Title: Effects of the copper IUD on composition of the vaginal microbiota in the olive baboon

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Abstract:

Objectives: Determine the feasibility of copper intrauterine devices (Cu-IUD) in baboons and impact on vaginal microbiota.

Study design: Vaginal swabs were taken before insertion (pre-IUD), during IUD use (IUD), and after removal (post-IUD) and microbiota assessed by 16S rRNA-encoding gene sequence analysis.

Results: No animals showed physical changes or discomfort during pre-IUD, IUD, or post-IUD phases. There were significant vaginal bacterial community differences in the post-IUD phase.

Conclusions: The baboon is a viable model for Cu-IUD investigation, with Cu-IUD removal, but not insertion, altering vaginal microbiota.

Implications: A baboon model of Cu-IUD allows investigation into the intersection of Cu-IUDs and human reproductive tract disorders and pathogens.

Introduction

A non-human primate (NHP) model for copper intrauterine devices (Cu-IUD) that mimics all aspects of human Cu-IUD usage, including both the device and transcervical insertion, has been elusive. Macaques have a tortuous cervix, which prevents transcervical insertion, and their small size may make device tolerance challenging. The olive baboon (*Papio anubis*) is larger and has a straight cervical canal, making it an ideal animal model for intrauterine contraception. A NHP model for Cu-IUDs will permit studies regarding the impact of Cu-IUDs on the biology of the female reproductive tract and the relationship between intrauterine contraception and infectious agents (*e.g. Chlamydia trachomatis*, HIV) that may be ethically impossible in humans.

Our laboratory has previously developed a NHP model of levonorgestrel-releasing intrauterine system (LNG-IUS) with olive baboons, which assessed changes to the vaginal microbiome in baboons with LNG-IUS. We found inter-individual differences, but no consistent device-associated shift in microbial communities [1]. Animals with LNG-IUSs had a moderate shift towards more stable microbial communities over time [1]. In this study, we assessed physiologic changes in a cohort of 5 baboons with Cu-IUD and analyzed populations of the vaginal microbiota over 24 weeks.

Methods

Regulatory approval

The Institutional Review Committee at the Institute of Primate Research (IPR) in Nairobi, Kenya (NIH Office of Laboratory Animal Welfare foreign assurance A5796)

approved this study. This study received an off-site exemption from the University of Michigan University Institutional Animal Care and Use Committee. An export permit was obtained from the Kenyan Wildlife Service and import permits were obtained from the US CDC [1].

Study site, population, design, sampling

This study was conducted at the IPR (for IPR description, see [1]). Five wild-caught sexually mature female olive baboons (*P. anubis*) were utilized in this study, quarantined, examined, housed individually, and vaginally sampled as previously described [1]. Study timecourse was as follows: pre-IUD phase weeks 0-4, weekly sampling; Cu-IUDs inserted according to package insert after week 4 sampling; Cu-IUD phase weeks 5-20, sampling every 4 weeks; Cu-IUDs removed after week 20 sampling; post-IUD phase weeks 21-24, weekly sampling.

Microbiome DNA preparation, sequencing, and analysis

DNA extraction from vaginal swabs was performed with the PowerMag Soil DNA Isolation Kit (Mo Bio, Carlsbad, CA, USA), and amplification of the V4 region of the bacterial 16S rRNA gene, sequencing (Illumina MiSeq), sequence processing and analysis were performed as previously described [1].

Results

Clinical signs

All baboons retained the Cu-IUD for the planned duration (from week 5-20). No animals showed any physical changes or discomfort (*e.g.* changes in weight, temperature, pulse, respiration, and frequency of discharge) during the pre-IUD (weeks 0-5), IUD (weeks 6-

20), or post-IUD (weeks 21-24) phases of the study, nor did the IUD appear to disrupt the menstrual cycle (data not shown).

