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Dominance of Arcobacter in the white filaments from the thermal sulfidic spring of Fetida Cave (Apulia, southern Italy)

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1 Dominance of *Arcobacter* in the white filaments from the thermal sulfidic spring

2 of Fetida Cave (Apulia, southern Italy)

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Abstract

23 The thermal spring of Fetida Cave, a still active sulfuric acid cave opening

24 at ~~the~~ sea level and located in Santa Cesarea Terme, southeastern Salento

25 (Apulia region, Southern Italy) hosts abundant floating white filaments. The

26 white filaments were mainly composed of sulfur crystals surrounded by

27 microbial mass of the phyla *Epsilonbacteraeota*, *Proteobacteria*, *Bacteroidetes*,

28 and *Patescibacteria*. The most abundant genus in the white filaments collected

from the waters in the innermost part of the cave dominated by sulfidic exhalations was *Arcobacter*. This abundance can be related to the higher concentration of sulfide dissolved in water, and low oxygen and pH values. Conversely, lower *Arcobacter* abundances were obtained in the filaments collected in the entrance and middle part of the cave, where sulfidic water mixes with seawater, as the cave is subjected to tides and the mixing of fresh (continental) with marine water. The geochemical analysis of water ~~geochemistry~~ and atmospheric gases confirmed these environmental constraints. In fact, higher concentrations of H_2S in the air and water were recorded closest to the spring upwelling in the innermost part of the cave, and the lower ones near the cave entrance. The metabolic versatility of *Arcobacter* might provide a competitive advantage in the colonization of water bodies characterized by high sulfide, low oxygen, and dynamic fluid movement.

1. Introduction

Hypogene sulfuric acid speleogenesis (SAS) caves are underground karst systems formed in carbonate areas where acidic fluids, derived from the interactions with deep-seated sulfates and/or sulfides, rise through deeply rooted geological structures (Audra et al., 2009). In particular, SAS caves form when host rock dissolution is mainly related to the interaction with the sulfuric acid produced by the oxidation of hydrogen sulfide. Around 25% of the known worldwide SAS systems are located in Italy, especially along the Italian Apennine Chain, and some of them are still in active conditions (D'Angeli et al., 2019a).

Within SAS caves, hydrogen sulfide provides a rich energy source for chemolithotrophic microorganisms, which support chemosynthetic primary production for the growth of heterotrophic organisms (Jones et al., 2008; Bizic et al., 2020). In association with this, SAS caves typically host conspicuous microbial biofilms and mats that are visible on the walls, ceilings, and in the

61 water. Those covering the cave walls and ceilings have variable morphologies
62 and colors in the form of viscous snottites and vermiculations (Jones et al.,
63 2010; D'Angeli et al., 2019b). In the water, that has a milky appearance (due to
64 elemental sulfur), the biofilms are in the form of white filaments that are typically
65 visible as either rock-attached streamers or sediment surface biofilms. These
66 two morphologies were first referred to as feathery biofilms and cotton biofilms,
67 respectively, by Macalady et al. (2007) in Frasassi Cave. Analogous white
68 filaments were observed and described in a series of other SAS caves in
69 Acquasanta Terme, Capo Palinuro, Monte Sellaro, Cassano allo Ionio, and
70 Santa Cesarea Terme (D'Angeli et al., 2019a,b) and also in Romania (Bizic et
71 al., 2020).

72 The microbiology and composition of white filaments was previously
73 studied, but limited to a few caves and springs. The caves with major research
74 efforts were Frasassi (Macalady et al., 2006, 2008; Engel, 2007), Movile
75 (Hutchens et al., 2004; Chen et al., 2009; Kumaresan et al., 2014; Bizic et al.,
76 2020), and Lower Kane (Engel et al., 2003, 2004, 2010). A few individual
77 reports on other caves and springs can be found in the literature (Mattison et
78 al., 1998; Engel et al., 2001; Elshahed et al., 2003; Barton and Luiszer, 2005;
79 Reigstad et al., 2011; Rossmassler et al., 2012). Among these, the microbiology
80 of the water streamers has been the most extensively studied through
81 molecular methods (16S rRNA clone library) but also through microscopy and
82 culture-based experiments (Hose and Pisarowicz, 1999; Engel et al., 2004;
83 Hamilton et al., 2015). Sulfur-oxidizing microorganisms belonging to *Gamma*-,
84 *Beta*- and *Epsilonproteobacteria*, reclassified as *Epsilonbacteraeota* (Waite et
85 al., 2017) dominate the water streamer microbial communities. Additionally,
86 members of *Deltaproteobacteria*, associated with sulfur-reduction processes
87 were identified in lower abundance (Macalady et al., 2006). Among the different
88 environmental factors possibly affecting these biofilms, the water flow (shear
89 stress) and the ratio sulfide/oxygen were reported to be the major ones
90 influencing white filament morphologies (i.e. long rock-attached streamers or
91 shorter sediment biofilm) and microbial diversity (Macalady et al., 2008).

92 The present work is focused on the analysis of white filaments from Fetida
93 Cave, a still active sulfuric acid cave opening at ~~the~~ sea level and located in
94 Santa Cesarea Terme, southeastern Salento (Apulia region, Southern Italy)

(D'Angeli et al., 2019b; 2021) (Fig. 1). The cave hosts abundant microbial biofilms on cave walls and ceiling as vermiculations and gypsum moonmilk. A previous study described the mineralogy, geochemistry, and microbial diversity associated with the different biofilms found in Fetida Cave (D'Angeli et al., 2019b).

The microbial communities featuring Fetida Cave water are characterized by the presence of abundant floating white filaments and are related with the constant mixing between the thermal sulfidic fluids, rising from below, and seawater entering from outside that leads to variable contents of dissolved H_2S along the cave (D'Angeli et al., 2021). The purpose of the present work is to extend the knowledge on the microbial communities composing white filaments in this unique SAS cave environment. In this regard, gaseous composition of the cave atmosphere and water geochemistry as well as morphological (through FESEM) and microbiological (using Illumina sequencing of 16S rRNA gene) analyses were performed to get deep into the diversity of the white floating filaments in Fetida Cave (Fig. 1) and the possible predicted metabolic functions associated with their development.

