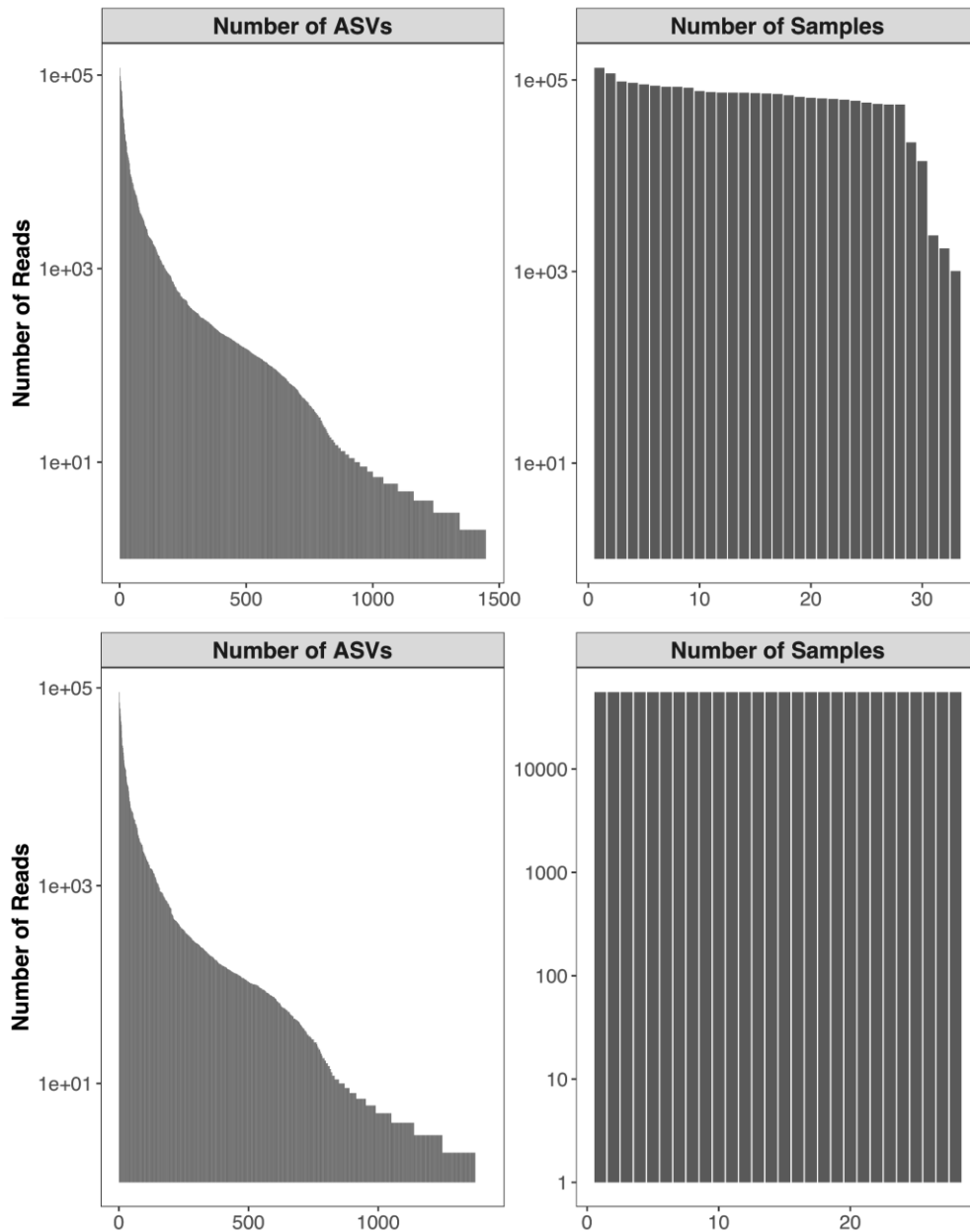
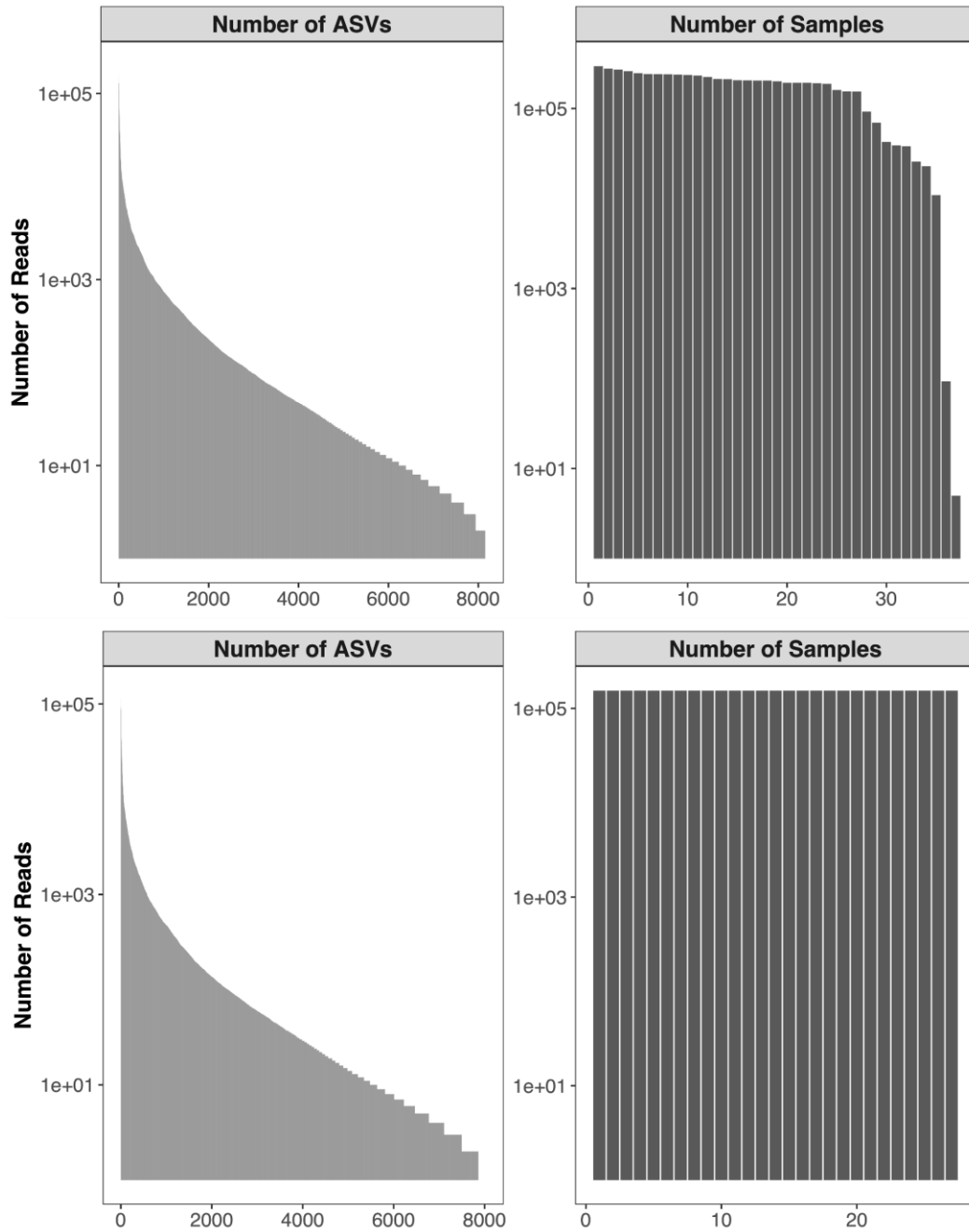


