Characterization of
Complex Microbial Communities
Developed in a
Single-Stage Chemostat Model
## Of the Human Distal Gut

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Ecology of the distal gut

The human gut microbiota is a complex microbial ecosystem

Function and behaviour of this ecosystem best studied as a whole

Several methods to study the gut microbiota, including both *in vivo* and *in vitro* models
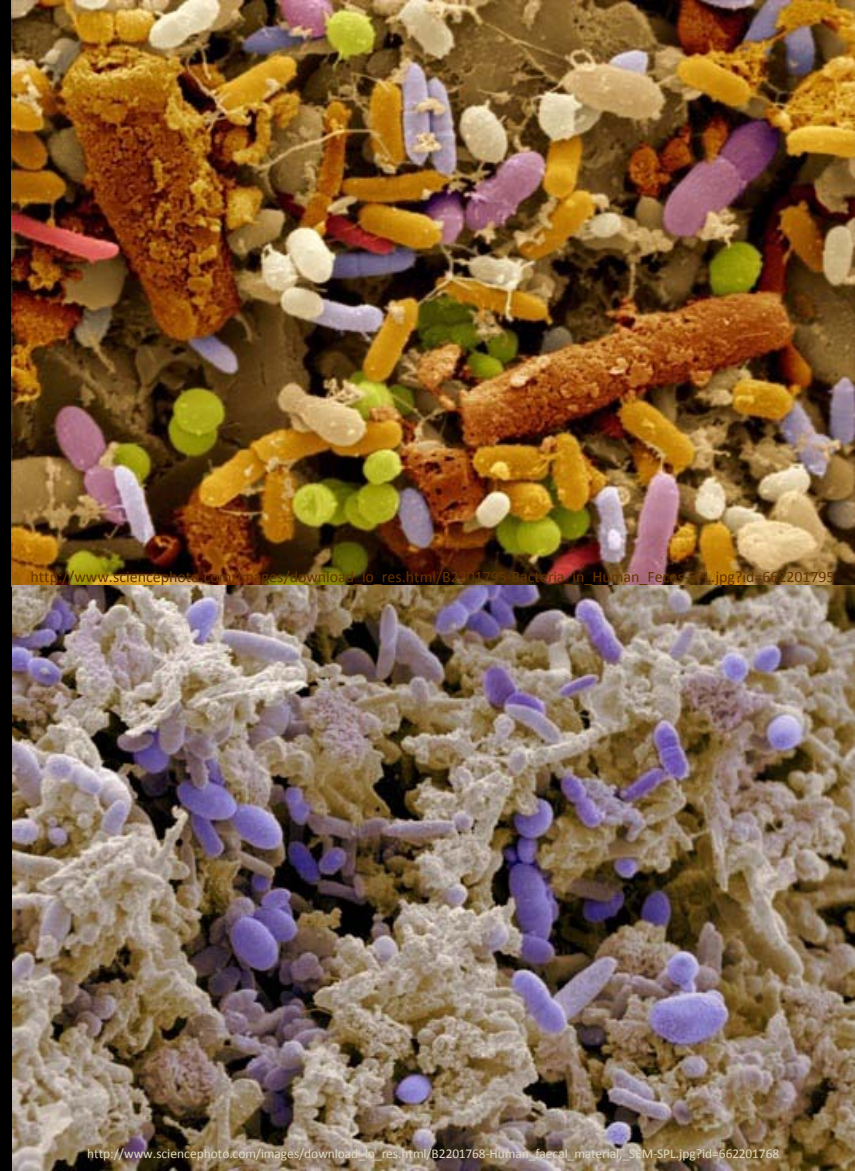
Drawbacks of *in vivo* models

Unique, host-specific community profiles
Often derive data from end-point measurements
Mechanistic studies often confounded
Often require specialist facilities
Expensive and time-consuming
Low compliance and high drop-out rates
Experiments must pass research ethics approval

Macfarlane, G.T. and Macfarlane, S. (2007) Curr Opin Biotechnol. , 18(2):156–62.

Advantages of
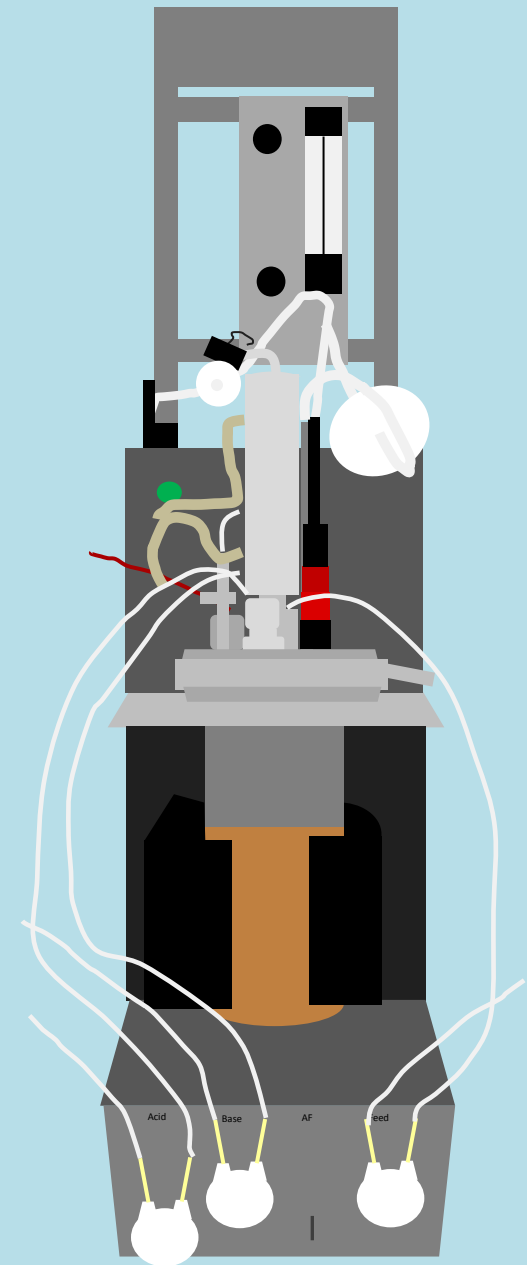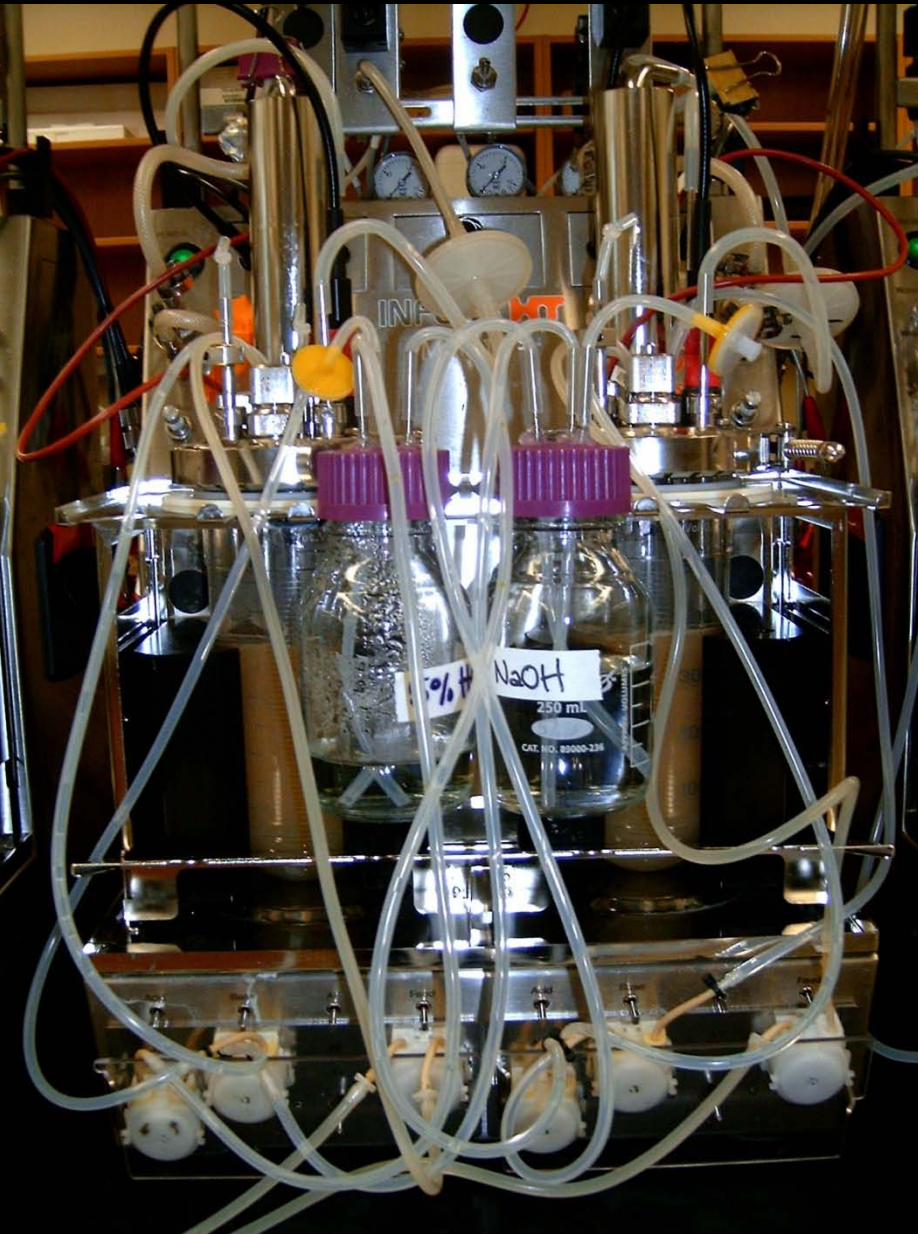# *in vitro* studies

