Supplementary Information

A cascade of localized transitions drives system-wide microbial collapse

Supplementary Text

Rise and fall of key populations in the microbial community

Community analysis focusing on the 20 most abundant genera in the reactors (**Extended Data Fig. 3**). The perturbed reactor exhibited multiple shifts in dominant genera throughout the perturbation period, starting from a diverse community where most populations were more or less stable for the first 10 days and culminating in a community dominated by four genera after the tipping point. Two methanogens, *Methanolinea* and *Methanothrix,* declined from Day 3 onward and almost completely vanished on Day 24 and 40, respectively. Their decline correlated with the first drop in CH_4 production from 62% to 49% over the first 16 days. In addition, a methanogenic population of Methanoregulaceae seemed to be resilient to the increased loading for the first 22 days and was likely important for the maintained, although lower, CH_4 production of the reactor from Day 16 and up to the tipping point. After the tipping point at Day 38, this population and *Methanothrix* were low abundant and further declined toward Day 40, correlating with the second drop in CH_4 production.

In addition to the methanogens, other abundant genera also showed massive temporal changes. Rikenellaceae is one of the dominant populations. Rikenellaceae increased steadily from the start, with a boost around Day 17-20, making it the most abundant genus in the community between Days 20-31, after which it declined rapidly towards the tipping point on Day 38. Rikenellaceae are commonly found in anaerobic digestion systems[1] and have been associated with a fermentative or acetogenic metabolism[2]. The Lutispora population (midas_g_1251, a firmicutes bacterium) was very low abundant up until Day 21 and thereafter gained momentum and became one of the four dominating genera after the tipping point. Lutispora is associated with anaerobic digestion, primarily degrading protein sources and producing acetate[3]. Lactobacillaceae, a family known for fermentation of simple carbohydrates into lactate, acetate, and ethanol, were low abundant for the first 21 days, whereafter it increased slowly for the next 15 days before a rapid and massive increase from the tipping point and onwards. From Day 43, Lactobacillaceae was the most abundant genus in the reactor. Its abundance profile corresponds with the increased hydrolysis of microalgae observed in the reactor (**Fig. 5**), which made simple carbohydrates available for this family to ferment. Peptoclostridium, typically amino acid degraders, increased from the start and was quite dominant between Days 12-21; however, it plummeted markedly from Days 22-27. In contrast, the control reactor exhibited a steadier microbial composition throughout the whole period.

Cascading pattern throughout all microbial genera

Viewing the entire set of genera present in the reactors as a whole (**Extended Data Fig. 4**, n=886), as opposed to only the OTUs with significant causal effects on methane production (**Fig. 2A**), we observe five sets of temporal variations. Cluster I and IV are most abundant at the beginning of the perturbation period and decline within the first 10-15 days. Cluster II peaks just before the tipping point, cluster III after the tipping point, and cluster V consists of populations with varying peak timing, but all have a broad, long-lasting presence in the reactor. In contrast, the control reactor did not show similarly defined transitions.

Supplementary references

1 Mills, S., Yen Nguyen, T. P., Ijaz, U. Z. & Lens, P. N. L. Process stability in expanded granular sludge bed bioreactors enhances resistance to organic load shocks. *Journal of Environmental Management* **342**, 118271 (2023). https://doi.org:https://doi.org/10.1016/j.jenvman.2023.118271

2 Nakasaki, K., Nguyen, K. K., Ballesteros, F. C., Maekawa, T. & Koyama, M. Characterizing the microbial community involved in anaerobic digestion of lipid-rich wastewater to produce methane gas. *Anaerobe* **61**, 102082 (2020). https://doi.org:https://doi.org/10.1016/j.anaerobe.2019.102082

3 Stackebrandt, E. The family Gracilibacteraceae and transfer of the genus Lutispora into Gracilibacteraceae. *The Prokaryotes*, 149-151 (2014).

Source data file (separate file)

This data file contains the data presented in **Figs. 2a**, **2b**, **4b** and **4c**, providing information on the microbial OTUs and metabolites depicted (ordered from top to bottom in each respective figure). This data file also contains detailed information on the network properties depicted in **Fig. 3a**.