

A pilot study evaluating microbiome profiles of breast tissue, vaginal samples and stool in patients with benign breast lesions (fibroadenoma).

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Supplementary Table S1. Differential abundance analysis: stool vs vagina Top20 genera identified using ALDEx2.

	Adjusted p-value (BH)	CLR difference	Effect size	Median relative abundance (STOOL)	Median relative abundance (VAGINA)
<i>Lactobacillus</i>	0.1668447	25.323184	9.6898664	-5.941868	19.431656
<i>Limosilactobacillus</i>	0.1668447	15.106244	3.3757505	-4.039981	10.915473
Unknown	0.2143677	8.238108	2.1192248	-4.195824	4.310929
<i>Ureaplasma</i>	0.2068777	13.589120	1.9824705	-5.852583	8.732323
<i>Peptoniphilus</i>	0.2418115	8.711247	1.6599221	-5.959120	2.273110
<i>Aedoeadaptatus</i>	0.2269395	8.513598	1.6349620	-6.058055	1.676057
<i>Gardnerella</i>	0.2377118	8.154383	1.5418844	-5.677130	1.665725
<i>Aerococcus</i>	0.2373499	8.952793	1.5276558	-6.109153	2.025857
<i>Haemophilus</i>	0.2339822	8.743495	1.4555296	-5.648236	1.859914
<i>Finegoldia</i>	0.2475833	9.192047	1.4495948	-5.573163	2.105988
<i>Anaerococcus</i>	0.2359456	8.376608	1.4382986	-5.742936	1.840489
<i>Metamycoplasma</i>	0.2299010	8.905622	1.4062579	-5.741551	1.915571
<i>Fannyhessea</i>	0.2381156	7.969131	1.3574176	-5.686830	1.761841
<i>Neoolivea</i>	0.2582659	8.161924	1.3526283	-6.095320	2.004217
<i>Prevotella</i>	0.2389105	8.827038	1.2070174	-5.842212	1.804215
<i>Peptostreptococcus</i>	0.3562846	5.712844	0.9254535	-3.472116	1.834591
<i>Streptococcus</i>	0.4656157	4.005101	0.5589027	3.718532	7.020322
<i>Hoyieseu</i>	0.5639260	4.125105	0.5548811	-1.165775	2.008690
<i>Veillonena</i>	0.6626646	4.400498	0.4580224	1.707585	1.863994
<i>Enterococcus</i>	0.6749974	2.079112	0.2562920	1.463757	2.077269

Supplementary Table S2. Differential abundance analysis: stool vs fibroadenoma Top20 genera identified using ALDEx2.

