Paper Comparison

	Paper 1 (higher alpha)	**Paper 4 (longitudinal)**
Variable region	V3-V4 region	V3-V4 region
Number of Donors	2	19
Age range	aged 14–29 years	Ages 26-74
Sample size	n=15 Four subjects had CD, and 11 had UC - Eleven (3 CD and 8 UC) subjects completed the blinded phase of the study - Three (3 UC) subjects were ineligible to continue into the open-label phase because they did not respond to FMT treatment in the blinded phase of the study and 1 CD subject did not want to continue into the open-label portion of the study. - Seven (2 CD and 5 UC) subjects completed the open-label phase of the study - **6** (2 CD and 4 UC) subjects completed long-term follow-up	n= 19 - 11 recipients with a history of 2 or more recurrences of *C. difficile* infections without inflammatory bowel disease (CDI-only), 3 UC recipients with recurrent *C. difficile*infections (CDI + UC), and 5 UC recipients without a history of *C. difficile* infections (UC-only)
When was illumina sequencing done	Performed on the	16S ribosomal RNA (rRNA) gene sequencing was performed on the pre-FMT, 1-week post-FMT, and 3-months post-FMT recipient fecal samples along with those collected from the healthy donors
Location	Boston USA	Stony Brook, NY

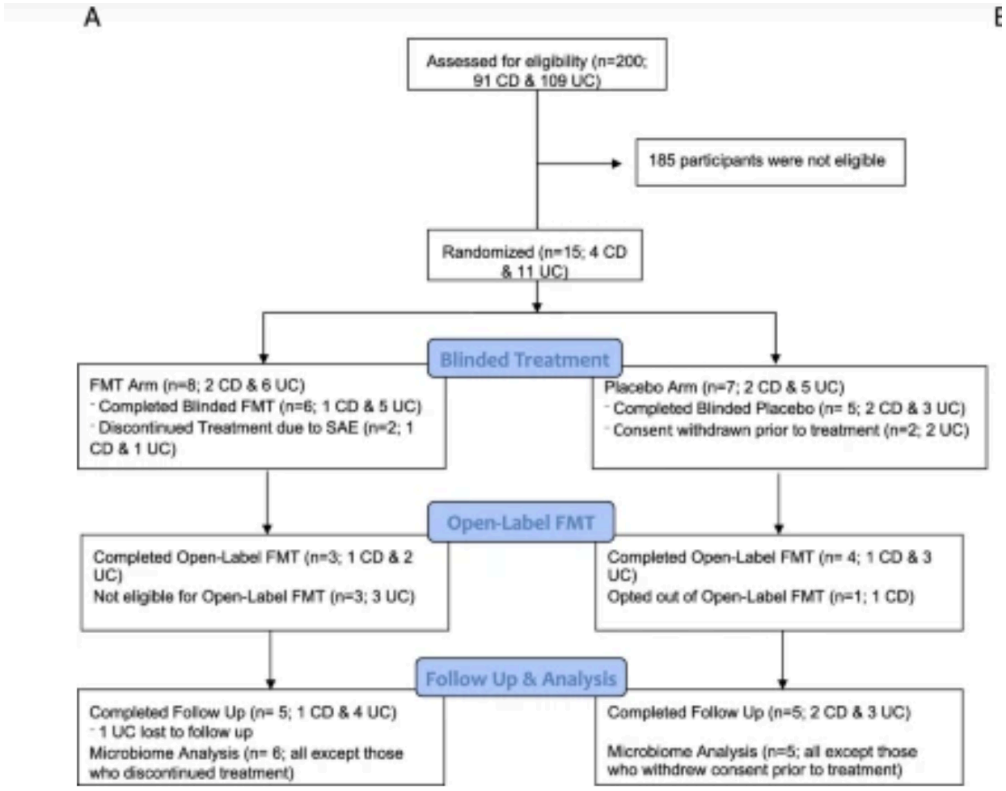
Timepoints of Samples	Subjects submitted stool samples during screening, baseline, after antibiotics but before FMT, then weekly during blinded and open-label treatment, and during follow-up. These samples were stored at − 80 °C.	Pre, 1 week post, 3 months post

- **PCDAI score:** can range from zero to 100 with higher scores indicating more active disease. At the time of each patient visit the attending physician is asked to categorize disease activity as inactive, mild, moderate, or severe
- **PUCAI score**→ simple, noninvasive tool that tells a doctor how mild or severe your child's UC symptoms are.

