

**Supplementary material associated with**

**Optimizing DNA extraction protocols for the diet analysis of a baleen whale (*Eubalaena australis*)**

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**Table S1.** List of SRW samples used in this study and associated metadata.

Sample	Date collected	Location	Storage
SA-1	24/10/2006	South Africa – St Helena Bay	Ethanol
SA-2	Unknown	South Africa – St Helena Bay	Ethanol
SA-3	10/12/1992	South Africa – St Helena Bay	Ethanol
NZ-1	15/8/2017	New Zealand – Auckland Islands	Frozen
NZ-2	8/8/2017	New Zealand – Auckland Islands	Frozen

**Table S2.** PCR conditions for 18S rDNA and Crust16S mtDNA.

Table S2. PCR conditions for 18S rDNA and Crust16S mtDNA.					
Amplicon	PCR Mixture (20 uL)	PCR Conditions			
		PCR Stage	Temperature	Time	PCR cycles
18S	2x Amplitaq Gold 360 Mastermix	Initial denaturation	95°C	8 min	x 43
	Primer-F (0.1mM)	Denaturation	95°C	20 s	
	Primer-R (0.1mM)	Annealing	58°C	20 s	
	PNA clamp (0.5mM)*	Elongation	72°C	30 s	
	DNA (2uL)	Final elongation	72°C	8 min	
Crust16S	2x Amplitaq Gold 360 Mastermix	Initial denaturation	95°C	10 min	x 45
	Primer-F (0.2mM)	Denaturation	95°C	30 s	
	Primer-R (0.2mM)	Annealing	51°C	30 s	
	DNA (2uL)	Elongation	72°C	45 s	
		Final elongation	72°C	10 min	
*Whale blocking PNA clamp: CGACCGTCTTCTCAGC-Lys					

**Table S3.** Classification of target and non-target organisms captured by 18S rDNA and Crust16S mtDNA.

		Target	Non-Target
		Metazoa (free-floating marine zooplankton within this kingdom)	Amoebozoa, Cryptophyceae, Discoba, Metamonada, Opisthokonta, Rhodophyta, Sar and Viridiplantae
18S rDNA	Kingdom		

	Class	<b>Metazoa:</b> Polychaeta, Hexanauplia, Malacostraca, Ostracoda, Thecostraca, Sagittoidea, Ascidiacea, Cnidaria, Hydrozoa, Scyphozoa, Ctenophora, Lophotrochozoa, Bivalvia, Gastropoda, Protostomia, Eurotatoria	<b>Metazoa:</b> Palaeacanthocephala, Clitellata, Arachnida, Collembola, Insecta, Cestoda and Trematoda
Crust16S mtDNA	Class	<b>Metazoa:</b> Malacostraca, Thecostraca	<b>Metazoa:</b> Arachnida, Collembola, Eurotatoria, Gymnolaemata, Hydrozoa, Insecta, Mammalia

**Table S4.** Summary of target zOTU richness captured by each extraction protocol for the 18S rDNA dataset.

Extraction protocol	Mean zOTU richness	SD
Ph1P.1B	3.8	3.25
Ph1P.2B	3.67	2.28
Ph1S.1B	3.04	2.67
Ph1S.2B	3.27	3.11
Ph24P.1B	4.16	3.75
Ph24P.2B	4.16	3.7
Ph24S.1B	2.58	3.53
Ph24S.2B	3.24	2.81
Soil.1B	4.6	3.8
Soil.2B	4.73	4.37
Stool.1B	4.69	2.79
Stool.2B	3.51	2.65

**Table S5.** *Post hoc* pairwise comparison of differences in target zOTU richness derived from different protocols from the 18S rDNA dataset.

Protocols	Estimate	Std Error	z value	Pr(> z )
Ph1P.2B-Ph1P.1B	-0.13333	0.4486	-0.297	1
Ph1S.1B-Ph1P.1B	-0.75556	0.4486	-1.684	0.8758
Ph1S.2B-Ph1P.1B	-0.53333	0.4486	-1.189	0.9899
Ph24P.1B-Ph1P.1B	0.35556	0.4486	0.793	0.9997
Ph24P.2B-Ph1P.1B	0.35556	0.4486	0.793	0.9997
Ph24S.1B-Ph1P.1B	-1.22222	0.4486	-2.725	0.2146
Ph24S.2B-Ph1P.1B	-0.55556	0.4486	-1.238	0.9859
Soil.1B-Ph1P.1B	0.8	0.4486	1.783	0.8278

Soil.2B-Ph1P.1B	0.93333	0.4486	2.081	0.6369	
Stool.1B-Ph1P.1B	0.88889	0.4486	1.981	0.7065	
Stool.2B-Ph1P.1B	-0.28889	0.4486	-0.644	1	
Ph1S.1B-Ph1P.2B	-0.62222	0.4486	-1.387	0.9662	
Ph1S.2B-Ph1P.2B	-0.4	0.4486	-0.892	0.9992	
Ph24P.1B-Ph1P.2B	0.48889	0.4486	1.09	0.9952	
Ph24P.2B-Ph1P.2B	0.48889	0.4486	1.09	0.9952	
Ph24S.1B-Ph1P.2B	-1.08889	0.4486	-2.427	0.3883	
Ph24S.2B-Ph1P.2B	-0.42222	0.4486	-0.941	0.9987	
Soil.1B-Ph1P.2B	0.93333	0.4486	2.081	0.6368	
Soil.2B-Ph1P.2B	1.06667	0.4486	2.378	0.4217	
Stool.1B-Ph1P.2B	1.02222	0.4486	2.279	0.4923	
Stool.2B-Ph1P.2B	-0.15556	0.4486	-0.347	1	
Ph1S.2B-Ph1S.1B	0.22222	0.4486	0.495	1	
Ph24P.1B-Ph1S.1B	1.11111	0.4486	2.477	0.355	
Ph24P.2B-Ph1S.1B	1.11111	0.4486	2.477	0.3546	
Ph24S.1B-Ph1S.1B	-0.46667	0.4486	-1.04	0.9968	
Ph24S.2B-Ph1S.1B	0.2	0.4486	0.446	1	
Soil.1B-Ph1S.1B	1.55556	0.4486	3.468	0.0258	*
Soil.2B-Ph1S.1B	1.68889	0.4486	3.765	<0.01	**
Stool.1B-Ph1S.1B	1.64444	0.4486	3.666	0.0134	*
Stool.2B-Ph1S.1B	0.46667	0.4486	1.04	0.9968	
Ph24P.1B-Ph1S.2B	0.88889	0.4486	1.981	0.7056	
Ph24P.2B-Ph1S.2B	0.88889	0.4486	1.981	0.707	
Ph24S.1B-Ph1S.2B	-0.68889	0.4486	-1.536	0.9309	
Ph24S.2B-Ph1S.2B	-0.02222	0.4486	-0.05	1	
Soil.1B-Ph1S.2B	1.33333	0.4486	2.972	0.1163	
Soil.2B-Ph1S.2B	1.46667	0.4486	3.269	0.0499	*
Stool.1B-Ph1S.2B	1.42222	0.4486	3.17	0.0671	.
Stool.2B-Ph1S.2B	0.24444	0.4486	0.545	1	
Ph24P.2B- Ph24P.1B	0	0.4486	0	1	
Ph24S.1B- Ph24P.1B	-1.57778	0.4486	-3.517	0.0221	*
Ph24S.2B- Ph24P.1B	-0.91111	0.4486	-2.031	0.6727	
Soil.1B-Ph24P.1B	0.44444	0.4486	0.991	0.9979	
Soil.2B-Ph24P.1B	0.57778	0.4486	1.288	0.9808	
Stool.1B-Ph24P.1B	0.53333	0.4486	1.189	0.9899	
Stool.2B-Ph24P.1B	-0.64444	0.4486	-1.437	0.9563	
Ph24S.1B- Ph24P.2B	-1.57778	0.4486	-3.517	0.0223	*
Ph24S.2B- Ph24P.2B	-0.91111	0.4486	-2.031	0.6724	

Soil.1B-Ph24P.2B	0.44444	0.4486	0.991	0.9979	
Soil.2B-Ph24P.2B	0.57778	0.4486	1.288	0.9807	
Stool.1B-Ph24P.2B	0.53333	0.4486	1.189	0.9899	
Stool.2B-Ph24P.2B	-0.64444	0.4486	-1.437	0.9563	
Ph24S.2B- Ph24S.1B	0.66667	0.4486	1.486	0.9446	
Soil.1B-Ph24S.1B	2.02222	0.4486	4.508	<0.01	***
Soil.2B-Ph24S.1B	2.15556	0.4486	4.805	<0.01	***
Stool.1B-Ph24S.1B	2.11111	0.4486	4.706	<0.01	***
Stool.2B-Ph24S.1B	0.93333	0.4486	2.081	0.6366	
Soil.1B-Ph24S.2B	1.35556	0.4486	3.022	0.1025	
Soil.2B-Ph24S.2B	1.48889	0.4486	3.319	0.0426	*
Stool.1B-Ph24S.2B	1.44444	0.4486	3.22	0.0578	.
Stool.2B-Ph24S.2B	0.26667	0.4486	0.594	1	
Soil.2B-Soil.1B	0.13333	0.4486	0.297	1	
Stool.1B-Soil.1B	0.08889	0.4486	0.198	1	
Stool.2B-Soil.1B	-1.08889	0.4486	-2.427	0.3871	
Stool.1B-Soil.2B	-0.04444	0.4486	-0.099	1	
Stool.2B-Soil.2B	-1.22222	0.4486	-2.725	0.2141	
Stool.2B-Stool.1B	-1.17778	0.4486	-2.625	0.265	

**Table S6.** Summary of target zOTU richness captured by each extraction protocol for the Crust16S mtDNA dataset.

Extraction protocol	Mean zOTU richness	SD
Ph1P.1B	8.31	5
Ph1P.2B	6.31	5.22
Ph1S.1B	5.33	3.05
Ph1S.2B	7.6	6.06
Ph24P.1B	7.58	6.02
Ph24P.2B	7.6	5
Ph24S.1B	3.82	3.68
Ph24S.2B	5.73	2.67
Soil.1B	7.18	4.3
Soil.2B	6.62	7.15
Stool.1B	4.07	4.23
Stool.2B	4.98	3.35

**Table S7.** *Post hoc* pairwise comparison of differences in target zOTU richness derived from different protocols from the Crust16S mtDNA dataset.

