

Supplementary Tables, Figures and File

The urinary and vaginal microbiota in postmenopausal women with and without overactive bladder

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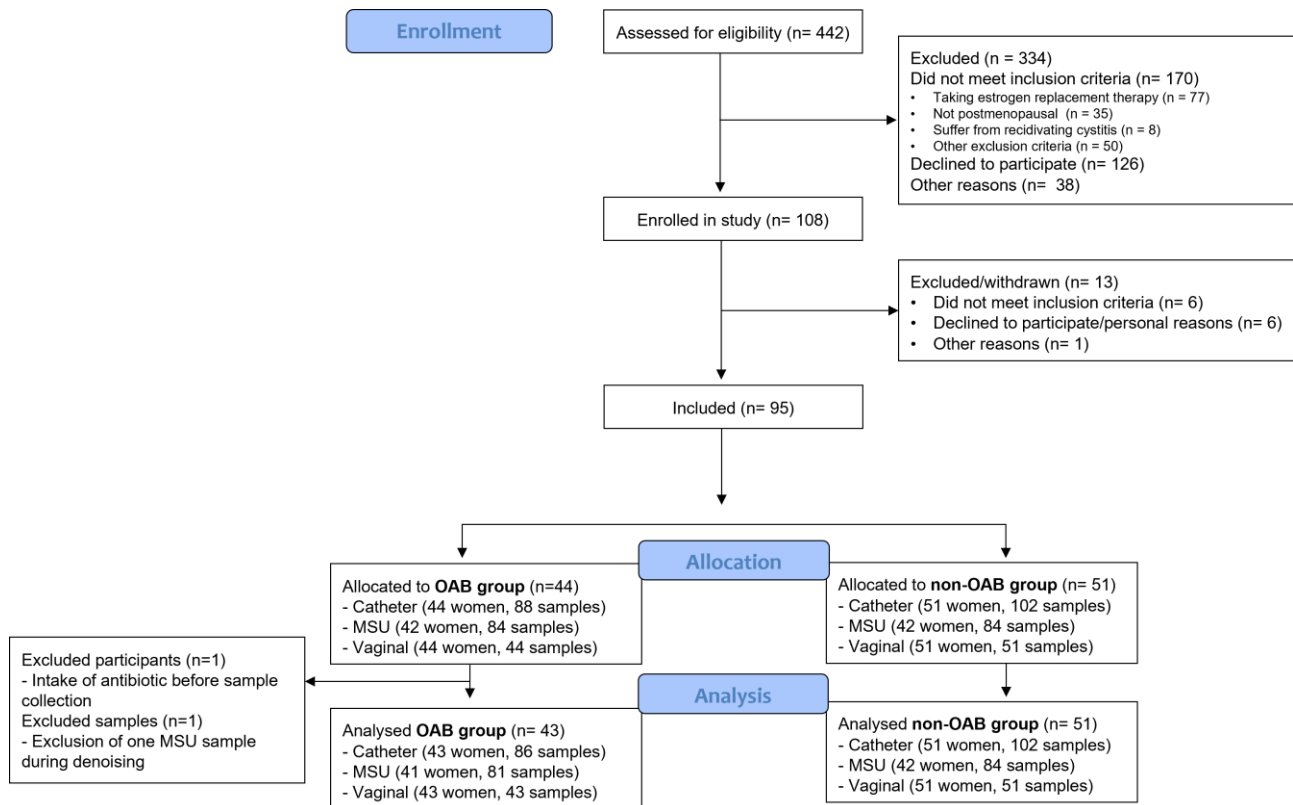
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Supplementary Fig. S1. CONSORT flowchart of participants and participant samples from women with and without overactive bladder (OAB). From each woman, duplicate catheterized urine samples, duplicate midstream urine (MSU) samples, and one vaginal sample were proceeded to microbiota analysis. After allocation, two women with OAB and nine women without OAB did not deliver an MSU sample but delivered catheter and vaginal samples. One woman was, after inclusion, excluded due to intake of antibiotics before sample collection. During the bioinformatic analysis, one of the duplicates of one MSU sample was lost due to poor quality.

	Catheter and vaginal samples (n = 94) *		MSU samples (n = 83) *	
Current or previous diseases	OAB (n=40) ^Δ	Non-OAB (n=49) ^Δ	OAB (n=38) ^Δ	Non-OAB (n=42) ^Δ
Diseases, n (%)				
Central nervous system				
- Stroke	1 (2.5)	1 (2.0)	1 (2.6)	1 (2.4)
- Migraine	3 (7.5)	3 (6.1)	3 (7.9)	2 (4.8)
- Parkinson's	1 (2.5)	0 (0.0)	1 (2.6)	0 (0.0)
Cardiovascular				
- Arteriosclerosis	1 (2.5)	1 (2.0)	1 (2.6)	1 (2.4)
- Hypertension	8 (20.0)	9 (18.4)	7 (18.4)	9 (21.4)
- Increased cholesterol	12 (30.0)	9 (18.4)	12 (31.6)	8 (19.0)
- Arrhythmia	1 (2.5)	0 (0.0)	1 (2.6)	0 (0.0)
- Others	0 (0.0)	1 (2.0)	0 (0.0)	0 (0.0)
Lung / pulmonary				
- Chronic obstructive pulmonary disease	2 (5.0)	1 (2.0)	1 (2.6)	1 (2.4)
- Asthma	4 (10.0)	0 (0.0)	4 (10.5)	0 (0.0)
- Others	1 (2.5)	0 (0.0)	1 (2.6)	0 (0.0)
Gastrointestinal tract				
- Constriction of cardiac sphincter	1 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)
- Diverticula	0 (0.0)	1 (2.0)	0 (0.0)	1 (2.4)
- Gastroesophageal reflux disease	0 (0.0)	1 (2.0)	0 (0.0)	0 (0.0)
Musculoskeletal				
- Osteoarthritis	14 (35.0)	3 (6.1)	13 (34.2)	3 (7.1)
- Rheumatoid arthritis	0 (0.0)	2 (4.1)	0 (0.0)	2 (4.8)
- Osteoporosis	7 (17.5)	7 (14.3)	7 (18.4)	7 (16.7)
- Osteopenia	0 (0.0)	1 (2.0)	0 (0.0)	1 (2.4)
- Back disease	2 (5.0)	1 (2.0)	1 (2.6)	1 (2.4)
- Localized pain in musculoskeletal system	0 (0.0)	4 (8.2)	0 (0.0)	4 (9.5)
- Hip fracture	1 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)
- Meniscus injury	0 (0.0)	1 (2.0)	0 (0.0)	0 (0.0)
Urogenital				
- Lichen sclerosus	1 (2.5)	4 (8.2)	1 (2.6)	3 (7.1)
- Prolaps	1 (2.5)	1 (2.0)	1 (2.6)	1 (2.4)
- Renal calculi	1 (2.5)	0 (0.0)	1 (2.6)	0 (0.0)
Endocrine				
- Diabetes mellitus 2	1 (2.5)	0 (0.0)	1 (2.6)	0 (0.0)
- Thyroid disease ^Δ	2 (5.0)	1 (2.0)	2 (5.3)	0 (0.0)
Cancer				
- Skin cancer	1 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)
- Vulva cancer	1 (2.5)	0 (0.0)	1 (2.6)	0 (0.0)
- Malignant melanoma	0 (0.0)	1 (2.0)	0 (0.0)	0 (0.0)
Others				
- Erythema nodosum	0 (0.0)	1 (2.0)	0 (0.0)	0 (0.0)

