



UNIVERSITÀ POLITECNICA DELLE MARCHE
Repository ISTITUZIONALE

Benthic deep-sea fungi in submarine canyons of the Mediterranean Sea

This is a pre print version of the following article:

Original

Benthic deep-sea fungi in submarine canyons of the Mediterranean Sea / Barone, G.; Rastelli, E.; Corinaldesi, C.; Tangherlini, M.; Danovaro, R.; Dell'Anno, A.. - In: PROGRESS IN OCEANOGRAPHY. - ISSN 0079-6611. - 168:(2018), pp. 57-64.

Availability:

This version is available at: 11566/266051 since: 2022-05-31T16:50:39Z

Publisher:

Published

DOI:

Terms of use:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. The use of copyrighted works requires the consent of the rights' holder (author or publisher). Works made available under a Creative Commons license or a Publisher's custom-made license can be used according to the terms and conditions contained therein. See editor's website for further information and terms and conditions.

This item was downloaded from IRIS Università Politecnica delle Marche (<https://iris.univpm.it>). When citing, please refer to the published version.

(Article begins on next page)

Manuscript Details

Manuscript number	PROOCE_2018_117
Title	Benthic deep-sea fungi in submarine canyons of the Mediterranean Sea
Article type	Full Length Article

Abstract

Fungi are ubiquitous components of microbial assemblages in aquatic ecosystems, but their quantitative relevance, ecological role and diversity in benthic deep-sea ecosystems are still largely unknown. Here, we investigated patterns and drivers of benthic fungal abundance, biomass and diversity from 200 to 1000 m depth in three submarine canyons of the Mediterranean Sea (Tricase, Crotone and Squillace canyons). The Crotone and Squillace canyons, which are close to the coast and influenced by river inputs, showed significantly higher fungal abundance, biomass and diversity (as operational taxonomic units, OTUs) compared with the Tricase canyon that was far from the coast and without nearby estuaries. Fungal biomass, ranging from 0.17 to 5.78 $\mu\text{C g}^{-1}$, and abundance increased with increasing carbohydrate concentrations in the sediments, suggesting that deep-sea fungi have a role in the utilisation of this component of the organic matter. A total of 1742 fungal OTUs, belonging to all fungal phyla known to date, were found and Ascomycota represented the dominant phylum. However, only 36% of the reads belonged to known genera. In particular, Tricase and Crotone canyons hosted the highest proportion of unknown fungal taxa, suggesting that deep-sea sediments can harbour a high number of novel fungal lineages. Our findings also reveal that fungal assemblage composition in the investigated canyons was influenced by trophic and thermo-haline conditions, which may promote a high turnover diversity of benthic deep-sea fungal assemblages. Overall results reported here indicate that the submarine canyons of the Mediterranean Sea can represent hot-spots of abundant and highly diversified fungal assemblages and pave the way for a better understanding of the ecological role of fungi in the largest ecosystem on Earth.

Keywords	Benthic deep-sea ecosystems; fungal abundance; fungal diversity; submarine canyons; Mediterranean Sea
Manuscript category	Biological Oceanography
Corresponding Author	Antonio Dell'Anno
Corresponding Author's Institution	Università Politecnica delle Marche
Order of Authors	Giulio Barone, Eugenio Rastelli, cinzia corinaldesi, Michael Tangherlini, Roberto Danovaro, Antonio Dell'Anno
Suggested reviewers	Gaëtan Burgaud, Jan Pawlowski, Takuro Nunoura, Lucia Bongiorno
Opposed reviewers	Virginia Edgcomb

Submission Files Included in this PDF

File Name [File Type]

Barone et alii_Cover Letter.doc [Cover Letter]

Barone et alii_highlights.doc [Highlights]

Barone_et alii_main text_final.doc [Manuscript File]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
All data are included in the text



UNIVERSITÀ
POLITECNICA
DELLE MARCHE

—
Dipartimento
di Scienze
della Vita
e dell'Ambiente
DISVA

Ancona, 08.05.2018

Dear Editor,

please find enclosed the main text and figures of the manuscript entitled: “*Benthic deep-sea fungi in submarine canyons of the Mediterranean Sea*” by Giulio Barone et alii, submitted for consideration to *Progress in Oceanography* within the special issue “**Ecology and functioning of Mediterranean submarine canyons**”.

—
This is an original manuscript not submitted or presented elsewhere in which we investigated, for the first time, the quantitative relevance and diversity of fungi in deep-sea sediments of submarine canyons of the Mediterranean Sea. In this study, we show that fungi are an important component within the benthic deep-sea food webs and that they are highly diversified. Our results also indicate that different environmental characteristics encountered in the different canyons investigated can have a major role in influencing fungal diversity and assemblage composition. We think that this work expands our knowledge on the ecology and diversity of fungi inhabiting Mediterranean submarine canyons and we hope that it might be of interest for your journal.

For any requests, please do not hesitate to contact me at the address and numbers reported here below.

Looking forward to hearing from you soon, we remain.

Best regards

On behalf of all co-authors

Antonio Dell’Anno

Prof. Antonio Dell’Anno
Department of Life and Environmental Sciences (DISVA)
Università Politecnica delle Marche, 60131, Ancona, Italy.
Phone number: +39 0712204328
E-mail: a.dellanno@univpm.it

HIGHLIGHTS

- Submarine canyons host abundant and diverse fungal communities
- Fungal abundance, biomass and diversity are driven by carbohydrate concentrations
- Deep-sea sediments can harbour a high number of novel fungal taxa
- Thermohaline and trophic conditions may promote a high turnover diversity of fungi

1
2
3
4 **1 Benthic deep-sea fungi in submarine canyons of the Mediterranean Sea**

5
6
7
8 2
9 3 Giulio Barone¹, Eugenio Rastelli², Cinzia Corinaldesi³, Michael Tangherlini², Roberto
10 4 Danovaro^{1,2}, Antonio Dell'Anno^{1*}

11 5
12 6
13
14 7 *¹Department of Life and Environmental Sciences, Polytechnic University of Marche, 60131*
15 8 *Ancona Italy*

16 9 *²Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy*

17 10 *³Department of Sciences and Engineering of Materials, Environment and Urbanistics,*
18 11 *Polytechnic University of Marche, 60131 Ancona, Italy*

19
20
21
22
23
24
25
26
27
28 16 *Address for correspondence: a.dellanno@univpm.it
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

20 **Running title:** Fungal abundance and diversity in Mediterranean canyons

61
62
63 **Abstract**
64

65
66 Fungi are ubiquitous components of microbial assemblages in aquatic ecosystems, but their
67
68 quantitative relevance, ecological role and diversity in benthic deep-sea ecosystems are still
69
70 largely unknown. Here, we investigated patterns and drivers of benthic fungal abundance,
71
72 biomass and diversity from 200 to 1000 m depth in three submarine canyons of the
73
74 Mediterranean Sea (Tricase, Crotone and Squillace canyons). The Crotone and Squillace
75
76 canyons, which are close to the coast and influenced by river inputs, showed significantly
77
78 higher fungal abundance, biomass and diversity (as operational taxonomic units, OTUs)
79
80 compared with the Tricase canyon that was far from the coast and without nearby estuaries.
81
82 Fungal biomass, ranging from 0.17 to 5.78 $\mu\text{gC g}^{-1}$, and abundance increased with increasing
83
84 carbohydrate concentrations in the sediments, suggesting that deep-sea fungi have a role in
85
86 the utilisation of this component of the organic matter. A total of 1742 fungal OTUs,
87
88 belonging to all fungal phyla known to date, were found and Ascomycota represented the
89
90 dominant phylum. However, only 36% of the reads belonged to known genera. In particular,
91
92 Tricase and Crotone canyons hosted the highest proportion of unknown fungal taxa,
93
94 suggesting that deep-sea sediments can harbour a high number of novel fungal lineages. Our
95
96 findings also reveal that fungal assemblage composition in the investigated canyons was
97
98 influenced by trophic and thermo-haline conditions, which may promote a high turnover
99
100 diversity of benthic deep-sea fungal assemblages. Overall results reported here indicate that
101
102 the submarine canyons of the Mediterranean Sea can represent hot-spots of abundant and
103
104 highly diversified fungal assemblages and pave the way for a better understanding of the
105
106 ecological role of fungi in the largest ecosystem on Earth.
107
108
109

110
111
112 **Key Words:** Benthic deep-sea ecosystems, fungal abundance, fungal diversity, submarine
113
114 canyons, Mediterranean Sea
115
116
117
118
119
120

121
122
123 **1. Introduction**
124
125

126 49 Deep-sea ecosystems represent more than 65% of the world's surface and >95% of the
127
128 50 global biosphere (Herring, 2002), and host yet undiscovered biodiversity and a significant
129 51 portion of the world's genetic diversity (Danovaro et al., 2017). In benthic deep-sea
130
131 52 ecosystems, biomass is dominated by bacteria and archaea, followed by unicellular
132
133 53 eukaryotes and small metazoans (<0.5 mm in size, meiofauna). These organisms are
134
135 54 essential for carbon cycling and nutrient regeneration, and thus vital for sustaining
136
137 55 oceanic production (Dell'Anno and Danovaro, 2005; Sogin et al., 2006; Jørgensen and
138
139 56 Boetius, 2007; Danovaro et al., 2015; Danovaro et al., 2017). Recent findings, based on
140
141 57 culture-dependent and independent approaches, revealed that fungi are present in deep-
142
143 58 sea environments across a variety of ecosystem types spanning from hypersaline anoxic
144
145 59 basins (Bernhard et al., 2014; Edgcomb et al., 2017) to cold seeps (Nagahama et al.,
146
147 60 2011; Thaler et al., 2012), from hydrothermal vents (Burgaud et al., 2009; Burgaud et al.,
148
149 61 2010; Xu et al., 2017) to surface and subsurface sediments (Orsi et al., 2013; Pachiadaki
150
151 62 et al., 2016). Fungi have also been reported as the dominant unicellular eukaryotic group
152
153 63 in the marine snow in bathypelagic waters with biomass similar to that of prokaryotes
154
155 64 (Bochdansky et al., 2017).

156
157 65 Theoretical estimates suggest that fungi can be the most diversified component of
158
159 66 unicellular eukaryotes on Earth, with more than 5 million species of which only 5% have
160
161 67 been described (Hawksworth, 1997; Blackwell, 2011). This gap applies in particular to
162
163 68 open ocean ecosystems where a significant fraction of fungal diversity is still unknown
164
165 69 (Jeffries et al., 2016). Recent studies suggest that a variety of environmental factors (e.g.
166
167 70 temperature, salinity, nutrients) can influence the diversity and assemblage composition
168
169 71 of fungi in marine ecosystems (Li et al., 2016; Tisthammer et al., 2016). However, drivers
170
171
172
173
174
175
176
177
178
179
180

181
182
183 72 controlling the distribution and diversity of fungi in benthic deep-sea ecosystems remain
184
185 73 to date largely unexplored.

186
187 74 Fungi in terrestrial and freshwater ecosystems are among the main decomposers of
188
189 75 organic matter and play a key role in the processing of the most refractory fraction of
190
191 76 organic carbon (Carlile et al. 2001; Clipson et al. 2006; Hwang et al. 2006; Dighton,
192
193 77 2007). Since deep-sea ecosystems can contain relatively high amounts of organic carbon
194
195 78 (Pusceddu et al., 2009), fungi might play a key role in C cycling also in these ecosystems
196
197 79 (Hyde et al., 1998; Burgaud et al., 2009; Cathrine and Raghukumar, 2009; Jebaraj et al.,
198
199 80 2010).

200
201
202 81 In this study, we investigated the abundance, biomass and taxonomic composition of
203
204 82 fungal assemblages along the continental margins of the Central Mediterranean Sea.

