Microbial dynamics in newly diagnosed and treatment naïve IBD patients in the Mediterranean

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Supplemental materials and methods

DNA Extraction: DNA was extracted using the QIAamp DNA fast stool mini kit automated on the QIAcube (Qiagen, Hilden, Germany). Material is transferred to 0.70 mm Garnet Bead tubes (Dianova, Hamburg, Germany) filled with 1.1 ml ASL lysis buffer. Bead beating is performed using the a SpeedMill PLUS (Analytik Jena, Jena, Germany) for 45 s at 50 Hz. Samples are then heated to 95°C for 5 min with subsequent continuation of the manufacturer's protocol. DNA binds specifically to the QIAamp silica-gel membrane while contaminants pass through. PCR inhibitors are removed by the combined action of a unique adsorption resin and an optimized buffer. The approximate amount of DNA is between 10-40 µg per sample.

Bacterial 16S rRNA Gene Sequencing: Variable regions V1-V2 of the 16S rRNA gene are amplified using the primer pair 27F-338R in a dual-barcoding approach according to Caporaso *et al.* 2012 [1]. DNA is diluted 1:10 prior PCR, and 3 µl of this dilution are finally used for amplification. PCR-products are verified using the electrophoresis in agarose gel. PCR products are normalized using the SequalPrep Normalization Plate Kit (Thermo Fischer Scientific, Waltham, MA, USA), pooled in an equimolar fashion and sequenced on the Illumina MiSeq v3 2×300 bp (Illumina Inc., San Diego, CA, USA). Demultiplexing after sequencing was based on 0 mismatches in the barcode sequences via *bcl2fastq*.

Quality control, classification, and binning of sequences: Data processing was performed using the *DADA2* version 1.10 [2] workflow for big datasets (https://benjjneb.github.io/dada2/bigdata.html) resulting in abundance tables of **A**mplicon **S**equence **V**ariants (**ASV**s). All sequencing runs were handled separately for error correction, read merging, and combined chimera detection. ASVs underwent taxonomic annotation using the naïve Bayesian classifier implemented in DADA2 using the Ribosomal Database Project 16 release as a taxonomic classification database [3, 4]. ASV sequences were aligned via NAST-alignment to the SILVA core database and filtered for informative sites (constant gaps, constant bases) in *mothur* [5-8]. Phylogenetic tree construction on ASV alignment generated was carried out using *FastTree 2.1* using the CAT substitution model with Γ-correction and improved accuracy, employing more minimum evolution rounds for initial tree search [-spr 4], more exhaustive tree search [-mlacc 2], and a slower initial tree search [-slownni] [9].

Statistical methods for microbiome analyses: Microbiome data was rarefied to 11800 reads/sample to ensure comparable and sufficient coverage across samples during analysis (average Good's coverage 99.88% ± 0.001 SD).

Alpha diversity: Phylogenetic measures of alpha diversity [Nearest Taxon Index (NTI), and Net Relatedness Index (NRI)] were derived using the *picante* package, based on 999 permutations against a null model preserving relative species richness within the communities [10, 11] [$NRI=-1 \times (MPD_{observed}-mean(MPD_{random}))/SD(MPD_{random})$; $NTI=-1 \times (MNTD_{observed}-mean(MNTD_{random}))/SD(MNTD_{random})$]. Both metrics are phylogenetic effect sizes, for which positive values indicate phylogenetic clustering, values close to zero indicate neutral or random community assembly, and negative values indicate phylogenetic overdispersion, either over the whole phylogenetic tree (NRI) or across the closest related species/tips of the phylogenetic tree (NTI).

Network analyses: To generate co-abundance networks we employed the *SparCC* algorithm as implemented in *mothur* (50 samplings, 100 iterations, 10,000 permutations, *P*-value adjustment via

Benjamini-Hochberg procedure) was used to generate co-abundance networks based on ASV abundances within the study cohorts (shared among 10% samples) at a *P*-value cutoff of $P_{FDR} \leq 0.05$ and a correlation strength above $|R| \geq 0.25$ [12].

Supplemental figures

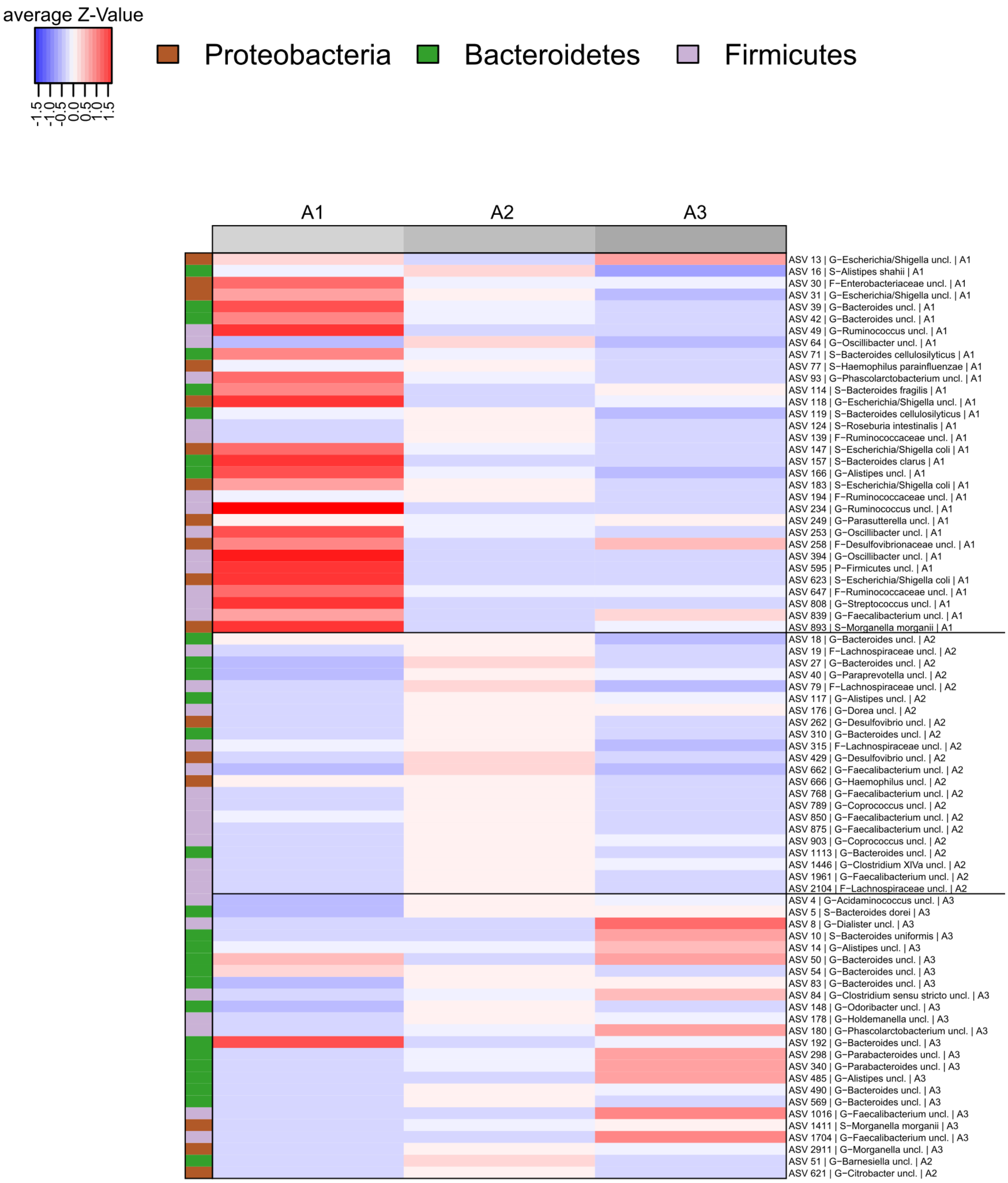


Figure S1: Heatmap visualizing significant differentially abundant ASVs in CD patients with respect to age subgroups following the Montreal classification (A1: <16 yrs., A2: 17-40 yrs., A3: >40 yrs., Table S3). Differential abundance was tested via *DESeq2* and only functions significant after p-value adjustment are displayed.

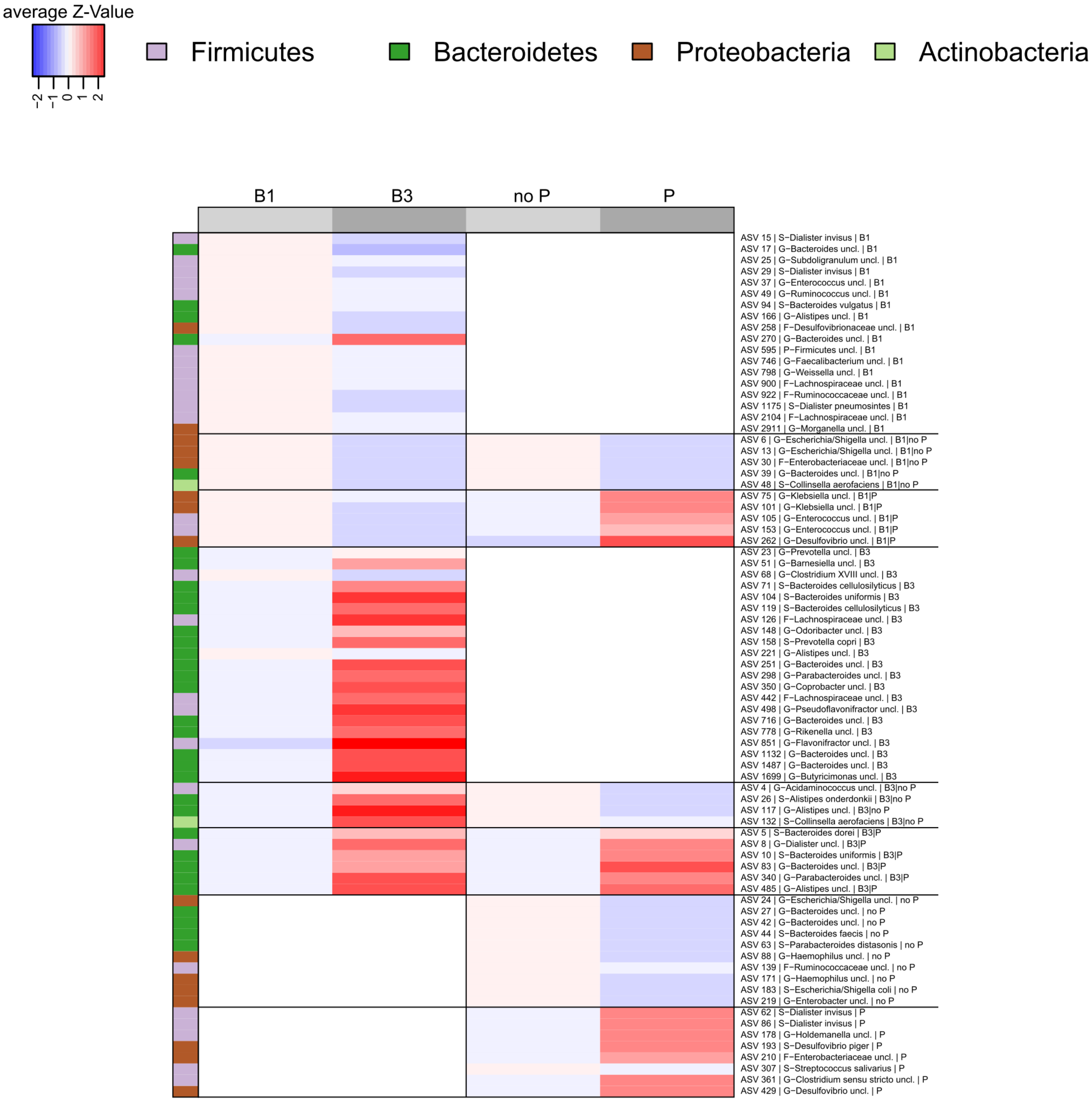


Figure S2: Heatmap visualizing significant differentially abundant ASVs in CD patients with respect to disease behavior subgroups following the Montreal classification (B1: non-stricturing/non-penetrating, B3: penetrating, Table S3), while using the classification of perianal disease as a separate subtype (P:

perianal disease manifestation, no-P: no perianal disease). Differential abundance was tested via DESeq2 and only functions significant after p-value adjustment are displayed.

