

High Fiber Dietary Intervention Induces Change in Role of *Faecalibacterium Prausnitzii* in Gut Microbiome Ecosystem

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Introduction

- ❖ The gut microbiome impacts response to immune checkpoint blockade (ICB) treatment in melanoma patients (1).
- ❖ A habitual high-fiber diet was associated with significantly improved progression-free survival (PFS) in melanoma patients on ICB (2) and bacteria associated with response to ICB are fiber-responsive taxa (3).
- ❖ A high-fiber diet intervention (HFDI) was therefore conducted in melanoma survivors to prospectively evaluate the effect of a HFDI on the microbiome.
- ❖ The relative abundance of keystone fiber-responsive, pro-ICB response taxa (such as *Faecalibacterium prausnitzii*) did not change with a HFDI. However, increasing evidence from microbial ecology supports that it is not the abundance of individual taxa that is important, rather the role the taxa play in supporting the overall ecosystem (7).
- ❖ Prior studies have shown that *F. prausnitzii* is a sentinel species for Short Chain Fatty Acid (SCFA) production (8). SCFAs are taken up by the host as well as cross-fed to other intestinal microbiota, supporting a healthy microbial ecosystem (9).
- ❖ Network analysis is a way of characterizing associations and ultimately potential interactions (5). Network analysis of the gut microbiota throughout a HFDI allows the opportunity to examine how *F. prausnitzii* changes its microbial associations and putative role in the gut microbial ecosystem.

