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# Copper intrauterine device increases vaginal concentrations of inflammatory anaerobes and depletes lactobacilli compared to hormonal options in a randomised trial

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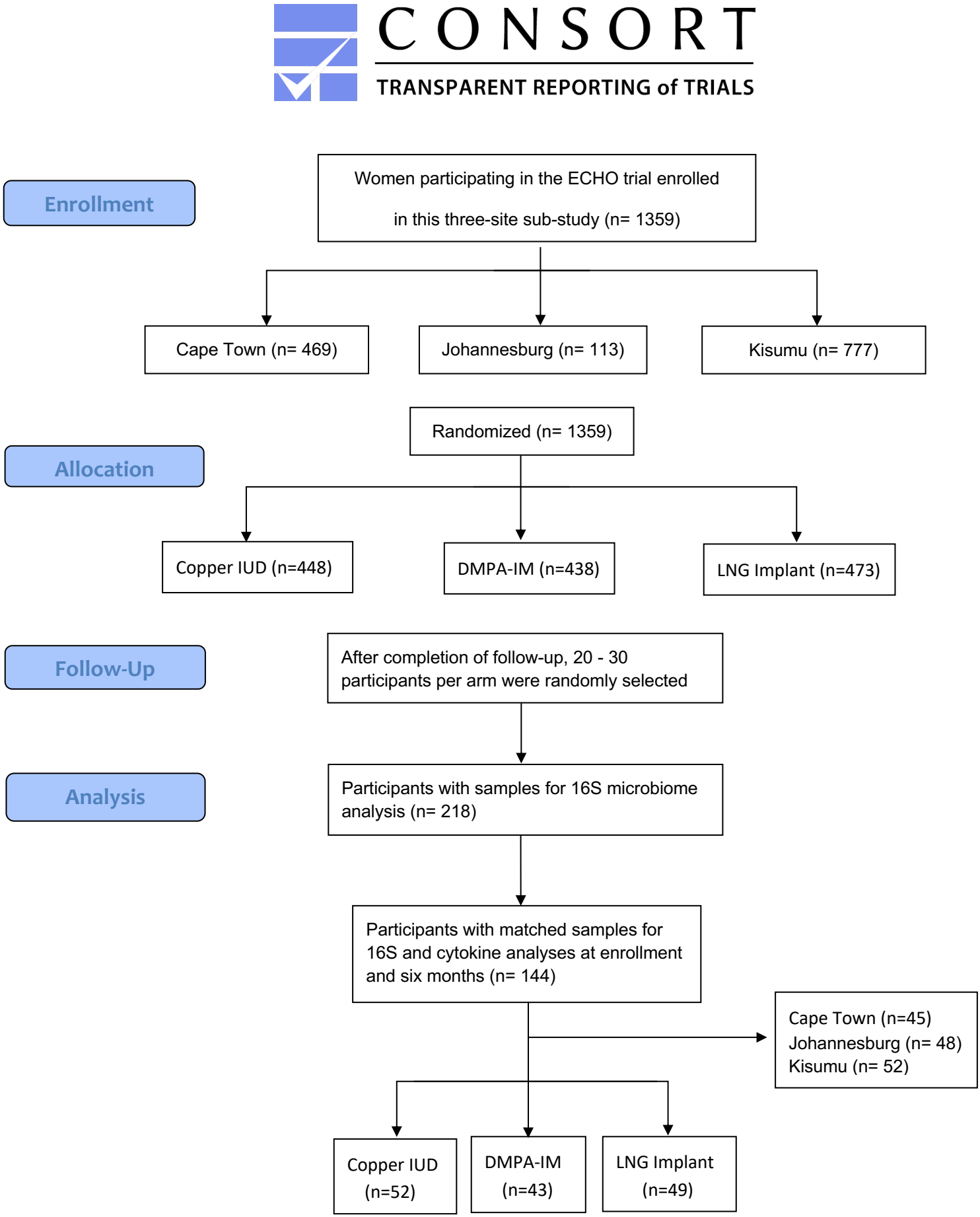



Figure S1. CONSORT flow diagram of participants in this study.

