Supplementary Information

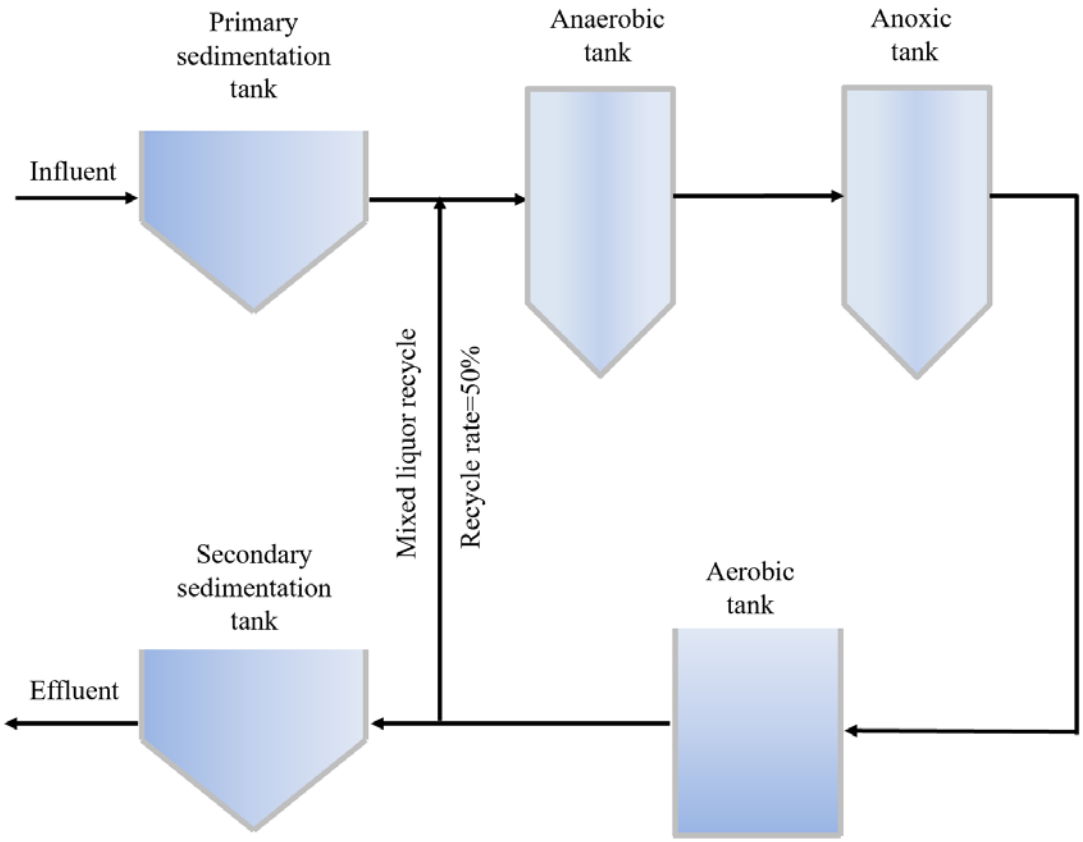

Fig. S1 Schematic diagram of biological treatment process in the full-scale A2O wastewater treatment plant.

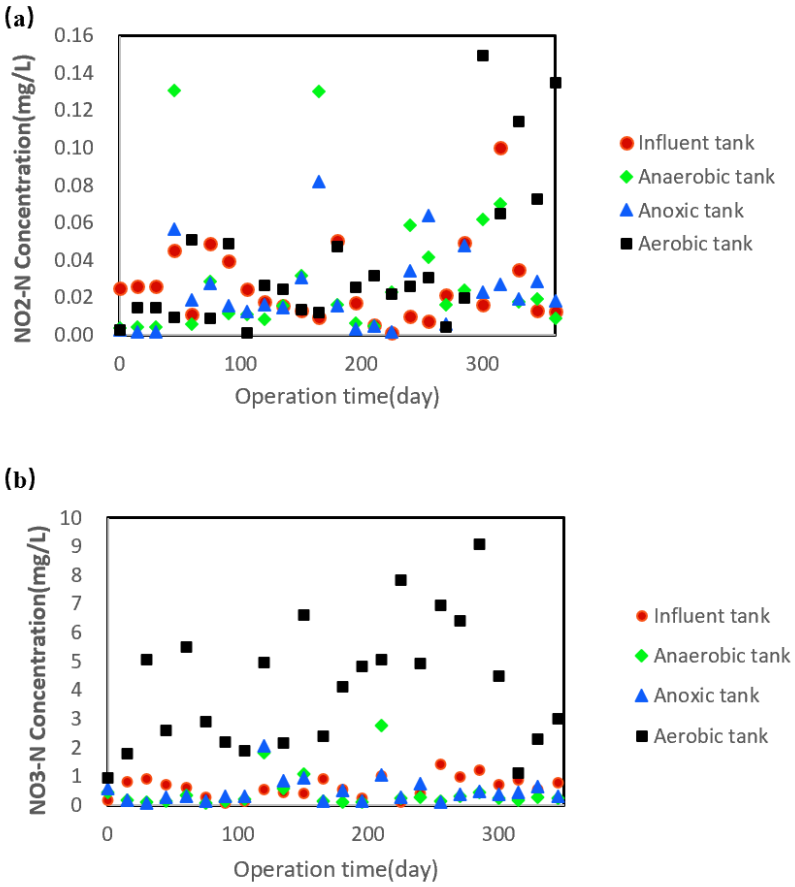

Fig. S2 Water quality of influent and three tanks during one-year sampling. (a) Nitrite Nitrogen (NO_2-N), (b) Nitrate nitrogen (NO_3-N).

