Supplementary information for:

A Full-Scale Comparative Study of Conventional and Side-Stream Enhanced Biological Phosphorus Removal Processes

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Table S1 Key process parameters for the typical secondary biological treatment train.

Zone	Cell	Size (L×W×D) (ft)	Volume (gal)	HRT (h)	SRT (days)
Anaerobic	Cell 1	27×27×15	81,194	0.7	0.7 h
	Cell 2	27×27×15	82,726		
Anoxic	Cell 3	27×27×15	81,946	0.7	0.7 h
	Cell 4	27×27×15	81,946		
Aerobic	Cell 5	218×55×15	1,357,523	6.1	4.5
Total	-	-	1,685,335	7.5	5.4

Table S2 Sampling plan during full-scale pilot testing at the Rock Creek Facility.

Parameter /Test	Minimum frequency /Sampling Date	Sampling site
Flow	Daily	PE, UFAT, AB, WAS, RAS, CELL
pH	Daily	PE, SE, AB
COD	Daily	PE, SE
sCOD	1/week	SE
Alkalinity	4/week	PE, SE, RAS
TSS	3/week	PE, SE, AB, CELL
VSS	1/week	PE, SE, AB
SVI	4/week	AB
TP	1/week	PE, SE
PO_4-P	Daily	PE, SE, CELL
TKN	1/week	PE
NH_4-N	Daily	PE, SE
NO_3-N, NO_2-N	1/week	PE, SE, CELL
SRT	3/week	AB
ORP	1/week	CELL
VFAs	3/week	PE, UFAT
P release-uptake testing	a	AB
FISH, DAPI	b	AB
Glycogen	c	AB
PHAs	c	AB
Amplicon sequencing	d	AB

PE: primary effluent; SE: secondary effluent; UFAT: unified fermentation and thickening process; AB: aeration basins (AB4 and AB5); WAS: wasted activated sludge; RAS: return activated sludge; CELL: Cell 1, 2, 3, 4, mid and end of Cell 5 in each train;
a: May 18, May 25, June 8, June 15, June 22, June 29, July 6, July 13, July 21, and August 3;
b: April 26, June 22, July 7, July 20, August 2, August 30, and October 26;
c: June 21 and August 3;
d: April 7, April 26, June 22, July 20, August 2, August 17, August 30, and October 26.

Table S3 Probes for FISH analysis for quantification of functionally relevant microbial populations.

	Probe	Sequence	Specificity	Formamide %	References
Most Bacteria	EUB338	GCTGCCTCCCGTAGGAGT	Most bacteria	35	(Bonkowski, 2004)
	EUB338 II	GCAGCCACCCGTAGGTGT	*Verrucomicrobiales* and other bacteria	35	(Daims et al., 1999)
	EUB338 III	GCTGCCACCCGTAGGTGT	*Planctomycetales* and other bacteria	35	(Daims et al., 1999)
PAO	PAO462b	CCGTCATCTRCWCAGGGTATTAAC	Most Accumulibacter	35	(Zilles et al., 2002)
	PAO651	CCCTCTGCCAAACTCCAG	Most Accumulibacter	35	(Crocetti et al., 2000)
	PAO846b	GTTAGCTACGGYACTAAAAGG	Most Accumulibacter	35	(Zilles et al., 2002)
	Tet1-266	CCCGTCGTCGCCTGTAGC	*Tetrasphaera* clade 1	25	(Nguyen et al., 2011a)
	Tet2-892	TAGTTAGCCTTGCGGCCG	*Tetrasphaera* clade 2A	5	(Nguyen et al., 2011a)
	Tet2-174	GCTCCGTCTCGTATCCGG	*Tetrasphaera* clade 2B	20	(Nguyen et al., 2011a)
	Tet3-654	GGTCTCCCCTACCATACT	*Tetrasphaera* clade 3	35	(Nguyen et al., 2011a)
GAO	GAOQ431	TCCCCGCCTAAAGGGCTT	Some Competibacter	35	(Crocetti et al., 2002)
	GAOQ989	TTCCCCGGATGTCAAGGC	Some Competibacter	35	(Crocetti et al., 2002)
	GB742	CTCAGCGTCAGTGTGGCC	Most Competibacter	35	(Kim et al., 2010)
	TFO_DF218	GAAGCCTTTGCCCCTCAG	*Defluvicoccus* Cluster 1	35	(Wong et al., 2004)
	TFO_DF618	GCCTCACTTGTCTAACCG	*Defluvicoccus* Cluster 1	35	(Wong et al., 2004)
	DF988	GATACGACGCCCATGTCAAGG	*Defluvicoccus* Cluster 2	35	(Meyer et al., 2006)
	DF1020	CCGGCCGAACCGACTCCC	*Defluvicoccus* Cluster 2	35	(Meyer et al., 2006)