205
206 83 Continental margins are characterised by open slopes and submarine canyons, which are
207
208 84 essential for C cycling and nutrient regeneration processes at a global scale (Bousquet et
209
210 85 al., 2000; Dickens, 2003). In particular, submarine canyons can channel large amounts of
211
212 86 organic matter photosynthetically produced from the continental shelf down to deep-sea
213
214 87 ecosystems (Monaco et al., 1999; Sánchez-Vidal et al., 2008; Allen and Durrieu de
215
216 88 Madron, 2009; Puig et al., 2014). For this reason, we selected three submarine canyons
217
218 89 characterised by different environmental conditions and investigated fungal abundance,
219
220 90 biomass and diversity at depths ranging from 200 to 1000 m. To identify the factors
221
222 91 potentially controlling their quantitative importance and diversity in deep-sea sediments,
223
224 92 we explored the role of environmental conditions, including the organic matter quality
225
226 93 and quantity.

227
228
229
230 94
231
232 95
233
234 96 **2. Materials and methods**

241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300

97 *2.1. Study area and sampling design*

98 Sediment sampling was carried out in the Ionian Sea (Central Mediterranean Sea) during
99 the oceanographic cruise “SAND 2016” held on board of the research vessel R/V
100 Minerva Uno in May 2016. Sediment samples were collected within the main axis of
101 three canyons located along the SE Italian margin at 200, 500 and 1000 m depths (Figure
102 1). One of the investigated canyon (hereafter defined “Tricase”) located along the
103 Apulian margin, is far from any continental freshwater inputs. The other two investigated
104 canyons are located along the Calabrian margin and were close to river estuaries. The
105 Northern canyon, extending for about 30 km, is located in front of the Crotona
106 municipality (canyon “Crotona”) and its head is close to a river mouth. The head of the
107 canyon “Squillace” is close to the coastline in front of the Squillace municipality and is
108 characterised by the presence of sporadic, but intense river inputs. Sediment samples
109 were collected at each benthic site by independent multiple corer deployments.
110 The top 1 cm of each sediment sample was used for the analysis of the quantity and
111 biochemical composition of organic matter, fungal abundance (based on q-PCR analysis
112 of 18S rRNA genes), biomass and diversity. At each station, temperature and salinity of
113 bottom waters were measured using CTD casts.

114
115 *2.2. Quantity and biochemical composition of organic matter*

116 The three major biochemical classes of organic compounds (proteins, carbohydrates and
117 lipids) in deep-sea sediments were determined according to previously described
118 procedures (Danovaro, 2010). Protein, carbohydrate and lipid concentrations were
119 determined spectrophotometrically and expressed as albumin, glucose and tripalmitin
120 equivalents, respectively. All analyses were carried out in 3 replicates. Protein,
121 carbohydrate and lipid concentrations were then converted to carbon equivalents

301
302
303 122 (conversion factors: 0.49, 0.40 and 0.75 gC g⁻¹, respectively) to determine biopolymeric C
304
305 123 content (Dell'Anno et al., 2002).
306
307
308 124

309 125 *2.3. Fungal biomass*

310 126 To detect and quantify fungi in the sediment samples, fluorescence in-situ hybridisation
311
312 127 (FISH) coupled with Calcofluor white staining (which targets chitin, cellulose and
313
314 128 carboxylated polysaccharides) have been used following procedures previously described
315
316 129 (Bochdansky et al., 2016). The FISH reaction was performed using the Pan-Fungal probe
317
318 130 PF2 (5'-CTC TGG CTT CAC CCT ATT C-3') Cy-3 labelled (Kempf et al., 2000).
319
320 131 Briefly, about 1 g of sediment was first treated using 4 ml of a mix containing EDTA,
321
322 132 Tween 80, sodium-pyrophosphate and methanol and ultrasounds treatment to separate
323
324 133 fungi from the sediment matrix. After centrifugation, sediment samples were washed
325
326 134 twice with PBS buffer and then treated with increasing concentrations of ethanol (50, 80
327
328 135 and 96%, for 3 min each). The sediment was then suspended in 500 µl hybridisation
329
330 136 buffer containing 0.9 M NaCl, 0.01% w/v SDS, 20 mM Tris-HCl pH 7.2, 30 %v/v
331
332 137 formamide and 1 µM PF2 (Kempf et al., 2000), then incubated for 3 h at 46°C in the
333
334 138 dark. Samples were then transferred in sterile tubes containing pre-warmed washing
335
336 139 buffer (20 mM Tris-HCl pH 8.0, 0.01% w/v SDS, 5 mM EDTA, 0.112M NaCl) and
337
338 140 incubated for 30 minutes at 48°C. After centrifugation and resuspension of the sediment
339
340 141 samples with 0.2 µm pre-filtered water, aliquots of the slurry (n=3) were filtered on 0.2
341
342 142 µm polycarbonate filters (Millipore) conditions. Filters were then stained with 0.5 mM
343
344 143 Calcofluor white and incubated in the dark for 5 min. Subsequently, slides were washed
345
346 144 with 0.02 µm pre-filtered water and analysed under epifluorescence microscopy. The
347
348 145 whole filter was examined, and length and width measures were taken for each fungal-
349
350 146 like structure. Then, the average width and cumulative length were converted to a
351
352
353
354
355
356
357
358
359
360

361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420

147 cylinder with half-spheres at ends, and the biovolume was converted into fungal biomass,
148 assuming 1 μm^3 of fungal biovolume equivalent to 1 pg C (Damare and Raghukumar
2008).

151 *2.4 DNA extraction and purification for molecular analysis*

152 The DNA was extracted and purified from the sediment samples using the PowerSoil
153 DNA isolation kit (QIAGEN)) following the manufacturer's instruction with slight
154 modifications to remove extracellular DNA (based on three subsequent washing steps)
155 before DNA extraction (Danovaro, 2009; Danovaro et al., 2016).

157 *2.5 Quantitative real-time PCR of fungal 18S rRNA gene sequences*

158 DNA extracted from two sediment samples collected at each study site by independent
159 multiple corer deployments was used for quantitative real-time PCR (qPCR) analysis
160 which was performed as described in Taylor et al. (2016) with slight modifications.
161 Briefly, fungi-specific primers FR1 5'-AIC CAT TCA ATC GGT AIT-3' and FF390 5'-
162 CGA TAA CGA ACG AGA CCT-3' (Prevost-Boure et al., 2011) were used with the
163 Sensi-FAST SYBR Q-PCR kit (Bioline, London, UK). The 15 μl reactions contained 8 μl
164 Sensi-FAST master mix, 1 μl of each primer (final concentration 1 μM), 1 μl of DNA
165 template and 5 μl nuclease-free molecular-grade water (Taylor and Cunliffe, 2016). A
166 Bio-Rad iQ5 was used to perform qPCR. The following qPCR thermal cycles were used:
167 94°C for 3min, then 40 cycles of 94 °C for 10 s, annealing at 50 °C for 15 s, elongation at
168 72°C for 20 s and acquisition of fluorescence data at 82°C. Standard curves were
169 generated using known concentration of *Aspergillus niger* 18S rDNA.

171 *2.6 Fungal diversity*

421
422
423 172 DNA extracted from two sediment samples collected at each study site by independent
424
425 173 multiple corer deployments was amplified using the primer set ITS1F (5'-
426
427 174 GGAAGTAAAAGTCGTAACAAGG-3') and ITS2 (5'-
428
429 175 GCTGCGTTCTTCATCGATGC-3') which amplify the internal transcribed spacer-1
430
431 176 (ITS1) region of the fungal rRNA gene (Walters et al., 2015). Amplicons were
432
433 177 sequenced on an Illumina MiSeq platform by LGC group (Berlin, Germany) following
434
435 178 Earth Microbiome Project protocols ([http://www.earthmicrobiome.org/emp-standard-](http://www.earthmicrobiome.org/emp-standard-protocols/)
436
437 179 [protocols/](http://www.earthmicrobiome.org/emp-standard-protocols/)). Barcodes and ITS1 primer pairs were removed before demultiplexing. Paired-
438
439 180 end sequences were then merged with FLASH (Magoč and Salzberg, 2011). Merged
440
441 181 sequences were quality filtered using the SEARCH tool (Edgar, 2010) to remove
442
443 182 sequences with expected error >1.0 and analysed with the QIIME software package
444
445 183 (Caporaso et al., 2010). Operational taxonomic units (OTUs) were assigned with a
446
447 184 threshold of 98.5% pairwise identity as indicated by the UNITE fungal ITS database
448
449 185 (<http://unite.ut.ee/>). Then, OTUs were classified taxonomically against the UNITE
450
451 186 database (<http://unite.ut.ee/>, Version 7.1, November 20, 2016). To allow a proper
452
453 187 comparison among samples, we followed the approach by Gihring et al. (2012) with
454
455 188 sample normalisation to 2500 randomly-selected sequences (corresponding to the lowest
456
457 189 read count obtained in our samples). Rarefaction curves highlighted that 2500 sequences
458
459 190 used for the comparison among all samples were generally sufficient to describe the
460
461 191 fungal diversity in the different benthic deep-sea ecosystems investigated (Figure S1).
462
463
464
465
466
467

468 193 *2.7 Statistical analyses*

470 194 Two-way analysis of variance (ANOVA) was performed to test for differences in organic
471
472 195 matter content, fungal abundance, biomass and OTU richness among canyons and depths.
473
474 196 When significant differences were encountered, post-hoc tests were also carried out.
475
476
477
478
479
480

481
482
483 197 ANOSIM analysis was performed to test for the presence of statistical differences in the
484
485 198 trophic conditions at the seafloor between canyons. Permutational multivariate analysis of
486
487 variance (PERMANOVA) was used based on Bray-Curtis similarity matrix and
488 199
489 visualised using cluster analysis to test for differences in fungal community composition
490 200
491 among canyons and depths. Distance-based multivariate analysis for a linear model
492 201
493 (DistLM) forward (Anderson, 2008) was performed to identify potential factors
494 202
495 influencing fungal abundance, biomass, OTU richness and assemblage composition. P
496 203
497 values were obtained with 9,999 permutations of residuals under the reduced model
498 204
499 (Anderson, 2008). Temperature, salinity and trophic resources (as protein, carbohydrate
500 205
501 and lipid concentrations) were used as predictor variables. Distance-based redundancy
502 206
503 analysis (dbRDA) was finally used to visualise the relationships between fungal
504 207
505 assemblage composition of the different canyon systems and thermo-haline and trophic
506 208
507 variables. All statistical analyses were performed using Primer 6+ software.
508 209
509
510
511 210
512
513 211
514 212
515

516 213 **3. Results and discussion**

517
518 214 The thermo-haline conditions of bottom waters of the benthic systems investigated in the
519 215
520 present study changed across depths and canyons, with temperature values ranging from
521 216
522 13.77 to 15.20 °C, and salinity values ranging from 38.75 to 38.93 (Table 1). Lowest
523 217
524 temperature and salinity values were generally observed at the greatest depth (i.e. 1000
525 218
526 m). Also, the analysis of organic matter quantity in the sediments revealed differences
527 219
528 among the investigated canyons (Tables 1, TableS1), with concentrations of proteins and
529 220
530 carbohydrates significantly higher in Crotona and Squillace canyons than in Tricase
531 221
532 canyon ($p < 0.05$ and $p < 0.01$, for proteins and carbohydrates, respectively). The highest
533 222
534 organic matter content in the sediments of Crotona and Squillace canyons is likely due to
535
536
537
538
539
540

541
542
543 223 their proximity to the coast and the presence of nearby river inputs which amplify the
544
545 224 magnitude of organic matter exported from the water column and settling on the seafloor
546
547
548 225 (Lopez-Fernandez et al., 2013).