2. Methods

2.1. Field sampling

Microbiological and air sampling was performed in different sites inside the cave (Fig. 1). In particular, four microbiological samples were collected in sites FC1, FC4, FC6, and FC7, moving from the innermost part of the cave towards the coastline. D'Angeli et al. (2019b) described three different typologies of water filaments based on their location, *i.e.*, floating on the water surface (named F-float), sedimented on the bottom of the water (F-sed), and attached to the cave rocks (F-stream). The four samples analyzed in this study are different from those described by D'Angeli et al. (2019) but belong to the F-float category described in this previous paper. Microbial sampling for this paper was done during a field trip in June 2017 (summer conditions with calm sea), while the samples analyzed in the study by D'Angeli et al. (2019) were collected in October 2015 and December 2017 (during winter, when marine conditions were

rougher). The samples were collected using sterile scalpels, stored in sterile tubes at 4°C until arrival at the laboratory. Five replicas from each sampling point were taken for molecular biology as well as for field emission scanning electron microscopy (FESEM). The samples for molecular biology were preserved in Lifeguard preservation solution (Qiagen, Hilden, Germany) and then held at -80°C until analysis, whereas samples for microscopy were fixed in-situ with 2.5% glutaraldehyde in 0.1M cacodylate-buffer (pH 7.4).

Gas samples were taken into the cave in sites P1, P2, P3, P4 (Fig. 1, Table A1 in Appendix A - Supplementary data), and outside to take external air and soil in several locations, both in June 2017 and in May 2018. They were collected using a handheld pump and 1 L Tedlar bags (Fig. 1). For each sample two replicas were taken, filling only 2/3 of the entire bag to avoid bag damage and/or explosion during transport. Immediately after the collection, 12 Tedlar bags (for the two fieldworks in June 2017 and May 2018) were stored in a rigid luggage and analyzed within 48 hours at the stable isotopes laboratories of Museo Nacional de Ciencias Naturales in Madrid and University of Almeria. CO_2 and CH_4 molar fractions and $\delta^{13}C$ in both gases were measured with a CRDS spectrometer (G2201-i analyser, Picarro Inc., USA) with a precision of 200 ppb (±0.05 of reading) and 10 ppb (±0.05 of reading) for $^{12}CO_2$ and $^{13}CO_2$, respectively, resulting in a precision better than 0.16 ‰ for $\delta^{13}C$-CO_2 after 5 min of analysis. The measurements of methane isotopologues ($^{12}CH_4$ and $^{13}CH_4$) reached a precision of 5 ppb (±0.05 of reading) and 1 ppb (±0.05 of reading) for $^{12}CH_4$ and $^{13}CH_4$, respectively. The precision for $\delta^{13}C$-CH_4 was better than 1.15 ‰ after 5 min of analysis. $\delta^{13}C$-isotope values were referenced to the Vienna PeeDee Belemnite (V-PDB). Three in-house standards with certified CO_2 and CH_4 concentrations and known $\delta^{13}C$ values for each gas were processed to verify the proper functioning of the CRDS analyzer. Fernández-Cortès et al. (2018) reported further details on the methodological procedures and quality results. Spot measurements of CO_2 concentration and levels of other key gases (H_2S and O_2) were also taken in the same locations for air sampling using handheld devices (XP200, Lufft) and a multigas monitor (MX6 iBrid, Industrial Scientific), respectively.

In addition, during June 2017 and May 2018 field trips, water samples (SCC1, SCB1, SCAfen, SCA1) were collected in the same location of

161 microbiological samplings (Fig. 1, Table 1A in Appendix A - Supplementary
162 data). The results of a comprehensive water monitoring campaign that began in
163 October 2015 and ended in November 2018 have been published in the
164 framework of a hydrogeological-geochemical study (D'Angeli et al., 2021).
165 Nevertheless, in this work, we will consider only the results obtained during the
166 microbiological and gas sampling (i.e. June 2017 and May 2018). Water
167 parameters such as temperature (T °C), pH, electrical conductivity (EC), and
168 total dissolved solids (TDS) were measured using a multiparametric probe
169 Hanna HI991001 (relative accuracies at 25 °C: ±0.5 °C, ±0.02 pH, ±2%
170 EC/TDS). Two replicas of water were collected in each sampling site in 250-ml
171 HDPE bottles, and one was acidified with 65% HNO_3, and stored at 4°C until
172 analysis at the laboratory of Politecnico di Torino.

173 The δ^2H and $\delta^{18}O$ in a third replica of water collected during May 2018 were
174 measured simultaneously at University of Almeria by cavity ringdown
175 spectroscopy (CRDS) by a L2140-i Picarro water isotope analyzer interfaced
176 with an A0211 high-precision vaporizer (Picarro Inc., USA), coupled with a
177 Picarro micro-combustion module (MCM®) to remove combustible organic
178 compounds from water samples. Each sample was injected 10 times into the
179 vaporizer, which was heated to 110 °C. Memory effects from previous samples
180 were avoided by rejecting the first three analyses, so values for the final 7
181 injections were averaged with a typical in-sample precision (±1σ) of ±0.04‰ for
182 $\delta^{18}O$ and ±0.18‰ for δ^2H. The results were normalized against V-SMOW by
183 analyzing internal standards before and after each set of twenty samples and
184 are given as per mil (‰). ^{13}C measurements of the dissolved inorganic carbon
185 in water samples ($\delta^{13}C$-DIC) were also obtained by using the same G2201-i
186 analyzer coupled with an Automate FX sample preparation device. NBS-18
187 (Carbonatite) and IAEA-603 (calcite) were used to calibrate the AutoMate-
188 CRDS system and referring the results to the Vienna Pee Dee Belemnite (V-
189 PDB). Three replicates per sample were analyzed together with two internal
190 standards run before and after the water sample set, reaching an average
191 precision (±1σ) of ±0.10 ‰.

