

1 Attention Deficit Hyperactivity Disorder and the gut 2 microbiome: An ecological perspective 3

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32 **Abstract**

33

34 Attention Deficit Hyperactivity Disorder (ADHD) is an increasingly prevalent
35 neuropsychiatric disorder characterized by hyperactivity, inattention, and impulsivity.
36 Symptoms emerge from underlying deficiencies in neurocircuitry, and recent research
37 has suggested a role played by the gut microbiome. The gut microbiome is a complex
38 ecosystem of interdependent taxa with an exponentially complex web of interactions
39 involving these taxa, plus host gene and reaction pathways, some of which involve
40 neurotransmitters with roles in ADHD neurocircuitry. Studies have analyzed the
41 ADHD gut microbiome using macroscale metrics such as diversity and composition,
42 and have proposed several biomarkers. Few studies have delved into the complex
43 underlying dynamics ultimately responsible for the emergence of such metrics, leaving
44 a largely incomplete, sometimes contradictory, and ultimately inconclusive picture.

45

46 We aim to help complete this picture by venturing beyond taxa *abundances* and into taxa
47 *relationships* (i.e. cooperation and competition), using a publicly available gut
48 microbiome dataset from 30 Control (15 female, 15 male) and 28 ADHD (15 female, 13
49 male) undergraduate students. We conduct our study in two parts. We first perform
50 the same macroscale analyses prevalent in ADHD gut microbiome literature (diversity,
51 differential, biomarker, and composition) to observe the degree of correspondence, or
52 any new trends. We then estimate two-way ecological relationships by producing
53 Control and ADHD Microbial Co-occurrence Networks (MCNs), using SparCC
54 correlations ($p < 0.01$). We perform community detection to find clusters of taxa
55 estimated to mutually cooperate along with their centroids, and centrality calculations
56 to estimate taxa most vital to overall gut ecology. We conclude by summarizing our
57 results, and provide conjectures on how they can guide future experiments, some
58 methods for improving our experiments, and general implications for the field.

59

60 **Introduction**

61

62 Attention Deficit Hyperactivity Disorder (ADHD) is a significant mental health problem
63 with a current 3.4% prevalence worldwide (1). In the United States, ADHD affects one
64 in 10 children (a 43% increase over the last 15 years) (2), and 3 -16% of adults (3) with
65 that percentage increasing over the past 20 years. Individuals with ADHD face many
66 practical challenges, including risk for low academic achievement, lower employment
67 status, and incarceration (4). Symptoms of hyperactivity, impulsivity, and inattention
68 characterize ADHD (5). Underlying ADHD behavioral symptoms are deficits in the
69 neurocognitive mechanisms of both executive function (EF) and emotional regulation
70 (ER) (6), including and extending beyond prefrontal-striatal networks (7). EF refers to a
71 set of cognitive control processes, includes one's ability to focus on relevant information
72 while suppressing irrelevant distractors. ER generally ascribes to one's ability to
73 effectively cope with emotionally charged circumstances (both negative and positive).
74 Many medications have been developed to combat the disorder by influencing the
75 underlying neurocircuitry (8).

76

77 The pathogenesis of ADHD is thought to be multifactorial, with heritability estimates at
78 roughly 70-90% (9). These genetic connections suggest some dependency on underlying
79 metabolic reactions, directly or indirectly involving gene products. In the meantime, the
80 new and exciting field of microbiome research has made its way into the mental health
81 domain. Our gut is home to a plethora of bacteria, fungi, and other microbial
82 organisms, whose collective genomes comprise our gut *microbiome*. Studies estimate
83 that the average number of bacterial cells in humans matches or exceeds that of host
84 cells (10,11). Each bacterium has unique genetic material that produces different sets of
85 metabolites, which interact with each other and host metabolites downstream (12),
86 creating a complex host-microbiome web of interactions. It has become increasingly
87 important to pay attention to the symbiotic relationship between the gut microbiome

88 and brain development and function, often referred to as the *gut-brain-microbiome axis*
89 (13). This axis is a bidirectional communication network, providing gut microbiota and
90 metabolites an avenue for influencing brain development and function (14-18). One
91 proposed mechanism through which gut microbiota may affect our neurobiology is by
92 altering the levels of *neurotransmitters*, including dopamine and serotonin (5-HT) (19),
93 which fuel brain regions that mediate cognition and emotion. Although serotonin is
94 also produced in the brain, up to 90% of serotonin is synthesized in the gut (20).