549
550 226 The amount of organic matter in deep-sea sediments represents a significant factor
551
552 227 influencing the abundance and distribution of benthic assemblages (Danovaro et al.,
553
554 228 2014). Fungal abundance, expressed as number of fungal 18S rDNA copies ranged from
555
556 229 1.4×10^6 to 5.1×10^7 copies g⁻¹ and was significantly lower in Tricase ($0.38 \pm 0.04 \times 10^7$
557
558 230 copies g⁻¹) than in Crotona and Squillace canyons (2.7 ± 0.5 and $1.3 \pm 0.4 \times 10^7$ copies
559
560 231 g⁻¹, respectively; $p < 0.01$; Figure 2a). Our results fall within previously reported ranges for
561
562 232 deep-sea sediments of the Pacific Ocean (3.5×10^6 - 5.2×10^7 28S rDNA copies g⁻¹; Xu
563
564 233 et al., 2014), providing the first evidence of the quantitative importance of fungi also in
565
566 234 benthic deep-sea ecosystems of the Mediterranean Sea. In all canyons, the 18S rDNA
567
568 235 copy number changed significantly with water depth, with highest values at the
569
570 236 shallowest depth in Crotona and Squillace canyons and at 500 m depth in Tricase
571
572 237 canyons.

573
574
575 238 Fungal biomass ranged from 0.17 to 5.78 $\mu\text{gC g}^{-1}$, with values significantly lower in the
576
577 239 sediments of Tricase ($0.63 \pm 0.14 \mu\text{gC g}^{-1}$) than in Crotona and Squillace canyons ($2.40 \pm$
578
579 240 0.43 and $2.73 \pm 0.49 \mu\text{gC g}^{-1}$, respectively; $p < 0.01$) (Figure 2b). The distribution of
580
581 241 fungal biomass along the bathymetric gradients within each canyon was similar to that of
582
583 242 18S rDNA copy number. Data on fungal biomass are practically no existent for deep-sea
584
585 243 surface sediments (Damare and Raghukumar, 2008). However, the fungal biomass values
586
587 244 reported here are similar to those of other benthic components reported at equal depths in
588
589 245 the whole Mediterranean Sea (Gambi et al., 2017) suggesting that fungi can represent a
590
591 246 significant component of benthic biomass in deep-sea sediments.

592
593
594 247 We found a significant relationship between fungal abundance and biomass (Figure S2).
595
596
597
598
599
600

601
602
603 248 From the slope of this relationship, we estimated that 1 μg of fungal biomass could be
604
605 249 equivalent to 7.8×10^6 fungal 18S rDNA copies. Although such relationship should be
606
607
608 250 view with caution and needs to be better refined with a broader spatial scale investigation,
609
610 251 it can provide useful information on the quantitative relevance of deep-sea fungi based on
611
612 252 copy number determinations (Taylor and Cunliffe, 2016).

613
614 253 Significant positive relationships between carbohydrate concentrations and fungal
615
616 254 abundance and biomass were found ($r=0.715$ and $r=0.893$, both $p<0.01$, for the
617
618 255 abundance and biomass, respectively; Figure 3). Also, multivariate multiple regression
619
620 256 analysis provided evidence that carbohydrate concentration in the sediment was the
621
622 257 primary factor explaining the distribution of the abundance and biomass of fungi in the
623
624 258 benthic deep-sea ecosystems investigated (Table S2). Since fungi are osmotrophic (i.e.
625
626 259 feed by secreting enzymes into the environment to degrade organic matter externally
627
628 260 before taking the resulting metabolites into the cell; Richards and Talbot, 2013; Richards
629
630 261 et al., 2015), our results suggest that they could be highly specialized in the utilisation of
631
632 262 carbohydrates which are typically characterised by a highly recalcitrant fraction,
633
634 263 especially in benthic deep-sea ecosystems (Dell'Anno et al., 2000; Dell'Anno et al.,
635
636 264 2013).

637
638
639 265 Our results also show that the clustering of the 1203476 fungal ITS sequences (obtained
640
641 266 after quality check) allowed us to identify a total of 1742 fungal OTUs, belonging to all
642
643 267 fungal phyla known to date. Ascomycota represented the dominant phylum (accounting
644
645 268 for 68% of the total reads), followed by Basidiomycota (10%) and Chytridiomycota (4%).
646
647 269 The dominance of such phyla has been consistently reported in other benthic deep-sea
648
649 270 ecosystems (Zhang et al., 2016).

650
651
652 271 The number of fungal OTUs we found in the sediments of the different canyons was
653
654 272 similar compared with that reported in other deep-sea ecosystems (Zhang et al., 2016).
655
656
657
658
659
660

661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720

273 The Tricase canyon displayed a significantly lower OTU number (range: 64-71 OTUs)
274 compared to Crotone and Squillace canyons (range: 113-325 and 173-221 OTUs,
275 respectively; $p < 0.01$; Figure 4).

276 In our dataset, the OTUs affiliating to currently known fungal families were represented
277 by only 19-38% of the total reads (Figure 5). The classified fungal OTUs affiliated to 206
278 genera belonging to 132 families, 66 orders and 27 classes.

279 At all benthic sites, Pleosporales was the most represented fungal order (accounting for
280 ca. 20% of the total reads in each sample). This group is commonly present in marine
281 environment and can account for a relevant fraction of the fungal diversity (up to 18% of
282 all OTUs and sequences) in benthic deep-sea ecosystems (Li et al., 2016). Moreover,
283 members belonging to the Pleosporales order are known to be adapted to high hydrostatic
284 pressure (Nagano and Nagahama, 2012), possibly contributing to the ecological success
285 of such taxon in deep-sea ecosystems.

286 Most of the fungi that we successfully classified were affiliated to genera such as
287 *Aspergillus*, *Penicillium*, *Epicoccum*, *Cryptococcus* and *Candida* previously encountered
288 in other deep-sea environments (Nagahama et al., 2003; Edgcomb et al., 2011; Rédou et
289 al., 2014). However, these genera represented overall only ca. 36% of the total reads,
290 indicating that the majority of fungal taxa belonged to genera not represented in UNITE
291 database (Kõljalg et al., 2013).

292 The majority of fungal OTUs were unclassified below the order level and overall
293 represented up to 69% of the total sequences. The quantitative relevance of unclassified
294 sequences in our study was much higher than that reported for coastal sediments (Picard,
295 2017), indicating that deep-sea ecosystems might harbour a higher richness of novel
296 fungal lineages compared with shallow benthic ecosystems.

721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780

297 The composition of fungal assemblage in the sediments of the Tricase canyon was
298 significantly different ($p < 0.01$) from that of the other canyons, which otherwise showed
299 no significant differences (Figure 5). These results suggest that submarine canyons far
300 from the coastline and lacking river inputs can host distinct fungal assemblages from
301 those close to river estuaries.

302 The analysis of the turnover (β -)diversity highlighted that the similarity of the fungal
303 assemblage composition among different sites was very low (Table S3 and Figure 6).
304 Indeed, the within-canyon similarity (i.e., the similarity of fungal assemblage
305 composition among samples collected at a different depth within the same canyon) was
306 on average 11%, while the inter-canyon comparisons resulted in an average similarity of
307 7% (Table S3). Moreover, the Tricase canyon showed the highest percentage of unique
308 OTUs (i.e., OTUs found in Tricase but not in Squillace nor Crotone canyons; Table S4).
309 Overall, the three canyons shared only 46 out of 1742 OTUs, that cumulatively accounted
310 for only 22% of the total sequences. Twenty-seven of these 46 shared OTUs (overall
311 accounting for 14% of the total sequences) were not classified, while the others shared
312 OTUs (each of them contributing for $\leq 0.45\%$ of the total sequences) included taxa
313 belonging to *Epicoccum nigrum*, *Illyonectria robusta*, *Trichoderma bissettii*,
314 *Cryptococcus victoriae*, *Aspergillus sydowii*, *Fusarium sp.*, *Penicillium halotolearns* and
315 *Thermomyces lanuginosus*.

316 Distance-based redundancy analysis highlighted that the fungal assemblage composition
317 in the sediments of the different canyons was related to an array of factors including
318 organic matter content (as carbohydrates and lipid concentrations, $r = -0.624$ and $r = 0.434$,
319 respectively) and temperature ($r = 0.980$) and salinity ($r = -0.560$; Figure 7). These results
320 confirm that also in the deep-sea sediments investigated trophic availability and thermo-
321 haline conditions are important drivers of fungal assemblage composition (Hanson et al.,

781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840

322 2008; McGuire et al., 2010; Li et al., 2016; Taylor and Cunliffe, 2016; Tisthammer et al.,
323 2016). Our findings also suggest that changes in the thermo-haline and trophic conditions
324 among submarine canyons may promote a high turnover diversity of benthic deep-sea
325 fungal assemblages.

326 Overall results of the present study indicate that the submarine canyons of the
327 Mediterranean Sea host abundant and highly diversified fungal assemblages most of
328 which still unidentified and pave the way for a better understanding of the ecological role
329 of fungi in the largest ecosystem on Earth.

330
331 **Acknowledgments:** This study has been conducted in the framework of the National Flag
332 Project RITMARE (Marine Italian Research, www.ritmare.it) and supported by the EU
333 H2020 MERCES (Marine Ecosystem Restoration in Changing European Seas) project (Grant
334 Agreement No. 689518) and DG ENV project IDEM (Implementation of the MSFD to the
335 Deep Mediterranean Sea; contract EU No 11.0661/2017/750680/SUB/EN V.C2).

336
337 **Author Contributions:** R.D., C.C., and A.D. conceived the study. G.B. participated in the
338 oceanographic cruise for collecting sediment samples and performed laboratory analyses.
339 G.B., E.R., M.T. and A.D. contributed to data elaboration and interpretation. G.B., E.R., and
340 A.D. wrote the first draft of the manuscript. All authors contributed to results discussion and
341 finalization of the manuscript.

342
343 **Conflict of interest:** All the other authors declare no competing financial interests.

344

841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900

346 **References**

347 Allen, S.E., Durrieu de Madron, X., 2009. A review of the role of submarine canyons in
348 deep-ocean exchange with the shelf. *Ocean Science* 5, 607–620. doi:10.5194/os-5-607-
349 2009

350 Anderson, D.R., 2008. Model based inference in the life sciences: A primer on evidence,
351 Model Based Inference in the Life Sciences: A Primer on Evidence. Springer New York,
352 New York, NY. doi:10.1007/978-0-387-74075-1

353 Bernhard, J.M., Kormas, K., Pachiadaki, M.G., Rocke, E., Beaudoin, D.J., Morrison, C.,
354 Visscher, P.T., Cobban, A., Starczak, V.R., Edgcomb, V.P., 2014. Benthic protists and
355 fungi of Mediterranean deep hypersaline anoxic basin redoxcline sediments. *Frontiers in*
356 *Microbiology* 5, 360. doi:10.3389/fmicb.2014.00605

357 Blackwell, M., 2011. The Fungi: 1, 2, 3 ... 5.1 million species? *American Journal of Botany*
358 98, 426–438. doi:10.3732/ajb.1000298

359 Bochdansky, A.B., Clouse, M.A., Herndl, G.J., 2016. Eukaryotic microbes, principally fungi
360 and labyrinthulomycetes, dominate biomass on bathypelagic marine snow. *The ISME*
361 *Journal* 11, 362–373. doi:10.1038/ismej.2016.113

362 Bousquet, P., Peylin, P., Ciais, P., Le Quere, C., Friedlingstein, P., Tans, P.P., 2000. Regional
363 changes in carbon dioxide fluxes of land and oceans since 1980. *Science* 290, 1342–1346.
364 doi:10.1126/science.290.5495.1342

365 Burgaud, G., Arzur, D., Durand, L., Cambon-Bonavita, M.-A., Barbier, G., 2010. Marine
366 culturable yeasts in deep-sea hydrothermal vents: species richness and association with
367 fauna. *FEMS Microbiology Ecology* 73, 121–133. doi:10.1111/j.1574-
368 6941.2010.00881.x