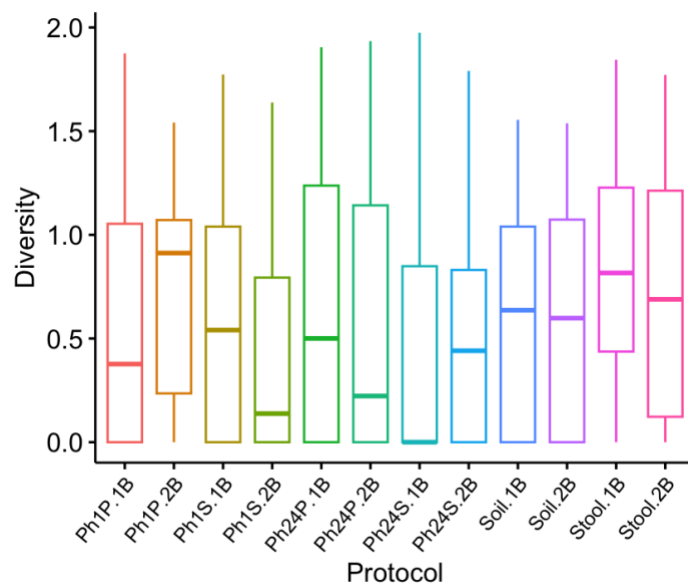
Protocols	Estimate	Std Error	z value	Pr(> z )
Ph1P.2B-Ph1P.1B	-2	0.99471	-2.011	0.6869
Ph1S.1B-Ph1P.1B	-2.97778	0.99471	-2.994	0.1109

Ph1S.2B-Ph1P.1B	-0.71111	0.99471	-0.715	0.9999	
Ph24P.1B- Ph1P.1B	-0.73333	0.99471	-0.737	0.9999	
Ph24P.2B- Ph1P.1B	-0.71111	0.99471	-0.715	0.9999	
Ph24S.1B- Ph1P.1B	-4.48889	0.99471	-4.513	<0.01	***
Ph24S.2B- Ph1P.1B	-2.57778	0.99471	-2.591	0.2845	
Soil.1B-Ph1P.1B	-1.13333	0.99471	-1.139	0.9929	
Soil.2B-Ph1P.1B	-1.68889	0.99471	-1.698	0.8698	
Stool.1B-Ph1P.1B	-4.24444	0.99471	-4.267	<0.01	**
Stool.2B-Ph1P.1B	-3.33333	0.99471	-3.351	0.038	*
Ph1S.1B-Ph1P.2B	-0.97778	0.99471	-0.983	0.9981	
Ph1S.2B-Ph1P.2B	1.28889	0.99471	1.296	0.9799	
Ph24P.1B- Ph1P.2B	1.26667	0.99471	1.273	0.9825	
Ph24P.2B- Ph1P.2B	1.28889	0.99471	1.296	0.9798	
Ph24S.1B- Ph1P.2B	-2.48889	0.99471	-2.502	0.3382	
Ph24S.2B- Ph1P.2B	-0.57778	0.99471	-0.581	1	
Soil.1B-Ph1P.2B	0.86667	0.99471	0.871	0.9994	
Soil.2B-Ph1P.2B	0.31111	0.99471	0.313	1	
Stool.1B-Ph1P.2B	-2.24444	0.99471	-2.256	0.5074	
Stool.2B-Ph1P.2B	-1.33333	0.99471	-1.34	0.9738	
Ph1S.2B-Ph1S.1B	2.26667	0.99471	2.279	0.4912	
Ph24P.1B- Ph1S.1B	2.24444	0.99471	2.256	0.5081	
Ph24P.2B- Ph1S.1B	2.26667	0.99471	2.279	0.4915	
Ph24S.1B- Ph1S.1B	-1.51111	0.99471	-1.519	0.9357	
Ph24S.2B- Ph1S.1B	0.4	0.99471	0.402	1	
Soil.1B-Ph1S.1B	1.84444	0.99471	1.854	0.7875	
Soil.2B-Ph1S.1B	1.28889	0.99471	1.296	0.9798	
Stool.1B-Ph1S.1B	-1.26667	0.99471	-1.273	0.9825	
Stool.2B-Ph1S.1B	-0.35556	0.99471	-0.357	1	
Ph24P.1B- Ph1S.2B	-0.02222	0.99471	-0.022	1	
Ph24P.2B- Ph1S.2B	0	0.99471	0	1	
Ph24S.1B- Ph1S.2B	-3.77778	0.99471	-3.798	<0.01	**
Ph24S.2B- Ph1S.2B	-1.86667	0.99471	-1.877	0.7742	

Soil.1B-Ph1S.2B	-0.42222	0.99471	-0.424	1	
Soil.2B-Ph1S.2B	-0.97778	0.99471	-0.983	0.9981	
Stool.1B-Ph1S.2B	-3.53333	0.99471	-3.552	0.02	*
Stool.2B-Ph1S.2B	-2.62222	0.99471	-2.636	0.259	
Ph24P.2B- Ph24P.1B	0.02222	0.99471	0.022	1	
Ph24S.1B- Ph24P.1B	-3.75556	0.99471	-3.776	<0.01	**
Ph24S.2B- Ph24P.1B	-1.84444	0.99471	-1.854	0.7875	
Soil.1B-Ph24P.1B	-0.4	0.99471	-0.402	1	
Soil.2B-Ph24P.1B	-0.95556	0.99471	-0.961	0.9984	
Stool.1B- Ph24P.1B	-3.51111	0.99471	-3.53	0.0215	*
Stool.2B- Ph24P.1B	-2.6	0.99471	-2.614	0.2719	
Ph24S.1B- Ph24P.2B	-3.77778	0.99471	-3.798	<0.01	**
Ph24S.2B- Ph24P.2B	-1.86667	0.99471	-1.877	0.7743	
Soil.1B-Ph24P.2B	-0.42222	0.99471	-0.424	1	
Soil.2B-Ph24P.2B	-0.97778	0.99471	-0.983	0.9981	
Stool.1B- Ph24P.2B	-3.53333	0.99471	-3.552	0.0202	*
Stool.2B- Ph24P.2B	-2.62222	0.99471	-2.636	0.2589	
Ph24S.2B- Ph24S.1B	1.91111	0.99471	1.921	0.7458	
Soil.1B-Ph24S.1B	3.35556	0.99471	3.373	0.0366	*
Soil.2B-Ph24S.1B	2.8	0.99471	2.815	0.1732	
Stool.1B- Ph24S.1B	0.24444	0.99471	0.246	1	
Stool.2B- Ph24S.1B	1.15556	0.99471	1.162	0.9917	
Soil.1B-Ph24S.2B	1.44444	0.99471	1.452	0.9529	
Soil.2B-Ph24S.2B	0.88889	0.99471	0.894	0.9992	
Stool.1B- Ph24S.2B	-1.66667	0.99471	-1.676	0.8797	
Stool.2B- Ph24S.2B	-0.75556	0.99471	-0.76	0.9998	
Soil.2B-Soil.1B	-0.55556	0.99471	-0.559	1	
Stool.1B-Soil.1B	-3.11111	0.99471	-3.128	0.0762	.
Stool.2B-Soil.1B	-2.2	0.99471	-2.212	0.5408	
Stool.1B-Soil.2B	-2.55556	0.99471	-2.569	0.2972	
Stool.2B-Soil.2B	-1.64444	0.99471	-1.653	0.889	
Stool.2B-Stool.1B	0.91111	0.99471	0.916	0.999	

**Table S8.** *Post hoc* pairwise comparison of significant differences in Shannon diversity of target species derived from different protocols from the 18S rDNA dataset.

Protocols	Estimate	Std. Error	Z-value	Pr(> z )	
Stool.1B-Ph1S.2B	0.3891827	0.0788487	4.936	<0.01	***
Stool.2B-Ph1S.2B	0.2919968	0.0788487	3.703	0.0117	*
Stool.1B-Ph24S.1B	0.3765767	0.0788487	4.776	<0.01	***
Stool.2B-Ph24S.1B	0.2793909	0.0788487	3.543	0.0205	*
Stool.1B-Ph24S.2B	0.3221138	0.0788487	4.085	<0.01	**

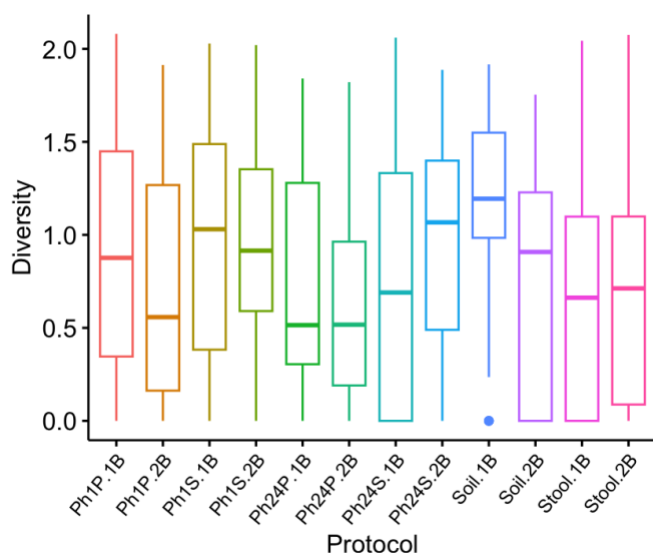


**Figure S1.** Comparison of Shannon diversity of target species captured by the different extraction protocols in the 18S rDNA dataset.

**Table S9.** *Post hoc* pairwise comparison of significant differences in Shannon diversity of target species derived from different protocols from the Crust16S mtDNA dataset.

Protocols	Estimate	Std. Error	Z-value	Pr(> z )	
Soil.1B-Ph1P.2B	0.41254	0.122292	3.373	0.036	*
Soil.1B-Ph24P.1B	0.430272	0.122292	3.518	0.0226	*
Soil.1B-Ph24P.2B	0.51634	0.122292	4.222	<0.01	**
Stool.1B-Soil.1B	-0.515106	0.122292	-4.212	<0.01	**
Stool.2B-Soil.1B	-0.437476	0.122292	-3.577	0.0181	*





**Figure S2.** Comparison of Shannon diversity of target species captured by the different extraction protocols in the Crust16S mtDNA dataset.

**Table S10.** *Post hoc* pairwise comparison of significant differences in target species abundance composition derived from different protocols from the 18S rDNA dataset.

	Observed statistic	Free Stepdown Adjusted P-Value
Ph24S.1B vs Stool.1B	119.757	0.005 **
Ph24S.1B vs Soil.2B	104.095	0.010 **
Stool.1B vs Soil.2B	102.376	0.010 **
Stool.1B vs Soil.1B	99.982	0.010 **
Ph1P.2B vs Ph24S.1B	97.004	0.010 **
Ph1P.2B vs Soil.2B	92.456	0.015 *
Ph1P.2B vs Stool.1B	92.327	0.015 *
Stool.2B vs Soil.2B	90.866	0.015 *
Ph24S.1B vs Soil.1B	89.635	0.015 *
Ph1P.1B vs Stool.1B	88.172	0.015 *
Ph1P.2B vs Soil.1B	87.412	0.015 *
Stool.2B vs Soil.1B	86.460	0.015 *
Ph1P.1B vs Soil.2B	86.076	0.015 *
Ph1P.1B vs Ph24S.1B	85.243	0.015 *
Ph1S.1B vs Stool.1B	85.224	0.015 *
Ph24S.2B vs Stool.1B	80.202	0.020 *
Ph1P.1B vs Soil.1B	77.748	0.030 *
Ph24P.1B vs Stool.1B	74.187	0.035 *
Ph1P.2B vs Ph24P.1B	73.204	0.035 *
Ph24P.2B vs Ph24S.1B	73.140	0.035 *
Ph24P.2B vs Stool.2B	72.983	0.035 *
Ph24P.2B vs Stool.1B	72.282	0.035 *
Ph1P.1B vs Stool.2B	70.222	0.045 *

**Table S11.** *Post hoc* pairwise comparison of significant differences in target species presence-absence composition derived from different protocols from the 18S rDNA dataset.