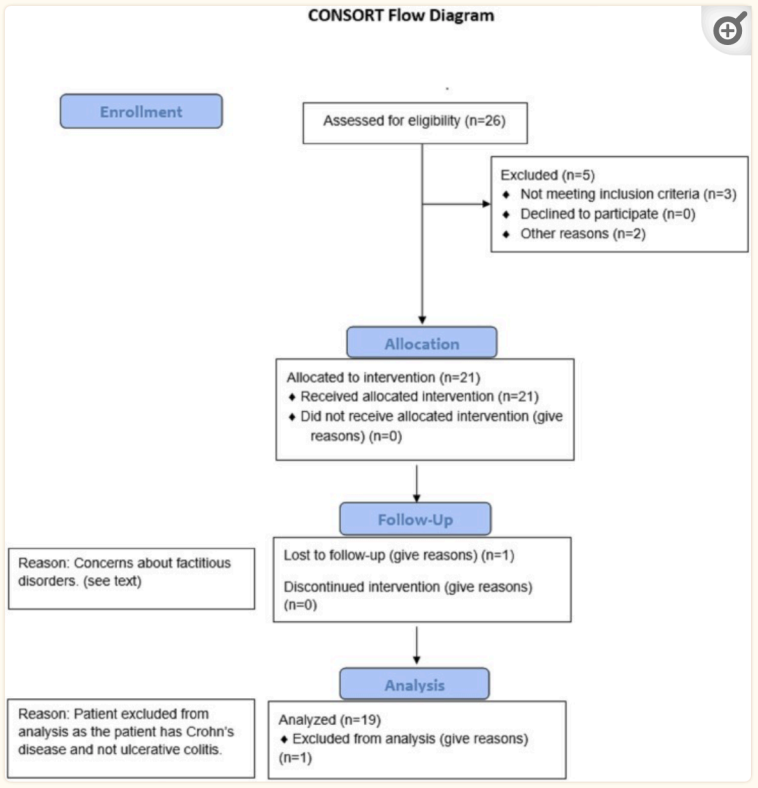
Paper 1:
We extracted DNA using a Powersoil DNA extraction kit (Qiagen). 16S rDNA libraries were prepared using primers targeting the V3-V4 region and sequenced by the Broad Institute Genomic Platform, using paired-end 250-bp reads on an Illumina HiSeq. We analyzed 16S data using Qiime2, DADA2, Phyloseq in R, and custom Python scripts43,44,45. We calculated alpha diversity using the Shannon index, Peilou evenness, and the Faith PD score within the Qiime2 environment. Beta diversity was calculated using Bray Curtis dissimilarity scores using Phyloseq. To test differences in alpha diversity at various timepoints, between responders and non-responders, we used the Mann–Whitney test and Bonferroni corrections with a corrected p-value cutoff of 0.05. To test correlations between alpha diversity and clinical disease variables, such as PUCAI, we calculated Pearson correlations, adjusted by Bonferroni corrections when multiple tests were performed. We assigned taxonomic labels to 16S sequences using the SILVA database46. We used SourceTracker2 to estimate the sources of various bacteria after FMT using the flag "-p-no-loo"28. We used the pre-antibiotic sample, the post-antibiotics sample, and the known donor as sources for each participant. To test correlations between engraftment and clinical disease variables, we calculated Pearson correlations between the engraftment score from SourceTracker2 at week 2 and week 7 (the early and late timepoints where we had sampling from all subjects) and the clinical variables. We also included the other donor, not used in that participant's FMT, as a negative control. To differentiate the participants who achieved high engraftment from those who did not, we used ANCOM-BC within the Qiime2 environment35,47. To create abundance time series of specific taxa, we extracted relative abundance tables from Qiime2 at the species and genus levels and graphed time series using custom python scripts.