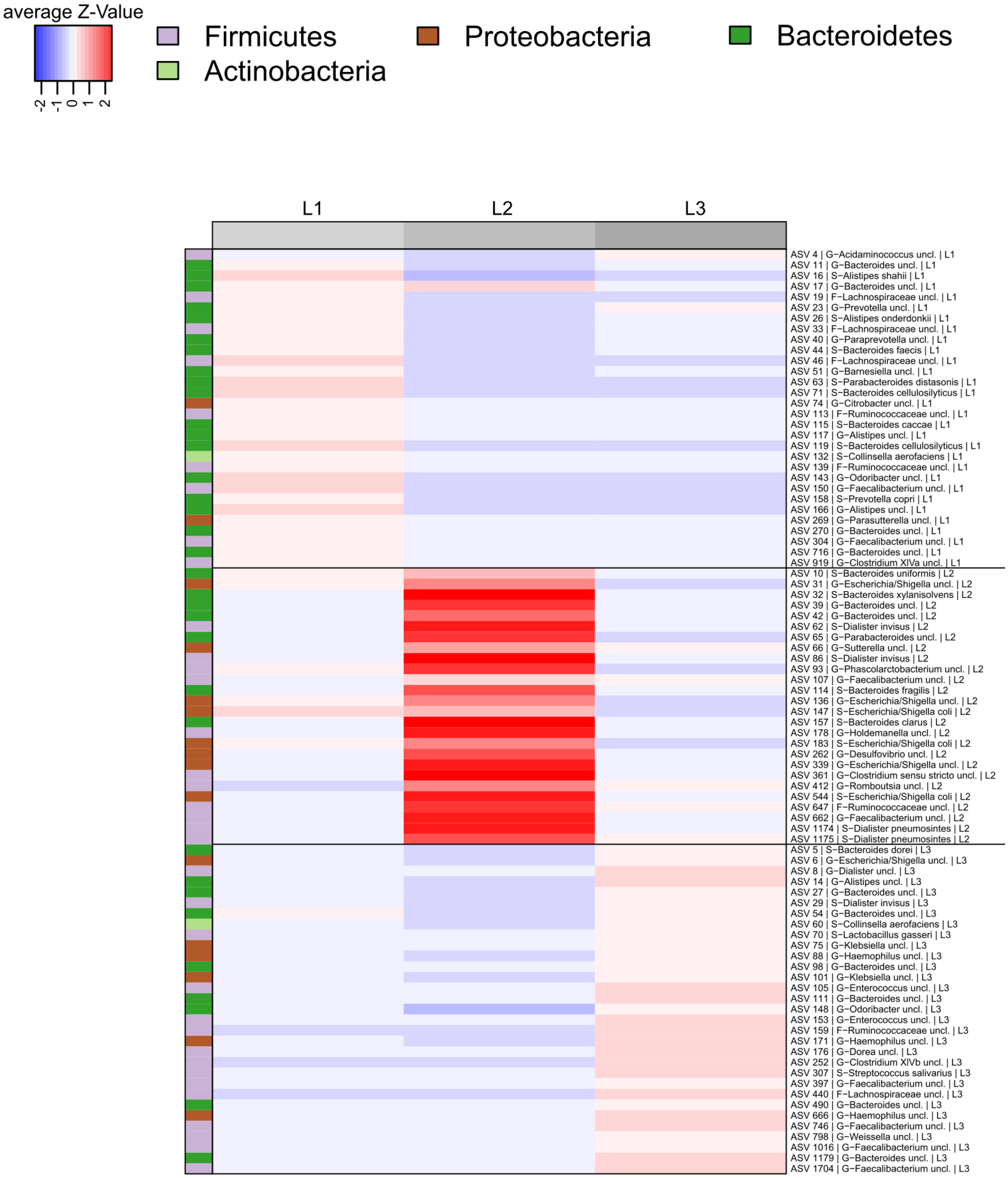


Figure S3: Heatmap visualizing significant differentially abundant ASVs in CD patients with respect to disease location subgroups following the Montreal classification (L1: ileal, L2: colonic, L3: ileocolonic, Table S3). Differential abundance was tested via DESeq2 and only functions significant after p-value adjustment are displayed.

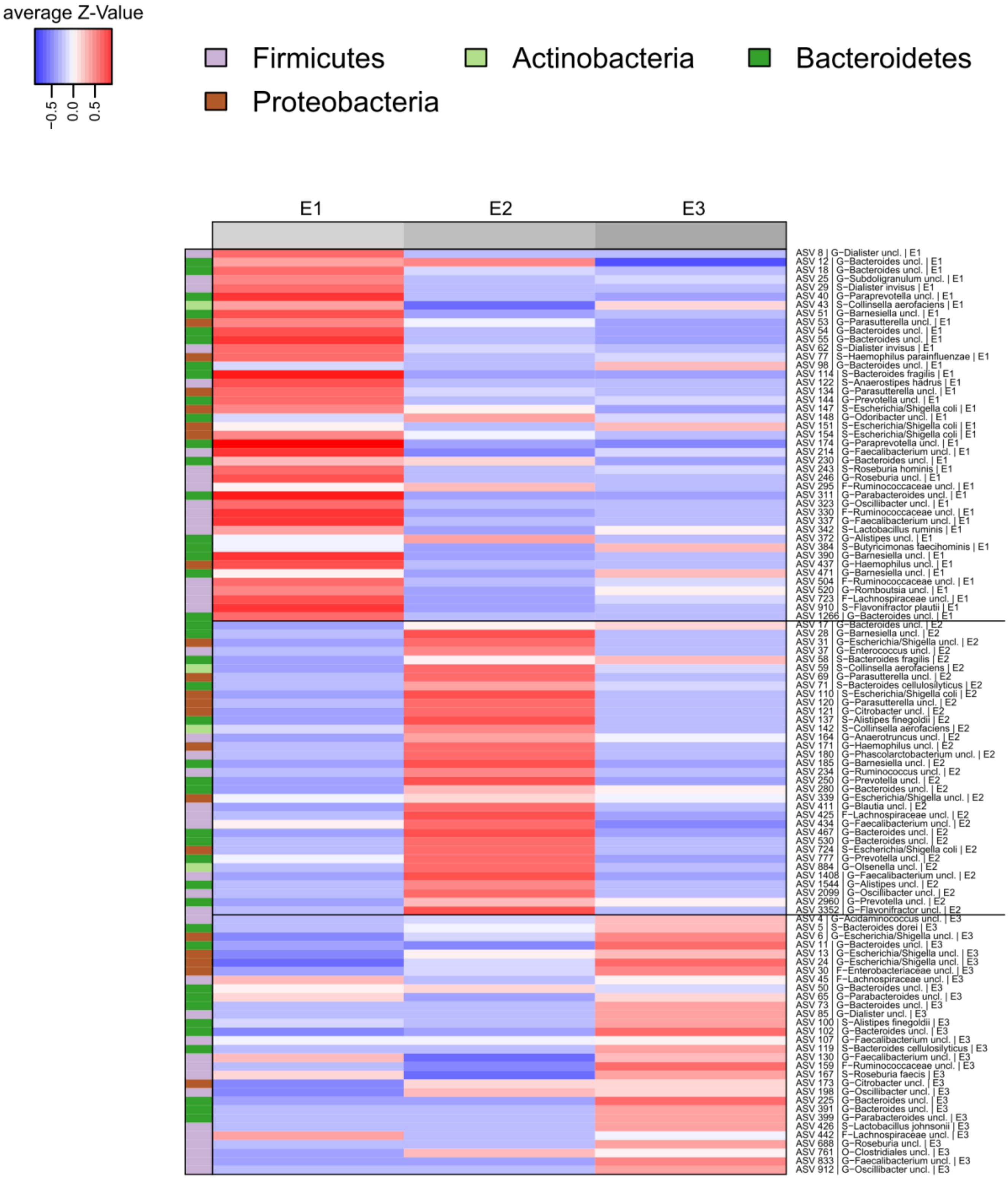


Figure S4: Heatmap visualizing significant differentially abundant ASVs in UC patients with respect to age subgroups following the Montreal classification (E1: Ulcerative proctitis, E2: left sided UC (distal UC), E3: extensive UC (pancolitis), Table S3). Differential abundance was tested via DESeq2 and only functions significant after p-value adjustment are displayed.

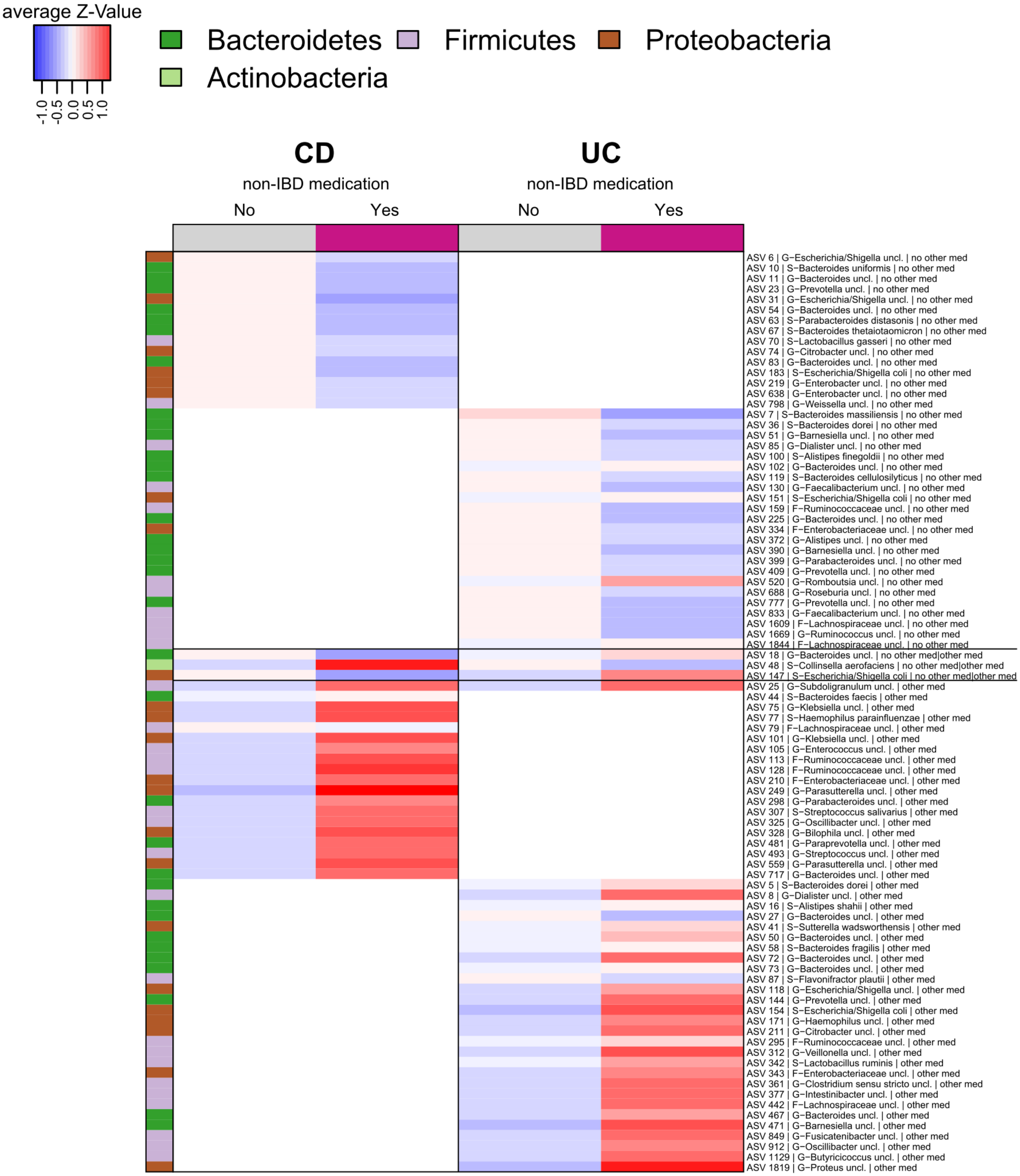


Figure S5: Heatmap visualizing significant differentially abundant ASVs in CD and UC patients with respect to prior antibiotic treatments (Table S5). Differential abundance was tested via DESeq2 and only functions significant after p-value adjustment are displayed.

