High Schistosoma mansoni infection intensity is associated with distinct gut microbiome and low levels of systemic cytokines in children along the Albert-Nile, Northern Uganda

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Abstract

Background

Schistosomiasis is a chronic neglected disease that affects millions of people in sub Saharan Africa, with a range of impacts on both host immune responses and the gut microbiome. The gut microbiota plays a fundamental in role in the host's nutrition, metabolism, protection against pathogens, and modulation of host immunity. There is a need to understand the role of the gut microbiome in pathophysiology of *Schistosoma mansoni* infection and how this influences the host's immune response.

Methodology:

A cross sectional study was carried out on 140 faecal samples collected from school children aged 10-15years residing in the schistosomiasis endemic hot spots of the Albert-Nile, Pakwach district, Northern Uganda. The samples were categorised by *S. mansoni* infection intensity based on the Kato Katz test. Faecal DNA was isolated and microbiome composition was determined by 16S rRNA V3-V4 sequencing. Plasma Th1/Th2 profiling of 13 cytokines was carried out on the Luminex platform and compared with respect to *S. mansoni* infection intensities.

Results

The genera *Phascolarctobaterium* and *Prevotella_7* were significantly enriched (padj < 0.05, LDA > 3.0) in the high *S. mansoni* infection intensity group whereas, *Ruminobacter* and *Alloprevotella* were enriched in the Low infection intensity group. We observed significantly lower systemic Th1/Th2 cytokine levels between the high intensity infection and the control samples (padj < 0.05). Linear regression analysis using all cytokines as covariates showed that the genus *Alloprevotella, Streptococcus, Gastranaerophilales* and *Ruminobacter* were associated with systemic IL6 response.

Conclusion

There are alterations in the gut microbiome of *S. mansoni* infected children with distinct genera that discriminate the high and low infection intensity that could be potentially used as biomarkers. There is an association between the gut microbiome and systemic cytokine response whose mechanism in chronic disease pathophysiology can be further investigated.

Introduction

Schistosomiasis is a chronic neglected tropical disease which is caused by blood flukes of the genus Schistosoma. The three most clinically important species are *Schistosoma mansoni*, and *Schistosoma japonicum*, which cause hepato-intestinal schistosomiasis, and *Schistosoma haematobium*, which causes urinary schistosomiasis (Colley et al., 2014; McManus et al., 2018).

On a global scale, schistosomiasis currently impacts over one billion people, with over a quarter of those currently infected in 78 countries and more than 780 million people at risk of infection, most of whom are in Sub-Saharan Africa (Hotez et al., 2014; Lo et al., 2022; Díaz et al., 2023). In Sub-Saharan Africa, the control of schistosomiasis has mainly focused on morbidity reduction through mass drug administration with Praziquantel, intermediate snail host molluscicide, health education, and water sanitation and hygiene (WASH) programs (Campbell et al., 2018; Li et al., 2019).

Schistosoma mansoni chronically infects humans for over 10 years if untreated, and it is

associated with systemic morbidities that include malnutrition, anemia, physical and/or cognitive impairment and stunted development in children (King and Dangerfield-Cha, 2008). Humans are the definitive hosts of *S. mansoni*, and harbor the adult male and female worms in the mesenteric vasculature where they mate, and lay approximately 350 eggs per worm pair in the blood each day. These eggs then translocate through the intestinal wall into the gut and are excreted in the faeces (Schwartz and Fallon 2018). The process of intestinal egg granuloma formation and excretion by *S. mansoni* is mediated by the immune system (Hams, Aviello, and Fallon 2013). In addition, during the course of infection, the different schistosome stages induce significant alterations in the immune response, both during the acute and chronic phase of infection (Pearce and MacDonald, 2002; Hesse et al., 2004; Stadecker et al., 2004).

The schistosome-specific immune response is modulated from a Th1, inflammatory and cell-mediated, to an antibody-dependent Th2 response (Caldas et al., 2008). In schistosomiasis endemic populations, there is a differential pattern of immune responses against worm-derived antigens and egg-derived antigens regardless of the endemic population sampled (Williams et al., 1994). This is seen as early high-level responses to soluble egg antigens (SEA) which subsequently decrease as infections become chronic (Caldas et al., 2008). In the chronic phase of infection, the Th2 response is moderated and granulomas, which form around newly deposited eggs, reduce in size as the infection progresses (Pearce and MacDonald, 2002). Furthermore, reports on humans acquiring immune resistance to schistosome reinfection have been associated with Th2-type responses and production of parasite-specific IgE, eosinophils and cytokines such as IL-5 and IL-4 (Ganley-Leal et al., 2006; Oliveira et al., 2012; Negrão-Corrêa et al., 2014).

Chronic schistosomiasis is characterized by a decline in active granulomatous lesions, increased pro-fibrotic process and systemic immune suppression (Lundy and Lukacs, 2013). However, in the absence of praziquantel treatment, *Schistosoma* eggs accumulate in host tissues over several years leading to liver fibrosis and granulomas as the main drivers of disease pathophysiology (Fallon 2000; Hams, Aviello, and Fallon 2013). The host gut microbiota have been implicated in influencing the immunological events that lead to intestinal granulomas in schistosomiasis (Holzscheiter et al., 2014). The gut microbiota plays a fundamental role in the host's nutrition, metabolism, protection against pathogens, and modulation of host immunity (Bäckhed et al., 2005; Hill and Artis, 2010; Hou et al., 2022; Maciel-Fiuza et al., 2023). The gut microbiota and their metabolites have also been associated with inflammatory bowels diseases, hepatocellular carcinoma, cardiovascular diseases, chronic kidney diseases, and cirrhosis (Yu and Schwabe, 2017; Jansen et al., 2021; Wang et al., 2021; Shan et al., 2022; Liu et al., 2023). Additionally, gut dysbiosis has been reported in adolescents and school aged children infected with *S. mansoni* (Schneeberger et al., 2018), S. *haematobium* (Kay et al., 2015; Ajibola et al., 2019; Osakunor et al., 2020)d *japonicum* (Jiang et al., 2021; Zhou et al., 2022). The schistosome infection alters the diversity of the host's intestinal flora which affects the host's metabolism and immune system function that eventually lead to more severe complications (Carding et al., 2015).

In this study, we investigated the association between the gut microbiome and Th1/Th2 plasma cytokine profiles in school aged children (10–15 years) that had different *S. mansoni* infection intensities. This approach will enable understanding the role of systemic host immune responses against *S. mansoni* infections and regulation of the gut microbiome homeostasis in a chronic infection. This will in turn enable determination of non-invasive microbial biomarkers that can be used in the detection of Schistosomiasis and also offer potential probiotic markers as alternative treatment.

Materials and Methods

3.1 Ethics statement

The study protocol was reviewed by the institutional review board of the Ministry of Health, Vector Control Division Research and Ethics Committee (Reference No. VCDREC106) and approved by the Uganda National Council of Science and Technology (Reference No. UNCST HS 118). The study was conducted with guidance from the district health officials, including the selection and training of the village health teams that were involved in the mobilization and recruitment of the school aged children into the study. The objectives, potential risks and benefits of the study were explained to the parents/

guardians who signed informed consent, and later explained to the school age children in English and Alur dialect who provided assent for participation into the study. Written formal consent from parents and written assent from the children were obtained. If a child was observed to have *S. mansoni* eggs in their stool, they were offered free treatment, which consisted of praziquantel at a dosage of 40mg/kg administered by trained Ministry of Health personnel, assisted by the district health worker.