Consort diagrams

Paper 1:



Paper 4:



Paper 1:

Methods from "Higher alpha diversity and Lactobacillus blooms are associated with better engraftment after fecal microbiota transplant in inflammatory bowel disease"

This clinical trial investigated the use of Fecal Microbiota Transplant (FMT) in treating young individuals (aged 5-30 years) with mild to moderate Crohn's Disease (CD) or Ulcerative Colitis (UC).

Study Design: A single-center, randomized, double-blind, placebo-controlled trial was carried out at Boston Children's Hospital.

- **Participant Recruitment & Eligibility:**
  - Potential participants were identified from the Boston Children's Hospital IBD Center and through external referrals.
  - **Inclusion Criteria:**
    - Age: 5-30 years old.
    - Disease Activity: Mild to moderate CD (Pediatric Crohn's Disease Activity Index (PCDAI) score > 10 but ≤ 30) or UC (Pediatric Ulcerative Colitis Activity Index (PUCAI) score > 9 but < 30).
    - Inflammation Evidence: Visual or histological confirmation within 105 days before randomization.

        - Infectious Disease Screening: Negative results for Hepatitis B (HBV), Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV).
        - Pregnancy Test: Negative urine test for individuals of childbearing potential.
        - Other: Ability to swallow capsules and absence of food allergies.
    - **Exclusion Criteria:**
        - Severe CD: Presence of complications like fistulizing disease, abscess, obstruction, or fevers.
        - Recent Medication Adjustments: Changes in biologics, 5-ASA, steroids, or immunomodulators within 4 weeks of enrollment.
        - Medical Conditions: Toxic megacolon, drug allergies (vancomycin, metronidazole, or polymyxin), history of aspiration or gastroparesis, upper gastrointestinal tract surgeries, esophageal dysmotility, swallowing dysfunction.
        - Other: Recent systemic antibiotic use (within 6 weeks), active *Clostridioides difficile* infection, prior FMT experience, known food allergies, and inability or unwillingness to receive a retention enema.
- **Randomization and Blinding:**
    - Participants were randomly assigned to either the FMT group or the placebo group using a 1:1 ratio and a predetermined block randomization procedure.
    - An independent study team member managed the randomization list to ensure treatment allocation concealment.
    - The placebo arm was incorporated to control for potential placebo effects observed in previous FMT trials.
- **Intervention Protocol:**
    - **FMT Arm:**
        - Antibiotic Pretreatment (7 days): This aimed to reduce existing gut microbiota and potentially facilitate donor microbiota engraftment.
            - Metronidazole: Weight-based dosing (maximum 500 mg) twice daily.
            - Vancomycin and Polymyxin: 125 mg and 62.5 mg capsules, respectively, administered three times daily, with dosage determined by Body Surface Area.
        - Induction Enema (Day 0): 120 mL of fecal matter in saline solution administered as a retention enema, encouraging retention for as long as possible.
        - Weekly FMT Capsules (7 weeks): 30 capsules taken orally once a week on an empty stomach.
    - **Placebo Arm:**
        - Placebo capsules and enema were administered, visually matching the FMT arm to maintain blinding.
- **FMT Material Sourcing:** Fecal material was obtained from OpenBiome, a non-profit stool bank, ensuring standardized processing and safety protocols.

