Supporting information

For

Understanding the linkage between elevation and activated sludge bacterial community along a 3600 m elevational gradient in China

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List of supporting information:

Table S1. Geographic location of WWTPs investigated in this study.

WWTP	City	Elevation (masl)	Latitude (N)	Longitude (E)	Urban population density[a] （person/km^2）
SH	Shanghai	9	31.1	121.1	3754
XM	Xiamen	14	24.3	118.1	3216
NJ	Nanjing	18	32.0	118.5	3341
NC	Nanchang	20	28.4	115.5	3276
HF	Hefei	26	31.5	117.1	3459
WH	Wuhan	30	30.4	114.2	2818
QD	Qingdao	60	36.2	120.3	1750
ZZ	Zhengzhou	110	34.4	113.4	3681
CC	Changchun	218	43.5	125.2	1792
CQ	Chongqing	261	29.3	104.3	1474
XA	Xian	383	34.2	108.6	1877
CDS	Chengdu	500	30.3	104.0	2600
TY	Taiyuan	800	37.5	112.3	2010
GY	Guiyang	1280	26.4	106.4	3444
LZ	Lianzhou	1520	36.0	103.5	2281
KM	Kunming	1900	25.0	102.4	2399
XN	Sining	2272	36.4	101.5	1846
LJ	Lijiang	2400	26.5	100.1	1962
GEM	Golmud	2780	36.5	95.0	1690
CDX	Qamdo	3200	31.1	97.1	1105
LS	Lhasa	3660	29.4	91.1	889

[a] Urban population density was calculated based on urban population divided by urban area around the WWTPs under study.

Table S2. Operational parameters of WWTPs investigated in this study.

WWTP	DO	SRT	Temp	HRT	Process	pH	TC	MLSS	F/M	organic loading rate (kg/(m^3d)) of				
	(mg/L)	(d)	(°C)	(h)			(10^3 m^3/d)	(mg/L)	(g COD/g MLSS d^{-1})	COD	BOD	TN	NH_4-N	TP
SH	2.9	16	27.4	9.4	AAO	6.9	70	5406	0.102	0.980	0.550	0.128	0.080	0.013
XM	3	14	25.4	10.5	AAO	6.5	56	3811	0.104	0.790	0.395	0.121	0.090	0.011
NJ	2.9	15	28.2	14.2	AO	6.5	35	5734.9	0.075	0.620	0.431	0.097	0.060	0.010
NC	3.3	14	23.9	8.6	CASS	6.7	65	3061.1	0.15	0.790	0.458	0.142	0.090	0.009
HF	2.4	14	26.2	13.4	AO	7	87	7220.4	0.063	0.720	0.455	0.158	0.102	0.010
WH	3.7	11	26.3	14.5	AO	6.7	78	5907.1	0.088	0.750	0.521	0.090	0.067	0.005
QD	2.5	13	20.9	8.7	AAO	7.1	100	4389.5	0.114	0.700	0.502	0.111	0.074	0.020
ZZ	4.1	15	25.3	11.2	AAO	7.2	22	5532.3	0.068	0.710	0.377	0.077	0.059	0.012
CC	5.4	15	18.1	18.5	OD	7.1	85	2029.3	0.051	0.240	0.103	0.076	0.044	0.008
CQ	2.5	14	28.6	10.6	AAO	6.3	23	3886.8	0.115	0.510	0.447	0.066	0.043	0.010
XA	2.2	13	22.6	4.7	CASS	7.3	56	3235.1	0.319	1.620	1.031	0.154	0.080	0.020
CDS	2.8	14	26.6	8.3	AAO	6.4	60	2383.4	0.153	0.740	0.364	0.098	0.071	0.011
TY	3.1	14	26	9.3	AAO	7.2	88	7609.5	0.09	1.200	0.687	0.247	0.169	0.008
GY	2.4	13	23.1	13.5	AO	6.5	74	5679.9	0.083	0.770	0.471	0.059	0.039	0.008
LZ	2.5	11	22	7.2	AAO	7.3	107	4313.2	0.136	1.100	0.588	0.157	0.093	0.016
KM	2.4	12	22.4	9.1	AAO	7.1	53	3599	0.128	0.870	0.462	0.087	0.052	0.010
XN	2.3	13	24.2	19.1	OD	6.7	61	3653	0.056	0.380	0.205	0.045	0.035	0.003
LJ	2.7	11	25.7	12.3	AO	6.9	58	3565	0.09	0.550	0.322	0.105	0.060	0.005
GEM	2.7	14	22.8	12.6	AAO	6.6	35	2931.7	0.1	0.530	0.294	0.061	0.048	0.005
CDX	1.8	12	21.1	13.2	AAO	6.8	25	2403	0.103	0.450	0.248	0.055	0.040	0.002
LS	1.5	14	20.6	12.8	CASS	7	28	1812.8	0.1	0.410	0.182	0.054	0.034	0.002

TC, treatment capacity; F/M, food/microorganism ratio.