Methods

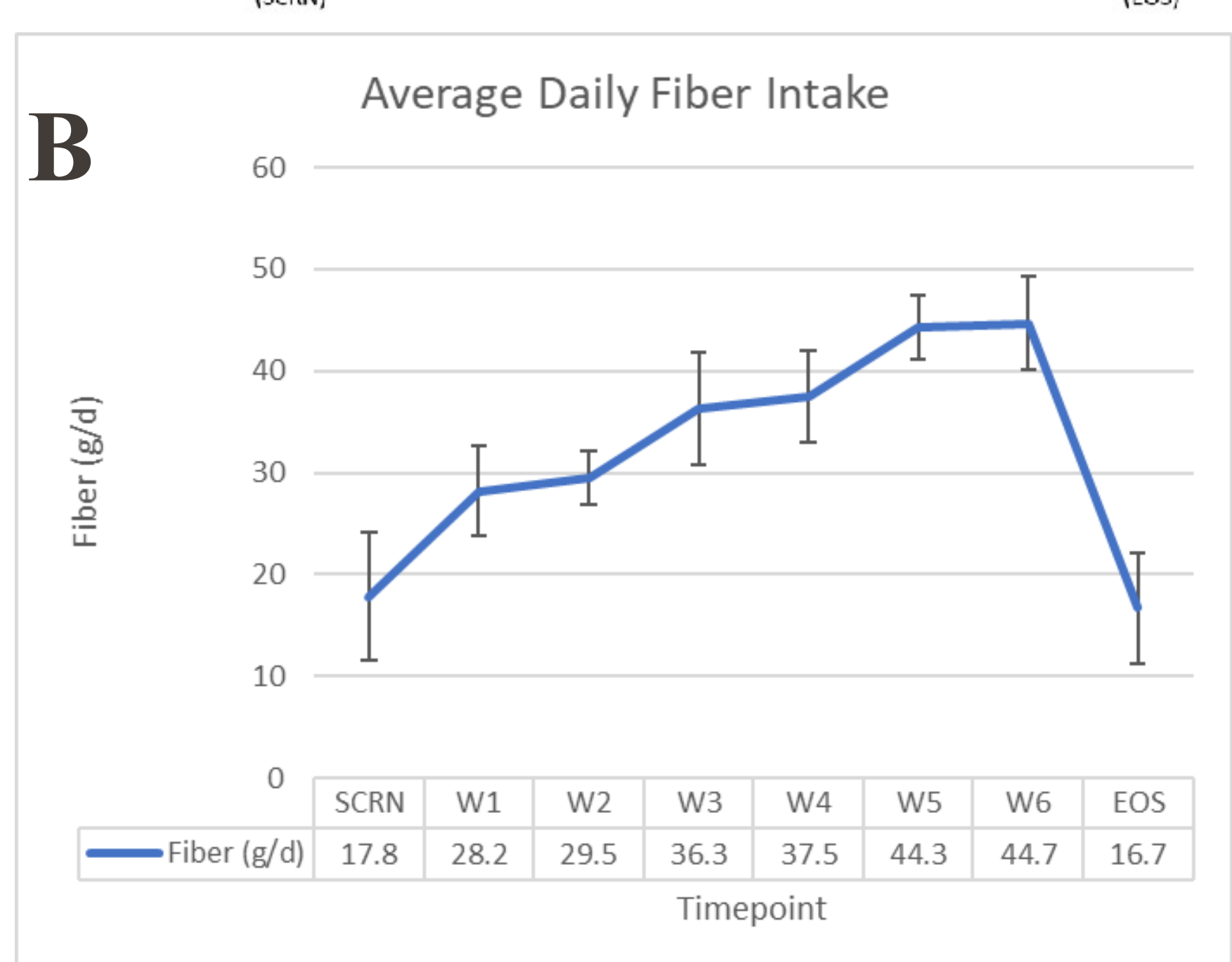
