Shiftwork, functional bowel symptoms, and the microbiome (#57966)

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Shiftwork, functional bowel symptoms, and the microbiome

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Background. There are about 15 million Americans working full-time on evening, night, or rotating shifts. Between 48% and 81.9% of those working rotating or night shifts report abdominal pain, constipation, diarrhea and other symptoms of functional bowel disorders. The basis for this high prevalence of functional bowel disorders, including irritable bowel syndrome (IBS), among shift workers is unknown. Animal studies, however, suggest that circadian disruption, similar to that in shift workers, may contribute to the development of GI complaints among shift workers by altering the composition and normal diurnal rhythmicity of the resident intestinal microbes.

Methods. Fifty-one full time staff nurses who worked either 12-hour day or night shifts completed demographic information, and the Rome III IBS module. They also collected two samples of gut microbiota before the beginning and at the end of their last work shift on day 14, using validated field-tested methods consistent with the Human Microbiome Project. After DNA extraction, 16S rRNA sequencing and assignment to the genus level was completed, samples were then compared to determine if there were 1) differences in the diversity and profile of the microbiome by shift type; 2) if there were differences in the microbiome by time of day for collection; and 3) whether there were differences in the diversity and profile of the microbiome of nurses with IBS and those without IBS.
Results. There were no differences in alpha or beta diversity of gut microbiota when specimens from day and night shift nurses were compared. There were however marginal differences in beta diversity when specimens collected at the beginning and end of the shifts were compared, with seven OTUs being differentially abundant when collected from day shift workers in the evening. There were also three OTUs to be differentially abundant in participants reporting IBS symptoms.

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Abstract

Background. There are about 15 million Americans working full-time on evening, night, or rotating shifts. Between 48% and 81.9% of those working rotating or night shifts report abdominal pain, constipation, diarrhea and other symptoms of functional bowel disorders. The basis for this high prevalence of functional bowel disorders, including irritable bowel syndrome (IBS), among shift workers is unknown. Animal studies, however, suggest that circadian disruption, similar to that in shift workers, may contribute to the development of GI complaints among shift workers by altering the composition and normal diurnal rhythmicity of the resident intestinal microbes

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Introduction

There are about 15 million Americans working full-time on evening, night, or rotating shifts, or other irregular employer-arranged schedules; 4.7% on evening shifts, 3.2% on night shifts, 3.1% on irregular schedules, and 2.5% on rotating shifts (United States Department of Labor Bureau of Labor Statistics 2005). Night shift work is associated with increased mortality, higher risk of cardiovascular disease, cancer, diabetes,

hypertension, chronic fatigue, sleep problems and higher body weight (Gu 2015; Jia 2013; Myers 2015; Pan 2011; Rajaratnam 2011; Vyas 2012). Night and rotating shift workers also report a higher prevalence of Irritable Bowel Syndrome (IBS), abdominal pain, constipation and diarrhea than do day shift workers (Caruso 2004; Knutsson 2010; Nojkov 2010; Wells 2012). In fact, between 48% and 81.9% of those working rotating or night shifts report abdominal pain, constipation, diarrhea and other symptoms of functional bowel disorders (Nojkov 2010; Saberi 2010).

The basis for this high prevalence of functional bowel disorders, including IBS, among shift workers is unknown. However, some studies suggest that inappropriate nutrition or irregularity in the timing of meals (Bilski 2006; Lowen 2010), and psychological disorders (Zhen 2006) may contribute to the high prevalence of functional bowel symptoms among workers on rotating or night shifts.

Other studies strongly suggest that sleep deprivation or sleep disturbances are associated with the presence and severity of functional bowel symptoms reported by resident physicians and nurses (Jarrett 2000; Saberi 2010; Wells 2012). Moreover, animal studies suggest that circadian disruption, similar to that in shift workers, contributes to the development of GI complaints among shift workers by altering the composition and normal diurnal rhythmicity of the resident intestinal microbes (De Bacquer 2009). Interestingly, gut microbiota community composition and diversity are malleable and sensitive to changes in diet and other environmental factors (Voigt 2014), including activity and sleep patterns. For example, Thaiss and colleagues (Thaiss 2014)

82 “jet lagged” a group of mice by subjecting them to an 8-hour advance for three days
83 before allowing them to revert to their usual schedule for three more days, then
84 subjecting them to another 8 hour advance for three days. Mice exposed to 4 weeks of
85 this schedule lost their usual pattern of physical activity, and consumed food at irregular
86 intervals. Significantly, this environmentally induced disruption of daily activity patterns
87 (jet lag schedule) was associated with a loss of diurnal rhythmicity of microbiota
88 composition in mice.
89
90 Thaiss and colleagues (Thaiss 2014) also found similar changes in human microbiota
91 composition in two volunteers who flew from the US to Israel (an 8-10 hour advance).
92 Samples collected at baseline (one day pre-flight), during jet lag (one day after landing),
93 and during recovery (2 weeks after landing) showed rapid changes in the composition of
94 the microbiota. During jet lag (the first 24-hours after landing), there was a higher
95 relative representation of Firmicutes, which reversed upon recovery from jet lag (2
96 weeks later). Although some studies have demonstrated no differences in composition
97 of the gut microbiome when samples from lean and obese individuals were compared
98 (Ley 2006; Turnbaugh 2009), other studies in humans have demonstrated that
99 Firmicutes are associated with a higher propensity for obesity and metabolic disease
100 (Finucane 2014; Ley 2006), conditions that are more common in night and rotating shift
101 workers (De Bacquer 2009; Suwazono 2008).
102
103 Finally, multiple studies have linked reduced microbial diversity and richness in
104 microbial communities to IBS symptoms. For example, Krogius-Kurikka and

105 colleagues.(Krogius-Kurikka 2009) reported that fecal samples from patients with
106 diarrhea-predominant IBS were enriched with Proteobacteria and Firmicutes but had
107 reduced Actinobacteria and Bacteroidetes compared to healthy controls. Other studies
108 (Bhattarai Y. 2017; Salonen 2010) have shown an increase in the Firmicutes-to-
109 Bacteriodetes-ratio, a decrease in some types of Firmicutes families (Lactobacilli,
110 Faecalibacterium) and the Actinobacteria population (Bifidobacteria, Collinsella), and an
111 increase in some Firmicutes families (Veillonella, Streptococci, and Ruminococcus spp.)
112 and in Proteobacteria (Enterobacteriaceae spp.). In addition, low microbial richness, an
113 absence of Methanobacteriales, and enrichment with Bacteroides enterotypes are
114 associated with more severe IBS symptoms (Tap 2017). Not only is the composition of
115 the gut microbiota altered in patients with IBS, these imbalances in the microbial
116 community or dysbiosis, occur more frequently in patients with IBS compared to healthy
117 individuals. Reduced diversity was observed in nearly three-fourths of the IBS patients
118 studied by Casén and colleagues (Casén 2015) compared to 16% in normal individuals
119 (Collins 2014; Jeffery 2012; Karantosos 2010).
120
121 While these studies suggest that shiftwork alters gut microbiota and that alterations in
122 gut microbiota are common in patients with IBS, they do not demonstrate whether these
123 alterations are associated with the somatic symptoms experienced by many rotating
124 and night shift workers. Therefore, the present study is designed as the initial step in
125 determining if there are differences in 1) composition and diversity of the microbiome of
126 night shift workers compared to day shift workers; and 2) the composition and diversity
127 of microbiome among night shift workers experiencing functional bowel symptoms (e.g.,

bloating, lower abdominal pain, constipation and diarrhea) compared to night shift workers not experiencing functional bowel symptoms.