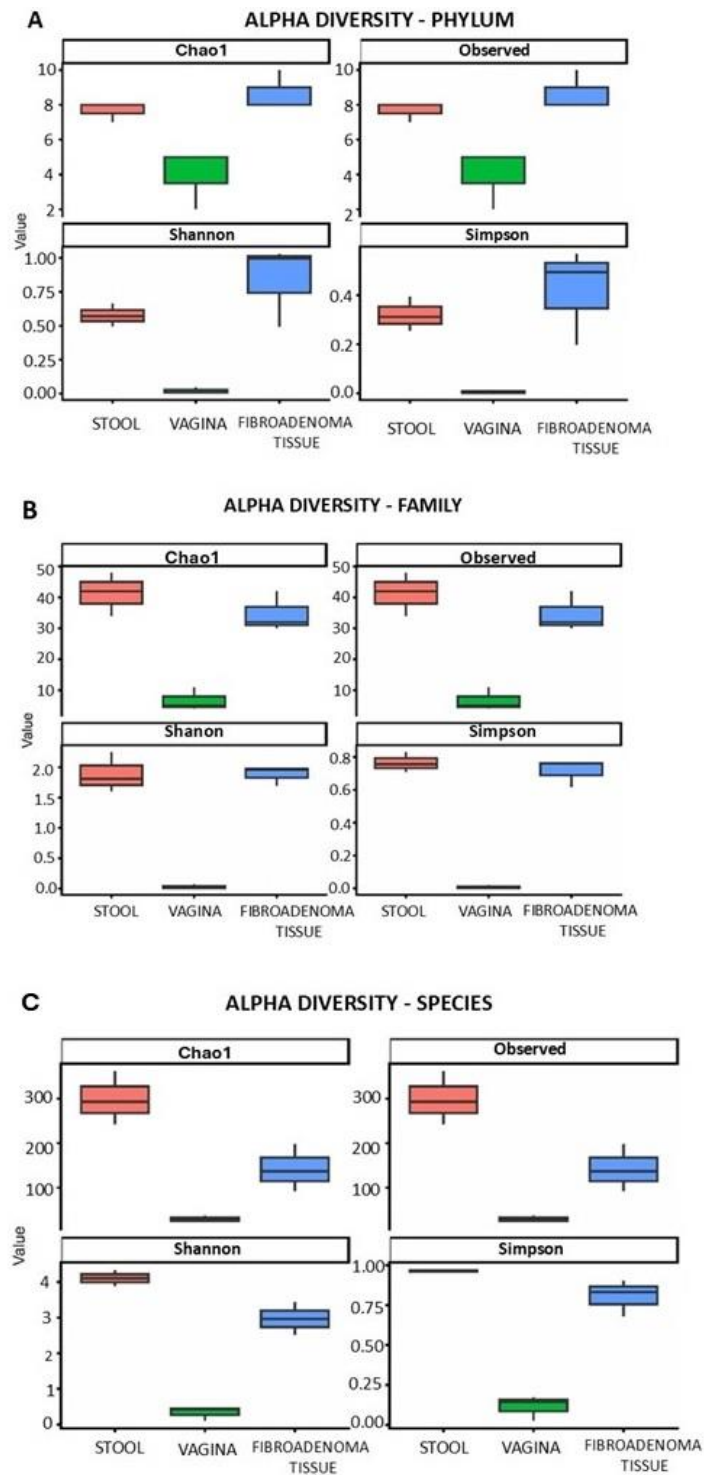
	Adjusted p-value (BH)	CLR difference	Effect size	Median relative abundance (STOOL)	Median relative abundance (FIBROADENOMA)
Unknown	0.2340075	14.8543778	6.5418205	-3.469722	11.428722
<i>Sporosarcina</i>	0.2340075	18.8434996	6.4852175	-4.990030	14.544054
<i>Cutibacterium</i>	0.2340075	15.0486893	5.6889089	-5.240301	10.010154
<i>Bradyrhizobium</i>	0.2340075	18.4342970	5.1297551	-5.338177	13.237141
<i>Staphylococcus</i>	0.2340075	14.6739159	5.0267930	-5.268063	8.527847
<i>Neolivea</i>	0.2340075	13.1640987	4.4667059	-5.257006	8.183215
<i>Lactobacillus</i>	0.2340075	17.1808430	4.3702572	-4.976531	11.045787
<i>Acinetobacter</i>	0.2340075	13.4355521	3.1549479	-5.114038	8.843915
<i>Enterococcus</i>	0.2340075	6.6552006	2.0114218	2.179092	8.766060
<i>Bacteroides</i>	0.5407943	-2.5030914	-0.9586384	10.999765	7.979348
<i>Faecalibacterium</i>	0.6106217	-1.5154442	-0.8313315	11.856264	10.338165
<i>Clostridium</i>	0.5257604	1.0938428	0.8108607	8.341589	9.223531
<i>Phocaeicola</i>	0.4766812	-1.2729469	-0.7683584	9.405678	8.304117
<i>Lachnospira</i>	0.7302552	0.6381642	0.3821024	8.364848	8.381790
<i>Blautia</i>	0.8233059	0.7531947	0.3669136	10.152805	11.141215
<i>Agathobacter</i>	0.6543422	0.9806593	0.3074397	7.558630	8.482451
<i>Ruminococcus</i>	0.7366588	-0.1789342	-0.1569721	10.866274	10.447636
<i>Mediterraneibacter</i>	0.6983022	0.2444892	0.0423543	7.729141	8.000555
<i>Vescimonas</i>	0.8245325	-0.1359337	-0.0183548	7.793182	8.389868
<i>Coproccoccus</i>	0.8127893	0.0199746	0.0102140	8.008406	8.735811

Supplementary Table S3. Differential abundance analysis: vagina vs fibroadenoma Top20 genera identified using ALDEx2.