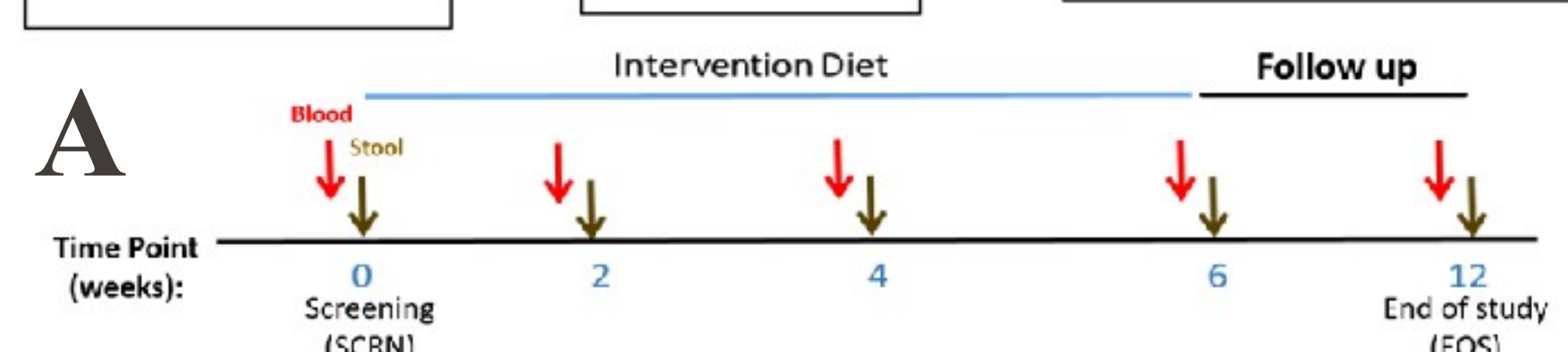
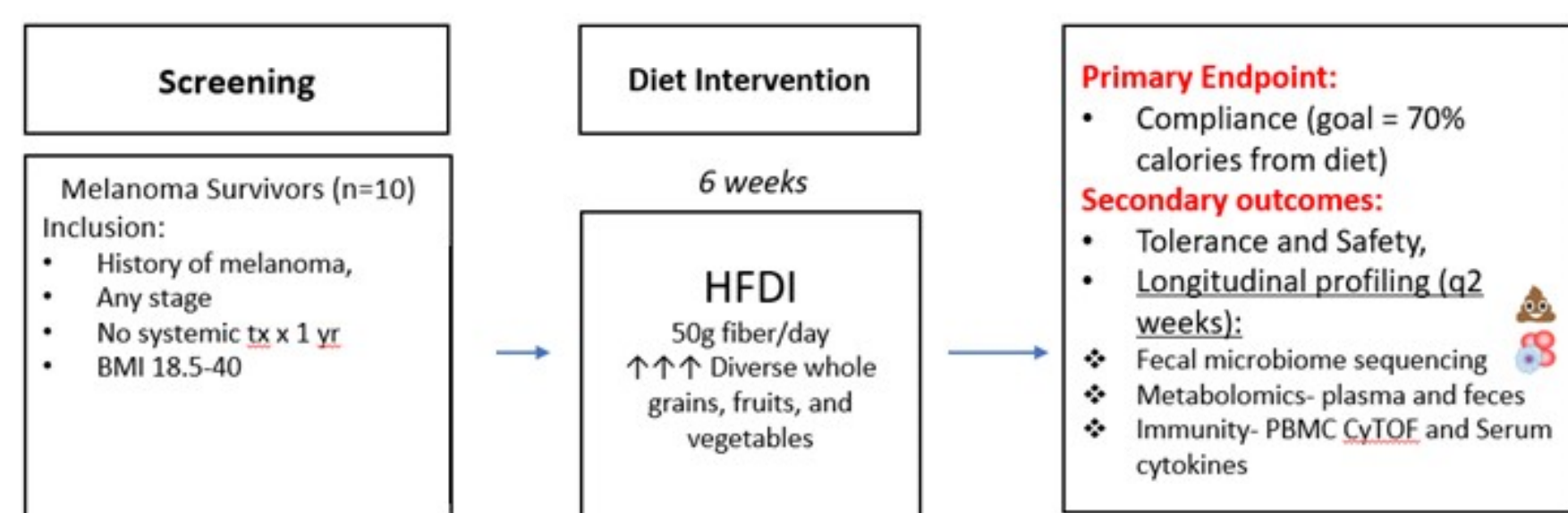