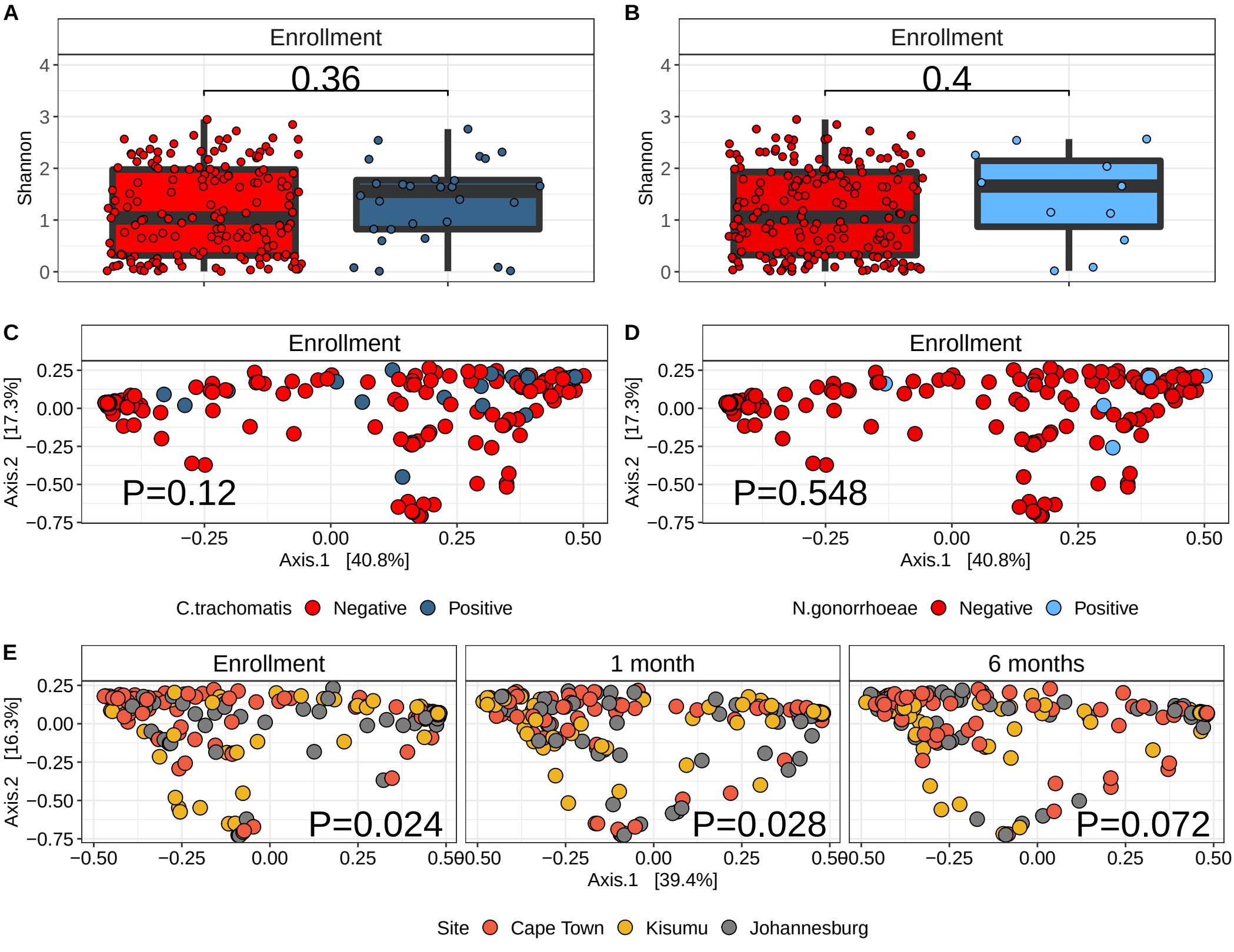

A
Enrollment
0.36
Shannon
B
Enrollment
0.4
Shannon
C
Enrollment
P=0.12
Axis.2 [17.3%]
Axis.1 [40.8%]
C.trachomatis Negative Positive
D
Enrollment
P=0.548
Axis.2 [17.3%]
Axis.1 [40.8%]
N.gonorrhoeae Negative Positive
E
Enrollment
1 month
6 months
P=0.024
P=0.028
P=0.072
Axis.2 [16.3%]
Axis.1 [39.4%]
Site Cape Town Kisumu Johannesburg

**Figure S2. The effect of STIs and study site on vaginal bacterial profiles. A.** Shannon diversity boxplots of samples that tested positive (n=28) or negative (n=165) for *Chlamydia trachomatis*. **B.** Shannon diversity boxplots of samples that tested positive (n=11) or negative (n=182) for *Neisseria gonorrhoeae*. **C.** PCoA ordination (Bray-Curtis distance) of relative abundance transformed bacterial abundance at enrollment, samples are colored by *Chlamydia trachomatis* or **D.** Neisseria *gonorrhoeae* test results. **E**. PCoA ordination (Bray-Curtis distance) of relative abundance transformed bacterial abundance across all timepoints, colored by study site. Boxplot center lines indicate the median, while the hinges indicate the first and third quartiles, and whiskers extend to 1.5 * IQR from the given hinge. Two-tailed P values were calculated with a Wilcoxon Rank Sum test for pairwise comparisons or a PERMANOVA p value from 100 0 permutations for multivariate analysis.


CST
I−B
III−A
IV−A
IV−B
IV−D
Shannon entropy
3
2
1
0
BV Intermediate Negative
Cape Town Kisumu Johannesburg
Lactobacillus iners
Gardnerella vaginalis
Lactobacillus crispatus/gallinarum
Lachnocurva vaginae
Atopobium vaginae
Sneathia amnii
Megasphaera sp.
Prevotella sp.
Sneathia sanguinegens
Mycoplasma hominis
Gardnerella sp.
Lactobacillus crispatus
Lactobacillus jensenii
Lactobacillus gasseri
Prevotella amnii
Veillonella montpellierensis
Lactobacillus sp.
Streptococcus agalactiae/pyogenes
Dialister sp.
Prevotella bivia/denticola
Bifidobacterium breve
Sneathia sp.
Streptococcus anginosus/milleri/mitis
Streptococcus sp.
Fastidiosipila sp.
Relative abundance
50.0
10.0
1.0
0.1