3.2 Sample collection

The stool samples used in this study were obtained from the schistosomiasis cross sectional study that was carried out among school aged children in communities along the Albert-Nile in Pakwach district, Northern Uganda (Mulindwa et al. 2022). Briefly, screening of children aged 10–15 years for schistosomiasis by POC-CCA was carried out at 6 sites of Alwi, Panyigoro, Kivuje, Nyakagei, Kayonga and Dei in Pakwach district. Following written formal consent from the parents and written assent from the children who accepted to participate in the study, anthropometric measurements and stool samples was collected. The stool samples were processed using Kato-Katz double thick smears (Katz et al., 1972) using a 41.7 mg template. The duplicate smears from each child were examined under a microscope and all *S. mansoni* ova seen were identified and counted using tally counters to determine the intensity of infection according to WHO guidelines (WHO, 2013). A scoop of the stool (approximately 5grams) was immediately snap frozen and cryo-preserved in liquid nitrogen. In addition, approximately 4mL of venous blood was collected in BD Vacutainer EDTA tubes centrifuged at 5000 rpm in order to separate the sample into packed cells and plasma. The plasma was aliquoted into cryotubes and placed in liquid nitrogen for subsequent use in serological assays including quantification of cytokines using the Luminex assay (R&D systems). For anthropometry, the age, height and weight measurements were taken for which the Height for Age Z-score were determined using the WHO 2007 R package that incorporates the WHO child growth standards (De Onis et al., 2007). Stunting was defined as HAZ < -2standard deviations (SDs).

3.3 DNA isolation and 16S rRNA gene sequencing

For this study, a total of 140 participants stool samples had their total DNA extracted and used for the microbiome analysis. Briefly the snap frozen stool was thawed and approximately 0.25g of stool were homogenized and subjected to the DNA isolation protocol using the QIAamp PowerFecal DNA Kit (Qiagen, Germany) according to the manufacturer's instructions. The DNA concentration was quantified with a Qubit 4.0 fluorometer using the dsDNA High sensitivity kit (Thermo Fisher Scientific, USA). The DNA samples were shipped on dry ice for library preparation and sequencing at the Beijing Genomics Institute (BGI, Hong Kong). Briefly, 30ng of qualified DNA template and the 16S rRNA V3-V4 fusion primers (338F: ACTCCTACGGGAGGCAGCAG, 806R: GGACTACHVGGGTWTCTAAT) were added for the Polymerase chain reaction (PCR) using the Phanta Max Super-Fidelity Kit (Vazyme, Nanjing, China). All PCR products were purified using the Agencourt AMPure XP beads, dissolved in Elution Buffer and labeled for the library construction. Library size and concentration were determined by Agilent 2100 Bioanalyzer. The Single-stranded PCR products were circularized using the MGIEasy Circularization kit (MGI-Tech, Shenzhen, China) and thereafter sequenced on the DNA nanoball (DNB) platform generating sequencing read length of PE300.

3.4 Taxonomic identification and abundance enrichment

Read filtering. The raw fastq reads were quality filtered and trimmed using cutadapt (Marcel, 2011) to generate high quality clean reads. Filtering thresholds were specified as follows; truncated reads whose average Phred quality values were less than 20 with a 25bp sliding window, removed reads whose length was less than 75% of the original lengths after truncation, removed reads that were contaminated with adapter sequences (15 bp overlapped by reads and adapter with maximal 3 bp mismatch), and removed reads with ambiguous bases and low-complexity (reads with 10 consecutive same bases). Consensus sequence tags were generated from the overlapping clean paired end reads using FLASH (v1.2.11) (Magoč and Salzberg, 2011) considering a minimum overlap length of 15bp and mismatching ratio of overlapped region < = 0.1.

OTU clustering and taxonomy annotation. The sequence tags were then clustered into Operational Taxonomic Unit (OTU), with a 97% threshold using UPARSE (Edgar, 2013) and the chimeric sequences removed by UCHIMEv4.2.40 (Edgar et al.,

2011) through mapping to gold database (v20110519). All the sequence tags were mapped to OTU representative sequence using USEARCH (v7.0.1090) (Edgar and Bateman, 2010). Taxonomic assignments to the OTUs was then carried out using RDP classifier (v2.2) (Wang et al., 2007) in order to calculate abundance. To determine the difference in OTU composition between *S. mansoni* infection intensity groups, ANOSIM (Somerfield et al., 2021) was performed based on the Bray-Curtis dissimilarity distance matrices.

Phylogenetic and diversity analysis. From the cleaned reads, Amplicon Sequence Variants (ASVs) were obtained using dada2 Bioconductor package (Callahan et al., 2016) with default parameters for the *filterAndTrim*, *derepFastq*, *learnErrors*, *dada*, *mergePairs*, *makeSequenceTable*, and *removeBimeraDenovo* functions. Annotation of ASVs was done using Silva NR99 v138.1 16S/18S ribosomal RNA database (Quast et al., 2013). A maximum likelihood phylogenetic tree was made using DECIPHER (Wright, 2016) and phangorn (Schliep, 2011) R packages and the resulting tree was annotated using iTOL web utility tool (Letunic and Bork, 2024). The heatmap around the tree was plotted using normalised abundances per tree node for the different classes of bacteria. Alpha diversity metrics were computed from raw abundance counts using phyloseq R package (McMurdie and Holmes, 2013).

Differential analysis. Differential abundance for enriched microbiome markers was done using the R microbiomeMarker package (Cao et al., 2022). LefSe analysis was performed on filtered and log transformed abundance counts using a p-value cutoff of 0.05, LDA score cutoff equal to 2 and pre-sample normalisation of the sum of the values to 1e + 06. Differential abundance analysis for genera was also performed using DESeq2 (specified as RLE) and EdgeR (specified as QLFT) with significance p-value cutoff = 0.05 and Benjamini-Hochberg correction and sample collection site considered as a confounder. A combined list of the differentially abundant genera from the 3 methods (LefSe, DESeq2 and EdgeR) was then compiled. The differential output was used to comparatively determine the significantly different genera in, the *S. mansoni* infection intensity groups (High, Low and Control), the Sex (Males vs Females), the HAZ status (Normal vs stunted), and the sites of sample collection. Pairwise Wilcoxon test was conducted to determine if the differences between groups is significant (alpha = 0.05) and the Kruskal Wallis test was used to determine differences in multiple groups (p value $\leq$ 0.05).

3.5 Th1/Th2 Cytokine profile analysis

The plasma levels of GMCSF, IFNG, IL1B, IL2, IL4, IL5, IL6, IL9, IL10, IL12P70, IL13, TNFA, TNFB were quantified using the Human Th1/Th2 Luminex bead-based assay (R&D Systems, Cat No. LKTM008B) on the Magpix- Milliplex map System (Luminex technologies). The experimental protocol was carried out following the manufacturer's instructions. Briefly, the antibody-beads were aliquoted onto the 96 well plate, 50µL of standards and samples were dispensed into the 96 well plate. Then added 50µL of the microparticle cocktail to each well and incubated for 2 hours at room temperature on an orbital microplate shaker at 800rpm. Washed the plate and then added 50µL of Biotin-antibody cocktail and incubated for 1 hour at room temperature with shaking at 800rpm. Washed the plate then added streptavidin-PE and incubated for 30 minutes at room temperature with shaking at 800rpm. Washed and the array plate was then read using the Luminex MAGPIX system and the cytokine Median Fluorescence Intensity (MFI) determined.

Results

4.1 Population characteristics

The study was carried out in Northern Uganda, Pakwach district, where participant recruitment was done in five sites, four of which were previously designated as high prevalence hotspots of *S. mansoni* infection (Adriko et al. 2018; Kittur et al. 2017; Mulindwa et al. 2022) and are located within 2km of the Albert-Nile (Figure 1). A total of 140 participants were included in this study of which 43% (60/140) were females and 57% (80/140) were males (Table 1). The participants were aged between 10-15 years belonging to the same Alur tribe of the Nilotic Ethnic group and depend on similar staple food. Anthropometric measurements were carried out and the height for age Z-score (HAZ) was determined and it was observed that approximately 69% (96/140) of the study participants were stunted (HAZ < -2 Standard deviations). The measure of *S.*

mansoni infection intensity among the participants was done by determining the stool egg counts by Kato Katz method and normalising these to WHO standard Eggs per Gram (EPG). The *S. mansoni* infection intensity was then categorised into High (>100 EPG), Low (1-100 EPG) and Control (0 EPG). 41% (58/140) of the participants had high infection intensity, 26% (37/140) had low infection intensity and 32% (45/140) were uninfected negative controls. Except for Alwi site which only had uninfected control samples, the rest of the sites had distribution of high, low and negative controls (Figure 1, Table 1).