Generally inexpensive

Easy to set-up

Frequent and simple sampling

Strict control of factors that influence the environment

Useful for mechanistic studies

Lack some ethical considerations

Macfarlane, G.T. and Macfarlane, S. (2007) Curr Opin Biotechnol. , 18(2):156–62.

Twin-vessel single-stage chemostat

Distal gut communities can be modeled *in vitro*
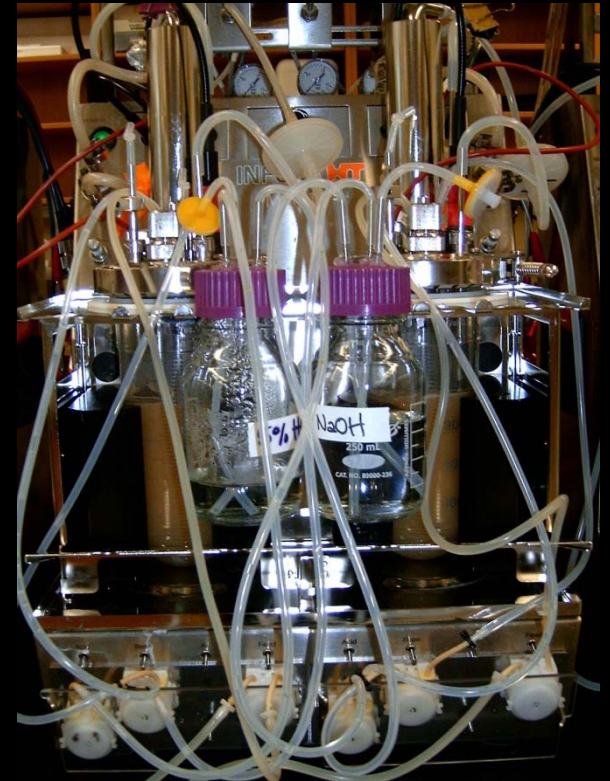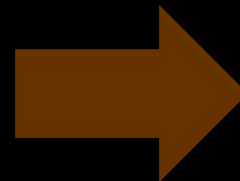
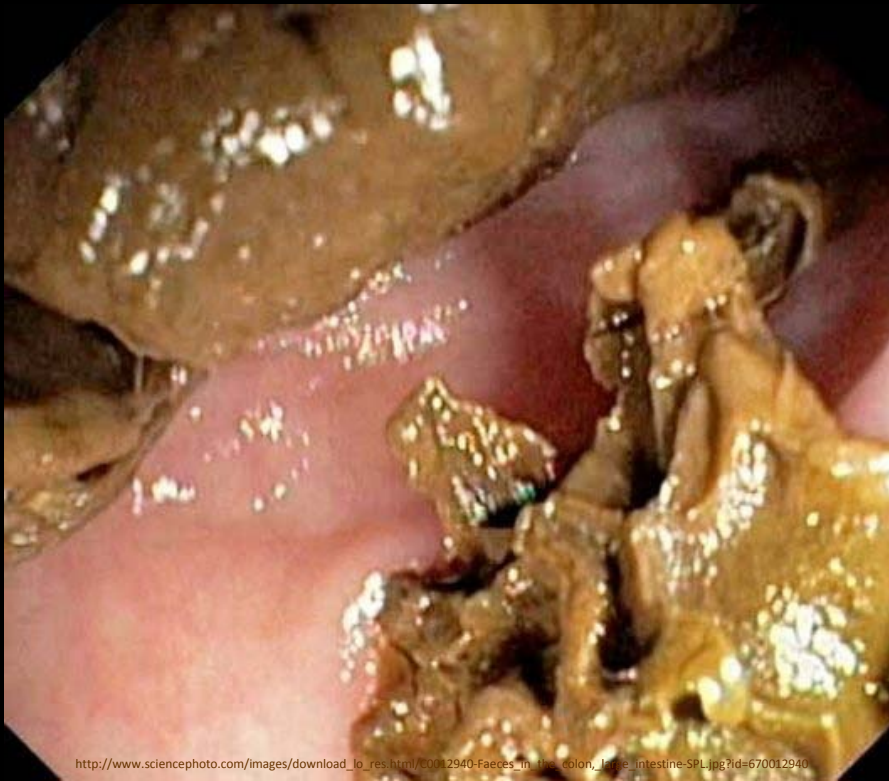Model one segment of the GI tract

Host-free system

Can assess the effect of a treatment on community

Full characterization of community required

Hypothesis

We can use our single-stage chemostat model of the human distal gut to develop and characterize microbial communities that are stable, reproducible, and biologically significant

