

Supplementary Data S1

Table S3. Sequencing and alignment statistics. Per- sample Sequencing statistics. First column: sample identifier; columns 2 – 8: absolute counts and percentages for surviving reads after QIIME2 DADA2 implementation.

| Sample ID | # reads | # filtered | % filtered | #denoised | #merged | % input merged | # non-chimeric | % non-chimeric |
|-----------|---------|------------|------------|-----------|---------|----------------|----------------|----------------|
| GF001 | 231491 | 185240 | 80.02 | 180450 | 162434 | 70.17 | 29364 | 12.68 |
| GF002 | 116476 | 100044 | 85.89 | 96985 | 81764 | 70.2 | 14184 | 12.18 |
| GF003 | 161391 | 92846 | 57.53 | 88966 | 72289 | 44.79 | 14854 | 9.2 |
| GF005 | 114766 | 68667 | 59.83 | 66672 | 57001 | 49.67 | 12334 | 10.75 |
| GF006 | 562794 | 466442 | 82.88 | 462373 | 448227 | 79.64 | 80677 | 14.34 |
| GF007 | 326515 | 244888 | 75 | 237602 | 201279 | 61.64 | 34224 | 10.48 |
| GF008 | 397607 | 324455 | 81.6 | 317400 | 283517 | 71.31 | 39431 | 9.92 |
| GF009 | 333530 | 260659 | 78.15 | 254086 | 227032 | 68.07 | 35659 | 10.69 |
| GF011 | 170922 | 109083 | 63.82 | 105968 | 89256 | 52.22 | 16354 | 9.57 |
| GF013 | 237362 | 152243 | 64.14 | 149908 | 133753 | 56.35 | 19783 | 8.33 |
| GF014 | 261668 | 188878 | 72.18 | 184449 | 165642 | 63.3 | 26937 | 10.29 |
| GF015 | 230576 | 165929 | 71.96 | 159360 | 133221 | 57.78 | 23402 | 10.15 |
| GF016 | 193680 | 152264 | 78.62 | 148534 | 129415 | 66.82 | 20055 | 10.35 |
| GF017 | 114634 | 91437 | 79.76 | 89322 | 78433 | 68.42 | 14261 | 12.44 |
| GF018 | 308471 | 206063 | 66.8 | 201720 | 179528 | 58.2 | 24067 | 7.8 |
| GF019 | 115357 | 70592 | 61.19 | 68056 | 57458 | 49.81 | 11893 | 10.31 |
| GF020 | 160711 | 131920 | 82.09 | 127230 | 107228 | 66.72 | 15528 | 9.66 |
| GF023 | 529083 | 396583 | 74.96 | 386683 | 340346 | 64.33 | 46300 | 8.75 |
| GF024 | 230965 | 175989 | 76.2 | 172092 | 152201 | 65.9 | 19051 | 8.25 |
| GF025 | 162860 | 100236 | 61.55 | 98024 | 87500 | 53.73 | 17937 | 11.01 |
| GF026 | 218906 | 182049 | 83.16 | 179875 | 167673 | 76.6 | 18952 | 8.66 |
| GF027 | 143433 | 113873 | 79.39 | 110143 | 92647 | 64.59 | 15974 | 11.14 |
| GF028 | 94414 | 61405 | 65.04 | 60290 | 54662 | 57.9 | 11210 | 11.87 |
| GF029 | 132150 | 85591 | 64.77 | 83411 | 69582 | 52.65 | 13896 | 10.52 |
| GF031 | 179228 | 114629 | 63.96 | 110840 | 92695 | 51.72 | 18549 | 10.35 |
| GF032 | 132171 | 112612 | 85.2 | 108874 | 89634 | 67.82 | 10717 | 8.11 |
| GF033 | 100392 | 60239 | 60 | 58222 | 47369 | 47.18 | 12359 | 12.31 |
| GF037 | 132711 | 114604 | 86.36 | 111251 | 94933 | 71.53 | 11830 | 8.91 |
| GF040 | 76093 | 56656 | 74.46 | 54048 | 44317 | 58.24 | 9451 | 12.42 |
| GF041 | 148500 | 114001 | 76.77 | 110058 | 90977 | 61.26 | 12654 | 8.52 |
| GF42 | 152338 | 66761 | 43.82 | 64666 | 56515 | 37.1 | 10246 | 6.73 |
| GF043 | 118209 | 91221 | 77.17 | 88029 | 74415 | 62.95 | 12185 | 10.31 |
| GF044 | 122886 | 99292 | 80.8 | 96877 | 84394 | 68.68 | 14150 | 11.51 |
| GF048 | 163577 | 130821 | 79.98 | 127298 | 110997 | 67.86 | 13179 | 8.06 |
| GF053 | 277318 | 226460 | 81.66 | 222631 | 200009 | 72.12 | 21170 | 7.63 |
| GF054 | 150487 | 123219 | 81.88 | 119727 | 105211 | 69.91 | 15823 | 10.51 |
| GF060 | 221267 | 159046 | 71.88 | 156271 | 139574 | 63.08 | 20261 | 9.16 |
| GF061 | 108981 | 80233 | 73.62 | 77302 | 63396 | 58.17 | 12543 | 11.51 |
| GF062 | 124553 | 102948 | 82.65 | 99564 | 83780 | 67.26 | 11971 | 9.61 |
| GF063 | 251719 | 172639 | 68.58 | 168425 | 143991 | 57.2 | 19272 | 7.66 |