Week-to-week vaginal microbial community structure variation

16S rRNA gene sequence analysis of vaginal swabs was used to compare baboon bacterial communities, both between individual animals and within an individual animal over time. We first analyzed community structure within and between baboons by θ_{YC} dissimilarity coefficient, a measure of both the presence of specific operational taxonomic units (OTUs) and their relative abundance [2]. Examination of the average θ_{YC} distance showed high within-animal week-to-week variation in community structure (Fig. S1). The greatest shift in bacterial community composition, indicated by the highest θ_{YC} distances, occurred between week 0 and week 1, when the animals were moved from group housing to single cages. Community stability, measured by week-to-week θ_{YC} distances, did not change significantly between pre-IUD, IUD, and post-IUD timepoints (Fig. 1).

Microbial community composition and clustering

The most frequent bacterial phyla across all baboons were Bacteroidetes, Fusobacteria, Proteobacteria, and Firmicutes (Fig. 1). When we compared the pre-IUD, IUD, and post-IUD timepoints by analysis of molecular variance (AMOVA) [3] on θ_{YC} distances, there was a significant difference between post-IUD communities and both pre-IUD communities (p-value: 0.003) and IUD communities (p-value: <0.001), but not a significant difference between pre-IUD and IUD communities (p-value: 0.126) (Fig. 2). Differentially abundant OTUs identified by LEfSe [4] included OTU1 (Leptotrichiaceae),

which was higher in the post-IUD timepoints relative to both pre-IUD and IUD (Fig. S2, Table S1).

When we followed community structure over time, communities within an individual were more similar then those found in different animals (Fig. S3).

Discussion

This study was designed to test the feasibility of Cu-IUD placement in baboons and assess vaginal microbiota changes due to Cu-IUD placement. We found no clinical changes to baboons (*e.g.* temperature, weight, menstrual cycle, discharge) and a slight but statistically significant alteration of the vaginal microbiota after Cu-IUD removal. As we have demonstrated with the LNG-IUS in the baboon, we conclude that the olive baboon is a feasible model for Cu-IUD studies.

The mechanism behind the change in vaginal microbiota is unclear, as is the biological significance. Further investigation is necessary to determine why removal of the Cu-IUD altered community structure while insertion of the Cu-IUD did not. The lack of change between the pre-IUD phase and IUD phase is consistent with recently published work in humans, although the human study did not extend to after IUD removal [5].

Animal models of Cu-IUD are limited. In mice, insertion of a copper wire into the uterine horn [6] is limited by the reliance on surgery, the vastly different estrus cycle and reproductive tract physiology from humans, and the more limited range of naturally-occurring sexually-transmitted pathogens relative to humans and NHP. NHP studies of Cu-IUD have been successfully performed in the pigtail macaque [7], but surgery is

necessary for device insertion, which carries significant risks to the animal. While the baboon is not the primary NHP model for HIV, it is a viable model [8], and studies wishing to evaluate the interaction of IUDs with HIV are feasible. Moreover, baboons are used for models of endometriosis [9] and their high vaginal pH and diverse microbial communities may model aspects of bacterial vaginosis [10]. Our study opens a new avenue of research into the intersection of the Cu-IUD and human reproductive tract disorders and pathogens.

Acknowledgements

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172 **Figure Legends:**

173 Figure 1. Bacterial communities exhibit inter-animal variation, but no pattern in change
174 between pre-IUD, IUD, and post-IUD periods. Week-to-week change in θYC values in
175 each animal is matched to the bar chart of microbial communities at each timepoint.
176 Microbial communities are color coded as indicated, and are grouped by phylum
177 indicated. LEfSe analysis performed on each individual animal by grouping communities
178 found in the pre-IUD, IUD, and post-IUD timepoints found: animal 3903 had significant
179 difference in relative abundance between the pre-IUD and IUD phases; animal 3919 had
180 a significant difference in relative abundance between the IUD and post-IUD timepoints;
181 animals 3919 and 4047 had shifts in relative abundance of microbial communities
182 between the pre-IUD and post-IUD periods.