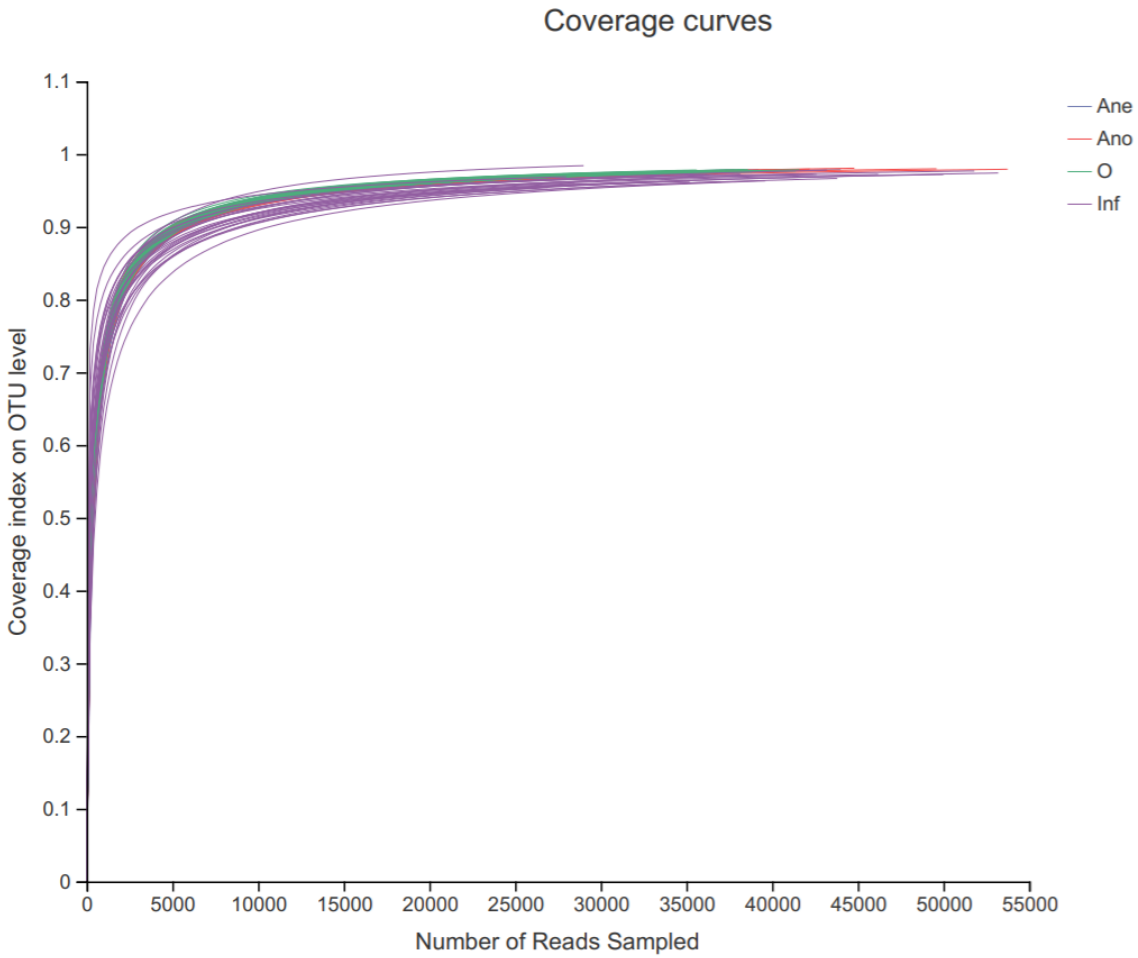

Fig. S3 Rarefaction curve Coverage curves.