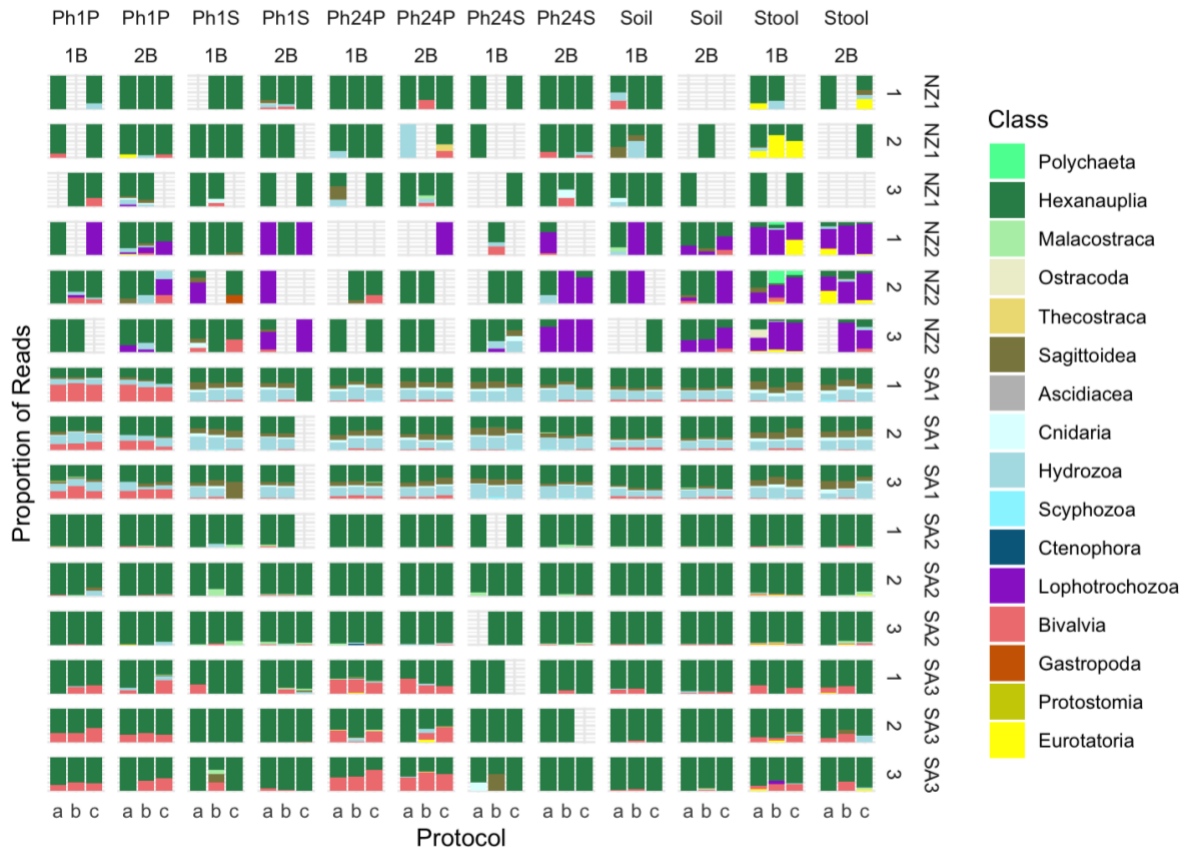
	Observed statistic	Free Stepdown Adjusted P-Value
Ph24S.1B vs Stool.1B	106.41	0.020 *
Ph24S.1B vs Soil.2B	88.10	0.085 .

**Table S12.** *Post hoc* pairwise comparison of significant differences in target species abundance composition derived from different protocols from the Crust16S mtDNA dataset.

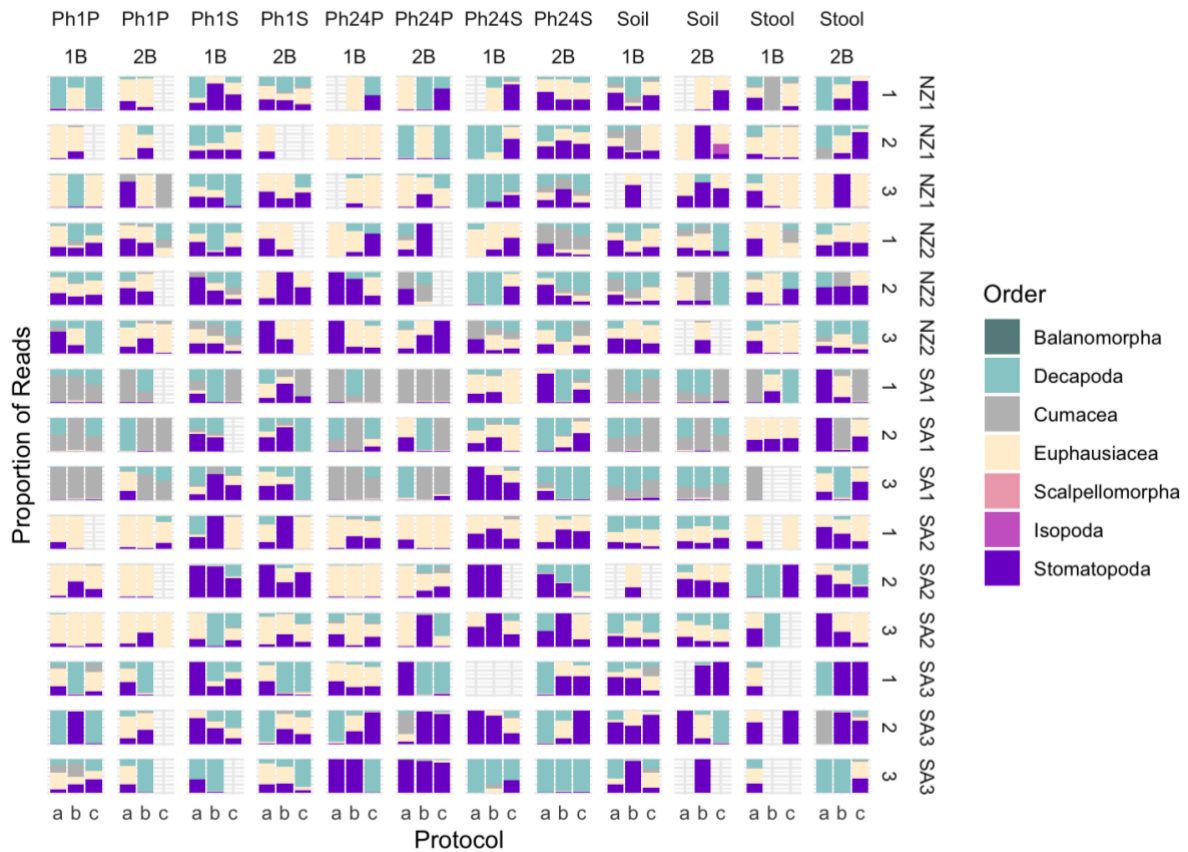
	Observed statistic	Free Stepdown Adjusted P-Value
Ph1P.1B vs Ph24S.1B	294.24	0.005 **
Ph1P.1B vs Ph1S.1B	288.09	0.005 **
Ph24P.2B vs Ph24S.1B	256.05	0.005 **
Ph1P.1B vs Ph24S.2B	246.96	0.005 **
Ph1P.1B vs Stool.1B	242.08	0.005 **
Ph24P.1B vs Ph24S.1B	241.21	0.005 **
Ph24S.1B vs Soil.2B	237.03	0.005 **
Ph1S.1B vs Ph24P.2B	227.09	0.005 **
Ph24S.1B vs Soil.1B	215.57	0.005 **
Ph24P.2B vs Ph24S.2B	214.92	0.005 **
Ph24P.1B vs Ph24S.2B	210.41	0.005 **
Ph24P.1B vs Stool.1B	209.99	0.005 **
Ph1S.1B vs Soil.2B	208.56	0.005 **
Ph1S.2B vs Ph24S.1B	205.96	0.005 **
Ph1P.2B vs Ph1S.1B	205.50	0.005 **
Ph24P.2B vs Stool.1B	205.26	0.005 **
Ph1S.1B vs Ph24P.1B	200.29	0.005 **
Ph1P.2B vs Ph24S.1B	196.09	0.005 **
Soil.2B vs Stool.1B	195.56	0.005 **
Ph1P.1B vs Ph1S.2B	192.19	0.005 **
Ph1S.2B vs Stool.1B	192.05	0.005 **
Ph24P.1B vs Stool.2B	185.47	0.015 *
Ph24S.2B vs Soil.2B	181.22	0.015 *
Ph1P.1B vs Soil.1B	179.37	0.015 *
Ph1S.1B vs Soil.1B	176.46	0.015 *
Ph1S.1B vs Stool.2B	174.91	0.015 *
Ph1P.2B vs Ph24S.2B	174.44	0.015 *
Ph24S.2B vs Stool.1B	164.37	0.025 *
Ph24S.2B vs Soil.1B	159.14	0.030 *
Ph24S.1B vs Ph24S.2B	157.91	0.035 *
Soil.1B vs Stool.2B	156.80	0.040 *
Ph24S.2B vs Stool.2B	155.29	0.045 *
Ph1P.2B vs Stool.1B	154.75	0.045 *

**Table S13.** *Post hoc* pairwise comparison of significant differences in target species presence-absence compositional data derived from different protocols from the Crust16S mtDNA dataset.

	Observed statistic	Free Stepdown Adjusted P-Value
Ph1P.1B vs Ph1S.1B	208.62	0.005 **
Ph1P.1B vs Ph24S.2B	196.72	0.005 **
Ph24S.1B vs Soil.1B	172.21	0.010 **
Ph1P.1B vs Stool.1B	171.36	0.010 **
Ph24S.2B vs Stool.1B	169.95	0.010 **
Ph1S.1B vs Ph24P.2B	167.64	0.010 **
Soil.1B vs Stool.2B	165.83	0.015 *
Soil.1B vs Stool.1B	165.51	0.015 *
Ph1P.1B vs Ph24S.1B	163.52	0.015 *
Ph24P.1B vs Ph24S.1B	159.26	0.020 *
Ph1S.1B vs Stool.1B	156.88	0.025 *
Ph1S.2B vs Ph24S.1B	154.47	0.030 *
Ph24P.1B vs Ph24S.2B	154.29	0.030 *
Ph24P.2B vs Ph24S.2B	154.19	0.030 *
Ph24P.2B vs Ph24S.1B	153.15	0.030 *
Ph1S.2B vs Stool.1B	150.18	0.030 *
Ph1S.1B vs Ph24P.1B	146.13	0.040 *
Ph24P.1B vs Stool.1B	145.82	0.040 *
Ph24S.1B vs Ph24S.2B	145.23	0.040 *
Ph24P.1B vs Stool.2B	141.64	0.045 *
Ph1P.2B vs Ph1S.1B	141.57	0.045 *
Ph1S.1B vs Ph24S.1B	141.17	0.045 *
Ph24S.2B vs Soil.2B	137.85	0.045 *
Ph1S.1B vs Soil.2B	136.51	0.050 *
Ph24S.2B vs Stool.2B	136.42	0.050 *
Ph24P.2B vs Stool.1B	136.34	0.050 *



**Figure S3.** Proportion of reads of classes of target organisms captured by each individual sample by the 18S rDNA amplicon. Samples are arranged based on biological sample/replicate (NZ1 – SA3 on right y-axis), extraction replicate (1, 2, 3 on right y-axis), PCR replicate (a, b, c on bottom x-axis), and extraction protocol (top x-axis).



**Figure S4.** Proportion of reads for different orders of subphylum Crustacea captured by each individual sample by the Crust16S mtDNA amplicon. Samples are arranged based on biological sample/replicate (NZ1 – SA3 on right y-axis), extraction replicate (1, 2, 3 on right y-axis), PCR replicate (a, b, c on bottom x-axis), and extraction protocol (top x-axis).

