

Additional methods

qPCR of prokaryotes and fungi

Bacterial abundance in the soil samples was measured by quantifying the V3 region of 16S rDNA as described previously [1]. Briefly, the PCR mixture contained 1 µl of the forward EUB338 and 1 µl of the reverse EUB518 primer [2] (0.2 µM final concentration of both primers), 12.5 µl of the Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix (Agilent), 8.5 µl of molecular grade water and 2 µl of 10x diluted cDNA or 100x diluted DNA template. The total amount of DNA added to the qPCR reaction was around 1 ng and the total amount of cDNA added was in the range of 3-20 ng. PCR was performed as follows: 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 10s, annealing at 56°C for 10s, and extension at 72°C for 13s and a final dissociation curve. The standard curve was made from DNA isolated from *E.coli* [3]. Fungal abundance in the soil samples was measured by quantifying the ITS2 region as described previously [4]. The PCR mixture contained 1 µl of the forward primer gITS7 [5], 1 µl of the reverse primer ITS4 [6] (0.2 µM final concentration of both primers), 10 µl of Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix (Agilent), 7 µl of RNA free water and 1 µl of 10x diluted cDNA or 100x diluted DNA template. PCR conditions were 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s, 56 °C for 20 s, 72 °C for 45 s, an extension step of 72 °C for 6 min and a final dissociation curve. The standard curve was made from a plasmid that contained the ITS fragment of *Aureobasidium pullulans*.

Preparation for sequencing of prokaryotic and fungal communities

The sequencing to determine the composition of prokaryotic and fungal communities for the 16S rRNA and ITS2 gene and transcripts were done as described by Borg Dahl et al. [7]. For 16S, we used the 314F and 806R primers [8-10]. For ITS2 region, we used the primers gITS7 [5] and ITS4 [6]. The PCR amplification was done in two steps. The first PCR mixture contained 15.75 µl molecular grade water, 5 µl PCR BIO 5 x reaction buffer, 0.25 µl PCR BIO Hifi Polymerase (PCR Biosystems, London, UK), 1 µl of each forward and reverse primer (0.2 µM final concentration) and 2 µl of diluted template (see above). The PCR conditions were the following: 95 °C for 1 min followed by 30 cycles of 95 °C for 15 seconds, 56°C for 15 seconds and 72°C for 30 seconds. Then there was a final extension at 70 °C for 3 min. PCR products were checked on agarose gels. First PCR was performed in technical triplicates to minimize PCR bias [11], which were pooled after the first PCR. After pooling technical replicates, PCR products were purified by Agencourt AMPure XP (Beckman Coulter). Addition of adaptors and indices was done in a second PCR reaction that contained 15.75 µl molecular grade water, 5 µl PCR BIO 5 x reaction buffer, 0.25 µl PCR BIO Hifi Polymerase (PCR biosystems), 1 µl of each of the forward and reverse primer that contained adaptor and index and 2 µl of the cleaned PCR product (see above). The PCR conditions were the following: 95 °C for 1 min followed by 15 cycles of 95 °C for 15 seconds, 56°C for 15 seconds and 72°C for 30 seconds. Then there was a final extension at 70 °C for 3 min. The PCR product were checked on a gel and purified using Agencourt AMPure XP (Beckman Coulter).

The cleaned and indexed PCR products were pooled by adding equal amounts of DNA to a 1.5 ml tube. The sample pool was concentrated using the DNA clean-and-concentrator-5 kit (Zymo Research, Irvine, California, USA) and prepared for sequencing using the MiSeq reagent kit v2 (500 cycles) and paired-end sequenced on the Illumina MiSeq platform (Illumina Inc., San Diego, California, USA) at the Section of Microbiology, University of Copenhagen.

Annotation

For 16S cDNA and DNA, raw reads were trimmed of adaptor, indices and primer sequences using *cutadapt* [12]. Only read pairs having both primers were retained. Trimmed sequences were merged (*assemble_pairs*) and clustered in OTUs (*cluster_otus*) with *UPARSE-OTU* algorithm [13] (97% sequence similarity, custom *BioDSL* script, <https://github.com/maasha/BioDSL>). Taxonomic annotation of OTUs was performed with *mothur* [14] naïve Bayes' classifier using Ribosomal Database Project database (*RDP*, trainset 16 [15], https://www.mothur.org/wiki/RDP_reference_files). This implementation has been described [16] OTUs affiliated to mitochondria, chloroplasts, Eukaryotes or not affiliated to a Domain were removed (0.33% of total OTUs).

For ITS2 region cDNA and DNA, raw sequence reads were treated as above. Trimmed sequences were processed using *PIPITS* workflow [17]. Primer-free sequences were merged using *PEAR* [18] and quality-filtered using *FASTX-Toolkit* (Hannon 2010). ITS2 sequences were extracted using *ITSx* [19] and clustered into OTUs using *VSEARCH* and a (97% sequence similarity). Representative OTU sequences were chimera-checked using *UNITE-UCHIME* reference dataset. Taxonomy of chimera-free OTUs was assigned with *RDP* classifier [16] against the *UNITE* fungal ITS database version 7.2 [20]. OTUs without Domain affiliation were removed (2.73% of total OTUs).

References

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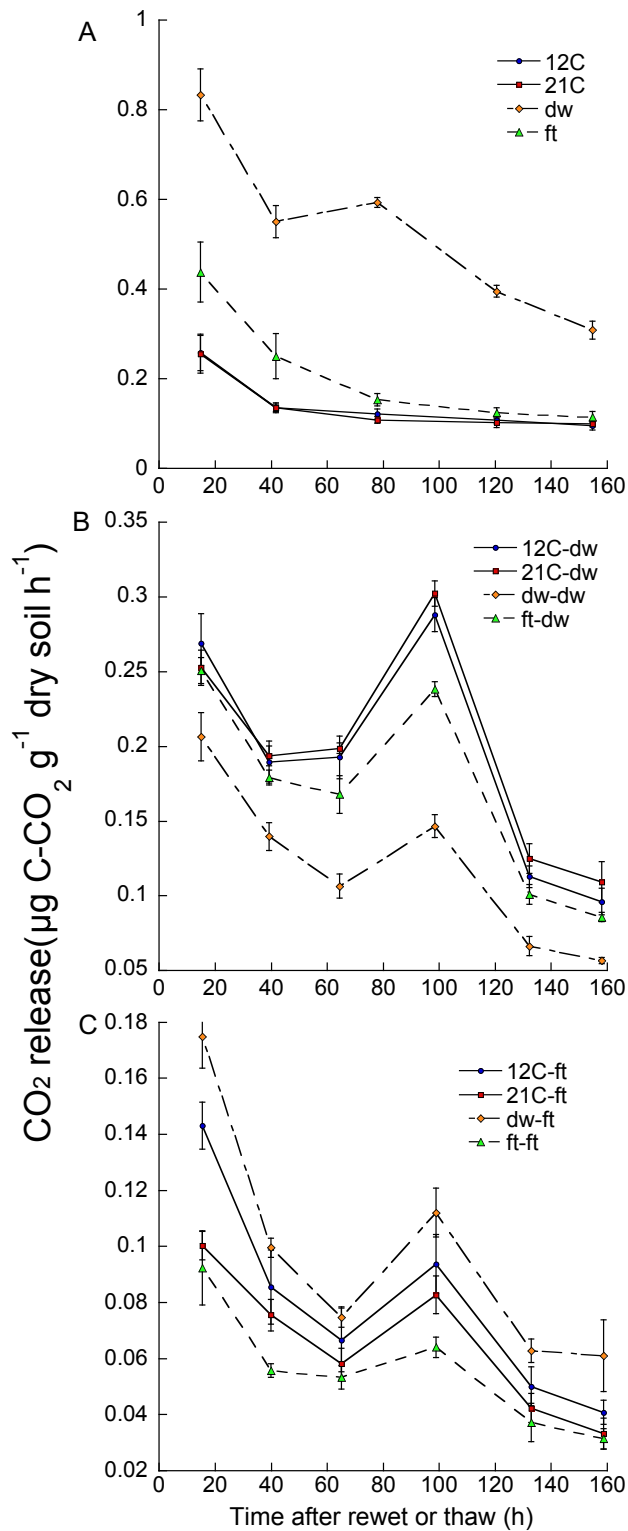


Fig. S1 CO₂ release following rewetting or thawing for Experiment 1 (A), Experiment 2 (B) and Experiment 3 (C). A presents the CO₂ release Experiment 1, which tested the effect of drying-rewetting versus freezing-thawing. B presents the CO₂ release in Experiment 2, which tested the legacy of freezing-thawing on drying-rewetting. C presents the CO₂ release in Experiment 3, which tested the legacy of drying-rewetting on freezing-thawing. 12°C indicates pre-incubation at 12°C, while 21°C indicates pre-incubation at 21°C. DW indicates drying-rewetting cycle. FT indicates freezing-thawing cycle.

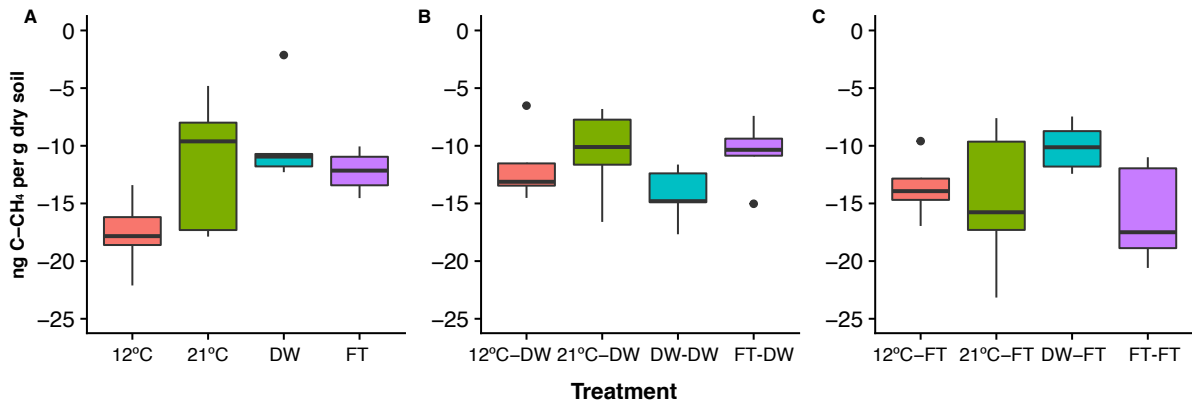


Fig. S2 CH₄ uptake at 12°C by soil samples during a week upon rewetting and thawing. A presents the CH₄ uptake in Experiment 1, which tested the effect of drying-rewetting versus freezing-thawing. B presents the CH₄ uptake in Experiment 2, which tested the legacy of freezing-thawing on drying-rewetting. C presents the CH₄ uptake in Experiment 3, which tested the legacy of drying-rewetting on freezing-thawing. 12°C indicates pre-incubation at 12°C, while 21°C indicates pre-incubation at 21°C. DW indicates drying-rewetting cycle. FT indicates freezing-thawing cycle.