**Figure S3. The taxonomic composition, clinical BV status, and distribution across study sites of each CST.**

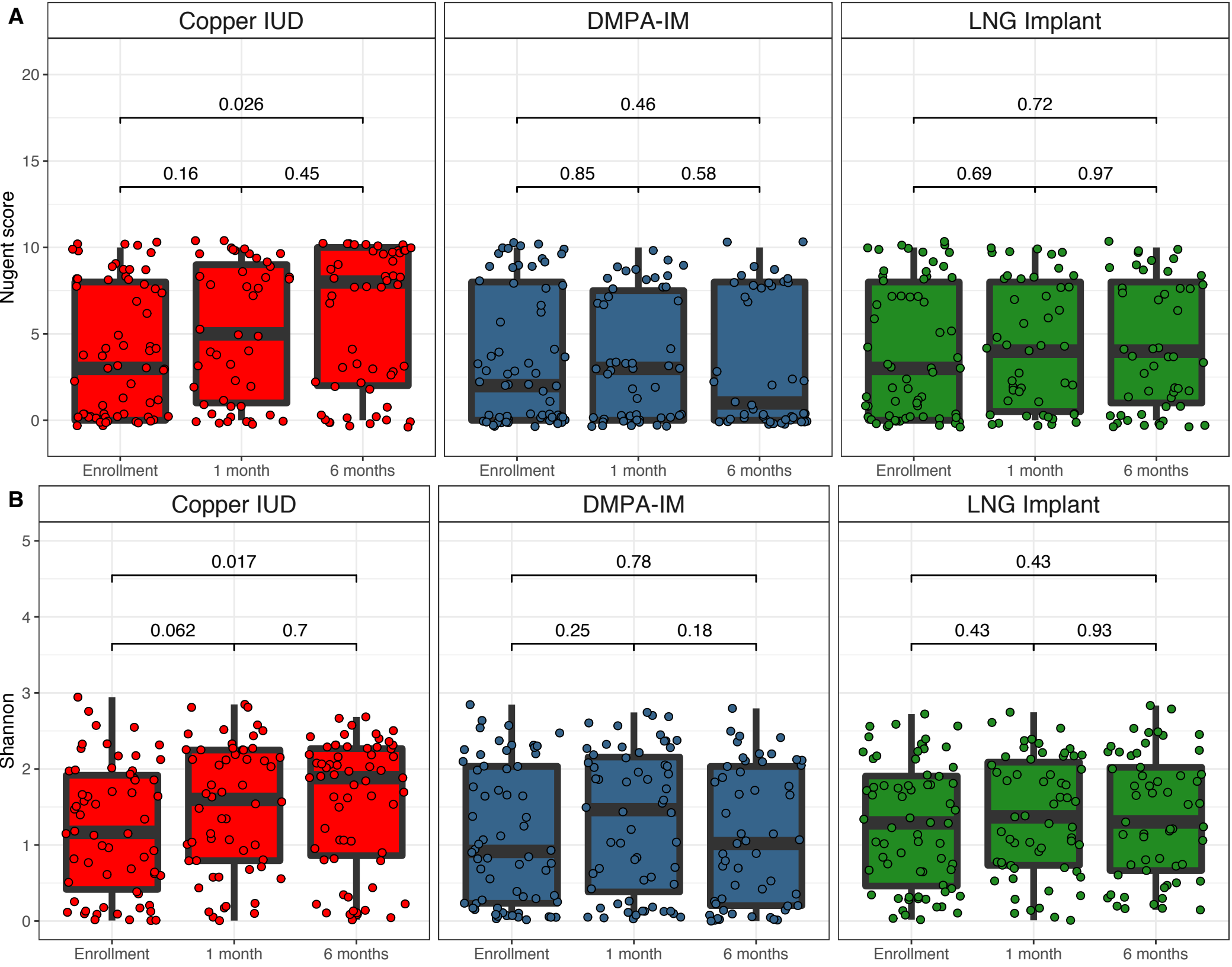

A
Copper IUD
DMPA-IM
LNG Implant
Nugent score
20
15
10
5
0
0.026
0.16
0.45
0.46
0.85
0.58
0.72
0.69
0.97
Enrollment
1 month
6 months
B
Shannon
5
4
3
2
1
0
0.017
0.062
0.7
0.78
0.25
0.18
0.43
0.43
0.93

**Figure S4. Contraceptive use significantly alters the clinical and molecular measures of diversity of vaginal bacterial communities across 218 participants. A**. Longitudinal Nugent Score boxplots displayed for each contraceptive Cu-IUD (n = 67); DMPA-IM (n = 67); LNG implant (n = 65). **B**. Longitudinal Shannon diversity boxplots stratified by each time point Cu-IUD (n = 70); DMPA-IM (n = 74); LNG implant (n = 74). Boxplot center lines indicate the median, while the hinges indicate the first and third quartiles, and whiskers extend to 1.5 * IQR from the given hinge. Two-tailed P values were calculated using a Wilcoxon Rank Sum test.

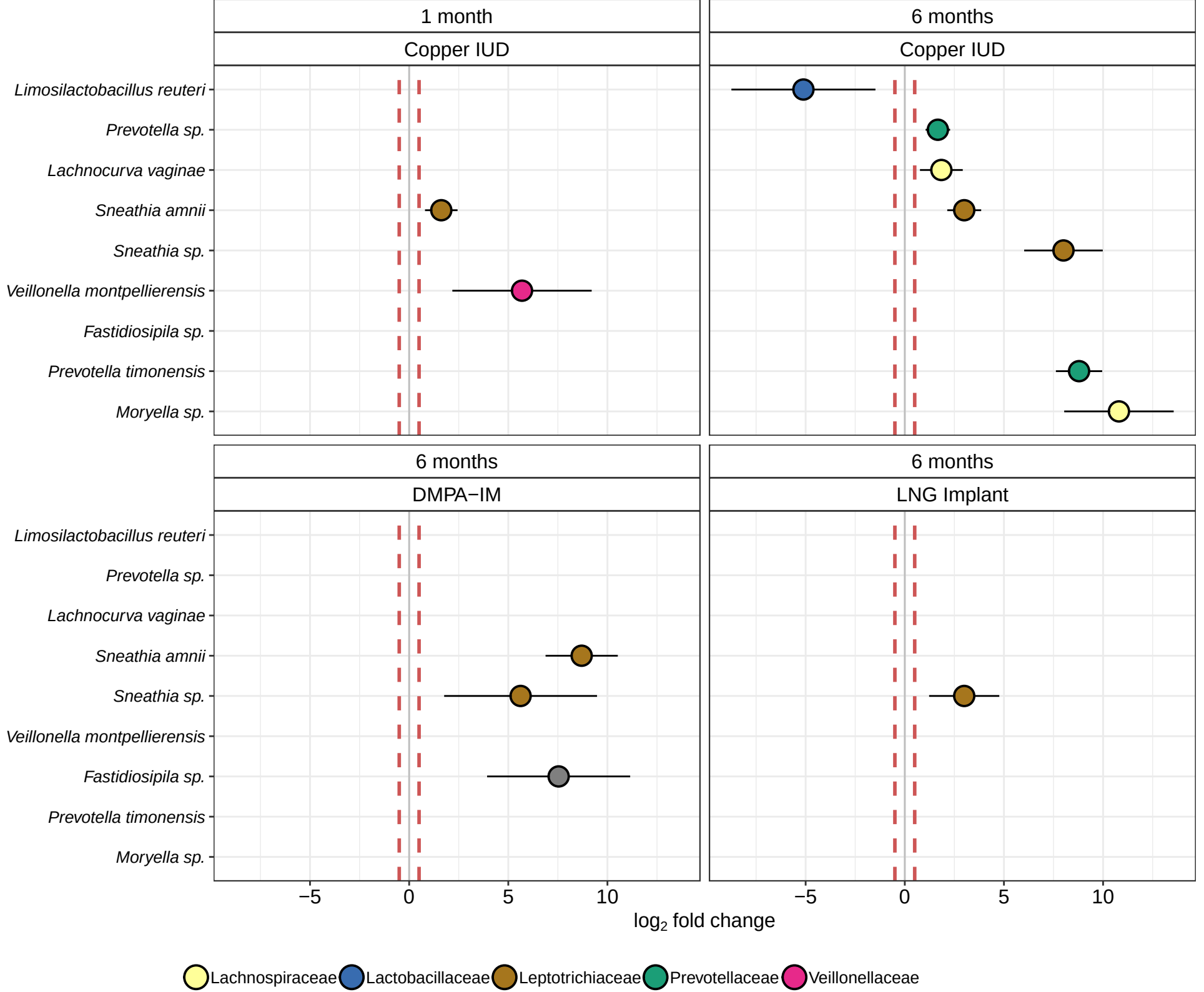

1 month
Copper IUD
6 months
Copper IUD
6 months
DMPA−IM
6 months
LNG Implant
Limosilactobacillus reuteri
Prevotella sp.
Lachnocurva vaginae
Sneathia amnii
Sneathia sp.
Veillonella montpellierensis
Fastidiosipila sp.
Prevotella timonensis
Moryella sp.
−5
0
5
10
log2 fold change
Lachnospiraceae
Lactobacillaceae
Leptotrichiaceae
Prevotellaceae
Veillonellaceae

