Microbiome Survey of the Inflamed and Noninflamed Gut at Different Compartments Within the Gastrointestinal Tract of Inflammatory Bowel Disease Patients

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Background: We aimed to contrast the mucosal microbiota in Crohn's disease (CD) and ulcerative colitis (UC).

Methods: We assessed the concept of localized dysbiosis by comparing the bacterial communities of inflamed and noninflamed mucosa of patients with inflammatory bowel disease (IBD) and by analysis of the microbiota composition at distinct gut compartments (ileum, cecum, mid-colon, and rectum). We performed 16S rDNA sequencing to analyze population structures. Quality control and operational taxonomic unit classification of reads were performed using mothur with statistical analyses executed in the R package, phyloseq.

Results: There was no variation in any phyla or genera comparing inflamed to noninflamed mucosa within CD (or UC) or when comparing different gut compartments within CD (or UC). There were differences between the inflamed and noninflamed mucosa between CD and UC: analysis of the inflamed IBD gut at the phylum level indicated that Bacteroidetes ($P = 0.002$) and Fusobacteria ($P < 0.05$) were detected more frequently in inflamed CD mucosa than in inflamed UC mucosa. Conversely, Proteobacteria and Firmicutes ($P < 0.05$) were more frequently observed in the inflamed UC mucosa. At the genus level, the abundance of *Faecalibacterium* ($P \leq 0.05$), *Bacteroides* ($P = 0.003$), and *Pseudomonas* ($P < 0.001$) were significantly different between the inflamed CD and UC and the abundance of 13 genera were significantly different within the noninflamed mucosa. The noninflamed UC mucosa was the most different from non-IBD mucosa.

Conclusions: Dramatic shifts of microbial communities were not observed between the noninflamed and inflamed mucosa within CD (or UC) although both the inflamed (and noninflamed) mucosa was different between CD and UC.

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Key Words: Crohn's disease, ulcerative colitis, gut dysbiosis, microbiome, bioinformatics

Inflammatory bowel disease (IBD) represents a spectrum of chronic, intestinal immune-mediated inflammatory diseases of unknown origin. It is thought to occur in genetically susceptible individuals exposed to particular environmental, dietary, and microbial triggers collectively perpetuating immune dysregulation.[1] In the past decade, the theory of gut dysbiosis (abnormal dominance of particular microbes and a parallel depletion of others) has developed as a pivotal pathogenic focus in IBD. A growing body of literature profiling the microbial community structure in IBD has identified characteristic compositional shifts of the gut microbiota with respect to bacterial microbes and more recently viral and fungal counterparts.[2,3] For example, a number of alterations of the bacterial microbiota that have been associated with IBD include a reduction of α-diversity and a decrease in Firmicutes, Clostridia, Ruminococcaceae, *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, *Roseburia*, *Odoribacter*, *Anaerostipes*, and *Faecalibacterium prausnitzii*.[4] Additionally, an increase of Gammaproteobacteria and the presence of (adherent-invasive) *Escherichia coli*, *Fusobacterium*, and *Clostridium difficile* have been observed.[5–9] Although the gut microbiome likely plays a role in the development of IBD, the interactive role in disease is poorly understood and is further complicated by inconsistencies among the literature.[10] An important consideration is whether the sample was of mucosal biopsy or stool origin—extreme microbial differences are recognized between the 2 communities.[8] Furthermore, there is much to be learned about the contrast in pathogenesis between CD and UC, and it is plausible that differences in the gut microbiome may be important.

Relatively few studies have specifically investigated the compositional differences among inflamed and noninflamed mucosa of the gastrointestinal tract in CD or UC.[11–13] Likewise, there is a paucity of studies profiling taxonomic shifts between distinct anatomical locations of the gastrointestinal tract. In the present study, we aimed to address these gaps and determine whether the signatures of microbial dysbiosis in CD and UC vary between distinct mucosal inflammatory states and anatomical sites of the gastrointestinal tract.

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To our knowledge, this is the largest 16S rDNA amplicon-based analysis investigating plausible microbial distinctions between both inflammatory states and locations of the gastrointestinal tract.

MATERIALS AND METHODS

Patients and Acquisition of Tissue Specimen

In all, 3 to 4 biopsies were collected from CD, UC, and non-IBD participants at colonoscopy from the ileum, cecum, mid-colon, and/or rectum for a total of 166 specimens. The specimens were histologically defined as inflamed or noninflamed as per the clinical pathologist. Non-IBD participants were undergoing colonoscopy either for screening or for other gastrointestinal complaints unrelated to IBD. Biopsies were immediately snap frozen in cryovials without preservative in liquid nitrogen and transferred to a −70°C freezer. Participant characteristics are described in Table 1.

DNA Extraction

Genomic DNA was extracted from tissue samples using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The DNA concentration and purity were assessed by microspectrophotometry (Beckman DU/800; Beckman Coulter, Inc., Fullerton, CA). Genomic DNA was diluted to a concentration of 20 ng/μL.