Figure 1: A. Schema of HFDI study. **B.** Average daily fiber intake for the patients on the study. The dietary fiber intake was ramped up weekly from baseline (17.8 ± 6.28 g/d) to week 6 (44.7 ± 4.56 g/d) which reverted at EOS (16.7 ± 5.48 g/d)

- ❖ Ten melanoma survivors were enrolled to a 6-week HFDI, targeting 50 grams of fiber daily, derived from legumes, whole grains, vegetables and fruit with all meals provided from MDACC Bionutrition Research Core.
- ❖ Metagenomic sequencing was conducted on DNA extracted from fecal samples. Sequence data were processed with MetaPhlan3 (4) to construct microbiome composition profiles and abundances at each timepoint from screening (SCRn) to week 6 (W6).
- ❖ NetCoMi (Network Construction and Analysis for Microbiome Data) (6) was used to construct microbial association networks. For SparCC association network visualization, taxa abundance data was filtered to taxa that appear in at least 3 patients out of the 10 melanoma survivors and only the 450 most weighted edges were displayed. Clusters of microbial species are calculated using fast greedy optimization.

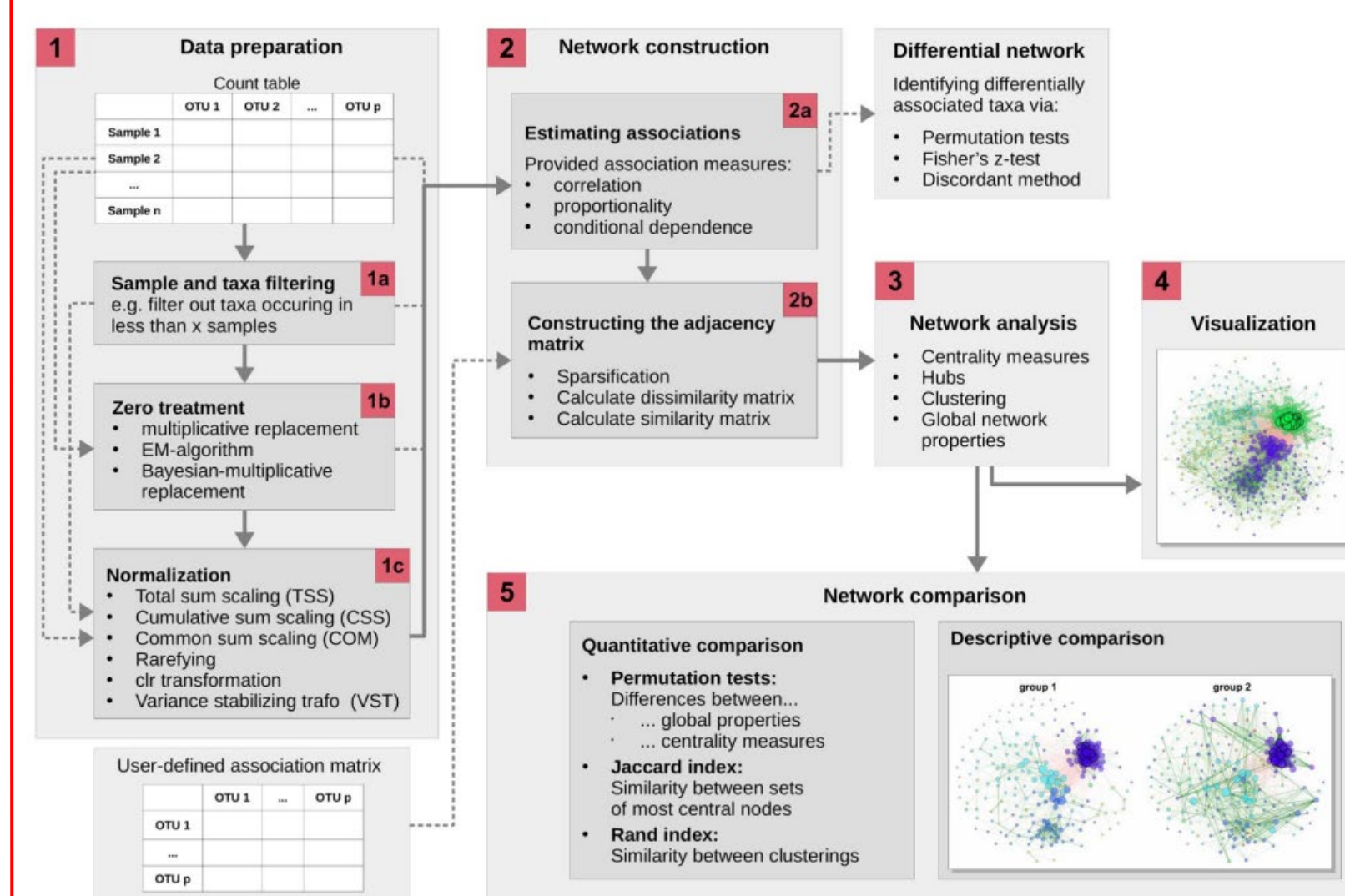


Figure 2: The workflow above illustrates how NetCoMi constructs, analyzes, and compares networks from taxa abundance data. NetCoMi is a computational tool that uses relative taxa abundance data to estimate abundance-based associations between taxa. It enables the visual construction, analysis, and plotting of microbial association networks as well as quantification of network differences across groups (5).

