

1 **Fungi characteristics of biofilms from sewage and greywater in small diameter gravity sewers**

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13 The number of pages, tables, and figures in this supplementary data are 11, 3, 7, respectively.

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Table S1 Composition of synthetic greywater.

Substance	Concentration (mg/L)	Substance	Concentration ($\mu\text{g/L}$)
Glucose	80	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	73.5
Peptone	80	$\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$	30.43
CH_3COONa	54.1	$\text{Al}_2(\text{SO}_4)_3 \cdot 16\text{H}_2\text{O}$	11.78
NaHCO_3	91.3	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	4.83
KCl	57.4	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	51.25
KNO_3	7.2	H_3BO_3	0.58
NH_4Cl	19	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.27
$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	15.12	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.88
Edible oil	30	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.39
Sodium dodecyl benzene sulfonate	5	KI	0.03
pH	~ 7	EDTA	20

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Table S2 Network OTU taxonomy of sewage SDGS biofilms (Fig. 6a).

Label	Kingdom	Genus	Species	Abundance (%)
OTU1	Fungi	Unclassified	Unclassified	6.09
OTU115	Fungi	Unclassified	Unclassified	0.00
OTU12	Fungi	<i>Fusicolla</i>	Unclassified	0.54
OTU132	Fungi	Unclassified	Unclassified	0.00
OTU14	Fungi	Unclassified	Unclassified	0.39
OTU15	Fungi	Unclassified	Unclassified	0.41
OTU150	Fungi	Unclassified	Unclassified	0.00
OTU16	Fungi	<i>Mucor</i>	<i>Mucor irregularis</i>	0.43
OTU17	Fungi	Unclassified	Unclassified	0.24
OTU18	Fungi	<i>Cladosporium</i>	<i>Cladosporium halotolerans</i>	0.11
OTU19	Fungi	Unclassified	Unclassified	0.21
OTU20	Fungi	Unclassified	Unclassified	0.29
OTU22	Fungi	<i>Debaryomyces</i>	Unclassified	0.25
OTU23	Fungi	<i>Mucor</i>	<i>Mucor circinelloides</i>	0.49
OTU24	Fungi	Unclassified	Unclassified	0.18
OTU25	Fungi	<i>Rhodotorula</i>	<i>Rhodotorula diobovata</i>	0.13
OTU27	Fungi	<i>Candida</i>	<i>Candida tropicalis</i>	0.12
OTU29	Fungi	<i>Cladosporium</i>	<i>Cladosporium delicatulum</i>	0.03
OTU3	Fungi	<i>Rhodotorula</i>	<i>Rhodotorula mucilaginosa</i>	2.04

OTU30	Fungi	Unclassified	Unclassified	0.13
OTU31	Fungi	<i>Exophiala</i>	<i>Exophiala oligosperma</i>	0.08
OTU34	Fungi	Unclassified	Unclassified	0.03
OTU37	Fungi	Unclassified	Unclassified	0.09
OTU38	Fungi	Unclassified	Unclassified	0.11
OTU42	Fungi	<i>Rhinocladiella</i>	<i>Rhinocladiella similis</i>	0.02
OTU47	Fungi	Unclassified	Unclassified	0.02
OTU48	Metazoa	Unclassified	Unclassified	0.05
OTU49	Fungi	Unclassified	Unclassified	0.01
OTU5	Fungi	<i>Fusarium</i>	Unclassified	0.03
OTU53	Fungi	Unclassified	Unclassified	0.04
OTU54	Fungi	Unclassified	Unclassified	0.01
OTU56	Metazoa	Unclassified	Unclassified	0.03
OTU57	Fungi	Unclassified	Unclassified	0.01
OTU6	Fungi	<i>Trichoderma</i>	Unclassified	0.35
OTU60	Fungi	<i>Arthrotrrys</i>	<i>Arthrotrrys oligospora</i>	0.01
OTU620	Fungi	Unclassified	Unclassified	0.01
OTU7	Metazoa	Unclassified	Unclassified	1.42
OTU72	Fungi	Unclassified	Unclassified	0.02
OTU73	Fungi	Unclassified	Unclassified	0.00
OTU752	Fungi	<i>Rhodotorula</i>	Unclassified	0.05
OTU77	Fungi	<i>Penicillium</i>	Unclassified	0.01

OTU8	Fungi	<i>Fusicolla</i>	Unclassified	1.09
OTU82	Fungi	Unclassified	Unclassified	0.01
OTU84	Fungi	<i>Mucor</i>	<i>Mucor irregularis</i>	0.01
OTU9	Fungi	Unclassified	Unclassified	0.00
OTU91	Fungi	Unclassified	Unclassified	0.01
OTU938	Fungi	Unclassified	Unclassified	0.01
OTU95	Fungi	Unclassified	Unclassified	0.01
OTU98	Fungi	Unclassified	Unclassified	0.01

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Table S3 Network OTU taxonomy of greywater SDGS biofilms (Fig. 6b).