Materials & Methods

Subjects

Participants in this study included 51 full-time staff nurses who worked 12-hour day or night shifts at a large university hospital. Registered nurses were eligible to participate if they were between the ages of 18 and 65 and did not report a history of inflammatory bowel disease (e.g., Crohn's disease or ulcerative colitis) or other chronic disorder affecting the GI track (e.g., GI cancer). Those with recent antibiotic exposure were asked to delay their enrollment in the study for two weeks.

As expected, the sample was predominantly female (96%), with a mean age of 32.9 ± 10.0 years and a range of 21-59 years. Just under half of the participants reported working straight day shifts (47%), with the remainder of the sample working either straight night shifts (51%) or rotating shifts (2%). For purposes of the analysis, the nurse who reported working rotating shifts was categorized as working night shift since she worked straight nights during the two-week data-gathering period. Although only three participants (5.8%) reported a prior diagnosis of IBS, a total 18 participants (35%) met criteria for the diagnosis of IBS using the Rome III criteria. Participant BMIs ranged from 18.2 to 39.5 with a mean BMI of 26.7 $\pm$ 5.4. As illustrated in Table 1, there were no significant differences by shift type in terms of age, BMI, diagnosis of IBS or type of IBS.

Instruments

Data for this study was obtained using a variety of subjective and objective measures. A Demographic Questionnaire and Brief Health History was used to collect information about participant age, and the usual shift worked. Participants were also asked to report any previous diagnosis of inflammatory bowel disorders or chronic diseases affecting the GI, and to list current medications and supplements used. The IBS module from the Rome III Questionnaire (2006) consists of 10 questions that ask subjects to rate the frequency of recurrent abdominal pain or discomfort, onset of pain associated with a change in frequency of stools, and the onset of pain associated with a change in the form of stools. This module is considered the gold standard for assessing functional bowel symptoms.

Samples of gut microbiota were collected just before the beginning and just after the end participants' work shift at the end of the two week data-collection period using validated, field-tested methods consistent with the Human Microbiome Project (Methé 2012). Four specimens (two each time) were collected using the Elution-swap system (Copan). The rectal swabs were stored in 1 mL of Amies transport medium (Copan) and immediately frozen and stored until DNA extraction. Prior to extraction, fecal material (200 mg) was suspended in 500-µl lysozyme (20 mg/ml in 20 mM Tris-HCl pH 8, 2 mM EDTA, 1.2% w/v Triton X-100) and incubated at 37°C for 2 h using the QIAamp® DNA Stool Mini Kit (Qiagen, Inc., Valencia, CA).

Procedure

After obtaining approval from the Emory University's IRB (MOD001-IRB00089064) and Emory Healthcare's Nursing Research Council, emails describing the study were sent to all staff nurses. Those interested in participating were instructed to contact the research team to schedule an appointment to provide informed consent, review study procedures, and complete the demographic and Rome III questionnaire. After written informed consent was obtained, the participant's work schedule was then reviewed to determine an appropriate date to collect samples of gut microbiota at the beginning and end of the participant's shift.

Data Analysis

DNA extraction & 16S rRNA sequencing_DNA extraction and 16S sequencing was performed by Omega Bioservices (Norcross, GA, USA) using a standard protocol. DNA was isolated using Omega Biotek Mag-Bind® Universal Pathogen DNA Kit. The V3-V4 region of the bacterial 16S rRNA gene sequences were amplified using the primer pair containing the gene-specific sequences and Illumina adapter overhang nucleotide sequences. The full length primer sequences are: 16S Amplicon PCR Forward Primer (5'- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and 16S Amplicon PCR Reverse Primer (5'- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC) . For amplicon PCR, each 25 µL of polymerase chain reaction (PCR) reaction contained 12.5 ng of sample DNA as input, 12.5 µL 2x KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Wilmington, MA) and 5 µL of 1 µM of each primer. PCR reactions were carried with an initial denaturation step performed at 95°C for 3min followed by 25

cycles of denaturation (95°C, 30 s), annealing (55°C, 30 s) and extension (72°C, 30 sec), and a final elongation of 5 min at 72°C. PCR product was cleaned up from the reaction mix with Mag-Bind RxnPure Plus magnetic beads (Omega Bio-tek, Norcross, GA). A second index PCR amplification, used to incorporate barcodes and sequencing adapters into the final PCR product, was performed in 25 µL reactions, using the same master mix conditions as described above. Cycling conditions were as follows: 95°C for 3 minutes, followed by 8 cycles of 95°C for 30", 55°C for 30" and 72°C for 30". A final, 5 minutes' elongation step was performed at 72°C. The libraries were normalized with Mag-Bind® EquiPure Library Normalization Kit ((Omega Bio-tek, Norcross, GA) then pooled. The pooled library ~600 bases in size was checked using an Agilent 2200 TapeStation and sequenced (2 x 300 bp paired-end read setting) on the MiSeq (Illumina, San Diego, CA). Sequence data was submitted to the National Center for Bioinformatic Information Short Read Archive database: accession PRJNA687007.

Processing of sequence data and assignment to Operational Taxonomic Units (OTUs)
Data processing, including demultiplexing, QC filtering, contamination and sample mislabeling data checks, OTU representation, taxonomy assignment via a reference database (Caporaso 2010; Wang 2007), and phylogeny and diversity analysis (Lozupone 2007; Lozupone 2006; Lozupone 2005) was done using R packages dada2 (Callahan 2016; McMurdie 2013).