Table S3. Wastewater treatment performance of WWTPs investigated in this study.

WWTP	Influent concentration (mg/L) of						Effluent concentration (mg/L) of					Removal (%) of				
	COD	BOD	TN	NH_4-N	TP	C/N	COD	BOD	TN	NH_4-N	TP	COD	BOD	TN	NH_4	TP
SH	385.4	215.6	50.2	31.2	5.0	7.7	39.2	8.5	13.6	1.5	0.3	89.8	96.1	97.0	95.2	94.0
XM	347.5	173.0	53.1	39.5	4.8	6.5	35.5	7.9	12.5	1.7	0.4	89.7	95.4	96.8	95.7	91.7
NJ	367.5	255.0	57.5	35.6	6.1	6.4	25.8	8.3	14.7	2.0	0.3	92.9	96.7	96.5	94.4	95.1
NC	281.4	164.0	50.9	32.2	3.4	5.5	36.3	9.1	13.6	1.6	0.2	87.1	94.5	96.9	95.0	94.1
HF	402.2	254.0	88.0	57.1	5.5	4.6	27.6	9.7	13.2	1.5	0.5	93.1	96.2	98.3	97.4	90.9
WH	451.6	314.5	54.6	40.5	2.8	8.3	40.4	10.1	12.6	1.8	0.1	91.1	96.8	96.7	95.6	96.4
QD	254.3	181.8	40.3	26.7	7.2	6.3	43.5	6.5	14.8	2.3	0.4	82.9	96.4	94.3	91.4	94.4
ZZ	329.0	176.0	36.0	27.4	5.8	9.1	47.0	5.0	14.9	2.0	0.1	85.7	97.2	94.4	92.7	98.3
CC	186.3	79.6	58.3	34.1	6.3	3.2	39.8	6.9	12.7	1.6	0.1	78.6	91.3	97.3	95.3	98.4
CQ	226.7	197.6	29.0	18.8	4.4	7.8	27.6	8.8	13.1	1.8	0.4	87.8	95.5	93.8	90.4	90.9
XA	318.0	202.0	30.2	15.6	4.0	10.5	45.8	7.8	13.8	1.3	0.1	85.6	96.1	95.7	91.7	97.5
CDS	256.0	126.0	34.0	24.7	3.7	7.5	46.1	6.5	12.4	1.7	ND[a]	82.0	94.8	95.0	93.1	NA[b]
TY	463.7	266.3	95.7	65.3	3.1	4.8	43.7	6.7	14.3	1.5	ND	90.6	97.5	98.4	97.7	NA
GY	431.8	265.0	33.4	22.0	4.3	12.9	36.2	10.5	15.3	1.2	ND	91.6	96.0	96.4	94.5	NA
LZ	328.9	176.4	47.0	28.0	4.9	7	28.0	8.8	18.7	1.6	0.3	91.5	95.0	96.6	94.3	93.9
KM	330.4	175.0	33.0	19.6	3.9	10	28.7	6.3	16.8	1.6	ND	91.3	96.4	95.2	91.8	NA
XN	301.0	163.0	36.0	27.8	2.4	8.4	30.3	7.2	14.3	1.8	ND	89.9	95.6	95.0	93.5	NA
LJ	284.2	164.8	54.0	30.7	2.8	5.3	29.4	4.6	13.6	3.0	ND	89.7	97.2	94.4	90.2	NA
GEM	277.2	154.6	32.0	25.0	2.6	8.7	26.7	8.7	10.7	3.8	ND	90.4	94.4	88.1	84.8	NA
CDX	245.0	136.3	30.0	22.0	1.1	8.2	33.9	9.1	8.8	4.8	ND	86.2	93.3	84.0	78.2	NA
LS	218.0	97.0	29.0	18.0	0.9	7.5	46.7	7.6	9.1	5.4	ND	78.6	92.2	81.4	70.0	NA

[a]ND, not detected; [b]NA, not analyzed.

Table S4. Numerical values assigned to the treatment processes according to the number of separate biological tanks.

Treatment process	Biological tanks	Numerical value
AAO	AnT,AxT, AT	3
AO	AxT, AT	2
OD	AnT,SAAnT	2.5
CASS	SAAnAxT	1.5

AT, aerobic tank; AnT, anearobic tank; AxT, anoxic tank; SAAnT, simultaneous aerobic and anoxic tank; SAAnAxT, simultaneous aerobic, anaerobic, and anoxic tank.