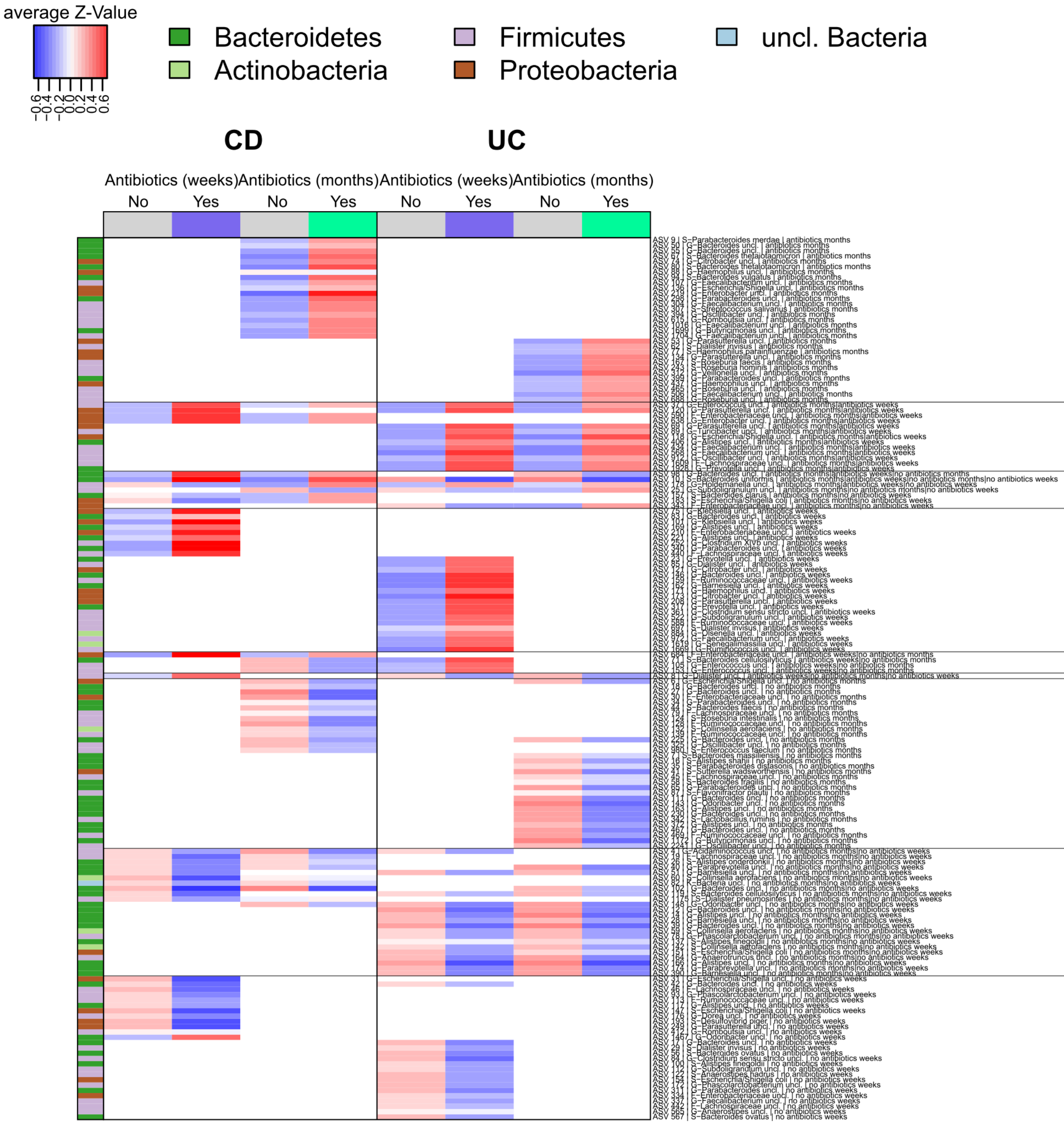


Figure S6: Heatmap visualizing significant differentially abundant ASVs in CD and UC patients with respect to prior antibiotic treatments (Table S5). Differential abundance was tested via DESeq2 and only functions significant after p-value adjustment are displayed.

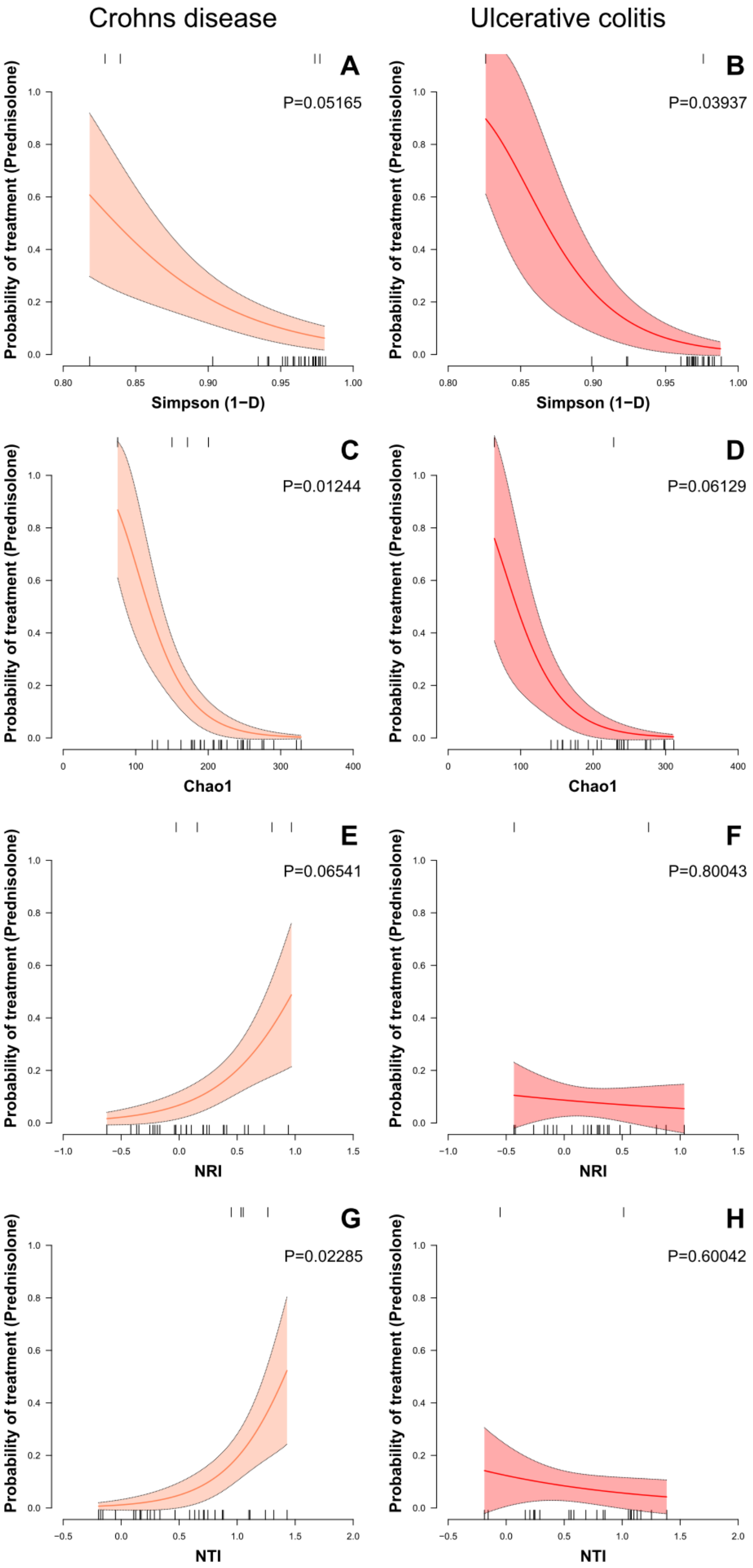

Crohns disease
Ulcerative colitis
A
P=0.05165
B
P=0.03937
Probability of treatment (Prednisolone)
1.0
0.8
0.6
0.4
0.2
0.0
0.80
0.85
0.90
0.95
1.00
Simpson (1−D)
C
P=0.01244
D
P=0.06129
0
100
200
300
400
Chao1
E
P=0.06541
F
P=0.80043
−1.0
−0.5
0.0
0.5
1.0
1.5
NRI
G
P=0.02285
H
P=0.60042
−0.5
0.0
0.5
1.0
1.5
2.0
NTI

Figure S7: Prediction of treatment probabilities (future treatment with mesalazine, azathioprine, anti-TNF, prednisolone) based on the underlying alpha diversity in CD and UC patients. As single treatment, only corticosteroid treatments are predictable from Simpson diversity (**A**: DF=1,29, Dev.=3.7872, *P*=0.05165, P_{FDR}=0.24177; **B**: DF=1,23, Dev.=4.2448, *P*=0.03937, P_{FDR}=0.61291), Chao1 species richness (**C**: DF=1,29, Dev.=6.2466, *P*=0.01244, P_{FDR}=0.20830; **D**: DF=1,23, Dev.=3.5021, *P*=0.06129, P_{FDR}=0.61291), NRI (**E**: DF=1,29, Dev.=3.3945, *P*=0.06541, P_{FDR}=0.24177; **F**: DF=1,23, Dev.=0.06391, *P*=0.8004, P_{FDR}=1.0000), and NTI (**G**: DF=1,29, Dev.=5.1799, *P*=0.02285, P_{FDR}=0.20830; **H**: DF=1,23, Dev.=0.27436, *P*=0.6004, P_{FDR}=1.0000). Ticks on the bottom of the graph indicate samples without treatment, ticks on the top indicate samples with future treatment.