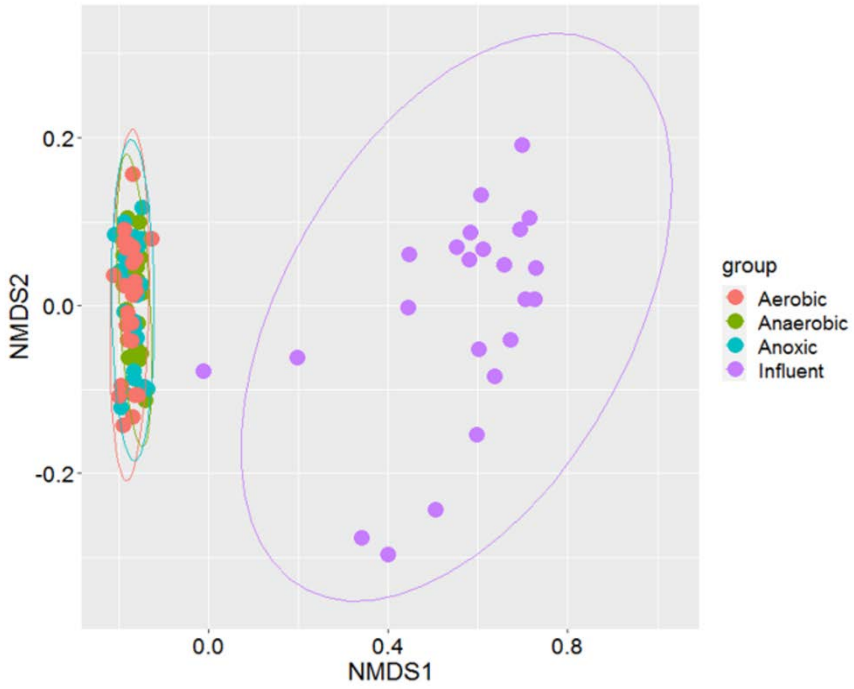

Fig. S4 Beta diversity was measured by NMDS using the Bray-Curtis distance.

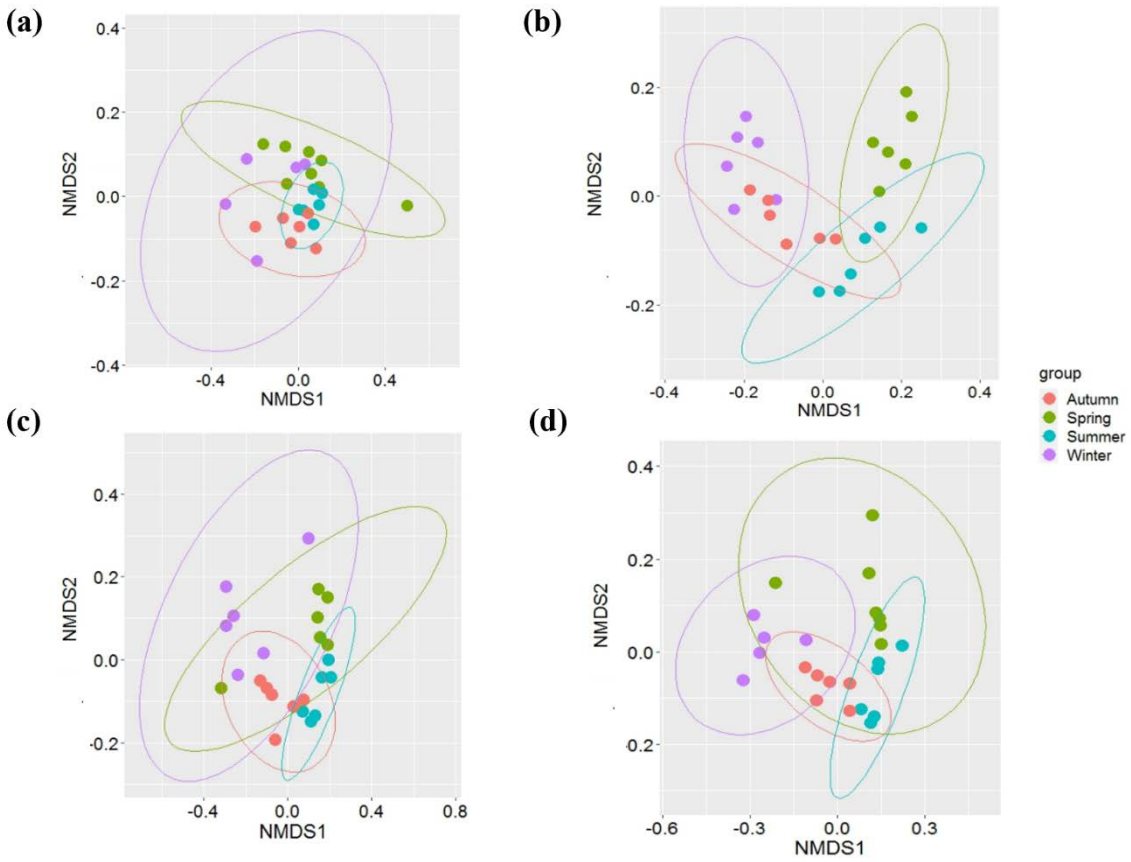

Fig. S5 NMDS based on Bray-Curtis distance showing the relationships between community grouping pattern and season. (a) Influent, (b) Anoxic tank, (c) Anaerobic tank, and (d) Aerobic tank.