Supplementary Table S1. Self-reported current or previous diseases by participants *Eleven women did not deliver a midstream urine (MSU) sample, thus a total of 83 women were included in all subsequent analyses. Opposite, from 94 women we collected both a catheterized urine and vaginal sample. ^ΔThree women in the overactive bladder (OAB) group and two women in the non-OAB group did not report current or previous diseases, thus 40 OAB and 49 non-OAB were included in all samples, and 38 OAB and 42 non-OAB in MSU samples. Percentages are calculated based on each disease

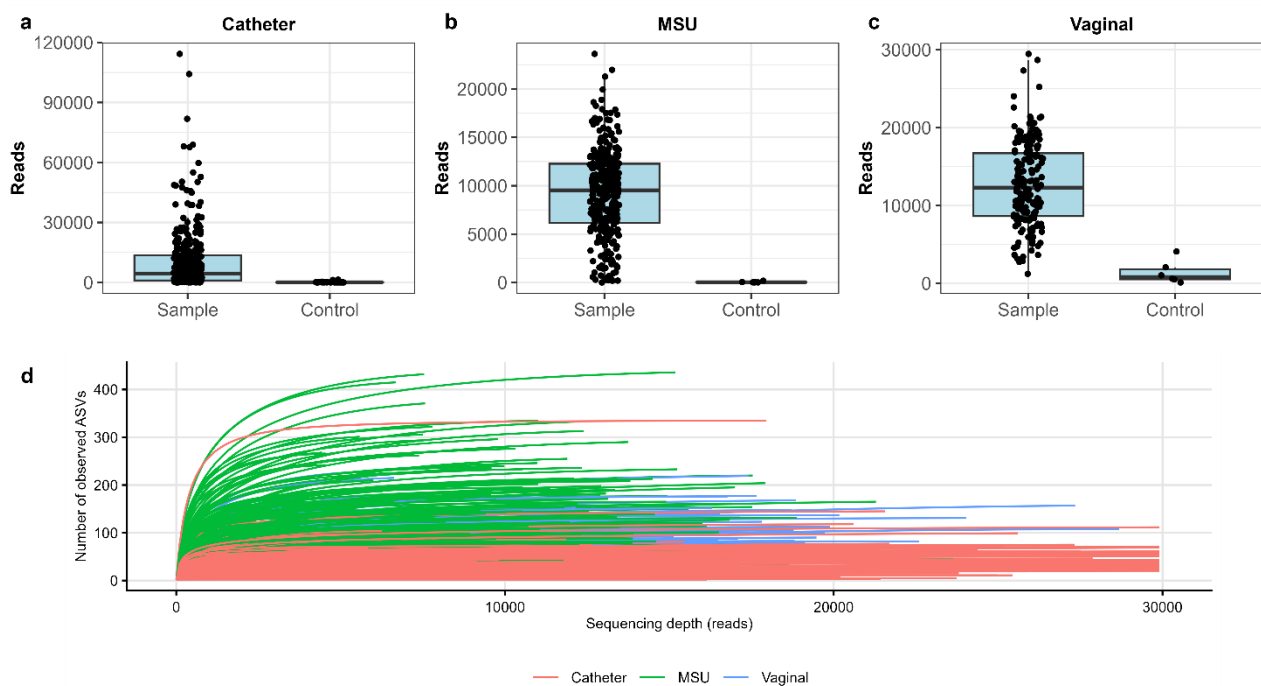
group.^Δ Two women in the OAB group reported thyroid disease: one reported hypothyroidism and the other unknown. One woman in the non-OAB group reported thyroid disease, however, it is unknown if it is hypothyroidism or hyperthyroidism.