Table S5. Number of OTUs at different abundant levels.

N_reads per OTU	>=10000	>=1000	>=100	>=10	>=1
Total samples					
N_OTUs	13	43	890	12495	19345
Percentage of OTUs	0.07%	0.22%	4.60%	64.59%	100%
N_reads	117838	161329	317624	482275	503366
Percentage of Reads	23.41%	32.05%	63.10%	95.81%	100%
Individual samples					
Averaged N_OTUs	0	0	40	626	1064
Averaged Percentage of OTUs	0	0.00%	3.76%	58.83%	100%
Averaged N_reads	0	0	3564	7375	7990
Averaged Percentage of Reads	0	0.00%	44.61%	92.30%	100%

Table S6. Alpha diversity indices of bacterial communities of 63 activated sludge samples.

Lable	Shannon-H[a]	Evenness[b]	No. of OTUs[c]	No. of reads[d]
SH1	6.209	0.8796	1362	8,261
SH2	6.225	0.8322	1388	7,854
SH3	6.206	0.8578	1358	7,765
XM1	5.942	0.8925	1003	8,010
XM2	5.997	0.8968	1068	8,845
XM3	5.983	0.9209	1051	7,852
NJ1	6.147	0.8862	1268	7,952
NJ2	6.174	0.8673	1309	8,554
NJ3	6.133	0.8965	1248	8,622
NC1	5.903	0.8075	960	9,068
NC2	5.968	0.8333	1033	8,581
NC3	5.948	0.8525	1010	8,346
HF1	6.067	0.8244	1157	7,247
HF2	6.077	0.8376	1170	8,050
HF3	6.052	0.8421	1137	7,267
WH1	6.059	0.8774	1146	7,856
WH2	6.092	0.9054	1191	8,851
WH3	6.046	0.8934	1129	7,740
QD1	6.159	0.8192	1286	6,925
QD2	6.192	0.8091	1336	7,988
QD3	6.128	0.8215	1241	7,440
ZZH1	5.972	0.9053	1038	9,390
ZZH2	5.985	0.8393	1053	8,148
ZZH3	5.945	0.8402	1006	8,264
CC1	5.98	0.8071	1047	7,522
CC2	5.935	0.8492	995	8,745
CC3	5.929	0.8241	989	7,877
CQ1	6.111	0.797	1217	8,020
CQ2	6.12	0.824	1230	9,165
CQ3	6.171	0.8105	1304	8,960
XA1	5.914	0.8113	972	8,045
XA2	5.995	0.8043	1065	7,263
XA3	5.975	0.7998	1041	7,989
CDS1	5.924	0.8584	983	7,104
CDS2	5.959	0.8118	1022	7,754
CDS3	5.928	0.8205	987	8,005

Lable	Shannon-H[a]	Evenness[b]	No. of OTUs[c]	No. of reads[d]
TY1	6.124	0.8406	1235	7,055
TY2	6.139	0.8307	1257	7,537
TY3	6.108	0.8586	1213	7,660
GY1	6.107	0.9229	1211	6,650
GY2	6.119	0.8666	1228	7,655
GY3	6.142	0.8876	1261	8,444
LZ1	5.967	0.8559	1032	7,480
LZ2	5.955	0.8455	1018	8,110
LZ3	5.971	0.8745	1036	9,564
KM1	5.884	0.8447	940	7,795
KM2	5.933	0.8346	993	8,633
KM3	5.902	0.8136	959	7,828
XN1	5.875	0.7802	931	8,657
XN2	5.838	0.8022	895	7,577
XN3	5.85	0.8084	906	8,654
LJ1	5.832	0.7438	889	7,357
LJ2	5.813	0.7645	871	8,585
LJ3	5.822	0.7367	880	8,526
GEM1	5.818	0.7057	876	7,160
GEM2	5.797	0.6833	857	6,971
GEM3	5.793	0.6925	853	6,540
CDX1	5.766	0.6563	830	8,020
CDX2	5.755	0.6621	821	8,525
CDX3	5.732	0.6356	802	7,257
LS1	5.739	0.6263	808	7,823
LS2	5.728	0.6158	799	7,368
LS3	5.746	0.6321	814	8,550

Table S7. Spearman correlation coefficients between diversity indices and partial environmental variables at elevations below 1200 masl.