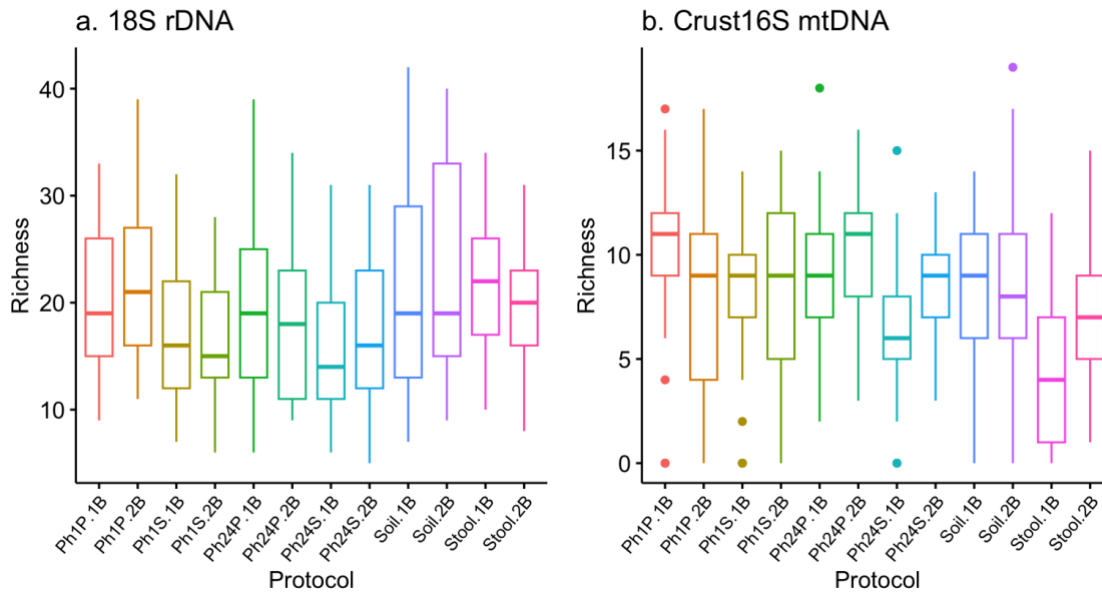
## Secondary Analyses – Full dataset containing both non-target and target taxa

The 18S rDNA and Crust16S mtDNA OTU tables generated from the GHAP pipeline were both analysed for species richness, Shannon diversity and composition in their raw formats, after removing any OTUs that were not matched to the phylum level or higher. There were 433 OTUs retained in the 18S rDNA dataset and 77 OTUs in the Crust16S mtDNA dataset. R results for the statistical models for the different analyses may be found below.

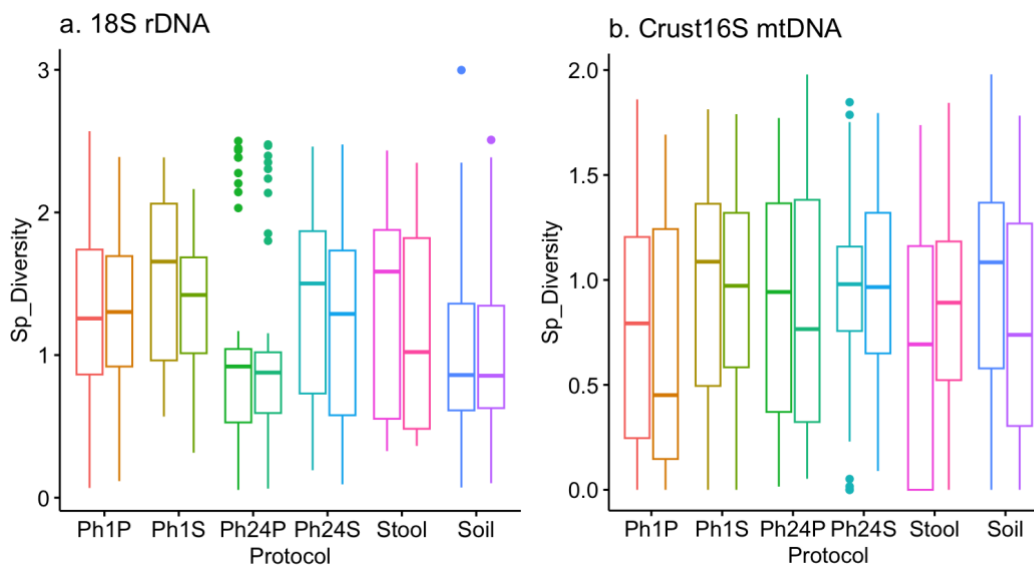
### *zOTU Richness and Shannon diversity*

For 18S rDNA extraction protocol had a highly significant effect on zOTU richness and Shannon diversity in the lmer models ( $P < 0.001$  for both). For Crust16S mtDNA, extraction protocol also had a very significant influence on zOTU richness ( $P < 0.001$ ) and a slightly less, but still significant effect on Shannon diversity ( $P < 0.05$ ).

*Lmer model:*  $\text{lmer}(\text{Richness/Diversity} \sim \text{Protocol} + (1|\text{Sample/Ext\_reps}), \text{data} = 16S/18S)$



**Figure S5.** Comparison of zOTU richness as captured by each protocol in the raw datasets for (a) 18S rDNA and (b) Crust16S mtDNA



**Figure S6.** Comparison of Shannon diversity as captured by each protocol in the raw datasets for (a) 18S rDNA and (b) Crust16S mtDNA

**Table S14.** Summary of zOTU richness captured by each extraction protocol for the raw 18S rDNA dataset.

Extraction protocol	Mean zOTU richness	SD
Ph1P.1B	20	7.09
Ph1P.2B	22.5	7.95
Ph1S.1B	16.8	6.05
Ph1S.2B	16.5	5.59
Ph24P.1B	20.2	8.97
Ph24P.2B	18.4	7.14

Ph24S.1B	15.7	6.55
Ph24S.2B	16.8	6.61
Soil.1B	21.7	9.76
Soil.2B	22.7	9.83
Stool.1B	22.4	6.5
Stool.2B	19.5	5.92

**Table S15.** *Post hoc* pairwise comparison of differences in zOTU richness derived from different protocols from the raw 18S rDNA dataset.

Protocols	Estimate	Std Error	z value	Pr(> z )	
Ph1P.2B-Ph1P.1B	2.48889	1.1778	2.113	0.6139	
Ph1S.1B-Ph1P.1B	-3.24444	1.1778	-2.755	0.2006	
Ph1S.2B-Ph1P.1B	-3.53333	1.1778	-3	0.109	
Ph24P.1B-Ph1P.1B	0.17778	1.1778	0.151	1	
Ph24P.2B-Ph1P.1B	-1.62222	1.1778	-1.377	0.9679	
Ph24S.1B-Ph1P.1B	-4.33333	1.1778	-3.679	0.0124	*
Ph24S.2B-Ph1P.1B	-3.24444	1.1778	-2.755	0.1997	
Soil.1B-Ph1P.1B	1.62222	1.1778	1.377	0.968	
Soil.2B-Ph1P.1B	2.62222	1.1778	2.226	0.5294	
Stool.1B-Ph1P.1B	2.4	1.1778	2.038	0.6673	
Stool.2B-Ph1P.1B	-0.57778	1.1778	-0.491	1	
Ph1S.1B-Ph1P.2B	-5.73333	1.1778	-4.868	<0.01	***
Ph1S.2B-Ph1P.2B	-6.02222	1.1778	-5.113	<0.01	***
Ph24P.1B-Ph1P.2B	-2.31111	1.1778	-1.962	0.7186	
Ph24P.2B-Ph1P.2B	-4.11111	1.1778	-3.49	0.0244	*
Ph24S.1B-Ph1P.2B	-6.82222	1.1778	-5.792	<0.01	***
Ph24S.2B-Ph1P.2B	-5.73333	1.1778	-4.868	<0.01	***
Soil.1B-Ph1P.2B	-0.86667	1.1778	-0.736	0.9999	
Soil.2B-Ph1P.2B	0.13333	1.1778	0.113	1	
Stool.1B-Ph1P.2B	-0.08889	1.1778	-0.075	1	
Stool.2B-Ph1P.2B	-3.06667	1.1778	-2.604	0.2781	
Ph1S.2B-Ph1S.1B	-0.28889	1.1778	-0.245	1	
Ph24P.1B-Ph1S.1B	3.42222	1.1778	2.906	0.1386	
Ph24P.2B-Ph1S.1B	1.62222	1.1778	1.377	0.9679	
Ph24S.1B-Ph1S.1B	-1.08889	1.1778	-0.925	0.9989	
Ph24S.2B-Ph1S.1B	0	1.1778	0	1	
Soil.1B-Ph1S.1B	4.86667	1.1778	4.132	<0.01	**
Soil.2B-Ph1S.1B	5.86667	1.1778	4.981	<0.01	***
Stool.1B-Ph1S.1B	5.64444	1.1778	4.792	<0.01	***
Stool.2B-Ph1S.1B	2.66667	1.1778	2.264	0.5026	
Ph24P.1B-Ph1S.2B	3.71111	1.1778	3.151	0.0708	.
Ph24P.2B-Ph1S.2B	1.91111	1.1778	1.623	0.9012	
Ph24S.1B-Ph1S.2B	-0.8	1.1778	-0.679	0.9999	

Ph24S.2B-Ph1S.2B	0.28889	1.1778	0.245	1	
Soil.1B-Ph1S.2B	5.15556	1.1778	4.377	<0.01	***
Soil.2B-Ph1S.2B	6.15556	1.1778	5.226	<0.01	***
Stool.1B-Ph1S.2B	5.93333	1.1778	5.038	<0.01	***
Stool.2B-Ph1S.2B	2.95556	1.1778	2.509	0.3331	
Ph24P.2B- Ph24P.1B	-1.8	1.1778	-1.528	0.9332	
Ph24S.1B- Ph24P.1B	-4.51111	1.1778	-3.83	<0.01	**
Ph24S.2B- Ph24P.1B	-3.42222	1.1778	-2.906	0.1383	
Soil.1B-Ph24P.1B	1.44444	1.1778	1.226	0.987	
Soil.2B-Ph24P.1B	2.44444	1.1778	2.075	0.6406	
Stool.1B-Ph24P.1B	2.22222	1.1778	1.887	0.7678	
Stool.2B-Ph24P.1B	-0.75556	1.1778	-0.641	1	
Ph24S.1B- Ph24P.2B	-2.71111	1.1778	-2.302	0.4753	
Ph24S.2B- Ph24P.2B	-1.62222	1.1778	-1.377	0.9679	
Soil.1B-Ph24P.2B	3.24444	1.1778	2.755	0.2004	
Soil.2B-Ph24P.2B	4.24444	1.1778	3.604	0.0163	*
Stool.1B-Ph24P.2B	4.02222	1.1778	3.415	0.0314	*
Stool.2B-Ph24P.2B	1.04444	1.1778	0.887	0.9992	
Ph24S.2B- Ph24S.1B	1.08889	1.1778	0.925	0.9989	
Soil.1B-Ph24S.1B	5.95556	1.1778	5.056	<0.01	***
Soil.2B-Ph24S.1B	6.95556	1.1778	5.906	<0.01	***
Stool.1B-Ph24S.1B	6.73333	1.1778	5.717	<0.01	***
Stool.2B-Ph24S.1B	3.75556	1.1778	3.189	0.0641	.
Soil.1B-Ph24S.2B	4.86667	1.1778	4.132	<0.01	**
Soil.2B-Ph24S.2B	5.86667	1.1778	4.981	<0.01	***
Stool.1B-Ph24S.2B	5.64444	1.1778	4.792	<0.01	***
Stool.2B-Ph24S.2B	2.66667	1.1778	2.264	0.5025	
Soil.2B-Soil.1B	1	1.1778	0.849	0.9995	
Stool.1B-Soil.1B	0.77778	1.1778	0.66	1	
Stool.2B-Soil.1B	-2.2	1.1778	-1.868	0.779	
Stool.1B-Soil.2B	-0.22222	1.1778	-0.189	1	
Stool.2B-Soil.2B	-3.2	1.1778	-2.717	0.2184	
Stool.2B-Stool.1B	-2.97778	1.1778	-2.528	0.3197	

**Table S16.** *Post hoc* pairwise comparison of differences in Shannon diversity derived from different protocols from the raw 18S rDNA dataset.