	MSU samples (n = 83)	
	OAB (n=41)	Non-OAB (n=42)
Demographics and clinical parameters		
Age, mean ±SD	63.8±6.0	61.2±6.1
Body mass index ^Δ (kg/m ²), median (Q1-Q3)	25.5 (22.0-30.1)	24.4 (22.4-27.0)
Smoking status, n (%)		
-Never smoked	16 (39.0)	20 (47.6)
-Former smoker	18 (43.9)	19 (45.2)
-Smoker	7 (17.1)	3 (7.1)
Number of births, median (QR1-QR3)	2 (2-2)	2 (2-3)
Cesarean, n (%)	6 (16.2)	6 (15.8)
Years since last menstruation, median (QR1-QR3)	12.5 (8.5-20.0)	9.0 (6.0-16.8)
Intercourse [°] , n (%)	4 (9.8)	3 (7.1)
Vaginal dryness, n (%)	23 (56.1)	23 (54.8)
Vaginal pH [□] , median (QR1-QR3)	5.3 (5.0-5.5)	5.3 (5.0-6.0)
Urinary parameters		
Urine pH [°] , mean ± SD	5.9±0.6	6.0±0.7
OAB type, n (%)		
- Wet	24 (58.5)	-
- Dry	17 (41.5)	-
Urinary incontinence type, n (%)		
- UUI	10 (24.4)	-
- MUI	14 (34.1)	-
- SUI	6 (14.6)	6 (14.3)
- Overflow	0 (0.0)	0 (0.0)
- No urinary incontinence	11 (26.8)	36 (85.7)
ICIQ-OAB score (0-16), median (QR1-QR3)	7.0 (6.0-9.0)	4.0 (3.0-4.0)
ICIQ-UI-SF score (0-21), median (QR1-QR3)	9.0 (6.0-14.0)	4.0 (0.0-6.0)
Nutritional components in diet⁺		
Energy, kJ, mean ± SD	6800±1200	7065±1582
Lipid, g, median (QR1-QR3)	60.3 (50.7-67.7)	61.0 (51.8-78.8)
Carbohydrate, g, mean ± SD	183±44.4	181±49.9
Protein, g, mean ± SD	69.1±14.5	71.4±19.6

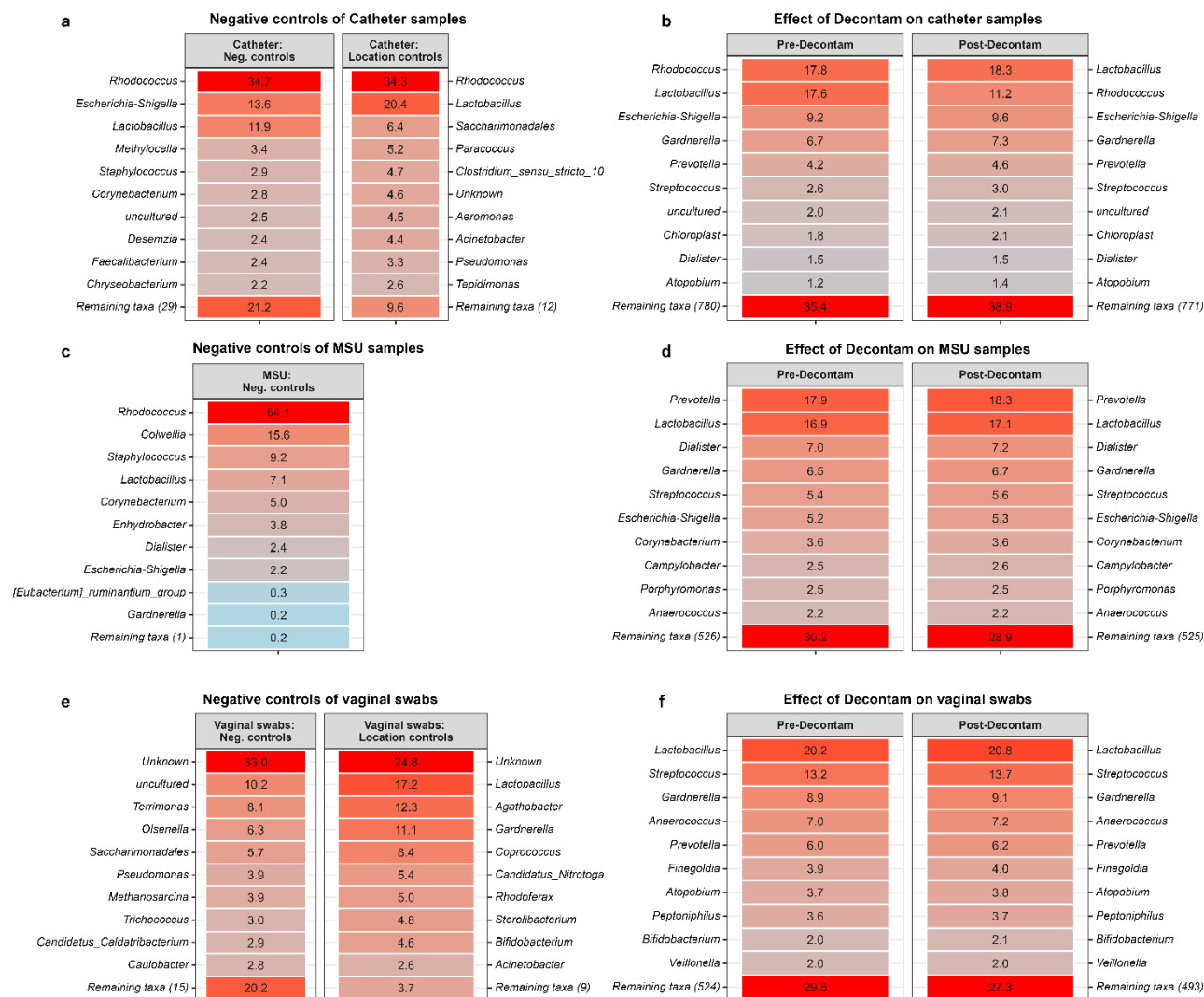
Supplementary Table S2. Comparison of demographics, clinical features, and bladder symptom parameters between women with and without OAB who delivered a midstream urine (MSU) sample. ^ΔThree women with OAB did not report weight and height, and we could therefore not calculate body mass index for them. [□]It was not possible to measure the vaginal pH of all the women (OAB n = 29, non-OAB 34). [°]Urine pH was not measured for all women (OAB n = 37, and non-OAB n = 42). [°]Intercourse: The women were asked, “Have you had intercourse within the last three days?”. ⁺Diet data are displayed as an average of the three days. Data do not include nutrient intake from supplements. OAB: Overactive bladder. ICIQ-OAB: International Consultation on Incontinence Questionnaire - Overactive Bladder. ICIQ-UI-SF: International Consultation on Incontinence Questionnaire - Urinary Incontinence Short Form. UUI: urge urinary incontinence, MUI: mixed urinary incontinence, SUI: stress urinary incontinence.

