

Reed Bed Use Within Scotch Whisky Distilleries to Treat Wastewater: New Solutions to Help Maximise Performance

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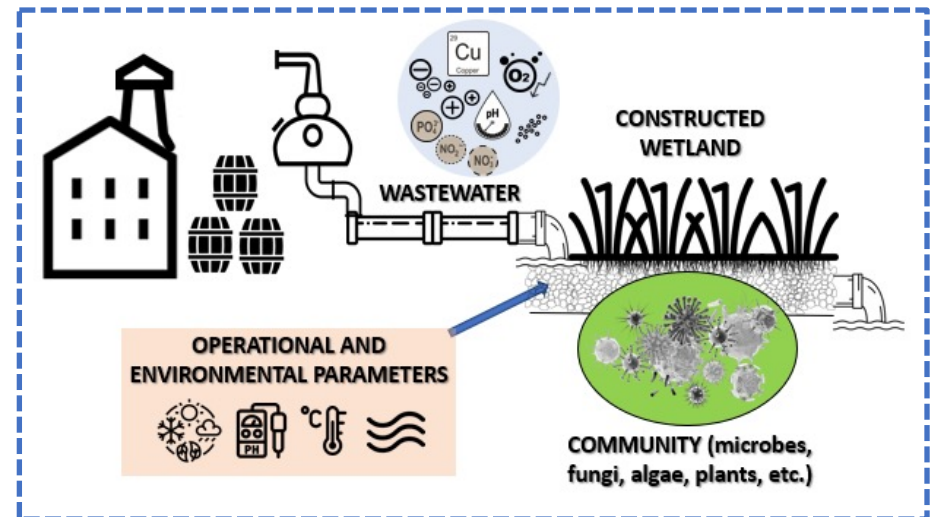
Supervisors: Dr Mark Taggart, Dr Lucio Marcello, Dr Barbara Morrissey, Dr Paul Gaffney

- Natural and constructed wetlands/reed bed systems can act as ‘filtration’ systems to clean water, including treating whisky distillery byproducts
- Among distilleries in Scotland, while constructed versions of these systems are in use at some sites, there is significant scope to better understand and optimise their performance

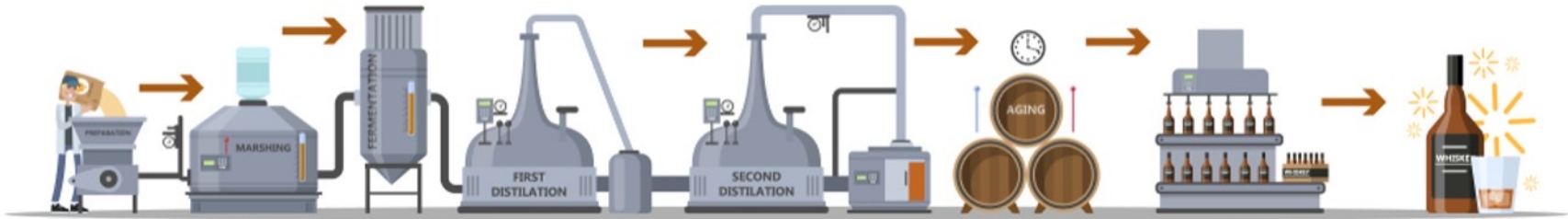
4 year aim of the project:

To establish relationships between:

- ✓ Treatment performance
- ✓ Environmental DNA community
- ✓ Operational and environmental parameters



And in so doing, designing new solutions to help maximise the performance of constructed wetlands/reed bed systems.



Anaerobic
Digestion



Spent Lees

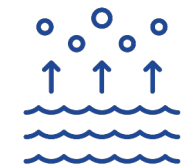


Constructed
Wetlands



Advanced
Filtration

Parameters	Unit	Laureate Spent Lees	Chevalier Spent Lees
pH		3.96	3.68
Total Suspended Solids	µg/L	2400	1800
Turbidity	NTU	3.2	3.0
Conductivity	µS	340	300
Total Organic Carbon	mg/L	921	1105
Ammonia	µg/L	247	163
Total Oxidised Nitrogen	µg/L	5	6
Soluble Reactive Phosphorus	µg/L	136	163
Dissolved Cu	µg/L	90168	79273
Dissolved Al	µg/L	908	525
Dissolved Zn	µg/L	1231	1237



Evaporation



Robust for use with heavy metals



Low energy input



Efficient treatment



Provides natural habitat



Simple operation



Land requirement due to low hydraulic loading rate
Not suitable for large scale

Clogging can lead to insufficient oxygen in the system, thus disturbance of aerobic processes



Decrease of performance in colder weather





What is the best way to capture diversity from wetlands?



What is the best sequencing platform and pipeline for genus-level identification? (Miseq & DADA2 vs. MinION & EPI2ME)



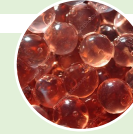
What gives spent lees of their toxic characteristics against wetland bacteria and plants? (Cu/Al concentration, pH)



What are the very simple 'passive' processes that need very little workforce input to reduce the toxicity of spent lees?



What is the relationship between bacterial diversity, season, and wetland performance over time?



Which wetland substrate gives the highest bacterial diversity and treatment performance?



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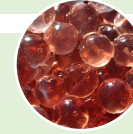
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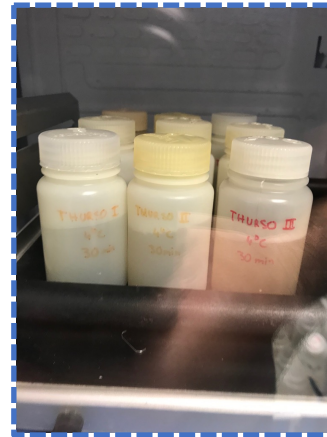
What is the relationship between bacterial diversity, season, and wetland performance over time?



Which wetland substrate gives the highest bacterial diversity and treatment performance?