Variable	Richness	Evenness	OTUs
Elevation	-0.225	-0.418	-0.011
Latitude	0.346	-0.099	-0.038
Longitude	0.44	0.11	0.066
influent TN	0.258	0.143	0.011
influent NH_4-N	0.22	0.308	0.022
influent COD	0.275	0.571	0.099
influent BOD5	0.544	0.242	0.132
influent TP	0.396	0.06	0.099
pH	-0.006	-0.039	-0.395
SRT	0.253	0.297	0.169
Treatment Process	0.322	0.045	0.455
DO	-0.152	0.256	-0.259
Temp	0.39	0.269	0.516
MLSS	0.489	0.225	0.203

Table S8. Spearman correlation coefficients between bacterial phyla and partial environmental variables at elevations above 1200 masl.

	Influent NH_4-N	Influent TP	Treatment process	DO	Temperature
OD1	0.335	0.952*	0.064	0.482	0.452
Gemmatimonadetes	0.383	0.833	0.268	0.337	0.5
Chlorobi	0.048	0.548	0.268	0.337	0.643
TM7	0.271	0.898*	0.128	0.37	0.371
Lentisphaerae	-0.096	0.548	0.23	-0.157	0.238
Spirochaetes	0.339	0.361	-0.168	0.171	0.06
Cyanobacteria	0.12	0.429	0.192	0.024	-0.024
Acidobacteria	-0.277	0.144	-0.045	0.2	0.443
Firmicutes	-0.144	-0.786	-0.281	-0.108	-0.214
Deltaproteobacteria	0.395	0.929*	0.153	0.699	0.286
Epsilonproteobacteria	0.359	0.595	0.562	0.337	0.5
Planctomycetes	0.53	0.683	0.019	0.418	0.563
Nitrospira	0.241	0.659	-0.238	0.642	0.719
Verrucomicrobia	-0.072	0.619	0.498	0.133	0.286
Chloroflexi	0.795	0.455	0.629	0.521	0.635
Actinobacteria	-0.252	0.024	-0.038	0.06	0.19
Alphaproteobacteria	-0.108	0.262	0.077	0.12	0.643
Gammaproteobacteria	-0.168	-0.143	0.294	-0.325	-0.143
Betaproteobacteria	-0.287	-0.905*	-0.204	-0.374	-0.429
Bacteroidetes	-0.287	-0.905*	-0.204	-0.374	-0.429

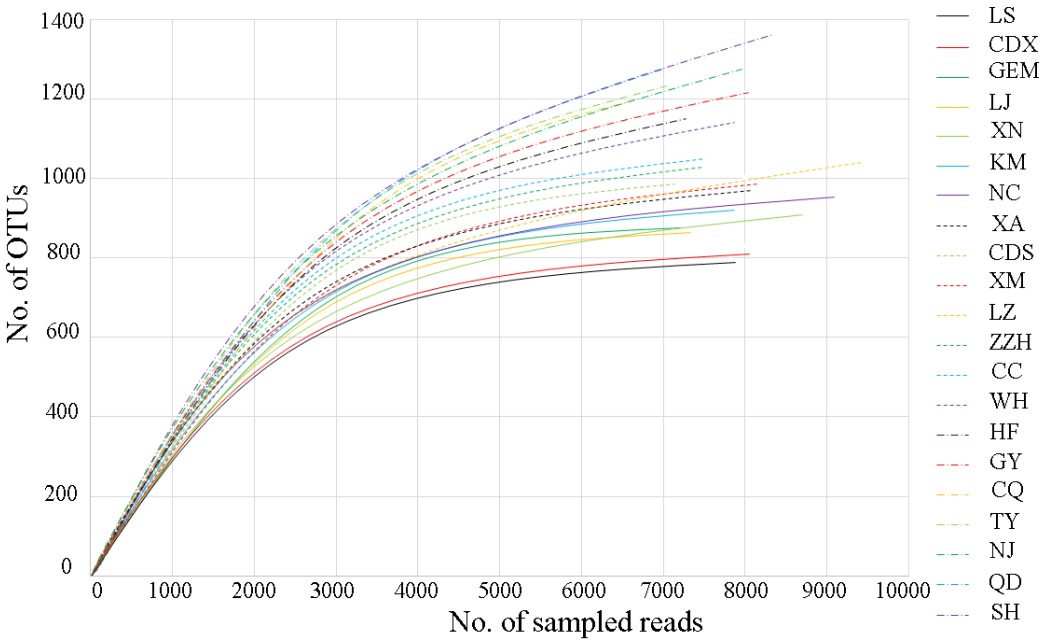


Fig. S1. Rarefaction analysis of one sample from each wastewater treatment plant.