	MSU samples (n = 83)	
	OAB (n=41)	Non-OAB (n=42)
Fluid intake		
Fluid intake 24-hour (mL), median (QR1-QR3)	1900 (1586-2070)	2347 (1906-2862)
Fluid intake night (mL), median (QR1-QR3)	0 (0-67)	0 (0-103)
Urinary voiding		
24-hour urine volume (mL), mean $\pm$ SD	1855 $\pm$ 560	2136 $\pm$ 655
Nocturnal urine volume (mL), median (QR1-QR3)	500 (335-625)	538 (334-741)
Average voided volume (mL), mean $\pm$ SD	204 $\pm$ 64	281 $\pm$ 80
24-hour frequency, median (QR1-QR3)	9.0 (7.7-11.0)	8.0 (6.4-9.3)
Daytime urinary frequency, median (QR1-QR3)	8.0 (7.0-9.3)	6.8 (5.8-7.7)
Nocturnal frequency, median (QR1-QR3)	1.3 (0.7-2.0)	1.0 (0.3-1.3)
24-hour incontinence episodes, median (QR1-QR3)	0.7 (0.0-2.0)	0.0 (0.0-0.0)
Incontinence episodes night, median (QR1-QR3)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
24-hour max volume (mL), median (QR1-QR3)	480 (308-550)	600 (442-651)
Daytime max volume (mL), median (QR1-QR3)	440 (300-500)	500 (429-600)

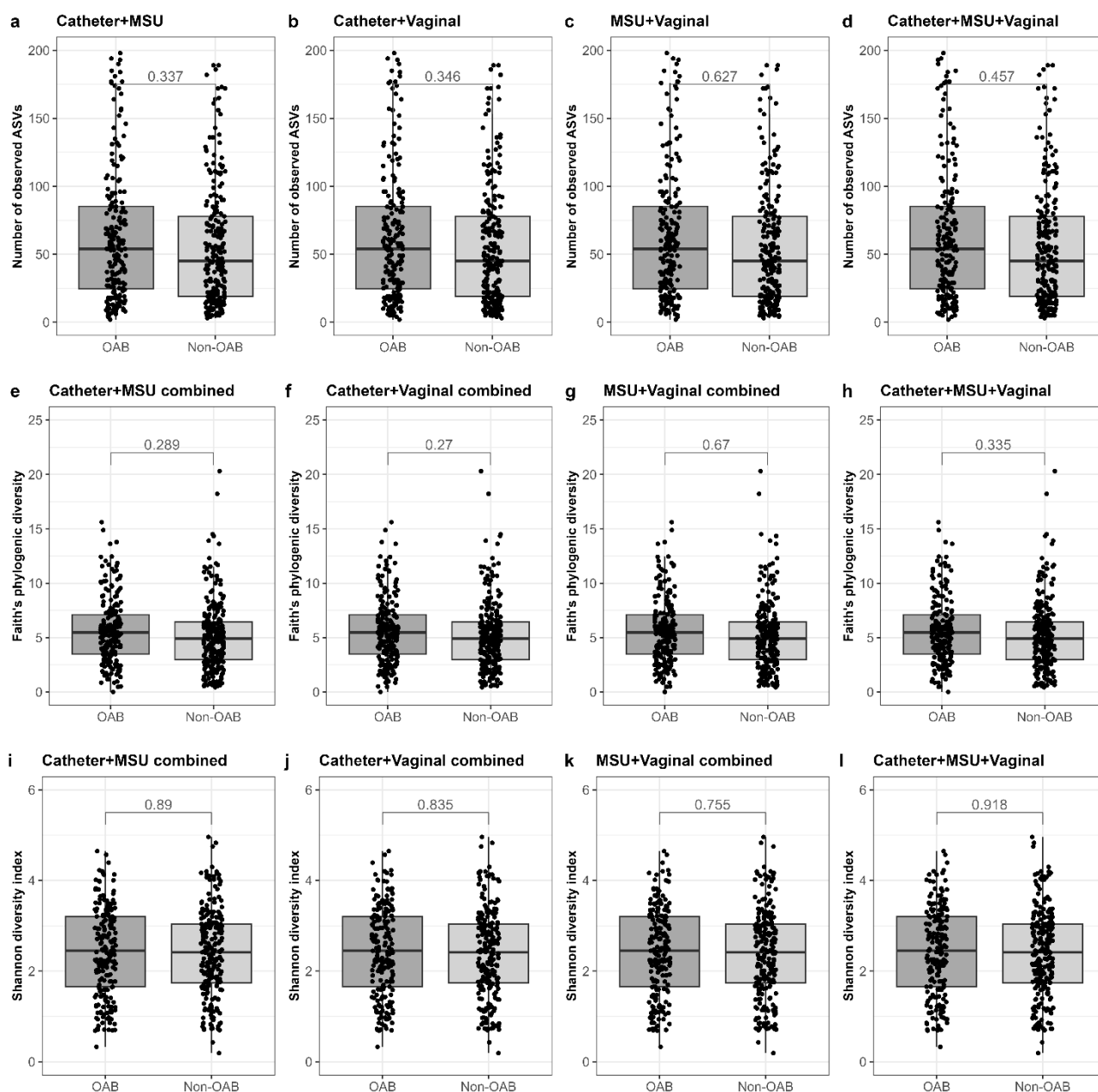
Supplementary Table S3. Comparison of fluid intake and urinary voiding information between women with and without OAB who delivered a midstream urine (MSU) sample. Two women only fulfilled the diary for two days, and the parameters were averaged for these days.