183

184 Figure 2. Principal Coordinates Analysis (PCoA) shows no distinct clustering of OTUs
185 with regard to IUD insertion but changes after IUD removal. Baboons at each timepoint
186 are represented by a circle, colored to indicate pre-IUD, IUD, and post-IUD periods.
187 Axes indicate percentage of variation by the plotted principal coordinates. Operational
188 Taxonomic Units (OTUs) of related bacteria are overlaid on the data. Significant
189 differences (in OTU1 Leptotrichaceae) were seen by AMOVA when post-IUD timepoints
190 were compared to both pre-IUD and IUD timepoints.

191

192 **Supplemental Figure Legends:**

193 Figure S1. Averaged week-to-week θYC values do not change significantly between pre-
194 IUD, IUD, and post-IUD periods.

195

196 Figure S2. Principal Coordinates Analysis (PCoA) show animals are most similar to
197 themselves and change little over 24 weeks. Baboons at each timepoint are represented
198 by a circle, colored to indicate individual animals. Axes indicate percentage of variation
199 by the plotted principal coordinates. Operational Taxonomic Units (OTUs) of related
200 bacteria are overlaid on the data. No significant differences were seen by AMOVA.

201

202 Figure S3. Significant differences by LEfSe in 10 OTUs with abundance greater than
203 0.001 in any group. OTU number and p-value (by LEfSe) are indicated on the Y-axis and
204 the relative abundance is indicated on the X-axis. LEfSe analysis was a 3-way
205 comparison between timepoints pre-IUD, IUD, and post-IUD. When further analyzed by
206 2-way ANOVA with multiple comparisons: OTU0001 was significantly different from
207 pre-IUD and IUD; OTU0004 was significantly different in post-IUD relative to IUD; and
208 OTU0007 was significantly different in post-IUD relative to IUD. Our cutoff for
209 inclusion was OTUs with a relative abundance greater than 0.001 in any group.

210

211 Table S1. OTU identities for groups with significant differences in abundance between
212 timepoints determined by LEfSE.