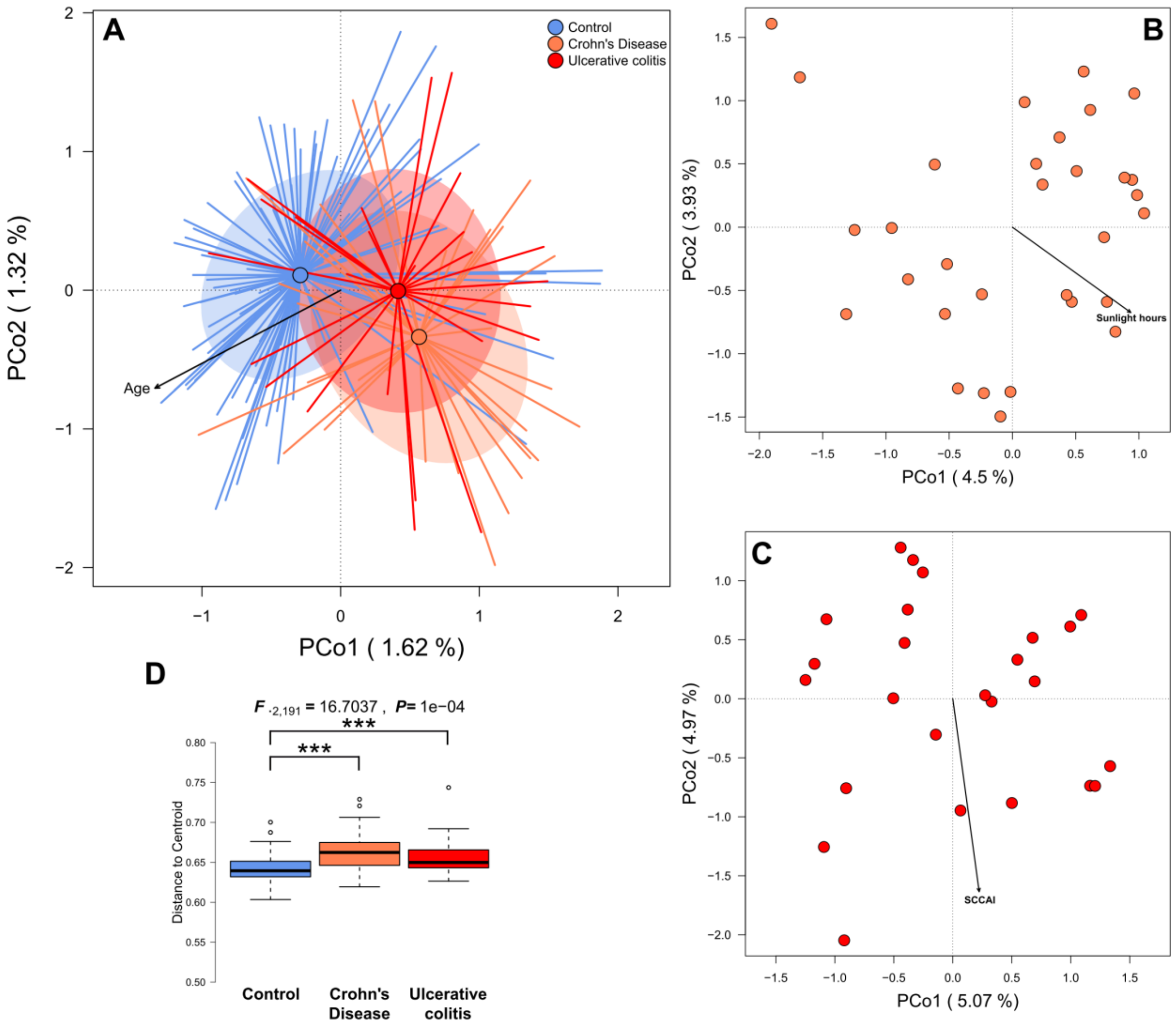
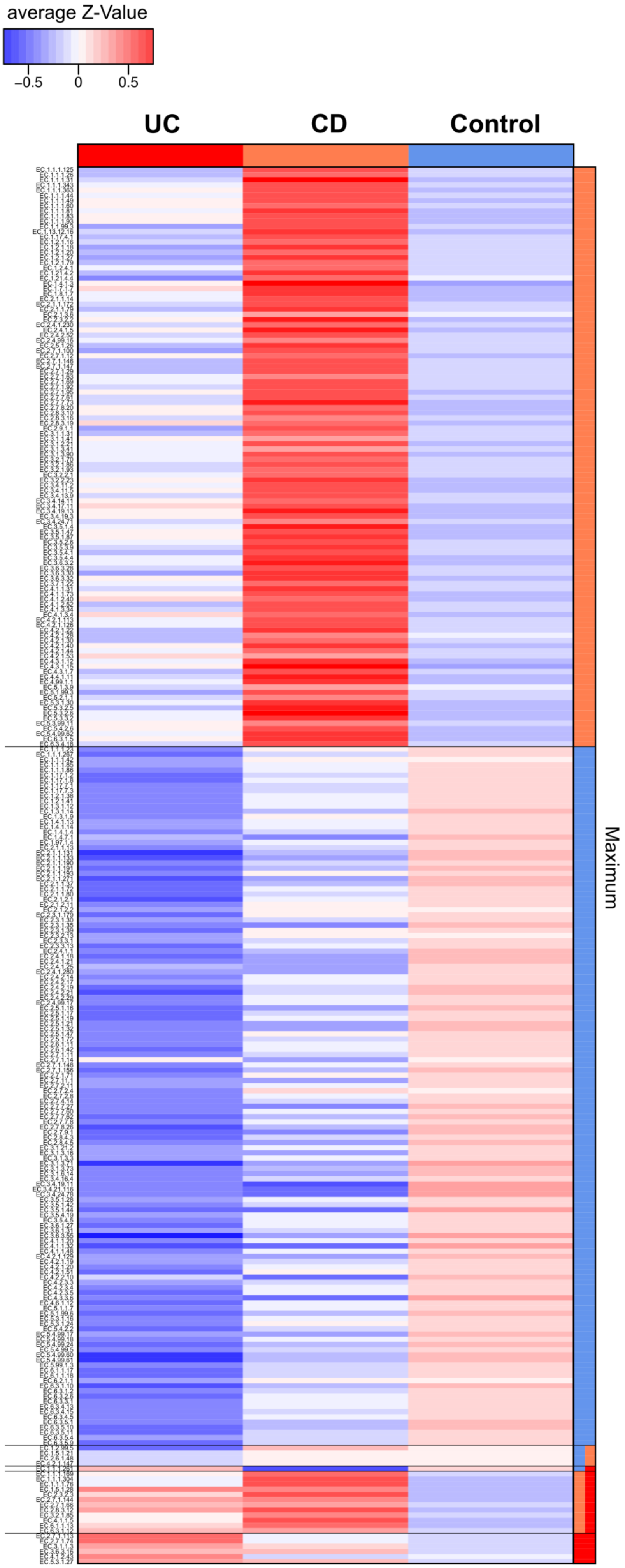


Figure S8: Principle coordinate analyses of Jaccard distance visualizing the differences between the disease cohorts and including nominal significant factors and continuous variables (A), as well as separate betadiversity analyses of only CD- (B) and UC (C) patients. (D) Boxplots visualize the community

variability within each health group measured as distance to the centroid, which is lowest in healthy individuals.


average Z-Value
−0.5
0
0.5
UC
CD
Control
Maximum
EC.1.1.1.125
EC.1.1.1.26
EC.1.1.1.31
EC.1.1.1.343
EC.1.1.1.363
EC.1.1.1.44
EC.1.1.1.49
EC.1.1.1.60
EC.1.1.1.81
EC.1.1.1.83
EC.1.1.1.93
EC.1.1.99.3
EC.1.13.12.16
EC.1.17.4.1
EC.1.2.1.16
EC.1.2.1.18
EC.1.2.1.20
EC.1.2.1.27
EC.1.2.1.79
EC.1.2.4.1
EC.1.21.4.2
EC.1.21.4.4
EC.1.4.1.3
EC.1.7.1.7
EC.1.8.1.7
EC.2.1.1.14
EC.2.1.1.172
EC.2.1.1.79
EC.2.1.3.6
EC.2.3.2.2
EC.2.4.1.230
EC.2.4.1.5
EC.2.4.2.52
EC.2.4.99.16
EC.2.5.1.26
EC.2.7.1.100
EC.2.7.1.12
EC.2.7.1.146
EC.2.7.1.147
EC.2.7.1.29
EC.2.7.1.63
EC.2.7.1.69
EC.2.7.1.92
EC.2.7.1.95
EC.2.7.7.61
EC.2.7.7.73
EC.2.7.8.20
EC.2.8.3.10
EC.2.8.3.16
EC.2.8.3.19
EC.2.9.1.1
EC.3.1.1.31
EC.3.1.1.41
EC.3.1.2.21
EC.3.1.3.41
EC.3.1.3.90
EC.3.2.1.70
EC.3.2.1.86
EC.3.2.1.93
EC.3.2.2.1
EC.3.2.2.23
EC.3.4.11.2
EC.3.4.11.5
EC.3.4.13.9
EC.3.4.14.11
EC.3.4.17.11
EC.3.4.19.13
EC.3.4.19.3
EC.3.4.24.71
EC.3.5.1.4
EC.3.5.1.47
EC.3.5.1.87
EC.3.5.2.6
EC.3.5.3.9
EC.3.5.4.1
EC.3.5.4.4
EC.3.6.3.2
EC.3.6.3.28
EC.3.6.3.30
EC.3.6.3.32
EC.3.7.1.22
EC.4.1.1.31
EC.4.1.1.73
EC.4.1.2.40
EC.4.1.2.52
EC.4.1.3.34
EC.4.1.3.4
EC.4.2.1.113
EC.4.2.1.126
EC.4.2.1.22
EC.4.2.1.28
EC.4.2.1.30
EC.4.2.1.40
EC.4.2.1.44
EC.4.2.1.53
EC.4.3.1.12
EC.4.3.1.15
EC.4.3.1.7
EC.4.4.1.11
EC.4.99.1.1
EC.5.1.3.9
EC.5.1.99.3
EC.5.2.1.1
EC.5.3.1.30
EC.5.3.2.5
EC.5.3.2.6
EC.5.3.3.2
EC.5.3.99.11
EC.5.4.2.6
EC.5.4.99.62
EC.6.3.1.5
EC.6.3.4.18
EC.1.1.1.23
EC.1.1.1.267
EC.1.1.1.42
EC.1.1.1.85
EC.1.1.1.86
EC.1.17.1.2
EC.1.17.1.8
EC.1.17.7.1
EC.1.17.7.3
EC.1.2.1.38
EC.1.2.1.41
EC.1.3.1.12
EC.1.3.1.14
EC.1.3.1.9
EC.1.4.1.13
EC.1.4.1.14
EC.1.4.1.4
EC.1.4.7.1
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EC.2.1.1.131
EC.2.1.1.133
EC.2.1.1.190
EC.2.1.1.191
EC.2.1.1.193
EC.2.1.1.271
EC.2.1.1.37
EC.2.1.1.72
EC.2.1.1.80
EC.2.1.2.1
EC.2.1.2.11
EC.2.1.2.2
EC.2.3.1.179
EC.2.3.1.30
EC.2.3.1.35
EC.2.3.1.39
EC.2.3.2.13
EC.2.3.3.1
EC.2.3.3.13
EC.2.4.1.1
EC.2.4.1.18
EC.2.4.1.21
EC.2.4.1.25
EC.2.4.1.280
EC.2.4.2.14
EC.2.4.2.17
EC.2.4.2.19
EC.2.4.2.21
EC.2.4.2.29
EC.2.4.99.17
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EC.2.5.1.19
EC.2.5.1.21
EC.2.5.1.32
EC.2.5.1.47
EC.2.5.1.72
EC.2.6.1.11
EC.2.6.1.42
EC.2.7.1.11
EC.2.7.1.14
EC.2.7.1.148
EC.2.7.1.156
EC.2.7.1.71
EC.2.7.11.1
EC.2.7.2.11
EC.2.7.2.4
EC.2.7.2.8
EC.2.7.4.14
EC.2.7.7.27
EC.2.7.7.60
EC.2.7.7.62
EC.2.7.7.8
EC.2.7.8.26
EC.2.7.9.1
EC.2.8.4.3
EC.2.8.4.5
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EC.3.5.4.5
EC.3.6.1.27
EC.3.6.1.31
EC.3.6.3.55
EC.4.1.1.20
EC.4.1.1.32
EC.4.1.1.48
EC.4.2.1.129
EC.4.2.1.19
EC.4.2.1.20
EC.4.2.1.51
EC.4.2.2.10
EC.4.2.3.3
EC.4.2.3.4
EC.4.2.3.5
EC.4.3.3.6
EC.4.6.1.12
EC.5.1.1.7
EC.5.1.99.6
EC.5.3.1.16
EC.5.3.1.24
EC.5.4.2.2
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EC.5.4.99.18
EC.5.4.99.24
EC.5.4.99.5
EC.5.4.99.60
EC.5.4.99.61
EC.5.99.1.3
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EC.6.2.1.1
EC.6.3.1.10
EC.6.3.1.2
EC.6.3.2.6
EC.6.3.3.1
EC.6.3.4.13
EC.6.3.4.15
EC.6.3.4.5
EC.6.3.5.1
EC.6.3.5.10
EC.6.3.5.11
EC.6.3.5.4
EC.6.3.5.9
EC.1.2.99.5
EC.1.5.1.21
EC.2.6.1.48
EC.4.2.1.147
EC.1.1.1.261
EC.1.1.1.169
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EC.3.6.3.16
EC.4.1.2.43
EC.5.3.1.27

Figure S9: Heatmap visualizing significant differentially abundant functions between healthy controls, CD and UC patients (Table S10). Functions are represented as the PiCRUST2 imputed abundance of single enzymes categories/EC categories (Enzyme Commission number). Differential abundance was tested via DESeq2 and only significantly different functions, after FDR adjustment, are displayed.