Statistical Analysis

220 All data elements collected were evaluated for completeness (extent of missing data
221 values and reasons for missing data as can be determined) and accuracy (relative to
222 minimizing typos and inaccuracies from data collection methods) to maximize data
223 quality and integrity. Additionally, all data was analyzed for descriptive statistical
224 summaries and underlying distributions to provide initial estimates of the measures of
225 centrality (means and medians), and variance (standard deviation, interquartile range,
226 minimum and maximum).
227
228 We compared the Chao1 (measuring species richness) and Shannon (measuring both
229 richness and evenness) diversity indices of cases and controls since loss of taxonomic
230 diversity in general is an indicator of disease state in many ecological systems. We
231 used Principal Coordinates Analysis (PCoA) to visualize clustering of the data based on
232 distances computed using the Bray-Curtis (measuring difference of samples based on
233 relative abundance of species) and Jaccard (measuring difference of samples based on
234 presence-absence of species) metrics.
235
236 Given that this is a pilot study with 51 subjects, the primary focus was computing initial
237 estimates for (a) differences between day and night shift workers and (b) differences
238 between subjects with and without IBS symptoms relative to microbiome diversity.
239 These analyses will consist primarily of two-group comparisons (t-tests, chi-square
240 tests) between day versus night shift workers and between subjects with and without
241 IBS symptoms, using analysis of variance and general linear model procedures. For the
242 two gut microbiome samples obtained in the morning and two gut microbiome samples

obtained in the evening for each subject, the Linear Decomposition Model (LDM) (Hu 2020; Zhu in press) will be used to evaluate differences between these two times, as well as how the differences depend on the differences between subjects (e.g., day shift vs. night shit; IBS vs. no IBS). LDM helps to evaluate the proportion of variance in the outcome (gut microbiome measurements) explained within subjects (morning vs evening) compared to the proportion of variance explained between subjects (shift worked and presence of IBS). To analyze the associations between shift type and the composition of the participants' gut microbiome, we analyzed the fecal samples of night and day shift workers first compared the alpha diversity and beta diversity of the microbiome communities as well as the relative abundance and presence-absence of individual genera by shift type, then evaluated whether there were differences in the microbiome when the first specimen was collected in the evening and the second specimen in the morning (night shift nurses) versus having the first specimen collected in the morning and the second one in the evening (day shift nurses). Finally, we examined whether there were any differences in the alpha diversity and beta diversity of the microbiome communities as well as the relative abundance and presence-absence of individual genera of nurses with IBS compared to those without IBS.

Results

As shown in Figure 1, there were no significant differences in alpha diversity between day and night shift nurses (p=0.411 based on Chao1 index and p=0.242 based Shannon index). Nor were there differences in Beta diversity by shift type (p=0.476 and p=0.625 by the PERMANOVA method based on Bray-Curtis and Jaccard distances,

267 respectively (Figure 2). Nor were there differences in relative abundance and presence-
268 absence data across all genera (p=0.489 and p=0.824 by the LDM method).
269
270 However, Figure 3 shows an increase of the log Chao1 index from the beginning to the
271 end of the shift for day-shift workers while a decrease for night-shift workers; so does
272 the Shannon index. We found that the change of both alpha diversity indices from the
273 beginning to the end of the shift was significantly different between day shift and night
274 shift workers (p=0.034 for Chao1 and p=0.08 for Shannon), although the change among
275 the pooled workers was not significantly different (p=0.473 for Chao1 and p=0.236 for
276 Shannon) possibly due to the cancellation of effects with opposite directions. In terms of
277 beta diversity, samples obtained from the same participants tend to cluster together and
278 samples from the same time on sample participants tend to cluster together (See Figure
279 4). Now the change of the beta diversity metrices from the beginning to the end of the
280 shift among the pooled workers was marginally significant or significant (p=0.056 and
281 0.014 by PERMANOVA based on Bray-Curtis and Jaccard, respectively). Marginally
282 significant and significant findings were also noted by the LDM (p=0.035 and 0.063 by
283 LDM based on relative abundance and presence-absence data, respectively). However,
284 there is not enough evidence to confirm that the change was significantly different
285 between day shift and night shift workers possibly due to the small sample size,
286 although there was suggestive evidence (p=0.192 and 0.118 by PERMANOVA based
287 on Bray-Curtis and Jaccard, respectively; p=0.320 and 0.134 by the LDM based on the
288 relative abundance and presence-absence data.) In addition, the LDM based on relative
289 abundance data revealed seven OTUs to be differentially abundant; ASV_455(S5-A14a,

more abundant at the beginning of the shift), ASV_2527(Ruminoccaceae_NK4A214_group, more abundant at the end), ASV_1304(Ruminococcus_1, more abundant at the end), ASV_221 (Mobiluncus, more abundant at the beginning), ASV_7(Campylobacter), ASV_130(Alistipes, more abundant at the end), and ASV_62(Agathobacter, more abundant at the end).

Finally, as shown in Figure 5 there were no significant difference in alpha diversity when comparing participants without and with IBS symptoms (p=0.849 for Chao 1, p=0.484 for Shannon, by the LDM method). Although there were no differences in beta diversity by whether or not the participant had symptoms of IBS (p=0.206 and p=0.213 by the PERMANOVA method based on Bray-Curtis and Jaccard distances), there were significant differences based on the LDM results. Specifically, three OTUs were detected to be differentially abundant (ASV_1160 (Flavonifractor), ASV_1134 (Oscillibacter) and ASV_2379 (Ruminiclostridium_9) by the LDM method based on relative abundance data (p=0.2). There were significant differences (p=0.03) by the LDM based on presence-absence, which detected three OTUs (ASV_1160 (Flavonifractor), ASV_2379 (Ruminiclostridium_9) and ASV_47 (Escherichia/Shigella)) to be significantly more abundant in participants reporting IBS symptoms.

Discussion

The findings of this pilot study suggest that there are no differences in the richness and diversity of species when samples from nurses working day and night shifts were compared. There were however some changes in both alpha and beta diversity metrics when specimens collected at the beginning and end of the shifts were compared and

315 there was also some evidence that the changes were different for day shift and night
316 shift workers, with increased alpha diversity noted at the end of the day shift and
317 decreased alpha diversity noted at the end of the night shift. Seven OTUs were found
318 to be differentially abundant between the beginning and end of the shifts for the entire
319 sample. In addition, there were three OTUs to be differentially abundant in participants
320 reporting IBS symptoms.
321
322 Studies comparing the effects of shift work on the gut microbiome are limited and
323 somewhat contradictory. For example, a study of 10 male security guards who worked
324 both day and night shifts found there were no significant differences in in alpha or beta
325 diversity within and across-subject variation for both shifts (Mortas 2020). In contrast,
326 slight changes in microbial abundance and diversity were noted when 22 subjects, aged
327 20-35 years, delayed their sleep period for 2-4 hours (Liu 2020). Although there have
328 been studies comparing circadian variation in the gut microbiota in mice (Thaiss 2014)
329 and another describing the results samples collected during multiple time points over
330 several days by two subjects (Thaiss 2014), our study is the first to compare the
331 richness and diversity of gut microbiota collected from 51 human participants at two
332 different time points in 24 hours.
333
334 Even though there are numerous studies that have reported increased and/or
335 decreased amounts of various gut bacteria among patients with IBS (Bhattarai Y. 2017;
336 Casén 2015; Pittayanon 2019; Salonen 2010; Tap 2017), a recent systematic review
337 found only nine studies that discussed differences in alpha-diversity in patients with IBS