95 Connections between the gut microbiome and neurotransmitters, EF/ER, and
96 *neuropsychiatric disorders (NPDs)* characterized by EF/ER disorders are already well-
97 established. In rodents, anxiety and social behavior have been linked to the gut
98 microbiome that can be attributed to altered neurotransmission in the hippocampus
99 and amygdala (21). In humans, associations between microbiome composition and ER
100 have been shown (18). It has also been established that the gut microbiome can release
101 dopamine and 5-HT, impacting ER (22,23). Connections on the cognitive axis related
102 to EF are less well-established in humans, though some theories are beginning to
103 emerge (24). In humans, dopamine influences EF (25). In rodents, the gut microbiome is
104 linked to dopamine (26), and EF-like behavior (27). The Autism Spectrum Disorder
105 (ASD) (28), which is associated with impaired EF (29), has been linked to the gut
106 microbiome (30). In animal studies, the gut microbiome has been associated with
107 anxiety-related disorders such as depression (31-36). People with stress-related
108 diseases have responded positively to probiotics (37,38). Connections between the gut
109 microbiome and another neuropsychiatric disorder (NPD) characterized by EF/ER
110 dysfunction such as ADHD would further support the impact of the gut microbiome on
111 EF/ER. It could also help to explain the large amount of symptomatic overlap that
112 exists between ADHD with other NPDs, particularly ASD (39-41), and could even
113 provide differentiating factors (42) to help address the current diagnosis challenges due
114 to this overlap (43), and new potential options for treatment (44). The fact that
115 individuals with ADHD suffer from gastrointestinal (GI) dysfunction, including

116 childhood digestive difficulties and low-grade inflammation (45) as well as constipation
117 (46,47), only further suggests a potential role of the gut microbiome in this disorder.

118 There are limited studies that implicate the gut microbiome on clinically diagnosed
119 ADHD, and recent efforts have been made to survey and summarize their results (48-
120 51). Two in particular published this year (49,50) contained findings from every
121 published study involving ADHD and the gut microbiome. Based on this, we make the
122 following observations about the current state of ADHD and gut microbiome research:

123 **1. Diversity results are contradictory and inconclusive.** Even with closely age-matched
124 gut microbiome studies using the same Shannon index (52) to measure alpha-diversity,
125 one set (mean age 11.9 years) revealed a lower level of alpha-diversity in ADHD
126 patients (53), another (mean age 9.3 years) revealed higher alpha-diversity (54), a third
127 (ages 6-10) reported no difference at all (55), and a fourth (10- and 15-year-olds) (56)
128 reported higher alpha-diversity in ADHD 15-year-olds, but no difference in ADHD 10-
129 year-olds. Within these same four studies, the first (53) reported a beta-diversity
130 difference between ADHD and Control, while the other three reported no difference
131 (54-56). With a mean age only slightly higher (20.2), a fifth study found no alpha-
132 diversity difference, but a beta-diversity difference (57).

133 **2. Many biomarkers have been proposed, some contradictory, others mixed depending
134 on taxonomic level, and others inconclusive.** Proposed ADHD biomarkers include:
135 increased *Collinsella* (58) (phylum Actinobacteria), increased *Fusobacterium* (54)
136 (Fusobacteria), decreased *Lachnospiraceae* (59), *Lactobacillus* (54,60), and *Ruminococcus*
137 *gnavus* (59) (all Firmicutes), decreased *Prevotella/Porphyromonadaceae* (53) and increased
138 *Paraprevotella xylaniphila*, *Odoribacteriaceae* and member species *Odoribacter splanchnicus*
139 (59) (all Bacteroidetes), decreased *Haemophilus* (57) and increased *Neisseria* (53),
140 *Sutterella stercoricanis* (54), and *Desulfovibrio* (61) (all Proteobacteria).