Label	Kingdom	Genus	Species	Abundance (%)
OTU1	Fungi	Unclassified	Unclassified	1.08
OTU10	Fungi	<i>Exophiala</i>	<i>Exophiala equina</i>	0.94
OTU105	Fungi	Unclassified	Unclassified	0.00
OTU11	Unassigned	Unassigned	Unassigned	0.82
OTU115	Fungi	Unclassified	Unclassified	0.00
OTU122	Fungi	Unclassified	Unclassified	0.01
OTU13	Fungi	Unclassified	Unclassified	0.32
OTU14	Fungi	Unclassified	Unclassified	0.15
OTU142	Fungi	Unclassified	Unclassified	0.00
OTU16	Fungi	<i>Mucor</i>	<i>Mucor irregularis</i>	0.04
OTU18	Fungi	<i>Cladosporium</i>	<i>Cladosporium halotolerans</i>	0.33
OTU181	Fungi	Unclassified	Unclassified	0.00
OTU19	Fungi	Unclassified	Unclassified	0.09
OTU21	Metazoa	Unclassified	Unclassified	0.44
OTU22	Fungi	<i>Debaryomyces</i>	Unclassified	0.05
OTU23	Fungi	<i>Mucor</i>	<i>Mucor circinelloides</i>	0.07
OTU25	Fungi	<i>Rhodotorula</i>	<i>Rhodotorula diobovata</i>	0.09
OTU28	Fungi	<i>Gibberella</i>	<i>Gibberella intricans</i>	0.20
OTU29	Fungi	<i>Cladosporium</i>	<i>Cladosporium delicatulum</i>	0.12

OTU3	Fungi	<i>Rhodotorula</i>	<i>Rhodotorula mucilaginosa</i>	0.75
OTU315	Fungi	Unclassified	Unclassified	0.00
OTU32	Fungi	<i>Cystobasidium</i>	<i>Cystobasidium slooffiae</i>	0.11
OTU329	Fungi	Unclassified	Unclassified	0.01
OTU33	Fungi	<i>Fusarium</i>	Unclassified	0.12
OTU34	Fungi	Unclassified	Unclassified	0.09
OTU36	Fungi	<i>Aspergillus</i>	<i>Aspergillus sydowii</i>	0.07
OTU39	Fungi	Unclassified	Unclassified	0.08
OTU4	Fungi	<i>Cutaneotrichosporon</i>	Unclassified	1.83
OTU40	Fungi	Unclassified	Unclassified	0.10
OTU41	Fungi	<i>Alternaria</i>	Unclassified	0.02
OTU44	Fungi	Unclassified	Unclassified	0.04
OTU448	Fungi	Unclassified	Unclassified	0.51
OTU45	Fungi	Unclassified	Unclassified	0.07
OTU47	Fungi	Unclassified	Unclassified	0.01
OTU5	Fungi	<i>Fusarium</i>	Unclassified	1.66
OTU50	Fungi	Unclassified	Unclassified	0.05
OTU52	Fungi	Unclassified	Unclassified	0.03
OTU527	Fungi	<i>Fusarium</i>	Unclassified	0.15
OTU53	Fungi	Unclassified	Unclassified	0.02
OTU54	Fungi	Unclassified	Unclassified	0.01
OTU55	Fungi	<i>Chaetomium</i>	<i>Chaetomium homopilatum</i>	0.02

