

Supplemental File 1 (Supplemental Figures)

SF1: Oral & Fecal Sample Distributions

SF2: NF-EN vs scFOS-EN Fecal - Alpha Diversity

SF3: NF-EN vs scFOS-EN Fecal – LOESS plots for significant genera

SF4: Genus-level results of linear mixed models of repeated measures (LMMRM)

SF5: Changes in Oral Microbial Dynamics in NF-EN vs scFOS-EN

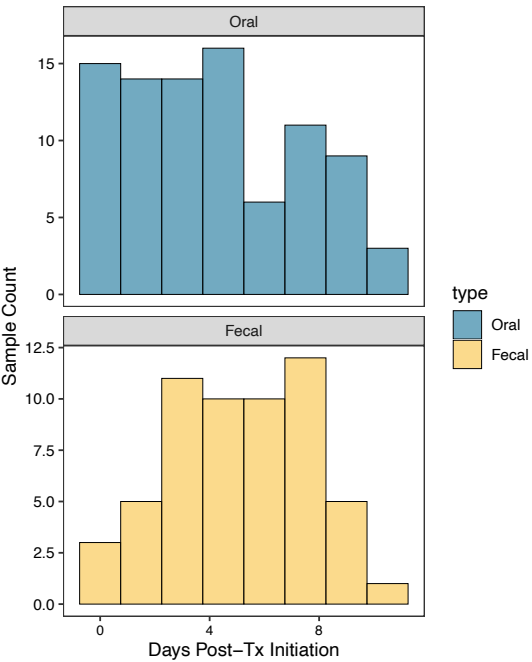
SF6: Relationship between early *Enterobacteriaceae* expansion and commensals impacted by scFOS-EN

SF7: Characteristics of fecal samples used for NF-EN and scFOS-EN Networks

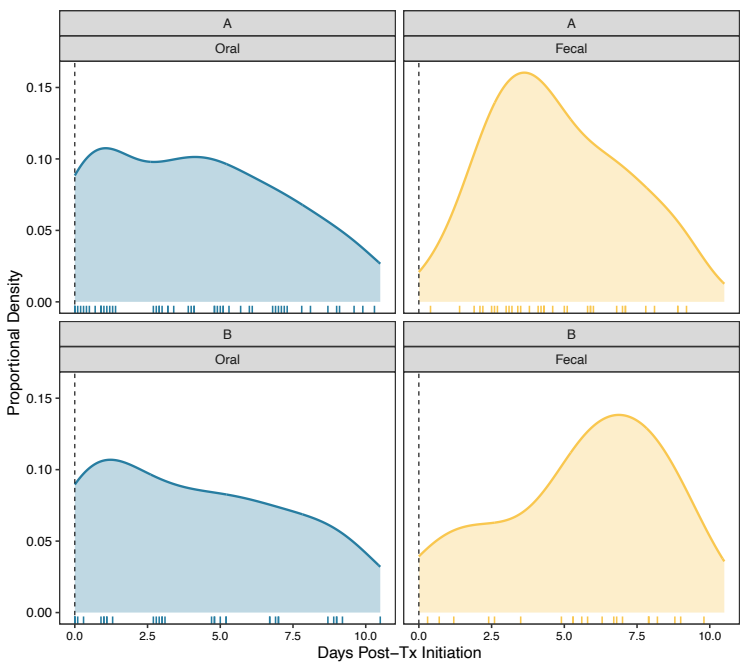
SF8: MicNet Dashboard NF-EN and scFOS-EN Network topography results

Supplemental Figure 1

A



B



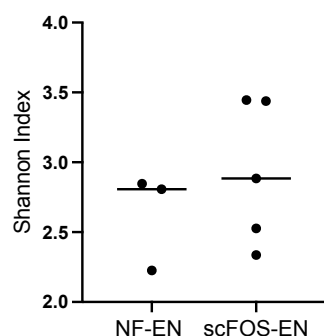
SF1: Oral & Fecal Sample Distributions

A) Distribution of numbers of oral (top) and fecal (bottom) samples by collection time for samples included in final analysis . B) Proportional density of oral (left) and fecal (right) samples by collection time for scFOS-EN (Group A, top) and NF-EN (Group B, bottom)