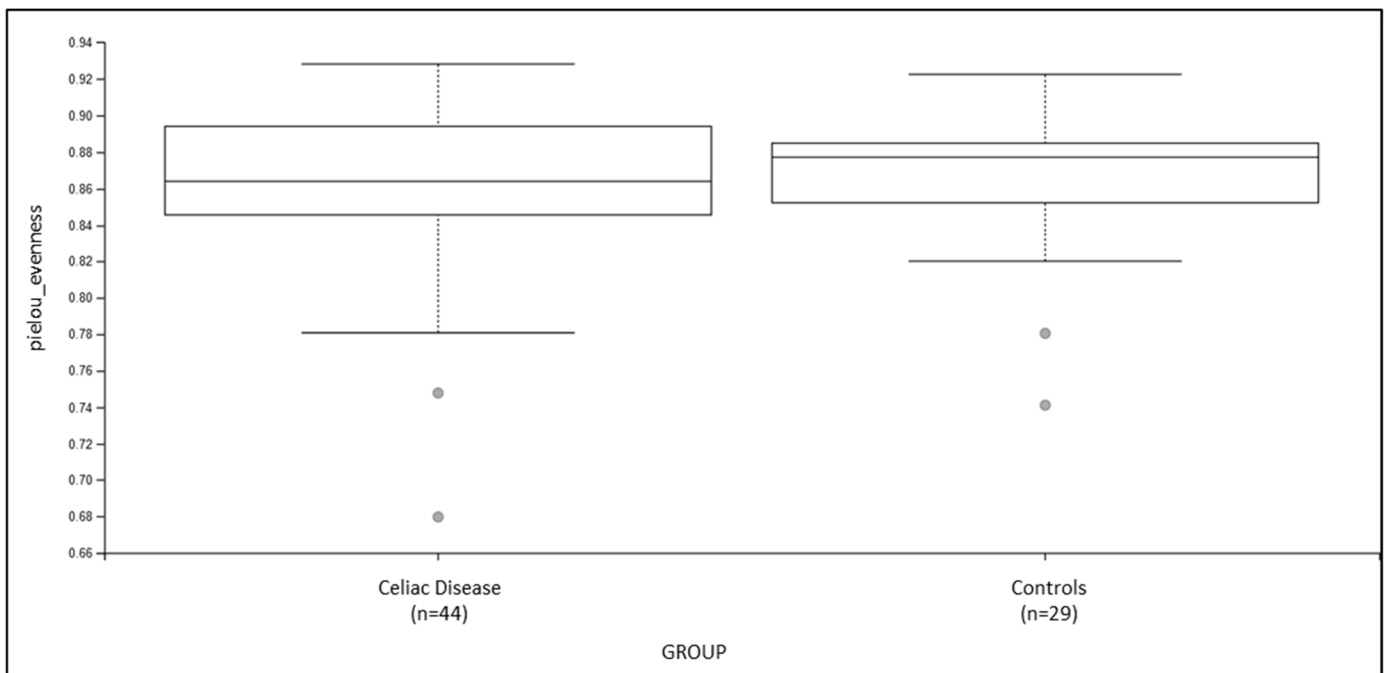
| | | | | | | | | |
|-----------------------------|--------|--------|-------|--------|--------|-------|-------|-------|
| GF100 | 193574 | 137465 | 71.01 | 134793 | 119066 | 61.51 | 20845 | 10.77 |
| GF104 | 242022 | 204730 | 84.59 | 200227 | 172240 | 71.17 | 22828 | 9.43 |
| GF105 | 85311 | 66930 | 78.45 | 64861 | 54781 | 64.21 | 9410 | 11.03 |
| GF106 | 293200 | 229475 | 78.27 | 225622 | 200511 | 68.39 | 26301 | 8.97 |
| GF108 | 166732 | 138265 | 82.93 | 135627 | 122736 | 73.61 | 17572 | 10.54 |
| GF111 | 367450 | 286188 | 77.88 | 278236 | 236417 | 64.34 | 27837 | 7.58 |
| 10C | 115538 | 50658 | 43.85 | 47763 | 36555 | 31.64 | 7386 | 6.39 |
| 11C | 333911 | 148888 | 44.59 | 143856 | 123882 | 37.1 | 21454 | 6.43 |
| 12C | 161464 | 75287 | 46.63 | 74187 | 68880 | 42.66 | 10316 | 6.39 |
| 1C | 221871 | 87400 | 39.39 | 85387 | 74812 | 33.72 | 11885 | 5.36 |
| 2C | 409927 | 193644 | 47.24 | 191101 | 180275 | 43.98 | 31608 | 7.71 |
| 4C | 402377 | 196069 | 48.73 | 190090 | 164806 | 40.96 | 26791 | 6.66 |
| 5C | 292050 | 120821 | 41.37 | 116659 | 96068 | 32.89 | 14975 | 5.13 |
| 6C | 276142 | 111430 | 40.35 | 108799 | 98575 | 35.7 | 18544 | 6.72 |
| 7C | 324335 | 139489 | 43.01 | 135453 | 117216 | 36.14 | 16909 | 5.21 |
| 8C | 541443 | 256702 | 47.41 | 249813 | 216613 | 40.01 | 30584 | 5.65 |
| CON652SGR-06-09-2021 | 193330 | 130727 | 67.62 | 123196 | 93798 | 48.52 | 15729 | 8.14 |
| CON653SGR-20-10-2021 | 218667 | 153358 | 70.13 | 147132 | 118670 | 54.27 | 23230 | 10.62 |
| CON658SGR-06-09-2021 | 183060 | 135549 | 74.05 | 128358 | 102087 | 55.77 | 14598 | 7.97 |
| CON659SGR-06-09-2021 | 238868 | 188386 | 78.87 | 181525 | 152661 | 63.91 | 17866 | 7.48 |
| CON660SGR-08-09-2021 | 159146 | 115942 | 72.85 | 112243 | 90153 | 56.65 | 13693 | 8.6 |
| CON661SGR-06-10-2021 | 208649 | 133871 | 64.16 | 128914 | 105663 | 50.64 | 21426 | 10.27 |
| CON662SGR-06-10-2021 | 109757 | 83858 | 76.4 | 79721 | 60245 | 54.89 | 13221 | 12.05 |
| CON663SGR-06-10-2021 | 268645 | 191306 | 71.21 | 185499 | 158310 | 58.93 | 18426 | 6.86 |
| CON664SGR-07-10-2021 | 122688 | 75899 | 61.86 | 73489 | 62444 | 50.9 | 13775 | 11.23 |
| CON665SGR-08-10-2021 | 172435 | 129878 | 75.32 | 125546 | 103873 | 60.24 | 16773 | 9.73 |
| CON666SGR-13-10-2021 | 167347 | 115243 | 68.86 | 112648 | 100970 | 60.34 | 15717 | 9.39 |
| CON667SGR-14-10-2021 | 124097 | 83706 | 67.45 | 81486 | 68817 | 55.45 | 11746 | 9.47 |
| CON668SGR-30-12-2021 | 246404 | 182035 | 73.88 | 179215 | 165255 | 67.07 | 23999 | 9.74 |
| CON669SGR-30-12-2021 | 202119 | 159507 | 78.92 | 157157 | 144839 | 71.66 | 29815 | 14.75 |
| CON670SGR-30-12-2021 | 260862 | 198693 | 76.17 | 195730 | 180693 | 69.27 | 25709 | 9.86 |
| CON671SGR-30-12-2021 | 302717 | 205184 | 67.78 | 201414 | 181428 | 59.93 | 30546 | 10.09 |
| D01SGR-18-03-2021 | 295998 | 218052 | 73.67 | 211333 | 184354 | 62.28 | 27848 | 9.41 |
| D02SGR-22-04-2021 | 145683 | 101912 | 69.95 | 98894 | 84615 | 58.08 | 15908 | 10.92 |
| D03SGR-22-04-2021 | 197762 | 123744 | 62.57 | 118392 | 92796 | 46.92 | 15489 | 7.83 |
| D04SGR-23-06-2021 | 122531 | 89905 | 73.37 | 86722 | 72313 | 59.02 | 12583 | 10.27 |