Sequencing

The bacterial 16S rRNA genes were amplified with primers 967 to 985 (CAACGCGARGAACCTTACC) and 1078 to 1061 (ACAACACGAGCTGACGAC) targeting the V6 hypervariable region (58 bp) as described elsewhere.[14] The reads were generated as part of a multiplexed sequencing run.[15] Sequencing was conducted at Research and Testing Laboratory (Lubbock, TX; http://Researchandtesting.com) using the Illumina HiSeq platform.

Comparative Sequence Analysis

Paired-end reads (2 × 100 bp) were assembled with the FLASH assembler[16] within the galaxy framework.[17] Contig assembly generated 23,789,945 raw reads. Quality control and taxonomic profiling were conducted using mothur (v.1.34.0),[18] a suite of tools for microbial community investigation. Barcodes and primers were removed, and low-quality reads were filtered based on an average quality score <20, having read lengths >80 bp, containing hompolymers >8 nucleotides, containing any ambiguous base calls or identified as a chimeric artifact. Reads were aligned against the 16S rDNA SILVA database.[19] Sequencing noise was reduced by clustering reads that differ by only 1 bp. The remaining reads were binned into species-level (≥97% sequence similarity) operational taxonomic units (OTUs) using the average neighbor algorithm, and taxonomic classification was performed using the SILVA database with Ribosomal Database Project taxonomy and a 50% minimum bootstrap.[20]

Statistical Analysis

Analyses were executed using the R package (http://www.r-project.org) phyloseq.[21] Samples with <1000 reads, and OTUs present in <5 samples were removed. OTUs were normalized to relative abundance. The data were filtered to include only taxa with a mean abundance of $>10^{-3}$ across all samples. Statistical significance of community differences was tested using customized R-scripts; the Kruskal–Wallis one-way analysis of variance was applied to microbiota data and community estimates to compare median similarities. *P*-values were corrected for multiple comparisons using false discovery rate and were considered significant at $P < 0.05$.

RESULTS

Community Characteristics of the Microbiota

Filtering out low-quality, chimeric and non-bacterial reads generated 22,913,986 high-quality 16S rDNA reads with an average amplicon length of 74 bp. Sequences were clustered into 13,040 OTUs based on their shared sequence similarity at a 97% threshold (3% sequence divergence). The average number of reads per sample was 181,806; 40 of the 166 samples were

TABLE 1. Patient and Biopsy Characteristics at the Time of Sampling

Disease (N)	Average Disease Duration, yrs	Male/Female	Biopsy Location (N)	Inflamed/Noninflamed
CD (15)	10.24 ± 6.9	8/7	Ileum (13)	9/4
			Cecum (5)	2/3
			Mid-colon (24)	6/18
			Rectum (15)	1/14
UC (21)	10.14 ± 8.8	8/13	Ileum (19)	1/18
			Cecum (20)	1/19
			Mid-colon (22)	10/12
			Rectum (21)	14/7
non-IBD (7)	-	3/4	Ileum (6)	—
			Cecum (6)	—
			Mid-colon (8)	—
			Rectum (7)	—

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excluded from statistical analysis because of insufficient read numbers (<1000). The average coverage for OTU characterization based on Good's coverage was 99.5%.

Using a number of different measures (Shannon, Simpson, inverse-Simpson) to survey bacterial α-diversity, we found that overall there were significant differences between particular inflammatory states within each disease group using each diversity index (Fig. 1). Significant differences ($P < 0.05$) were observed when comparing noninflamed tissues from CD and UC and likewise between inflamed tissues of UC to non-IBD. In general, the diversity of inflamed tissues of UC (or non-IBD) was higher than inflamed tissues of CD, although these trends did not reach statistical significance. Comparable diversity was observed between anatomical sites of a specific disease (CD or UC) regardless of inflammatory state and anatomical sites when compared between diseases (data now shown). However, microbial diversity was noticeably lowest in the cecum when comparing gastrointestinal tract sites of CD. Trends were observed in cecal (UC > non-IBD > CD) and mid-colonic diversity (non-IBD > UC > CD).

Significant differences ($P < 0.05$) in community richness (Chao1 and ACE) when considering the inflammatory state of tissue were observed and presented a noteworthy trend (CD non-inflamed > CD inflamed > UC non-inflamed > non-IBD > UC inflamed). Significant differences ($P < 0.05$) of community richness were observed between non-inflamed tissues of CD and UC, and inflamed UC and non-IBD tissues. We observed an interesting pattern in richness within the CD cohort: richness tended to be lowest in the ileum and increased distally to the mid-colon. Furthermore, within each gut compartment (ileum, cecum, mid-colon, and rectum) richness was higher in IBD compared with controls and greater in CD than in UC.

