

# Supplementary material

## Characterization of the spatial variation of microbial communities in a decentralized subtropical wastewater treatment plant using passive methods

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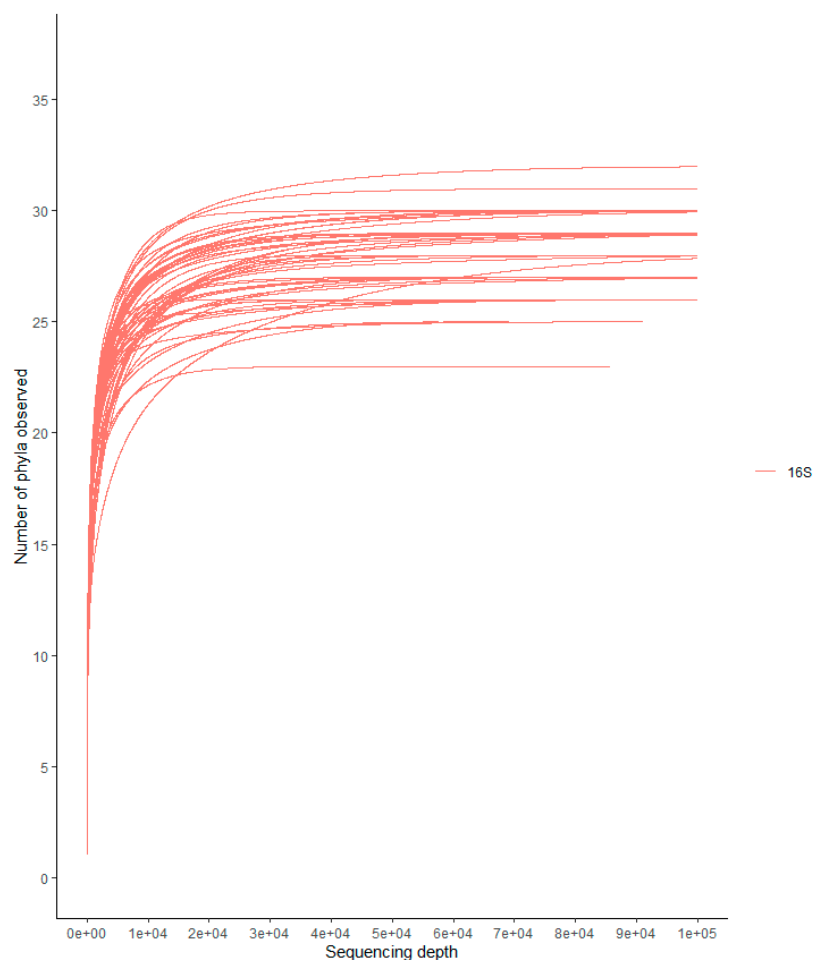
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**Table S1.** Removal efficiencies of the overall system and for stage

Parameter	Removal efficiency (%)				Mass reduction (mg/L)			
	ST	UAF	HFCW	OS	ST	UAF	HFCW	OS
BOD <sub>5</sub>	33 ± 07	57 ± 17	64 ± 08	90 ± 05	201.6 ± 60.2	243.2 ± 105.1	103.3 ± 22.1	548.1 ± 117.0
COD	35 ± 05	55 ± 16	67 ± 10	90 ± 05	330.8 ± 92.8	345.2 ± 136.9	171.0 ± 37.0	847.1 ± 181.0
TSS	73 ± 04	41 ± 15	78 ± 05	97 ± 00	257.7 ± 65.2	38.8 ± 15.0	42.5 ± 11.4	339.0 ± 73.3
TNK	12 ± 11	12 ± 03	36 ± 07	51 ± 05	42.3 ± 37.7	36.2 ± 8.4	94.6 ± 25.9	173.0 ± 20.3
NH <sub>4</sub>	07 ± 10	08 ± 04	35 ± 08	45 ± 04	11.4 ± 15.7	10.9 ± 6.9	46.3 ± 13.5	68.5 ± 7.2
ON	17 ± 13	17 ± 03	37 ± 07	57 ± 08	32.7 ± 25.6	25.3 ± 2.4	48.3 ± 12.8	106.2 ± 17.0
NO <sub>2</sub>	53 ± 32	44 ± 15	63 ± 08	91 ± 05	1.90 ± 1.82	0.45 ± 0.22	0.33 ± 0.08	2.7 ± 1.7
NO <sub>3</sub>	44 ± 15	67 ± 14	74 ± 13	95 ± 02	0.12 ± 0.05	0.10 ± 0.04	0.03 ± 0.02	0.2 ± 0.1



