

## **Supplemental information**

**Supplemental Table S1.** Summary of Illumina sequencing results.

Sample	Loading conditions		Number of sequences	Number of OTUs	$C_x^a$	Diversity indices <sup>b</sup>			
	Volumetric load (mgCOD L <sup>-1</sup> d <sup>-1</sup> )	HRT (d)				Chao1	Shannon	1/Simpson	
Piggery wastewater			41262±418	96±15	0.998±0.0004	208±28	1.77±0.12	1.76±0.10	
	Seed		33235±168	473±0	0.986±0.0001	919±0	4.54±0.29	7.61±2.20	
Biofilm	Unstable	704	6	30581±1922	350±20	0.988±0.0014	1046±113	4.56±0.05	7.93±0.10
	Stable	704	6	20656±418	269±81	0.986±0.0027	998±177	3.29±0.37	3.58±0.99
	Deteriorative	1,407	3	50017±418	264±8	0.995±0.0005	494±83	2.03±0.12	1.84±0.11
Sludge	Unstable	704	6	23661±1148	437±33	0.991±0.0009	680±29	2.83±0.03	2.96±0.01
	Stable	704	6	34694±2732	955±35	0.980±0.0001	1375±22	3.78±0.12	4.44±0.16
	Deteriorative	1,407	3	30581±1922	933±43	0.985±0.0014	1173±93	3.69±0.21	4.01±0.33

<sup>a</sup>Calculated from the equation  $C_x = 1 - (n/N)$ , where “n” is the number of singleton OTUs and N is the total number of sequences.

<sup>b</sup>Each index was calculated based on an equal number of sequences ( $n=9,838$ ). All data presented are based on the average of two replicates.

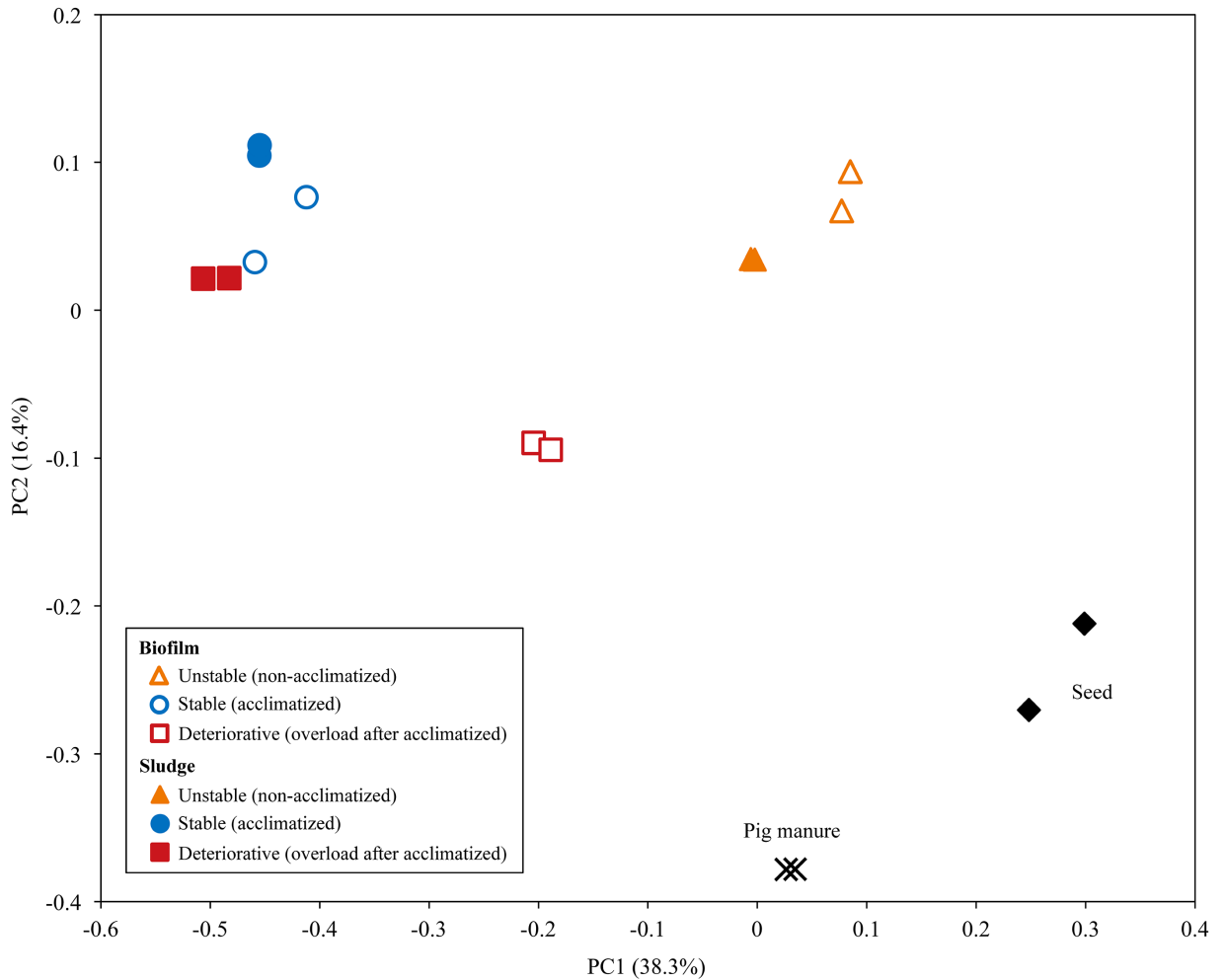
**Supplemental Table S2.** Ten most abundant operational taxonomic units (OTUs) in sludge under unstable, stable, and deteriorative conditions.

