



Archaea are prominent members of the prokaryotic communities colonizing common forest mushrooms

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1 Archaea are prominent members of the prokaryotic communities colonizing common forest
2 mushrooms

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21 **Abstract**

22 In this study the abundance and composition of prokaryotic communities associated with the
23 inner tissue of fruiting bodies of *Suillus bovinus*, *Boletus pinophilus*, *Cantharellus cibarius*,
24 *Agaricus arvensis* *Lycoperdon perlatum* and *Piptoporus betulinus* were analyzed using
25 culture-independent methods. Our findings indicate that archaea and bacteria colonize the
26 internal tissues of all investigated specimens and that archaea are prominent members of the
27 prokaryotic community. The ratio of archaeal 16S rRNA gene copy numbers to those of
28 bacteria was >1 in the fruiting bodies of four out of six fungal species included in the study.
29 The largest proportion of archaeal 16S rRNA gene sequences belonged to thaumarchaeotal
30 classes Terrestrial group and Miscellaneous Crenarchaeotic Group (MCG) and
31 Thermoplasmata. Bacterial communities showed characteristic compositions in each fungal
32 species. Bacterial classes Gammaproteobacteria, Actinobacteria, Bacilli and Clostridia were
33 prominent among communities in fruiting body tissues. Bacterial populations in each fungal
34 species had different characteristics. The results of this study imply that fruiting body tissues
35 are an important habitat for abundant and diverse populations of archaea and bacteria.

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38 Keywords: bacteria, archaea, mushroom, qPCR, sequencing

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43 **Introduction**

44 Bacteria colonize the tissues of fruiting bodies of basidiomycetes (Swartz 1929, Danell *et al.*
45 1993, Dahm *et al.* 2005, Timonen and Hurek 2006, Pent *et al.* 2017) and ascomycetes
46 (Barbieri *et al.* 2007, Quandt *et al.* 2015). Bacteria and fungi have a partnership throughout
47 the fungal life cycle; bacteria may even be necessary for the formation of fruiting bodies
48 (Cho *et al.* 2003) and they may supplement the fruiting body with nutrients, such as fixed
49 nitrogen (Barbieri *et al.* 2010). Association between archaea and ectomycorrhizal fungal
50 hyphae has been observed in boreal forest soil environment (Bomberg *et al.* 2003, Bomberg
51 and Timonen 2007). Archaea are detected more frequently and their populations are more
52 diverse on tree roots colonized by ectomycorrhizal fungi than on uncolonized roots or humus
53 (Bomberg and Timonen 2009). However, currently there is no information available on
54 whether the association of archaea with fungal hyphae extends from the mycorrhiza to the
55 fruiting bodies of the fungi.

56 Bacteria colonizing the fruiting body tissues of basidiomycetes have been studied mainly
57 using culture-based techniques and microscopy (Li and Castellano 1987, Danell *et al.* 1993,
58 Dahm *et al.* 2005, Timonen and Hurek 2006). Recently, Pent *et al.* (2017) performed the first
59 comprehensive study of fruiting body bacteriomes using high throughput sequencing in
60 parallel with culture-based approach. Most of the culturable bacteria recovered from fruiting
61 bodies have been *Pseudomonas* spp. (Danell *et al.* 1993, Rangel-Castro *et al.* 2002, Pent *et*
62 *al.* 2017), while other groups, such as *Burkholderia* (Pent *et al.* 2017), *Paenibacillus*
63 (Timonen and Hurek 2006), *Xanthomonas* spp., *Streptomyces* spp., *Bacillus* spp. (Danell *et*
64 *al.* 1993) and *Azospirillum* (Li and Castellano 1987) have been found less consistently.
65 Recent molecular studies have elucidated the internal microbiomes of some ascomycetes
66 indicating that Alphaproteobacteria are predominant members in microbial communities
67 (Barbieri *et al.* 2007, Barbieri *et al.* 2010, Antony-Babu *et al.* 2014, Quandt *et al.* 2015).

68 Archaea from temperate environments are notoriously hard to grow in cultures, therefore
69 previous culture-based studies of fruiting body-associated prokaryotes have not been able to
70 touch upon the diversity and abundance of them. Despite the obvious evidence of bacterial
71 colonization of fruiting bodies, not much is known yet about the fruiting body tissue as a
72 habitat for archaea. Quantitative estimates of bacterial abundance have been based on the
73 recovery of culturable bacteria from tissues of fruiting bodies of basidiomycetes. In some
74 cases no or a very low number of culturable bacteria have been recovered (Dahm *et al.* 2005,
75 Timonen and Hurek 2006).

76 We hypothesized that archaea colonize the internal tissue of the fruiting body, not just
77 mycorrhizas or hyphae in forest soils. The purpose of this study was to quantify and
78 characterize archaeal communities in the internal tissue of fruiting bodies of six different
79 species of common forest mushrooms, using culture-independent techniques, quantitative
80 PCR and 16S rRNA gene sequencing. In parallel, we used the same methods to determine the
81 abundance and community composition bacteria colonizing the internal tissue of fruiting
82 body.

83

84 **Materials and methods**

85 *Sample collection*

86 Sample materials were obtained from fruiting bodies of three species of mycorrhizal fungi:
87 *Boletus pinophilus*, *Suillus bovinus* and *Cantharellus cibarius* and three species of
88 saprophytic fungi: *Agrarius arvensis*, *Lycoperdon perlatum* and *Piptoporus betulinus*. Six
89 specimens of each species were collected. All specimens were young (ca. 4-8 days old) and
90 without larvae. All specimens were collected from southern Finland from locations specified

91 in Table 1. After collection, the fruiting bodies were stored at +4°C (1-2 days) until further
92 processing in the laboratory. Fruiting body tissue for DNA-based analysis was collected from
93 the interior of each specimen by first splitting the fruiting body in two halves without
94 touching the exposed tissue and checking for any traces or damage by burrowing animals.
95 Then two flawless, approximately 0.05 g tissue pieces were cut from the exposed interior at
96 the base of the cap of the fruiting body using a sterile scalpel. The tissue samples were placed
97 in a sterile microcentrifuge tube. Samples were immediately frozen at -20°C until DNA
98 extraction.

99

100 *DNA extraction*

101 Tissue samples were defrosted in room temperature and homogenized in a 1.5 ml
102 microcentrifuge tube with sterile glass beads or silicic acid (Sigma Aldrich) and 100-200 µl
103 of bead beating buffer solution (Ultra Clean Soil DNA Isolation Kit, MoBio Laboratories)
104 using a sterile acid-washed pestle. DNA was extracted from the homogenized fruiting body
105 tissue with Ultra Clean Soil DNA Isolation Kit (MoBio Laboratories) following the
106 manufacturer's protocol. Two replicate DNA samples originating from the same specimen
107 were pooled before further analyses. Concentration of extracted DNA was determined with
108 Nanodrop spectrophotometer (NanoDrop Spectrophotometer ND-1000, V3.5.2).

