Supplementary Methods

MBRA Design and Operation

As described in the Methods, MBRAs were fabricated from DSM Somos Watershed XC 11122 via stereolithography (FineLine prototyping). Six reactors with an internal volume of 25 mL were designed into a single strip with 200mm x 47 mm x 36 mm dimensions (Fig. 1). Reactors were drawn with 25 x 25 x 40 mm dimensions, including 10 mm radial blends on the bottom corners (to prevent buildup of cells and other insoluble materials), and 5 mm radial blends on the top. Reactors were spaced 32.5 mm center to center to match dimensions on the stir plate. Three 5.55 mm diameter holes (influent, effluent, sampling) were placed into the top of each reactor, and spaced 16 mm apart center to center. Holes were threaded to fit conventional leur connectors. Inner walls of the reactors were placed 2 mm from the bottom of the strip, 5 mm from the top of the strip, 5 mm from the side of the strip, and 3.25 mm into the strip. A 1.25 x 16 x 31 mm intrusion was designed into the bottom corners of the MBRA for fixing into a custom built acrylic block that held the reactors upright and properly aligned with the stir-plate. A CAD file (.stl) that can be used directly for fabrication via stereolithography is available upon request.

Media (described in the materials and methods section) was transferred from the source bottles through a combination of 1/8 in inner diameter (ID) C-flex tubing (Cole-Parmer) tubing and 0.89 mm ID 2-stop Tygon lab tubing supplied to the reactors via a 24-channel peristaltic pump (205S/CA, Watson-Marlow). Waste

was removed from the reactors through a combination of 1/8 in ID C-flex tubing tubing and 1.14 mm ID 2-stop Tygon lab tubing drawn from the reactors via the same 24-channel peristaltic pump. Reactors were stirred using magnetic stir bars driven by independent magnets on a 60-spot magnetic stir plate (VarioMAG HP 60, Vario-MAG USA).

Pure culture competition experiments

Pure culture continuous-flow competition experiments were performed in CBM medium. One liter of CBM media contained: 22.5 g trypticase peptone (VWR), 1 g ammonium sulfate, and 1.5 g tris base (Invitrogen) and was adjusted to pH 7.5 and autoclaved at 121° C for 30 min. This media is a variation of the *C. difficile* sporulation medium published by Wilson *et al.* (1). Five ml of brain heart infusion broth (Difco, BD BioSciences, San jose, CA) supplemented with 5 g/l yeast extract (Difco, BD BioSciences, San jose, CA) (BHIS) was inoculated from fresh BHIS streak plates with selected *C. difficile* strains and grown overnight. Overnight cultures were then sub-cultured into 50 ml CBM media (1:100 dilution) and allowed to grow at 37° C for 6 hr before inoculation into the reactors. To inoculate, 5 ml media was removed from each reactor and replaced with 5 ml 10:1 mixed *C. difficile* subculture (10 non-027 strain:1 027 strain, based on culture OD). The peristaltic pump was started immediately after inoculation and was operated to ensure a retention time of 2 hurs. 1 ml samples were removed from the reactors at the time of inoculation and at 24 hrs. 10 µl of the culture samples were serially diluted and plated on BHIS agar media with and without

selective antibiotic (25 μg/ml rifampicin or 10 μg/ml erythromycin) to enumerate CFU/ml of the different strains. The antibiotics used are selective for the NAP1 strains; therefore, non-NAP1 strain enumeration was determined by subtracting the number of colonies on selection plates from the number of colonies on the non-selective plates (total bacteria). The remainder of the 1 ml samples were pelleted and frozen at -20° C for qPCR analysis as described in the main text. There are 6-8 total replicates of each competition, including three biological replicates.

Table S1. Competitive indices of ribotype 027 strains at selected time points after *C. difficile* inoculation as determined by quantitative PCR.

027 CIs in Fecal Community Background Competitions			
	Days post *C. difficile* inoculation		
	3	7	11
CD2015 (027) + CD3014 (001)	0.06[+]	0.75[+]	8.09[+]
	0.52[+]	1.19[+]	3.74[+]*
	0.22[+]	0.49[+]	0.88[+]
	3.30[+]	22.39[+]*	ND
	3.19	18.59	ND
	0.11	3.36	36.17
CD3017 (027) + CD1014 (014)	119.15	36.93	3.83
	11.88	21.91	33.05
	17.15	22.84	18.13
	232.86	3993.21	ND
	8.15	86.92*	23.43[¢]
CD4015 (027) + CD2048 (053)	8.11[+]	4.97[+]	24.59[+]
	11.16[+]	34.62[+]	52.47[+]
	8.44[+]	1.41[+]	18.34[+]
	138.94*	593.38*	ND
	20.21	21.26	40.32
CD4010 (027) + CD4004 (002)	19.43	183.55	107.20*
	15.24	30.34	9.14*
	12.97	31.27	50.09*
	27.22	627.14*	ND
	43.71*	764.94*	1386.61*

ND= not determined

* Ratio calculated based upon limit of detection for non-027 ribotype

[+] CI's calculated 1 day later than the day indicated than the column heading.