**Figure S5. Fold changes in bacterial abundance after one and six months of contraceptive use, relative to enrollment abundance.** Fold changes are $\log_2$ transformed and were calculated using DESeq2. Vertical dashed lines indicate a 0.5 fold change. Each point represents the arithmetic mean $\log_2$ fold change and solid horizontal lines represent the standard error. Taxa with two-tailed Wald P < 0.05 after adjustment for multiple comparisons (Benjamini and Hochberg) are shown; Cu-IUD (n = 55); DMPA-IM (n = 45); LNG implant (n = 53).

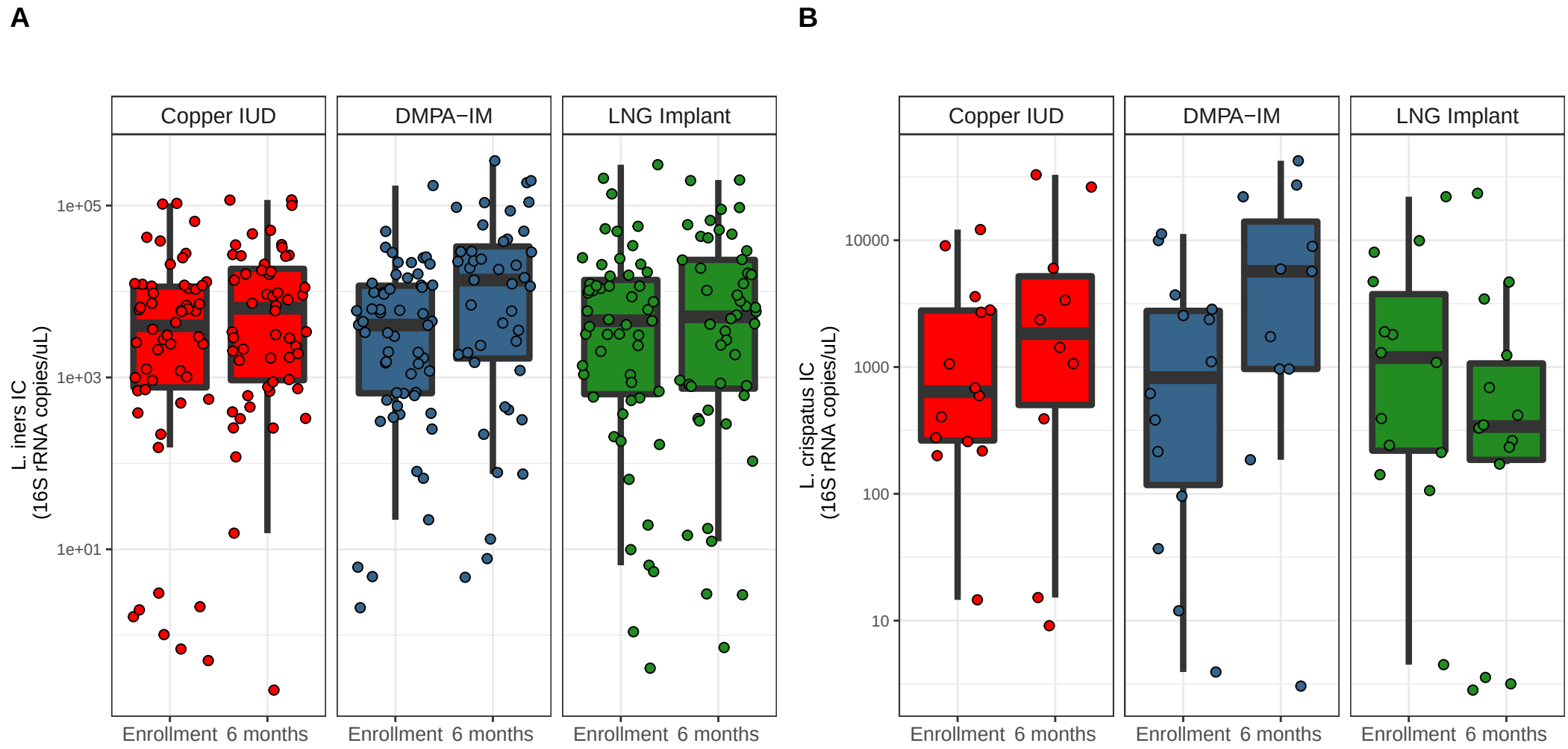

A
Copper IUD
DMPA−IM
LNG Implant
L. iners IC
(16S rRNA copies/uL)
1e+05
1e+03
1e+01
Enrollment
6 months
B
Copper IUD
DMPA−IM
LNG Implant
L. crispatus IC
(16S rRNA copies/uL)
10000
1000
100
10
Enrollment
6 months

**Figure S6. The effect of contraceptive use on common lactobacilli.** Inferred concentrations of **A.** *L. iners* (Cu-IUD (n = 66); DMPA-IM (n = 67); LNG implant (n = 66)) and **B.** *L. crispatus* (Cu-IUD (n = 18); DMPA-IM (n = 23); LNG implant (n = 23)) at enrollment and after six months of contraceptive use. Boxplot center lines indicate the median, while the hinges indicate the first and third quartiles, and whiskers extend to 1.5 * IQR from the given hinge.