4.2 Taxonomic composition of gut microbiome with *S. mansoni* infection

Following quality control analysis of the sequenced reads, 138 samples with clean reads were retained with a total number of 9,359,877 reads and an average of 67,825 ± 416 paired reads per sample. Following tag connection, a total of 9,082,761 tags were obtained with an average 65,817± 417 tags per sample and average tag length of 415± 10bp. OTU (Operational Taxonomic Unit) clustering obtained a total of 1853 OTUs with an average 541± 86 OTU per sample. 78% (1452/1853) OTU were shared among the High, Low and Control *S. mansoni* infection intensity groups whereas 3.5%, 1% and 4% OTU were unique to the High intensity, Low Intensity and Control groups respectively (Figure S1A). Principal component analysis on the OTUs did not show any form of distinct clustering of the samples when grouped by *S. mansoni* infection intensity (Figure S1B). However, to test whether differences in the OTU abundance between the infection intensity groups was significantly greater than the difference within the groups, an analysis of similarity (ANOSIM) was carried out and the distance between groups determined based on Bray-Curtis dissimilarity. There was more difference in the taxa within High and Low intensity than between them as shown by the negative test statistic R (R = -0.036, Figure S1C). However, there was a difference between the High and Control groups (R = 0.015, Figure S1D).

4.3 Abundance and diversity of gut microbiome in different *S. mansoni* infection intensities

To determine the microbiome abundance in the infection intensity groups, we considered the taxa from the annotated ASVs generated by dada2 (Table S1). In the 138 stool samples, 265 bacterial genera from 14 unique phyla were detected. At the phylum level (Figure 2A), the Firmicutes, Bacteroidota, Spirochaetota and Proteobacteria were the most abundant (>90%). There was a slightly higher mean abundance of Firmicutes in the control samples (46%) as compared to the *S. mansoni* high infection intensity samples (44%) with the lowest being in the low infection intensity samples (40%). The high infection intensity samples had lower mean levels of Bacteroidota (39%) in comparison to the low infection intensity and control samples which had 41%. The Spirochaetota were most abundant in both the high and low infection intensity samples (10%) and had low abundance in the control samples (8%). The fourth most abundant phyla in the samples were the Proteobacteria with highest mean level being in the Low intensity samples (8%) and lowest in the control samples (3%) with the high intensity samples at 6%. At the genus level (Figure 2B), we analysed the top 20 most abundant genera and observed that *Prevotella* was most abundant with the Control group having 43%, low intensity at 42% and High intensity at 40% abundance. *Treponema*, *Faecalibacterium* and *Blautia* followed next in abundance in the groups. The distribution and frequency of the top phyla and class taxa among the 138 samples (Figure S2) was also compared with respect to abundance in the High intensity, Low intensity and Control groups (Figure 2C). We observed that the taxon abundances were generally high in individuals with high infection intensity more so Clostridia, Spirocheata, Bacteriodota and Gammaproteobacteria classes.

The gut microbiome diversity was determined by analysis of the alpha (species diversity within sample) and beta (differences between samples in species complexity) diversity metrics. The alpha diversity mean metrics were high for all the groups (Figure 3), observed (272±7.6), Chao1 (273±7.6), ACE (273±7.7), Fisher (41±1.4), Shannon (4.4±0.05); indicating that there was species diversity within the same groups. However, we observed higher mean indices for all the alpha diversity metrics in the infected children in comparison to the control group. But there was no significant difference among the groups in all the alpha diversity metrics (Kruskal-Wallis Rank sum test p-value > 0.5, c^2 > 1.0). The beta diversity analysis was used to evaluate the differences in species complexity between the samples. For this, Permanova analysis with the Bray-Curtis distance, unweighted and weighted UniFrac distances (Figure S3A) were used to compare groups. Whereas there was a significant difference in the species between the groups reported by the Bray-Curtis distance (p=0.056), there

was no difference reported by the weighted and unweighted Unifrac distances which implies that there was minimum beta diversity between the groups. Clustering of samples based on similar Bray-Curtis values showed no distinct grouping based on *S. mansoni* infection intensity (Figure S3B).

4.4 Differential genus enrichment in gut microbiome of *S. mansoni* infected children

In order to identify bacteria taxa that are significantly enriched in the high intensity, low intensity and uninfected control groups, LefSe, DESeq2 and EdgeR analyses were carried out. From this we identified 25 (Table S2) genera that were significantly differentially enriched (padj < 0.05, LDA > 3.0) of which 12 genera had over 70% coverage (abundance counts) in all the samples (Table 2).

The abundance of the genera *Ruminobacter* (padj 0.005, Log2Fc 2.0), *Alloprevotella* (padj 0.0015, LDA 3.8), *Phascolarctobaterium* (padj 0.0007, LDA 3.6) and *Prevotella_7* (padj 0.0054, LDA 3.3) were significantly enriched in the infected groups (Figure 4.i). There was no difference in the genera abundance between the females and males (Figure 4.ii) whereas the *Gastranaerophilales* and *Lachnospiraceae_ND3007* were significantly different between the stunted and normal children. *Ruminobacter*, *Alloprevotella*, *Prevotella_7* and *Streptococcus* abundance were significantly different between the sampling sites (Kruskal test p.adj < 0.05, Figure 4.iv). In order to determine if there was an association between the significantly differentiated genera with the *S. mansoni* infection intensity, a fixed linear regression model analysis with site as a covariate was carried out. We observed a positive association (p value < 0.05) with infection intensity in the genera, *Ruminobacter* (Fstat = 4.05, R^2 = 0.02), *Alloprevotella* (Fstat = 9.71, R^2 = 0.06), *Phascolarctobaterium* (Fstat = 17.8, R^2 = 0.11) and *Prevotella_7* (Fstat = 9.38, R^2 = 0.06) (Figure S4, Table S3).

4.5 Systemic inflammatory cytokines associated with gut microbiome

The plasma levels of the Th1 (IFNG, IL2, IL10, IL12P70, TNFA, TNFB) and Th2 (GMCSF, IL1B, IL4, IL5, IL6, IL9, IL13) cytokines were determined in children with high (n=56) and low (41) *S. mansoni* infection intensity and compared with uninfected control (n=45) children (Figure 5). We observed significantly lower plasma cytokine levels between the high intensity infection and control samples (padj < 0.05) in the Th1 cytokines TNFA, TNFB, IL2, IL12 and IL10. Except for IL2, there was no difference in levels of the Th1 cytokines between the low intensity and control samples although there were trends towards diminished responses in the low intensity samples (Figure 5). Similarly, there were significantly lower Th2 responses in the high intensity *S. mansoni* infected children compared to the controls while the low intensity infections were significantly associated with reduced levels of IL1B, IL4, IL5, and IL13 (Figure 5). In order to determine if the serum cytokine levels observed corroborates with the expressed gene transcripts, we used our previously published gene expression data to extract the read counts of 176 cytokine associated genes (Namulondo et al., 2023). We obtained count data for 44 samples which were part of this current study of which, 16 were High intensity, 12 low intensity and 16 control samples (Table S4). Principal component analysis on the gene read counts showed that there was overall variance of 18% with 50% (8/16) of the high intensity samples having PC1, PC2 < 0 while 43% (7/16) of the controls having PC1, PC2 > 1 (Figure 6A). These data are indicative of an inverse cytokine gene expression relationship between the high intensity *S. mansoni* infections compared to the Low intensity infection and uninfected controls (Figure 6B). Therefore, this implied that the low levels of plasma cytokines observed in the high intensity *S. mansoni* infections were possibly due to the low expression levels of the cytokine genes involved in immune response pathways (Namulondo et al., 2023).

In order to determine if there was an association between the significantly abundant microbial genera and the cytokine levels in the groups, a fixed linear regression analysis of the genus against one specific cytokine using all the other cytokines as covariates was carried out (Table 2, Table S5). The genus *Alloprevotella*, which is enriched in *S. mansoni* infected (Low intensity) children was associated with Th2 response cytokines of IL6 (Figure 6C), IL13 (Figure S5A) and IL1 (Figure S5B). The genus *Streptococcus* which is enriched in infected children (Low intensity) was strongly associated with IL6 (Figure 6D) IL10 (Figure S5C) and TNFA (Figure S5D). The *Gastranaerophilales* which were highly enriched in the uninfected control groups, was significantly associated with the Th2 response, IL6 cytokines (Figure 6E) and weakly

associated with GMCSF (Figure S5E). The genus *Ruminobacter* which is abundant in the infected (Low intensity) group, was highly associated with IFNG (Figure S5F), IL9 (Figure S5G) and weakly associated with IL6 (Figure 6F). The genus *bacteroides* which is enriched in the control group were highly associated with the Th2 response, TNFA (Figure S5H) and weakly with TNFB (Figure S5I) cytokines.