Table S4. Feature distribution per sample (“per sample” column) and global feature distribution (“per feature”). The number of high quality sequence count ranges from 7386 to 80677, while feature abundance ranges from 1 to 24426 counts.

| | <i>Per sample</i> | <i>Per feature</i> |
|--------------------------------|-------------------|--------------------|
| <i>Min. frequency</i> | 7386 | 1 |
| <i>1st quartile</i> | 13210 | 2 |
| <i>Median frequency</i> | 16841 | 4 |
| <i>3rd quartile</i> | 23273 | 18 |
| <i>Max. frequency</i> | 80677 | 24426 |
| <i>Mean frequency</i> | 19684 | 89 |

Alpha diversity analysis.

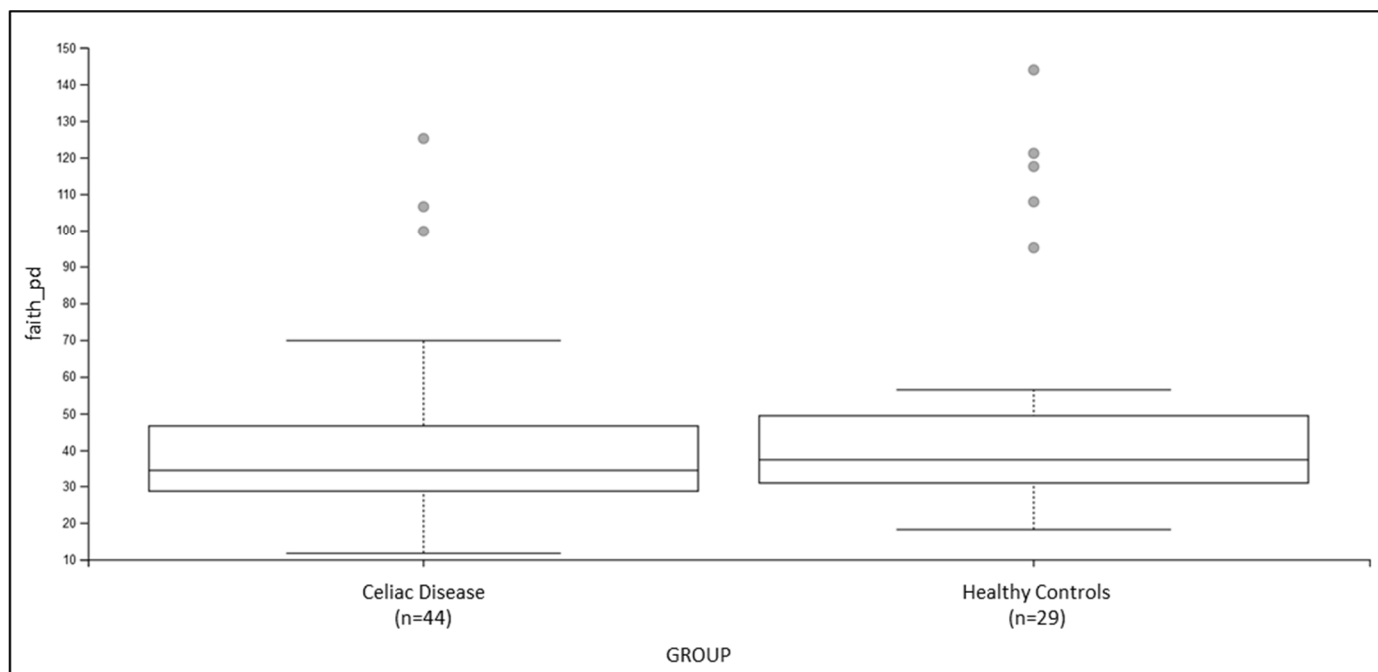
We investigated Alpha diversity by calculating four different measures: Pielou’s evenness (1.4.1), Faith’s Phylogenetic Distance (1.4.2), Number of Observed Features (1.4.3) and Shannon’s entropy (1.4.4). For each index, the following outcomes are provided: boxplot for group-specific index distribution (x-axis: 2 groups, “Celiac Disease” and “Healthy Controls”; y-axis: alpha-diversity index); Kruskal-Wallis test results (H statistics; p-value) for the global comparison of group-specific distribution; Kruskal-Wallis test results for pairwise group comparisons. Boxplot 1.4.4 is also reported in the main text, Figure 1. Analyses were performed through the “QIIME2 Diversity” module, using “10296” as cut-off for reads sampling depth (see main text for further details).