192

193 *2.2. Field emission scanning electron microscopy with Energy Dispersive X-ray*
194 *Spectroscopy (FESEM-EDS)*

195   The morphology of the white filaments was studied by FESEM using a FEI
196   Teneo FESEM (FEI Company, Eindhoven, The Netherlands) with secondary
197   electron detection mode and an acceleration voltage of 5 kV for high resolution
198   images and 10 kV for elemental microanalysis. Before FESEM observations,
199   the fixed samples were washed in cacodylate-buffer, post-fixed in 1% osmium
200   tetroxide and dehydrated by serial dilutions in ethanol and acetone.
201   Subsequently, samples were dried in a Leica EM CPD300 critical point dryer at
202   34.5 °C and then sputter coated with a gold thin film, as described by De la
203   Torre Noetzel et al. (2018).
204
205   *2.3. DNA extraction, sequencing and functional prediction*
206   Genomic DNA extraction was carried out using the FastDNA SPIN Kit for
207   Soil (MP Biomedicals, Illkirch, France). DNA quantification was measured by
208   means of a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA) and the DNA
209   concentration values of FC1, FC4, FC6, and FC7 samples were 22.1, 217.6,
210   22.0 and 31.5 ng/μL, respectively.
211   High-throughput sequencing of extracted DNA was performed by Macrogen
212   (Seoul, Korea). We targeted the V3-V4 hypervariable regions of the 16S rRNA
213   gene using the primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-
214   GACTACHVGGGTATCTAATCC-3'). PCR amplification reaction per sample
215   consisted of 5 μL of each primer (1μM), 12.5 μL of 2x KAPA HiFi HotStart
216   ReadyMix (Roche) and 2.5 μL of DNA template (5 ng/μL), for a total of 25
217   μL/sample. The PCR program was run as follows: primary denaturation at 95°C
218   for 3 min, followed of 30 cycles beginning with a denaturalization step at 95°C
219   for 30 s, a second step of annealing at 56°C for 30 s and a third step of
220   elongation at 72°C for 1 min. The PCR program concluded with an elongation
221   step at 72°C for 5 min. Illumina MiSeq platform was used for 2 × 250 paired-end
222   sequencing, following the Illumina protocol for library Nextera XT Index Kit
223   preparation.
224   Raw data were checked for quality using FastQC
225   (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Amplicon
226   Sequence Variant (ASV)-based analyses were conducted with the QIIME2
227   platform (Bolyen et al., 2019). First, DADA2 (Callahan et al., 2016) filtered the

228 raw data according to the quality, generating an amplicon sequence variant
229 (ASV) table. Afterwards, taxonomic assignment was implemented using the
230 feature-classifier classify-sklearn (Bokulich et al., 2018) and the SILVA
231 database 132 version (Quast et al., 2013). Finally, alpha diversity analysis was
232 carried out for evenness and richness measurement of microbial communities
233 using the following metrics: Chao1, Shannon and Simpson indices, as well as
234 Pielou's evenness measure (Chao, 1984; Shannon and Weaver, 1949;
235 Simpson, 1949; Pielou, 1966).

236 Taxonomic distribution and relative abundance of the microbial community
237 were depicted in heat-maps using R package *gplots* (Warnes et al., 2015).
238 Samples were arranged through dendrograms based on the taxonomic
239 abundance and representativeness. Prediction of functionality in the microbial
240 community based on 16S rRNA gene data was carried out using FAPROTAX
241 (Louca et al., 2016).

242

243 **3. Results and discussion**

244 *3.1. Gaseous composition of the cave atmosphere and water geochemistry*

245 The values of measured CO_2 and CH_4 concentrations and their stable
246 isotopic compositions ($\delta^{13}C\text{-}CO_2$ and $\delta^{13}C\text{-}CH_4$) in Fetida Cave environment,
247 above-cave soil, and local exterior atmosphere are shown in Table 1. The mean
248 concentrations of CO_2 and CH_4 in the local external atmosphere were 437.4 ±
249 17.3 ppmv and 2.00 ± 0.02 ppmv, respectively. CO_2 concentration of cave air
250 varied within a narrow range between 460 and 650 ppmv, i.e. it usually ranges
251 between 100 and 200 ppmv higher than the local atmospheric background
252 outside. CH_4 concentration of cave air (2.13 ± 0.09 ppmv, on average) is slightly
253 higher than those recorded for the local external atmosphere, but with maximum
254 values around 2 - 2.2 ppmv in the inner locations closer to the acid springs. H_2S
255 concentrations in the air of Fetida Cave ranged from 0.8 to 2.4 ppmv, with the
256 highest concentrations closest to the acid springs (P2 and P1) and the lower
257 concentrations at the entrance and middle cave locations (P3 and P4), due to
258 an efficient exchange and mixing with H_2S-free outdoor atmosphere.

259 At these cave-air levels of H_2S, the spectral lines of the CRDS spectrometer
260 avoid the strongly absorbing H_2S spectral lines and, therefore, the measurable

261 effect on the reported CO_2 concentrations can be considered negligible. The
262 changes in the $^{12}CO_2$ and $^{13}CO_2$ with an addition of H_2S ranging from 0.8 to 2.4
263 ppm would be 0.5–0.75 % and less than 7%, respectively, according to
264 Malowany et al. (2015). In the case of a well-ventilated atmosphere as Fetida
265 Cave, with CO_2 contents slightly higher than local exterior atmosphere and
266 $^{13}CO_2$ roughly being 1% of the $^{12}CO_2$ molar fraction, these percentages entail a
267 total variation for CO_2 concentration of only 4.1 ppm, on average (±3.7 ppm for
268 $^{12}CO_2$ and ±0.4 ppm for $^{13}CO_2$). The small interferences of H_2S on CO_2
269 measurements by CRDS were confirmed through the field CO_2 measurements
270 obtained with the handheld XP200 logger equipped with a NDIR probe.

271 However, the H_2S spectral lines, that partially overlap with some spectral
272 features of the CO_2 used in the CRDS system, turns into some relative changes
273 on $^{12}CO_2$ with respect to $^{13}CO_2$ that provokes a remarkable interference on the
274 CO_2 isotopic measurements (Malowany et al., 2015). Thus, H_2S in air samples
275 from Fetida Cave is enough to influence measurements by an unreal increase
276 in the $^{12}CO_2$ concentration and a more significant decrease in the $^{13}CO_2$
277 concentration, resulting in quite depleted $\delta^{13}C$-CO_2 values. Consequently, the
278 carbon isotopic values for CO_2 of cave air were not reported in Table 1.