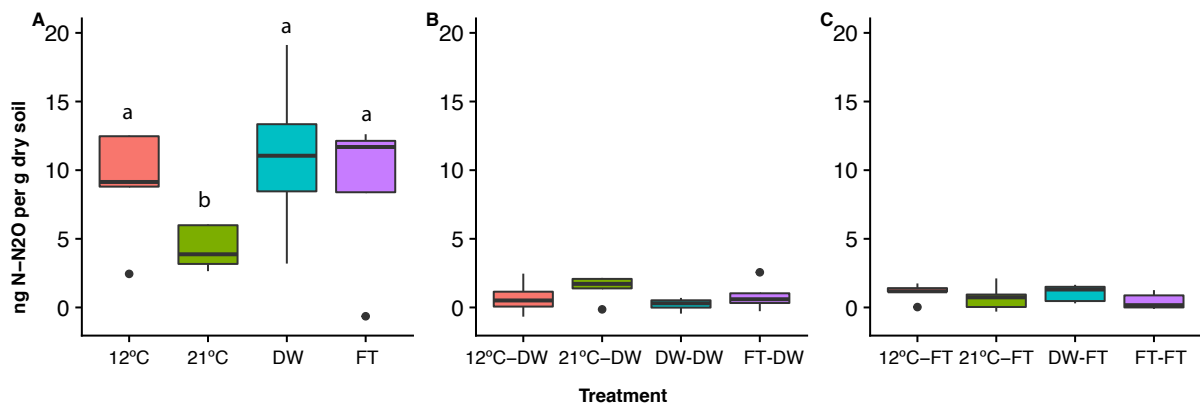


Fig. S3 N₂O released by soil samples incubated at 12°C during a week upon rewetting and thawing. A presents the N₂O released in Experiment 1, which tested the effect of drying-rewetting versus freezing-thawing. B presents the N₂O released in Experiment 2, which tested the legacy of freezing-thawing on drying-rewetting. C presents the N₂O released in Experiment 3, which tested the legacy of drying-rewetting on freezing-thawing. 12°C indicates pre-incubation at 12°C, while 21°C indicates pre-incubation at 21°C. DW indicates drying-rewetting cycle. FT indicates freezing-thawing cycle. Different superscript letters denote significant different rates from the post hoc test at P < 0.05.

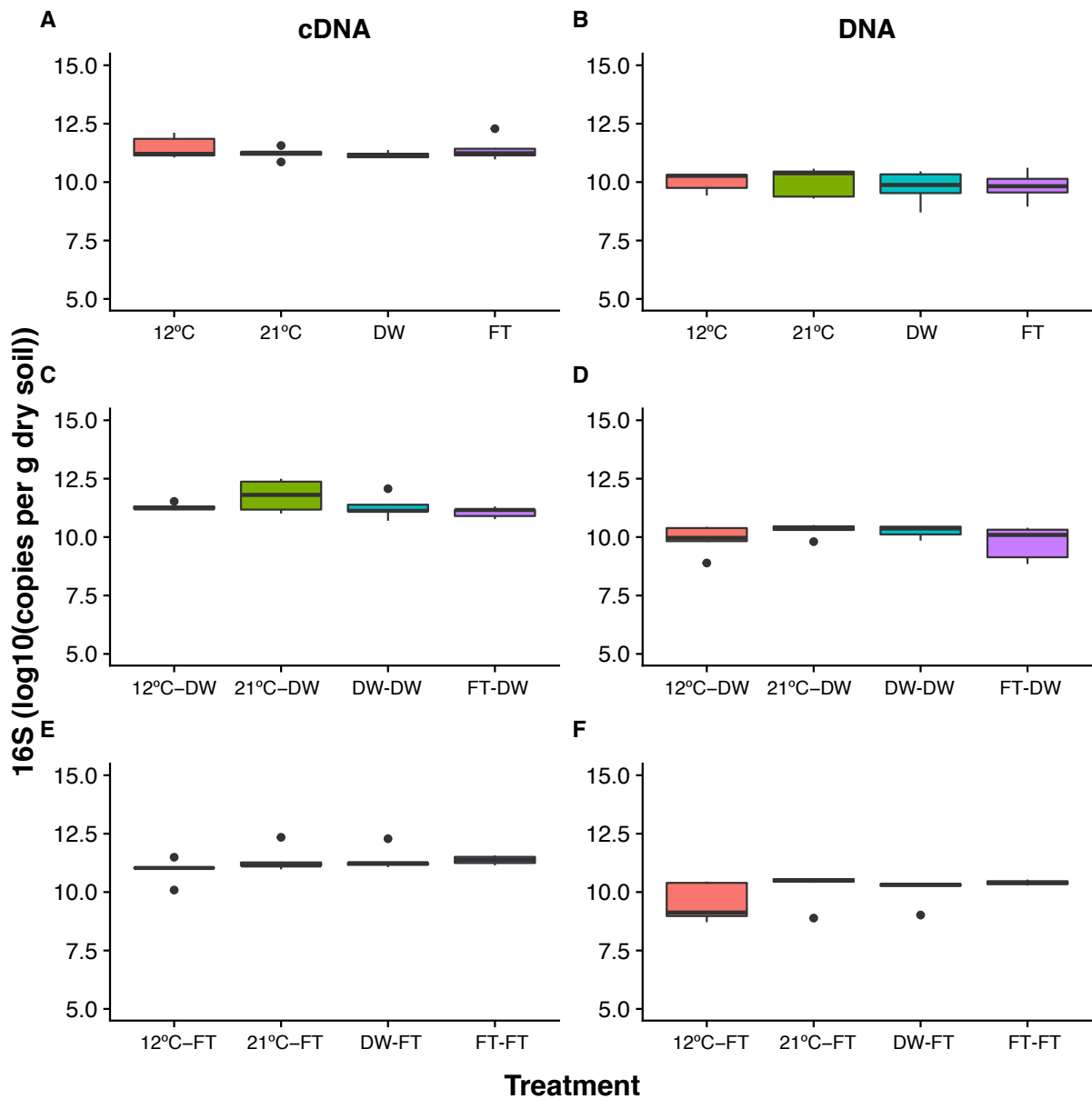


Fig S4 Abundance of 16S cDNA reads (A,C,E) and DNA reads (B,D,F) according to treatments as measured by qPCR. The treatments are described in fig. 1. A,B presents the abundance in Experiment 1, which tested the effect of drying-rewetting versus freezing-thawing. C, D presents the abundance in Experiment 2, which tested the legacy of freezing-thawing on drying-rewetting. E, F presents the abundance in Experiment 3, which tested the legacy of drying-rewetting on freezing-thawing. 12°C indicates pre-incubation at 12°C, while 21°C indicates pre-incubation at 21°C. DW indicates a drying-rewetting cycle. FT indicates a freezing-thawing cycle. Different superscript letters denote differences at $P < 0.05$ for a post hoc test for the mixed model.