Protocols	Estimate	Std Error	z value	Pr(> z )
Ph1P.2B-Ph1P.1B	0.0104359	0.0926887	0.113	1
Ph1S.1B-Ph1P.1B	0.2779648	0.0926887	2.999	0.1096



Ph1S.2B-Ph1P.1B	0.1382541	0.0926887	1.492	0.9431	
	-				
Ph24P.1B-Ph1P.1B	0.2363032	0.0926887	-2.549	0.3091	
	-				
Ph24P.2B-Ph1P.1B	0.2632176	0.0926887	-2.84	0.1627	
Ph24S.1B-Ph1P.1B	0.0810135	0.0926887	0.874	0.9993	
	-				
Ph24S.2B-Ph1P.1B	0.1262406	0.0926887	-1.362	0.9707	
	-				
Soil.1B-Ph1P.1B	0.2181565	0.0926887	-2.354	0.4382	
Soil.2B-Ph1P.1B	-0.21896	0.0926887	-2.362	0.4331	
Stool.1B-Ph1P.1B	0.0768936	0.0926887	0.83	0.9996	
	-				
Stool.2B-Ph1P.1B	0.0630501	0.0926887	-0.68	0.9999	
Ph1S.1B-Ph1P.2B	0.2675289	0.0926887	2.886	0.1463	
Ph1S.2B-Ph1P.2B	0.1278182	0.0926887	1.379	0.9676	
	-				
Ph24P.1B-Ph1P.2B	0.2467391	0.0926887	-2.662	0.2458	
	-				
Ph24P.2B-Ph1P.2B	0.2736535	0.0926887	-2.952	0.1235	
Ph24S.1B-Ph1P.2B	0.0705776	0.0926887	0.761	0.9998	
	-				
Ph24S.2B-Ph1P.2B	0.1366765	0.0926887	-1.475	0.9477	
	-				
Soil.1B-Ph1P.2B	0.2285924	0.0926887	-2.466	0.3611	
	-				
Soil.2B-Ph1P.2B	0.2293959	0.0926887	-2.475	0.3557	
Stool.1B-Ph1P.2B	0.0664577	0.0926887	0.717	0.9999	
Stool.2B-Ph1P.2B	-0.073486	0.0926887	-0.793	0.9997	
	-				
Ph1S.2B-Ph1S.1B	0.1397108	0.0926887	-1.507	0.9391	
Ph24P.1B-Ph1S.1B	-0.514268	0.0926887	-5.548	<0.01	***
	-				
Ph24P.2B-Ph1S.1B	0.5411825	0.0926887	-5.839	<0.01	***
	-				
Ph24S.1B-Ph1S.1B	0.1969513	0.0926887	-2.125	0.6044	
	-				
Ph24S.2B-Ph1S.1B	0.4042054	0.0926887	-4.361	<0.01	***
	-				
Soil.1B-Ph1S.1B	0.4961214	0.0926887	-5.353	<0.01	***
	-				
Soil.2B-Ph1S.1B	0.4969248	0.0926887	-5.361	<0.01	***
	-				
Stool.1B-Ph1S.1B	0.2010713	0.0926887	-2.169	0.5723	
	-				
Stool.2B-Ph1S.1B	0.3410149	0.0926887	-3.679	0.0126	*
	-				
Ph24P.1B-Ph1S.2B	0.3745572	0.0926887	-4.041	<0.01	**

Ph24P.2B-Ph1S.2B	- 0.4014717	0.0926887	-4.331	<0.01	**
Ph24S.1B-Ph1S.2B	- 0.0572406	0.0926887	-0.618	1	
Ph24S.2B-Ph1S.2B	- 0.2644946	0.0926887	-2.854	0.1581	
Soil.1B-Ph1S.2B	- 0.3564106	0.0926887	-3.845	<0.01	**
Soil.2B-Ph1S.2B	- 0.3572141	0.0926887	-3.854	<0.01	**
Stool.1B-Ph1S.2B	- 0.0613605	0.0926887	-0.662	1	
Stool.2B-Ph1S.2B	- 0.2013042	0.0926887	-2.172	0.5706	
Ph24P.2B- Ph24P.1B	- 0.0269144	0.0926887	-0.29	1	
Ph24S.1B- Ph24P.1B	- 0.3173167	0.0926887	3.423	0.0304	*
Ph24S.2B- Ph24P.1B	- 0.1100626	0.0926887	1.187	0.99	
Soil.1B-Ph24P.1B	- 0.0181467	0.0926887	0.196	1	
Soil.2B-Ph24P.1B	- 0.0173432	0.0926887	0.187	1	
Stool.1B-Ph24P.1B	- 0.3131968	0.0926887	3.379	0.0352	*
Stool.2B-Ph24P.1B	- 0.1732531	0.0926887	1.869	0.7785	
Ph24S.1B- Ph24P.2B	- 0.3442311	0.0926887	3.714	0.011	*
Ph24S.2B- Ph24P.2B	- 0.136977	0.0926887	1.478	0.9469	
Soil.1B-Ph24P.2B	- 0.0450611	0.0926887	0.486	1	
Soil.2B-Ph24P.2B	- 0.0442576	0.0926887	0.477	1	
Stool.1B-Ph24P.2B	- 0.3401112	0.0926887	3.669	0.0128	*
Stool.2B-Ph24P.2B	- 0.2001675	0.0926887	2.16	0.5791	
Ph24S.2B- Ph24S.1B	- 0.2072541	0.0926887	-2.236	0.5238	
Soil.1B-Ph24S.1B	- -0.29917	0.0926887	-3.228	0.0571	.
Soil.2B-Ph24S.1B	- 0.2999735	0.0926887	-3.236	0.0552	.
Stool.1B-Ph24S.1B	- 0.0041199	0.0926887	-0.044	1	
Stool.2B-Ph24S.1B	- 0.1440636	0.0926887	-1.554	0.9251	
Soil.1B-Ph24S.2B	- 0.0919159	0.0926887	-0.992	0.9979	
Soil.2B-Ph24S.2B	- 0.0927194	0.0926887	-1	0.9977	
Stool.1B-Ph24S.2B	- 0.2031342	0.0926887	2.192	0.5552	
Stool.2B-Ph24S.2B	- 0.0631905	0.0926887	0.682	0.9999	

Soil.2B-Soil.1B	0.0008035	0.0926887	-0.009	1
Stool.1B-Soil.1B	0.2950501	0.0926887	3.183	0.0648
Stool.2B-Soil.1B	0.1551064	0.0926887	1.673	0.8805
Stool.1B-Soil.2B	0.2958536	0.0926887	3.192	0.0638
Stool.2B-Soil.2B	0.1559099	0.0926887	1.682	0.8766
Stool.2B-Stool.1B	0.1399437	0.0926887	-1.51	0.9383

**Table S17.** Summary of zOTU richness captured by each extraction protocol for the raw Crust16S mtDNA dataset.

Extraction protocol	Mean zOTU richness	SD
Ph1P.1B	10.5	3.41
Ph1P.2B	7.71	4.66
Ph1S.1B	8.18	2.94
Ph1S.2B	8.58	3.91
Ph24P.1B	9.18	3.46
Ph24P.2B	9.98	3.15
Ph24S.1B	6.36	2.9
Ph24S.2B	8.64	2.18
Soil.1B	8.44	3.64
Soil.2B	8.56	4.7
Stool.1B	4.51	3.74
Stool.2B	7.02	3.08

**Table S18.** *Post hoc* pairwise comparison of differences in zOTU richness derived from different protocols from the raw Crust16S mtDNA dataset.

Protocols	Estimate	Std Error	z value	Pr(> z )	
Ph1P.2B-Ph1P.1B	-2.75556	0.72663	-3.792	<0.01	**
Ph1S.1B-Ph1P.1B	-2.28889	0.72663	-3.15	0.0703	.
Ph1S.2B-Ph1P.1B	-1.88889	0.72663	-2.6	0.2793	
Ph24P.1B-Ph1P.1B	-1.28889	0.72663	-1.774	0.8323	
Ph24P.2B-Ph1P.1B	-0.48889	0.72663	-0.673	0.9999	
Ph24S.1B-Ph1P.1B	-4.11111	0.72663	-5.658	<0.01	***
Ph24S.2B-Ph1P.1B	-1.82222	0.72663	-2.508	0.3337	
Soil.1B-Ph1P.1B	-2.02222	0.72663	-2.783	0.1857	
Soil.2B-Ph1P.1B	-1.91111	0.72663	-2.63	0.2627	
Stool.1B-Ph1P.1B	-5.95556	0.72663	-8.196	<0.01	***
Stool.2B-Ph1P.1B	-3.44444	0.72663	-4.74	<0.01	***
Ph1S.1B-Ph1P.2B	0.46667	0.72663	0.642	1	
Ph1S.2B-Ph1P.2B	0.86667	0.72663	1.193	0.9897	
Ph24P.1B-Ph1P.2B	1.46667	0.72663	2.018	0.6809	