[¢]CI's calculated 1 day earlier than the day indicated than the column heading. Shaded rows indicate replicates where *C. difficile* was added to the MBRAs post-cessation of clindamycin dosing 24 hrs later than the other replicates.

Table S2. Primers used for qPCR

Target Gene	Primer sequences (Forward & Reverse)	Citation
C. difficile tcdA	**F:** AGC TTT CGC TTT AGG CAG TG	This study
	R: ATG GCT GGG TTA AGG TGT TG	
Bacterial 16S *rRNA*	**F:** ACT CCT ACG GGA GGC AGC AG	(2)
	R: ATT ACC GCG GCT GCT GG	
C. difficile thyA	**F:** GAT GGC CAG CCT GCT CAT ACA ATA	This study
	R: TGT TTC ATC AGC CCA GCT ATC CCA	
C. difficile thyX	**F:** CCA GTT GGG ACA GAC GAA AT	This study
	R: TGA ACA AGC CCT TGA AAT ACC	

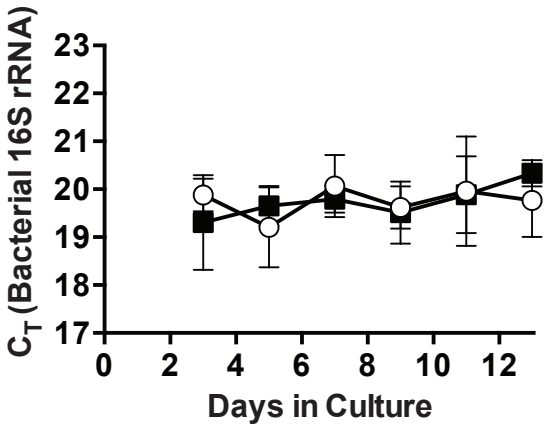

Figure S1. Bacterial abundance does not change significantly in clindamycin-treated reactors. We measured the relative abundance of 16S rRNA gene copies in bioreactor samples from triplicate clindamycin-treated (open circles) and mock-treated reactors (closed-squares) using quantitative PCR with previously described broad-range 16S rRNA gene qPCR primers (2). We have reported the average cycle threshold (C_T, ± standard deviation) where the qPCR reactions began amplifying linearly. We did not determine absolute quantification of 16S rRNA gene copies in the samples because the sample populations are composed of mixtures of bacteria with different 16S rRNA gene copy numbers. Clindamycin or mock-treatment began on day 2.5 in culture and continued twice daily through day 6.

