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# Characteristics of the Gut Microbiome and Its Relationship With Peripheral CD4$^+$ T Cell Subpopulations and Cytokines in Rheumatoid Arthritis

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This study investigated the association between intestinal microbiota abundance and diversity and cluster of differentiation (CD)4$^+$ T cell subpopulations, cytokine levels, and disease activity in rheumatoid arthritis RA. A total of 108 rheumatoid arthritis (RA) patients and 99 healthy control (HC) subjects were recruited. PICRUSt2 was used for functional metagenomic predictions. Absolute counts of peripheral CD4$^+$ T cell subpopulations and cytokine levels were detected by flow cytometry and with a cytokine bead array, respectively. Correlations were analyzed with the Spearman rank correlation test. The results showed that the diversity of intestinal microbiota was decreased in RA patients compared to HCs. At the phylum level, the abundance of Firmicutes, Fusobacteriota, and Bacteroidota was decreased while that of Actinobacteria and Proteobacteria was increased and at the genus level, the abundance of *Faecalibacterium*, *Blautia*, and *Escherichia-Shigella* was increased while that of *Bacteroides* and *Coprococcus* was decreased in RA patients compared to HC subjects. The linear discriminant analysis effect size indicated that *Bifidobacterium* was the most significant genus in RA. The most highly enriched Kyoto Encyclopedia of Genes and Genomes pathway in RA patients was amino acid metabolism. The relative abundance of *Megamonas*, *Monoglobus*, and *Prevotella* was positively correlated with CD4$^+$ T cell counts and cytokine levels; and the relative numbers of regulatory T cells (Tregs) and T helper (Th17)/Treg ratio were negatively correlated with disease activity in RA. These results suggest that dysbiosis of certain bacterial lineages and alterations in gut microbiota metabolism lead to changes in the host immune profile that contribute to RA pathogenesis.

**Keywords: rheumatoid arthritis, gut microbiota, cytokines, CD4, T cells, immune system diseases, DAS28**

## INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by irreversible peripheral joint damage (England et al., 2018). The etiopathogenic mechanism of RA involves abnormal immune activation with various autoantibodies, an imbalance of lymphocyte subpopulations, and cytokine dysregulation.

Alterations in the gut microbiome profile are associated with immune dysfunction in several rheumatic diseases (Li and Wang, 2021). The abundance of intestinal microbiota is known to differ between RA patients and healthy subjects (Vaahtovuo et al., 2008; Scher et al., 2013; Taneja, 2014). Moreover, gut dysbiosis may negatively affect immune function in RA (Li and Wang, 2021). Interaction between gut microbiota and the host is important for maintaining immune homeostasis (Chu and Mazmanian, 2013). Gut bacteria have been shown to influence the polarization of lymphocyte subpopulations and their cytokines and thereby regulate immune functions (Abdulla et al., 2021).

Dysbiosis in the gut microbiome is associated with various autoimmune diseases, and the detailed mechanisms of how changes in the abundance and diversity of these microorganisms contribute to disease pathogenesis in the host is unknown (Li et al., 2017). To address this question, in this study we investigated the associations between gut microbiota community composition and metabolic pathways, cluster of differentiation (CD)$4^+$ T cell subpopulations, cytokine levels, and disease activity in RA.

## MATERIALS AND METHODS

### Study Population

A total of 108 RA patients (73 females, 35 males) were recruited at the Department of Rheumatology, Second Hospital of Shanxi Medical University (Zhao et al., 2019) between December 2018 and August 2019. No medication was provided to patients, and the RA group specimens were collected on the day of hospitalization. The median age was 52.7 years and the age range was 21–70 years. We also recruited 99 healthy controls (HCs) (68 females, 31 males) from our health examination center (Li Y. et al., 2021). The mean age of healthy volunteers was 56.56 years. There were no statistically significant differences (*t*-test) in the composition of the 2 groups with regard to age, and sex. All patients met the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for RA (Lee and Kim, 2017). Exclusion criteria were subjects who had another autoimmune-related or gastrointestinal tract or inflammatory bowel disease, take any probiotics or antibiotics within 1 month, serious infection, or a malignant tumor within the previous 6 months (Zhang et al., 2021). Each participant signed the consent form. This study was approved by the institutional ethics committee of the Second Affiliated Hospital of Shanxi Medical University (approval no. 2019-YX-107).

We recorded all clinical symptoms and laboratory measures of disease activity including C-reactive protein, erythrocyte sedimentation rate, swollen joint count, and tender joint count; these were used to calculate the disease activity score (DAS28) (Li et al., 2020).

### Fecal Sample Collection and 16S rRNA Gene Sequencing

Fresh fecal samples were collected and immediately stored at −80°C in a sterile box for microbial DNA extraction (Li Y. et al., 2021). After obtaining consent, peripheral blood samples were obtained from 106 patients and 109 patients were tested for cytokine levels. Microbial DNA was extracted from about 250 mg of fecal sample using the QIAamp PowerFecal DNA Kit (Qiagen, Valencia, CA, United States) according to the manufacturer's instructions. It was then quantified by agarose gel electrophoresis on a NanoDrop One spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States) (Ahmad et al., 2019; Li Y. et al., 2021). The primers 341F (CCTACGGGNGGCWGCAG) and 805 R (GACTACHVGGGTATCTAATCC) were used to PCR amplify the V3–V4 region of the 16S rRNA gene (Liang et al., 2017) using KAPA HiFi HotStart Ready Mix (Roche, Indianapolis, IN, United States). The resultant amplicons were sequenced on a MiSeq platform (Illumina, San Diego, CA, United States).

QIIME2 was used to process representative sequence clusters with a similarity cutoff of 100% (Ahmad et al., 2019). Operational taxonomic units (OTUs) were partitioned into taxonomic lineages in the SILVA 16S rDNA database (Frisbee et al., 2019). Based on the rarefied OTUs, α and β diversity were calculated using the R package "PhyloSeq." Shannon (also known as Shannon-Weaver index) (Ott et al., 2014; Gabriel et al., 2019), simpson, and invSimpson index (richness and evenness) and richness (observed OTUs, chao1, ACE) (Ramadan et al., 2021) was used to measure alpha-diversity. Biomarker species were identified based on linear discriminant analysis effect size (LefSe).

### Metagenome Functional Predictions

Microbial functions were predicted using PICRUSt2 (Chen et al., 2020), which contains reference genomes and gene families and provides interoperability with OTU picking and a denoising algorithm that incorporates the Benjamini–Hochberg correction (Muraoka et al., 2020), allowing assignment of OTUs to reference genomes and linking of taxonomic information to Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations (Douglas et al., 2020). PICRUSt2 was used to assess the potential metabolic functions of the gut microbiome in RA (Suárez-Moo et al., 2020).

### Flow Cytometry

$CD4^+$ T cells in whole blood collected in a 3-ml anticoagulant tube were detected using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, United States) (Kojima et al., 1976; Niu et al., 2020). Four multicolor monoclonal antibodies were used for immunofluorescence labeling of circulating $CD4^+$ T cell subpopulations. An 80-μl volume of blood was combined with 10 μl ionomycin, 10 μl phorbol 12-myristate 13-acetate, and 1 μl GolgiStop and incubated at 37°C for 5 h (Niu et al., 2020).