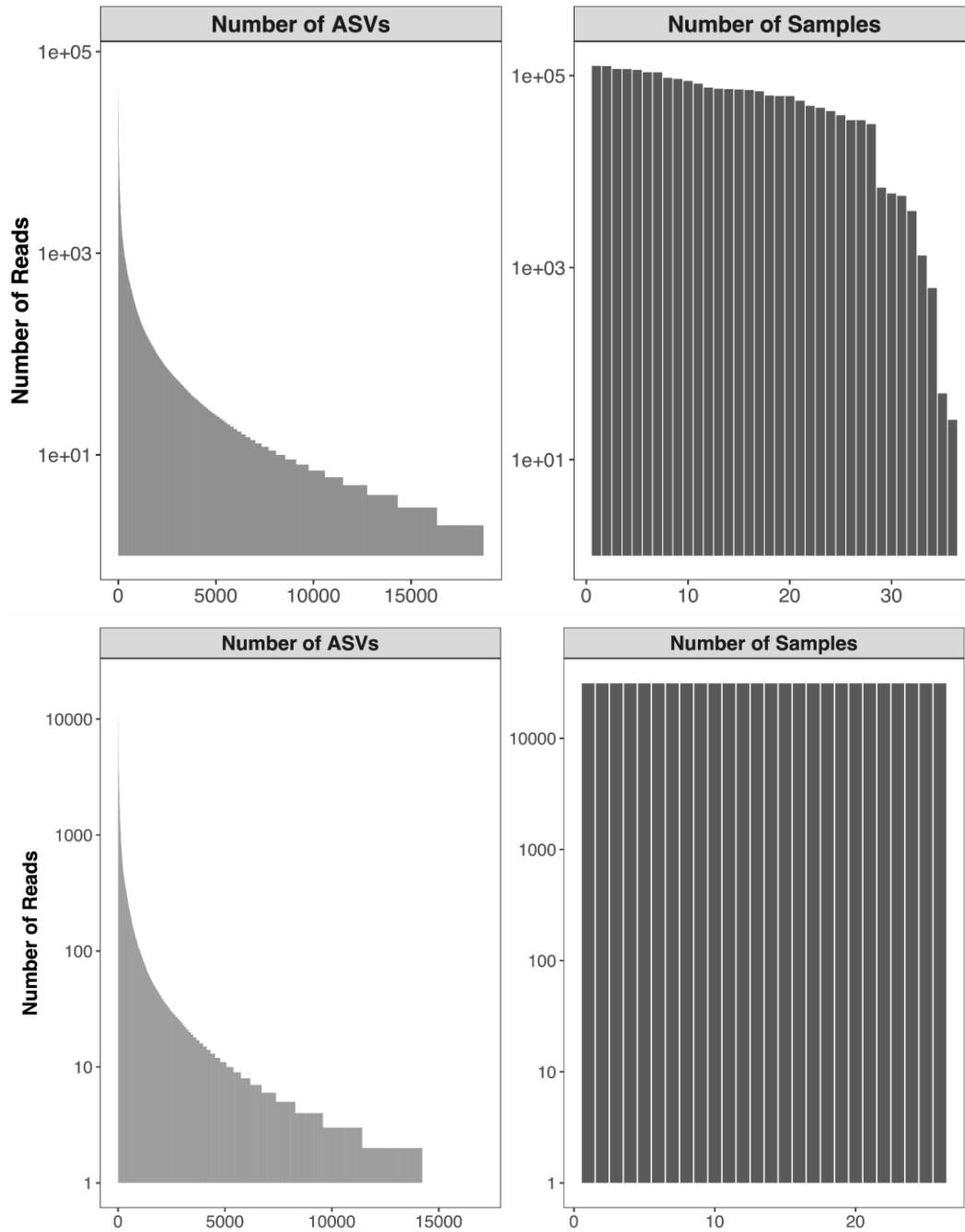
**Supplemental Figures**



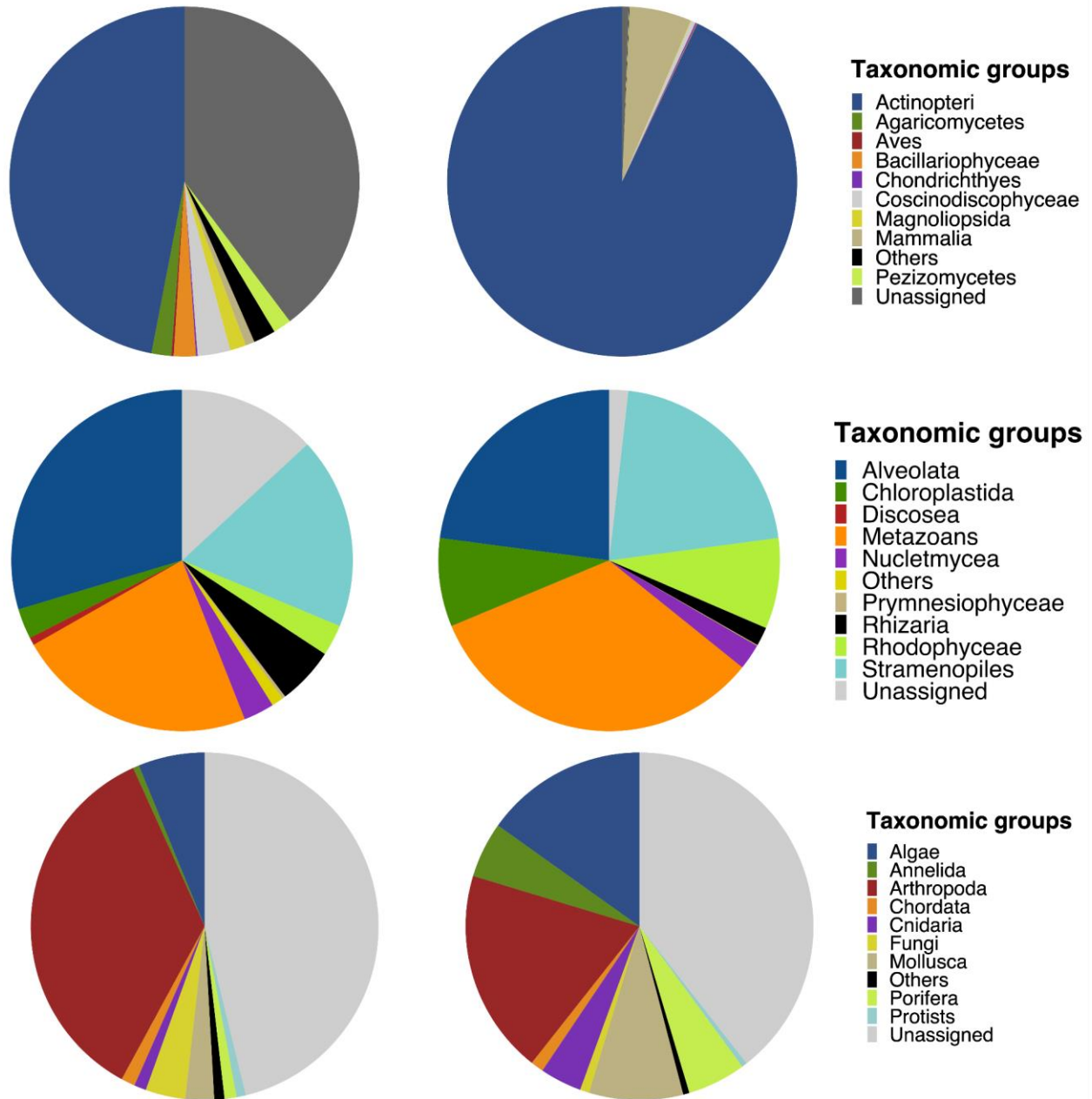
**Fig. S1.** Sequencing depth plots for the 12S dataset. The y-axis of the plots on the left have been log transformed. *Top:* Raw data for n = 33 samples, as the two field controls for Toboggan (Hermitage) and one field control for Saint-Pierre were removed by the SAMBA pipeline as a result of no data. *Bottom:* Curated data (n = 26) following the removal of one Saint-Pierre bottom sample and all negative controls.