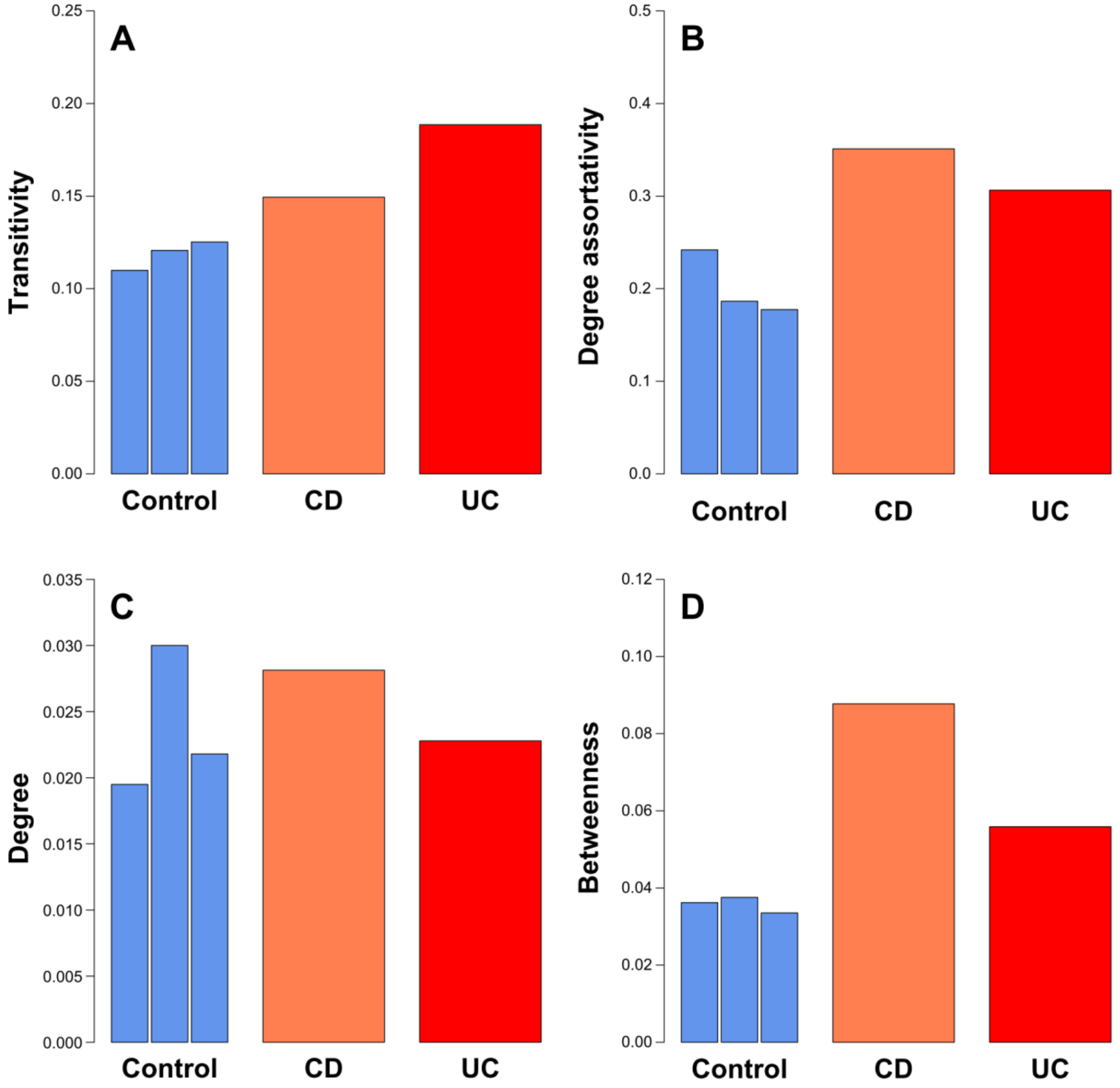

Figure S10: Global network descriptors for each health condition specific network (including subsamples of the control based networks), like **(A)** average transitivity, which describes the clustering and denseness of the respective network. (**B**) Degree assortativity describes preferential attachment of bacteria with similarly connected bacteria, while average degree and betweenness describe the **(C)**

average number of connections of nodes in the network and **(D)** the average of shortest paths in the networks.