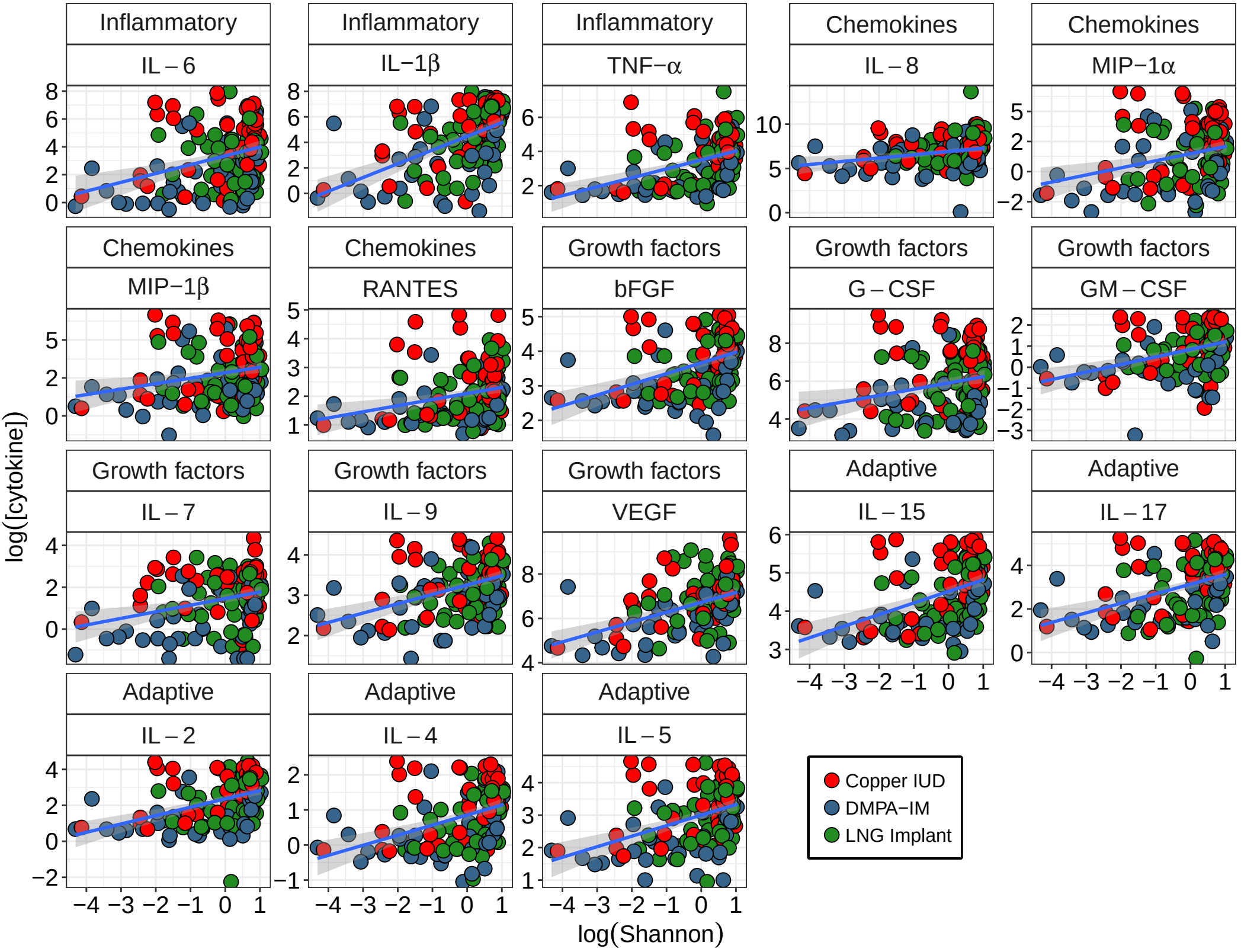

Inflammatory
IL – 6
Inflammatory
IL−1β
Inflammatory
TNF−α
Chemokines
IL – 8
Chemokines
MIP−1α
Chemokines
MIP−1β
Chemokines
RANTES
Growth factors
bFGF
Growth factors
G – CSF
Growth factors
GM – CSF
Growth factors
IL – 7
Growth factors
IL – 9
Growth factors
VEGF
Adaptive
IL – 15
Adaptive
IL – 17
Adaptive
IL – 2
Adaptive
IL – 4
Adaptive
IL – 5
log([cytokine])
log(Shannon)
Copper IUD
DMPA−IM
LNG Implant

**Figure S7. Bacterial Shannon index correlates with the abundance of several classes of cytokines after six months of contraceptive use.** Cytokine abundance and diversity index values were $\log_e$ transformed prior to model generation. The class of cytokine is indicated above each subplot. Participants are colored by randomization arm. Two-tailed P values were derived from the t-value of the generalized linear model. Data were collected after six months of contraceptive use. Models with $p < 0.05$ after adjustment for multiple comparisons (Benjamini and Yekutieli) are shown, shaded regions around the regression line indicate the 95% confidence interval.

