Unravelling diversity and metabolic potential of microbial consortia at each stage of leather sewage treatment†

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Diss/SD: the ratio of average dissimilarity among groupings (DNA and RNA communities) and corresponding standard deviation, Contr%: contribution to the overall percentage dissimilarity between DNA and RNA communities, Cum%: cumulative percentage dissimilarity between DNA and RNA communities.

Table S2. qPCR cycling conditions used for the quantification of abundance of

total/active bacteria and archaea in activated sludge.

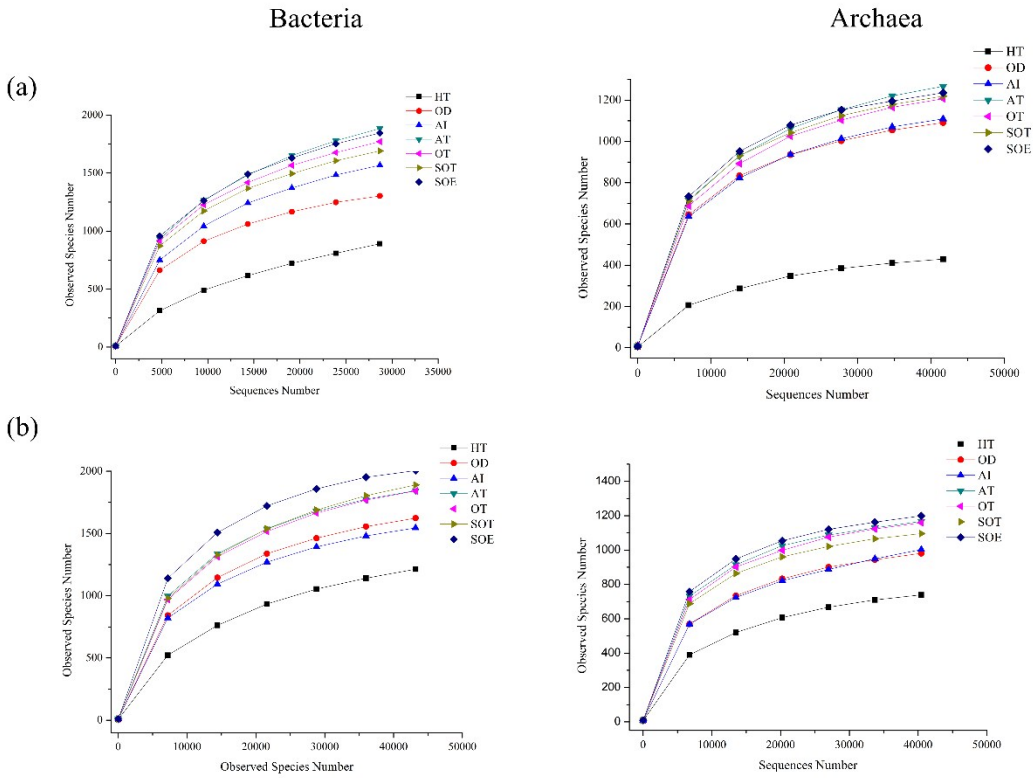
Supporting Information S1: Illumina sequencing

For Illumina HiSeq sequencing, DNA and cDNA were amplified with the primer sets Arch519F/Arch915R and 515F/806R targeting the V4 regions of the 16S rRNA genes for archaea and bacteria, respectively. [1, 2] Barcodes modified in the 5' terminus of the forward primer were used to distinguish different samples. All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs) under the following PCR conditions: initial denaturation at 94°C for 5 min; 35 cycles at 94°C for 50 s, 40°C for 30 s and 72°C for 90 s; and a final extension at 72°C for 5 min. All amplicons were purified with Qiagen Gel Extraction Kit (Qiagen, Germany) for the generation of regular sequencing libraries. At last, the library was sequenced on an Illumina HiSeq2500 platform at Novogene Bioinformatics Technology (Beijing, China).

Supporting Information S2: qPCR assays

qPCR was optimized to using SYBR® Premix Ex TaqTM II (Takara, Japan) in a total volume of 25 μl reaction mixtures consist of 12.5 μl SYBR® Premix Ex TaqTM II (Takara, Japan), 0.5μl of each primer (10μM), 0.5 μl of Rox Dye II, 2 μl of genomic DNA and 9 μL ddH_2O. Cycling conditions are summarized in Table S2. Fluorescence detection was performed at the annealing step.

qPCR calibration curves were constructed with the 10-fold dilutions of linearized clone QSD1 (KX223813) containing bacteria 16S rRNA genes in the range of 5.54×10^2–5.5×10^9 copies and ArchaeaQ1 (KX254344) containing archaea 16S rRNA genes in the range of 1.03×10^4–1.03×10^{10} copies. Controls without template were applied to check for cross contamination. All the standard curves had a correlation coefficient $r^2 > 0.99$ in all the assays. The efficiency of qPCR was 97% and 105% for archaea and bacteria, respectively.