4°C No Enzyme
→
30°C No Enzyme
→
30°C with Enzyme
→



Filtering the buffer



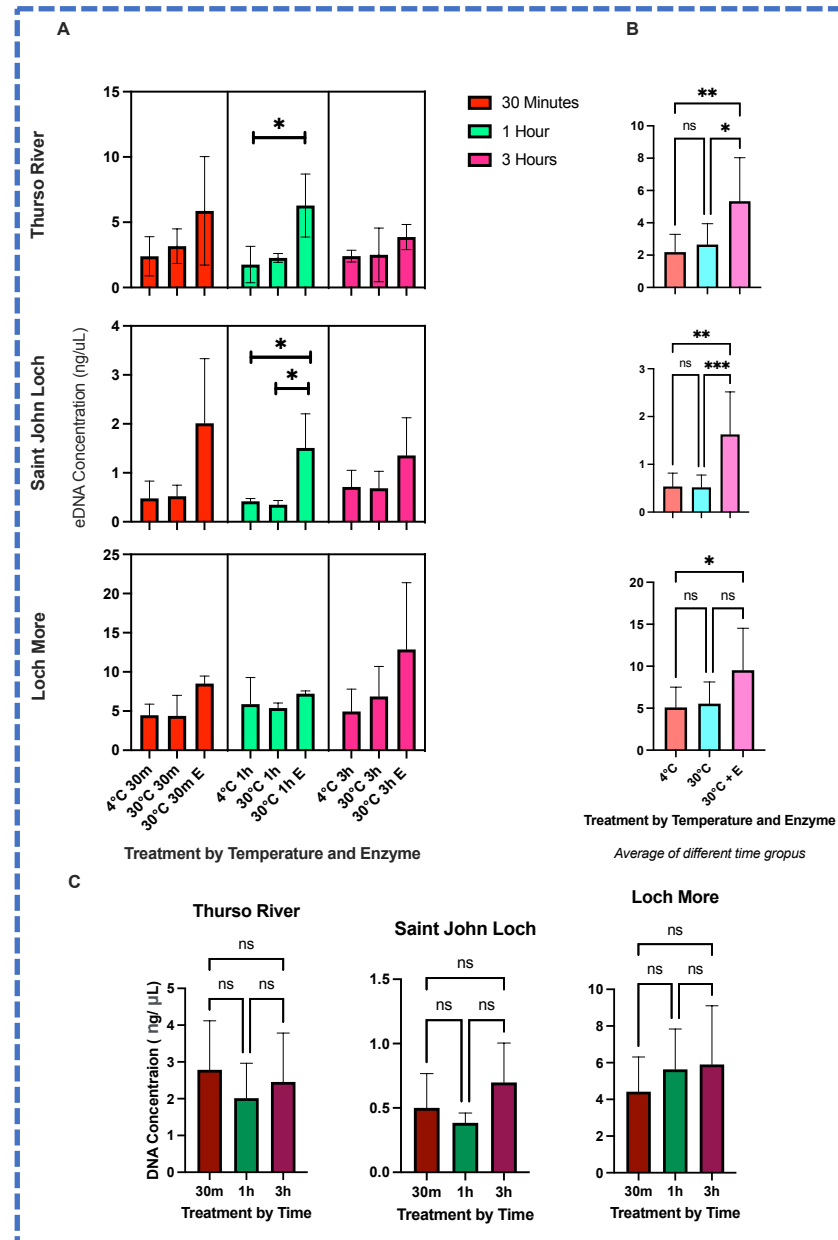
eDNA extraction



Concentration measurement

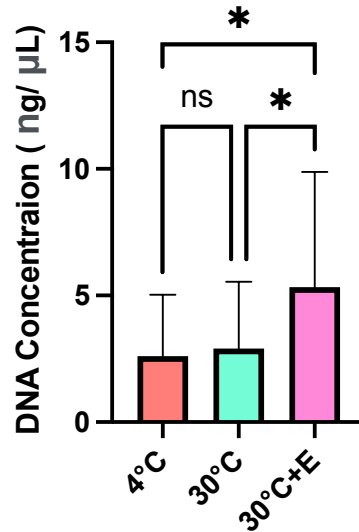
18g of pea gravel from 3
different natural wetlands
+
75 ml PBS

30 min, 1h, 3h
agitation at 150 rpm



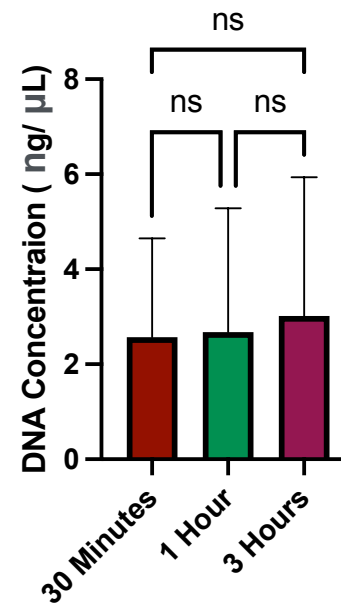
Enzyme treatment increased the eDNA yield