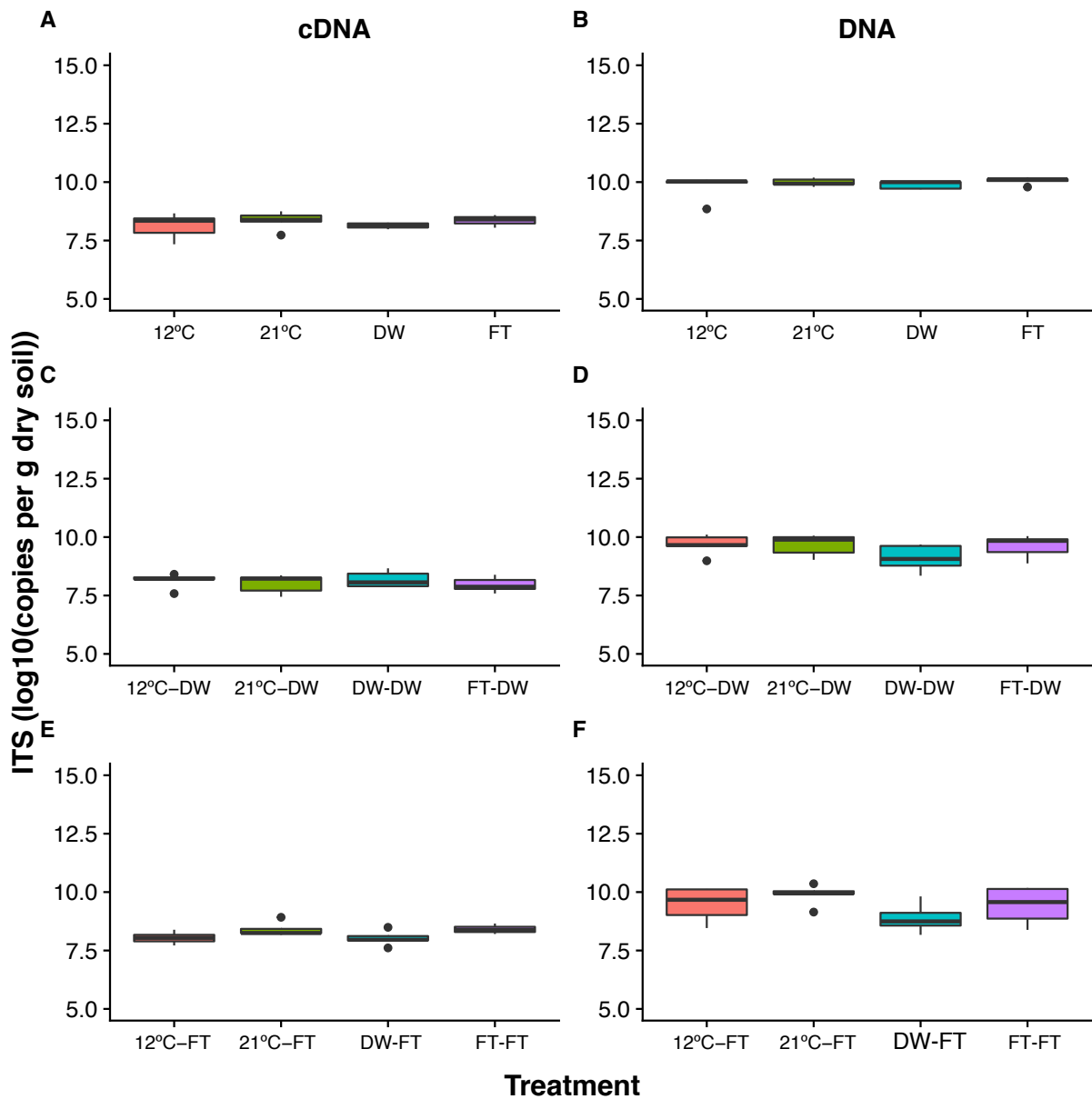


Fig. S5 Abundance of ITS cDNA reads (A,C,E) and DNA reads (B,D,F) according to treatments as measured by qPCR. The treatments are described in fig. 1. A,B presents the abundance in Experiment 1, which tested the effect of drying-rewetting versus freezing-thawing. C,D presents the abundance in Experiment 2, which tested the legacy of freezing-thawing on drying-rewetting. E,F presents the abundance in Experiment 3, which tested the legacy of drying-rewetting on freezing-thawing. 12°C indicates pre-incubation at 12°C, while 21°C indicates pre-incubation at 21°C. DW indicates a drying-rewetting cycle. FT indicates a freezing-thawing cycle. Different superscript letters denote differences at $P < 0.05$ for a post hoc test for the mixed model.

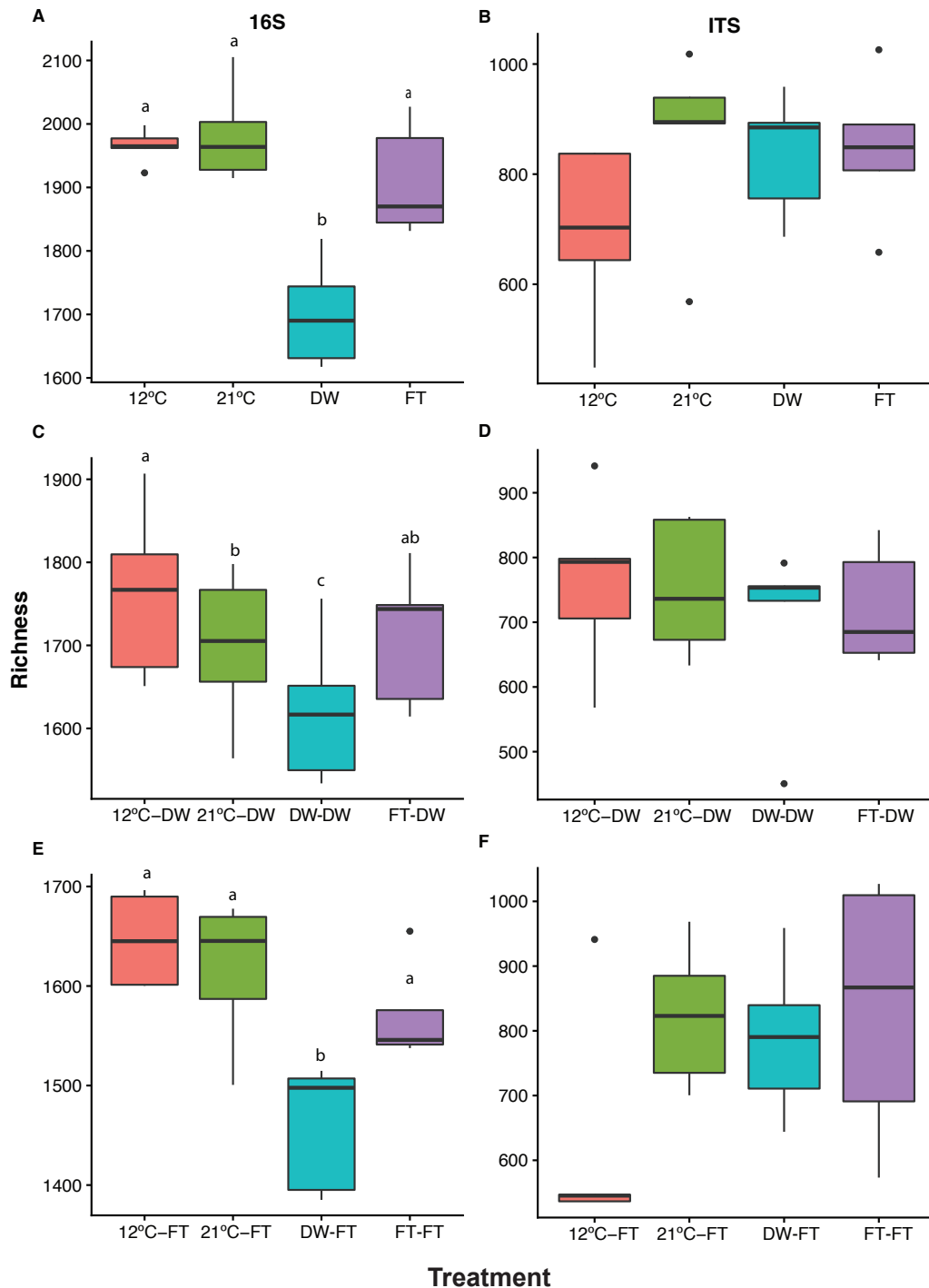


Fig. S6 Richness of 16S rRNA (A,C,E) and ITS amplicon profiles (B,D,F) for cDNA according to treatments. The treatments are described in figure 1. A,B presents the richness in Experiment 1, which tested the effect of drying-rewetting versus freezing-thawing. C,D presents the richness in Experiment 2, which tested the legacy of freezing-thawing on drying-rewetting. E,F presents the richness in Experiment 3, which tested the legacy of drying-rewetting on freezing-thawing. 12°C indicates pre-incubation at 12°C, while 21°C indicates pre-incubation at 21°C. DW indicates a drying-rewetting cycle. FT indicates a freezing-thawing cycle. Different superscript letters denote differences at $P < 0.05$ for a post hoc test for the mixed model.

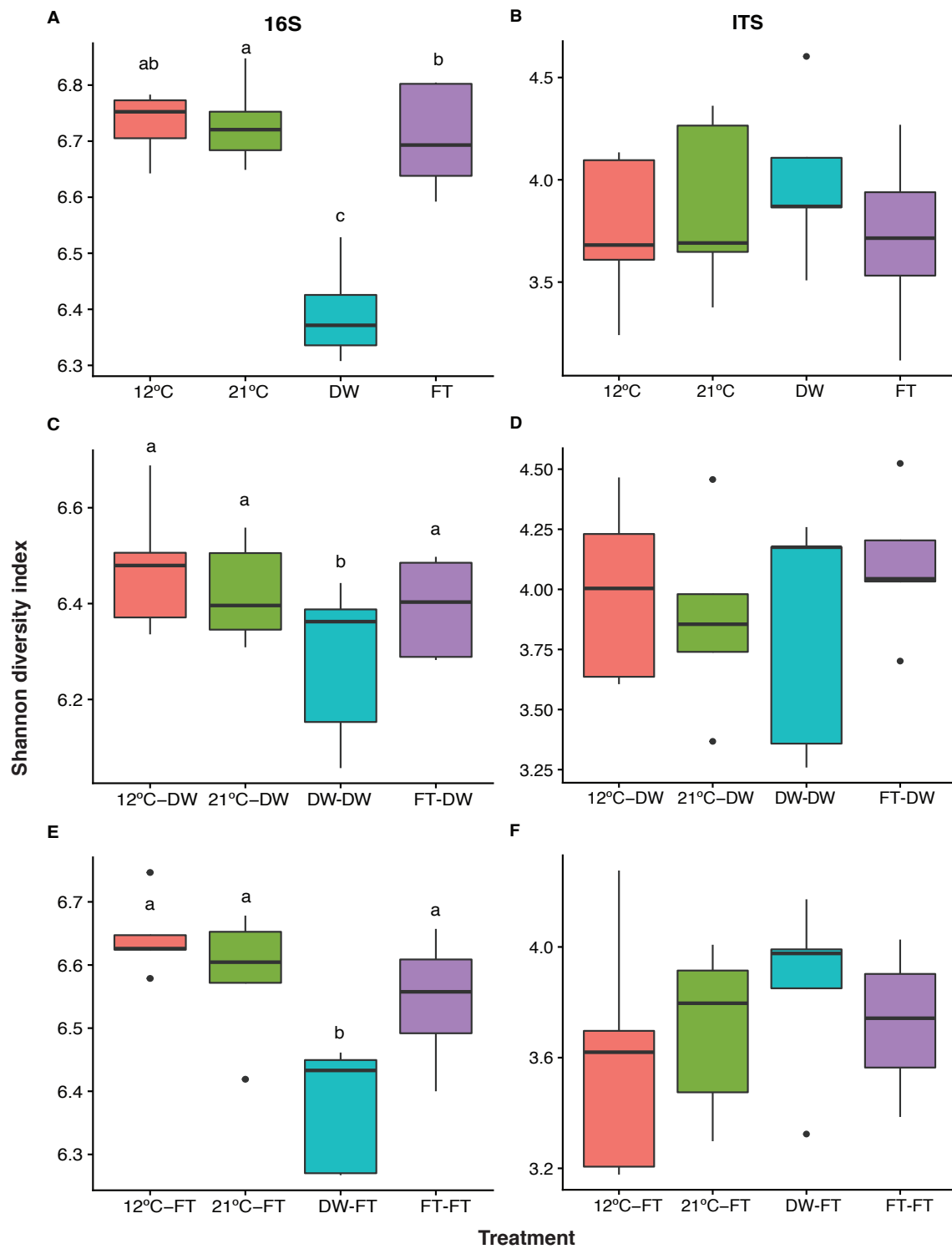


Fig. S7 Shannon-Weaver diversity index of 16S rRNA (A,C,E) and ITS amplicon profiles (B,D,F) for cDNA according to treatments. The treatments are described in figure 1. A,B presents the r Shannon diversity index in Experiment 1, which tested the effect of drying-rewetting versus freezing-thawing. C,D presents the Shannon diversity index in Experiment 2, which tested the legacy of freezing-thawing on drying-rewetting. E,F presents the Shannon diversity index in Experiment 3, which tested the legacy of drying-rewetting on freezing-thawing. 12°C indicates pre-incubation at 12°C, while 21°C indicates pre-incubation at 21°C. DW indicates a drying-rewetting cycle. FT indicates a freezing-thawing cycle. Different superscript letters denote differences at $P < 0.05$ for a post hoc test for the mixed model.

