|  |  |  |  |
| --- | --- | --- | --- |
| Categorical Variables | | | |
| **Variables** | **Levels** | **Count** | **%** |
| **Probiotic treatment** | Yes | 63 | 67.0 |
| No | 31 | 33.0 |
| **Diet** | Formula | 23 | 24.5 |
| Breastmilk | 38 | 40.4 |
| Formula & Breastmilk | 33 | 35.1 |
| **Delivery** | Vaginal | 32 | 34.0 |
| Caesarean | 62 | 66.0 |
| **NEC** | Yes | 5 | 5.3 |
| No | 89 | 94.7 |
| **Sepsis** | Yes | 3 | 3.2 |
| No | 91 | 96.8 |
| **Antenatal antibiotics** | Yes | 52 | 55.3 |
| No | 42 | 44.7 |
| **Neonatal antibiotics** | Yes | 83 | 88.3 |
| No | 11 | 11.7 |
| **Chorioamnionitis** | Yes | 28 | 29.8 |
| No | 66 | 70.2 |
| **Preeclampsia** | Yes | 13 | 13.8 |
| No | 81 | 86.2 |
| **Maternal Diabetes** | Yes | 19 | 20.2 |
| No | 75 | 79.8 |
| **Continuous Variables** | | | |
| **Variable** | **mean/median** | | |
| **Gestational age at birth** | 30.8/30.1 weeks | | |
| **Gestational age at collection** | 36.0/36.0 weeks | | |

*Table 1. Overview of the demographic data for the preterm-infant cohort that underwent 16 rRNA gene amplicon sequencing.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **ID** | **1** | **2** | **3** | **4** | **5** | **6** |
| **Probiotics** | Yes | Yes | Yes | No | No | No |
| **Diet** | Formula | Formula | Formula | Breastmilk | Breastmilk & Formula | Breastmilk |
| **NEC** | No | Yes | No | No | No | No |
| **Sepsis** | No | No | No | No | No | No |
| **Delivery** | Caesarean | Caesarean | Caesarean | Caesarean | Vaginal | Caesarean |
| **Antenatal antibiotics** | Yes | Yes | No | No | No | No |
| **Neonatal antibiotics** | Yes | Yes | Yes | No | No | No |
| **Chorioamnionitis** | No | Yes | Yes | No | No | No |
| **Maternal diabetes** | No | No | No | No | Yes | No |
| **Preeclampsia** | No | No | No | No | No | No |
| **ROP** | No | Yes | Yes | No | No | No |
| **Gestational age at birth** | 26 | 24 | 25 | 34 | 37 | 34 |
| **Gestational age at collection** | 31 | 36 | 35 | 35 | 39 | 35 |

*Table 2. Overview of the demographic data for the six preterm-infants who had samples that underwent shotgun metagenomic sequencing.*

Chart, bar chart

Description automatically generated

*Figure 1. Bar plot of relative abundance of taxa at the genus level from the probiotic Infloran.*

*Chart, box and whisker chart

Description automatically generated*

*Figure 2. A: Boxplots comparing the Shannon Index for probiotic-treated and non-treated infants, B: Boxplots comparing the Richness for probiotic-treated and non-treated infants. Annotation for probiotic-treated; PT: non-treated; NT.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *contrast* | *estimate* | *SE* | *z.ratio* | *p.value* |
| Treated – Non-treated | 0.52 | 0.25 | 2.12 | 0.03 |

*Table 3. Tukey’s pairwise comparison from generalized linear mixed effects modelling, comparing probiotic-treated to non-treated infants.*

|  |  |  |
| --- | --- | --- |
| **envfit.** | | |
| *Variable* | *r2* | *p* |
| Gestational\_Age\_at\_Birth | 0.06 | 0.66 |
| **Probitoic\_Treatment** | **0.03** | **0.04** |
| Feeding\_Type | 0.02 | 0.90 |
| NEC | < 0.01 | 0.59 |
| **Sepsis** | **0.33** | **0.04** |
| Mode\_of\_Delivery | 0.02 | 0.53 |
| Neonatal\_Antibiotics | < 0.01 | 0.90 |
| Chorioamnionitis | 0.02 | 0.59 |
| Preeclampsia | < 0.01 | 0.80 |
| ROP | < 0.01 | 0.74 |
| Batch | < 0.01 | 1.00 |
| Diabetes | < 0.01 | 0.77 |
| Antenatal\_Antibiotics | 0.01 | 0.59 |

*Table 4. Results (r2 and p value) of the envfit analysis from the vegan package.*

*Chart, bar chart

Description automatically generated*

*Figure 3. Bar chart representing the relative-abundance of Bifidobacterium across samples and between treatment groups, where NICU represents those treated with probiotics and SCN those not treated.*

*Chart

Description automatically generated*

*Figure 4. Bar chart representing the relative-abundance of Lactobacillus across samples and between treatment groups, where NICU represents those treated with probiotics and SCN those not treated.*

*Chart, bar chart

Description automatically generated*

*Figure 5. Bar chart representing the relative-abundance of Bifidobacterium and Lactobacillus across samples collected at > 36 weeks gestation (post probiotic-treatment) in the treatment group.*