141 More mixed results have been reported with respect to the following taxa:

142 **Clostridiales (Firmicutes).** This order (57) was reported as increased in studies
143 involving ADHD children and adolescents, but another study involving 18-24 month-
144 olds (60) found members of this order as lower.

145 **Ruminococcaceae (Firmicutes).** This family was reported as elevated in ADHD by one
146 study (57) along with member genus *Ruminococcus*, but member genus *Faecalibacterium*
147 was reported as reduced in two others (55,59).

148 **Veillonellaceae (Firmicutes).** Within the same study (59), family *Veillonellaceae* and
149 genus *Veillonella* were reduced in ADHD, but member species *V. parvula* was elevated.

150 **Bacteroidaceae (Bacteroidetes).** *Bacteroidaceae* was found as elevated in ADHD by one
151 study (53), member genus *Bacteroides* was reduced in another among 18-month-olds
152 (60), member species *B. uniformis* (54), *B. ovatus* (54) and *B. coccace* (59) were all reported
153 as elevated, and member species *B. coprocola* was reported as reduced (54).

154 For one particular taxon, results have been contradictory:

155 ***Bifidobacterium* (Actinobacteria).** Perhaps no greater mystery currently exists than the
156 role of genus *Bifidobacterium*. One Dutch study found a nominal increase in
157 *Bifidobacterium* with average ADHD and Control subject ages of 19.5 and 27.1 years,
158 respectively (62). A longitudinal study (3 months, six months and 13 years) made a
159 somewhat contradictory observation of reduced *Bifidobacterium* during infancy, but not
160 at age 13 (60). A third study (58) reported reduced *Bifidobacterium* (specifically *B.*
161 *longum* and *B. adolescentis*) in ADHD children (mean age: 9.3) that actually reversed
162 after micro-nutrient treatment, where elevated *Bifidobacterium* was observed at high
163 ADHD-Rating Scale IV (ADHD-RS-IV, (63)) scores.

164 The current picture of the role played by the gut microbiome in ADHD is therefore still
165 unclear. Most of the effort to connect ADHD to the gut microbiome has involved (1)
166 macroscale population metrics such as diversity, and/or (2) taxa abundances. These
167 properties are in reality emergent from a complex and interdependent interaction web
168 involving taxa, their gene products, and those of the host (64). Diversity and abundance

169 therefore ignore many underlying details behind their measurements, helping to
170 explain the current incomplete picture. Venturing deeper into this web is critical to
171 completing more of this picture. Two studies have attempted this task, both using
172 multi-omics. One (59) reported differences ADHD neurotransmitter pathways. A
173 second (62) uncovered a connection between *Bifidobacterium* and cyclohexadienyl
174 dehydratase (CDT) abundances.

175 We have thus far only scratched the surface of this large and exponentially complex
176 interaction web, and every completed piece has value. Multi-omics will continue to be
177 critical, bridging an important gap between taxa, products, and metabolic reactions. We
178 aim to complete another piece, that involves *ecological relationships* between taxa.
179 Microbial taxa have been shown to demonstrate a wide variety of ecological
180 relationships, including cooperation (65,66) and competition (67), that ultimately impact
181 collective functionality of the ecosystem and macroscale properties (64). We estimate
182 these relationships for Control and ADHD datasets and report results; including
183 relationships, communities, driver taxa (or 'centroids') of these communities, and taxa
184 central to overall gut ecology. Results can offer guidance on potential taxa to target for
185 further multi-omics or laboratory experiments. The ultimate goal is to increase depth
186 of knowledge about connections between the influence of the gut microbiome on an
187 NPD that impacts millions of individuals worldwide.

