



Temporal Changes in Gut Microbial Network Structure and Edge Rewiring in Pediatric Ulcerative Colitis: A PROTECT Cohort Study

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ABSTRACT: Pediatric ulcerative colitis is associated with changes in the gut microbiome. However, how microbial associations reorganize over time during treatment remains less well understood. This study aimed to examine longitudinal changes in microbial networks and their association with initial treatment strategies and clinical remission. We analyzed longitudinal data from the PROTECT cohort, including 132 patients with samples collected at weeks 0, 4, and 12. Of these, 90 also had samples available at week 52. Genus-level microbial association networks were constructed using centered log-ratio (CLR) transformed abundances and Spearman correlations, with a threshold of $|\rho| \geq 0.40$. Topological and spectral measures, Jaccard distance, changes in edge identity, and bacterial genus centrality were examined. Between-group comparisons were assessed using patient-level bootstrap resampling. In the longitudinal subcohort, network density decreased from 0.1439 at week 0 to 0.0930 at week 52. Clustering coefficient and spectral radius also declined. The CS-IV group showed the highest Jaccard distance from baseline at week 52 (0.8053), compared with CS-Oral (0.7380) and 5-ASA (0.6827). Patients who achieved corticosteroid-free remission at week 52 showed partial recovery in network density between weeks 4 and 12, while density continued to decline in the non-remission group. However, bootstrap comparisons did not provide clear statistical evidence of differences between remission groups. These findings indicate longitudinal changes in microbial network organization and edge identity during treatment.

KEYWORDS: pediatric ulcerative colitis, gut microbiome, longitudinal network analysis, network rewiring, clinical remission, PROTECT cohort.

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1. INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory condition affecting the colon. There is evidence to show that changes in the gut microbiota play a role in disease pathogenesis. Changes have been found in both the composition and diversity of the gut microbiota, which are linked to intestinal inflammation and response to treatment (Gevers et al., 2014; Hall et al., 2017; Pascal et al., 2017; Vich Vila et al., 2018). Longitudinal studies have shown that microbial communities in patients with inflammatory bowel disease (IBD) vary considerably over time (Halfvarson et al., 2017; Lloyd-Price et al., 2019).

The PROTECT cohort provides an opportunity to look at these changes in children with UC (Hyams et al., 2019). Schirmer et al. (2018) found changes in microbiome composition and temporal variation associated with disease severity and clinical outcomes. However, measuring abundance and diversity does not give a full picture of how associations between bacterial taxa change during treatment.

Network analysis can be used to understand the structure of the microbiome (Wu et al., 2024). Nodes are used to represent bacterial genera, and edges represent statistical relationships between them. Changes in network topology and modularity have been identified in IBD patients (Baldassano & Bassett, 2016). Chen et al. (2020) identified differences in microbial associations related to disease status. This suggests that dysbiosis may involve changes in taxonomic composition as well as changes in microbial associations.

Despite knowing that the microbiome varies over time and that microbial networks can change, there is limited understanding of how network topology, spectral properties, edge identity, and genus degree centrality change together during treatment of UC in children. There is also a need to understand their associations with initial treatment strategies and clinical remission. Having the

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same number of edges in two networks does not mean they have the same structure. They could connect different bacterial genera. By examining which edges are persistent, lost, and gained, we can identify changes that may not be captured by global network measurements.

The aim of this study is to understand how microbial association networks change over time in children with UC from the PROTECT cohort and how these changes relate to initial treatment groups and clinical outcomes. We investigate changes in network topology and spectral properties, measure how many edges persist, disappear, or emerge, compare network reorganization across initial treatment groups, and examine whether network changes are associated with corticosteroid-free remission at week 52 (W52). We also look at changes in genus degree centrality.

This study addresses the following research questions:

RQ1: How do microbial network topology, spectral properties, and edge identity change over time?

RQ2: How does microbial network reorganization differ between initial treatment groups?

RQ3: Are changes in microbial network organization associated with corticosteroid-free remission at W52?

The main contribution of this study is the combined analysis of network topology, spectral properties, and edge rewiring across multiple visits within the PROTECT cohort. We examine not only how many connections exist within the microbial networks, but also which connections persist, disappear, or emerge over time. We then compare these changes across initial treatment groups and according to clinical remission. This adds to previous taxonomic analyses by providing additional information about microbial network organization during treatment.

2. MATERIALS AND METHODS

2.1. Study Cohort and Clinical Data

This study used longitudinal microbiome data from children with ulcerative colitis (UC) in the PROTECT cohort (Hyams et al., 2019; Schirmer et al., 2018). There were 949 fecal samples available in total. Among these, 135 patients had microbiome data recorded at weeks 0 (W0), 4 (W4) and 12 (W12). Three patients were excluded because their clinical outcome was not recorded at week 52 (W52). The remaining 132 patients, providing 396 fecal samples, were used for the analysis.

Corticosteroid-free remission at W52 was the primary clinical outcome. Of the 132 patients, 53 achieved remission and 79 did not. Patients were divided into three groups based on their initial treatment: intravenous corticosteroids (CS-IV; $n = 51$), oral corticosteroids (CS-Oral; $n = 43$) and 5-aminosalicylic acid (5-ASA; $n = 38$). The main cohort contained 66 females and 66 males, with a mean age of 12.18 ± 3.29 years.