Figure 1

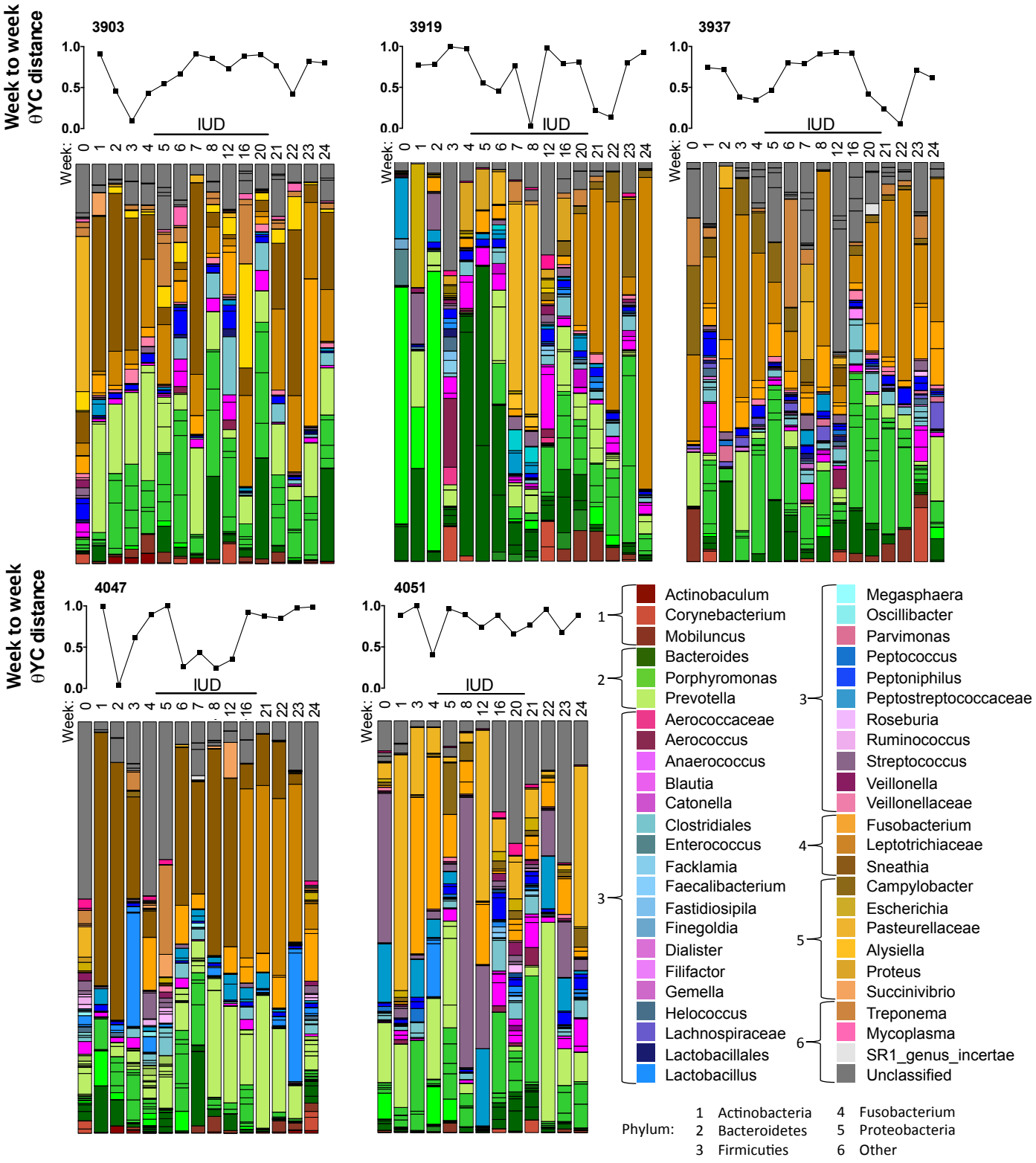

Week to week θYC distance
3903
3919
3937
4047
4051
IUD
Week:
Actinobaculum
Corynebacterium
Mobiluncus
Bacteroides
Porphyromonas
Prevotella
Aerococcaceae
Aerococcus
Anaerococcus
Blautia
Catonella
Clostridiales
Enterococcus
Facklamia
Faecalibacterium
Fastidiosipila
Finegoldia
Dialister
Filifactor
Gemella
Helococcus
Lachnospiraceae
Lactobacillales
Lactobacillus
Megasphaera
Oscillibacter
Parvimonas
Peptococcus
Peptoniphilus
Peptostreptococcaceae
Roseburia
Ruminococcus
Streptococcus
Veillonella
Veillonellaceae
Fusobacterium
Leptotrichiaceae
Sneathia
Campylobacter
Escherichia
Pasteurellaceae
Alysiella
Proteus
Succinivibrio
Treponema
Mycoplasma
SR1_genus_incertae
Unclassified
Phylum:
1 Actinobacteria
2 Bacteroidetes
3 Firmicuties
4 Fusobacterium
5 Proteobacteria
6 Other