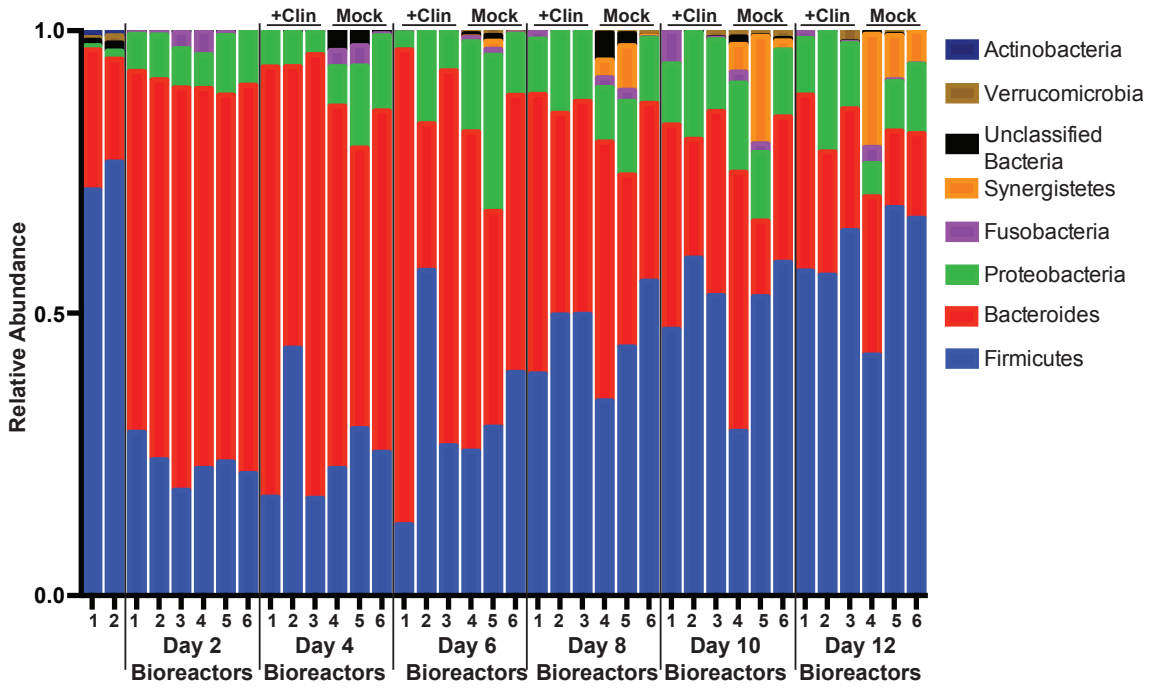

Figure S2. Comparison of the phylum level composition of bioreactor samples across time in culture and in response to clindamycin-treatment. As described in Figure 3, we analyzed the 16S rRNA gene abundances in samples from 3 replicate mock-treated and 3 replicate clindamycin-treated reactors on days 2, 4, 6, 8, 10 and 12 in culture and compared the composition of these communities to duplicate samples of the initial fecal inoculum. We classified each sequence to the phylum level with at least 80% confidence (sequences <80% were designated “Unclassified Bacteria”) and then plotted the relative abundance of each phylum across the samples. The reactors are labeled as in Figures 2,4, and 5.

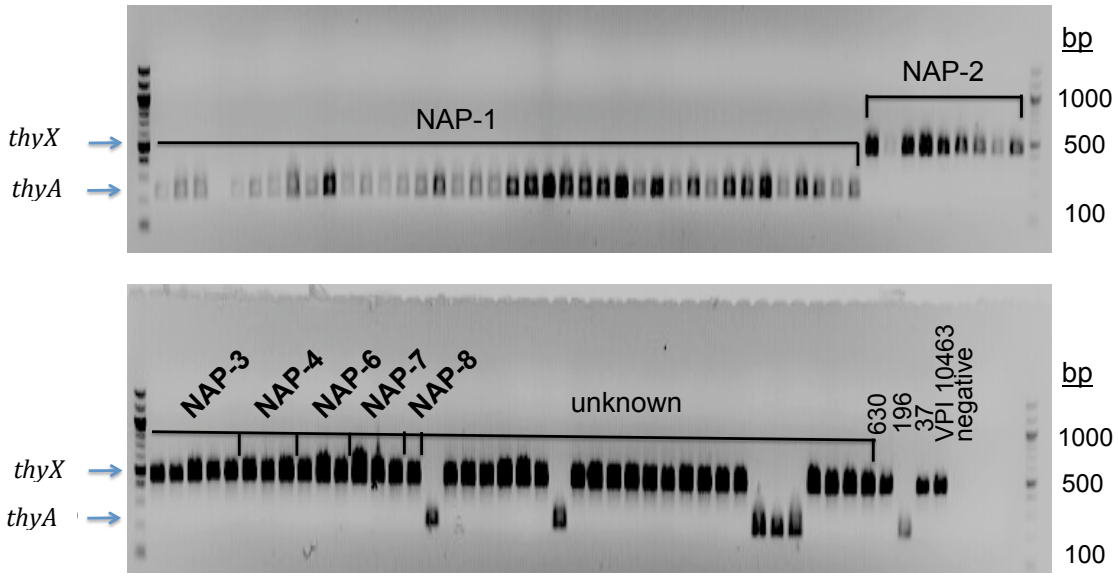

Figure S3. PCR screen of DNA samples from 88 strains of *C. difficile* for detection of insert containing *thyA* or the uninterrupted *thyX*.