Discussion

The gut microbiome plays a significant role in human health through fermentation of food (Wong et al., 2006), protection against pathogen colonization, promoting immune homeostasis and immune responses (Pickard et al., 2017), and synthesis of vitamins (Hillman et al., 2017; Rowland et al., 2018). Gut microbiota dysbiosis is often caused by changes in the health status (such as infections and inflammation), environmental factors such as diet (high sugar low fibre), xenobiotics (antibiotics, drugs) and hygiene (Hrncir, 2022).

We conducted a cross sectional study on 138 faecal DNA samples from school aged children residing in high *S. mansoni* prevalence and transmission hotspots along the Albert-Nile in Pakwach district, Uganda (Mulindwa et al. 2022). These children presented with varied *S. mansoni* infection intensities which were categorised according to World Health Organisation standards as high, low and uninfected controls. We observed a high abundance of the phyla Firmicutes, Bacteroidota, Spirochaetota, Proteobacteria and Actinobacteriota in these children, with subtle differences in the abundance of each of these phyla in the infected groups in comparison to the uninfected controls. The Firmicutes are the most abundant phyla in the human gut followed by Bacteroidota, Actinobacteria and Proteobacteria (Laterza et al., 2016; Rinninella et al., 2019; Hou et al., 2022). Interestingly we observed Spirochaetota instead of the Actinobacteria as the third most abundant phyla among these children. This disparity in the order of abundance could be due to the sampled population ethnicity (Brooks et al., 2018; Dehingia et al., 2019; Byrd et al., 2020; Abdill et al., 2022) and environmental factors such as diet (Hrncir, 2022). The Firmicutes and Bacteriodota had the highest abundance which is consistent with what has been observed in the healthy microbiome (Huttenhower et al., 2012; Lloyd-Price et al., 2016). However, the Spirochaetota and Proteobacteria had a relatively higher abundance in the infected compared to the control children, which was indicative of dysbiosis that has also been associated with Inflammatory bowel disease (Sokol and Seksik, 2010; Morgan et al., 2012). The Spirochaetota contain pathogenic species which include *Leptospira spp* that cause Leptospirosis (Gonalves-De-Albuquerque et al., 2012), *Borrelia spp* which causes Lyme disease (Koutantou et al., 2024) and Relapsing fever (Cutler et al., 2010; Tang et al., 2024), *Treponema spp* which causes Syphilis and yaws (Mitjà et al., 2015) and *Brachyspira spp* which causes colonic Spirochetosis (Norris, 2019). The Spirochaetota pathogens are mainly zoonotic (*Leptospira, Brachyspira*) or transmitted by a tick vector (*Borrelia*) and given the Pakwach Alur community mixed farming practices (dependent on cattle herding, crop farming and fishing), these could be the source of the pathogenic taxa observed. At the genus level, *Prevotella* followed by *Treponema*, *Faecalibacterium* and *Blautia* were the most abundant. *Prevotella* plays a pivotal role in invoking immune response in chronic inflammatory diseases whereby its increased abundance is associated with Th17 mediated mucosal inflammation (Larsen, 2017). *Faecalibacterium* are considered to be ubiquitous in the gastrointestinal tracts (GITs) of healthy humans (Tap et al., 2009; De Filippis et al., 2020) where they are essential for gut GIT homeostasis (Miquel et al., 2013). However reduced levels of *Faecalibacterium* in the gut have been associated with inflammatory conditions (Martín et al., 2023). The measure of species diversity in the gut microbiomes of the children was assessed using the alpha and beta diversity indices (Chiu and Chao, 2016) which were high and indicative of rich and diverse species microbiome within these groups. We observed slightly higher alpha diversity indices in the *S. mansoni* infected children in comparison to the controls, which is consistent with the fact that helminth infections have been shown to alter the gut microbiome diversity (Loke and Lim, 2015; Mutapi, 2015; Zaiss and Harris, 2016).

The differential abundance of the bacterial genera in the *S. mansoni* infected children compared to the uninfected controls showed that, *Ruminobacter*, *Alloprevotella*, *Phascolarctobaterium*, *Prevotella_7*, *Prevotella*, *Prevotellaceae_NK3B31*, *Erysipelotrichaceae* and *Streptococcus* were significantly enriched in the infected groups. On the other hand, the genera that were significantly enriched in the uninfected control children included, *Gastranaerophilales*, *Bacteroides*,

Lachnospiraceae_ND3007 and *Parabacteroides*. *Alloprevotella*, *Prevotella* and *Bacteroides* have previously been shown to be elevated in the faecal microbiome of urinogenital *S. haematobium* infected children in Zimbabwe (Kay et al., 2015; Osakunor et al., 2020) and adolescents in Nigeria (Ajibola et al., 2019). In addition, *Alloprevotella*, *Prevotella* and *Bacteroides* were enriched in the gut microbiome of patients with chronic *S. japonicum* infection in China (Zhou et al., 2022; Guo et al., 2023). On the other hand, *Erysipelotrichaceae* was found to be decreased in *S. haematobium* adolescents (Ajibola et al., 2019). *Alloprevotella*, *Prevotella, Ruminobacter and Phascobacterium* belong to a group of gram negative anaerobes which encode a broad spectrum of enzymes that hydrolyze dietary fibres into short chain fatty acids (SCFAs) (Kaoutari et al., 2013). The concentrations of the SCFAs in blood influence host cell responses (Kim et al., 2016) depending on the type and amount of dietary fibre ingested by the host, and also helminthic infection (Piekarska et al., 2011; Zaiss and Harris, 2016; Llinás-Caballero and Caraballo, 2022). In this study we observed that the children with high *S. mansoni* intensity infections coupled with the high levels of the *Prevotellaceae* microbiota could have culminated in high SCFAs in the gut which resulted in the low systemic cytokine levels observed in comparison to the low infection intensity and control groups. This is probably due to the fact that SCFAs produce anti-inflammatory effects by inhibiting Nuclear factor-κB (NFKB) (Tedelind et al., 2007; Li et al., 2018), a signalling pathway that mediates the transcription of various cytokines such as, TNFA, TNFB, IL1B, IL2, IL3, IL5, IL12, and IL18 (Pahl, 1999; Liu and Malik, 2006; Vallabhapurapu and Karin, 2009). Furthermore, SCFAs promote the proliferation of Regulatory T cells, Tregs (Smith et al., 2013), which prevent inflammatory reactions in the gut by producing IL10 (Barbi et al., 2014), which we postulate resulted in the low inflammatory response in the children with high intensity infections. Except for IFNG and IL10, we observed significantly lower levels of the Th1/Th2 cytokines assayed in the high intensity infection in comparison to the low infection intensity and control samples. A similar phenomenon has been observed in helminth infected participants with suppressed Th1 and Th17 response (McSorley et al., 2011). Similarly, low levels of IL6, IL10 and TNFA have been associated with severe hepatic fibrosis in *S. mansoni* chronically exposed individuals (Mutengo et al., 2018). The dampened cytokine response in the children with high *S. mansoni* infection intensity, was indicative of high Schistosome egg deposition in the gut. The intestinal homeostasis is maintained by gut mucosal networks which includes, gut associated lymphoid tissues, secretory IgA, mucosal immune cells (Th1, Th2, Th17, Tregs), cytokines (such as IL10, IL6), chemokines (such as CCR9) and commensal bacteria (McGhee et al., 1992; Kurashima and Kiyono, 2017). The schistosome egg deposition in the gastro intestinal tract (GIT) contributes to gut dysbiosis and immunopathology observed in chronic schistosomiasis, through dampening Th1 immune responses and advancing Th2 response (Brunet et al., 1998; Wilson et al., 2007). These gut microbiota activate dendritic cells that induce the production of pathogen- or antigen-specific inflammatory Th1 and Th17 cells via IL1B, IL6, and IL23 (Wu et al., 2010; Rosser et al., 2014; Tokuhara et al., 2019). We investigated how the variation in systemic cytokine response with *S. mansoni* infection could be associated with the gut microbiome dysbiosis in the children. We identified the cytokines IL1, IL6, IL13, IFNG, IL9, IL6, IL10, TNFA as highly associated with dysbiosis under schistosome infection. However, IL6 had the highest frequency of association with *Alloprevotella*, *Ruminobacter*, *Gastranaerophilales* and *Streptococcus.* IL6 is released by mucosal immune cells and has been shown to be associated with gut microbiome dysbiosis (Wu et al., 2022). In addition, genotyping and expression quantitative trait loci (eQTL) analysis carried out on the children within this cohort showed that *IL6* gene contains haplotypes and eQTL single nucleotide polymorphisms (SNPs) that influence IL6 expression and are associated with worm burden (Nyangiri et al., 2023). This implies that IL6 plays a pivotal role in chronic *S. mansoni* pathology and influencing gut microbial dysbiosis.