Figure 2

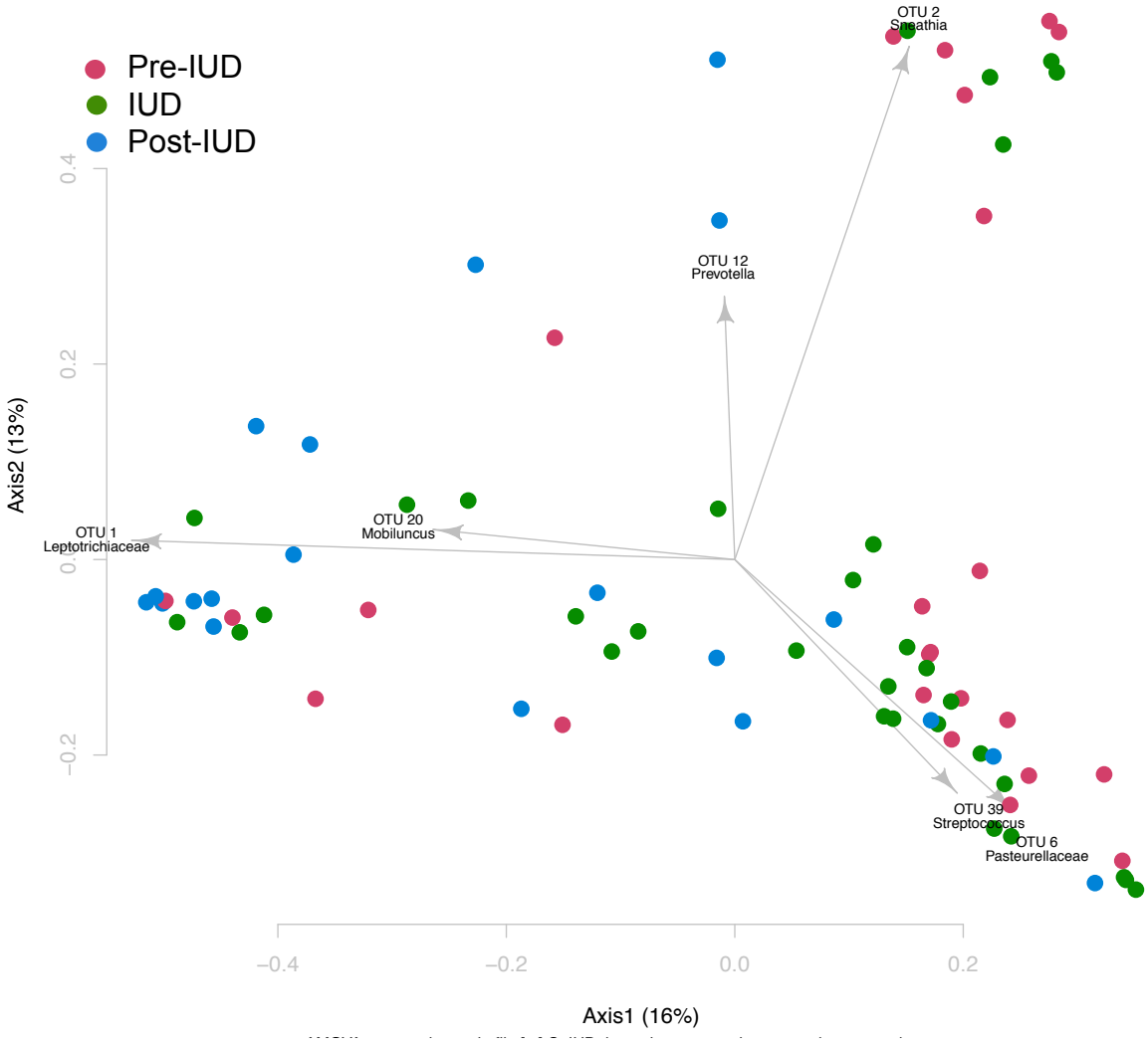

Pre-IUD
IUD
Post-IUD
OTU 2
Sneathia
OTU 12
Prevotella
OTU 20
Mobiluncus
OTU 1
Leptotrichiaceae
OTU 39
Streptococcus
OTU 6
Pasteurellaceae
Axis2 (13%)
Axis1 (16%)
0.4
0.2
0.0
−0.2
−0.4
−0.2
0.0
0.2