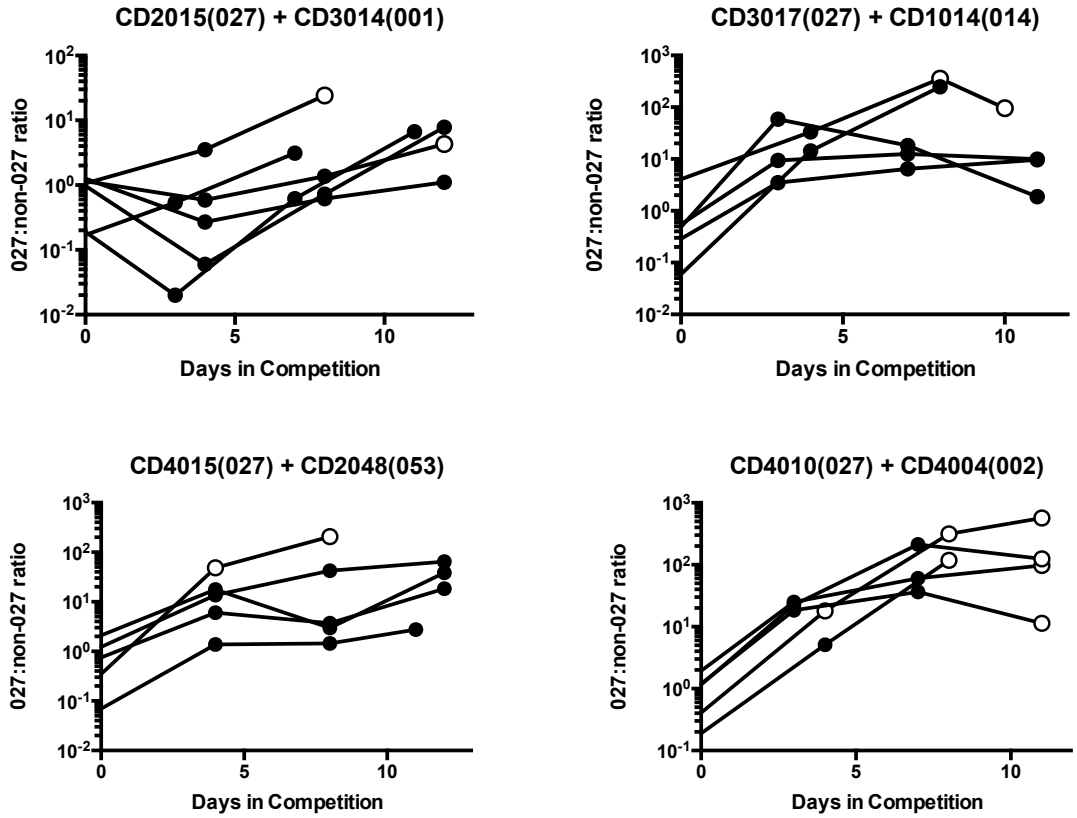

Figure S4. Ratios of ribotype 027:non-027 *C. difficile* strains over time in MBRA competitions. Each plot represents a different competition pair of one ribotype 027 and one non-027 ribotype strain; ribotypes indicated in parentheses above. Each line in the plots represents a replicate reactor, combined from three independent experiments. Where the non-027 ribotype strain was below the limit of detection, the ratio was determined by substituting in the highest C_T value in the linear range (detection limit) for the non-027 value (open circles).