Supplemental Figure 2

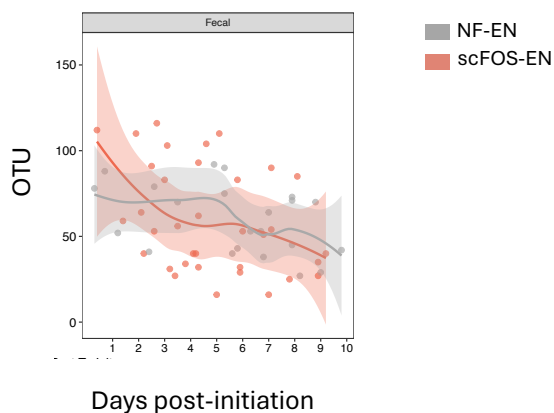
A

Gut Microbial Community Richness
Day 0-2 post-formula initiation



B

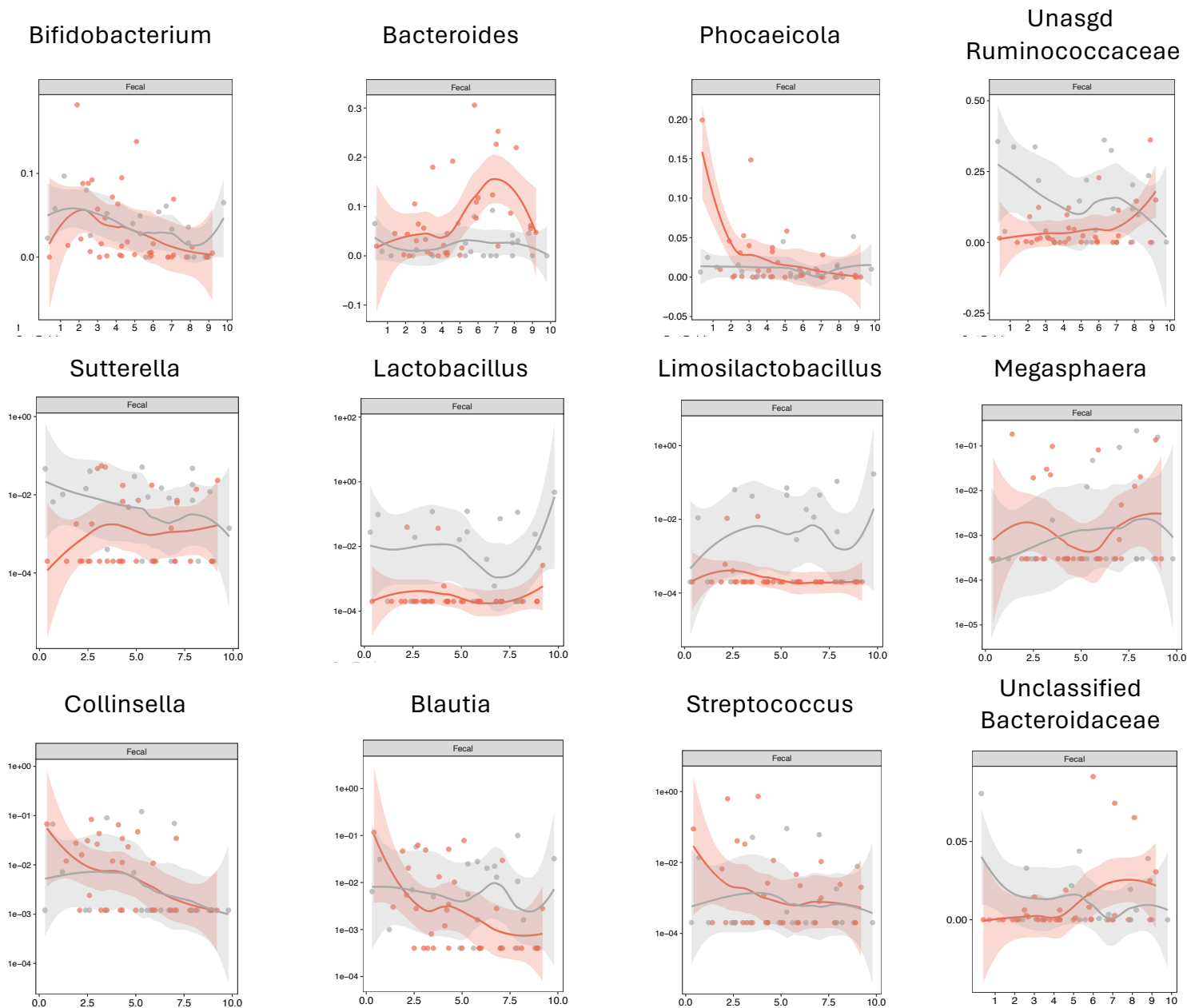
Gut microbiota
OTU counts over Time



SF2: NF-EN vs scFOS-EN Fecal - Alpha Diversity

A) Baseline community richness between groups compared by mann-whitney test of Shannon indexes from fecal samples collected within 60h of study EN initiation, when intervention effect is expected to be minimal (days 0-2) B) Locally-estimated scatterplot smoothing (LOESS) of OTUs (α -diversity) from NF-EN and scFOS-EN fecal samples over study period.

Supplemental Figure 3



SF3: NF-EN vs scFOS-EN Fecal – LOESS plots for significant genera

LOESS visualization of commensal microbes identified to significantly differ over study period (see main Figure 2 and SF4 for analysis from statistical analysis from linear mixed models of repeated measures (LMMRM)).

