

1 **Impact of anaerobic co-digestion between**
2 **sewage sludge and carbon-rich organic**
3 **waste on microbial community resilience**

4 Supplementary Data

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15 **Table S1.** Statistical analysis of alpha diversity indices between control community and
 16 community at different OLR increase. Statistically significant differences were *italicised*. The
 17 calculation was performed using Student's t-test in Excel.

| Index | Based on 16S rRNA gene | | | Based on <i>mcrA</i> gene | | |
|------------------|------------------------|------------------|------------------|---------------------------|------------------|------------------|
| | <i>P</i> - value | | | | | |
| | 0 vs. 86 | 0 vs. 171 | 0 vs. 240 | 0 vs. 86 | 0 vs. 171 | 0 vs. 240 |
| Observed species | 0.081 | <i>0.003</i> | <i>0.009</i> | 0.082 | <i>0.017</i> | <i>0.014</i> |
| Chao 1 value | 0.133 | <i>0.003</i> | <i>0.016</i> | 0.227 | <i>0.038</i> | <i>0.015</i> |
| Shannon | 0.162 | <i>0.009</i> | <i>0.002</i> | 0.379 | <i>0.048</i> | <i>0.005</i> |
| Simpson | 0.260 | <i>0.028</i> | <i>0.0004</i> | 0.083 | 0.066 | <i>0.001</i> |

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19 **Table S2.** Percentage decrease (%) in alpha diversity indices as a function of OLR increase.
 20 The calculation was normalized against the values from the control digester. The data were
 21 the mean of three, three and two datasets at OLR% increase of 86%, 171% and 240%,
 22 respectively.

| Index | Based on 16S rRNA gene | | | Based on <i>mcrA</i> gene | | |
|------------------|------------------------|------|------|---------------------------|------|------|
| | OLR % | | | | | |
| | 86 | 171 | 240 | 86 | 171 | 240 |
| Observed species | 6.7 | 29.0 | 25.5 | 15.8 | 24.0 | 36.7 |
| Chao 1 value | 6.6 | 28.8 | 23.7 | 14.4 | 23.4 | 35.9 |
| Shannon | 3.4 | 25.8 | 17.4 | 2.4 | 7.6 | 24.6 |
| Simpson | 0.6 | 14.3 | 6.4 | 2.4 | 3.9 | 13.5 |

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24 **Table S3.** Pearson's correlation of different microbial groups (order level) (i.e. hydrolytic,
 25 fermentative, acetogenic and methanogenic) and environmental variables (i.e. TOA, pH,
 26 alkalinity, OLR) and performance variables (i.e. VS removal, COD removal and biogas
 27 production) with statistically significant correlations *italicised*.

| Selected orders | | OLR % | Alkalinity | pH | TOA | VS removal | COD removal | Biogas production |
|----------------------------|--------------------------|--------------|--------------|--------------|--------|---------------|----------------|----------------------|
| <i>Clostridiales</i> | Pearson's correlation | 0.598 | -0.751 | -0.258 | 0.08 | 0.697 | 0.687 | 0.742 |
| | <i>p</i> -value | <i>0.03</i> | <i>0.003</i> | 0.391 | 0.79 | <i>0.008</i> | <i>0.009</i> | <i>0.004</i> |
| <i>Synergistales</i> | Pearson's correlation | -0.831 | 0.795 | 0.705 | -0.283 | -0.628 | -0.266 | -0.602 |
| | <i>p</i> -value | <i>0.001</i> | <i>0.001</i> | <i>0.007</i> | 0.348 | <i>0.02</i> | 0.379 | <i>0.001</i> |
| <i>Spirochaetales</i> | Pearson's correlation | -0.627 | 0.661 | 0.155 | -0.177 | -0.269 | -0.527 | -0.787 |
| | <i>p</i> -value | <i>0.02</i> | <i>0.01</i> | 0.611 | 0.557 | 0.374 | 0.06 | <i>0.001</i> |
| <i>Syntrophobacterales</i> | Pearson's correlation | -0.32 | 0.444 | 0.394 | -0.07 | -0.494 | -0.03 | -0.204 |
| | <i>p</i> -value | 0.280 | 0.127 | 0.182 | 0.802 | 0.08 | 0.906 | 0.503 |
| <i>Methanomicrobiales</i> | Pearson's correlation | -0.32 | 0.444 | 0.394 | -0.07 | -0.494 | -0.03 | -0.204 |
| | <i>p</i> -value | 0.280 | 0.127 | 0.182 | 0.802 | 0.08 | 0.906 | 0.503 |
| <i>Methanosarcinales</i> | Pearson's correlation | -0.253 | 0.187 | 0.440 | 0.026 | -0.407 | 0.122 | -0.03 |
| | <i>p</i> -value | 0.403 | 0.540 | 0.131 | 0.930 | 0.167 | 0.69 | 0.900 |
| <i>Methanobacteriales</i> | Pearson's correlation | 0.028 | -0.111 | -0.420 | 0.449 | -0.529 | -0.392 | -0.226 |
| | <i>p</i> -value | 0.927 | 0.717 | 0.152 | 0.123 | 0.06 | 0.184 | 0.456 |

