of the SRB-affiliated reads were not used for contigs and bins assembly due to the high taxonomic diversity in conjunction with low absolute abundance (based on metagenomic analysis, SRB comprised =<0.1% community share, which is a typical number for a rare biosphere taxon). To check whether it is true or not, raw reads of soil metagenomes were mapped onto corresponding contigs, and unmapped reads were used to assemble new sets of contigs. The latter were then inspected for the presence of SRB. Results are shown in figure 12.

Figure 12. Read abundance of typical SRB classes in sets of contigs, assembled from dropped reads. Samples t6 and 28 were excluded from this analysis based on the low content of SM members.

In most samples, quantity of dropped reads affiliated with SRB were higher or almost identical to that of assembled into initial contigs. This indirectly confirms the idea of SRB rarity and small population sized in soils. Genomes of diverse but nonabundant species have less chances to be assembled in a typical shotgun metagenome analysis pipeline. We decided to concatenate all contigs assembled from dropped reads and perform binning on that aggregated dataset. Such an approach yielded an additional group of 37 bins, of which only two passed quality control. They were added to the main MAGs dataset (samples d_009 and d_019). Interestingly, MAG d_019 was the only bin affiliated with Chloroflexota phylum obtained so far. No SRB were assembled as well. Other MAGs were investigated for the presence of dissimilatory sulfate reduction genes. Gene and pathway diversity is shown in figure 13.

Figure 13. The presence of sulfur cycle genes and corresponding pathways in all assembled MAGs.

Closest to SRB were MAGs within Binatota phylum

Seven MAGs were affiliated within Binatota, a candidate phylum. The closest taxon in terms of AAI was *Desulfacinum infernum* for all Binatota MAGs. Although they do not contain Dsr genes, all of them have SELENEBP1 gene, encoding methanethiol oxidase which converts methanethiol to H2S. Three of them also contain 1/3 or 2/3 genes that encode assimilatory sulfite reductase (Asr); six contain several genes for different enzymes able to produce sulfite from taurine (tauD), dimethylsulfone (ssuE) and alkanesulfonates (ssuD). Two MAGs have incomplete gene clusters for thiosulfate and sulfur disproportionation (hydB, phsA). Most of the MAGs also encoded dimethylsulfone monooxygenase, an enzyme that produces methanesulfonic acid, which also can be converted to sulfite by toluene monooxygenase tmoF, presented in one of the bins. No AprAB genes were found in this group, but sat gene appeared in all. Binatota members are widespread, though known only from metagenomic binning data. Analysis of Binatota genomes study revealed great metabolic versatility and methylotrophy (including the use of sulfomethanes and dimethylsulfone) as a common trait for more the one hundred Binatota-related MAGs (Murphy et al., 2021). It seems that members of the taxa are able to couple methylotrophy to sulfite reduction in studied soils.

Acidobacteriota members are predominant sulfate-reducers in studied Technosols

We obtained 8 Acidobacteriota bins, five are unclassified Terriglobia members, two belong to Pyrinomonadales (Blastocattelia class), and one belongs to a Candidate order Acidoferrales. Four MAGs encoded the complete set of genes required for dissimilatory sulfate reduction (sat, AprAB, DsrAB, DsrC) and two encoded 1/3 subunits of Asr and 1/2 of Dsr complex. Several Acidobacteriota MAGs show capability to liberate sulfite from organosulfonates using ssuE, ssuD and tauD, as in the case of Binatota, but also via sulfolactate lyase (suyAB) gene cluster in case of 13_10 MAG. The latter also encoded taurine-oxoglutarate transaminase which converts taurine to sulfoacetaldehyde. MAG t6_1 encoded capacity for production of methanethiol from methionine. All of that expands the distribution of organosulfur metabolism across

Technosol sulfur cycle microbiota beyond only Binatota group. Dissimilatory sulfate reduction is known for acidobacteriota from metagenomic and metatranscriptomic surveys, and recent study confirmed that they can couple degradation of complex plant biopolymers to sulfate reduction (Dyksma and Pester, 2023). Because of that, these bacteria often encode multiple glycosyl hydrolases domains. In accordance, acidobacterial MAGs in this study also encoded different families of glycosyl hydrolases (figure 14).

Figure 14. Abundance of glycosyl hydrolases and transferases families in acidobacterial genomes recovered in this study.

Several conditions could favor acidobacterial sulfate-reducers in such an environment. Firstly, acidobacteria can easily switch between anaerobic and aerobic metabolism, which is a useful trait in soils with regular flooding events. Secondly, due to the extreme level of contamination, hypoxic conditions, and overall metabolic suppression in Atamanskoe soils (Zamulina et al., 2021), slow-growing taxa (like Acidobacteriota) gain a selective advantage over other less-oligotrophic plant material degraders. Finally, many acidobacterial classes are acidotolerant, and 4/6 MAGs in this study were assembled from low pH soil metagenomes. Acidobacteria appears to be an important driver of C and S cycles in such habitats as peats (Hausmann et al., 2018), thawing permafrost (Woodcroft et al., 2018), revegetated acidic mine wasteland (Li et al., 2022) and, as it was shown in this study, in Technosols. Interestingly, acidobacterial bins (d2_35, 13_56, d2_130_2) were the only to encode ttr cluster genes such as ttrA and ttrB, which indicates potential tetrathionate reduction.

Technosol sulfur oxidizers are remarkably multifunctional

A total of 31 MAGs (mostly, Pseudomonadota) encoded complete or almost complete SoxAB genes clusters (KEGG pathway reconstruction confirmed the full or one-block-missing Sox cluster). Among them, 9 MAGs also encoded sat, AprAB, DsrAB, and were also confirmed to have genetic capacity of dissimilatory sulfate reduction. Although Dsr genes in typical sulfur oxidizers are often engaged in oxidation pathways, we have recently isolated filamentous bacterium capable of both elemental sulfur oxidation and sulfate reduction in laboratory conditions (unpublished data). Four MAGs encoded only Dsr genes along the Sox cluster, which may indicate both sulfite reduction to sulfate or sulfur compounds oxidation by a reverse Dsr pathway. Over half of SOB group also encoded a variety of organosulfonate monooxygenases and oxidoreductases. Moreover, the presence of sulfite oxidoreductases in their genomes (SUOX, SorA, SoeA, SoeB) suggests that they can utilize them to oxidize sulfite formed from organosulfonates. Several taxa showed incomplete sets of DMSO (dimethylsulfoxide) and DMSP (Dimethylpropiothetin) metabolism genes: bins 13_10, d4_12, 42_42, 42_6 and d3_36 could potentially transform DMSO from dimethylsolfone, while microbes under d2_14, 13_32_2, 13_37, d3_108, d3_99 and d2_76 bins can possibly transform metabolites of DMSP to Methylthiopropanoyl-CoA or methanethiol (which can in turn me metabolized by other member of community into sulfide or sulfite.

Several recovered MAGs were affiliated with taxa rarely mentioned in the context of sulfur oxidation metabolism. *Pseudomonas yamanorum* (MAG 42_83) and member of candidate Casimicrobiaceae family (MAG d2_57_2) were found to encode Sox genes, while Gemmatimonadales bacterium (MAG d3_36_2) genome, although possessed incomplete SoxAB cluster, contained SELENEBP1 and met genes. Excellent quality MAG d4_12 was classified only on phyla level (Pseudomonadota) and encoded Sox genes, SELENEBP1, ssuD, sfnG and aprAB+sat genes, again showing great sulfur-metabolizing multifunctionality. Finally, MAGs d2_130_2 (Acidoferrales), d_019 (Limnocylindria), d4_19 (*Sulfuriferula multivorans*) and d2_36 (Terriglobia) can possibly oxidize thiosulfate via Dox pathway.