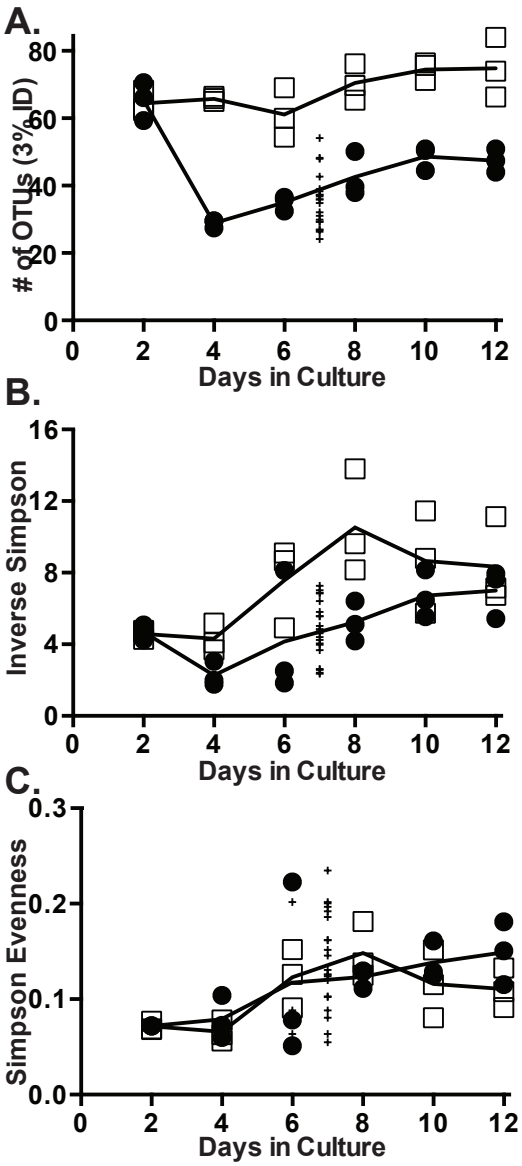

Figure S5. Comparison of the community structure on day 7 from clindamycin-treated reactors used for *C. difficile* competition experiments to triplicate mock-treated and clindamycin-treated reactors infected with CD2015. As described in Fig. 4, we analyzed the 16S *rRNA* gene abundances (binned into OTUs with 3% sequence identity) from three mock-treated and clindamycin-treated communities on days 2, 4, 6, 8, 10 and 12 in culture and compared these to samples from all competitions described (except one replicate each of 2015/3014 and 4010/4004

competitions; these are not plotted due to technical failures of the sample analysis) on day 7 just prior to the addition of *C. difficile*. We plotted the number of OTUs (A), inverse Simpson microbial diversity indicator (B), and Simpson evenness indicator (C) in the clindamycin (closed circles), mock-treated (open squares), and competition (plus symbols) communities. The solid lines represent the mean values for the triplicate clindamycin-treated and mock-treated samples at each time point, which are reproduced from Fig. 4.

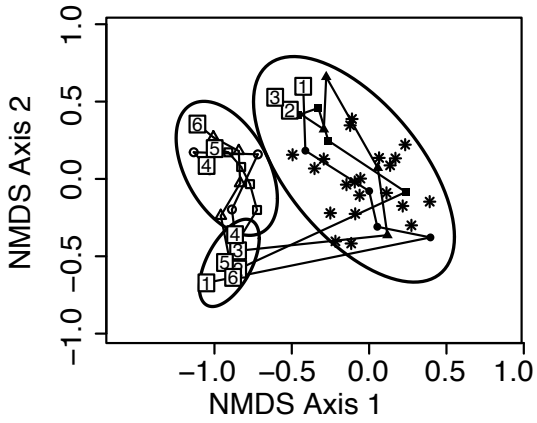

Figure S6. Similar community structure changes were observed in response to clindamycin-treatment in competition bioreactor communities. As described in Fig. 5, we plotted the Bray-Curtis dissimilarity (3% OTUs) between samples using nonmetric multidimensional scaling (NMDS). Samples were plotted from three clindamycin-treated replicates (1-3; closed symbols) and three mock-treated replicates (4-6; open symbols) every two days from day 2 (pre-treatment) through day 12. The numbers in boxes indicate the points for the indicated reactors on day 2 and day 12 and correspond to the reactor numbers indicated in Fig. 2. Intervening time points are represented by symbols and are connected in sequential order by lines. Asterisks represent the day 7 samples from the competition bioreactor communities described in Fig. S4. The ellipses indicate the 95% confidence intervals for the indicated groups (pre-treatment; mock-treatment; clindamycin-treatment.) The plot stress was 0.201.