- 901
902
903 369 Burgaud, G., Le Calvez, T., Arzur, D., Vandenkoornhuysse, P., Barbier, G., 2009. Diversity of
904
905 370 culturable marine filamentous fungi from deep-sea hydrothermal vents. *Environmental*
906
907 371 *Microbiology* 11, 1588–1600. doi:10.1111/j.1462-2920.2009.01886.x
908
909 372 Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K.,
910
911 373 Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights,
912
913 374 D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M.,
914
915 375 Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T.,
916
917 376 Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community
918
919 377 sequencing data. *Nature Methods* 7, 335–336. doi:10.1038/nmeth.f.303
920
921 378 Carlile MJ, Watkinson SC, Gooday GW (2001) *The fungi*, 2nd edn. Academic Press, San
922
923 379 Diego □
924
925 380 Cathrine, S.J., Raghukumar, C., 2009. Anaerobic denitrification in fungi from the coastal
926
927 381 marine sediments off Goa, India. *Mycological Research* 113, 100–109.
928
929 382 doi:10.1016/j.mycres.2008.08.009
930
931 383 Clipson, N., Otte, M., Landy E., 2006. Biogeochemical roles of fungi in the marine and
932
933 384 estuarine habitats. In: Gadd GM (ed) *Fungi in biogeochemical cycles*. Cambridge
934
935 385 university press, New York, pp 436–461
936
937 386 Damare, S., Raghukumar, C., 2008. Fungi and macroaggregation in deep-sea sediments.
938
939 387 *Microb Ecol* 56, 168–177. doi:10.1007/s00248-007-9334-y
940
941 388 Danovaro, R., 2009. *Methods for the Study of Deep-Sea Sediments, Their Functioning and*
942
943 389 *Biodiversity*. CRC Press.
944
945 390 Danovaro, R., Carugati, L., Berzano, M., Cahill, A.E., Carvalho, S., Chenuil, A., Corinaldesi,
946
947 391 C., Cristina, S., David, R., Dell’Anno, A., Dzhenbekova, N., Garcés, E., Gasol, J.M.,
948
949 392 Goela, P., Féral, J.-P., Ferrera, I., Forster, R.M., Kurekin, A.A., Rastelli, E., Marinova,
950
951 393 V., Miller, P.I., Moncheva, S., Newton, A., Pearman, J.K., Pitois, S.G., Reñé, A.,
952
953
954
955
956
957
958
959
960

961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020

394 Rodríguez-Ezpeleta, N., Saggiomo, V., Simis, S.G.H., Stefanova, K., Wilson, C., Martire,
395 Lo, M., Greco, S., Cochrane, S.K.J., Mangoni, O., Borja, A., 2016. Implementing and
396 Innovating Marine Monitoring Approaches for Assessing Marine Environmental Status.
397 *Frontier in Marine Science* 3, 233–25. doi:10.3389/fmars.2016.00213

398 Danovaro, R., Corinaldesi, C., Dell’Anno, A., Snelgrove, P.V.R., 2017. The deep-sea under
399 global change. *Curr. Biol.* 27, R461–R465. doi:10.1016/j.cub.2017.02.046

400 Danovaro, R., Corinaldesi, C., Rastelli, E., Dell’Anno, A., 2015. Towards a better
401 quantitative assessment of the relevance of deep-sea viruses, Bacteria and Archaea in the
402 functioning of the ocean seafloor. *Aquatic Microbial Ecology.* 75, 81–90.
403 doi:10.3354/ame01747

404 Danovaro, R., Snelgrove, P.V.R., Tyler, P., 2014. Challenging the paradigms of deep-sea
405 ecology. *Trends in Ecology & Evolution* 29, 465–475. doi:10.1016/j.tree.2014.06.002

406 Dell’Anno, A., Fabiano, M., Mei, M.L., Danovaro, R., 2000. Enzymatically hydrolysed
407 protein and carbohydrate pools in deep-sea sediments: estimates of the potentially
408 bioavailable fraction and methodological considerations. *Marine Ecology Progress Series*
409 196, 15–23. doi:10.3354/meps196015

410 Dell’Anno, A., Mei, M.L., Pusceddu, A., Danovaro, R., 2002. Assessing the trophic state and
411 eutrophication of coastal marine systems: a new approach based on the biochemical
412 composition of sediment organic matter. *Marine Pollution Bulletin* 44, 611–622.

413 Dell’Anno, A., Danovaro, R., 2005. Ecology: Extracellular DNA plays a key role in deep-sea
414 ecosystem functioning. *Science* 309, 2179. doi:10.1126/science.1117475

415 Dell’Anno, A., Pusceddu, A., Corinaldesi, C., Canals, M., Heussner, S., Thomsen, L.,
416 Danovaro, R., 2013. Trophic state of benthic deep-sea ecosystems from two different
417 continental margins off Iberia. *Biogeosciences*, 10, 2945–2957. doi:10.5194/bg-10-2945-
418 2013

1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080

419 Dickens, G.R., 2003. Rethinking the global carbon cycle with a large, dynamic and
420 microbially mediated gas hydrate capacitor. *Earth and Planetary Science Letters* 213,
421 169–183. doi:10.1016/S0012-821X(03)00325-X

422 Dighton J., 2007. Nutrient cycling by saprotrophic fungi in terrestrial habitats. In: Kubicek
423 CP, Druzhinina IS (eds) *The Mycota IV, environmental and microbial relationships*, 2nd
424 edn. Springer, Berlin, pp 287–300

425 Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST.
426 *Bioinformatics* 26, 2460–2461. doi:10.1093/bioinformatics/btq461

427 Edgcomb, V.P., Beaudoin, D., Gast, R., Biddle, J.F., Teske, A., 2011. Marine subsurface
428 eukaryotes: the fungal majority. *Environmental Microbiology* 13, 172–183.
429 doi:10.1111/j.1462-2920.2010.02318.x

430 Edgcomb, V.P., Pachiadaki, M.G., Mara, P., Kormas, K.A., Leadbetter, E.R., Bernhard, J.M.,
431 2017. Gene expression profiling of microbial activities and interactions in sediments
432 under haloclines of E. Mediterranean deep hypersaline anoxic basins. *The ISME Journal*
433 1–15. doi:10.1038/ismej.2016.58

434 Fernandez-Arcaya, U., Ramirez-Llodra, E., Aguzzi, J., Allcock, A.L., Davies, J.S.,
435 Dissanayake, A., Harris, P., Howell, K., Huvenne, V.A.I., Macmillan-Lawler, M., Martín,
436 J., Menot, L., Nizinski, M., Puig, P., Rowden, A.A., Sanchez, F., Van den Beld, I.M.J.,
437 2017. Ecological Role of Submarine Canyons and Need for Canyon Conservation: A
438 Review. *Frontiers in Marine Science*. 4, 69–26. doi:10.3389/fmars.2017.00005

439 Gihring, T.M., Green, S.J., Schadt, C.W., 2012. Massively parallel rRNA gene sequencing
440 exacerbates the potential for biased community diversity comparisons due to variable
441 library sizes. *Environmental Microbiology* 14, 285–290. doi:10.1111/j.1462-
442 2920.2011.02550.x

1081
1082
1083 443 Hanson, C.A., Allison, S.D., Bradford, M.A., Wallenstein, M.D., Treseder, K.K., 2008.
1084
1085 444 Fungal Taxa Target Different Carbon Sources in Forest Soil. *Ecosystems* 11, 1157–1167.
1086
1087 doi:10.1007/s10021-008-9186-4
1088 445
1089
1090 446 Hawksworth, D.L., 1997. The fascination of fungi: Exploring fungal diversity. *Mycologist*
1091
1092 447 11, 18–22. doi:10.1016/S0269-915X(97)80062-6
1093
1094 448 Hwang, J., Druffel, E.R.M., Bauer, J.E., 2006. Incorporation of aged dissolved organic
1095
1096 449 carbon (DOC) by oceanic particulate organic carbon (POC): An experimental approach
1097
1098 450 using natural carbon isotopes. *Marine Chemistry* 98, 315–322.
1099
1100 451 doi:10.1016/j.marchem.2005.10.008
1101
1102 452 Hyde, K.D., Jones, E.B.G., Leñaño, E., Pointing, S.B., Poonyth, A.D., Vrijmoed, L.L.P., 1998.
1103
1104 453 Role of fungi in marine ecosystems. *Biodiversity and Conservation* 7, 1147–1161.
1105
1106 454 doi:10.1023/A:1008823515157
1107
1108
1109 455 Jebaraj, C.S., Raghukumar, C., Behnke, A., Stoeck, T., 2010. Fungal diversity in oxygen-
1110
1111 456 depleted regions of the Arabian Sea revealed by targeted environmental sequencing
1112
1113 457 combined with cultivation. *FEMS Microbiology Ecology* 71, 399–412.
1114
1115 458 doi:10.1111/j.1574-6941.2009.00804.x
1116
1117 459 Jeffries, T.C., Curlevski, N.J., Brown, M.V., Harrison, D.P., Doblin, M.A., Petrou, K., Ralph,
1118
1119 460 P.J., Seymour, J.R., 2016. Partitioning of fungal assemblages across different marine
1120
1121 461 habitats. *Environmental Microbiology Reports* 8, 235–238. doi:10.1111/1758-2229.12373
1122
1123
1124 462 Jørgensen, B.B., Boetius, A., 2007. Feast and famine — microbial life in the deep-sea bed.
1125
1126 463 *Nature Reviews Microbiology* 5, 770–781. doi:10.1038/nrmicro1745
1127
1128 464 Kempf, V.A.J., Trebesius, K., Autenrieth, I.B., 2000. Fluorescent in situ hybridization allows
1129
1130 465 rapid identification of microorganisms in blood cultures. *Journal of Clinical Microbiology*
1131
1132 466 38, 830–838.

1141
1142
1143 467 Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates,
1144
1145 468 S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T.,
1146
1147
1148 469 Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M.,
1149
1150 470 Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B.,
1151
1152 471 Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, K.,
1153
1154 472 Saag, L., Saar, I., Schübler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor,
1155
1156 473 D.L., Telleria, M.T., Weiss, M., Larsson, K.-H., 2013. Towards a unified paradigm for
1157
1158 474 sequence-based identification of fungi. *Molecular Ecology* 22, 5271–5277.
1159
1160 475 doi:10.1111/mec.12481
1161
1162 476 Li, W., Wang, M.M., Wang, X.G., Cheng, X.L., Guo, J.J., Bian, X.M., Cai, L., 2016. Fungal
1163
1164 477 communities in sediments of subtropical Chinese seas as estimated by DNA
1165
1166 478 metabarcoding. *Nature Publishing Group* 1–9. doi:10.1038/srep26528
1167
1168
1169 479 Lopez-Fernandez, P., Calafat, A., Sanchez-Vidal, A., Canals, M., Flexas, M.M., Cateura, J.,
1170
1171 480 Joan B. Company, 2013. Multiple drivers of particle fluxes in the Blanes submarine
1172
1173 481 canyon and southern open slope: Results of a year round experiment. *Progress in*
1174
1175 482 *Oceanography* 118, 95–107. doi:10.1016/j.pocean.2013.07.029
1176
1177 483 Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve
1178
1179 484 genome assemblies. *Bioinformatics* 27, 2957–2963. doi:10.1093/bioinformatics/btr507
1180
1181 485 McGuire, K.L., Bent, E., Borneman, J., Majumder, A., Allison, S.D., Treseder, K.K., 2010.
1182
1183 486 Functional diversity in resource use by fungi. *Ecology* 91, 2324–2332. doi:10.1890/09-
1184
1185 487 0654.1
1186
1187
1188 488 Monaco, A., Durrieu de Madron, X., Radakovitch, O., Heussner, S., Carbonne, J., 1999.
1189
1190 489 Origin and variability of downward biogeochemical fluxes on the Rhone continental
1191
1192 490 margin (NW mediterranean). *Deep-Sea Research Part I* 46, 1483–1511.
1193
1194 491 doi:10.1016/S0967-0637(99)00014-X
1195
1196
1197
1198
1199
1200

1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239
1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260

492 Nagahama, T., Hamamoto, M., Nakase, T., Takaki, Y., Horikoshi, K., 2003. *Cryptococcus*
493 *surugaensis* sp. nov., a novel yeast species from sediment collected on the deep-sea floor
494 of Suruga Bay. *International Journal of Systematic and Evolutionary Microbiology* 53,
2095–2098. doi:10.1099/ijs.0.02712-0