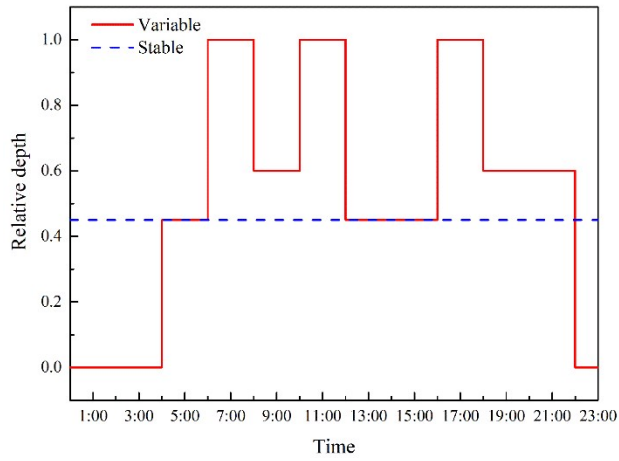
OTU6	Fungi	<i>Trichoderma</i>	Unclassified	1.20
OTU63	Fungi	Unclassified	Unclassified	0.01
OTU66	Fungi	Unclassified	Unclassified	0.01
OTU67	Fungi	Unclassified	Unclassified	0.08
OTU7	Metazoa	Unclassified	Unclassified	0.37
OTU71	Fungi	<i>Aspergillus</i>	<i>Aspergillus intermedius</i>	0.02
OTU72	Fungi	Unclassified	Unclassified	0.01
OTU73	Fungi	Unclassified	Unclassified	0.00
OTU74	Fungi	<i>Penicillium</i>	<i>Penicillium citrinum</i>	0.00
OTU75	Fungi	<i>Meira</i>	<i>Meira nashicola</i>	0.01
OTU752	Fungi	<i>Rhodotorula</i>	Unclassified	0.04
OTU79	Fungi	<i>Aspergillus</i>	Unclassified	0.01
OTU80	Fungi	Unclassified	Unclassified	0.01
OTU86	Fungi	Unclassified	Unclassified	0.01
OTU9	Fungi	Unclassified	Unclassified	1.03
OTU938	Fungi	Unclassified	Unclassified	0.00
OTU95	Fungi	Unclassified	Unclassified	0.00
OTU97	Fungi	Unclassified	Unclassified	0.00

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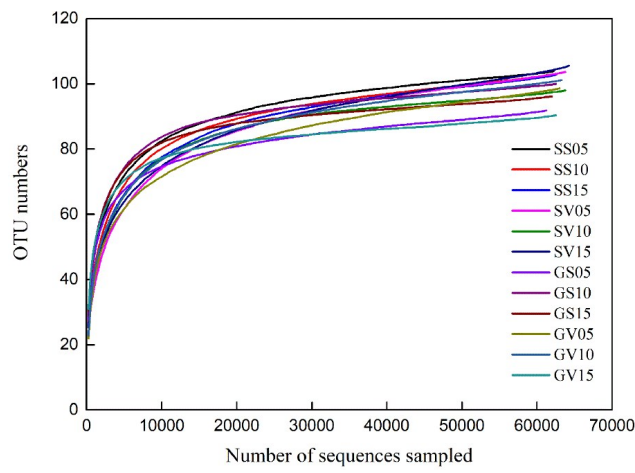


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Fig. S1 Relative depth of flow conditions in sewers.

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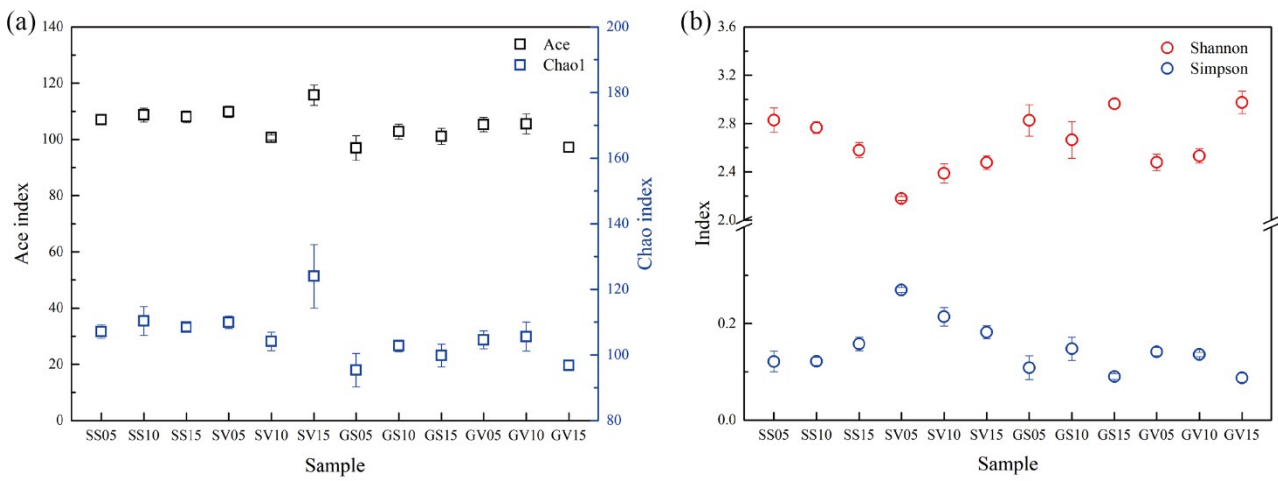


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Fig. S2 Average rarefaction curves in this experiment.

