Supplementary material

For Thesis: “Beyond the surface: enhancing freshwater pond ecosystem assessment through eDNA metabarcoding”, Claire Robertson.

Supplementary material: Chapter 2

16S	18S	COI	pITS	fITS	Step 2
95’C 2:00 95’C 0:15 + 50’C 0:30 + 72’C 0:30 + x 30 72’C 10:00	95’C 2:00 95’C 0:15 + 57’C 0:30 + 72’C 0:30 + X 30 72’C 10:00	94’C 3:00 94’C 0:30 + 46’C 0:30 + 65’C 2:30 + x 30 65’C 5:00	95’C 2:00 95’C 0:15 + 55’C 0:30 + 72’C 0:30 + X 35 72’C 10:00	95’C 2:00 95’C 0:15 + 55’C 0:30 + 72’C 0:30 + X 35 72’C 10:00	95’C 2:00 95’C 0:15 + 55’C 0:30 + 72’C 0:30 x 8 72’C 10:00

Table S2.1: PCR conditions for the five step 1 assays and for step 2

Pond type	**pH**	**Temp (°C)**	**TDS (ppm)**	**ORP (mV)**	**TDN (mgN/l)**	**NH4 (mg/l)**	**SRP (ug/l)**	**DOC (mg/l)**
MP:EP	2.85*	-3.76**	2.76*	-2.51	-0.36	-2.06	1.01	2.08
MP:GP	2.98**	-2.19	1.57	0.63	-6.08**	-2.89*	-2.64	-1.52
MP:SP	-0.85	1.00	-1.21	1.38	-0.55	2.60	-0.15	-2.22
GP:EP	0.52	-2.00	1.49	-2.91*	2.43	0.53	3.37*	3.44**
GP:SP	1.14	-0.47	-0.13	1.77	-2.59	0.72	-1.86	-3.20*
EP:SP	1.41	-1.97	1.03	-0.70	-0.78	1.05	0.55	-0.67

Table S2.2: Results of post-hoc test results (Dunn test-Z value), one or two asterisks indicate significance at the p<0.05 and p<0.01 level respectively.

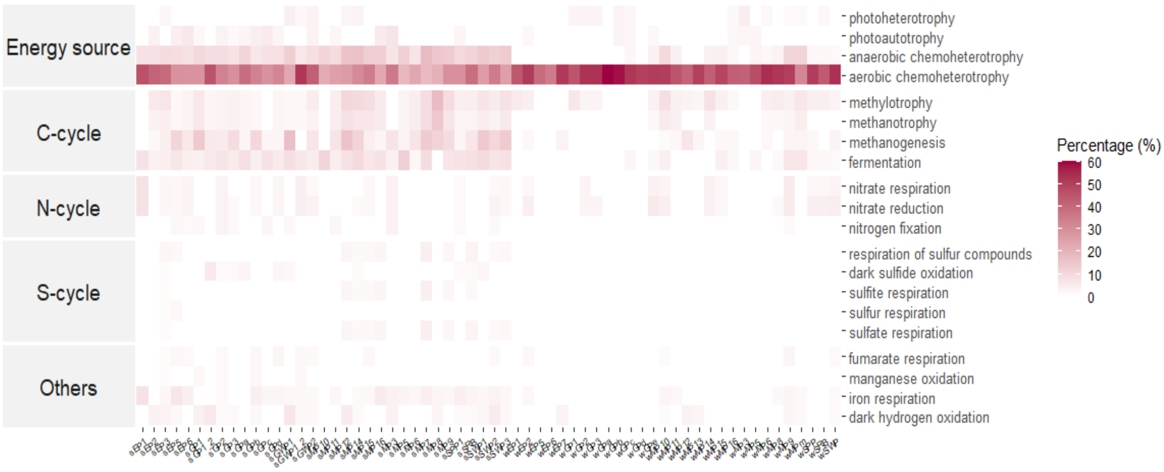


Figure S2.1: Functional diversity plot of prokaryotic organisms in the 16S dataset by sample.

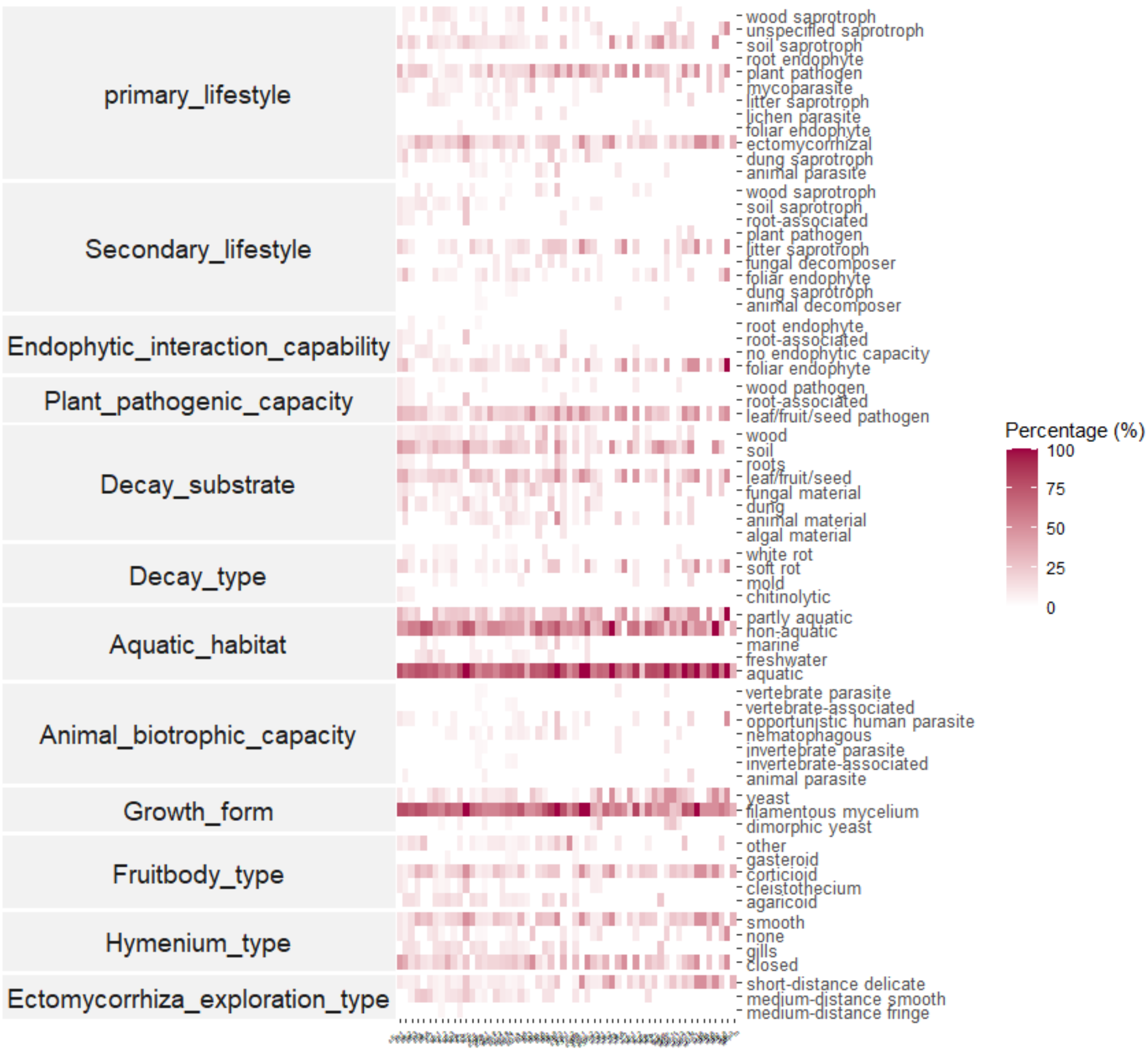


Figure S2.2: Functional diversity in the fITS dataset by sample

	Merged (read abundance %)	Separate (mean read abundance %) n=16	Separate (standard deviation)
Bacteria phyla			
Myxococcia	0.88	0.05	0.11
Methanosarcinia	1.53	0.07	0.08
Desulfobacteria	1.40	0.09	0.16
Cyanobacteriia	0.00	0.23	0.59
Desulfuromonadia	1.37	0.13	0.26
Methanomicrobia	0.98	0.25	0.42
Verrucomicrobiae	1.80	0.33	0.36
Campylobacteria	0.35	0.94	1.15
Alphaproteobacteria	2.90	1.64	1.09
Bacteroidia	3.14	7.20	4.14
Actinobacteria	20.85	23.57	12.09
Gammaproteobacteria	62.49	64.88	14.53
Animalia class			
Bivalvia	0	1.66	5.43
Chrysophyceae	0.79	2.00	5.11
Arachnida	0.26	2.64	10.57
Malacostraca	2.79	3.29	8.72
Dinophyceae	5.27	2.89	8.13
Bacillariophyceae	16.07	3.27	7.58
Branchiopoda	1.32	8.61	17.57
Cryptophyceae	5.14	12.17	17.14
Insecta	4.34	14.67	27.34
Maxillopoda	2.92	17.41	30.56
Ostracoda	2.50	15.05	23.76
Clitellata	15.02	14.91	29.25
Copepoda	53.97	16.32	25.12
Others	10.01	0.43	1.28
Green plant or algae class			
Others	0.74	18.63	24.41
Alismatales	0.00	0.12	0.42
Chaetopeltidales	0.00	0.17	0.60

Chaetophorales	0.00	0.18	0.42
Fabales	3.59	0.00	0.00
Oedogoniales	0.00	0.42	1.10
Rosales	0.00	0.85	3.07
Poales	5.12	0.49	1.77
Chlorellales	0.00	0.91	2.31
Fagales	13.46	0.12	0.44
Lamiales	0.00	1.21	4.36
Apiales	41.72	0.00	0.00
Asterales	0.00	5.18	18.66
Malphigiales	23.88	4.34	11.52
Sphaeropleales	0.00	9.15	22.32
Brassicales	0.00	9.91	25.78
Cucurbitales	0.00	13.63	33.59
Chlamydomonadales	11.49	34.70	32.61

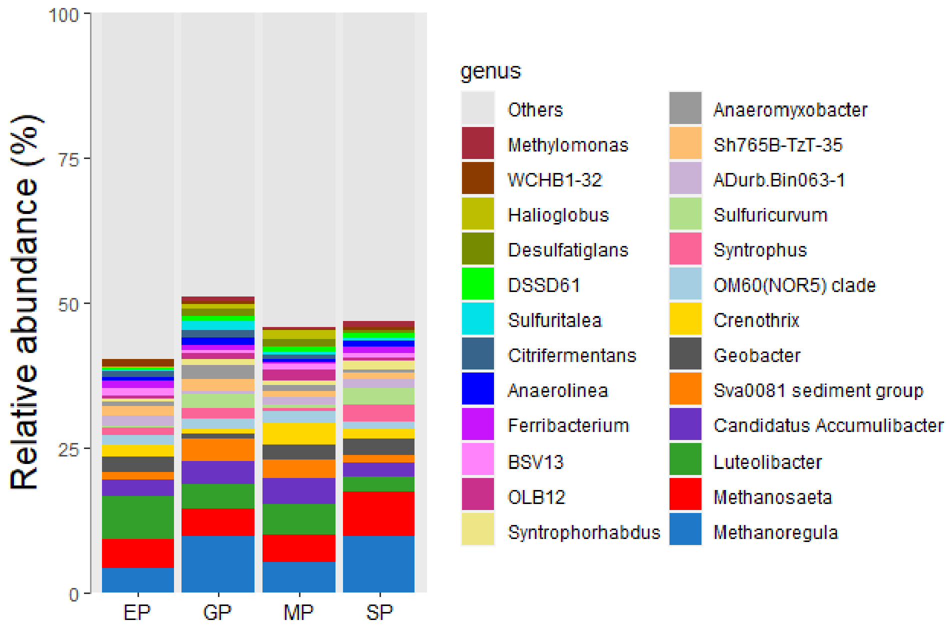
Table S2.3: Comparison of percentage eDNA read abundance of different taxa in the merged Main Pond water sample (1020ml), and mean (and SD) percentage eDNA read abundance in 16 separate water samples from the Main Pond (940ml each).

Table S2.4 Number of sediment and water samples collected from Pinkhill Meadow in June 2020.