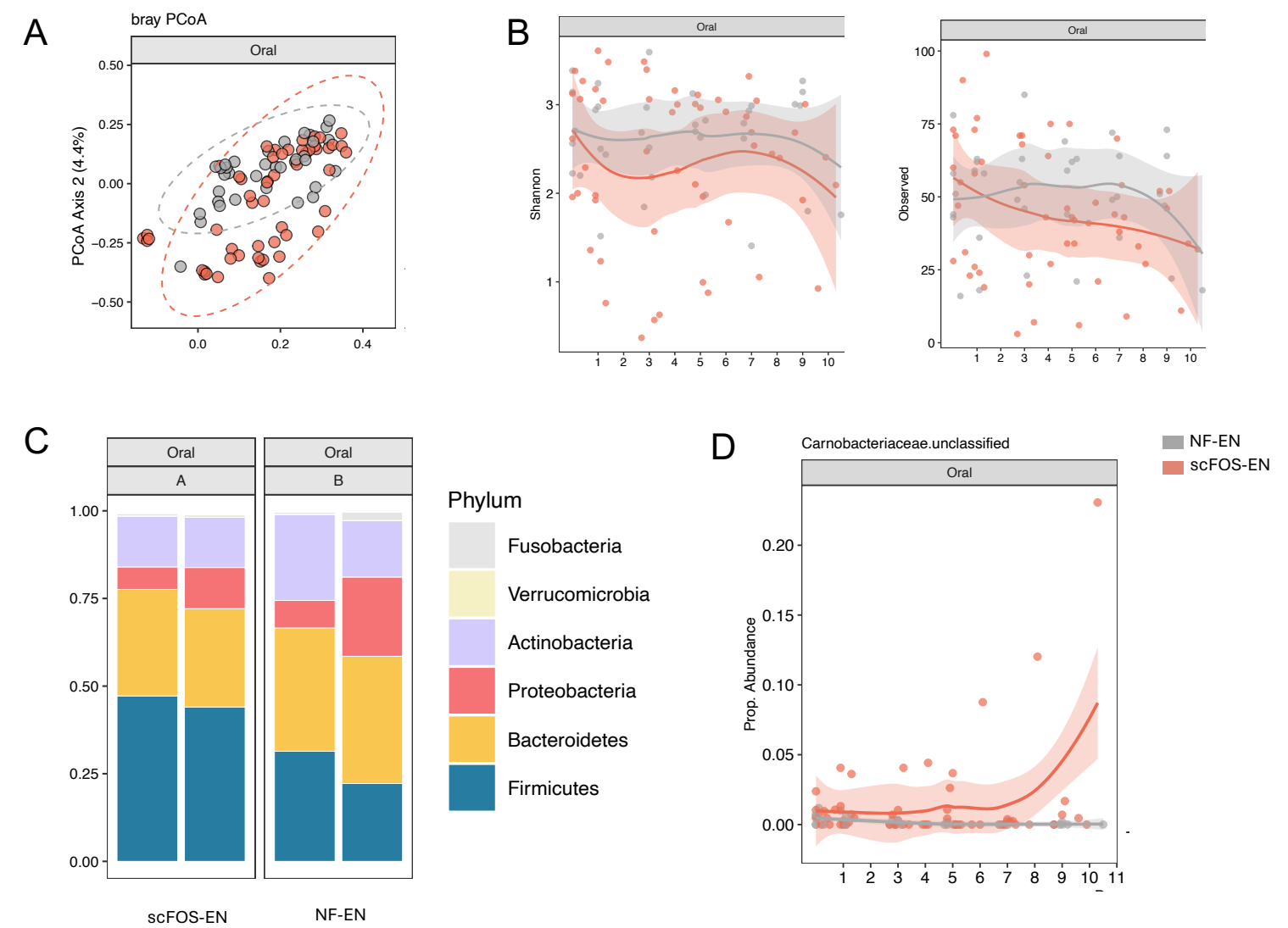
Supplemental Figure 4

Fecal Summary Table	scFOS-EN+		NF-EN		Difference (scFOS-NF) Between Groups	
	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
Bacteroidaceae.unclassified	0.011 (0.003, 0.018)	0.008	-0.002 (-0.01, 0.005)	0.5488	0.013 (0.002, 0.024)	0.024
Bacteroides	0.011 (0.003, 0.02)	0.005	-0.002 (-0.009, 0.006)	0.707	0.013 (0.002, 0.024)	0.027
Bifidobacterium	-0.009 (-0.013, -0.005)	0.000	-0.003 (-0.007, 0)	0.064	-0.006 (-0.011, -0.001)	0.026
Blautia	-0.003 (-0.006, 0)	0.043	0.001 (-0.002, 0.004)	0.490	-0.004 (-0.008, 0)	0.059
Collinsella	-0.005 (-0.008, -0.002)	0.003	-0.002 (-0.005, 0.001)	0.124	-0.002 (-0.007, 0.002)	0.310
Klebsiella	0.017 (0.005, 0.028)	0.004	0.001 (-0.01, 0.012)	0.889	0.016 (0, 0.031)	0.052
Lactobacillus	-0.001 (-0.01, 0.008)	0.827	0.01 (0.001, 0.02)	0.032	-0.011 (-0.025, 0.002)	0.092
