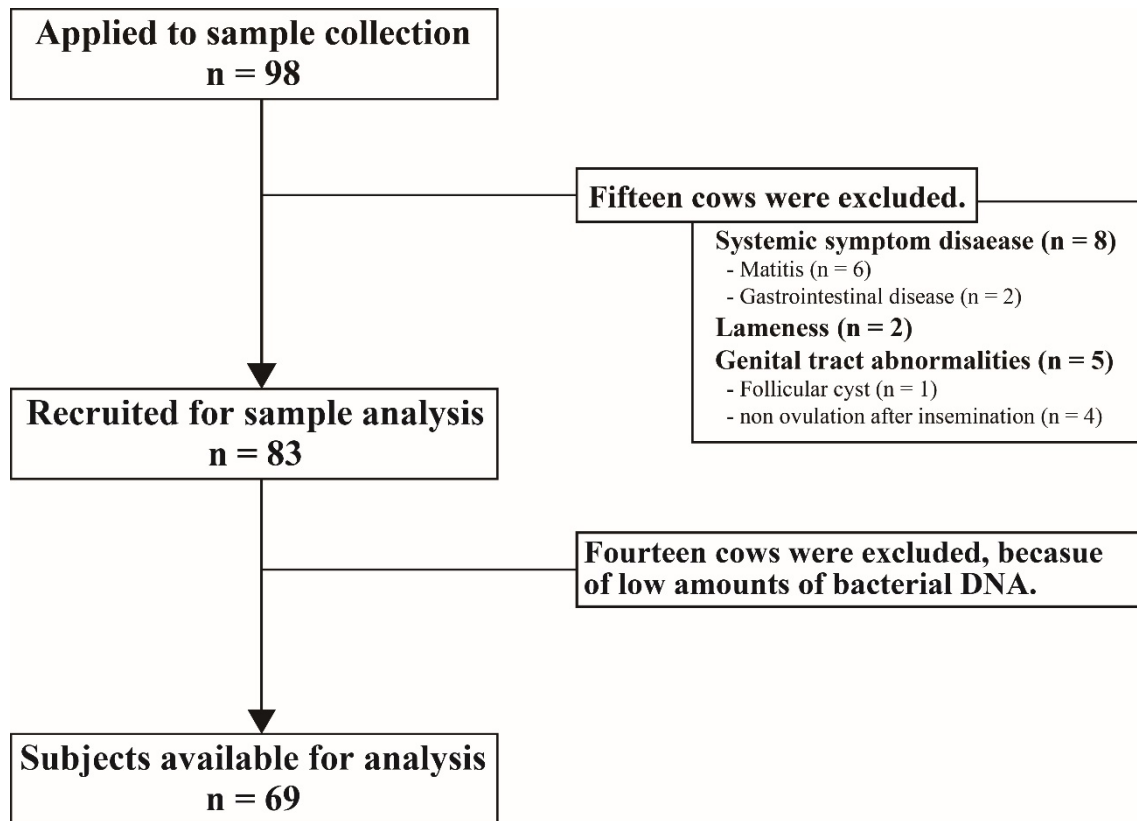
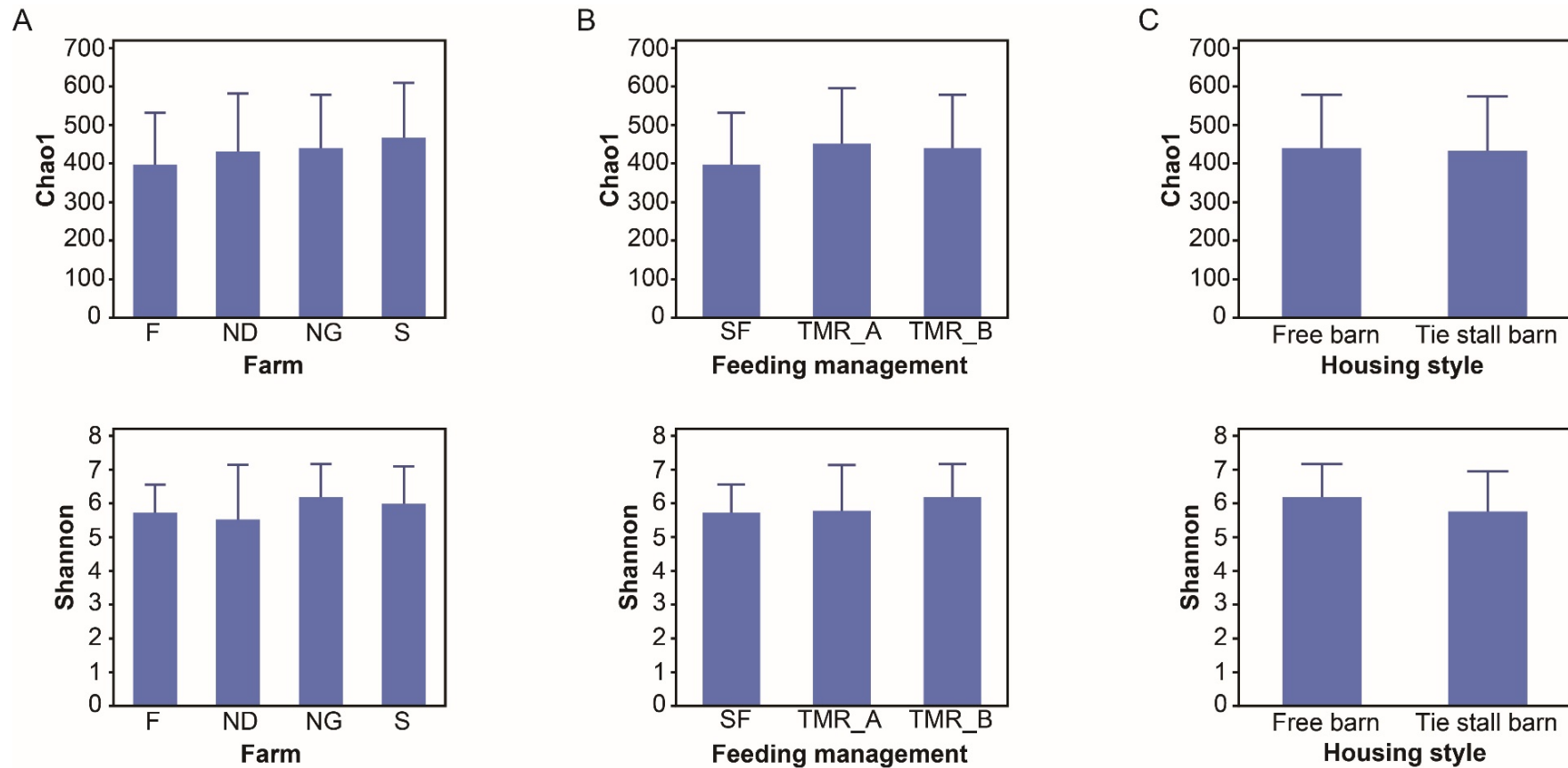