Pond name	**Sample code**	**Pond type**	**No. Sediment samples**	**No. Water samples**
Main pond	MP	Main pond (MP)	17	18 (17 separate, 1 merged)
Semi-permanent pond	SPP	Surface water pond (SP)	2	1 (merged)
Surface water pond	SWP	Surface water pond (SP)	3	1 (merged)
Experimental pond 1	EP1	Experimental pond (EP)	1	1
Experimental pond 2	EP2	Experimental pond (EP)	1	1
Experimental pond 3	EP3	Experimental pond (EP)	1	1
Experimental pond 4	EP4	Experimental pond (EP)	1	1
Experimental pond 5	EP5	Experimental pond (EP)	1	1

Experimental pond 6	EP6	Experimental pond (EP)	1	1
Experimental pond 7	EP7	Experimental pond (EP)	1	1
Groundwater pond	GWP	Gravel pond (GP)	2	1 (merged)
Gravel pond 1	GP1	Gravel pond (GP)	1	1
Gravel pond 2	GP2	Gravel pond (GP)	1	1
Gravel pond 3	GP3	Gravel pond (GP)	1	1
Gravel pond a	GPa	Gravel pond (GP)	1	1
Gravel pond b	GPb	Gravel pond (GP)	1	1
Gravel pond c	GPc	Gravel pond (GP)	1	1
Gravel pond d	GPd	Gravel pond (GP)	1	1
Gravel pond e	GPe	Gravel pond (GP)	1	1
Gravel pond f	GPf	Gravel pond (GP)	1	1
Southern reedbed pond	SRB	Gravel pond (GP)	1	1
Blanks				
Field blanks	FB	-	0	1
Extraction blank	EB	-	1	1

Note: 2 PCR blanks were also included per primer pair



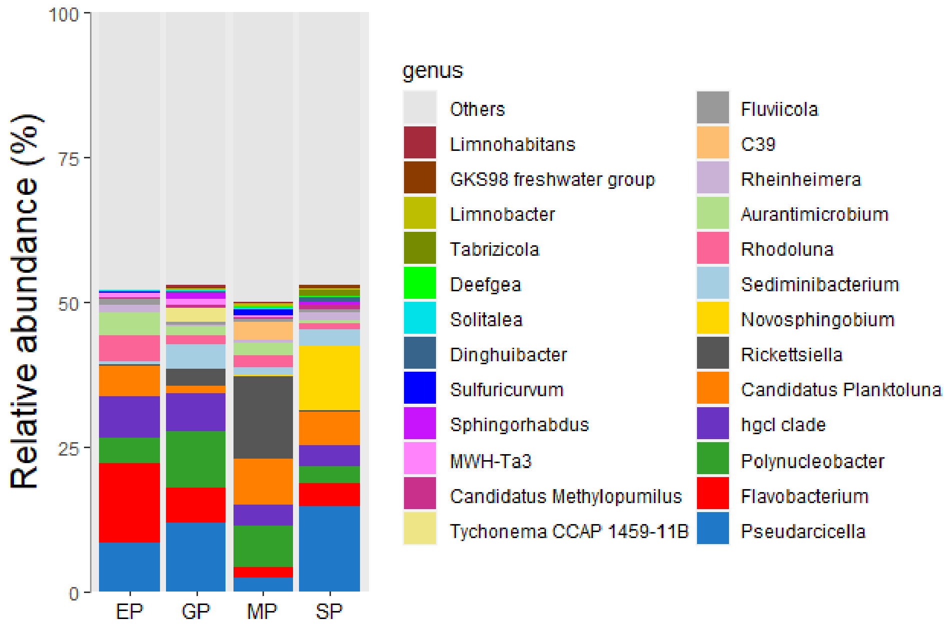
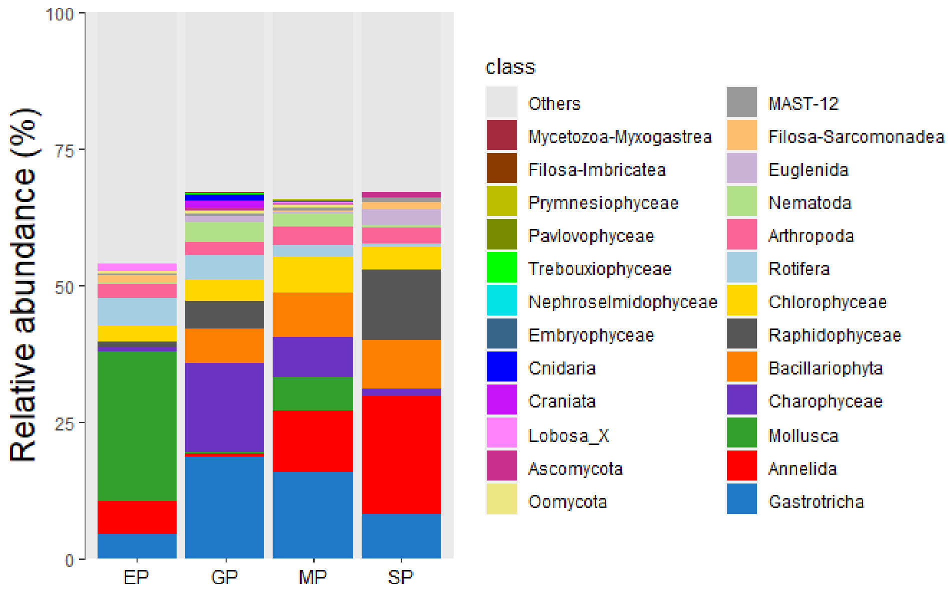


Figure S2.3: 25 most abundant bacteria and archaean genera by relative read abundance, across different pond types (x axis). Top: sediment samples (n=33), bottom: water samples (n=29).



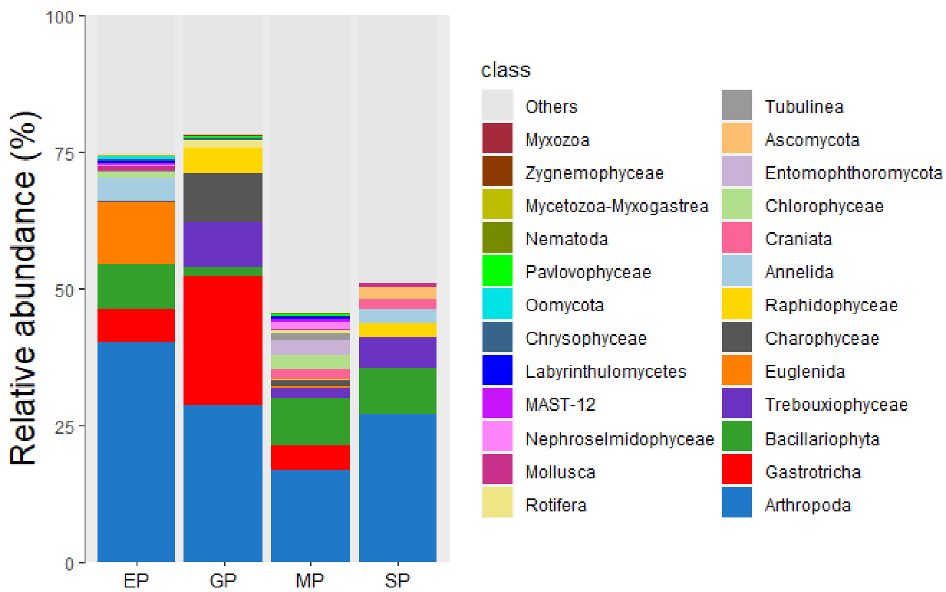


Figure S2.4: 25 most abundant Eukaryote classes by relative read abundance across (top) sediment samples (n=34) and (bottom) water samples (n=31)

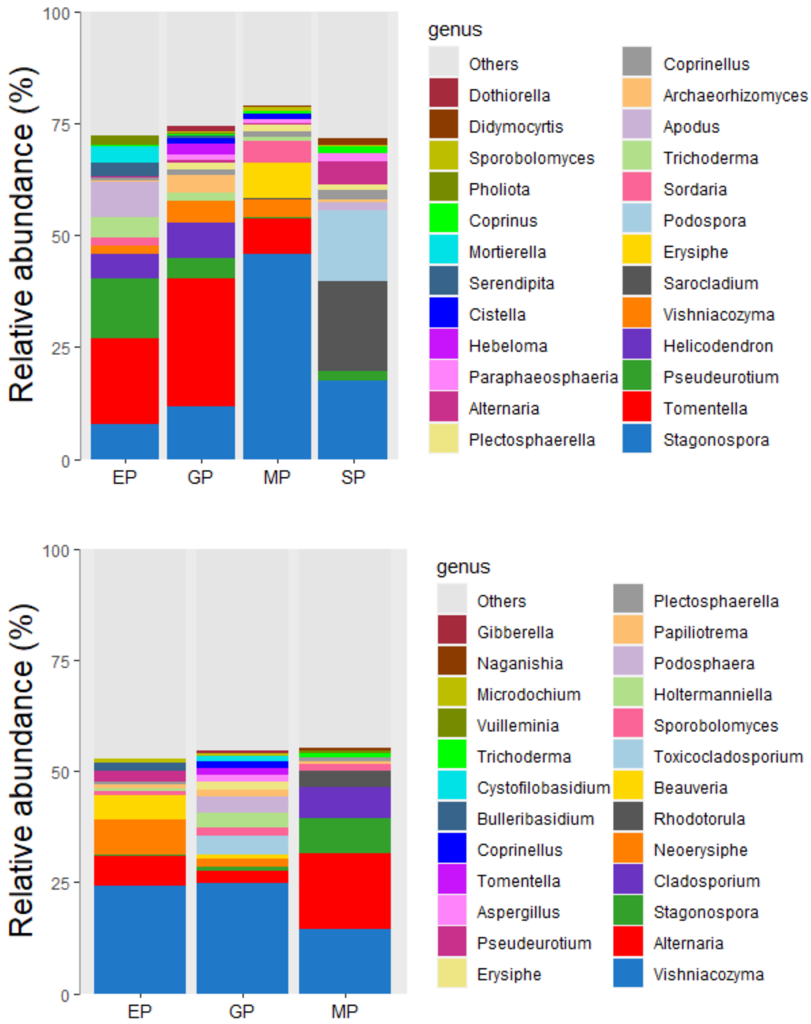


Figure S2.5: Top 25 Fungal genera by relative read abundance across different pond types, in (top) sediment samples (n=33) and (bottom) water samples (n=23).

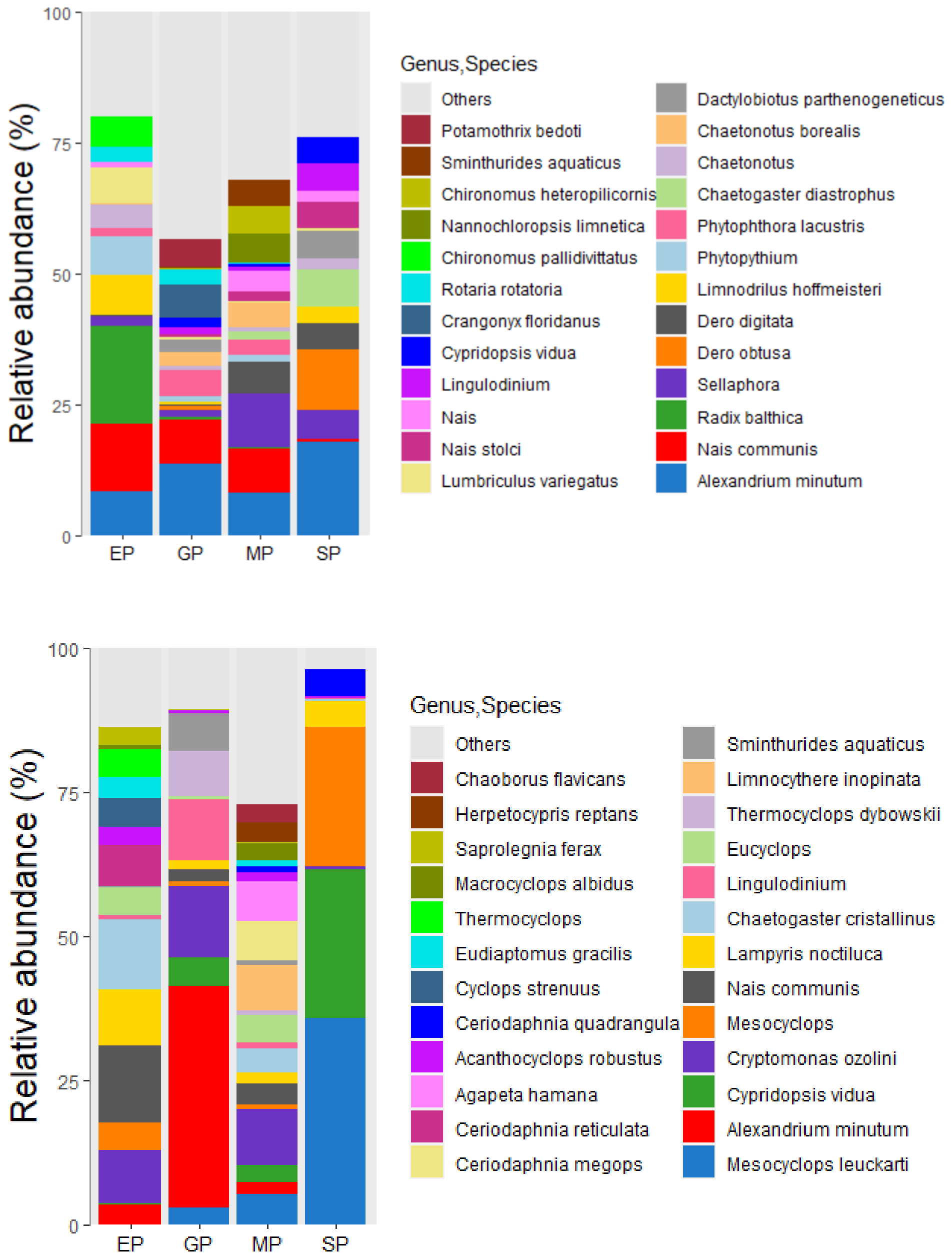


Figure S2.6: 25 most abundant animal species by relative read abundance in different pond types in (top) sediment samples (n=36) and (bottom) water samples (n=34).