**Figure S1.** Rarefaction curve of 16S rRNA gene samples.

**Table S2.** Percentage of reads classified as bacterial phyla > 1% using Greengenes 13\_8. Comparison between the three treatment stages (ST, UAF and HFCW) for nitrogen degrading families.

Phyla > 1%	Greengenes %		
	ST	UAF	HFCW
Proteobacteria	33.92%	37.81%	59.85%
Acidobacteria	2.05%	1.78%	3.96%
Actinobacteria	5.68%	5.41%	7.61%
Bacteroidetes	13.66%	8.81%	9.04%
Caldiserica	5.69%	13.97%	-
Chloroflexi	1.62%	1.12%	4.45%
Cyanobacteria	-	-	3.06%
Firmicutes	25.49%	20.81%	4.23%
Synergistetes	2.51%	4.05%	-
Gemmatimonadetes	-	-	1.15%
Nitrospirae	-	-	1.98%
Un Bacteria	6.59%	3.93%	1.01%
Others	2.78%	2.32%	3.66%

**Table S3.** Percentage of reads classified as bacteria phyla > 1% using Greengenes 13\_8. Vertical variations within the UAF.

Phyla > 1%	Greengenes %	
	Surface	Bottom
Proteobacteria	40.79%	33.27%

Acidobacteria	1.35%	2.43%
Actinobacteria	4.57%	6.68%
Bacteroidetes	10.02%	6.96%
Caldiserica	15.31%	11.93%
Chloroflexi	1.31%	-
Firmicutes	19.73%	22.44%
Synergistetes	3.16%	5.41%
Un Bacteria	2.21%	6.53%
Others	1.54%	4.34%

**Table S4.** Percentage of reads classified as bacteria phyla > 1% using Greengenes 13\_8. Vertical variations within the HFCW.

Phyla > 1%	GreenGenes %	
	10 cm	50 cm
Proteobacteria	58.65%	61.03%
Acidobacteria	4.75%	3.18%
Actinobacteria	10.02%	5.20%
Bacteroidetes	6.00%	12.07%
Chloroflexi	2.81%	6.08%
Cyanobacteria	5.83%	-
Firmicutes	3.37%	5.08%
Gemmatimonadetes	1.70%	-
Nitrospirae	2.97%	-
Spirochaetes	-	1.21%
Un Bacteria	-	1.20%
Others	3.89%	4.94%

**Table S5.** Percentage of reads classified as bacteria phyla > 1% using Greengenes 13\_8. Longitudinal variations within the HFCW (Inlet, Middle and Outlet).

Phyla > 1%	GreenGenes %		
	Inlet	Middle	Outlet
Proteobacteria	60.08%	60.28%	59.15%
Acidobacteria	3.48%	4.30%	4.15%
Actinobacteria	5.85%	8.07%	9.05%
Bacteroidetes	11.02%	9.08%	6.85%
Chloroflexi	4.26%	5.98%	3.12%
Cyanobacteria	4.47%	1.27%	3.35%
Firmicutes	3.45%	3.38%	5.94%
Gemmatimonadetes	-	1.55%	1.22%
Nitrospirae	2.56%	1.65%	1.67%
Spirochaetes	-	-	1.00%
Un Bacteria	-	-	1.14%
Others	4.84%	4.43%	3.37%

**Table S6.** Percentage of reads classified as bacteria family > 1% using Greengenes 13\_8. Comparison between the three treatment stages (ST, UAF and HFCW) regarding the nitrogen degrading families.