Ph24P.2B-Ph1P.2B	2.26667	0.72663	3.119	0.0769	.
Ph24S.1B-Ph1P.2B	-1.35556	0.72663	-1.866	0.781	
Ph24S.2B-Ph1P.2B	0.93333	0.72663	1.284	0.9813	
Soil.1B-Ph1P.2B	0.73333	0.72663	1.009	0.9975	
Soil.2B-Ph1P.2B	0.84444	0.72663	1.162	0.9916	
Stool.1B-Ph1P.2B	-3.2	0.72663	-4.404	<0.01	***
Stool.2B-Ph1P.2B	-0.68889	0.72663	-0.948	0.9986	
Ph1S.2B-Ph1S.1B	0.4	0.72663	0.55	1	
Ph24P.1B-Ph1S.1B	1	0.72663	1.376	0.9681	
Ph24P.2B-Ph1S.1B	1.8	0.72663	2.477	0.3544	
Ph24S.1B-Ph1S.1B	-1.82222	0.72663	-2.508	0.3345	
Ph24S.2B-Ph1S.1B	0.46667	0.72663	0.642	1	
Soil.1B-Ph1S.1B	0.26667	0.72663	0.367	1	
Soil.2B-Ph1S.1B	0.37778	0.72663	0.52	1	
Stool.1B-Ph1S.1B	-3.66667	0.72663	-5.046	<0.01	***
Stool.2B-Ph1S.1B	-1.15556	0.72663	-1.59	0.913	
Ph24P.1B-Ph1S.2B	0.6	0.72663	0.826	0.9996	
Ph24P.2B-Ph1S.2B	1.4	0.72663	1.927	0.7428	
Ph24S.1B-Ph1S.2B	-2.22222	0.72663	-3.058	0.0926	.
Ph24S.2B-Ph1S.2B	0.06667	0.72663	0.092	1	
Soil.1B-Ph1S.2B	-0.13333	0.72663	-0.183	1	
Soil.2B-Ph1S.2B	-0.02222	0.72663	-0.031	1	
Stool.1B-Ph1S.2B	-4.06667	0.72663	-5.597	<0.01	***
Stool.2B-Ph1S.2B	-1.55556	0.72663	-2.141	0.5932	
Ph24P.2B- Ph24P.1B	0.8	0.72663	1.101	0.9948	
Ph24S.1B- Ph24P.1B	-2.82222	0.72663	-3.884	<0.01	**
Ph24S.2B- Ph24P.1B	-0.53333	0.72663	-0.734	0.9999	
Soil.1B-Ph24P.1B	-0.73333	0.72663	-1.009	0.9975	
Soil.2B-Ph24P.1B	-0.62222	0.72663	-0.856	0.9995	
Stool.1B-Ph24P.1B	-4.66667	0.72663	-6.422	<0.01	***
Stool.2B-Ph24P.1B	-2.15556	0.72663	-2.967	0.1184	
Ph24S.1B- Ph24P.2B	-3.62222	0.72663	-4.985	<0.01	***
Ph24S.2B- Ph24P.2B	-1.33333	0.72663	-1.835	0.7989	
Soil.1B-Ph24P.2B	-1.53333	0.72663	-2.11	0.6165	
Soil.2B-Ph24P.2B	-1.42222	0.72663	-1.957	0.7231	
Stool.1B-Ph24P.2B	-5.46667	0.72663	-7.523	<0.01	***
Stool.2B-Ph24P.2B	-2.95556	0.72663	-4.067	<0.01	**
Ph24S.2B- Ph24S.1B	2.28889	0.72663	3.15	0.071	.
Soil.1B-Ph24S.1B	2.08889	0.72663	2.875	0.1493	

Soil.2B-Ph24S.1B	2.2	0.72663	3.028	0.1009	
Stool.1B-Ph24S.1B	-1.84444	0.72663	-2.538	0.3155	
Stool.2B-Ph24S.1B	0.66667	0.72663	0.917	0.999	
Soil.1B-Ph24S.2B	-0.2	0.72663	-0.275	1	
Soil.2B-Ph24S.2B	-0.08889	0.72663	-0.122	1	
Stool.1B-Ph24S.2B	-4.13333	0.72663	-5.688	<0.01	***
Stool.2B-Ph24S.2B	-1.62222	0.72663	-2.233	0.5256	
Soil.2B-Soil.1B	0.11111	0.72663	0.153	1	
Stool.1B-Soil.1B	-3.93333	0.72663	-5.413	<0.01	***
Stool.2B-Soil.1B	-1.42222	0.72663	-1.957	0.7224	
Stool.1B-Soil.2B	-4.04444	0.72663	-5.566	<0.01	***
Stool.2B-Soil.2B	-1.53333	0.72663	-2.11	0.6153	
Stool.2B-Stool.1B	2.51111	0.72663	3.456	0.0269	*

**Table S19.** *Post hoc* pairwise comparison of differences in Shannon diversity derived from different protocols from the raw Crust16S mtDNA dataset.

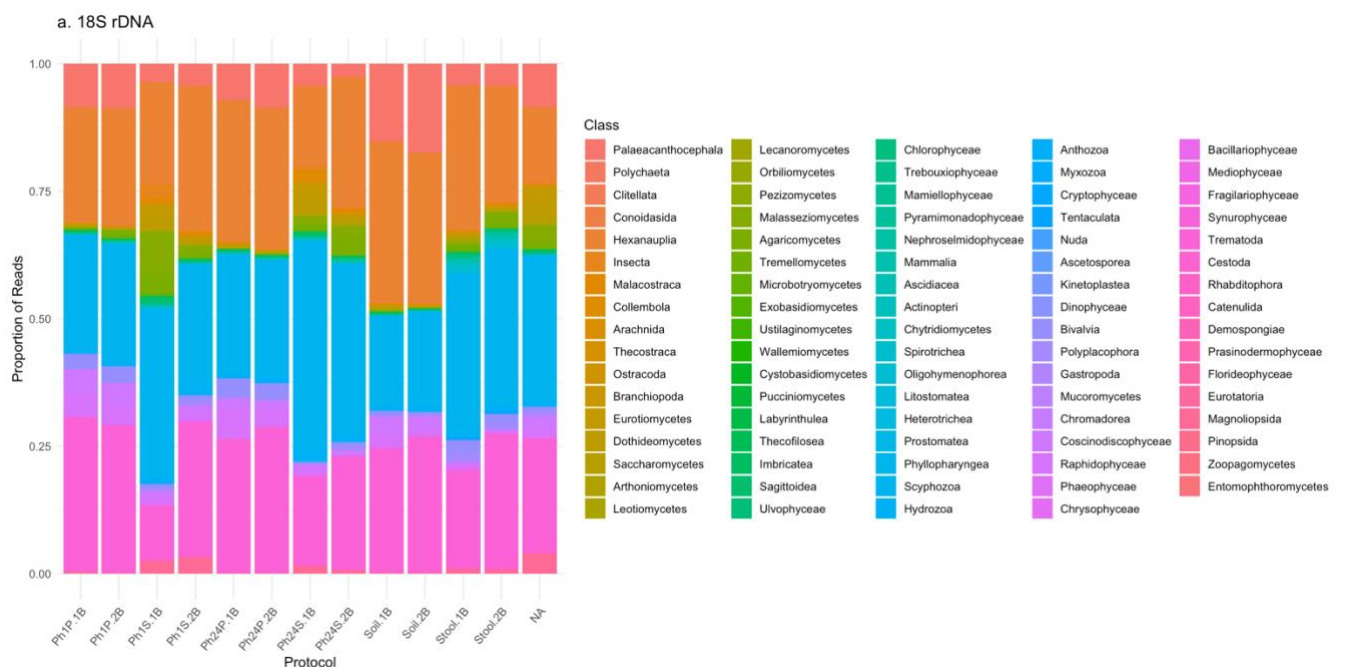
Protocols	Estimate	Std Error	z value	Pr(> z )
	-			
Ph1P.2B-Ph1P.1B	0.1513174	0.107553	-1.407	0.962
Ph1S.1B-Ph1P.1B	0.1113204	0.107553	1.035	0.997
Ph1S.2B-Ph1P.1B	0.0909475	0.107553	0.846	1
Ph24P.1B-Ph1P.1B	0.0898855	0.107553	0.836	1
Ph24P.2B-Ph1P.1B	0.010241	0.107553	0.095	1
Ph24S.1B-Ph1P.1B	0.1200061	0.107553	1.116	0.994
Ph24S.2B-Ph1P.1B	0.1108741	0.107553	1.031	0.997
Soil.1B-Ph1P.1B	0.1522184	0.107553	1.415	0.961
Soil.2B-Ph1P.1B	-0.048796	0.107553	-0.454	1
	-			
Stool.1B-Ph1P.1B	0.1529458	0.107553	-1.422	0.959
Stool.2B-Ph1P.1B	0.048904	0.107553	0.455	1
Ph1S.1B-Ph1P.2B	0.2626378	0.107553	2.442	0.377
Ph1S.2B-Ph1P.2B	0.242265	0.107553	2.253	0.511
Ph24P.1B-Ph1P.2B	0.241203	0.107553	2.243	0.518
Ph24P.2B-Ph1P.2B	0.1615584	0.107553	1.502	0.94
Ph24S.1B-Ph1P.2B	0.2713235	0.107553	2.523	0.326
Ph24S.2B-Ph1P.2B	0.2621916	0.107553	2.438	0.381
Soil.1B-Ph1P.2B	0.3035358	0.107553	2.822	0.17
Soil.2B-Ph1P.2B	0.1025215	0.107553	0.953	0.999
	-			
Stool.1B-Ph1P.2B	0.0016283	0.107553	-0.015	1
Stool.2B-Ph1P.2B	0.2002215	0.107553	1.862	0.782
	-			
Ph1S.2B-Ph1S.1B	0.0203729	0.107553	-0.189	1
	-			
Ph24P.1B-Ph1S.1B	0.0214349	0.107553	-0.199	1

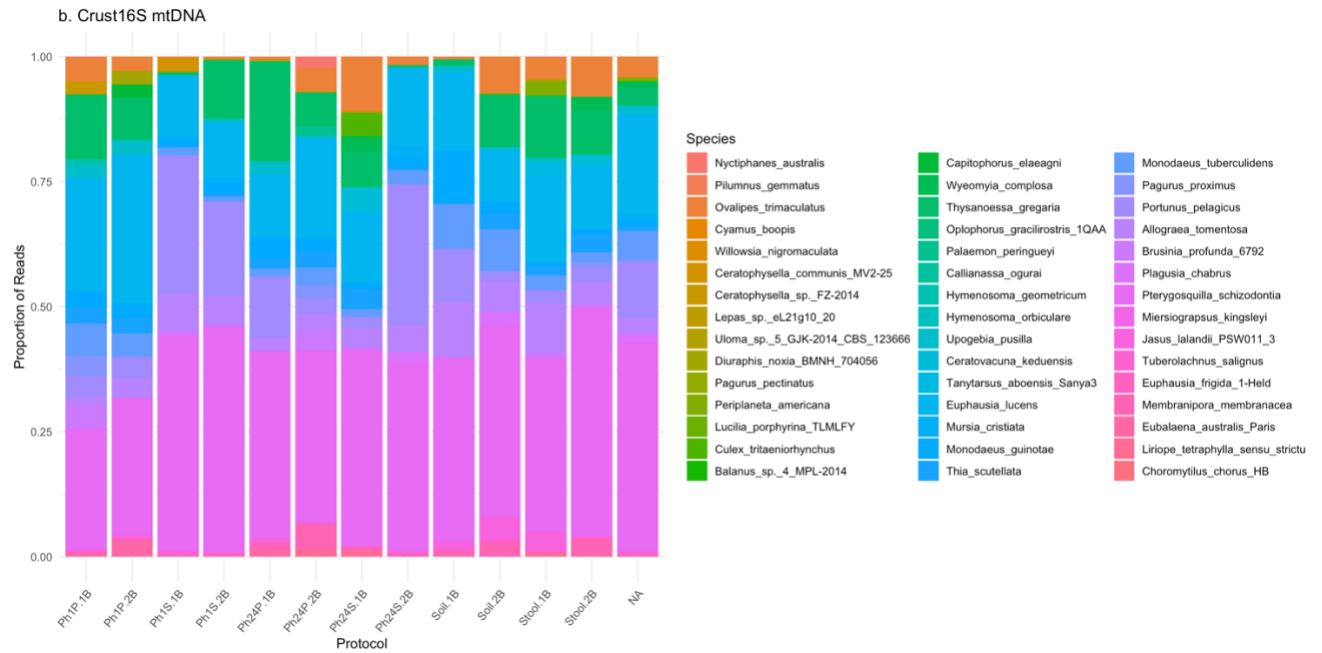
	-			
Ph24P.2B-Ph1S.1B	0.1010794	0.107553	-0.94	0.999
Ph24S.1B-Ph1S.1B	0.0086857	0.107553	0.081	1
	-			
Ph24S.2B-Ph1S.1B	0.0004463	0.107553	-0.004	1
Soil.1B-Ph1S.1B	0.040898	0.107553	0.38	1
	-			
Soil.2B-Ph1S.1B	0.1601164	0.107553	-1.489	0.944
	-			
Stool.1B-Ph1S.1B	0.2642662	0.107553	-2.457	0.367
	-			
Stool.2B-Ph1S.1B	0.0624164	0.107553	-0.58	1
Ph24P.1B-Ph1S.2B	-0.001062	0.107553	-0.01	1
	-			
Ph24P.2B-Ph1S.2B	0.0807065	0.107553	-0.75	1
Ph24S.1B-Ph1S.2B	0.0290585	0.107553	0.27	1
Ph24S.2B-Ph1S.2B	0.0199266	0.107553	0.185	1
Soil.1B-Ph1S.2B	0.0612708	0.107553	0.57	1
	-			
Soil.2B-Ph1S.2B	0.1397435	0.107553	-1.299	0.979
	-			
Stool.1B-Ph1S.2B	0.2438933	0.107553	-2.268	0.5
	-			
Stool.2B-Ph1S.2B	0.0420435	0.107553	-0.391	1
Ph24P.2B-	-			
Ph24P.1B	0.0796445	0.107553	-0.741	1
Ph24S.1B-	-			
Ph24P.1B	0.0301206	0.107553	0.28	1
Ph24S.2B-	-			
Ph24P.1B	0.0209886	0.107553	0.195	1
Soil.1B-Ph24P.1B	0.0623328	0.107553	0.58	1
	-			
Soil.2B-Ph24P.1B	0.1386815	0.107553	-1.289	0.981
	-			
Stool.1B-Ph24P.1B	0.2428313	0.107553	-2.258	0.508
	-			
Stool.2B-Ph24P.1B	0.0409815	0.107553	-0.381	1
Ph24S.1B-	-			
Ph24P.2B	0.1097651	0.107553	1.021	0.997
Ph24S.2B-	-			
Ph24P.2B	0.1006331	0.107553	0.936	0.999
Soil.1B-Ph24P.2B	0.1419774	0.107553	1.32	0.977
Soil.2B-Ph24P.2B	-0.059037	0.107553	-0.549	1
	-			
Stool.1B-Ph24P.2B	0.1631868	0.107553	-1.517	0.936
Stool.2B-Ph24P.2B	0.038663	0.107553	0.359	1
Ph24S.2B-	-			
Ph24S.1B	-0.009132	0.107553	-0.085	1