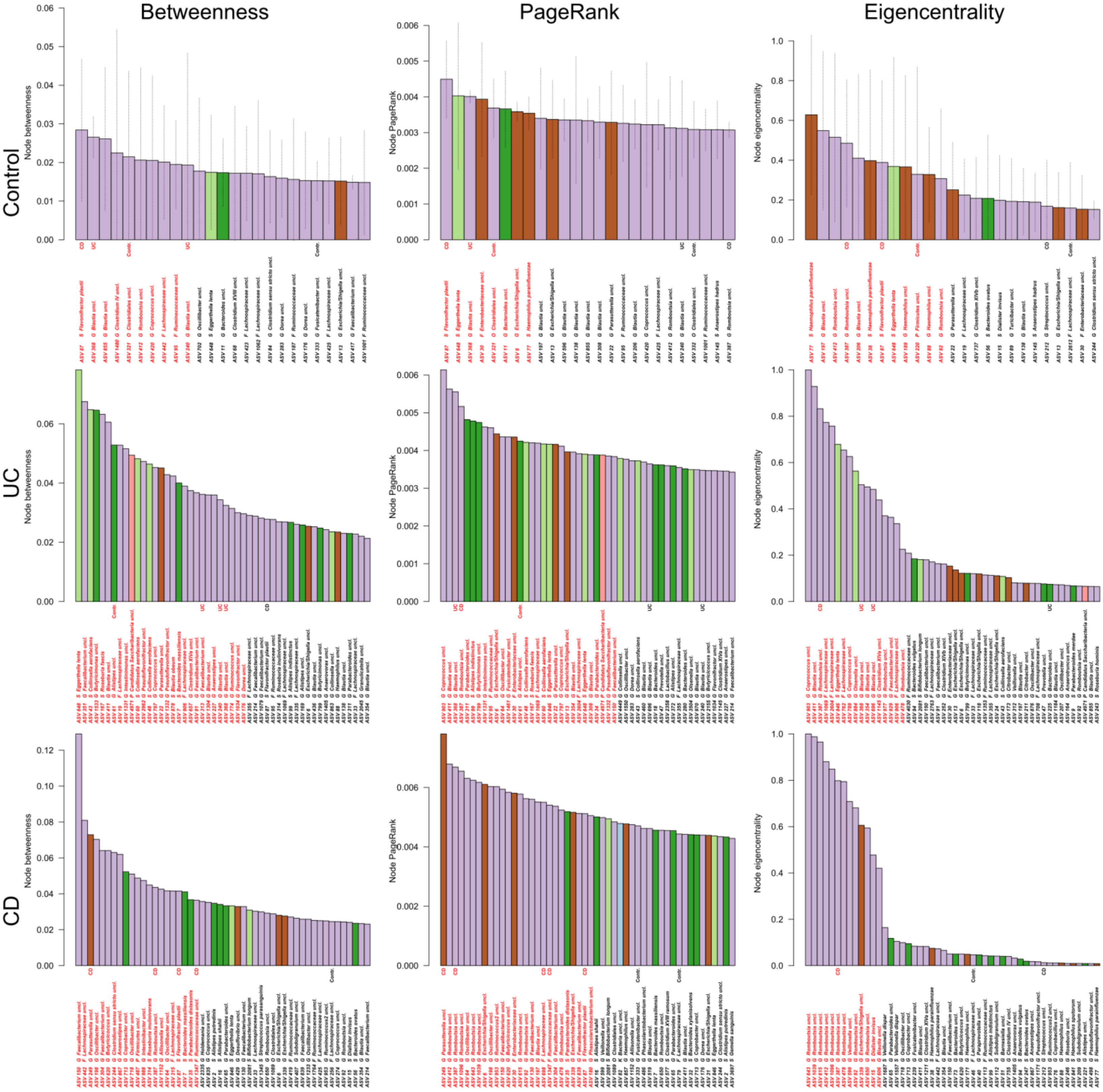
Betweenness
PageRank
Eigencentrality
Control
UC
CD
Node betweenness
Node PageRank
Node eigencentrality
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.000
0.001
0.002
0.003
0.004
0.005
0.006
0.0
0.2
0.4
0.6
0.8
1.0
0.08
0.10
0.12
CD
UC
Contr.
ASV 87 S Flavonifractor plautii
ASV 368 G Blautia uncl.
ASV 855 G Blautia uncl.
ASV 1480 G Clostridium IV uncl.
ASV 321 O Clostridiales uncl.
ASV 412 G Romboutsia uncl.
ASV 420 G Coprococcus uncl.
ASV 442 F Lachnospiraceae uncl.
ASV 95 F Ruminococcaceae uncl.
ASV 240 G Blautia uncl.
ASV 702 G Oscillibacter uncl.
ASV 648 S Eggerthella lenta
ASV 11 G Bacteroides uncl.
ASV 68 G Clostridium XVIII uncl.
ASV 423 F Lachnospiraceae uncl.
ASV 1062 F Lachnospiraceae uncl.
ASV 84 G Clostridium sensu stricto uncl.
ASV 283 G Dorea uncl.
ASV 187 F Ruminococcaceae uncl.
ASV 176 G Dorea uncl.
ASV 333 G Fusicatenibacter uncl.
ASV 425 F Lachnospiraceae uncl.
ASV 13 G Escherichia/Shigella uncl.
ASV 417 G Faecalibacterium uncl.
ASV 1001 F Ruminococcaceae uncl.
ASV 87 S Flavonifractor plautii
ASV 648 S Eggerthella lenta
ASV 368 G Blautia uncl.
ASV 30 F Enterobacteriaceae uncl.
ASV 321 O Clostridiales uncl.
ASV 11 G Bacteroides uncl.
ASV 6 G Escherichia/Shigella uncl.
ASV 77 S Haemophilus parainfluenzae
ASV 197 G Blautia uncl.
ASV 13 G Escherichia/Shigella uncl.
ASV 596 G Blautia uncl.
ASV 138 G Blautia uncl.
ASV 855 G Blautia uncl.
ASV 308 G Blautia uncl.
ASV 22 G Parasutterella uncl.
ASV 95 F Ruminococcaceae uncl.
ASV 206 G Blautia uncl.
ASV 420 G Coprococcus uncl.
ASV 425 F Lachnospiraceae uncl.
ASV 412 G Romboutsia uncl.
ASV 240 G Blautia uncl.
ASV 332 O Clostridiales uncl.
ASV 1001 F Ruminococcaceae uncl.
ASV 145 S Anaerostipes hadrus
ASV 387 G Romboutsia uncl.
ASV 77 S Haemophilus parainfluenzae
ASV 197 G Blautia uncl.
ASV 412 G Romboutsia uncl.
ASV 387 G Romboutsia uncl.
ASV 206 G Blautia uncl.
ASV 38 S Haemophilus parainfluenzae
ASV 87 S Flavonifractor plautii
ASV 648 S Eggerthella lenta
ASV 189 G Haemophilus uncl.
ASV 220 P Firmicutes uncl.
ASV 88 G Haemophilus uncl.
ASV 92 G Romboutsia uncl.
ASV 22 G Parasutterella uncl.
ASV 19 F Lachnospiraceae uncl.
ASV 737 G Clostridium XIVb uncl.
ASV 56 S Bacteroides ovatus
ASV 15 S Dialister invisus
ASV 89 G Turicibacter uncl.
ASV 138 G Blautia uncl.
ASV 145 S Anaerostipes hadrus
ASV 212 G Streptococcus uncl.
ASV 13 G Escherichia/Shigella uncl.
ASV 2612 F Lachnospiraceae uncl.
ASV 30 F Enterobacteriaceae uncl.
ASV 244 G Clostridium sensu stricto uncl.
ASV 648 S Eggerthella lenta
ASV 351 G Faecalibacterium uncl.
ASV 43 S Collinsella aerofaciens
ASV 1533 G Butyricimonas uncl.
ASV 167 S Roseburia faecis
ASV 411 G Blautia uncl.
ASV 61 G Alistipes uncl.
ASV 19 F Lachnospiraceae uncl.
ASV 1331 G Intestinimonas uncl.
ASV 4971 P Candidatus Saccharibacteria uncl.
ASV 60 S Collinsella aerofaciens
ASV 2962 G Pseudoflavonifractor uncl.
ASV 48 S Collinsella aerofaciens
ASV 797 G Coprococcus uncl.
ASV 22 G Parasutterella uncl.
ASV 1332 G Pseudoflavonifractor uncl.
ASV 878 S Blautia obeum
ASV 7 S Bacteroides massiliensis
ASV 906 F Lachnospiraceae uncl.
ASV 657 G Clostridium XIVa uncl.
ASV 91 G Faecalibacterium uncl.
ASV 313 G Faecalibacterium uncl.
ASV 1304 G Blautia uncl.
ASV 227 G Anaerostipes uncl.
ASV 240 G Blautia uncl.
ASV 368 G Blautia uncl.
ASV 774 G Ruminococcus uncl.
ASV 1034 G Flavonifractor uncl.
ASV 176 G Dorea uncl.
ASV 355 F Lachnospiraceae uncl.
ASV 214 G Faecalibacterium uncl.
ASV 1079 G Faecalibacterium uncl.
ASV 87 S Flavonifractor plautii
ASV 95 F Ruminococcaceae uncl.
ASV 314 S Roseburia inulinivorans
ASV 1069 F Lachnospiraceae uncl.
ASV 99 S Alistipes indistinctus
ASV 335 F Lachnospiraceae uncl.
ASV 169 G Alistipes uncl.
ASV 6 G Escherichia/Shigella uncl.
ASV 436 G Blautia uncl.
ASV 799 G Butyricimonas uncl.
ASV 1405 G Anaerovorax uncl.
ASV 863 G Collinsella uncl.
ASV 88 G Haemophilus uncl.
ASV 138 G Blautia uncl.
ASV 311 G Parabacteroides uncl.
ASV 33 F Lachnospiraceae uncl.
ASV 2045 G Granulicatella uncl.
ASV 354 G Blautia uncl.
ASV 903 G Coprococcus uncl.
ASV 411 G Blautia uncl.
ASV 368 G Blautia uncl.
ASV 387 G Romboutsia uncl.
ASV 311 G Parabacteroides uncl.
ASV 99 S Alistipes indistinctus
ASV 799 G Butyricimonas uncl.
ASV 1331 G Intestinimonas uncl.
ASV 95 F Ruminococcaceae uncl.
ASV 6 G Escherichia/Shigella uncl.
ASV 64 G Oscillibacter uncl.
ASV 1461 G Fusicatenibacter uncl.
ASV 30 F Enterobacteriaceae uncl.
ASV 61 G Alistipes uncl.
ASV 48 S Collinsella aerofaciens
ASV 167 S Roseburia faecis
ASV 1069 F Lachnospiraceae uncl.
ASV 60 S Collinsella aerofaciens
ASV 846 S Eggerthella lenta
ASV 22 G Parasutterella uncl.
ASV 797 G Coprococcus uncl.
ASV 13 G Escherichia/Shigella uncl.
ASV 354 G Blautia uncl.
ASV 2604 F Lachnospiraceae uncl.
ASV 648 S Eggerthella lenta
ASV 308 G Blautia uncl.
ASV 34 G Parabacteroides uncl.
ASV 4971 P Candidatus Saccharibacteria uncl.
ASV 1332 G Pseudoflavonifractor uncl.
ASV 150 G Faecalibacterium uncl.
ASV 4449 G Collinsella uncl.
ASV 1550 G Oscillibacter uncl.
ASV 283 G Dorea uncl.
ASV 43 S Collinsella aerofaciens
ASV 460 G Blautia uncl.
ASV 696 G Blautia uncl.
ASV 18 G Bacteroides uncl.
ASV 47 G Prevotella uncl.
ASV 2358 G Lactobacillus uncl.
ASV 372 G Alistipes uncl.
ASV 355 F Lachnospiraceae uncl.
ASV 280 G Bacteroides uncl.
ASV 3504 G Olsenella uncl.
ASV 970 G Blautia uncl.
ASV 240 G Blautia uncl.
ASV 2155 G Butyricicoccus uncl.
ASV 1034 G Flavonifractor uncl.
ASV 1145 G Clostridium XIVa uncl.
ASV 227 G Anaerostipes uncl.
ASV 214 G Faecalibacterium uncl.
ASV 903 G Coprococcus uncl.
ASV 411 G Blautia uncl.
ASV 387 G Romboutsia uncl.
ASV 1069 F Lachnospiraceae uncl.
ASV 2604 F Lachnospiraceae uncl.
ASV 846 S Eggerthella lenta
ASV 762 F Lachnospiraceae uncl.
ASV 789 G Coprococcus uncl.
ASV 884 G Olsenella uncl.
ASV 368 G Blautia uncl.
ASV 354 G Blautia uncl.
ASV 696 G Blautia uncl.
ASV 1145 G Clostridium XIVa uncl.
ASV 977 G Romboutsia uncl.
ASV 929 G Faecalibacterium uncl.
ASV 906 F Lachnospiraceae uncl.
ASV 478 G Romboutsia uncl.
ASV 4030 F Ruminococcaceae uncl.
ASV 94 S Bacteroides vulgatus
ASV 2081 S Bifidobacterium longum
ASV 150 G Faecalibacterium uncl.
ASV 2763 F Lachnospiraceae uncl.
ASV 91 G Faecalibacterium uncl.
ASV 252 G Clostridium XIVb uncl.
ASV 30 F Enterobacteriaceae uncl.
ASV 13 G Escherichia/Shigella uncl.
ASV 6 G Escherichia/Shigella uncl.
ASV 799 G Butyricimonas uncl.
ASV 33 F Lachnospiraceae uncl.
ASV 118 G Escherichia/Shigella uncl.
ASV 1353 F Ruminococcaceae uncl.
ASV 355 F Lachnospiraceae uncl.
ASV 24 G Escherichia/Shigella uncl.
ASV 43 S Collinsella aerofaciens
ASV 173 G Citrobacter uncl.
ASV 312 G Veillonella uncl.
ASV 197 G Blautia uncl.
ASV 211 G Citrobacter uncl.
ASV 876 G Oscillibacter uncl.
ASV 708 F Lachnospiraceae uncl.
ASV 47 G Prevotella uncl.
ASV 225 G Bacteroides uncl.
ASV 1184 G Blautia uncl.
ASV 207 G Oscillibacter uncl.
ASV 164 G Anaerotruncus uncl.
ASV 9 S Parabacteroides merdae
ASV 92 G Romboutsia uncl.
ASV 4971 P Candidatus Saccharibacteria uncl.
ASV 855 G Blautia uncl.
ASV 243 S Roseburia hominis
ASV 150 G Faecalibacterium uncl.
ASV 442 F Lachnospiraceae uncl.
ASV 249 G Parasutterella uncl.
ASV 198 G Oscillibacter uncl.
ASV 304 G Faecalibacterium uncl.
ASV 620 G Butyricicoccus uncl.
ASV 244 G Clostridium sensu stricto uncl.
ASV 867 G Anaerostipes uncl.
ASV 713 G Coprobacter uncl.
ASV 718 G Oscillibacter uncl.
ASV 487 P Firmicutes uncl.
ASV 988 G Intestinibacter uncl.
ASV 314 S Roseburia inulinivorans
ASV 387 G Romboutsia uncl.
ASV 1152 G Allisonella uncl.
ASV 64 G Oscillibacter uncl.
ASV 315 F Lachnospiraceae uncl.
ASV 87 S Flavonifractor plautii
ASV 7 S Bacteroides massiliensis
ASV 35 S Parabacteroides distasonis
ASV 1347 F Ruminococcaceae uncl.
ASV 2335 G Holdemania uncl.
ASV 835 G Coprococcus uncl.
ASV 2 S Alistipes putredinis
ASV 16 S Alistipes shahii
ASV 65 G Parabacteroides uncl.
ASV 846 S Eggerthella lenta
ASV 429 G Desulfovibrio uncl.
ASV 130 G Faecalibacterium uncl.
ASV 2081 S Bifidobacterium longum
ASV 33 F Lachnospiraceae uncl.
ASV 1345 S Streptococcus parasanguinis
ASV 650 G Romboutsia uncl.
ASV 1089 O Clostridiales uncl.
ASV 6 G Escherichia/Shigella uncl.
ASV 339 G Escherichia/Shigella uncl.
ASV 419 F Ruminococcaceae uncl.
ASV 407 G Subdoligranulum uncl.
ASV 839 G Faecalibacterium uncl.
ASV 1233 G Oscillibacter uncl.
ASV 1764 F Ruminococcaceae uncl.
ASV 425 F Lachnospiraceae uncl.
ASV 953 G Ruminococcus2 uncl.
ASV 256 F Lachnospiraceae uncl.
ASV 274 G Coprococcus uncl.
ASV 92 G Romboutsia uncl.
ASV 15 S Dialister invisus
ASV 56 S Bacteroides ovatus
ASV 354 G Blautia uncl.
ASV 214 G Faecalibacterium uncl.
ASV 249 G Parasutterella uncl.
ASV 412 G Romboutsia uncl.
ASV 387 G Romboutsia uncl.
ASV 1096 G Romboutsia uncl.
ASV 64 G Oscillibacter uncl.
ASV 643 G Romboutsia uncl.
ASV 1039 G Romboutsia uncl.
ASV 6 G Escherichia/Shigella uncl.
ASV 198 G Oscillibacter uncl.
ASV 953 G Ruminococcus2 uncl.
ASV 150 G Faecalibacterium uncl.
ASV 620 G Butyricicoccus uncl.
ASV 30 F Enterobacteriaceae uncl.
ASV 615 G Romboutsia uncl.
ASV 92 G Romboutsia uncl.
ASV 130 G Faecalibacterium uncl.
ASV 197 G Blautia uncl.
ASV 698 F Lachnospiraceae uncl.
ASV 1347 F Ruminococcaceae uncl.
ASV 304 G Faecalibacterium uncl.
ASV 478 G Romboutsia uncl.
ASV 35 S Parabacteroides distasonis
ASV 339 G Escherichia/Shigella uncl.
ASV 839 G Faecalibacterium uncl.
ASV 87 S Flavonifractor plautii
ASV 180 G Phascolarctobacterium uncl.
ASV 16 S Alistipes shahii
ASV 599 G Veillonella uncl.
ASV 2081 S Bifidobacterium longum
ASV 1089 O Clostridiales uncl.
ASV 82 K Bacteria uncl. uncl.
ASV 857 G Haemophilus uncl.
ASV 1152 G Allisonella uncl.
ASV 333 G Fusicatenibacter uncl.
ASV 486 G Phascolarctobacterium uncl.
ASV 519 G Blautia uncl.
ASV 7 S Bacteroides massiliensis
ASV 650 G Romboutsia uncl.
ASV 577 S Clostridium XVIII ramosum
ASV 65 G Parabacteroides uncl.
ASV 79 F Lachnospiraceae uncl.
ASV 411 G Blautia uncl.
ASV 32 S Bacteroides xylanisolvens
ASV 713 G Coprobacter uncl.
ASV 176 G Dorea uncl.
ASV 31 G Escherichia/Shigella uncl.
ASV 846 S Eggerthella lenta
ASV 244 G Clostridium sensu stricto uncl.
ASV 2 S Alistipes putredinis
ASV 3697 S Gemella sanguinis
ASV 643 G Romboutsia uncl.
ASV 1039 G Romboutsia uncl.
ASV 615 G Romboutsia uncl.
ASV 412 G Romboutsia uncl.
ASV 1096 G Romboutsia uncl.
ASV 387 G Romboutsia uncl.
ASV 478 G Romboutsia uncl.
ASV 599 G Veillonella uncl.
ASV 92 G Romboutsia uncl.
ASV 339 G Escherichia/Shigella uncl.
ASV 650 G Romboutsia uncl.
ASV 15 S Dialister invisus
ASV 606 G Blautia uncl.
ASV 1687 S Veillonella dispar
ASV 65 G Parabacteroides uncl.
ASV 1557 S Veillonella dispar
ASV 718 G Oscillibacter uncl.
ASV 713 G Coprobacter uncl.
ASV 239 G Fusicatenibacter uncl.
ASV 411 G Blautia uncl.
ASV 1211 G Clostridium XIVa uncl.
ASV 38 S Haemophilus parainfluenzae
ASV 442 F Lachnospiraceae uncl.
ASV 354 G Blautia uncl.
ASV 150 G Faecalibacterium uncl.
ASV 73 G Bacteroides uncl.
ASV 620 G Butyricicoccus uncl.
ASV 177 G Haemophilus uncl.
ASV 46 F Lachnospiraceae uncl.
ASV 40 G Paraprevotella uncl.
ASV 113 F Ruminococcaceae uncl.
ASV 99 S Alistipes indistinctus
ASV 112 G Subdoligranulum uncl.
ASV 51 G Barnesiella uncl.
ASV 755 G Clostridium IV uncl.
ASV 1405 G Anaerovorax uncl.
ASV 94 S Bacteroides vulgatus
ASV 247 S Bacteroides ovatus
ASV 867 G Anaerostipes uncl.
ASV 1171 G Pseudoflavonifractor uncl.
ASV 212 G Streptococcus uncl.
ASV 953 G Ruminococcus2 uncl.
ASV 76 G Coprobacillus uncl.
ASV 88 G Haemophilus uncl.
ASV 246 G Roseburia uncl.
ASV 841 S Haemophilus sputorum
ASV 209 G Subdoligranulum uncl.
ASV 221 G Alistipes uncl.
ASV 498 G Pseudoflavonifractor uncl.
ASV 77 S Haemophilus parainfluenzae

Figure S11: Centrality measures of the 50 most important nodes of the respective importance measures (Betweenness, Eigenvalue centrality, PageRank). Red letters highlight network members with a higher importance than expected by chance, based on a Z-test against 10’000 randomized networks ($P \leq 0.05$). Highlighted are the associations of the respective ASVs as detected by differential abundance analysis as well ($P_{\mathrm{FDR}} \leq 0.05$, Table S12).

