HUMAN RESULTS:

1. Overview of findings
   1. Dominate assemblages/generalist taxa “hubs”
2. Temporally stable and variable assemblages have different ‘key taxa’
   1. temporally stable and persistent assemblages represent robust associations
      1. See more lachnospiriceae in least variable
      2. Example of potentially mucosally associated community?
   2. variable assemblages represent transient/fragile associations
      1. Bifido, e. copro, r. bromii, and porphymo.. more distinctly in variable assemblages
      2. Seem to be driven by some taxa; discuss potential causes in discussion…

*B. dorei* Otu3 also had the largest generality score (\*\*\*cite methods). This aligns with existing literature suggesting that Bacteroides taxa are generalists and are able to exploit many distinct niches (\*\*\*cite). \*\*\*using a 0.05 threshold, F. prausnitzii Otu2 in 3rd most number of asssemblages…\*\*\*…Most dominate assemblage (averaged over time) was A50 (average abundance = 0.05 \*\*\*use percent or ratio thoughout \*\*\*remove percents?) which was largely dominated by B. dorei (94.7%). This assemblage increased monotonically over the five days. \*\*\*relevance? The next most dominate assemblage was A58, which largely contained Lachnospiraceae Otu9 (89%) along with Blautia faecis Otu23 (1.5%) and B. dorei Otu3 (1.5%). Third most abundant was A55, largely contained A. rectalis Otu1 (86%), Roseburia Otu12 (1.5%) and B. dorei (1.3%). \*\*\*cite association between A. rectalis and B. dorei? \*\*\*roseburia in lachno…

…using z-score…; some potential mucosal associaotins, and interfold lachno in stable; taxa-- (more associated with lumen??) and varying potentially with diet—may be driving variable assemblages…

Many stable assemblages contained associations between (taxa largely present in assemblages…) \*\*\*acetate producers and consumers\*\*\*. Assemblage A\*\*\* contained Phascolarcto and … \*\*\*potentially succinate cross-feeding. Additionally, Bacteroides and A. Rectalis. \*\*\*potential mucosal association. Assemblage A4 was largely dominated by a single taxon, Ruminococcus Otu17. May represent more metabolically independent taxon…\*\*\* Many *Ruminococcus* species are keystone species that can degrade many complex polysaccharides [48]. This may allow them to utilize resources others cannot.

The temporally stable assemblage A56 contains *Blautia faecis* Otu23 which produces lactate and acetate as the major end products of glucose fermentation [51], and *Agathobacter rectalis* Otu1, which has been seen to utilize acetate to generate butyrate [52]. \*\*\*…Similarly, A23 and A15 contain *Blautia* Otu9 acetate producer. A23 contains acetate consumers Otu2 Faecalibacterium prausnitzii [53] and Otu22 Eubacterium coprostanoligenes. A15 contains acetate consumers Otu12 Roseburia and Otu1 Agathobacter rectalis [52].

…notable example with phascolarcto\*\*\*. Assemblage A4 was largely dominated by a single taxon, Ruminococcus Otu17. May represent more metabolically independent taxon…\*\*\* Many *Ruminococcus* species are keystone species that can degrade many complex polysaccharides [48]. This may allow them to utilize resources others cannot. \*\*\*mention eg (known succinate producer and consumer, don’t need to say ‘cross-feeding..’)

Phascolarctobacterium faecium needs succinate from other bacteria to complete its metabolism; lacks pathway to produce succinate (\*\*\*cite review)

Next we sought to characterize more variable assemblages. Specifically, we focused on assemblages that were largely dominate on one day, with an abundance of at least 3 times the abundance on all other days (Figure5 ?B?\*\*\*). These represent more fragile and transient associations, potentially caused by changes in diet or other perturbations. Assemblages A21 and A51 both contained Bifido, which was largely abundant on days \*\*\* (Fig 5C), and is likely causing the corresponding assemblages to be more variable. (\*\*\*fiber discussion in discussion section…). …bifido has been seen to increase with increased fiber intake… Assemblages A5 and A54 contained Rumino bromii Otu10, with larger abundances on days … (\*\*\*RS discussion). Also see presence of Eubacterium… and specialized otu\*\*\*…

\*\*\*…Next, we focused on highly variable assemblages, given by those that differed on one day by a 3x increase, compared to all other days. These may characterize associations that vary over time, potentially due to perturbations such as a change in diet. …The variability in A21 and A51 both seemed to be driven by the varying abundance of Bifidobacterium Otu6 over time. Bifidobacterium may increase due to consumption of dietary fiber [45], and (\*\*\*potential cross-feeding?). A5 and A54 bloom on days 1, 2 and 3 potentially due to increased abundance of *Ruminococcus bromii* Otu10, a keystone species and resistant starch degrader [46].