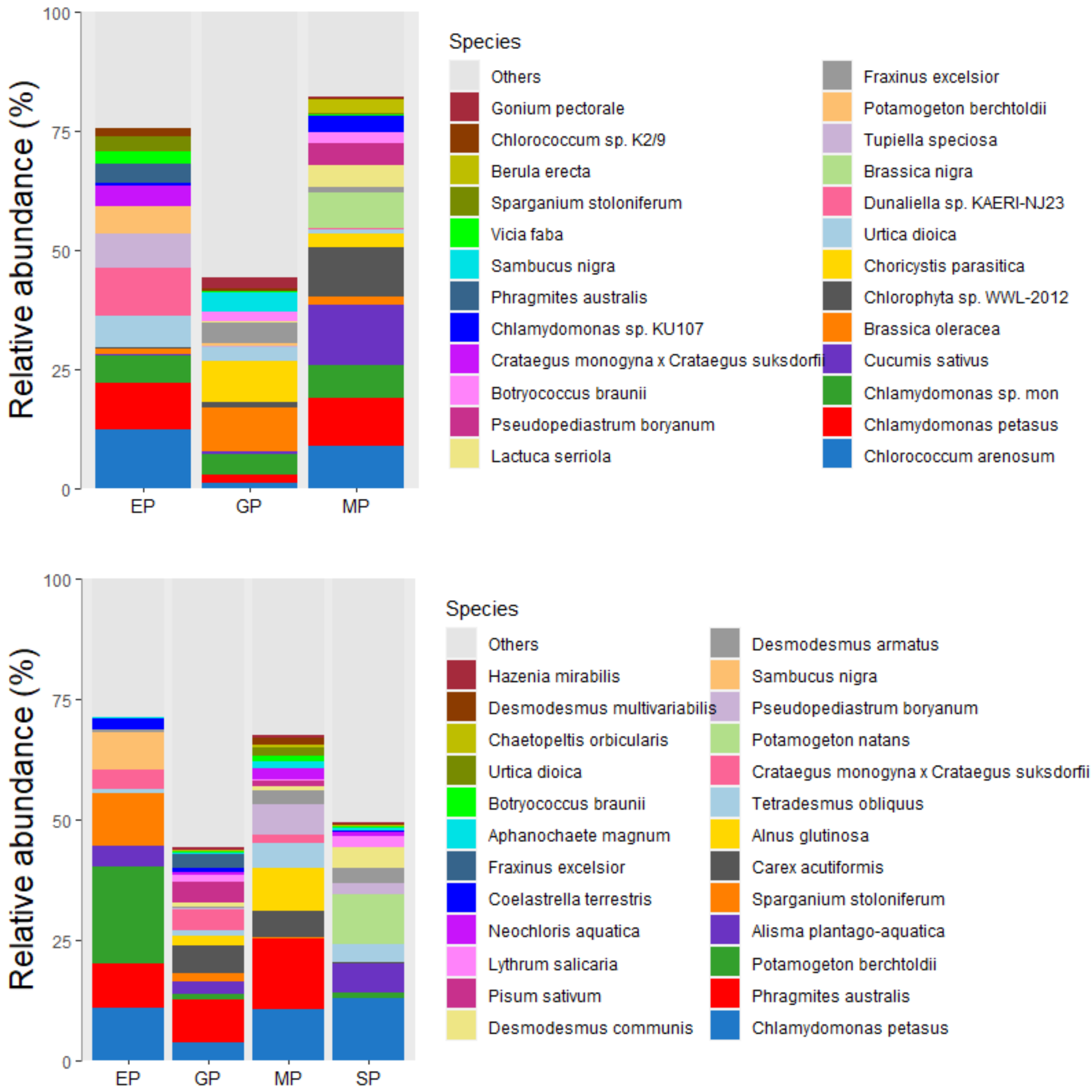


Figure S2.7: Top 25 green plant and algae species by relative read abundance across different pond types in (top) sediment samples (n=35) and (bottom) water samples (n=27).

Supplementary material: Chapter 3

Paper	**Geographic location**	**Top taxa reported (% of total read abundance)**	**Sample type**	**Primer region**
Sadeghi et. al. 2021	60 lakes, S Ontario, Canada	SAR (23%), Maxillopoda (10%), Spirotrichaea (9%) Cryptophyceae (8%) Chrysophyceae (4%)	Water (500ml per lake)	18S V9
Zheng et. al. 2020	Drinking water reservoir, China	Arthropoda (6.63% to 79.19%), Ochrophyta (5.60% to 35.16%), Ciliophora (1.81% to 10.93%), and Cryptomonadales (0.25% to 11.48%)	Water (500ml every month from 4 different depths)	18S V9
Debroas et. al. 2017	25 lakes and 4 rivers, European, Arctic and Himalayas	Chlorophyta (18.1%), Bacillariophyta (4.4%), Chrysophyceae (5.2%), Cryptomonadales (5.2%) and Dikarya (fungi) (5.5%)	Water (a variety of sampling methods)	18S V4
Banerji et. al. 2018	Lake, Ohio Canada	Copopda (55.27%), Dinoflagellata (10.2%), Cryptophyta (7.46%), Fungi (5%+), Chlorophyta (2.53%)	Water (4 sample sites, sampled for 4 months, 214 samples. 100ml from each)	18S V4
Mikhailov et. al. 2021	Lake Baikal, Siberia	Dinoflagellata (5-30%), Ciliophora (10-30%), Chlorophyta (5-30%), Chrysophyceae (2.5-30%)	Water (1 sample site, 8 time points, 200ml from 6 depths combined into 1.2l)	18S V9
Mikhailov et. al. 2018	Lake Baikal, Siberia	Dinoflagellata (11–56%), unclassified Eukaryota (3.4–45%), Chrysophyceae (4–31%),	Water (27 sample sites, 200ml from 6 depths	18S V3

		Chlorophyta (0.7–41%), Ciliophora (1.3–28%), Fungi (0.2–45%), Cryptophyta (1.6–17%).	combined into 1.2l)	
Macingo et. al. 2019	31 mountainous "pools, ponds and lakes", Greece	Chrysophyceae (10-65%), Dinophyceae (10-90%), Spirotrichaea (3-65%)	Water (1l per waterbody)	18S V3
Yi et. al. 2017	Lake Baikal, Siberia, Russia	Sediment: Chrysophyceae (10.2%), Ciliophora (9.5%) Metazoa (5.9%), Cercozoa (4.5%) Water: Chrysophyceae (13.2%), Ciliophora (10.1%), Metazoa (5.4%), Cercozoa (5.1%)	Water and sediment, 8 sites	18S V9
Mitsi et. al. 2023	Sanabria Lake, Spain	Ochrophyta (25-32.5%), Ciliophora (10-20%), Dinoflagellata (0-10%), Cryptophyta (0-10%)	Water, sediment and biofilms (10 sites)	18S V4
Pearman et. al. 2023	296 lakes across New Zealand, from 1ha – 30,000 ha	Dinophyceae (29.7%), Chlorophyceae (6.7%), Clitellata (8.0%), Ostracoda (6.5%)	Sediment, 1 site per lake	18S V4
Capo et. al. 2016	2 lakes in Greenland and France	Results from most recent layer (2000-2012): Fungi (~20%), Dinophyceae (7-20%), Chlorophyta (7-14%), Cercozoa (7-14%)	Sediment cores, ~2000 years	18S V7
Wilden, Traunspurger and Geisen 2021	Lake Ohrid, Albania/North Macedonia	Annelida (36%), Arthopoda (30%), Ochrophyta (15%), Ciliophora (11%)	Sediment, 20 samples, and periphyton, 10 samples	18S V4

Table S3.1: Review of metabarcoding studies using 18S primer region in lakes

Paper	Geographic region	Top taxa reported (% of total read abundance)	Sample type	Primer region
Cruaud et. al. 2019	Saint-Charles River, Quebec, Canada	Ciliophora (16-27%), Cryptophyceae (10-24%), Chrysophyceae (10-18%), Dinoflagellata (5-13%)	Water, 1 site, 34 dates across 1 year	18S V4
Cruaud et. al. 2020	Saint-Charles River, Quebec, Canada	Cryptophyceae (38%), Chrysophyceae (24%), Ciliopohora (12%), Fungi (5.6%)	Water, 25 sites, 11km stretch	18S V4
Li et. al. 2020	Shaying River, Henan, China	Ochrophyta (29%), Cryptophyta (27.5%), Ciliophora (11.6%), Chytridiomycota (7.5%)	Water, 18 sites across 40,000km^2 area	18S V9
Lu et. al. 2020	Upper Yangtze River, China	Cryptophyta (52.4%), Fungi (24.3%) and Alveolata (15.26%)	Water, 24 sites along 2,300km stretch	18S, region unknown
Xu et. al. 2020	Xiaoqing River, China	Ochrophyta (25-55%), Chloroplastida (10-30%), Ciliophora (2.5-20%), Cryptomonadales (0-10%)	Water, 5 sites along 240km stretch	18S V4
Yang et. al. 2022	Hangjiang River, China	Water: Ochrophyta (34.7%), Cilliophora (2-30%) Sediment: Arthropoda (2-50%), Ciliophora (2-25%)	Water and sediment at 15 sites along 625km stretch	18S V4
Xie et. al. 2016	Nanfei River, China	Ciliophora (21.6%), Annelida (14.0%), Arthropoda (11.5%), Rotifera (8.8%), Ochrophyta (8.6%), Chlorophyta (7.4%)	Sediment, 18 sites in 1446km^2 catchment	18S V9
Hindshaw, Lindsay & Boyd (2017)	Streams in Svalbard	Ciliophora (0-63%), Chlorophyta (0-48%), Basidiomycota (0-75%), Bacillariophyta (0-43%)	Sediment, 2 sites within 1km^2	18S V1-V3

Table S3.2: Review of metabarcoding studies using 18S primer region in rivers

Paper	**Geographic location**	**Top taxa reported (% total read abundance)**	**Sample type**	**Primer region**
Nakatsu et. al. 2019	Lake Michigan	"Proteobacteria (45.6 ± 5.9%), Actinobacteria (26.8 ± 8.9%), and Bacteroidetes (22.8 ± 6.1%) (mean ± SD)"	Water, 7 locations, 3 timepoints	16S V3-V4
Jiao et. al. 2021	13 urban lakes, Nanjing, China (range of trophic states)	Water: Betaproteobacteria (10-40%), Gammaproteobacteria (2-40%), Actinobacteria (4-30%), Bacteroidetes (2-30%) Sediment: Chloroflexi (10-30%), Actinobacteria (5-30%), Betaproteobacteria (5-30%), Deltaproteobacteria (5-10%)	Water and sediment, 39 sample sites, 2 timepoints	16S V4
Ruuskanen et. al. 2018	Lake Hazen, Arctic Canada	Proteobacteria (38%), Bacteroidetes (10%), Chloroflexi (7%), Actinobacteria (7%), Acidobacteria (8%)	Sediment, 4 sites, 2 timepoints	16S V3-V4
Zhang et. al. 2018	Lake Bosten, China	Lake centre: Proteobacteria (43.5 ± 9.8%), Firmicutes (19.5 ± 22.6%), and Chloroflexi (14.9 ± 1.8%) Lake edge: Firmicutes (25.0 ± 33.3%), Proteobacteria (22.4 ± 8.5%), and Chloroflexi (16.8 ± 10.3%)	Sediment, 2 sites, 2 timepoints	16S V1-V3

Liu et. al. 2020	Lake Poyang, China (seasonal lake)	Cyanobacteria (50-60%), Actinobacteria (15%), Proteobacteria (10-15%), Bacteroidetes (5-7%)	Water, 12 sites	16S V3-V4