109

110 *Quantitative PCR*

111 The abundances of bacterial and archaeal 16S rRNA genes in fruiting bodies were
112 determined using quantitative PCR (qPCR). All qPCR reactions were run in triplicate and no-
113 template-control reactions, where DNA template was replaced with an equal volume of

114 ultrapure water, were run in duplicate. Each 20 μ l reaction mixture for archaeal 16S rRNA
115 gene quantification consisted of 1x Dynamo Flash SYBR Green mastermix (Thermo), 0.5
116 μ M (final concentration) of primers Arch349F 5'-GYGCASCAGKCGMGAAW-3' and
117 539R 5'-GCBGGTDTTACCGCGGCGGCTGRCA-3' (Takai and Horikoshi 2000), 5 μ L of
118 diluted template DNA and nuclease-free water up to 20 μ L. A standard curve was generated
119 using a dilution series of a commercially prepared plasmid consisting of a vector pUC57
120 (length 2710 bp) and a 894 bp insert (GenScript), which was synthesized according to DNA
121 sequence of a 16S rRNA gene fragment belonging to an uncultivated 1.1c-group
122 Thaumarchaeota (NCBI accession number [AM903348.1](#)). The concentrations of standards
123 ranged from 3×10^6 copies per reaction to 3×10^2 copies per reaction. For eubacterial 16S
124 rRNA gene quantification, 25 μ l PCR reactions consisted of 1x Maxima SYBR green
125 mastermix (Thermo), 0.3 μ M (final concentration) of each primer Eub338 5'-
126 ACTCCTACGGGAGGCAGCAG-3' and Eub518 5'-ATTACCGCGGCTGCTGG-3' (Fierer
127 *et al.* 2005), 5 μ L of diluted template DNA and ultrapure water up to 25 μ L. Template DNA
128 was substituted with nuclease free water in negative controls. A standard curve was generated
129 using a 10-fold dilution series ranging from 3×10^6 to 30 copies per reaction of a plasmid
130 containing a 16S rRNA gene fragment from *Burkholderia glathei*. The plasmid was prepared
131 by amplifying a 16S rRNA gene fragment from DNA extracted from a pure culture of
132 *Burkholderia glathei* by PCR, using primers 25f and 1492R (Hurek *et al.* 1997) as described
133 above. The fragment was ligated into a pJet 2.1 cloning vector and cloned using GeneJet
134 cloning kit (Thermo Scientific). Plasmid DNA from a culture of transformed cells was
135 purified with GeneJet Plasmid Miniprep Kit (Thermo Scientific) and quantified with
136 Nanodrop spectrophotometer (Thermo Scientific). All qPCR products were verified by melt
137 curve analysis and by running one of the triplicate reactions on an ethidium bromide (0.2
138 μ g/ml) stained 1.2 % agarose gel.

139

140 *Sequencing*

141 DNA samples from three specimens of each fungal species were selected for sequencing
142 archaeal and bacterial 16S rRNA gene amplicons. *L. perlatum* was left out due to an
143 insufficient amount of sequencing template.

144 To prepare the archaeal 16S rRNA gene amplicons for sequencing, the original qPCR
145 products were run on 2 % agarose gel prepared with 1x SB buffer and stained with ethidium
146 bromide (0.2 µg/ml). DNA bands were excised from the gel and purified using GeneJET gel
147 extraction kit (Thermo Scientific). The purified DNA fragments were additionally cleaned
148 using Agencourt AMPure XP magnetic particles (Beckman Coulter) according to the
149 manufacturer's protocol. Sequencing libraries were generated by ligating Illumina flowcell
150 adapters and 9-base barcode sequences using a 2-step protocol adapted from Spencer *et al.*
151 (2016): adapters were ligated into original PCR products by amplification with
152 miseq_A349_F1 and miseq_A539_R1 primers (Supplementary Table 1). The first ligation
153 PCR reaction consisted of 1x Dynamo Flash SYBR Green mastermix (Thermo), 0.5 µM of
154 each primer F1 and R1, 2 µL of original PCR product and ultrapure water up to 20 µL.
155 Thermal cycling was done at 95°C for 7 min., 15 cycles at 95° 10 s. 56° 30 s., then 72° for 5
156 min. The products with adapters and barcodes were run on a gel, excised, extracted from the
157 gel and purified with Agencourt AMPure XP magnetic particles (Beckman Coulter)
158 following the manufacturer's protocol. The second part of the of the adapters and barcode
159 sequences were ligated in a subsequent PCR reaction, that consisted of 1x Dynamo Flash
160 SYBR Green masterimix (Thermo), 0.25 µM of each primer miseq_uni_F2 and
161 miseq_uni_R2_bcxxx (where xxx stands for a code corresponding to a unique 9 nucleotide
162 barcode) (Supplementary Table 1), 2 µL of original PCR product and ultrapure water up to

163 20 μ L. Thermal cycling was done at 95°C for 7 min., 8 cycles at 95° for 10 s. 56° for 30 s.,
164 then 72° for 5 min. PCR products were held at +4°C after completion of thermal cycling. The
165 products were cleaned as described after the first ligation reaction and quantified using Qubit
166 2.0 fluorometer (Life Technologies, Thermo Fisher Scientific Inc.). Amplicons were pooled
167 in equimolar quantities into one amplicon library. Sequencing using Illumina MiSeq was
168 done at Macrogen Inc. in Seoul, South Korea.

169 V1 – V3 regions of bacterial 16S rRNA genes were sequenced using Illumina MiSeq at the
170 Institute of Biotechnology at the University of Helsinki. Prior to sequencing, a two-step PCR
171 was used to amplify V1-V3 regions of 16S rRNA genes, using the primers F27 (Chung *et al.*
172 2004) and pD' (Edwards *et al.* 1989), amended with partial TruSeq adapter sequences at their
173 5' ends. Sterile water instead of template DNA was added into PCR control samples.

174

175 *Bioinformatics*

176 Archaeal 16S rRNA gene sequences were analyzed using QIIME software package, version
177 1.8.0 (Caporaso *et al.* 2010). Paired-end reads of archaeal 16S rRNA gene amplicons from
178 Illumina MiSeq sequencing were joined with SeqPrep program (URL:
179 <https://github.com/jstjohn/SeqPrep>). Reads were subsequently quality filtered with
180 `split_libraries_fastq.py` command using default settings, except that the maximum
181 unacceptable Phred quality score was set at 19. Reads passing quality filtering were clustered
182 into OTUs using `pick_open_reference_otus.py` workflow command. OTUs were clustered at
183 97% similarity level. Representative OTU sequences were aligned and checked for presence
184 of chimeras using Chimera Slayer. Taxonomic classification of OTUs was done using
185 BLAST algorithm (Altschul *et al.* 1990) and Silva database, release 111 as a reference
186 database (Pruesse *et al.* 2007).

187 Bacterial 16S rRNA gene sequences were joined using Pear 0.9.10 (Zhang *et al.* 2014).
188 Reads were subjected to quality filtering and phiX removal using bbdduk.sh script provided by
189 BBTools 37.02. The reads were subsequently subjected to the UPARSE pipeline for OTU
190 calling implemented in usearch version 9.2.64 using the standard parameter minsize 2 with
191 the cluster_otu functionality (Edgar 2013). OTU taxonomic classification was performed
192 using assign_taxonomy.py script with standard parameters provided by Qiime version 1.9.1
193 (Caporaso *et al.* 2010), using Silva database release 128 as a reference database (Quast *et al.*
194 2013). OTU sequences were aligned using Sina version 1.2.11 (Pruesse *et al.* 2012) and Silva
195 database release 128 as a reference database. The processed sequence data was normalized
196 using cumulative-sum scaling (CSS) (Paulson *et al.* 2013) in metagenomeSeq R package
197 (Paulson *et al.*).

198 An additional analysis was performed for the terrestrial group Thaumarchaeota from this
199 study to investigate their similarity to 1.1c thaumarchaeotal sequences retrieved from fungal
200 samples by (Bomberg *et al.* 2010). To investigate the pairwise similarity (%), the selected
201 sequence fragments from our study were aligned with 16S rRNA gene sequences from 1.1c
202 Thaumarchaeota from the previous study using Geneious software version 6.1.5 (Kearse *et*
203 *al.* 2012).