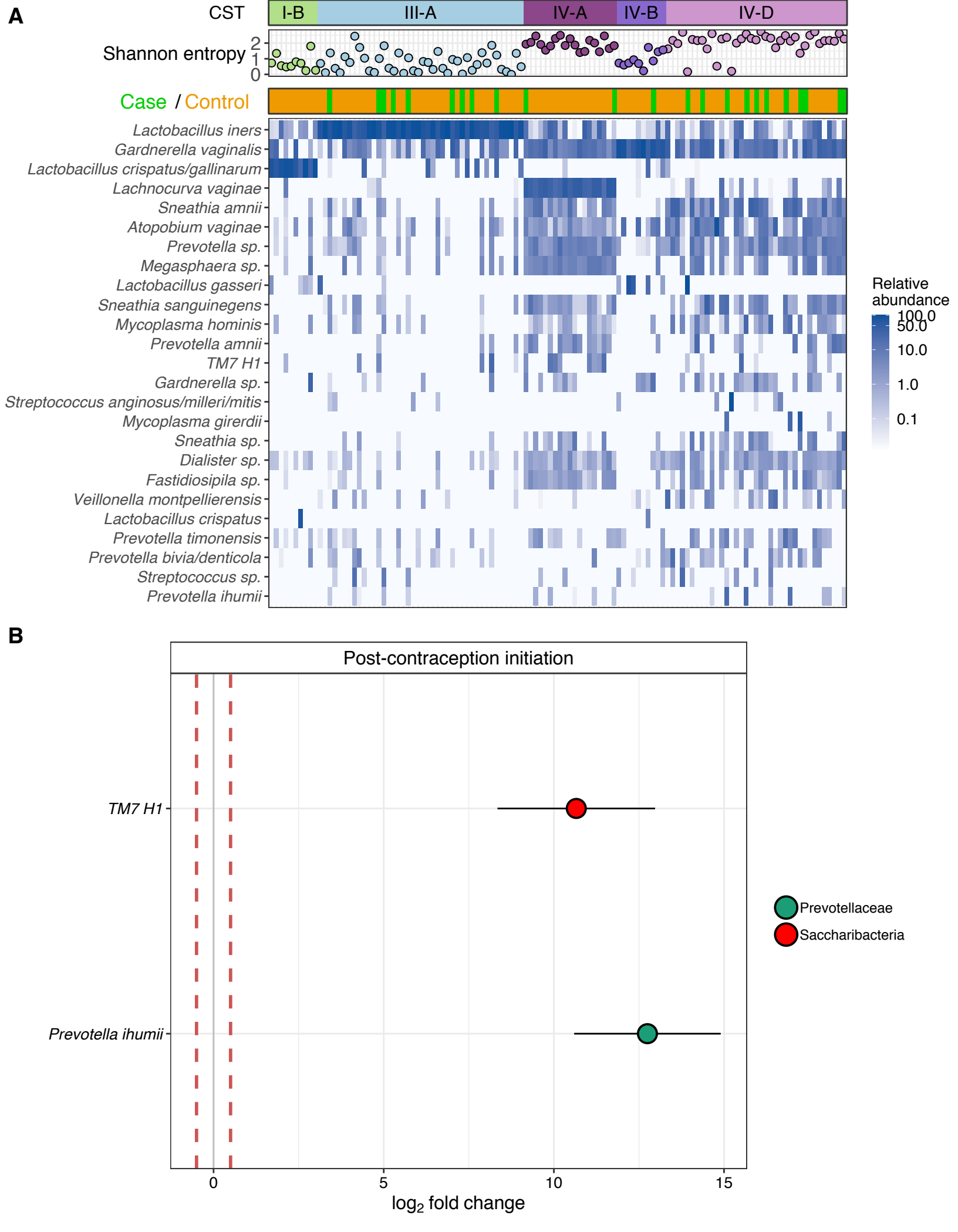

A
CST
I-B
III-A
IV-A
IV-B
IV-D
Shannon entropy
2
1
0
Case / Control
Lactobacillus iners
Gardnerella vaginalis
Lactobacillus crispatus/gallinarum
Lachnocurva vaginae
Sneathia amnii
Atopobium vaginae
Prevotella sp.
Megasphaera sp.
Lactobacillus gasseri
Sneathia sanguinegens
Mycoplasma hominis
Prevotella amnii
TM7 H1
Gardnerella sp.
Streptococcus anginosus/milleri/mitis
Mycoplasma girerdii
Sneathia sp.
Dialister sp.
Fastidiosipila sp.
Veillonella montpellierensis
Lactobacillus crispatus
Prevotella timonensis
Prevotella bivia/denticola
Streptococcus sp.
Prevotella ihumii
Relative abundance
100.0
50.0
10.0
1.0
0.1
B
Post-contraception initiation
TM7 H1
Prevotella ihumii
0
5
10
15
log2 fold change
Prevotellaceae
Saccharibacteria

**Figure S8. Bacterial community profiles and shifts in bacterial abundance in cases and controls. A**. Relative abundance heatmap of the top 25 taxa across all participants in the case/control analysis (n=118). Community state types are indicated in the top bar. Shannon diversity is displayed in the dotplot and colored by CST. Case/control status is indicated in the middle bar (Case: green; control: orange). **B**. Fold changes in bacterial abundance in case (n=23), relative to control (n=91), participants. Fold changes are $\log_2$ transformed and were calculated using DESeq2. Vertical dashed lines indicate a 0.5 fold change. Each point represents the arithmetic mean $\log_2$ fold change and solid horizontal lines represent the standard error. Taxa with two-tailed Wald P < 0.05 after adjustment for multiple comparisons (Benjamini and Hochberg) are shown.