Table S3.3: Review of metabarcoding studies using the 16S primer region in lakes

Paper	**Geographic location**	**Top taxa reported (% overall read abundance)**	**Sample type**	**Primer region**
Gweon et. al. 2019	River Thames, UK	Bacteroidetes (15-35%), Proteobacteria (~30%), Actinobacteria (5-10%), Verrucomicrobia (5%) Actinobacteria and Bacteroidetes were found in higher proportions in water than sediment/biofilm samples.	Water, sediment, biofilm, 12 sites	16S V3-V4
Read et. al. 2015	River Thames, UK	Actinobacteria (12.5-65%), Bacteroidetes (10-80%), Proteobacteria (12.5-50%), Verrucomicrobia (5-10%)	Water, 23 sites	16S V1-V3
Doherty et. al. 2017	Amazon River, Brazil	Actinobacteria (25.8%), Other Alphaproteobacteria (10-30%), Bacteroidetes (5-20%)	Water, 5 sites, 2 depths, 3 timepoints	16S V1-V2
Li et. al. 2020	Shayang River, China	Proteobacteria (28%), Bacteroidetes (24%), Cyanobacteria (21%), Actinobacteria (14%), Verrucomicrobia (7%)	Water, 18 sites across 40,000km^2 area	16S V3
Cruaud et. al. 2020	Saint-Charles River, Quebec, Canada	Actinobacteria (35.8%), Bacteroidetes (17.6%), Betaproteobacteria (15.8%), Verrucomicrobia (11.7%)	Water, 25 sites, 11km stretch	16S V3-V4

Liu et. al. 2018	Yangtze River, China	Water: Proteobacteria (15-90%), Actinobacteria (2.5-70%), Bacteroidetes (2.5-75%) Sediment: Proteobacteria (30-70%), Bacteroidetes (2-60%), Acidobacteria (1-20%), Chloroflexi (1-10%), Actinobacteria (1-5%)	Water and sediment, 50 sites, 4300km stretch, 2 timepoints	16S V4-V5
Wu et. al. 2019	Jinchuan River, China (urban river, heavily polluted)	Proteobacteria (42.28%), Chloroflexi (13.95%), Acidobacteria (8.94%), Bacteroidetes (7.80%), and Firmicutes (5.58%)	Sediment, 16 sample sites	16S V4
Gibbons et. al. 2014	Tongue River, Montana, USA	Proteobacteria (51%), Acidobacterium (6.7%), Bacteroidetes (6%), Planctomycetes (5%)	Sediment, 6 sites along 134km stretch, 4 timepoints	16S V4
Yuan et. al. 2023	Lacang River, Yunnan, China (heavily dammed for hydropower)	Proteobacteria (25-55%), Actinobacteria (5-40%), Acidobacteria (5-15%), Chloroflexi (7-30%)	Sediment, 15 sites, 750km reach, 2 timepoints	16S V3-V4
Liu et. al. 2022	Yarlung Tsangpo River, Tibet	Proteobacteria (40%), Bacteroidetes (19%), Firmicutes (17%), Actinobacteria (14%)	Sediment, 81 sites across ~1500km stretch	16S V4-V5

Table S3.4: Review of metabarcoding studies using the 16S primer region in rivers

16S Step 1	18S Step 1	ITS2 Step 1	Step 2

95'C 2:00 95'C 0:15 + 50'C 0:30 + 72'C 0:30 + x 30 72'C 10:00	95'C 2:00 95'C 0:15 + 60'C 0:30 + 72'C 0:30 + X 30 72'C 10:00	95'C 2:00 95'C 0:15 + 55'C 0:30 + 72'C 0:30 + X 35 72'C 10:00	95'C 2:00 95'C 0:15 + 55'C 0:30 + 72'C 0:30 x 8 72'C 10:00

Table S3.5: PCR conditions for all primer pairs

Date	Hydrological connectivity	Thames River level at Farmoor (m)
22/01/2020	Site flooded. MP, SPP, SWP, GPs, SRB and GWP all one continuous waterbody. EPs 1-4 one waterbody, EPs 5-7 another. Some parts icy.	1.006
17/03/2020	All ponds separate, as they are in Chapter 3, Fig 1	1.047
22/07/2020	All ponds separate, as they are in Fig 1. No water in EP2, EP3, EP4 or MP17.	0.938
30/09/2020	All ponds separate, as they are in Fig 1. No water in EP2, EP3, EP4, MP1 or MP2.	0.910
25/11/2020	All ponds separate, as they are in Fig 1.	0.923

Table S3.6: Hydrological conditions from observation and River Thames water level on sampling dates. Water level data provided by an Environment Agency gauge and extracted from https://riverlevels.uk/thames-stanton-harcourt-farmoor on 9th February 2022

Pond name	**Sample code**	**Pond type**	**No. Sediment samples**	**No. Water samples**
Main pond	MP	Main pond (MP)	17	18 (17 separate, 1 merged)
Semi-permanent pond	SPP	Surface water pond (SP)	2	1 (merged)
Surface water pond	SWP	Surface water pond (SP)	3	1 (merged)

Experimental pond 1	EP1	Experimental pond (EP)	1	1
Experimental pond 2	EP2	Experimental pond (EP)	1	1
Experimental pond 3	EP3	Experimental pond (EP)	1	1
Experimental pond 4	EP4	Experimental pond (EP)	1	1
Experimental pond 5	EP5	Experimental pond (EP)	1	1
Experimental pond 6	EP6	Experimental pond (EP)	1	1
Experimental pond 7	EP7	Experimental pond (EP)	1	1
Groundwater pond	GWP	Gravel pond (GP)	2	1 (merged)
Gravel pond 1	GP1	Gravel pond (GP)	1	1
Gravel pond 2	GP2	Gravel pond (GP)	1	1
Gravel pond 3	GP3	Gravel pond (GP)	1	1
Gravel pond a	GPa	Gravel pond (GP)	1	1
Gravel pond b	GPb	Gravel pond (GP)	1	1
Gravel pond c	GPc	Gravel pond (GP)	1	1
Gravel pond d	GPd	Gravel pond (GP)	1	1
Gravel pond e	GPe	Gravel pond (GP)	1	1
Gravel pond f	GPf	Gravel pond (GP)	1	1
Southern reedbed pond	SRB	Gravel pond (GP)	1	1
Blanks				
Field blanks	FB	-	0	1
Extraction blank	EB	-	1	1

Note: 2 PCR blanks were also included per primer pair, per sampling event.

Table S3.9 Number of sediment and water samples collected from Pinkhill Meadow in each sample event (Jan, Mar, July, Sept, Nov).

Image S3.1: 22nd Jan 2020

Image S3.2: 17th March 2020

Image S3.3: 22nd July 2020

Image S3.4: 28th September 2020

Image S3.5: 25th November 2020

Images S3.1-3.5: Representative photos of climatic, hydrological and vegetation conditions at the site on sampling days. The two poles (Osprey platforms) provide a reference point. Image S3.5 is taken from the vantage point of the pole in image S3.4.

Measurement	**Df**	**F**	**p value**
Temperature (°C)	4	493.6	<0.001 ***
Conductivity (µS/cm)	4	7.34	<0.001 ***
Total Dissolved Solids (ppm)	4	5.38	<0.001 ***
pH	4	13.9	<0.001 ***
Oxidative-Reductive Potential (mV)	4	158.6	<0.001 ***

Table S3.7: Results of ANOVAs of abiotic variables by sample month for abiotic variables

Measurement	**Df**	**Kruskal-Wallis Chi Squared**	**P value**
Temperature	3	5.35	0.148
Conductivity	3	10.51	0.015 *
TDS	3	13.46	0.0037 **
pH	3	44.56	<0.001 ***
ORP	3	0.95	0.813

SRP	3	11.44	0.0096 **
TDN	3	20.0	<0.001 ***
NH4	3	12.91	0.0048 **
Chlorophyll	3	12.01	0.007 **
DOC	3	22.46	<0.001 ***
TP	3	4.68	0.197
SS	3	2.97	0.397

Table S3.8: Results of Kruskal-Wallis tests of chemical and abiotic variables by pond type

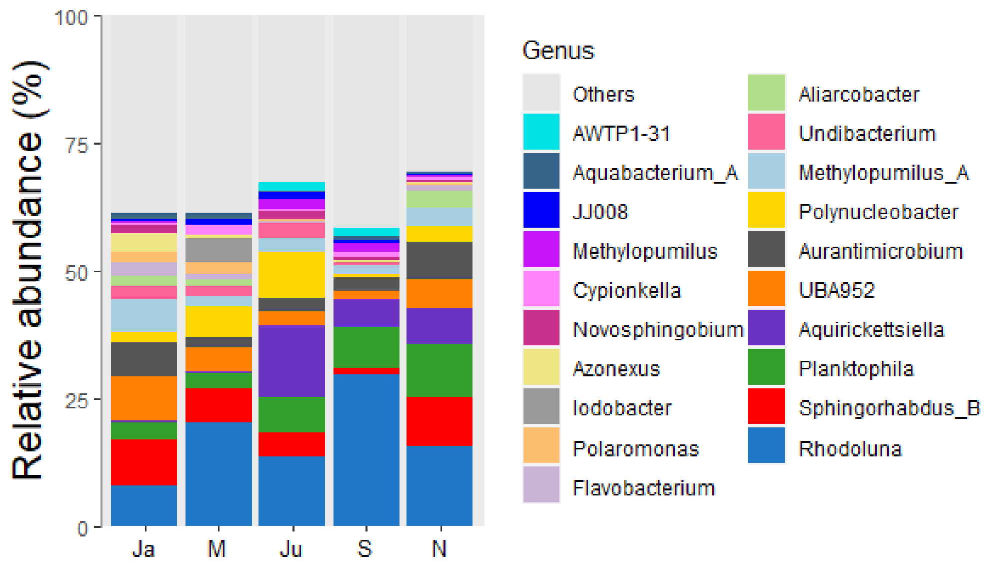


Figure S3.1 a) Top 20 bacterial and archaean genera by read abundance per month in water samples, n=120

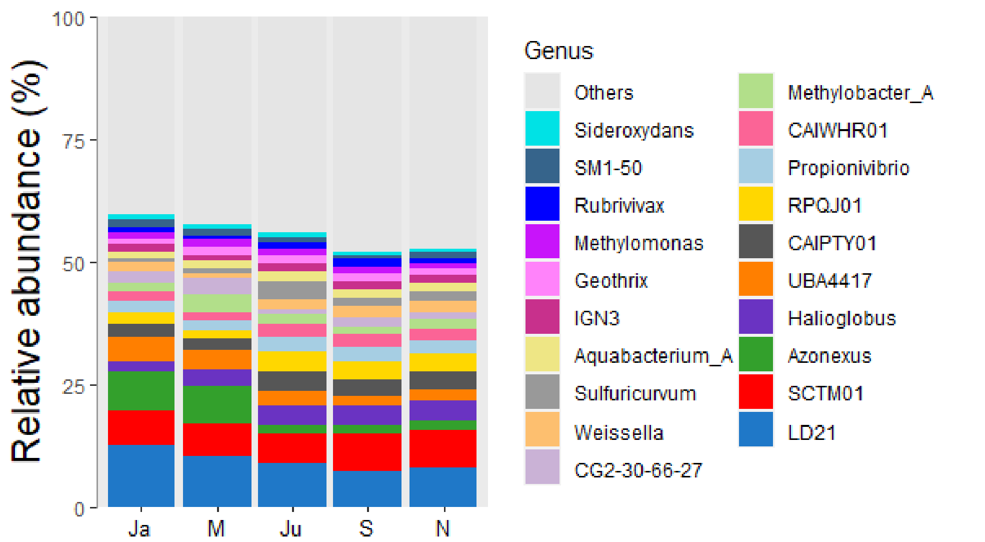


Figure S3.1 b) Top 20 bacterial and archaean genera by read abundance per month in sediment samples, n=160

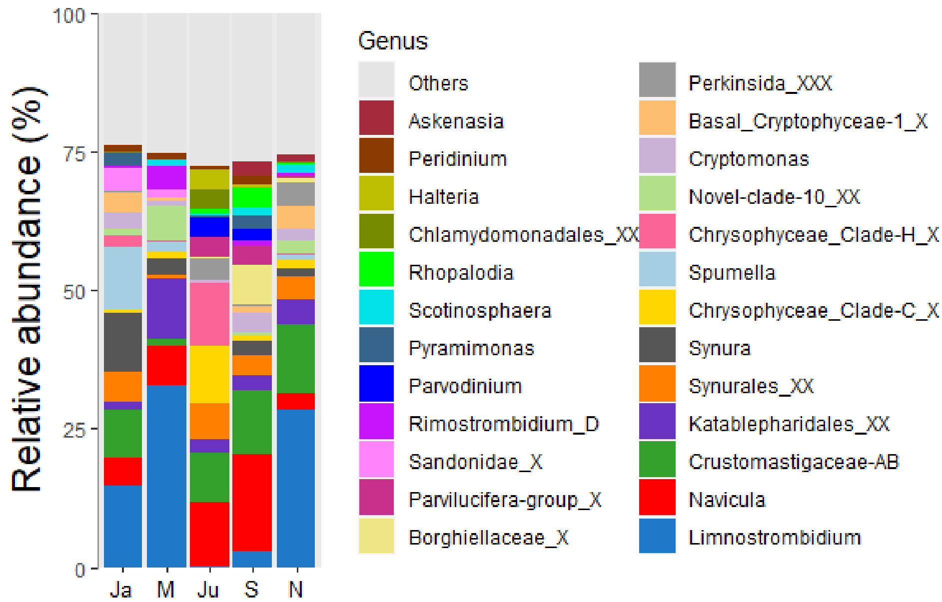


Figure S3.2 a) Top 25 microbial eukaryote genera by read abundance per month in water samples, n=149