Goals

1) Compare the colonization process in two identical vessels

2) Determine how long it takes both vessels to reach steady-state

3) Characterize diversity (including richness and evenness) of the fecal and chemostat communities

Experimental Design
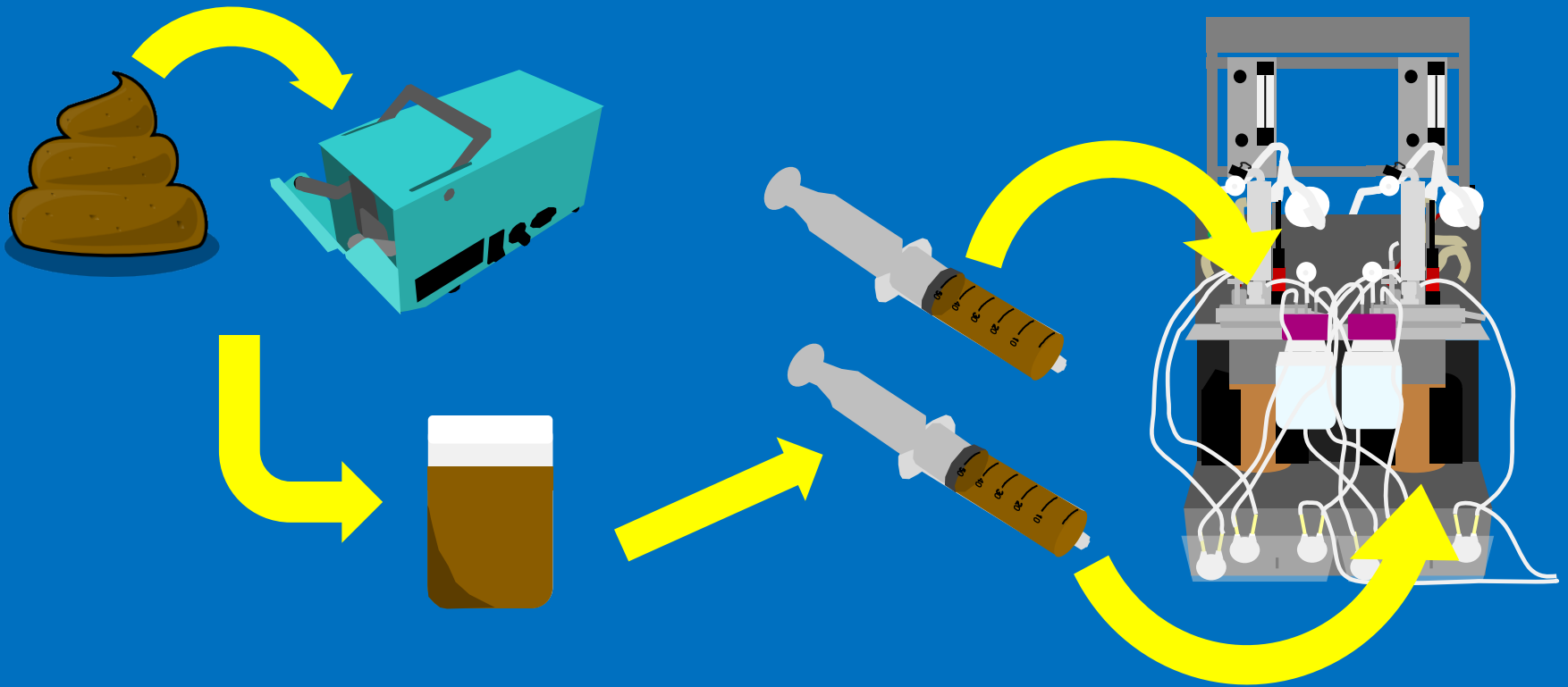
Sample daily, archive samples at -80□C

DNA extraction - combination of bead beating, the Omega Bio-Tek E.Z.N.A.® Stool DNA Kit, and the Promega Maxwell®16 DNA Purification Kit

DGGE - amplified the V3 variable region of the 16S gene

Analysis of DGGE gels

- Statistical analysis software: GeneTools (Syngene)
- Similarity indices: 0-1
  - 0 = no bands shared between profiles
  - 1 = all bands shared between profiles
- Percentage similarity (aka correlation coefficient) = similarity index multiplied by 100

	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 9	Lane 10	Lane 11	Lane 12	Lane 13	Lane 14
Lane 2	1.000	0.753	0.863	0.849	0.816	0.822	0.712	0.730	0.735	0.739	0.809	0.823
Lane 3	0.753	1.000	0.906	0.892	0.889	0.898	0.856	0.805	0.639	0.598	0.670	0.711
Lane 4	0.863	0.906	1.000	0.979	0.960	0.977	0.871	0.870	0.720	0.687	0.747	0.785
Lane 5	0.849	0.892	0.979	1.000	0.960	0.981	0.881	0.887	0.755	0.718	0.771	0.803
Lane 6	0.816	0.889	0.960	0.960	1.000	0.974	0.896	0.879	0.721	0.673	0.723	0.757
Lane 7	0.822	0.898	0.977	0.981	0.974	1.000	0.893	0.886	0.727	0.686	0.727	0.761
Lane 9	0.712	0.856	0.871	0.881	0.896	0.893	1.000	0.939	0.738	0.685	0.646	0.697
Lane 10	0.730	0.805	0.870	0.887	0.879	0.886	0.939	1.000	0.817	0.757	0.656	0.694
Lane 11	0.735	0.639	0.720	0.755	0.721	0.727	0.738	0.817	1.000	0.973	0.828	0.820
Lane 12	0.739	0.598	0.687	0.718	0.673	0.686	0.685	0.757	0.973	1.000	0.866	0.851
Lane 13	0.809	0.670	0.747	0.771	0.723	0.727	0.646	0.656	0.828	0.866	1.000	0.985
Lane 14	0.823	0.711	0.785	0.803	0.757	0.761	0.697	0.694	0.820	0.851	0.985	1.000