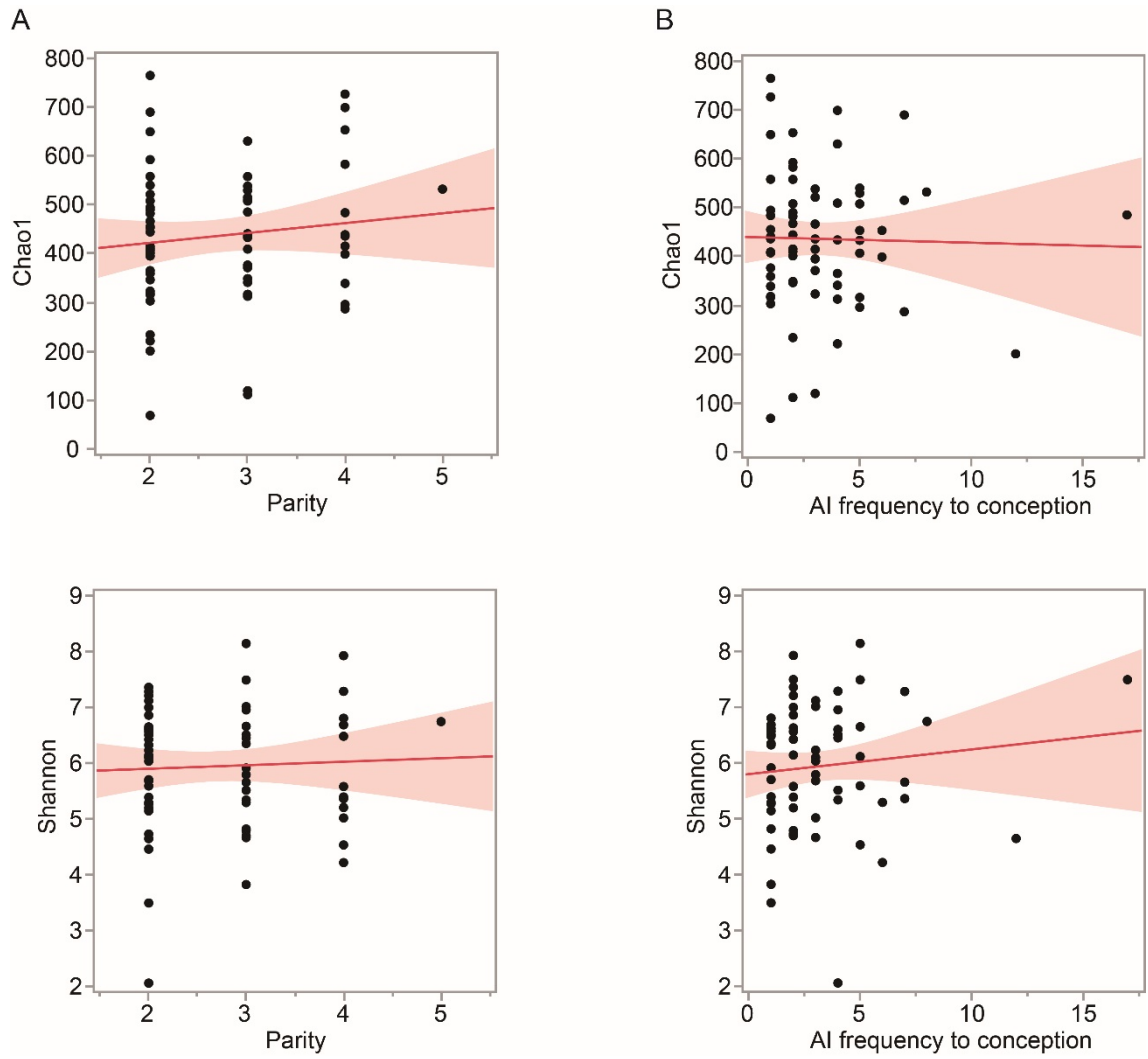




