|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Supplemental Table 1.** Statistical analyses of alpha diversity measures. |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
|   |   |   |   |   | ANCOVA | Tukey's post-hoc pairwise comparisons | Paired test |
|   | Lean Intact1 | Lean Neutered1 | Obese Neutered1 | Obese Neutered After Energy Restriction1 | p-value for overall test2 | Lean Intact vs. Lean Neutered3 | Lean Neutered vs. Obese Neutered3 | Lean Intact vs.ObeseNeutered3 | Obese Before vs. Obese After Energy Restriction4 |
|
| Faith's PD | 13.46 ± 1.05 | 11.87 ± 1.12 | 13.31 ± 0.76 | 13.62 ± 0.89 | ***0.008*** | ***0.015*** | **0.02** | 0.975 | 0.493 |
| Chao | 434.73 ± 44.78 | 398.32 ± 64.83 | 427.08 ± 41.99 | 413.29 ± 44.88 | 0.179 | 0.206 | 0.28 | 0.969 | 0.644 |
| Shannon | 5.84 ± 0.5 | 5.52 ± 0.69 | 5.77 ± 0.33 | 5.49 ± 0.99 | 0.319 | 0.432 | 0.351 | 0.992 | 0.458 |
| Observed Species | 283.49 ± 27.74 | 254.86 ± 33.08 | 272 ± 17.16 | 273.59 ± 21.98 | 0.083 | 0.083 | 0.218 | 0.823 | 0.907 |
|  |  |  |  |  |  |  |  |  |  |
| 1Values are unadjusted mean ± SEM |  |  |  |  |  |  |  |  |
| 2ANCOVA for group comparisons in mean, adjusted for age and gender as covariates for 1st 3 independent groups (Lean Intact, Lean Neutered, Obese Neutered) |
| 3Tukey's HSD (honest significant difference) test for post-hoc pairwise comparisons on ANCOVA adjusted means |  |
| 4Significance assessed by two-tailed paired Student's T-test.   |  |  |  |  |  |
| n=8 per group |  |  |  |  |  |  |  |  |  |

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| **Supplemental Table 2**. Scaled variable importance measures of Partial Least Squares Discriminant Analysis (PLS-DA) for comparisons made in Figure 2. Bolded italicized values are above the 90th percentile cutoff, indicating their importance for discriminating groups within each model. Scaled variable importance values are not equivalent to percentile rank. |
|  |  |  |  |
|   | Lean Intact vs. Lean Neutered | Lean Neutered vs. Obese Neutered | Obese Before vs. Obese After Energy Restriction |
|
| **Actinobacteria** |   |   |   |
| *Adlercreutzia* | 15.95 | 60.96 | 28.14 |
| *Bifidobacterium* | 45.23 | 43.98 | 58.45 |
| *Bulleidia* | 43.01 | 60.98 | 50.62 |
| *Collinsella* | 15.52 | 7.01 | 35.78 |
| **Bacteroidetes** |   |   |   |
| *Alistipes* | 41.75 | 0 | 36.69 |
| *Bacteroides* | ***100*** | 16.74 | 39.75 |
| *Parabacteroides* | 32.42 | 3.48 | 38.21 |
| *Prevotella* | 38.19 | ***100*** | 47.61 |
| **Firmicutes** |   |   |   |
| *Acidaminococcus* | 59.11 | ***92.11*** | ***100*** |
| *Anaerotruncus* | 52.08 | 38.71 | 40.82 |
| *Bacillus* | 2.54 | 60.44 | ***99.57*** |
| *Blautia* | 39.5 | 66.27 | 46 |
| *Catenibacterium* | 6.97 | 12.15 | 44.73 |
| *Clostridium* | 43.04 | 68.07 | 70.04 |
| *Coprococcus* | 52.01 | 52.37 | 84.52 |
| *Dialister* | 20.59 | 70.93 | 78.49 |
| *Dorea* | 1.61 | 80.03 | ***90.33*** |
| *Eubacterium* | ***84.35*** | 56.24 | 5.41 |
| *Faecalibacterium* | ***86.25*** | 77.26 | 58.2 |
| *Lactobacillus* | 49.46 | 67.98 | 88.84 |
| *Megamonas* | 53.09 | 57.91 | 48.74 |
| *Megasphaera* | 65.7 | 33.75 | 46.01 |
| *Oscillospira* | 29.74 | 4.36 | 19.04 |
| *Phascolarctobacterium* | ***84.36*** | ***92.48*** | ***98.68*** |
| *Roseburia* | 41.97 | 60.29 | 10.96 |
| *Ruminococcus* | 60.5 | 54.46 | 52.99 |
| *Sarcina* | 5.39 | 0.18 | ***98.69*** |
| *Staphylococcus* | 0 | 71.46 | ***94.61*** |
| *Syntrophomonas* | 71.79 | 45.28 | 15.74 |
| **Fusobacteria** |   |   |   |
| *Fusobacterium* | 71.08 | 52.67 | 7.67 |
| **Proteobacteria** |   |   |   |
| *Succinivibrio* | 23.83 | 32.34 | 30.17 |
| *Sutterella* | ***90.4*** | 68.29 | 6.61 |