496 Nagahama, T., Takahashi, E., Nagano, Y., Abdel-Wahab, M.A., Miyazaki, M., 2011.
497 Molecular evidence that deep-branching fungi are major fungal components in deep-sea
498 methane cold-seep sediments. *Environmental Microbiology* 13, 2359–2370.
499 doi:10.1111/j.1462-2920.2011.02507.x

500 Nagano, Y., Nagahama, T., 2012. Fungal diversity in deep-sea extreme environments. *Fungal*
501 *Ecology* 5, 463–471. doi:10.1016/j.funeco.2012.01.004

502 Orsi, W., Biddle, J.F., Edgcomb, V.P., 2013. Deep Sequencing of Subseafloor Eukaryotic
503 rRNA Reveals Active Fungi across Marine Subsurface Provinces. *PLoS ONE* 8, e56335–
504 10. doi:10.1371/journal.pone.0056335

505 Pachiadaki, M.G., Rédou, V., Beaudoin, D.J., Burgaud, G., Edgcomb, V.P., 2016. Fungal and
506 Prokaryotic Activities in the Marine Subsurface Biosphere at Peru Margin and
507 Canterbury Basin Inferred from RNA-Based Analyses and Microscopy. *Frontiers in*
508 *Microbiology* 7, 364–17. doi:10.3389/fmicb.2016.00846

509 Puig, P., Palanques, A., Martín, J., 2014. Contemporary Sediment-Transport Processes in
510 Submarine Canyons. *Annual Review of Marine Science* 6, 53–77. doi:10.1146/annurev-
511 marine-010213-135037

512 Pusceddu, A., Dell’Anno, A., Fabiano M., Danovaro R., 2009. Quantity and bioavailability of
513 sediment organic matter as signatures of benthic trophic status. *Marine Ecology Progress*
514 *Series*, 375, 41–52. doi:10.3354/meps07735

515 Rédou, V., Ciobanu, M.-C., Pachiadaki, M.G., Edgcomb, V.P., Alain, K., Barbier, G.,
516 Burgaud, G., 2014. In-depth analyses of deep subsurface sediments using 454-

1261
1262
1263 517 pyrosequencing reveals a reservoir of buried fungal communities at record-breaking
1264
1265 518 depths. *FEMS Microbiology Ecology* 90, 908–921. doi:10.1111/1574-6941.12447
1266
1267 519 Richards, T.A., Leonard, G., Mahé, F., del Campo, J., Romac, S., Jones, M.D.M., Maguire,
1268
1269 F., Dunthorn, M., de Vargas, C., Massana, R., Chambouvet, A., 2015. Molecular diversity
1270 520 and distribution of marine fungi across 130 European environmental samples. *Proc. R.*
1271
1272 521 *Soc. B* 282, 20152243–10. doi:10.1098/rspb.2015.2243
1273
1274 522
1275
1276 523 Richards, T.A., Talbot, N.J., 2013. Horizontal gene transfer in osmotrophs: playing with
1277
1278 524 public goods. *Nature Reviews Microbiology* 11, 720–727. doi:10.1038/nrmicro3108
1279
1280 525 Sánchez-Vidal, A., Pasqual, C., Kerhervé, P., Calafat, A., Heussner, S., Palanques, A.,
1281
1282 526 Durrieu de Madron, X., Canals, M., Puig, P., 2008. Impact of dense shelf water cascading
1283
1284 527 on the transfer of organic matter to the deep western Mediterranean basin. *Geophysical*
1285
1286 528 *Research Letters* 35, 117–125. doi:10.1029/2007GL032825
1287
1288 529 Sogin, M.L., Morrison, H.G., Huber, J.A., Welch, D.M., Huse, S.M., Neal, P.R., Arrieta,
1290
1291 530 J.M., Herndl, G.J., 2006. Microbial diversity in the deep sea and the underexplored “rare
1292
1293 531 biosphere.” *Proceedings of the National Academy of Sciences of the United States of*
1294
1295 532 *America* 103, 12115–12120. doi:10.1073/pnas.0605127103
1296
1297 533 Taylor, J.D., Cunliffe, M., 2016. Multi-year assessment of coastal planktonic fungi reveals
1298
1299 534 environmental drivers of diversity and abundance. *The ISME Journal* 10, 2118–2128.
1300
1301 535 doi:10.1038/ismej.2016.24
1302
1303 536 Thaler, A.D., Van Dover, C.L., Vilgalys, R., 2012. Ascomycete phylotypes recovered from a
1304
1305 537 Gulf of Mexico methane seep are identical to an uncultured deep-sea fungal clade from
1306
1307 538 the Pacific. *Fungal Ecology* 5, 270–273. doi:10.1016/j.funeco.2011.07.002
1308
1309 539 Tisthammer, K.H., Cobian, G.M., Amend, A.S., 2016. Global biogeography of marine fungi
1310
1311 540 is shaped by the environment. *Fungal Ecology* 19, 39–46.
1312
1313 541 doi:10.1016/j.funeco.2015.09.003
1314
1315
1316
1317
1318
1319
1320

1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380

542 Walters, W., Hyde, E.R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A.,
543 Gilbert, J.A., Jansson, J.K., Caporaso, J.G., Fuhrman, J.A., Apprill, A., Knight, R., 2015.
544 Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed
545 Spacer Marker Gene Primers for Microbial Community Surveys. *mSystems* 1, e00009–
546 15–11. doi:10.1128/mSystems.00009-15

547 Xu, W., Guo, S., Pang, K.-L., Luo, Z.-H., 2017. Fungi associated with chimney and sulfide
548 samples from a South Mid-Atlantic Ridge hydrothermal site: Distribution, diversity and
549 abundance. *Deep-Sea Research Part I* 123, 48–55. doi:10.1016/j.dsr.2017.03.004

550 Zhang, X.-Y., Wang, G.-H., Xu, X.-Y., Nong, X.-H., Wang, J., Amin, M., Qi, S.-H., 2016.
551 Exploring fungal diversity in deep-sea sediments from Okinawa Trough using high-
552 throughput Illumina sequencing. *Deep-Sea Research Part I* 116, 99–105.
553 doi:10.1016/j.dsr.2016.08.004

Captions of figures

Figure 1. Study area and sampling location (a). Details of benthic sites investigated within Tricase (a), Crotone (b) and Squillace (c) canyons. Bathymetry has been obtained from EMODnet (<http://portal.emodnet-bathymetry.eu>). Maps elaborated with QGIS.

Figure 2. Fungal abundance, expressed as 18S rDNA copy number (a), and biomass (b) in the different benthic sites of the Tricase, Crotone and Squillace canyons. Mean values and standard deviations are reported.

Figure 3. Relationships between carbohydrate concentrations in the sediments of the different canyons investigated and fungal abundance (a) and biomass (b)

Figure 4. OTU number in the different benthic sites within Tricase, Crotone and Squillace canyons. Mean values and standard deviations are reported.

Figure 5. Taxonomic composition (at the family level on data normalized to 2500 sequences) of the benthic fungal assemblages in the different canyons investigated. To better visualise differences among the investigated sites the output of cluster analysis is also reported.

Figure 6. Network visualisation based on the output of SIMPER analysis carried out on fungal community composition among the nine sites investigated. Line width is proportional to similarity values.

Figure 7. Output of the distance-based redundancy analysis (dbRDA) carried out on fungal community composition in the different benthic deep-sea sites in relation with thermo-haline and trophic conditions.

1441
1442
1443
1444
1445
1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500

580 **Table 1.** Temperature, salinity and protein (PRT), carbohydrate (CHO), lipid (LIP) and
581 biopolymeric C concentrations in the different sites of the Tricase, Crotone and Squillace
582 canyons. Mean values and standard deviations (\pm) are reported.

583

Canyon	Water depth m	Temperature °C	Salinity	PRT mg g ⁻¹	CHO mg g ⁻¹	LIP mg g ⁻¹	Biopolymeric C mg g ⁻¹
Tricase	200 m	14.58±0.01	38.8±0.01	1.91±0.55	2.21±0.59	0.82±0.28	2.43±0.72
	500 m	14.23±0.05	38.75±0.01	2.42±0.73	2.37±0.29	1.22±0.4	3.05±0.78
	1000 m	13.85±0.01	38.8±0.01	0.77±0.52	2.1±0.25	1.72±0.68	2.51±0.87
Crotone	200 m	15.07±0.12	38.91±0.01	2.87±0.24	3.56±0.23	1.61±0.76	4.04±0.78
	500 m	14.4±0.03	38.88±0.01	2.09±0.48	2.44±0.23	0.48±0.19	2.36±0.47
	1000 m	13.77±0.02	38.76±0.01	2.22±0.29	2.18±0.11	0.3±0.1	2.19±0.26
Squillace	200 m	14.78±0.06	38.82±0.01	2.21±0.36	3.77±0.59	0.6±0.31	3.04±0.64
	500 m	14.64±0.05	38.92±0.01	3.5±0.78	3.08±0.22	0.28±0.05	3.16±0.5
	1000 m	13.78±0.01	38.76±0.01	2.96±0.34	2.61±0.21	0.66±0.58	2.99±0.68

584

1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
586
1528
1529
587
1530
1531
588
1532
1533
589
1534
1535
590
1536
1537
591
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560

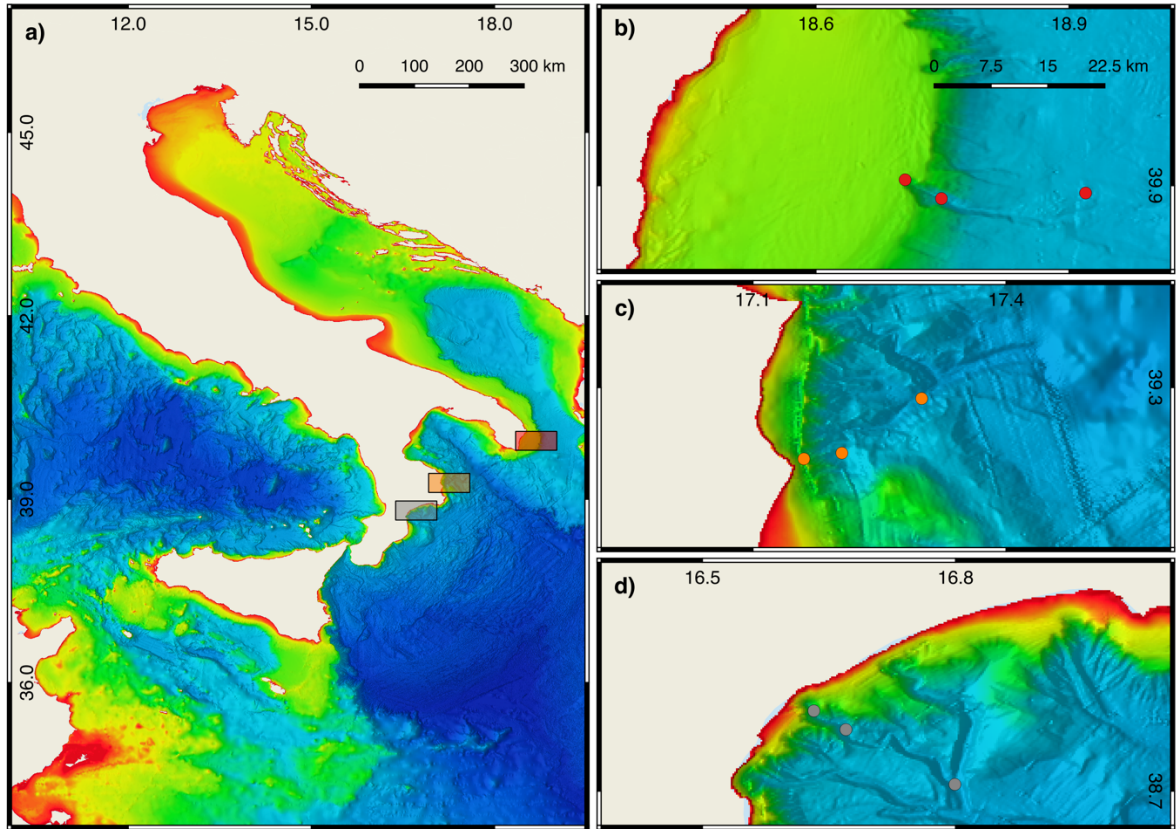


Figure 1

1561
 1562
 1563
 1564 593
 1565
 1566 594
 1567
 1568 595
 1569
 1570 596
 1571
 1572 597
 1573
 1574 598
 1575
 1576 599
 1577
 1578 600
 1579
 1580 601
 1581
 1582 602
 1583
 1584 603
 1585
 1586 604
 1587
 1588 605
 1589
 1590 606
 1591
 1592 607
 1593
 1594 608
 1595
 1596 609
 1597
 1598 610
 1600
 1601
 1602 611
 1603
 1604 612
 1605
 1606
 1607
 1608
 1609
 1610
 1611
 1612
 1613
 1614
 1615
 1616
 1617
 1618
 1619
 1620