Fluorescein isothiocyanate-conjugated anti-CD4 antibody was added to the tube, followed by incubation for 30 min in the dark at room temperature (20–25°C). The cells were immobilized using Cytofix/Cytoperm reagent and incubated at 4°C for 30 min; they were then labeled with allophycocyanin (APC)-conjugated anti-interferon (IFN)-γ, phycoerythrin (PE)-conjugated anti-interleukin (IL)-17A, and PE-conjugated anti-IL-4 antibodies to detect Th1, Th17, and Th2 cells, respectively, followed by incubation for 30 min in the dark at room temperature (20–25°C). To detect regulatory T cells (Tregs), 80 μl of anticoagulant-treated blood was labeled with APC-conjugated anti-CD25 and PE-conjugated anti-forkhead box (Fox) P3 antibodies. After washing, the samples were analyzed by flow cytometry. The percentages and absolute counts of $CD4^+$ T cell subpopulations were determined using BD Multitest software (BD Biosciences) (Niu et al., 2021).

A flow cytometric bead array (Jiangsu, China) was used to analyze the levels of IL-2, IL-4, IL-6, IL-10, IL-17, tumor necrosis factor (TNF)-α, and IFN-γ, followed by flow cytometry analysis (Chen et al., 2012). The standard curve of each cytokine was in the range of 1–5,000 pg/ml.

## Statistical Analysis

Alpha diversity was compared between groups with Welch's *t*-test. Correlations were evaluated with the Spearman rank correlation test (Anuradha et al., 2017). Differences with $P < 0.05$ were considered statistically significant.

# RESULTS

## Clinical Characteristics of the Study Population

A total of 207 stool samples were collected. The detailed clinical characteristics of the study population are shown in **Table 1**.

## Disease Duration Is Associated With Decreased Microbial Diversity

We obtained 13,147 high-quality OTUs including 12 phyla, 19 classes, 42 orders, 72 families, and 189 genera. The Observed, ACE, Chao1 (richness) and Shannon (richness and evenness) indices showed difference in RA patients compared to HCs ($P < 0.05$) (**Figure 1**), although there was no difference in Simpson index between the 2 groups (**Figure 1**). Above all, gut microbiota was markedly less diverse in composition in RA patients than in the HC group. These indexs lay a foundation for species analyzed. The weighted-UniFrac distance principal coordinate analysis based on OTUs showed that the gut microbial community structure between RA and HC groups differed (**Figure 2**). Helper T cell (Th)2 (**Figure 3A**) and Th17 (**Figure 3B**) counts were negatively correlated with α diversity in RA patients ($P < 0.05$ and $< 0.01$, respectively).

Gut microbial community structure differed between RA and HC groups, as revealed by phylum and genus Bray–Curtis distances (**Figures 4A**, **5A**). At the phylum level, the relative abundance of *Firmicutes*, *Fusobacteriota*, and *Bacteroidota* was decreased ($P < 0.05$) whereas that of *Actinobacteria* and *Proteobacteria* was increased ($P < 0.05$) in RA patients compared to HCs (**Figures 4B,C**). At the genus level, the abundance of *Faecalibacterium*, *Blautia*, *Terrisporobacter*, *Escherichia-Shigella*, and *Fusicatenibacter* was increased while that of *Bacteroides*, *Coprococcus*, and *Parabacteroides* was decreased ($P < 0.05$) in RA patients compared to HCs (**Figures 5B**, **6**).

## Gut Flora Is Altered in Rheumatoid Arthritis

A total of 28 differentially abundant taxa were observed by LEfSe analysis (Mollazadeh et al., 2019). The abundance of phylum *Actinobacteria*, including the genus *Bifidobacterium*, was increased in RA patients compared to HCs. The genus *Dialister* was most closely associated with RA. The *Bacteroidaceae* and *Marinifilaceae* families in phylum *Bacteroidota* were expanded in the HC group; and *Sutterella* and *Escherichia-Shigella* in phylum *Proteobacteria*, which formed separate clusters, were expanded in the RA and HC groups, respectively (**Figures 7A,B**).

## Functional Analysis of Gut Microbes in Rheumatoid Arthritis

PICRUSt2 was used to infer the functions of the significantly different taxa between RA patients and HCs based on 16S rRNA gene sequences. KEGG pathways that were significantly enriched in RA patients included amino acid metabolism (e.g., alanine, aspartate, and glutamate), amino sugar and nucleotide sugar metabolism, β-alanine metabolism, ATP-binding cassette (ABC) transporters, glycolysis/gluconeogenesis, NOD-like receptor signaling

**TABLE 1** | Clinical characteristics of the study population.

| | RA ($n = 108$) | HC ($n = 99$) |
|---|---|---|
| Age, years, mean (median) | 52.56 (53) | 50.44 (50) |
| Female | 67.6% | 68.7% |
| **Disease activity parameters** | | |
| ESR, mm/h, mean (median) | 56.55 (43), 3–124 | |
| CRP, mg/l, mean (median) | 33.01 (13.75), 0–325 | |
| TJC-28, mean (median) | 10.09 (6.5), 0–28 | |
| SJC-28, mean (median) | 0.19 (0), 0–2 | |
| DAS28, mean (median) | 4.51 (4.45), 1.9–7.1 | |
| **Lymphocyte subpopulations** | | |
| Th1, mean (median) | 162.51 (131.38), 11.25–549.26 | |
| Th2, mean (median) | 7.14 (6.23), 3.12–19.06 | |
| Th17, mean (median) | 8.84 (6.76), 1.69–19.22 | |
| Tregs, mean (median) | 31.58 (28.19), 7.93–87.75 | |
| **Cytokines** | | |
| IL-2, mean (median) | 4.67 (3.03), 0–30.55 | |
| IL-4, mean (median) | 5.53 (2.805), 0–82.48 | |
| IL-6, mean (median) | 28.00 (15.77), 0–93.63 | |
| IL-10, mean (median) | 7.01 (5.62), 0–30.2 | |
| IL-17, mean (median) | 18.20 (9.575), 0–57.16 | |
| TNF-α, mean (median) | 6.60 (5.62), 0–27.02 | |
| INF-γ, mean (median) | 10.39 (5.535), 0–77.83 | |