Family > 1%	GreenGenes %		
	ST	UAF	HFCW
<i>Microbacteriaceae</i>	2.40%	3.58%	1.35%
<i>Mycobacteriaceae</i>	2.20%	4.11%	2.35%

<i>Propionibacteriaceae</i>	9.15%	3.41%	-
<i>Bacillaceae</i>	2.04%	1.96%	-
<i>Nitrospiraceae</i>	1.19%	2.12%	6.10%
<i>Bradyrhizobiaceae</i>	-	2.07%	3.55%
<i>Thermodesulfobibrionaceae</i>	2.45%	-	1.28%
<i>Hyphomicrobiaceae</i>	3.59%	5.37%	10.15%
<i>Caulobacteraceae</i>	-	1.18%	1.79%
<i>Rhizobiaceae</i>	1.86%	3.76%	2.46%
<i>Rhodospirillaceae</i>	2.10%	3.60%	6.55%
<i>Comamonadaceae</i>	23.85%	11.64%	13.05%
<i>Rhodocyclaceae</i>	30.96%	39.23%	28.00%
<i>Pseudomonadaceae</i>	9.76%	4.03%	1.53%
<i>Xanthomonadaceae</i>	5.09%	7.41%	15.49%
<i>Chromatiaceae</i>	1.07%	4.97%	4.51%
Others	2.27%	1.56%	1.85%

**Table S7.** Percentage of reads classified as bacteria family > 1% using Greengenes 13\_8. Vertical variations of nitrogen degrading families within the UAF.

Family > 1%	GreenGenes %	
	Surface	Bottom
<i>Microbacteriaceae</i>	2.48%	5.50%
<i>Mycobacteriaceae</i>	2.04%	7.69%
<i>Propionibacteriaceae</i>	3.26%	3.68%
<i>Bacillaceae</i>	2.51%	-
<i>Nitrospiraceae</i>	1.03%	4.00%
<i>Bradyrhizobiaceae</i>	1.41%	3.20%
<i>Hyphomicrobiaceae</i>	4.18%	7.42%
<i>Caulobacteraceae</i>	-	1.84%
<i>Rhizobiaceae</i>	3.26%	4.63%
<i>Rhodospirillaceae</i>	2.10%	6.20%
<i>Comamonadaceae</i>	9.53%	15.30%
<i>Rhodocyclaceae</i>	51.72%	17.62%
<i>Pseudomonadaceae</i>	4.91%	2.52%
<i>Xanthomonadaceae</i>	3.65%	13.93%
<i>Chromatiaceae</i>	5.55%	3.96%
Others	2.37%	2.52%

**Table S8.** Percentage of reads classified as bacteria family > 1% using Greengenes 13\_8. Vertical variations of nitrogen degrading families within the HFCW.

Family > 1%	GreenGenes %	
	10 cm	50 cm
<i>Microbacteriaceae</i>	1.58%	1.04%
<i>Mycobacteriaceae</i>	3.20%	1.25%
<i>Nitrospiraceae</i>	9.47%	1.71%
<i>Thermodesulfobibrionaceae</i>	-	2.27%
<i>Bradyrhizobiaceae</i>	4.46%	2.35%
<i>Hyphomicrobiaceae</i>	10.67%	9.49%
<i>Caulobacteraceae</i>	2.51%	-

<i>Rhizobiaceae</i>	3.07%	1.67%
<i>Rhodospirillaceae</i>	9.30%	2.96%
<i>Comamonadaceae</i>	14.41%	11.27%
<i>Rhodocyclaceae</i>	11.71%	49.20%
<i>Pseudomonadaceae</i>	2.02%	-
<i>Xanthomonadaceae</i>	23.08%	5.61%
<i>Chromatiaceae</i>	1.74%	8.11%
Others	2.77%	3.08%

**Table S9.** Percentage of reads classified as bacteria family removal > 1% using Greengenes 13\_8. Longitudinal variations of nitrogen degrading families within the HFCW (Inlet, Middle and Outlet).