279 Fortunately, the H_2S levels of Fetida Cave have a negligible interference on
280 the CRDS-reported $\delta^{13}C$-CH_4 for cave air samples, similar to what reported in
281 Malowany et al. (2015) described for standard gases in laboratory
282 measurements, allowing for comparison of results of both concentration and
283 carbon isotopic signal for this gas. Despite the fact that CH_4 concentrations of
284 cave air are slightly higher than those recorded for the local atmosphere, the
285 $\delta^{13}C$-CH_4 varies within a narrow range between -45.1 ‰ and -47.5 ‰ and is not
286 linearly related to the inverse CH_4 concentration. These $\delta^{13}C$-CH_4 values
287 evidence that CH_4 in cave air is not locally generated by biogenic processes
288 since if this would be the case it should be significantly enriched in ^{12}C relative
289 to the external atmosphere. Methane present in cave air, however, is mainly
290 sourced by the atmospheric CH_4 from ventilation through the sole entrance, i.e.
291 bypassing the soil zone. This suggests that the subterranean atmosphere is
292 greatly diluted by inputs from the outside atmosphere, which is also
293 corroborated by low CO_2 concentrations and O_2 levels close to 21%.

294   Soil-CO_2 concentrations show a remarkable difference between the two
295 field surveys (Table 1) ~~that~~ depending on the prevailing soil microbial respiration
296 rate each time, which, in turn, is mainly controlled by soil temperature and
297 moisture. However, in all samples nearly constant $\delta^{13}C$-CO_2 values (-19.73 ±
298 0.98 ‰, on average) prevail being relatively heavier than the expected
299 composition range for CO_2 derived from C3 organic matter. These $\delta^{13}C$-CO_2
300 values of soil air likely the ~~results~~ of the upward diffusion of CO_2 to the open
301 atmosphere and the effects of a kinetic fractionation on the residual CO_2 from
302 the soil zone. Contrary to what has been observed in the cave-air CH_4, an
303 intense microbial oxidation of CH_4 in soil provokes the residual methane to
304 become isotopically depleted in ^{12}C, a fact that was particularly evident in the
305 first air sampling campaign.

306   Overall, the gaseous composition of the external soil denotes its evident
307 disconnection from the cave atmosphere. Consequently, the isotopic
308 composition of CO_2 and CH_4 in cave air confirms a prevailing gas exchange
309 pathway with the atmospheric background source, ruling out both the biogenic
310 CH_4 and soil-derived CO_2 sources or a remarkable deep-sourced input for both
311 gases.

312   In general, the collected waters show mean temperature higher than 20°C,
313 and pH ranges between 6.89 (in the innermost portion -SCC1 and SCB1- of the
314 cave which is influenced by rising sulfuric acid fluids) and 7.38 (in the sampling
315 sites close to the entrance of the cave). The higher concentration of [HS^-]
316 dissolved in water was observed in the innermost sampling sites SCB1 and
317 SCC1 (Table 2). In addition, SCB1 showed the most mineralized waters with
318 higher values of temperature and lower value of pH. Detailed information on the
319 water geochemistry can be found in D'Angeli et al. (2021).

320   The stable isotope analyses of water from Fetida Cave revealed δ^2H values
321 that ranged from −4 to +7 ‰, approximately, with an average of +2.85 ‰, and
322 $\delta^{18}O$ values from −0.5 to +1.1‰, with an average of +0.39 ‰ (Table 3). The
323 isotopic composition of water samples from the thermal spring of Fetida Cave,
324 reported by Santaloia et al. (2016), was 0.60 and 0.05 ‰ for δ^2H and $\delta^{18}O$,
325 respectively. This single previous data practically coincides with the average
326 isotopic composition of the water sampled in the end passage of the cave

327 (SCC1-FC1 and SCB1-FC4 sampling locations), where the main inputs of deep
328 acid water are observed.

329 The isotopic data pairs of thermal water from Fetida Cave define a Local
330 Water Line (LWL) (Fig. 2), which is also aligned with the only isotopic data for
331 local meteoric water, as the ones reported by Santaloia et al. (2016) for a cold
332 borehole located in the cave's water recharge area. The isotopic data for local
333 meteoric water roughly matches the average isotopic composition of
334 precipitation in the area, according to data for St. Maria di Leuca reported by
335 Longinelli and Selmo (2003). Here, the LWL is fitted with a slope of 5.83 and
336 deuterium excess of 0.29. This linear function is almost equal to the LWL fitted
337 by Santaloia et al. (2016) (slope: 5.68 and deuterium excess: -0.69), but which
338 tends to positive $\delta^{18}O$ with respect to the GMWL (Clark and Fritz, 1997) and the
339 "regional" MWL for Southern Italy (Longinelli and Selmo, 2003).

340 Both the thermal water of Fetida Cave and the previous spring water
341 reported by Santaloia et al. (2016) have δ^2H and $\delta^{18}O$ values along a line
342 between the meteoric samples (cold well) and the seawater, suggesting a
343 mixing of fresh, thermal and marine waters. The low deuterium excess of
344 samples depends on the contribution of seawater, similar to what has been
345 described for other sulfide-bearing waters due to the variable contribution of
346 saline formation waters (Toscani et al., 2001). The positive $\delta^{18}O$-shift with
347 respect to the GMWL also indicates the prevailing influence of a mixing process
348 with seawater. Similar precipitation-seawater mixing lines have been isotopically
349 defined to assess some other coastal processes as groundwater discharge in a
350 hypersaline lagoon (Rocha et al., 2015) or the precise identification of river
351 plumes within the Great Barrier Reef (Munksgaard et al., 2012).

352 There is a spatial gradient in the mixing process with seawater, in this way
353 the $\delta^{18}O$-δ^2H data pairs of cave water are closer to those of meteoric water as
354 we move away from the coastline (Fig. 2 and Table 3). As a reference,
355 Santaloia et al. (2016) reported a mean isotopic composition of water from
356 nearby non-thermal water boreholes as -32.34±1.86, -5.57±0.15 and -
357 12.43±1.27 for δ^2H, $\delta^{18}O$ and $\delta^{13}C$-DIC, respectively (Table 3). The relative
358 position of the data pairs on the precipitation-seawater mixing lines will vary
359 over time and this will be determined by the tides controlling the sea level in
360 each specific sampling period, as well as the degree of meteoric water recharge

361 at that time (previous rainfall). The δ^{13}C-DIC values also show a gradient with
362 distance from the shoreline, with more negative values in the innermost areas of
363 the cave (< -2.5 ‰ in SCC1-FC1 and SCB1-FC4 locations - Table 3) indicating
364 an input of seepage water that is, in any case, distinguishable from seawater.
365 The more negative δ^{13}C-DIC values coincide with areas with more abundance
366 of bacteria suggesting processes of biomediated CO_2-fixation.