compared to normal controls (Pittayanon 2019). Slightly over half of the studies (55.6%) reported a significant decrease in the richness and diversity in patients with IBS (Carroll 2011; Carroll 2012; Liu 2016; Pozuelo 2015; Rangel 2015), whereas the remaining four studies (Carroll 2012; Durban 2012; Rigsbee 2012; Tap 2017), like our current study, revealed no differences in alpha-diversity compared to healthy controls. Like other studies comparing patients with IBS symptoms to healthy controls, our pilot study found increased Firmicutes (Chong 2019), specifically Flavonifractor, Oscillibacter, and Ruminiclostudium among participants with IBS (Casén 2015). The increased abundance of E. coli/ Shigella possibly reflects the suspected relationship between Shigellosis and IBS (Youn 2016).

IBS is estimated to have a world-wide prevalence of 10-15% (Canavan 2014; Sperber 2017). Prevalence rates of IBS are typically higher among nurses, with rates ranging from 17.4% in China (Liu 2014) to 45.2% in Nigeria (Akere 2014). The prevalence rate of IBS among study participants was 35%, quite similar to the prevalence rate reported among nurses at the University of Michigan Medical Center (36.6%) (Nojkov et al. 2010). However, unlike Nojkov et al's study of hospital staff nurses (Nojkov et al. 2010), and other studies of shift workers (Kim 2013), there were no differences in the prevalence of IBS symptoms among day and night shift nurses in our study.

This study is limited by a number of factors. First, our study population consisted of a convenience sample of nurses that might not be representative of the larger nursing workforce or the larger population of shift workers. The overall participation rate was

relatively low, which raises concerns about how representative the participants were of the total population of nurses who were invited to participate. Additionally, given the focus of the study, nurses who experienced IBS symptoms may have been more likely to participate than those who did not experience IBS symptoms. Finally, the severity of IBS symptoms and quality of life was not assessed, two factors which may have been impacted by gut microbiome diversity and richness.

Conclusions

There were no differences between in the richness and diversity when samples of the gut microbiome from nurses working day and night shifts were compared. However, when specimens collected at the beginning and ends of the shifts were compared, there were some differences in alpha and beta diversity. Three OTUs were more common in participants reporting IBS symptoms.

References

2006. *The Rome III Adult Criteria for Functional Gastrointestional Disorders*. McLean, VA: Degnon Associates.

Akere A, Akande, K.O. 2014. Association between Irritable Bowel Syndrome and Shift Work: Prevalence and Associated Factors among Nurses. *Journal of Gastroenterology and Hepatology Research* 3:1328-1331.

Bhattarai Y. M, Pedrogo, D.A., Kashyap, P.C. 2017. Irritable bowel syndrome: A gut microbiota-related disorder. *American Journal of Physiology: Gastrointestiinal and Liver Physiology* 312:52-62. 10.1152/ajpgi.0038.2016

Bilski B. 2006. Influence of shift work on diet and gastrointestional complaints among nurses: A pilot study. *Medical Practice* 57:15-19.

Callahan BJ, McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P. 2016. DADA2: High-resolution sample inference from Illumnia amplicon data. *Nature Methods* 13:581-183. 10.1038/nmeth.3869

Canavan C, West, J., Card, T. 2014. The epidemiology of irritable bowel syndrome. *Clinical Epidemiology* 6:71-80. 10.2147/CLEP.S40245

393 Caporaso JG, Bittinger, K., Bushman, F.D., DeSantis, T.Z., Anderson, G.L., Knight, R.
394 2010. PyNAST: a flexible tool for aligning sequences to a template alignment.
395 *Bioinformatics* 26:266-267.
396 Carroll IM, Ringel-Kulka, T, Keku, T.,O, Chang, Y-H, Packey, C.D., Sarto, R.B., Ringel,
397 Y. 2011. Molecular analysis of the luminal-and muscosal-associated intestinal
398 microbiota in patients with diarrhea-predominant irritable bowel syndrome. *American*
399 *Journal of Physiology, Gastrointestinal and Liver Physiology* 301:G799-G807.
400 Carroll IM, Ringel-Kulka, T, Siddle, J.P., Ringel, Y. 2012. Alterations in the composition
401 and diversity of the intestional microbiota in patients with diarrhea-predominant irritable
402 bowel syndrome. *Neurogastroenterology Motility* 24:521-530.
403 Caruso C, Lusk, S., Gillespie, B. 2004. Relationship of work schedules to
404 gastroinstestional diagnosis, symptoms, and medication use in auto factory workers.
405 *American Journal of Internal Medicine* 46:586-598.
406 Casén C, Vebø, H.C., Sekelja, ., Hegge, F.T., Kalsson, M.K., Ciemniejewska, E.,
407 Dzankovic, S., Frøyland, C., Nestestog, R., Engstarand, L., Munkholm, P., Nielsen,
408 O.H., Rogler, G., Simrén, Öhman, L., Vatn, M.H., Rudi, K. 2015. Deviations in human
409 gut microbiota: A novel diagnostic test for determining dysbiosis in patients with IBS or
410 IBD. *Alimentary Pharmacology and Theraputics* 42:71-83.
411 Chong PPC, V.K. Looi, C.Y., Wong, W.F., Madhavan, P., Yong, V.C. 2019. The
412 Microbiome and Irritable Bowel Syndrome-A Review on the Pathophysiology, Current
413 Research, and Future Therapy. *Frontiers in Microbiology* 1o:1-23.
414 10.3389/fmicb.2019/01136
415 Collins SM. 2014. A role for the gut microbiota in IBS. *Nature Reviews Gastroenterology*
416 *& Hepatology* 11:497-505. 10.1038/nrgastro.2014.40
417 De Bacquer D, van Risseghem, M., Clays, E., Kittel, F., De Backer, G., Braeckman, L.
418 2009. Rotating shift work and the metabolic syndrome: A prospective study.
419 *International Journal of Epidemiology* 38:848-854.
420 Durban A, Abellan, J.J., Jimenez-Hernandez, N., Salgardo, P., Ponce, M., Ponce, J.,
421 Garrigues, V., Latorre, A., Moya, A. 2012. Structural laterations of faecal and mucosa-
422 associated communities in irritable bowel syndrome. *Enviromental Microbiology Reports*
423 4:242-247.
424 Finucane MM, Sharpton, T.J., Laurent, T.J., Pollard, K.S. 2014. A taxonomic signature
425 of obesity in the microbiome? Getting to the guts of the matter. *PLOS One* 9:e84689.
426 Gu F, Han, J., Laden, F., Pan, A., Caporaso, N.E., Stampfer, M.J., Kawachi, I.,
427 Rexrode, K.M., Willett, W.C., Hankinson, S.E., Speizer, F.E., Schernhammer, E.S.
428 2015. Total and Cause-Specific Mortality of U.S., Nurses Working Rotating Night Shifts.
429 *American Journal of Preventive Medicine* 48:241-252.
430 Hu YJ, Saggen, F.A. 2020. Testing hypotheses about the microbiome using the Lindear
431 Decomposition Model (LDM). *Bioinformatics* 36:4106-4115.
432 https://doi.org/10.1093/bioinformatics/ntaa260
433 Jarrett M, Heitkemper, M., Cain, K.C., Burr, R.I., Hertig, V. 2000. Sleep disturbance
434 influences gastrointestonal symptoms in women with irritable bowel syndrome.
435 *Digestive Diseases and Sciences* 45:952-959.
436 Jeffery JB, O'Toole, P.W., Öhman, L., Claesson, M., Deane, J., Quigley, E.M.M.,
437 Simrén, M. 2012. An irritable blowel syndrome subtype defined by species-specific