Within this cohort, 90 patients had microbiome data available for all four time points (W0, W4, W12 and W52), providing 360 fecal samples. This subcohort contained 45 females and 45 males, with a mean age of 12.47 ± 3.25 years. At W52, 35 patients achieved corticosteroid-free remission and 55 did not. Of these, 33 patients were in the CS-IV group, 30 in the CS-Oral group and 27 in the 5-ASA group.

The two cohorts were used for different analyses. The main cohort ($n = 132$) was used to select the parameters for network construction and to examine how microbial networks changed according to clinical outcome at W52. The subcohort ($n = 90$) was used to examine network changes across all four time points and compare them between the initial treatment groups. The same patients were included at each relevant time point within each analysis.

The demographic and clinical characteristics, treatment distribution, clinical outcomes, and sampling structure are provided (see Table 1).

Table 1. Demographic and clinical characteristics and longitudinal sampling structure of the PROTECT cohort

Characteristic	Primary cohort ($n = 132$)	Longitudinal subcohort ($n = 90$)
Patients, n	132	90
Fecal samples included, n	396	360
Age, mean $\pm$ SD (years)	12.18 ± 3.29	12.47 ± 3.25
Female, n (%)	66 (50.0%)	45 (50.0%)
Male, n (%)	66 (50.0%)	45 (50.0%)
Initial treatment strategy		
CS-IV, n	51	33
CS-Oral, n	43	30
5-ASA, n	38	27
Clinical outcome at W52		
Corticosteroid-free remission, n	53	35
No remission, n	79	55

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Samples included by time point		
W0, n	132	90
W4, n	132	90
W12, n	132	90
W52, n	Not included*	90

Note: SD = standard deviation; CS-IV = intravenous corticosteroids; CS-Oral = oral corticosteroids; 5-ASA = 5-aminosalicylic acid. W0, W4, W12, and W52 indicate sampling weeks. Only W0, W4, and W12 samples were included in the primary cohort analysis. The 90-patient longitudinal subcohort was part of the primary cohort and additionally included W52 samples. Recorded antibiotic exposure was considered a potential confounding factor in the microbial network analyses. Its influence was examined in a separate sensitivity analysis, as described in Section 2.8.

2.2. Microbiome Data Preprocessing

Taxonomic data were aggregated from operational taxonomic units (OTUs) to the bacterial genus level using taxonomic information encoded in the variable names. Taxa without genus-level identification were excluded. Prevalence thresholds of 5% and 10% were tested to remove rare taxa. The 5% threshold was chosen because it retained more bacterial genera than the higher threshold. Prevalence filtering was performed separately for the primary cohort and the longitudinal subcohort, retaining 81 and 82 genera, respectively. This resulted in the same set of genera being used within each cohort at each time point and across comparison groups. Bacterial abundances were converted to relative abundances. For sample i and genus j , relative abundance was calculated as:

$$p_{ij} = \frac{x_{ij}}{\sum_{k=1, \dots, m} x_{ik}} \quad (1)$$

where x_{ij} was the abundance of genus j in sample i , and m was the number of genera retained after filtering.

A pseudocount $\varepsilon = 10^{-6}$ was added before the logarithmic transformation to handle zero values:

$$q_{ij} = p_{ij} + 10^{-6} \quad (2)$$

The centered log-ratio (CLR) transformation was then applied:

$$CLR(q_{ij}) = \ln(q_{ij}) - \frac{1}{m} \sum_{k=1, \dots, m} \ln(q_{ik}) \quad (k = 1, \dots, m) \quad (3)$$

This transformation was used to account for the compositional nature of microbiome data. The resulting CLR-transformed matrices (samples $\times$ genera) were used for microbial network inference at W0, W4, and W12, and additionally at W52 for the 90-patient longitudinal subcohort.

2.3. Microbial Network Construction and Parameter Selection

Microbial association networks were constructed separately for each time point and analytical group using genus-level CLR-transformed abundance data. Associations between bacterial genera were estimated using Spearman rank correlations. Each network was represented as an undirected graph, with bacterial genera as nodes and statistical associations as edges.

An edge between genera i and j was included when the absolute Spearman correlation met the selected threshold:

$$A_{ij} = \begin{cases} 1, & |\rho_{ij}| \geq \tau, i \neq j \\ 0, & \text{otherwise} \end{cases} \quad (4)$$

Here, ρ_{ij} denoted the Spearman correlation between genera i and j , and τ was the correlation threshold. The diagonal of the adjacency matrix was set to zero. Thresholds of 0.30, 0.40, 0.50, and 0.60 were evaluated. Edge count, density, average degree, clustering coefficient, connected components, isolated nodes, and temporal edge stability were assessed for each threshold.

We calculated the Jaccard similarity between the edge sets of two visits as:

$$J(E_a, E_b) = \frac{n(E_a \cap E_b)}{n(E_a \cup E_b)} \quad (5)$$

where E_a and E_b denote the edge sets at two time points, and $n(\cdot)$ denoted the number of edges in a set.