**Fig. S2** Sequencing depth plots for the 18S dataset. The y-axis of the plots on the left have been log transformed. *Top*: Raw data for n = 37 samples. *Bottom*: Curated data (n = 27) following the removal of all negative controls.

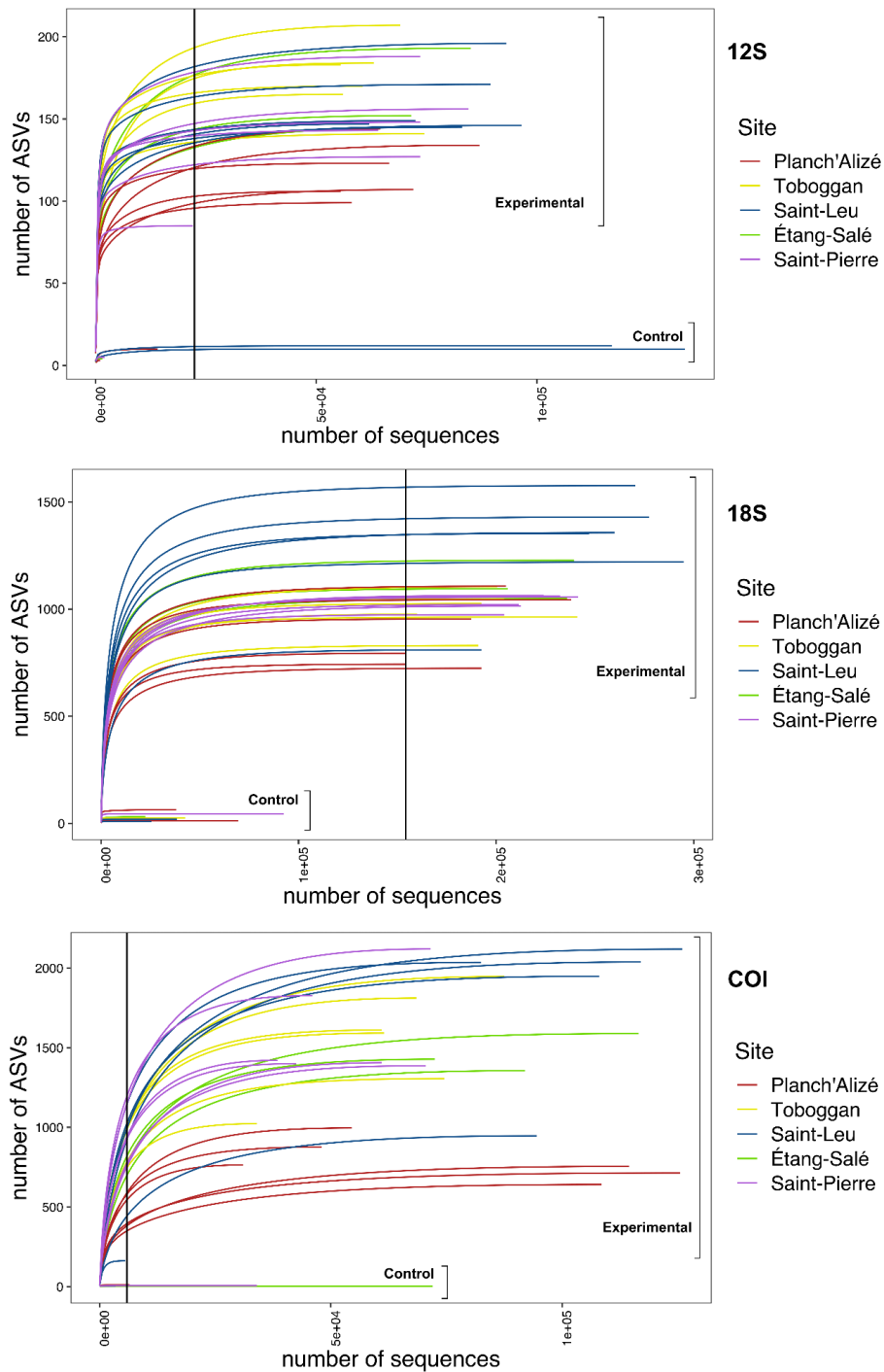


**Fig. S3** Sequencing depth plots for the COI dataset. The y-axis of the plots on the left have been log transformed. *Top*: Raw data for n = 37 samples. *Bottom*: Curated data (n = 26) following the removal of all negative controls and one surface sample from Saint-Leu.