438 alterations in faecal microbiota. *Gut Pathology* 61:997-1006. 10.1136/gut/nl-2011-
439 301501
440 Jia Y, Lu, Y., Wu, K., Lin, Q., Wei, S., Zhu, M., Huang, S., Chen, J. 2013. Does night
441 work increase the risk of breast cancer? A systematic review and meta-analysis of
442 epidemiological studies. *Cancer Epidemiology* 37:197-206.
443 Karantosos T, Markoutsaki, T., Gazouli, M., Anagnou, N.P., Karamanolis, D.G. 2010.
444 Current insights in to the pathophysiology of irritable bowel syndrome. *Gut Pathology* 2.
445 10.1186/1757-4749-2-3
446 Kim HI, Choi, J.Y., Kim, S-E., Jung, H-K., Shim, K-N, Yoo, K. 2013. Impact of shiftwork
447 on irritable bowel sydrome and functional dyspepsia. *Journal of Korean Medical*
448 *Sciences* 28:431-437. 10.3346/jkms.2013.28.3.431
449 Knutsson A, Boggild, H. 2010. Gastrointestional disorders among shift workers.
450 *Scandinavian Journal of Work, Environment and Health* 36:85-95.
451 Krogius-Kurikka L, Lura, A., Malinen, E., Aarnikunnas, J., Tuimala, J., Paulin, L.,
452 Makivuokko, H., Kajander, K., Palva, A. 2009. Microbial community analysis reveals
453 high level phylogentetic alterations in the overall gastrointestional microbiota of
454 diarrhoea-predominant irritable bowel sufferers. *BMC Gastroenterology* 9:95.
455 10.1186/1471-230X-9-95
456 Ley RE, Turnbaugh, P.J., Klein, S., Gordon, J.I. 2006. Microbial ecology: Human gut
457 microboes associated with obesity. *Nature* 444:1022-1023.
458 Liu L, Xiao, Q-f., Zhang, Y-l., Yao, S-k. 2014. A cross-sectional study of irritable bowel
459 syndrome in nurses in China: Prevalence and associated psychological and lifestyle
460 factors. *Journal of Zheijang University Science B* 15:590-597. 10.1631/jzus.B1300159
461 Liu Y, Zhang, L., Wang, X., Zhang, J., Jiang, R., Wang, X., Wang, K., Liu, Z., Xia, Z.,
462 Xu, Z., Nie, Y., Lv, X., Wu, X., Zhu, H., Duan, L. 2016. Similar fecal microbiota
463 signatures in patients with diarrhea-predominant irritable bowel syndrome and patients
464 with depression. *Clinical Gastroenterology and Hepatology* 14:1602-1611.e1605.
465 Liu Z, Wei, Z-Y, Chen, J., Chen, K., Mao, X., Liu, Q., Sun, Y., Zhang, Z., Zhang, Y.,
466 Dan, Z., Tang, J., Qin, L., Chen, J-H., Liu, X. 2020. Acute Sleep-Wake Cycle Shifts
467 Results in Community Alteration of Human Gut Microbiome. *mSphere* 5:e00914-00919.
468 Lowen A, Moreno, C., Holmback, U., Nannernas, M., Tucker, P. 2010. Eating and shift
469 work-effects on habits, metabolism and performance. *Scandinavian Journal of Work,*
470 *Environment and Health* 36:150-162.
471 Lozupone CA, Hamady, M., Kelley, S.T., Knight, R. 2007. Quanitative and qualitative
472 beta diversity measures lead to different insights into factors that structure microbial
473 communities. *Applied and Enviornmental Microbiology* 73:1576-1585.
474 Lozupone CA, Hamady, M., Knight, R. 2006. UniGrac--an online tool for comparing
475 microbial community diversity in a phylogenetic context. *BMC Bioinformatics* 7:371-378.
476 Lozupone CAK, R. 2005. UniFrac: a new phylogenetic method for comparing microbial
477 communities. *Applied and Enviornmental Microbiology* 71:8228-8235.
478 McMurdie PJ, Holmes, S. 2013. Phyloseq: an R package for reproducible interactive
479 analysis and graphics of microbiome census data. *PLOS One* 8:e61217.
480 https://doi.org/10.1371/journal.pone.0061217
481 Methé BA, Nelson, K.E., Pop, M., Creasy, H.H., Giglio, M.G., Huttenhower, C, Gevers,
482 D., et al. 2012. A framework for human microbiome research. *Nature* 486:215-221.

Mortas H, Bilici, S., Karakan, T. 2020. The circadian disruption of night work alters gut microbiota consistent with elevated risk for future metabolic and gastrointestinal pathology. *Chronobiology International* 37:1067-1081. 10.1080/07420528.2020.1778717

Myers JA, Haney, M.F., Griffiths, R.F., Pierse, N.F., Powell, D.M.C. 2015. Fatigue in air medical clinicians undertaking high-acuity patient transports. *Prehospital Emergency Care* 19:36-43.

Nojkov B, Rubenstein JH, Chey WD, and Hoogerwerf WA. 2010. The Impact of Rotating Shift Work on the Prevalence of Irritable Bowel Syndrome in Nurses. *American Journal of Gastroenterology* 105:842-847.