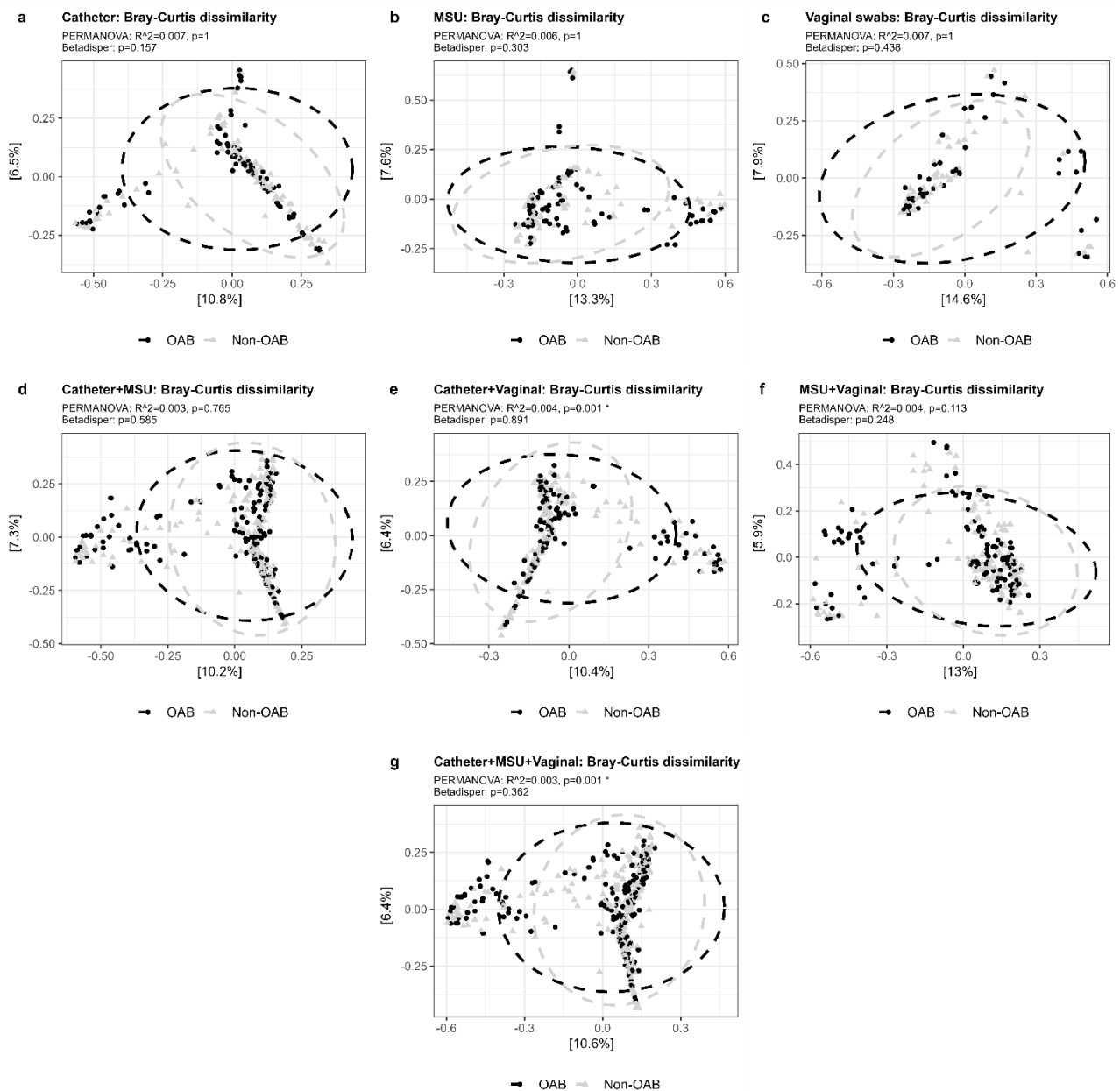
Supplementary Fig. S2. Quality assessment of sequencing run. a-c) Number of reads for the individual samples in a) catheter samples, b) Midstream urine (MSU) samples, and c) vaginal swabs compared to their respective negative controls. Controls consist of location controls, DNA extraction controls, and sterile, nuclease-free water. d) Rarefaction curve, depicting the number of unique ASVs dependent on sequencing depth, with colors depicting sample type and each line representing a unique participant sample.



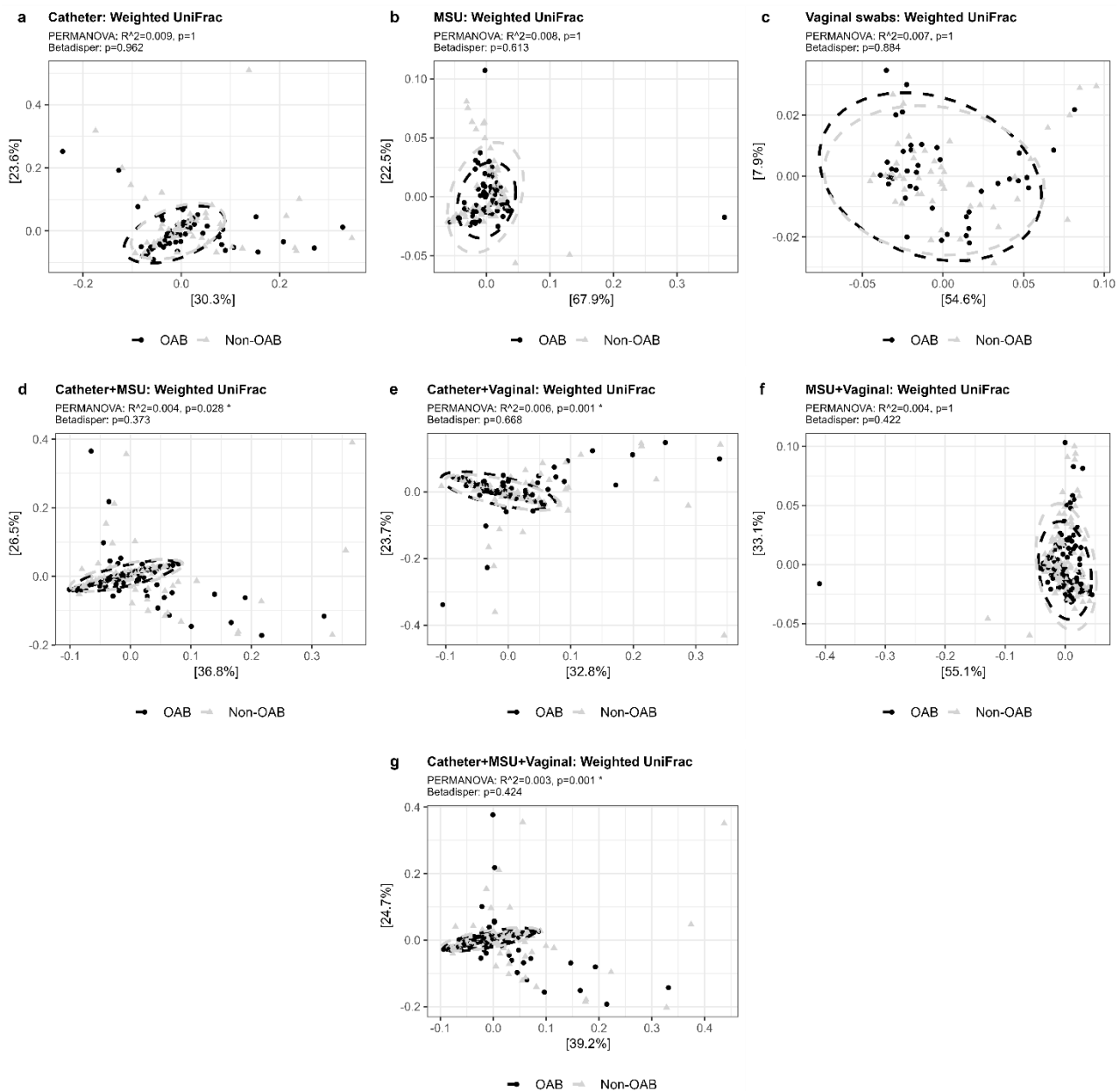
Supplementary Fig. S3. Influence of contaminating bacteria on the relative abundance of individual genera before and after decontamination. Summarized relative abundance of genera in the different negative controls and in samples before and after contaminant removal with the Decontam package for catheter urine (a and b), midstream urine (MSU) (c and d), and vaginal swabs (e and f). Relative abundance is plotted as heatmaps, with the 10 most abundant genera listed. Numbers represent the percentage relative abundance of the genera.