367 An enrichment in hydrogen isotopes based on the ^{2}H exchange between H-
368 bearing species (H_2O–H_2S) has been previously reported in shallow
369 groundwater mixed with H_2S enriched gases under volcanic and hydrothermal
370 settings (Chiodini et al., 2000; Hsu and Yeh, 2020). In contrast, our current data
371 set for Fetida Cave waters shows no evidence of an isotopic exchange of water
372 with the hydrogen in H_2S linked to rising sulfuric acid fluids. This effect would
373 lead to an increase in the δ^2H content of the water and, graphically, the isotopic
374 data pairs would lie along straight lines parallel to the ordinate axis (δ^2H-axis), a
375 behavior that is not observed in Figure 2.

376

377 *3.2. Morphology of Fetida Cave white filaments*

378 Filamentous morphologies were observed in the four samples of Fetida
379 Cave, as depicted in Figure 3. A dense net of partially corroded filaments
380 embedded in extracellular polymeric substances is shown in Figure 3A. Cells
381 resembling *Arcobacter* morphology were evidenced in this figure. The structures
382 and morphologies are similar to those previously reported by D'Angeli et al.
383 (2019b) for filaments collected in this cave. These authors reported that the
384 damaged structures can be associated with the constant exposure to rising
385 acidic sulfidic water. Mineral grains were attached and/or entrapped by the
386 filaments (Figs. 3B and D). D'Angeli et al. (2019b) concluded that the grains
387 were sulfur crystals based on EDS spectra. The detailed morphology of the
388 dense tangled masses of septate filaments observed in Figure 3C closely
389 resemble those reported by Bauermeister et al. (2012) and Flot et al. (2014) for
390 *Thiothrix* filaments.

391

392 *3.3. Microbial community composition*

393   Sequence quality control and construction of feature table using QIIME2
394 and DADA2 resulted in a total 180,528 features or sequences for the four
395 samples, clustering in a total of 5,209 amplicon sequence variants (ASVs). The
396 mean length of sequences was 453.6 bp, with a range oscillating between 274–
397 468 bp length. The most representative sample was FC6, with 62,628 features,
398 whereas FC4 presented only 30,826. Besides, the number of ASVs found in the
399 four samples varied in the 704–1,902 interval, where FC6 was observed to have
400 the higher value and FC4 the sample with the lower number. Samples FC6 and
401 FC1 presented 1,662 and 941 ASVs, respectively.

402   Summarizing the structure of the bacterial communities, assessing the
403 number of taxonomic groups (richness), and the distribution of abundances of
404 these groups (evenness) leads to a better understanding of the bacterial
405 ecology. Bacterial community composition was measured through Shannon,
406 Simpson and Chao indices, and Simpson's and Pielou´s evenness. Thus,
407 resulted values from alpha-diversity analysis (Table A2 in Appendix A -
408 Supplementary data) showed the sample FC6 both more diverse and even,
409 followed by FC7, since the values in every analyzed metric were differentially
410 higher in these samples than in FC1 and FC4.

411   Table A3 (Appendix A - Supplementary data) shows that the microbial
412 communities of the white filaments from Fetida Cave were almost totally
413 composed of *Bacteria*, ranging from 97.9% (in FC6) up to 99.8% (in FC1).
414 *Archaea* exhibited a low percentage ranging from 0.2% (in FC1) to 2.1% (in
415 FC6) and were composed exclusively of *Woesearchaeia*. This distribution is
416 similar to those reported for other caves (Itcus et al., 2018; Jurado et al., 2020).

417   Engel (2007) compared the 16S rRNA gene sequences retrieved from
418 microbial mats from six active sulfidic caves (including Frasassi, Movile and
419 Lower Kane caves) and revealed a diverse range of microorganisms among
420 which the phyla *Epsilonbacteraeota*, *Proteobacteria*, and *Bacteroidetes*, were
421 identified in all the caves. This agrees with our data on Fetida Cave (Table A4 in
422 Appendix A - Supplementary data), in which the microbial mats resulted to be
423 mainly composed of these three phyla with total relative abundances varying
424 between 57.2 and 82.7%. Moreover, if the phylum *Patescibacteria* is also
425 considered, the four samples under analysis reached between 72.6 and 88.2%
426 of the total microbial community composition. This indicates that the microbial

427 mats populating the acidic sulfidic waters have a remarkable homogeneity in
428 phyla distribution in different geographical settings. Less abundant phyla were
429 *Planctomycetes*, *Spirochaetes*, and *Chloroflexi*, which were also detected in the
430 caves studied by Engel (2007).

431 The phyla distribution presented in Table A4 (Appendix A - Supplementary
432 data) is similar to Engel's (2007) findings. In fact, five additional bacterial phyla
433 rarely exceed 1% of relative abundance in at least one of the white filaments
434 investigated: *Actinobacteria, Calditrichaeota, Deferribacteres, Firmicutes,* and
435 *Lentisphaerae*.

436 In the sulfidic caves studied by Engel (2007) the archaeal phylum
437 *Euryarchaeota* (class *Methanomicrobia*) was identified, however, in the
438 filaments from Fetida Cave *Nanoarchaeaeota* (class *Woesearchaeia*) was
439 present, although the relative abundance of *Archaea* in Fetida Cave was
440 scarce. The very low contribution of *Archaea* must be related with unfavorable
441 environmental conditions. Patin et al. (2014) sampled a West Florida Shelf blue
442 hole and found that *Woesearchaeia* comprised up to 40% of the water column
443 community below the oxycline around 100 m, which also featured elevated
444 sulfide levels. The metabolic pathways and potential biogeochemical roles of
445 *Woesearchaeia* likely include a strict anaerobic lifestyle and possible syntrophy
446 with a sulfate-reducing gammaproteobacterial clade (Castelle and Banfield,
447 2018).

448 Figure 4 shows the heat-map of the bacterial classes occurring in the white
449 filaments. These data were roughly in accordance with those of D'Angeli et al.
450 (2019b) on Fetida Cave microbial communities, in which a high abundance of
451 members related to sulfur metabolism and belonging to *Gammaproteobacteria,*
452 *Deltaproteobacteria*, and *Epsilonbacteraeota* were found in white filaments.
453 D'Angeli et al. (2019b) investigated floating and sedimented white filaments that
454 were generally dominated by *Gammaproteobacteria* (12–34%), followed by
455 *Deltaproteobacteria* (8–15%), *Alphaproteobacteria* (4–10%), and
456 *Epsilonbacteraeota* (3–12%). Archaeal sequences accounted for a maximum of
457 6% and were mainly affiliated with the phylum *Woesearchaeota*.