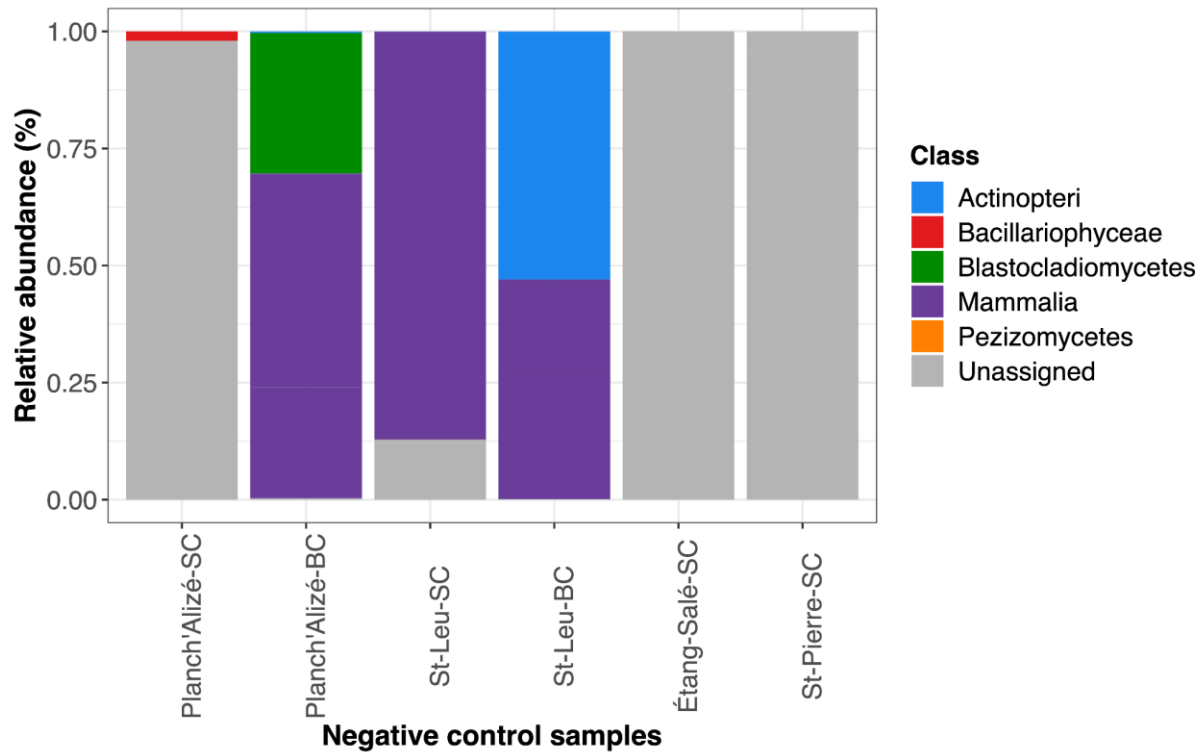
**Fig. S4** Pie plots illustrate the percentage of ASVs (left) and sequences (right) assigned to the top 10 most abundant taxonomic groups for the 12S gene (*top*), 18S gene (*middle*) and COI gene (*bottom*). The total number of groupings is 21 for 12S, 32 for 18S and 36 for COI.

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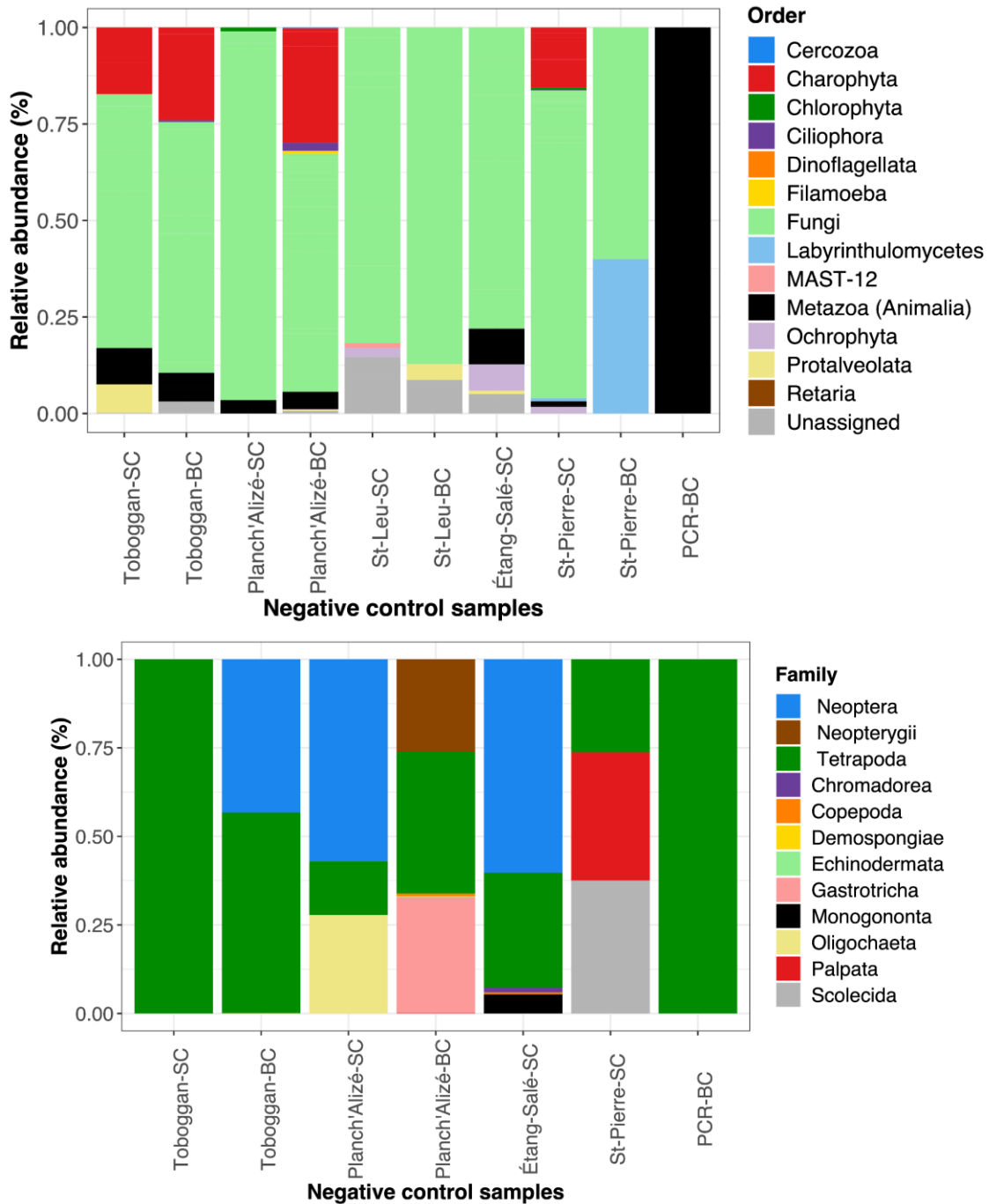


**Fig. S5** Plots of rarefaction curves for all samples for 12S (*top*), 18S (*middle*) and COI (*bottom*), prior to removal of non-target taxa, normalization and contamination resolution. The black line through the x-axis indicates the sample elimination threshold at the experimental sample with the fewest number of sequences, which was 22,392 for 12S, 150,000 for 18S and 5,917 for COI.