Conclusion

In this study we have shown that children in *S. mansoni* endemic hotspots present with different infection intensities which are associated with the gut microbiome and systemic immune response. The microbial and immune response profiles in children with high *S. mansoni* infection intensities could be explored as biomarkers that can be used in screening of such individuals who can be prioritized for treatment. The association between the gut microbiome and systemic cytokine response was profound and this mechanism of interaction in Schistosomiasis chronic disease pathophysiology needs to be further investigated. The microbiota that are differentially enriched in individuals who are not infected with Schistosomes could be explored for probiotic intervention among the infected children in order to improve their health status.

Declarations

Data availability

The 16SrRNA Fastq read data for the 138 samples will be deposited to EGA under the TrypanoGEN+ project accession link https://ega-archive.org/studies/EGAS00001007173

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Ethics approval and consent to participate

The study protocol was reviewed by the institutional review board of the Ministry of Health, Vector Control Division Research and Ethics Committee (Reference No. VCDREC106) and approved by the Uganda National Council of Science and Technology (Reference No. UNCST HS 118). The study was conducted with guidance from the district health officials, including the selection and training of the village health teams that were involved in the mobilization and recruitment of the school aged children into the study. The objectives, potential risks and benefits of the study were explained to the parents/guardians who signed informed consent, and later explained to the school age children in English and Alur dialect who provided assent for participation into the study. Written formal consent from parents and written assent from the children were obtained. If a child was observed to have *S. mansoni* eggs in their stool, they were offered free treatment, which consisted of praziquantel at a dosage of 40mg/kg administered by trained Ministry of Health personnel, assisted by the district health worker.

Consent for publication

Not applicable

Authors contributions

JM, BN, RB, HN, AM, EM contributed to conception and design of the study; JM, JN, MPK, ON supervised sample and metadata collection; JM, IB, CM analysed the microbiome data; JM, GC, CM2, ST, AS, IS analysed the cytokine data; JM wrote the manuscript. All authors read and approved the final manuscript

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Tables

Table 1. Summary of study participant demographics and sample phenotype. The total number of samples and their categories by Sex, Age, Site, *S.mansoni* infection intensity and Height for Age Z-score

			S. mansoni intensity (Kato-Katz, EPG)			*Height for Age Z-score (HAZ)*	
Group	Sub group	Total (N)	High [>100]	Low [<100]	Control [0]	Normal [> -2 SD]	Stunted [< -2SD]
Sex	Females	60	23	18	19	23	37
	Males	80	35	19	26	21	59
Age	10	33	11	8	14	9	24
	11	17	5	6	6	8	9
	12	29	14	3	12	13	16
	13	25	11	10	4	4	21
	14	24	10	7	7	5	19
	15	12	7	3	2	5	7
Site	Alwi	11	0	0	11	9	2
	Dei	26	12	9	5	3	23
	Kayonga	23	9	4	10	5	18
	Kivuje	30	11	8	11	6	24
	Nyakagei	33	20	10	3	14	19
	Panyigoro	17	6	6	5	7	10

Table 2. Summary of genera that are differentially abundant and associated with *S. mansoni* infection intensity and plasma cytokine levels. Bacterial genus, in which group the genus is Enriched categorized by *S.mansoni* infection intensity (High, Low, Control), the P-adjusted values obtained from the differential abundance analysis (Lefse, DESeq2, EdgeR), the Sex (Male, Female) bias observed by Wilcoxon P-adjusted value, Height for Age (Stunted, Normal) bias observed by Wilcoxon P-adjusted value, linear regression test of association between genus and S.mansoni infection intensity as observed by Pvalue and linear regression test of association between genus and plasma cytokine levels as observed by Pvalue. *signifies Pvalues that are considered significant.

Genus	Enriched group	Differential abundance (p.adj)	Sex (Wilcoxon test p.adj)	HAZ (Wilcoxon test p.adj)	Site (Kruskal test p.adj)	Association Sm Intensity (lm pvalue)	Association Cytokine (lm pvalue)
Phascolarctobacterium	High	0.0007	0.24	0.76	0.11	4.2E-05*	NA
Alloprevotella	Low	0.0015	0.53	0.59	0.025*	0.0022*	IL13 (0.01) IL1 (0.05) IL6 (0.04)
Prevotella_7	High	0.0054	0.6	0.39	0.042*	0.0026*	NA
Prevotellaceae_NK3B31	Low	0.0091	0.29	0.8	0.29	0.0033*	NA
Ruminobacter	Low	0.0054	0.82	0.15	0.016*	0.0460*	IFNg (0.04) IL9 (0.05) IL6 (0.08)
Gastranaerophilales	Control	0.0193	0.34	0.04*	0.23	0.0723*	GMCSF (0.09) IL6 (0.05)
Bacteroides	Control	0.0427	0.26	0.344	0.225	0.0875*	TNFb (0.06) TNFa (0.03)
Prevotella	Low	0.0129	0.42	0.89	0.17	0.1198	NA
Lachnospiraceae ND3007	Control	0.03	0.071*	0.058*	0.177	0.1781	NA
Erysipelotrichaceae_g__	High	0.0306	0.47	0.93	0.62	0.3891	NA
Streptococcus	Low	0.0024	0.48	0.48	0.014*	0.6209	IL10 (0.04) IL12 (0.07) IL6 (0.02) TNFa (0.03)
Parabacteroides	Control	0.0037	0.89	0.29	0.51	0.8992	NA