458 Conversely, in our samples, the phylum *Epsilonbacteraeota* (12.9–53.7)
459 dominated the filaments, followed by *Proteobacteria* (18.0–39.4%). Among
460 *Proteobacteria*, *Gammaproteobacteria* (10.0–16.4%), *Deltaproteobacteria* (5.9–

461 15.9), and *Alphaproteobacteria* (2.2–7.7%) were the most abundant classes.
462 *Woesearchaeota* ranged between 0.2 and 2.1%. The different phyla and
463 classes distribution can be attributed, among other reasons, to the diverse
464 environmental conditions and the period of sampling (2015 *vs* 2017) as well as
465 to different methodological procedures, including the use of different primer
466 pairs in D'Angeli et al. (2019b) that might provide slightly different but
467 complementary results (Wasimuddin et al., 2020).

468 The most abundant class was *Campylobacteria*, with relative abundances
469 of 44.0 and 53.7% in samples FC1 and FC4, respectively, that decreased (12.9
470 and 15.3%) in FC6 and FC7. With relative abundances above 15% appeared
471 *Gammaproteobacteria* (F1 and F7) and *Deltaproteobacteria* (FC6 and FC7).
472 The abundances of *Bacteroidia* ranged between 10.4 and 12.3% in FC4, FC6,
473 and FC7. *Gracilibacteria* was the class with abundances above 5% (FC6 and
474 FC7). Other classes reached abundances below 5%.

475 Figure 5 depicts the heat-map of the genera found in the microbial mats.
476 The genus *Arcobacter* attained 35.3% of relative abundance in FC1 and 45.8%
477 in FC4. Relative abundances above 5% were found for the genera *Arcobacter*
478 in FC6, *Sulfurimonas* in FC7, and *Halothiobacillus* in FC1, whereas the genera
479 *Arcobacter* in FC7, *Saprospira* in FC4, *Sulfurimonas* in FC1, FC4, and FC6,
480 *Sulfurovum* in FC1, FC4, and FC7, *Thioflexothrix* in FC4, *Candidatus* Thiobios
481 in FC7, and *Spirochaeta* in FC4 were identified between 5 and 2%. In the same
482 range, unidentified members of the family *Rhodobacteraceae* and order
483 *Campylobacterales* were found in FC1, of the class WS6 (*Dojkabacteria*) in FC6
484 and a gammaproteobacterium in FC7. Genera with relative abundances around
485 1% were *Thermomarinilinea* in FC1, FC4, and FC6, *Sulfurovum* in FC6,
486 *Desulfocapsa* in FC6 and FC7, MSBL7 in FC6, *Peredibacter* in FC7, and
487 *Hydrogenovibrio* in FC1. In addition, a number of uncultured genera and
488 families were unevenly distributed among the four samples. It must be noticed
489 that the genus *Beggiatoa* was only retrieved in sample FC7, with a low relative
490 abundance (0.7%) and *Thiothrix* reached even lower abundances (0.1-0.2%) in
491 samples FC1, FC6 and FC7.

492 The high abundance of *Arcobacter* (45.8% in FC4 and 35.3% in FC1) is a
493 novelty respect to previous studies on SAS caves (Macalady et al. 2006;
494 D'Auria et al., 2018). This abundance in the two samples taken in the innermost

part of the cave can be related to the higher concentration of [HS$^-$] dissolved in water and lower pH values.

Recently, Talà et al. (2021) studied the prokaryotic communities in Zinzulùsa, a submerged coastal cave at 6 km from Fetida Cave. In samples from submerged black crusts on the walls and bottom sediments (depths 1.9 m to 2.4 m) *Arcobacter* were lower than 0.01% and 0.07%, respectively. No filaments were reported. Anaerobic, sulfate-reducing bacteria (mainly *Thermodesulfovibrio* and *Fervidobacterium*) dominated the black crusts, but they were absent in Fetida Cave. This seems to indicate that Zinzulùsa anchialine waters were not favorable for the growth of *Arcobacter*.

Sievert et al. (2007) stated that *Candidatus* Arcobacter sulfidicus tolerates higher concentrations of H$_2$S and grows at very low oxygen concentrations, which allows an efficient competition with other sulfur-oxidizing bacteria. This bacterium produces sulfur filament mats in high sulfidic waters (Wirsen et al. 2002). The sulfur-oxidizer genus *Arcobacter* includes free-living species with the ability to fix CO$_2$, and grow chemolithotrophically via sulfur-oxidation linked to denitrification. He et al. (2020) reported that *Arcobacter* showed a relative abundance of 24.1% in the oligotrophic, anoxic, and sulfidic bottom layer (100 m) of Sansha Yongle Blue Hole, China. Therefore, the metabolic versatility of *Arcobacter* might provide a competitive advantage in the colonization of oligotrophic environments characterized by high sulfide, low oxygen, and dynamic fluid movement.

In a 60 m deep sulfide-rich groundwater, Deja-Sikora et al. (2019) found that the representations of *Beta-* and *Deltaproteobacteria* were small, while the *Epsilonbacteraeota* genera *Sulfurimonas, Sulfurovum*, and *Arcobacter* were very abundant (nearly 77%). Conversely, in waters collected from greater depths (148–300 m), the dominance of *Betaproteobacteria* and sulfate/sulfur-reducing *Deltaproteobacteria* was evident. The authors correlated the shift in microbial communities to depth, and changing nitrogen and oxygen contents. Macalady et al. (2008) indicated that high sulfide to oxygen ratio (> 150) promoted the intensive growth of *Epsilonbacteraeota* (e.g., *Sulfurovum* and *Arcobacter* among others) in the sulfidic water of the Frasassi Cave. Hotaling et al. (2019) found that the microbiome of H$_2$S-rich stream waters in southern Mexico was composed of *Acidithiobacillus, Sulfuricurvum, Sulfurimonas,*

Thiomonas, and *Arcobacter*, where *Arcobacter* reached a relative abundance of 2.1%.