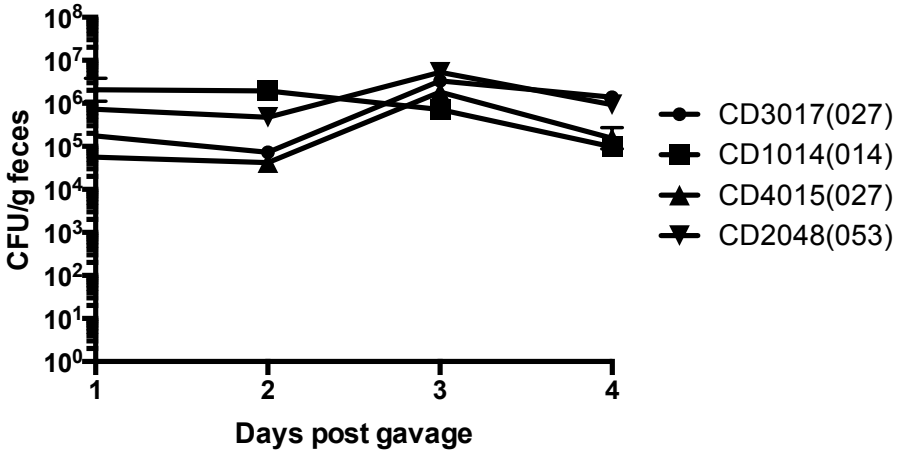

Figure S7. Levels of *C. difficile* strains across time in mouse model of infection as determined by plating from fecal pellets. After antibiotic treatment, mice were gavaged with spores from each individual ribotype. Fecal samples were collected daily for four days. For each time point, fecal samples were weighed, suspended in sterile water, heat killed for 30 min at 65°C, and plated on BHIS containing 0.1% taurocholic acid. Plotted here are the mean CFU/g feces from replicate mice for each strain group (with standard deviations where applicable; some data points were lost due to technical failure; two groups lost a triplicate mouse after day 1).

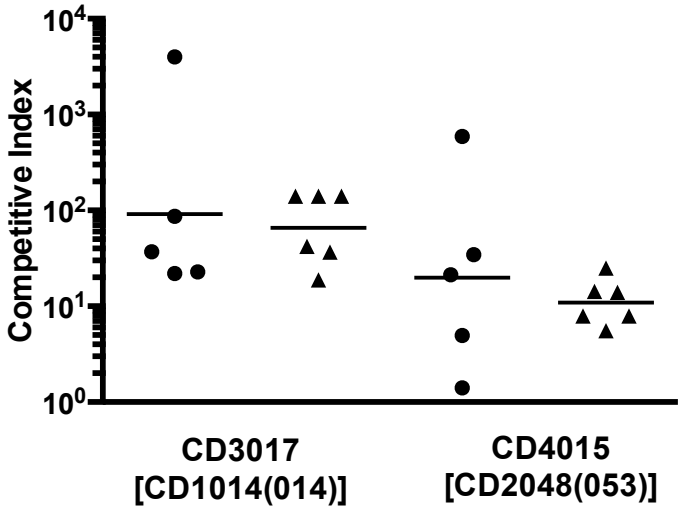

Figure S8. Competitive indices (CI) of two competition pairs of ribotype 027 and non-027 *C. difficile* strains in the MBRA (circles) and Mouse (triangles) models. When comparing the relative differences between the CI's of the competition pairs in each model, the geometric mean CI of the CD3017/CD1014 competition pair is higher than the CD4015/CD2048 competition pair in both models.

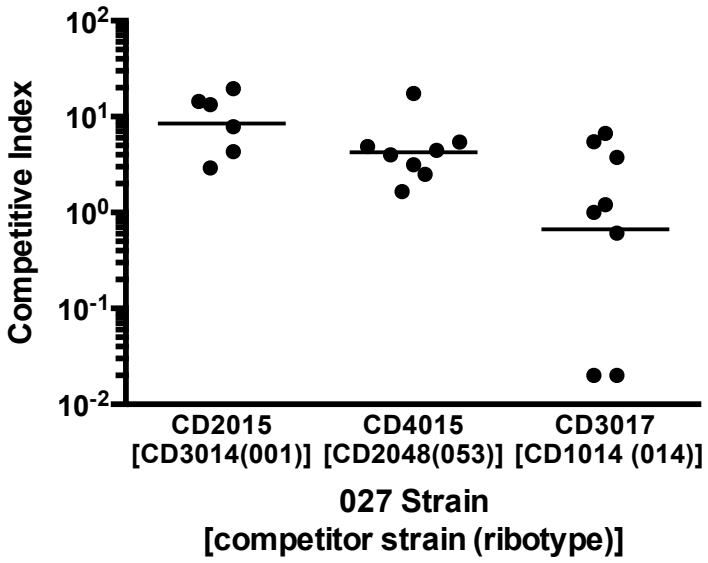

Figure S9. Competitive Indices of ribotype 027 strains relative to non-027 strains in pure, continuous-flow competition experiments. Mid-log cultures of each strain were mixed 1:10 (027:non-027) and inoculated into continuous-flow bioreactors containing CBM that were operated with a retention time of 2 hrs. Competitive indices were calculated by diving the ratio at 24 hrs by the starting ratio. Each black circle represents an individual replicate competition of one 027 strain and one non-027 strain. Geometric means are plotted as black bars.

References

1. **Wilson KH**, **Kennedy MJ**, **Fekety FR**. 1982. Use of sodium taurocholate to enhance spore recovery on a medium selective for Clostridium difficile. Journal of Clinical Microbiology **15**:443–446.
2. **Fierer N**, **Jackson JA**, **Vilgalys R**, **Jackson RB**. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. **71**:4117–4120.