Figures

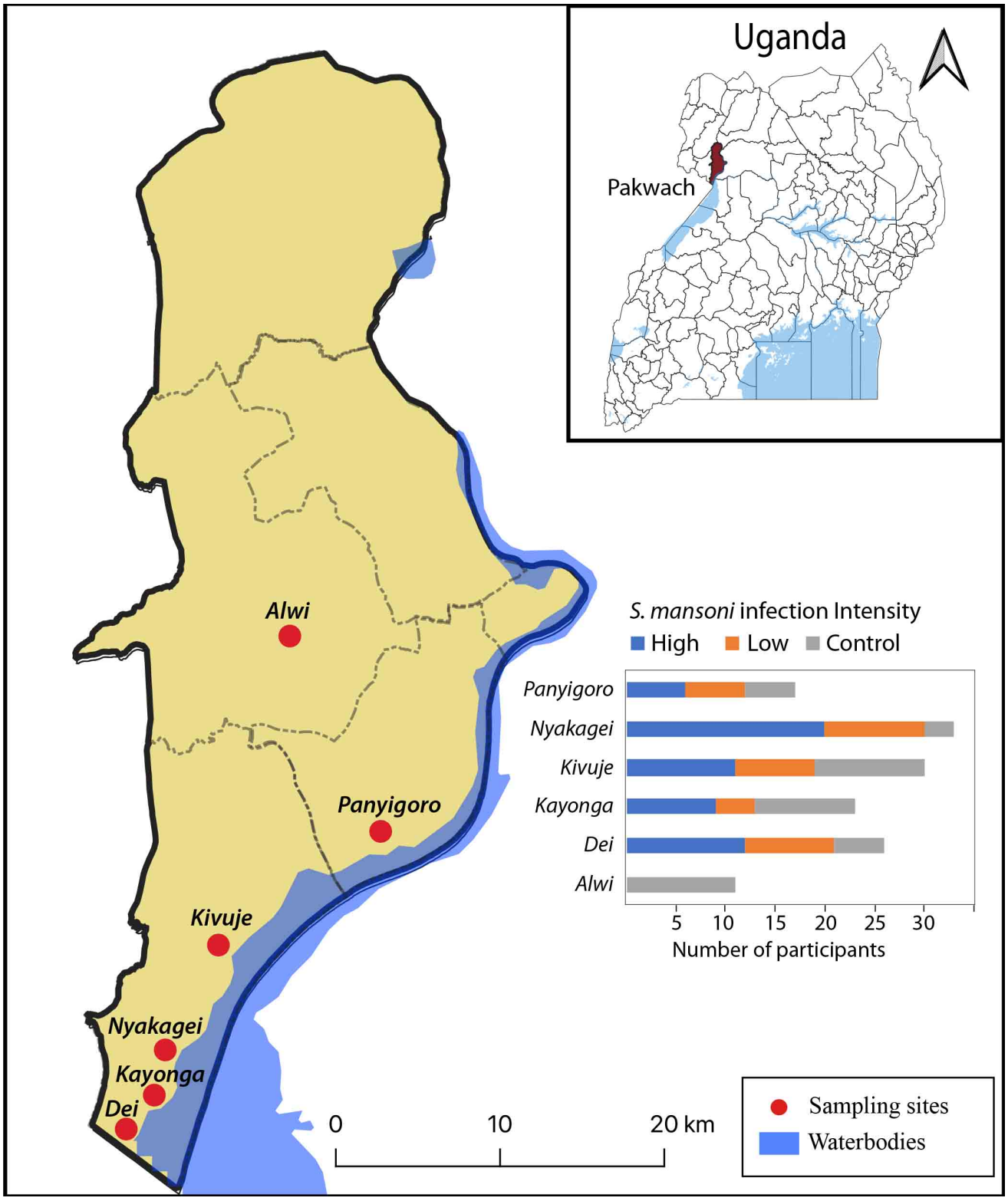

Figure 1

Map showing the study participant sampling sites and *S. mansoni* infection intensities by site. The *S. mansoni* infection intensities were based on the Kato Katz test and the normalised eggs per gram (EPG) categorised as High (EPG > 100), Low (EPG 1-100) and Control (EPG=0). The base map of Pakwach district located along the Albert-Nile in West Nile region of Uganda, was obtained from Uganda Bureau of statistics (2012), http://purl.stanford.edu/vg894mz3698, and is in public domain with no restrictions on use.

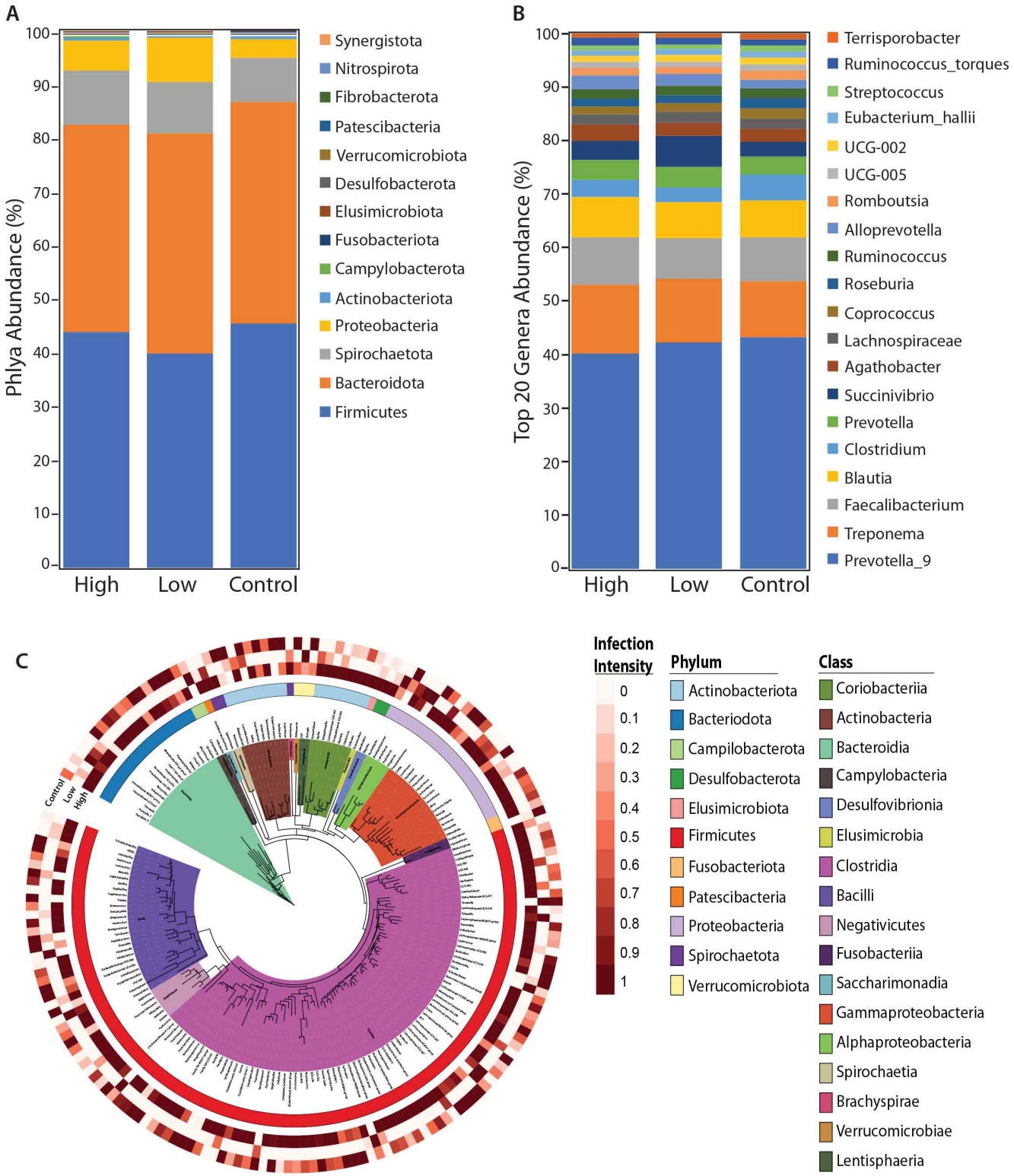

Figure 2

Comparison of gut microbiome composition in uninfected children and those with *S. mansoni* high and low infection intensities. **A**. Percentage distribution of the 14 phyla identified from the annotated Amplicon Sequence Variants (ASVs) in the children's gut microbiome. **B.** Percentage distribution of the Top 20 genera identified from ASVs in the children's gut microbiome. **C.** Maximum likelihood circular phylogenetic tree of the abundance of bacterial phyla and classes overlaid with a heatmap of the normalised abundances per tree node for the different classes of bacteria.