Soil.1B-Ph24S.1B	0.0322123	0.107553	0.3	1
Soil.2B-Ph24S.1B	-0.168802	0.107553	-1.569	0.92
	-			
Stool.1B-Ph24S.1B	0.2729518	0.107553	-2.538	0.316
	-			
Stool.2B-Ph24S.1B	0.0711021	0.107553	-0.661	1
Soil.1B-Ph24S.2B	0.0413442	0.107553	0.384	1
	-			
Soil.2B-Ph24S.2B	0.1596701	0.107553	-1.485	0.945
	-			
Stool.1B-Ph24S.2B	0.2638199	0.107553	-2.453	0.37
	-			
Stool.2B-Ph24S.2B	0.0619701	0.107553	-0.576	1
	-			
Soil.2B-Soil.1B	0.2010143	0.107553	-1.869	0.779
	-			
Stool.1B-Soil.1B	0.3051641	0.107553	-2.837	0.164
	-			
Stool.2B-Soil.1B	0.1033143	0.107553	-0.961	0.998
	-			
Stool.1B-Soil.2B	0.1041498	0.107553	-0.968	0.998
Stool.2B-Soil.2B	0.0977	0.107553	0.908	0.999
Stool.2B-Stool.1B	0.2018498	0.107553	1.877	0.774

### Taxonomic composition

For both 18S rDNA and Crust16S mtDNA there was a significant effect of extraction protocol on composition based on both abundance and presence-absence ( $P < 0.001$  in all cases). Pairwise protocol comparisons may be found below after the figure.





**Figure S7.** Relative abundance of (a) unique classes detected in the raw 18S rDNA dataset and (b) unique species detected in the raw Crust16S mtDNA dataset.

**Table S20.** *Post hoc* pairwise comparison of significant differences in species abundance compositional data derived from different protocols from the raw 18S rDNA dataset.

Protocols	Observed statistic	Adjusted P-value
Soil.1B vs Stool.1B	1147.8	0.005 **
Soil.2B vs Stool.1B	1098.2	0.005 **
Ph1P.2B vs Ph24S.1B	938.4	0.005 **
Soil.1B vs Stool.2B	931.9	0.005 **
Ph1P.1B vs Stool.1B	930.5	0.005 **
Ph1S.1B vs Stool.1B	921.4	0.005 **
Ph1P.2B vs Stool.1B	914.2	0.005 **
Ph24S.1B vs Stool.1B	911.9	0.005 **
Soil.2B vs Stool.2B	907.5	0.005 **
Ph24P.1B vs Stool.1B	893.5	0.005 **
Ph1P.1B vs Ph24S.1B	847.6	0.005 **
Ph1P.2B vs Ph1S.1B	835	0.005 **
Ph24S.1B vs Soil.2B	828.3	0.005 **
Ph1S.1B vs Soil.1B	810	0.005 **
Ph24P.2B vs Stool.1B	809.9	0.005 **
Ph1S.1B vs Soil.2B	802.6	0.005 **
Ph24S.1B vs Soil.1B	799.3	0.005 **
Ph1P.1B vs Stool.2B	781.1	0.005 **
Ph1S.2B vs Stool.1B	776.6	0.005 **
Ph24S.2B vs Soil.1B	774.2	0.005 **
Ph1P.1B vs Ph1S.1B	771.2	0.005 **
Ph1P.2B vs Stool.2B	770.4	0.005 **



Ph24S.2B vs Stool.1B	769	0.005	**
Ph24S.2B vs Soil.2B	766.1	0.005	**
Ph24P.1B vs Stool.2B	733.8	0.005	**
Ph1S.1B vs Ph24P.1B	720	0.005	**
Ph1P.2B vs Ph24S.2B	718.7	0.005	**
Ph24P.1B vs Ph24S.1B	713.5	0.005	**
Ph1S.1B vs Ph24P.2B	710.2	0.005	**
Ph24P.2B vs Ph24S.1B	707.4	0.005	**
Ph24P.2B vs Stool.2B	688.5	0.005	**
Ph1S.1B vs Stool.2B	684.9	0.005	**
Ph1S.2B vs Soil.1B	682.8	0.005	**
Ph24S.1B vs Stool.2B	676.4	0.005	**
Ph1S.2B vs Soil.2B	660.3	0.005	**
Ph1P.1B vs Ph24S.2B	654.4	0.005	**
Ph1P.2B vs Ph1S.2B	647.5	0.005	**
Ph24P.1B vs Ph24S.2B	618.5	0.005	**
Ph24P.2B vs Ph24S.2B	580.3	0.005	**
Ph1S.2B vs Ph24P.1B	574.9	0.005	**
Ph1S.2B vs Stool.2B	572.5	0.005	**
Ph24S.2B vs Stool.2B	567.7	0.005	**
Ph1P.1B vs Ph1S.2B	552.4	0.005	**
Ph1P.2B vs Soil.2B	537	0.005	**
Ph1S.2B vs Ph24P.2B	512.4	0.005	**
Ph1P.1B vs Soil.2B	502.7	0.005	**
Ph1P.2B vs Soil.1B	485.9	0.005	**
Ph1P.1B vs Soil.1B	473.8	0.005	**
Ph24S.1B vs Ph24S.2B	459.4	0.005	**
Ph24P.2B vs Soil.1B	439.9	0.005	**
Ph24P.2B vs Soil.2B	433	0.005	**
Ph1S.2B vs Ph24S.1B	414.7	0.005	**
Ph1S.1B vs Ph24S.1B	401.3	0.005	**
Ph24P.1B vs Soil.2B	398.9	0.005	**
Ph24P.1B vs Soil.1B	389.2	0.01	**
Ph1P.2B vs Ph24P.1B	384.1	0.01	**
Ph1S.1B vs Ph1S.2B	370	0.015	*
Ph1S.1B vs Ph24S.2B	369.3	0.015	*
Ph1P.1B vs Ph24P.1B	339.8	0.03	*
Ph1P.2B vs Ph24P.2B	337.9	0.03	*
Ph1S.2B vs Ph24S.2B	324.3	0.05	*

Stool.1B vs Stool.2B	322.1	0.05	*
Ph1P.1B vs Ph24P.2B	288.6	0.16	
Soil.1B vs Soil.2B	260.6	0.28	
Ph1P.1B vs Ph1P.2B	252	0.28	
Ph24P.1B vs Ph24P.2B	207.2	0.28	

**Table S21.** *Post hoc* pairwise comparison of significant differences in species presence-absence compositional data derived from different protocols from the raw 18S rDNA dataset.

<b>Protocols</b>	<b>Observed statistic</b>	<b>Adjusted P-value</b>	
Soil.1B vs Stool.1B	882.6	0.005	**
Soil.2B vs Stool.1B	797.3	0.005	**
Ph1P.1B vs Stool.1B	756.1	0.005	**
Ph1S.1B vs Stool.1B	750.1	0.005	**
Ph1P.2B vs Stool.1B	718.5	0.005	**
Soil.1B vs Stool.2B	718.2	0.005	**
Ph24P.1B vs Stool.1B	712	0.005	**
Ph24P.2B vs Stool.1B	679.7	0.005	**
Soil.2B vs Stool.2B	673.4	0.005	**
Ph24S.2B vs Stool.1B	662.8	0.005	**
Ph24S.1B vs Stool.1B	655.5	0.005	**
Ph1S.2B vs Stool.1B	651.8	0.005	**
Ph1P.1B vs Stool.2B	634.4	0.005	**
Ph1P.2B vs Stool.2B	628.1	0.005	**
Ph24P.1B vs Stool.2B	584.8	0.005	**
Ph1P.2B vs Ph24S.1B	583.8	0.005	**
Ph24P.2B vs Stool.2B	579	0.005	**
Ph1P.2B vs Ph1S.1B	576.8	0.005	**
Ph1S.1B vs Soil.2B	558.6	0.005	**
Ph24S.1B vs Soil.2B	554	0.005	**
Ph24S.1B vs Soil.1B	545	0.005	**
Ph1S.1B vs Soil.1B	538.9	0.005	**
Ph1S.1B vs Stool.2B	538.2	0.005	**
Ph1P.1B vs Ph24S.1B	526.9	0.005	**
Ph1P.2B vs Ph24S.2B	508.1	0.005	**
Ph1P.1B vs Ph1S.1B	493.8	0.005	**
Ph24S.2B vs Stool.2B	487.2	0.005	**
Ph24S.2B vs Soil.2B	484.2	0.005	**
Ph24S.2B vs Soil.1B	481.3	0.005	**
Ph24S.1B vs Stool.2B	472.2	0.005	**
Ph1S.2B vs Stool.2B	471.4	0.005	**
Ph1P.2B vs Ph1S.2B	469.4	0.005	**
Ph1S.1B vs Ph24P.2B	466.9	0.005	**