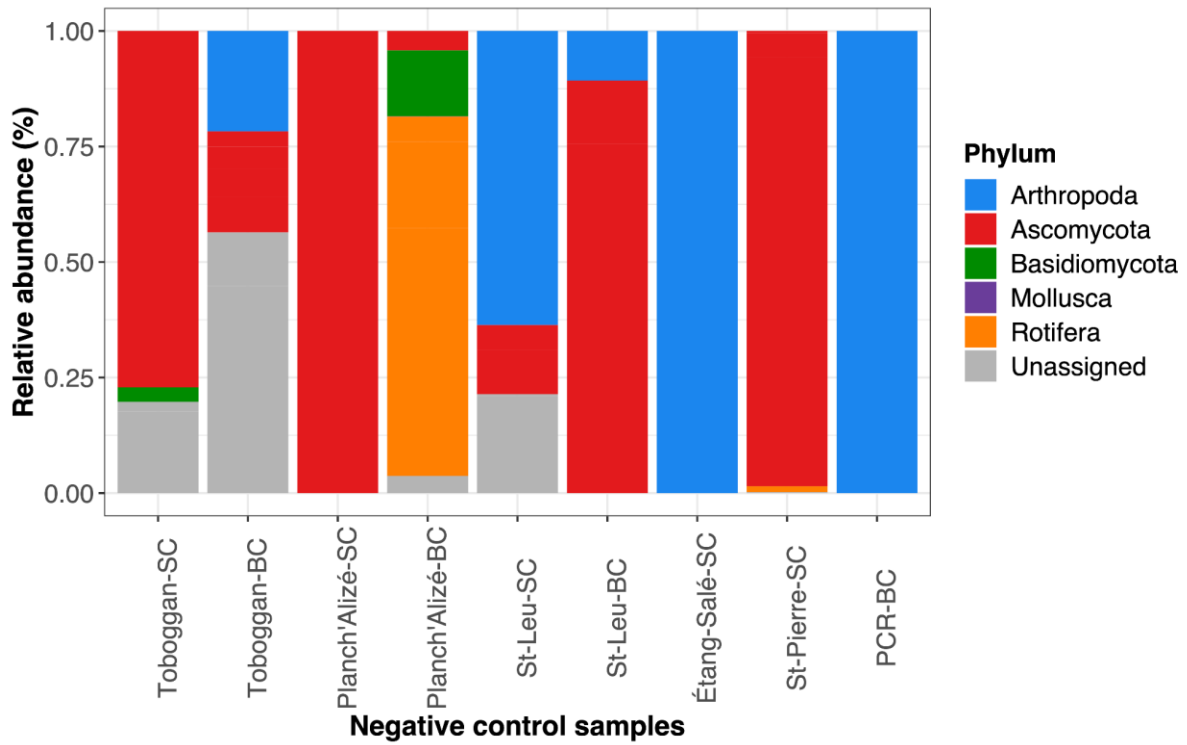
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**Fig. S6** Relative abundance of sequences assigned to 38 ASVs within the remaining negative field controls (n = 6) for the 12S dataset. SC refers to ‘Surface Control’, while BC refers to ‘Bottom Control’.

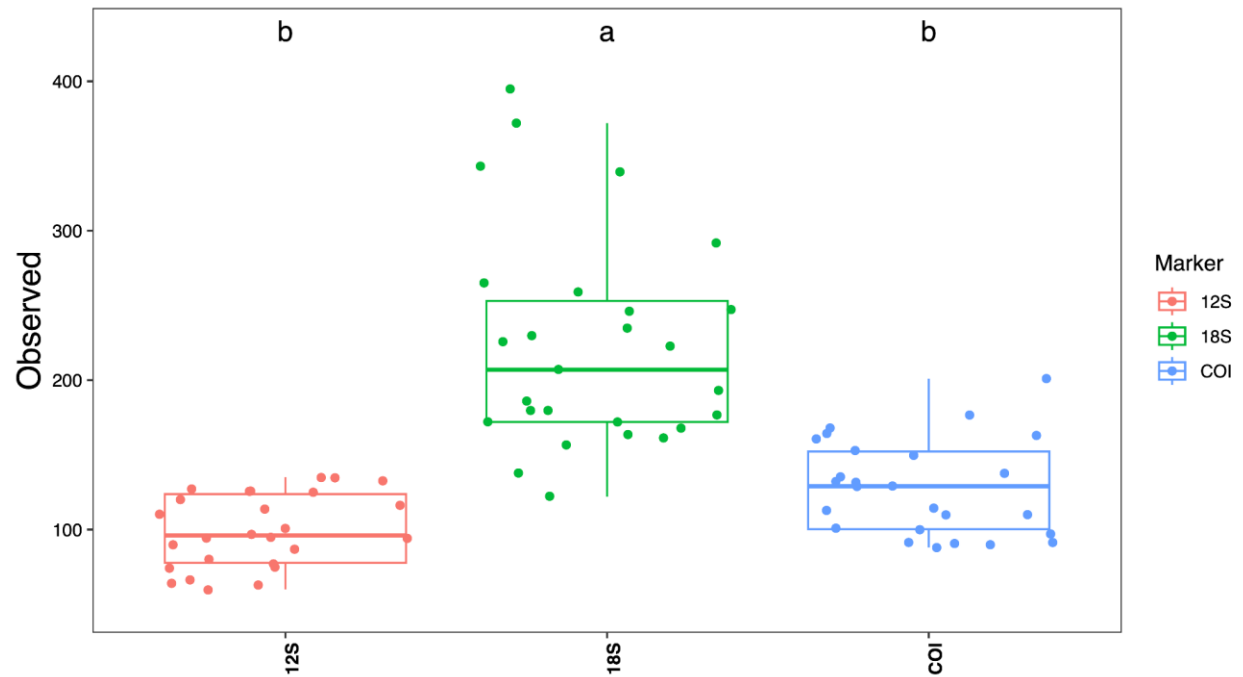


**Fig. S7** Relative abundance of sequences assigned to every ASVs identified in the 18S negative field controls. *Top*: ASVs (n = 187) in all 10 samples. *Bottom*: only metazoan ASVs (n = 20) in seven samples. . SC refers to ‘Surface Control’, BC refers to ‘Bottom Control’ and PCR-BC refers to the ‘PCR control’.