Threshold sensitivity was evaluated using data from 132 patients at W0, W4, and W12. The threshold $|\rho| \geq 0.40$ was selected because it gave the highest mean Jaccard similarity (0.5316), while maintaining moderate network density and fragmentation.

2.4. Longitudinal Analysis of Microbial Networks

Ninety patients were included in the longitudinal analysis. The same 82 bacterial genera were used to create a microbial network at each time point, based on CLR-transformed abundances and Spearman correlations ($|\rho| \geq 0.40$). Network density, edge count, average degree, clustering coefficient, modularity, connected components, isolated nodes, and spectral radius were computed. Positive and negative correlations were counted separately.

Changes in edge identity were evaluated between successive visits (W0–W4, W4–W12, and W12–W52). Edges were classified as persistent, lost, or gained. Their overall similarity was measured using the Jaccard index described in Section 2.3. We measured the rewiring fraction by dividing the total number of lost and gained edges by the number of edges in the union of the two sets:

$$R(E_a, E_b) = \frac{n(E_a \setminus E_b) + n(E_b \setminus E_a)}{n(E_a \cup E_b)} = 1 - J(E_a, E_b) \quad (6)$$

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These measures were used to assess changes in microbial edge identity and network stability over time.

2.5. Longitudinal Network Analysis by Initial Treatment

To examine whether microbial network reorganization differed according to initial treatment, the longitudinal subcohort of 90 patients was divided into three groups: intravenous corticosteroids (CS-IV; $n = 33$), oral corticosteroids (CS-Oral; $n = 30$), and 5-aminosalicylic acid (5-ASA; $n = 27$). All 90 patients had microbiome samples at W0, W4, W12, and W52. They remained in their initial treatment groups throughout the study. For each group and visit, we constructed a network using the same 82 bacterial genera and the parameters described in Sections 2.2–2.3. Next, network density, average degree, clustering coefficient, connected components, isolated nodes, and spectral radius were calculated.

Changes in edge identity relative to baseline were measured using Jaccard distance:

$$D_J(E_0, E_t) = 1 - \frac{n(E_0 \cap E_t)}{n(E_0 \cup E_t)} \quad (7)$$

where E_0 and E_t represent the edge sets at baseline and follow-up, respectively. Larger Jaccard distances indicate greater changes in edge identity. We also counted the edges that persisted, disappeared, or appeared between consecutive visits.

To estimate uncertainty, we performed 500 patient-level bootstrap resamples. Within each treatment group, patients were sampled with replacement, and their four visits were kept together. We rebuilt the networks for every resample and calculated 95% confidence intervals for changes from W0 and differences between groups.

We treated these comparisons as observational associations, not evidence that the treatments caused the network changes. Antibiotic exposure was examined separately in Section 2.8.

2.6. Association Between Microbial Network Reorganization and Clinical Remission

We examined whether changes in microbial network organization were associated with corticosteroid-free remission at W52. The analysis included 132 patients with microbiome samples at W0, W4, and W12 and clinical outcome information at W52. Of these, 53 achieved remission and 79 did not.

Microbial networks were constructed separately for each group and time point using the same 81 bacterial genera, CLR-transformed abundances, Spearman correlations, and a threshold of $|\rho| \geq 0.40$. Network organization was assessed through density, clustering coefficient, spectral radius, connected components, and isolated nodes.

Changes in edge identity were measured using Jaccard distance from the baseline network at W0, as defined in Section 2.5. Persistent, lost, and gained edges were also identified between consecutive visits.

Network trajectories were compared between the two clinical groups. Uncertainty was assessed using 500 patient-level bootstrap resamples, with patients sampled within each group and all three visits retained together. For changes from baseline and between-group contrasts, 95% confidence intervals were calculated from the bootstrap distributions. The analysis was conducted at the group level and was not intended to predict remission in individual patients.

2.7. Identification of Bacterial Genera and Changes in Microbial Associations

Changes in degree centrality and edge identity were examined to identify bacterial genera showing the largest structural changes over time. The analysis was performed separately for the Remission ($n = 53$) and No remission ($n = 79$) groups, using networks from the 132-patient cohort. We applied $|\rho| \geq 0.40$ at W0, W4, and W12.

Normalized degree centrality at time t for each genus i was computed as follows:

$$C_i(t) = \frac{k_i(t)}{N-1} \quad (8)$$

where $k_i(t)$ is the number of edges connected to genus i , and N is the total number of network nodes. Changes in centrality between two visits were calculated as:

$$\Delta C_i = C_i(t_2) - C_i(t_1) \quad (9)$$

Centrality changes were examined for W0–W4, W4–W12, and the overall W0–W12 interval. Positive values indicated an increase in centrality, while negative values indicated a decrease. Genera with the largest absolute changes were selected for tabular summaries and graphical presentation.

For the W0–W12 comparison, edges were classified into three categories:

$$\text{Persistent} = E_0 \cap E_{12}; \text{Lost} = E_0 - E_{12}; \text{Gained} = E_{12} - E_0 \quad (10)$$

Centrality changes were visualized over time, together with a selected subnetwork of gained and lost edges in the Remission group. This analysis was descriptive. It was not used to establish causal relationships or statistically significant differences at the individual genus or edge level.