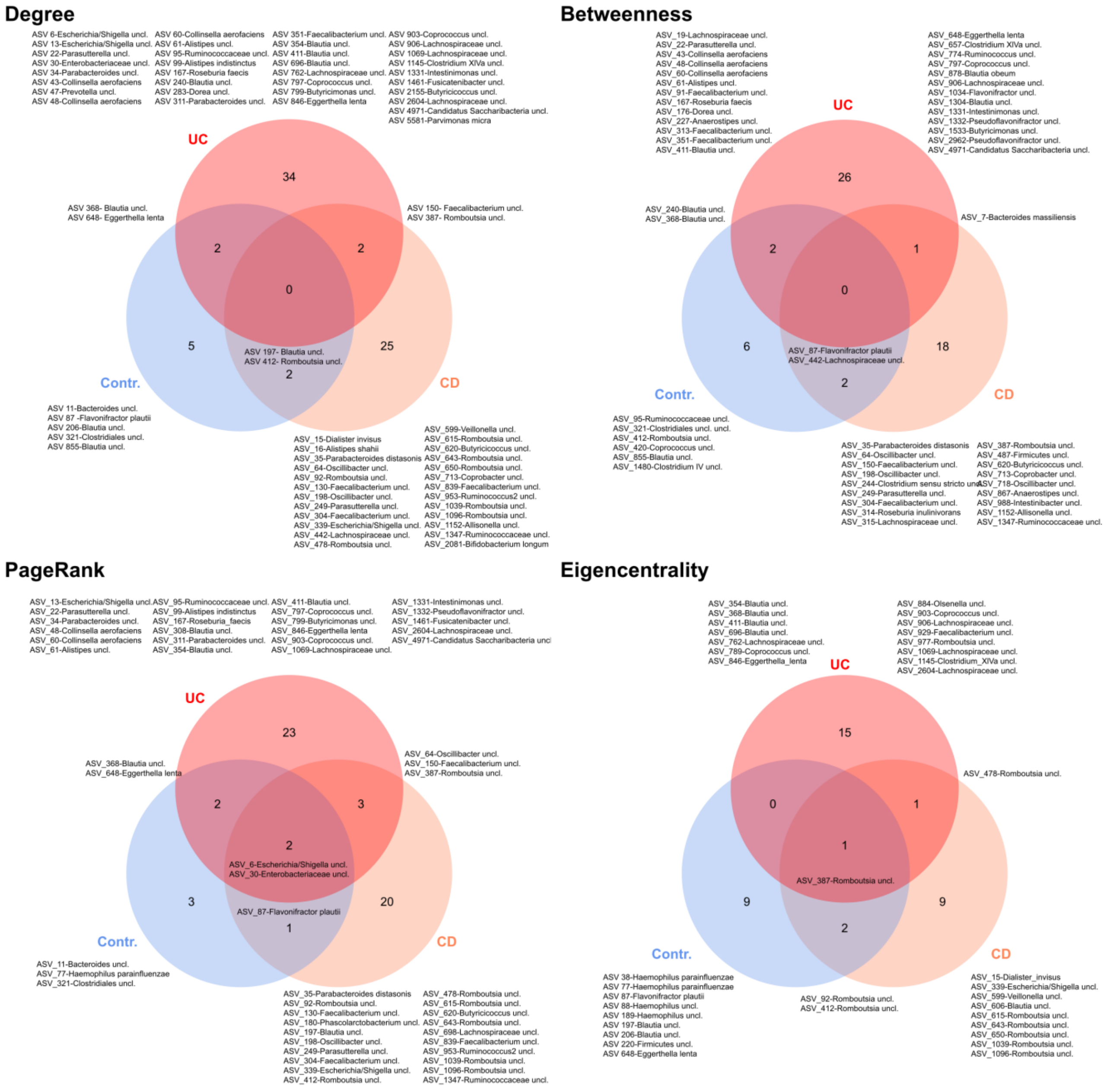
Degree
ASV 6-Escherichia/Shigella uncl.
ASV 13-Escherichia/Shigella uncl.
ASV 22-Parasutterella uncl.
ASV 30-Enterobacteriaceae uncl.
ASV 34-Parabacteroides uncl.
ASV 43-Collinsella aerofaciens
ASV 47-Prevotella uncl.
ASV 48-Collinsella aerofaciens
ASV 60-Collinsella aerofaciens
ASV 61-Alistipes uncl.
ASV 95-Ruminococcaceae uncl.
ASV 99-Alistipes indistinctus
ASV 167-Roseburia faecis
ASV 240-Blautia uncl.
ASV 283-Dorea uncl.
ASV 311-Parabacteroides uncl.
ASV 351-Faecalibacterium uncl.
ASV 354-Blautia uncl.
ASV 411-Blautia uncl.
ASV 696-Blautia uncl.
ASV 762-Lachnospiraceae uncl.
ASV 797-Coprococcus uncl.
ASV 799-Butyricimonas uncl.
ASV 846-Eggerthella lenta
ASV 903-Coprococcus uncl.
ASV 906-Lachnospiraceae uncl.
ASV 1069-Lachnospiraceae uncl.
ASV 1145-Clostridium XIVa uncl.
ASV 1331-Intestinimonas uncl.
ASV 1461-Fusicatenibacter uncl.
ASV 2155-Butyricicoccus uncl.
ASV 2604-Lachnospiraceae uncl.
ASV 4971-Candidatus Saccharibacteria uncl.
ASV 5581-Parvimonas micra
UC
34
ASV 368- Blautia uncl.
ASV 648- Eggerthella lenta
2
ASV 150- Faecalibacterium uncl.
ASV 387- Romboutsia uncl.
2
0
5
ASV 197- Blautia uncl.
ASV 412- Romboutsia uncl.
2
25
Contr.
CD
ASV 11-Bacteroides uncl.
ASV 87 -Flavonifractor plautii
ASV 206-Blautia uncl.
ASV 321-Clostridiales uncl.
ASV 855-Blautia uncl.
ASV_15-Dialister invisus
ASV_16-Alistipes shahii
ASV_35-Parabacteroides distasonis
ASV_64-Oscillibacter uncl.
ASV_92-Romboutsia uncl.
ASV_130-Faecalibacterium uncl.
ASV_198-Oscillibacter uncl.
ASV_249-Parasutterella uncl.
ASV_304-Faecalibacterium uncl.
ASV_339-Escherichia/Shigella uncl.
ASV_442-Lachnospiraceae uncl.
ASV_478-Romboutsia uncl.
ASV_599-Veillonella uncl.
ASV_615-Romboutsia uncl.
ASV_620-Butyricicoccus uncl.
ASV_643-Romboutsia uncl.
ASV_650-Romboutsia uncl.
ASV_713-Coprobacter uncl.
ASV_839-Faecalibacterium uncl.
ASV_953-Ruminococcus2 uncl.
ASV_1039-Romboutsia uncl.
ASV_1096-Romboutsia uncl.
ASV_1152-Allisonella uncl.
ASV_1347-Ruminococcaceae uncl.
ASV_2081-Bifidobacterium longum
Betweenness
ASV_19-Lachnospiraceae uncl.
ASV_22-Parasutterella uncl.
ASV_43-Collinsella aerofaciens
ASV_48-Collinsella aerofaciens
ASV_60-Collinsella aerofaciens
ASV_61-Alistipes uncl.
ASV_91-Faecalibacterium uncl.
ASV_167-Roseburia faecis
ASV_176-Dorea uncl.
ASV_227-Anaerostipes uncl.
ASV_313-Faecalibacterium uncl.
ASV_351-Faecalibacterium uncl.
ASV_411-Blautia uncl.
ASV_648-Eggerthella lenta
ASV_657-Clostridium XIVa uncl.
ASV_774-Ruminococcus uncl.
ASV_797-Coprococcus uncl.
ASV_878-Blautia obeum
ASV_906-Lachnospiraceae uncl.
ASV_1034-Flavonifractor uncl.
ASV_1304-Blautia uncl.
ASV_1331-Intestinimonas uncl.
ASV_1332-Pseudoflavonifractor uncl.
ASV_1533-Butyricimonas uncl.
ASV_2962-Pseudoflavonifractor uncl.
ASV_4971-Candidatus Saccharibacteria uncl.
UC
26
ASV_240-Blautia uncl.
ASV_368-Blautia uncl.
2
ASV_7-Bacteroides massiliensis
1
0
6
ASV_87-Flavonifractor plautii
ASV_442-Lachnospiraceae uncl.
2
18
Contr.
CD
ASV_95-Ruminococcaceae uncl.
ASV_321-Clostridiales uncl. uncl.
ASV_412-Romboutsia uncl.
ASV_420-Coprococcus uncl.
ASV_855-Blautia uncl.
ASV_1480-Clostridium IV uncl.
ASV_35-Parabacteroides distasonis
ASV_64-Oscillibacter uncl.
ASV_150-Faecalibacterium uncl.
ASV_198-Oscillibacter uncl.
ASV_244-Clostridium sensu stricto uncl.
ASV_249-Parasutterella uncl.
ASV_304-Faecalibacterium uncl.
ASV_314-Roseburia inulinivorans
ASV_315-Lachnospiraceae uncl.
ASV_387-Romboutsia uncl.
ASV_487-Firmicutes uncl.
ASV_620-Butyricicoccus uncl.
ASV_713-Coprobacter uncl.
ASV_718-Oscillibacter uncl.
ASV_867-Anaerostipes uncl.
ASV_988-Intestinibacter uncl.
ASV_1152-Allisonella uncl.
ASV_1347-Ruminococcaceae uncl.
PageRank
ASV_13-Escherichia/Shigella uncl.
ASV_22-Parasutterella uncl.
ASV_34-Parabacteroides uncl.
ASV_48-Collinsella aerofaciens
ASV_60-Collinsella aerofaciens
ASV_61-Alistipes uncl.
ASV_95-Ruminococcaceae uncl.
ASV_99-Alistipes indistinctus
ASV_167-Roseburia_faecis
ASV_308-Blautia uncl.
ASV_311-Parabacteroides uncl.
ASV_354-Blautia uncl.
ASV_411-Blautia uncl.
ASV_797-Coprococcus uncl.
ASV_799-Butyricimonas uncl.
ASV_846-Eggerthella lenta
ASV_903-Coprococcus uncl.
ASV_1069-Lachnospiraceae uncl.
ASV_1331-Intestinimonas uncl.
ASV_1332-Pseudoflavonifractor uncl.
ASV_1461-Fusicatenibacter uncl.
ASV_2604-Lachnospiraceae uncl.
ASV_4971-Candidatus Saccharibacteria uncl
UC
23
ASV_368-Blautia uncl.
ASV_648-Eggerthella lenta
2
ASV_64-Oscillibacter uncl.
ASV_150-Faecalibacterium uncl.
ASV_387-Romboutsia uncl.
3
2
ASV_6-Escherichia/Shigella uncl.
ASV_30-Enterobacteriaceae uncl.
3
ASV_87-Flavonifractor plautii
1
20
Contr.
CD
ASV_11-Bacteroides uncl.
ASV_77-Haemophilus parainfluenzae
ASV_321-Clostridiales uncl.
ASV_35-Parabacteroides distasonis
ASV_92-Romboutsia uncl.
ASV_130-Faecalibacterium uncl.
ASV_180-Phascolarctobacterium uncl.
ASV_197-Blautia uncl.
ASV_198-Oscillibacter uncl.
ASV_249-Parasutterella uncl.
ASV_304-Faecalibacterium uncl.
ASV_339-Escherichia/Shigella uncl.
ASV_412-Romboutsia uncl.
ASV_478-Romboutsia uncl.
ASV_615-Romboutsia uncl.
ASV_620-Butyricicoccus uncl.
ASV_643-Romboutsia uncl.
ASV_698-Lachnospiraceae uncl.
ASV_839-Faecalibacterium uncl.
ASV_953-Ruminococcus2 uncl.
ASV_1039-Romboutsia uncl.
ASV_1096-Romboutsia uncl.
ASV_1347-Ruminococcaceae uncl.
Eigencentrality
ASV_354-Blautia uncl.
ASV_368-Blautia uncl.
ASV_411-Blautia uncl.
ASV_696-Blautia uncl.
ASV_762-Lachnospiraceae uncl.
ASV_789-Coprococcus uncl.
ASV_846-Eggerthella_lenta
ASV_884-Olsenella uncl.
ASV_903-Coprococcus uncl.
ASV_906-Lachnospiraceae uncl.
ASV_929-Faecalibacterium uncl.
ASV_977-Romboutsia uncl.
ASV_1069-Lachnospiraceae uncl.
ASV_1145-Clostridium_XIVa uncl.
ASV_2604-Lachnospiraceae uncl.
UC
15
0
ASV_478-Romboutsia uncl.
1
1
ASV_387-Romboutsia uncl.
9
2
9
Contr.
CD
ASV 38-Haemophilus parainfluenzae
ASV 77-Haemophilus parainfluenzae
ASV 87-Flavonifractor plautii
ASV 88-Haemophilus uncl.
ASV 189-Haemophilus uncl.
ASV 197-Blautia uncl.
ASV 206-Blautia uncl.
ASV 220-Firmicutes uncl.
ASV 648-Eggerthella lenta
ASV_92-Romboutsia uncl.
ASV_412-Romboutsia uncl.
ASV_15-Dialister_invisus
ASV_339-Escherichia/Shigella uncl.
ASV_599-Veillonella uncl.
ASV_606-Blautia uncl.
ASV_615-Romboutsia uncl.
ASV_643-Romboutsia uncl.
ASV_650-Romboutsia uncl.
ASV_1039-Romboutsia uncl.
ASV_1096-Romboutsia uncl.