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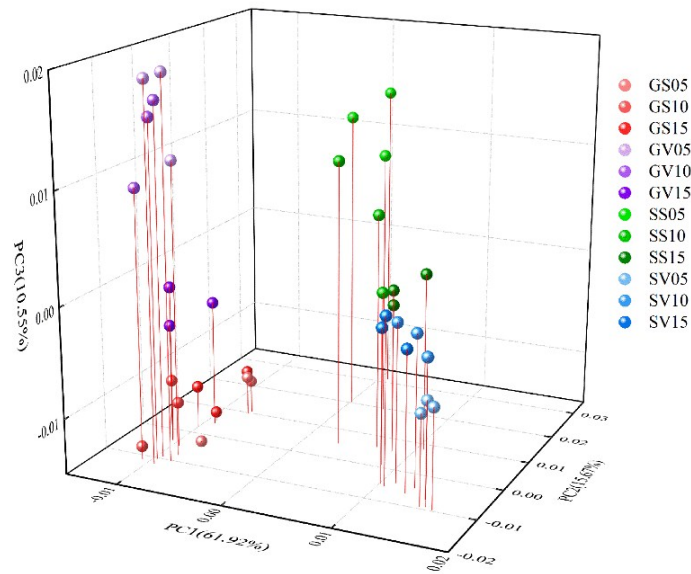


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Fig. S3 Alpha-diversity index of fungi samples.

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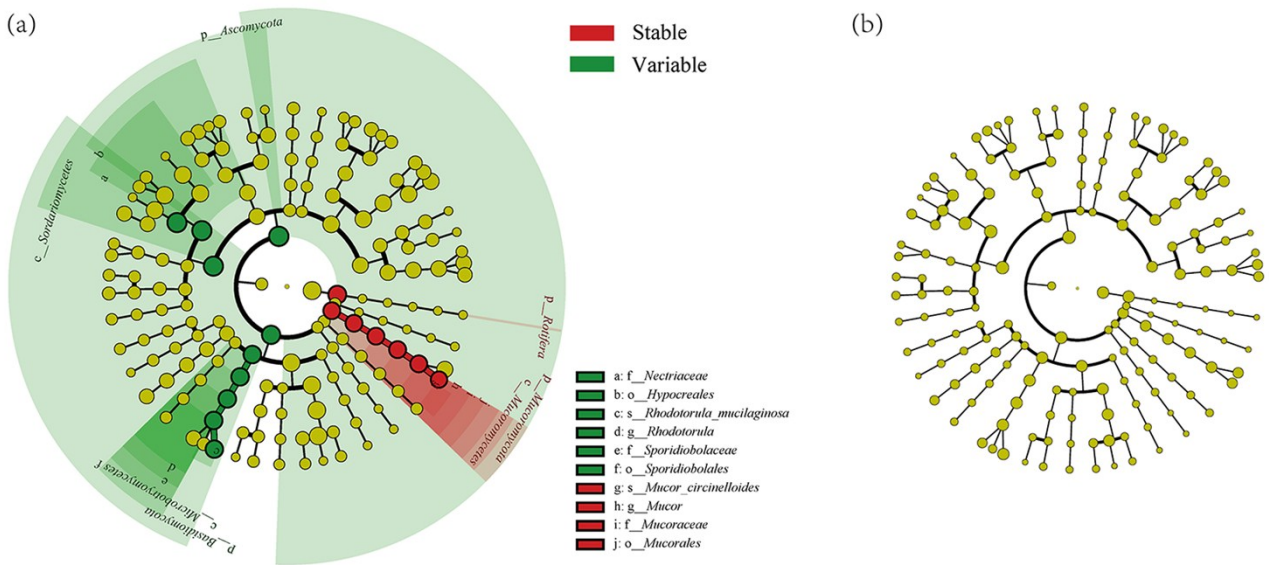


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Fig. S4 PCA diagram of fungi in SDGS biofilms.