204

205 *Statistical analyses*

206 Differences in archaeal and bacterial 16S rRNA gene copy abundances determined by qPCR
207 in different fruiting bodies were analyzed using the nonparametric Kruskal-Wallis test, and
208 Wilcoxon signed rank sum test for post hoc comparisons. Tests were performed using the R
209 package Stats (R Core Team 2015), with functions Kruskal.test and wilcox.test for non-
210 paired samples. A regression analysis was used to model the effect of fungal species (n=6) on

211 the ratio of archaeal to bacterial 16S rRNA gene copies (R). *L. perlatum* was used as
212 reference group in the analysis. The ratio R was modelled as: $R = \beta_0 + \beta_1 X_1 + \varepsilon$, where $\beta_0 =$
213 reference group, $X_1 = A. arvensis$, $X_2 = B. pinophilus$, $X_3 = C. cibarius$, $X_4 = P. betulinus$,
214 $X_5 = S. bovinus$ and ε is the error term. The model was constructed in R environment using
215 the function `lm` in the package `Stats` (R Core Team 2015). Differences in bacterial
216 communities in the fruiting bodies of fungal species were determined by distance-based
217 Redundancy Analysis (db-RDA) using the function `capscale` in R package `vegan` (Oksanen et
218 al. 2017). In the db-RDA, fungal species were used as explanatory variable to constrain the
219 normalized 16S rRNA gene sequence data. The Bray-Curtis dissimilarity index was used to
220 measure between-sample dissimilarity. The significance of differences between bacterial
221 communities in each fungal species was calculated by the function `adonis` in R package
222 `vegan` (Oksanen *et al.* 2017), with 999 permutations.

223

224 *Nucleotide sequence accession number*

225 Raw sequence data have been deposited to the National Center of Biotechnology
226 Information's Sequence Read Archive under study accession number **SRP073783**.

227

228

229 **Results**

230 *Quantities of archaeal and bacterial 16S rRNA gene copies*

231 The quantity of archaeal rRNA gene copies ranged from 3.0×10^6 (in *L. perlatum*) to 3.2×10^8
232 (in *S. bovinus*) copies per gram (fw) of fruiting body tissue. Copy numbers varied

233 significantly between different species (Kruskal-Wallis chi-squared = 22.638, df = 5, p =
234 0.0004) (Figure 1a). Bacterial 16S rRNA gene copy numbers ranged from 5.9×10^6 (in *B.*
235 *pinophilus*) to 1.9×10^8 copies per gram (in *P. betulinus*). Variations in bacterial copy
236 numbers between species were also significant (Kruskal-Wallis chi-squared = 21.988, df = 5,
237 p-value = 0.0005) (Figure 1b). Archaeal 16S rRNA gene copy abundance exceeded that of
238 bacterial in all six specimens of *S. bovinus* and *B. pinophilus*. In *C. cibarius*, archaeal and
239 bacterial 16S copy abundance were roughly equal in half of the specimens (3), while in the
240 other half of the specimens, bacterial 16S rRNA gene copy abundance clearly exceeded
241 archaeal copy abundance (Supplementary Figure 1). Fungal species had a significant effect
242 on the ratio of archaeal to bacterial 16S rRNA gene copy abundance (regression analysis, $p <$
243 0.001 , Supplementary Table 2); in *S. bovinus* the ratio was 15.5 times higher and in *B.*
244 *pinophilus* 22.7 times higher than in *L. perlatum*, which was chosen as a reference group in
245 the analysis because it had the lowest ratio of 0.4 (Figure 2).

246

247 *Sequences of archaeal 16S amplicons*

248 Sequencing of PCR amplicons amplified with Archaea-specific primers yielded a total of
249 12737 good quality archaeal 16S sequences, which clustered into 57 OTUs at 97% similarity
250 level. The quality of sequences and thus, sequencing depth varied considerably between
251 samples. Taxonomically classifiable archaeal sequences were distributed in 4-6 archaeal
252 classes depending on the fungal species (Figure 3a). Archaeal communities in fruiting bodies
253 of all fungal species were clearly dominated by thaumarchaeotal classes Terrestrial group,
254 *Thermoplasmata*, and Miscellaneous Crenarchaeotal Group (MCG) while archaea of Marine
255 group I, Soil Crenarchaeotic group (SCG) and Sc-EA05 Thaumarchaeota represented smaller
256 proportions of the communities.

257 Sequences classified in this study as Terrestrial group Thaumarchaeota had highest (78-99 %)
258 similarities to 1.1c thaumarchaeotal sequences, which were retrieved from mycorrhizosphere
259 samples by Bomberg *et al.* (2010). The highest match (99 % identity) to the sequences from
260 our study originated from a pine mycorrhiza. In comparison, Terrestrial group
261 thaumarchaeotal 16S rRNA gene sequences from this study had 63-75 % similarities with
262 representatives of common soil thaumarchaeotal groups: *Nitrosotalea devanaterra* (group
263 1.1a) and *Nitrososphaera viennensis* (group 1.1b) (Supplementary Table 3).

264

265 *Sequences of bacterial 16S amplicons*

266 Sequencing yielded 1647881 sequences that passed quality filtering and they clustered into
267 177 bacterial OTUs at 97% similarity level. Bacterial communities of all fungal species
268 formed loose groups showing that they had characteristic bacterial populations. Fungal
269 species explained 30 % of the total variation in the bacterial communities (Figure 4). The
270 populations of fruiting bodies of mycorrhizal fungi did not cluster together apart from those
271 of saprophytic fungi. Bacterial orders with highest relative abundances in the entire data set
272 (Pseudomonadales and Bacillales) were present in all fruiting bodies, but their relative
273 abundances showed considerable variation between fungal species (Figure 3b), and
274 sometimes even between the specimens of the same species. Bacterial community
275 compositions of fruiting bodies of *S. bovinus* differed significantly (adonis, $p=0.036$) from
276 the compositions of other fungal species. Compared to other fruiting bodies, *S. bovinus* had
277 higher relative abundance of Enterobacteriales, Clostridiales and Dehalococcoidia. Orders
278 Pseudomonadales and Bacillales together formed a major proportion of bacterial
279 communities in *A. arvensis* (87 %), *B. pinophilus* (50 %) and *P. betulinus* (46 %).
280 Lactobacillales were particularly abundant in *B. pinophilus*, contributing to the high relative

281 abundance of the class Bacilli in this species, while *A. arvensis* was heavily dominated by
282 Bacillales. *P. betulinus* had particularly high proportion of Corynebacteria. In *C. cibarius*
283 bacterial community had higher relative abundance of Sphingobacteriales (24 %),
284 Rhizobiales (13 %), Caulobacterales (11 %) and Burkholderiales (10 %) than other fungal
285 species.

286

287 **Discussion**

288 The results of this study indicate that both archaea and bacteria are abundant in the internal
289 tissues of fruiting bodies, based on enumeration by qPCR. We observed significant variations
290 in the abundance of archaeal and bacterial 16S rRNA gene copies between different fungal
291 species. To our knowledge our data represent the first estimates of archaeal and bacterial
292 abundance in fruiting bodies of fungi obtained using culture-independent approach. The
293 quantities of 16S rRNA gene copies do not correspond to cell numbers as such; according to
294 the ribosomal RNA operon copy number database (rrnDB) version 4.4.4 (Stoddard *et al.*
295 2015) the number of 16S rRNA gene copies in sequenced archaeal genomes varies from 1 to
296 4 and 1 to 15 in bacteria. Here, the archaea:bacteria 16S rRNA gene copy number ratios ≥ 1
297 still indicate that archaea form a significant proportion of prokaryotic biomass in fruiting
298 body tissues of some fungi. Such high ratios of archaeal versus bacterial 16S rRNA gene
299 copy abundances are not common in terrestrial habitats, although in archaea-rich marine
300 sediments archaeal abundances exceeding that of bacteria have been observed (Lipp *et al.*
301 2008, Lloyd *et al.* 2013). In contrast to previous culture-based studies, our new data show
302 that bacteria are abundant in the internal tissues of fruiting bodies, such as in *S. bovinus*,
303 where the numbers of culturable bacteria were very low (Timonen and Hurek 2006).