188 This work involves two parts, conducted on a publicly available, gender-matched
189 dataset of 16S gut microbiome sequences. The first involves performing the same
190 macroscale analyses currently prevalent in ADHD gut microbiome literature, to note
191 how this dataset compares, as well as any new and interesting trends. Metrics will
192 include alpha- and beta-diversity, Sparse Partial Least Squares Discriminant Analysis
193 (sPLS-DA, (68)) to estimate Control and ADHD differentiation degree, biomarker
194 analysis using Linear discriminant analysis Effect Size (LEfSe, (69)), and QIIME (70)
195 normalized abundance compositional profiles.

196 In the second part we estimate ecological relationships (71) within Control and ADHD
197 gut microbiomes. We first use Microbial Co-occurrence Networks (MCNs, (72)) to
198 estimate these relationships (73), and then perform cluster analysis using the Affinity
199 Propagation (AP, (74)) algorithm to discover communities of mutually supporting taxa,
200 as well as driver or ‘centroid’ nodes of these communities. Finally we perform
201 centrality analysis using the Ablatio Triadum (ATria, (75)) algorithm, to estimate taxa
202 most significant to the overall ecosystem.

203

204 **Materials and Methods**

205

206 We provide more details on the methods we use for analysis. Our entire downstream
207 analysis pipeline has been built using Plugin-Based Microbiome Analysis (PluMA, (76))
208 and is available for download within its publicly available pipeline pool.

209

210 **Cohort**

211 We start from a publicly available dataset (Accession Number: PRJNA656791) of gut
212 microbiome samples from an undergraduate student population. Full sequencing
213 details are provided in the BioProject description; 16S rRNA (V3-V4 region) sequencing
214 was used, following steps corresponding to standard Illumina protocols (77). Each
215 deidentified sample provides gender and ADHD assessment based on Adult ADHD
216 Self Report Scale (ASRS) score (Control, ADHD Combined, ADHD Inattentive, or
217 ADHD Hyperactivity) in its title. For both subscales they used an ASRS score of 17 as
218 an ADHD threshold, which also follows published practices (78). The project released
219 58 samples: 30 Control and 28 ADHD, with 15 females in both groups. We summarize
220 statistics in Table 1. Of the ADHD cohort, 17 were ADHD-combined (inattentive and
221 hyperactive), five ADHD-hyperactive, and six ADHD-inattentive (Table 1). Analyzed
222 with a *t*-distribution, we found no significant impact of gender ($p > .2$).

223

Table 1. Cohort analyses

Groups	Total No	Gender	Total No	Inattentiveness	Hyperactivity	Combined
				ASRS Scores \pm SD*		
Control	30	Female	15	11.2 \pm 4.3	9.6 \pm 4.9	20.8 \pm 7.2
		Male	15	9.8 \pm 3.4	9.9 \pm 4.4	19.7 \pm 6.8
ADHD	28	Female	15	22.6 \pm 6.8	19.5 \pm 5.3	42.1 \pm 10.1
		Male	13	18.2 \pm 3.8	17.3 \pm 4.7	35.5 \pm 6.1

* *t*-test revealed no significant impact of gender ($p > .2$).

To establish an initial set of taxa we took these sequences and compiled, clustered, and analyzed them using QIIME 1.9.1 (70), (similarity threshold of 97%, GreenGenes reference database (79)). We removed all singletons and scarce taxa (present in less than 50% of the samples) for both groups to produce our final set for analysis.

224

225 **Part I: Traditional Macroscale Analyses**

226 We first perform macroscale analyses on this ADHD dataset that have been performed
227 on other ADHD datasets, compare and contrast our results with those in the literature,
228 and take note of any new and interesting observations.

229 **Diversity analysis.** Alpha- and beta-diversity plots were constructed using
230 QIIME (version 1.9.1), with default metrics: observed_species (unique taxa count),
231 Chao1 (80), and PD_whole_tree (phylogenetic diversity), and default parameters.