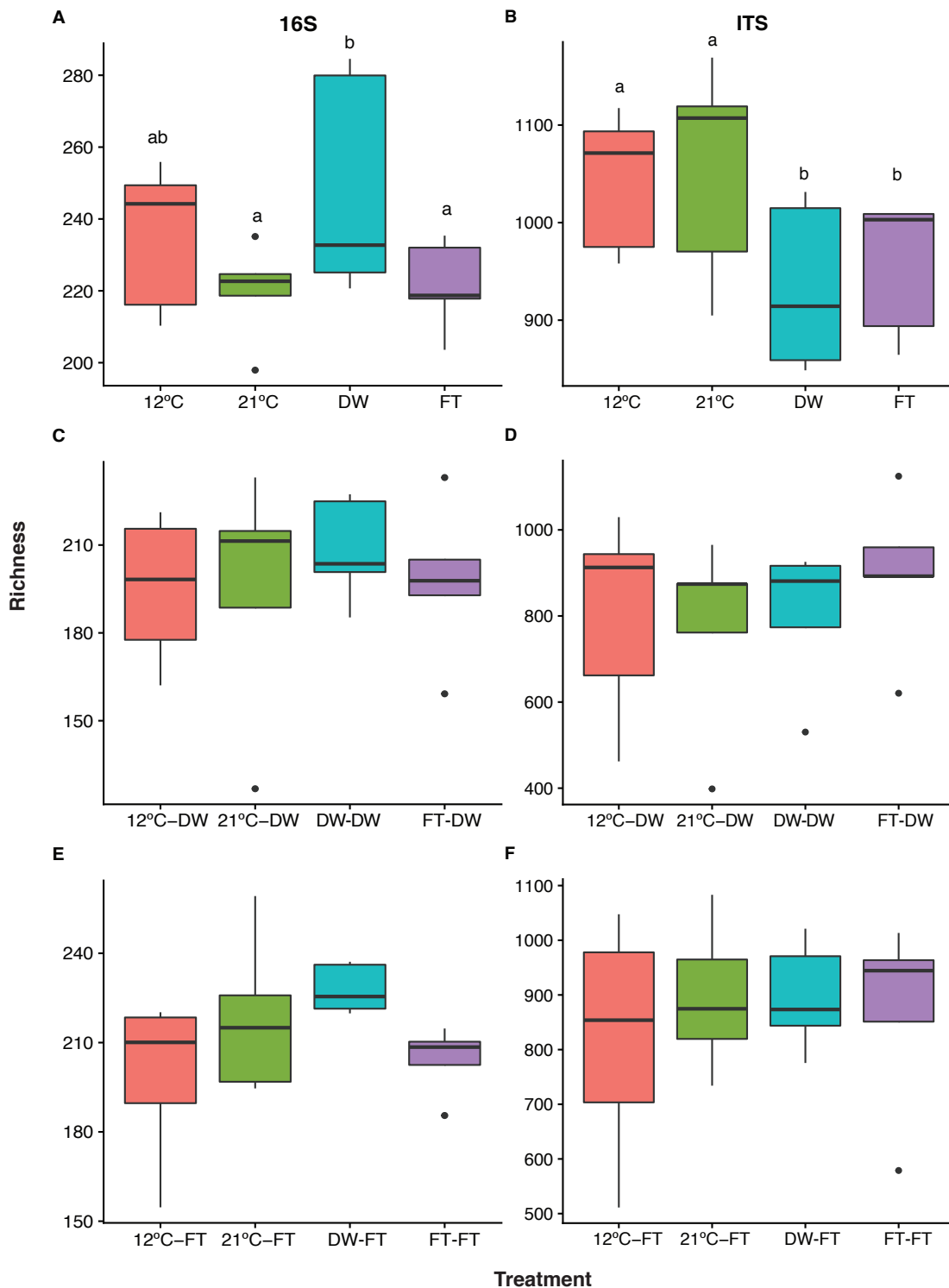


Fig. S8 Richness of 16S (A,C,E) and ITS amplicon profiles (B,D,F) for DNA according to treatments. The treatments are described in figure 1. A,B presents the richness in Experiment 1, which tested the effect of drying-rewetting versus freezing-thawing. C,D presents the richness in Experiment 2, which tested the legacy of freezing-thawing on drying-rewetting. E,F presents the richness in Experiment 3, which tested the legacy of drying-rewetting on freezing-thawing. 12°C indicates pre-incubation at 12°C, while 21°C indicates pre-incubation at 21°C. DW indicates a drying-rewetting cycle. FT indicates a freezing-thawing cycle. Different superscript letters denote differences at $P < 0.05$ for a post hoc test for the mixed model.

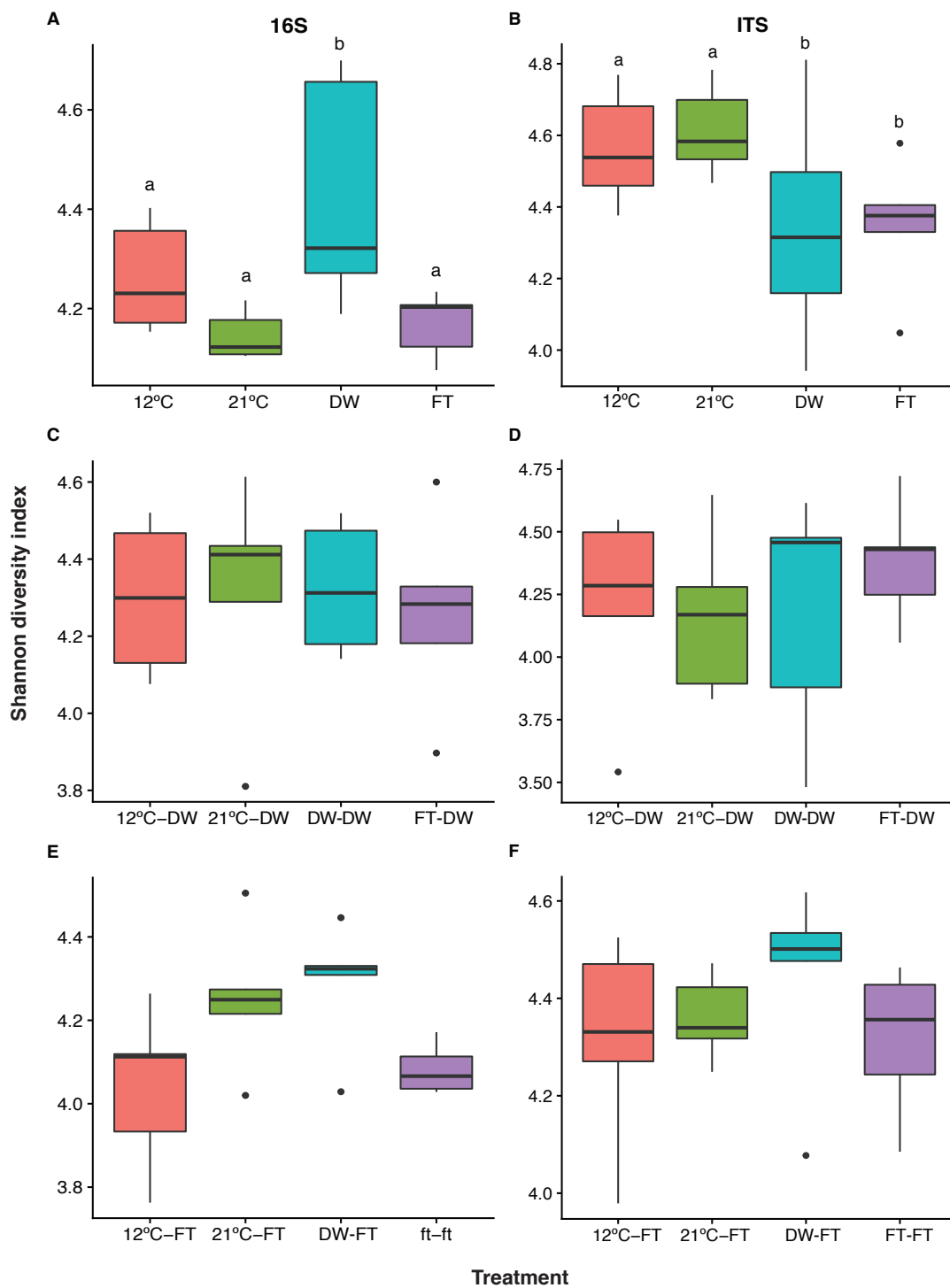


Fig. S9 Shannon diversity index of 16S (A,C,E) and ITS amplicon profiles for DNA (B,D,F) according to treatments. The treatments are described in figure 1. A,B presents the r Shannon diversity index in Experiment 1, which tested the effect of drying-rewetting versus freezing-thawing. C,D presents the Shannon diversity index in Experiment 2, which tested the legacy of freezing-thawing on drying-rewetting. E,F presents the Shannon diversity index in Experiment 3, which tested the legacy of drying-rewetting on freezing-thawing. 12°C indicates pre-incubation at 12°C, while 21°C indicates pre-incubation at 21°C. DW indicates a drying-rewetting cycle. FT indicates a freezing-thawing cycle. Different superscript letters denote differences at $P < 0.05$ for a post hoc test for the mixed model.