2.8. Sensitivity Analysis for Antibiotic Exposure

A sensitivity analysis was performed to assess whether the network findings were affected by recorded antibiotic exposure. Patients with antibiotic use documented in at least one sample during the study visits were excluded. In the four-visit subcohort (W0, W4,

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W12, and W52), 13 of the 90 patients were excluded, leaving 77 patients. In the three-visit cohort (W0, W4, and W12), 19 of the 132 patients were excluded, leaving 113.

Microbial networks were reconstructed using the same bacterial genera (82 and 81, respectively), CLR-transformed abundances, Spearman correlations, and the threshold of $|\rho| \geq 0.40$. Network density and Jaccard distance from W0 were then compared between the original analyses and those excluding patients with recorded antibiotic exposure.

The sensitivity analysis was interpreted descriptively and was not intended to estimate a causal effect of antibiotic use.

3. RESULTS

3.1. Temporal Reorganization of the Gut Microbiome Network

Table 2. Global topological and spectral properties of microbial networks across study visits.

Metric	W0	W4	W12	W52
Nodes	82	82	82	82
Edges	478	373	354	309
Network density	0.1439	0.1123	0.1066	0.0930
Average degree	11.6585	9.0976	8.6341	7.5366
Clustering coefficient	0.4821	0.4181	0.3686	0.3163
Modularity	0.2298	0.1707	0.1574	0.1922
Connected components	17	25	23	25
Isolated nodes	15	20	19	19
Spectral radius	25.8905	23.4965	22.3038	20.4558
Positive edges	444	363	350	298
Negative edges	34	10	4	11

Longitudinal analysis showed a progressive decline in microbial network connectivity from W0 to W52. The number of edges decreased from 478 to 309, while network density fell from 0.1439 to 0.0930. Along with the spectral radius (25.8905 to 20.4558), the clustering coefficient also decreased from 0.4821 to 0.3163. The main topological and spectral measurements are presented in the corresponding table (see Table 2).

Increased network fragmentation was indicated by the number of connected components rising from 17 at W0 to 25 at W52. Up to W12, modularity decreased; however, at W52, it increased slightly. At all four time points, positive correlations continued to be more prevalent than negative ones (see Figure 1).

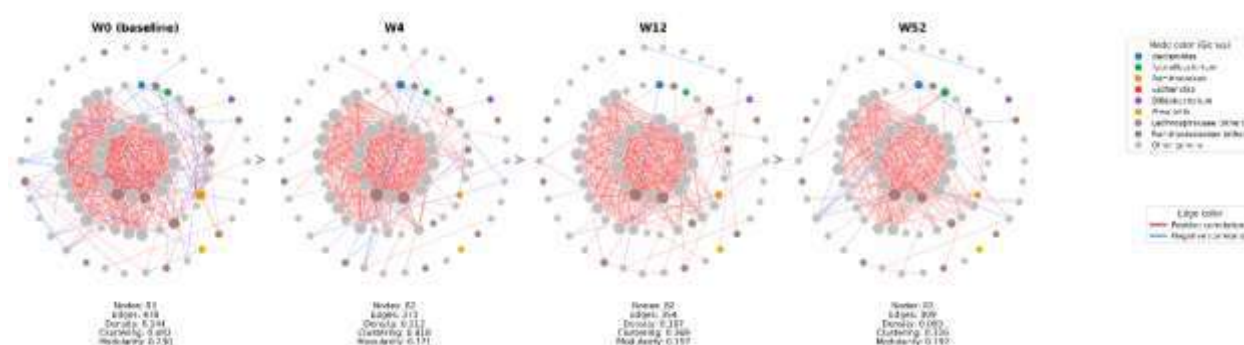


Figure 1. Microbial association networks during weeks 0, 4, 12 and 52. Node colors represent the selected taxonomic groups, while edge colors distinguish positive from negative correlations.

During successive visits, changes in the edges were observed. From week 0 to week 4, 291 edges remained, 187 disappeared, and 82 new edges appeared, resulting in a Jaccard similarity of 0.5196. The highest similarity was found between week 4 and week 12 (0.5839), while the lowest was between week 12 and week 52 (0.4766). This latter interval also showed the highest rewiring fraction (0.5234) (see Figure 2).

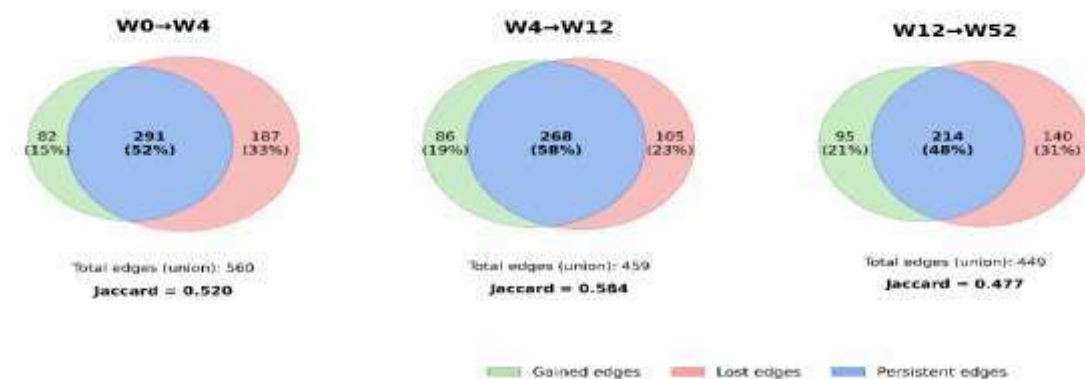


Figure 2. Changes in microbial network edges between consecutive visits.