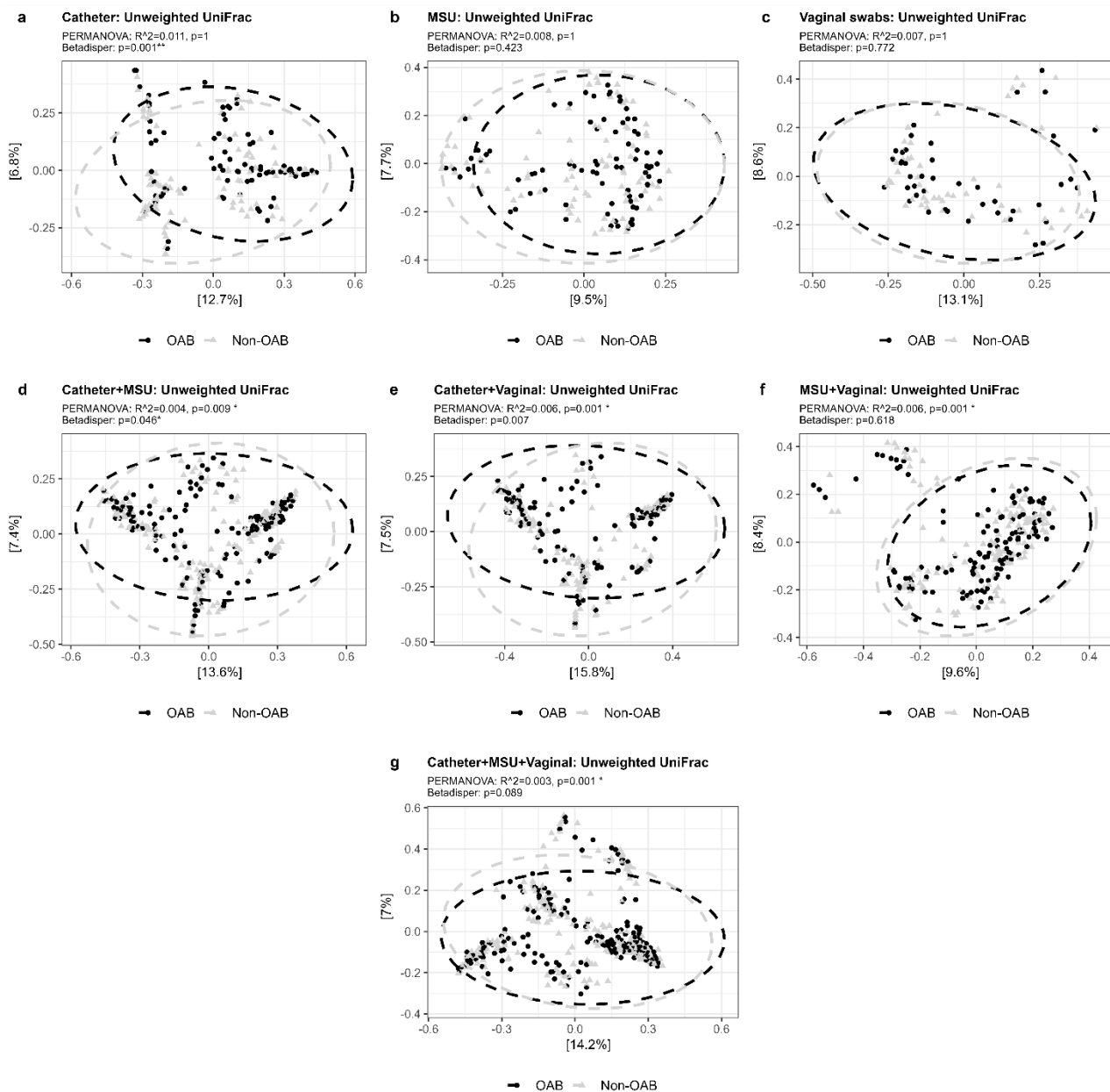
Supplementary Fig. S4. Alpha-diversity utilizing different combinations of the three sample types in accordance with overactive bladder (OAB) status. Comparison of women with and without OAB in the following combinations: Catheter + MSU, Catheter + Vaginal, MSU + Vaginal, Catheter + MSU + Vaginal analyzed by a-d) ASV richness, e-h) Faith's phylogenetic diversity, and i-l) Shannon diversity index.