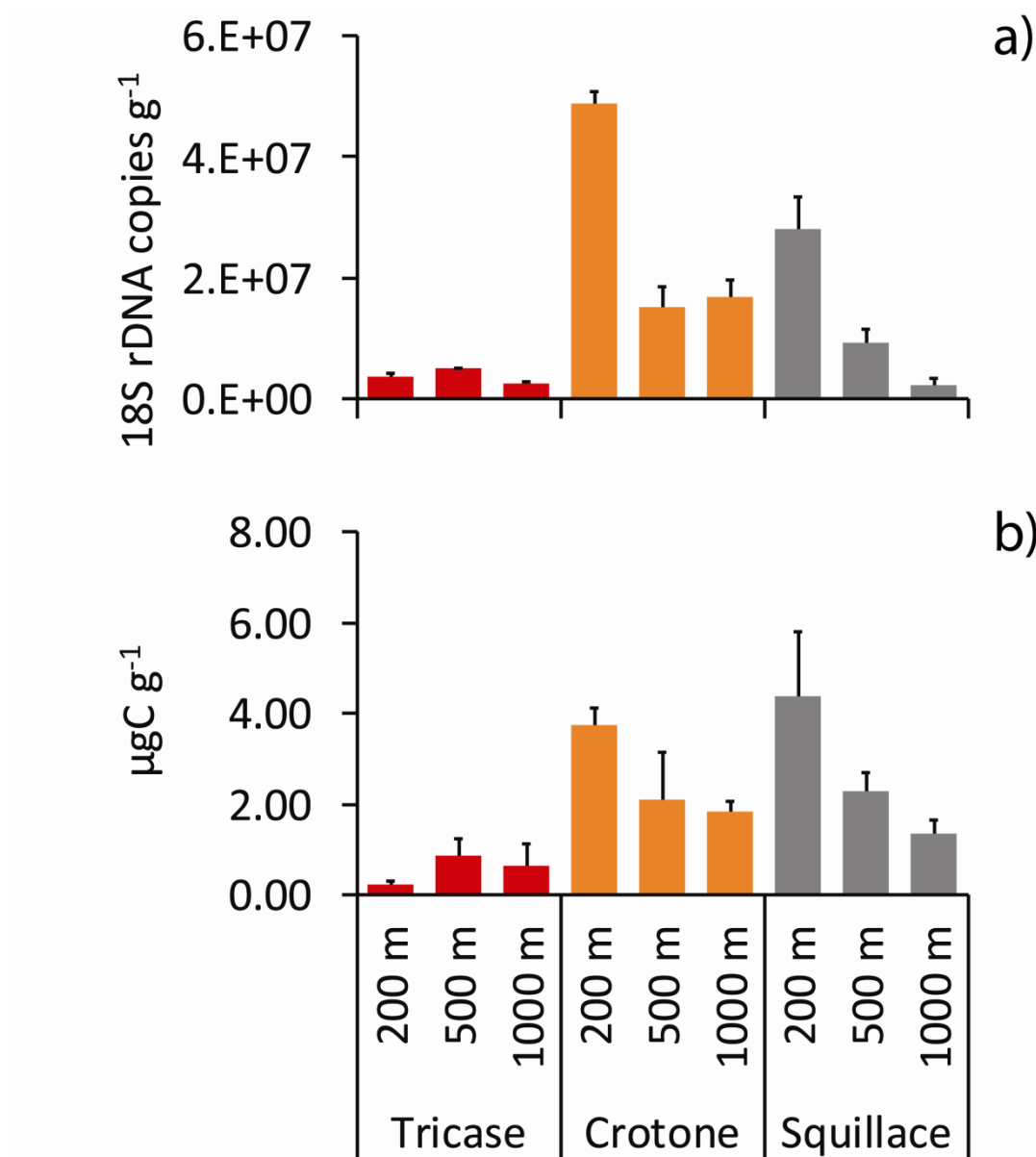
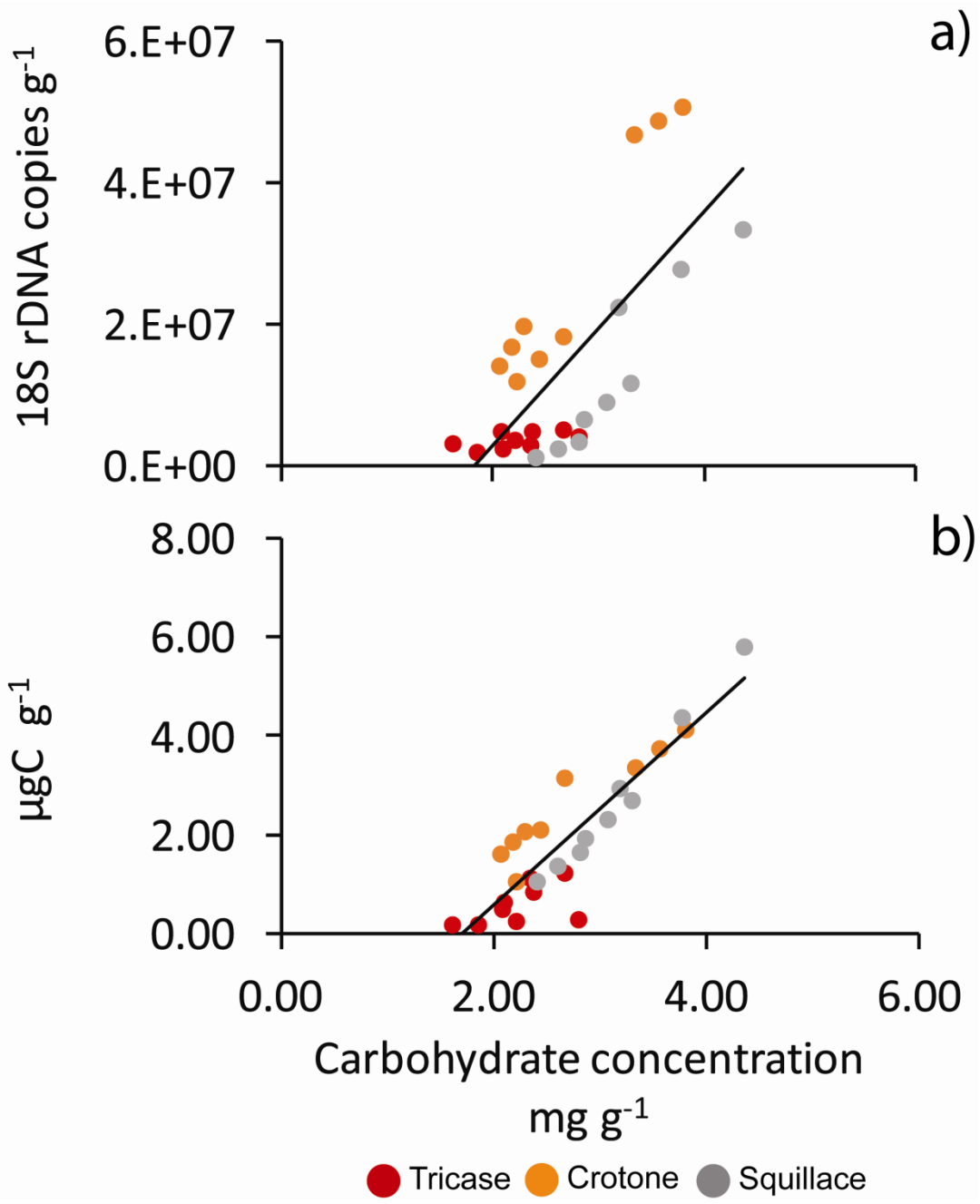


Figure 2



614

615 Figure 3

1681
1682
1683
1684 617
1685
1686 618
1687
1688 619
1689
1690 620
1691
1692 621
1693
1694 622
1695
1696 623
1697
1698 624
1699
1700 625
1701
1702 626
1703
1704 627
1705
1706 628
1707
1708 629
1709
1710 630
1711
1712 631
1713
1714 632
1715
1716 633
1717
1718 634
1719
1720 635
1721
1722
1723
1724 636
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740