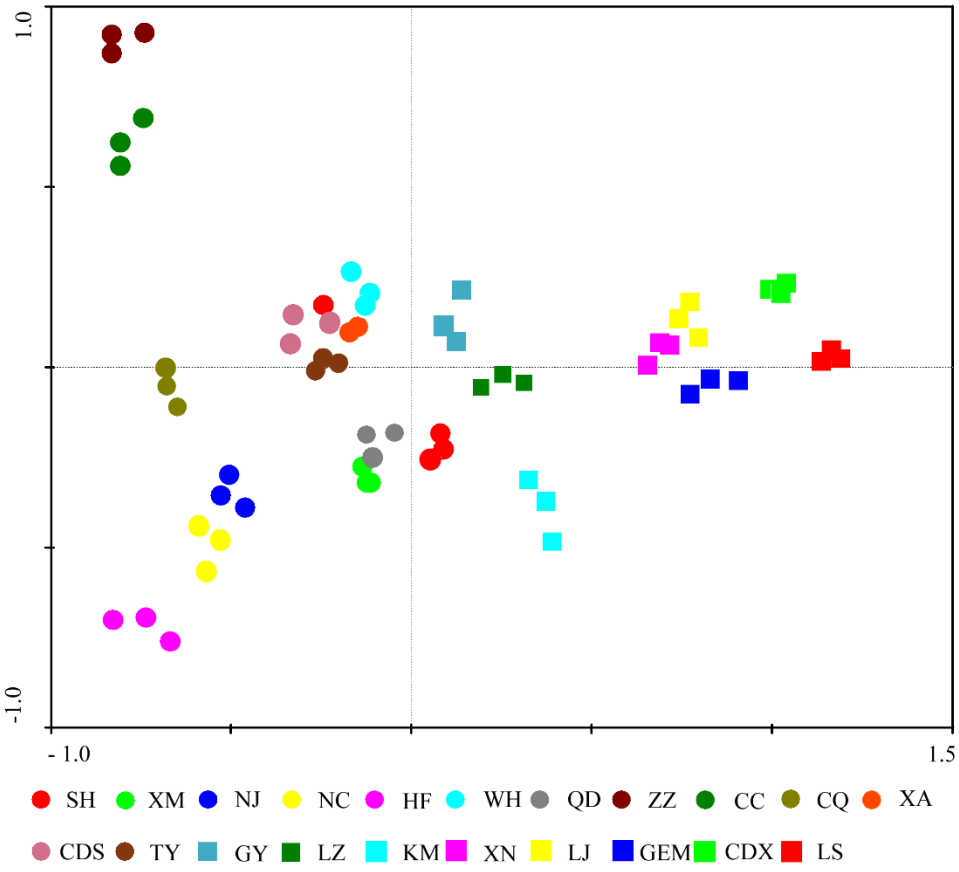


Fig. S2. PCoA analysis with a UniFrac distance matrix comparing all the 63 samples.