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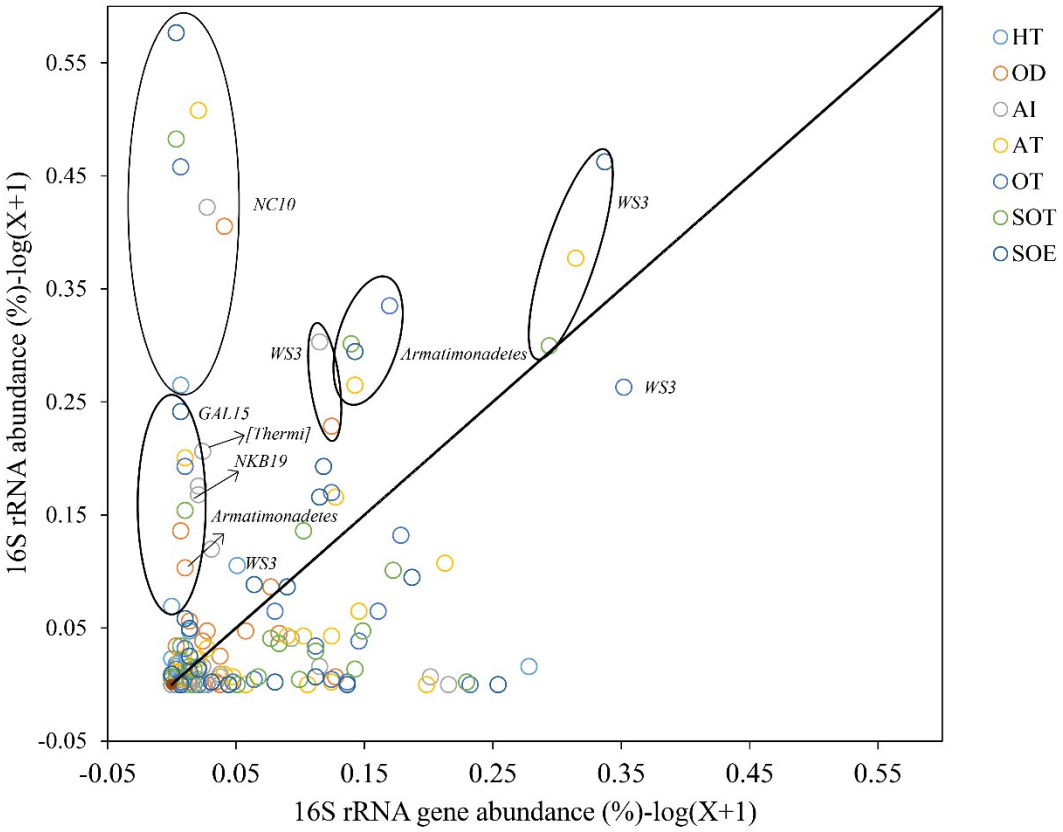

Fig. S2 Relationships between 16S rRNA and 16S rRNA gene frequencies of bacteiral taxa. Only taxa relative abundance below 1% in both total and active communities were shown. The solid line is the y=x line and the solid arrows point to the phylotype.