Limosilactobacillus	0 (-0.003, 0.003)	0.857	0.006 (0.003, 0.009)	0.000	-0.006 (-0.01, -0.002)	0.003
Megasphaera	-0.003 (-0.009, 0.003)	0.301	0.007 (0.001, 0.012)	0.028	-0.01 (-0.018, -0.002)	0.025
Phocaeicola	0.008 (0, 0.016)	0.053	-0.016 (-0.024, -0.009)	0.000	0.024 (0.013, 0.035)	0.000
Ruminococcaceae.unclassified	-0.003 (-0.005, -0.002)	0.000	-0.001 (-0.002, 0.001)	0.486	-0.003 (-0.005, 0)	0.023
Streptococcus	-0.021 (-0.035, -0.007)	0.002	0 (-0.013, 0.013)	0.9951	-0.021 (-0.04, -0.002)	0.035
Sutterella	0 (-0.002, 0.002)	0.98	-0.002 (-0.003, 0)	0.035	0.002 (0, 0.004)	0.134

SF4: Genus-level results of linear mixed models of repeated measures (LMMRM)

Estimates demonstrated as change in r.a. per day (Δ r.a./day) for changes over time within each group and differences in rate of change over time across groups (last 2 columns). Data from all NF-EN and scFOS-EN fecal samples (n=22 and n=35 respectively).