A



Treatment by Temperature and Enzyme

B



Treatment by Time

Agitation time did not affect the eDNA yield

Average of all three sites



Concentration measurement



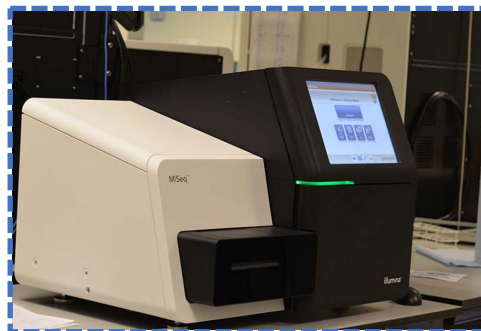
Amplicon PCR of bacterial 16s
rRNA



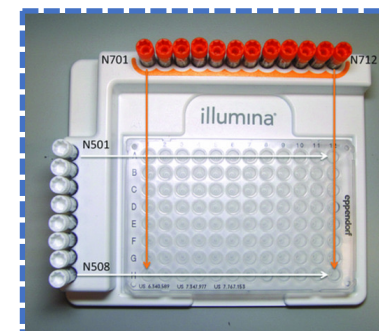
Size selective purification



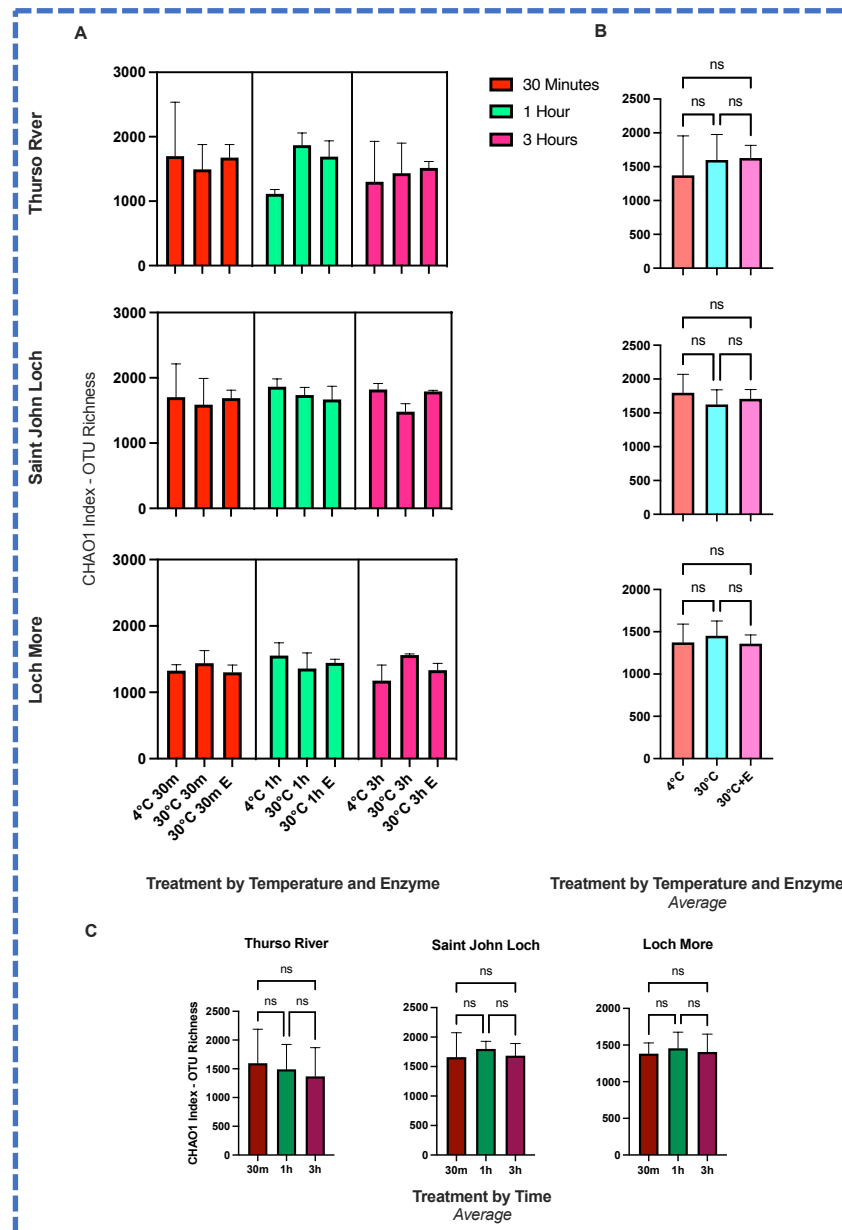
Taxonomic analysis



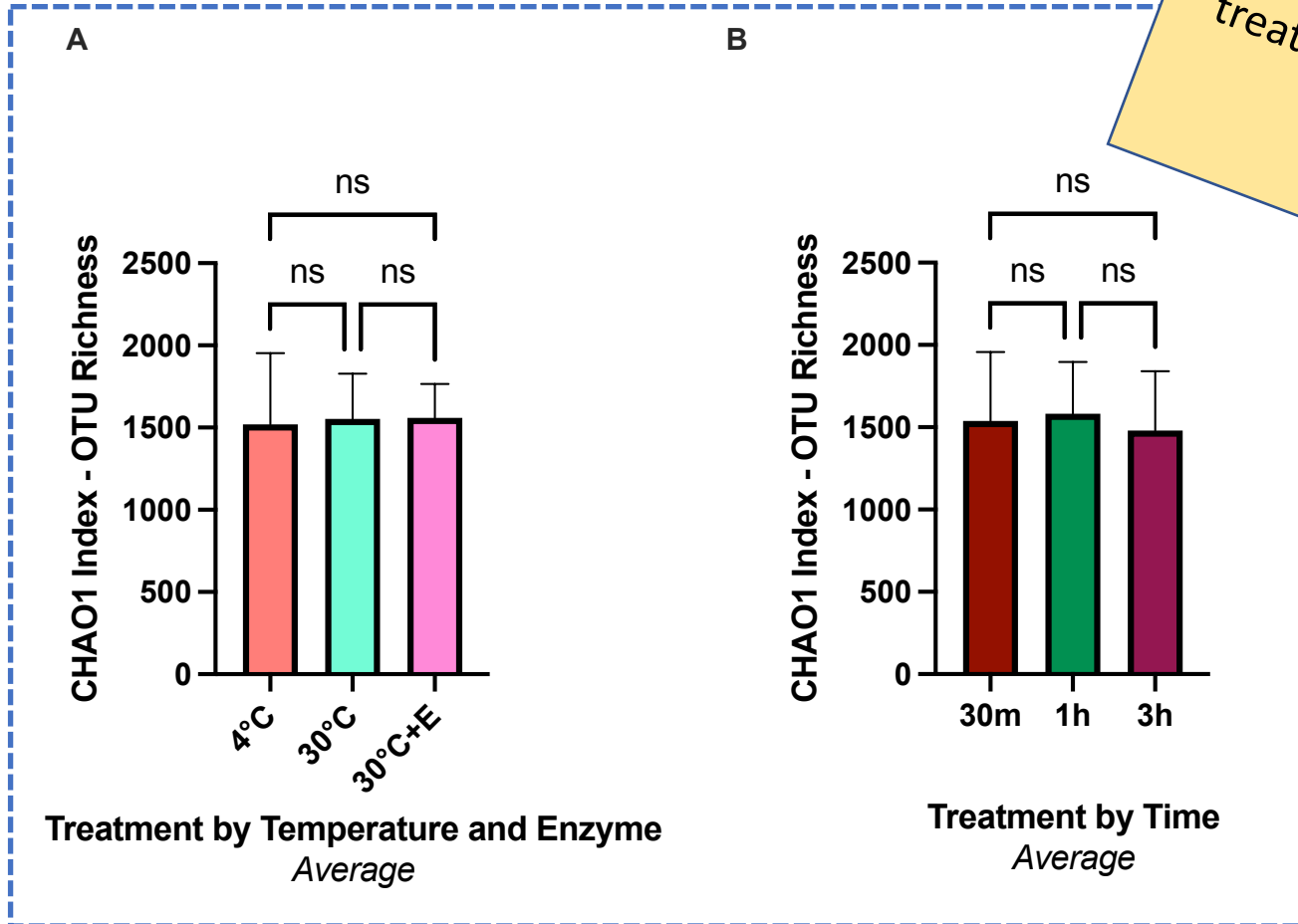
Library preparation and
sequencing



Index PCR



No difference
between
treatments



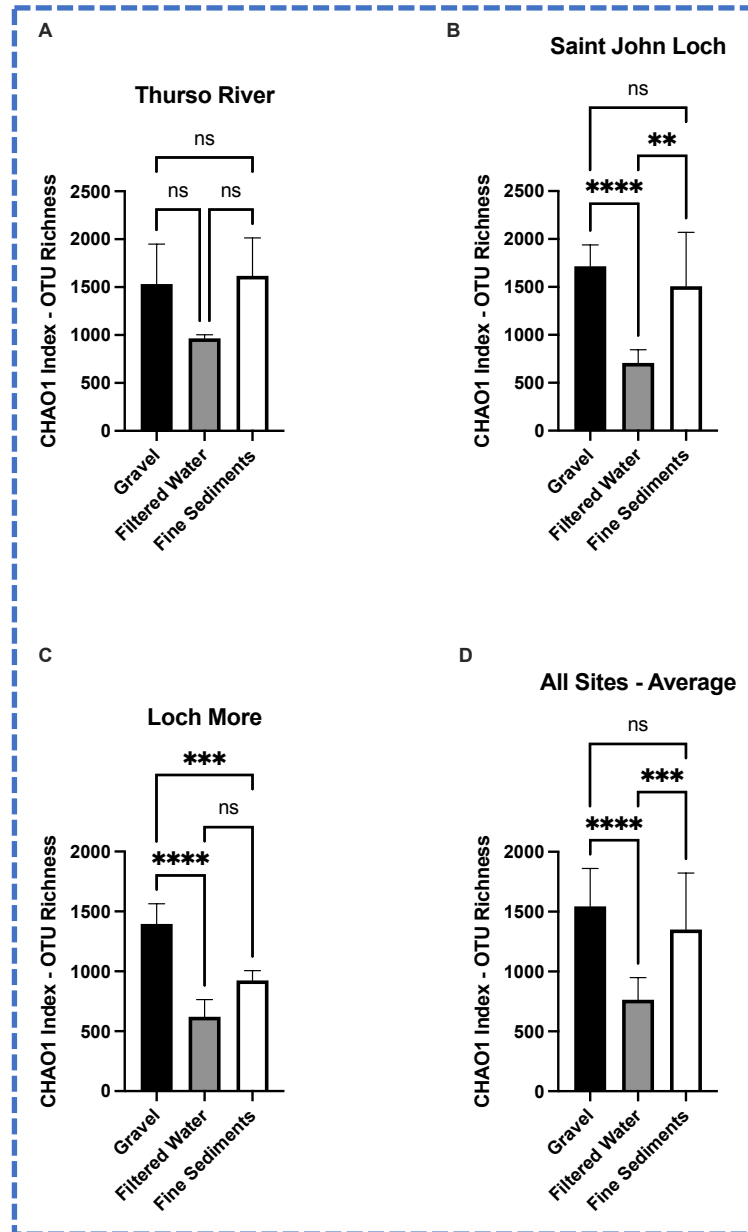