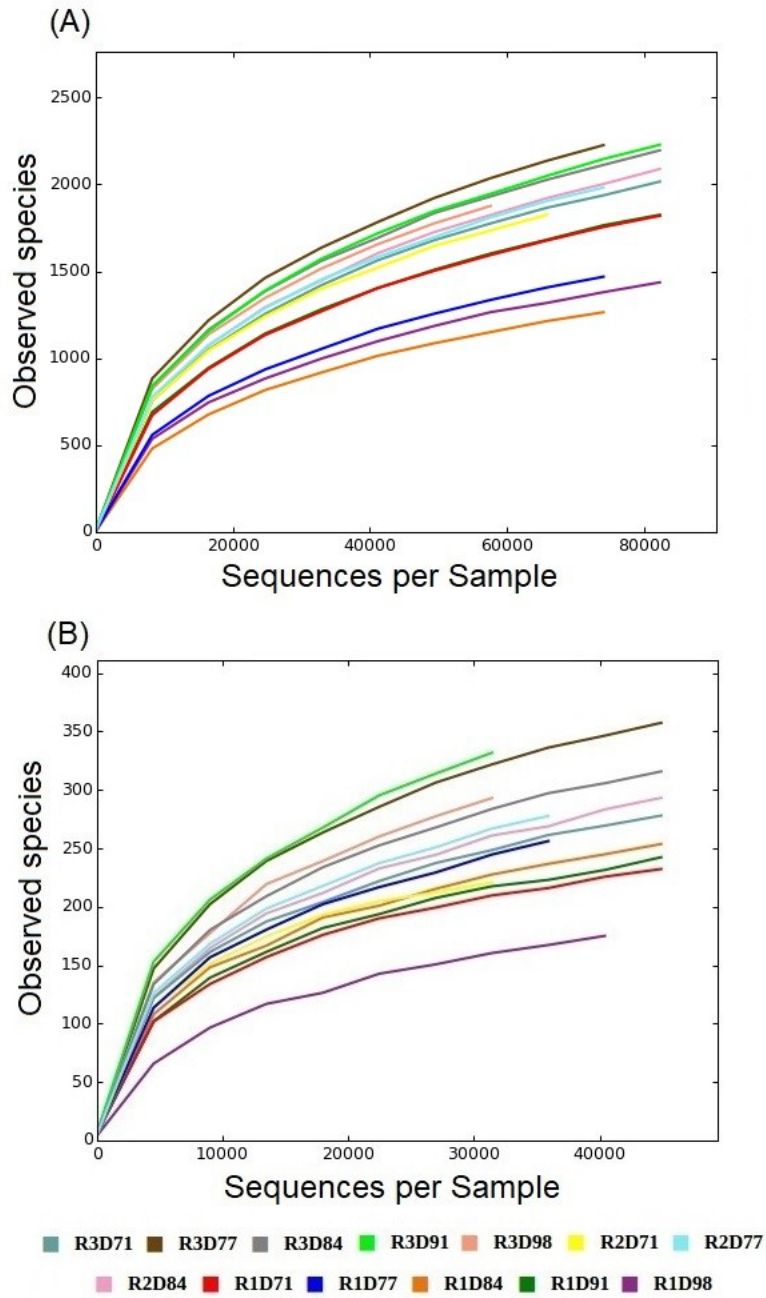
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30 **Table S4.** Statistical analysis of Pearson's correlation of different microbial groups (i.e.
 31 hydrolytic, fermentative, acetogenic and methanogenic) with statistically significant
 32 differences *italicised*.