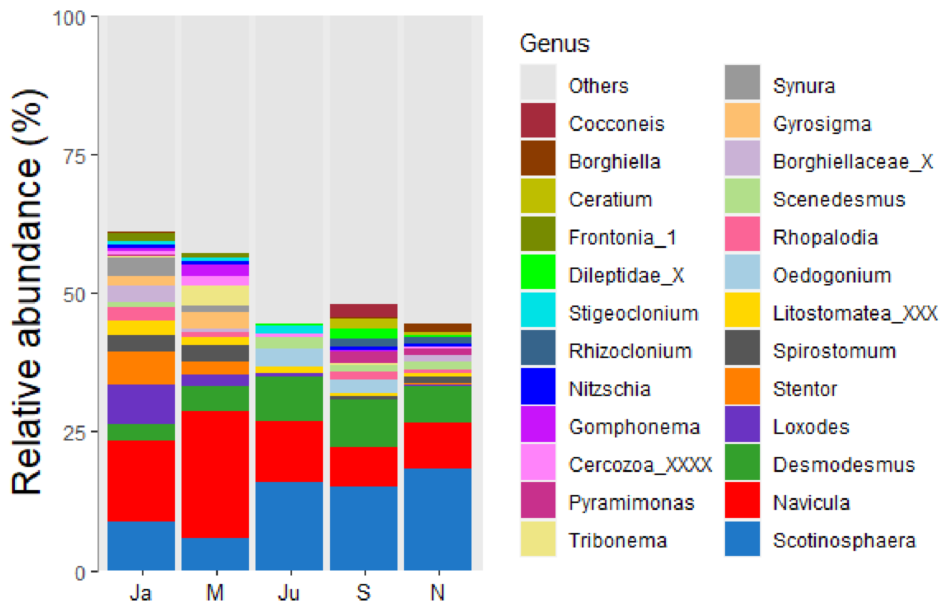


Figure S3.2 b) Top 25 microbial eukaryote genera by read abundance per month in sediment samples, n=145

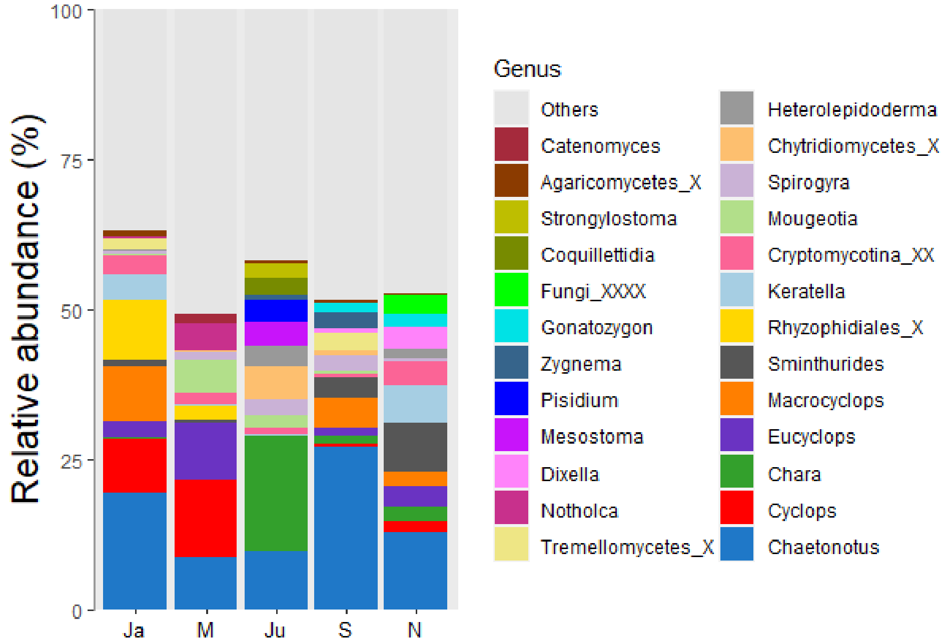


Figure S3.3 a) Top 25 multicellular eukaryote genera in water samples by read abundance per month n=144

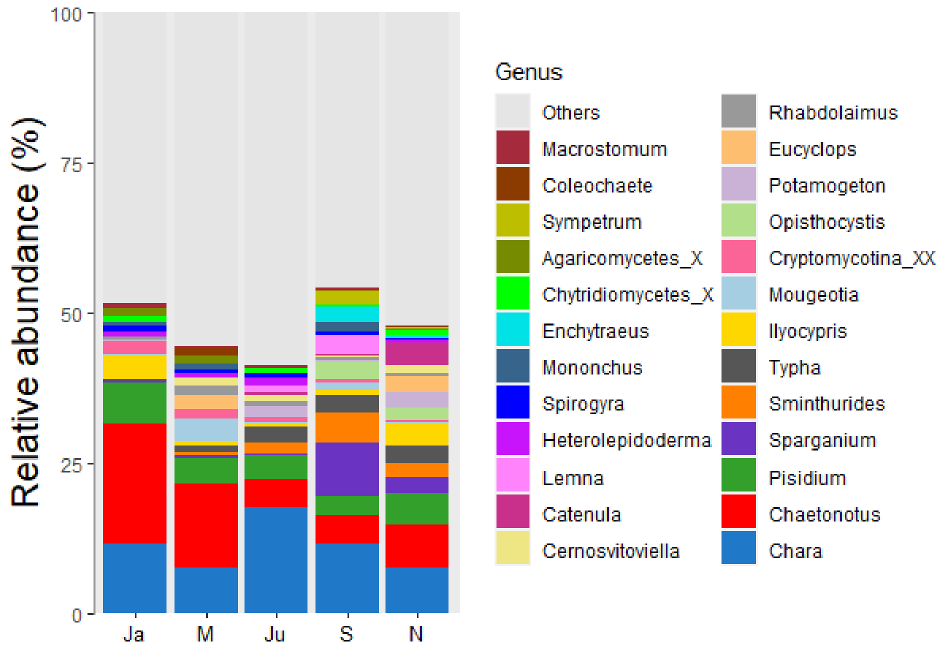


Figure S3.3 b) Top 25 multicellular eukaryote genera in sediment samples by read abundance per month n=147

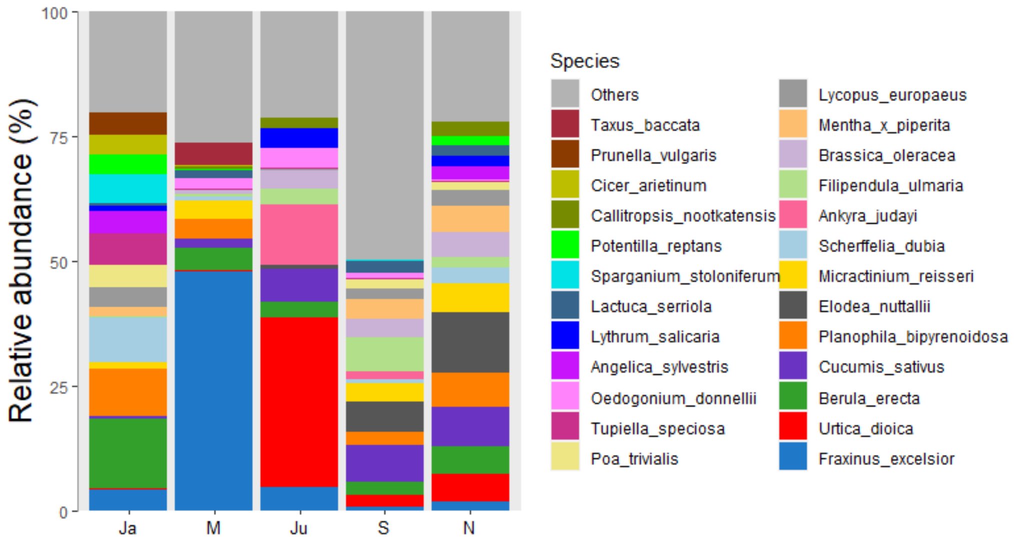


Figure S3.4 a) Top 25 higher plant and green algae species in water samples by read abundance per month n=143

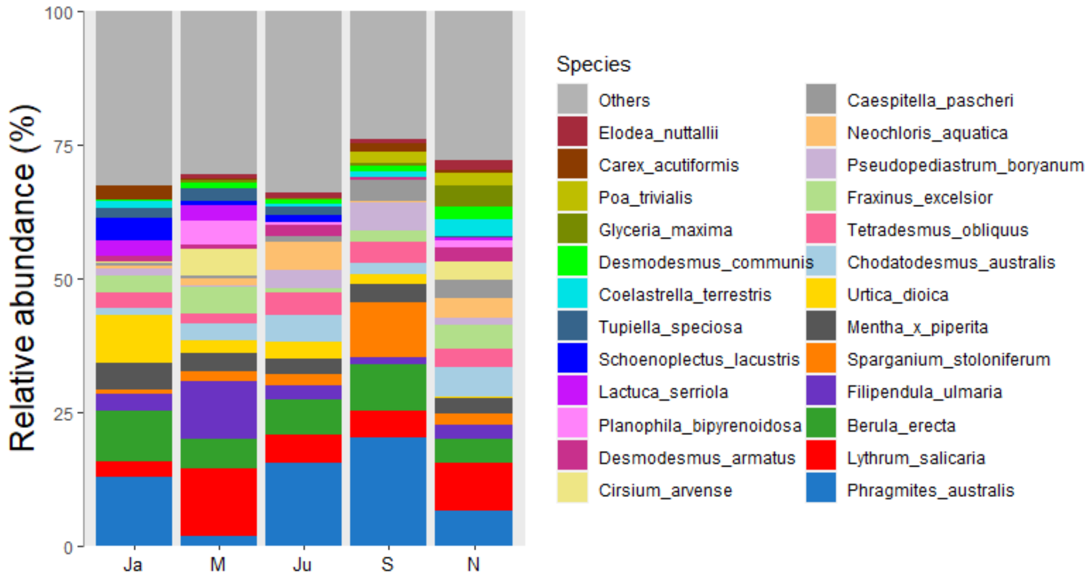


Figure S3.4 b) Top 25 higher plant and green algae species in sediment samples by read abundance per month n=180

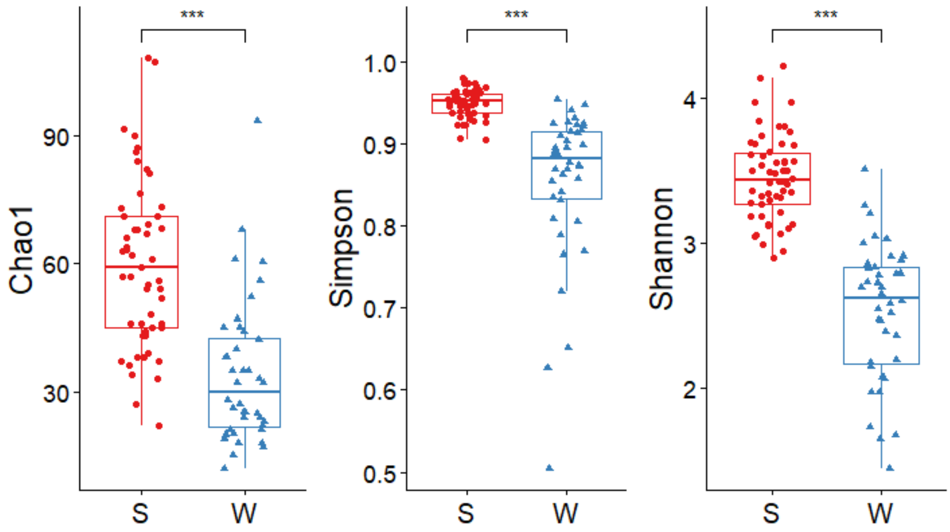


Figure S3.5 a) Boxplots of Chao, Simpson and Shannon indices of alpha diversity of bacteria and archaea communities with t test comparison. Red = sediment samples (n=140), blue = water samples (n=120). One, two or three asterisks are visual representations of p values below 0.05, 0.01 and 0.001 respectively.