Additional methods of DGGE analysis
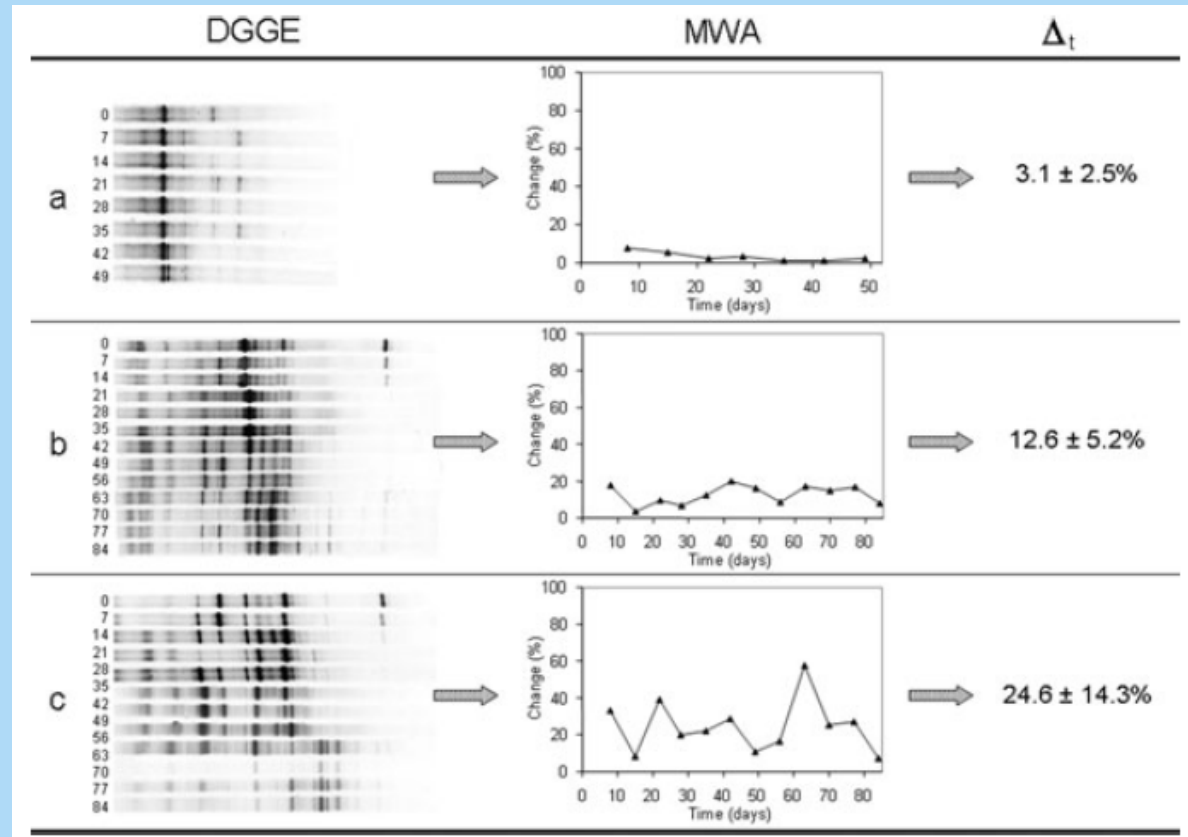
Dynamics

Shannon's diversity index

Range-weighted richness

Shannon's equitability index

Functional organization

Dynamics (Dy)

- **Changes within the community over a fixed time frame**

- **Moving-window correlation analysis**

- **Low Δt : 0-5% (most stable)**
- **Medium Δt : 5-15%**
- **High Δt : ≥ 15% (least stable)**

Chemostat communities at steady state once a low Δt value (0-5%) was maintained by the community

Possemiers *et al*. (2004) FEMS Microbiol Ecol. 49(3):495-507. Wittebolle *et al*. (2008) J Appl Microbiol. 107(2):385-94. Wittebolle *et al*. (2009) Water Res. 43(17):4149-58.

Shannon's diversity index (H')

- **Measure of community diversity**
  - **Richness (number of species present)**
  - **Evenness (relative abundance)**

$$H' = - \sum_{i=1}^{S} (p_i \ln p_i)$$

where:
- H' = the value of the Shannon diversity index
- p_i = the proportion of the ith species
- ln = the natural logarithm of p_i
- S = total number of species in the community (richness)
- $\sum$ = sum from species 1 to species S

- **Values range from 1.5 (low species richness and evenness) to 3.5 (high species evenness and richness) in natural systems**
- **Increase in H' may be the result of an increase in species richness, an increase in species evenness, or an increase in both**

Gafan G.P., *et al*. (2005) J Clin Microbiol., 43(8):3971-8.

Range-weighted richness (Rr)

- **Measure of community richness**

- **Environments with broader carrying capacities have a higher probability of hosting a larger number of bands with a wide variability in GC content**

$$Rr = N^2 \times D_g$$

where:
- Rr = Range-weighted richness
- N = total number of bands in the pattern
- D_g = denaturing gradient comprised between the first and last band of the pattern

- Low Rr: ≤ 10

- Medium Rr: 10-30

- High Rr: ≥ 30

Marzorati *et al.* (2008) Environ Microbiol. 10(6):1571-81.

Shannon's equitability index (E_H)

- Measure of community evenness
- Increases in the evenness result in an increase in community diversity

$$E_H = H'/H_{max} = H'/\ln S$$

where:
- E_H = Shannon's equitability index
- H' = Shannon index
- H_{max} = $\ln S$
- S = total number of species in the community (richness)
- $\ln$ = the natural logarithm of S

- E_H values range from $0 - 1$
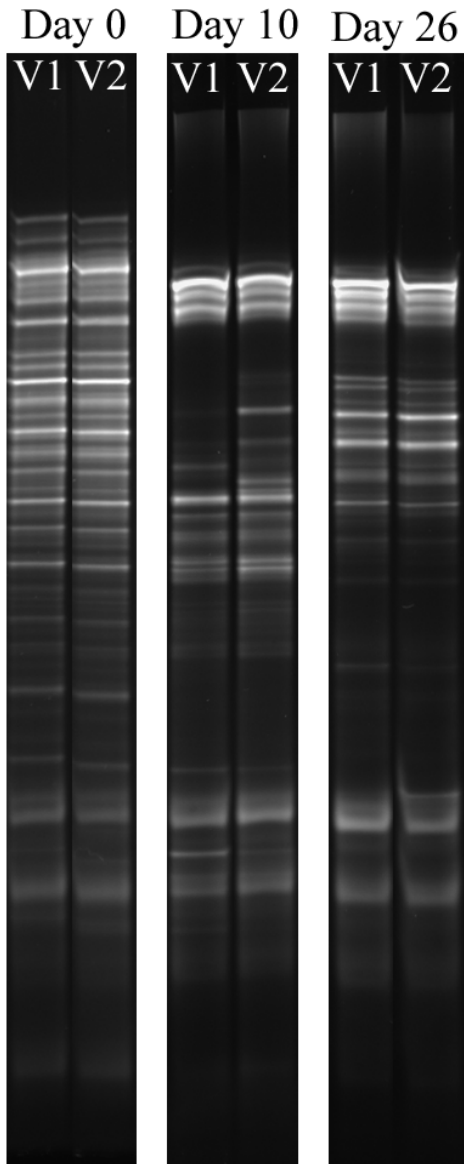 - 0 = complete community unevenness
  - 1 = complete community evenness

Pielou, E. C. (1975) Ecological diversity. Wiley, New York.