Kruskal-Wallis test (pairwise):

| Group 1 | Group 2 | H | p-value | q-value |
|----------------|------------------|--------|---------|---------|
| Celiac Disease | Healthy_Controls | 0.0996 | 0.75 | 0.75 |

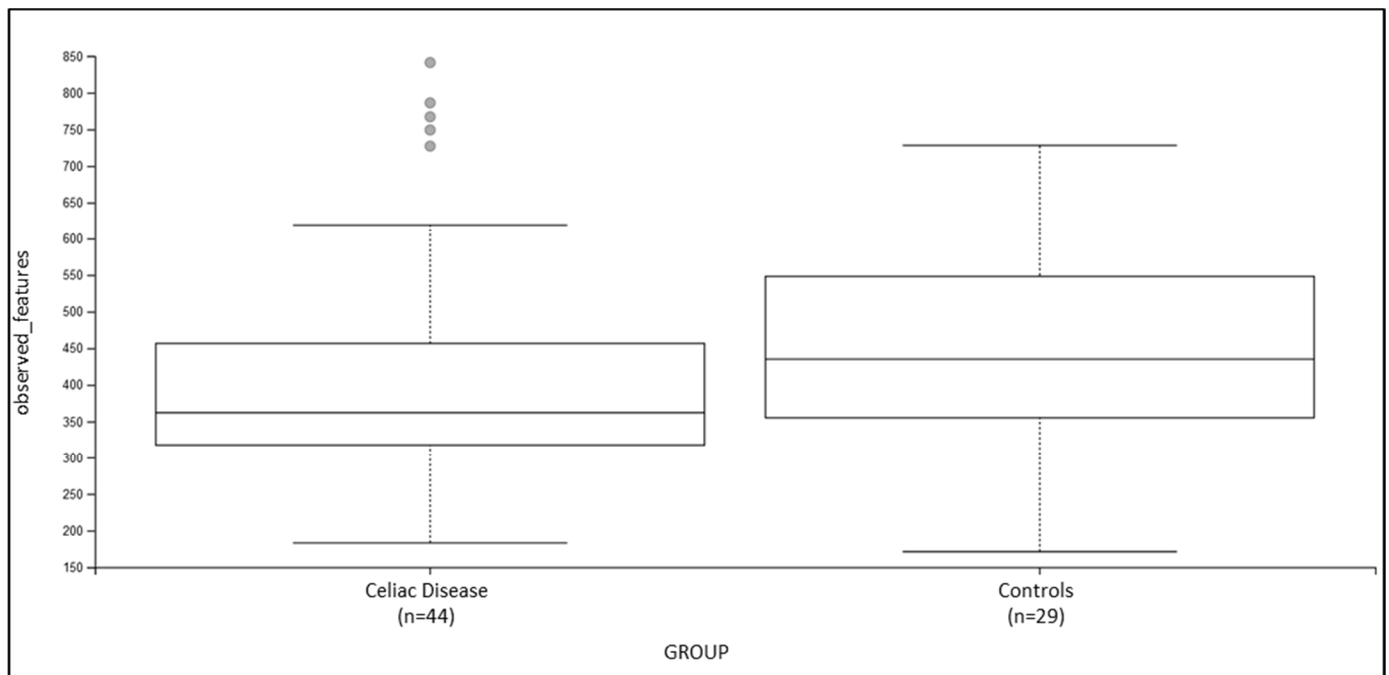
Figure S1. Pielou’s evenness.



Kruskal-Wallis test (pairwise):

| Group 1 | Group 2 | H | p-value | q-value |
|----------------|------------------|-------|---------|---------|
| Celiac Disease | Healthy_Controls | 0.897 | 0.34 | 0.34 |