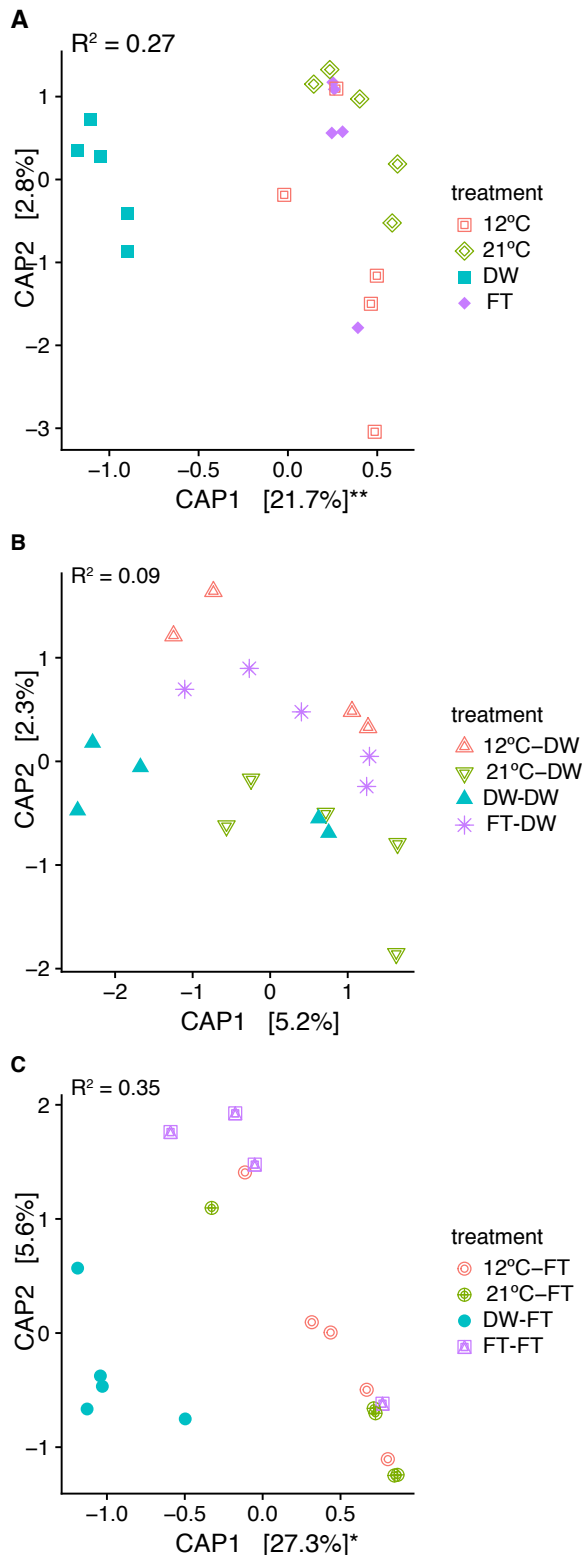


Fig. S10 Partial distance-based redundancy analysis of prokaryotes for DNA on Bray-Curtis dissimilarity using capscale ordination. A is Experiment 1 where we tested if a drying-rewetting (DW) or a freezing-thawing (FT) cycle leave different legacies in the prokaryote community. B is from Experiment 2 where we tested how the different legacies affected the microbial response to a drying-rewetting cycle. C is from Experiment 3 where we tested how different legacies affected the microbial communities after an additional FT cycle. 12°C indicates pre-incubation at 12°C, while 21°C indicates pre-incubation at 21°C (see Fig. 1). Significance of axes is tested with a permutation test by axis: ** $P < 0.01$; *** $p < 0.001$.

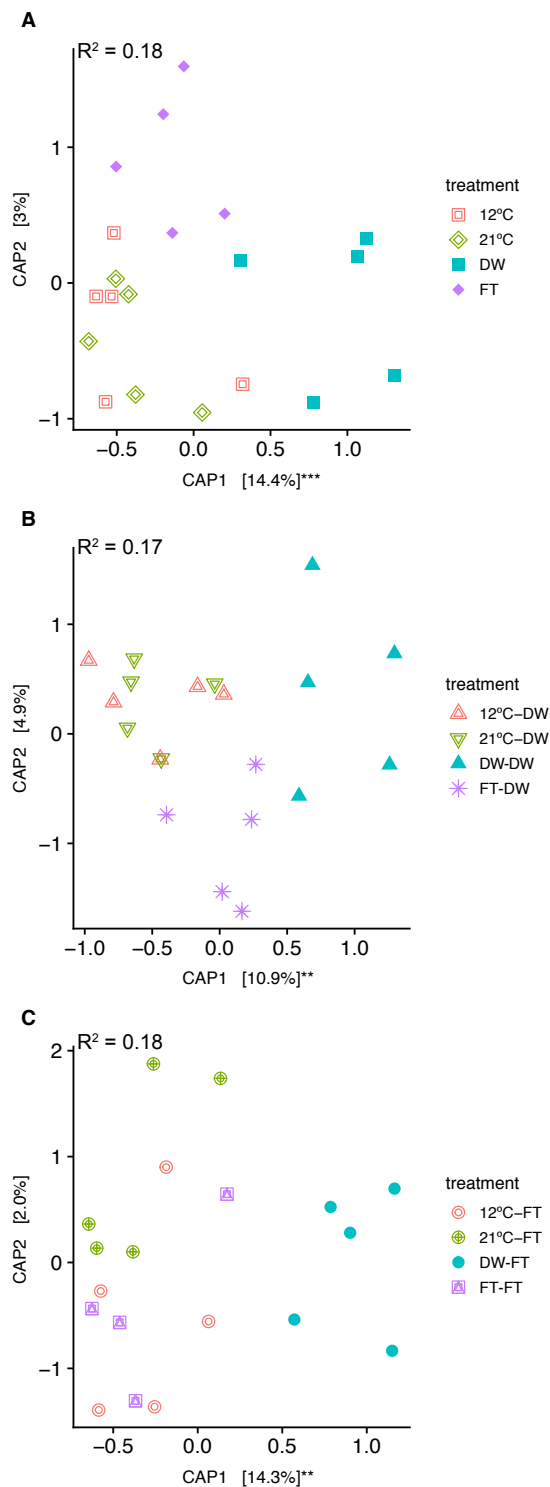


Fig. S11 Partial distance-based redundancy analysis of ITS for DNA on Bray-Curtis dissimilarity using capscale ordination. A is Experiment 1 where we tested if a drying-rewetting (DW) or a freezing-thawing (FT) cycle leave different legacies in the prokaryote community. B is from Experiment 2 where we tested how the different legacies affected the microbial response to a drying-rewetting cycle. C is from Experiment 3 where we tested how different legacies affected the microbial communities after an additional FT cycle. 12°C indicates pre-incubation at 12°C, while 21°C indicates pre-incubation at 21°C (see Fig. 1). Significance of axes is tested with a permutation test by axis: ** $P < 0.01$; *** $p < 0.001$.

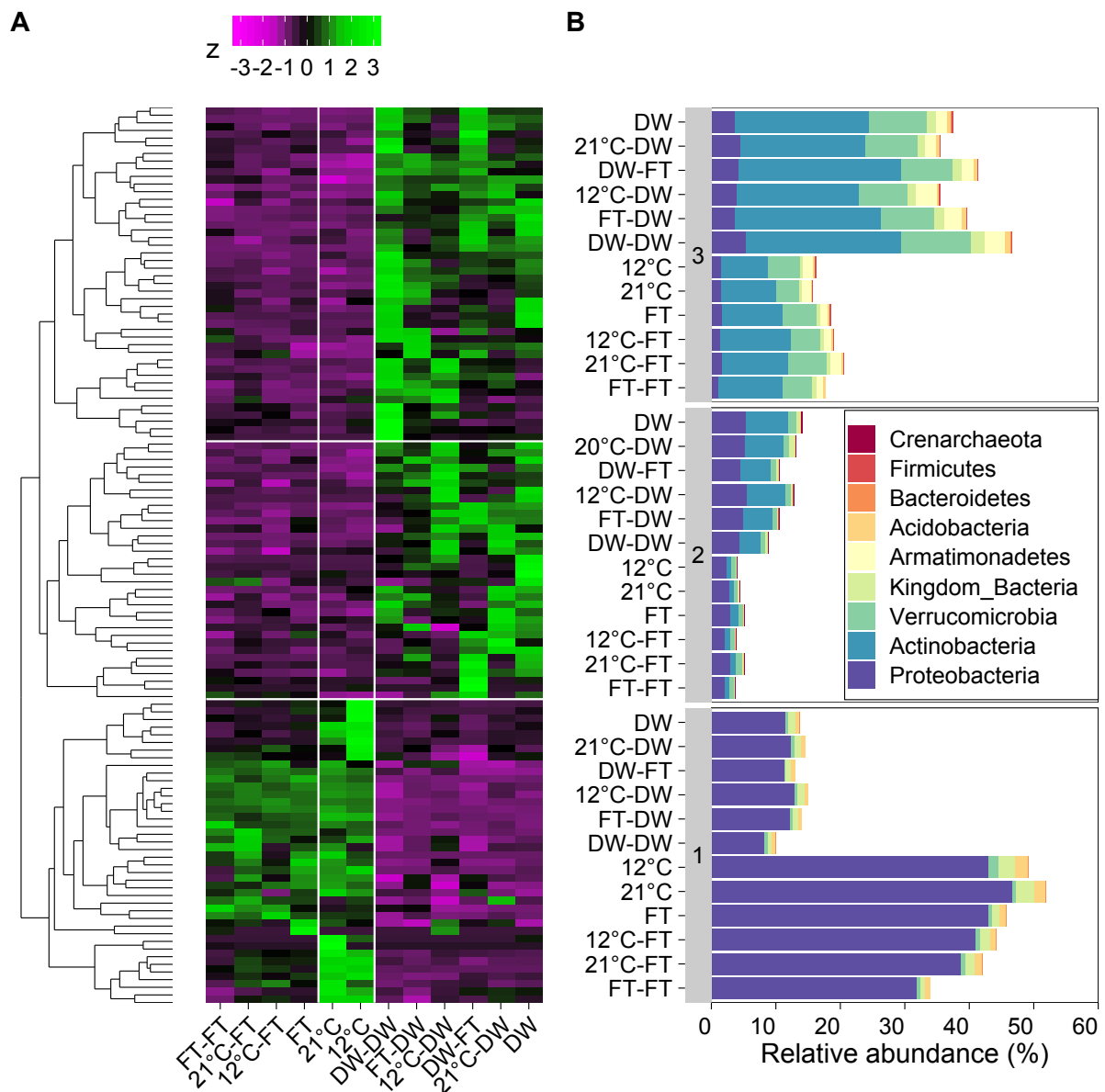


Fig. S12 Response groups of 16S amplicons at DNA level. **A)** Heatmap of the z-score per OTU per treatment compared to the mean relative abundance. **B)** Barplot with the relative abundance per phylum per response group. Response groups are labeled with numbers and correspond to the groups in A, 1 is the bottom group, 3 is the top group, the rest is also shown in order.