Family > 1%	GreenGenes %		
	Inlet	Middle	Outlet
<i>Microbacteriaceae</i>	1.12%	1.80%	1.15%
<i>Mycobacteriaceae</i>	1.60%	2.01%	3.69%
<i>Nitrospiraceae</i>	8.31%	5.00%	4.40%
<i>Thermodesulfobionaceae</i>	-	1.23%	2.12%
<i>Bradyrhizobiaceae</i>	2.19%	5.05%	3.70%
<i>Hyphomicrobiaceae</i>	6.82%	13.07%	11.36%
<i>Caulobacteraceae</i>	1.55%	2.23%	1.62%
<i>Rhizobiaceae</i>	1.23%	3.17%	3.30%
<i>Rhodospirillaceae</i>	3.51%	7.47%	9.52%
<i>Comamonadaceae</i>	12.52%	12.24%	14.60%
<i>Rhodocyclaceae</i>	23.21%	29.07%	33.09%
<i>Pseudomonadaceae</i>	-	2.66%	1.28%
<i>Xanthomonadaceae</i>	26.10%	10.61%	6.89%
<i>Chromatiaceae</i>	9.12%	2.27%	-
Others	2.71%	2.12%	3.27%

**Table S10.** Percentage of reads classified as bacteria family > 1% using Greengenes 13\_8. Comparison between the three treatment stages (ST, UAF and HFCW) for organic matter degrading families.

Family > 1%	GreenGenes %		
	ST	UAF	HFCW
<i>Bacteroidaceae</i>	6.07%	2.82%	1.96%
<i>Cytophagaceae</i>	-	-	3.05%
<i>Chitinophagaceae</i>	-	-	5.53%
<i>Porphyromonadaceae</i>	9.79%	6.07%	1.82%
<i>Saprospiraceae</i>	-	-	1.11%
<i>Acidaminobacteraceae</i>	-	1.70%	-
<i>Clostridiaceae</i>	16.36%	17.97%	5.20%
<i>Lachnospiraceae</i>	9.12%	2.60%	1.33%
<i>Lactobacillaceae</i>	4.15%	-	-
<i>Mogibacteriaceae</i>	1.36%	2.47%	-
<i>Ruminococcaceae</i>	16.45%	4.95%	1.13%
<i>Acetobacteraceae</i>	-	1.06%	6.66%
<i>Methylocystaceae</i>	1.37%	3.36%	6.98%
<i>Rhodobacteraceae</i>	1.02%	1.21%	2.56%
<i>Shingomonadaceae</i>	-	-	3.18%

<i>Hydrogenophilaceae</i>	-	1.19%	4.62%
<i>Enterobacteriaceae</i>	5.74%	1.62%	-
<i>Methylophilaceae</i>	-	-	2.60%
<i>Methylococcaceae</i>	-	1.19%	5.06%
<i>Moraxellaceae</i>	3.59%	1.20%	-
<i>Sinobacteraceae</i>	1.12%	1.91%	15.82%
<i>Desulfobacteraceae</i>	1.02%	2.68%	2.79%
<i>Desulfobulbaceae</i>	2.55%	2.00%	1.41%
<i>Desulfomicrobiaceae</i>	1.01%	-	-
<i>Desulfovibrionaceae</i>	6.81%	3.46%	-
<i>Geobacteraceae</i>	2.01%	4.42%	2.48%
<i>Syntrophaceae</i>	6.55%	28.82%	17.45%
<i>Syntrophobacteraceae</i>	-	3.88%	2.45%
Others	3.91%	3.44%	4.82%

**Table S11.** Percentage of reads classified as bacteria family > 1% using Greengenes 13\_8 involved in organic degradation at vertical variations within the UAF.