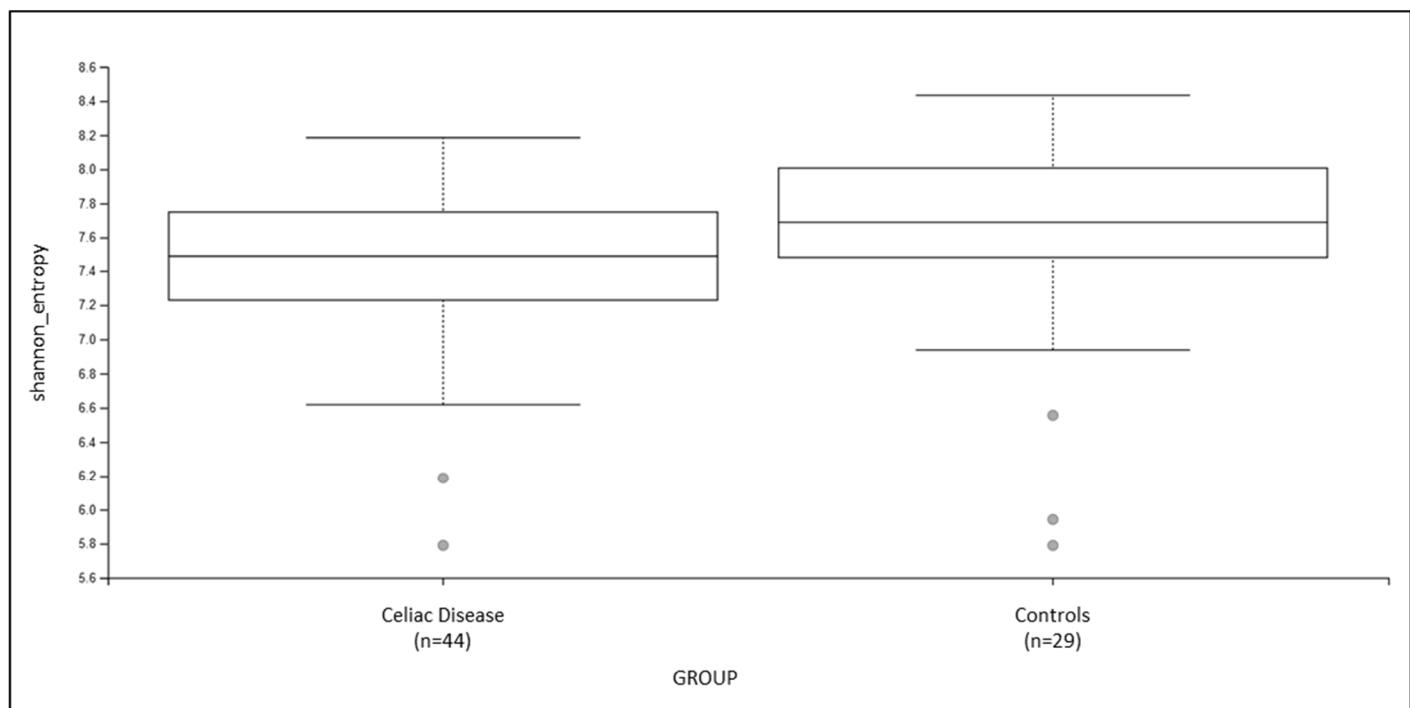
Figure S2. Faith's Phylogenetic Distance.



Kruskal-Wallis test (pairwise):

| Group 1 | Group 2 | H | p-value | q-value |
|----------------|------------------|------|---------|---------|
| Celiac Disease | Healthy Controls | 2.86 | 0.091 | 0.091 |

Figure S3. Number of Observed Features.



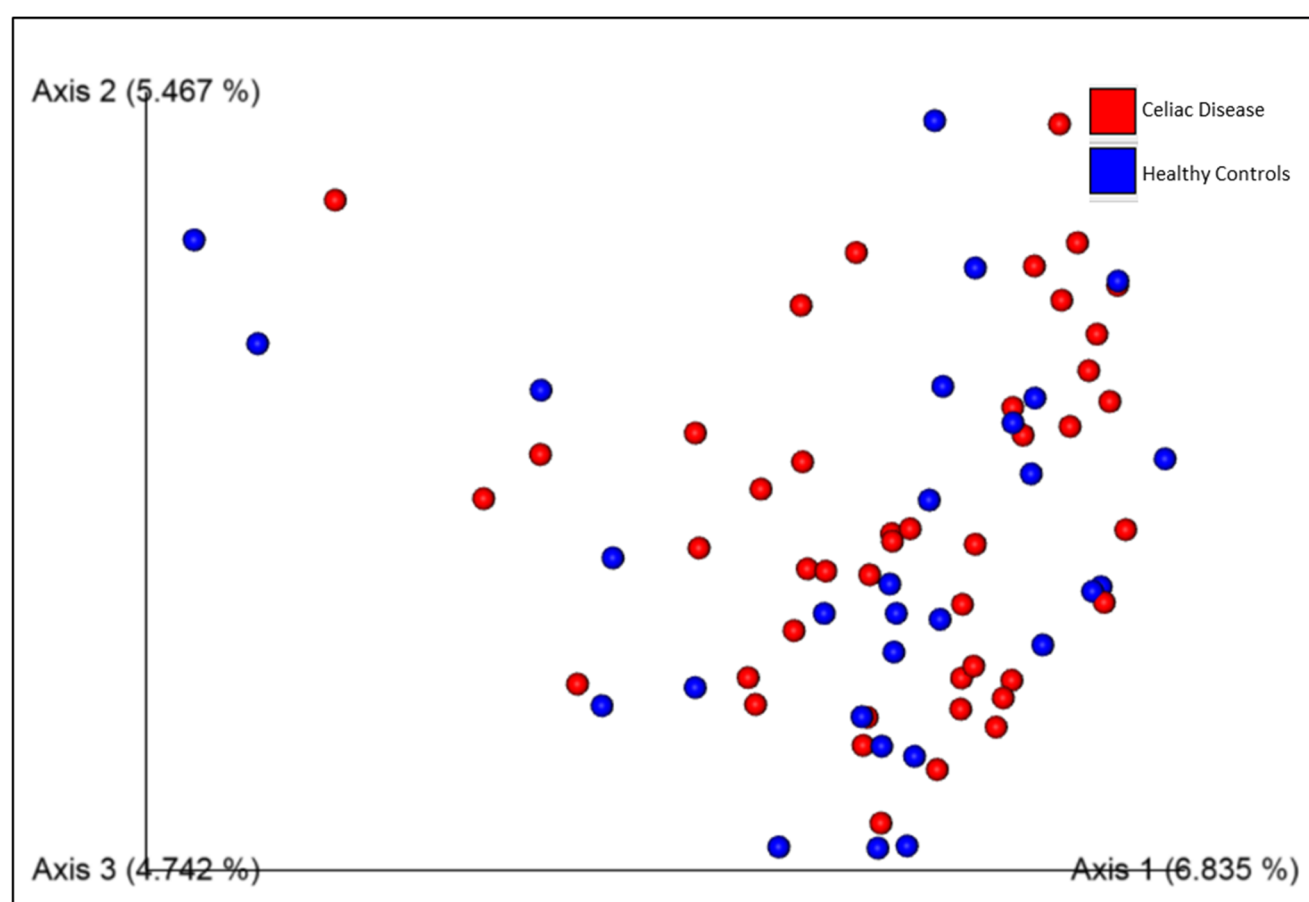
Kruskal-Wallis test (pairwise):

| Group 1 | Group 2 | H | p-value | q-value |
|----------------|------------------|-------|---------|---------|
| Celiac Disease | Healthy_Controls | 3.212 | 0.073 | 0.073 |

Figure S4. Shannon entropy.