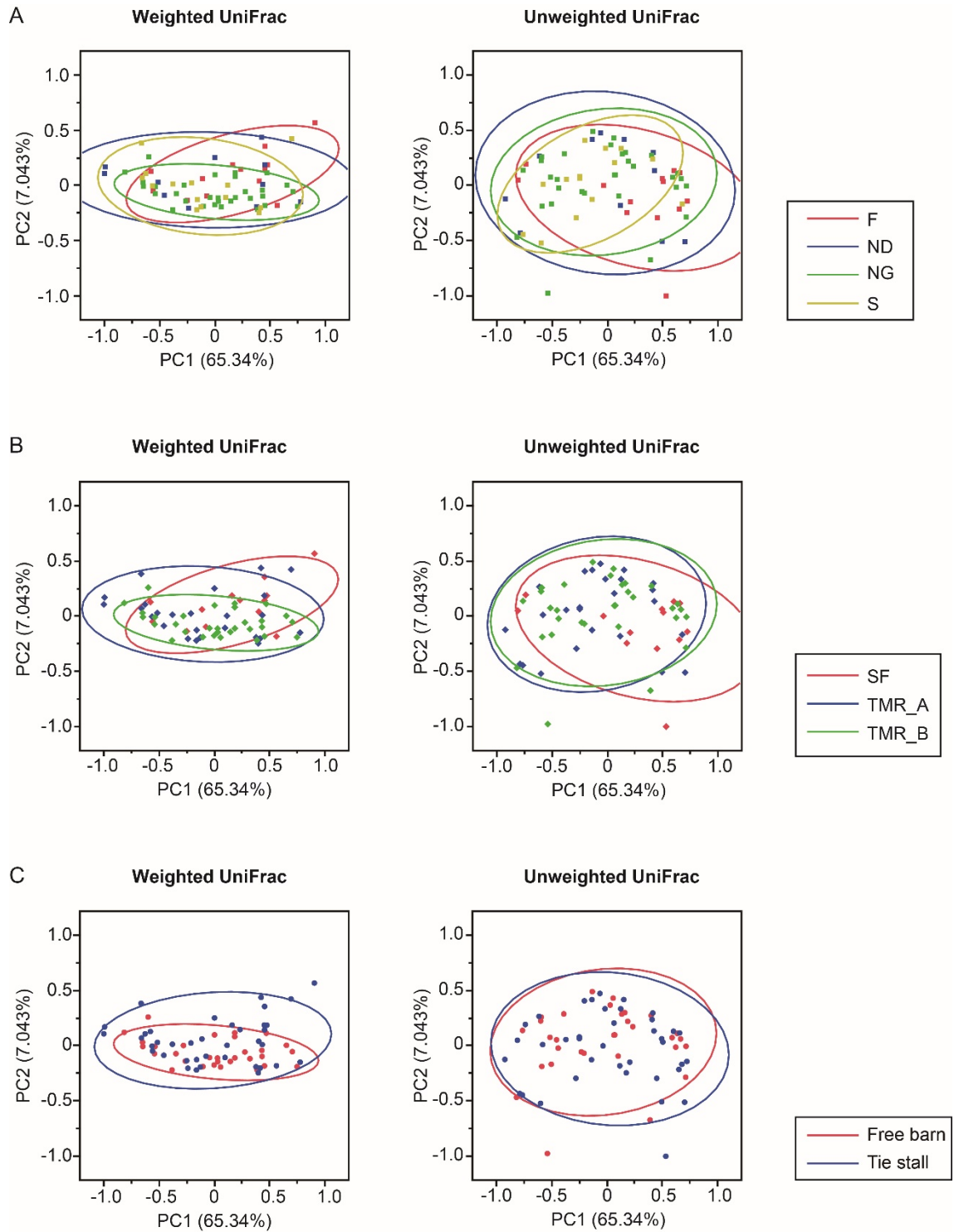
**Fig. S1.** Flow chart of the sample selection procedure. Of all the cows used for sample collection (n = 98), 15 were excluded and 14 were lost during analysis. Sixty-nine samples were investigated in this study.