Functional organization (Fo)

- **Measures the structure of the community in terms of its evenness**

- **Pareto-Lorenz (PL) evenness curves**

- **Community less evenly structured the more the curve deviates from the 45° diagonal**

- **Curves interpreted by scoring the y-axis projection of the intercept of the curve with a 20% x-axis line**
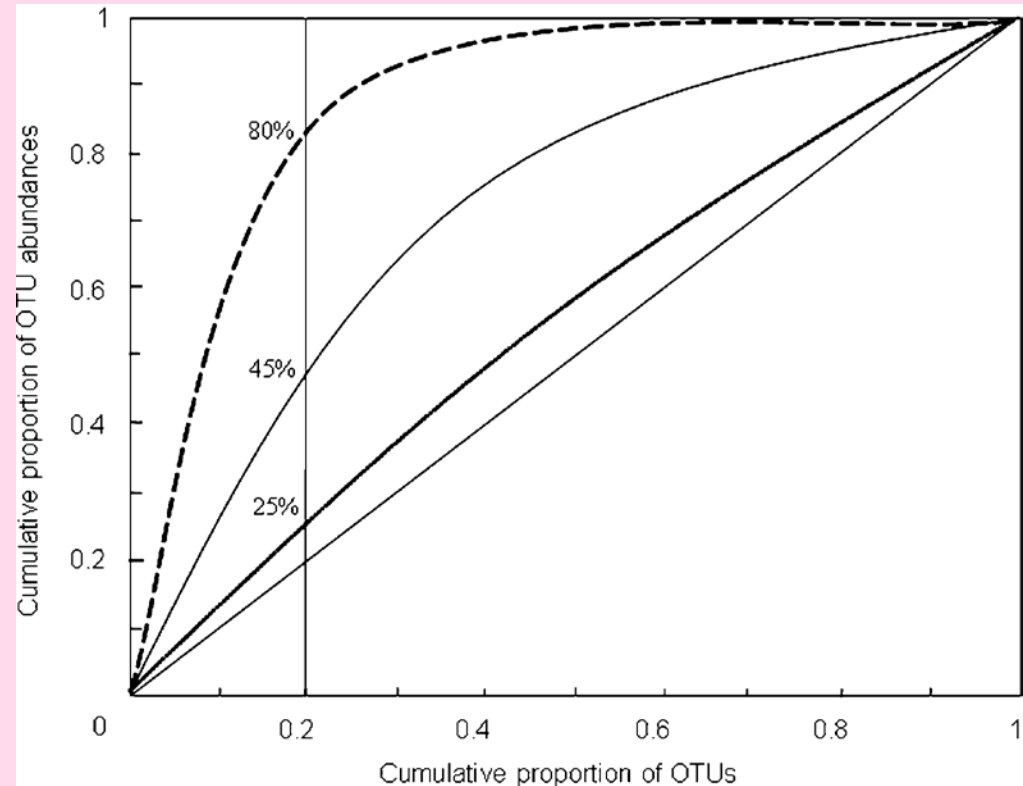
- low Fo values: 20-35% (high evenness)
- medium Fo values: 35-70% (medium evenness)
- high Fo values: ≥ 70% (low evenness)

Possemiers *et al.* (2004) FEMS Microbiol Ecol. 49(3):495-507. Wittebolle *et al.* (2008) J Appl Microbiol. 107(2):385-94. Wittebolle *et al.* (2009) Water Res. 43(17):4149-58.

Comparison of two vessels

Similarity between inocula: 91.3%

Average correlation coefficient: 94.7±2.0% between days 10-28
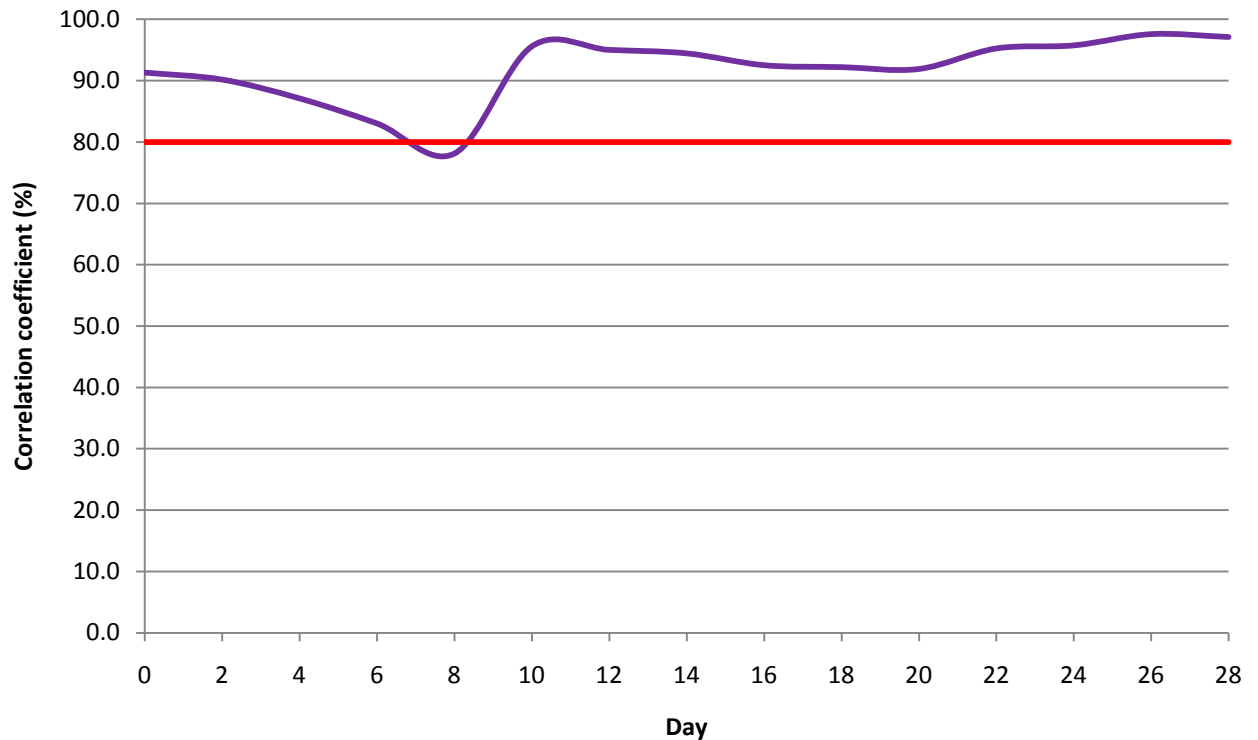
Steady-state vs. inocula
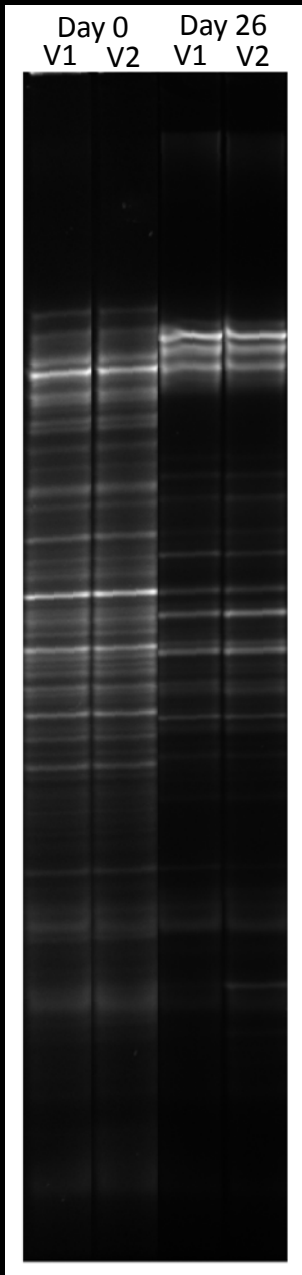
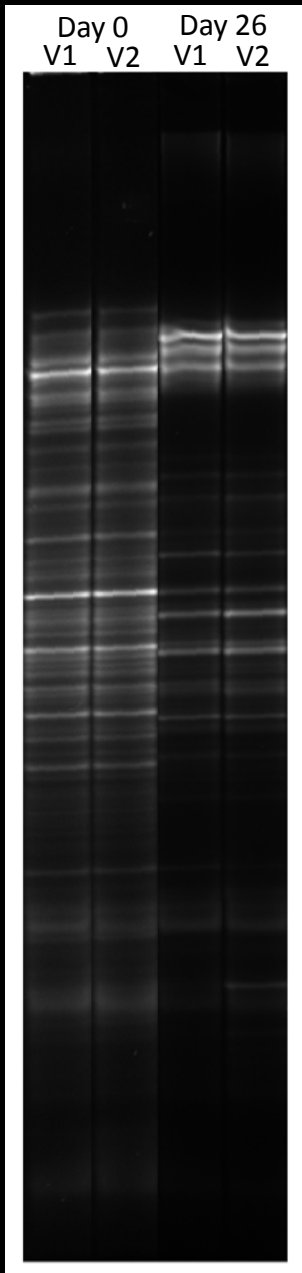Both vessels were similar to the starting material