Prop207	GCTCCAAAAGCGCAAGGTCC GAAG	*Candidatus* Propionivibrio aalborgensis	35	(Albertsen *et al.*, 2016)

Table S4 Summary of PO_4-P performance data during the pilot test.

	Phase I-A			Phase I-B		
	PE	AB4 SE	AB5 SE	PE	AB4 SE	AB5 SE
Mean (mg/L)	1.73	0.67	0.59	2.07	0.40	0.10
Geometric Mean (mg/L)	1.62	0.28	0.19	2.02	0.27	0.09
Std. Dev (mg/L)	0.72	1.02	0.99	0.48	0.32	0.08
Skew	1.72	2.29	2.43	1.45	0.95	2.08
Minimum (mg/L)	0.79	0.04	0.03	1.25	0.04	0.03
Maximum (mg/L)	4.44	4.20	4.28	4.07	1.50	0.41
3.84% [mg/L]	0.98	0.04	0.03	1.39	0.06	0.04
50% [mg/L]	1.49	0.24	0.17	1.98	0.36	0.07
90% [mg/L]	2.50	2.05	1.60	2.58	0.78	0.24
3.84%/50%	0.65	0.16	0.18	0.71	0.16	0.52
90%/50%	1.68	8.40	9.18	1.30	2.15	3.20
PO_4-P < 0.1 mg/L	0%	25%	45%	0%	23%	71%
PO_4-P < 0.5 mg/L	0%	71%	71%	0%	64%	100%
PO_4-P < 1.0 mg/L	7%	78%	80%	0%	93%	100%
PO_4-P < 1.5 mg/L	51%	87%	89%	7%	99%	100%
PO_4-P < 2.0 mg/L	78%	89%	91%	51%	100%	100%

Table S Error! No text of specified style in document. Summary of PO_4-P release and uptake profiles during full-scale pilot testing (Phase I, A2O and SSRC configurations), units in mg P/L.

Configuration (Phase)	Influent[a]	Effluent	P release	P uptake	Net P removal	Removal efficiency
Phase I-A						
AB4	4.4	1.0	3.3	6.7	3.4	77%
AB5	4.4	1.0	3.0	6.4	3.4	77%
Phase I-B						
AB4	3.9	0.4	5.9	9.4	3.5	89%
AB5	3.9	0.1	6.2	10.0	3.8	97%

[a]: includes PO_4-P contained in PE, UFAT, and RAS.

Table S6 Influent COD/P and VFA/P ratios in A2O and S2EBPR configuration during pilot testing.

Source	COD/TP	COD/PO_4-P	VFA/TP	VFA/PO_4-P	HAc/HPr
PE	72±9	80±22	4.6±3.7	5.3±3.9	2.6±2.1

UFAT	NA	NA	NA	208±180	1.3±0.4

Units: g COD/g P; values shown are mean± standard deviation. HAc: acetate; HPr: propionate; NA: not available.

Table S7 Distribution and load of VFA and PO_4-P in the aerobic zone of A2O and S2EBPR configurations during Phase I-B.