Differential Networks Show Shifts in Microbial Associations in Response to HFDI

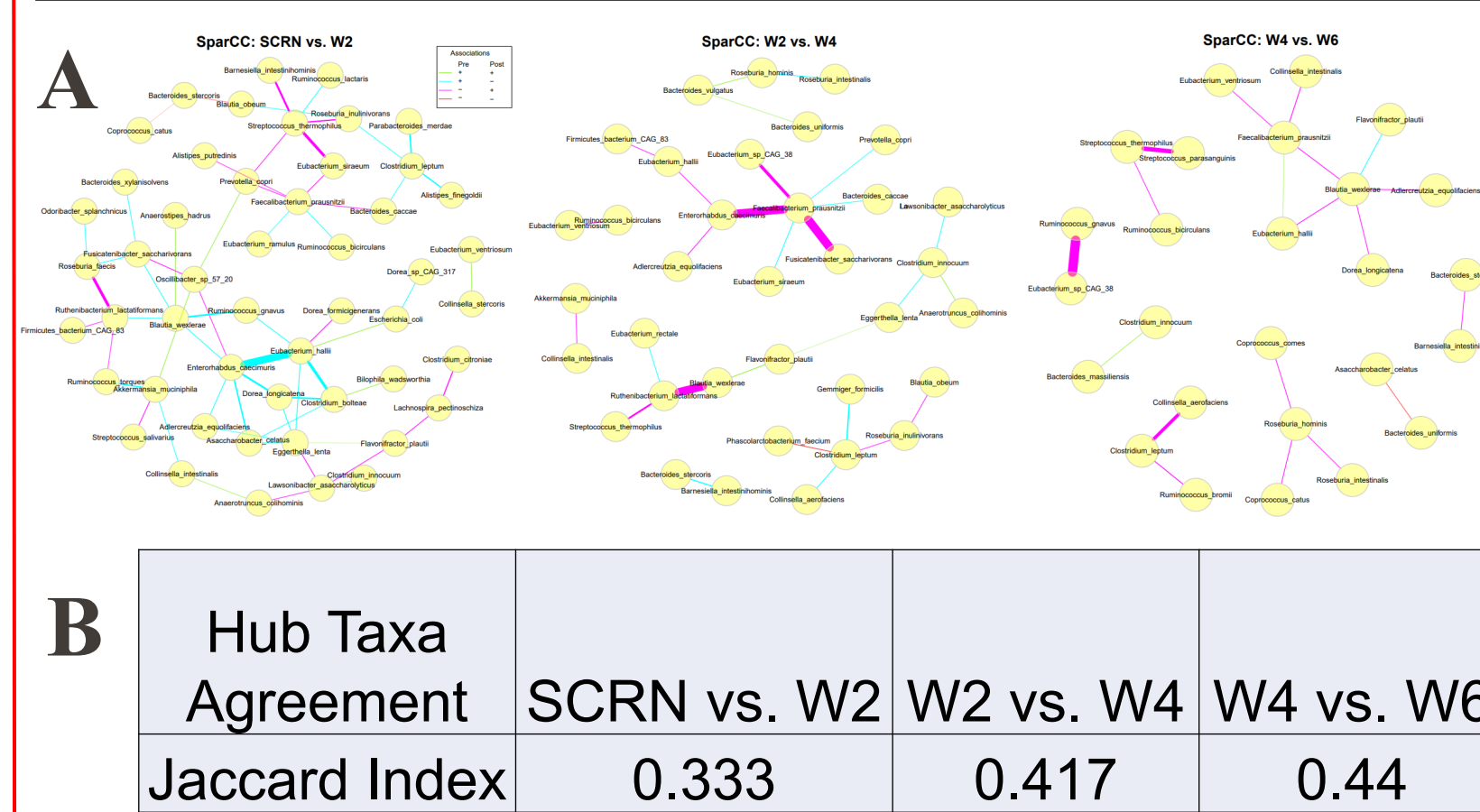


Figure 3: A. Differential networks show shifts in microbial associations in response to HFDI. **B.** The Jaccard Index is a measure of agreement between two timepoints. The greatest dissimilarity is observed between baseline and week 2 when the most dramatic increase in fiber occurs and then levels over time.

F. Prausnitzii Becomes Tightly Associated with SCFA-Producing Majority-Firmicutes Cluster with HFDI While Other Clusters Become More Loosely Associated

