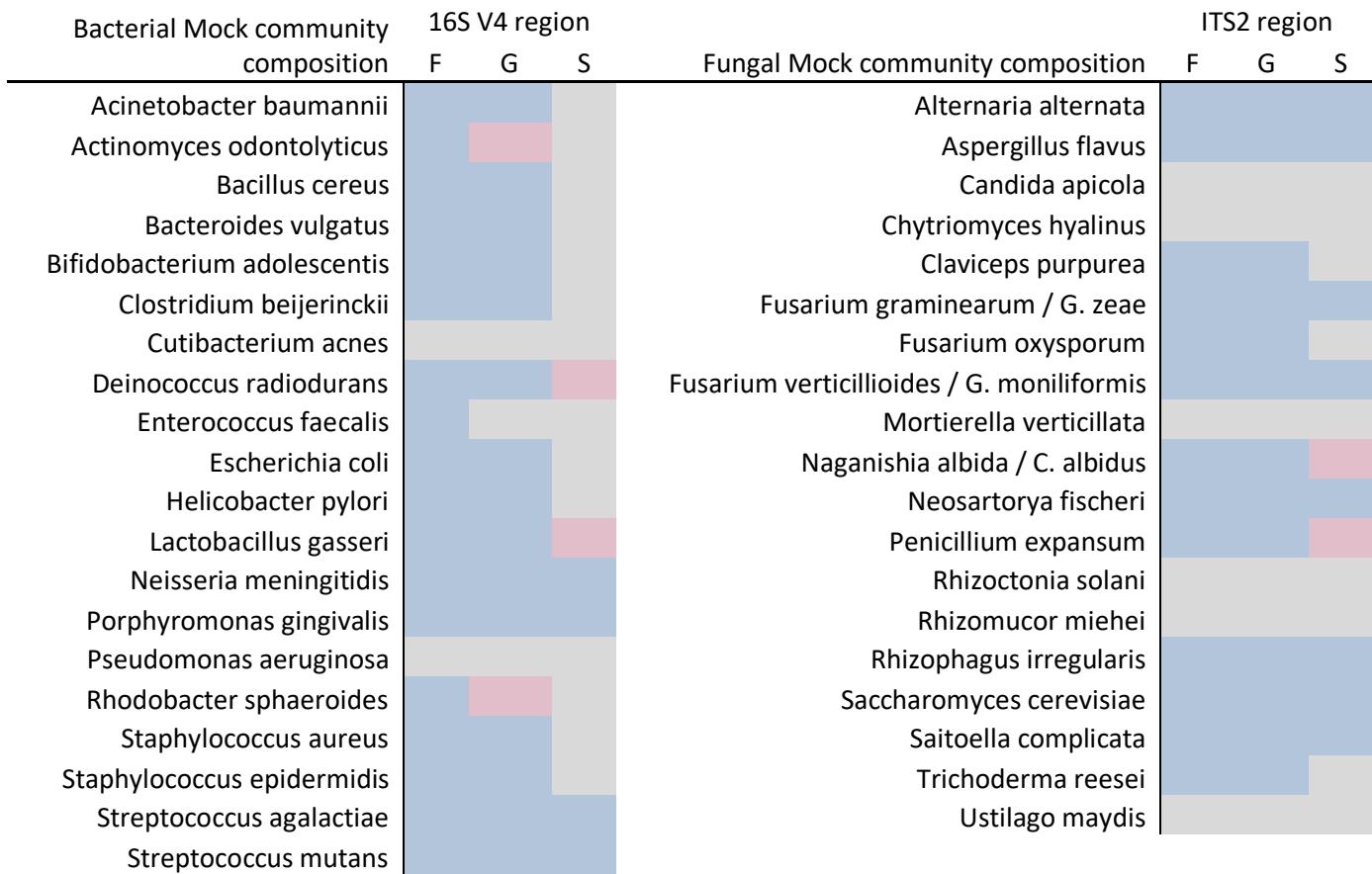


**Fecal sample collection methods and time of day impact microbiome composition and short chain fatty acid concentrations**

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Supplementary Table 1. Sequences of each bacterial and fungal library. Filtering involved all bioinformatic processing including quality filtering, merging, and trimming, as well as decontamination and low prevalence filtering of ASVs. Richness and diversity estimates are of a single technical replicate sequenced for each gene region