According to Macalady et al. (2006) in Frasassi Cave white filaments are dominated by filamentous *Gammaproteobacteria* with *Beggiatoa*-like (cottony) or *Thiothrix*-like (feathery) cell morphologies and abundant sulfur inclusions. *Beggiatoa*-related clones were present in both biofilm types (cottony and feathery) and formed a monophyletic clade within the *Thiotrichaceae*. This clade accounted for almost half of the total sequences retrieved from the cottony biofilm. *Betaproteobacteria* and *Epsilonbacteraeota* related to *Thiobacillus*, *Arcobacter*, and other sulfur-oxidizing groups were retrieved in clone libraries but constituted a small fraction of the biomass in both biofilm types. In Fetida Cave, *Beggiatoa* was only found in sample FC7 with a minority abundance (0.7%) and *Thiothrix* was even lower (0.1–02%) in three samples (FC1, FC6, and FC7).

D'Angeli et al. (2019b) found *Arcobacter* in different samples of white filaments inside the cave, either floating or sedimented, but the higher relative abundance was 2.1% in a white filament at the bottom of the water stream close to the location of the rising H_2S-rich fluids. Outside the cave, the presence of *Arcobacter* in white filaments is missing or insignificant (relative abundance 0.2%). The authors suggested that this can be due to the selection imposed on the microbial diversity by the peculiar physico-chemical characteristics of the water inside the cave with a higher concentration of H_2S, slower water flow and absence of light.

Arcobacter was also found in Monte Conca, a Sicilian gypsum cave with an active sulfidic spring in the inner part of the cave. Other important genera were *Sulfurovum, Sulfurimonas,* and *Thiovirga*. The spring generates a small pool where in summer, sulfur-oxidizing bacteria reached up to 95%, and in winter represented 13.6% of the total population. *Arcobacter* relative abundances were 2.5 to 4.6% in summer and absent or insignificant in winter (0–0.2%) (Davis et al., 2020). These changes in abundances denote seasonal variations.

Apart from *Arcobacter*, other sulfur-oxidizing genera identified in the white filaments were *Sulfurimonas, Halothiobacillus, Thiothrix, Thioflexothrix, Thiomicrospira, Sulfurovum*, *Hydrogenovibrio*, *Candidatus* Thiobios, and *Beggiatoa*. These have been found in many of the investigated sulfide waters

563 (Brigmon et al., 2003; Engel, 2007; Boden et al., 2012; Rossmassler et al.,
564 2012; Han and Perner, 2015; Fomenkov et al., 2017; Jiang et al., 2017; Deja-
565 Sikora et al., 2019; Davis et al., 2020).

566 Villanueva Alvarez (2005) studied the occurrence and bacterial succession
567 in sulfur-rich blooms in the Ebro delta river. She reported that the bacterial
568 succession started with a dominance of *Beggiatoa*, followed by a *Spirillum*-
569 bloom composed of a high population of *Arcobacter* and after the dominance of
570 *Spirillum*-like cells a spirochaetal bloom was observed in which *Halothiobacillus*
571 and *Thiomicrospira* were detected. The succession was related to the
572 occupation of microniches at different oxygen and sulfide concentrations. These
573 could be the factors inducing the abundant population of *Arcobacter* in Fetida
574 Cave.

575 Sulfur-reducing bacteria, such as *Desulfocapsa* were also present in the
576 filaments from Fetida Cave, which indicates that microorganisms mediate both
577 sulfur oxidation and sulfur reduction within the white filaments and stream.
578 *Desulfocapsa* has been previously found in Lower Kane and Frasassi caves
579 (Engel, 2007). Other sulfur-reducing bacteria were the lineage MSBL7 (Häusler
580 et al., 2014; Nigro et al., 2020) and *Spirochaetes* (Berlanga et al., 2008;
581 Dubinina et al., 2011).

582 Within the *Patescibacteria*, Wrighton et al. (2016) identified RubisCO genes,
583 with a central role in CO_2 fixation in members of the *Dojkabacteria* (WS6) and
584 *Parcubacteria* (*Candidatus* Moranbacteria). *Candidatus* Moranbacteria are
585 relatively abundant in groundwaters (Probst et al., 2018). Other *Patescibacteria*
586 (*Gracilibacteria*) have limited metabolism and were predicted to be symbionts,
587 possibly episymbionts (Sieber et al., 2019).

588 The anaerobic ammonium-oxidating SM1A02 lineage (*Planctomycetes*) was
589 found in an anaerobic biological reactor, using ammonium and sulfate as the
590 substrate to start sulfate-reducing ammonium oxidation (Zhang et al., 2019).
591 Other anaerobic bacteria include *Thermomarinilinea* (*Chloroflexi*) first isolated
592 from a submarine hot spring (Nunoura et al., 2013).

593 The identification of the genus *Peredibacte*r and an uncultured bacterium
594 from the family *Micavibrionaceae* is remarkable, which points to a well-
595 established trophic chain in the cave. These bacteria belong to the *Bdellovibrio*-
596 and-like organisms (BALOs), composed of *Bdellovibrionaceae*, *Bacteriovorax*,

Peredibacter, and *Micavibrio*, and are widespread obligatory predators of other Gram-negative bacteria, including cyanobacteria (Davidov et al., 2006; Cai et al., 2014). In addition, *Saprospira*-like organisms have been reported to behave as predators of bacteria and algae (McIlroy and Nielsen, 2014).

3.4. 16S rRNA gene-based metabolic inference

The most relevant ecological roles of microbial communities in Fetida Cave were analyzed by FAPROTAX. Almost 25% of ASVs were assigned to at least one group of the 43 found in the analysis; the remaining percentage could not be assigned to any group (Fig. 6). This database is not exhaustive; therefore, only small proportions of ASVs were assigned.

The major predicted ecological functions were chemoheterotrophy (7.6–26.6%), respiration of sulfur compounds (8.5–18.8%), dark oxidation of sulfur compounds (5.8–15.8%), and fermentation (3.3–14.7%). Chemoheterotrophy was predicted to be greater in FC4, in which *Proteobacteria* (*Delta-* and *Gammaproteobacteria*), and *Bacteroidetes* were the most abundant involved phyla. In addition, the abundance of sequences assigned to the respiration of sulfur compounds and sulfate respiration was higher in FC6 and, slightly, in FC7. However, FC6 was the sample with the fewest predicted sequences within the dark oxidation of sulfur compounds group. Respiration of sulfur compounds was mainly assigned to the order *Desulfobacteral*es while dark oxidation of sulfur compounds was related to the genera *Sulfurimonas, Halothiobacillus*, and *Beggiatoa.* In a similar way, the abundance of *Desulfobacteral*es was related to the higher abundance of sulfite respiration and thiosulfate respiration predicted functions in FC6 and FC7, but there was no linkage with the sulfur respiration. FAPROTAX predictions also identified *Halothiobacillus* as the main taxon assigned to dark thiosulfate oxidation, dark sulfide oxidation and dark sulfur oxidation. Dark oxidation of sulfur compounds was not predicted by the FAPROTAX analysis for the genus *Arcobacter*, probably due to the absence of metabolic information on *Arcobacter* in the database.