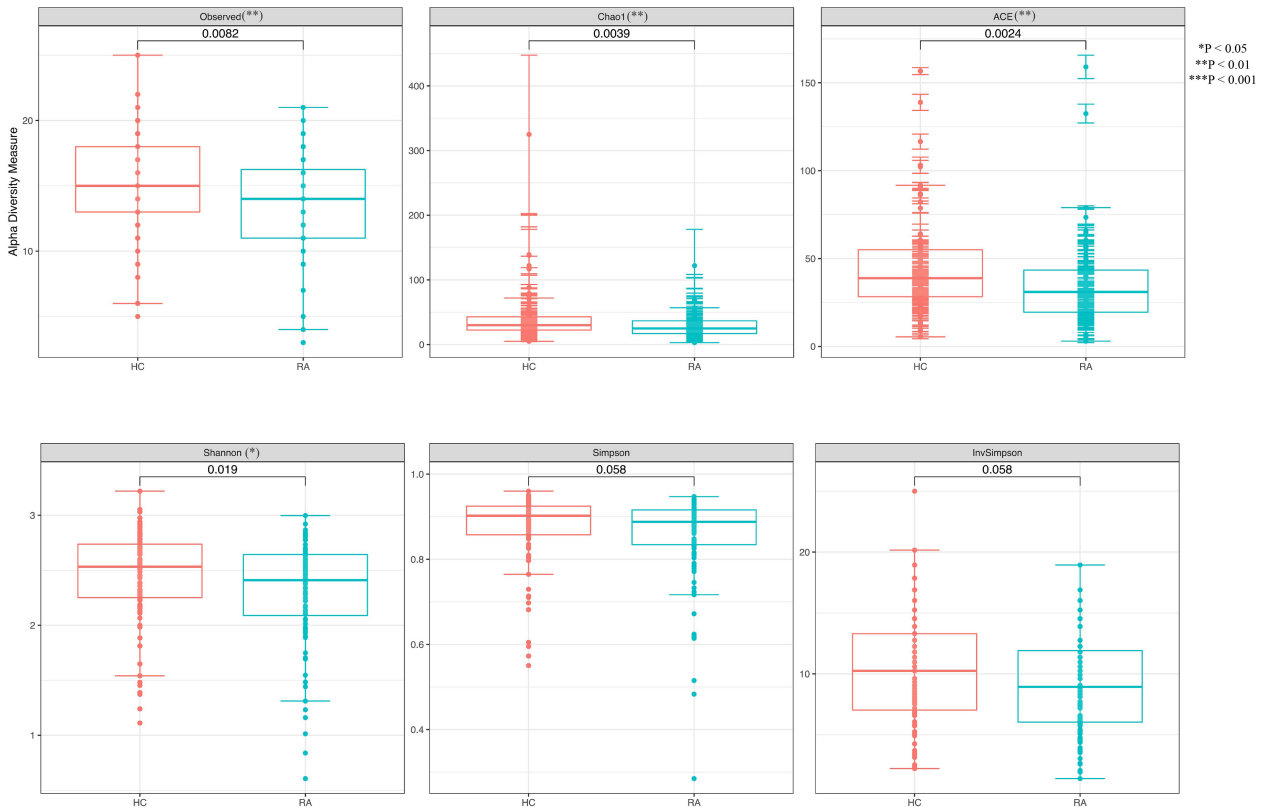


**FIGURE 1 |** Differences in α diversity of the gut microbiome between RA patients and HCs. $^{**}P < 0.01$, $^{***}P < 0.001$.


**FIGURE 2 |** β Diversity of the gut microbiome in RA patients and HCs. Principal coordinate analysis plot generated from the weighted UniFrac analyze. The x and y axes represent the first and second coordinates, respectively; and values in parentheses show the percentage of the community variation explained by each coordinate. Blue and red symbols represent bacteria in the RA and control groups, respectively.

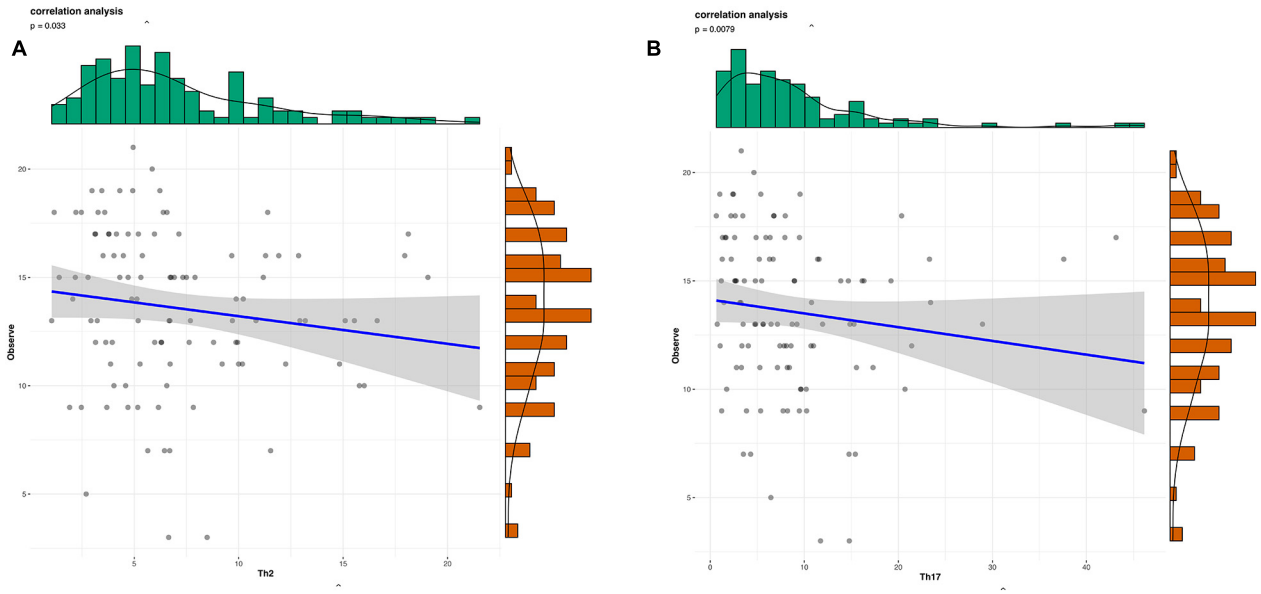


**FIGURE 3 |** Scatterplots of the correlation between cytokine levels and α diversity. **(A,B)** Correlation between Th1 **(A)** and Th17 **(B)** and Observed value (richness).