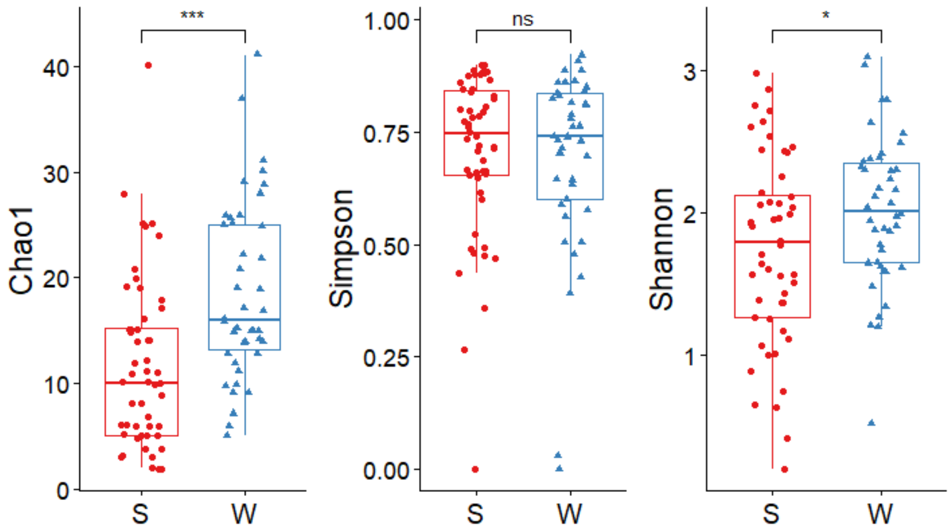


Figure S3.5 b) Boxplots of Chao, Simpson and Shannon indices of alpha diversity of microbial eukaryote communities with t test comparison. Red = sediment samples (n=140), blue = water samples (n=120). One, two or three asterisks are visual representations of p values below 0.05, 0.01 and 0.001 respectively, ns = "not significant".

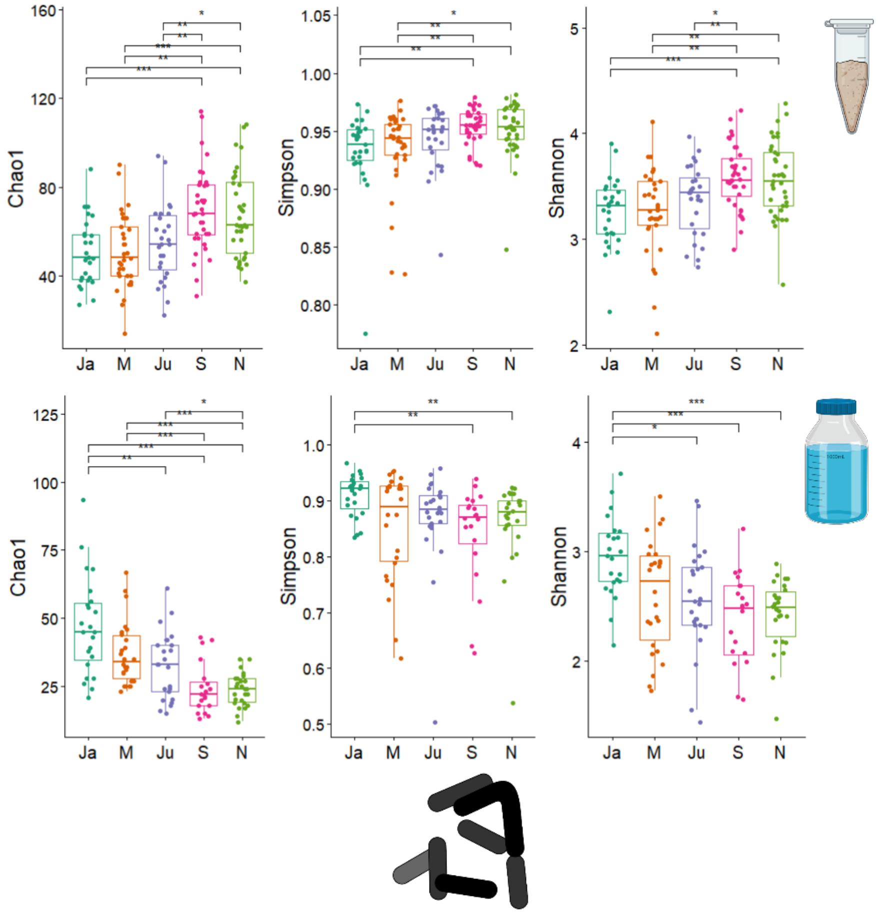


Figure S3.6: Boxplots of Chao, Simpson and Shannon indices of alpha diversity of bacteria communities with Wilcoxon rank-sum comparison. Top: sediment samples, bottom: water samples. Dark green = January (n=79), orange = March (n=85), purple = June (n=85), pink = September (n=83), light green = November (n=87). One, two or three asterisks are visual representations of p values below 0.05, 0.01 and 0.001 respectively.

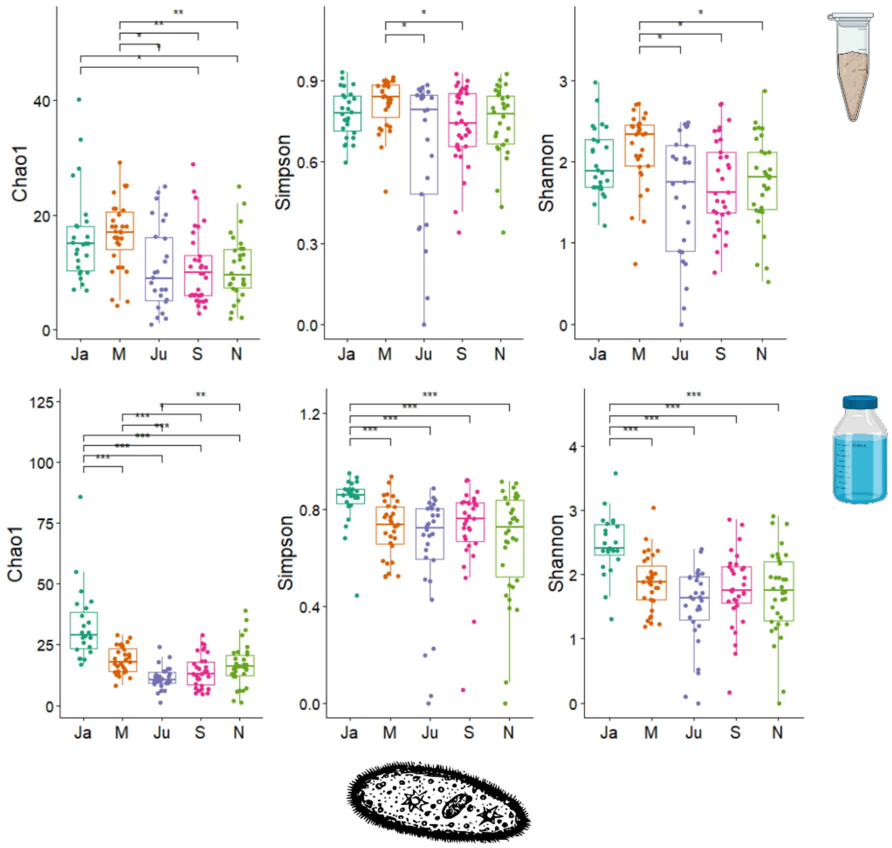


Figure S3.7: Boxplots of Chao, Simpson and Shannon indices of alpha diversity of microbial eukaryote communities with Wilcoxon rank-sum comparison. Top: sediment samples, bottom: water samples. Dark green = January (n=79), orange = March (n=85), purple = June (n=85), pink = September (n=83), light green = November (n=87). One, two or three asterisks are visual representations of p values below 0.05, 0.01 and 0.001 respectively.

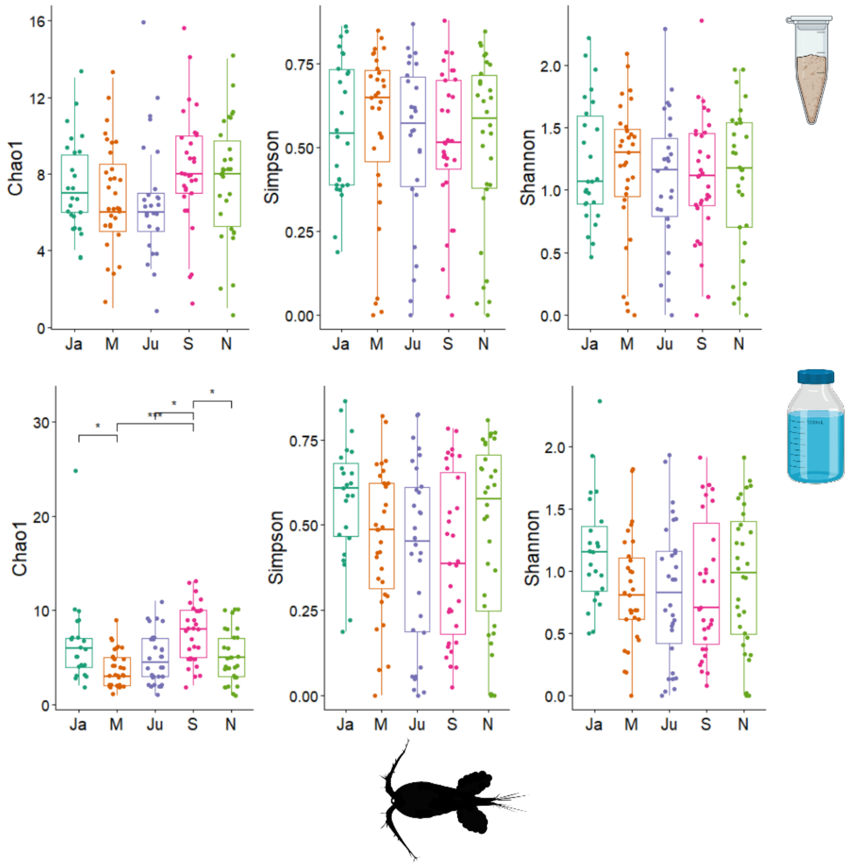


Figure S3.8: Boxplots of Chao, Simpson and Shannon indices of alpha diversity of multicellular eukaryote communities with Wilcoxon rank-sum comparison. Top: sediment samples, bottom: water samples. Dark green = January (n=79), orange = March (n=85), purple = June (n=85), pink = September (n=83), light green = November (n=87). One, two or three asterisks are visual representations of p values below 0.05, 0.01 and 0.001 respectively.