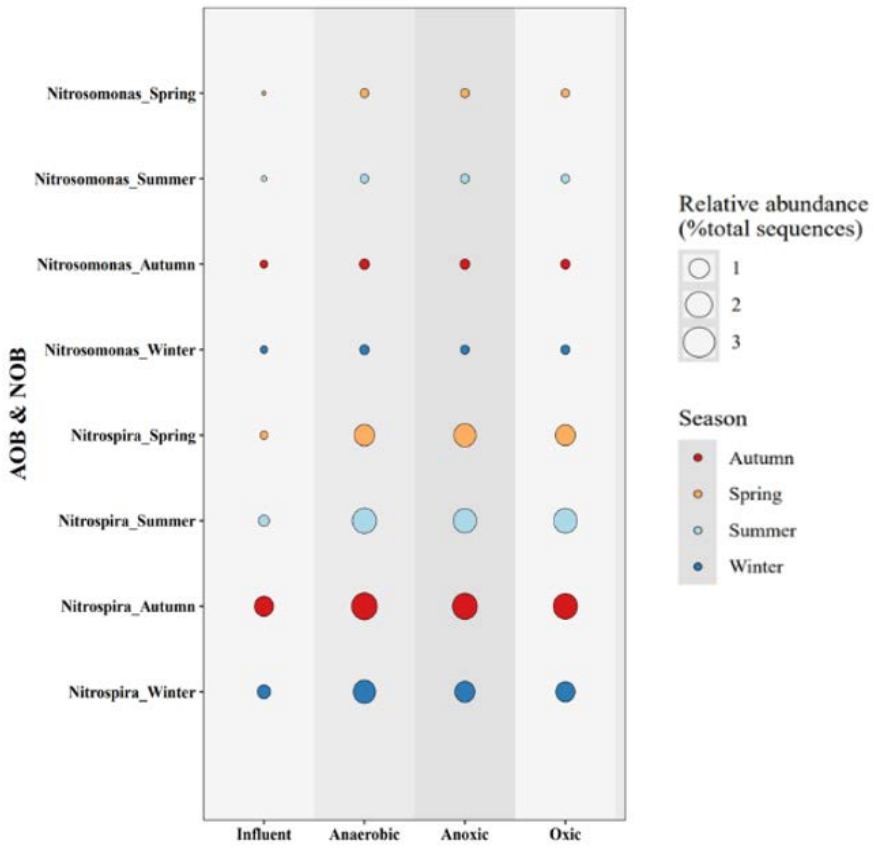

Fig. S6 Seasonal variation in functional bacteria for nitrification (AOB & NOB).

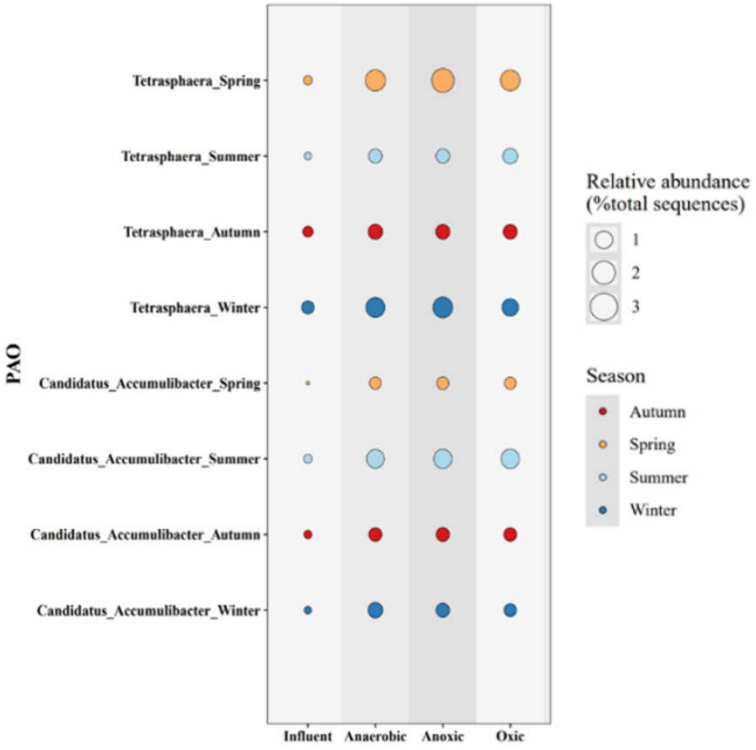

Fig. S7 Seasonal variation in functional bacteria for dephosphorization (PAO).