Picture of the sulfur cycle in Atamanskoe soils

Results of our study suggest the following view on the sulfur cycle processes in Atamanskoe technosols. The initial contamination enriched the sediment and water of the former lake with sulfates of heavy metals, primarily zinc sulfate. This created a selective advantage for sulfate reducers, sulfur- and iron bacteria, especially acidophilic and acid-tolerant species, as the contamination also led to a significant decrease in pH. Over time, sulfate reduction resulted in the accumulation of metal sulfides in the sediment. At the same time, the drying out of the area and the gradual accumulation and decomposition of reed plant residues initiated soil formation processes. Flooding-evaporation cycles in the area, in turn, favor spore-forming sulfate reducers (Such as *Desulfosporosinus*), facultative anaerobes, and allow different sulfur cyclers to thrive in such conditions. Acidic, dried-out, and well-aerated areas of the Atamasnkoe territory with a variety of restored sulfur compounds create favorable conditions for aerobic sulfur-oxidizers such as *Thiobacillus* and *Sulfuriferula*. This is supported by both cultural and molecular data. It can be hypothesized that in such quantities, chemo-litho-autotrophic sulfur bacteria contribute significantly to biomass and organic carbon input during carbon dioxide fixation. Considering the diversity of sulfate reducers in these soils, as well as the selective advantage of sulfate reduction and the high intensity of horizontal transfer of Dsr genes (Anantharaman et al., 2018), a high frequency of the latter can be assumed under these conditions. Presumably, this explains the presence of sulfate-reducing acidobacteria, since they can switch between aerobic and anaerobic conditions, exposed to the excess of electron acceptors (oxygen and sulfate), low pH levels, and surrounded by complex plant polysaccharides. It is possible that typical sulfate reducers can utilize short-chain organic acids produced during the metabolism of plant biomass by Acidobacteriota. Besides the complete conversion of sulfates to sulfides and vice versa – which can take presumably weeks given typical growth rate of SOB and SRB – hidden network of diverse and rapid sulfur transformations should take place in the studied area. This can be concluded from the omnipresence of diverse genes that encode capacity for sulfite, thiosulfate, tetrathionate and, most importantly, organosulfonates transformations. Moreover, recent estimates show that utilization of small organosulfur molecules by soil microbiota takes minutes to hours (Ma et al., 2021). Over time and multiple flooding-evaporation cycles, different forms of sulfur accumulate in the soil. Thus, a dynamic equilibrium between oxidation and reduction of sulfur compounds is established in the Atamanskoe soils, which is the cause of the observed high diversity of corresponding microbial groups.

Conclusions

The sulfur cycle exhibits immense diversity in terms of underlying taxa, metabolic reactions, and interconnections with other biogeochemical cycles. In recent years, researchers have begun to recognize that the terrestrial sulfur cycle differs from that of water environments. Our study aligns with this understanding and offers insights into the behavior of sulfur cycle bacteria in a unique soil environment of technogenic origin. We demonstrate that elevated concentrations of xenobiotic pollutants like HM and PAH, along with extreme levels of bulk sulfur and fluctuating redox conditions, have given rise to a distinctive microbial community involved in sulfur transformation. This community is composed of: 1) known, but recently overlooked taxa that carry out typical reactions, as seen with sulfate-reducing acidobacteria; 2) known taxa engaging in a multitude of oxidative, reductive, and organosulfur transformations, such as various sulfur oxidizing alpha-, gamma-, and betaproteobacteria; 3) previously unidentified taxa that have not been successfully cultured in a laboratory setting, like Binatota, Casimicrobiaceae, and Acidoferrales bins, which may theoretically possess completely new genes associated with sulfur compound metabolism; 4) multiple taxa capable of collectively replenishing pools of readily consumable compounds, such as sulfite and thiosulfate. Consequently, these taxa contribute to the cryptic biogeochemical cycling of compounds with high turnover rates but low absolute abundance. Furthermore, we demonstrate that sulfate-reducers – a significant part of microbial community according to the taxonomic profiling of metagenomes and culture-dependent analysis – can still represent only a small fraction of overall microbiome and require additional efforts to

characterize it taxonomically and functionally. We conclude that sulfate-reducers indeed are members of soil rare biosphere and do not reach abundance comparable to that of aquatic habitats.

Studies on microbial sulfur cycles continue to uncover an inexhaustible source of new information, revealing the adaptive and diverse nature of microbial communities. It is important to acknowledge that we have only explored a small fraction of terrestrial ecosystems thus far. Therefore, further research in this field holds immense significance, both for practical and fundamental implications, such as mitigating greenhouse gas emissions and deepening our fundamental understanding of microbial behavior in nature.