Family > 1%	GreenGenes %	
	Surface	Bottom
<i>Bacteroidaceae</i>	3.68%	1.27%
<i>Porphyromonadaceae</i>	6.38%	5.51%
<i>Acidaminobacteraceae</i>	2.59%	-
<i>Clostridiaceae</i>	16.37%	20.85%
<i>Lachnospiraceae</i>	1.53%	4.52%
<i>Mogibacteriaceae</i>	1.82%	3.63%
<i>Ruminococcaceae</i>	3.89%	6.86%
<i>Acetobacteraceae</i>	-	1.18%
<i>Methylocystaceae</i>	3.88%	2.42%
<i>Hydrogenophilaceae</i>	1.53%	-
<i>Enterobacteriaceae</i>	1.97%	-
<i>Methylococcaceae</i>	1.36%	-
<i>Moraxellaceae</i>	1.54%	-
<i>Rhodobacteraceae</i>	-	2.50%
<i>Sinobacteraceae</i>	1.34%	2.92%
<i>Desulfobacteraceae</i>	3.71%	-
<i>Desulfobulbaceae</i>	1.80%	2.35%
<i>Desulfovibrionaceae</i>	2.51%	5.16%
<i>Geobacteraceae</i>	5.12%	3.16%
<i>Syntrophaceae</i>	28.83%	28.81%
<i>Syntrophobacteraceae</i>	5.62%	-
Others	4.52%	8.84%

**Table S12.** Percentage of reads classified as bacteria > 1% using Greengenes 13\_8 involved in organic degradation of vertical variations within the HFCW depth.

Family > 1%	GreenGenes %	
	10 cm	50 cm
<i>Bacteroidaceae</i>	1.00%	2.73%
<i>Cytophagaceae</i>	6.14%	-

<i>Prophyromonadaceae</i>	-	2.67%
<i>Chitinophagaceae</i>	10.44%	-
<i>Saprospiraceae</i>	1.25%	1.00%
<i>Clostridiaceae</i>	5.50%	4.95%
<i>Lachnospiraceae</i>	-	1.65%
<i>Ruminococcaceae</i>	-	1.38%
<i>Acetobacteraceae</i>	9.42%	4.40%
<i>Methylocystaceae</i>	9.03%	5.30%
<i>Rhodobacteraceae</i>	3.27%	1.97%
<i>Sphingomonadaceae</i>	5.33%	1.41%
<i>Xanthobacteraceae</i>	1.42%	-
<i>Hydrogenophilaceae</i>	2.52%	6.33%
<i>Methylophilaceae</i>	2.10%	3.00%
<i>Enterobacteriaceae</i>	1.21%	-
<i>Methylococcaceae</i>	6.84%	3.61%
<i>Sinobacteraceae</i>	15.98%	15.69%
<i>Desulfobacteraceae</i>	1.11%	4.17%
<i>Desulfobulbaceae</i>	-	2.07%
<i>Geobacteraceae</i>	2.75%	2.26%
<i>Syntrophaceae</i>	6.96%	26.04%
<i>Syntrophobacteraceae</i>	2.52%	2.40%
Others	5.20%	5.43%

**Table S13.** Percentage of reads classified as bacteria family > 1% using Greengenes 13\_8. Longitudinal variations of organic matter degrading families within the HFCW (Inlet, Middle and Outlet).