Beta diversity analysis.

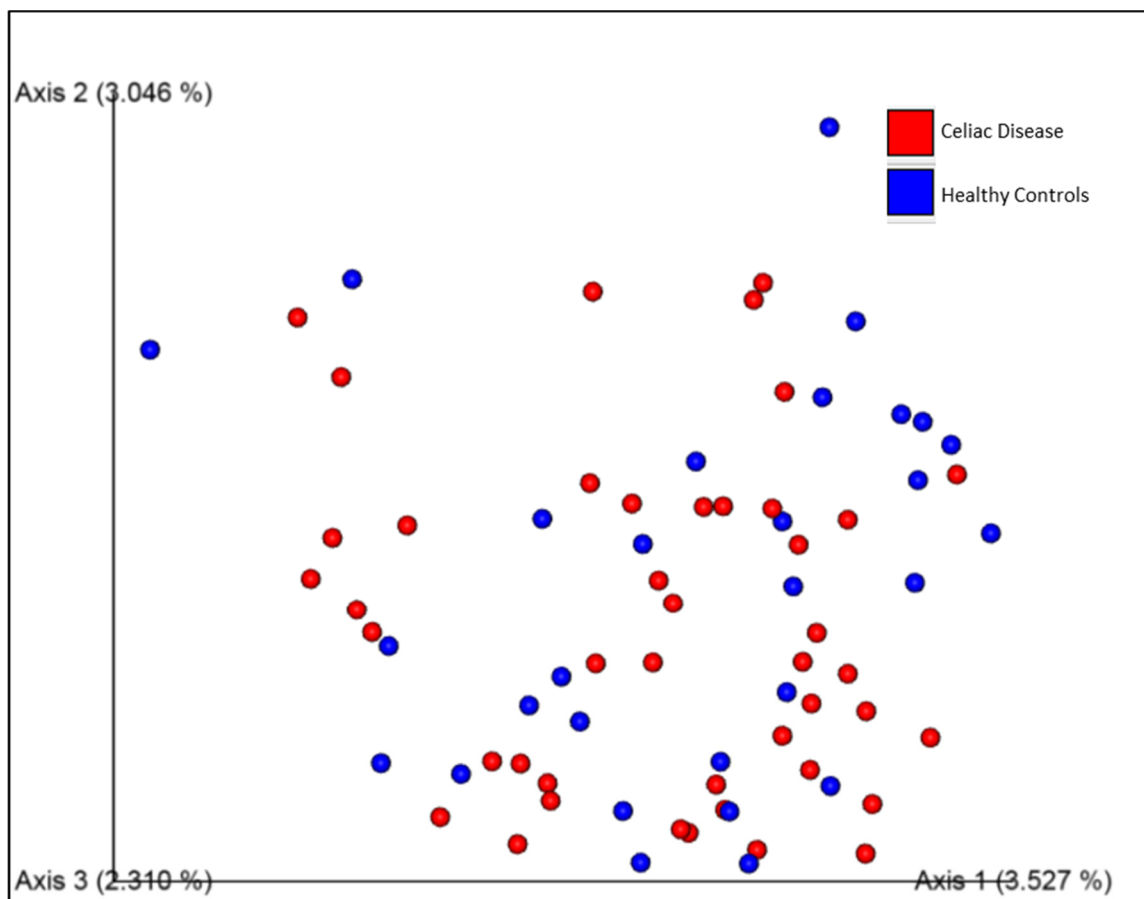
Diversity between the two groups has been evaluated by considering four popular dissimilarity/distance methods: Bray-Curtis (1.5.1), Jaccard (1.5.2), Unweighted and Weighted Unifrac (1.5.3 and 1.5.4). For each method, the following outcomes are provided: a screenshot of three-dimensional Principal Coordinate Analysis (“3D-PCoA”) plot; a table summarizing PERMANOVA test results for pairwise group comparisons. PCoA plots have been obtained by using the Emperor web-application within QIIME2 website (<https://view.qiime2.org/>); red dots indicate “Celiac Disease” samples and blue: “Healthy Controls. Furthermore, each table show details on comparing groups and their size, number of permutations, pseudo-F statistics, p-values and Benjamini-Hochberg adjusted p-value. PERMANOVA verifies the hypothesis that distances among samples with one class group differ from distances of these samples from samples of other groups. PCoA plot obtained by using Unweighted Unifrac distance is also reported in Main Text, as Figure 2.



Kruskal-Wallis test (pairwise)

| Group 1 | Group 2 | Sample size | Permutations | pseudo-F | p-value | q-value |
|----------------|------------------|-------------|--------------|----------|---------|---------|
| Celiac Disease | Healthy Controls | 73 | 999 | 1.561 | 0.005 | 0.005 |