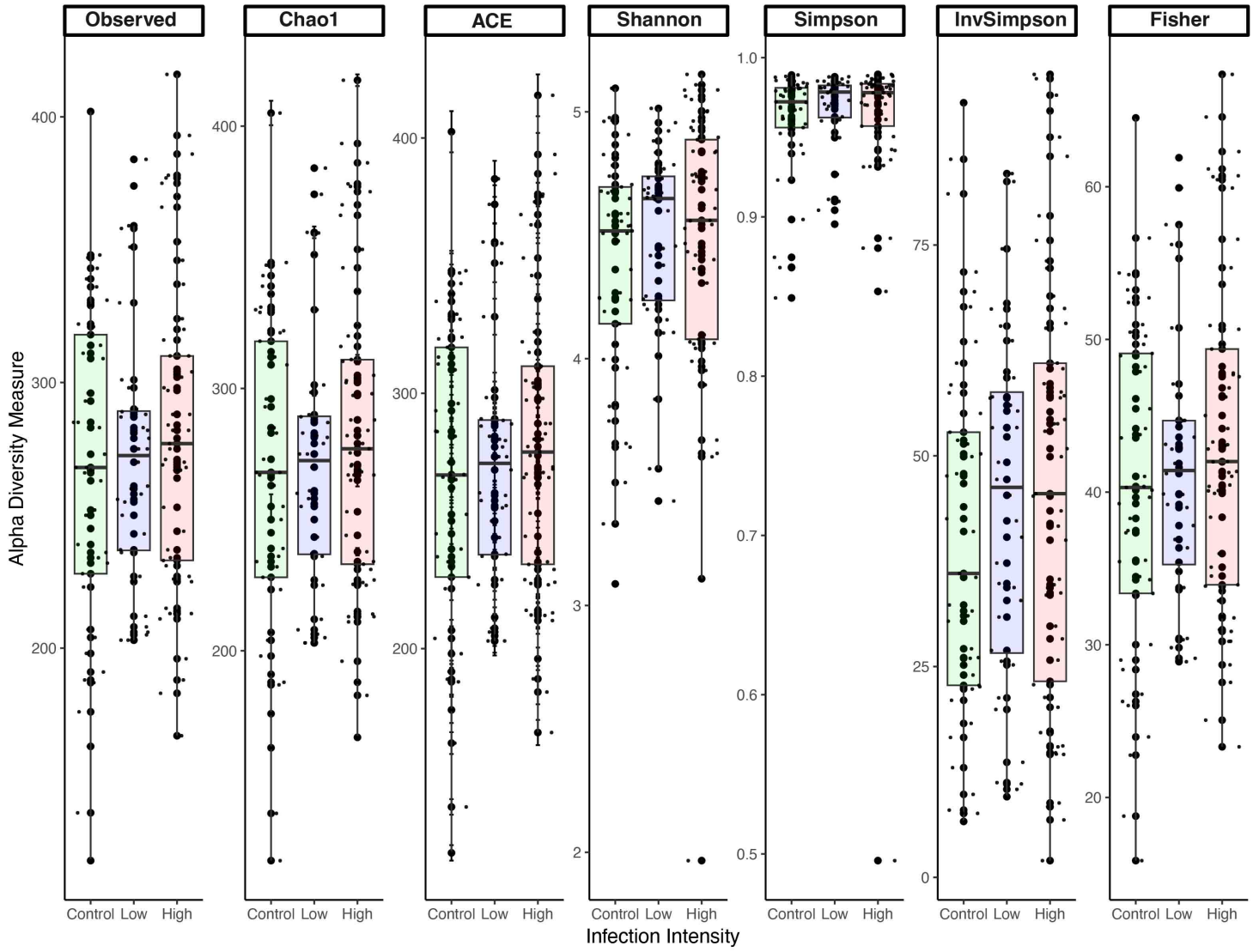

Figure 3

Alpha diversity analysis showing the weighted average of diversity in the samples with categorised *S. mansoni* infection intensity. A comparison of alpha diversity indices of Observed index, Chao1 index, ACE index, Shannon index, Simpson index, InvSimpson index and Fisher index.

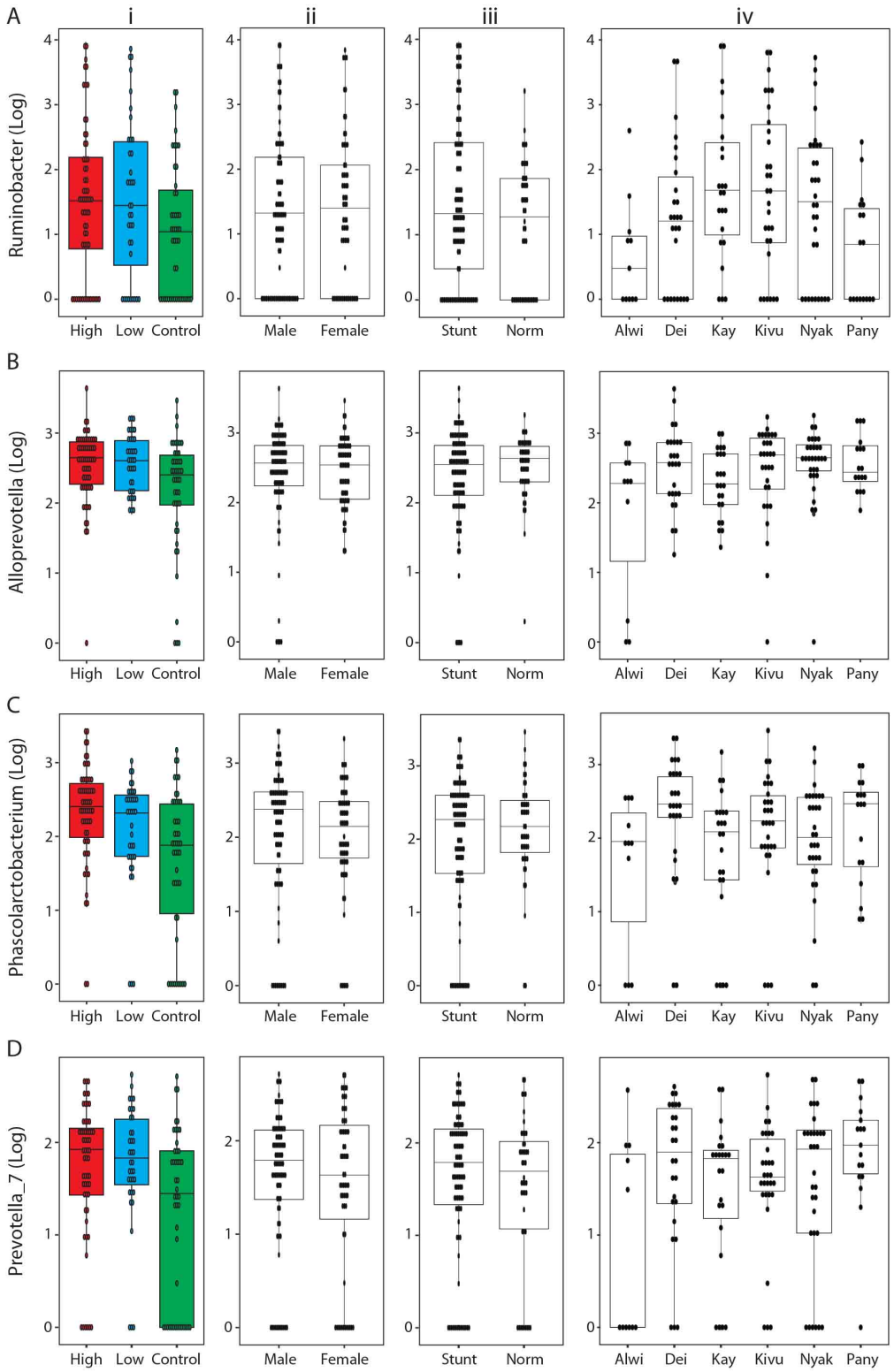

Figure 4

Comparison of Abundance of genera with **i**. Infection intensity, **ii**. Sex, **iii**. Stunting and **iv**. Sampling site (Alwi, Dei, Kayonga, Kivuje, Nyakagei, Panyigoro). This comparison was done on the most significantly differentiated genera based on infection intensities, that is **A.** *Ruminobacter*, *B. Alloprevotella*, C. *Phascolarctobaterium* and D. *Prevotella_7*.