304 This study shows that archaeal communities in fruiting body tissues are diverse based on
305 sequencing of 16S rRNA genes. Fruiting bodies included in this study were colonized by
306 archaeal classes that are commonly found in both aquatic and terrestrial environments. The
307 metabolic potential and roles of these organisms in the prokaryotic community inside the
308 fruiting bodies remains unknown at this point due to lack of cultured representatives or
309 genomic information. A metagenomic assembly of representatives from the “Soil
310 Crenarchaeotic Group” (SCG) suggested, that these archaea might participate in both
311 nitrification and denitrification (Butterfield *et al.* 2016). Some of the dominant groups, such
312 as the ubiquitous MCG group, are diverse both phylogenetically and metabolically (Kubo *et*
313 *al.* 2012, Meng *et al.* 2014). In marine sediments the MCG group archaea may derive energy
314 from mineralization of proteins (Lloyd *et al.* 2013), degradation of aromatic compounds
315 (Meng *et al.* 2014), and possibly also from physically and chemically recalcitrant organic
316 matter, such as membrane lipids (Takano 2010). Marine group I thaumarchaeota are mostly
317 pelagic mixotrophs also with versatile metabolic potential, including aerobic ammonia
318 oxidation and hydrolysis of urea (Swan *et al.* 2014). Thermoplasmata were the only
319 euryarchaeal class present in the fruiting bodies. Sequences belonging to archaea of this class
320 (order Thermoplasmatales) have been recovered from forest soil (Burke *et al.* 2012) as well
321 as from freshwater habitats (Jurgens *et al.* 2000, Fillol *et al.* 2015). Thermoplasmatales may
322 have methanogenic potential (Paul *et al.* 2012), but their activities are still mostly unknown.
323 In this study, sequencing depth within replicates of same species as well as between different
324 specimens varied considerably and this also likely affected strongly the observed numbers of
325 archaeal OTUs. For this reason statistical assessment of differences between archaeal
326 communities was not performed, as the results would not represent accurately the natural
327 variation between the communities.

328 Although our results give the first glimpse of the diversity of archaea colonizing internal
329 tissues of fruiting bodies, the short length (< 200 bp) of the 16S rRNA gene fragments set
330 limits to taxonomic resolution and comparisons with uncultivated archaea found in specific
331 habitats. Nevertheless, the short 16S rRNA gene sequences of the terrestrial group
332 Thaumarchaeota from this study had high % identities with sections of longer sequences of
333 mycorrhizosphere associated 1.1c Thaumarchaeota previously found by Bomberg *et al.*
334 (2003, 2010). This implies that archaea from mycorrhizal roots and external hyphae might
335 effectively colonize the fruiting bodies as well. It has been hypothesized previously that the
336 group 1.1c Thaumarchaeota are involved in carbon cycling through uptake and turnover of
337 single-carbon compounds (such as methane, methanol or carbon dioxide) and they may carry
338 out this role also in fruiting bodies as well (Timonen and Bomberg 2009, Bomberg *et al.*
339 2010).

340 Bacterial communities between different fungal species showed species-specific
341 characteristics, although only the bacterial community of *S. bovinus* was statistically
342 significantly different from the others in this study. Fungal genus was a significant factor
343 affecting the composition of bacterial community in a study comprising fruiting bodies of
344 eight genera within the class Agaricomycetes (Pent *et al.* 2017). There were large variations
345 in relative abundances of certain bacterial taxa within biological replicates, such as in the
346 case of Corynebacteriales. Because of this, we have focused the discussion of the results on
347 bacterial groups that appeared evenly in biological replicates to avoid spurious conclusions.
348 The variation between biological replicates may be caused by uneven distribution of bacteria
349 within the fungal tissue or variation between individual fruiting bodies. Soil properties may
350 also have an effect on the composition of bacterial community in fruiting body tissue (Pent *et*
351 *al.* 2017). In this study replicates for each species originated from the same general area and
352 therefore there should be no major differences in soil properties that could have an effect on

353 the composition of bacterial communities, although we can not exclude the possible effect of
354 minor differences within the sampling locations.

355 Class Enterobacteriaceae (orders Pseudomonadales and Enterobacteriales) was a predominant
356 bacterial group in all fruiting bodies. They were also predominant groups among bacteria
357 recovered through cultivation from fruiting bodies of *C. cibarius* and *S. bovinus* by Pent et al
358 (2017). Enterobacteria and Pseudomonads can act as mycorrhiza helper bacteria facilitating
359 interaction between plant roots and mycorrhizal fungi (Frey-Klett *et al.* 2007). In this study,
360 we also found a high relative abundance of Bacilli in fruiting bodies of *A. arvensis*, *P.*
361 *betulinus* and *B. pinophilus*, whereas they formed only < 2% of the community in *C. cibarius*
362 and *S. bovinus*. Bacilli have been recovered from inner tissues of fruiting bodies through
363 cultivation (Danell *et al.* 1993, Zagriadskaia *et al.* 2014). In line with our findings, Pent *et al.*
364 (2017) found a low relative abundance of Bacilli in *S. bovinus* and none in *C. cibarius* by
365 sequencing bacterial 16S rRNA genes. Orders Clostridiales and Dehalococcoides had
366 particularly high relative abundances in *S. bovinus*. There are no previous reports of finding
367 Dehalococcoides in fungal fruiting bodies while Pent *et al.* (2017) had detected Clostridiales
368 in some of their fruiting body material. Clostridiales are obligate anaerobes and their role
369 may be related to cellulose degradation (de Boer *et al.* 2005). Dehalococcoides are obligate
370 organohalide respiring bacteria (Löffler *et al.* 2013) and their presence is likely linked to
371 degradation of organohalogen produced by the host. Basidiomycetes fungi are capable of *de*
372 *novo* synthesis of halogenated organic compounds making them a major source of
373 organohalogen in terrestrial environments (deJong and Field 1997). In our study, *C. cibarius*
374 had higher relative abundance of Sphingobacteriales than in other fungal species. Pent *et al.*
375 (2017) found sequences of these bacteria from *C. cibarius* tissue, but were not able to culture
376 them, which may explain why these bacteria have not been recovered from fruiting body
377 tissues by cultivation in earlier culture-based studies. Also Alphaproteobacterial orders

378 Rhizobia and Caulobacteria and Betaproteobacterial order Burkholderiales had higher
379 relative abundances in *C. cibarius* than in other fruiting bodies. Alphaproteobacteria were
380 prominent groups in ascomycete *Elaphomyces granulatus* based on relative abundance of
381 16S rRNA gene sequences (Quandt *et al.* 2015). Rhizobia and Burkholderiales were also
382 found in bacterial 16S rRNA gene sequence libraries from *C. cibarius* and *S. bovinus* in the
383 study by Pent *et al.* (2017). To our knowledge, Caulobacteria have not been detected by
384 sequencing or cultivation in fruiting bodies yet. These three orders may have a role in glucan
385 degradation, as suggested by Eichorst and Kuske (2012). Bacteria belonging to these classes
386 are adapted in low-nutrient environments and they may have a role in supplementing
387 nutritional demands of the host by fixing nitrogen, (Li and Castellano 1987, Barbieri *et al.*
388 2010, Sellstedt and Richau 2013), or solubilizing phosphate for the use of the fungus (Pavic
389 *et al.* 2013).