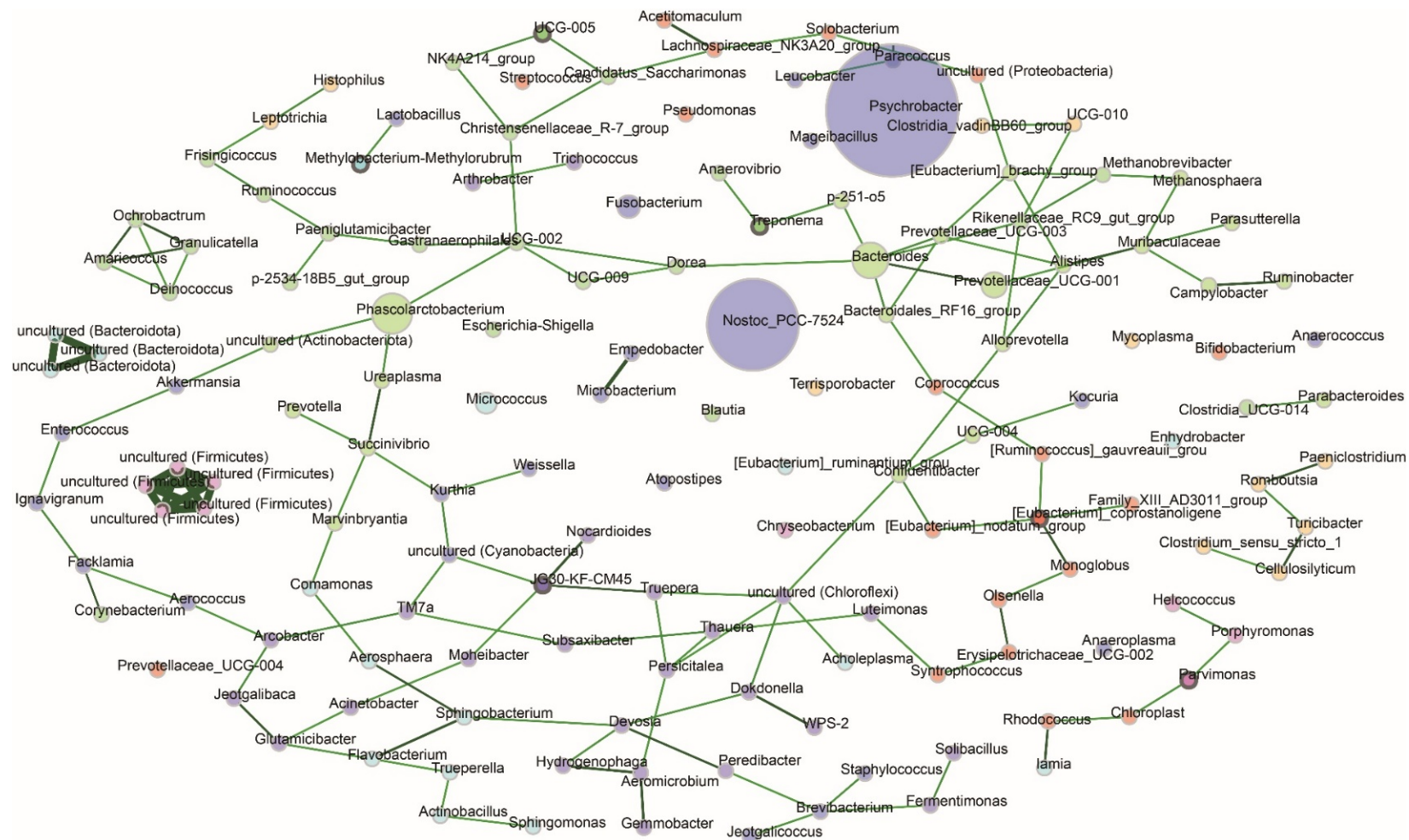
**Fig. S2.** Analysis of the alpha diversity metrics, Chao1 and Shannon, according to (A) farm, (B) feeding management, and (C) housing style. After analysis using the Kruskal–Wallis test for comparison of  $\geq 3$  groups, Tukey’s HSD test was performed. No statistically significant differences were observed among the groups.