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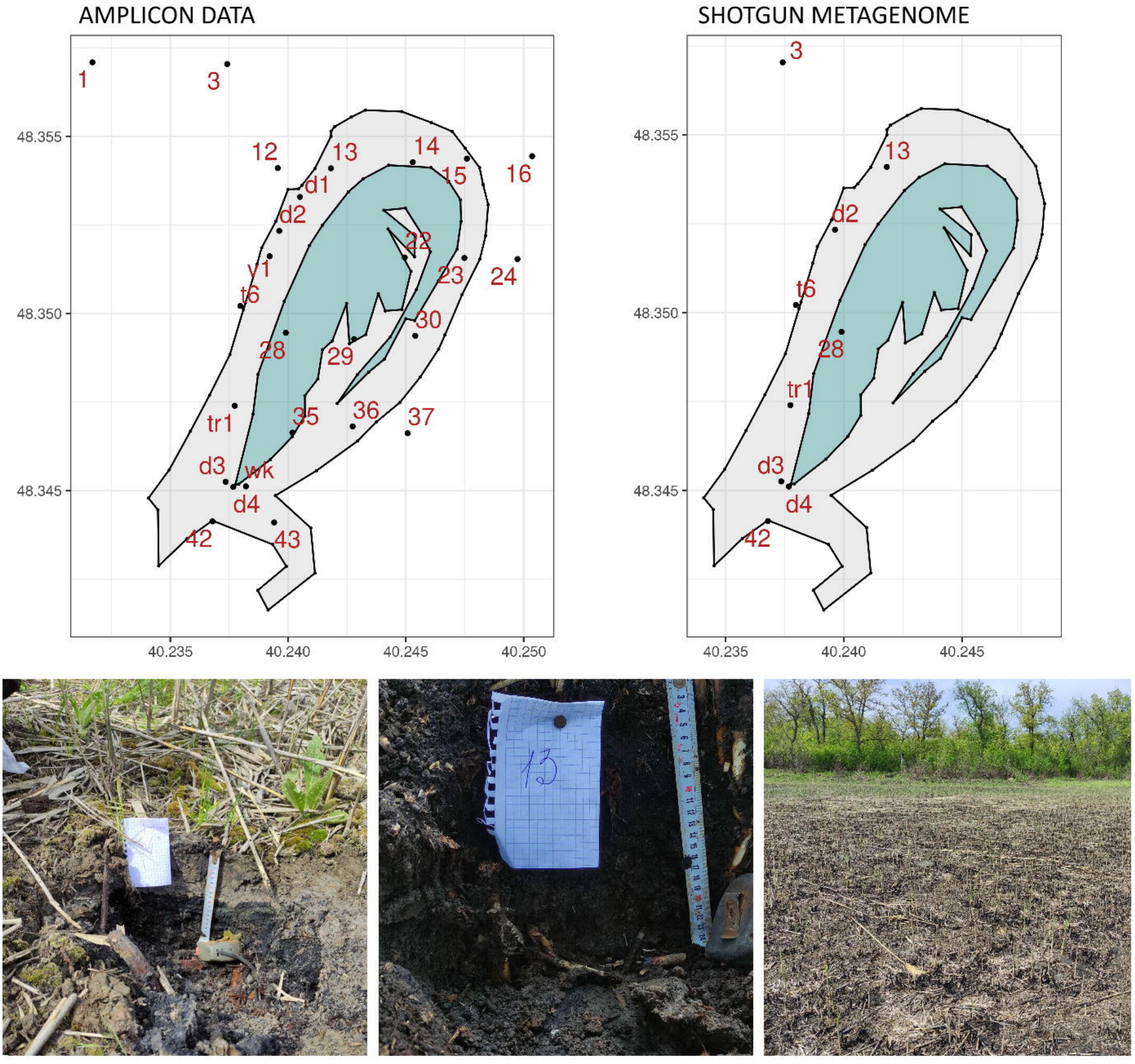
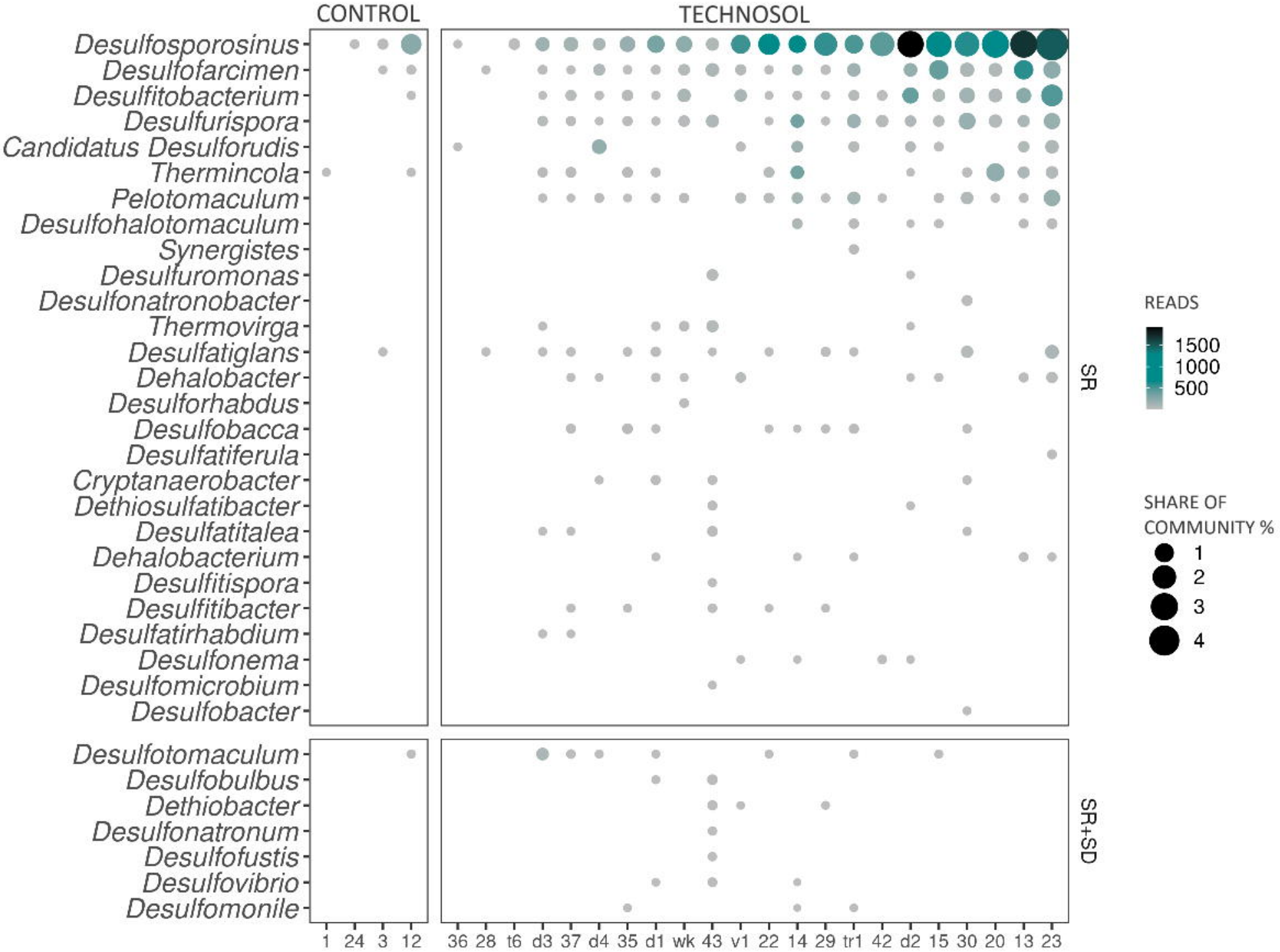
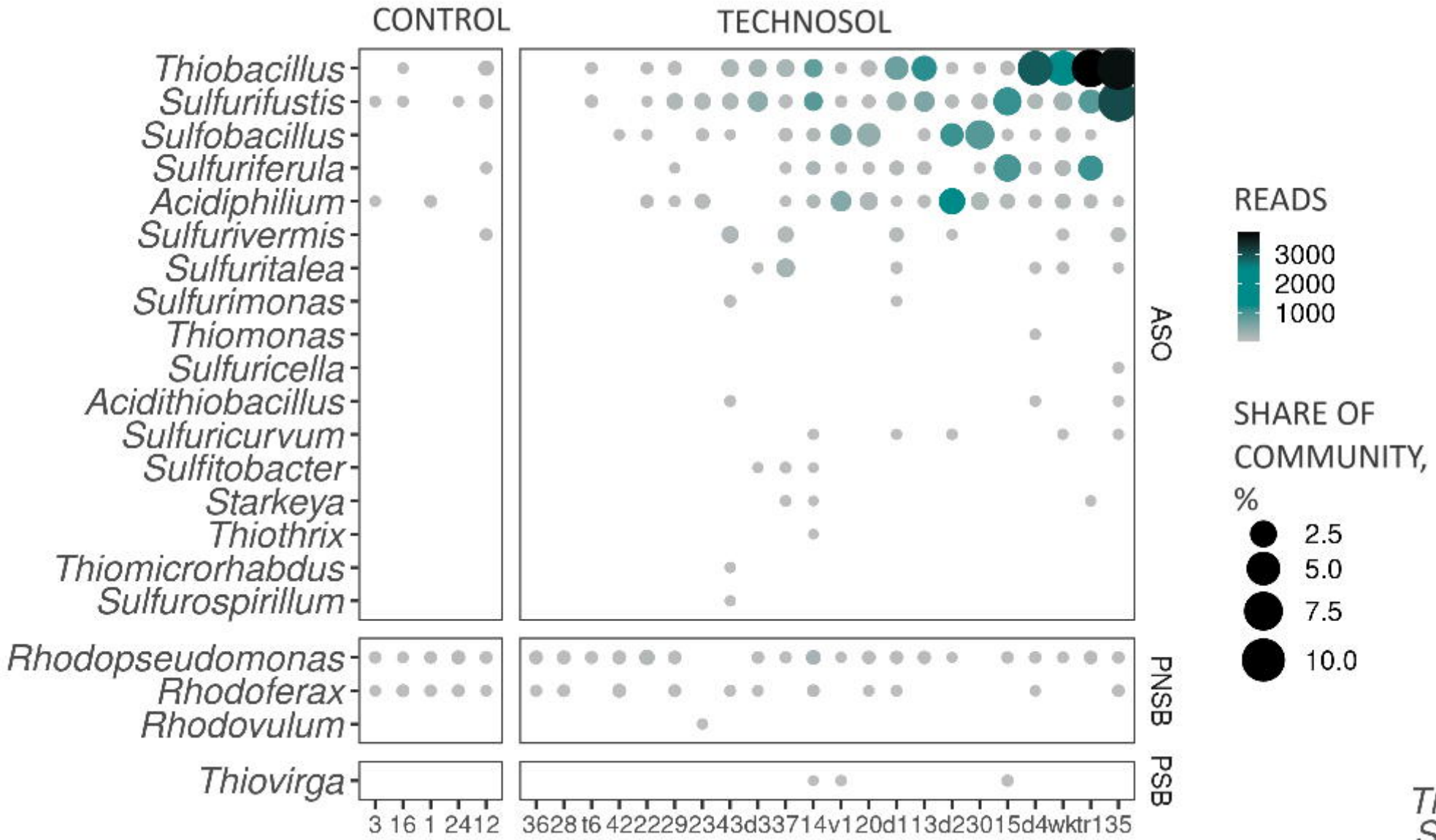
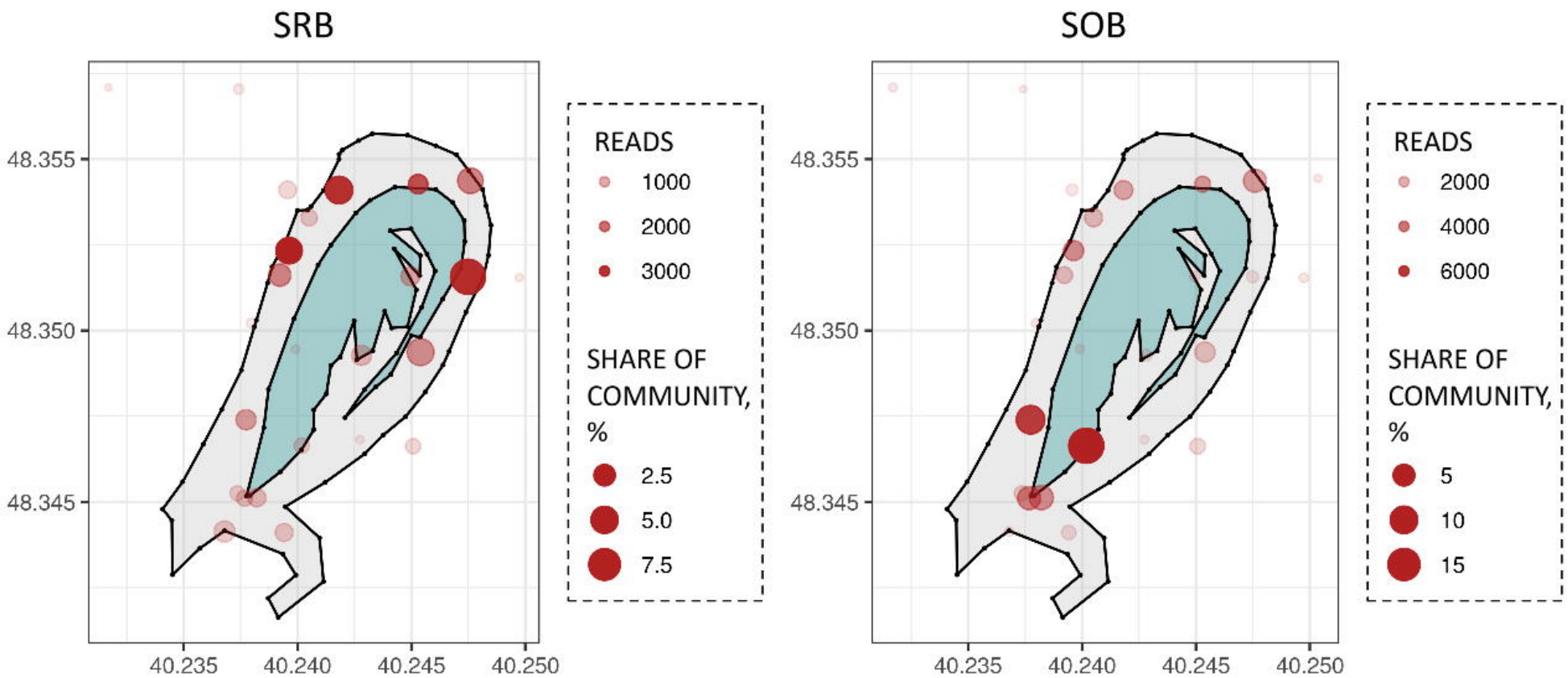
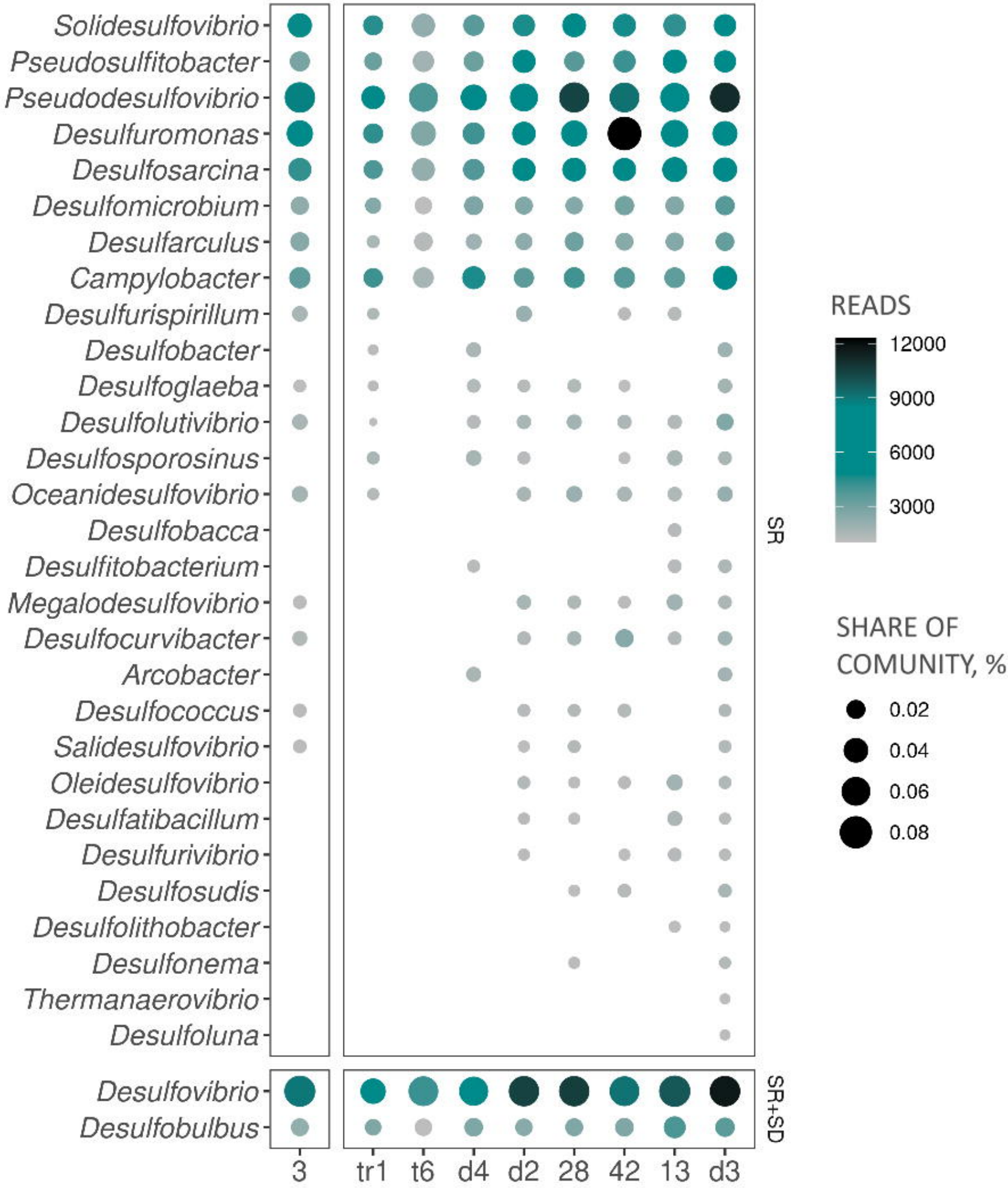
44. Ma, Qingxu, et al. "Substrate control of sulphur utilisation and microbial stoichiometry in soil: Results of 13C, 15N, 14C, and 35S quad labelling." The ISME Journal 15.11 (2021): 3148-3158.