3.2. Microbial Network Reorganization by Initial Treatment

The 12 networks constructed across three treatment groups and four visits showed differences in connectivity and edge organization. In the CS-IV group, the density decreased between W0 (0.2147) and W4 (0.1072). It then increased to 0.1141 at W52. In the CS-Oral group, the density varied slightly between 0.1900 and 0.1930 in the initial 4 weeks but then decreased to 0.1349 at W52. Smaller variations were observed in the 5-ASA group, where the values were 0.1915 (W0) and 0.1873 (W52) (see Table 3).

Table 3. Longitudinal network density and Jaccard distance by initial treatment strategy

Treatment	Week	Density	Jaccard distance from W0
CS-IV	W0	0.2147	0.0000
CS-IV	W4	0.1072	0.7259
CS-IV	W12	0.1310	0.7371
CS-IV	W52	0.1141	0.8053
CS-Oral	W0	0.1900	0.0000
CS-Oral	W4	0.1930	0.6625
CS-Oral	W12	0.1268	0.7431
CS-Oral	W52	0.1349	0.7380
5-ASA	W0	0.1915	0.0000
5-ASA	W4	0.1762	0.6699
5-ASA	W12	0.2057	0.6876
5-ASA	W52	0.1873	0.6827

Note: The Jaccard distances were calculated using the W0 as the reference set for each treatment group. The baseline value was zero by definition.

At W52, the CS-IV group showed the highest Jaccard distance from baseline (0.8053), followed by CS-Oral (0.7380) and 5-ASA (0.6827). Rewiring fractions for W12–W52 were 0.7814, 0.7496, and 0.6588, respectively (see Figure 3).

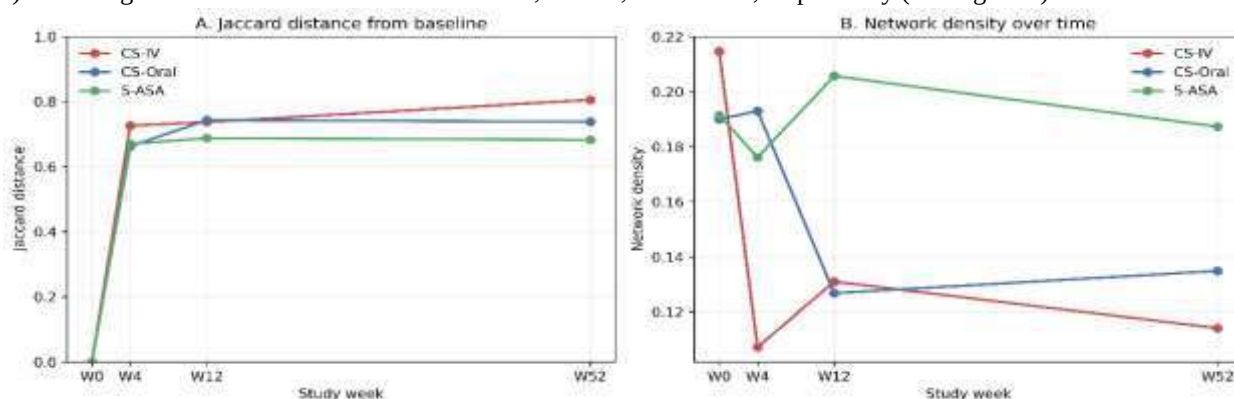


Figure 3. Longitudinal trajectories of microbial network organization by initial treatment. (A) Jaccard distance from baseline (W0). (B) Network density at W0, W4, W12, and W52 in the CS-IV, CS-Oral, and 5-ASA groups.

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A bootstrap analysis with 500 resamples was conducted and gave a mean Jaccard distance difference of 0.0703 between the CS-IV and 5-ASA groups for W52 (95% CI: 0.0119-0.1273). The mean density change from W0 to W52 in the CS-IV group was -0.0851 (95% CI: -0.1635 to -0.0138). Confidence intervals for the other Jaccard contrasts and between-group density contrasts included zero. These comparisons remained exploratory because no adjustment for multiple comparisons was applied.

3.3. Association Between Microbial Network Reorganization and Clinical Remission

The analysis included 132 patients classified by corticosteroid-free remission at W52: Remission ($n = 53$) and No remission ($n = 79$). Network density decreased in both groups from W0 to W4. Between W4 and W12, density increased from 0.1019 to 0.1256 in the Remission group, while it declined from 0.1077 to 0.0997 in the No remission group. Clustering also increased in the Remission group (0.3892–0.5035), whereas the No remission network had more connected components at W12 (25 versus 13) (see Table 4).

Table 4. Network density and Jaccard distance by corticosteroid-free remission status at W52

Clinical outcome	Week	Density	Jaccard distance from W0
Remission	W0	0.1481	0.0000
Remission	W4	0.1019	0.6387
Remission	W12	0.1256	0.6205
No remission	W0	0.1537	0.0000
No remission	W4	0.1077	0.5140
No remission	W12	0.0997	0.5845

Note: Jaccard distances were calculated relative to each group's W0 network. Clinical outcomes were assessed at W52, whereas microbiome samples in this analysis were evaluated through W12.