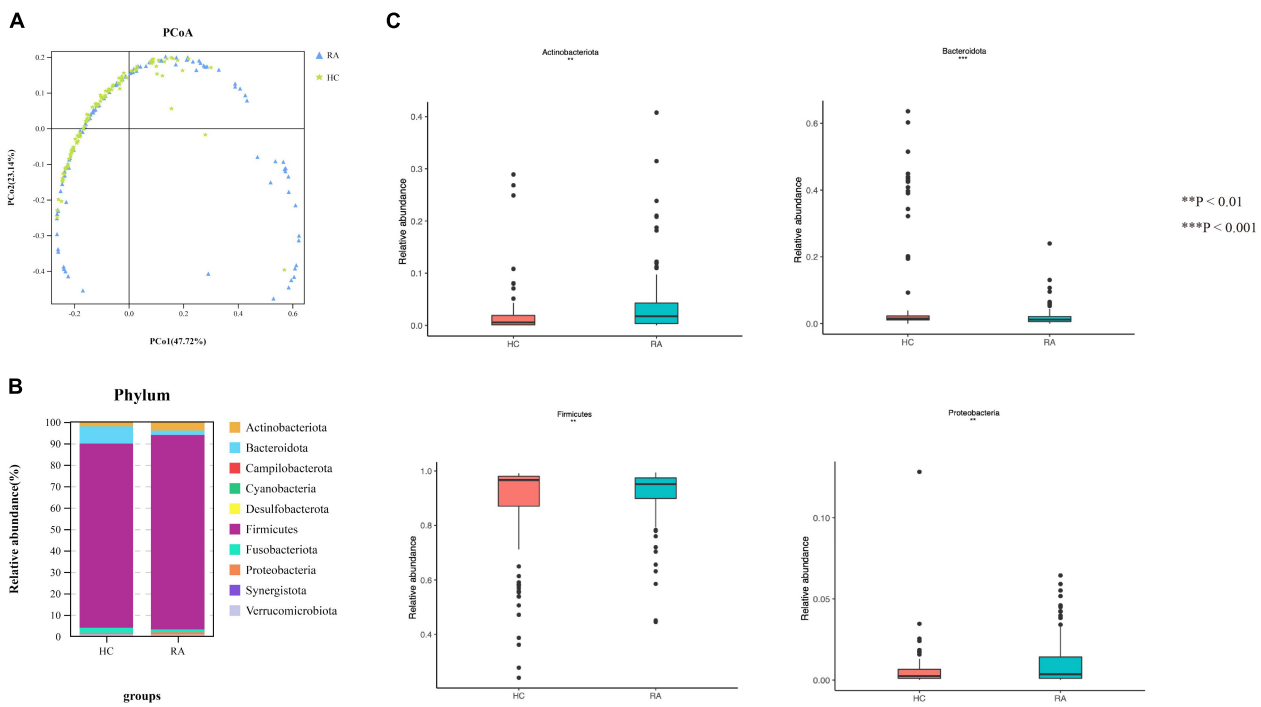


**FIGURE 4 |** Microbiota community composition of RA patients at the phylum level. **(A)** Principal coordinate analysis plots at the phylum level for RA patients and HCs. The x and y axes represent the first and second coordinates, respectively, and values in parentheses show the percentages of the community variation explained by each coordinate. Blue and green symbols represent bacterial phyla in the RA and control groups, respectively. **(B)** Relative abundance of phyla in all subjects and in each group. **(C)** Phylum-level comparison of the relative abundance of gut microbiota between RA patients and HCs. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.001$.

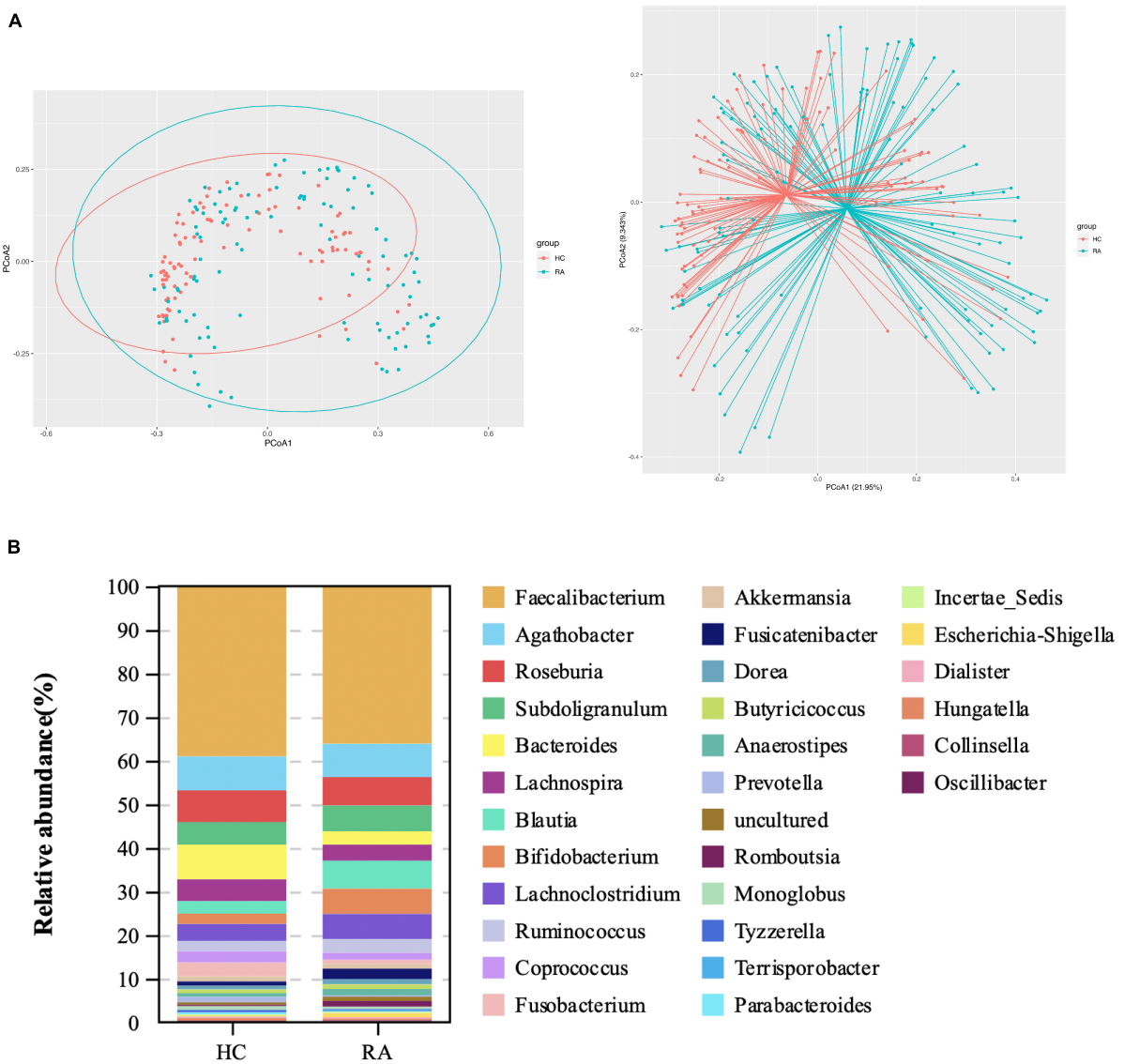


**FIGURE 5 |** Microbiota community composition of RA patients at the genus level. **(A)** Principal coordinate analysis plots generated from the Bray–Curtis analysis. The x and y axes represent the first and second coordinates, respectively, and values in parentheses show the percentages of the community variation explained by each coordinate. Blue and red symbols represent bacterial genera in the RA and control groups, respectively. **(B)** Relative abundance of genera in all subjects and in each group.

pathway, and p53 signaling pathway (**Figure 7C**). The metabolic pathway of ABC transporters was negatively correlated with the absolute numbers of Tregs ($P < 0.01$), while IL-6 level and NOD-like receptor signaling pathway showed positive correlations with the absolute number of Th1 cells ($P < 0.05$) (**Figures 8A,B**).

## Relationship Between Gut Microbiota and CD4$^+$ T Cell Subpopulations and Cytokine Levels

The relative abundance of *Megamonas* and *Monoglobus* was positively correlated with the absolute numbers of Th1 and Th2 cells, which are CD4$^+$ T cell subsets ($P < 0.01$). *Verrucomicrobiota* was positively correlated with the absolute number of Tregs ($P < 0.01$), while *Firmicutes* was negatively correlated with the absolute number of Th17 cells ($P < 0.05$) (**Figure 9A**).

Gut microbiota abundance was closely associated with cytokine levels. The relative abundance of *Megamonas* and *Monoglobus* showed significant positive correlations with the levels of IL-10, IL-2, IL-4, TNF-α, and IFN-γ ($P < 0.01$ or $< 0.05$). *Muribaculaceae* and *Odoribacter* were positively correlated with IL-17, and TNF-α, and IFN-γ levels ($P < 0.01$ or $< 0.05$) and *Prevotella* was positively correlated with IL-4 level (**Figure 9B**).