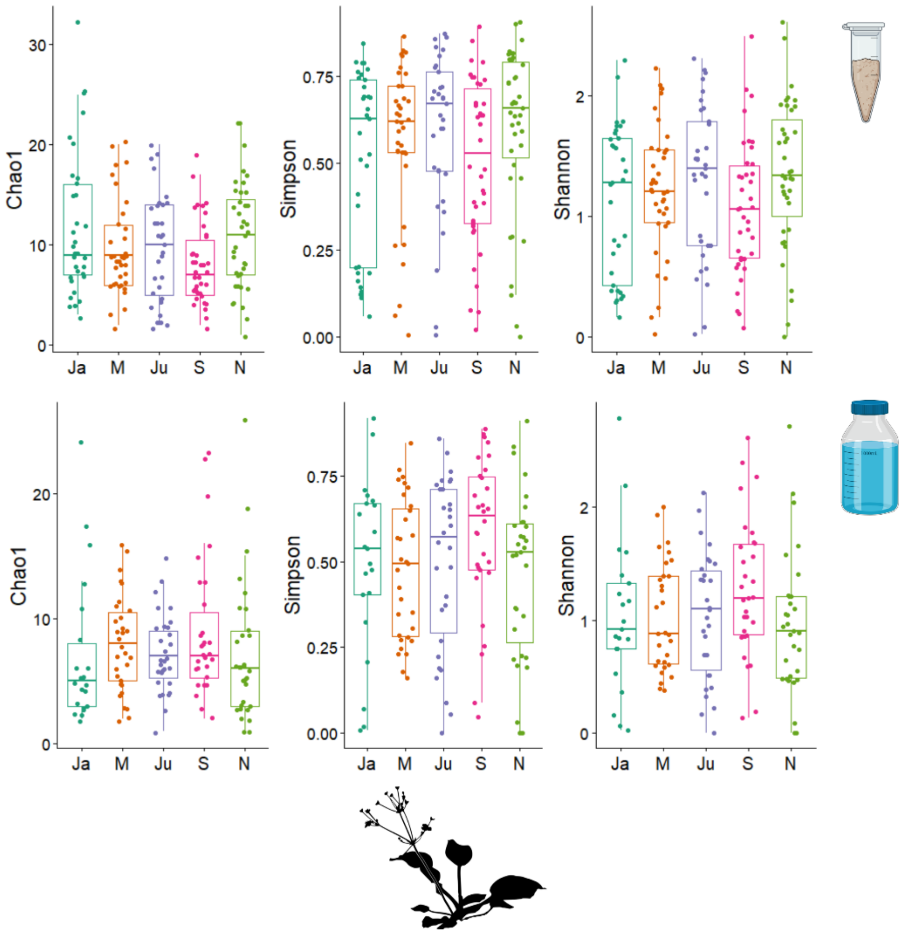
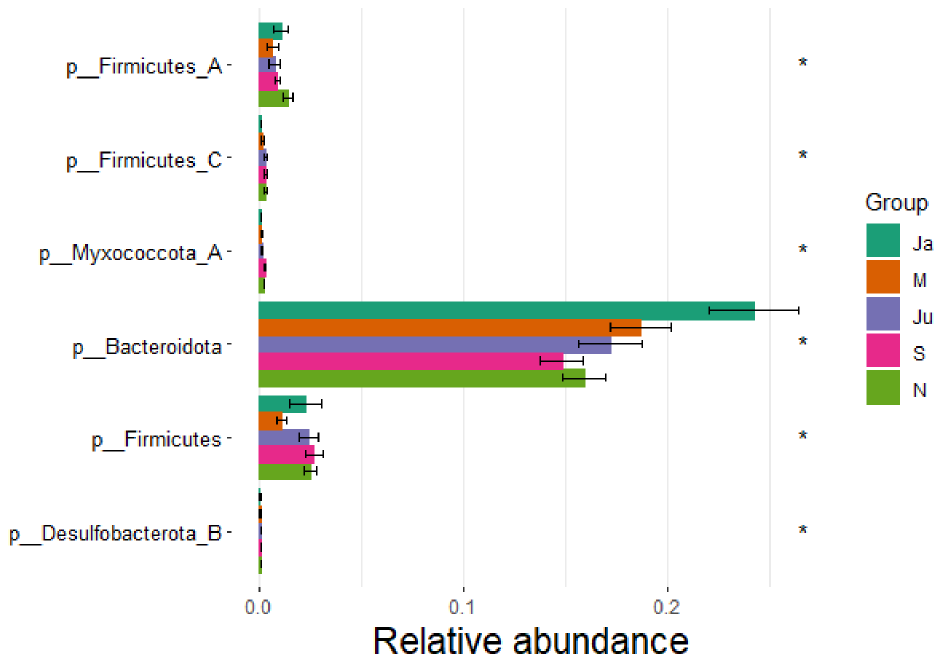
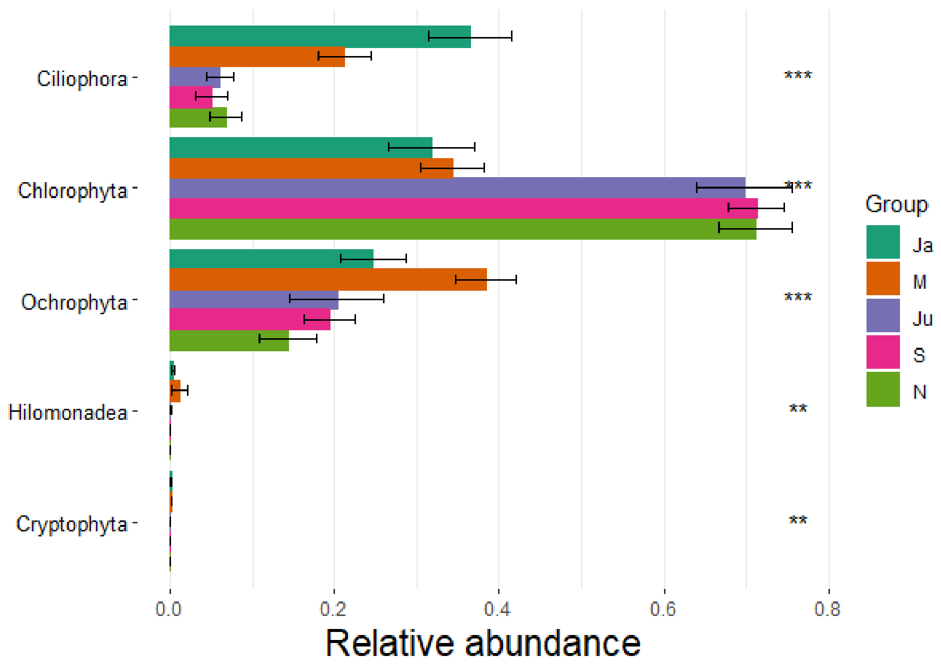
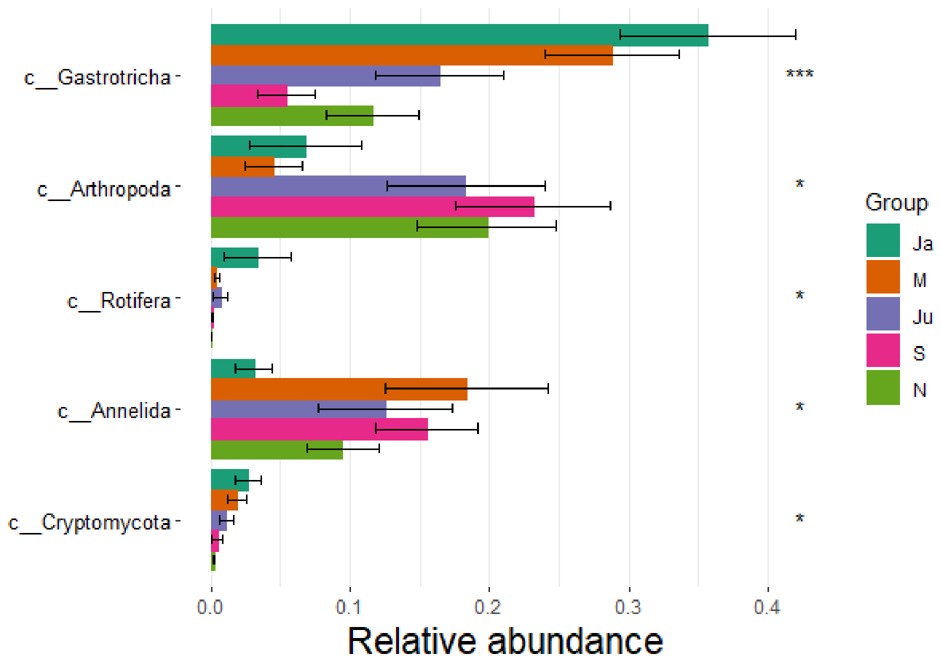


Figure S3.9: Boxplots of Chao, Simpson and Shannon indices of alpha diversity of bacteria communities with Wilcoxon rank-sum comparison. Top: sediment samples, bottom: water samples. Dark green = January (n=79), orange = March (n=85), purple = June (n=85), pink = September (n=83), light green = November (n=87). One, two or three asterisks are visual representations of p values below 0.05, 0.01 and 0.001 respectively.


p__Firmicutes_A
p__Firmicutes_C
p__Myxococcota_A
p__Bacteroidota
p__Firmicutes
p__Desulfobacterota_B
*
*
*
*
*
*
0.0
0.1
0.2
Relative abundance
Group
Ja
M
Ju
S
N


Ciliophora
Chlorophyta
Ochrophyta
Hilomonadea
Cryptophyta
***
***
***
**
**
0.0
0.2
0.4
0.6
0.8
Relative abundance
Group
Ja
M
Ju
S
N



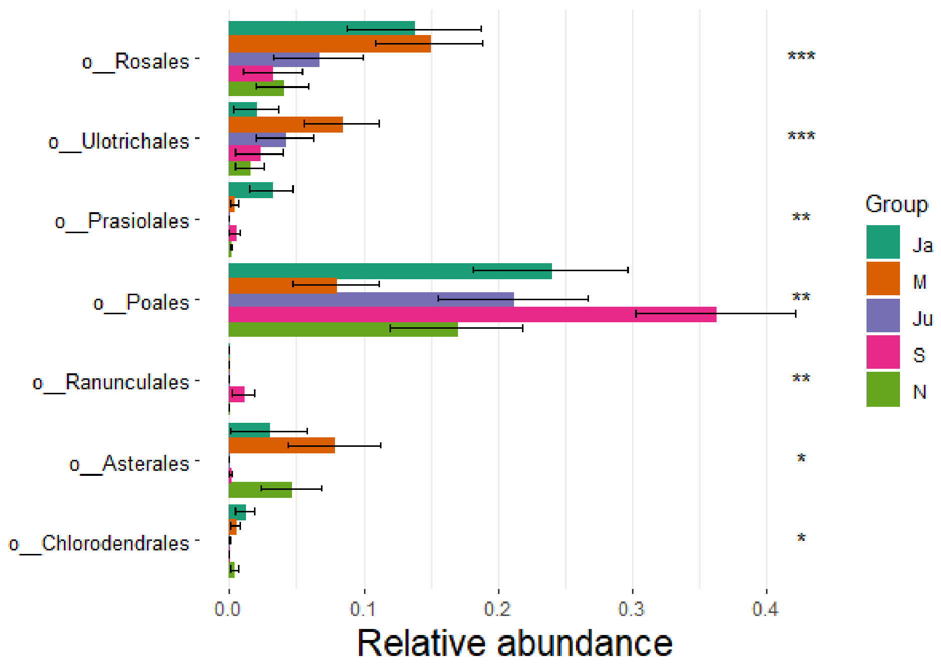
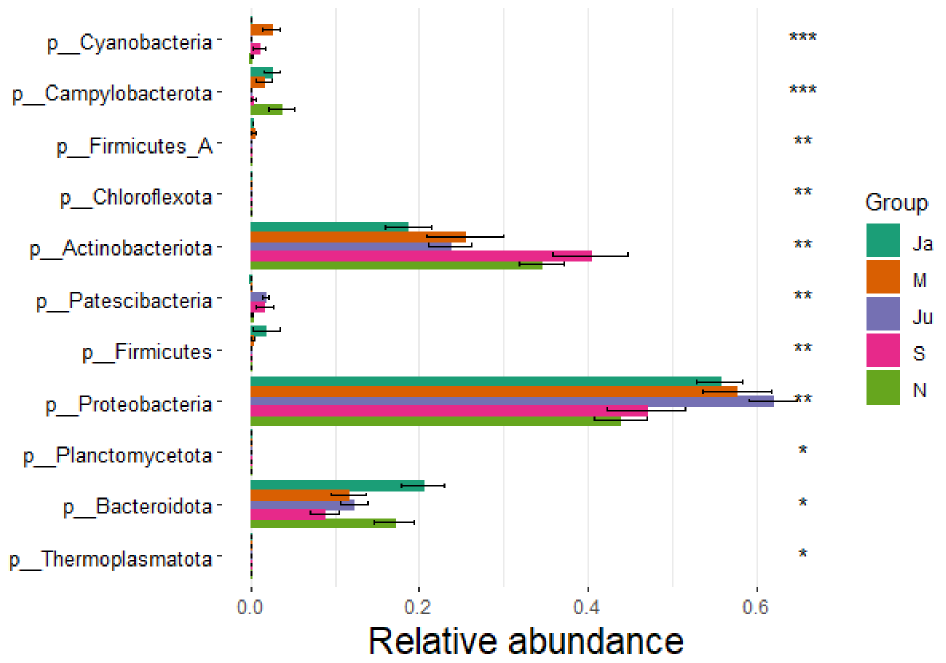
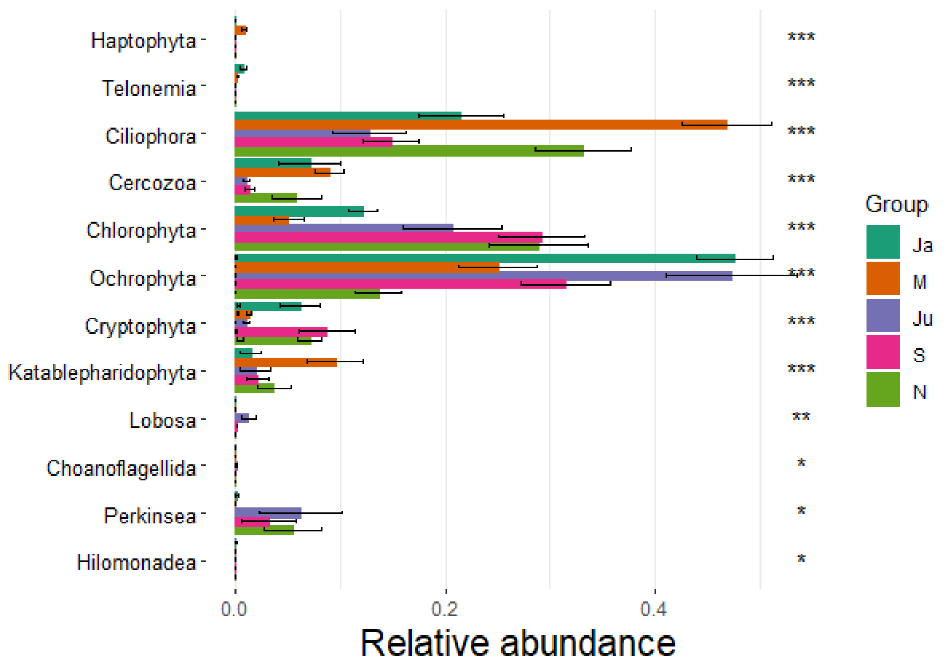
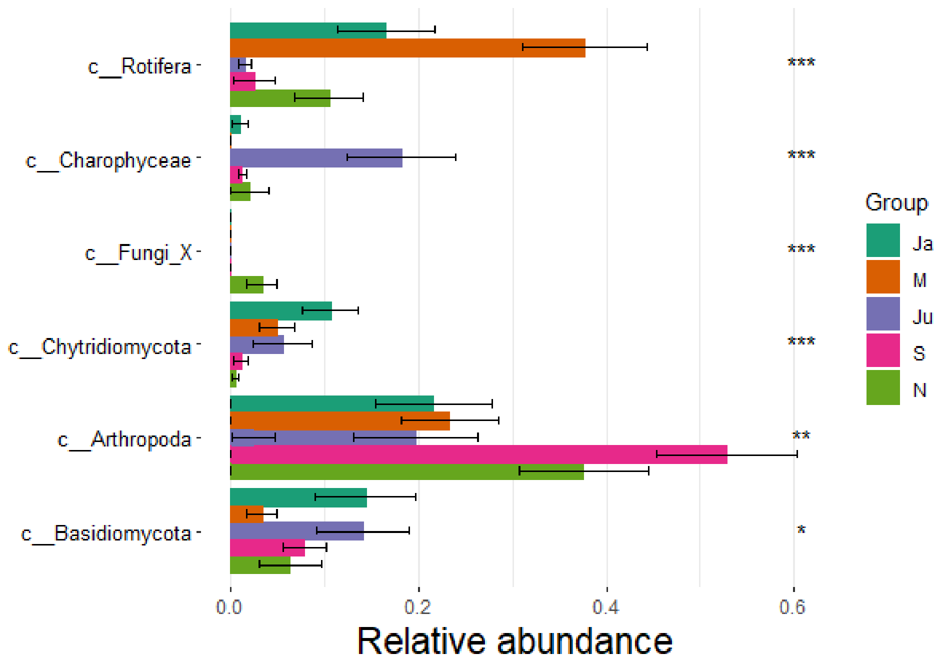