**Table S1. Primer constructs targeting the V3-4 region of the 16S rRNA gene used in this study.**

| Primer name | Index set origin | Index name | Index in sample sheet | Illumina adapter | Index | Frameshift | Linker | Primer sequence - FRAMESHIFTED |
|---|---|---|---|---|---|---|---|---|
| F1_MetaIndex | TruSeq i5 | D501 | TATAGCCT | AATGATACGGCGACCACCGAGATCTACAC | TATAGCCT | G | TCGTCGGCAGCGTC | AATGATACGGCGACCACCGAGATCTACAC TATAGCCT G TCGTCGGCAGCGTC |
| F2_MetaIndex | TruSeq i5 | D502 | ATAGAGGC | AATGATACGGCGACCACCGAGATCTACAC | ATAGAGGC | GT | TCGTCGGCAGCGTC | AATGATACGGCGACCACCGAGATCTACAC ATAGAGGC GT TCGTCGGCAGCGTC |
| F3_MetaIndex | TruSeq i5 | D503 | CCTATCCT | AATGATACGGCGACCACCGAGATCTACAC | CCTATCCT | CGA | TCGTCGGCAGCGTC | AATGATACGGCGACCACCGAGATCTACAC CCTATCCT CGA TCGTCGGCAGCGTC |
| F4_MetaIndex | TruSeq i5 | D504 | GGCTCTGA | AATGATACGGCGACCACCGAGATCTACAC | GGCTCTGA | ATGA | TCGTCGGCAGCGTC | AATGATACGGCGACCACCGAGATCTACAC GGCTCTGA ATGA TCGTCGGCAGCGTC |
| F5_MetaIndex | TruSeq i5 | D505 | AGGCGAAG | AATGATACGGCGACCACCGAGATCTACAC | AGGCGAAG | TGCGA | TCGTCGGCAGCGTC | AATGATACGGCGACCACCGAGATCTACAC AGGCGAAG TGCGA TCGTCGGCAGCGTC |
| F6_MetaIndex | TruSeq i5 | D506 | TAATCTTA | AATGATACGGCGACCACCGAGATCTACAC | TAATCTTA | CAGTGG | TCGTCGGCAGCGTC | AATGATACGGCGACCACCGAGATCTACAC TAATCTTA CAGTGG TCGTCGGCAGCGTC |
| F7_MetaIndex | TruSeq i5 | D507 | CAGGACGT | AATGATACGGCGACCACCGAGATCTACAC | CAGGACGT | CCTGTGG | TCGTCGGCAGCGTC | AATGATACGGCGACCACCGAGATCTACAC CAGGACGT CCTGTGG TCGTCGGCAGCGTC |
| F8_MetaIndex | TruSeq i5 | D508 | GTACTGAC | AATGATACGGCGACCACCGAGATCTACAC | GTACTGAC | | TCGTCGGCAGCGTC | AATGATACGGCGACCACCGAGATCTACAC GTACTGAC TCGTCGGCAGCGTC |
| F9_MetaIndex | TruSeq Amplicon | A501 | TGAACCTT | AATGATACGGCGACCACCGAGATCTACAC | TGAACCTT | G | TCGTCGGCAGCGTC | AATGATACGGCGACCACCGAGATCTACAC TGAACCTT G TCGTCGGCAGCGTC |
| F10_MetaIndex | Nextera i5 | N501 | TAGATCGC | AATGATACGGCGACCACCGAGATCTACAC | TAGATCGC | GT | TCGTCGGCAGCGTC | AATGATACGGCGACCACCGAGATCTACAC TAGATCGC GT TCGTCGGCAGCGTC |
| F11_MetaIndex | Nextera i5 | N502 | CTCTCTAT | AATGATACGGCGACCACCGAGATCTACAC | CTCTCTAT | CGA | TCGTCGGCAGCGTC | AATGATACGGCGACCACCGAGATCTACAC CTCTCTAT CGA TCGTCGGCAGCGTC |
| F12_MetaIndex | Nextera i5 | N503 | TATCCTCT | AATGATACGGCGACCACCGAGATCTACAC | TATCCTCT | ATGA | TCGTCGGCAGCGTC | AATGATACGGCGACCACCGAGATCTACAC TATCCTCT ATGA TCGTCGGCAGCGTC |
| F13_MetaIndex | Nextera i5 | N504 | AGAGTAGA | AATGATACGGCGACCACCGAGATCTACAC | AGAGTAGA | TGCGA | TCGTCGGCAGCGTC | AATGATACGGCGACCACCGAGATCTACAC AGAGTAGA TGCGA TCGTCGGCAGCGTC |
| F14_MetaIndex | Nextera i5 | N505 | GTAAGGAG | AATGATACGGCGACCACCGAGATCTACAC | GTAAGGAG | CAGTGG | TCGTCGGCAGCGTC | AATGATACGGCGACCACCGAGATCTACAC GTAAGGAG CAGTGG TCGTCGGCAGCGTC |
| F15_MetaIndex | Nextera i5 | N506 | ACTGCATA | AATGATACGGCGACCACCGAGATCTACAC | ACTGCATA | CCTGTGG | TCGTCGGCAGCGTC | AATGATACGGCGACCACCGAGATCTACAC ACTGCATA CCTGTGG TCGTCGGCAGCGTC |
| F16_MetaIndex | Nextera i5 | N507 | AAGGAGTA | AATGATACGGCGACCACCGAGATCTACAC | AAGGAGTA | | TCGTCGGCAGCGTC | AATGATACGGCGACCACCGAGATCTACAC AAGGAGTA TCGTCGGCAGCGTC |
| R13_MetaIndex | TruSeq Amplicon | A701 | ATCACGAC | CAAGCAGAAGACGGCATACGAGAT | GTCGTGAT | A | GTCTCGTGGGCTCGG | CAAGCAGAAGACGGCATACGAGAT GTCGTGAT A GTCTCGTGGGCTCGG |
| R14_MetaIndex | TruSeq i7 | D701 | ATTACTCG | CAAGCAGAAGACGGCATACGAGAT | CGAGTAAT | TC | GTCTCGTGGGCTCGG | CAAGCAGAAGACGGCATACGAGAT CGAGTAAT TC GTCTCGTGGGCTCGG |
| R15_MetaIndex | TruSeq i7 | D702 | TCCGGAGA | CAAGCAGAAGACGGCATACGAGAT | TCTCCGGA | CTA | GTCTCGTGGGCTCGG | CAAGCAGAAGACGGCATACGAGAT TCTCCGGA CTA GTCTCGTGGGCTCGG |
| R16_MetaIndex | TruSeq i7 | D703 | CGCTCATT | CAAGCAGAAGACGGCATACGAGAT | AATGAGCG | GATA | GTCTCGTGGGCTCGG | CAAGCAGAAGACGGCATACGAGAT AATGAGCG GATA GTCTCGTGGGCTCGG |
| R17_MetaIndex | TruSeq i7 | D704 | GAGATTCC | CAAGCAGAAGACGGCATACGAGAT | GGAATCTC | ACTCA | GTCTCGTGGGCTCGG | CAAGCAGAAGACGGCATACGAGAT GGAATCTC ACTCA GTCTCGTGGGCTCGG |
| R18_MetaIndex | TruSeq i7 | D705 | ATTCAGAA | CAAGCAGAAGACGGCATACGAGAT | TTCTGAAT | TTCTCT | GTCTCGTGGGCTCGG | CAAGCAGAAGACGGCATACGAGAT TTCTGAAT TTCTCT GTCTCGTGGGCTCGG |
| R19_MetaIndex | TruSeq i7 | D706 | GAATTCGT | CAAGCAGAAGACGGCATACGAGAT | ACGAATTC | CACTTCT | GTCTCGTGGGCTCGG | CAAGCAGAAGACGGCATACGAGAT ACGAATTC CACTTCT GTCTCGTGGGCTCGG |
| R20_MetaIndex | TruSeq i7 | D707 | CTGAAGCT | CAAGCAGAAGACGGCATACGAGAT | AGCTTCAG | | GTCTCGTGGGCTCGG | CAAGCAGAAGACGGCATACGAGAT AGCTTCAG GTCTCGTGGGCTCGG |
| R21_MetaIndex | TruSeq i7 | D708 | TAATGCGC | CAAGCAGAAGACGGCATACGAGAT | GCGCATTA | A | GTCTCGTGGGCTCGG | CAAGCAGAAGACGGCATACGAGAT GCGCATTA A GTCTCGTGGGCTCGG |
| R22_MetaIndex | TruSeq i7 | D709 | CGGCTATG | CAAGCAGAAGACGGCATACGAGAT | CATAGCCG | TC | GTCTCGTGGGCTCGG | CAAGCAGAAGACGGCATACGAGAT CATAGCCG TC GTCTCGTGGGCTCGG |
| R23_MetaIndex | TruSeq i7 | D710 | TCCGCGAA | CAAGCAGAAGACGGCATACGAGAT | TTCGCGGA | CTA | GTCTCGTGGGCTCGG | CAAGCAGAAGACGGCATACGAGAT TTCGCGGA CTA GTCTCGTGGGCTCGG |
| R24_MetaIndex | TruSeq i7 | D711 | TCTCGCGC | CAAGCAGAAGACGGCATACGAGAT | GCGCGAGA | GATA | GTCTCGTGGGCTCGG | CAAGCAGAAGACGGCATACGAGAT GCGCGAGA GATA GTCTCGTGGGCTCGG |

| Primer name | Sequence | Marker gene | Target region |
|---|---|---|---|
| V4_806R_Nextera | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGG**ACTACNVGGGTWTCTAAT** | 16S rRNA | V3-V4 |
| V3_357F_Nextera | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCC**TACGGGNGGCWGCAG** | 16S rRNA | V3-V4 |