V1 Day 0 vs. V2 Day 0: 96.3%

V1 Day 26 vs. V2 Day 26: 96.0%

V1 Day 0 vs. V1 Day 26: 40.2%

V2 Day 0 vs. V2 Day 26: 39.3%

DGGE detects bacterial populations with > 1% abundance

Steady-state vs. inocula

Day 0 V1 V2   Day 26 V1 V2

Why only 40% similar?

- 1/3 of fecal bacteria are dead cells

  Ben Amor K. (2004) PhD thesis. Wageningen University, The Netherlands.

- Stressed cells

  Hart A.L. *et al.* (2002). Gut Ecology, Informa Healthcare, United Kingdom, pg 172.

- Missing essential interactions with host or other bacteria

  Macfarlane, G.T. and Macfarlane, S. (2007) Curr Opin Biotechnol. , 18(2):156–62.
  Hart A.L. *et al.* (2002). Gut Ecology, Informa Healthcare, United Kingdom, pg 172.

- Lack essential nutrients or particular culture conditions

  Hart A.L. *et al.* (2002). Gut Ecology, Informa Healthcare, United Kingdom, pg 172.

- Loss of heterogeneity in system

  Sanchez O., *et al.* (2008) Microbial Ecology Research Trends, Nova publishers; pgs 87-114.
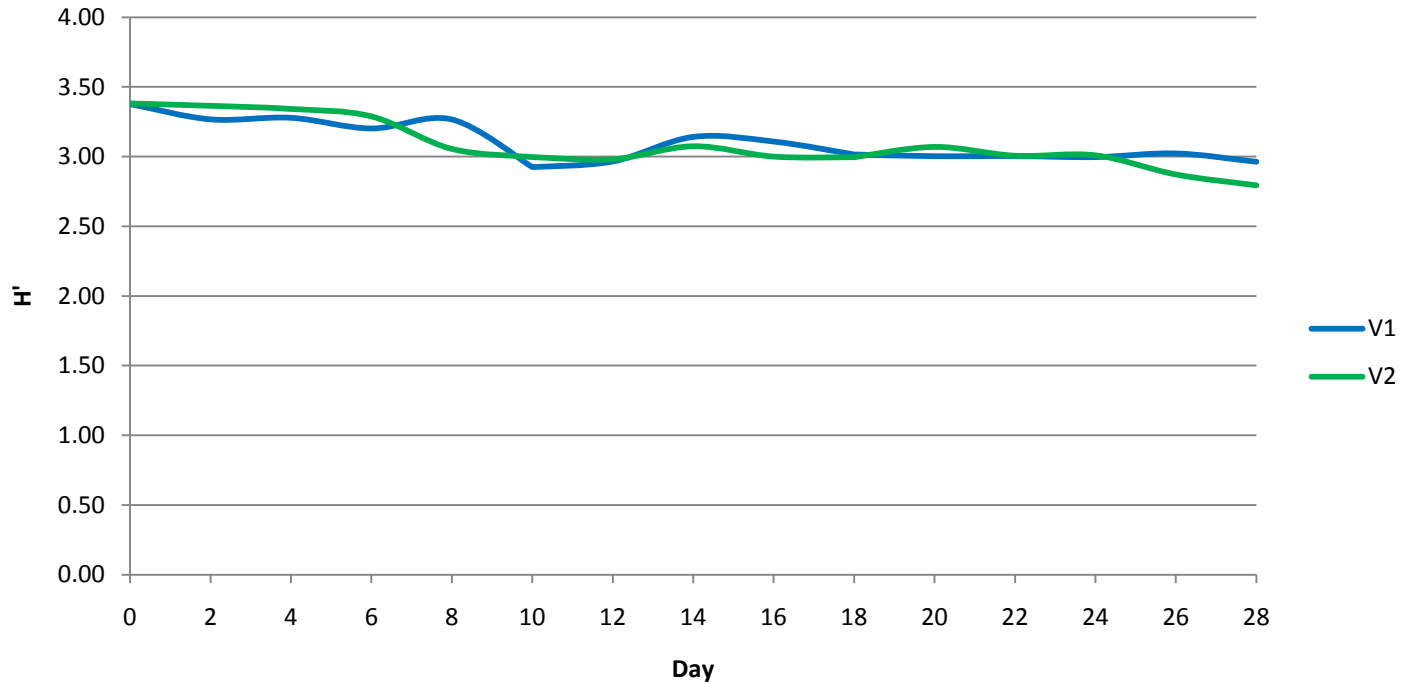
Dynamics (Dy)
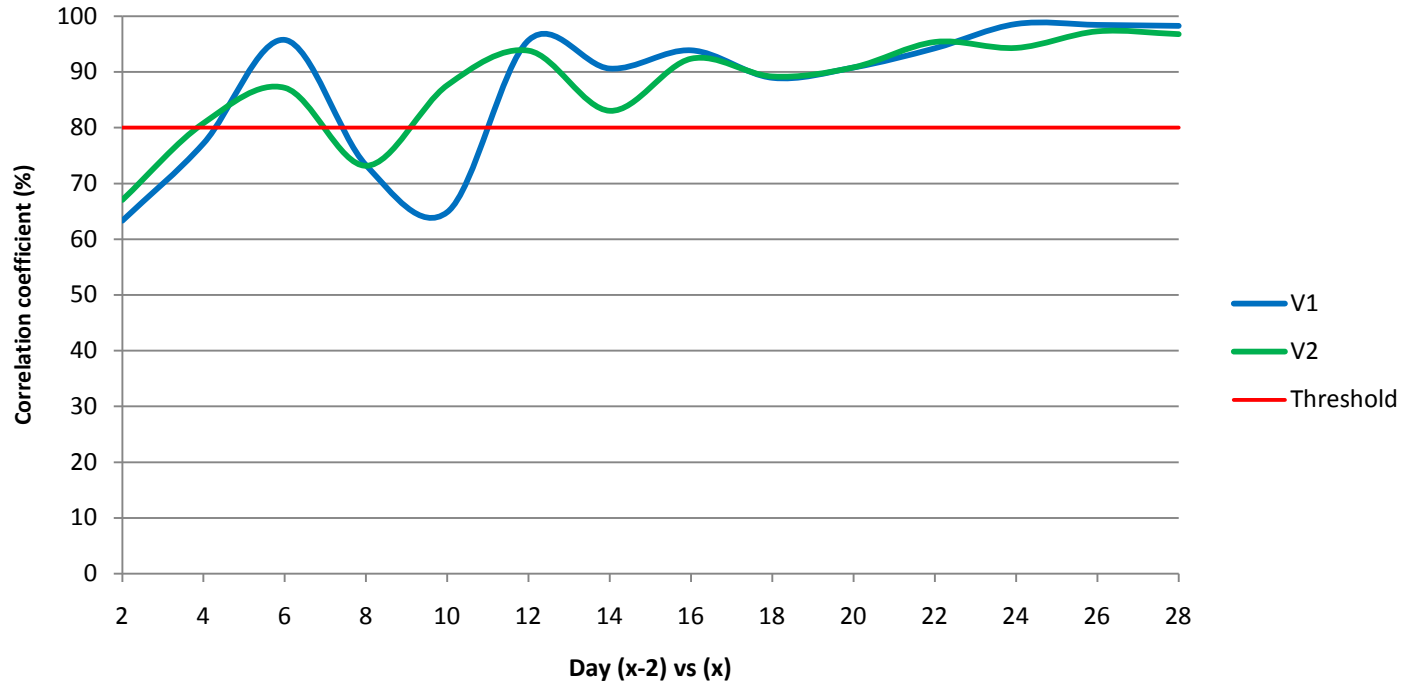
V1 and V2 had similar low dynamics ($\Delta t < 5\%$, $p > 0.10$)