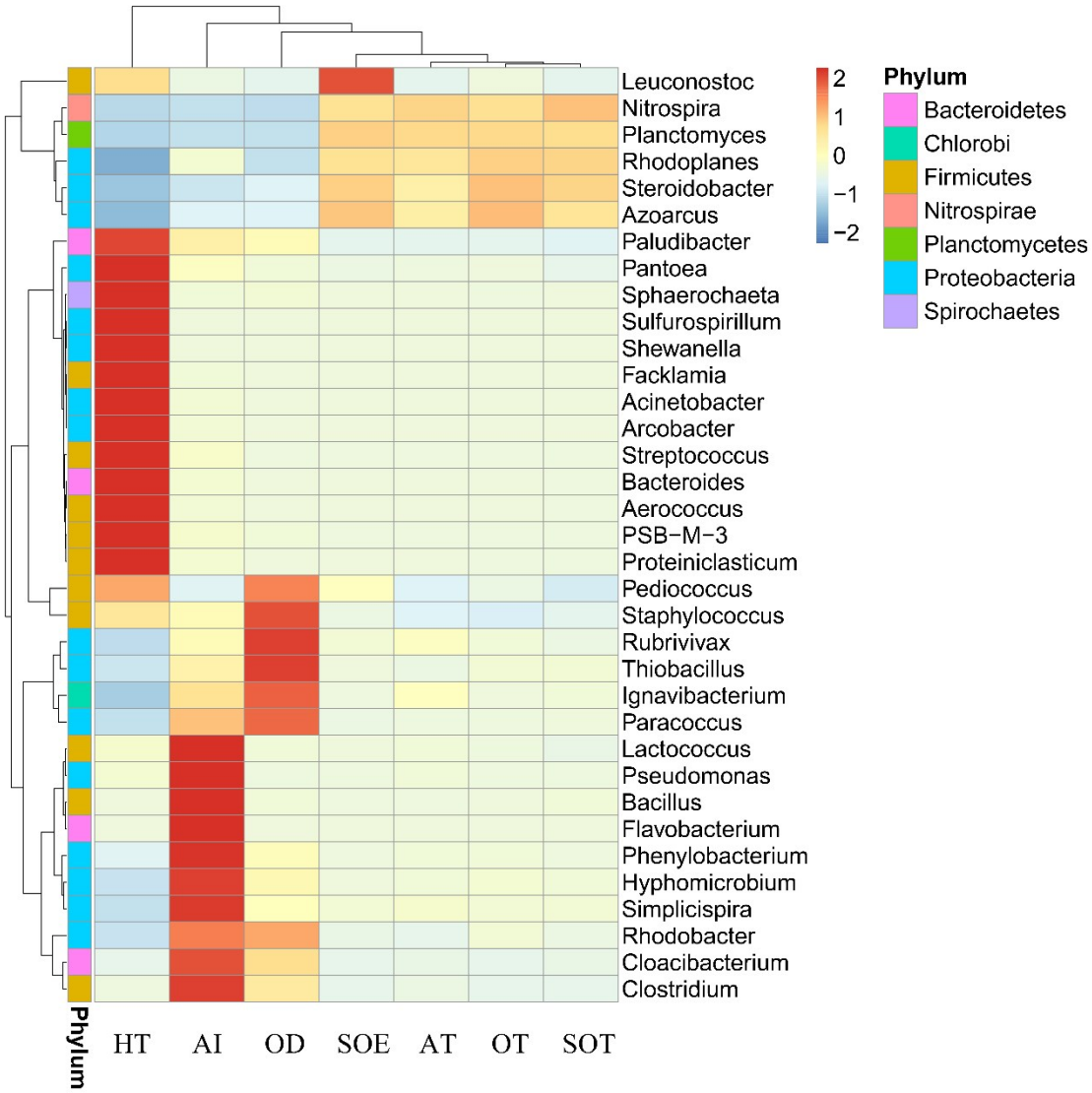

Fig. S3 Heatmap of the top 35 bacterial genera in DNA community.