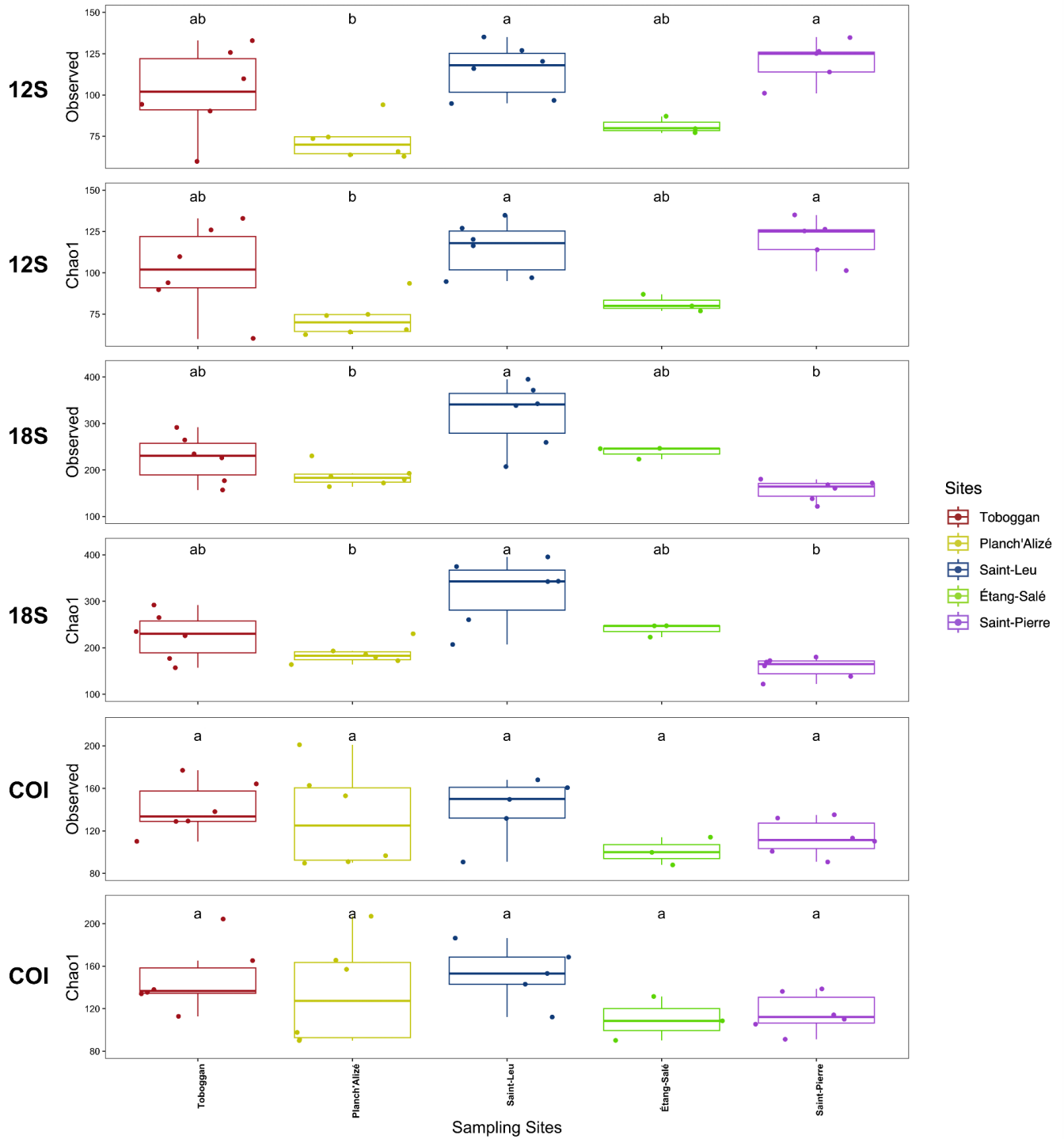
**Fig. S8** Relative abundance sequences assigned to 39 ASVs within the negative field controls (n = 10) for the COI dataset. SC refers to 'Surface Control', BC refers to 'Bottom Control' and PCR-BC refers to the 'PCR control'.





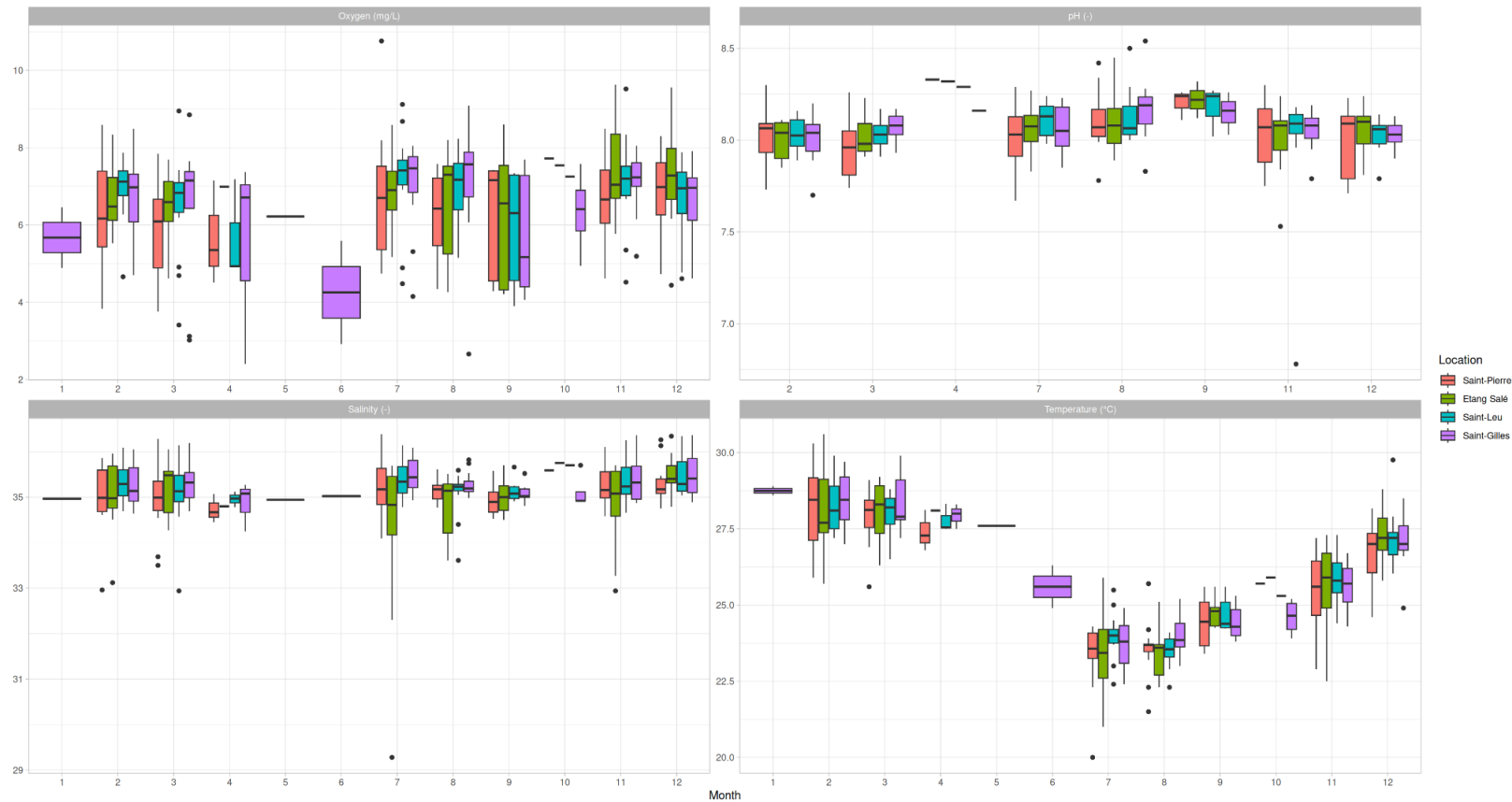
**Fig. S9** Observed richness for three marker datasets (12S, 18S, COI) results from ANOVA and Tukey Honest Significant Difference (HSD) tests Tukey testing results at  $p < 0.01$ .