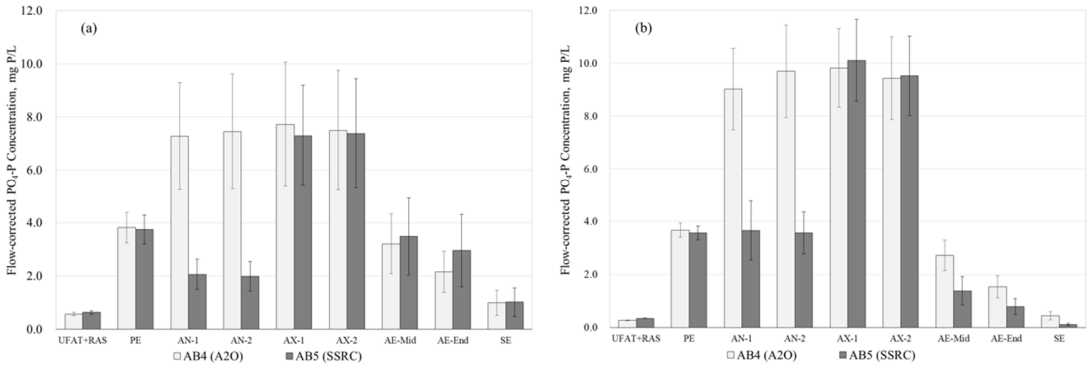
Location	VFA (kgCOD/d)				PO_4-P (kgP/d)				VFA/PO_4-P (g COD/g P)
	PE	UFAT	SSR	Total	PE	UFAT	SSR	Total	
AB4 (AN)	429	403	NA	832	85	1	NA	86	9.7
AB5 (SSR+MS)	428	403	180	1011	84	1	NA	85	11.9

AN: anaerobic zone; SSR: estimated VFA production in side-stream reactor; MS: mainstream; NA: not available.

Table S8 Average retention time in A2O and S2EBPR configurations during pilot testing at the Rock Creek Facility.

Configuration	Anaerobic HRT (h)		Anaerobic SRT (h)		Aerobic SRT (d)	
	I-A	I-B	I-A	I-B	I-A	I-B
AB4	0.6	0.8	0.6	0.8	4.6	4.5
AB5	1.6	2.1	1.6	NA	4.8	4.5

NA: not available.



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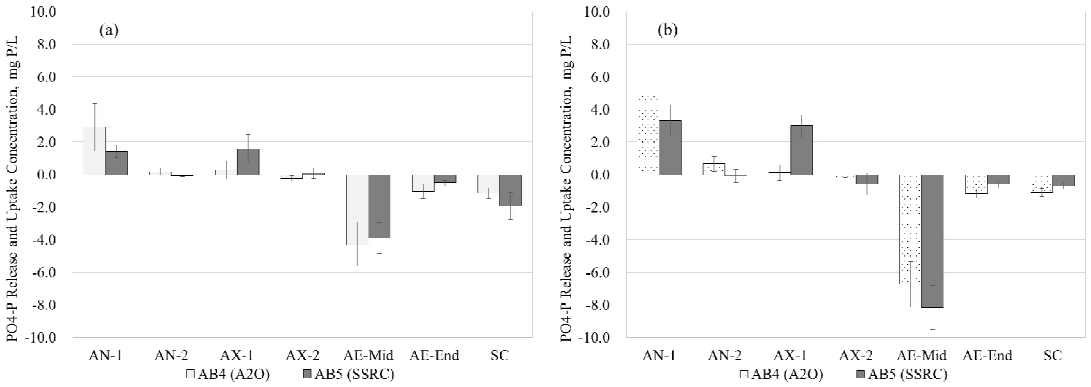