Bacterial Population Comparisons

Bacterial Community Structure of the Mucosal Microbiota

To compare the overall structure of the mucosal microbiota between (1) CD, UC, and non-IBD; (2) inflammatory states; (3) anatomical sites of the gastrointestinal tract; and (4) patients, we visualized Bray–Curtis distances between samples using principle coordinate analysis (PCoA; Fig. 2). The results of PCoA indicate a difference in structure of the microbiota between CD, UC, and non-IBD. Although to an extent, overlap of all disease groups was observed, samples tended to cluster together based on disease. PCoA of inflammatory states overlapped and could not be well separated; however, cluster analysis of only inflamed tissues from CD and UC shows clear separation as did the noninflamed tissues (data not shown). Inflamed and noninflamed biopsies of CD or UC share the characteristic that there are no structural patterns apparent. There was also no separation based on the anatomical site of the biopsy. Cluster analysis indicates that the overall gut microbiota composition are more dependent on interindividual variation rather than disease. Our results indicate that tissues from the same participant are most similar regardless of the inflammatory state and/or sampling location of the specimen.

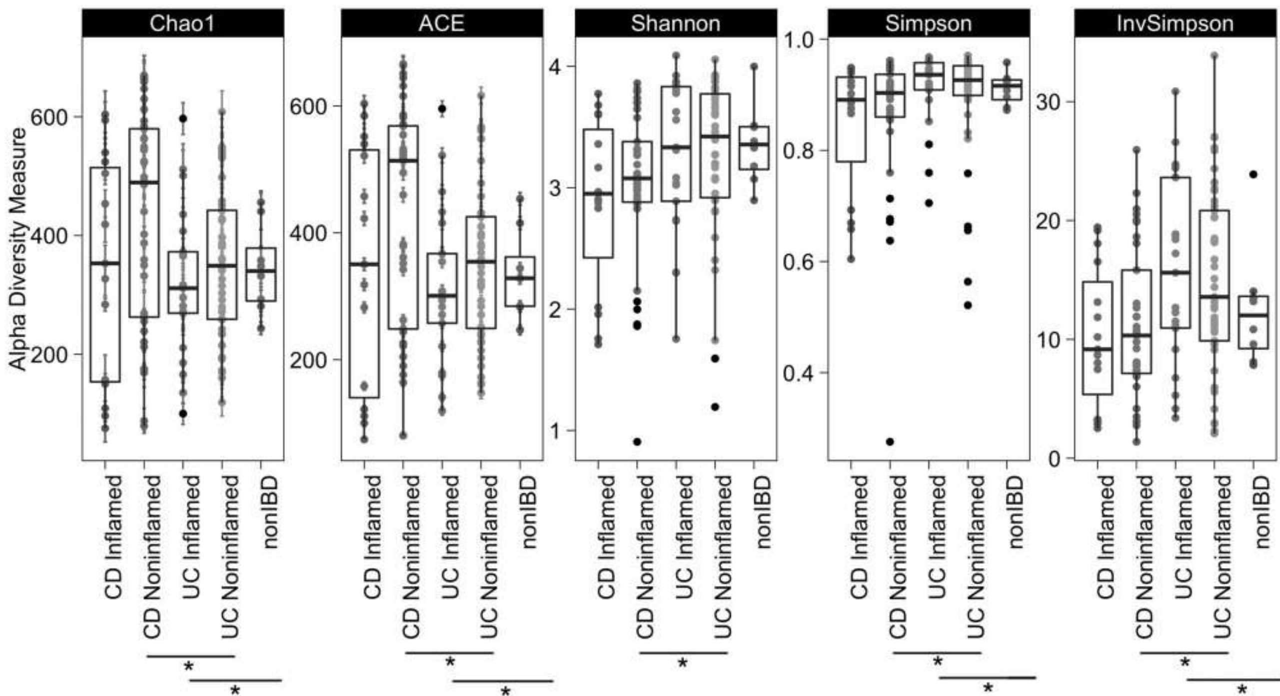

FIGURE 1. Plot of community richness (ACE, Chao1) and diversity (Shannon, Simpson, inverse-Simpson) variation between disease group inflammatory states. Differences considered significant at *$P < 0.05$.

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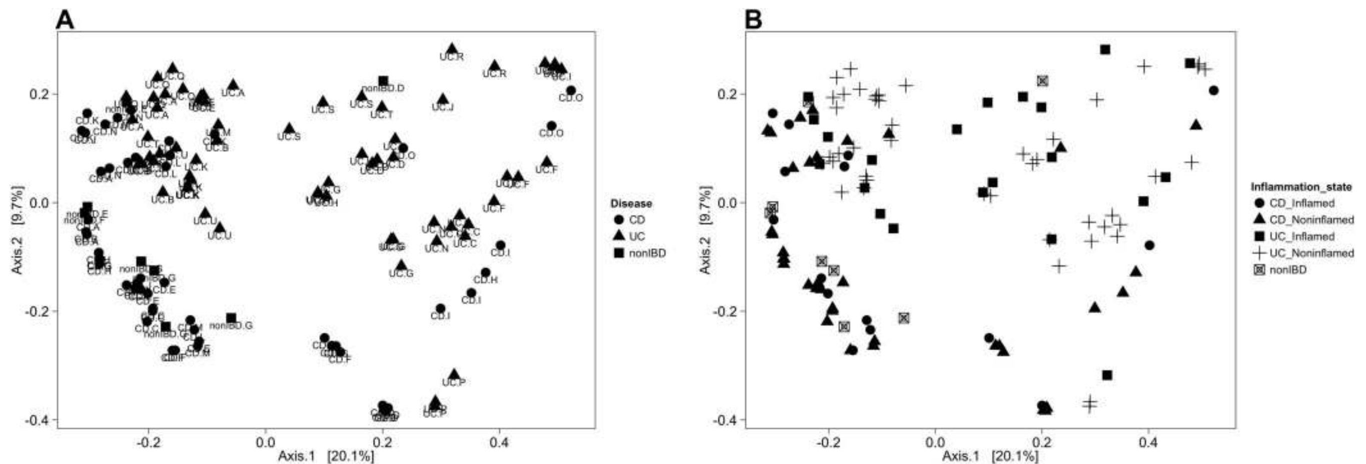