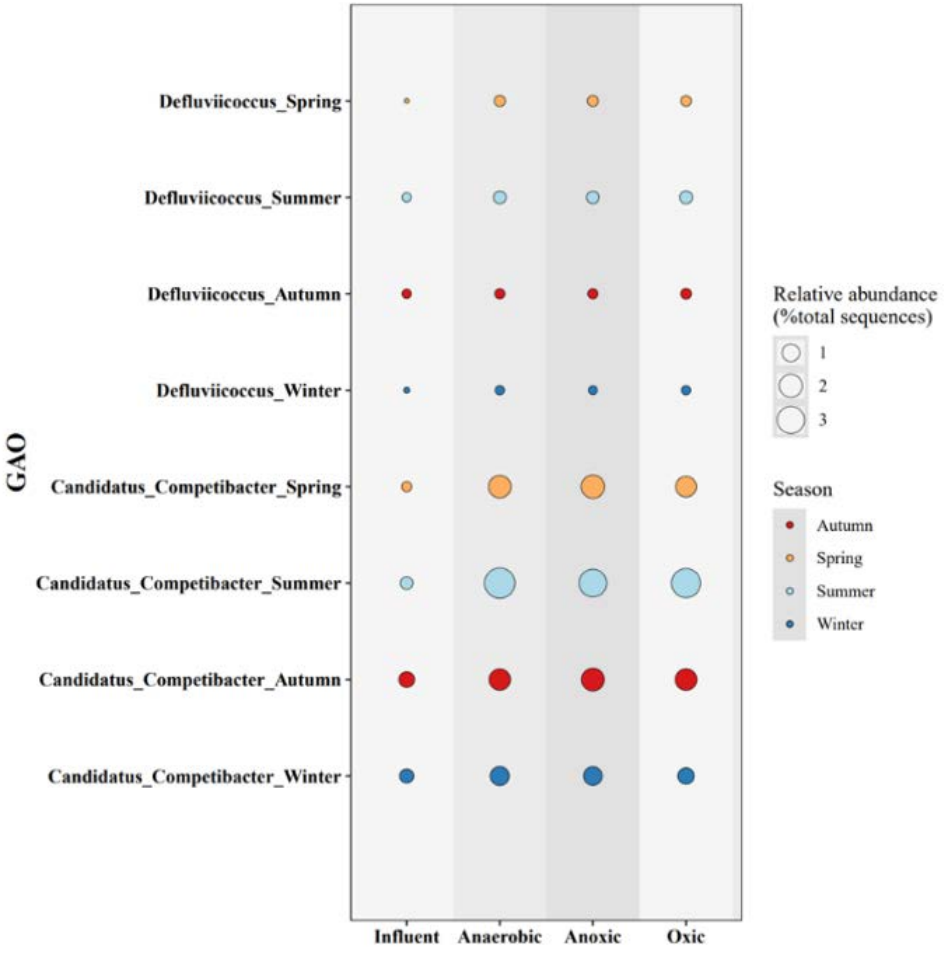

Fig. S8 Seasonal variation in functional bacteria.

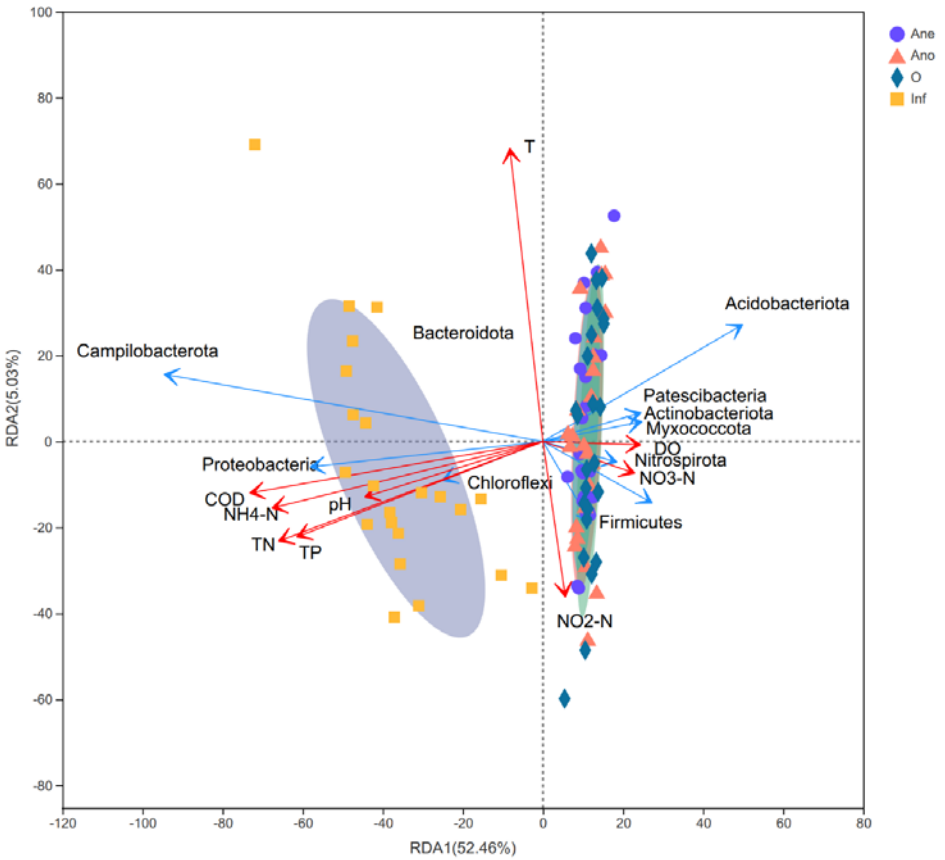

Fig. S9 RDA-CCA plot showing the WWTP activated sludge and influent wastewater microbial community correlation with environmental variables.