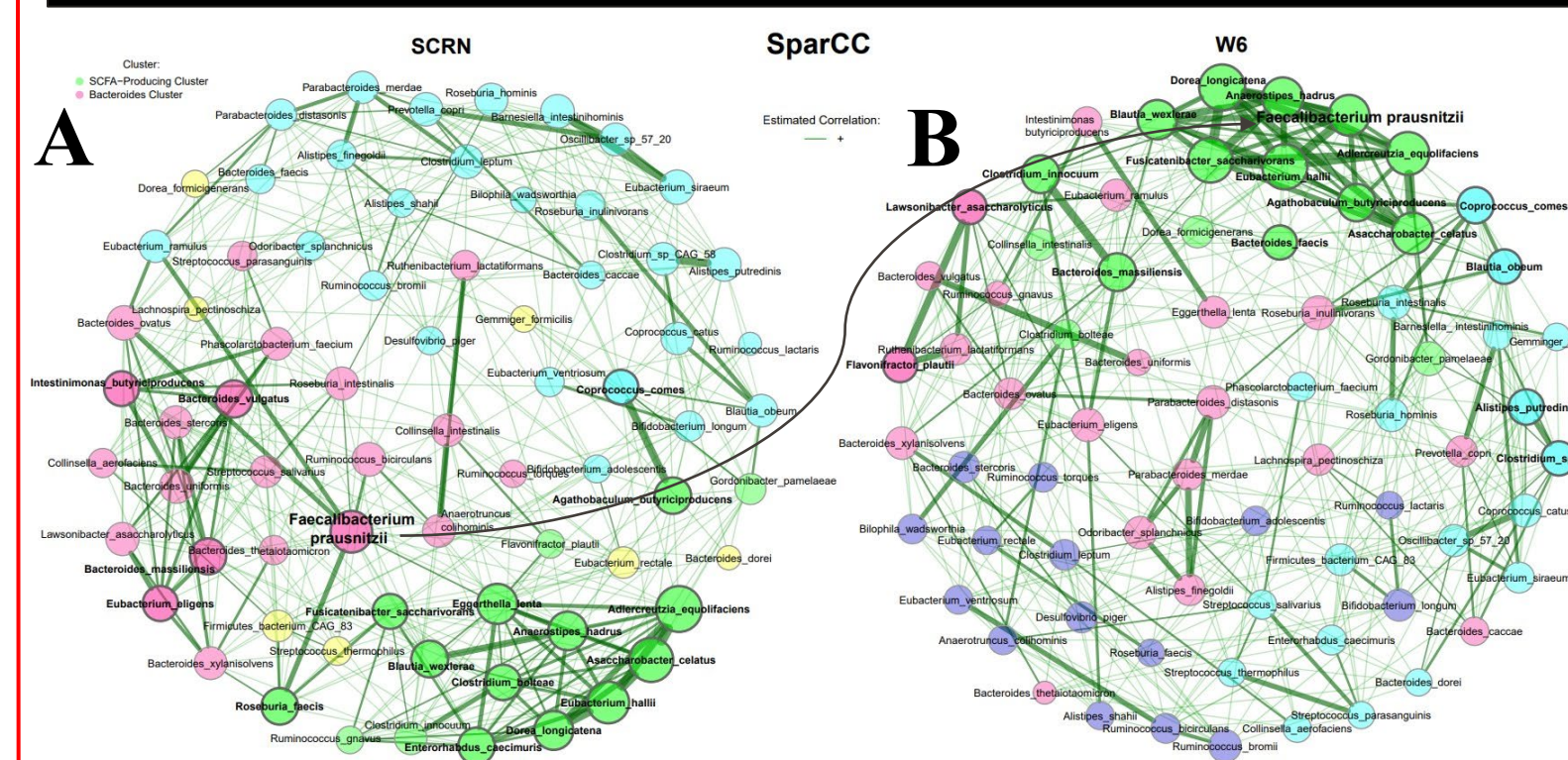


Figure 4: A. Prior to HFDI at SCRn, the microbial network contains two tightly associated clusters: green (key SCFA-producing Firmicutes) and pink (dominated by Bacteroides genus) and a loose (blue) network of other loosely associated commensal bacteria. *F. prausnitzii* is a hub (top 25% most central nodes in bold) in the pink cluster. **B.** After the HFDI, taxa within the green SCFA-producing cluster have become even more tightly associated with each other while the pink cluster becomes more loosely associated. *F. prausnitzii* has switched clusters and is now a hub taxa in the SCFA-producing cluster as denoted by the arrow.

Dynamic Shifts in Associations of *F. Prausnitzii* with Fiber Ramp Up Support Role As Keystone Cross-feeding Taxa