FIGURE 2. PCoA based on the overall structure of the mucosal microbiota in all biopsy samples. Each data point represents an individual sample. PCoA was calculated using Bray–Curtis distances. Symbol is indicative of (A) disease group and (B) inflammatory state of the biopsies. Data points are labeled by subject disease type.

Overall Taxonomic Classification of 16S Reads

The filtered data included 131 OTUs (clustered at >97% sequence identity) that were assigned to 6 bacterial phyla. The Firmicutes (47.0%) and Bacteroidetes (36.0%) were the most diverse phyla as the bulk of genera identified belonged to one of these groups. We identified the genus *Bacteroides* to be the most abundant bacterial genus accounting for 28.7% of reads. Proteobacteria represented 13.1% of reads, of which *Escherichia–Shigella* (which are indistinguishable as a 16S-based phylotype) accounted for 5.6%. The remaining phyla were detected in a much lesser capacity: Verrucomicrobia (1.9%), Actinobacteria (1.2%), and Fusobacteria (0.3%). A total of 0.5% of reads could not be mapped to the phylum level of taxonomy.

Mucosal Microbial Community Analysis in Distinct Inflammatory States of IBD

A taxonomy-based comparison was performed to accurately determine if a localized dysbiosis occurs in the inflamed tissue of IBD participants relative to noninflamed (healthy) tissue in diseased patients. In both CD and UC, phylum-level analyses (Table 2) revealed no significant variation in any phyla. Furthermore, the relative abundance of all genera was comparable among inflamed and noninflamed tissue in either disease group. *Escherichia–Shigella* was numerically more abundant in inflamed CD tissue, *Pseudomonas* in noninflamed CD tissue, and *Sporacetigenium* in noninflamed UC tissue (data not shown).

Taxonomic Comparisons Between the Microbiota of IBD Inflamed Tissues

The Bacteroidetes and Fusobacteria were more commonly detected in inflamed CD tissue relative to inflamed UC tissue, which demonstrated a corresponding increase in Firmicutes and Proteobacteria ($P \leq 0.05$). Comparison of noninflamed tissue revealed similar patterns, with the exception of Fusobacteria that was found to be similar between both groups.

Overall, 131 OTUs representing 34 classified genera were detected in our data. The abundance of 13 genera (*Clostridium*, *Enterococcus*, *Faecalibacterium*, *Phascolarctobacterium*, *Sporacetigenium*, *Streptococcus*, *Turicibacter*, *Alistipes*, *Bacteroides*, *Haemophilus*, *Klebsiella*, and *Actinomyces*) was significantly different after correction for multiple testing between the noninflamed tissues of CD and UC (Table 2). The differential abundance of only 3 genera (*Faecalibacterium*, *Bacteroides*, and *Pseudomonas*) reached statistical significance among inflamed tissues of CD and UC.

Taxonomic Comparisons Between the Microbiota of IBD Tissues to Non-IBD

We next surveyed the bacterial community distributions of IBD to non-IBD (Table 2). Analysis at the phylum level suggests that regardless of the inflammatory nature of the tissue, the microbial communities in CD are more similar to non-IBD, whereas UC was less similar to non-IBD. For example, only the abundance of Fusobacteria was nominally increased in inflamed CD tissues ($P = 0.04$), although this did not remain significant following correction for multiple testing. A significant decrease in Bacteroidetes and a proportional increase in Firmicutes and Proteobacteria were observed between both inflamed and noninflamed UC to non-IBD; the differences in abundance were more dramatic between noninflamed UC tissues and non-IBD.

The noninflamed UC group was the most different based on overall community structure. At a nominal significance ($P \leq 0.05$), the abundance of 15 genera were different, with 10 (*Actinomyces*, *Alistipes*, *Bacteroides*, *Dorea*, *Marinobacter*, *Oscillibacter*, *Parabacteroides*, *Phascolarctobacterium*, *Prevotella*, and *Pseudomonas*) maintaining significance after false discovery rate correction for multiple comparisons ($P \leq 0.05$; Table 2). Likewise with the inflamed UC group, a total of 11 genera demonstrated nominal significance with only 3 (*Alistipes*, *Parabacteroides*, and *Pseudomonas*) holding significance after correction.