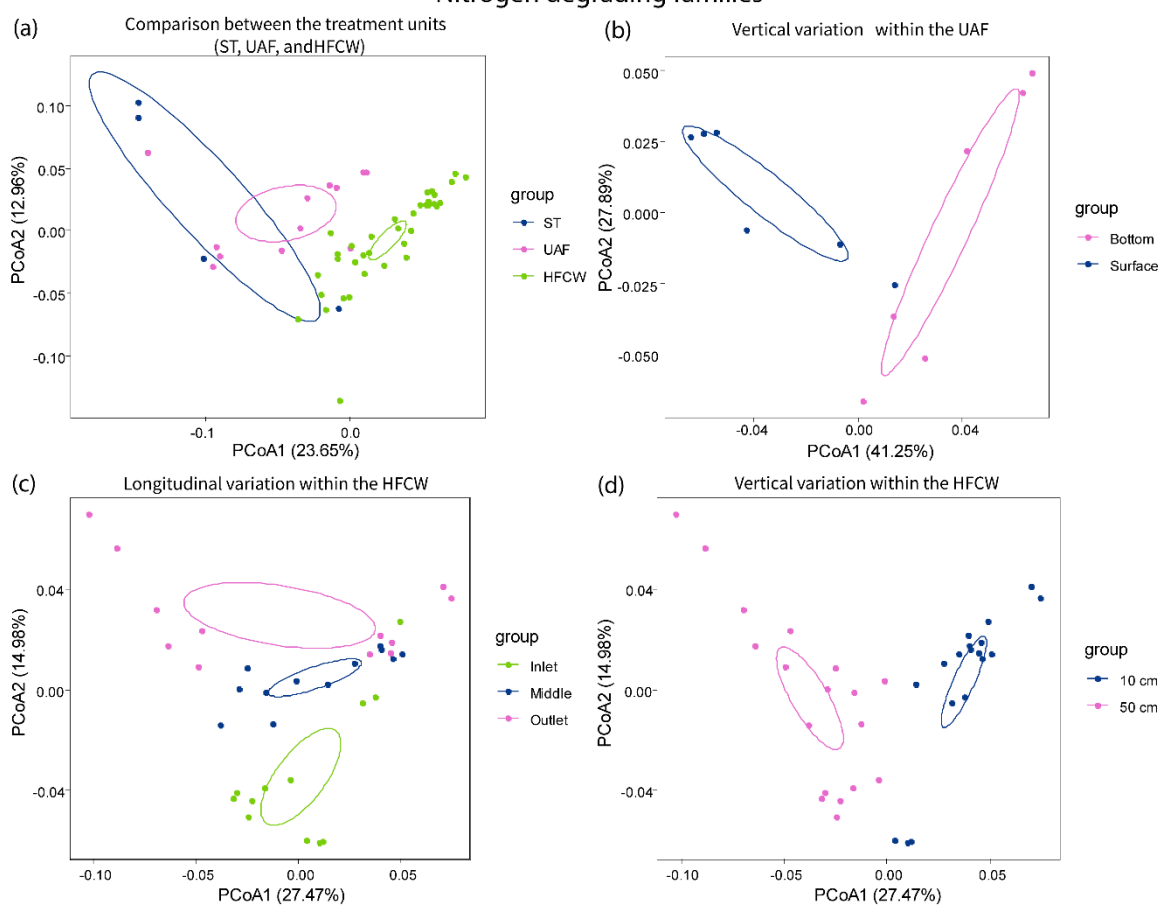
Family > 1%	GreenGenes %		
	Inlet	Middle	Outlet
<i>Bacteroidaceae</i>	4.16%	-	-
<i>Cytophagaceae</i>	1.47%	3.26%	4.69%
<i>Chitinophagaceae</i>	6.38%	5.44%	4.62%
<i>Prophyromonadaceae</i>	3.08%	-	1.23%
<i>Saprospiraceae</i>	-	1.87%	1.03%
<i>Acidaminobacteraceae</i>	-	-	1.12%
<i>Clostridiaceae</i>	4.08%	4.26%	7.47%
<i>Lachnospiraceae</i>	-	1.28%	2.10%
<i>Mogibacteriaceae</i>	-	-	1.27%
<i>Ruminococcaceae</i>	4.08%	-	1.52%
<i>Acetobacteraceae</i>	1.12%	8.17%	4.55%
<i>Methylocystaceae</i>	7.15%	7.49%	6.81%
<i>Rhodobacteraceae</i>	6.69%	2.37%	3.71%
<i>Sphingomonadaceae</i>	1.73%	3.11%	4.93%
<i>Xanthobacteraceae</i>	-	-	1.48%
<i>Hydrogenophilaceae</i>	7.08%	4.13%	2.20%
<i>Methylophilaceae</i>	-	3.22%	3.92%
<i>Enterobacteriaceae</i>	-	1.05%	1.25%
<i>Methylococcaceae</i>	8.21%	3.84%	2.60%
<i>Sinobacteraceae</i>	13.70%	17.43%	16.69%
<i>Desulfobacteraceae</i>	2.51%	2.53%	3.39%