- **Standard of Care Medications:** Participants continued their prescribed IBD medications throughout the study, managed by their primary healthcare provider.
- **Open-label FMT:**
    - After 8 weeks, participants in both groups were unblinded.
    - Those in the FMT group who showed a positive response and all individuals from the placebo group were eligible for an additional 8 weeks of open-label FMT (weekly capsules only).
- **Outcome Measures:**
    - **Primary Outcomes:**
        - Safety: Incidence of any treatment-related adverse events (grade 2 or higher) at 8 weeks post-FMT.
        - Tolerability: Proportion of participants reporting any adverse events of grade 2 or higher at 8 weeks.
    - **Secondary Outcomes:**
        - Clinical Response:
            - CD: ≥ 12.5 point decrease in PCDAI score.
            - UC: ≥ 20 point decrease in PUCAI score.
            - Remission in either CD or UC defined as PCDAI or PUCAI score < 10.
            - Assessed between weeks 2 and 7 post-FMT.
        - Inflammatory Biomarkers: Fecal calprotectin, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels.
        - Microbiome Composition: Analyzed for changes in diversity and taxonomic profiles.
        - Symptom Improvement: Self-reported by participants.
        - Donor Microbe Engraftment: Assessed using 16S rRNA gene sequencing.
- **Sample Collection & Microbiome Analysis:**
    - Stool samples were collected at various points: screening, baseline, after antibiotics but before FMT, weekly during the blinded and open-label phases, and during follow-up.
    - DNA Extraction & Sequencing: DNA was extracted from stool samples, and the V3-V4 region of the 16S rRNA gene was amplified and sequenced using an Illumina HiSeq platform.
    - Bioinformatic Analysis:
        - Sequence data were processed using Qiime2 and DADA2 pipelines.
        - Alpha Diversity: Calculated using Shannon Index, Pielou evenness, and Faith's Phylogenetic Diversity metrics.
        - Beta Diversity: Assessed using Bray-Curtis dissimilarity.
        - Taxonomic Classification: Performed using the SILVA database.
        - Donor Engraftment Estimation: SourceTracker2 software was used to determine the proportion of donor microbiota present in recipient samples.

        - Statistical Analyses: Mann-Whitney tests, Pearson correlations, and ANCOM-BC were employed to identify significant differences and associations between clinical and microbial variables.

Paper 4:

Methods from "Longitudinal microbiome analysis of single donor fecal microbiota transplantation in patients with recurrent Clostridium difficile infection and/or ulcerative colitis"

This study explored the impact of Fecal Microbiota Transplantation (FMT) on the gut microbiome of patients suffering from recurrent *Clostridioides difficile* infections (CDI) and/or Ulcerative Colitis (UC).

Study Design: This research employed an open-label, prospective design, meaning both researchers and participants were aware of the treatment being administered. Notably, there was no placebo control group. The study focused on analyzing changes in the gut microbiome following FMT over time.

- **Participant Recruitment & Eligibility:**
    - A total of 26 potential participants with recurrent CDI, with or without UC, were referred by community physicians to the Stony Brook University outpatient gastroenterology clinic between November 2013 and June 2016.
    - **Inclusion Criteria:**
        - Recurrent CDI: Individuals experiencing two or more CDI recurrences despite antibiotic treatment, confirmed by three positive stool tests.
        - UC: Patients with medication-refractory UC, necessitating treatment escalation beyond mesalamine alone.
    - **Exclusion Criteria:**
        - Upcoming Abdominal Surgery: Individuals scheduled for abdominal surgery within 12 weeks.
        - Pregnancy
        - Severe Anemia: Hemoglobin levels below 6 g/dL.
        - Grade 1 Neutropenia: Absolute Neutrophil Count below 1500.
        - Medical History: Graft vs. host disease diagnosis, major abdominal surgery within the past 3 months.
        - Recent Treatments: Investigational drug administration within the past 2 months, TNF-α antagonist use within 2 weeks of the intended transplantation date.
        - Recent Infections: Bacteremia within the past 4 weeks (28 days).
        - Inability to Consent: For adults aged 18 years and older, the inability to provide informed consent led to exclusion.
- **Donor Selection and Screening:**