Figure S1

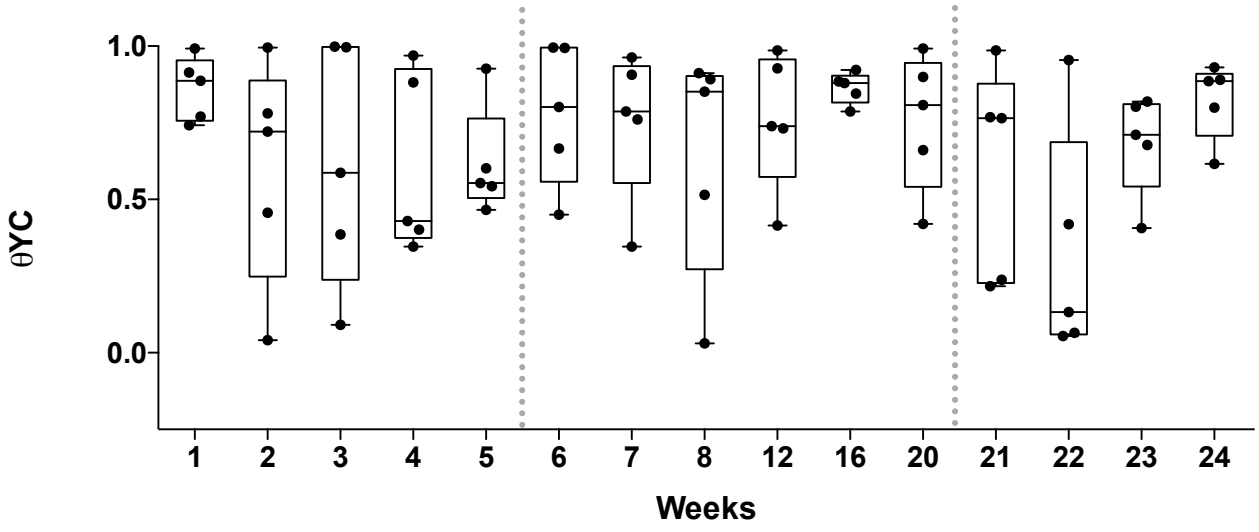


Figure S2