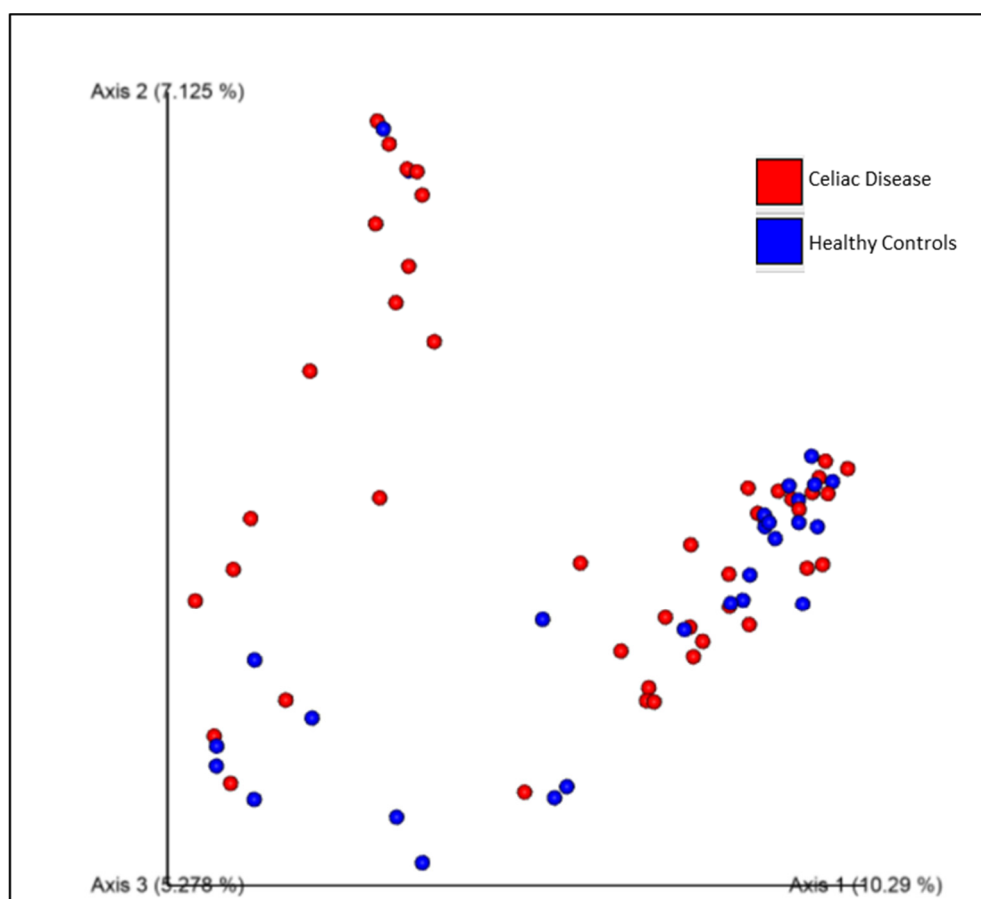
Figure S5. Bray-Curtis.



Kruskal-Wallis test (pairwise)

| Group 1 | Group 2 | Sample size | Permutations | pseudo-F | p-value | q-value |
|----------------|------------------|-------------|--------------|----------|---------|---------|
| Celiac Disease | Healthy Controls | 73 | 999 | 1.190 | 0.004 | 0.004 |

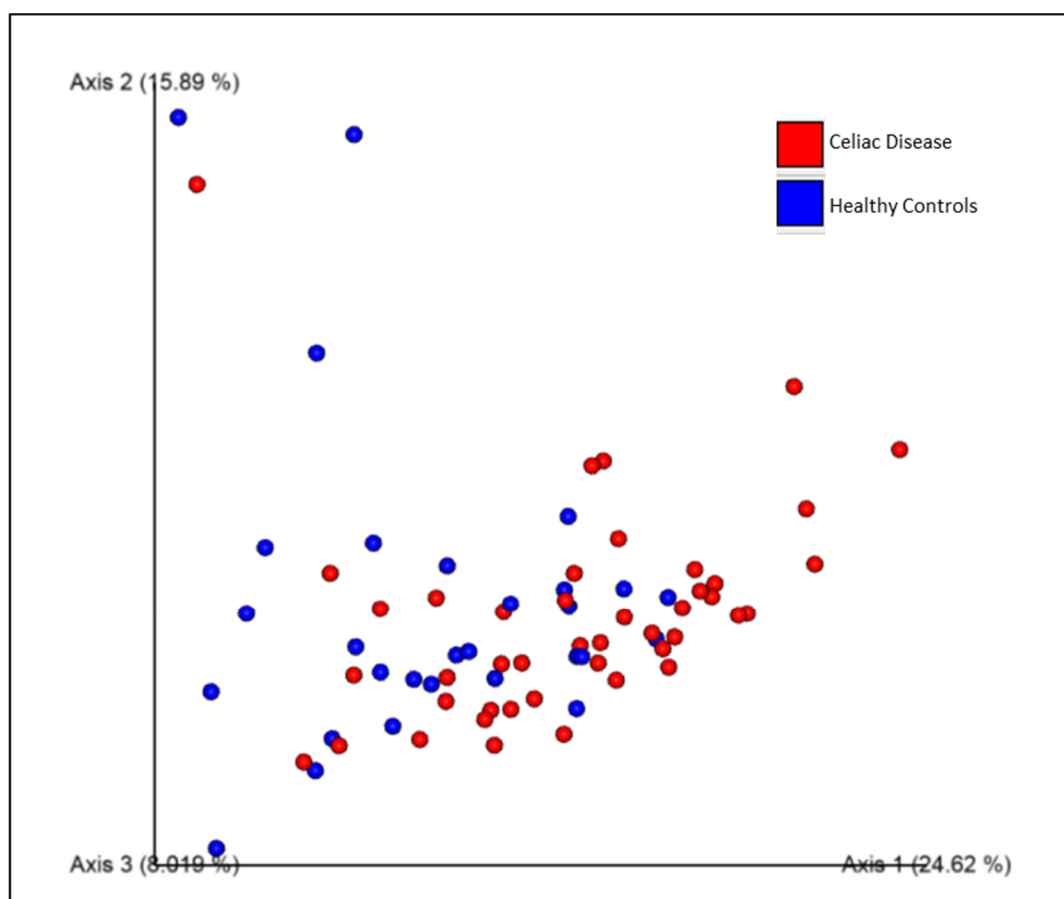
Figure S6. Jaccard.