<b>Gene region</b>	<b>16S, V4</b>		<b>ITS2</b>	
Sample sequences post filtering	4,303,623		8,416,581	
Average reads per sample	53,387		150,296	
Minimum read count	5,387		1,073	
Maximum read count	243,233		1,431,685	
ASVs post filtering	904		70	
<b>Technical replicates for each gene region</b>				
Observed ASVs	285	279	37	39
Shannon	3.55	3.63	0.3	2.4



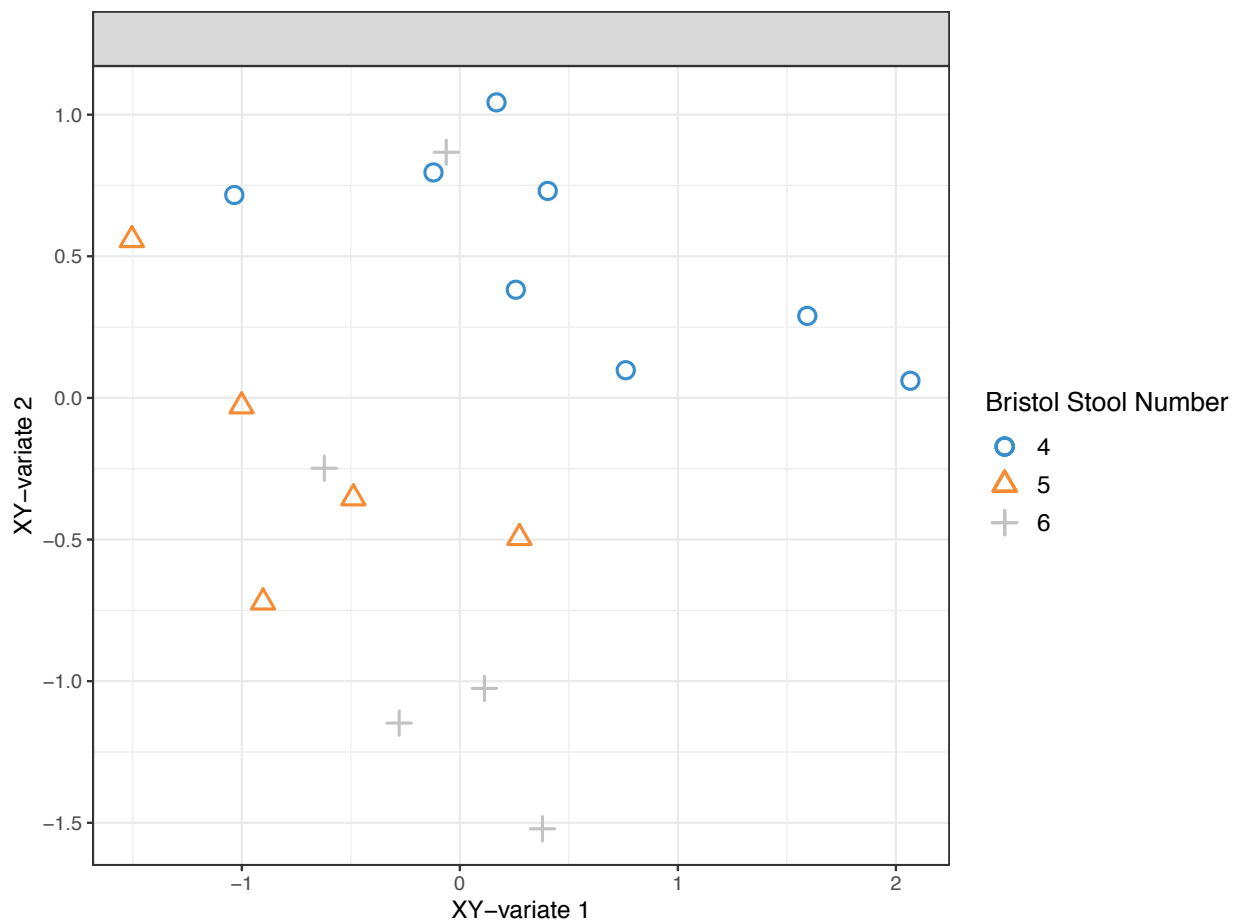
Supplementary Figure 1. Occurrence of bacterial species of the mock community successfully identified (blue), incorrect classification but resolved correctly at a higher rank (pink), or not identified (grey). Ranks are family (F), genus (G), and species (S)

Supplementary Table 2. Percent coefficient of variation of SCFA concentrations and bacterial diversity estimates among three aliquots collected from a single stool (aliquots) and from three whole stools collected from separate bowel movements (whole). Bolded values indicate the more variable measure per individual. Chao1 and Faith phylogenetic distance was calculated on rarefied counts.