**Table S2. Model statistics and prediction accuracy for bacterial inferred concentration-cytokine models at six months of use. Two-tailed P values were derived from the t-value of the generalized linear model. All reported p-values were corrected using the method of Benjamini and Yekutieli.**

| | Cu-IUD altered bacterial concentrations | | | | |
|---|---|---|---|---|---|
| **Dependent Variable** | **Beta Coefficient** | **Standard Error** | **t-statistic** | **adjusted P values[A]** | **MAE[B]** |
| **IL-1b** | 0.284437682 | 0.057507091 | 4.946132335 | 0.000227745 | 1.653177992 |
| **IL-6** | 0.069260858 | 0.058029524 | 1.193545177 | 1 | 1.863821072 |
| **TNF-a** | 0.107664527 | 0.036351009 | 2.961802962 | 0.043723603 | 1.071315093 |
| **IL-8** | 0.059867929 | 0.044195567 | 1.354613897 | 1 | 1.334564626 |
| **Eotaxin** | 0.021097176 | 0.020241201 | 1.042288773 | 1 | 0.609637209 |
| **IP-10** | -0.179729921 | 0.058474543 | -3.073643857 | 0.035444947 | 1.741603382 |
| **MCP-1** | -0.038790204 | 0.046255676 | -0.838604195 | 1 | 1.410128288 |
| **MIP-1a** | 0.04073915 | 0.061690302 | 0.660381762 | 1 | 2.030856538 |
| **MIP-1b** | 0.002469727 | 0.046111404 | 0.053559998 | 1 | 1.572466249 |
| **RANTES** | 0.026200389 | 0.024263262 | 1.079837846 | 1 | 0.706788327 |
| **IL-2** | 0.105696104 | 0.029817931 | 3.544716246 | 0.013240493 | 0.897198347 |
| **IL-4** | 0.062342787 | 0.022011828 | 2.832240321 | 0.053353902 | 0.663017303 |
| **IL-5** | 0.064939225 | 0.023027912 | 2.820022234 | 0.053353902 | 0.694607933 |
| **IL-15** | 0.071040818 | 0.020441089 | 3.475392997 | 0.013424683 | 0.607675222 |
| **IL-17** | 0.104269615 | 0.031673895 | 3.291973286 | 0.020609517 | 0.982535341 |
| **IFN-γ** | 0.000630958 | 0.009763007 | 0.064627453 | 1 | 0.226975286 |
| **IL-7** | 0.038000216 | 0.032113913 | 1.183294468 | 1 | 1.001492231 |
| **IL-9** | 0.034683665 | 0.016741953 | 2.071661845 | 0.320940762 | 0.50654532 |
| **bFGF** | 0.080274034 | 0.021184101 | 3.789352827 | 0.007430777 | 0.654816036 |
| **G-CSF** | 0.024382315 | 0.042518225 | 0.573455626 | 1 | 1.387148649 |
| **GM-CSF** | 0.072318632 | 0.027379448 | 2.641347297 | 0.080911786 | 0.742578516 |
| **PDGF-BB** | -0.006811303 | 0.018578917 | -0.366614635 | 1 | 0.483318725 |
| **VEGF** | 0.120309954 | 0.026217501 | 4.588917736 | 0.000512966 | 0.732224209 |
| **IL-1Ra** | -0.024743641 | 0.019766411 | -1.251802438 | 1 | 0.476912763 |
| **IL-10** | 0.019155186 | 0.024401277 | 0.785007508 | 1 | 0.684711968 |

[A] P value were adjusted using the method of Benjamini and Yekutieli (2001)

[B]Mean absolute error was calculated from fitting the specified model to a validation dataset, which was a random subset

**Distribution of sample counts across the primary and secondary analyses.**

**Table S3A.** Sample counts for the primary (pre-post) analysis.

| Study Site | Randomized Arm | n* | n** |
|---|---|---|---|
| Cape Town, South Africa | Cu-IUD | 27 | 24 |
| Cape Town, South Africa | DMPA-IM | 28 | 23 |
| Cape Town, South Africa | LNG Implant | 25 | 21 |
| Kisumu, Kenya | Cu-IUD | 20 | 17 |
| Kisumu, Kenya | DMPA-IM | 20 | 16 |
| Kisumu, Kenya | LNG Implant | 20 | 16 |
| Johannesburg, South Africa | Cu-IUD | 20 | 17 |
| Johannesburg, South Africa | DMPA-IM | 20 | 18 |
| Johannesburg, South Africa | LNG Implant | 20 | 18 |

*samples selected

**samples that passed QC

**Table S3B.** Sample counts for the secondary (case-control) analysis.

| Sample Type | Study Month | n** |
|---|---|---|
| Case | 1 month | 5 |
| Case | 3 months | 5 |
| Case | 6 months | 2 |
| Case | 9 months | 3 |
| Case | 12 months | 5 |
| Case | 15 months | 3 |
| Control | Enrollment | 4 |
| Control | 1 month | 17 |
| Control | 3 months | 19 |
| Control | 6 months | 11 |
| Control | 9 months | 14 |
| Control | 12 months | 18 |
| Control | 15 months | 12 |

**samples that passed QC

**Table S3C.** Sample counts that were included in both analyses due to the availability of all primary analysis samples and which were also included in the case-control analysis.

| Study Site | Randomized Arm | n** |
|---|---|---|
| Cape Town, South Africa | Cu-IUD | 5 |
| Cape Town, South Africa | DMPA-IM | 12 |
| Cape Town, South Africa | LNG Implant | 11 |
| Kisumu, Kenya | Cu-IUD | 4 |
| Kisumu, Kenya | DMPA-IM | 6 |
| Kisumu, Kenya | LNG Implant | 2 |
| Johannesburg, South Africa | Cu-IUD | 3 |
| Johannesburg, South Africa | DMPA-IM | 2 |
| Johannesburg, South Africa | LNG Implant | 2 |

**samples that passed QC