<i>Desulfobulbaceae</i>	-	1.41%	2.37%
<i>Desulfovibrionaceae</i>	-	-	1.32%
<i>Geobacteraceae</i>	2.66%	3.30%	1.44%
<i>Syntrophaceae</i>	21.07%	16.88%	13.77%
<i>Syntrophobacteraceae</i>	1.77%	3.35%	2.36%
Others	5.41%	5.58%	2.18%

**Table S14.** PerMANOVA and ANOSIM analyses for the vertical (UAF and HFCW) and longitudinal variations (HFCW) for nitrogen and organic matter degrading families.

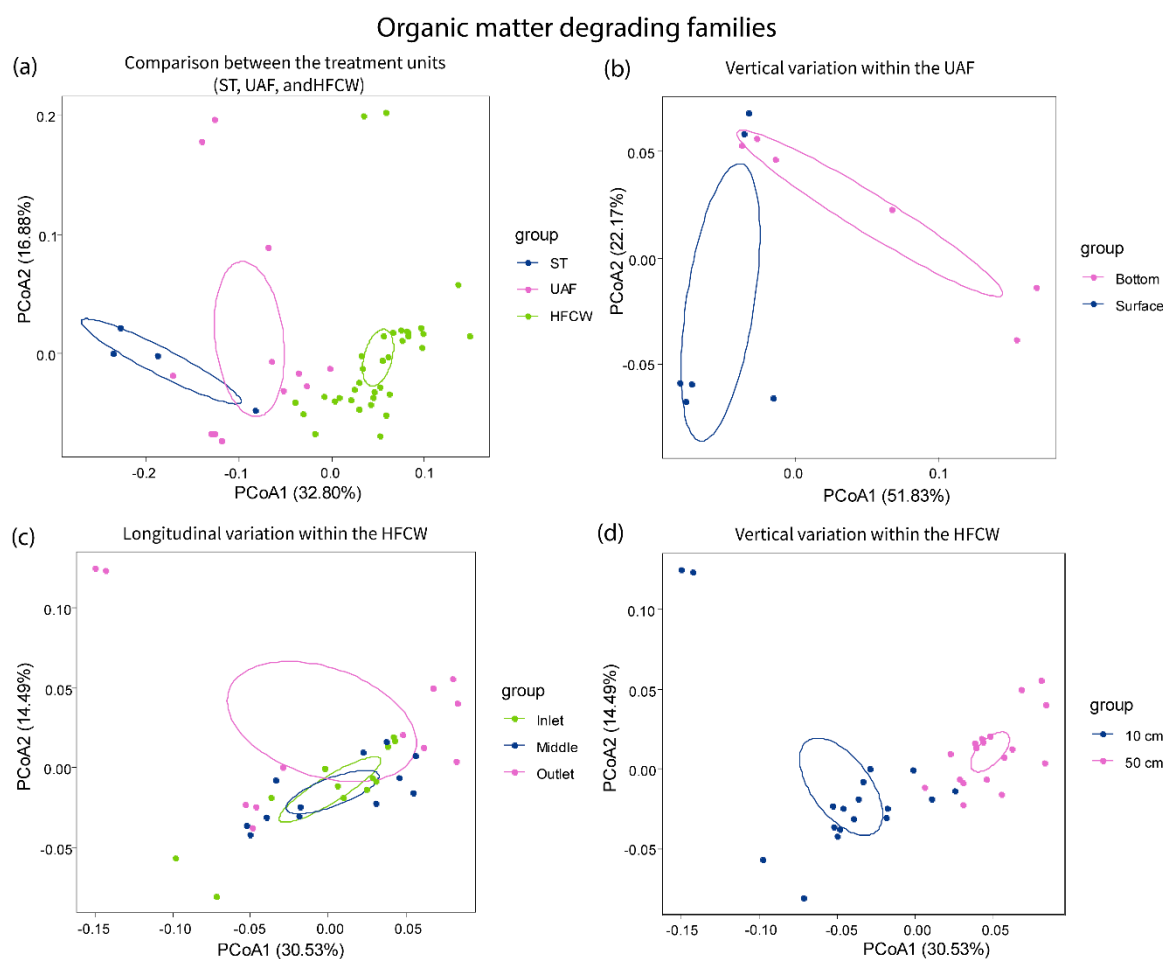
Bacteria	ANOSIM R	Significance
<b>Nitrogen related bacteria</b>	Unit: 0.5486	Unit: 0.001*
	UAF Depth: 0.4889	UAF Depth: 0.007*
	HFCW Longitudinal variations: 0.2549	HFCW Longitudinal variations: 0.001*
	HFCW Depth: 0.4933	HFCW Depth: 0.001*
<b>Organic matter related bacteria</b>	Unit: 0.6311	Unit: 0.001*
	UAF Depth: 0.3056	UAF Depth: 0.003*
	HFCW Longitudinal variations: 0.1996	HFCW Longitudinal variations: 0.008*
	HFCW Depth: 0.4575	HFCW Depth: 0.001*