Individuals	1	2	3	4	5	6
<b>Aliquots</b>						
acetic acid	<b>27.20</b>	11.57	18.12	5.96	<b>10.42</b>	<b>37.65</b>
propionic acid	<b>16.45</b>	4.21	11.52	3.59	6.43	16.86
butyric acid	<b>14.92</b>	15.30	11.25	3.84	7.02	<b>45.22</b>
valeric acid	<b>19.05</b>	3.52	20.05	5.94	7.65	<b>39.97</b>
Total	<b>22.02</b>	6.42	16.21	5.04	<b>8.54</b>	<b>25.22</b>
<b>Whole</b>						
acetic acid	13.18	<b>23.53</b>	<b>30.70</b>	<b>29.28</b>	2.36	29.72
propionic acid	2.25	<b>5.50</b>	<b>26.17</b>	<b>37.25</b>	<b>29.39</b>	<b>31.05</b>
butyric acid	6.79	<b>27.01</b>	<b>21.96</b>	<b>28.56</b>	<b>14.90</b>	41.73
valeric acid	7.14	<b>27.77</b>	<b>20.12</b>	<b>35.04</b>	<b>11.88</b>	20.89
Total	10.26	<b>20.32</b>	<b>28.76</b>	<b>29.53</b>	5.66	24.75
<b>Aliquots</b>						
Shannon	1.32	<b>6.51</b>	0.94	2.12	4.98	0.39
Chao1	9.73	<b>27.03</b>	7.85	<b>11.59</b>	<b>24.65</b>	<b>13.16</b>
Faith PD	4.50	6.61	10.67	<b>7.71</b>	<b>20.79</b>	<b>2.63</b>
<b>Whole</b>						
Shannon	<b>2.09</b>	3.32	<b>5.79</b>	<b>4.41</b>	<b>10.67</b>	<b>2.48</b>
Chao1	<b>13.79</b>	21.45	<b>34.99</b>	9.95	21.17	1.28
Faith PD	<b>10.05</b>	<b>17.83</b>	<b>16.37</b>	5.23	11.83	2.23

Supplementary Table 3. Taxa identified in significantly different abundance in the O or N methods compared to the F method.

	F vs N			F vs O	
	Pvalues	FDR		Pvalues	FDR
<b>Phyla</b>			<b>Phyla</b>		
Firmicutes	0.008	0.092	Actinobacteriota	0.013	0.160
Actinobacteriota	0.023	0.140	Bacteroidota	0.039	0.234
<b>Class</b>			<b>Class</b>		
Clostridia	0.003	0.059	Negativicutes	0.002	0.032
Lentisphaeria	0.031	0.229	Coriobacteriia	0.003	0.032
			Bacilli	0.030	0.185
			Bacteroidia	0.039	0.185
<b>Order</b>			<b>Order</b>		
Lachnospirales	0.001	0.056	Monoglobales	0.001	0.058
Oscillospirales	0.005	0.077	Coriobacteriales	0.003	0.068
Not_Assigned	0.006	0.077	Lactobacillales	0.010	0.131
Rhizobiales	0.017	0.174	Bacteroidales	0.042	0.381
Victivallales	0.031	0.250			
Monoglobales	0.041	0.254			
<b>Family</b>			<b>Family</b>		
Ruminococcaceae	0.001	0.041	Ruminococcaceae	0.000	0.003
Lachnospiraceae	0.001	0.041	Eggerthellaceae	0.000	0.003
Actinomycetaceae	0.002	0.047	UBA1381	0.001	0.035
Eggerthellaceae	0.008	0.138	Rikenellaceae	0.004	0.080
Oscillospiraceae	0.010	0.151	Actinomycetaceae	0.011	0.161
Rhizobiaceae	0.017	0.186	Streptococcaceae	0.015	0.184
Not_Assigned	0.018	0.186	Acutalibacteraceae	0.018	0.184
Victivallaceae	0.031	0.281	Veillonellaceae	0.025	0.221
UBA1381	0.041	0.327	Erysipelatoclostridiaceae	0.037	0.297



Supplementary Figure 2. Bacterial communities and metabolites group according to Stool form. SCFA and 16S ASV from three matching whole stool samples were combined using rCCA and plotted as correlation coefficients.