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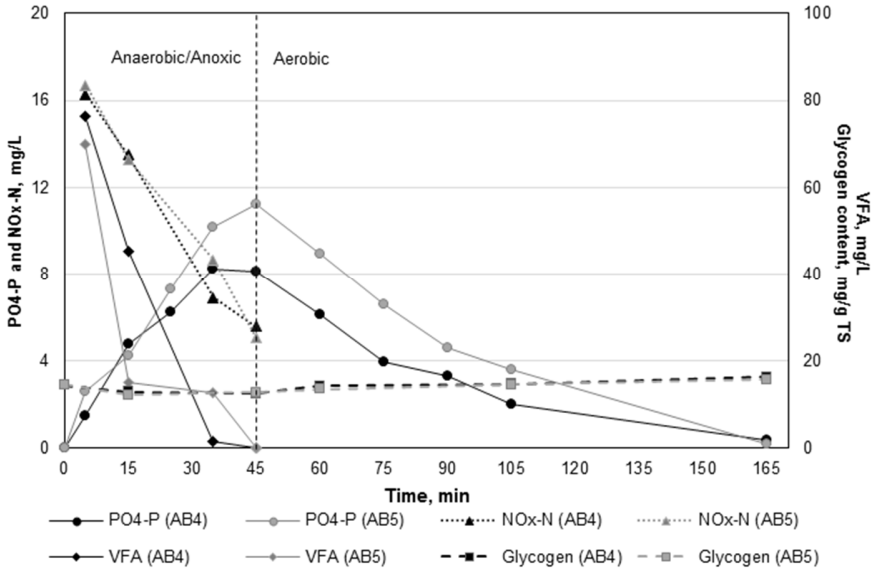

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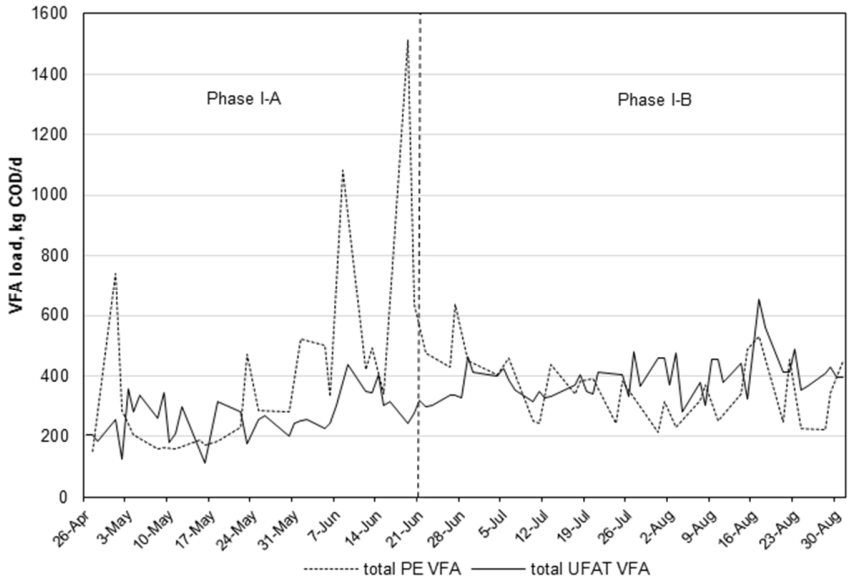

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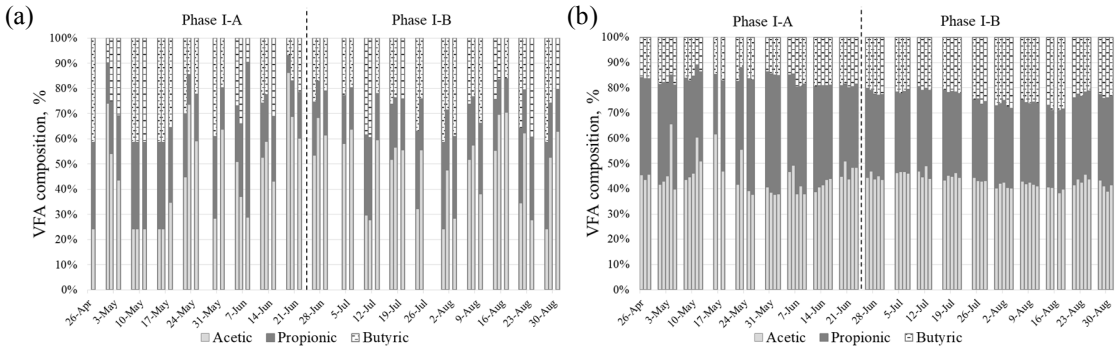