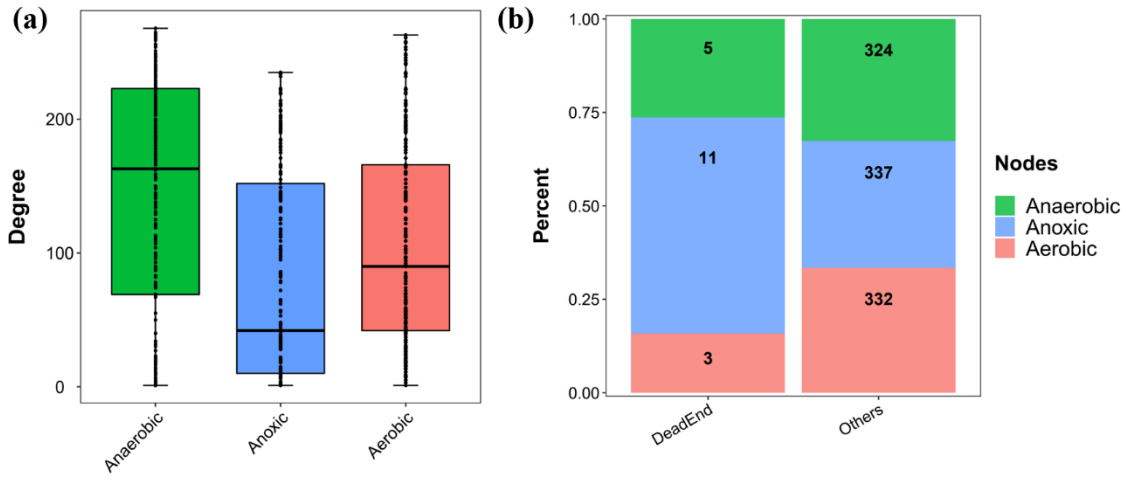

Fig. S10 (a) Distribution of the degrees of nodes, (b) numbers and percentage of dead ends (nodes with one degree) and other nodes in anaerobic, anoxic and aerobic networks.

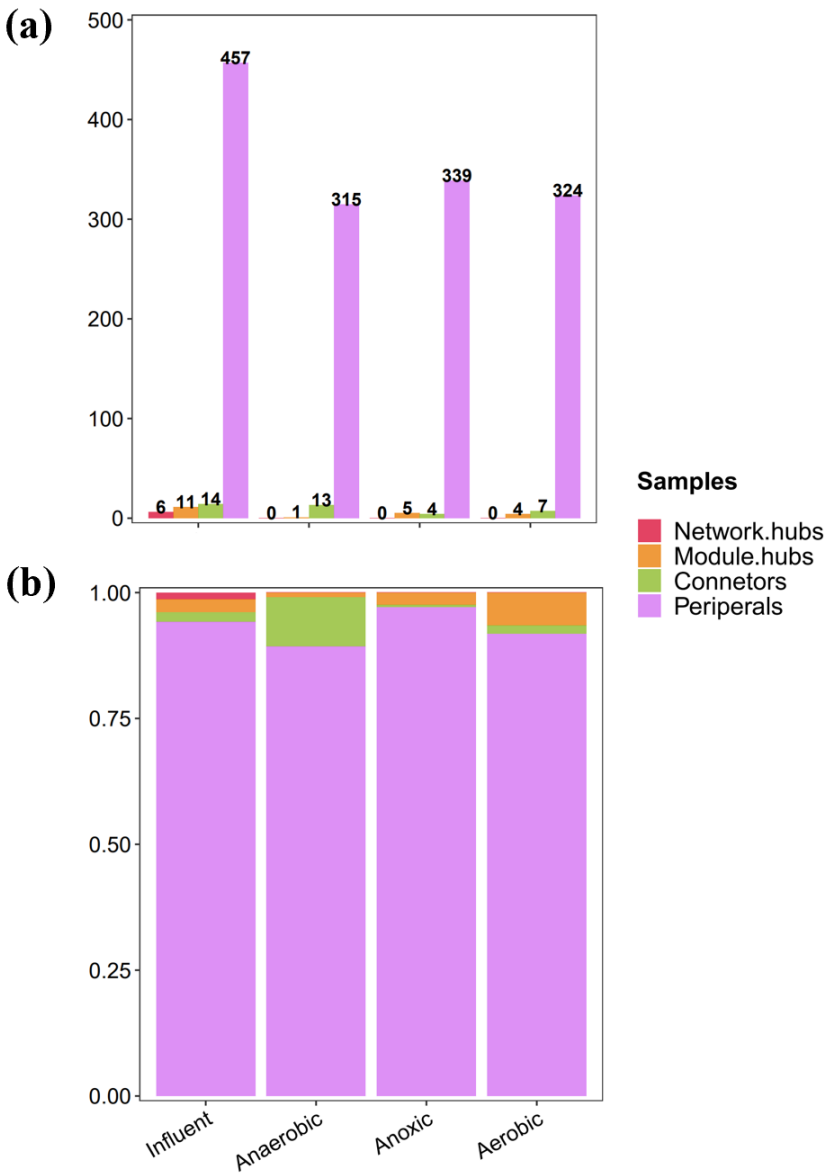

Fig. S11 (a) Numbers of nodes and (b) Relative Abundance of taxa in different parts (the peripherals, connectors, module hubs, and network hubs) of co-occurrence network in different tanks.