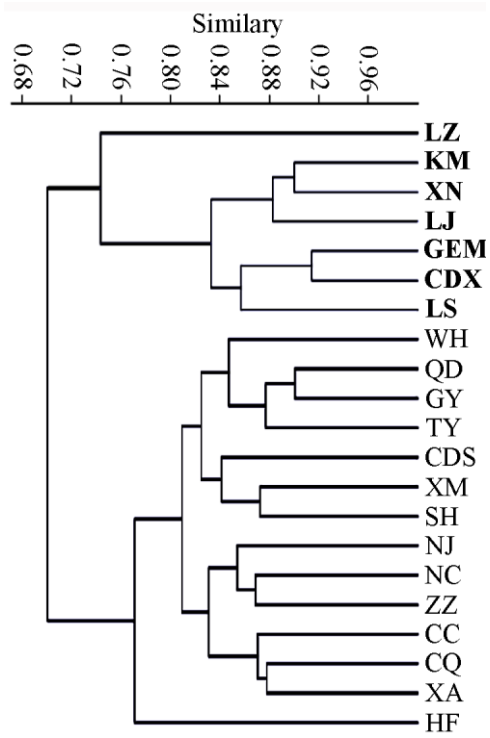
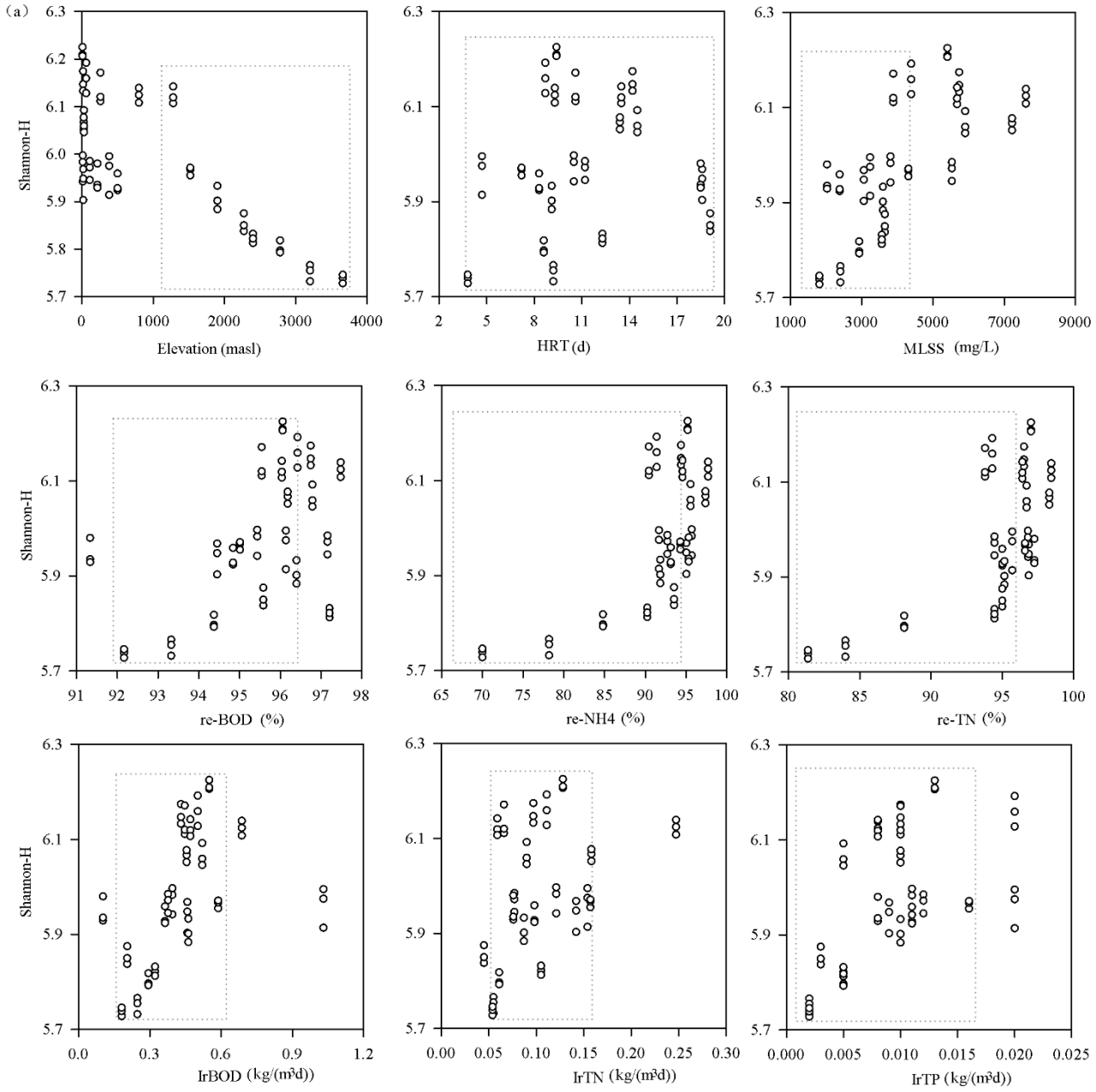