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TABLE 2. Microbial Distribution of Taxa (Phylum and Genus) Found to Be Differentially Abundant According to Disease and Inflammatory State of Biopsy

	Inflamed	Noninflamed	CD			UC		
	CD/UC	CD/UC	Inflamed	Noninflamed	Inflamed/Noninflamed	Inflamed	Noninflamed	Inflamed/Noninflamed
Firmicutes	a	c					↑[b]	
Clostridium		a						
Coprococcus				↓[a]				
Dorea							↓[a]	
Enterococcus		a						
Faecalibacterium	a	b						
Oscillibacter				↓[a]			↓[a]	
Phascolarctobacterium		a					↓[a]	
Sporacetigenium		a						
Streptococcus		c						
Turicibacter		a						
Bacteroidetes	b	c				↓[b]	↓[c]	
Alistipes		b				↓[a]	↓[a]	
Bacteroides	b	c					↓[a]	
Parabacteroides						↓[a]	↓[a]	
Prevotella							↓[a]	
Proteobacteria	a	c				↑[a]	↑[b]	
Haemophilus		b						
Klebsiella		b						
Marinobacter							↑[a]	
Pseudomonas	c	c				↑[a]	↑[a]	
Verrucomicrobia								
Actinobacteria								
Actinomyces		c					↑[a]	
Fusobacteria	a							

↑ and ↓ relative to non-IBD.
[a]$P < 0.05$.
[b]$P < 0.01$.
[c]$P < 0.001$.

In comparisons between bacterial communities of noninflamed CD relative to non-IBD, we found significant differences in *Coprococcus* and *Oscillibacter*. Particular communities of the inflamed CD mucosa demonstrated only nominal significance ($P \leq 0.05$), including *Alistipes*, *Coprococcus*, *Faecalibacterium*, *Marinobacter*, *Paracteroides*, and *Subdoligranulum*, although significance was not retained after false discovery rate correction.

Comparative Analysis of Gut Compartments Within CD and UC

In analyses comparing genera from distinct anatomical gastrointestinal tract sites within each disease group, there were no significant differences in the average abundances. Figure 3 shows that regardless of the genera in either CD or UC, their abundance was uniform across all sites with a few exceptions: *Escherichia–Shigella* in CD appears to be numerically highest in the cecum and *Pseudomonas* in CD also appears to be numerically highest in the rectum.

Comparative Analysis of Gut Compartments Between Diseases

We also used a taxonomy-based analysis to recognize particular phyla or genera found to be differentially represented in tissues of the ileum, cecum, mid-colon, or rectum between distinct disease groups (Fig. 4). We have demonstrated that the abundance of Bacteroidetes significantly varies between disease groups and that their degree of variability is dependent on particular compartments of the gastrointestinal tract. For example, the disproportional representation of Bacteroidetes is most clear in ileal and mid-colonic tissue, followed by rectal tissue. We have also shown the abundance of Firmicutes to vary between disease groups in ileal and mid-colonic tissue. Similarly, the Proteobacteria were disproportionally abundant