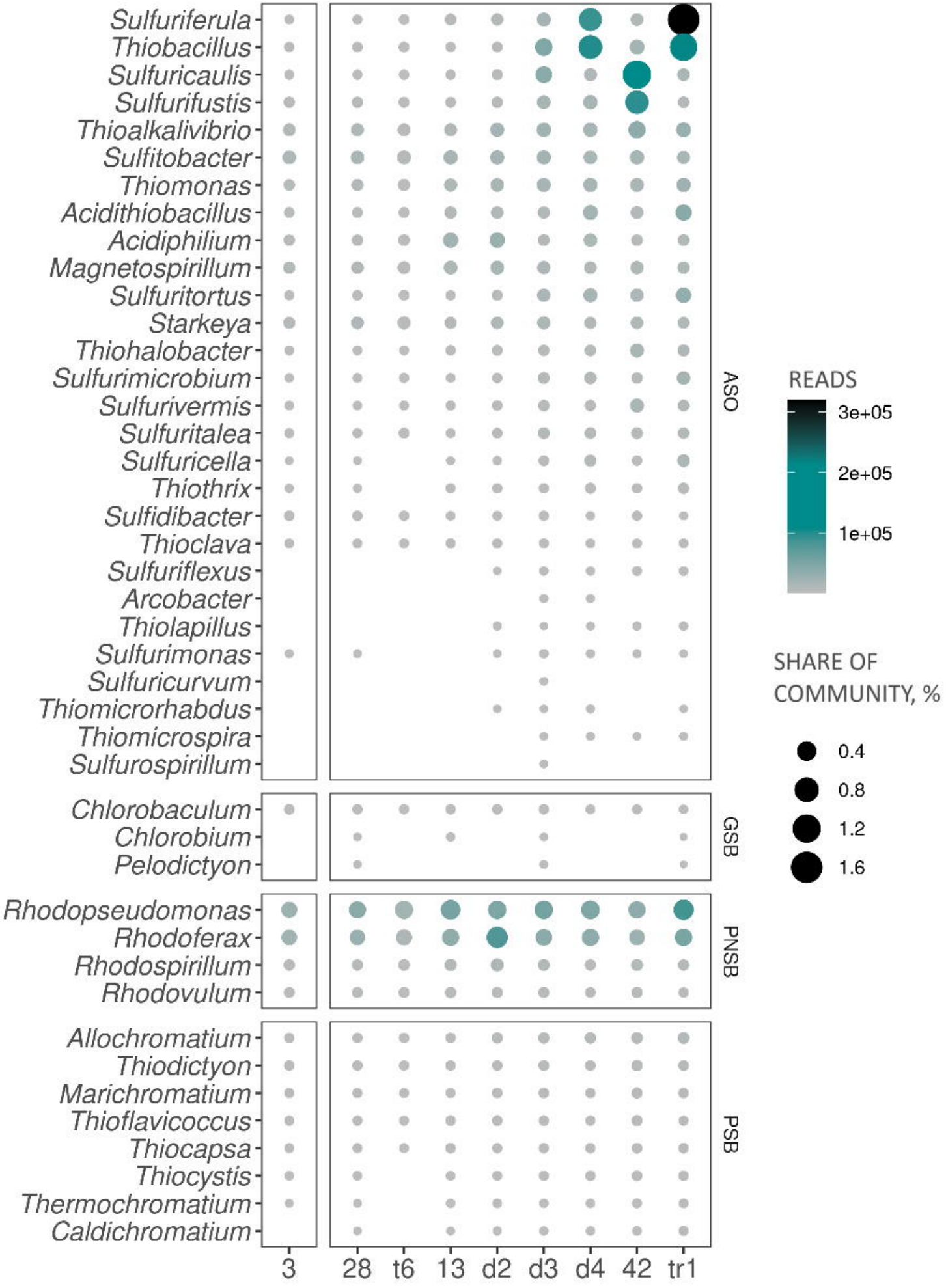

AMPLICON DATA
SHOTGUN METAGENOME
48.355
48.350
48.345
40.235
40.240
40.245
40.250
1
3
12
13
14
15
16
d1
d2
22
23
24
v1
t6
30
28
29
tr1
35
36
37
d3
wk
d4
42
43
13