Supplemental Figure 5

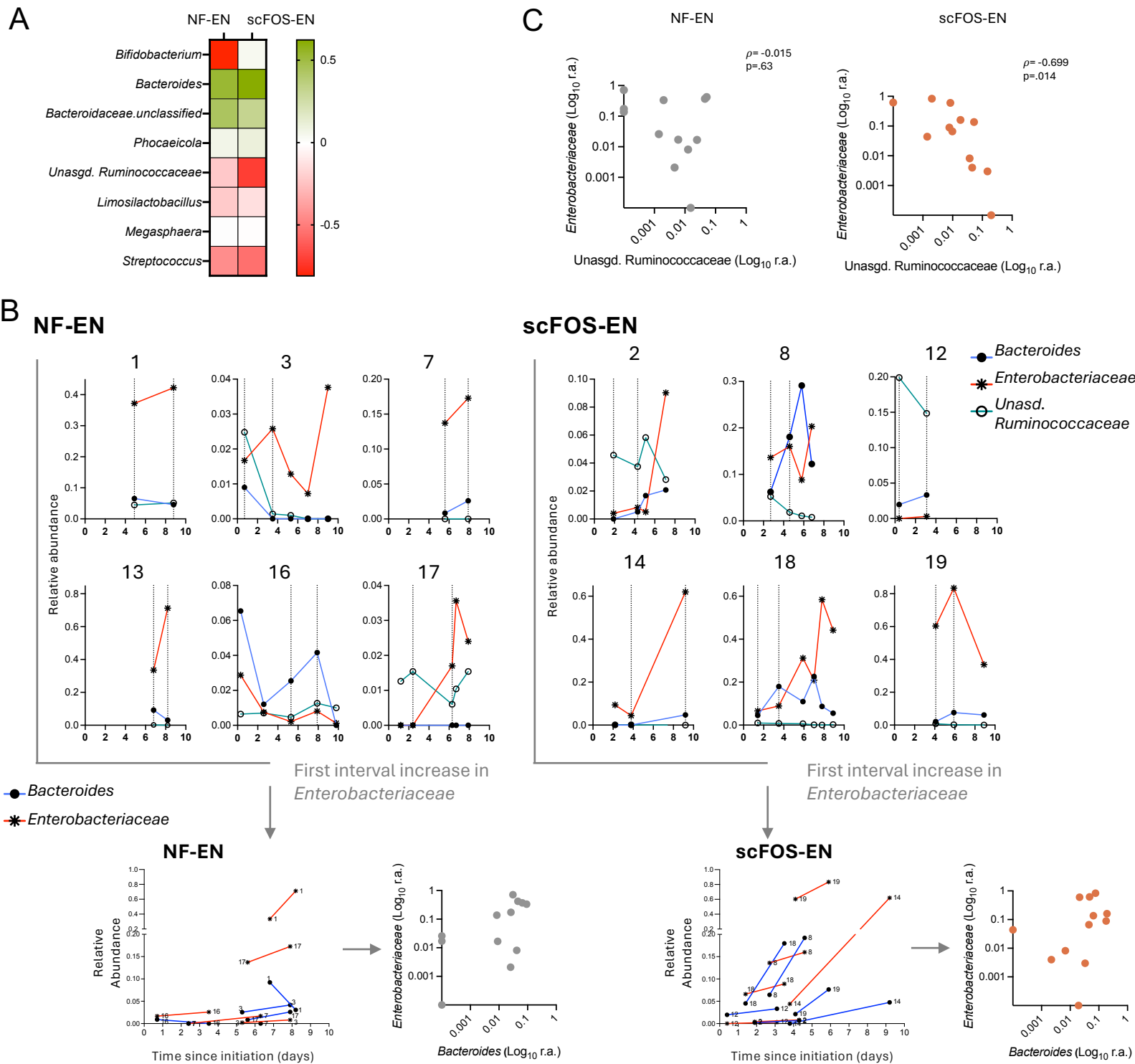


	scFOS-EN+		NF-EN		Difference (scFOS-NF) Between Groups	
	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
Carnobacteriaceae.unclassified	0.003 (0.001, 0.005)	0.0045	-0.001 (-0.003, 0.002)	0.6208	0.003 (0, 0.006)	0.0273
Fusobacterium	0 (-0.002, 0.002)	0.8237	0.003 (0, 0.005)	0.0190	-0.003 (-0.005, 0)	0.0552
Prevotellaceae.unclassified	0 (-0.004, 0.004)	0.8981	0.002 (-0.002, 0.007)	0.3067	-0.003 (-0.008, 0.003)	0.3953
Schaalia	0 (-0.003, 0.004)	0.8978	-0.004 (-0.008, -0.001)	0.0284	0.005 (-0.001, 0.01)	0.0826
Veillonella	-0.008 (-0.019, 0.003)	0.1493	-0.014 (-0.026, -0.002)	0.0238	0.006 (-0.01, 0.022)	0.4422

SF5: Changes in Oral Microbial Dynamics in NF-EN vs scFOS-EN

A) PcoA of Bray-Curtis distance measures B) LOESs of Shannon and OTU measures over study period (alpha-diversity) C) Taxonomic composition at phylum-level with day 0-2 represented in first columns of each group, days 2-10 in 2nd column. D) Results of LMMRM analysis at genus-level. Estimates demonstrated as change in r.a. per day (Δ r.a./day) for changes over time within each group and differences in rate of change over time across groups (last 2 columns). Data from all NF-EN & scFOS-EN samples (n=35 & n=53 respectively).