Figure S12: Overlapping and private ASVs with significant network positions among health condition specific networks. Centralities in the control cohort were based on the average of three independent

network calculations on three subsamples of the respective cohort to balance differences in cohort sizes (Table S12).

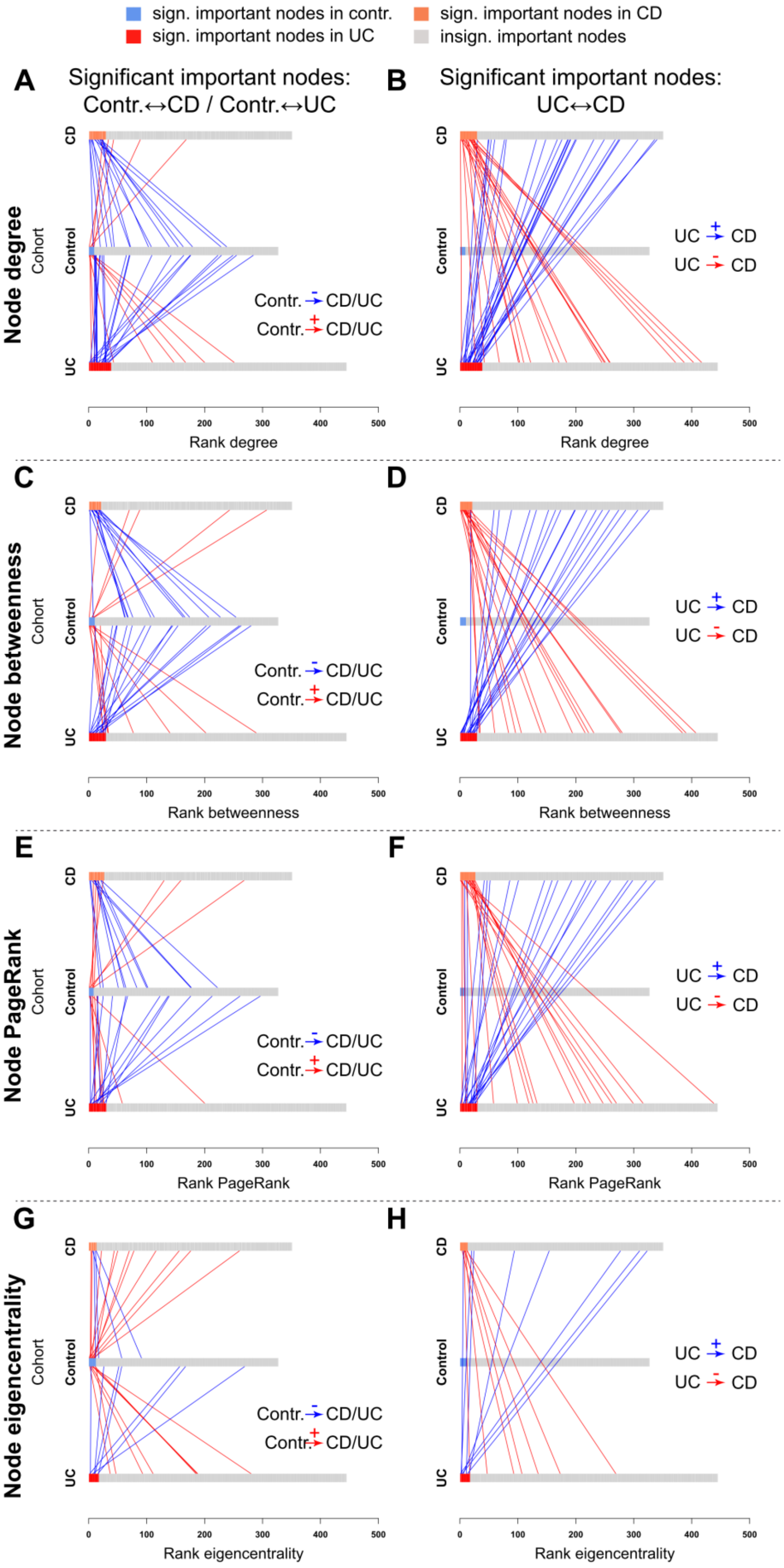
sign. important nodes in contr.
sign. important nodes in CD
sign. important nodes in UC
insign. important nodes
A
Significant important nodes:
Contr.↔CD / Contr.↔UC
B
Significant important nodes:
UC↔CD
Node degree
Cohort
CD
Control
UC
Contr. → CD/UC
Contr. → CD/UC
UC → CD
UC → CD
Rank degree
C
D
Node betweenness
Rank betweenness
E
F
Node PageRank
Rank PageRank
G
H
Node eigencentrality
Rank eigencentrality
0
100
200
300
400
500
-
+

Figure S13: The parallel coordinate plots illustrate the change of positions of significantly central bacteria from one health condition to the other. Single bars visualize nodes ordered by their ranked importance within the respective networks, based on **(A, B)** node degree, **(C, D)** node betweenness, **(E, F)** PageRank index, and eigenvalue centrality **(G, H)**. The first bar shows taxa ranked by their importance in CD specific networks, the second bar taxa ranked by average node importance in networks based on the cohort, and the third bar taxa ranked by their importance in UC specific networks. The change in network position/importance is indicated by lines connecting the same ASVs/nodes between the respective networks/bars. Plots **A, C, E, G** display significant nodes and their respective position/rank in Contr. and CD networks, or Contr., and UC networks. Change of taxa, which increase in importance (smaller rank) when found in a diseased community as compared to the controls is shown in blue, while taxa decreasing in importance (increased rank) in a diseased community are shown in red. Plots **B, D, F, H** display significant nodes and their change in position between CD and UC, with increases of importance (decreasing rank) from CD to UC highlighted in blue, while increases of importance from UC to CD are highlighted in red. Centralities in the control cohort were based on the average of three independent network calculations on three subsamples of the respective cohort to balance differences in cohort sizes.

Supplemental tables

Table S1: Results of differential ASV abundance tests via negative binomial generalized linear models (via *DESeq2,* LR test) with respect to IBD condition and smoking status, corrected for age and BMI. P-values were corrected for multiple testing via FDR-adjustment. ASVs are ordered by the cohort with their maximum abundance and overlaps with significant indicator species are shown. ASVs highlighted in grey are associated with more than one variable.

Table S2: Indicator species analysis abundance analyses of ASVs with respect to IBD- and smoking status ($P \leq 0.05$). ASVs are ordered by their association and overlaps with significant differentially abundant ASVs are highlighted.

Table S3: Differential abundance analyses of ASVs via *DESeq2* (LR test) with respect to IBD subtypes corrected for age and BMI ($P_{FDR} \leq 0.05$). ASVs are ordered by the cohort with their maximum abundance and overlaps with significant indicator species are shown. Subtypes follow the Montreal classification [13].

Table S4: Indicator species analysis abundance analyses of ASVs with respect to IBD subtypes ($P \leq 0.05$). ASVs are ordered by their association and overlaps with significant differentially abundant ASVs are highlighted. Subtypes follow the Montreal classification [13].

Table S5: Differential abundance analyses of ASVs via DESeq2 (LR test) with respect to medication with non-IBD related medication and prior antibiotic usage (within the last 6 weeks, within the last 6 months) corrected for age and BMI ($P_{FDR} \leq 0.05$). ASVs are ordered by the cohort with their maximum abundance and overlaps with significant indicator species are shown.

Table S6: Indicator species analysis abundance analyses of ASVs with respect to medication with non-IBD related medication and prior antibiotic usage (within the last 6 weeks, within the last 6 months) ($P \leq 0.05$). ASVs are ordered by their association and overlaps with significant differentially abundant ASVs are highlighted.

Table S7: Analysis of alpha diversity differences in CD and UC patients focusing on non-IBD related pharmaceutical treatments.

Table S8: Betadiversity analysis of CD and UC patients focusing on community variability with respect to anthropogenic and disease related variables, as estimated via a multivariate and permutative version of the Levene's test for homogeneity of variances.

Table S9: Betadiversity analysis of CD and UC patients focusing on anthropogenic and disease related variables (PERMANOVA).

Table S10: Analyses of betadiversity between CD and UC subtypes following the Montreal classification [13] (PERMANOVA).

Table S11: Differential abundance analyses of predicted functions (EC categories) via *DESeq2* (Wald test) with respect to health condition, corrected for age and BMI ($P_{FDR} \leq 0.05$). Functions are ordered by the

cohort with their maximum abundance. Each pairwise comparison is shown and only significant differences were evaluated for the direction of change as indicated by their maximum abundance.

Table S12: Differential abundance analyses of predicted pathways (MetaCyc pathways[14]) via *DESeq2* (Wald test) with respect to health condition, corrected for age and BMI ($P_{\mathrm{FDR}} \leq 0.05$). Pathways are ordered by the cohort with their maximum abundance. Each pairwise comparison is shown and only significant differences were evaluated for the direction of change as indicated by their maximum abundance.

Table S13: Overview of significantly central ASVs in disease specific ASV correlation networks for healthy controls (average of 3 subsets), CD- and UC patients. The number of connections (degree), their position between different nodes (Betweenness) and their general importance (Eigenvector-centrality and PageRank) were used measures of centrality and their significance was determined by a one-sided Z-Test of the observed values against a null distribution of 10’000 permuted networks. Associations to different anthropometric characteristics, as detected via indicators species analyses ($P \leq 0.05$) and differential abundance analyses ($P_{\mathrm{FDR}} \leq 0.05$), are included in the table. The color-code highlights in which networks ASVs are repeatedly important (🟩-important across all health conditions, 🟦-important only in control networks, 🟧**-**important only in the CD network, 🟥-important only in the UC network).

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