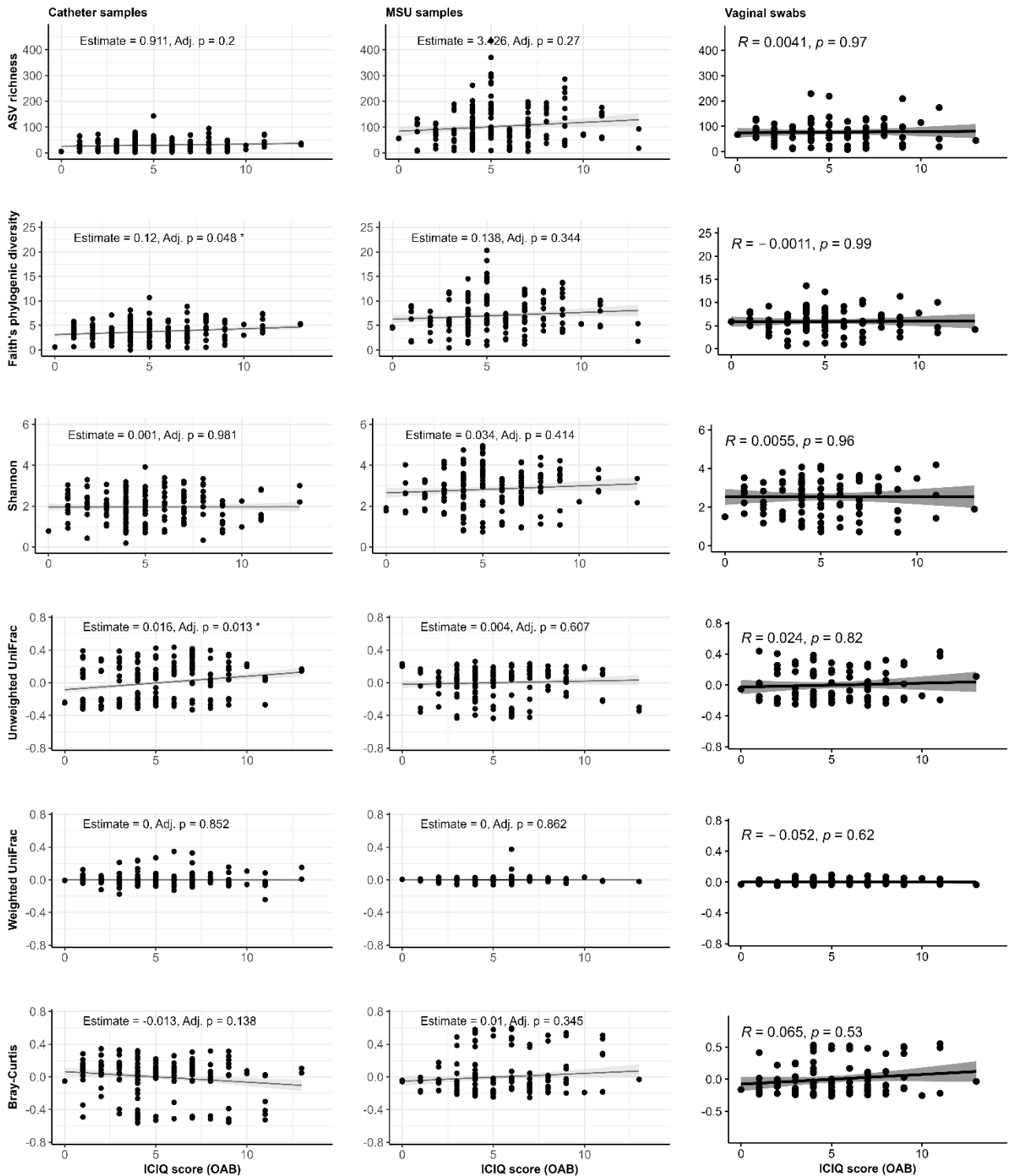
Supplementary Fig. S5. The difference in beta-diversity between women with and without OAB for combinations of sample types: a) Catheter, b) Midstream urine (MSU), c) Vaginal, d) Catheter+MSU, e) Catheter+vaginal, f) MSU+Vaginal, g) Catheter+MSU+Vaginal. The beta-diversity is presented using PCoA with **Bray-Curtis dissimilarities**, with ellipses depicting 95 % confidence intervals. Differences in beta-diversity were tested using PERMANOVA. The results of PERMANOVA are indicated with adjusted p-values and R^2 .