390 The internal environment in fruiting bodies reshapes the bacterial communities compared to
391 communities found e.g. in *Pinus sylvestris* mycosphere and in the surrounding uncolonized
392 soil. These environments are dominated by bacteria belonging to classes
393 Alphaproteobacteria, Actinobacteria and Acidobacteria (Timonen *et al.* 2017). Factors
394 affecting the composition of the prokaryotic community in the fruiting body tissue include
395 the presence of antimicrobial compounds excreted by the fungi (de Carvalho *et al.* 2015).
396 Also carbohydrate, crude protein, sugar and lipid contents between *Boletus edulis*, *A.*
397 *arvensis*, *C. cibarius* and *L. perlatum* can vary greatly (Barros *et al.* 2007, Barros *et al.* 2008,
398 Kalac 2009, Heleno *et al.* 2011), which could be a selecting factor for prokaryotic community
399 composition. The availability of different carbon sources inside the fruiting bodies as well as
400 the ability of colonizing prokaryotes to utilize the fungal storage sugars (such as trehalose
401 and mannitol) could explain at least some proportion of the variation seen in prokaryotic
402 community compositions. Also, the physical composition of the fruiting body, such as

403 porosity and moisture, may play a role in shaping the prokaryotic abundance and community
404 composition and distribution within the fruiting body. It is likely that the increased moisture
405 of degrading fruiting bodies with larval infestation and increased leakage of substrates from
406 fungal tissues could support more bacteria than young fruiting bodies. All fruiting bodies
407 analyzed in this study were relatively young and showed no signs of decay. However, even
408 small variations in fruiting body age may cause differences in the archaeal and bacterial 16S
409 copy abundances and community composition as the biochemical composition of the fruiting
410 body tissue changes over time (Citterio *et al.* 2001, Barbieri *et al.* 2010).

411 The view of archaeal biomass in ecosystems and their contribution to biogeochemical cycles
412 has changed radically in recent years - however, our understanding of their distribution in
413 different habitats is still developing. The mixture of both aquatic and terrestrial archaeal
414 classes in the communities colonizing the tissues of fruiting bodies suggests that present
415 habitat-based broad classification will likely be subject to change in the future, as archaeal
416 diversity in different habitats is further explored. The data from this study do not explain the
417 success of archaea in fruiting body tissues. The apparent enrichment of archaea in fruiting
418 body tissues of *B. pinophilus* and *S. bovinus* suggests that fungi-archaea associations must be
419 important in ecosystems to the extent that archaea remain associated with the fungi even
420 outside the soil environment to accompany fruiting bodies during their short life cycle
421 (approximately 2 weeks). It is not yet known whether the composition of the archaeal
422 population changes over the life cycle of the host and whether the archaeal activity affects the
423 host somehow. Such an abundance of archaea in this (or any) natural habitat shows that the
424 environment is important in shaping the composition of the microbial community associated
425 with it. Differences in bacterial abundance and in community composition between different
426 fungal species suggest that bacterial populations form a network of interactions between
427 themselves and the host. The composition of the community is likely a result of antagonistic

428 and antagonistic interactions between the host and microbes as well as between the microbes
429 themselves. A recent study by Schulz-Bohm *et al.* demonstrated the pervasive effect of
430 microbes to a life style of a saprotrophic fungus *Mucor hiemalis* (Schulz-Bohm *et al.* 2017).
431 An antibiotic-induced shift in microbial community composition altered the morphology,
432 secondary metabolite production and morphology of the fungus. These results suggest that
433 the network of interactions between fungi and bacteria may be more complex than is
434 previously thought and bacteria are important cohabitants for fungi.

435 Our findings transform our view of prokaryotic populations in fruiting bodies. We identify
436 fruiting bodies as a previously unknown habitat for temperate archaeal populations, where in
437 some cases archaeal abundance may exceed that of bacteria. We also show that fruiting
438 bodies of different fungal species harbor characteristic bacterial communities.

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632 Tables

633

634 Table 1. Fungal species included in the study and characterization of sample collection sites located in

635 Southern Finland.

636

Species	Sample code	Coordinates of sample collection sites	Site characteristics (no. of specimens collected)
<i>Suillus bovinus</i> (Fr.) Roussel	Sb	60°01' N, 23°34' E	Dry pine forest (6).
<i>Boletus pinophilus</i> Pilát & Dermek (Bp)	Bp	60°38' N, 25°20' E, 59°54' N, 23°43' E	Dry pine forest (5), mixed forest (1)
<i>Cantharellus cibarius</i> Fr.	Cc	60°01' N, 23°34' E 59°54' N, 23°43' E,	Dry pine forest (2), mixed forest (4)
<i>Agaricus arvensis</i> Schaeff.	Aa	60°11' N, 24°53' E	Grassy field (6)
<i>Lycoperdon perlatum</i> Pers.	Lp	60°13' N, 25°01' E	Mixed forest (6)
<i>Piptoporus betulinus</i> (Bull.) P. Karst.	Pb	59°54' N, 23°43' E	Mixed forest (6)

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642 Figure captions

643

644 Figure 1. Abundance of archaeal (A) and bacterial (B) 16S rRNA gene sequences in fruiting

645 bodies. Solid bars represent means (n=6, except for Lp n=3), and error bars standard errors.

646 Different letter above the bar indicates statistically significant difference (Wilcoxon signed
647 rank sum test, $p < 0.05$).

648

649 Figure 2. Ratio of archaeal to bacterial 16S rRNA gene copy abundance. The copy numbers
650 were determined using qPCR with domain-specific primers. Regression analysis determined
651 that *S. bovinus* and *B. pinophilus* (marked with an asterisk) have statistically significant ($p <$
652 0.001), increasing effect on the ratio of archaeal to bacterial 16S rRNA gene copy numbers.
653 Dashed line indicates a ratio of 1:1.

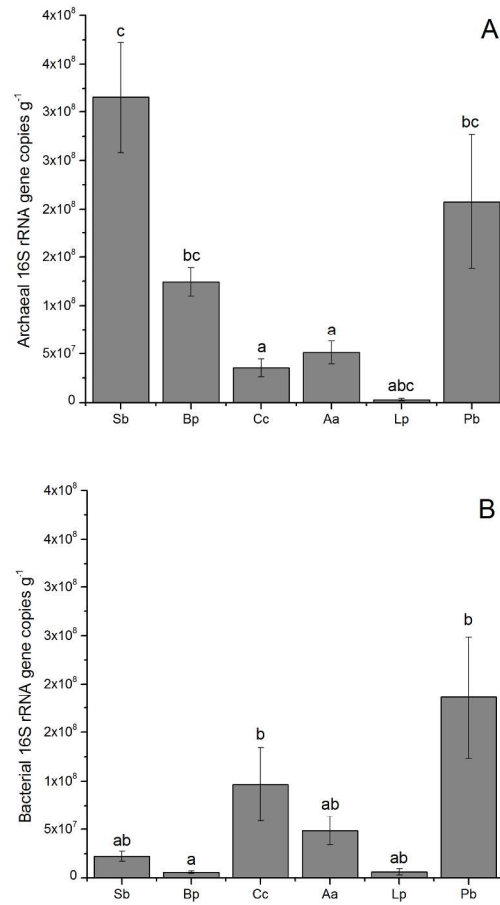
654

655 Figure 3. Taxonomic distribution of archaeal (A) and bacterial (B) 16S sequences in different
656 fungal species. Relative abundances are calculated from pooled sequences of three biological
657 replicate samples.