A54 was mostly present on day 2 and largely contained Rumino bromii Otu10 and Roseburia inulin Otu14, which were largely present in the first few days. … A38 contained specialized porphory… A60 was largely present on day 2. Assemblages A5 and A54 contained Rumino bromii Otu10, with larger abundances on days … (\*\*\*RS discussion). Also see presence of Eubacterium… and specialized otu\*\*\*…

MOUSE RESULTS:

We next sought to characterize changes in associations for taxa that are abundant/present on multiple diets. Plotted associations over diets for taxa present above \*\*\* on at least 2 different diets (\*\*\*Figure 7). Changes with Akkermansia may represent mucosally linked associations. Increases on HF may be due to mucosal thinning. In contrast, loss of associations on HFHF may be due to mucosal thickening, or direct utilization of the diet by Akkermansia (\*\*\*though this seems less so from literature; may be just that other taxa are also indep). Increased LP associations?\*\*\*. Turicibacter -> few associations on S1. Largely lactobacillus, could be due to high abundance of both taxa. Increased associations with added fat on HF and HFHF, could be to mitigate and change resources, as high fat has been shown to decrease turici. LP shows loss of associations. Similar to S1, but those that originally were associated are lower in abundance. Lactobacillus had few associations overall …makes sense since lower GI, implies not mixing much downstream either (also biofilm forming, \*\*\*but look up if single species biofilms or multi?). Some increased associations on HF. Many on LP – potentially to mitigate/adapt to new communities. Otus6 and 7 both mainly only present on HF and LP; could be part of cellulose cross-feeding communities. Some associations with Akkermansia, potentially mucosally associated…; overall seeing more associations on LP?...; …increased association and abundance of f. rodentium…?

Akkermansia Otu2 and Clostridium XIVa Otu7 were largely present in assemblages that significantly increased in abundance on all three dietary perturbations (HF, HFHF, and LP), but exhibited different associations on each diet. Akkermansia Otu2 lost its association with Cellosilyticim Otu\*\*\*, but gained associations with more taxa on HF (\*\*cite figure). On HFHF, Akkermansia increased largely on its own. Assemblage A52 contained largely only Akkermansia (…percentage) and increased significantly (BF>100). Consequently, it lost its associations with other taxa. Indicating metabolic independence and diet-specific advantages… On the LP, assemblage A52 also increased significantly, but so did many other Akkermansia containing assemblages, with multi-way associations. Consequently it gained associations…

Discussion… given metabolic independence; associatins on LP likely taxa benefitng from Akkermansia?...

Clostridium XIVa also largely increased on its own, on HF and LP. Lost associations on HF, but gained more on LP.

Turicibacter decreased on its own in HF (Assemblage A60; BF…), and gained associations with other taxa. Was not largely present in assemblages perturbed on LP. Had most associations on HF and HFHF. Lactobacillus had few associations on most diets. \*\*\*makes sense since upper GI, existed largely in assemblage on its own (\*\*\*cite full figure). Assemblage A67 decreased significantly on LP, containing largely just Lactobacillus. Some other lactobacillus containing assemblages increased. Accordingly, Lactobacillus associated with more taxa on LP.

Eubacterium copro… had fewest associations on HF and HFHF, and gained the most on LP.

\*\*\*figure notes:

* Include otu label
* Annotate main full figure with bayes factors (use root 10, 10, 100)

………………..