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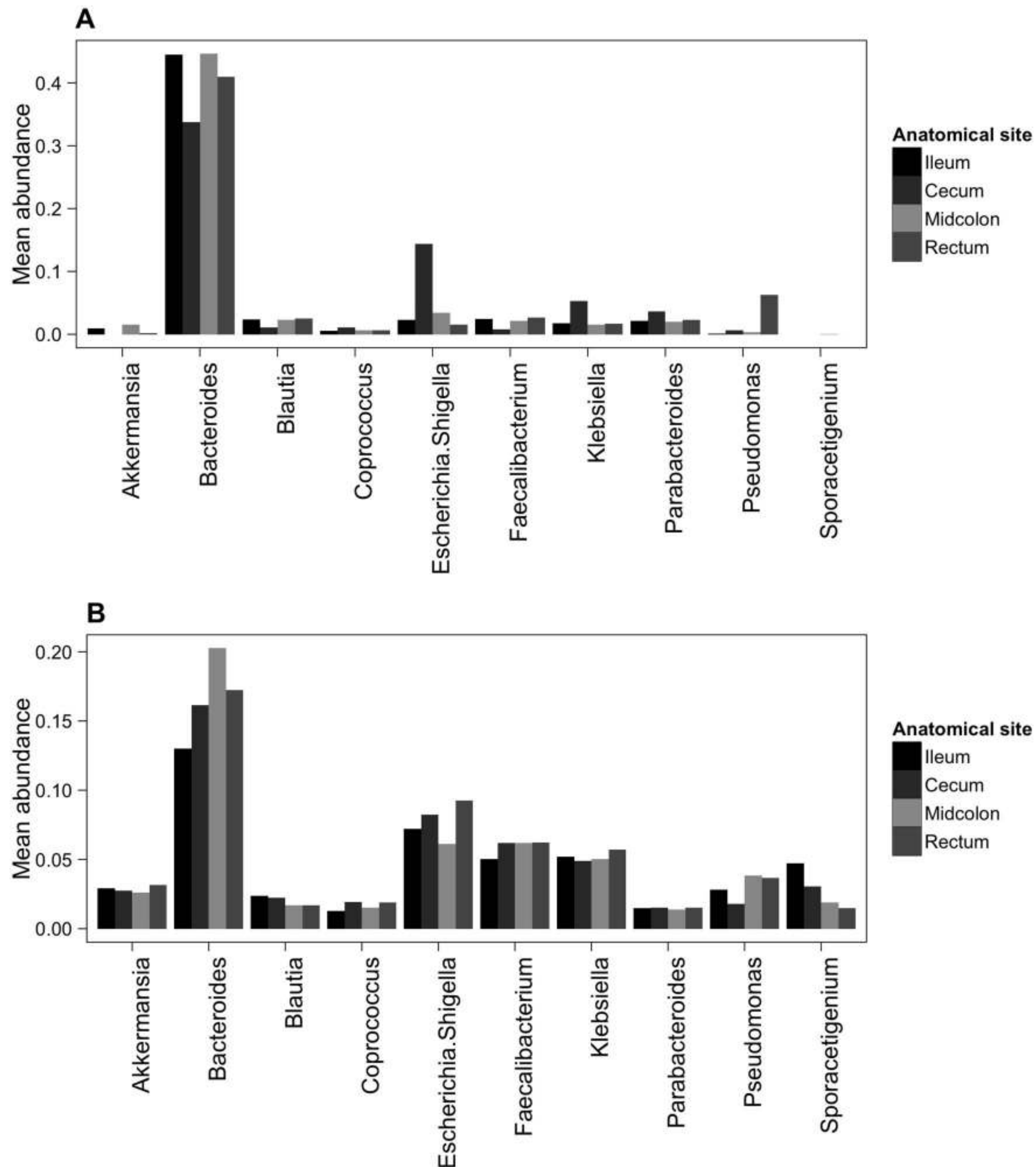

FIGURE 3. Genus-level comparisons between 4 anatomical sites of sampling (the ileum, cecum, mid-colon, and rectum). Genera shown represent the 10 most abundant genera of CD and UC biopsies. A, Average abundance of genera in CD. B, Average abundance of genera in UC.

within ileal, mid-colonic, and rectal tissue. Of importance, no significant variability was observed between disease groups within the cecal compartment. Additionally, we have identified significant differences between genera, including *Bacteroides* and *Pseudomonas* among the ileal, mid-colonic, and rectal tissues (Fig. 5).

DISCUSSION

An alteration of the microbiome composition and its interaction with the host immunological response likely plays a critical role in the pathogenesis of IBD; however, this relationship is poorly understood. This study shows important differences in the bacterial profiles of the mucosal microbiota between participants with CD, UC, or non-IBD controls based on analysis of mucosal tissue using a 16S rDNA amplicon-based analysis. To our knowledge, our study represents one of the largest 16S rDNA amplicon-based studies investigating the mucosal microbiota composition in distinct inflammatory states of particular compartments within the gastrointestinal tract of persons with IBD.

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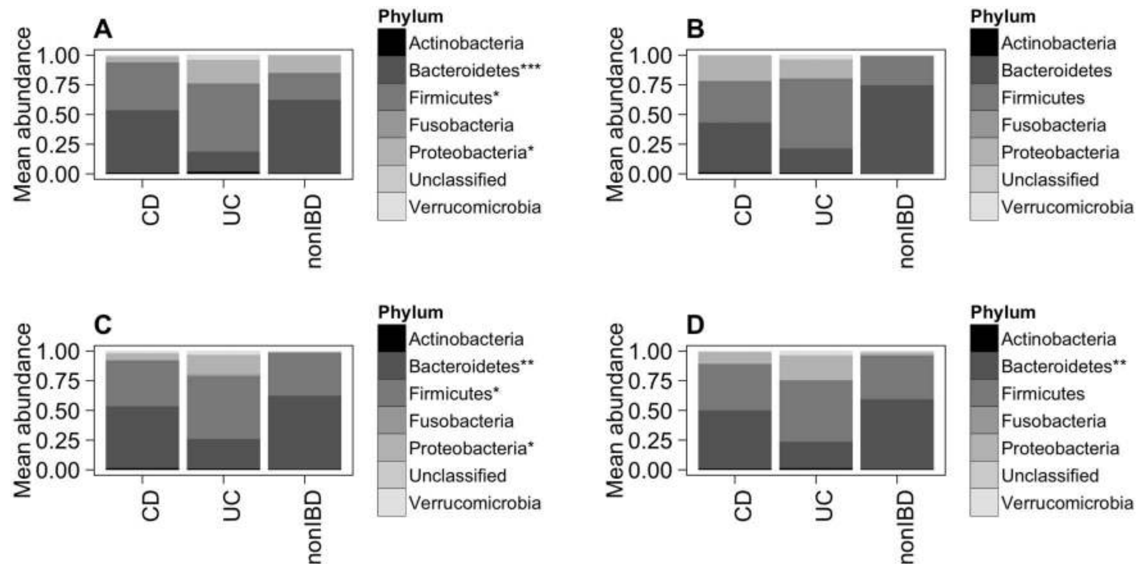