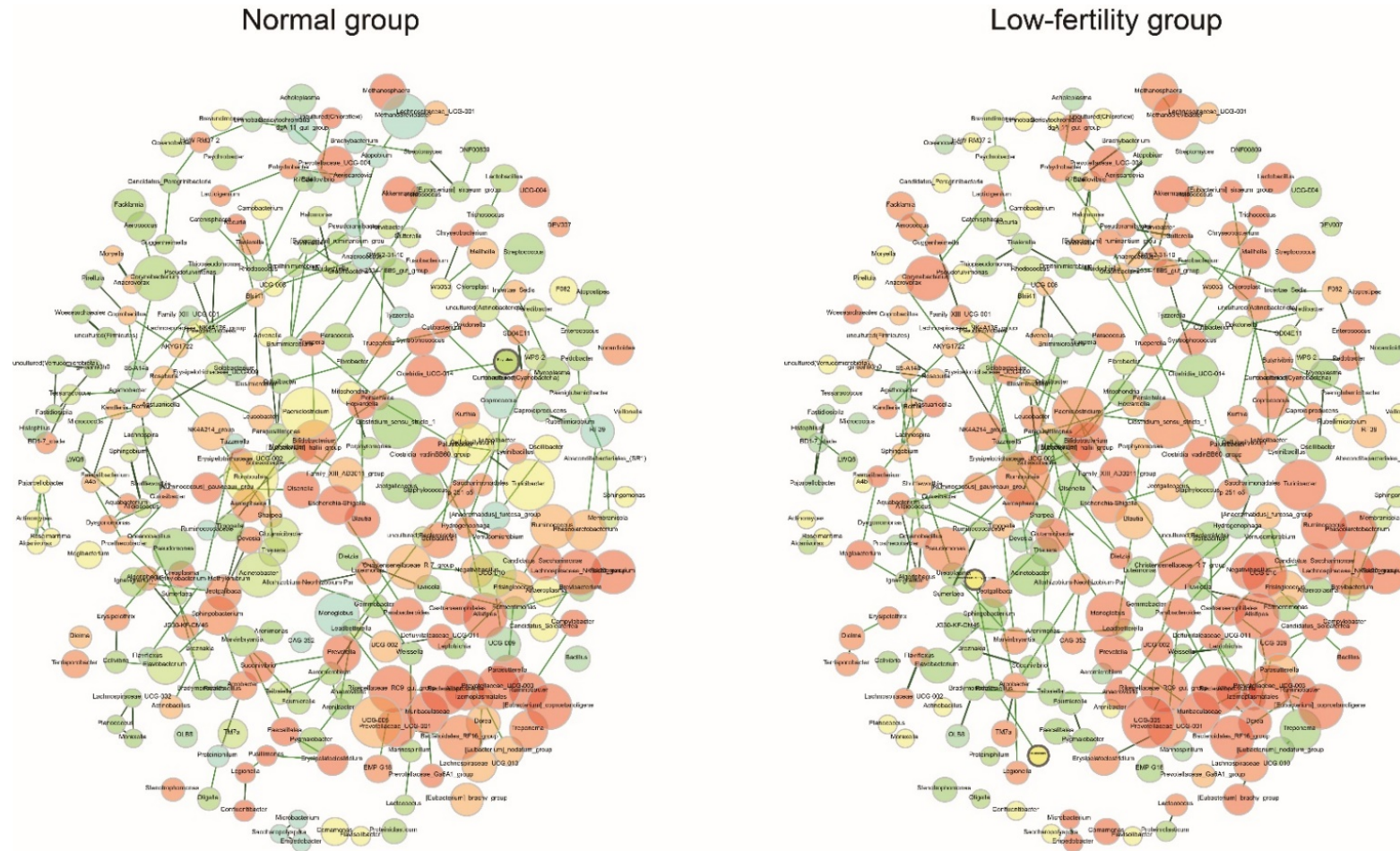
**Fig. S3.** Scatter plots of the alpha diversity metrics, Chao1 and Shannon, according to (A) parity and (B) AI frequency to conception. The linear lines are fitted to the plot graph. The red areas are the reliability of the fitted lines at 99%.