Average of all three sites

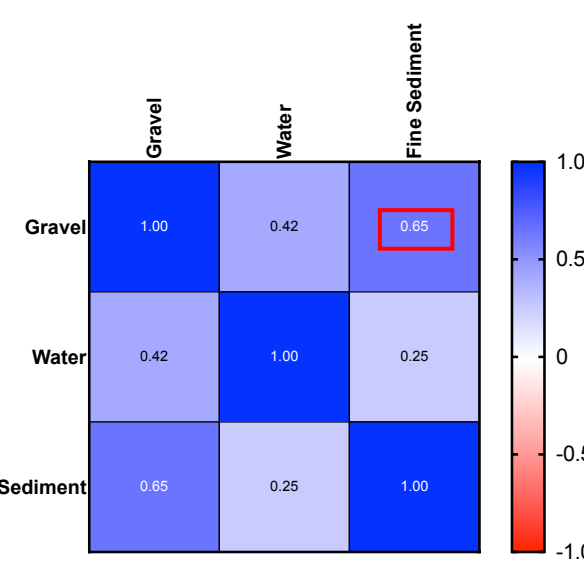
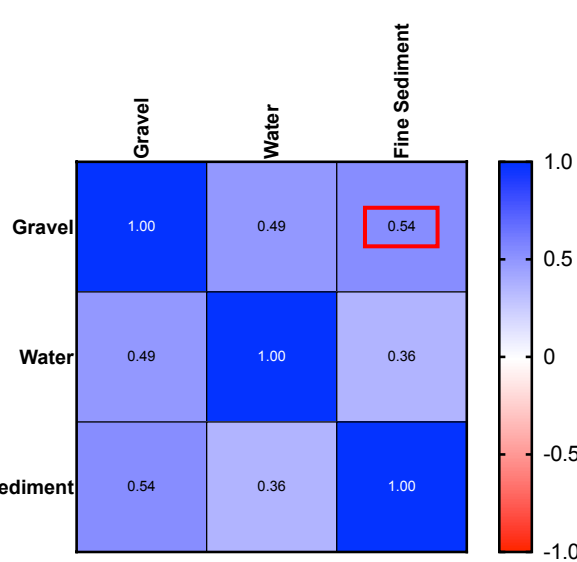
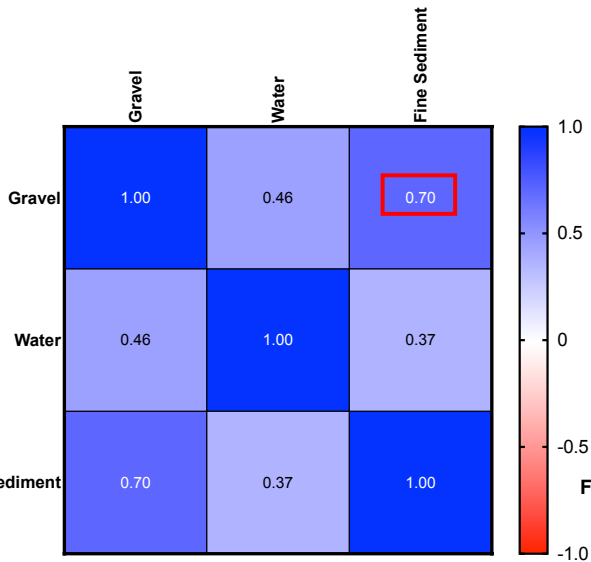
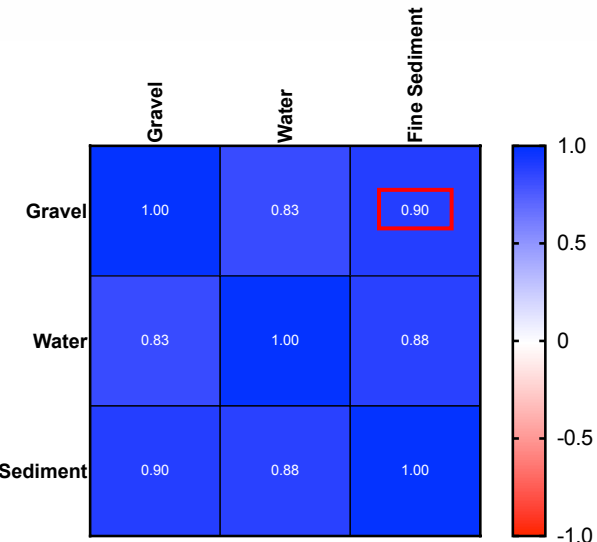
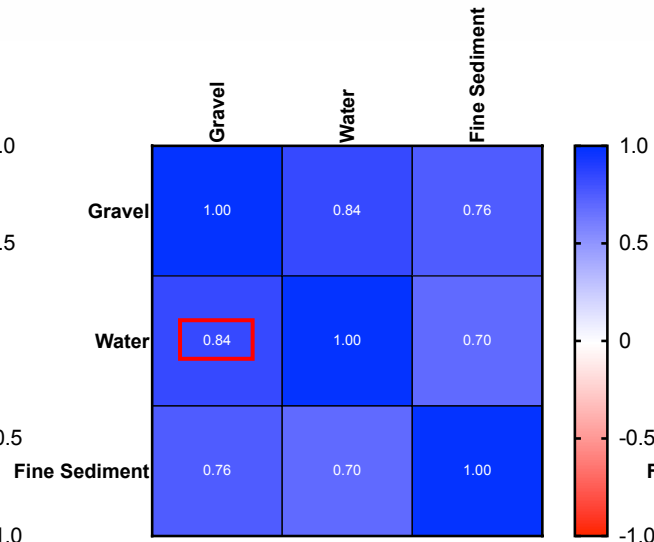
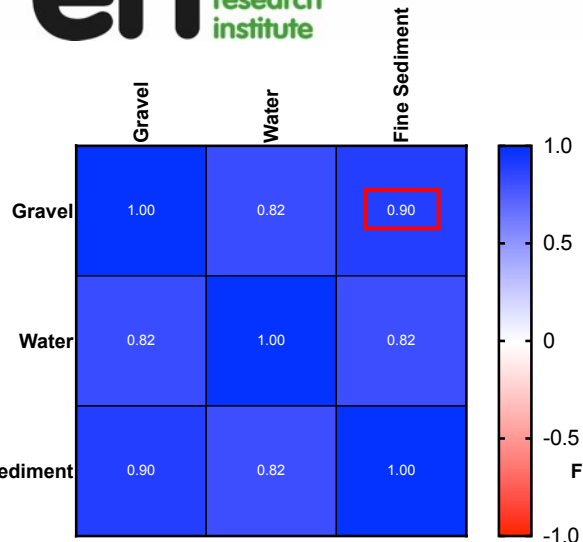
Enzyme treatment
caused more eDNA
yield from gravel
surfaces

Bacterial diversity did
not increase when
using enzyme/warmer
temperature

Considering the most
practical application,
agitation of gravels
with PBS at room
temperature for 30
min is enough for
eDNA isolation

OTU richness
Gravel > fine
sediment > water





Thurso River

Saint John Loch

Loch More

Alpha diversity
measures of
gravel>fine
sediment>filtered
water

Each layer of the
wetland should be
sampled for the
accurate taxonomic
profiling – Correlation
decreases towards
genus level

Proteobacteria is
present in all layers
with an abundance
above 10%

Bacteroidetes is
present in water layer
with an abundance
above 10%

Cyanobacteria is
present in water and
fine sediments layers
with an abundance
above 10%



What is the best way for capturing diversity from wetlands?