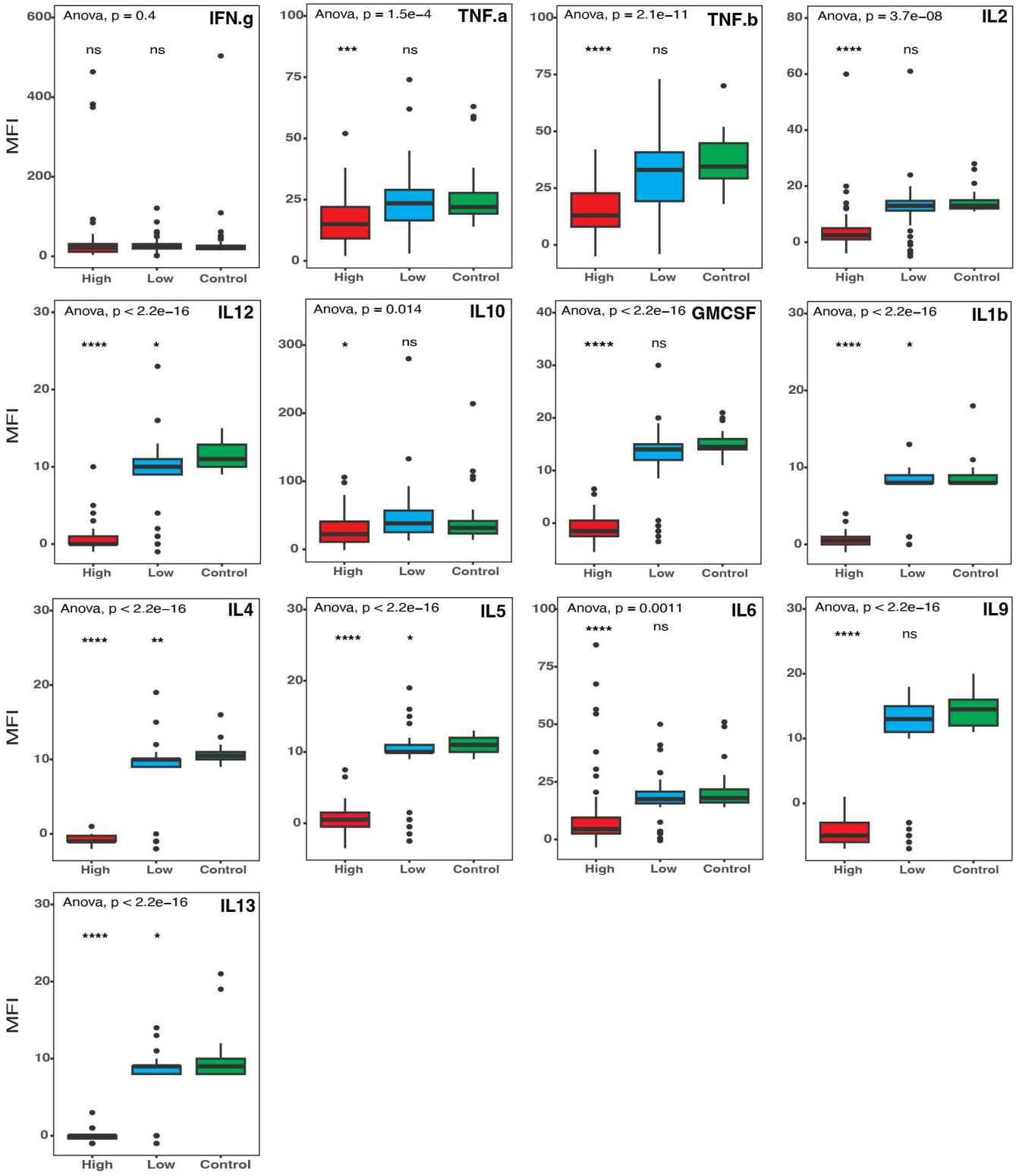

Figure 5

Comparison of plasma cytokine levels in the uninfected control and *S. mansoni* infected children with High and low intensities. The cytokine mean fluorescence intensity (MFI) for each individual was compared with their corresponding *S. mansoni* infection intensities. Wilcoxon test for statistical difference between high/ low intensity and uninfected control group was done, represented as non-significant (ns), significant p<0.05 (*) and highly significant p<1E-6 (****). The Luminex 13plex assay panel of Th1 cytokines, IFNG, TNFA, TNFB, IL2, IL12, IL10 and Th2 cytokines GMCSF, IL1B, IL4, IL5, IL6, IL9, IL13 was used.

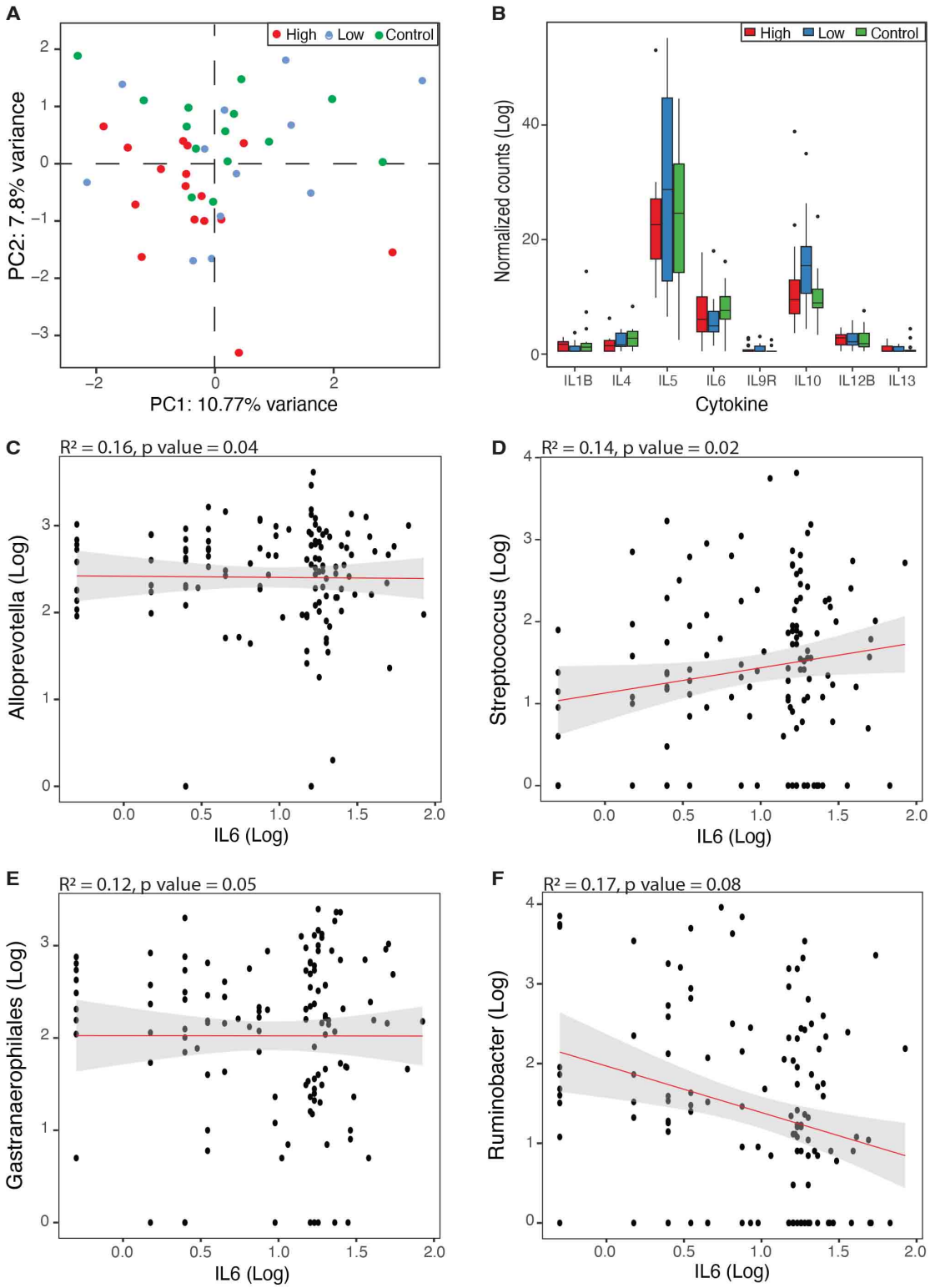

Figure 6

A. Principal component analysis on the 176 cytokine transcript read counts from 44 samples (16 High intensity, 12 low intensity and 16 control) represented in this study's microbiome data set. **B**. Plot of the Log transformed read counts in the control, high and low infection intensities versus the transcripts of selected cytokines transcripts. Linear regression association analysis of the cytokine IL6 plasma levels with the genus abundance in the gut microbiome genera of **C**. *Alloprevotella,* **D.** *Streptoccocus*, **E**. *Gastranaerophilales* and **F**. *Ruminobacter*.

Supplementary Files

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