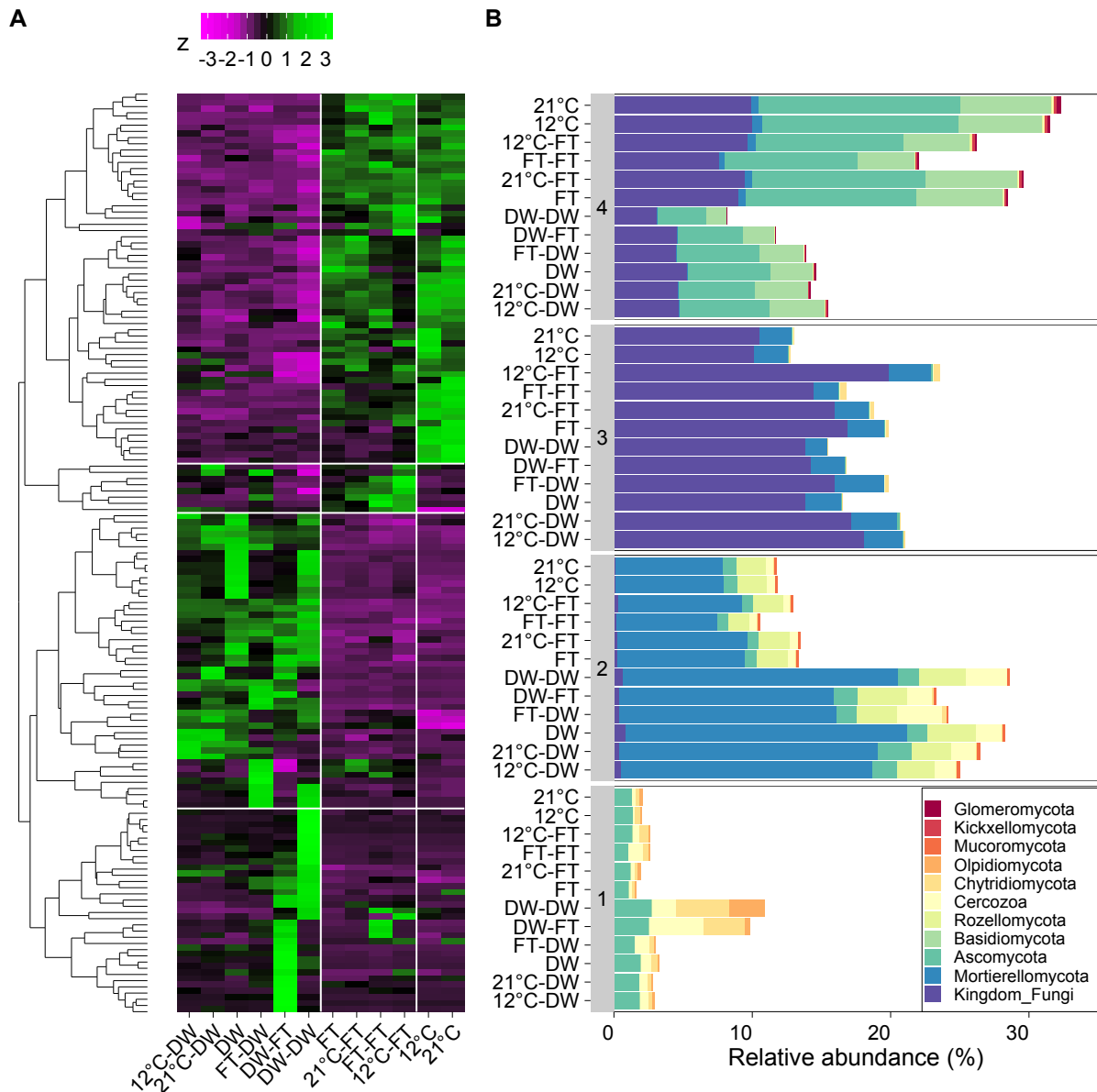


Fig. S13 Response groups of ITS amplicons at cDNA level. **A)** Heatmap of the z-score per OTU per treatment compared to the mean relative abundance. **B)** Barplot with the relative abundance per phylum per response group. Response groups are labeled with numbers and correspond to the groups in A, 1 is the bottom group, 4 is the top group, the rest is also shown in order.

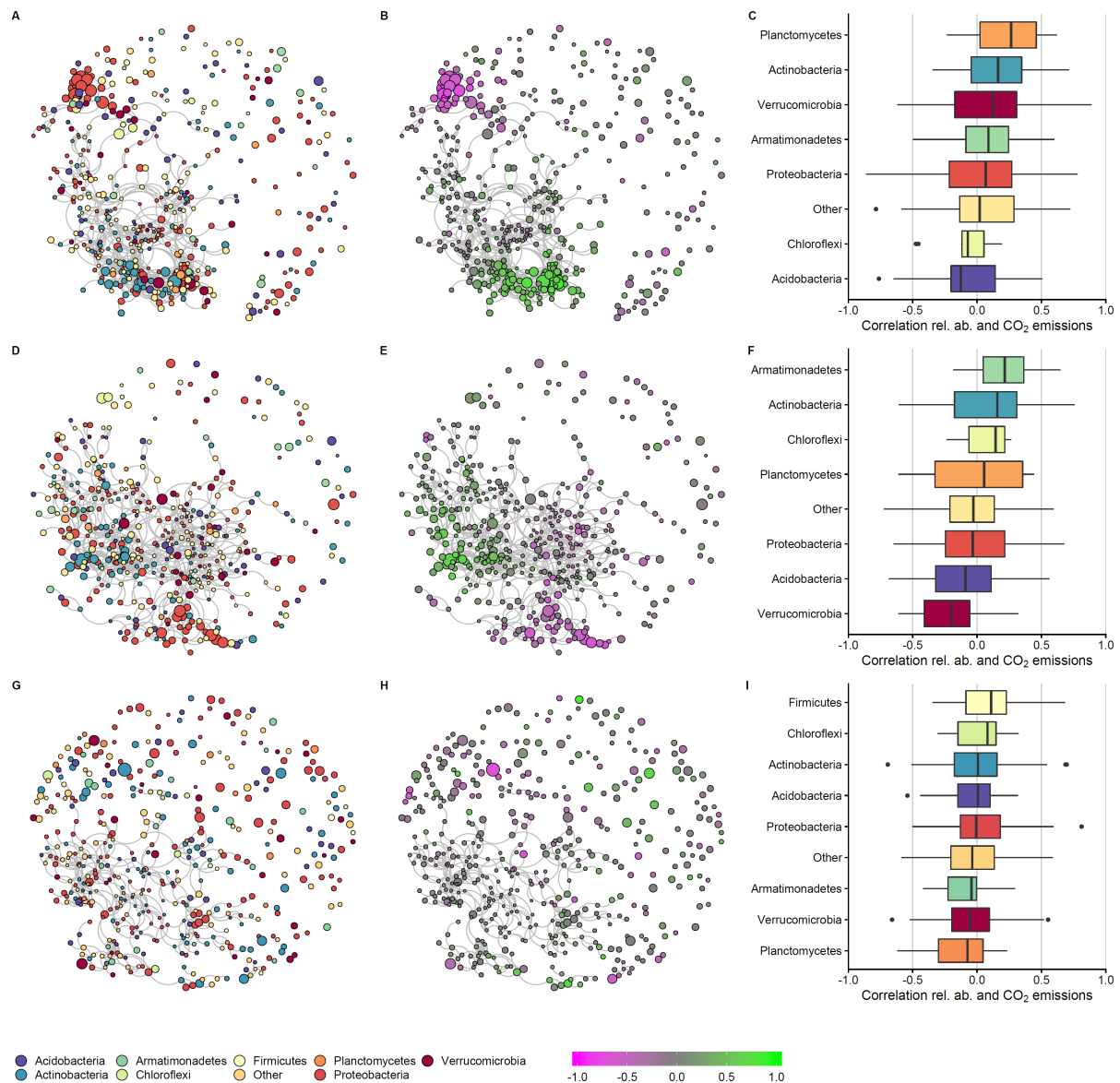


Fig. S14 Co-response networks of the 16S OTUs at DNA level and correlation between relative abundance and CO₂ emissions. A), B), C) show experiment 1, single treatments. D), E), F) show experiment 2, single treatments followed by a drought treatment. G), H), I), show experiment 3, single treatments followed by a freezing-thawing treatment. A), D), G) co-response network with phylum overlay, legend below panel G). Node sizes are indicative for the mean relative abundance. B), E), H) show the co-response network with correlation between relative abundance and CO₂ emissions, legend is shown below panel H). C), F), I), shows boxplots with the correlations per OTU per phylum.