Nojkov B, Rubenstein, J.H., Chey, W.D., Hoogerwerf, W.A. 2010. The impact of rotating shift work on the prevalence of irritable bowel syndrome in nurses. *American Journal of Gastroenterology* 105:842-847.

Pan A, Schernhammer, E.S., Sun, Q., Hu, F.B. 2011. Rotating night shift work and risk of type 2 diabetes: Two prospective cohort studies in women. *PLOS Med*.

Pittayanon R, Lau, J.T., Yuan, Y., Leontiadis, G.I., Tse, F., Surette, M., Moayyedi, P. 2019. Gut microbiota in patient with irritable bowel syndrome-a systematic review. *Gastroenterology* 157:97-108.

Pozuelo M, Panda, S., Santiago, A., Mendez., S., Accarino, A., Santos, J., Guarner, F., Azpiroz, F., Manichanh, C. 2015. Reduction of butyrate- and methane-producing microorganisms in patients with irritable bowel syndrome. *Scientific Reports* 5:12693.

Rajaratnam SMW, Barger, L.K., Lockley, S.W., Shea, S.A., Wang, W., Landrigan, C.P., O'Brien, C.S., Qadri, S., Sullivan, J.P., Cade, B.E., Epstein, L.J., White, D.P., Czeisler, C.A. 2011. Sleep disorders, health and safety in police officers. *Journal of the American Medical Association* 306:2567-2578.

Rangel I, Sundin, J., Fuentes, S., Repsilber, D., de Vos, W.M., Brummer, R.J. 2015. The relationship between faecal-associated and mucosal-associated microbiata in irritable bowel syndrome patients and healthy subjects. *Alimentary Pharmacology and Theraputics* 42:1211-1221.

Rigsbee L, Agans, R., Shankar, V., Kenche, H., Khamis, H.J., Michail, S.K., Paliy, O. 2012. Quantitative profiling of gut microbiota of children with diarrhea-predominant irritable bowel syndrome. . *American Journal of Gastroenterology* 107:1740-1751.

Saberi HR, Moravveji, A.R. 2010. Gastrointestional complaints in shift working and day-working nurses in Iran. *Journal of Circadian Rhythms* 8:1-4.

Salonen A, de Vos, W.M., Palva, A. 2010. Gastrointestinal microbiota in irritable bowel syndrome: Present state and perspectives. *Microbiology* 156:3205-3215. 10.1099/mic.0.043257-0

Sperber AD, Dumitrascu, D., Fukudo, S., Gerson, C., Ghoshal, U.C., Gwee,K.A., Pali, A., Hungin, S., Kang, J-Y., Min-hu, C., Schmulson, M., Bolotin, A., Friger, M., Whitehead, W. 2017. the global prevalence of IBS in adults remains elusive due to the hetrogeneity of studies: A Rome Foundation working team literature review. *Gut Pathology* 66:1075-1082.

Suwazono Y, Dochi, M., Sakata, K., Okubo, Y., Oishi, M., Tanaka, K., Kobayashi, E., Kido, T., Nogawa, K. 2008. A longitudinal study on the effect of shift work on weight gain in male Japanese workers. *Obesity* 16:1887-1893.

Tap J, Derrien, M., Törnblom, H., Brazeilles, R., Cools-Porter, S., Doré, J., Störsrud, S., Le Nevé, B., Öhman, L., Simrén, M. 2017. Identification of an Intestinal Microbiota Signature Associated with Severity of Irritable Bowel Syndrome. *Gastroenterology* 152:111-123. 10.1053/j.gastro.2016.09.049

Thaiss CA, Zeevi, D., Levy, M., Zilberman-Schapira, G., Suez, J.,Tengeter, A.C., Abramson, L., Katz, M.N., Korem, T., Zmora, N., Kuperman, Y., Biton, I., Gilad, S., Harmelin, A., Shapiro, H., Halpern, A., Segal, E., Elinav, E. 2014. Transkingdom Control of Microbiota Diurnal Oscillations Promotes Metabolic Homeostatis. *Cell* 159:514-529.

Turnbaugh PJ, Hamady, M., Yatsunenko, T., Cantarel, B.,L., Duncan, A., Ley, R.E., Sogin, M.L., Jones, W.J., Roe, B.A., Affourtit, J.P., Egholm, M., Henrissat, B., Health, A.C., Knight, R., Gordon, J.I. 2009. A core gut micrombiome in obese and lean twins. *Nature* 457:480-484.

United States Department of Labor Bureau of Labor Statistics. 2005. Workers on flexible and shift schedules in May 2004. *Available at* http://www.bls.gov/news.release/flex.toc.htm (accessed July 26 2015).

Voigt RM, Forsyth, C.B., Green, S.J., Mutlu, E., Engen, P., Vitaterna, M.H., Turek, F.W., Keshavarizian, A. 2014. Circadian disorganization alters intestional microbiota. *PLOS One* 9:1-17.

Vyas MV, Garg, A.X., Iansavichus, A.V., Costella, H., Donner, A., Laugsand, L.E., Janszky, I., Mrkobrada, M., Parraga, G., Hackam, D.G. 2012. Shift work and vascular events: Systematic review and meta-analyssis. *British Medical Journal* 345:1-11.

Wang H, Garrity, G.M., Tiedje, J.M., Cole, J.R. 2007. Naive Bayesian classified for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Enviornmental Microbiology* 73:5261-5270.

Wells MM, Roth, L., Chande, N. 2012. Sleep disruption secondary to overnight call shifts is associated with irritable bowel syndrome in residents: A cross sectional study. *The American Journal of Gastroenterology* 107:1151-1156.

Youn YH, Kim, H.C., Lim, H.C., Park, J.J., Kim, J-H., Park, H. 2016. Long-term clinical course of post-infectious irritable bowel syndrome after shifellosis: a 10-year follow up study. *Journal of Neurogastroenterology& Motility* 22:490-496. 10.5056/jnm15157

Zhen LW, Ann, G.K., Yu, H.K. 2006. Functional bowel disorders in rotating shift nurses may be related to sleep disturbances. *European Journal of Gastroenterology & Hepatology* 18:623-627.