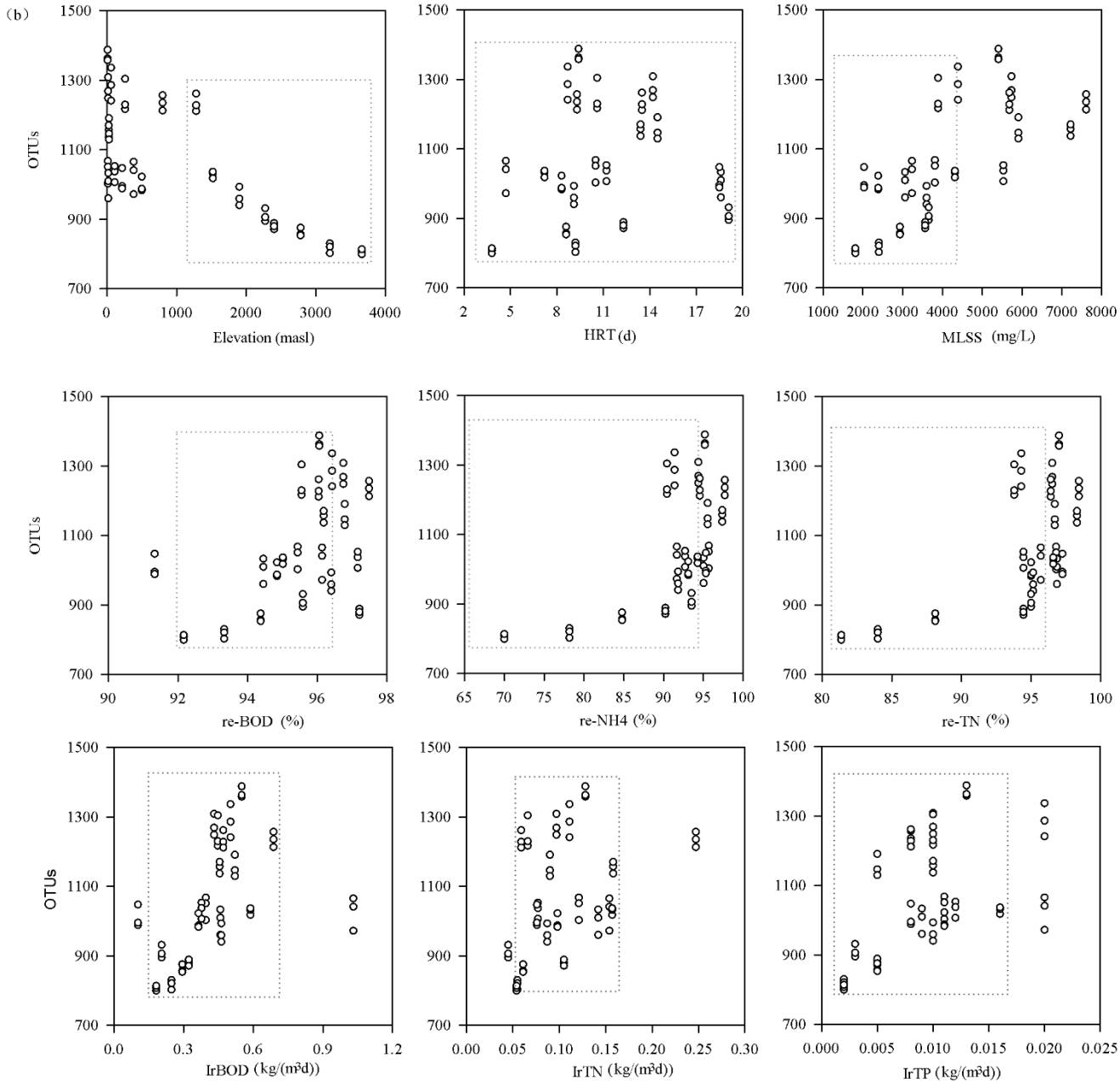