Ph1S.1B vs Ph24P.1B	455.6	0.005	**
Ph24P.1B vs Ph24S.1B	443.5	0.005	**
Ph1P.1B vs Ph24S.2B	435.3	0.005	**
Ph1S.2B vs Soil.1B	430.5	0.005	**
Ph24P.2B vs Ph24S.1B	429	0.005	**
Ph1S.2B vs Soil.2B	404.8	0.01	**
Ph24P.2B vs Ph24S.2B	383.8	0.01	**
Ph24P.1B vs Ph24S.2B	379.5	0.01	**
Ph1P.1B vs Soil.1B	366.6	0.015	*
Ph1P.2B vs Soil.1B	366.3	0.015	*
Ph1P.1B vs Ph1S.2B	365.8	0.015	*
Ph1P.2B vs Soil.2B	364.1	0.015	*
Ph1P.1B vs Soil.2B	359.1	0.015	*
Ph1S.2B vs Ph24P.1B	353.8	0.015	*
Ph24P.2B vs Soil.1B	353.1	0.015	*
Ph24P.1B vs Soil.1B	341.9	0.02	*
Ph24P.2B vs Soil.2B	333.8	0.03	*
Ph1S.2B vs Ph24P.2B	333.1	0.03	*
Ph24P.1B vs Soil.2B	326.2	0.03	*
Ph1P.2B vs Ph24P.1B	326.1	0.03	*
Ph24S.1B vs Ph24S.2B	322.7	0.03	*
Stool.1B vs Stool.2B	320.3	0.03	*
Ph1P.2B vs Ph24P.2B	314.1	0.03	*
Ph1S.1B vs Ph24S.1B	297.9	0.06	.
Ph1S.2B vs Ph24S.1B	290.3	0.085	.
Ph1S.1B vs Ph24S.2B	286.5	0.115	
Ph1P.1B vs Ph1P.2B	285.3	0.12	
Ph1S.1B vs Ph1S.2B	277.8	0.135	
Ph1P.1B vs Ph24P.1B	277.6	0.135	
Ph1S.2B vs Ph24S.2B	271	0.17	
Soil.1B vs Soil.2B	267.3	0.17	
Ph1P.1B vs Ph24P.2B	240.5	0.17	
Ph24P.1B vs Ph24P.2B	208.9	0.23	

**Table S22.** *Post hoc* pairwise comparison of significant differences in species abundance compositional data derived from different protocols from the raw Crust16S mtDNA dataset.

Protocols	Observed statistic	Adjusted P-value
Ph1P.1B vs Ph24S.1B	391.15	0.005 **

Ph1P.1B vs Ph1S.1B	387.24	0.005	**
Ph1P.1B vs Stool.1B	376.87	0.005	**
Ph24P.2B vs Ph24S.1B	363.66	0.005	**
Ph1S.1B vs Ph24P.2B	342.54	0.005	**
Ph24P.2B vs Ph24S.2B	338.53	0.005	**
Ph24P.2B vs Stool.1B	323.97	0.005	**
Ph1P.1B vs Ph24S.2B	320.2	0.005	**
Ph24S.1B vs Soil.2B	316.06	0.005	**
Ph1S.1B vs Soil.2B	309.19	0.005	**
Ph24P.1B vs Stool.1B	305.36	0.005	**
Ph24P.1B vs Ph24S.1B	303.61	0.005	**
Ph1S.1B vs Stool.2B	299.8	0.005	**
Ph24S.1B vs Ph24S.2B	297.16	0.005	**
Ph24S.2B vs Stool.2B	294.94	0.005	**
Ph24S.2B vs Soil.2B	292.27	0.005	**
Ph24P.2B vs Soil.1B	284.99	0.005	**
Ph1P.1B vs Soil.1B	284.9	0.005	**
Soil.2B vs Stool.1B	282.59	0.005	**
Ph1P.1B vs Ph1S.2B	274.48	0.005	**
Ph1P.2B vs Ph24S.1B	266.69	0.005	**
Ph1P.2B vs Ph1S.1B	265.48	0.005	**
Ph24S.2B vs Stool.1B	264.82	0.005	**
Ph24P.1B vs Ph24S.2B	261.89	0.005	**
Ph1S.2B vs Stool.2B	259.27	0.005	**
Ph24S.1B vs Soil.1B	255.93	0.005	**
Ph24P.1B vs Stool.2B	255.51	0.005	**
Ph1S.2B vs Ph24P.2B	254.74	0.005	**
Ph1S.1B vs Ph24S.1B	252.79	0.005	**
Soil.1B vs Stool.2B	251.8	0.01	**
Ph1S.2B vs Soil.2B	244.93	0.02	*
Ph24P.1B vs Ph24P.2B	243.82	0.02	*
Ph1S.1B vs Ph24P.1B	242.61	0.02	*
Ph1P.2B vs Ph24S.2B	241.39	0.02	*
Ph24P.2B vs Soil.2B	236.3	0.025	*
Ph1S.2B vs Ph24S.1B	235.38	0.025	*
Ph1P.2B vs Stool.1B	234.56	0.03	*
Ph24S.2B vs Soil.1B	233.03	0.03	*
Ph1P.1B vs Soil.2B	232.75	0.03	*
Ph1P.1B vs Stool.2B	232.5	0.03	*

Ph1S.1B vs Soil.1B	231.48	0.035	*
Ph1P.2B vs Soil.1B	229.91	0.035	*
Ph24P.1B vs Soil.2B	229.17	0.035	*
Ph24P.1B vs Soil.1B	227.92	0.05	*
Ph1S.2B vs Stool.1B	224.43	0.055	.
Ph1S.1B vs Stool.1B	223.77	0.055	.
Ph1P.1B vs Ph24P.1B	223.67	0.055	.
Ph1P.2B vs Ph24P.2B	220.43	0.055	.
Ph1P.2B vs Soil.2B	210.94	0.065	.
Ph1P.2B vs Stool.2B	207.78	0.08	.
Soil.1B vs Stool.1B	206.58	0.08	.
Ph24S.1B vs Stool.2B	205.5	0.08	.
Stool.1B vs Stool.2B	203.13	0.08	.
Ph1P.2B vs Ph1S.2B	178.75	0.38	
Ph1S.2B vs Soil.1B	174	0.435	
Ph1P.1B vs Ph24P.2B	164.56	0.57	
Soil.2B vs Stool.2B	164.36	0.57	
Ph1P.1B vs Ph1P.2B	153.27	0.57	
Ph24P.2B vs Stool.2B	152.52	0.57	
Ph1P.2B vs Ph24P.1B	148.41	0.57	
Soil.1B vs Soil.2B	145.65	0.57	
Ph1S.2B vs Ph24P.1B	135.87	0.57	
Ph24S.1B vs Stool.1B	132.87	0.57	
Ph1S.2B vs Ph24S.2B	130.74	0.57	
Ph1S.1B vs Ph1S.2B	117.67	0.57	
Ph1S.1B vs Ph24S.2B	88.58	0.57	

**Table S23.** *Post hoc* pairwise comparison of significant differences in species presence-absence compositional data derived from different protocols from the raw Crust16S mtDNA dataset.

Protocols	Observed statistic	Adjusted P-value
Ph1P.1B vs Stool.1B	295.19	0.005 **
Ph24S.2B vs Stool.1B	276.69	0.005 **
Ph24P.2B vs Stool.1B	255.21	0.005 **
Ph1P.1B vs Ph24S.2B	246.11	0.005 **
Ph24P.1B vs Stool.1B	242.46	0.005 **
Ph1P.1B vs Ph1S.1B	239.94	0.005 **
Ph1S.1B vs Stool.1B	233.26	0.005 **
Ph24P.2B vs Ph24S.1B	232.08	0.005 **
Ph1P.1B vs Ph24S.1B	226.7	0.005 **
Ph1S.1B vs Ph24P.2B	209.83	0.005 **

Ph24P.2B vs Ph24S.2B	208.61	0.005	**
Ph24P.1B vs Ph24S.1B	200.57	0.005	**
Ph24S.1B vs Ph24S.2B	200.48	0.005	**
Ph24S.2B vs Stool.2B	199.91	0.005	**
Ph24P.2B vs Soil.1B	198.65	0.005	**
Ph1S.2B vs Stool.1B	194.35	0.005	**
Ph1P.1B vs Ph1S.2B	193.97	0.005	**
Soil.1B vs Stool.1B	193.04	0.005	**
Ph1P.2B vs Ph24S.2B	190.75	0.005	**
Ph1P.1B vs Ph1P.2B	188.56	0.005	**
Ph1P.1B vs Ph24P.1B	188.47	0.005	**
Soil.2B vs Stool.1B	188.39	0.005	**
Ph1P.1B vs Stool.2B	187.92	0.005	**
Ph1P.1B vs Soil.1B	187.1	0.005	**
Soil.1B vs Stool.2B	185.57	0.005	**
Ph1S.1B vs Stool.2B	185.49	0.005	**
Ph24S.1B vs Soil.1B	177.4	0.005	**
Ph24P.2B vs Soil.2B	172.64	0.005	**
Ph1P.2B vs Ph1S.1B	171.81	0.005	**
Ph1S.1B vs Ph24S.1B	170.86	0.005	**
Ph24P.1B vs Stool.2B	165.28	0.005	**
Ph1P.1B vs Soil.2B	164.3	0.005	**
Ph1S.2B vs Ph24S.1B	156.37	0.005	**
Ph24S.2B vs Soil.2B	155.32	0.005	**
Ph24P.1B vs Soil.1B	154.99	0.005	**
Ph1S.2B vs Ph24P.2B	152.39	0.005	**
Ph24P.1B vs Ph24P.2B	149.03	0.005	**
Ph1S.2B vs Stool.2B	148.24	0.005	**
Ph24P.2B vs Stool.2B	146.63	0.005	**
Ph1P.2B vs Ph24P.2B	145.81	0.005	**
Ph1S.1B vs Soil.2B	142.58	0.005	**
Stool.1B vs Stool.2B	142.45	0.005	**
Ph1P.2B vs Ph24P.1B	140.63	0.005	**
Ph24S.1B vs Soil.2B	140.59	0.005	**
Ph1P.2B vs Soil.1B	135.9	0.005	**
Ph1P.2B vs Stool.2B	135.47	0.005	**
Ph1P.2B vs Ph24S.1B	130.21	0.01	**
Ph1S.1B vs Ph24P.1B	128.19	0.01	**
Soil.2B vs Stool.2B	121.85	0.01	**

Ph24P.1B vs Ph24S.2B	120.68	0.015	*
Ph1P.2B vs Soil.2B	120.27	0.015	*
Ph1P.2B vs Ph1S.2B	115.75	0.015	*
Ph24P.1B vs Soil.2B	112.63	0.025	*
Ph1P.2B vs Stool.1B	110.47	0.025	*
Ph24S.1B vs Stool.1B	108.95	0.025	*
Ph1S.2B vs Soil.1B	97.99	0.045	*
Ph1S.1B vs Soil.1B	96.2	0.055	.
Soil.1B vs Soil.2B	95.53	0.055	.
Ph1S.2B vs Soil.2B	95.21	0.055	.
Ph24S.2B vs Soil.1B	94.17	0.055	.
Ph1P.1B vs Ph24P.2B	90.25	0.07	.
Ph24S.1B vs Stool.2B	80.27	0.105	
Ph1S.2B vs Ph24S.2B	79.11	0.105	
Ph1S.2B vs Ph24P.1B	74.43	0.155	
Ph1S.1B vs Ph1S.2B	72.33	0.155	
Ph1S.1B vs Ph24S.2B	42.05	0.72	