Zhu Z, Satten, G.A., Hu, H.J. in press. Constraining PERMANOVA and LDM to within-set comparisons by project improves the efficiency of analysis of matched sets of microbiome data. *Microbiome*. 10.21203/rs.3.rs-38039

Table 1(on next page)

A Comparison of Day and Night Shift Participants

Table 1 Sample Description

	Day Shift (n=24)	Night Shift (n=27)	P Value
Age (mean)	32.4	33.3	0.73*
BMI (mean)	27.1	26.3	0.60*
BMI			
<20	1	3	0.41**
20-24.9	10	8	
25-29.9	5	10	
>30	8	6	
IBS (Rome III criteria)			
No	17	16	0.56**
Yes	7	11	
IBS Symptoms			
IBS with diarrhea	1	2	1.0**
IBS with constipation	1	1	
IBS mixed type	5	7	
IBS un-subtyped	0	1	

*Welsh two-sample t-test

** Fisher's exact test

Figure 1(on next page)

Alpha Diversity by Shift Type

Figure 1 Alpha diversity by shift type

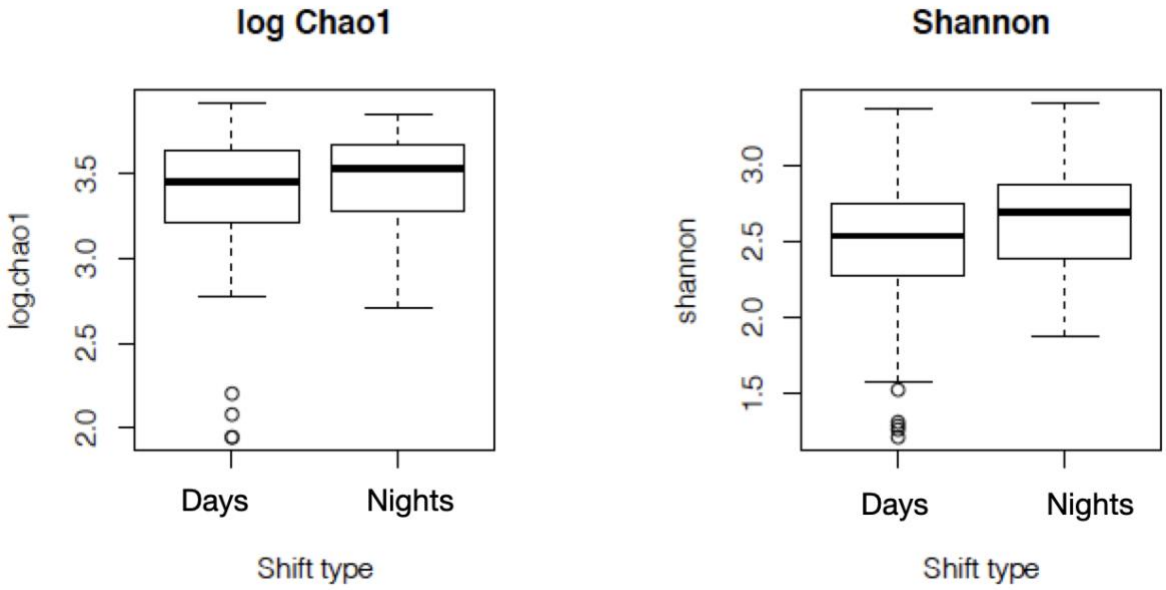


Figure 2(on next page)

PCoA Plot Comparing Beta Diversity by Shift Type

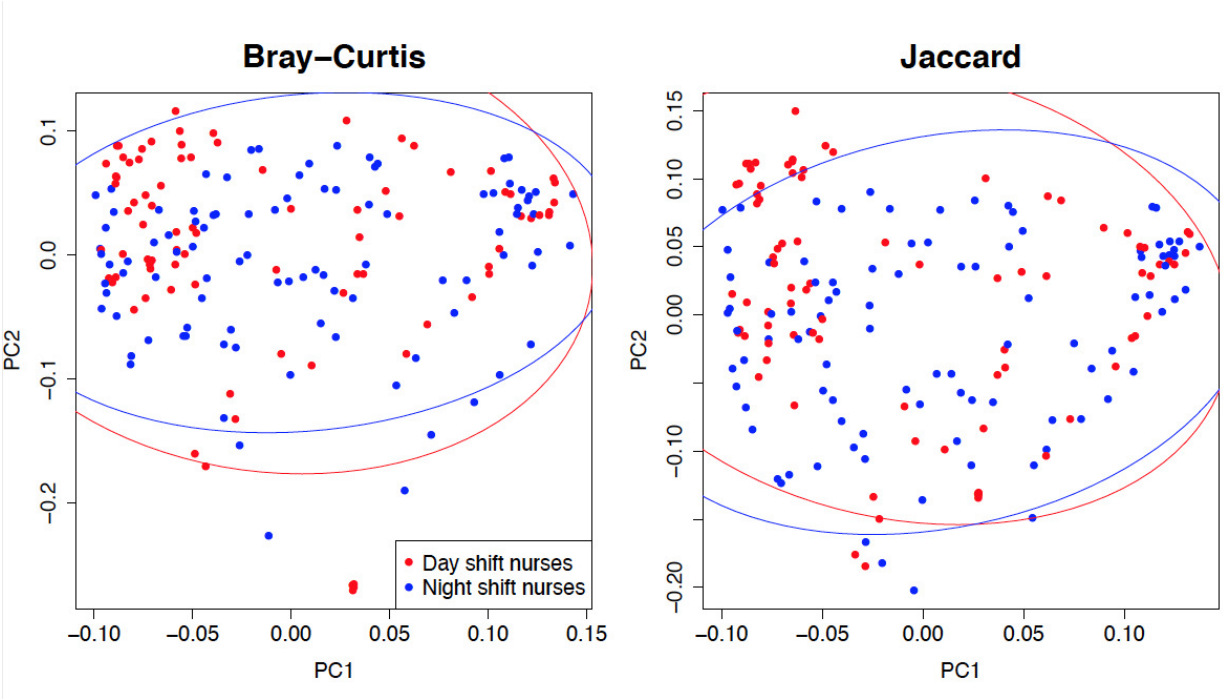
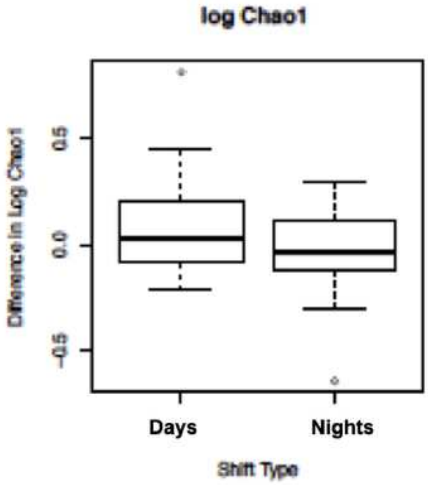

Bray–Curtis
PC2
PC1
0.1
0.0
−0.1
−0.2
−0.10
−0.05
0.00
0.05
0.10
0.15
Day shift nurses
Night shift nurses
Jaccard
PC2
PC1
0.15
0.10
0.05
0.00
−0.10
−0.20
−0.10
−0.05
0.00
0.05
0.10