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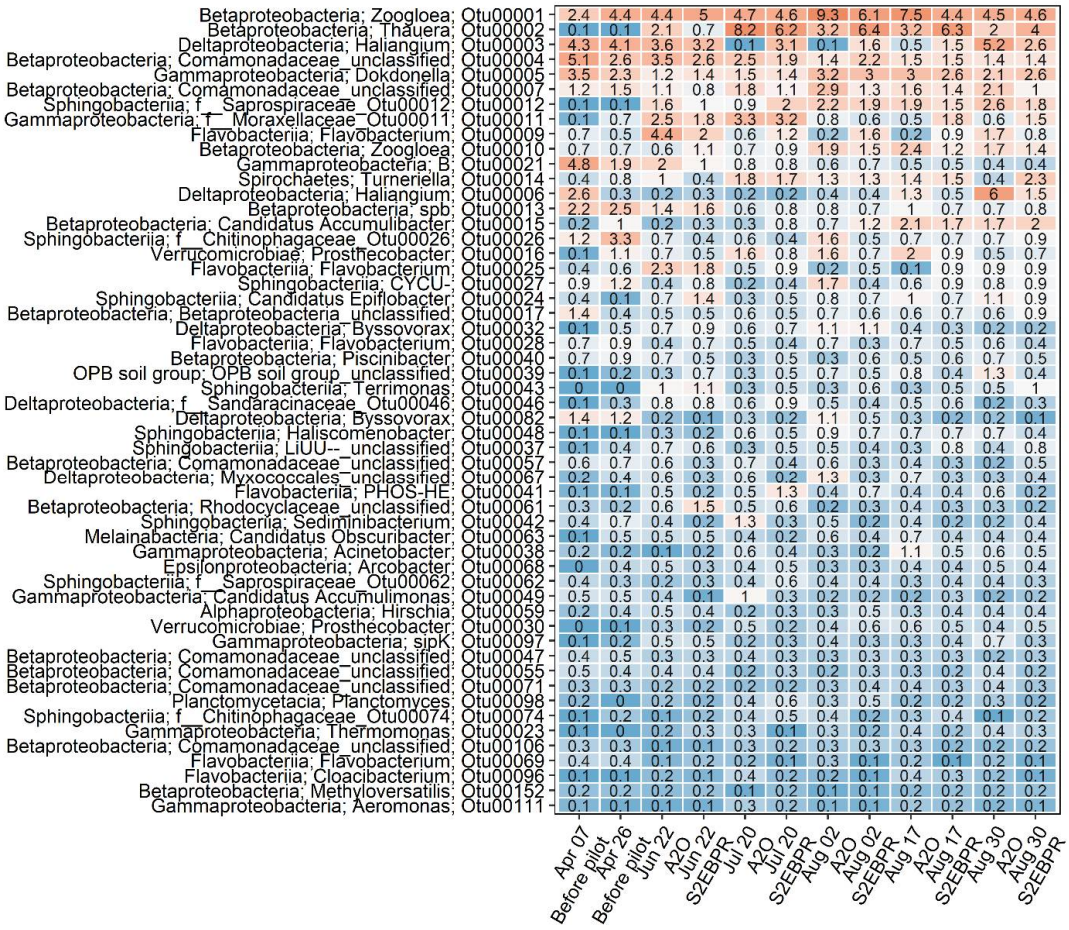

Figure S6 Time series heatmap of the core OTUs (n=57) found in both A2O and S2EBPR configurations during pilot testing.