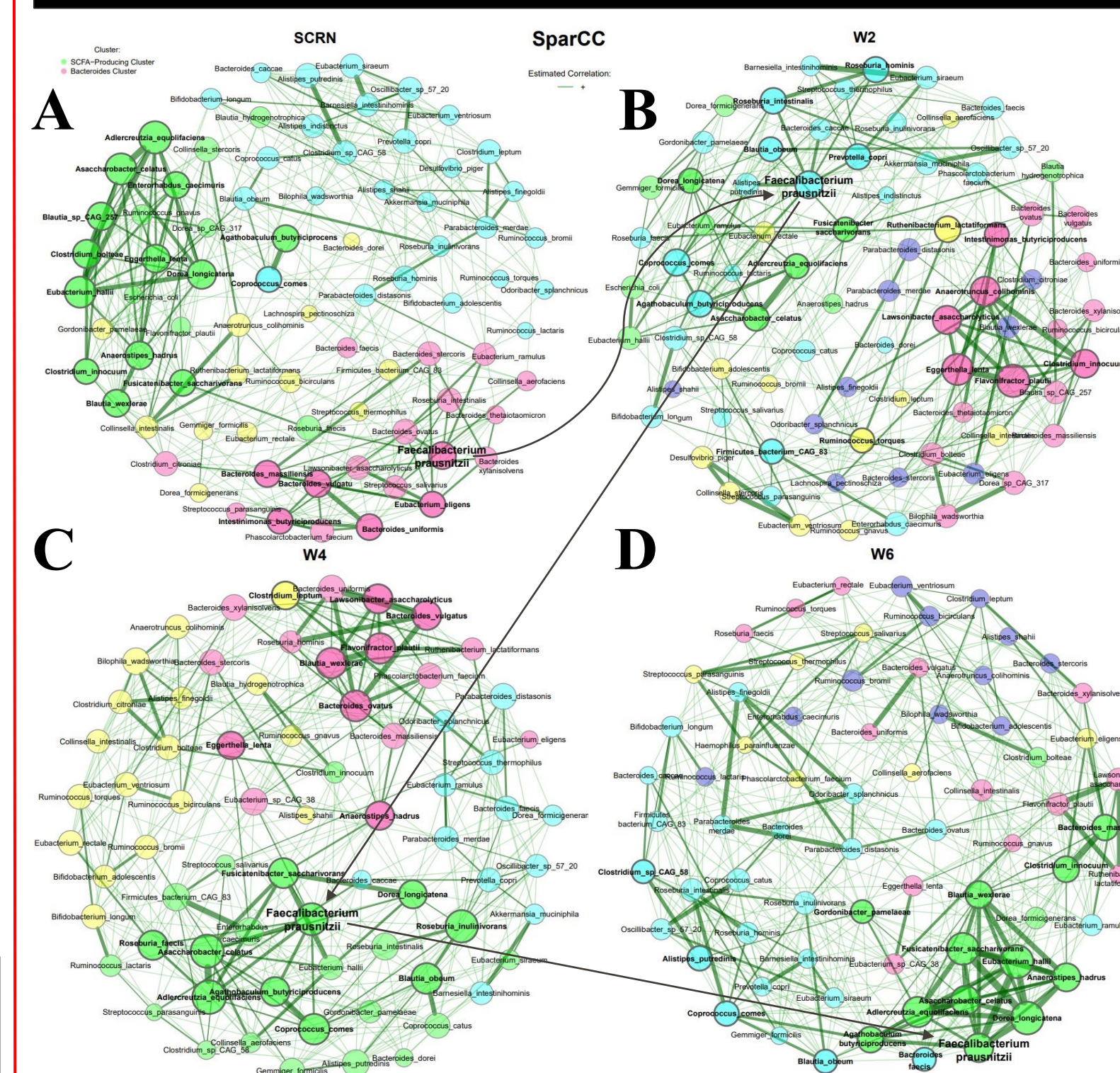


Figure 5: A. At SCRn there are clear cluster divisions **B.** By W2 these cluster divisions are less clear with significant overlap as fiber ramps up (>11 g/d increase). *F. prausnitzii* associates with taxa that were in neither the SCFA-processing cluster or its original pink cluster. Metagenomic data that *Faecalibacterium* and *Roseburia* both begin upregulating fiber-processing metabolic pathways at W2 are consistent with the associations between them. **C.** Cluster divisions reemerge by W4 with *F. prausnitzii* joining the SCFA-producing cluster. **D.** SCFA-producing cluster becomes even more tightly associated with itself and *F. prausnitzii* by W6 while other clusters are now only loosely associated.

Decreased Fiber Intake After W6 Causes Cluster Redivision by EOS and Removal of *F. Prausnitzii* from SCFA-Producing Cluster