Kruskal-Wallis test (pairwise)

| Group 1 | Group 2 | Sample size | Permutations | pseudo-F | p-value | q-value |
|----------------|------------------|-------------|--------------|----------|---------|---------|
| Celiac Disease | Healthy Controls | 73 | 999 | 1.286 | 0.064 | 0.064 |

Figure S7. Unweighted Unifrac.



Kruskal-Wallis test (pairwise)

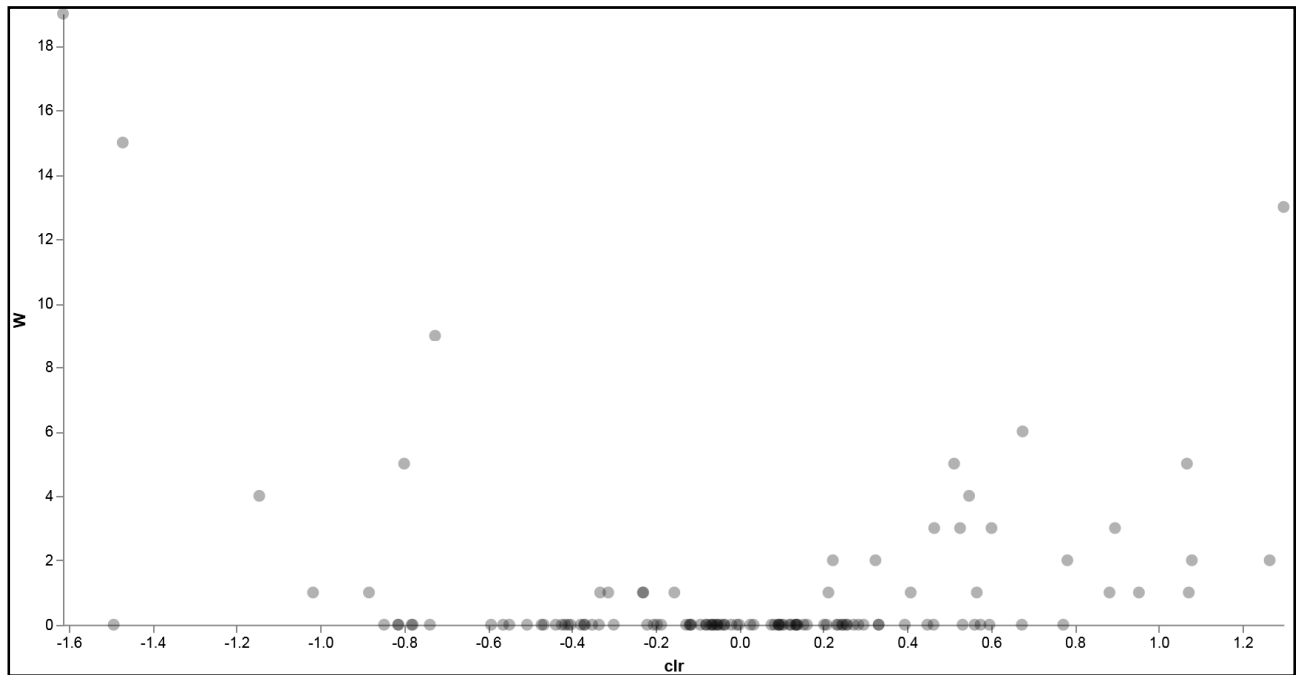
| Group 1 | Group 2 | Sample size | Permutations | pseudo-F | p-value | q-value |
|----------------|------------------|-------------|--------------|----------|---------|---------|
| Celiac Disease | Healthy Controls | 73 | 999 | 4.576 | 0.001 | 0.001 |

Figure S8. Weighted Unifrac.

ANCOM

QIIME2 ANCOM module was used to infer microbial genera or species that are differentially abundant across sample groups. Rare features and mitochondrial/plastid sequences were removed from the feature table (see Methods). Thus, features were collapsed according to QIIME2 taxonomical classification (based on GreenGenes resource) into “species-collapsed” (“level 7”) features, for a total of 118 features. ANCOM Volcano Plot and statistical results table are provided.

Significant features are placed on the top-right corner of the Volcano Plot; the first table shows significant features together with the corresponding W statistics, i.e., the number of sub-hypotheses that have passed for a certain feature (ANCOM compares pairs of feature relative abundances). The second table shows the percentile abundance of relevant features across the sample groups. For example, a value of 100 at 50% percentile indicates that the detected feature has a maximum sequence count of 100 in the 50% of samples of an investigated group.



| Feature | W |
|--|----|
| [A] k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales;f_Bifidobacteriaceae;g_Bifidobacterium;s_longum | 19 |
| [B] k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Coproccoccus;s_eutactus | 15 |
| [C] k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Anaerotruncus;s_ | 13 |

| Percentile | Group | [A] | [B] | [C] |
|------------|------------------|-------|--------|-------|
| W | | 19 | 15 | 13 |
| 0.0 | Celiac Disease | 1.0 | 1.0 | 1.0 |
| 25.0 | Celiac Disease | 1.0 | 1.0 | 1.0 |
| 50.0 | Celiac Disease | 1.0 | 1.0 | 1.0 |
| 75.0 | Celiac Disease | 34.25 | 1.0 | 41.75 |
| 100.0 | Celiac Disease | 165.0 | 146.0 | 129.0 |
| 0.0 | Healthy Controls | 1.0 | 1.0 | 1.0 |
| 25.0 | Healthy Controls | 3.0 | 1.0 | 1.0 |
| 50.0 | Healthy Controls | 47.0 | 1.0 | 1.0 |
| 75.0 | Healthy Controls | 75.75 | 107.75 | 1.0 |
| 100.0 | Healthy Controls | 482.0 | 243.0 | 400.0 |

Figure S9. Results for species-collapsed features ($n = 118$).