| Selected order | <i>Clostridiales</i> | <i>Synergistales</i> | <i>Spirochaetales</i> | <i>Syntrophobacterales</i> | <i>Methanomicrobiales</i> | <i>Methanosarcinales</i> | <i>Methanobacteriales</i> |
|----------------------------|----------------------|----------------------|-----------------------|----------------------------|---------------------------|--------------------------|---------------------------|
| <i>Methanobacteriales</i> | 0.7002 | 0.4486 | 0.9608 | 0.495 | 0.064 | 0.757 | - |
| <i>Methanosarcinales</i> | 0.3736 | <i>0.0117</i> | 0.283 | 0.078 | 0.069 | - | 0.757 |
| <i>Methanomicrobiales</i> | 0.059 | <i>0.0000143</i> | 0.957 | 0.053 | - | 0.069 | 0.064 |
| <i>Syntrophobacterales</i> | <i>0.0199</i> | 0.1224 | 0.3837 | - | 0.053 | 0.078 | 0.495 |
| <i>Spirochaetales</i> | <i>0.0349</i> | 0.3318 | - | 0.3837 | 0.957 | 0.283 | 0.9608 |
| <i>Synergistales</i> | <i>0.0208</i> | - | 0.3318 | 0.1224 | <i>0.0000143</i> | <i>0.0117</i> | 0.4486 |
| <i>Clostridiales</i> | - | <i>0.0208</i> | <i>0.0349</i> | <i>0.0199</i> | 0.059 | 0.3736 | 0.7002 |

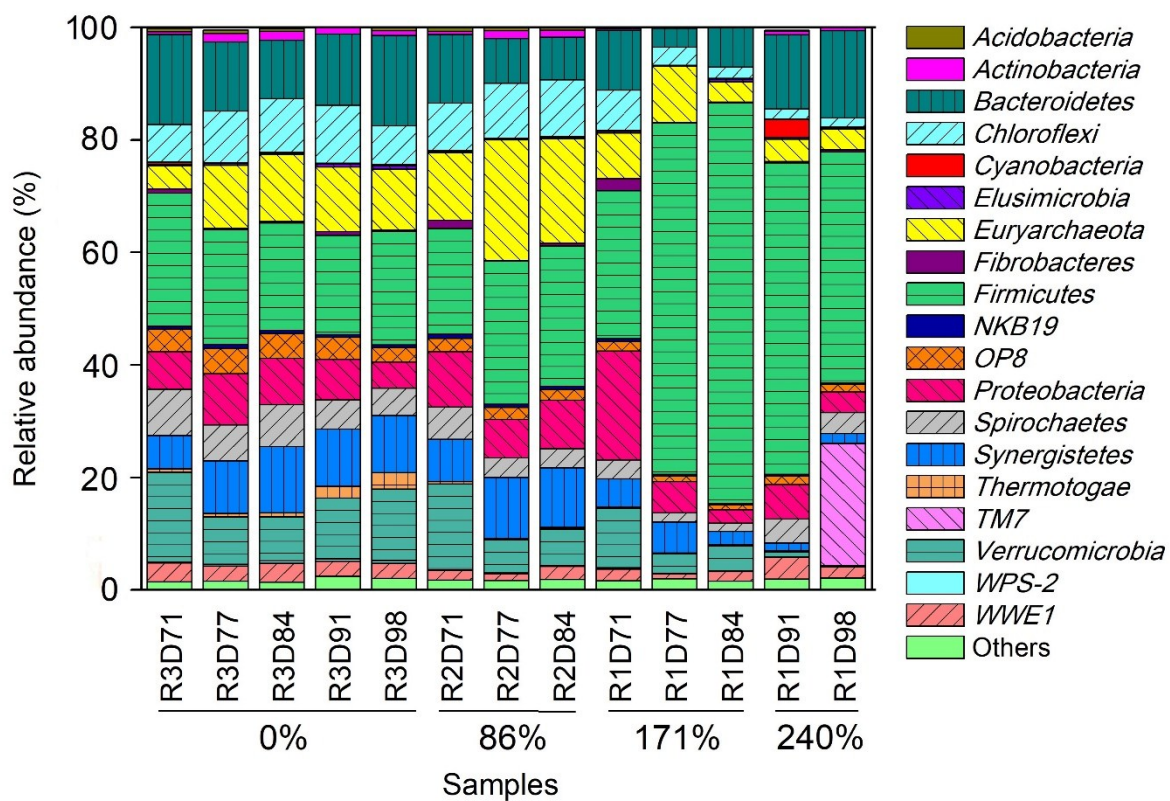
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35 **Figure S1.** Rarefaction curves of 16S rRNA marker gene amplicon sequences (A) and *mcrA*

36 marker gene amplicon sequences (B).



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38 **Figure S2.** Relative abundance of major phyla in the microbial community at different OLR
 39 increase. The data was retrieved from the analysis of 16S rRNA marker gene. The x-axis
 40 label shows ‘Digester’ and ‘Sampling date’. For example, “R3D71” refers to Digester R3 and
 41 sampling date of day 71.