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Fig. S5 Linear discriminant analysis effect size (LEfSe) of fungi in different flow conditions (a) and

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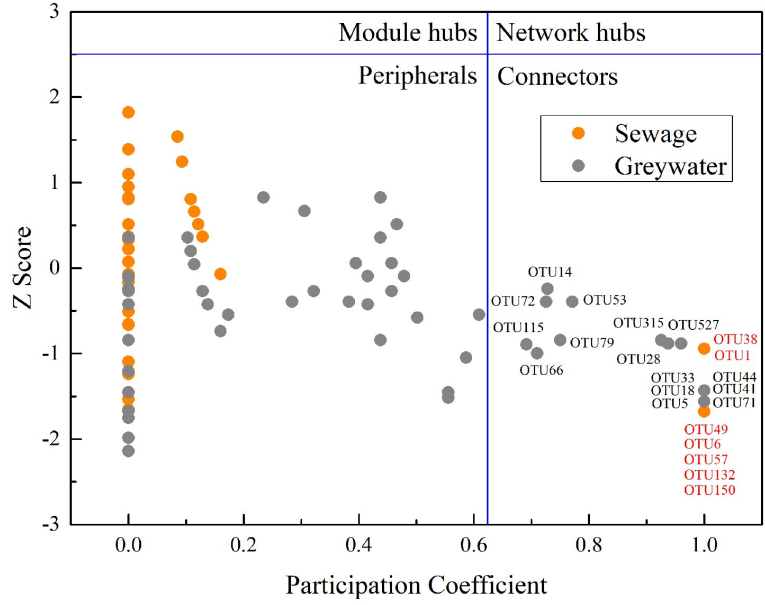
different slopes (b) (linear discriminant analysis (LDA) score > 4).

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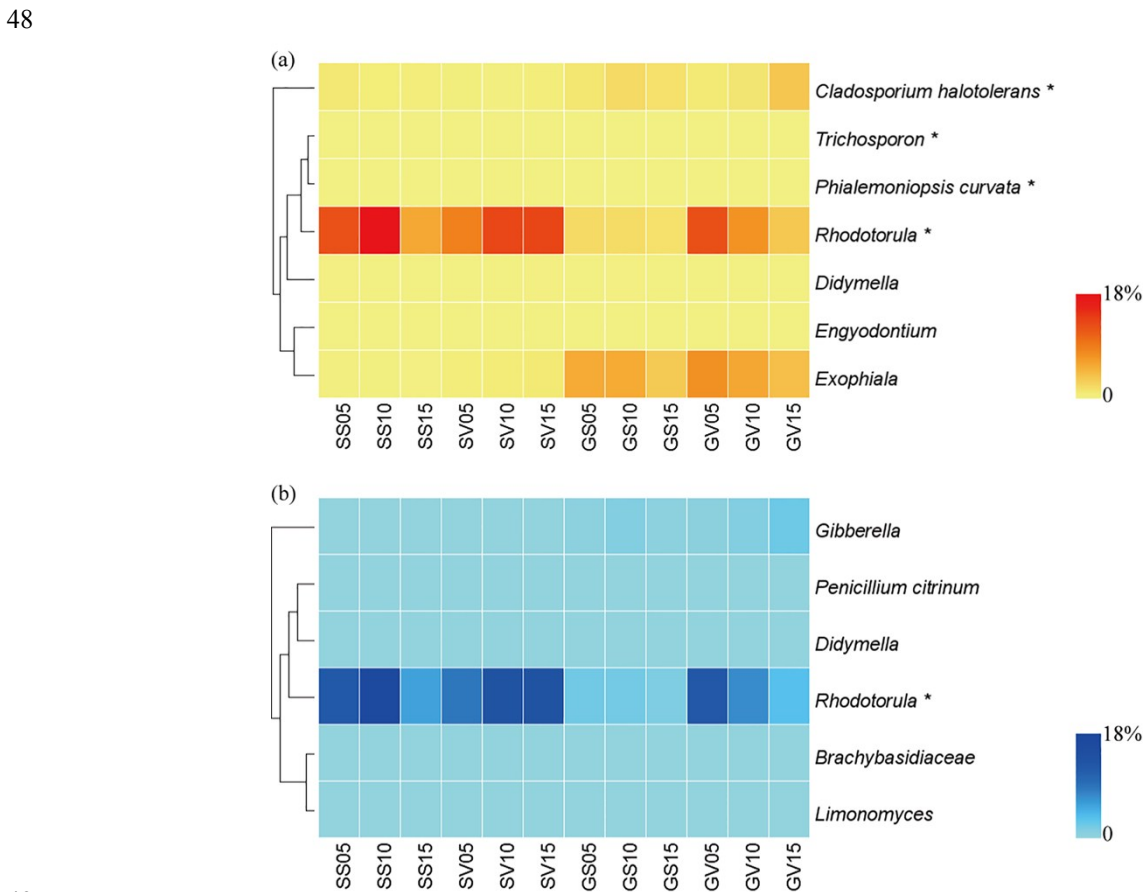
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47 Fig. S6 Z-P figure of fungi network in biofilms of small diameter gravity sewers.



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50 Fig. S7 The abundance heatmap of animal pathogen (a) and plant pathogen (b). * means
51 opportunistic human pathogen.