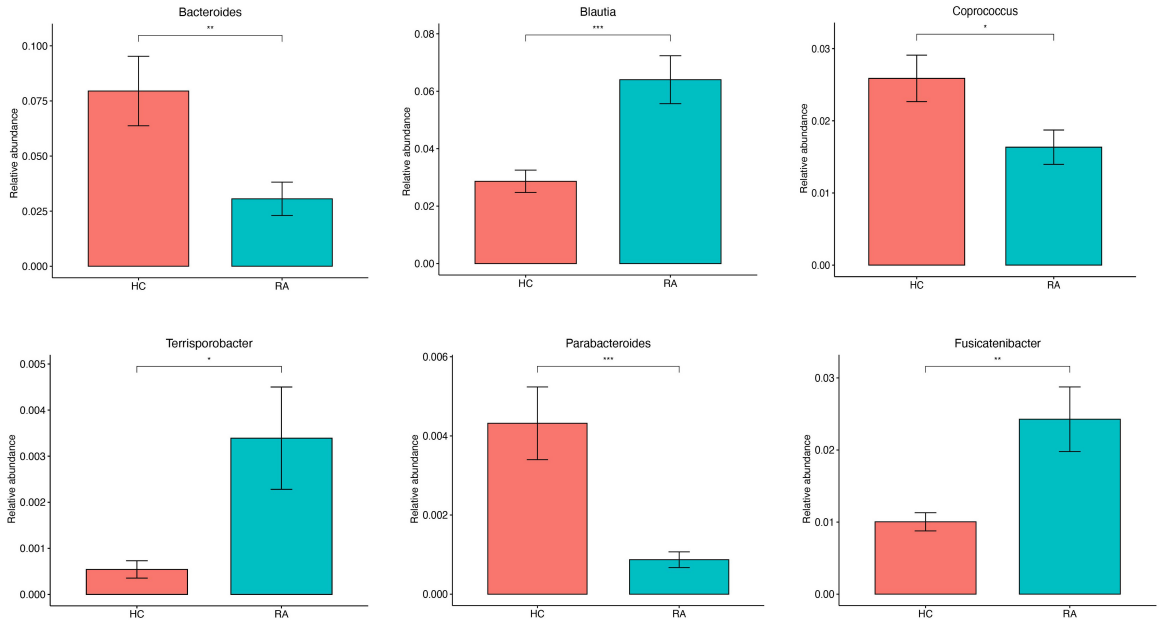


**FIGURE 6 |** Genus-level comparison of the relative abundance of gut microbiota between RA patients and HCs. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

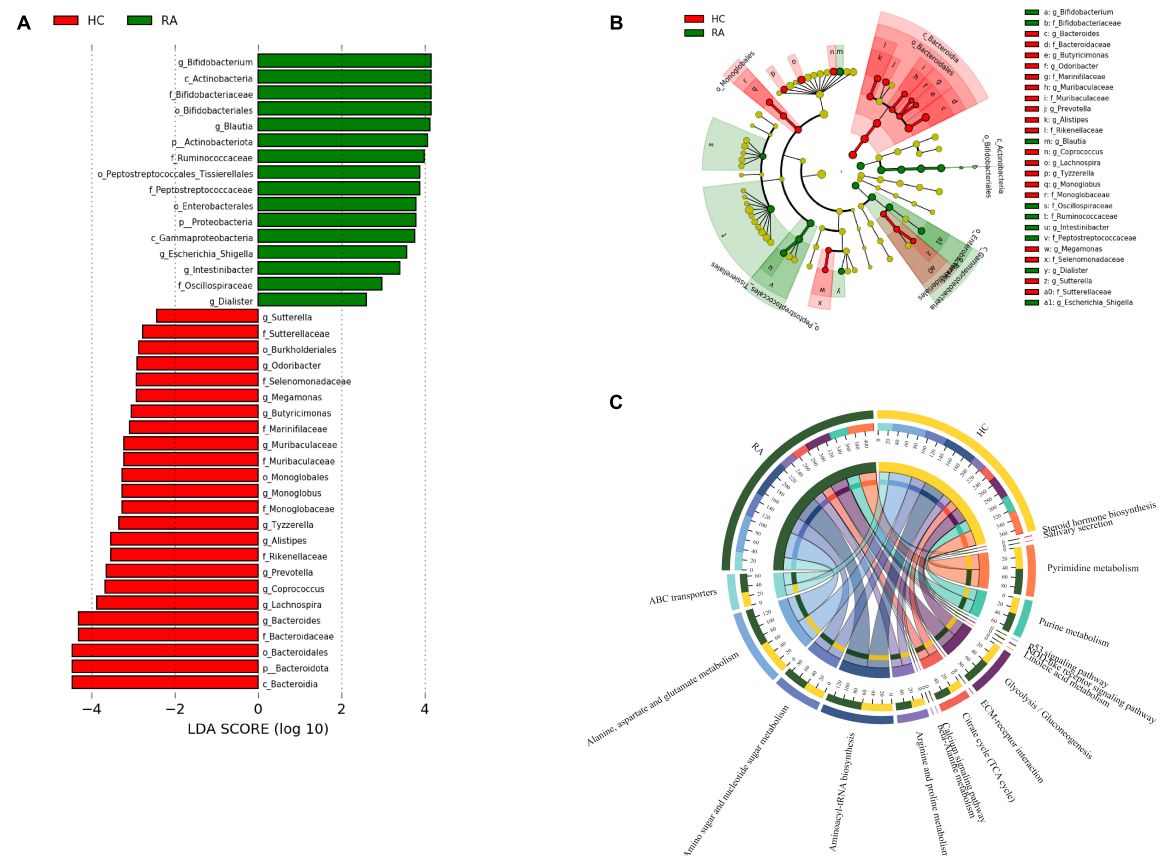


**FIGURE 7 |** Patients with RA characterized by expansion of rare microbial lineages. **(A,B)** LEfSe analysis was performed to identify differentially abundant taxa, which are highlighted on the phylogenetic tree in cladogram format **(A)** and for which the linear discriminant analysis scores are shown **(B)**. Red and green colors indicate an increase or decrease in taxa, respectively, in RA patients compared to HCs. **(C)** KEGG pathways significantly enriched in RA patients compared to HCs.