- V1 $\Delta t = 1.6 \pm 0.2\%$
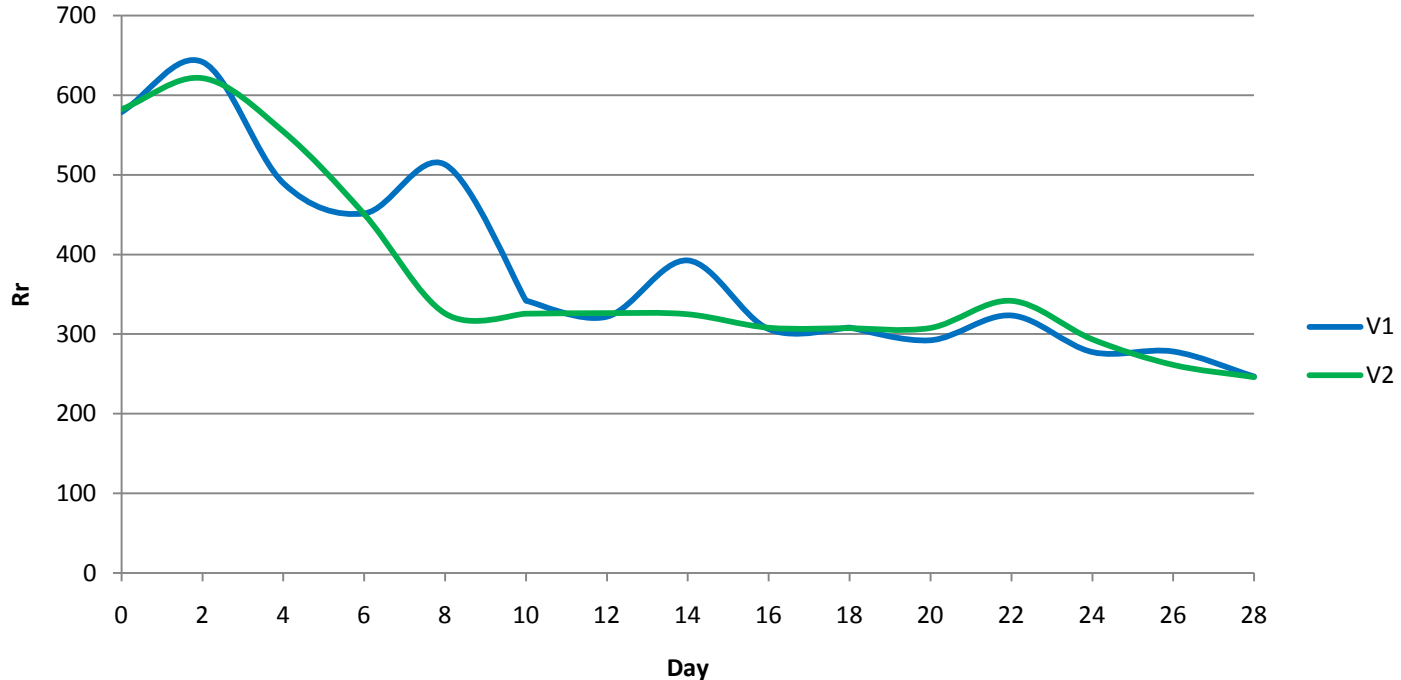- V2 $\Delta t = 3.9 \pm 1.6\%$

Diversity (H')

V1 and V2 had similar high diversity (p>0.10)
- V1 H' = 3.03±0.06
- V2 H' = 2.98±0.10

Richness (Rr)