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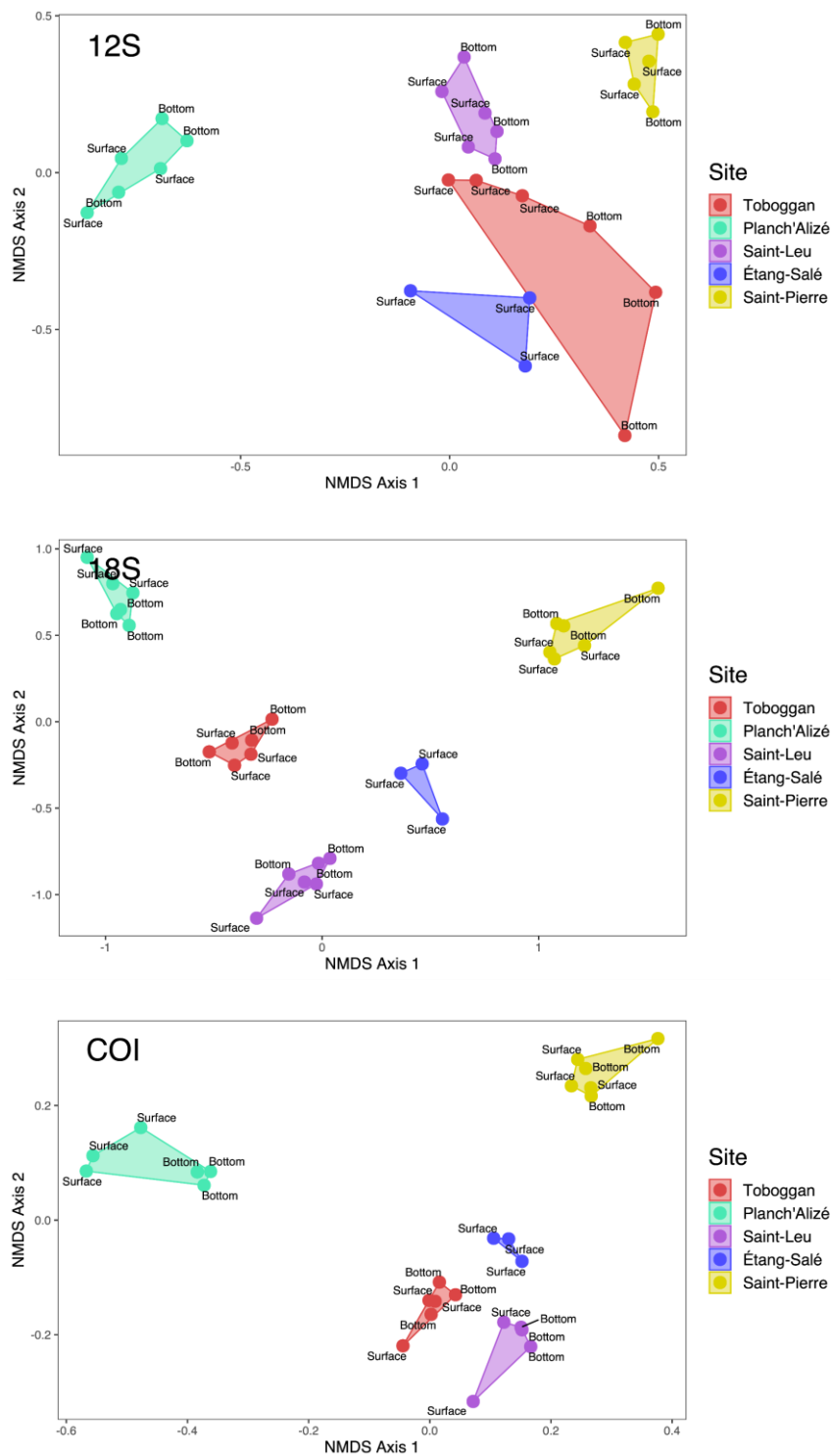


**Fig. S10** Observed and expected (Chao1) richness by site for the three marker datasets (12S, 18S, COI) results from ANOVA and Tukey Honest Significant Difference (HSD) tests, with Tukey testing results at  $p < 0.01$ .

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**Fig. S11:** Boxplot illustrating monthly trends in environmental parameters (oxygen, pH, salinity, temperature) were created using data collected between 2002 and 2024 from the back-reef depressions where sampling occurred. The data, sourced from the European Water Framework Directive, were gathered from designated locations identified by experts as representative of the back-reef depression where the parameter is measured and where the samples for this study were obtained



**Fig. S12** Non-metric multidimensional scaling (nMDS) plots for all taxa (metazoans and non-metazoans) based upon presence-absence data and Jaccard similarity distances for 12S (top), 18S (middle) and COI (bottom). Points indicate samples, while convex hulls identify the associations between samples for each site. Note: Data curation occurred as described in the

Cowart et al. Detecting local variations across metazoan communities in back-reef depressions of Reunion Island (Mascarene Archipelago) through environmental DNA survey text, with the exception of not removing non-target taxa. Contaminate ASVs (those ASVs present in the negative controls and experimental controls) remaining after normalization were 11 for 12S, 77 for 18S and 6 for COI; these were removed prior to generating the nMDS. This resulted in a final ASV count of 1,388 for 12S, 7,940 for 18S and 17,046 for COI.