What is the best sequencing platform and pipeline for genus-level identification? (Miseq & DADA2 vs. MinION & EPI2ME)



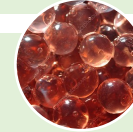
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What are the very simple 'passive' processes that need very little workforce input to reduce the toxicity of spent lees?



What is the relationship between bacterial diversity, season, and wetland performance over time?



What is the efficiency of the bacterial seeding method on a constructed wetland to increase treatment performance?



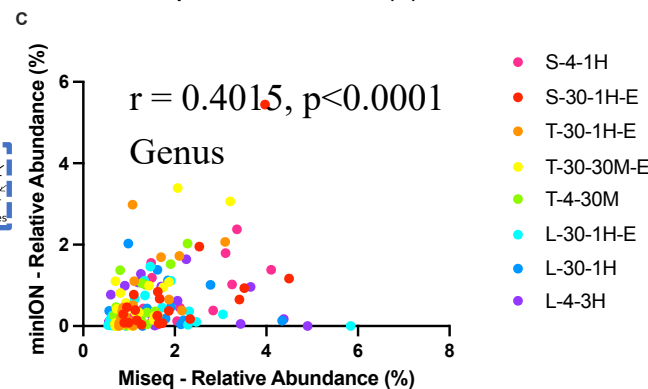
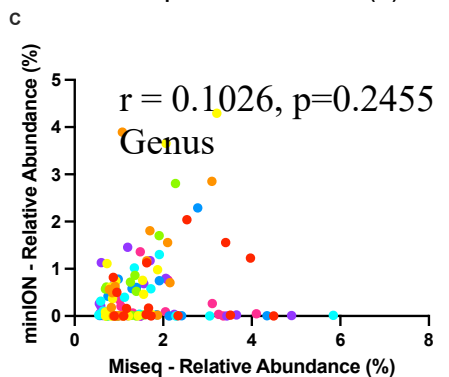
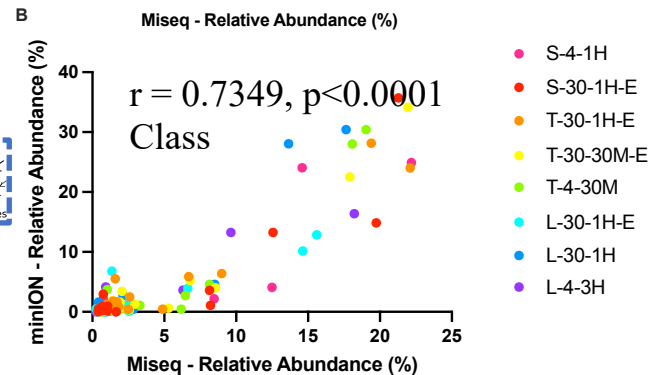
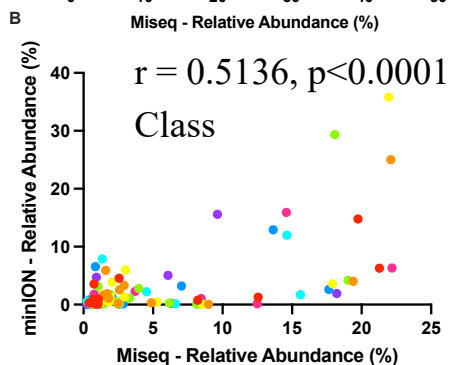
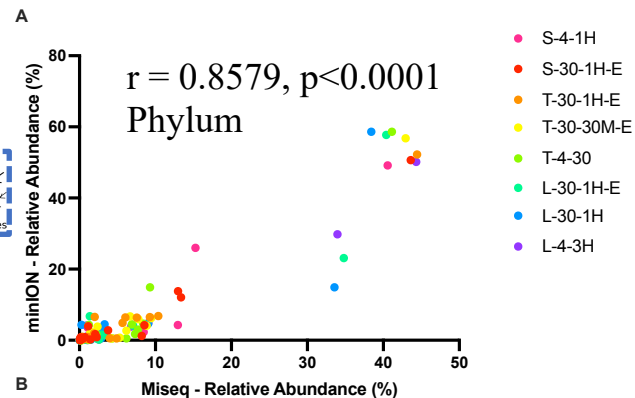
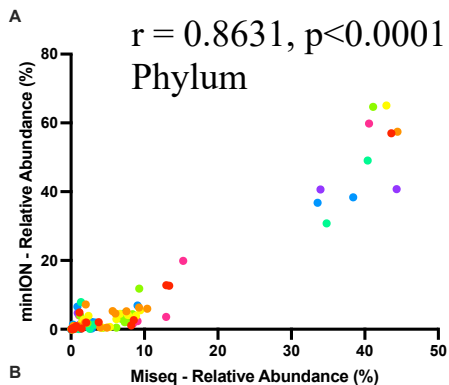
VS