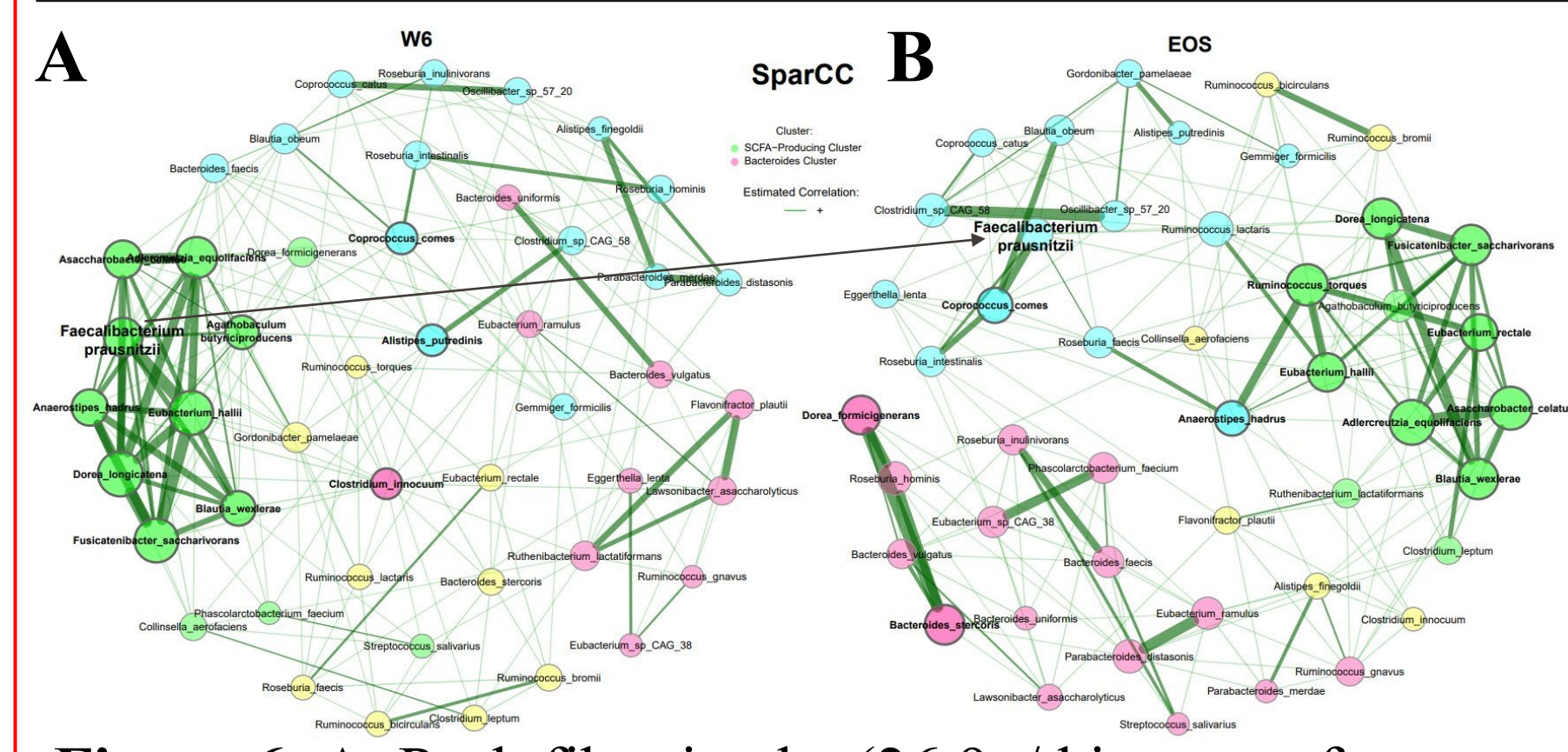


Figure 6: A. Peak fiber intake (26.9g/d increase from SCRn) at W6 shows *F. prausnitzii* firmly entrenched in the SCFA-producing cluster. **B.** 6 weeks after end of HFDI at EOS, patients return to habitual diet and daily fiber intake returns to SCRn levels (28.0 g/d drop). Cluster divisions re-emerge and *F. prausnitzii* is no longer associated with the green SCFA-producing cluster.

Results

- ❖ Response to high fiber slows down throughout HFDI (drastic changes from SCRn to W2, slows down by W4 and W6)
- ❖ SCFA-Producing Majority-Firmicutes cluster predominates gut and loosens associations within other clusters in response to HFDI
- ❖ *F. prausnitzii* switches to SCFA-Producing Majority-Firmicutes cluster in response to HFDI
- ❖ Removal from high fiber diet from W6 to EOS causes reemergence of clear cluster divisions and *F. prausnitzii* to no longer be associated with the SCFA-Producing Majority-Firmicutes cluster

Discussion

- ❖ Network analysis over time with a HFDI reveals dynamic shifts in microbial associations and a restructuring of the microbial ecosystem.
- ❖ The observed shifts in *F. prausnitzii* support its changing role in the ecosystem.
- ❖ With a HFDI, *F. prausnitzii* becomes tightly associated with SCFA-producing taxa. This suggests that *F. prausnitzii* may be using products generated from fiber metabolism to crossfeed with taxa in the SCFA-producing majority-Firmicutes cluster
- ❖ When the high fiber diet is withdrawn, cluster divisions reemerge and *F. prausnitzii* dissociates from the SCFA-producing cluster. This may signify the end of crossfeeding with the SCFA-producing cluster and the changing role of *F. prausnitzii* in the ecosystem

Next Steps

- ❖ We will conduct a metabolic and network analysis to understand the ecological role changes of *F. prausnitzii* from a metabolic perspective as well as to characterize the specific intermediates/products *F. prausnitzii* may use to change its role
- ❖ We will assess associations between SCFA-producing cluster taxa and pro-ICB response in melanoma patients

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