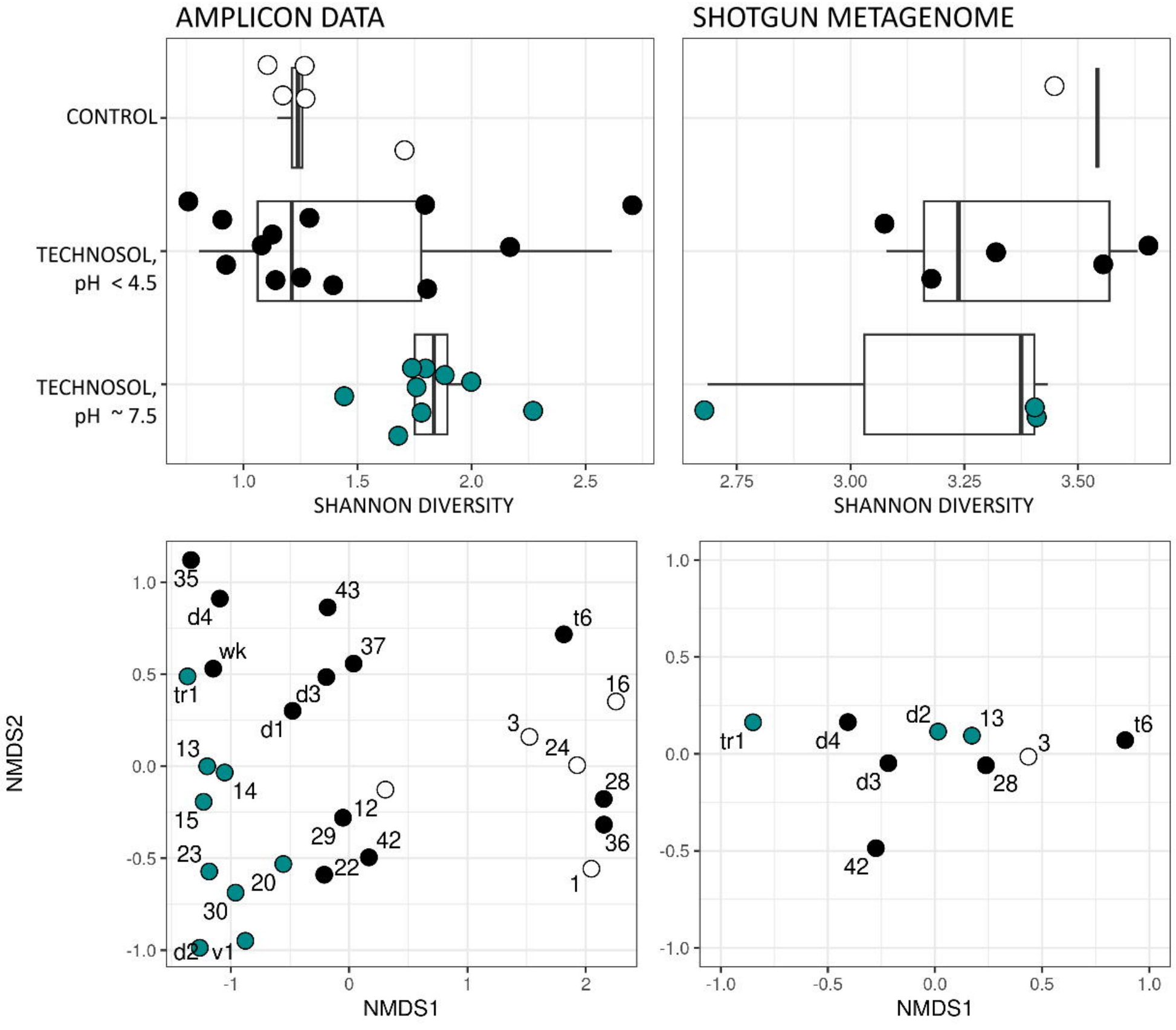

CONTROL
TECHNOSOL
Desulfosporosinus
Desulfofarcimen
Desulfitobacterium
Desulfurispora
Candidatus Desulforudis
Thermincola
Pelotomaculum
Desulfohalotomaculum
Synergistes
Desulfuromonas
Desulfonatronobacter
Thermovirga
Desulfatiglans
Dehalobacter
Desulforhabdus
Desulfobacca
Desulfatiferula
Cryptanaerobacter
Dethiosulfatibacter
Desulfatitalea
Dehalobacterium
Desulfitispora
Desulfitibacter
Desulfatirhabdium
Desulfonema
Desulfomicrobium
Desulfobacter
Desulfotomaculum
Desulfobulbus
Dethiobacter
Desulfonatronum
Desulfofustis
Desulfovibrio
Desulfomonile
SR
SR+SD
1 24 3 12
36 28 t6 d3 37 d4 35 d1 wk 43 v1 22 14 29 tr1 42 d2 15 30 20 13 23
READS
1500
1000
500
SHARE OF COMMUNITY %
1
2
3
4

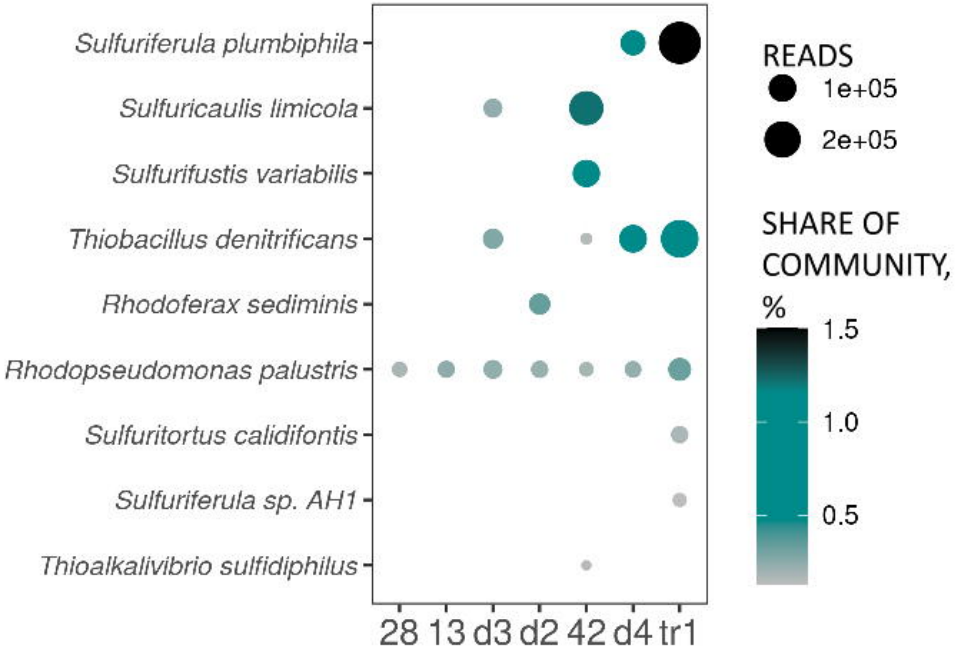

CONTROL
TECHNOSOL
Thiobacillus
Sulfurifustis
Sulfobacillus
Sulfuriferula
Acidiphilium
Sulfurivermis
Sulfuritalea
Sulfurimonas
Thiomonas
Sulfuricella
Acidithiobacillus
Sulfuricurvum
Sulfitobacter
Starkeya
Thiothrix
Thiomicrorhabdus
Sulfurospirillum
Rhodopseudomonas
Rhodoferax
Rhodovulum
Thiovirga
ASO
PNSB
PSB
3 16 1 2412
3628 t6 4222292343d33714v120d113d23015d4wktr135
READS
3000
2000
1000
SHARE OF COMMUNITY, %
2.5
5.0
7.5
10.0