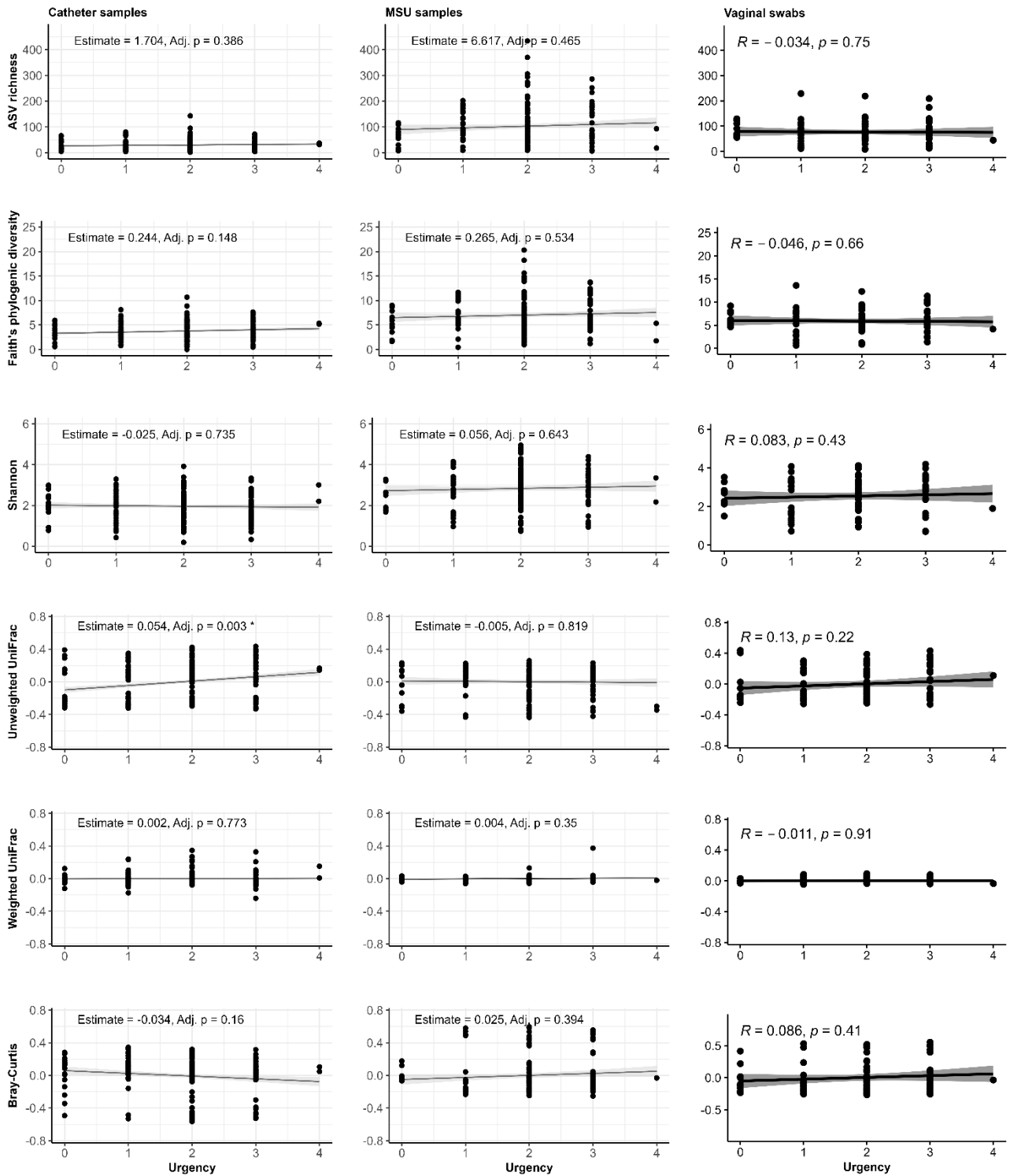
Supplementary Fig. S6. The difference in beta-diversity between women with and without OAB for combinations of sample types: a) Catheter, b) Midstream urine (MSU), c) Vaginal, d) Catheter+MSU, e) Catheter+vaginal, f) MSU+Vaginal, g) Catheter+MSU+Vaginal. The beta-diversity is presented using PCoA with **weighted UniFrac**, with ellipses depicting 95 % confidence intervals. Differences in beta-diversity were tested using PERMANOVA. The results of PERMANOVA are indicated with adjusted p-values and R^2 .



Supplementary Fig. S7. The difference in beta-diversity between women with and without OAB for combinations of samples: a) Catheter, b) Midstream urine (MSU), c) Vaginal, d) Catheter+MSU, e) Catheter+vaginal, f) MSU+Vaginal, g) Catheter+MSU+Vaginal. The beta-diversity is presented using PCoA with **unweighted UniFrac**, with ellipses depicting 95 % confidence intervals. Differences in beta diversity were tested using PERMANOVA. The results of PERMANOVA are indicated with adjusted p-values and R^2 .



Supplementary Fig. S8. Correlations between ICIQ-OAB score and alpha- and beta-diversity in catheter, midstream urine (MSU), and vaginal swab samples. The ICIQ-OAB score, ranging from 0-16, reflects the severity of OAB symptoms, with a higher score indicating greater severity. ICIQ-OAB: International Consultation on Incontinence Questionnaire – Overactive Bladder.



Supplementary Fig. S9. Correlations between urgency and alpha- and beta-diversity in catheter, midstream urine (MSU), and vaginal swab samples. The numbers 0-4 represent urgency responses of "Never", "Occasionally", "Sometimes", "Most of the time", and "All of the time", respectively. MSU: Midstream urine.

Overactive bladder ICIQ-OAB 08/04

Many people experience urinary symptoms some of the time. We are trying to find out how many people experience urinary symptoms, and how much they bother them. We would be grateful if you could answer the following questions, thinking about how you have been, on average, over the PAST FOUR WEEKS.

1. Please write in your date of birth:

Day Month Year

3a. *How often do you pass urine during the day?*

Every hour	☐	3
Every second hour	☐	2
Every third hour	☐	1
Every fourth hour or more	☐	0

3. *How much does this bother you?*

Please ring a number between 0 (not at all) and 10 (a great deal)

0	1	2	3	4	5	6	7	8	9	10
not at all										a great deal

4a. *During the night, how many times do you have to get up to urinate, on average?*

None	☐	0
One time	☐	1
Two times	☐	2
Three times	☐	3
Four times or more	☐	4

4b. *How much does this bother you?*

Please ring a number between 0 (not at all) and 10 (a great deal)

0	1	2	3	4	5	6	7	8	9	10
not at all										a great deal

5a. *Do you have to rush to the toilet to urinate?*

Never	☐	0
Occasionally	☐	1
Sometimes	☐	2
Most of the time	☐	3
All of the time	☐	4

5b. *How much does this bother you?*
Please ring a number between 0 (not at all) and 10 (a great deal)

0	1	2	3	4	5	6	7	8	9	10
not at all										a great deal

6a. *Does urine involuntary leak immediately after feeling the urge to urinate?*

Never	☐	0
Once a week or less often	☐	1
Two or three times a week	☐	2
Once daily	☐	3
Several times a day	☐	4
All the time	☐	5

6b. *How much does this bother you?*
Please ring a number between 0 (not at all) and 10 (a great deal)

0	1	2	3	4	5	6	7	8	9	10
not at all										a great deal

Thank you very much for answering these questions.