    - Participants were asked to identify potential donors from their personal network (spouses, family members, friends, or associates).
    - Potential donors underwent a rigorous screening process involving:
        - **Questionnaire:** Assessed for risk factors like HIV, Hepatitis B or C infections, recent exposure to these viruses, high-risk sexual behaviors, illicit drug use, recent tattoos or piercings, incarceration history, febrile illness, variant Creutzfeldt-Jakob disease risk factors, travel to developing countries, history of inflammatory bowel disease, gastrointestinal malignancy, recent antibiotic use, immunosuppressive medication use, autoimmune diseases, malnutrition, chronic pain, metabolic syndrome, or neurological disorders.
        - **Stool Testing:** Screened for *C. difficile* toxin, enteric pathogens (Salmonella, Shigella, Campylobacter, Yersinia, *Escherichia coli* O157), fecal parasites (Giardia, Cryptosporidium, Cyclospora, Isospora), and ova.
        - **Serologic Testing:** Tested for HIV types 1 and 2, Hepatitis A IgM, Hepatitis B surface antigen, Hepatitis B core antibody (IgG and IgM), Hepatitis B surface antibody, and syphilis (RPR and FTA-ABS).
    - Donors were excluded if they met any exclusion criteria, were unable to provide informed consent, tested positive in any screening test, or had a fever within two weeks of the FMT. Additionally, donors were advised to avoid foods that the recipient was allergic to for one week before the donation.
- **FMT Procedure:**
    - CDI Management: To prevent CDI recurrence while awaiting transplantation, participants received a reduced dose of oral vancomycin (typically 125 mg/day, 3 times a week) until 48 hours before the procedure.
    - Bowel Preparation: Recipients underwent a split-dose polyethylene glycol-based bowel preparation the day before the colonoscopy.
    - Transplantation: Using a colonoscope, approximately 250 ml of the processed donor stool filtrate was injected into the terminal ileum/cecum.
    - Post-Procedure: Recipients remained in a recovery position for one hour after the procedure before being discharged.
- **Stool Sample Collection and Analysis:**

    - Sample Collection: Stool samples were collected at three time points:
        - Pre-FMT: The day before the FMT procedure.
        - 1-week post-FMT
        - 3-months post-FMT
    - Donor stool samples were also collected using the same method.
    - Samples were stored in RNAlater (for nucleic acid extraction) and frozen at -80°C.
    - Food Diaries: Participants completed food diaries for the week preceding each stool sample collection.
    - Microbiome Analysis:

        - DNA Extraction: DNA was extracted from stool samples using the Zymo Research DNA MiniPrep kit.
        - 16S rRNA Gene Sequencing: The V3V4 region of the 16S rRNA gene was amplified using PCR with barcoded primers and sequenced on the Illumina MiSeq platform.
        - Bioinformatic Analysis:
            - Data Processing: Raw sequence data were processed and analyzed to identify and quantify bacterial taxa.
            - Diversity Analysis: Alpha diversity (within-sample diversity) and beta diversity (between-sample diversity) were calculated.
            - Statistical Analysis: Linear mixed models were used to assess the impact of FMT over time on the gut microbiome composition of participants with CDI-only, CDI + UC, and UC-only.
- **Other Measurements:**
    - Fecal Calprotectin: Measured as a marker of intestinal inflammation using the PhiCal Test ELISA.
    - UC Disease Activity: For UC patients, Mayo endoscopic subscores were determined during the FMT procedure. At the 3-month follow-up, Mayo scores were recorded.
- **Clinical Outcomes and Follow-Up:**
    - Participants received follow-up phone calls to monitor for adverse events and assess symptom resolution, stool frequency and consistency, weight changes, medication use, and any other relevant clinical changes.
    - Adverse events were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE).

Halfvarson dataset

- study the long-term dynamics of the gut microbiome from an IBD cohort of 137 individuals (49 Crohn's disease (CD), 60 UC, 4 lymphocytic colitis (LC), 15 collagenous colitis (CC)) and 9 healthy controls (HC). We sampled at three-month intervals, collecting 1–10 samples per individual for a total of 683 samples
- V4 region of the 16S rRNA gene for a total of 248 million 16S rRNA gene amplicons

Metadata Categories

Paper 1 (15 patients)

Run → Sequencing run

Geo_loc_name_country → country where sample was taken

Geo_loc_name_country_continent → continent where sample was taken

Geo_loc_name → city where sample was taken

Host → host species

Host_subject_id → ID

Host_disease → Crohn's disease (CD) or ulcerative colitis (UC)

Host_phenotype → responder vs non-responder (responders were defined as subjects with a drop in disease activity indexes of at least 12.5 (CD) or 20 (UC) or subjects in remission)

Paper 4 (19 patients)

Run → sequence run

DX → Disease (C. difficile infections without inflammatory bowel disease (CDI-only), 3 UC recipients with recurrent C. difficile infections (CDI + UC), and 5 UC recipients without a history of C. difficile infections (UC-only))

Group → DX+sample time point (pre, 1 week post treatment, 3 months post treatment)

Timepoint → time point post FMT of sample