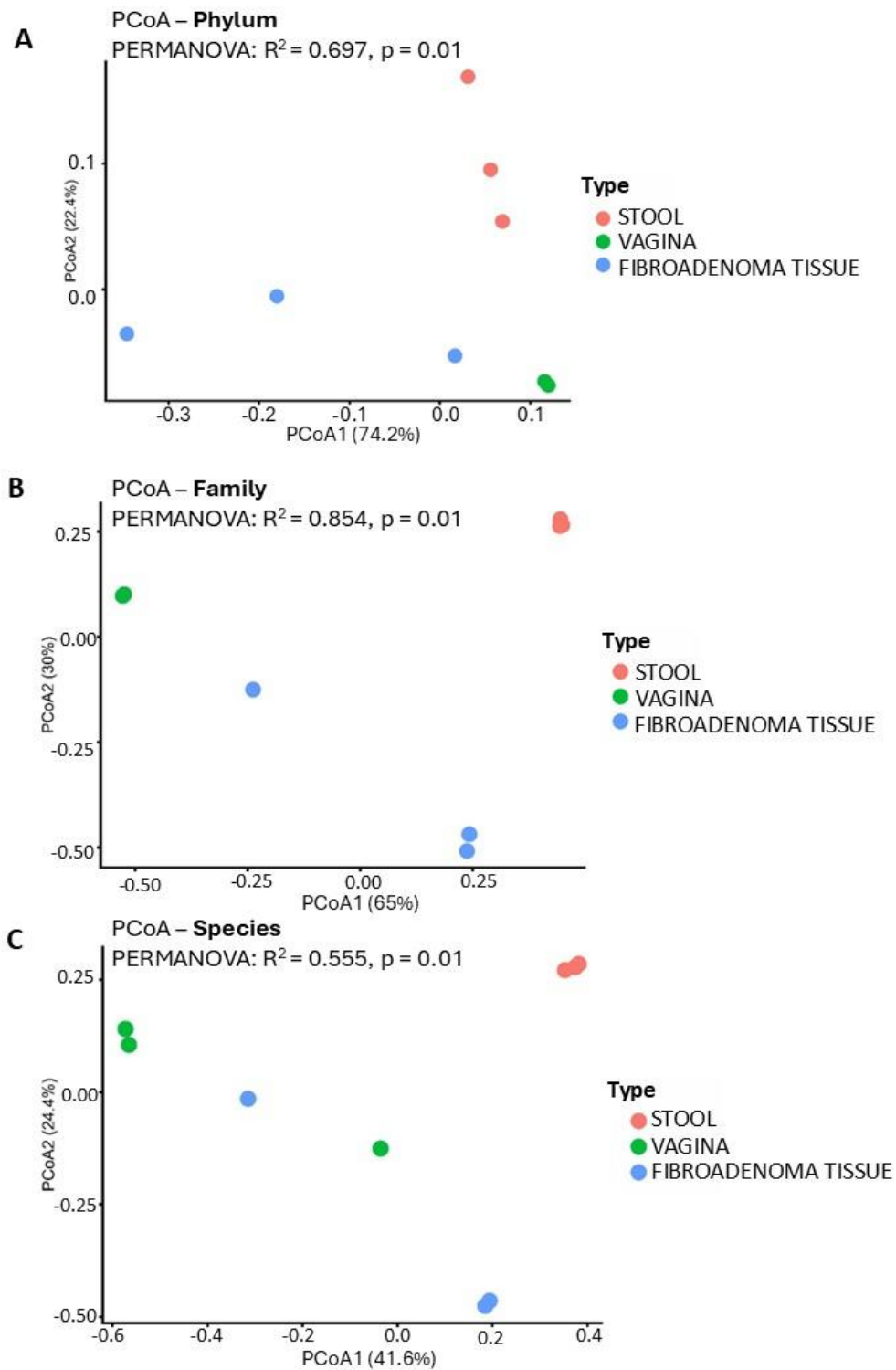
	Adjusted p-value (BH)	CLR difference	Effect size	Median relative abundance (VAGINA)	Median relative abundance (FIBROADENOMA)
<i>Lactobacillus</i>	0.2549058	-9.676434	-6.4048910	19.2198239	9.456659
<i>Sporosarcina</i>	0.2549058	11.728508	3.1380309	-0.0672515	12.696126
<i>Faecalibacterium</i>	0.2549058	8.199338	2.8572930	-0.0895106	8.439006
<i>Blautia</i>	0.2549058	8.572823	2.7790043	0.2749777	9.401453
<i>Bradyrhizobium</i>	0.2549058	11.173026	2.6428762	-0.1743473	11.666663
<i>Ruminococcus</i>	0.2549058	7.931797	2.5759960	0.3324657	8.566768
<i>Cutibacterium</i>	0.2549058	7.708973	2.2941899	0.0470812	8.168261
<i>Clostridium</i>	0.2549058	7.282556	2.2070120	0.0949147	7.327494
<i>Agathobacter</i>	0.2549058	6.776222	2.1887288	0.0539482	6.536635
<i>Coprococcus</i>	0.2563024	6.282970	2.1228855	0.3249208	6.859724
<i>Staphylococcus</i>	0.2549058	7.377429	2.1032776	-0.1115754	6.782057
<i>Bacteroides</i>	0.2561578	6.061320	2.0798460	0.0923825	5.909958
<i>Phocaeicola</i>	0.2549058	5.985063	1.8639854	0.4147654	6.017573
<i>Lachnospira</i>	0.2706710	6.771057	1.6594385	-0.2604134	6.541519
Unknown	0.2682780	5.308252	1.6197289	3.9309261	9.550408
<i>Akkermansia</i>	0.3854501	4.720395	1.2459580	-0.0109452	5.450785
<i>Acinetobacter</i>	0.4341699	6.091453	1.1170021	0.4542799	7.270548
<i>Enterococcus</i>	0.4482454	5.088038	0.9431172	1.5331601	7.174571
<i>Neoolivea</i>	0.5141209	4.010596	0.7338351	1.6360616	6.283502
<i>Agathobaculum</i>	0.6537723	4.991655	0.4931136	0.0198163	5.792409