Supplemental Figure 6

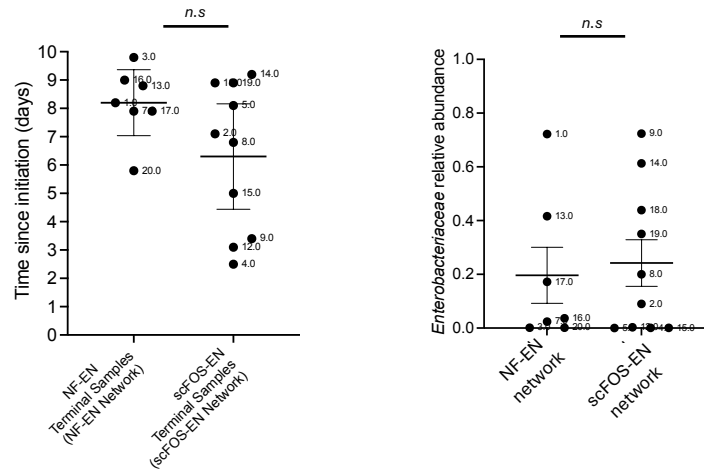


SF6: Relationship between early *Enterobacteriaceae* expansion and commensals impacted by scFOS-EN

A) Heat map of Spearman correlation coefficients of r.a. of *Enterobacteriaceae* and matched r.a.s of taxa found to differ in dynamics on scFOS-EN vs NF-EN comparisons from samples with 1st interval increase in *Enterobacteriaceae* (2 samples/patient). B) Interval changes in r.a. of *Enterobacteriaceae*, *Bacteroides*, and unasgd *Ruminococcaceae* over time in patients with *Enterobacteriaceae* expansion over study period with 1st interval increase demarcated. Bottom left plots show matched *Enterobacteriaceae* and *Bacteroides* values from 1st interval increase, with correlation plots of r.a.s demonstrated in bottom right. C) Correlation plots of *Enterobacteriaceae* and unasgd *Ruminococcaceae* using same methodology.