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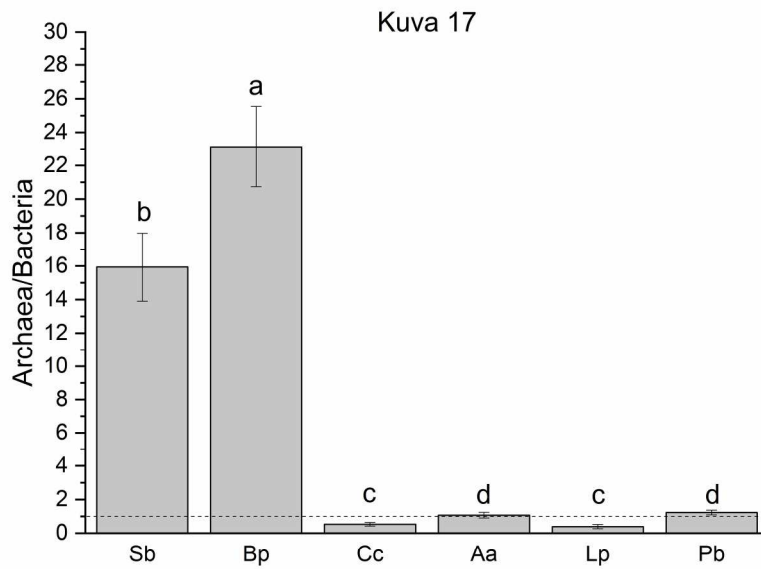
659 Figure 4. Distance-based Redundancy Analysis (db-RDA) of bacterial populations in fruiting
660 body tissues. The ordination is based on Bray-Curtis dissimilarity using fungal groups as
661 explanatory variables. The ellipses represent variation around the group centroids at 0.75
662 confidence interval.

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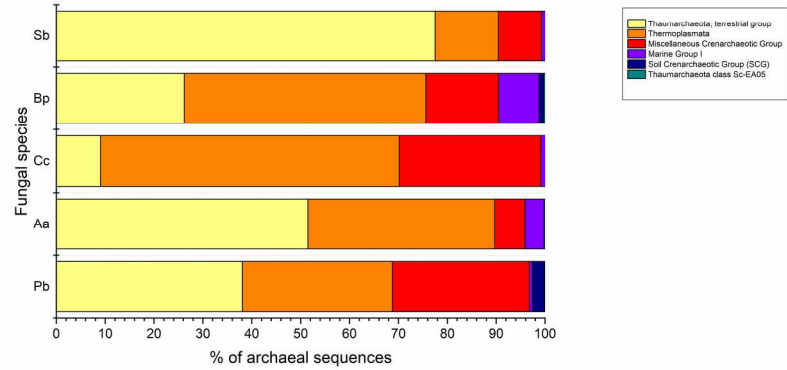
Abundance of archaeal (A) and bacterial (B) 16S rRNA gene sequences in fruiting bodies. Solid bars represent means ($n=6$, except for Lp $n=3$), and error bars standard errors. Different letter above the bar indicates statistically significant difference (Wilcoxon signed rank sum test, $p < 0.05$).

199x286mm (300 x 300 DPI)



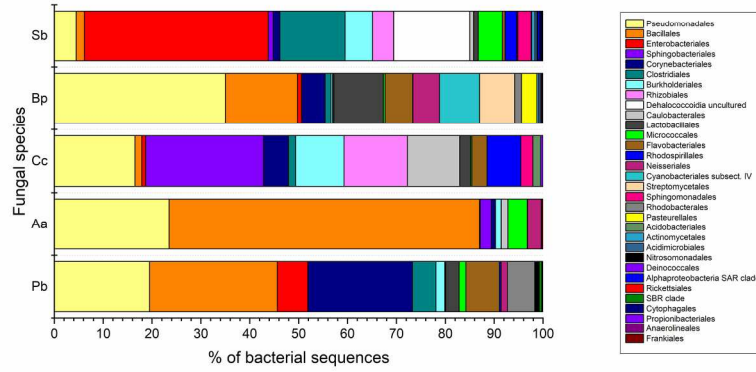
Ratio of archaeal to bacterial 16S rRNA gene copy abundance. The copy numbers were determined using qPCR with domain-specific primers. Regression analysis determined that *S. bovinus* and *B. pinophilus* (marked with an asterisk) have statistically significant ($p < 0.001$), increasing effect on the ratio of archaeal to bacterial 16S rRNA gene copy numbers. Dashed line indicates a ratio of 1:1.

199x139mm (300 x 300 DPI)



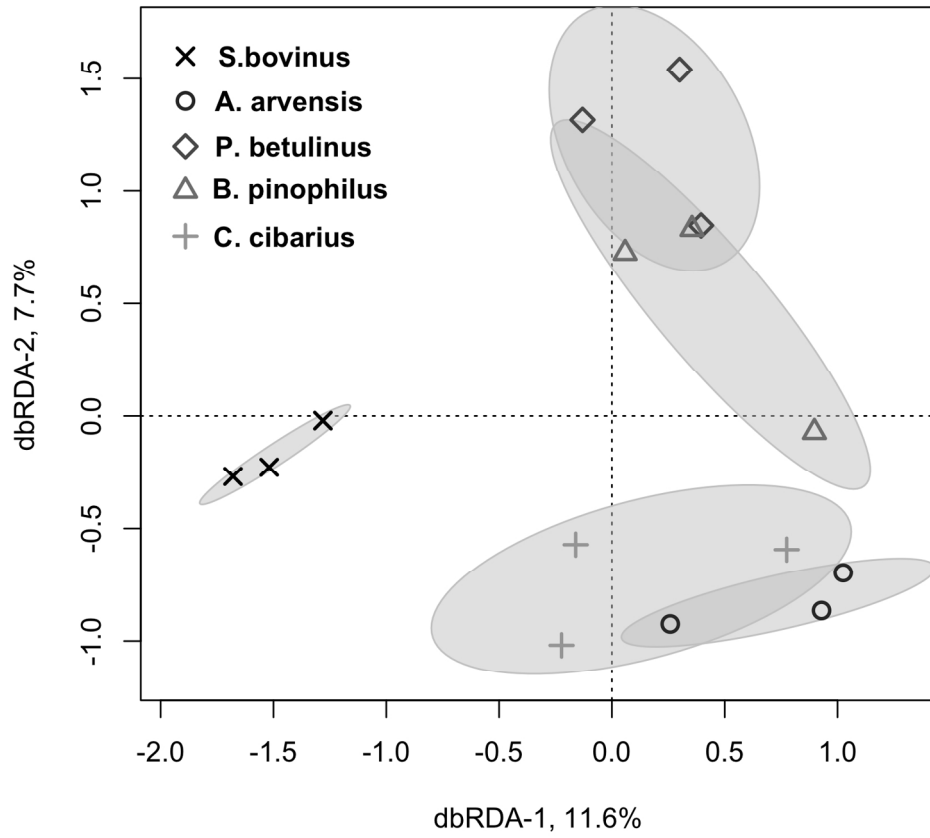
Taxonomic distribution of archaeal (A) and bacterial (B) 16S sequences in different fungal species. Relative abundances are calculated from pooled sequences of three biological replicate samples.

201x141mm (300 x 300 DPI)



Taxonomic distribution of archaeal (A) and bacterial (B) 16S sequences in different fungal species. Relative abundances are calculated from pooled sequences of three biological replicate samples.

201x141mm (300 x 300 DPI)



Distance-based Redundancy Analysis (db-RDA) of bacterial populations in fruiting body tissues. The ordination is based on Bray-Curtis dissimilarity using fungal groups as explanatory variables. The ellipses represent variation around the group centroids at 0.75 confidence interval.