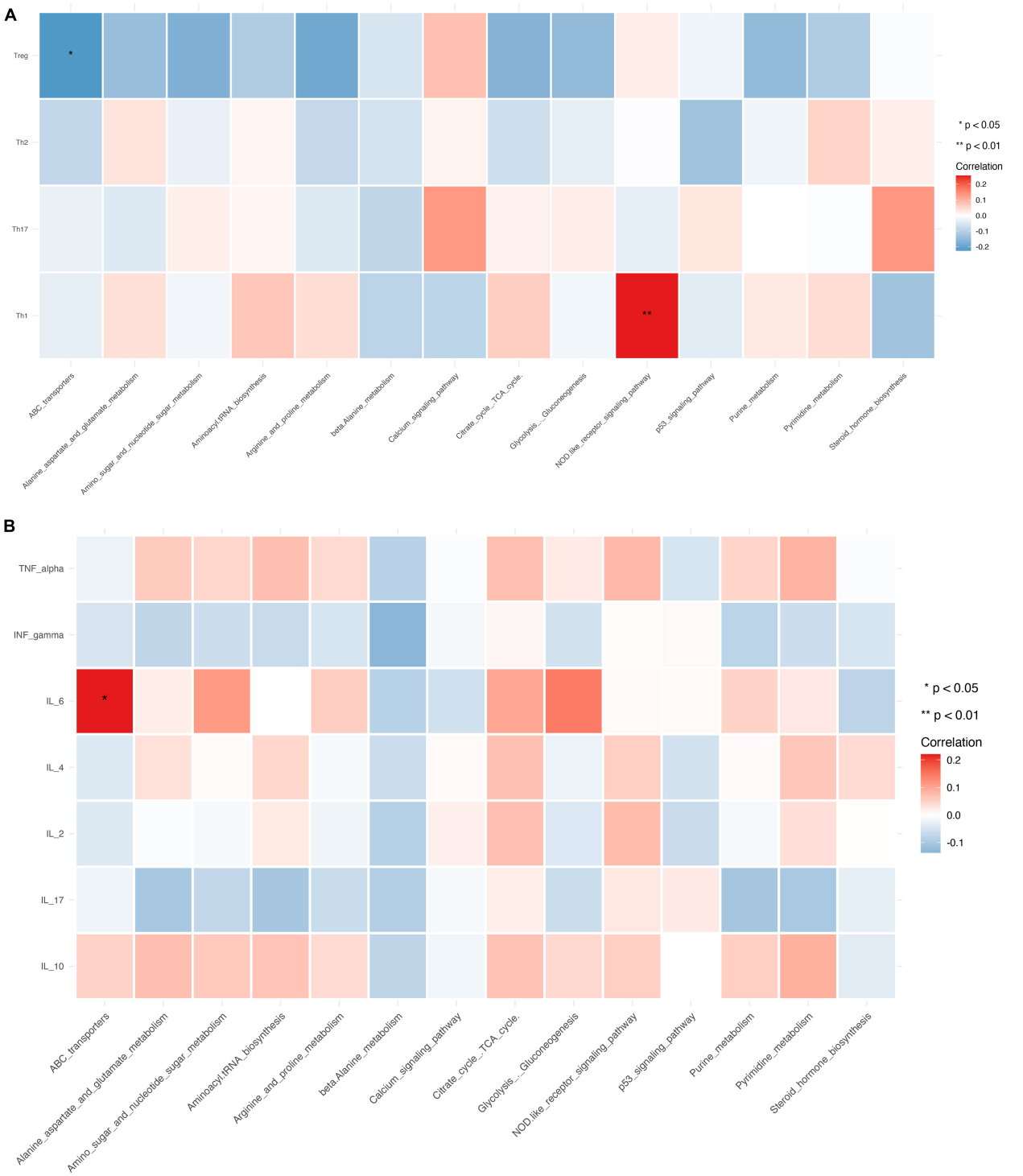


**FIGURE 8 |** Relationship between CD4$^+$ T cell subpopulations, cytokines, and metabolic pathways. **(A,B)** Heat map of the correlation between different metabolic pathways and CD4$^+$ T cell subpopulations **(A)** and cytokines. Colors indicate the Spearman rank correlation, with lighter and darker colors corresponding to weaker and stronger correlations, respectively. *$P < 0.05$, **$P < 0.01$.

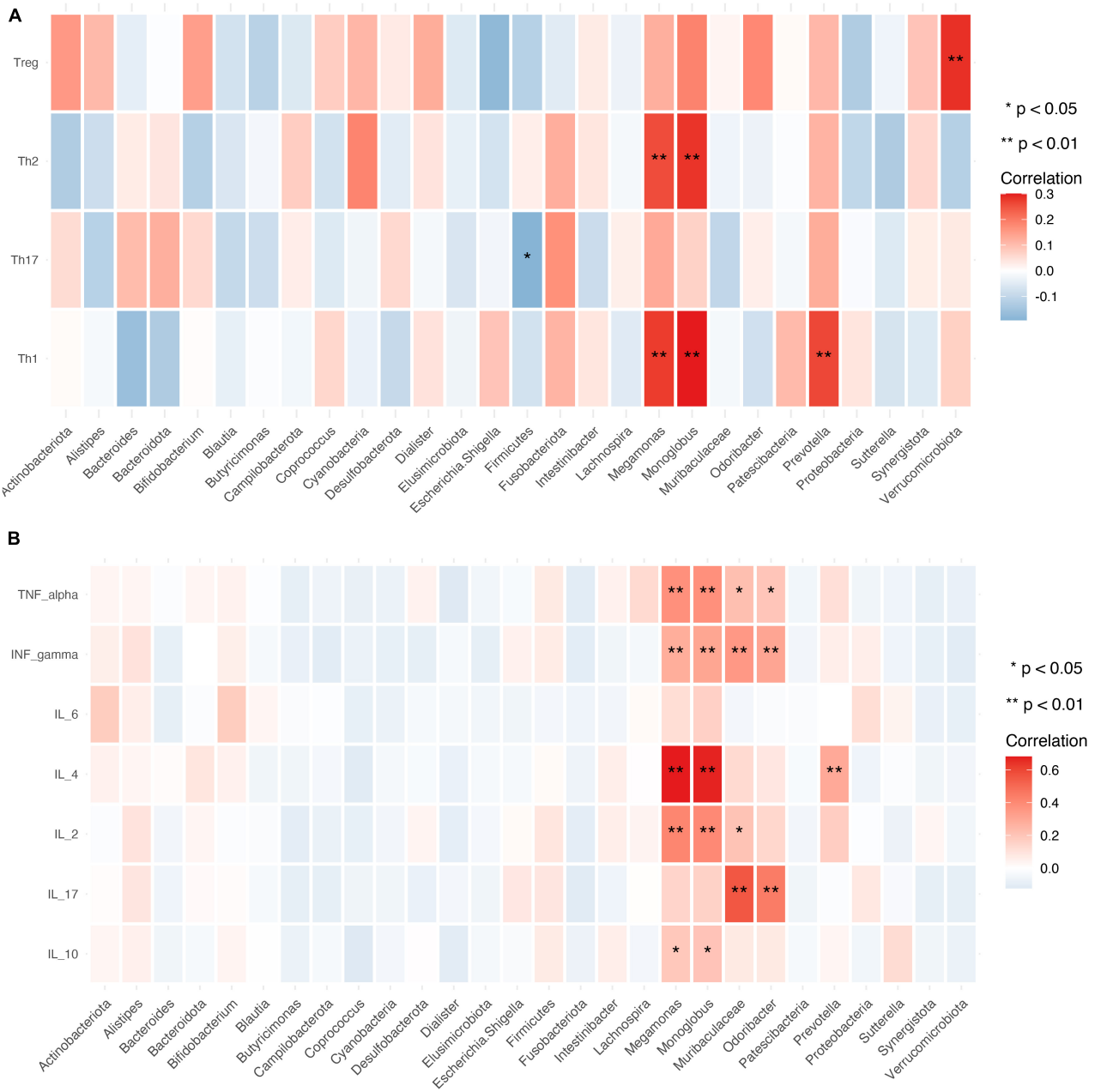


**FIGURE 9 |** Relationship between gut microbiota, CD4$^+$ T cell subpopulations, and cytokines. **(A,B)** Heat map of the correlation between different gut microbial species and CD4$^+$ T cell subpopulations **(A)** and cytokines **(B)**. Colors indicate the Spearman rank correlation, with lighter and darker colors corresponding to weaker and stronger correlations, respectively. *$P < 0.05$, **$P < 0.01$.