### Nitrogen degrading families





**Figure S2.** PCoA for nitrogen removal related bacterial families. **(a)** Comparison between the treatment units (ST, UAF, and HFCW), **(b)** Vertical variations within the UAF. **(c)** Longitudinal variations within the HFCW **(d)** Vertical variation within the HFCW.



**Figure S3.** PCoA for organic matter removal degrading families. **(a)** Comparison between the treatment units (ST, UAF, and HFCW), **(b)** Vertical variations within the UAF. **(c)** Longitudinal variations within the HFCW **(d)** Vertical variation within the HFCW.

**Table S15.** RDA for nitrogen degrading families (first four significant redundancy components).

Variable	RDA1	RDA2	RDA3	RDA4
pH	-0.3575	-0.4277	0.5001	-0.6627
Temperature	-0.7966	0.2480	-0.4519	0.3156
DO	-0.2291	0.4402	0.3653	-0.7876
EC	-0.6288	0.0047	0.5972	0.4980
BOD <sub>5</sub>	-0.6750	0.1776	-0.1297	-0.0452
COD	-0.6842	0.2126	-0.0877	-0.0178
TSS	-0.7106	0.0591	0.2101	-0.1142
TNK	-0.6066	0.4802	0.0281	0.1454
NH <sub>3</sub> -N	-0.5763	0.5560	0.0238	0.2618
ON	-0.6118	0.4157	0.0303	0.0616
NO <sub>2</sub>	-0.7105	0.0453	0.2438	-0.1911

**Table S16.** RDA for organic matter degrading families (first four significant redundancy components).

Variable	RDA1	RDA2	RDA3	RDA4
pH	-0.09092	-0.5845	-0.5187	-0.6173

<b>Temperature</b>	-0.92198	0.1128	0.3310	0.1663
<b>DO</b>	-0.21471	0.2501	-0.7993	-0.5025
<b>EC</b>	-0.51209	-0.5360	-0.3538	0.5703
<b>BOD<sub>5</sub></b>	0.8045	-0.0810	0.0813	-0.0156
<b>COD</b>	0.7913	-0.0327	0.0795	-0.0063
<b>TSS</b>	0.7276	0.1657	-0.0306	0.0782
<b>TNK</b>	0.6965	0.3853	0.3248	-0.1739
<b>NH3-N</b>	0.6432	0.4078	0.3468	-0.1765
<b>ON</b>	0.7152	0.3599	0.3012	-0.1676
<b>NO<sub>2</sub></b>	0.6855	0.1480	-0.0546	0.2050

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