- Purpose of PD drugs should be to specifically affect species that are involved in PD
  - $\circ$  Changes may not be reflected in  $\alpha$   $\beta$  diversity
- Boxplots of Alpha Diversity Measure may need to be analyzed through stats tests to determine significance → one-way ANOVA?
  - o Run the stats on boxplots → negatives are still results
- Beta diversity test weighted unifrac plot
- Do everything on R
- See if any of 7 diversity metrics are significant; if none, then show observed and shannons
- Aim 1 is done
- Aim 2??
  - o Done when informs us about next steps to do
  - o If some comparisons are interesting, focus more on those
- Move on to Aim 3, 4, 5
  - Understand which core microbiomes that we want to compare, how it overlaps
- Chris will provide code for comparisons two way ANOVA and generating plots
  - o 2 categorical variables independent on 1 dependent variable
- Aims 3, 4, 5 will take more thought
  - E.g. indicator taxa: generate table is easy, but have to think about comparing them; what does it actually mean if indicator table different between groups, or if certain groups are missing
- DESeq: need to already know which comparisons we want to do
  - o Group 2 vs group 3, 4, 5, 6, 7
  - o Control: 1 vs 3, 4, 5, 6, 7
    - If 1vs3 and 2vs3 are same, then not rlly interesting; not because of the treatment or PD
    - If positive value in a 2vs3 comp, it means more upreg in group 2
    - Assumption: bacteria have to be present in both treatments/groups
  - o 7 more of a for fun comparison, may not have biological significance
  - Shows upregulation of specific bacteria to see how it compares between groups
- Could just focus on ~5 bacteria of interest

## Which section?????:

- No more than 1 paragraph. Cite enough literature to justify hypothesis
- Explain novelty
- Research question -> Cite literature for hypothesis,-> state hypothesis in terms of this proposal -> mention impact (3-4 citations)

Research objective: half a page minimum

## Intro:

- Parkinson's -> microbiome -> drugs -> knowledge gap (no one has looked at effect of drugs w.r.t microbiome) small paragraph -> significance of research
- Audience = people who don't know topic. Explain PD, what is it?
- Transition into microbiome

- PD microbiome has been studied, has been found to be different. Some markers include ... certain bacteria/microbes
- Go into background of drugs a bit → there are dopaminergic drugs (focus of study) out there (don't need to go into detail for each individually)
- Write about significance of research; why important; what could we potentially find

Talking about any tools like QIIME or R, no abbreviations, and include references, Link: <a href="https://docs.giime2.org/2023.9/citation/">https://docs.giime2.org/2023.9/citation/</a>

For each aim: use point format for each aim; write out what the aim is as a sentence

- (e.g. Aim 2 = to assess and compare the ... between... with  $\alpha$  and  $\beta$  diversity  $\rightarrow$  under that, have a paragraph explaining why it's important to look at  $\alpha$  and  $\beta$  diversity, and how does it inform us)
- E.g. DEseq: why are we doing deseq, what info will it give us, why is that significant to studying PD
- E.g. "To determine if Hi-SEAS reliably mimics the microbial environment of the ISS, with the ultimate goal of assessing the utility of Hi-SEAS as an Earth-based analog for space-based studies. This will involve validating the Hi-SEAS model using indicator taxa analysis and differential abundance analysis to determine commonalities."

Need figure legends for any figures

Explaining aims: have citations from other papers that have used the same methods.