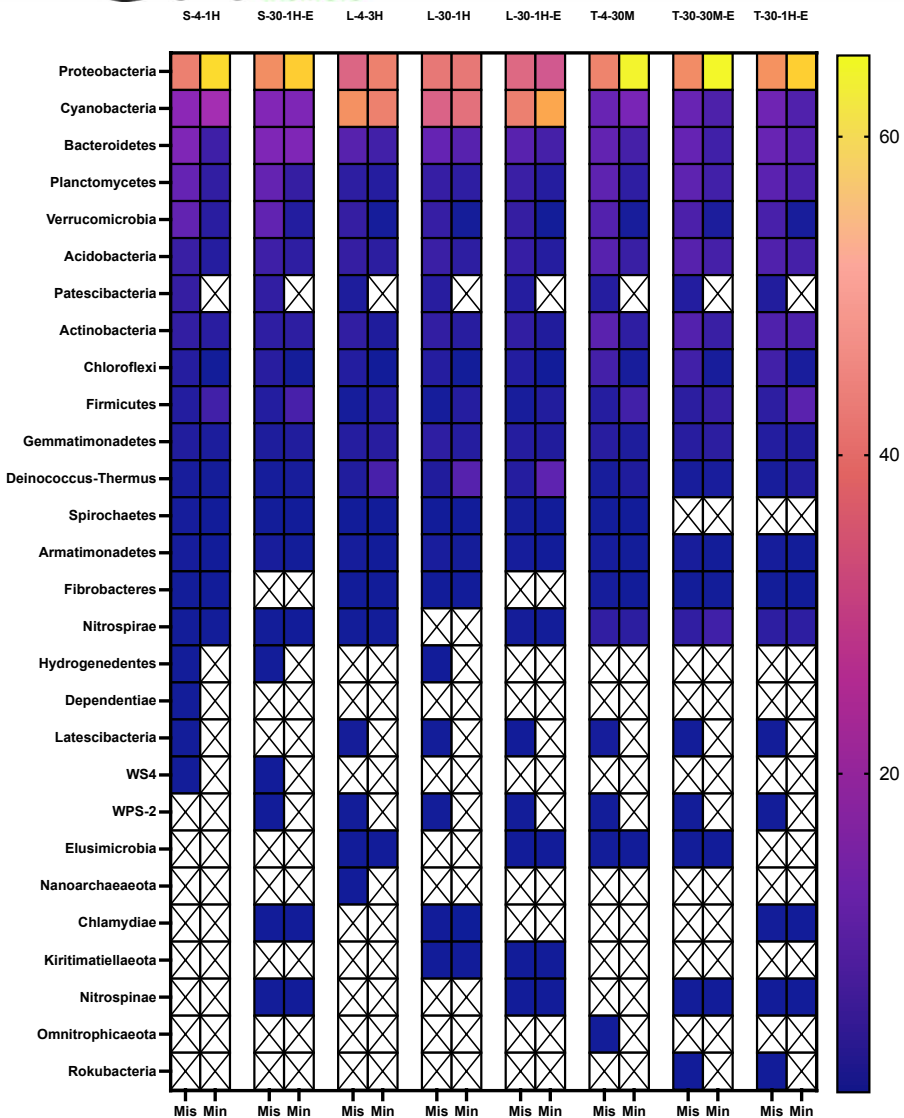


VS

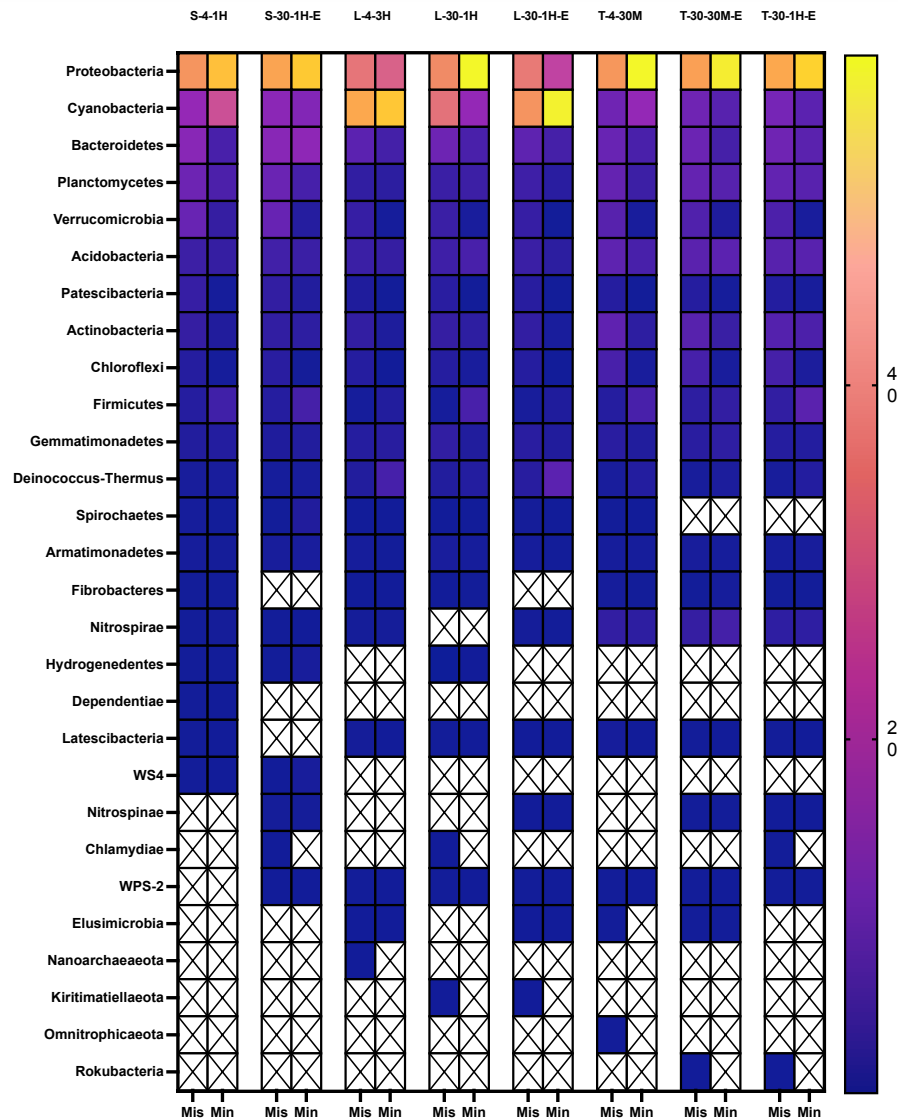


	Illumina Miseq - SILVA132					ONT minION-NCBI						ONT minION-SILVA132				
	Reads assigned to taxa (%)					Reads assigned to taxa (%)						Reads assigned to taxa (%)				
	Phylum	Class	Order	Family	Genus	Phylum	Class	Order	Family	Genus	Species	Phylum	Class	Order	Family	Genus
T1_4_30M	99.70%	99.20%	91.90%	75.00%	48.00%	96.60%	83.74%	71.09%	57.01%	30.76%	27.67%	99.59%	98.79%	94.10%	77.49%	45.75%
T2_4_30M	99.70%	98.40%	89.30%	80.00%	46.50%	94.23%	85.08%	68.48%	55.57%	29.09%	25.97%	99.60%	98.78%	94.10%	77.56%	45.72%
T3_4_30M	99.80%	99.50%	93.50%	82.90%	53.90%	99.39%	88.00%	76.80%	64.33%	41.08%	38.34%	99.59%	98.79%	94.15%	77.53%	45.66%
T1_30_30M	99.80%	99.50%	94.00%	79.90%	52.50%	96.43%	88.22%	80.14%	71.16%	54.40%	52.42%	99.43%	98.17%	93.76%	79.92%	50.28%
T2_30_30M	99.60%	98.80%	90.90%	80.60%	50.40%	95.66%	87.69%	79.72%	70.94%	54.54%	52.61%	99.43%	98.16%	93.78%	79.89%	50.22%
T3_30_30M	99.80%	99.60%	93.90%	82.40%	53.10%	96.50%	88.50%	80.63%	71.87%	55.54%	53.62%	99.43%	98.17%	93.75%	79.94%	50.25%
T1_30_1H_E	99.90%	99.50%	94.00%	81.50%	53.80%	96.46%	84.98%	73.69%	61.12%	37.69%	34.93%	99.42%	98.20%	94.29%	81.43%	50.49%
T2_30_1H_E	99.80%	98.80%	91.70%	80.70%	51.60%	96.27%	86.58%	77.05%	66.44%	46.66%	44.33%	99.43%	98.20%	94.31%	81.50%	50.50%
T3_30_1H_E	99.80%	99.60%	94.80%	82.30%	55.20%	97.28%	89.09%	81.03%	72.07%	55.35%	53.38%	99.43%	98.19%	94.31%	81.47%	50.42%
S2_30_1H_E	99.80%	99.50%	96.30%	89.20%	66.90%	97.35%	82.46%	67.81%	51.51%	21.12%	17.54%	99.62%	98.36%	93.31%	82.20%	53.81%
S3_30_1H_E	99.80%	99.50%	96.00%	88.00%	65.00%	97.36%	85.65%	74.13%	61.31%	37.41%	34.59%	99.61%	98.35%	93.35%	82.30%	53.80%