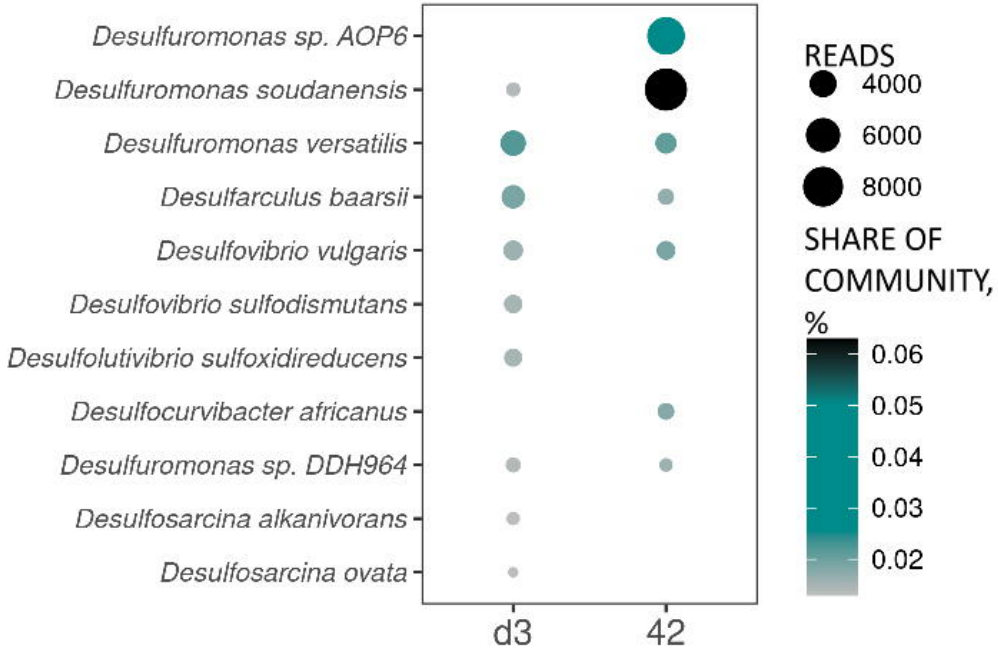

SRB
48.355
48.350
48.345
40.235
40.240
40.245
40.250
READS
1000
2000
3000
SHARE OF COMMUNITY, %
2.5
5.0
7.5
SOB
48.355
48.350
48.345
40.235
40.240
40.245
40.250
READS
2000
4000
6000
SHARE OF COMMUNITY, %
5
10
15

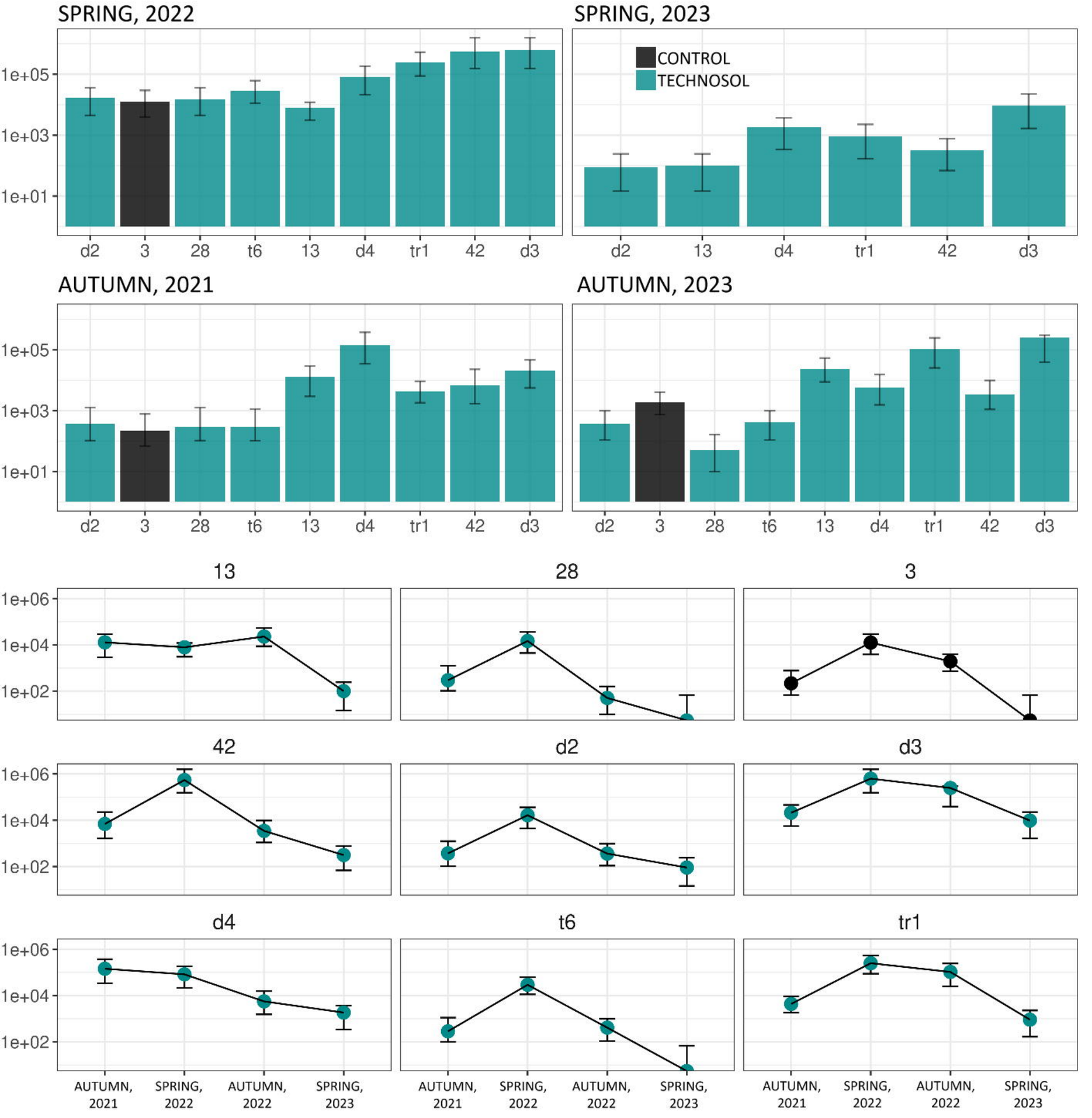

Solidesulfovibrio
Pseudosulfitobacter
Pseudodesulfovibrio
Desulfuromonas
Desulfosarcina
Desulfomicrobium
Desulfarculus
Campylobacter
Desulfurispirillum
Desulfobacter
Desulfoglaeba
Desulfolutivibrio
Desulfosporosinus
Oceanidesulfovibrio
Desulfobacca
Desulfitobacterium
Megalodesulfovibrio
Desulfocurvibacter
Arcobacter
Desulfococcus
Salidesulfovibrio
Oleidesulfovibrio
Desulfatibacillum
Desulfurivibrio
Desulfosudis
Desulfolithobacter
Desulfonema
Thermanaerovibrio
Desulfoluna
Desulfovibrio
Desulfobulbus
SR
SR+SD
3
tr1
t6
d4
d2
28
42
13
d3
READS
12000
9000
6000
3000
SHARE OF COMUNITY, %
0.02
0.04
0.06
0.08