Jaccard distance from baseline was higher in the Remission group at W4 (0.6387 versus 0.5140). This difference narrowed by W12, when distances were 0.6205 and 0.5845, respectively (see Figure 4).

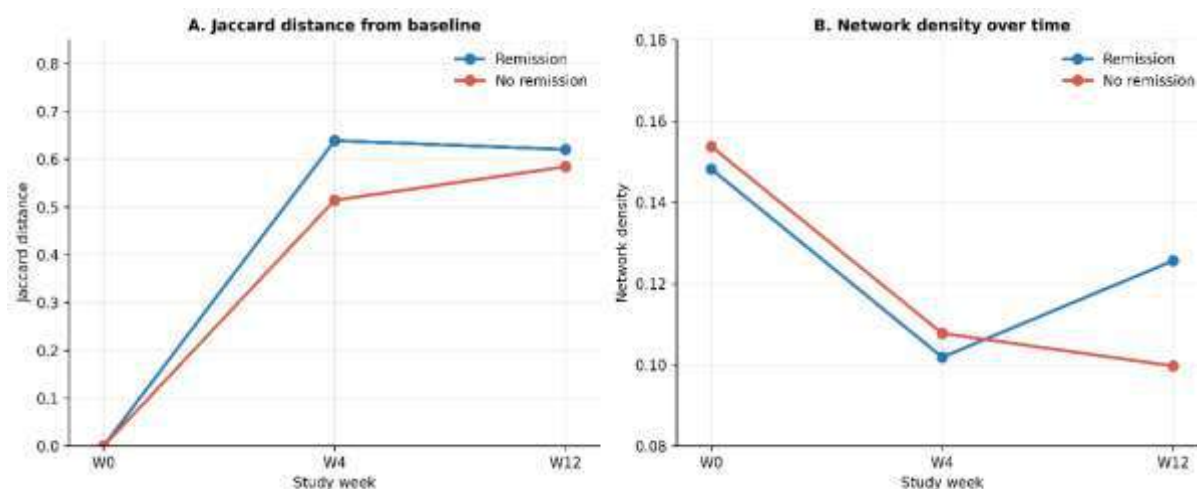


Figure 4. Longitudinal microbial network reorganization by clinical outcome. (A) Jaccard distance from W0. (B) Network density at W0, W4, and W12 in the Remission and No remission groups, defined by corticosteroid-free remission status at W52.

Bootstrap analysis using 500 resamples indicated a mean difference of 0.0450 between groups (95% CI: -0.0331 to 0.1225) for the change in density between W0 and W12. Additionally, the mean difference in Jaccard distance at W12 was 0.0419 (95% CI: -0.0642 to 0.1389).

3.4. Longitudinal Changes in Bacterial Genus Centrality and Microbial Associations

Overall, when comparing the network changes between week 0 and 12, the Remission group had a total of 244 edges retained, 236 edges removed, and 163 new edges formed, while the No remission group had 241 edges retained, 257 edges removed, and 82 new edges formed. In terms of degree centrality, there was an increase in centrality for *Eikenella* ($\Delta C = +0.2500$). However, *Campylobacter* (-0.2625), *Christensenella* (-0.2375), and *Pseudoramibacter_Eubacterium* (-0.2125) all decreased in centrality between week 0 and 12 in the Remission group. In the No remission group, the largest reported declines involved *Ruminococcus* (-0.3125), *Pediococcus* (-0.3000), and *Enterococcus* (-0.2500), whereas *Eikenella* increased ($+0.1875$).

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Between W0 and W4, *Pediococcus* and *Stenotrophomonas* lost centrality in the Remission group but partially recovered between W4 and W12. In the No remission group, *Ruminococcus* showed the largest decline during the first interval, while *Aggregatibacter* showed the largest increase during the second interval (see Table 5).

Table 5. Bacterial genera with the largest absolute changes in degree centrality by clinical outcome and time interval.

Group	Interval	Bacterial Genus	ΔC
Remission	W0–W4	<i>Pediococcus</i>	−0.3750
Remission	W0–W4	<i>Stenotrophomonas</i>	−0.2625
Remission	W0–W4	<i>Christensenella</i>	−0.2625
Remission	W0–W4	<i>Enterococcus</i>	−0.2375
Remission	W0–W4	<i>Campylobacter</i>	−0.2250
Remission	W4–W12	<i>Pediococcus</i>	+0.2000
Remission	W4–W12	<i>Stenotrophomonas</i>	+0.1875
Remission	W4–W12	<i>Varibaculum</i>	+0.1750
Remission	W4–W12	<i>cc_115</i>	−0.1750
Remission	W4–W12	<i>Enterococcus</i>	+0.1625
No remission	W0–W4	<i>Ruminococcus</i>	−0.3000
No remission	W0–W4	<i>Enterococcus</i>	−0.1875
No remission	W0–W4	<i>Oribacterium</i>	−0.1875
No remission	W0–W4	<i>Pediococcus</i>	−0.1875
No remission	W0–W4	<i>Leuconostoc</i>	−0.1750
No remission	W4–W12	<i>Aggregatibacter</i>	+0.2250
No remission	W4–W12	<i>Varibaculum</i>	+0.1500
No remission	W4–W12	<i>Eikenella</i>	+0.1375
No remission	W4–W12	<i>Cardiobacterium</i>	−0.1375
No remission	W4–W12	<i>Pseudomonas</i>	−0.1125

Changes in degree centrality and edge identity are illustrated (see Figure 5).