S1_4_1H	99.70%	99.20%	95.70%	85.70%	62.50%	97.79%	79.21%	60.09%	37.43%	17.28%	15.10%	99.45%	98.50%	89.98%	74.20%	47.30%
S2_4_1H	99.70%	98.90%	94.40%	84.60%	62.10%	97.90%	70.77%	49.98%	28.12%	10.12%	8.97%	99.44%	98.49%	89.90%	74.18%	47.35%
S3_4_1H	99.80%	99.60%	96.70%	88.90%	66.20%	97.38%	71.85%	57.44%	43.12%	22.36%	20.44%	99.45%	98.49%	89.94%	74.23%	47.27%
L1_4_3H	100.00%	99.40%	97.60%	64.30%	48.90%	97.19%	68.95%	55.55%	46.51%	25.18%	23.40%	99.29%	98.57%	92.94%	48.27%	29.10%
L3_4_3H	99.90%	99.60%	97.40%	66.90%	48.90%	98.66%	60.13%	45.42%	34.44%	15.28%	13.86%	99.29%	98.55%	92.96%	48.31%	29.06%
L2_30_1H	99.90%	99.30%	96.20%	69.00%	48.40%	98.95%	65.21%	50.67%	37.82%	18.04%	16.46%	99.60%	98.79%	94.13%	77.50%	45.69%
L3_30_1H	99.90%	99.50%	96.20%	70.30%	49.30%	98.32%	69.82%	55.03%	35.76%	16.36%	14.84%	99.50%	98.80%	94.12%	77.49%	45.67%
L1_30_1H_E	99.90%	99.50%	96.70%	68.00%	48.00%	98.70%	59.21%	43.58%	34.99%	15.73%	14.24%	99.47%	98.37%	93.78%	44.68%	27.62%
L3_30_1H_E	99.90%	99.60%	96.90%	67.90%	47.70%	98.39%	66.00%	47.99%	34.88%	15.64%	14.16%	99.47%	98.37%	93.77%	44.66%	27.55%
Average	99.81%	99.33%	94.71%	78.41%	53.95%	97.34%	78.06%	64.82%	51.82%	30.98%	28.84%	99.48%	98.45%	93.24%	72.24%	44.68%





Miseq-SILVA vs. MinION-NCBI



Miseq-SILVA vs. MinION-SILVA

MinION NCBI vs. Illumina SILVA132

Phylum level analysis displayed that the bacterial abundance data was strongly correlated between the two platforms; however, this correlation dropped to moderate at the class level and weak at the genus level

MinION SILVA132 vs. Illumina SILVA132

Phylum and class level analysis displayed that the bacterial abundance was strongly correlated between the two platforms. Genus level abundance was moderately correlated between the two platforms

*Thank
You*