FIGURE 4. Phylum-level comparisons between 3 disease groups (CD, UC, and non-IBD) within the (A) ileum (B) cecum (C) mid-colon and (D) rectum. Differences considered significant at $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Our principal and most surprising finding is that the mucosal microbiota of inflamed and noninflamed regions of the gastrointestinal tract in CD or UC, respectively, were indistinguishable, as virtually no taxa demonstrated disproportional abundances at a significant threshold nor were there significant diversity differences observed. Thus, as we were unable to recognize a specific microbe or group of microbes to consistently associate with the inflamed (or noninflamed) tissue in CD or in UC. Our results suggest that important localized changes in the mucosally associated microbiota do not exist between the inflamed and noninflamed gut. These findings argue against the hypothesis that inflammation drives gut dysbiosis and instead promotes the notion that gut dysbiosis is a prerequisite for inflammation. This is further supported by the clinical findings that resecting CD tissue will

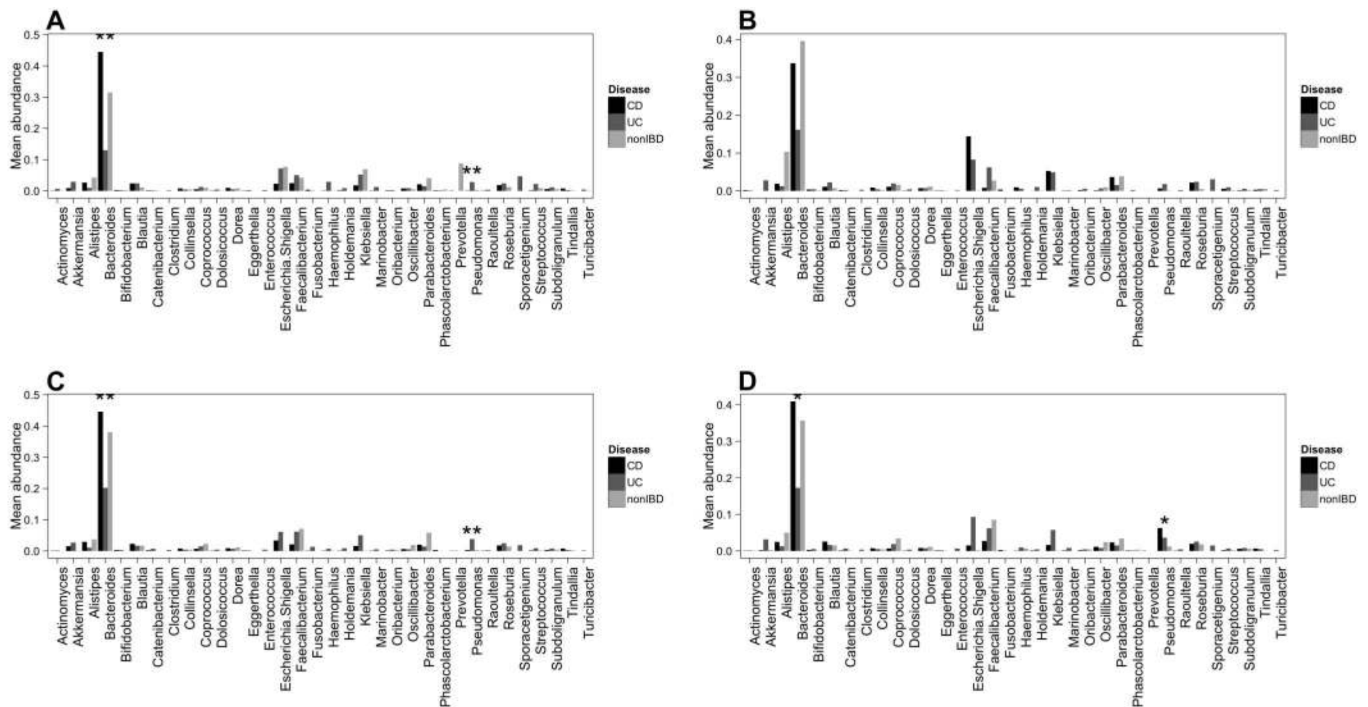

FIGURE 5. Genus-level comparisons between 3 disease groups (CD, UC, and non-IBD) within the (A) ileum (B) cecum (C) mid-colon and (D) rectum. Differences considered significant at $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

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lead to recurrences in the previously uninflamed tissue.[22] This would also occur in UC although segmental resections in UC have long been abandoned because of the very high recurrence in the tissue remaining behind. Although these microbial patterns may not be causal, they seem to be signature patterns for each disease. As the participants all had IBD for a lengthy duration and hence findings are associations and could not be construed as causal; it is noteworthy that within an individual, patterns were similar regardless of the inflammatory state. Since CD participants had consistent patterns as a group, the implication is that these really are unique to the entire extent of the distal gastrointestinal tract of a person with CD (or UC). Even if not causal, it is plausible that altering this pattern could impact on disease course.