Figure 3

Changes in Alpha Diversity from the Beginning to End of the Shift by Shift Type


log Chao1
Difference in Log Chao1
0.5
0.0
−0.5
Days
Nights
Shift Type

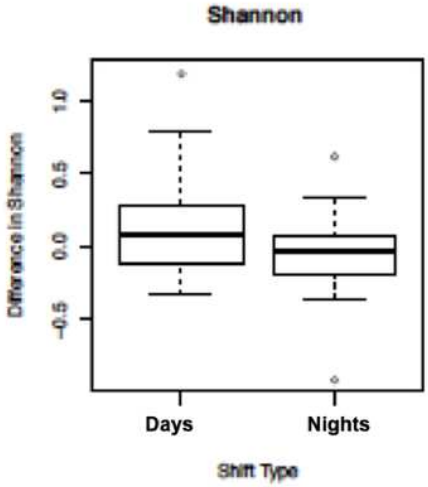

Shannon
Difference in Shannon
1.0
0.5
0.0
−0.5
Days
Nights
Shift Type

Figure 4

Principal Components Analysis by Participant ID

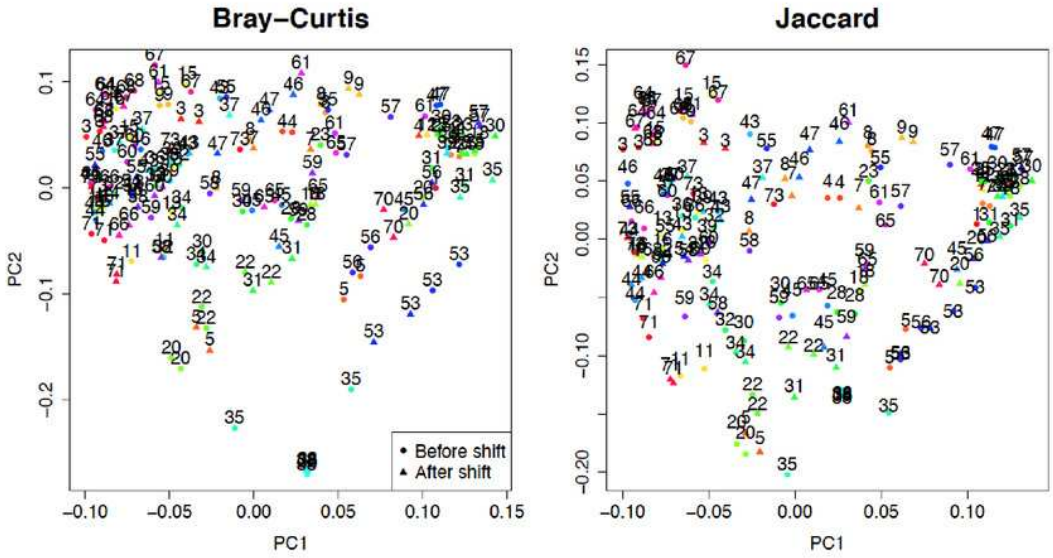
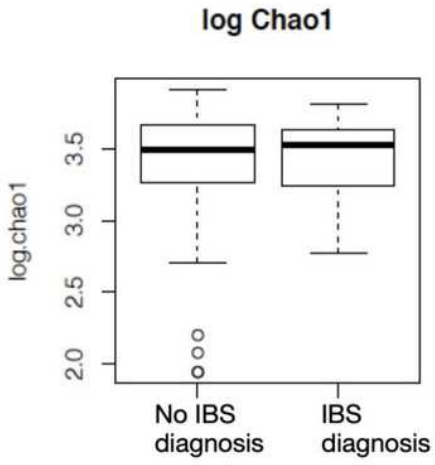

Bray–Curtis
Jaccard
PC1
PC2
Before shift
After shift

Figure 5

Alpha Diversity by Presence or Absence of IBS


log Chao1
log.chao1
2.0
2.5
3.0
3.5
No IBS diagnosis
IBS diagnosis

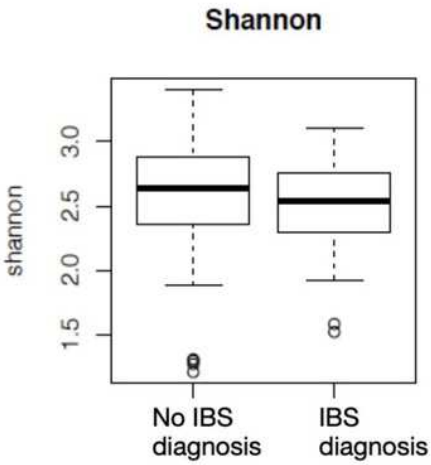
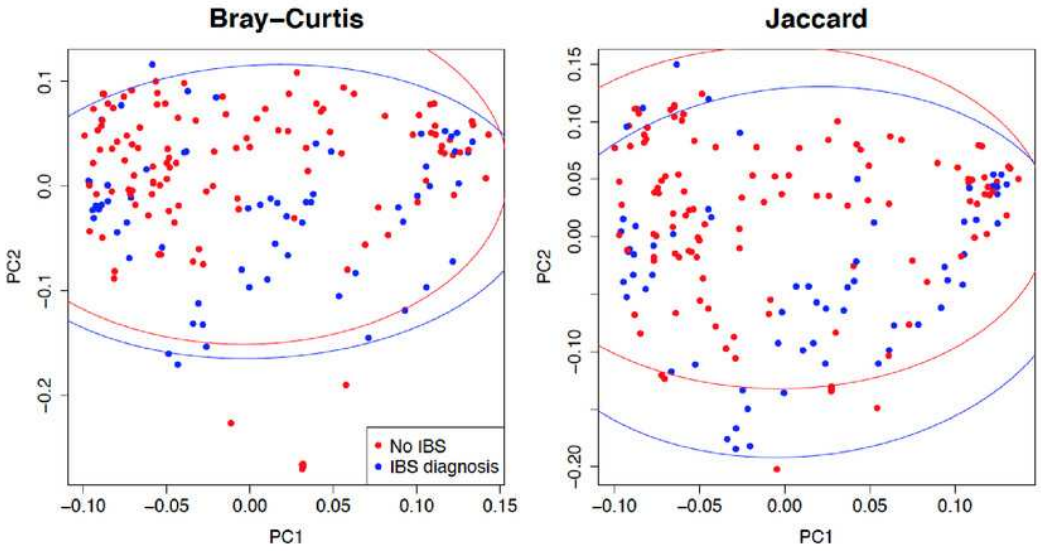

Shannon
shannon
1.5
2.0
2.5
3.0
No IBS diagnosis
IBS diagnosis

Figure 6

PCoA Plot Comparing Beta Diversity by Presence or Absence of IBS Symptoms


Bray–Curtis
Jaccard
PC1
PC2
No IBS
IBS diagnosis