Supplementary Table S4. Primer sequences and PCR amplification conditions used in this study.

Target microorganism	Primer	Sequence (5'-3')	Amplicon size (bp)	PCR conditions	Reference
<i>C. trachomatis</i>	CT1 CT5	GCCGCTTTGAGTTCTGCTTCCTC ATTTACGTGAGCAGCTCTCTCAT	1100	95°C 4 min; 40 cycles: 95°C 1 min, 55°C 1 min, 72°C 1,5 min; final extension 72°C 4 min	[21]
<i>M. genitalium</i>	MG1 MG2	AGTTGATGAAACCTTAACCCCTTGG CCGTTGAGGGGTTTTCCATTTTTGC	282	95°C 10 min; 35 cycles: 95°C 15 s, 54°C 30 s, 72°C 30 s; final extension 72°C 5 min	[20]
<i>M. hominis</i>	RNAH1 RNAH2	CAATGGCTAATGCCGGATACGC GGTACCGTCAGTCTGCAAT	334	95°C 10 min; 35 cycles: 95°C 15 s, 60°C 1 min, 72°C 1 min; final extension 72°C 5 min	[20]
<i>U. urealyticum</i>	UMS125 UMA226	GTATTTGCAATCTTTATATGTTTTCG CAGCTGATGTAAGTGCAGCATTAAATTC	448	95°C 10 min; 35 cycles: 95°C 15 s, 54°C 30 s, 72°C 30 s; final extension 72°C 5 min	[20]
<i>U. parvum</i>	UMS125 UMA226	GTATTTGCAATCTTTATATGTTTTCG CAGCTGATGTAAGTGCAGCATTAAATTC	403	95°C 10 min; 35 cycles: 95°C 15 s, 54°C 30 s, 72°C 30 s; final extension 72°C 5 min	[20]



Supplementary Figure S1. Alpha diversity of microbial communities at additional taxonomic levels. Boxplots show the Chao1 richness, Observed richness, Shannon diversity, and Simpson diversity indices calculated at the (A) phylum level, (B) family level and (C) species level. Boxes represent the interquartile range (IQR), and the horizontal line indicates the median. Statistical comparisons were performed using the Kruskal-Wallis test with Benjamini-Hochberg correction, followed by pairwise Wilcoxon rank-sum tests.



Supplementary Figure S2. Beta diversity of microbial communities at the (A) phylum, (B) family, and (C) species levels. PCoA based on Bray–Curtis dissimilarity illustrates the differences in microbial community composition among the three ecological niches. Each point represents an individual sample, and colors indicate sample type.