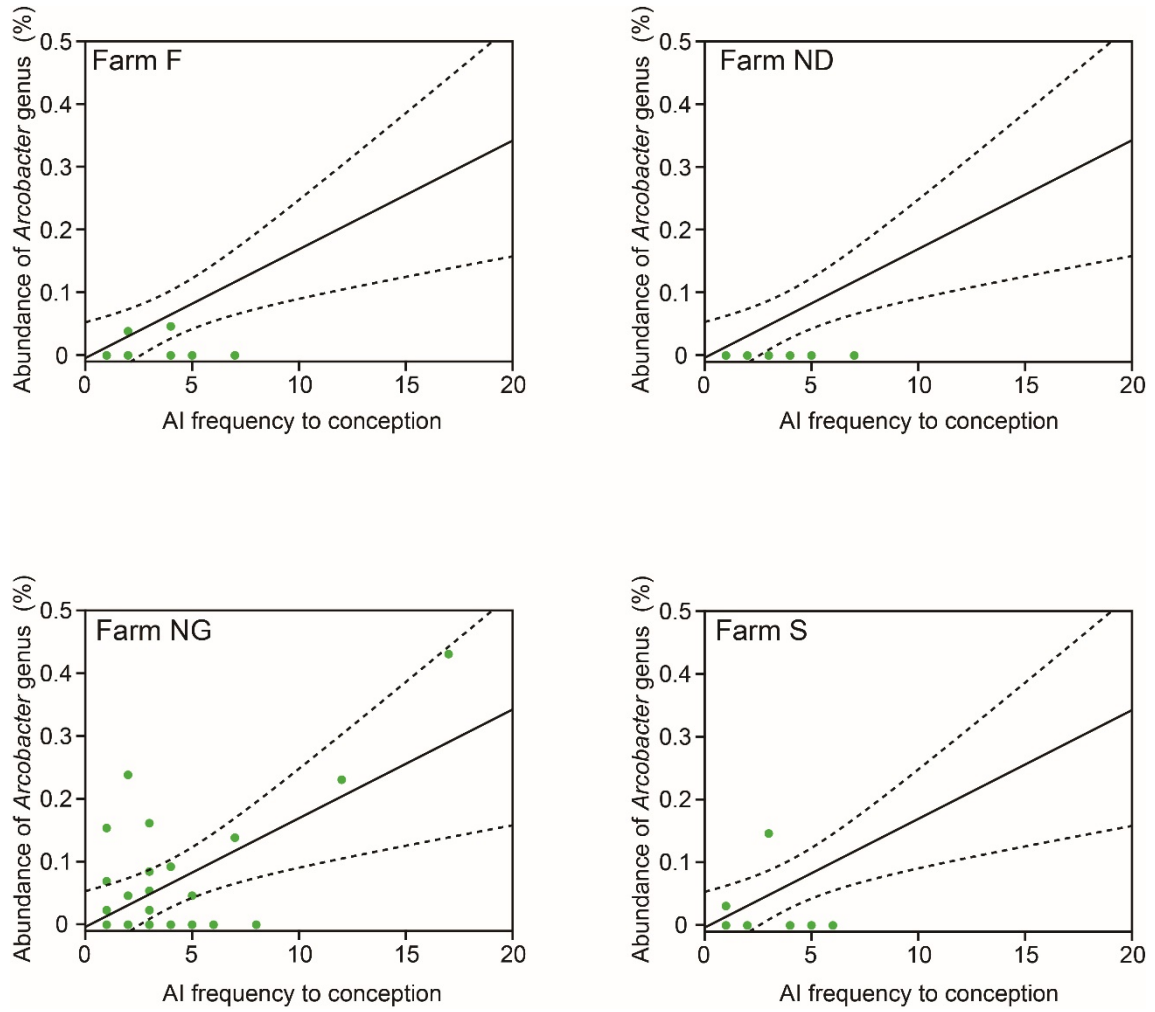
**Fig. S4.** Principal coordinate analysis of weighted and unweighted UniFrac. (A) Farm. (B) Feeding management. (C) Housing style. The ellipses of probability (90%) are shown on the graphs. In the rightmost boxes, the groups are indicated using different colors.