## Disease Activity in Rheumatoid Arthritis Is Closely Linked to Gut Microbiota, Lymphocyte Subpopulations, and Cytokine Levels

RA patients were classified according to disease activity into low (DAS28 $\geq$ 2.6 and $\leq$ 3.2), moderate (DAS28 $>$ 3.2 and $\leq$ 5.1), and high (DAS28 $>$ 5.1) activity groups. The relative abundance of *Bifidobacterium* and *Lachnospira* increased with disease activity (**Figure 10A**). The relative abundance of Blautia was higher in patients with high disease activity compared to those with moderate and low disease activity ($P < 0.01$; **Figure 10A**). *Muribaculaceae* abundance was positively correlated with disease activity ($P = 0.0084$; **Figure 10B**).

The number of Tregs and Th17/Tregs was inversely correlated with disease activity ($P = 0.033$ and 0.026; **Figure 10D**). The relative abundance of Th1/Th2 cells was higher patients with moderate disease activity than in those with low or high disease

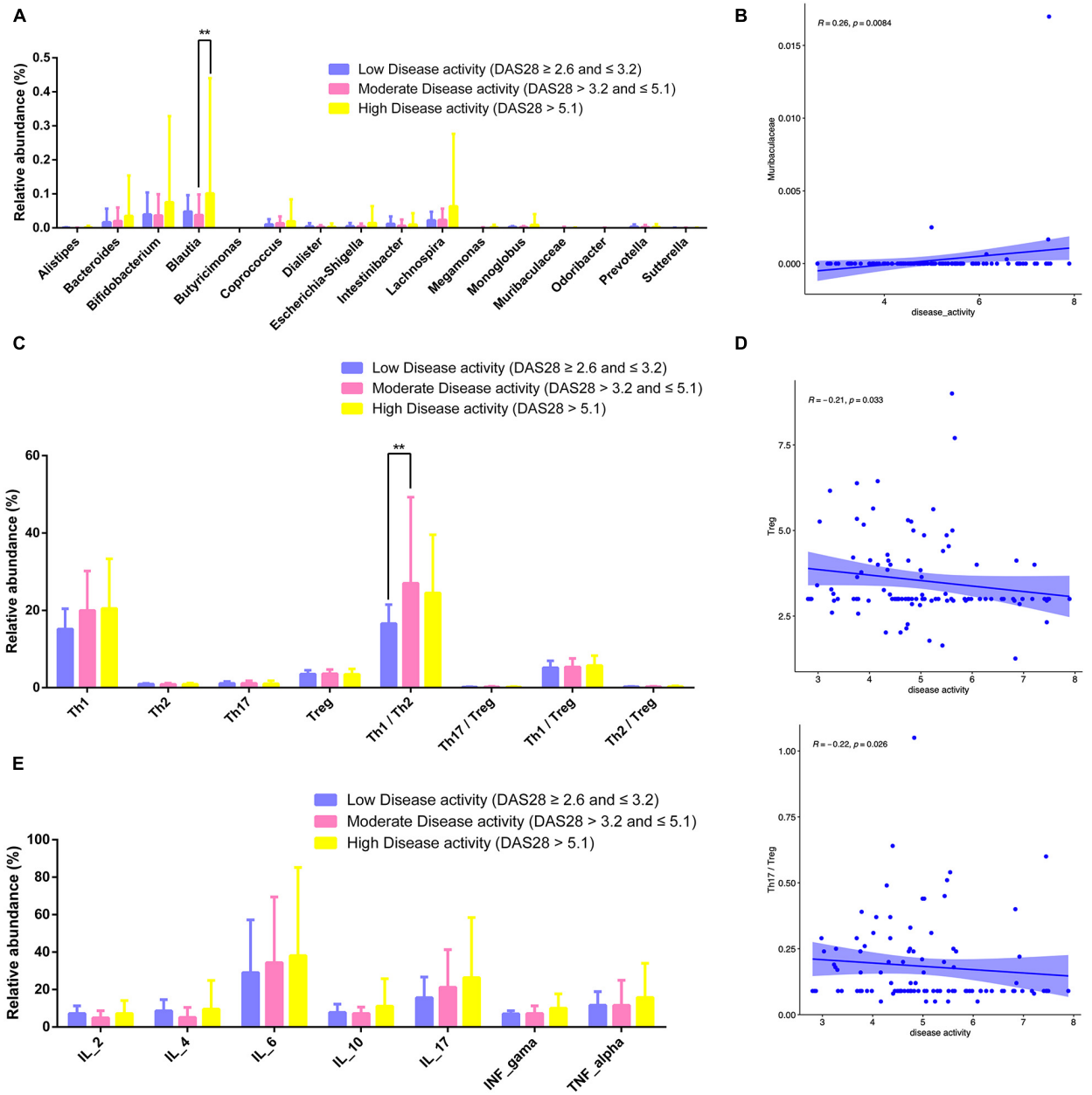


**FIGURE 10 |** Gut microbiota, CD4$^+$ T cell subpopulations, and cytokines in different RA disease activity groups. **(A)** Relative abundance of gut microbiota for different disease activity levels. **(B)** Scatter plot of the correlation between disease activity and Muribaculaceae. **(C)** Relative abundance of lymphocyte subpopulations for different disease activity levels. **(D)** Scatterplot of the correlation between disease activity and Treg counts Th17/Treg ratio. **(E)** Relative abundance of cytokines for different disease activity levels. $^{**}P < 0.01$.

activity ($P < 0.01$; **Figure 10C**). Relative abundance of cytokines for different disease activity levels has been shown in **Figure 10E**.

## DISCUSSION

The results of this study demonstrate that gut flora composition and α and β diversity were altered in RA patients, which is consistent with previous studies (Maeda and Takeda, 2019; Sun et al., 2019). RA patients significantly decreased α-diversity in the gut microbiome (Observed, Chao1, ACE, and Shannon), indicating that the richness and evenness of bacteria in RA groups were lower. Perturbation in the community was also found via the analysis of β-diversity (PCoA). As to beta diversity, PCoA results indicated that the composition of gut microbiota was significantly different in these two groups. At the phylum level, the profile of gut microbiota appeared dominated by *Firmicutes* and *Bacteroidetes* and the abundance

of *Firmicutes*, *Fusobacteriota*, and *Bacteroidota* was shown to be decreased in RA patients (Kasselman et al., 2018) whereas that of *Actinobacteria* was higher (Chen et al., 2016). *Firmicutes*, a butyrate-producing bacterium, regulates the differentiation of Tregs that inhibit inflammation; thus, a reduction in *Firmicutes* abundance and consequent decrease in butyrate levels can lead to inflammation in RA patients. *Actinobacteria* was reported increased in the rheumatoid arthritis, some of which were consistent with our findings (Chen et al., 2016). Actinobacteria can predict the RA status (Chen et al., 2016), However, another study is in contrast to our findings (Jeong et al., 2019). The role of *Actinobacteria* expression and function in RA needed to investigate further.