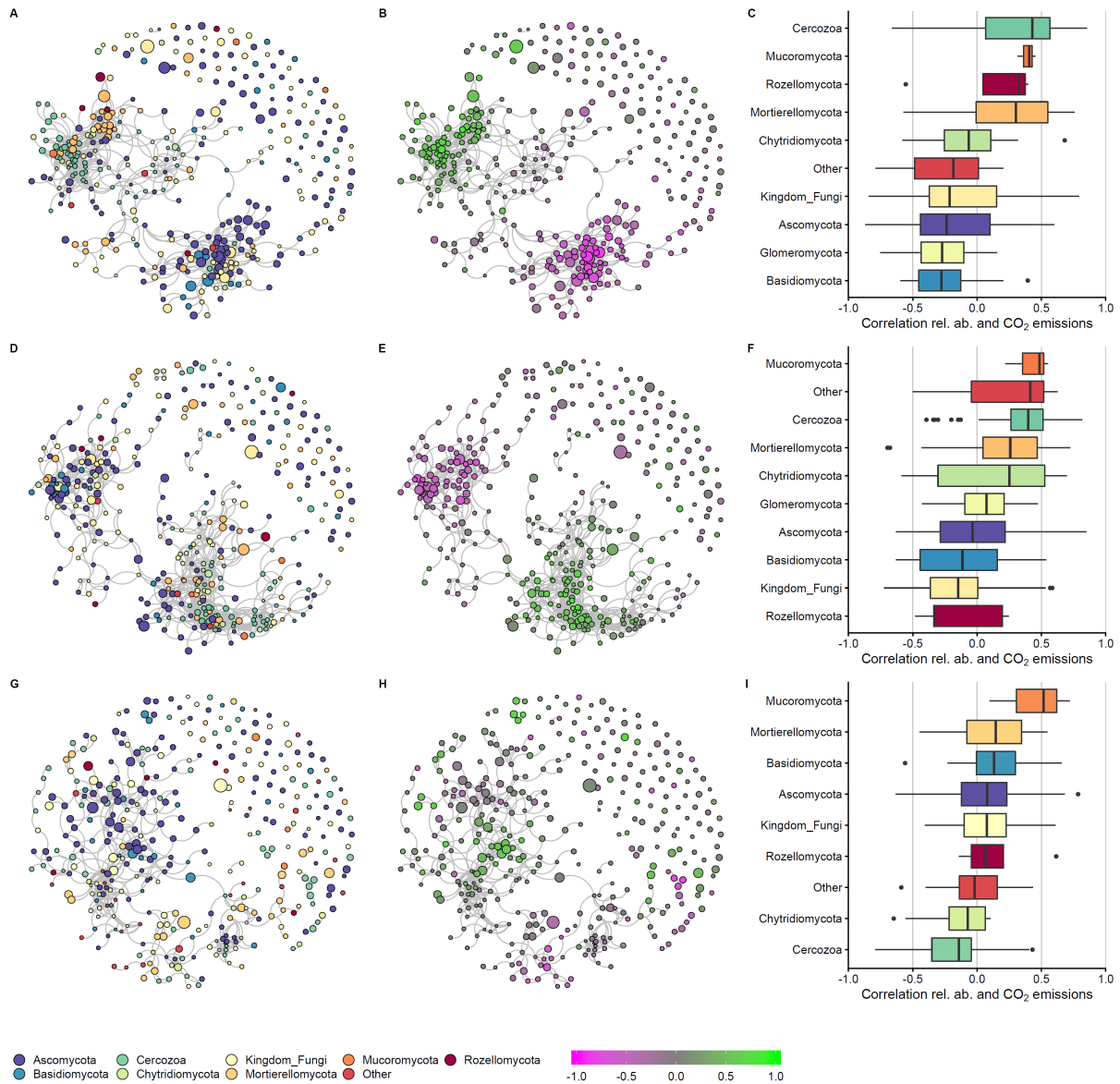


Fig. S15 Co-response networks of the ITS OTUs at DNA level and correlation between relative abundance and CO₂ emissions. A), B), C) show experiment 1, single treatments. D), E), F) show experiment 2, single treatments followed by a drought treatment. G), H), I), show experiment 3, single treatments followed by a freeze-thaw treatments. A), D), G) co-response network with phylum overlay, legend below panel G). Node sizes are indicative for the mean relative abundance. B), E), H) show the co-response network with correlation between relative abundance and CO₂ emissions, legend is shown below panel H). C), F), I), shows boxplots with the correlations per OTU per phylum.

Table S1 Summary table of 16S rRNA transcripts. One sample was excluded as the extraction of RNA failed.

Samples	Experiment	jar	treatment	block	#reads¹	notes
ANNELEIN-01	exp 1	85	DW	1	12127	
ANNELEIN-21	exp 1	86	FT	1	16214	
87B116SV3V4	exp 1	87	21°C	3	17307	
ANNELEIN-49	exp 1	88	12°C	4	16597	
8916SV3V4	exp 1	89	21°C	4	11731	
ANNELEIN-62	exp 1	90	DW	3	13882	
9116SV3V4	exp 1	91	FT	3	24196	
ANNELEIN-09	exp 1	92	12°C	3	11508	
ANNELEIN-28	exp 1	93	FT	5	15421	
ANNELEIN-33	exp 1	94	DW	4	12555	
9516SV3V4	exp 1	95	FT	4	12121	
9616SV3V4	exp 1	96	21°C	2	14028	
9716SV3V4	exp 1	97	21°C	5	22164	
87G116SV3V4	exp 1	98	12°C	2	24322	
ANNELEIN-26	exp 1	99	12°C	1	11612	
ANNELEIN-24	exp 1	121	DW	2	23655	
ANNELEIN-64	exp 1	121	FT	2	13961	
ANNELEIN-19	exp 1	122	21°C	1	17500	
ANNELEIN-17	exp 1	123	DW	5	17041	
ANNELEIN-57	exp 1	124	12°C	5	<u>9951</u>	
12516SV3V4	exp 2	125	DW-DW	2	14738	
12616SV3V4	exp 2	126	DW-DW	5	14461	
12116SV3V4	exp 2	121	DW-DW	3	15958	
13416SV3V4	exp 2	134	FT-DW	1	13185	
13516SV3V4	exp 2	135	FT-DW	2	18939	
14716SV3V4	exp 2	147	FT-DW	4	26822	
14816SV3V4	exp 2	148	FT-DW	5	30316	
ANNELEIN-11	exp 2	155	21°C-DW	2	12406	
ANNELEIN-12	exp 2	129	21°C-DW	3	12560	
ANNELEIN-14	exp 2	157	DW-DW	4	13962	
ANNELEIN-16	exp 2	137	21°C-DW	4	22432	
ANNELEIN-22	exp 2	131	12°C-DW	1	12527	
ANNELEIN-23	exp 2	145	12°C-DW	3	15278	
ANNELEIN-25	exp 2	159	12°C-DW	5	15350	
ANNELEIN-41	exp 2	133	FT-DW	3	11953	
ANNELEIN-46	exp 2	136	12°C-DW	2	13875	
ANNELEIN-54	exp 2	128	12°C-DW	4	12721	
ANNELEIN-55	exp 2	139	DW-DW	1	12497	
ANNELEIN-56	exp 2	112	21°C-DW	1	24993	
ANNELEIN-63	exp 2	126	21°C-DW	5	<u>12302</u>	
11616SV3V4	exp 3	116	21°C-FT	1	16385	
11916SV3V4	exp 3	119	FT-FT	3	12322	
12716SV3V4	exp 3	127	FT-FT	4	21221	
14316SV3V4	exp 3	143	12°C-FT	4	30984	
14616SV3V4	exp 3	146	FT-FT	5	16806	
14916SV3V4	exp 3	149	12°C-FT	2	25591	
15616SV3V4	exp 3	156	21°C-FT	4	32866	
ANNELEIN-12	exp 3	124	21°C-FT	5	7035	
ANNELEIN-15	exp 3	142	FT-FT	2	<u>9511</u>	
ANNELEIN-21	exp 3	130	12°C-FT	5	12895	

ANNELEIN-27	exp 3	123	DW-FT	2	17981	
ANNELEIN-29	exp 3	115	DW-FT	4	21743	
ANNELEIN-30	exp 3	140	DW-FT	3	15250	
ANNELEIN-31	exp 3	144	12°C-FT	1	14148	
ANNELEIN-32	exp 3	132	DW-FT	1	24244	
ANNELEIN-45	exp 3	154	12°C-FT	3	12157	
ANNELEIN-47	exp 3	111	DW-FT	5	11122	
ANNELEIN-48	exp 3	114	21°C-FT	3	23425	
ANNELEIN-61	exp 3	150	21°C-FT	2	13361	
		152	FT-FT	1		extraction failed

¹reads in italic and underlined. The rarefraction depth was determined by the number of reads in italic and underlined within the specific experiment (e.g. exp. 1, exp 2 or exp 3).

Table S2 Summary information of 16S rRNA gene amplicons. One sample was excluded as the extraction of DNA failed. One sample was excluded, because of too few reads.

samples	Experiment	jar	treatment	block	#reads¹	notes
121D16SV3V4	exp 1	121	DW	2	4444	
121D16SV3V4	exp 1	121	FT	2	6478	
122D16SV3V4	exp 1	122	21°C	1	7009	
123D16SV3V4	exp 1	123	DW	5	5832	
124D16SV3V4	exp 1	124	12°C	5	8062	
85D16SV3V4	exp 1	85	DW	1	6831	
86D16SV3V4	exp 1	86	FT	1	13009	
87D16SV3V4	exp 1	87	21°C	3	7993	
88D16SV3V4	exp 1	88	12°C	4	1722	
89D16SV3V4	exp 1	89	21°C	4	5151	
90D16SV3V4	exp 1	90	DW	3	3728	
91D16SV3V4	exp 1	91	FT	3	4211	
92D16SV3V4	exp 1	92	12°C	3	15953	
93D16SV3V4	exp 1	93	FT	5	5214	
94D16SV3V4	exp 1	94	DW	4	<u>1393</u>	
95D16SV3V4	exp 1	95	FT	4	4515	
96D16SV3V4	exp 1	96	21°C	2	3627	
97D16SV3V4	exp 1	97	21°C	5	2469	
98D16SV3V4	exp 1	98	12°C	2	3602	
99D16SV3V4	exp 1	99	12°C	1	11250	
125D16SV3V4	exp 2	125	DW-DW	2	<u>993</u>	
126D16SV3V4	exp 2	126	DW-DW	5	1499	
129D16SV3V4	exp 2	129	21°C-DW	3	1559	
112D16SV3V4	exp 2	112	21°C-DW	1	12332	
121D16SV3V4	exp 2	121	DW-DW	3	7040	
126D16SV3V4	exp 2	126	21°C-DW	5	3907	
128D16SV3V4	exp 2	128	12°C-DW	4	6124	
131D16SV3V4	exp 2	131	12°C-DW	1	7344	
133D16SV3V4	exp 2	133	FT-DW	3	1316	
134D16SV3V4	exp 2	134	FT-DW	1	7123	
135D16SV3V4	exp 2	135	FT-DW	2	7276	
136D16SV3V4	exp 2	136	12°C-DW	2	1588	
137D16SV3V4	exp 2	137	21°C-DW	4	9221	
139D16SV3V4	exp 2	139	DW-DW	1	1211	
145D16SV3V4	exp 2	145	12°C-DW	3	259	too few reads
147D16SV3V4	exp 2	147	FT-DW	4	2156	
148D16SV3V4	exp 2	148	FT-DW	5	3316	

155D16SV3V4	exp 2	155	21°C-DW	2	1978
157D16SV3V4	exp 2	157	DW-DW	4	6360
159D16SV3V4	exp 2	159	12°C-DW	5	1146
111D16SV3V4	exp 3	111	DW-FT	5	<u>1236</u>
114D16SV3V4	exp 3	114	21°C-FT	3	8305
115D16SV3V4	exp 3	115	DW-FT	4	2233
116D16SV3V4	exp 3	116	21°C-FT	1	12908
119D16SV3V4	exp 3	119	FT-FT	3	2836
123D16SV3V4	exp 3	123	DW-FT	2	2153
124D16SV3V4	exp 3	124	21°C-FT	5	12781
127D16SV3V4	exp 3	127	FT-FT	4	12930
130D16SV3V4	exp 3	130	12°C-FT	5	11902
132D16SV3V4	exp 3	132	DW-FT	1	2491
140D16SV3V4	exp 3	140	DW-FT	3	4507
142D16SV3V4	exp 3	142	FT-FT	2	1886
143D16SV3V4	exp 3	143	12°C-FT	4	5733
144D16SV3V4	exp 3	144	12°C-FT	1	2864
146D16SV3V4	exp 3	146	FT-FT	5	3339
149D16SV3V4	exp 3	149	12°C-FT	2	5400
150D16SV3V4	exp 3	150	21°C-FT	2	9868
154D16SV3V4	exp 3	154	12°C-FT	3	7840
156D16SV3V4	exp 3	156	21°C-FT	4	2578
		152	FT-FT	1	Extraction failed

¹reads in italic and underlined. The rarefraction depth was determined by the number of reads in italic and underlined within the specific experiment (e.g. exp. 1, exp 2 or exp 3).