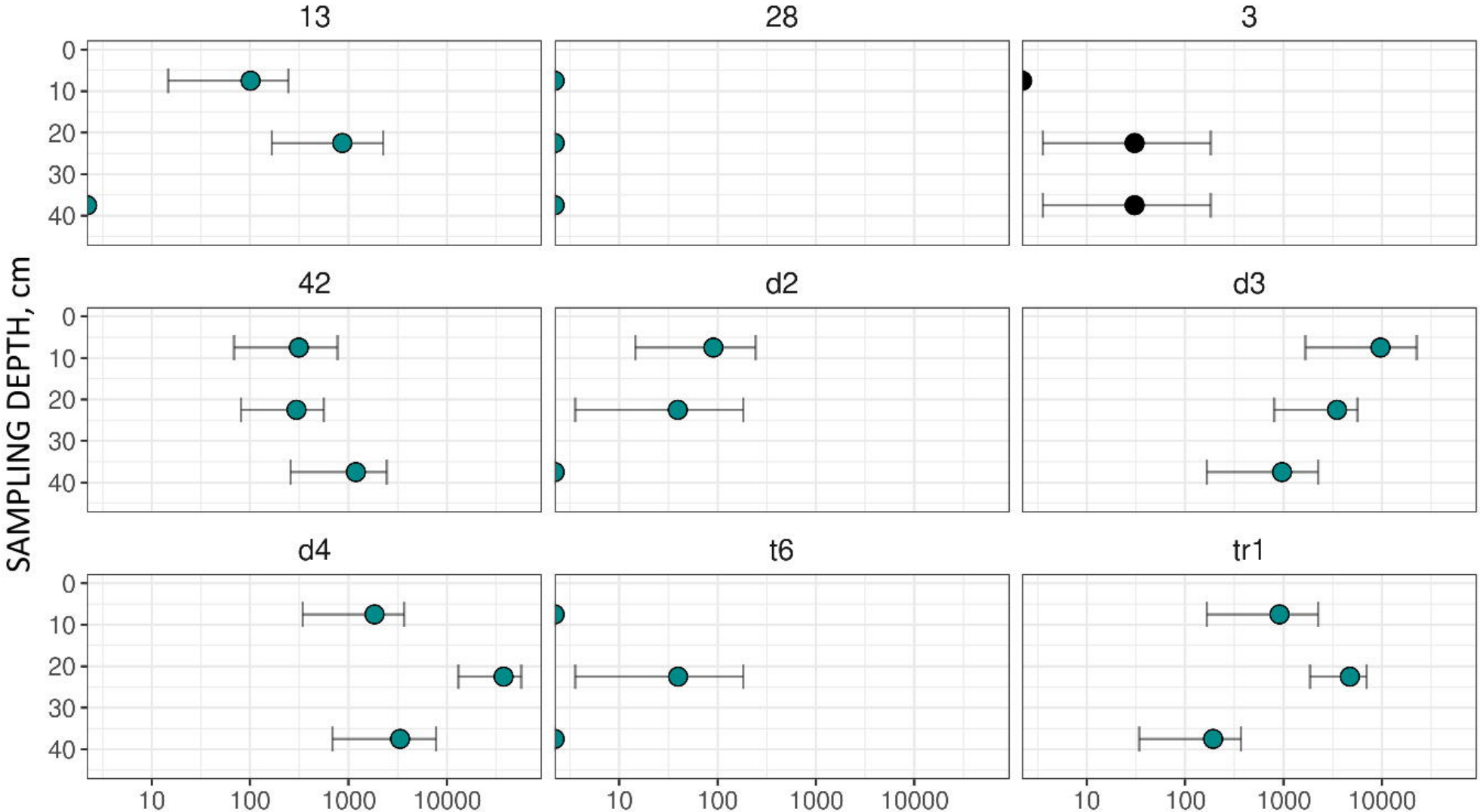

Sulfuriferula
Thiobacillus
Sulfuricaulis
Sulfurifustis
Thioalkalivibrio
Sulfitobacter
Thiomonas
Acidithiobacillus
Acidiphilium
Magnetospirillum
Sulfuritortus
Starkeya
Thiohalobacter
Sulfurimicrobium
Sulfurivermis
Sulfuritalea
Sulfuricella
Thiothrix
Sulfidibacter
Thioclava
Sulfuriflexus
Arcobacter
Thiolapillus
Sulfurimonas
Sulfuricurvum
Thiomicrorhabdus
Thiomicrospira
Sulfurospirillum
Chlorobaculum
Chlorobium
Pelodictyon
Rhodopseudomonas
Rhodoferax
Rhodospirillum
Rhodovulum
Allochromatium
Thiodictyon
Marichromatium
Thioflavicoccus
Thiocapsa
Thiocystis
Thermochromatium
Caldichromatium
ASO
GSB
PNSB
PSB
3
28
t6
13
d2
d3
d4
42
tr1
READS
3e+05
2e+05
1e+05
SHARE OF COMMUNITY, %
0.4
0.8
1.2
1.6

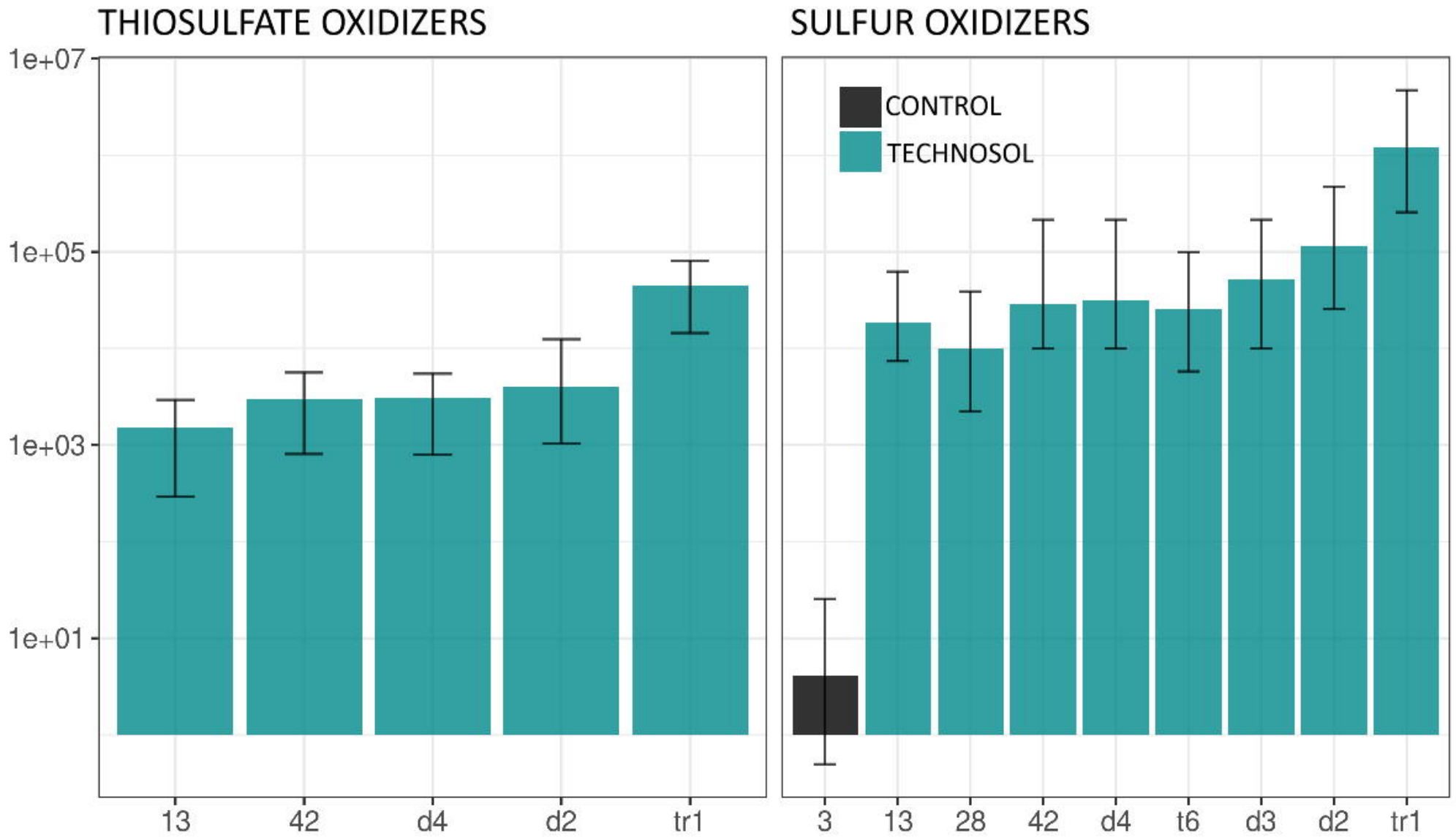

AMPLICON DATA
SHOTGUN METAGENOME
CONTROL
TECHNOSOL, pH < 4.5
TECHNOSOL, pH ~ 7.5
1.0
1.5
2.0
2.5
SHANNON DIVERSITY
2.75
3.00
3.25
3.50
SHANNON DIVERSITY
NMDS2
NMDS1
NMDS1
35
d4
43
t6
wk
37
tr1
d3
16
d1
3
24
13
14
28
15
12
29
42
36
23
22
20
1
30
d2
v1
tr1
d4
d2
13
t6
3
d3
28
42


Sulfuriferula plumbiphila
Sulfuricaulis limicola
Sulfurifustis variabilis
Thiobacillus denitrificans
Rhodoferax sediminis
Rhodopseudomonas palustris
Sulfuritortus calidifontis
Sulfuriferula sp. AH1
Thioalkalivibrio sulfidiphilus
28 13 d3 d2 42 d4 tr1
READS
1e+05
2e+05
SHARE OF COMMUNITY, %
1.5
1.0
0.5


Desulfuromonas sp. AOP6
Desulfuromonas soudanensis
Desulfuromonas versatilis
Desulfarculus baarsii
Desulfovibrio vulgaris
Desulfovibrio sulfodismutans
Desulfolutivibrio sulfoxidireducens
Desulfocurvibacter africanus
Desulfuromonas sp. DDH964
Desulfosarcina alkanivorans
Desulfosarcina ovata
d3 42
READS
4000
6000
8000
SHARE OF COMMUNITY, %
0.06
0.05
0.04
0.03
0.02