Supplemental Figure 7

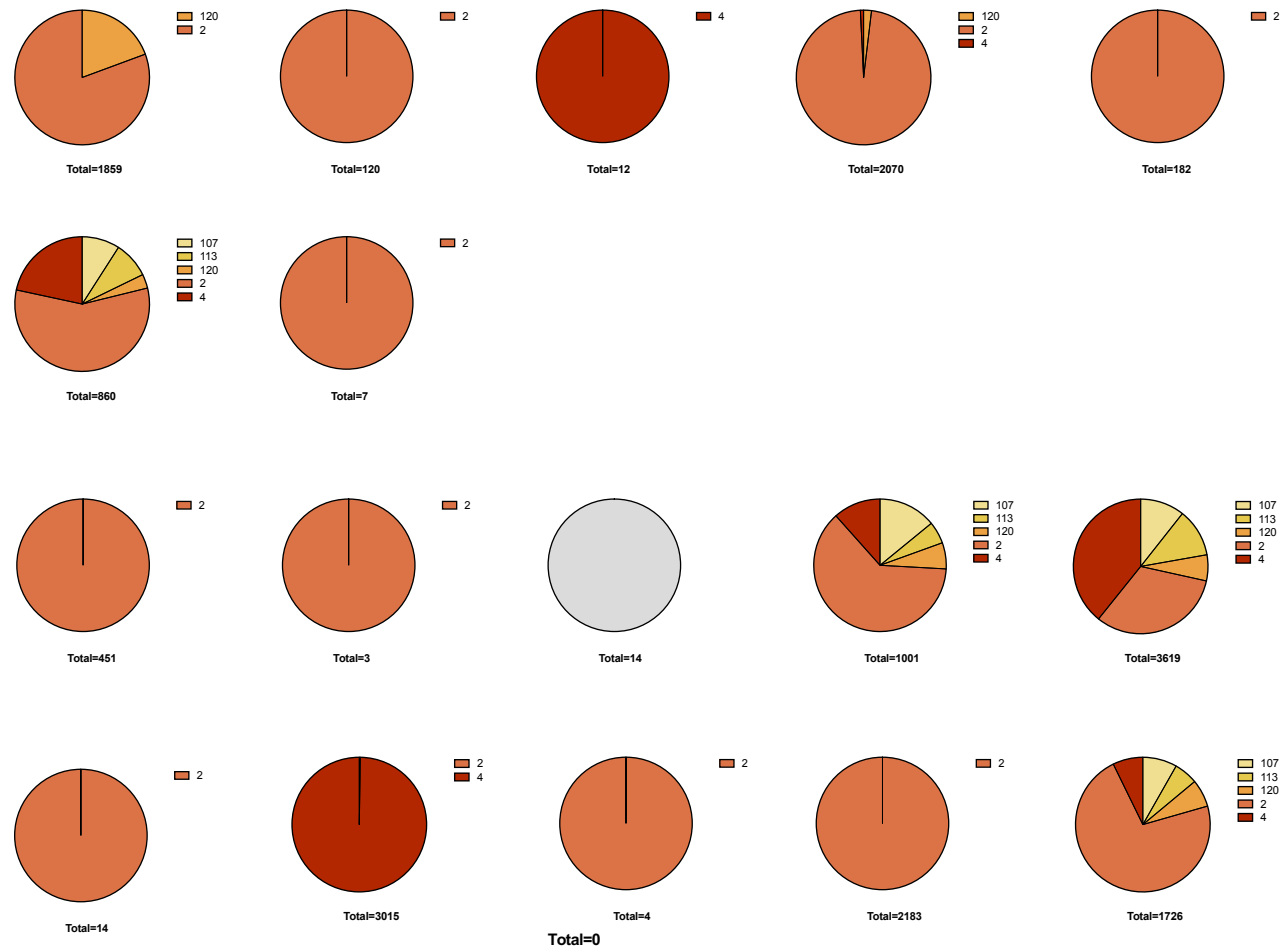
A



B

NF-EN

scFOS-EN



SF7: Characteristics of fecal samples used for NF-EN and scFOS-EN Networks

A) Comparison of sample collection timing (left) and *Enterobacteriaceae* relative abundance (right) between NF-EN and scFOS-EN fecal samples used in construction of networks. MW comparisons shows no significant difference in comparisons; data expressed as mean $\pm$ SEM, points labeled by patient ID. B) Distribution of absolute counts of Enterobacteriaceae ASVs in samples used for construction of networks (1 sample/patient). Total Enterobacteriaceae ASV counts listed below each pie chart; note that only ASVs identified in more than 1 sample were included in networks (ASV 2: *E/Shigella*; ASV 4: *Klebsiella*; ASV 120, 113, 107: all unasgd. *Enterobacteriaceae*).

Supplemental Figure 8

NF-EN Network Topography

scFOS-EN Network Topography

Large-scale metrics of network

Basic network information	
Nodes	35.0000
Total interactions	78.0000
Positive interactions	45.0000
Negative interactions	33.0000
Pos-Neg ratio	1.3636
Density	0.1294
Average degree	4.4000
Degree std	1.6423
Components	2.0000
Diameter	7.0000
Clustering coefficient	0.3724
Shortest average path length	2.7538
Modularity	0.5752
Small-world index	2.8864

Large-scale metrics of network

Basic network information	
Nodes	80.0000
Total interactions	337.0000
Positive interactions	160.0000
Negative interactions	177.0000
Pos-Neg ratio	0.9040
Density	0.1063
Average degree	8.4000
Degree std	4.2267
Components	2.0000
Diameter	5.0000
Clustering coefficient	0.3319
Shortest average path length	2.4200
Modularity	0.4390
Small-world index	2.9011

Basic structural balance information

Structural balance	
Percentage balanced	1.0000
Percentage unbalanced	0.0000
Triangles +++	0.4800
Triangles --+	0.5200
Triangles ++-	0.0000
Triangles ---	0.0000

Basic structural balance information

Structural balance	
Percentage balanced	1.0000
Percentage unbalanced	0.0000
Triangles +++	0.2411
Triangles --+	0.7589
Triangles ++-	0.0000
Triangles ---	0.0000

SF8: MicNet Dashboard NF-EN and scFOS-EN Network topography results

Results from MicNet Dashboard of NF-EN (left) and scFOS-EN (right) network analyses; outputs demonstrate global network features including information on network topology (top) and structural information on the connections between nodes (bottom).