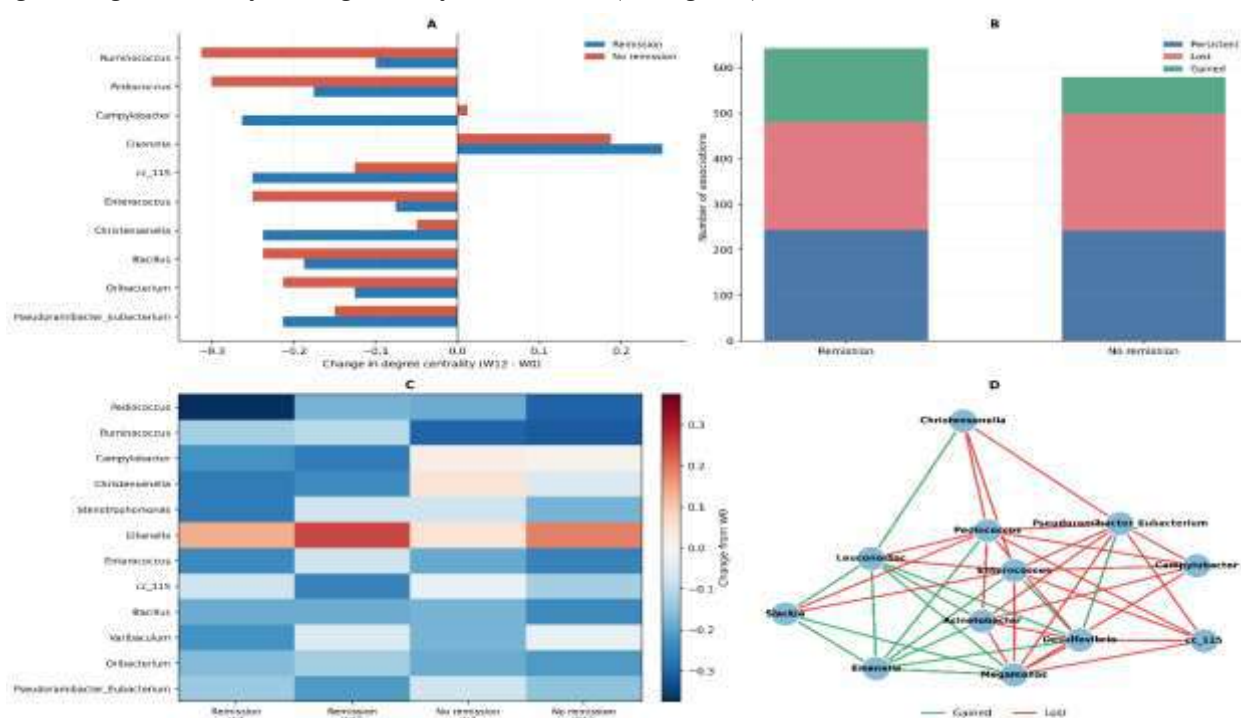


Figure 5. Longitudinal changes in bacterial genus centrality and microbial associations by clinical outcome. (A) W0–W12 centrality changes for the ten genera with the largest absolute shifts. (B) Persistent, lost, and gained edges from W0 to W12. (C) Heatmap of selected genus centrality changes at W4 and W12 relative to W0. (D) Selected subnetwork of gained (green) and lost (red) edges in the Remission group.

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These findings showed changes in both microbial edge identity and the structural positions of bacterial genera. However, genus- and edge-level differences were descriptive and were not individually tested for statistical significance.

3.5. Sensitivity Analysis for Antibiotic Exposure

After patients with recorded antibiotic exposure were excluded, the main network density trajectories remained broadly similar to those observed in the original analyses. In the treatment-stratified analysis, Jaccard distance from W0 to W52 remained highest in the CS-IV group (0.8195), followed by CS-Oral (0.7566) and 5-ASA (0.6995).

In the clinical outcome analysis, the Remission group showed a decline in network density at W4, followed by partial recovery at W12. Density continued to decrease in the No remission group. However, the between-group difference in Jaccard distance at W12 decreased from 0.0360 to 0.0112 after the exclusions. Overall, the main density patterns remained similar, while differences in edge identity showed some sensitivity to cohort composition (see Table 6).

Table 6. Sensitivity analysis for antibiotic exposure.