Wirsen et al. (2002) related *Candidatus* Arcobacter sulfidicus, a chemolithoautotrophic sulfur oxidizer, to CO_2 fixation and filamentous sulfur formation. This bacterium has the capacity to fix carbon via the reductive

tricarboxylic acid cycle (Hügler et al., 2005). More recently, Noguerola et al. (2015) reported that a member of the genus *Arcobacter* fixes CO_2 in the dark in the sulfidic redoxcline of a meromictic karstic lake, via the same cycle, and Evans et al. (2018) revealed that *Arcobacter*, associated with filamentous sulfur material, have the ability to fix CO_2.

Some previous studies on laboratory-grown strains of sulfur-oxidizing bacteria described a ^{13}C depletion in the fixed medium with respect to the ambient source of CO_2 and bicarbonates (Ruby et al., 1987). Turning the spotlight once more on our $\delta^{13}C$-DIC dataset (Table 3), an important difference in $\delta^{13}C$-DIC (around +8 ‰) is noticeable between the meteoric water and thermal water from Fetida Cave, particularly at the cave locations with greater evidence of water recharge from the upper vadose zone. These locations correspond to the innermost points of the cave, where the mixing with seawater is lower and a greater abundance of white filaments was also detected. Therefore, the *Arcobacter* from Fetida Cave might likely be involved in the ^{12}C fixation from the bicarbonates dissolved in the mixture of meteoric water (seepage water) and seawater, and this would cause a carbon isotopic fractionation in the bicarbonates resulting in higher $\delta^{13}C$-DIC values in relation to the typical isotopic values of bicarbonates in the seepage water.

Fermentation was predicted in FC4 associated to the genus *Spirochaeta.* The implication of *Spirochaeta* in the sulfur cycle was not predicted by FAPROTAX; however, the existence of sulfide-oxidizing and sulfur-reducing bacteria within the genus *Spirochaeta* has been described by Dubinina et al. (2011) in sulfide-rich water from a saline spring. Deja-Sikora et al. (2019) reported the coexistence of sulfur-oxidizing and sulfur-reducing bacteria in Polish sulfidic waters. In addition, many of the genera they found were the same as those retrieved from Fetida Cave. A similar coexistence was found in marine sediments (Ihara et al., 2019).

In light of the data shown, *Arcobacter* appears as a sulfur-oxidizing microorganism in coastal seawater that produces filaments trapping sulfur crystals and exhibits nitrogen fixation in concurrence with carbon dioxide fixation, as suggested by the water carbon isotope values.

The high variability in the composition of the white filaments, at the lower taxonomic levels, even in the same cave in different sampling periods, is due to changes in the water hydrochemistry and hydrodynamics, subjected to tidal water level fluctuation and the variable mixing of fresh continental with marine water.

Similar fluctuations were observed in Polish sulfide-rich mineral waters, since the composition of the bacterial communities strongly varied across the samples. However, most of the bacteria participating in the sulfur cycle were common in all sulfidic waters (Deja-Sikora et al., 2019). This was also found in Fetida Cave, where a certain homogeneity in phyla distribution and abundance can occur at higher taxonomic levels, due to the selection imposed by well-defined environmental conditions: the sulfide-rich water, and the bacterial groups participating in the sulfur cycle that were common to sulfidic waters.

CRediT authorship contribution statement

Valme Jurado, Ilenia D'Angeli, Tamara Martin-Pozas, Martina Cappelletti, Daniele Ghezzi, Jose Luis Gonzalez-Pimentel, Soledad Cuezva and Ana Zelia Miller: Investigation. Angel Fernández-Cortès: Investigation, writing - original draft. Jo De Waele: Conceptualization, review & editing, supervision. Sergio Sanchez-Moral: Conceptualization, Writing - original draft, Funding acquisition. Cesareo Saiz-Jimenez: Conceptualization, Supervision, Project administration, Funding acquisition, Writing – original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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FIGURE LEGENDS

Figure 1. The planimetry of Fetida Cave and the sampling locations (Image G) are shown on the right. Microbial (FC1, FC4, FC6, FC7) and water (SCA1, SCAfen, SCB1, SCC1) samples are reported in yellow, whereas gas samples are shown in red. The H_2S spring is indicated with a black line and corresponds to the SCB1-FC4 sampling location. The collected white floating filaments and their respective habitats (Images from A to F) are visible on the left. AE means anthropic entrance, whereas NE natural entrance.

Figure 2. Binary δ^2H–$\delta^{18}O$ diagram for thermal water samples from Fetida Cave, compared with the isotopic composition of meteoric and thermal spring, in accordance to Santaloia et al. (2016), and local rainfall. The local water meteoric line (black dashed line) is plotted in relation to the water meteoric line for southern Italy (Longinelli and Selmo, 2003) and the GMWL (Clark and Fritz, 1997).

Figure 3. White filaments from Fetida Cave. A: Sample FC1. Damaged filaments, extracellular polymeric substances and *Arcobacter*-like cells (in the center). B and C: Net of filaments from sample FC6. Magnification of C shows *Thiothrix*-like filaments. D: Filaments from sample FC7 and associated mineral grains of sulfur.

Figure 4. Heat-map analysis of Fetida Cave samples with taxonomic identifications of *Bacteria* at class level. The classes are described in the right column and their respective abundances included in the boxes. Colored left bar groups the classification at phylum level.

Figure 5. Heat-map analysis of Fetida Cave samples with taxonomic identifications of *Bacteria* at family/genus level. The families/genera are described in the right column and their respective abundances included in the boxes. Colored left bar groups the classification at order level.

1093   Figure 6. FAPROTAX analysis of white filaments from Fetida Cave with the
1094   predicted ecological functions based on 16S rRNA genes (Y axis). The size of
1095   the cycles indicates the relative abundance of the assigned ASVs