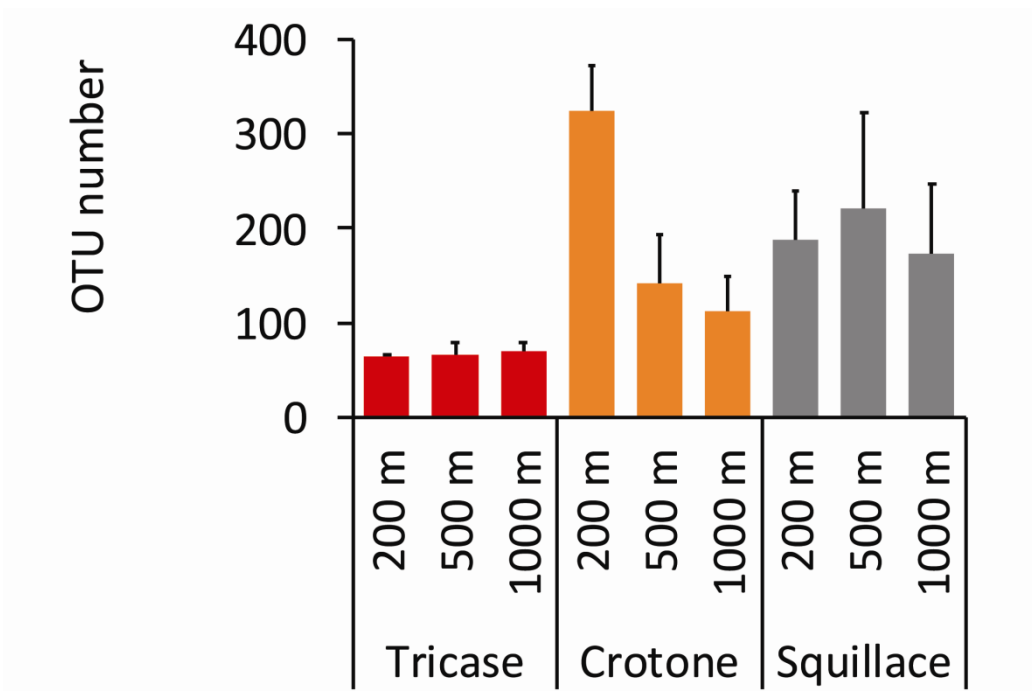


Figure 4

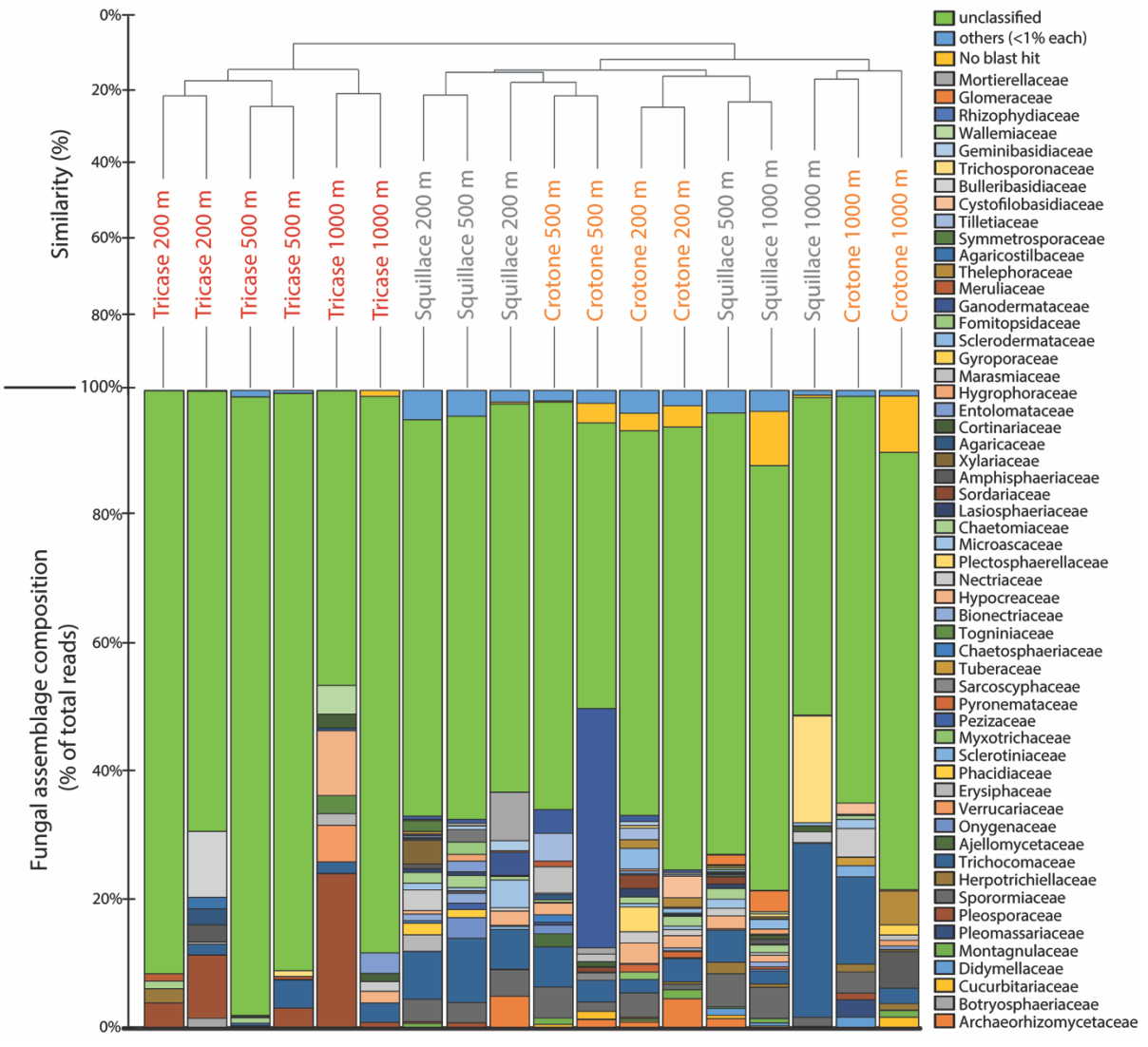
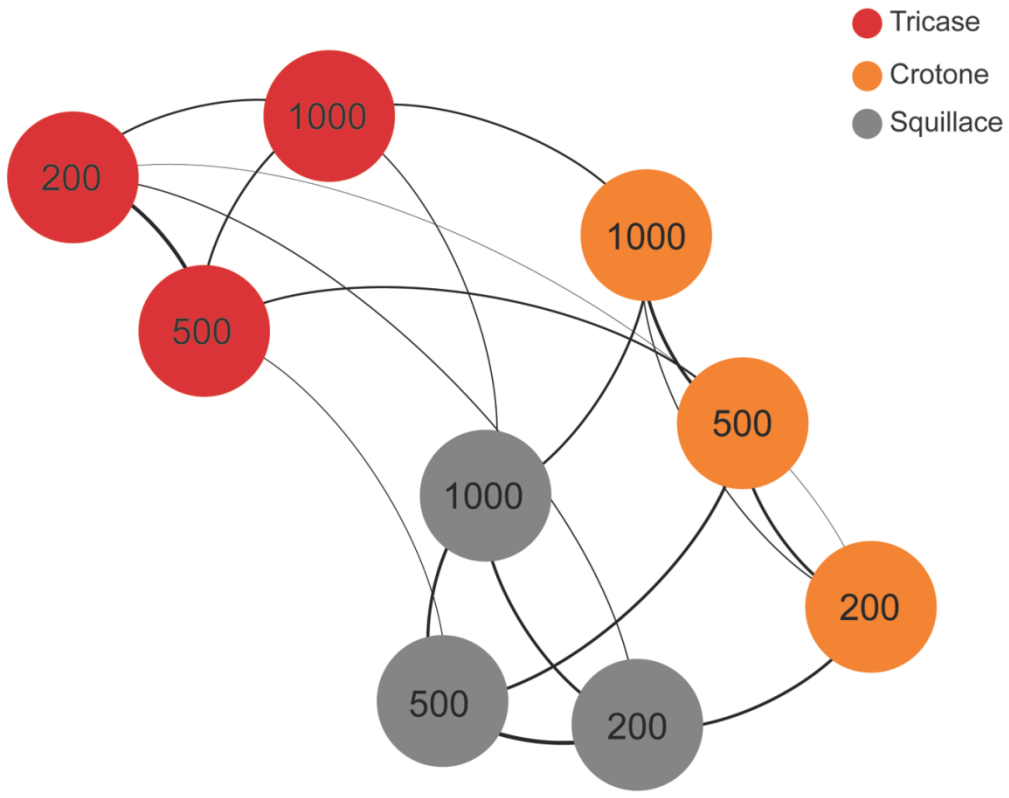


Figure 5

1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829
1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860



645
646 Figure 6
647
648
649
649
650
650
651

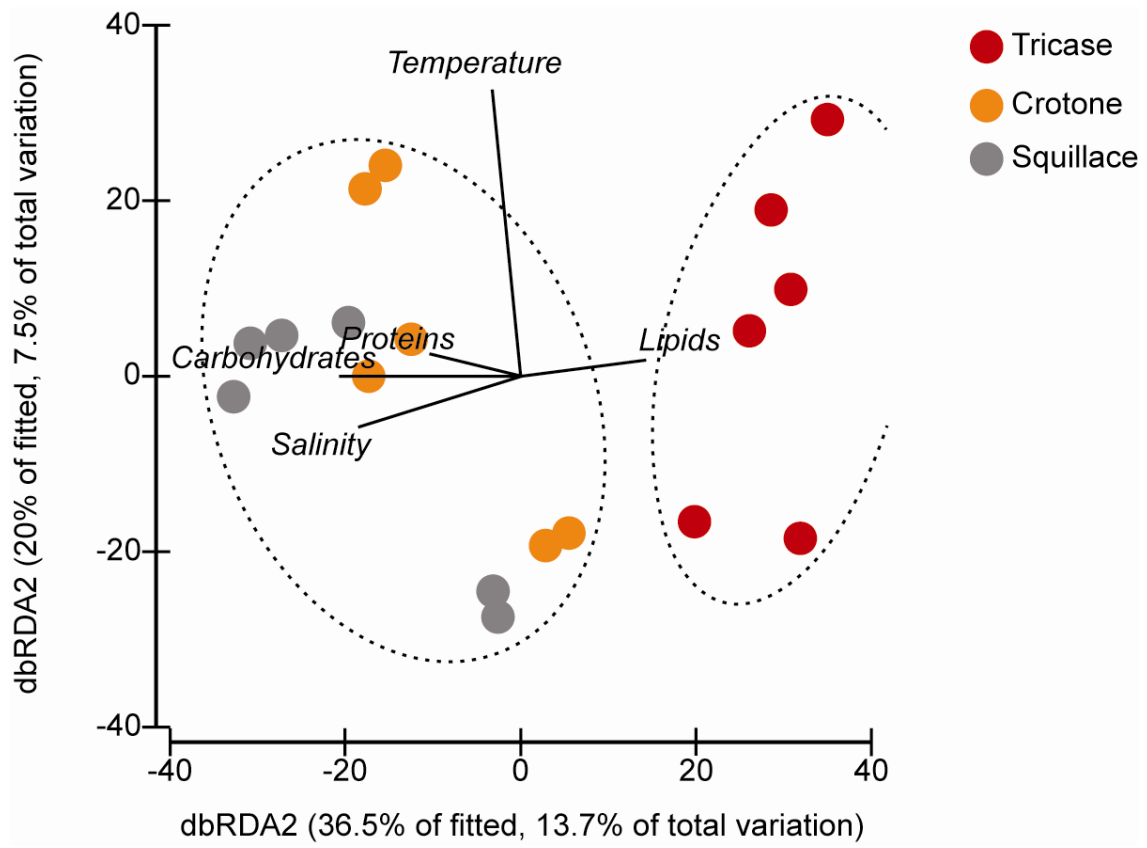


Figure 7

1921
1922
1923
1924
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935
1936
1937
1938
1939
1940
1941
1942
1943
1944
1945
1946
1947
1948
1949
1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980

Supplementary materials

666

667

668

Benthic deep-sea fungi in submarine canyons of the Mediterranean Sea

669

670

Giulio Barone, Eugenio Rastelli, Cinzia Corinaldesi, Michael Tangherlini, Roberto

671

Danovaro, Antonio Dell'Anno

672

673

674

675

676

677

Supplementary table S1-S4

678

679

Supplementary figures S1 and S2

1981
 1982
 1983
 1984
 1985
 1986
 1987
 1988
 1989
 1990
 1991
 1992
 1993
 1994
 1995
 1996
 1997
 1998
 1999
 2000
 2001
 2002
 2003
 2004
 2005
 2006
 2007
 2008
 2009
 2010
 2011
 2012
 2013
 2014
 2015
 2016
 2017
 2018
 2019
 2020
 2021
 2022
 2023
 2024
 2025
 2026
 2027
 2028
 2029
 2030
 2031
 2032
 2033
 2034
 2035
 2036
 2037
 2038
 2039
 2040

681
 682
 683
 684
 685
 686
 687
 688
 689

Table S1. Reported are the outputs of the ANOSIM and SIMPER analyses carried out to test for the differences and dissimilarity in sediment organic matter contents between the different canyons investigated and the variables responsible for the estimated differences. Reported are R which represents the sample statistic (global R) and P which is the probability level. **=P <0.01; ns = not significant

	ANOSIM		Dissimilarity	SIMPER		
	R	P		Explanatory variable	Explained variance (%)	Cumulative explained variance (%)
Tricase vs. Crotone	0.153	**	22.35	Proteins	40.78	40.78
				Lipids	33.48	74.26
				Carbohydrates	25.74	100
Tricase vs. Squillace	0.449	**	27.02	Proteins	43.85	43.85
				Carbohydrates	30.74	74.59
				Lipids	25.41	100
Crotone vs. Squillace	0.12	n.s.	16.82	Proteins	n.s	n.s
				Lipids	n.s	n.s
				Carbohydrates	n.s	n.s

2041
2042
2043
2044
2045
2046
2047
2048
2049
2050
2051
2052
2053
2054
2055
2056
2057
2058
2059
2060
2061
2062
2063
2064
2065
2066
2067
2068
2069
2070
2071
2072
2073
2074
2075
2076
2077
2078
2079
2080
2081
2082
2083
2084
2085
2086
2087
2088
2089
2090
2091
2092
2093
2094
2095
2096
2097
2098
2099
2100

691 **Table S2.** Output of the multivariate multiple regression analysis carried out for testing the
692 effects of organic matter content (proteins, carbohydrates and lipids), temperature and salinity
693 on fungal abundance (as 18S rDNA copies) and biomass. Reported are Pseudo-F and P
694 values (*<0.05; **<0.01; ***<0.001; ns>0.05) and the cumulative variance explained by the
695 significant variables.