Fig. S3. Clustering analysis based on Bray-Curtis distances on the OTU level among samples from 21 wastewater treatment plant.


(a)
Shannon-H
Elevation (masl)
HRT (d)
MLSS (mg/L)
re-BOD (%)
re-NH4 (%)
re-TN (%)
IrBOD (kg/(m³d))
IrTN (kg/(m³d))
IrTP (kg/(m³d))


(b)
OTUs
Elevation (masl)
HRT (d)
MLSS (mg/L)
re-BOD (%)
re-NH4 (%)
re-TN (%)
IrBOD (kg/(m³d))
IrTN (kg/(m³d))
IrTP (kg/(m³d))

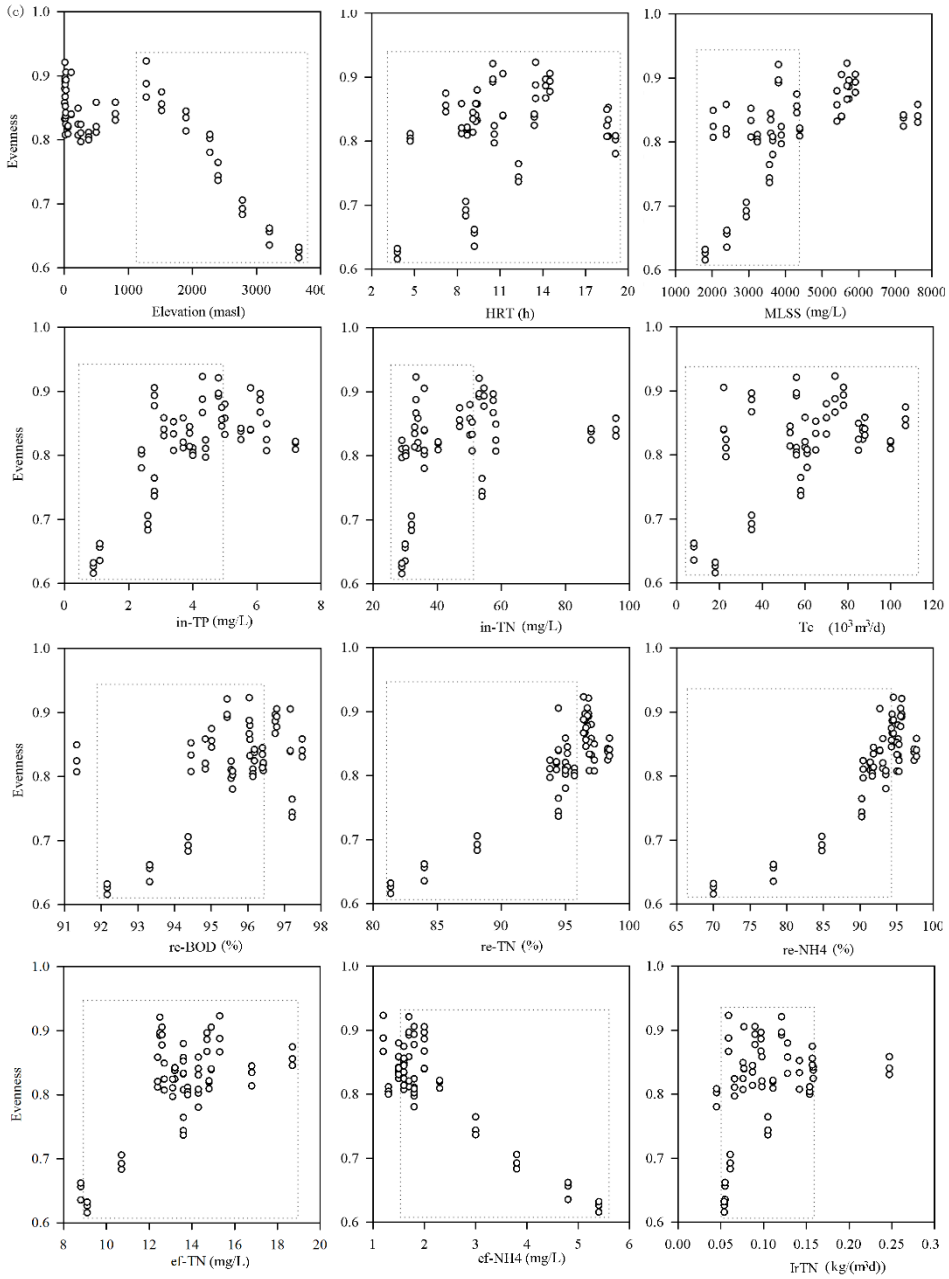


Fig. S4. Correlations between variables and bacterial community Shannon-H index (a), OTU richness (b), and evenness index (c). The ranges of variables corresponding to these high elevation WWTPs were pointed out by dotted rectangles.

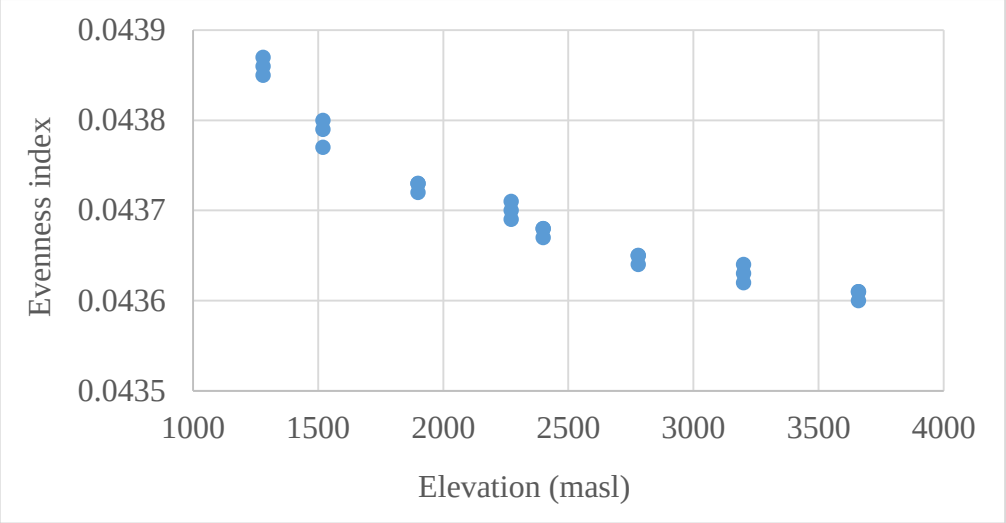


Fig. S5. Variation in bacterial community evenness at the phylum level.