Table S1 Significance tests on the differences between anaerobic sludge microbiota, anoxic sludge microbiota, aerobic sludge microbiota and influent wastewater microbiota.

Bacterial communities	Adonis [a)]		ANOSIM [b)]	
	F	*P*	*R*	*P*
Anaerobic sludge microbiota, influent wastewater microbiota	46.40	0.001	0.946	0.001
Anoxic sludge microbiota, influent wastewater microbiota	44.52	0.001	0.948	0.001
Aerobic sludge microbiota, influent wastewater microbiota	45.13	0.001	0.950	0.001
Anaerobic sludge microbiota, Anoxic sludge microbiota	0.034	0.969	0.413	0.979
Anoxic sludge microbiota, Aerobic sludge microbiota	0.028	0.895	0.431	0.971
Aerobic sludge microbiota, Anaerobic sludge microbiota	0.021	0.778	0.572	0.862

Notes: Both statistical approaches were tested at the OTU level based on Bray-Curtis distances. *P* values are of corresponding significance tests. a) Distance matrices were used as variance of Permutational multivariate analysis. *F* test was adopted to check the differences according to sequential sums of squares from permutations of the raw data. b) Analysis of similarities. Statistic *R* is conducted on the basis of the difference of mean ranks between groups and within groups. The significance of observed *R* is assessed by permuting the grouping vector to obtain the empirical distribution of *R* under the null model.

Table S2 The variations of the air temperature, water temperature and DO over the year.

Date	Air Temperature (℃)	Water Temperature (℃)	DO of Aerobic Tank (mg/L)	DO of Anoxic Tank (mg/L)	DO of Aeration Tank (mg/L)
Mar 1st, 2019	22	18.1	0.09	0.10	4.37
Mar 15th, 2019	21	18.3	0.09	0.11	4.65
Apr 1st, 2019	25	19.4	0.10	0.11	3.98
Apr 15th, 2019	27	19.8	0.08	0.10	4.23
May 1st, 2019	30	20.9	0.09	0.11	3.34
May 16th, 2019	31	21.1	0.09	0.12	3.86
Jun 1st, 2019	33	21.7	0.08	0.10	3.57
Jun 15th, 2019	34	21.8	0.09	0.10	4.32
Jul 1st, 2019	36	22.1	0.11	0.12	4.11
Jul 15th, 2019	35	23.0	0.10	0.12	3.87
Aug 1st, 2019	36	23.3	0.11	0.09	3.75
Aug 15th, 2019	37	22.7	0.09	0.09	3.23
Sep 1st, 2019	34	21.9	0.08	0.10	4.12
Sep 15th, 2019	33	21.5	0.08	0.09	4.23
Oct 1st, 2019	29	21.3	0.09	0.12	3.98
Oct 15th, 2019	31	21.0	0.09	0.11	3.76
Nov 1st, 2019	28	20.5	0.11	0.10	4.17
Nov 16th, 2019	25	20.6	0.10	0.10	3.71
Dec 1st, 2019	20	18.9	0.07	0.10	3.87
Dec 15th, 2019	17	17.8	0.10	0.12	4.35
Jan 1st, 2020	13	14.9	0.09	0.11	4.23
Jan 16th, 2020	8	13.5	0.11	0.11	3.65
Feb 1st, 2020	9	14.7	0.08	0.10	2.98
Feb 15th, 2020	12	15.3	0.08	0.09	2.73

Notes: Data of air temperature was obtained from Weather data website. Data of water temperature and DO were measured using a multi105 meterand a DO meter. See Material 2.1.

Table S3 Potential function of keystone species observed in ecological networks of microbial communities in different tanks.

Network	OTU	Topological position	Lowest taxonomic rank	Affiliate Phylum	Functional description *
Anaerobic tank	OTU4826	Connector	Roseiflexaceae	Chloroflexi	Fermentation, Short-chain fatty acids
	OTU757	Module hub	PHOS-HE36	Bacteroidetes	Rarely described
	OTU3664	Module hub	Solirubrobacterales	Actinobacteria	Short-chain fatty acids, Sugars
	OTU8321	Module hub	Microtrichaceae	Actinobacteria	Rarely described
	OTU1117	Module hub	Candidatus Competibacter	Proteobacteria	GAO, Denitrifier, Short-chain fatty acids
	OTU2035	Module hub	Ellin6067	Proteobacteria	Rarely described
	OTU9501	Module hub	Saprospiraceae	Bacteroidetes	Proteins/amino acids, Sugars
	OTU5586	Module hub	Sulfuritalea	Proteobacteria	Sulfate reduction, Denitrifier
	OTU2024	Module hub	Geothrix	Acidobacteria	Fermentation, Short-chain fatty acids
	OTU8352	Module hub	PeM15	Actinobacteria	Rarely described
	OTU8049	Module hub	Chitinophagales	Bacteroidetes	Rarely described
	OTU7814	Module hub	BSV26	Bacteroidetes	Rarely described
	OTU8918	Module hub	RBG-13-54-9	Chloroflexi	Rarely described
	OTU249	Module hub	Rhodocyclaceae	Proteobacteria	Denitrifier, Short-chain fatty acids, Proteins/amino acids, Sugars