Many significantly perturbed assemblages contained predominantly just a single taxon. \*\*\*those with only one taxon above 0.01 abundance…\*\*\* Assemblage A60 was significantly decreased on the HF diet and predominantly contained Otu3 Turicibacter (\*\*\*percentage). Indicates that it maybe more metabolically independent. Previous studies have also shown the decrease on HF diet (\*\*\*cite). A52 significantly increased on HFHF and LP diets. High fiber has been linked to mucosal thickening… (discussion? -> also low protein, and cellulose…). A57 increased on HF and LP and contained largely Otu7 clostridium. (\*\*\*both diets higher on cellulose… \*\*\*ref table). A67 signifcantly decreasd on LP and contained Otu1 lactobacillus…

Of the significantly perturbed assemblages, 19 contained just a single taxon (**Figure 6),** representing potentially metabolically independent taxa that rely specifically on resources from a given diet. \*\*\*examples…

[\*\*\*shorter discussion]… Many significantly perturbed assemblages also contained multi-way associations between many taxa (**Figure 6).** HF and LP had some similar increases?, may be cellulose-cross-feeding. Potential mucosal thinning on HF may be leading to increases in Akkermansia associated assemblages \*\*\*examples. Many assemblages increased on LP contained multiple taxa (\*\*\*may be overall higher degree of connectivity?…). Lack of resources in diet may be leading to more cross-feeding interactions –though more speculative. \*\*\*Also increased cellulose, and may be switching from amino acid to carb utilization…

[\*\*\*focus is here – suborganize (subthemes) and split into paragraphs…]…

\*\*\*some of these may be mucosally associated. Changes in association were seen with Akkermansia (\*\*\*also figure 7).

We next sought out to characterize taxa that underwent significant changes in their associations due to dietary changes. We saw Otu6 Eubacterium coprostanoligenes and Otu2 Akkermansia were part of many assemblages that were significantly perturbed due to dietary perturbations and underwent large changes in their associations.

More MOUSE:

1. Dietary perturbations alter associations between taxa
   1. HF causes significant decrease in assemblage containing Turicibacter
      1. See increase in associations from S1 to HF
      2. Also see some associations on HFHF
      3. Not strongly associated on LP
   2. HFHF causes significant increase in singleton Akkermansia assemblage; results in loss of associations in Akkermansia
      1. Increase in associations on HF
      2. Decrease on HFHF
      3. Increase in singleton assemblage, but also many others, seeing new associations on LP
      4. …potentially cross-feeding and mucosal associations
   3. LP causes decrease in lactobacillus singleton and increase in associations
      1. Few associations on S1 and HF; increase with E. copro, maybe due to abundance…
      2. Loss on HFHF
      3. Increase on LP; decreased in abundance, upper gi…

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DISCUSSION:

1. Model innovations allow us to outperform existing method
   1. Multi-way modeling, direct modeling of counts (and not binarizing or transforming data) allow us to better capture associations than current pairwise methods
   2. Bayesian variable selection, direct modeling of counts, and modeling contamination allow us to better recover the correct number of assemblages in data
   3. Generative model outperforms current GMMs
   4. Generation of new dataset…
2. Directly modeling time and perturbations give us insight into spatial-temporal dynamics
   1. Insights into human microbial spatial dynamics…, interpretation of results; emphasize how we envision the use of this tool
   2. Insights into effects of dietary perturbations and interpretation of results; emphasize how we envision the use of this tool
3. Strengths of mcspace compared to existing tools, especially in the context of interpreting results and modeling temporal dynamics
4. Limitations of technology and model
   1. Noisy copies of assemblages, likely due to sampling heterogeneity… can be improved technologically (with better control and calibration of particle sizes); and with modeling (embeddings and potentially utilizing multiple particle sizes)
5. Future work…

Modeling of temporal dynamics…

1. Multi-way associations give better biological insight then just pairwise associations
2. Modeling temporal dynamics allow us to uncover robust and transient associations over time, potentially due to perturbations such as dietary changes
3. Another useful feature of MCSPACE is its ability to explicitly model changes due to perturbations. MCSPACE enables the detection of significant changes resulting from perturbations such as changes in diet and provides quantitative measures for the evidence of change via Bayes factors.
4. Model provides the ability to determine shifts in associations over different conditions; gives insight into how taxa may adapt and perform various, context dependent functions