635x635mm (72 x 72 DPI)

Archaea are prominent members of the prokaryotic communities colonizing common forest mushrooms

Rinta-Kanto JM, Pehkonen, K, Sinkko H, Tamminen MV, Timonen S

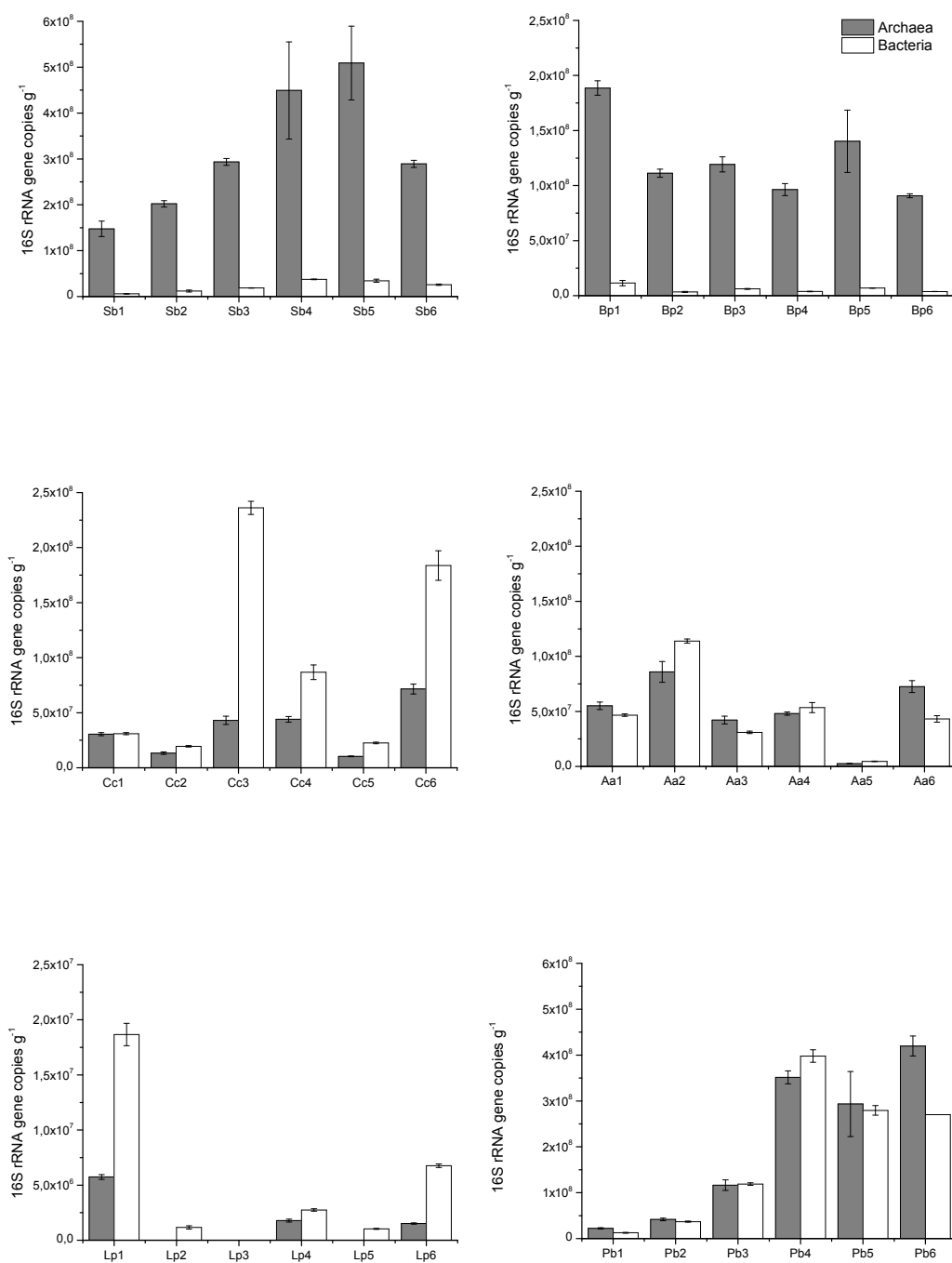
Supplementary information

Supplementary Table 1. Primers used for Illumina sequencing library preparation for sequencing of archaeal 16S rRNA gene fragments.

Primer name	Sequence
miseq_A349_F1	ACACGACGCTCTCCGATCTYRYRGGYGCASCAGKCGMGAAW
miseq_uni_F2	AATGATACGGCGACCACCGAGATCTACACTCTTCCCTACACGACGCTCTCCGATCT
miseq_A539_R1	GGAGTTCAGACGTGTGCTCTCCGATCTGCBGGDTTACCGCGGCGGCTGRCA
miseq_uni_R2_bc001	CAAGCAGAAGACGGCATAACGAGATTCGGTGCAGGCTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc002	CAAGCAGAAGACGGCATAACGAGATTTCCAGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc003	CAAGCAGAAGACGGCATAACGAGATGGAATGAAGTACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc004	CAAGCAGAAGACGGCATAACGAGATGAACTGGGGTACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc005	CAAGCAGAAGACGGCATAACGAGATACGGGTGAGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc006	CAAGCAGAAGACGGCATAACGAGATATGAAGTATGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc007	CAAGCAGAAGACGGCATAACGAGATACTATTGTGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc008	CAAGCAGAAGACGGCATAACGAGATGGCGGGAAAGTACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc009	CAAGCAGAAGACGGCATAACGAGATACACCTCGGGTACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc010	CAAGCAGAAGACGGCATAACGAGATCTCATTGGGGTACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc011	CAAGCAGAAGACGGCATAACGAGATGCTGCCGCGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc012	CAAGCAGAAGACGGCATAACGAGATCGATGGTGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc013	CAAGCAGAAGACGGCATAACGAGATCAAAGCTGGTACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc014	CAAGCAGAAGACGGCATAACGAGATCAGCGGCATGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc015	CAAGCAGAAGACGGCATAACGAGATCCGACAAATGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc016	CAAGCAGAAGACGGCATAACGAGATTAAGGGAGAGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc017	CAAGCAGAAGACGGCATAACGAGATTTGTGGCGCGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc018	CAAGCAGAAGACGGCATAACGAGATAGGTGCGGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc019	CAAGCAGAAGACGGCATAACGAGATAATGTCAAGGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc020	CAAGCAGAAGACGGCATAACGAGATGTTGCGAGGGTACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc021	CAAGCAGAAGACGGCATAACGAGATTATCAATCTGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc022	CAAGCAGAAGACGGCATAACGAGATGTTAACGCGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc023	CAAGCAGAAGACGGCATAACGAGATTTACTATACGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc024	CAAGCAGAAGACGGCATAACGAGATTGCCCGTGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc025	CAAGCAGAAGACGGCATAACGAGATTTGGACCTCGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc026	CAAGCAGAAGACGGCATAACGAGATGAGTTTGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc027	CAAGCAGAAGACGGCATAACGAGATAACAGTATTGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc028	CAAGCAGAAGACGGCATAACGAGATATCGCACCAGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc029	CAAGCAGAAGACGGCATAACGAGATCTAGAATCTGTGACTGGAGTTCAGACGTGTGCTCTCCGATCT
miseq_uni_R2_bc030	CAAGCAGAAGACGGCATAACGAGATCGCCAAAGGGGTACTGGAGTTCAGACGTGTGCTCTCCGATCT

Supplementary Table 2. Regression analysis for the square root-transformed ratio of the mean of archaeal to bacterial rRNA gene copies in different fungal species (n=6, except *L. perlatum* n=3). In the results, estimate for intercept = mean of *L. perlatum* (reference group).