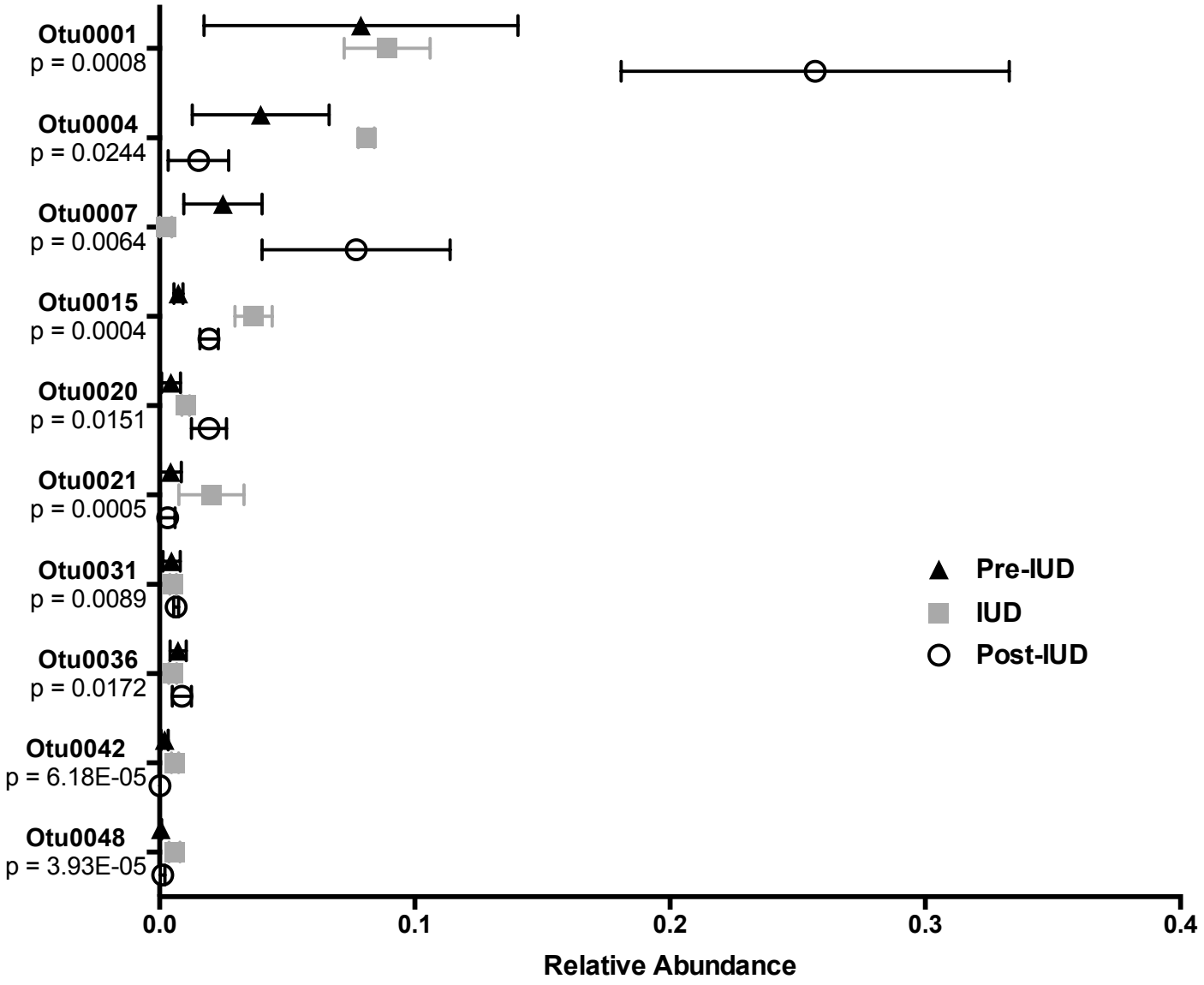
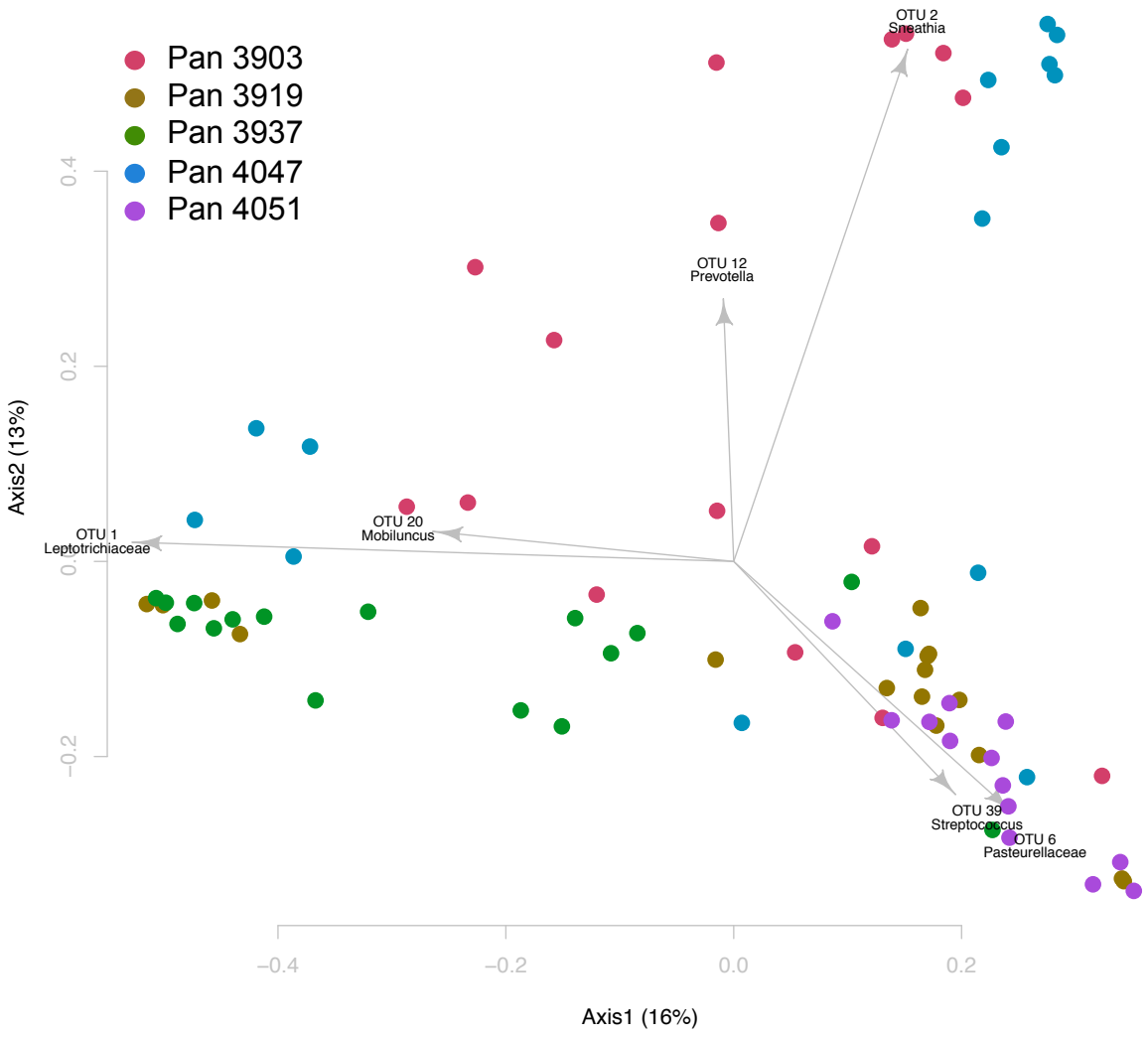