Table S3 Summary information of ITS transcripts. One sample was excluded as extraction of RNA failed.

Samples	Experiment	jar	treatment	block	#reads¹	notes
87B1ITS	exp 1	87	21°C	3	<u>43808</u>	
87G1ITS	exp 1	98	12°C	2	46085	
99ITS	exp 1	99	12°C	1	54218	
85ITS	exp 1	85	DW	1	54871	
97ITS	exp 1	97	21°C	5	54897	
95ITS	exp 1	95	FT	4	56656	
91E1ITS	exp 1	91	FT	3	57575	
86ITS	exp 1	86	FT	1	58505	
92ITS	exp 1	92	12°C	3	58950	
90ITS	exp 1	90	DW	3	61160	
88ITS	exp 1	88	12°C	4	64041	
123ITS	exp 1	123	DW	5	67095	
89ITS	exp 1	89	21°C	4	67349	
121ITS	exp 1	121	DW	2	68542	
94A4ITS	exp 1	94	DW	4	73545	
121ITS	exp 1	121	FT	2	75374	
124ITS	exp 1	124	12°C	5	78584	
96ITS	exp 1	96	21°C	2	80832	
122ITS	exp 1	122	21°C	1	84052	
93ITS	exp 1	93	FT	5	84315	
125ITS	exp 2	125	DW-DW	2	64591	
126ITS	exp 2	126	DW-DW	5	69839	
169ITS	exp 2	129	21°C-DW	3	37166	
112ITS	exp 2	112	21°C-DW	1	73254	

121ITS	exp 2	121	DW-DW	3	51821
126ITS	exp 2	126	21°C-DW	5	72121
128ITS	exp 2	128	12°C-DW	4	48482
151ITS	exp 2	131	12°C-DW	1	49123
133ITS	exp 2	133	FT-DW	3	45196
134ITS	exp 2	134	FT-DW	1	48821
135ITS	exp 2	135	FT-DW	2	46848
136ITS	exp 2	136	12°C-DW	2	<u>31222</u>
137ITS	exp 2	137	21°C-DW	4	58349
139ITS	exp 2	139	DW-DW	1	38456
145ITS	exp 2	145	12°C-DW	3	52733
147ITS	exp 2	147	FT-DW	4	51668
148ITS	exp 2	148	FT-DW	5	63177
155ITS	exp 2	155	21°C-DW	2	34026
157ITS	exp 2	157	DW-DW	4	44827
159ITS	exp 2	159	12°C-DW	5	62464
111ITS	exp 3	111	DW-FT	5	53144
114ITS	exp 3	114	21°C-FT	3	50555
115ITS	exp 3	115	DW-FT	4	58937
116ITS	exp 3	116	21°C-FT	1	66523
119ITS	exp 3	119	FT-FT	3	91976
123ITS	exp 3	123	DW-FT	2	<u>47409</u>
124ITS	exp 3	124	21°C-FT	5	49622
127ITS	exp 3	127	FT-FT	4	69343
130ITS	exp 3	130	12°C-FT	5	72387
132ITS	exp 3	132	DW-FT	1	48503
140ITS	exp 3	140	DW-FT	3	80918
142ITS	exp 3	142	FT-FT	2	70288
143ITS	exp 3	143	12°C-FT	4	60938
144ITS	exp 3	144	12°C-FT	1	48617
146ITS	exp 3	146	FT-FT	5	83555
149ITS	exp 3	149	12°C-FT	2	56948
150ITS	exp 3	150	21°C-FT	2	75872
154ITS	exp 3	154	12°C-FT	3	59064
156ITS	exp 3	156	21°C-FT	4	81723
		152	FT-FT	1	extraction failed

¹reads in italic and underlined. The rarefaction depth was determined by the number of reads in italic and underlined within the specific experiment (e.g. exp. 1, exp 2 or exp 3).

Table S4 Summary information of ITS rRNA gene amplicons. One sample was excluded as extraction of DNA failed.

Samples	Experiment	jar	treatment	block	#reads¹	notes
85DITS	exp 1	85	DW	1	38583	
86DITS	exp 1	86	FT	1	40634	
87DITS	exp 1	87	21°C	3	48703	
88DITS	exp 1	88	12°C	4	73634	
89DITS	exp 1	89	21°C	4	41252	
90DITS	exp 1	90	DW	3	41284	
91DITS	exp 1	91	FT	3	43162	
92DITS	exp 1	92	12°C	3	53437	
93DITS	exp 1	93	FT	5	62781	
94DITS	exp 1	94	DW	4	65077	
95DITS	exp 1	95	FT	4	53000	

96DITS	exp 1	96	21°C	2	65190
97DITS	exp 1	97	21°C	5	43755
98DITS	exp 1	98	12°C	2	53988
99DITS	exp 1	99	12°C	1	44253
121DITS	exp 1	121	DW	2	<u>34147</u>
121DITS	exp 1	121	FT	2	43342
122DITS	exp 1	122	21°C	1	69062
123DITS	exp 1	123	DW	5	68632
124DITS	exp 1	124	12°C	5	35471
125DITS	exp 2	125	DW-DW	2	<u>27405</u>
126DITS	exp 2	126	DW-DW	5	46875
129DITS	exp 2	129	21°C-DW	3	64838
112DITS	exp 2	112	21°C-DW	1	50390
121DITS	exp 2	121	DW-DW	3	38812
126DITS	exp 2	126	21°C-DW	5	59064
128DITS	exp 2	128	12°C-DW	4	67879
131DITS	exp 2	131	12°C-DW	1	50877
133DITS	exp 2	133	FT-DW	3	42426
134DITS	exp 2	134	FT-DW	1	33015
135DITS	exp 2	135	FT-DW	2	33621
136DITS	exp 2	136	12°C-DW	2	38061
137DITS	exp 2	137	21°C-DW	4	49652
139DITS	exp 2	139	DW-DW	1	60055
145DITS	exp 2	145	12°C-DW	3	61702
147DITS	exp 2	147	FT-DW	4	48916
148DITS	exp 2	148	FT-DW	5	47726
155DITS	exp 2	155	21°C-DW	2	55698
157DITS	exp 2	157	DW-DW	4	41167
159DITS	exp 2	159	12°C-DW	5	55751
111DITS	exp 3	111	DW-FT	5	55505
114DITS	exp 3	114	21°C-FT	3	<u>27217</u>
115DITS	exp 3	115	DW-FT	4	56027
116DITS	exp 3	116	21°C-FT	1	62409
119DITS	exp 3	119	FT-FT	3	54157
123DITS	exp 3	123	DW-FT	2	43212
124DITS	exp 3	124	21°C-FT	5	36133
127DITS	exp 3	127	FT-FT	4	60891
130DITS	exp 3	130	12°C-FT	5	73528
132DITS	exp 3	132	DW-FT	1	52195
140DITS	exp 3	140	DW-FT	3	56349
142DITS	exp 3	142	FT-FT	2	43386
143DITS	exp 3	143	12°C-FT	4	56935
144DITS	exp 3	144	12°C-FT	1	50280
146DITS	exp 3	146	FT-FT	5	43271
149DITS	exp 3	149	12°C-FT	2	49264
150DITS	exp 3	150	21°C-FT	2	59390
154DITS	exp 3	154	12°C-FT	3	36957
156DITS	exp 3	156	21°C-FT	4	64256
		152	FT-FT	1	Extraction failed

¹reads in italic and underlined. The rarefraction depth was determined by the number of reads in italic and underlined within the specific experiment (e.g. exp. 1, exp 2 or exp 3).

Table S5. Results of permutational multivariate analysis of variance using distance matrices using Adonis function on Bray-Curtis dissimilarities of 16S OTUs of cDNA and DNA. Exp.1, Exp.2, Exp.3 refer to the three experiments (See Fig. 1 for description of experiments). Bold P values indicate p-value < 0.05. sum sq is sum of squares.

Molecule	Experiment		DF	Sum sq	R2	F	P
cDNA	Exp.1	Treatment	3	0.54	0.30	2.29	0.002
		Residuals	16	1.26	0.70		
	Exp.2	Treatment	3	0.28	0.18	1.15	0.08
		Residuals	16	1.29	0.82		
	Exp.3	Treatment	3	0.43	0.22	1.41	0.009
		Residuals	15	1.51	0.82		
DNA	Exp.1	Treatment	3	0.69	0.27	1.93	0.003
		Residuals	16	1.91	0.73		
	Exp.2	Treatment	3	0.2992	0.09	0.50	0.86
		Residuals	15	2.9677	0.91		
	Exp.3	Treatment	3	1.01	0.35	2.65	0.012
		Residuals	15	1.91	0.65		

Table S6. Results of permutational multivariate analysis of variance using distance matrices using Adonis function on Bray-Curtis dissimilarities of ITS OTUs of cDNA and DNA. Exp.1, Exp.2, Exp.3 refer to the three experiments (See Fig. 1 for description of experiments). Bold P values indicate p-value < 0.05. sum sq is sum of squares.

Molecule	Experiment		DF	Sum sq	R2	F	P
cDNA	Exp.1	Treatment	3	0.375	0.21	1.34	<0.001
		Residuals	16	1.496	0.80		
	Exp.2	Treatment	3	0.26	0.13	0.78	0.005
		Residuals	16	1.78	0.87		
	Exp.3	Treatment	3	0.49	0.27	1.89	<0.001
		Residuals	15	1.29	0.73		
DNA	Exp.1	Treatment	3	0.32	0.18	1.2	0.002
		Residuals	16	1.44	0.82		
	Exp.2	Treatment	3	0.35	0.17	1.08	0.002
		Residuals	16	1.75	0.83		
	Exp.3	Treatment	3	0.32	0.18	1.08	0.003
		Residuals	15	1.48	0.82		