**Supplemental Tables**

**Table S1.** Mitochondrial Cytochrome c oxidase subunit I (COI), nuclear small ribosomal subunit 18S region V1-V2 (18S) and mitochondrial ribosomal rRNA 12S (12S) primers used for eDNA metabarcoding. Amplicon size is approximate.

Gene	Primer name	Sequences (5' – 3')	Reference	Amplicon size (bp)	Target taxa
COI	mICOLintF (forward)	GGW ACW GGW TGA ACW GTW TAY CCY CC	Leray et al. 2013b	313	Metazoans (vertebrates)
	jjgHCO2198 (reverse)	TAI ACY TCI GGR TGI CCR AAR AAY CA	Geller et al. 2013		
18S (region V1-V2)	SSUF04 (forward)	GCT TGT CTC AAA GAT TAA GCC	Blaxter et al. 1998	356	Metazoans (benthic invertebrates)
	SSUR22mod (reverse)	CCT GCT GCC TTC CTT RGA	Sinniger et al. 2016		
12S	MiFish-U-F (forward)	GTC GGT AAA ACT CGT GCC AGC	Miya et al. 2015	170 - 250	Metazoans (fishes)
	MiFish-U-R (reverse)	CAT AGT GGG GTA TCT AAT CCC AGT TTG	Miya et al. 2015		

**Table S2.** Negative control samples and associated ASVs remaining after first data curation steps. H-T is Toboggan, H-V is Planch’Alizé, SL is Saint-Leu, ES is Étang-Salé and SP is Saint-Pierre. SC and BC refer to “Surface Control” and “Bottom Control”, respectively, while “PCR” is the PCR control. “Neg total” refers to the total number of sequence reads across the negative controls, while “Grand total” refers to the total number of sequence reads across the entire dataset. ASVs highlighted in red were removed across the entire dataset as they were typically more abundant in the negative controls than the individual experimental samples. The exception was ASV940 in the 12S dataset, which was removed due to potential contamination as it is known to be absent at coral reef habitats in La Réunion.

12S dataset									
ASV	Taxonomy	H-V SC	H-V BC	SL SC	SL BC	ES SC	SP SC	Neg total	Grand total
940	Istiophoridae	0	18	0	18	0	0	36	26582
998	Pomacentridae	0	2	0	0	0	0	2	198590

18S dataset													
ASV	Taxonomy	H-T SC	H-T BC	H-V SC	H-V BC	SL SC	SL BC	ES SC	SP BC	SP SC	PCR	Neg total	Grand total
5859	Porifera	0	0	0	0	2	0	0	0	0	0	2	7351
5966	Porifera	0	0	2	0	0	0	0	0	0	0	2	122
6296	Echinodermata	0	0	0	0	2	0	0	0	0	0	2	1975
6637	Polychaeta	5	0	0	0	0	0	0	0	406	0	406	78886
7128	Copepoda	0	0	0	0	0	0	0	12	0	0	12	65190
7559	Polychaeta	0	0	2	0	0	0	0	0	0	0	2	10418

COI dataset													
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ASV	Taxonomy	H-T SC	H-T BC	H-V SC	H-V BC	SL SC	SL BC	ES SC	SP BC	SP SC	PCR	Neg total	Grand total
13665	Gastropoda	0	0	0	0	2	0	0	0	0	0	2	250



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**Table S3.** Observed species richness and estimated species richness (Chao2) calculations for each marker dataset, computed using the R packages iNEXT and vegan. ‘Total’ refers to all sites combined.

<b>12S dataset</b>			
<b>Site</b>	<b>Observed</b>	<b>Chao2</b>	<b>Chao SE</b>
Toboggan	260	483.34	56.29
Planch’Alizé	184	434.37	78.38
Saint-Leu	304	638.49	77.94
Étang-Salé	154	244.09	26.32
Saint-Pierre	275	518.37	59.07
<b>18S dataset</b>			
<b>Site</b>	<b>Observed</b>	<b>Chao2</b>	<b>Chao SE</b>
Toboggan	645	1222.05	86.23
Planch’Alizé	504	964.80	79.00
Saint-Leu	861	1671.00	107.90
Étang-Salé	430	682.03	44.57
Saint-Pierre	520	1132.27	97.91
<b>COI dataset</b>			
<b>Site</b>	<b>Observed</b>	<b>Chao2</b>	<b>Chao SE</b>
Toboggan	529	1427.83	145.66
Planch’Alizé	537	1552.00	162.14
Saint-Leu	448	1126.40	118.82
Étang-Salé	216	424.03	49.71
Saint-Pierre	399	885.29	88.41

**Table S4.** PERMANOVA results for all taxa (metazoans and non-metazoans) based upon Jaccard dissimilarity distances for the 12S, 18S and COI datasets. Significance values are 0.001 (\*\*\*), 0.01 (\*\*), 0.5 (\*) and 0.1 (.). Note: Data curation occurred as described in the text, with the exception of not removing non-target taxa. Contaminate ASVs (those ASVs present in the negative controls and experimental controls) remaining after normalization were 11 for 12S, 77 for 18S and 6 for COI; these were removed prior to statistical testing. This resulted in a final ASV count of 1,388 for 12S, 7,940 for 18S and 17,046 for COI.

Dataset	variable	Df	SS	R2	F-model	p-value
12S	Site	4	2.5494	0.32291	2.5534	0.001***
	Depth	1	0.2879	0.03646	1.1532	0.170
	Replicate	2	0.5169	0.06546	1.0353	0.349
	Site:Depth	3	0.8159	0.10335	1.0896	0.232
	Depth:Replicate	2	0.4865	0.06162	0.9745	0.563
	Site:Replicate	8	1.9906	0.25213	0.9968	0.531
	Residual	5	1.2481	0.15808		
	Total	25	7.8953	1.00000		
18S	Site	4	3.4507	0.40281	3.8570	0.001***
	Depth	1	0.2913	0.03400	1.3022	0.106
	Replicate	2	0.4531	0.05289	1.0130	0.410
	Site:Depth	3	0.8096	0.09451	1.2066	0.103
	Depth:Replicate	2	0.4558	0.05320	1.0189	0.396
	Site:Replicate	8	1.7640	0.20592	0.9859	0.548

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	Residual	6	1.3420	0.15665		
	Total	26	8.5666	1.00000		
COI	Site	4	2.8704	0.28116	2.0603	0.001***
	Depth	1	0.3814	0.03736	1.0951	0.187
	Replicate	2	0.6879	0.06738	0.9875	0.505
	Site:Depth	3	1.1241	0.11011	1.0758	0.185
	Depth:Replicate	2	0.6823	0.06684	0.9795	0.556
	Site:Replicate	8	2.7214	0.26657	0.9767	0.647
	Residual	5	1.741	0.17058		
	Total	25	10.2091	1.00000		