Otu0001
p = 0.0008
Otu0004
p = 0.0244
Otu0007
p = 0.0064
Otu0015
p = 0.0004
Otu0020
p = 0.0151
Otu0021
p = 0.0005
Otu0031
p = 0.0089
Otu0036
p = 0.0172
Otu0042
p = 6.18E-05
Otu0048
p = 3.93E-05
Pre-IUD
IUD
Post-IUD
0.0
0.1
0.2
0.3
0.4
Relative Abundance

Table S1

OTU	Size	Taxonomy
Otu0001	271779	Bacteria(100);Fusobacteria(100);Fusobacteria(100);Fusobacteriales(100);Leptotrichiaceae(100);unclassified(100);
Otu0004	89043	Bacteria(100);Bacteroidetes(100);Bacteroidia(100);Bacteroidales(100);Bacteroidaceae(100);Bacteroides(100);
Otu0007	71274	Bacteria(100);Bacteroidetes(100);Bacteroidia(100);Bacteroidales(100);Prevotellaceae(100);Prevotella(100);
Otu0015	31833	Bacteria(100);Firmicutes(100);Clostridia(100);Clostridiales(100);unclassified(100);unclassified(100);
Otu0020	23142	Bacteria(100);Actinobacteria(100);Actinobacteria(100);Actinomycetales(100);Actinomycetaceae(100);Mobiluncus(100);
Otu0021	22837	Bacteria(100);Bacteroidetes(100);Bacteroidia(100);Bacteroidales(100);Porphyromonadaceae(100);Porphyromonas(100);
Otu0031	9988	Bacteria(100);Firmicutes(100);Negativicutes(100);Selenomonadales(100);Veillonellaceae(100);unclassified(100);
Otu0036	7269	Bacteria(100);Firmicutes(100);Clostridia(100);Clostridiales(100);Clostridiales_Incertae_Sedis_XI(100);Anaerococcus(100);
Otu0042	6190	Bacteria(100);Firmicutes(100);Clostridia(100);Clostridiales(100);Peptostreptococcaceae(100);unclassified(100);
Otu0048	5547	Bacteria(100);Firmicutes(100);Clostridia(100);Clostridiales(100);Lachnospiraceae(100);Catonella(100);

Figure S3


Pan 3903
Pan 3919
Pan 3937
Pan 4047
Pan 4051
OTU 2
Sneathia
OTU 12
Prevotella
OTU 1
Leptotrichiaceae
OTU 20
Mobiluncus
OTU 39
Streptococcus
OTU 6
Pasteurellaceae
Axis2 (13%)
Axis1 (16%)
0.4
0.2
0.0
−0.2
−0.4
−0.2
0.0
0.2