At the genus level, the profile of gut microbiota appeared dominated by the abundance of *Faecalibacterium*, *Agathobacter*, *Roseburia*, *Subdoligranulum*, and *Bacteroidetes*. The relactive ahundnce of *Faecalibacterium* (phylum *Firmicutes*) showed reduced abundance in RA patients compared to HCs. *Faecalibacterium* produces butyrate, which maintains the integrity of the intestinal epithelial cell layer (Khan et al., 2012). *Collinsella* was also increased in RA patients. A recent study found that decreased *Faecalibacterium* and increased *Collinsella* abundance may enhance epithelial permeability, resulting in the entry of microbial products into the lamina propria and subepithelial space (Chen et al., 2016). Changes in the composition of gut microbiota are tightly linked with host immune status and local inflammation (Faber and Bäumler, 2014; Lee and Ko, 2016; Katayama et al., 2019). *Bacteroides*, which was decreased in RA patients compared to HCs, has been implicated in chronic inflammation and may cause osteomyelitis in RA (Sun et al., 2019). On the other hand, *Blautia* and *Lachnoclostridium* were increased in RA patients; *Lachnoclostridium* may contribute to the progression of inflammatory arthritis. *Dialister* has been linked to antidepressants drug along with *Coprococcus* and butyrate-producing *Faecalibacterium*. Whether there is reciprocal interaction in preventing autoimmune diseases requires further study. Above all, a correlation may exist between the abundance of these bacteria and RA patients.

The functions of gut microbiota showing altered abundance in RA patients included ABC transporters, glycolysis/gluconeogenesis, and NOD-like receptor signaling. ABC transporters enable the passage of endogenous and xenobiotic compounds through the cell membrane, which may be related to the development of rheumatic disease resistance and disease activity (Atisha-Fregoso et al., 2016). ABC transporters were shown to induce C-X-C chemokine receptor (CXCR) 4 overexpression in B cells and the production of various inflammatory cytokines such as TNF and IL-6 (Tsujimura et al., 2018). Glycolysis/gluconeogenesis plays an important role in maintaining adequate sugar sources (Cheng et al., 2017). The activation of NOD-like receptor signaling modulates the innate immune response and inflammation (Franca et al., 2016; Cheng et al., 2017; Root-Bernstein, 2020). On the other hand, steroid hormone biosynthesis (Cutolo et al., 2004) and p53 signaling were decreased in RA. Steroid hormone-related gene polymorphisms have been linked to bone erosion in rheumatoid arthritis (Sánchez-Maldonado et al., 2019). The p53 signaling pathway mediates the cellular response to stress, cell cycle, DNA repair, senescence, and apoptosis (Hou et al., 2016; Zhang et al., 2020). Bacteroides abundance was reduced in RA patients compared to HCs, suggesting that Bacteroides contributes to disease pathogenesis through the release of bioactive molecules such as butanoate, dicarboxylate, and glyoxylate (Wang et al., 2021). Notably, there was a close correlation between metabolic pathways and some genera (*Blautia*, *Escherichia-Shigella*, *Proteobacteria*, *Coprococcus*, and *Verrucomicrobiota*).

Intestinal microbiota are closely related to the immune profile of RA (Hu et al., 2017; Zhang et al., 2018; Li H. et al., 2021). *Lactobacillus casei* has been shown to alleviate arthritis by altering Treg/Th17 balance and modulating gut microbiota abundance and the plasma metabolome (Jhun et al., 2020; Fan et al., 2021). *Verrucomicrobia* (*Akkermansia muciniphila*) plays an anti-inflammatory role by regulating Treg differentiation and enhancing the production of short-chain fatty acids (Zhai et al., 2019). The study about *Verrucomicrobia* in RA is scarce, our finding may provide a new clue for the function of *Verrucomicrobia* in disease causation. It was reported that *Firmicutes-rich* suppresses inflammation and Th17 pathways (Natividad et al., 2015), suggesting that it has therapeutic potential in ulcerative colitis. *Bacteroides fragilis* and *Faecalibacterium prausnitzii* induce the secretion of IL-10 by $CD4^+$ T cells (Mazmanian et al., 2005; Sokol et al., 2008). Gut dysbiosis caused by segmented filamentous bacteria may reduce the number of anti-inflammatory Tregs and increase the risk of autoimmunity (Scher et al., 2013). IL-10 is an anti-inflammatory cytokine secreted by various lymphocytes including Th1, Th2, and Th17 cells and Tregs (Mollazadeh et al., 2019). The dysregulation of IL-10 has been reported in various inflammatory diseases such as lupus, arthritis, and psoriasis. In our study, IL-10 level and Th1 and Th2 counts were positively correlated with *Megamonas* and *Monoglobus* abundance; meanwhile, the relative abundance of *Prevotella* and *Monoglobus* was positively correlated with the absolute number of Th1 and Th2 cells and Il-4, IL-2, IL-10, TNF-α, and IFN-γ levels. Improving the balance of gut microbiota with *Blautia* along with Bifidobacterium and Ruminococcus was shown to alleviate inflammation by suppressing Tregs (Picchianti-Diamanti et al., 2018). Our results speculate that gut microorganisms influence RA by modulating the immune system. This requires further studying.

We found that the abundance of *Escherichia-Shigella*, *Monoglobus*, and *Lachnospira* increased while that of *Intestinibacter* and *Prevotella* decreased with disease activity in RA. An increase in RA disease activity was shown to be associated with elevated levels of TNF-α and IFN-γ, a higher Th17 count, and altered Treg balance (i.e., Th17/Treg and Th2/Treg ratios) (Edavalath et al., 2016). IL-6, IL-17, and IL-10 levels were higher in RA patients than in HCs and IL-17 and IL-10 were positively correlated with DAS28 score, suggesting that these cytokines can serve as markers for disease outcome and inflammatory response (Ge et al., 2015; Marwa et al., 2017; Dhaouadi et al., 2018). In our study, IL-6 level was significantly higher in RA patients with a high DAS28 score compared to those with lower scores. Meanwhile, high IL-6 level was shown to be an independent risk

factor for high disease activity in RA, as reflected by the DAS28 score (Park et al., 2016), and alters the Treg/Th17 balance by downregulating IL-6 (Samson et al., 2012). These results suggest that regulating cytokine levels and lymphocyte subpopulations may be an effective strategy in controlling disease activity in RA.

## CONCLUSION

The results of our study demonstrate that gut microbiota play an important role in the pathogenesis of RA, as evidenced by the observed correlations between gut microbiota and $CD4^+$ T cell counts, cytokine levels, and disease activity in RA patients compared to HCs. We found that gut microbiota composition was altered in RA, which was primarily associated with changes in metabolic signaling that could contribute to imbalances in $CD4^+$ T cell subpopulations and cytokines and affect disease activity. These findings provide novel insight into the possible pathogenic mechanisms of RA and suggest that the disease can potentially be regulated by affecting $CD4^+$ T cell subpopulations and cytokines through regulation of the gut microbiome profile.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Second Affiliated Hospital of Shanxi Medical University. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

QW designed the study and wrote the manuscript. S-XZ, M-JC, and JQ performed the experiments. QW and JQ analyzed the data. C-HW, X-FL, QY, and P-FH contributed to manuscript revision, read, and approved the submitted version. All authors contributed to the article and approved the submitted version.

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