Few studies have reported a localized dysbiosis within the inflamed gut.[23,24] Mylonaki et al[23] noted a reduction of *Bifidobacterium* and an increase of *E. coli* in the epithelial cell surface of inflamed UC colorectal mucosa compared with noninflamed UC mucosa. *Lactobacillus* and *Bacteroides* were found in similar abundances between the 2 groups. Similar observations were reported for inflamed CD colorectal mucosa. Although Sepehri et al[24] described an increase of Firmicutes and parallel reduction of Bacteroidetes in addition to significant differences in community richness and biodiversity, inflamed (and noninflamed) biopsies were not separated on the basis of the disease type (i.e., CD and UC). These studies used molecular methods that include fluorescent in situ hybridization, automated ribosomal intergenic spacer analysis, and terminal restriction fragment length polymorphism, which are less discriminatory methods compared with our approach yielding a greater sequencing depth and taxonomic resolution. Several additional studies have taxonomically surveyed the inflamed and noninflamed gut in IBD, most of which agree with our data indicating an absence of a localized dysbiosis.[12,13,25–27]

Although our study strengthens the notion that dysbiosis of the overall gut microbiota in CD or UC is testament to either disease, we also performed comparative analyses to determine the dysbiosis profiles between the inflamed gut of CD to UC and similarly with the non-inflamed gut. Our data indicated that both the inflamed and noninflamed gut has unique taxonomic profiles when compared across disease types and that, importantly, those differences are more apparent within the noninflamed gut. In active disease, CD and UC clinically differ based on the formation of strictures and fistulas in CD, hence it is theoretically compelling that the microbiotas of the inflamed mucosa are less different than the noninflamed mucosa, demonstrating no evidence of active disease. Functional divergence between the inflamed and noninflamed IBD mucosal microbiota has recently been identified.[11] In UC, carbohydrate and nucleotide metabolism were increased in the noninflamed mucosa, whereas amino acid and lipid metabolism were decreased; in CD, an increased abundance of genes for energy metabolism and nervous system pathways were reported in the inflamed mucosa. Since we observed greater community differences among the noninflamed or inflamed IBD gastrointestinal tracts than between the inflamed and noninflamed CD (or UC) mucosa, there may be additional systemic perturbations of host–bacteria interactions in CD (or UC) that is independent of the inflammation status of the mucosa.

A seminal study performed by Eckburg et al[28] demonstrated microbial homogeneity between mucosal sites of the large intestine of healthy individuals which has been replicated in both health and IBD.[29] Our data corroborates previously reported findings of gut microbial stability in CD and UC while considering distinct inflammatory regions of the bowel. Although all microbial populations demonstrated fairly consistent abundances across gut compartments, in CD, *Escherichia–Shigella* was notably highest in the cecum, and *Pseudomonas* in the rectum. We also compared the microbiota composition of gut anatomical sites between disease groups. Our main findings include the significant difference of *Bacteroides* and *Pseudomonas* among ileal, midcolonic, and rectal specimens.

This study confirms previously noted findings regarding the presence of particular bacteria. For example, a number of the microbes identified in this study are recognized to have the ability to adhere to the mucosa, invade intestinal epithelial cells, and possibly exacerbate inflammation, including *Escherichia*[5] and *Fusobacterium.*[30] The average abundance of both of these taxa was lowest in non-IBD participants. In fact, the presence of *Fusobacterium* was only identified in the rectal tissue of one non-IBD participant. Several groups of microbes including *Bifidobacterium*, *Lactobacillus*, and *Faecalibacterium* have been shown to exhibit protective effects via inflammatory cytokine regulation[31] or, conversely, stimulation of interleukin 10.[32] We also detected a few butyrate-producing bacteria in our data;[33] *Coprococcus* and *Oscillibacter* were reduced relative to non-IBD. Butyrate and other short-chain fatty acids are the result of bacterial metabolism.[34] Short-chain fatty acids are essential sources of energy for gut epithelial cells, and their reduction in IBD may perturb epithelial barrier integrity and modulate the gut immunological response thereby exacerbating disease.[34,35]

Our study presents a comprehensive analysis of the mucosal gut microbiota in IBD while investigating a possible localized dysbiosis in inflamed or noninflamed mucosa at distinct gut compartments. Although we conclude that the composition of the mucosal microbiota was not altered by the presence of inflammation, we were able to identify an interesting phenomenon whereby the microbiota of the noninflamed gut in IBD are more diverse than the microbiota of the inflamed gut. We have identified several microbes and suggest their disproportional abundances contribute to gut dysbiosis within the inflamed or noninflamed mucosa.

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