**Fig. S5.** Microbial co-occurrence network of the uterine microbiota. The network was constructed using the 150 taxonomical units with the highest variance. The cluster was inferred using the Louvain method. The network with the 150 edges with the highest weights is shown. Different node colors represent the clusters. Positively associated edges are shown in green lines in the network.



**Fig. S6.** Microbial co-occurrence network of the uterine microbiota of the normal and low-fertility groups. Each network was constructed using the 300 taxonomical units with the highest variance. The cluster was inferred using the Louvain method. The network with the 200 edges with the highest weights is shown. Different node colors represent the clusters. Positively associated edges are shown in green lines in the network. The networks of the normal and low-fertility groups are shown on the left and right, respectively.



**Fig. S7.** Relationship between *Arcobacter* abundance and AI frequency to conception in the four farms. The regression line with 99% reliability in farm NG (left bottom) was fitted to the scatter plots of farms F, ND, and S. The regression line with 99% reliability is indicated by a straight black line with an area sandwiched with two dotted black lines.

**Table S1.** Correlation analysis between alpha and beta diversity and parity and AI frequency to conception.

|                                    | Parity                                             |                | AI frequency to conception                         |                |
|------------------------------------|----------------------------------------------------|----------------|----------------------------------------------------|----------------|
|                                    | Spearman's rank correlation coefficient ( $\rho$ ) | <i>P</i> value | Spearman's rank correlation coefficient ( $\rho$ ) | <i>P</i> value |
| <i>Alpha diversity</i>             |                                                    |                |                                                    |                |
| Chao1                              | 0.0723                                             | 0.555          | -0.0044                                            | 0.972          |
| Shannon                            | 0.0174                                             | 0.887          | 0.1317                                             | 0.287          |
| <i>Beta diversity</i> <sup>1</sup> |                                                    |                |                                                    |                |
| Weighted UniFrac                   | -0.0082                                            | 0.828          | 0.0508                                             | 0.256          |
| Unweighted UniFrac                 | -0.0182                                            | 0.689          | 0.0109                                             | 0.824          |

<sup>1</sup>, The data were analyzed by the Mantel test (Spearman's rank correlation and 999 permutations).

**Table S2.** Comparison of weighted UniFrac indices among the two groups with respect to farm, feeding management, and housing style.

| Comparison groups         |     |                | Sample size | Permutations | pseudo-F | P-value | q-value |
|---------------------------|-----|----------------|-------------|--------------|----------|---------|---------|
| <i>Farm</i>               |     |                |             |              |          |         |         |
| F                         | vs. | ND             | 24          | 999          | 1.187222 | 0.274   | 0.3288  |
|                           |     | NG             | 44          | 999          | 2.00908  | 0.125   | 0.3288  |
|                           |     | S              | 27          | 999          | 2.325901 | 0.101   | 0.3288  |
| ND                        | vs. | NG             | 42          | 999          | 1.398976 | 0.231   | 0.3288  |
|                           |     | S              | 25          | 999          | 0.450809 | 0.666   | 0.666   |
| NG                        | vs. | S              | 45          | 999          | 1.508489 | 0.196   | 0.3288  |
| <i>Feeding management</i> |     |                |             |              |          |         |         |
| Separate feeding          | vs. | TMR_A          | 38          | 999          | 2.088741 | 0.132   | 0.144   |
|                           |     | TMR_B          | 44          | 999          | 2.00908  | 0.116   | 0.144   |
| TMR_A                     | vs. | TMR_B          | 56          | 999          | 1.831873 | 0.144   | 0.144   |
| <i>Housing style</i>      |     |                |             |              |          |         |         |
| Free barn                 | vs. | Tie stall barn | 69          | 999          | 1.528385 | 0.196   | 0.196   |

\*, Statistical analysis using a permutational multivariate analysis of variance (PERMANOVA).