OTU ID	Eukaryotic microorganisms		Accession No.	Identity (%)	Relative abundance <sup>a</sup> (%)	Fold change <sup>b</sup>
	Kingdom/ phylum	Species				
<b>Unstable (non-acclimatized)</b>						
117	<i>Fungi</i>	<i>Hyaloraphidium curvatum</i>	NG017172	84	53.14	2.1
6492	<i>Metazoa</i>	<i>Isohypsibius granulifer</i>	EF620403	98	25.38	71.3
11958	<i>Cercozoa</i>	<i>Rhogostoma minus</i>	LC032468	97	1.92	6.6
17603	<i>Metazoa</i>	<i>Polyarthra remata</i>	DQ297716	95	1.33	2.6
14625	<i>Fungi</i>	<i>Hyaloraphidium curvatum</i>	NG017172	82	1.23	2.4
4676	<i>Metazoa</i>	<i>Isohypsibius granulifer</i>	EF620403	95	1.13	90.2
16984	<i>Fungi</i>	<i>Hyaloraphidium curvatum</i>	NG017172	83	1.13	2.5
10633	<i>Metazoa</i>	<i>Isohypsibius granulifer</i>	EF620403	95	1.12	—
13663	<i>Fungi</i>	<i>Hyaloraphidium curvatum</i>	NG017172	83	0.98	5.2
12713	<i>Cercozoa</i>	<i>Cryothecomonas aestivalis</i>	AF290541	82	0.93	24.3
<b>Stable (acclimatized)</b>						
4403	<i>Metazoa</i>	<i>Habrotrocha bidens</i>	KM043258	100	43.22	8.6
17845	<i>Ciliphora</i>	<i>Campanella umbellaria</i>	AF401524	88	20.61	0.4
14179	<i>Opisthokont</i> *	<i>Salpingoeca tuba</i>	HQ026774	87	6.47	0.4
117	<i>Fungi</i>	<i>Hyaloraphidium curvatum</i>	NG017172	84	3.19	2.1
16432	<i>Metazoa</i>	<i>Habrotrocha bidens</i>	KM043258	100	2.79	8.3
1610	<i>Fungi</i>	<i>Mortierella clonocystis</i>	HQ667479	85	2.20	2.7
467	<i>Fungi</i>	<i>Paramicrosporidium vannellae</i>	JQ796368	86	2.08	1.2
3603	<i>Cercozoa</i>	<i>Orciraptor agilis</i>	KF207875	81	1.85	4.4
18072	<i>Metazoa</i>	<i>Encentrum astridae</i>	DQ297695	98	1.66	5.8
19347	<i>Ciliphora</i>	<i>Opercularia microdiscum</i>	AF401525	88	1.66	2.9
<b>Deteriorative (acclimatized but overloading)</b>						
17845	<i>Ciliphora</i>	<i>Campanella umbellaria</i>	AF401524	88	48.04	5.9
4403	<i>Metazoa</i>	<i>Habrotrocha bidens</i>	KM043258	100	14.80	6.6
16518	<i>Metazoa</i>	<i>Macrobotus sapiens</i>	DQ839601	96	5.47	1.5
16474	<i>Fungi</i>	<i>Hyaloraphidium curvatum</i>	NG017172	84	5.03	2.9
19310	<i>Dinoflagellata</i>	<i>Exuviaella pusilla</i>	DQ388459	91	3.26	4.9
3603	<i>Cercozoa</i>	<i>Orciraptor agilis</i>	KF207875	81	2.51	4.3
117	<i>Fungi</i>	<i>Hyaloraphidium curvatum</i>	NG017172	84	2.02	7.7
467	<i>Fungi</i>	<i>Paramicrosporidium vannellae</i>	JQ796368	86	1.97	4.7
19347	<i>Cercozoa</i>	<i>Opercularia microdiscum</i>	AF401525	88	1.52	10.3
16432	<i>Metazoa</i>	<i>Habrotrocha bidens</i>	KM043258	100	1.06	8.5

<sup>a</sup>Relative abundance represents average values of two independent determinations.

<sup>b</sup>Fold changes relative to the biofilm are shown.

\*Phylum level classification is uncertain, and classified as the unranked taxon *Opisthokont*.



**Supplemental Fig. S1.** Comparison of biofilm and sludge microbiomes during piggery wastewater treatment. A principal coordinate analysis (PCoA) scatter plot of 18S rRNA genes obtained via Illumina sequencing. Weighted UniFrac distances were calculated based on an equal number ( $n=9,838$ ) of sequences. Open and closed symbols indicate sludge and biofilm microbiomes, respectively, while the symbol type indicates reactor conditions.