Anoxic tank	OTU541	Module hub	Fluviicoccus	Proteobacteria	Aerobic heterotroph
Anoxic tank	OTU1117	Module hub	Candidatus Competibacter	Proteobacteria	GAO, Denitrifier, Short-chain fatty acids
	OTU1303	Module hub	Xanthobacter	Proteobacteria	Denitrifier, Fermentation, Short-chain fatty acids
	OTU622	Module hub	Saccharimonadales	Patescibacteria	Fermentation, Short-chain fatty acids
	OTU11836	Module hub	Rhodococcus	Actinobacteria	Breakdown of complex organic compounds
	OTU9501	Connector	Sapropiraceae	Bacteroidetes	Breakdown of complex organic compounds
	OTU10977	Connector	Prevotella	Bacteroidetes	Rarely described
	OTU14106	Connector	Nocardioides	Actinobacteria	Proteins/amino acids, Sugars
	OTU4686	Connector	Methylomonaceae	Proteobacteria	Fermentation, Short-chain fatty acids, Sugars
Aerobic tank	OTU14026	Connector	Bauldia	Proteobacteria	Breakdown of complex organic compounds
	OTU9845	Connector	Bdellovibrio	Proteobacteria	Rarely described
	OTU1161	Connector	OM27_clade	Proteobacteria	Rarely described
	OTU41	Connector	Opitutus	Verrucomicrobia	Rarely described
	OTU8794	Module hub	Leptotrichiaceae	Fusobacteria	Fermentation, Sugars
	OTU2095	Module hub	Oligoflexales	Proteobacteria	Rarely described
	OTU1736	Module hub	Omnitrophicaeota	Planctomycetes	Rarely described
	OTU7995	Module hub	Paludibaculum	Acidobacteria	Fermentation, Sugars
	OTU11656	Module hub	Burkholderiales	Proteobacteria	Denitrifier, Sulfate reduction, Breakdown of complex organic compounds
	OTU6821	Module hub	JG30-KF-CM45	Chloroflexi	Rarely described
	OTU2874	Module hub	Saccharimonadales	Patescibacteria	Fermentation, Sugars
Influent	OTU7358	Module hub	Microtrichaceae	Actinobacteria	Rarely described

OTU7734	Module hub	Candidatus Competibacter	Proteobacteria	GAO, Denitrifier, Short-chain fatty acids
OTU2688	Module hub	Ellin6067	Proteobacteria	Rarely described
OTU2091	Module hub	Saprospiraceae	Bacteroidetes	Proteins/amino acids, Sugars
OTU6340	Module hub	Omnitrophicaeota	Planctomycetes	Rarely described
OTU1795	Module hub	Paludibaculum	Acidobacteria	Fermentation, Sugars
OTU5846	Module hub	Burkholderiales	Proteobacteria	Denitrifier, Sulfate reduction, Breakdown of complex organic compounds
OTU1262	Module hub	JG30-KF-CM45	Chloroflexi	Rarely described
OTU7562	Module hub	Opitutus	Verrucomicrobia	Rarely described
OTU8863	Module hub	PHOS-HE36	Bacteroidetes	Rarely described
OTU8831	Module hub	Solirubrobacterales	Actinobacteria	Short-chain fatty acids, Sugars
OTU7473	Network hub	Sulfuritalea	Proteobacteria	Sulfate reduction, Denitrifier
OTU2452	Network hub	Sulfuritalea	Proteobacteria	Sulfate reduction, Denitrifier
OTU8714	Network hub	Geothrix	Acidobacteria	Fermentation, Short-chain fatty acids
OTU598	Network hub	PeM15	Actinobacteria	Rarely described
OTU8139	Network hub	Chitinophagales	Bacteroidetes	Rarely described
OTU7249	Network hub	BSV26	Bacteroidetes	Rarely described
OTU1028	Network hub	RBG-13-54-9	Chloroflexi	Rarely described

Notes: *: Potential functions of these keystone species were identified by a previous study (Nierychlo et al., 2020).

References

Nierychlo M, Andersen K S, Xu Y, Green N, Jiang C, Albertsen M, Dueholm M S, Nielsen P H (2020). MiDAS 3: An ecosystem-specific reference database, taxonomy and knowledge platform for activated sludge and anaerobic digesters reveals species-level microbiome composition of activated sludge. Water Research, 182: 115955