\*\*\*singletons allow for characterization of metabolically independent microbes, with diet specific changes; changes in communities – see what’s significantly affected by diet; saw changes in Akkermansia related communities, potentially due to cross-feeding or changes in host mucosal layer; increased associations of turici on hf and lacto on lp may be due to a switch to cross feeding when resources not available in diet…

…discussion -> A60 decreased from \*\*\* in S1 to \*\*\* in HF. The large presence of this assemblage, containing mainly Turicibacter Otu3, suggests Turicibacter may be largely metabolically independent and largely specifically affected by HF (and HFHF, little associations on LP)

…Discussion -> Akkermansia hfhf: , potentially indicating metabolic independence …relevance\*\*\*

\*\*\*cite, upper GI, likely absorbs protein. Large dominance in singleton assemblage on other diets, shows that it might be more isolated, and maintaining this isolation downstream. \*\*\*in discussion, may be gaining associations due to lacking resources from diet; potentially cross-feeding?... mcspace allows use to construct such hypotheses…

\*\*\*add refs with Akkermansia and fiber, mucosal layer…; link to diet table…; HF associated with thinning of mucosal layer, due to other taxa swiching, cellulose increase Akkermansia; as well as fiber (maybe indirectly)…; turici and lacto requiring others when not getting resources from diet…

[\*\*\*condense] Furthermore, our direct modeling of temporal dynamics and perturbations aides in interpreting results and is missing from existing models. This capability allows direct tracking of assemblages across time and different environmental conditions or perturbations, which is essential for elucidating microbial interactions. Moreover, this feature increases statistical power by using the entire dataset rather than training on one time-point or condition at a time. To demonstrate the utility of our approach in uncovering biologically meaningful and relevant insights into the spatial-temporal dynamics of the mammalian gut microbiome, we applied our model framework to two datasets: a previous time-series study of the human gut microbiome, and a new dataset tracking microbial co-localization changes in the murine gut due to three different dietary perturbations and over time. In the human gut microbiome, we found many biologically relevant assemblages displaying robust multi-way associations. By capturing multi-way spatial co-localizations, our method provides a more complete picture of co-localization dynamics as compared to approaches that only consider pairwise associations.

…we envision users to get … from our model…; allowing the for formulation of hypotheses and accelerating research in …

Roseburia inulinivorans:

<https://www.pnas.org/doi/epdf/10.1073/pnas.1000091107>

\*\*\*

When comparing least and most variable assemblages, we observed differences between dominant taxa in the least variable versus the highly variable assemblages. Temporally stable assemblages tended to contain more Lachnospiraceae taxa, while variable assemblages included taxa from various other families (\*\*\*cite figure). \*\*ideally have stats though… [can remove this] …stability in interfold and mucosal regions, opposed to transient associations on food particles in digesta…

…such insights not possible/difficult with existing methods, because…; When comparing the most stable and variable assemblages, we observed that stable assemblages were dominated by Lachnospiraceae taxa, while more variable groups consisted of a mix of different families. Stable associations likely occur in consistent environments like mucosal regions, whereas transient associations may form around digesta or food particles. These insights into spatial-temporal dynamics, particularly stable versus transient associations, are difficult to achieve with conventional methods.

\*\*\*Our model also provides the ability to determine shifts in associations over different conditions; gives insight into how taxa may adapt and perform various, context dependent functions.

Applied to our mouse study, this allowed us to determine which communities were significantly affected by dietary interventions. Some assemblages affected by dietary interventions were largely dominated by a single taxon. This allows us to hypothesize that these taxa may be more metabolically independent and are able to thrive from mainly the diet. When a dietary intervention leads to a decrease in the abundance of these taxa, these taxa may adapt. Interestingly, we saw a decrease in an assemblage containing Turicibacter Otu3 in the HF diet and a resulting increase in associations of Turicibacter. It is plausible that this is due to Turicibacter co-localizing with microbes to cross-feed and utilize, though less efficiently, resources from neighboring taxa, when deprived from essential metabolites from the diet. Similarly, we saw Lactobacillus decrease in abundance on LP diet and have a large increase in associations. Here again it is plausible that Lactobacillus is cross-feeding from neighboring taxa to utilize essential resources lacking in the LP diet.