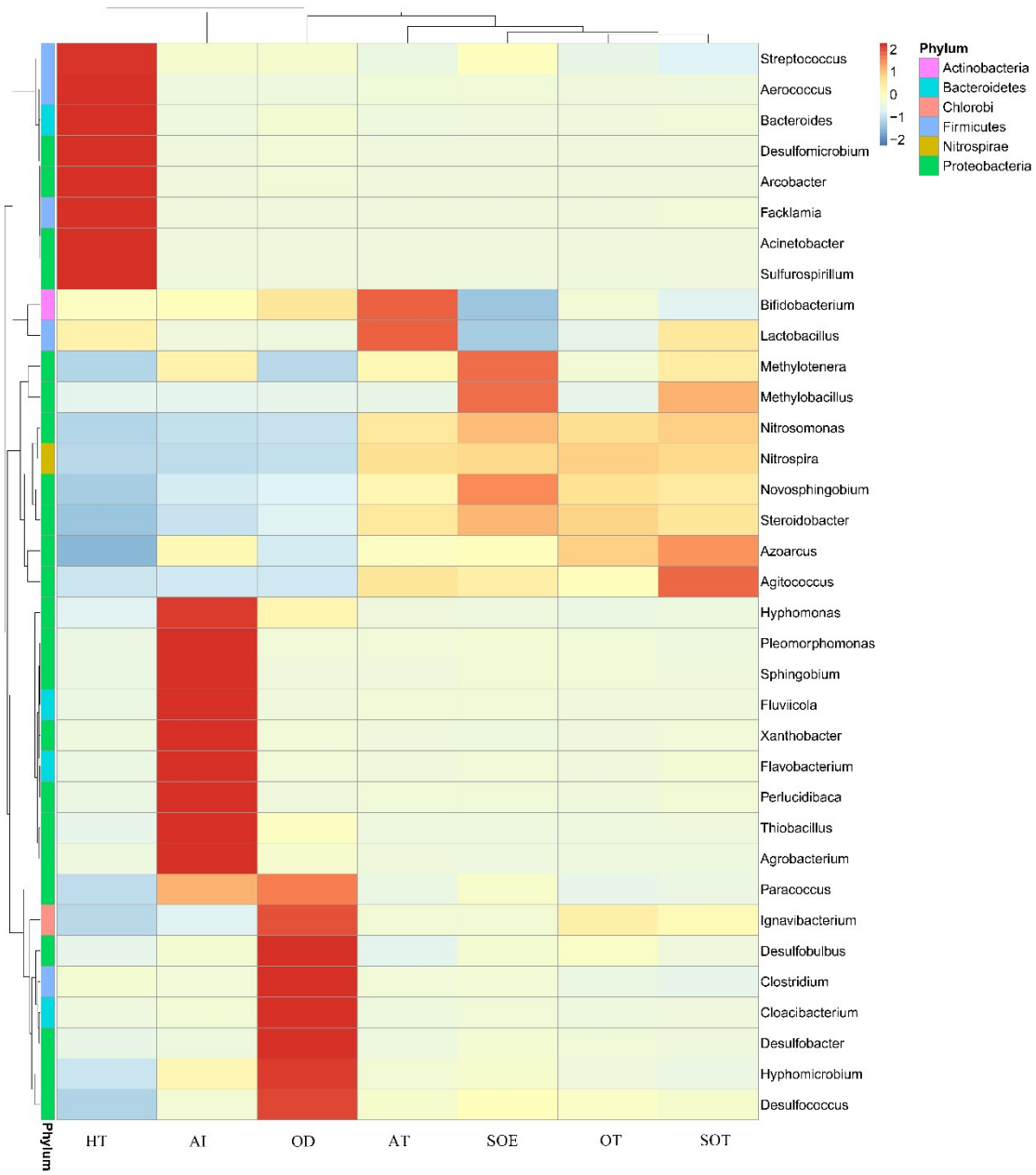

Fig. S4 Heatmap of the top 35 bacterial genera in RNA community.

Table S1 SIMPER analysis indicating several bacterial phylotypes cumulatively contributed to 95.72% of the dissimilarity between DNA and RNA communities.
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Bacterial phylotypes	SIMPER results		
	Diss/SD	Contr%	Cum%
Proteobacteria	3.26	35.3	35.3
Firmicutes	0.86	12.96	48.26
Chloroflexi	2.01	9.9	58.16
Bacteroidetes	2.09	7.38	65.54
Planctomycetes	1.56	5.78	71.31
Actinobacteria	1.68	3.87	75.18
Verrucomicrobia	0.85	3.74	78.91
Others	1.52	3.63	82.54
GN04	2.15	2.65	85.19
Cyanobacteria	1.14	2.32	87.51
Nitrospirae	1.67	2.02	89.53
Chlorobi	1.24	1.57	91.1
SR1	0.78	1.51	92.61
Acidobacteria	1.24	1.24	93.85
Gemmatimonadetes	1.48	0.95	94.8
NC10	3.94	0.92	95.72

Table S2 qPCR cycling conditions used for the quantification of abundance of total/active bacteria and archaea in activated sludge.

		Total bacteria	Total archaea
Amplification (×40 cycle)	Initial denaturalization	95°C 30s	95°C 30s
	Denaturalization	95°C 10s	95°C 15s
	Primers annealing	55°C 34s	53°C 30s
	Elongation	72°C 30s	72°C 30s
	Hold	16 °C	16 °C

References

1 N. Fierer, C. L. Lauber, K. S. Ramirez, J. Zaneveld, M. A. Bradford and R. Knight, *ISME J*, 2012, **6**, 1007-1017.

2 F. A. de Bok, R. C. van Leerdam, B. P. Lomans, H. Smidt, P. N. Lens, A. J. Janssen and A. J. Stams, *Appl. Environ. Microbiol.*, 2006, **72**, 7540-7547.