Analysis	Group	Week	Density (Original / No ABX)	Jaccard Distance (Original / No ABX)
RQ2	CS-IV	W12	0.1310 / 0.1629	0.7371 / 0.7542
RQ2	CS-IV	W52	0.1141 / 0.1325	0.8053 / 0.8195
RQ2	CS-Oral	W12	0.1268 / 0.1497	0.7431 / 0.7551
RQ2	CS-Oral	W52	0.1349 / 0.1578	0.7380 / 0.7566
RQ2	5-ASA	W12	0.2057 / 0.2138	0.6876 / 0.6986
RQ2	5-ASA	W52	0.1873 / 0.1939	0.6827 / 0.6995
RQ3	Remission	W12	0.1256 / 0.1361	0.6205 / 0.6207
RQ3	No remission	W12	0.0997 / 0.0978	0.5845 / 0.6095

Note. ABX = antibiotics; original = analysis before exclusions. The results of RQ2 were obtained from 90 patients before exclusions and 77 afterward (CS-IV: 33/27, CS-Oral: 30/25, and 5-ASA: 27/25). The results of RQ3 were obtained from 132 patients before exclusions and 113 afterward (Remission: 53/48 and No remission: 79/65). Jaccard distances were computed relative to the W0 network reconstructed for each corresponding analysis.

4. DISCUSSION

This study demonstrates that the microbial association networks in paediatric ulcerative colitis patients from the PROTECT cohort changed over time. The network density, clustering coefficient and spectral radius decreased from W0 to W52, whilst the number of connected components increased, suggesting a gradual change in the association networks. This agrees with previously described differences in association networks in IBD (Baldassano & Bassett, 2016; Chen et al., 2020), and longitudinal studies report large variability in gut microbial communities (Halfvarson et al., 2017; Lloyd-Price et al., 2019). The current study expands on findings by Schirmer et al. (2018), who studied the PROTECT cohort, by further investigating changes in association network topology and edge identity.

The association networks were different for each treatment group. At W52, the CS-IV group showed the most change in edge identity compared with baseline. The 5-ASA group had a more stable network density. This indicates that density alone might not be sufficient to understand the changes in association networks. Whilst the densities may be similar, there could still be loss or gain of edges within the network. There have previously been investigations into how microbial features correlate with treatment responses (Ananthakrishnan et al., 2017; Melograna et al., 2025; Tamburini et al., 2024; Zheng et al., 2025). However, the treatment groups may have differed in disease severity, meaning we cannot conclude that the network changes were directly due to treatment. The clinical analysis shows partial recovery in network density and clustering within the Remission group between W4 and W12, whereas the No remission group shows a continued decline in density. This aligns with literature regarding changes in microbiomes over time in relation to disease course (Schirmer et al., 2018). However, as the bootstrap confidence intervals include zero, we cannot conclude that there is a clear difference in network reorganisation between the groups.

Multiple bacterial genera showed changes in their degree centrality. These included *Eikenella*, *Campylobacter*, *Pediococcus* and *Ruminococcus*. In addition, the Remission group showed more gained edges between W0 and W12. This gives more detail about how the positions of bacterial genera have changed within association networks. This complements previous work on differential microbial associations in IBD (Baldassano & Bassett, 2016; Chen et al., 2020). However, it does not indicate any direct biological interactions or validated clinical biomarkers.

The sensitivity analysis shows similar trends in overall network density when patients with known antibiotic exposure were removed. However, some differences in edge identity are not as pronounced. This indicates that the comparisons may be somewhat cohort-dependent. In addition, the sample sizes for each treatment group were relatively small, and there could be confounding

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factors affecting the results. The association networks are correlational and therefore do not indicate direct interactions, meaning further validation will be needed to understand the biological and clinical relevance of these changes.

5. CONCLUSIONS

The PROTECT cohort analysis demonstrates changes in the microbial association networks in pediatric patients with ulcerative colitis. From W0 to W52, network density, clustering coefficient and spectral radius decreased whilst network fragmentation increased. Additionally, the identity of microbial connections changed.

There was variation in the network changes depending on the initial treatment group. The CS-IV group showed the greatest change in edge identity compared to baseline. Patients who achieved corticosteroid-free remission at W52 had a partial recovery in network density from W4 to W12. However, the No remission group continued to show a decline in density. Several bacterial genera had changes in degree centrality. The bootstrap results, however, did not provide enough evidence to demonstrate a clear difference between the two remission groups.

Overall, we observe similar trends in network density even after removing patients with recorded antibiotic exposure. This indicates that considering network-level characteristics alongside edge identity can provide additional information. Further work in independent cohorts will be required to investigate the biological and clinical relevance of these changes.

6. STUDY LIMITATIONS AND FUTURE RESEARCH

This study had some limitations. The microbial correlations were calculated using Spearman correlation at the group level and therefore represented statistical associations rather than direct biological interactions. There were small samples within each treatment group and unequal intervals between visits, which may have influenced the results. Disease severity and antibiotic use could have been confounding variables. There was insufficient statistical evidence to conclude that some differences between groups were significant.

Future work could include validating the findings in independent cohorts as well as looking into patient-specific networks. Bringing clinical data together with functional microbiome data could provide more insight into the biological relevance of network changes in microbial communities and how they relate to disease course.

DATA AVAILABILITY STATEMENT

The publicly available microbiome data used in this study were obtained from the PROTECT cohort (Schirmer et al., 2018). The processed OTU profiles and sample metadata were retrieved from Supplementary Table S6 of the original publication. The raw sequencing data are also available via the NCBI Sequence Read Archive (SRA) under BioProject PRJNA436359.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

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