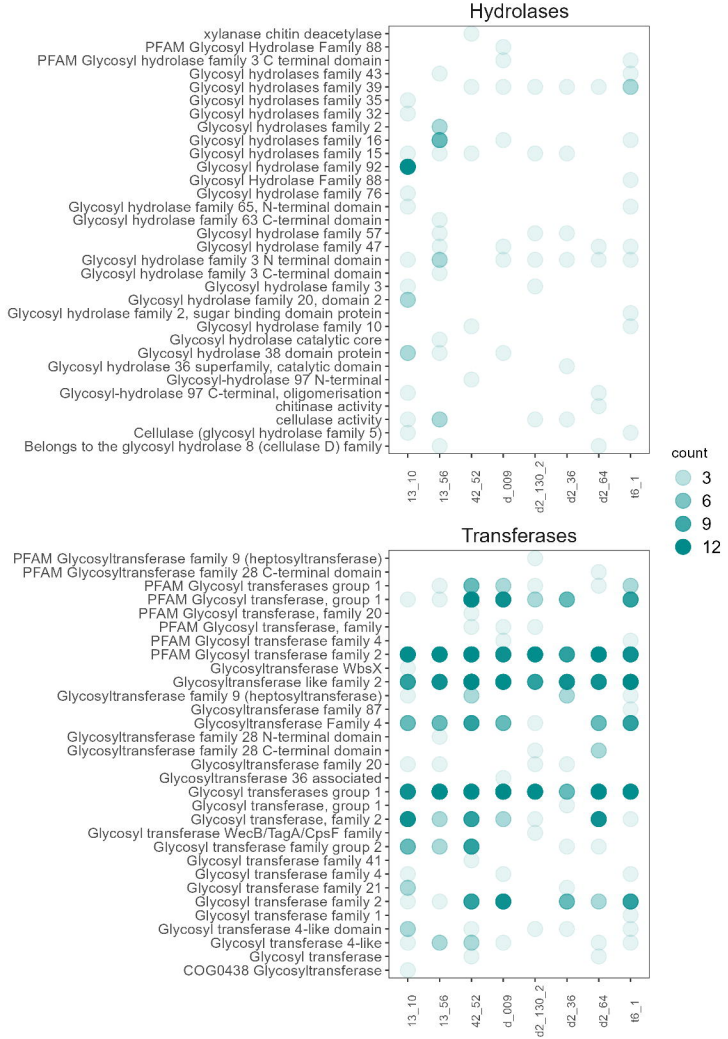

SPRING, 2022
SPRING, 2023
CONTROL
TECHNOSOL
AUTUMN, 2021
AUTUMN, 2023
1e+05
1e+03
1e+01
d2
3
28
t6
13
d4
tr1
42
d3
1e+06
1e+04
1e+02
AUTUMN, 2021
SPRING, 2022
AUTUMN, 2022
SPRING, 2023


13
28
3
42
d2
d3
d4
t6
tr1
SAMPLING DEPTH, cm
0
10
20
30
40
10
100
1000
10000


THIOSULFATE OXIDIZERS
SULFUR OXIDIZERS
1e+07
1e+05
1e+03
1e+01
CONTROL
TECHNOSOL
13
42
d4
d2
tr1
3
13
28
42
d4
t6
d3
d2
tr1

	13		42		d2		d3		d4		tr1	
	in the contigs	out of the contigs	in the contigs	out of the contigs	in the contigs	out of the contigs	in the contigs	out of the contigs	in the contigs	out of the contigs	in the contigs	out of the contigs
Desulfuromonadia	34280	10408	17223	35027	26091	11382	22339	26884	11330	19966	13630	12600
Desulfovibrionia	23629	7857	12218	17589	20526	9390	16604	21936	9220	13576	11344	9939
Thermotogae	2647			6568			4772	12084		5883		1168
Desulfobacteria	8120	2780	3875	5644	6304	2882	4905	8427	2748	5071	3062	3364
Desulfobulbia	5558	1635	2400	3476	3595	1721	3018	4191	1766	2456	3005	2008
Synergistia	2212		1988	2693	2276	1175	2378	3201	1100	1894		1243
Thermodesulfobacteria	1386											
Desulfarculia	1298							1159				

Acetobacteraceae
Acidiferrobacterales
Acidoferrales
Alphaproteobacteria
Betaproteobacteria
Binatota
Burkholderiales
Casimicrobiaceae
Gammaproteobacteria
Gemmatimonadales
Hyphomicrobiales
Limnocylindria
Nitrobacteraceae
Nitrosomonadales
Nitrospirales
Pseudomonadota
Pseudomonas yamanorum
Pyrinomonadales
Steroidobacterales
Sulfuricellaceae
Sulfuriferula
Terriglobia
DMSO metabolism
dmdD
dmdC
dmdB
ddhB
ddhA
Organosulfonate metabolism
xsc
toa
tmoF,tbuC,touF
tmoC,tbuB,touC
tauD
suyB
suyA
ssuE
ssuD
sfnG
msmB
msmA
cuyA
S oxidation
SUOX
sseA
soxZ
soxY
soxX
soxD
soxC
soxB
soxA
sorA
soeB
soeA
glpE
fccB
DoxD
DoxA
S reduction / disproportionation
ttrB
ttrA
sir
SELENBP1
sat
phsA/psrA
met
hydB
hydA
dsrB
dsrA
asrB
asrA
aprB
aprA
S transport
qmoC
qmoB
qmoA
dsrP
dsrM
dsrK
dsrJ
dsrH/TusB
dsrF/TusC
dsrE/TusB
dsrC
d4_78
42_11
d4_9
d2_130_2
42_7
d2_57
d2_76
d4_45
42_98
d2_10
d3_99
13_5
13_92
13_24
tr1_38
13_32
d2_114
tr1_27
d3_108
d2_57_2
42_6
13_37
42_54
d3_50
tr1_72
d4_42
42_42
42_94
d3_36_2
tr1_93
d_019
13_90
42_87
tr1_73
d2_22
13_32_2
42_23
d3_36
d4_12
42_83
t6_1
d_009
d2_14
tr1_59
d4_19
13_56
d2_36
d2_64
13_10
42_52
3-(Methylthio)acryloyl-CoA to Methanethiol
3-(Methylthio)propanoate to 3-(Methylthio)propanoyl-CoA
3-(Methylthio)propanoyl-CoA to 3-(Methylthio)acryloyl-CoA
Alkanesulfonate to SO3
Dimethyl sulfide to Dimethyl sulfoxide
Dimethylsulfone to Methanesulfonic acid
Dimethylsulfone to SO3
H2S to S0
L-cysteate to SO3
Methanesulfonic acid to SO3
Methanethiol to H2S
Methionine to Methanethiol
S transport
S(n) to H2S, S(n-1)
S2O3 to H2S and SO3
S2O3 to S4O6
S2O3 to SO3
S2O3 to SO4
S4O6 to S2O3
SO3 to H2S
SO3 to SO4
SO4 to adenylylsulfate
SO4 to SO3
Sulfoacetaldehyde to SO3
Sulfolactate to SO3
Taurine to SO3
Taurine to Sulfoacetaldehyde


Hydrolases
xylanase chitin deacetylase
PFAM Glycosyl Hydrolase Family 88
PFAM Glycosyl hydrolase family 3 C terminal domain
Glycosyl hydrolases family 43
Glycosyl hydrolases family 39
Glycosyl hydrolases family 35
Glycosyl hydrolases family 32
Glycosyl hydrolases family 2
Glycosyl hydrolases family 16
Glycosyl hydrolases family 15
Glycosyl hydrolase family 92
Glycosyl Hydrolase Family 88
Glycosyl hydrolase family 76
Glycosyl hydrolase family 65, N-terminal domain
Glycosyl hydrolase family 63 C-terminal domain
Glycosyl hydrolase family 57
Glycosyl hydrolase family 47
Glycosyl hydrolase family 3 N terminal domain
Glycosyl hydrolase family 3 C-terminal domain
Glycosyl hydrolase family 3
Glycosyl hydrolase family 20, domain 2
Glycosyl hydrolase family 2, sugar binding domain protein
Glycosyl hydrolase family 10
Glycosyl hydrolase catalytic core
Glycosyl hydrolase 38 domain protein
Glycosyl hydrolase 36 superfamily, catalytic domain
Glycosyl-hydrolase 97 N-terminal
Glycosyl-hydrolase 97 C-terminal, oligomerisation
chitinase activity
cellulase activity
Cellulase (glycosyl hydrolase family 5)
Belongs to the glycosyl hydrolase 8 (cellulase D) family
13_10
13_56
42_52
d_009
d2_130_2
d2_36
d2_64
t6_1
count
3
6
9
12
Transferases
PFAM Glycosyltransferase family 9 (heptosyltransferase)
PFAM Glycosyltransferase family 28 C-terminal domain
PFAM Glycosyl transferases group 1
PFAM Glycosyl transferase, group 1
PFAM Glycosyl transferase, family 20
PFAM Glycosyl transferase, family
PFAM Glycosyl transferase family 4
PFAM Glycosyl transferase family 2
Glycosyltransferase WbsX
Glycosyltransferase like family 2
Glycosyltransferase family 9 (heptosyltransferase)
Glycosyltransferase family 87
Glycosyltransferase Family 4
Glycosyltransferase family 28 N-terminal domain
Glycosyltransferase family 28 C-terminal domain
Glycosyltransferase family 20
Glycosyltransferase 36 associated
Glycosyl transferases group 1
Glycosyl transferase, group 1
Glycosyl transferase, family 2
Glycosyl transferase WecB/TagA/CpsF family
Glycosyl transferase family group 2
Glycosyl transferase family 41
Glycosyl transferase family 4
Glycosyl transferase family 21
Glycosyl transferase family 2
Glycosyl transferase family 1
Glycosyl transferase 4-like domain
Glycosyl transferase 4-like
Glycosyl transferase
COG0438 Glycosyltransferase
13_10
13_56
42_52
d_009
d2_130_2
d2_36
d2_64
t6_1