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	0.6109	0.2256	2.708	0.0116
<i>A. arvensis</i>	0.4057	0.2763	1.468	0.1536
<i>B. pinophilus</i>	4.1687	0.2763	15.088	1.12E-14
<i>C. cibarius</i>	0.0989	0.2763	0.358	0.7232
<i>P. betulinus</i>	0.4885	0.2763	1.768	0.0883
<i>S. bovinus</i>	3.3445	0.2763	12.104	2.03E-12



Supplementary Figure 1. Abundances of archaeal and bacterial 16S rRNA gene copies in biological replicates of fungal specimens included in this study. Labels on the x-axes correspond to the initial letters of the fungal species names and the number (1-6) identifies the biological replicate.

Supplementary Table 3: Percentage of similarity between sequences from this study compared with 1.1c thaumarchaeotal sequences from mycorrhizospheres (no background colour) and with other archaeal sequences (light grey background). The highest (red) and lowest (blue) similarity percentages in each taxonomic group of are coloured and bolded. Reference sequences are also compared (darker grey backgrounds).

Taxon and/or origin of sequence	Acc. No	AM903348	AM903346	AM903363	AM903372	DQ459996	DQ459997	DQ460000	DQ460001	DQ460005	DQ460010	FM202423	AF227638	CP007536	JN227488	DQ085097	U51469
Sporocarp, this study	OTU674	96,6	92	86	83,1	82,2	86,5	83,1	83,1	87,6	91	88,8	77,5	73	68,5	68,9	73,3
Sporocarp, this study	OTU3521	97,7	93,2	84,9	83,1	81,1	85,4	83,1	82	86,5	92,1	89,9	76,4	71,9	69,7	67,8	73,3
Sporocarp, this study	OTU4241	98,9	94,3	87,2	84,3	83,3	87,6	84,3	84,3	88,8	93,3	91	78,7	74,2	69,7	70	74,4
Sporocarp, this study	OTU4607	97,7	93,2	84,9	82	81,1	85,4	82	82	86,5	92,1	89,9	76,4	71,9	67,4	67,8	72,2
Sporocarp, this study	OTU4619	94,3	89,8	83,7	80,9	80	84,3	80,9	80,9	85,4	88,8	86,5	75,3	70,8	66,3	66,7	71,1
Sporocarp, this study	OTU4653	97,7	93,2	87,2	84,3	83,3	87,6	84,3	82	88,8	92,1	89,9	78,7	74,2	69,7	70	76,7
Sporocarp, this study	OTU5586	96,6	92	83,7	80,9	80	84,3	80,9	80,9	85,4	91	88,8	75,3	70,8	66,3	66,7	71,1
Sporocarp, this study	OTU5594	97,7	93,2	86	82	81,1	85,4	82	82	86,5	92,1	89,9	77,5	73	69,7	70	74,4
Sporocarp, this study	OTU6416	93,2	88,6	81,4	78,7	77,8	82	78,7	78,7	83,1	87,6	85,4	73	68,5	64	65,2	69,7
Sporocarp, this study	OTU8267	97,7	93,2	87,2	84,3	83,3	88,8	84,3	84,3	88,8	92,1	89,9	79,8	75,3	69,7	70	74,4
Sporocarp, this study	OTU8357	96,6	92	83,7	80,9	80	84,3	80,9	80,9	85,4	91	88,8	75,3	70,8	66,3	67,4	71,9
Sporocarp, this study	OTU8707	95,9	90,5	81,9	78,7	78,9	82,7	78,7	78,7	84	89,3	86,7	73,3	68	62,7	64	64
Sporocarp, this study	OTU8916	96,6	92	88,4	85,4	84,4	88,8	85,4	83,1	89,9	93,3	91	77,5	73	70,8	68,9	73,3
Sporocarp, this study	OTU8971	95,5	90,9	84,9	83,1	81,1	85,4	83,1	84,3	86,5	89,9	87,6	78,7	74,2	68,5	68,9	74,4
1.1c <i>Pinus sylvestris</i> mycorrhiza	AM903348	100	95,5	87,2	84,3	83,3	87,6	84,3	84,3	88,8	94,4	92,1	78,7	74,2	69,7	70	74,4
1.1c <i>Pinus sylvestris</i> mycorrhiza	AM903346	95,5	100	86	82	82,2	85,4	82	82	86,5	94,4	92,1	76,4	76,4	68,5	67,8	72,2
1.1c <i>Alnus glutinosa</i> fine root	AM903363	87,2	86	100	83,9	83	87,4	83,9	74,7	88,5	88,5	87,4	70,1	67,8	65,5	65,9	70,5
1.1c <i>Betula pendula</i> mycorrhiza	AM903372	84,3	82	83,9	100	87,8	94,4	100	84,3	95,5	85,4	85,4	74,2	70,8	74,2	73,3	75,6
1.1c <i>Pinus sylvestris</i> mycorrhiza	DQ459996	83,3	82,2	83	87,8	100	91,1	87,8	82,2	92,2	85,6	83,3	74,4	72,2	72,2	69,2	73,6
1.1c <i>Pinus sylvestris</i> mycorrhiza	DQ459997	87,6	85,4	87,4	94,4	91,1	100	94,4	84,3	98,9	88,8	88,8	78,7	75,3	74,2	74,4	77,8
1.1c <i>Piloderma fallax</i> mycorrhizosphere hyphal humus	DQ460000	84,3	82	83,9	100	87,8	94,4	100	84,3	95,5	85,4	85,4	74,2	70,8	74,2	73,3	75,6
1.1c <i>Paxillus involutus</i> mycorrhizosphere hyphal humus	DQ460001	84,3	82	74,7	84,3	82,2	84,3	84,3	100	85,4	80,9	78,7	78,7	75,3	71,9	73,3	75,6
1.1c <i>Suillus bovinus</i> mycorrhizosphere hyphal humus	DQ460005	88,8	86,5	88,5	95,5	92,2	98,9	95,5	85,4	100	89,9	89,9	77,5	74,2	75,3	75,6	78,9
1.1c <i>Suillus bovinus</i> mycorrhizosphere hyphal humus	DQ460010	94,4	94,4	88,5	85,4	85,6	88,8	85,4	80,9	89,9	100	97,8	75,3	73	68,9	71,1	
1.1c <i>Pinus sylvestris</i> mycorrhiza	FM202423	92,1	92,1	87,4	85,4	83,3	88,8	85,4	78,7	89,9	97,8	100	73	74,2	70,8	68,9	70
Uncultured crenarchaeote from tomato root	AF227638	78,7	76,4	70,1	74,2	74,4	78,7	74,2	78,7	77,5	75,3	73	100	95,5	71,9	72,2	72,2
1.1b Nitrososphaera viennensis	CP007536	74,2	76,4	67,8	70,8	72,2	75,3	70,8	75,3	74,2	75,3	74,2	95,5	100	70,8	68,9	68,9
1.1a Nitrosotalea devanaterri	JN227488	69,7	68,5	65,5	74,2	72,2	74,2	74,2	71,9	75,3	73	70,8	71,9	70,8	100	80	82,2
1.1a Nitrosopumilus maritimus	DQ085097	70	67,8	65,9	73,3	69,2	74,4	73,3	73,3	75,6	68,9	68,9	72,2	68,9	80	100	84,4
Cenarchaeum symbiosum	U51469	74,4	72,2	70,5	75,6	73,6	77,8	75,6	75,6	78,9	71,1	70	72,2	68,9	82,2	84,4	100