**Table S3.** Examination of the statistical method used for correlational analysis using the DAtest software.

| Method            | AUC  | FPR  | FDR  | Power | Score |
|-------------------|------|------|------|-------|-------|
| Quasi-Poisson GLM | 0.89 | 0.03 | 0    | 0.47  | 0.18  |
| Permutation       | 0.76 | 0.04 | 0    | 0.4   | 0.11  |
| Wilcox            | 0.76 | 0.03 | 0    | 0.27  | 0.07  |
| Log t-test        | 0.74 | 0.03 | 0    | 0.2   | 0.05  |
| t-test            | 0.85 | 0.03 | 0    | 0.13  | 0.05  |
| t-test - Rank     | 0.73 | 0.05 | 0    | 0.13  | 0.03  |
| Negbinom GLM      | 0.88 | 0.17 | 0.65 | 0.73  | -0.37 |
| Poisson GLM       | 0.91 | 0.7  | 0.9  | 1     | -0.49 |

**Table S4.** Differential abundance analysis of the AI-associated uterine microbiota using a quasi-Poisson generalized linear model.

| Bacterial taxa                                                                                              | (Intercept) | Predictor | P value           | log <sub>2</sub> FC | Adjusted P value |
|-------------------------------------------------------------------------------------------------------------|-------------|-----------|-------------------|---------------------|------------------|
| d_Bacteria; p_Campilobacterota; c_Campylobacteria; o_Campylobacterales; f_Arcobacteraceae; g_Arcobacter     | 1.17        | 0.17      | $1.5 \times 10^4$ | 0.2428              | 0.03             |
| d_Bacteria; p_Bacteroidota; c_Bacteroidia; o_Flavobacteriales; f_Weeksellaceae; g_Chryseobacterium          | 1.35        | 0.16      | $9.2 \times 10^3$ | 0.2367              | 0.58             |
| d_Bacteria; p_Bacteroidota; c_Bacteroidia; o_Chitinophagales; f_Chitinophagaceae; g-Taibaiella              | 0.55        | 0.14      | $8.6 \times 10^3$ | 0.2040              | 0.58             |
| d_Bacteria; p_Verrucomicrobiota; c_Verrucomicrobiae; o_Verrucomicrobiales; f_Akkermansiaceae; g_Akkermansia | 3.09        | 0.08      | 0.01              | 0.1205              | 0.58             |
| d_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rhodobacterales; f_Rhodobacteraceae; g_Paracoccus    | 2.05        | 0.14      | 0.02              | 0.2059              | 0.66             |
| d_Bacteria; p_Firmicutes; c_Clostridia; o_Oscillospirales; f_Ruminococcaceae; g_Ruminococcus                | 4.65        | 0.06      | 0.03              | 0.0843              | 0.83             |
| d_Bacteria; p_Firmicutes; c_Clostridia; o_Oscillospirales; f_Ruminococcaceae; g_Incertae_Sedis              | 0.70        | 0.11      | 0.03              | 0.1599              | 0.83             |

**Table S5.** Proportions of cows in the four farms to which the *Arcobacter*-hub-model fitted and unfitted.

|                                | Farm         |              |               |              | All farms     |
|--------------------------------|--------------|--------------|---------------|--------------|---------------|
|                                | F            | ND           | NG            | S            |               |
| <b>Cows fitted the model</b>   | 69.2% (9/13) | 54.5% (6/11) | 71.0% (22/31) | 64.3% (9/14) | 66.7% (46/69) |
| Healthy group                  | 61.5% (8/13) | 54.5% (6/11) | 54.8% (17/31) | 64.3% (9/14) | 58.0% (40/69) |
| Low-fertility group            | 7.7% (1/13)  | 0.0% (0/11)  | 16.1% (5/31)  | 0.0% (0/14)  | 8.7% (6/69)   |
| <b>Cows unfitted the model</b> | 30.8% (4/13) | 45.5% (5/11) | 29.0% (9/31)  | 35.7% (5/14) | 33.3% (23/69) |
| Healthy group                  | 0.0% (0/13)  | 18.2% (2/11) | 6.5% (2/31)   | 7.1% (1/14)  | 7.2% (5/69)   |
| Low-fertility group            | 30.8% (4/13) | 27.3% (3/11) | 22.6% (7/31)  | 28.6% (4/14) | 26.1% (18/69) |

The proportions were calculated based on the total number of cows in each farm.