Figure S3.10: Relative abundances (by read abundance) in pond sediments of 1. Prokaryotic phyla, 2. Microbial eukaryote phyla 3. Multicellular eukaryote phyla and 4. Green plant and algal orders over different sample months. Multiple Kruskal-Wallis tests with fdr p value correction, 1, 2 or 3 asterisks indicate significance at the p<0.05, p<0.01 and p<0.001 levels respectively.


p__Cyanobacteria
p__Campylobacterota
p__Firmicutes_A
p__Chloroflexota
p__Actinobacteriota
p__Patescibacteria
p__Firmicutes
p__Proteobacteria
p__Planctomycetota
p__Bacteroidota
p__Thermoplasmatota
***
***
**
**
**
**
**
**
*
*
*
0.0
0.2
0.4
0.6
Relative abundance
Group
Ja
M
Ju
S
N


Haptophyta
Telonemia
Ciliophora
Cercozoa
Chlorophyta
Ochrophyta
Cryptophyta
Katablepharidophyta
Lobosa
Choanoflagellida
Perkinsea
Hilomonadea
***
***
***
***
***
***
***
***
**
*
*
*
0.0
0.2
0.4
Relative abundance
Group
Ja
M
Ju
S
N



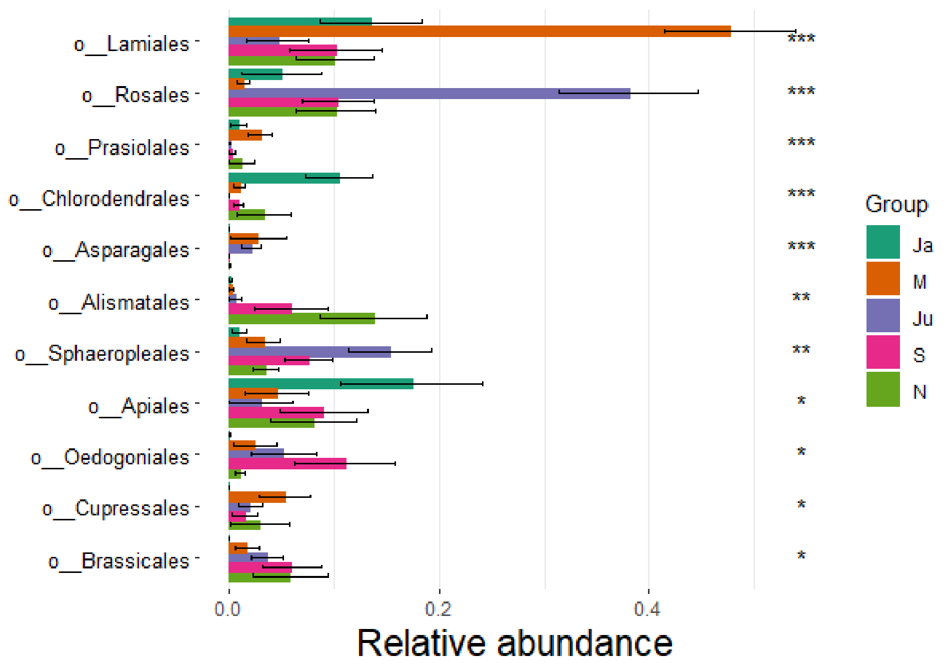


Figure S3.10: Relative abundances (by read abundance) in pond water of 1. Prokaryotic phyla, 2. Microbial eukaryote phyla 3. Multicellular eukaryote phyla and 4. Green plant and algal orders over different sample months. Multiple Kruskal-Wallis tests with fdr p value correction, 1, 2 or 3 asterisks indicate significance at the p<0.05, p<0.01 and p<0.001 levels respectively.

Supplementary material: Chapter 4

Figure S4.1: Read abundance of higher plant taxa (n=74) of different wetland functions (aquatic, wetland or terrestrial) within each pond. Left: sediment samples (n=31) and right: water samples (n=31).

16S Step 1	18S Step 1	ITS2 Step 1	Step 2
95’C 2:00	95’C 2:00	95’C 2:00	95’C 2:00
95’C 0:15 +	95’C 0:15 +	95’C 0:15 +	95’C 0:15 +
50’C 0:30 +	57’C 0:30 +	55’C 0:30 +	55’C 0:30 +
72’C 0:30 +	72’C 0:30 +	72’C 0:30 +	72’C 0:30
x 30	X 30	X 35	x 8
72’C 10:00	72’C 10:00	72’C 10:00	72’C 10:00

Table S4.1: PCR conditions for all primer pairs

Genus	Species	Common name	Habitat	Growth form	Cultivated?	Native?	Scarce?
Acer	Acer campestre	Field maple	Terrestrial	Tree			
Acer	Acer platanoides	Norway maple	Terrestrial	Tree		Non-native	
Aesculus	Aesculus hippocastanum	Horse chestnut	Terrestrial	Tree			
Agrostis	Agrostis capillaris	Common bent	Terrestrial				
Alisma	Alisma plantago-aquatica	Water plantain	Wetland				
Allium	Allium cepa	Onion	Terrestrial		Culivated		
Alnus	Alnus glutinosa	Alder	Wetland	Tree			
Alnus	Alnus glutinosa	Alder	Terrestrial	Tree			
Alopecurus	Alopecurus myosuroides	Slender meadow-foxtail	Terrestrial				
Arrhenatherum	Arrhenatherum elatius	False oat-grass	Terrestrial				
Bellis	Bellis perennis	Common daisy	Terrestrial				
Berula	Berula erecta	Lesser water parsnip	Wetland				
Betula		Birch	Terrestrial	Tree			
Brassica	Brassica oleracea	Cabbage/wild mustard	Terrestrial		Culivated		
Callitriche	Callitriche brutia	Pendunculate water starwort	Aquatic				
Callitriche	Callitriche obtusangula	Blunt-fruited water starwort	Aquatic				
Calystegia	Calystegia sepium	Hedge bindweed	Terrestrial				
Carpinus	Carpinus betulus	Hornbeam	Terrestrial	Tree			
Ceratophyllum	Ceratophyllum demersum	Hornwort	Aquatic				
Ceratophyllum	Ceratophyllum platyacanthum	Hornwort	Aquatic				
Ceratophyllum		Hornwort	Aquatic				
Conium	Conium maculatum	Hemlock	Wetland				
Crataegus	Crataegus monogyna x Crataegus suksdorfii	Hawthorn	Terrestrial	Tree			
Cucumis	Cucumis sativus	Cucumber	Terrestrial		Cultivated		
Dactylis	Dactylis glomerata	Cocksfoot grass	Terrestrial				
Epilobium		Willowherb	Wetland				
Eupatorium	Eupatorium cannabinum	Hemp agrimony	Wetland				
Filipendula	Filipendula ulmaria	Meadowsweet	Wetland				
Fraxinus	Fraxinus excelsior	Ash	Terrestrial	Tree			
Galium	Galium aparine	Cleavers	Terrestrial				
Glyceria	Glyceria notata	Plicate sweet grass	Wetland				
Glyceria	Glyceria fluitans	Floating sweet-grass	Wetland				
Glyceria	Glyceria maxima	Reed sweet-grass	Wetland				
Hedera	Hedera helix	Common ivy	Terrestrial				
Helosciadium	Helosciadium nodiflorum	Fool's watercress	Wetland				
Heracleum	Heracleum sphondylium	Hogweed	Terrestrial				
Hesperocyparis	Hesperocyparis arizonica	Arizona cypress	Terrestrial	Tree		Non-native	
Holcus	Holcus lanatus	Yorkshire fog	Terrestrial				
Juglans	Juglans regia	Walnut	Terrestrial	Tree			
Lolium	Lolium perenne	Perennial ryegrass	Terrestrial				
Lycopus	Lycopus europaeus	Gypsywort	Wetland				
Lythrum	Lythrum salicaria	Purple loosestrife	Wetland				
Medicago	Medicago sativa	Alfalfa	Terrestrial		Cultivated		
Medicago	Medicago sativa	Alfalfa	Terrestrial		Culivated		
Menyanthes	Menyanthes trifoliata	Bogbean	Wetland				
Musa		Banana	Terrestrial		Culivated	Non-native	
Nasturtium	Nasturtium officinale	Watercress	Wetland				
Nymphaea	Nymphaea alba	White water lily	Aquatic				
Nymphaea	Nymphaea odorata	American white water lily	Aquatic			Non-native	

Pastinaca	Pastinaca sativa	Parsnip	Terrestrial		Culivated		
Persicaria	Persicaria amphibia	Amphibious bistort	Wetland				
Phleum	Phleum pratense	Timothy	Terrestrial				
Plantago	Plantago lanceolata	Ribwort plantain	Terrestrial				
Poa	Poa trivialis	Rough meadow grass	Terrestrial				
Polygonum	Polygonum boreale	Northern knotgrass	Terrestrial				
Populus		Poplar	Wetland	Tree			
Potamogeton	Potamogeton natans	Broad-leaved pondweed	Aquatic				
Potamogeton	Potamogeton berchtoldii	Berchtold's pondweed	Aquatic				
Potamogeton		Pondweed	Aquatic				
Potamogeton	Potamogeton crispus	Curly-leaved pondweed	Aquatic				
Potamogeton	Potamogeton coloratus	Fen pondweed	Aquatic				Nationally Scarce
Potentilla	Potentilla reptans	Creeping cinquefoil	Terrestrial				
Prunus		Cherry/Plum	Terrestrial	Tree			
Quercus	Quercus infectoria	Aleppo Oak	Terrestrial	Tree		Non-native	
Rubus		Bramble	Terrestrial				
Rubus	Rubus silvaticus	Bramble	Terrestrial				
Rumex		Dock	Wetland				
Salix		Willow	Wetland	Tree			
Sambucus	Sambucus nigra	Elder	Terrestrial	Tree			
Sesamum	Sesamum indicum	Sesame	Terrestrial		Culivated	Non-native	
Solanum	Solanum dulcamara	Bittersweet	Wetland				
Sparganium	Sparganium stoloniferum	Bur-reed	Wetland				
Stachys	Stachys sylvatica	Hedge woundwort	Terrestrial				
Trifolium	Trifolium pratense	Red clover	Terrestrial				
Ulmus	Ulmus davidiana	Japanese elm	Terrestrial	Tree		Non-native	
Urtica	Urtica dioica	Common nettle	Terrestrial				
Urtica		Nettle	Terrestrial				
Vicia	Vicia faba	Broad bean	Terrestrial		Cultivated		

Table S4.2: List of higher plant (Embryophyta) taxa found from eDNA metabarcoding using ITS2 gene region in 31 ponds in lowland England.