Fungal abundance (18S rDNA copies)			
Variable	Pseudo-F	P	Cumulative variance %
Carbohydrates	11.556	***	31.6
Lipids	3.814	*	41.0
Proteins	1.771	ns	-
Salinity	1.654	ns	-
Temperature	0.667	ns	-

Fungal biomass			
Carbohydrates	98.421	***	79.7
Lipids	4.249	ns	-
Proteins	2.275	ns	-
Temperature	0.82	ns	-
Salinity	2.196	ns	-

696

2101
 2102
 2103
 2104
 2105
 2106
 2107
 2108
 2109
 2110
 2111
 2112
 2113
 2114
 2115
 2116
 2117
 2118
 2119
 2120
 2121
 2122
 2123
 2124
 2125
 2126
 2127
 2128
 2129
 2130
 2131
 2132
 2133
 2134
 2135
 2136
 2137
 2138
 2139
 2140
 2141
 2142
 2143
 2144
 2145
 2146
 2147
 2148
 2149
 2150
 2151
 2152
 2153
 2154
 2155
 2156
 2157
 2158
 2159
 2160

698 **Table S3.** Output of SIMPER showing the dissimilarity (turnover diversity) of fungal
 699 assemblage composition within the canyon and between the canyons investigated

700

Type of comparison			Turnover diversity (% Bray-Curtis dissimilarity)
within canyon	Tricase	200 m vs. 500 m	86.19
		200 m vs. 1000 m	91.97
		500 m vs. 1000 m	91.12
	Crotone	200 m vs. 500 m	89.03
		200 m vs. 1000 m	94.3
		500 m vs. 1000 m	87.88
	Squillace	200 m vs. 500 m	85.22
		200 m vs. 1000 m	88.92
		500 m vs. 1000 m	88.45
between canyons	200 m	Tricase vs. Crotone	97.01
		Tricase vs. Squillace	94.23
		Crotone vs. Squillace	88.7
	500 m	Tricase vs. Crotone	91.5
		Tricase vs. Squillace	95.42
		Crotone vs. Squillace	88.89
	1000 m	Tricase vs. Crotone	92.2
		Tricase vs. Squillace	94.52
		Crotone vs. Squillace	90.65

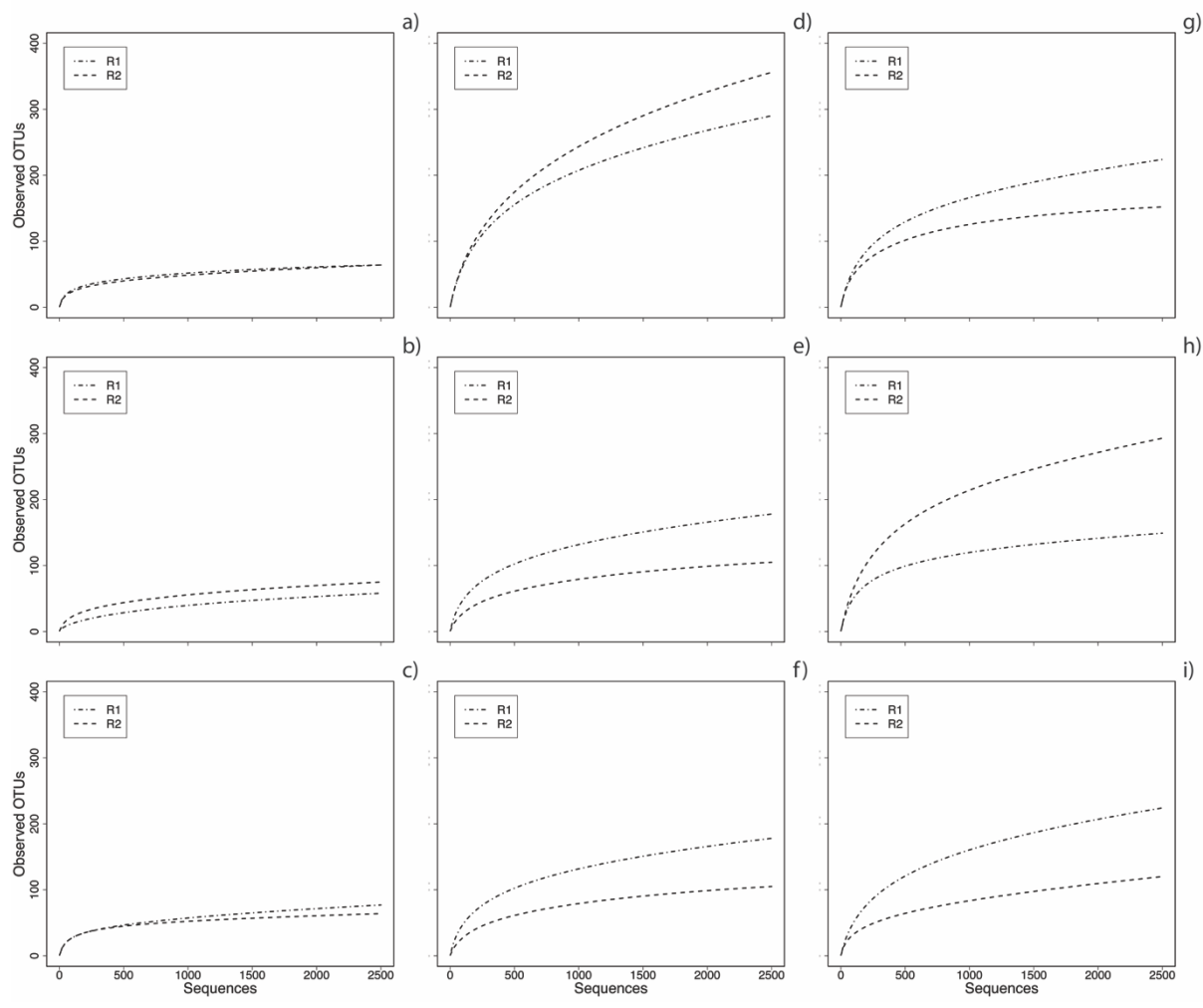
2161
2162
2163
2164
2165
2166
2167
2168
2169
2170
2171
2172
2173
2174
2175
2176
2177
2178
2179
2180
2181
2182
2183
2184
2185
2186
2187
2188
2189
2190
2191
2192
2193
2194
2195
2196
2197
2198
2199
2200
2201
2202
2203
2204
2205
2206
2207
2208
2209
2210
2211
2212
2213
2214
2215
2216
2217
2218
2219
2220

702 **Table S4.** Percentage of unique and shared OTUs between replicates of the same site, within
703 the canyon and between the canyons
704

Type of comparison			Shared %	Unique %
between replicates of the same site	Tricase	200 m	9.4	90.6
		500 m	15.7	84.3
		1000 m	10.2	89.8
	Crotone	200 m	12.5	87.5
		500 m	14.6	85.4
		1000 m	7.6	92.4
	Squillace	200 m	12.2	87.8
		500 m	7.0	93.0
		1000 m	6.8	93.2
	Average			10.7
within canyon	Tricase	200 vs. 500 m	18.8	90.0
		200 vs. 1000 m	14.0	93.9
		500 vs. 1000 m	13.8	92.5
	Crotone	200 vs. 500 m	12.2	91.1
		200 vs. 1000 m	19.8	94.8
		500 vs. 1000 m	19.9	91.2
	Squillace	200 vs. 500 m	29.3	86.3
		200 vs. 1000 m	18.6	89.2
		500 vs 1000 m	26.9	88.0
	Average			19.2
between canyons	Tricase vs. Crotone	200 m	3.8	96.2
		500 m	7.9	92.1
		1000 m	7.6	92.4
	Tricase vs. Squillace	200 m	6.6	93.4
		500 m	5.6	94.4
		1000 m	5.9	94.1
	Crotone vs. Squillace	200 m	10.3	89.7
		500 m	10.9	89.1
		1000 m	8.1	91.9
	Average			7.4

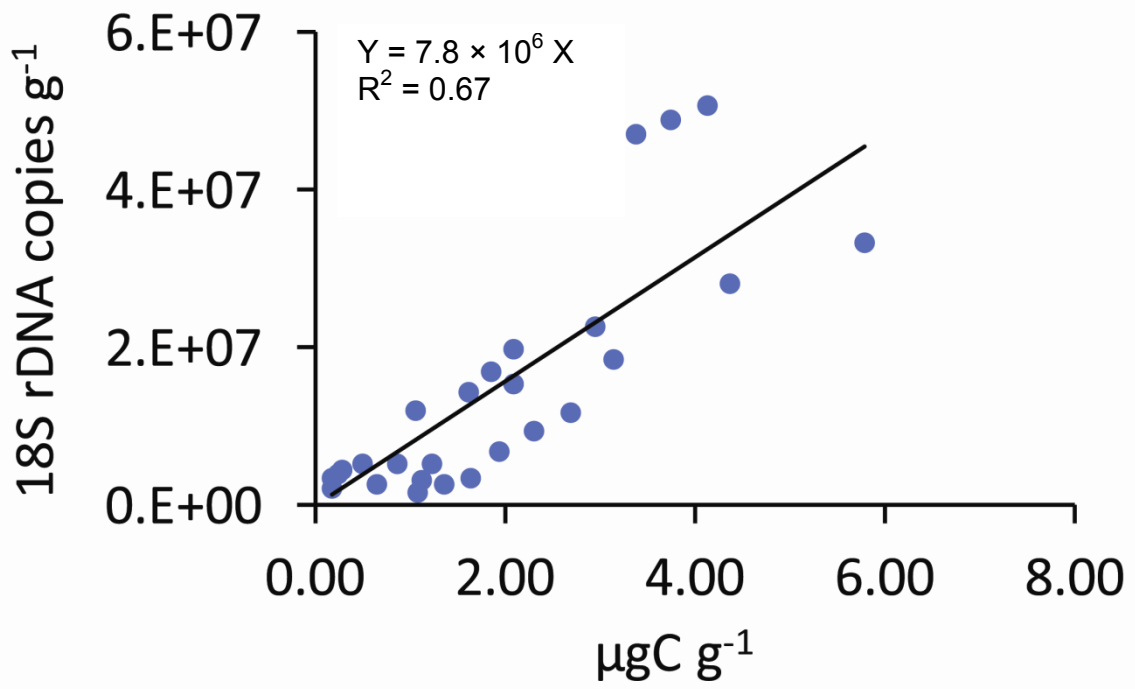
2221
2222
2223
2224
2225
2226
2227
2228
2229
2230
2231
2232
2233
2234
2235
2236
2237
2238
2239
2240
2241
2242
2243
2244
2245
2246
2247
2248
2249
2250
2251
2252
2253
2254
2255
2256
2257
2258
2259
2260
2261
2262
2263
2264
2265
2266
2267
2268
2269
2270
2271
2272
2273
2274
2275
2276
2277
2278
2279
2280

706 **Figure S1.** Rarefaction curves calculated for each of the two independent replicates (dashed
707 lines, 2500 sequences each) analysed in all benthic deep-sea sites of the canyons investigated.
708



2281
2282
2283
2284
2285
2286
2287
2288
2289
2290
2291
2292
2293
2294
2295
2296
2297
2298
2299
2300
2301
2302
2303
2304
2305
2306
2307
2308
2309
2310
2311
2312
2313
2314
2315
2316
2317
2318
2319
2320
2321
2322
2323
2324
2325
2326
2327
2328
2329
2330
2331
2332
2333
2334
2335
2336
2337
2338
2339
2340

721 **Figure S2.** Relationship between benthic fungal abundance (as 18S rDNA copies) and
722 biomass in the sediments of the three canyons



729
730
731
732
733
734
735
736
737
738
739