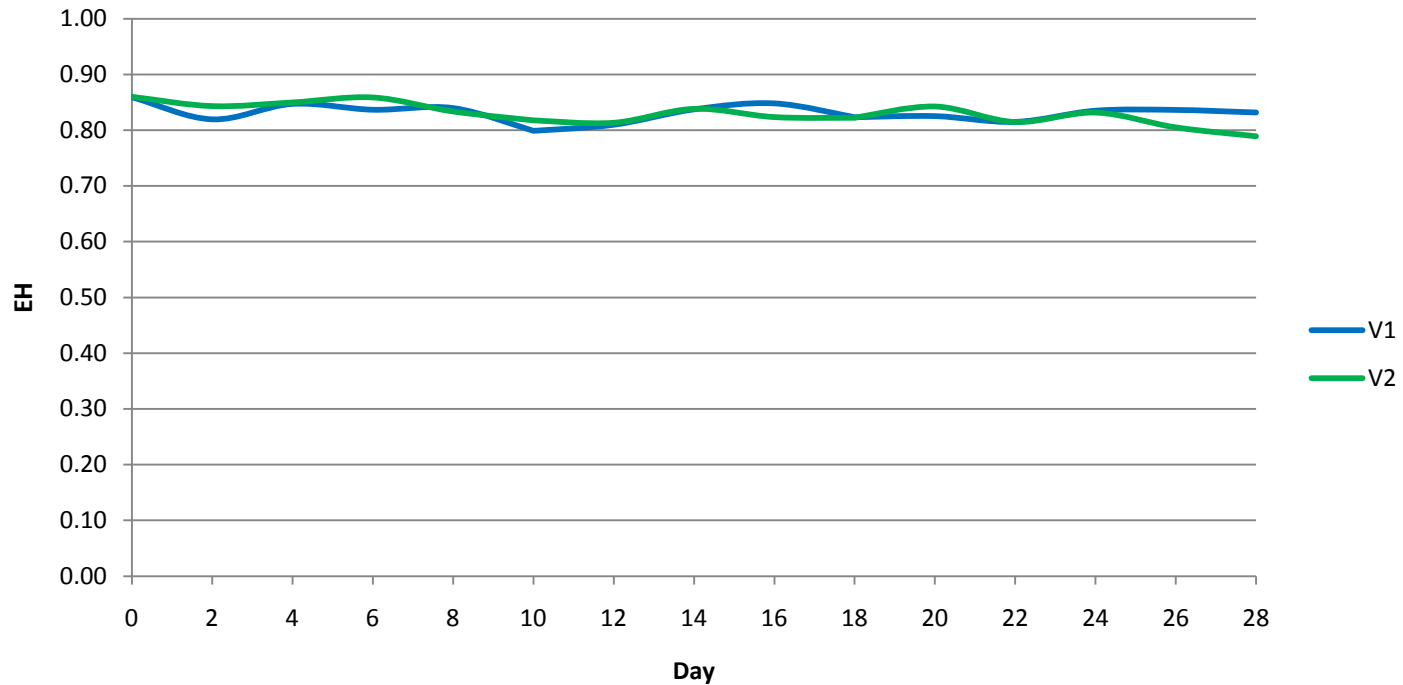V1 and V2 had similar high richness (Rr>30, p>0.10)
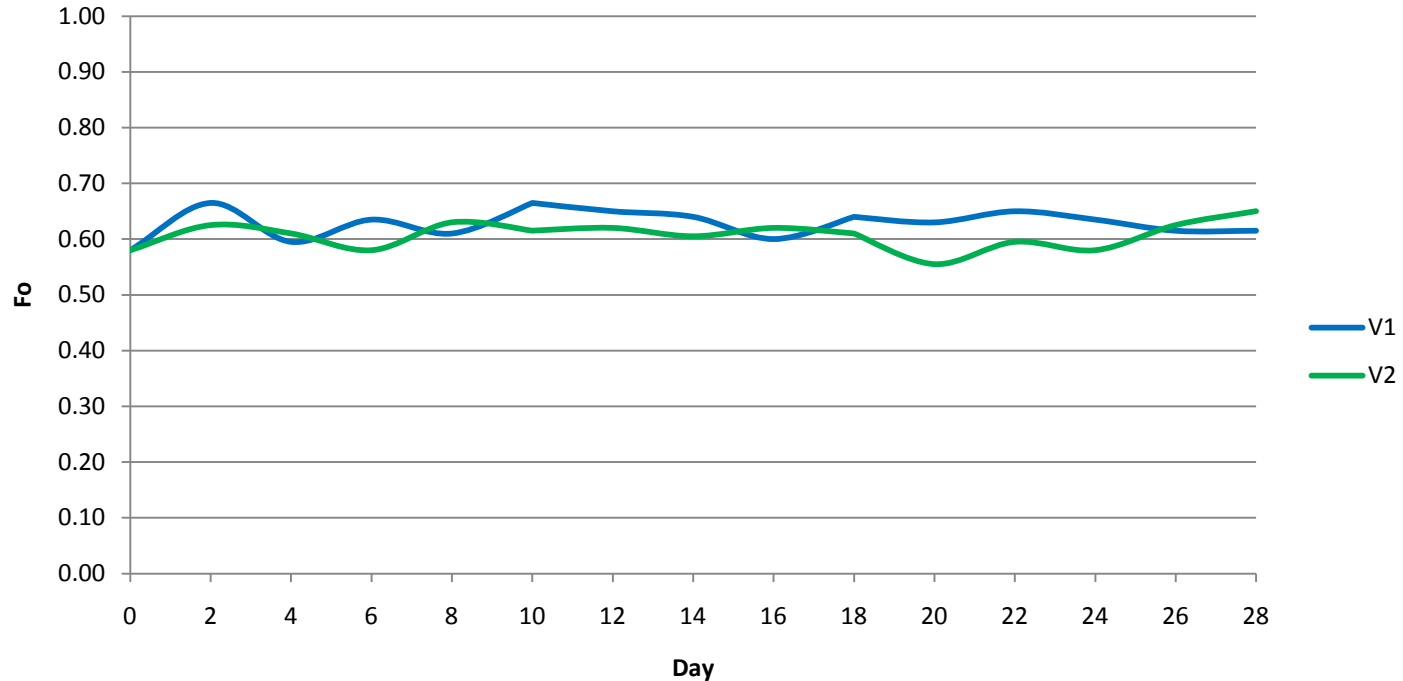– V1 Rr = 303.0±43.1
– V2 Rr = 299.0±31.6

Evenness (E_H)

V1 and V2 had similar high evenness (p>0.10)
- V1 E_H = 0.83±0.01
- V2 E_H = 0.82±0.02

Evenness (Fo)

V1 and V2 had similar medium evenness (35<Fo<70%, p>0.10)
- V1 Fo = 62.8±1.7%
- V2 Fo = 60.5±2.9%

SINGLE-STAGE VS. MULTI-STAGE CHEMOSTAT

Twin-SHIME multistage chemostat:

- 76% correlation 26 days post-inoculation
- Steady state 21 days post-inoculation, differences in profile band brightness

Twin-vessel single-stage chemostat:

- 97% correlation 26 days post-inoculation
- Steady state 26 days post-inoculation, little differences in profile band brightness

Multi-stage   Single-stage

den Abbeele *et al.* (2010) Appl Environ Microbiol. 76(15):5237-46.
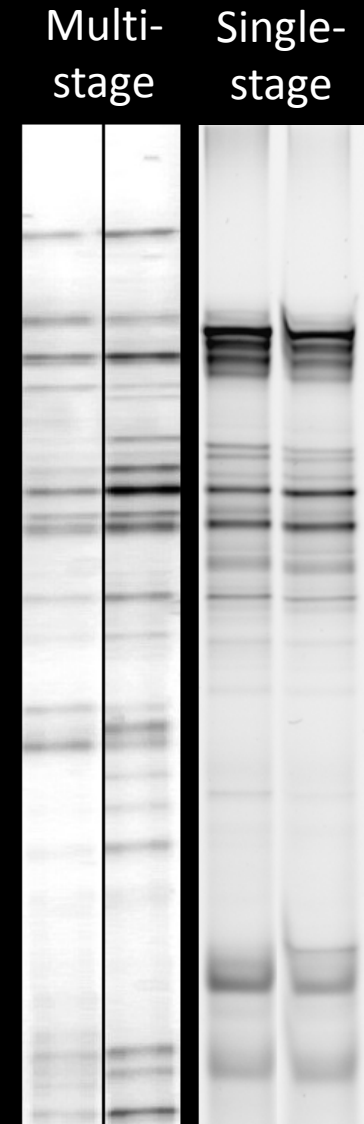
Conclusions

Our single-stage chemostat vessels can produce complex communities that are stable, reproducible, and diverse, reaching steady state 26 days post-inoculation

Communities can be used to conduct *in vitro* studies with exogenous stimuli

Acknowledgements

Thanks to the
NIH for
inviting me and
to everyone for
listening!

Questions?