232 **Discriminant analysis.** Our study uses Sparse Partial Least Squares
233 Discriminant Analysis (sPLS-DA, (68)), a sparse version of the Partial Least Squares
234 (PLS, (81)) method, as a supervised method for determining differentiation degree with
235 respect to taxa relative abundance (82).

236 **Biomarker analysis.** Our biomarker analysis used the Linear discriminant
237 analysis Effect Size (LEfSe, (69)) algorithm ($p < 0.05$, LDA effect size > 2).

238 **Compositional analysis.** We use QIIME 1.9.1 (70) to generate compositional bar
239 graphs, producing one bar per sample broken down by taxa percentages.

240

241 **Part II: Ecological Relationships**

242 **Co-occurrence network analysis.** We computed correlations based on taxa
243 relative abundances using SparCC (83) ($p < 0.01$), and built Microbial Co-occurrence
244 Networks (MCNs, (72)) using taxa as nodes and correlations as edges. MCNs were
245 visualized using Cytoscape (84) with layout produced by Fruchterman-Reingold (85).

246 **Clustering.** MCNs were clustered using Affinity Propagation (AP, (86)). AP has
247 been shown to operate efficiently and successfully on signed and weighted biological
248 networks without requiring an initial cluster count estimate, and additionally computes
249 the most representative or *centroid* node for each cluster.

250 **Centrality analysis.** We use Ablatio Triadium (ATria, (75)) for evaluating the
251 importance, or *centrality*, of taxa in our MCNs. ATria computes centrality for signed
252 and weighted networks through a modified economic payment model (87) that
253 calculates the influence of a node on all other nodes. ATria provides an alternative
254 perspective by considering relationships (not relative abundance) when computing
255 centrality, and unlike biomarker analysis does not compare sample sets. ATria
256 produces a ranked list of important taxa and runs iteratively; once a taxon is found as
257 central, ATria removes this taxon and its dependencies using social network theory (88).
258 Then it runs again to produce the next most important taxon, repeating until no edges
259 are left. Taxa not found as important are simply not ranked.

260

261 We analyze these ecological relationship at all taxonomic levels starting from phylum.
262 We first observe the upper three levels (phylum, class, and order) for an overview of
263 relationships between consistently abundant taxa. We then move to the lower three

264 levels (family, genus and species) which provide a finer level of granularity and enough
265 taxa to perform meaningful community analyses.

266

267

268 **Results**

269

270 **Part I. Traditional Macroscale Analyses**

271

272 **Diversity.** QIIME (70) alpha- and beta-diversity results produced no conclusive
273 differences between ADHD and Control (Fig. 1). Although Fig. 1A shows a marginal
274 Alpha diversity increase for ADHD using all three metrics: observed_species (count of
275 unique taxa), Chao1 (80), and PD_whole_tree (phylogenetic diversity), error bars clearly
276 indicate inconclusive results. Beta-diversity with unweighted and weighted Unifrac
277 (89) distance also shows no separation (Fig. 1B). This lack of alpha- and beta- diversity
278 differences matches several results from other datasets (55,56,62).

279

280 **Fig. 1. Alpha- and Beta- Diversity.** (a) Alpha-diversity of Control and ADHD samples
281 using (in order) the count of unique taxa, Chao1 richness (80), and phylogenetic
282 diversity. (b) Beta-diversity of Control and ADHD samples computed using
283 unweighted and weighted Unifrac (89) distance.

284

285

286 **Discriminant.** Discriminant analysis determines differentiation degree between
287 datasets, accounting for all variables in each set (90). Unsupervised and supervised
288 approaches can be used, with supervised having prior sample classification knowledge
289 (i.e., Control or ADHD). One ADHD gut microbiome study (53) attempted the
290 unsupervised method non-parametric multi-dimensional scaling (NMDS, (91)), but
291 could not differentiate the two groups. Limited studies have further decomposed
292 ADHD samples by subscale but these focus on diversity and composition, noting

293 inattention (elevated *Dialister* and reduced *Phascolarctobacterium* (57)) and hyperactivity
294 (lower alpha-diversity and elevated *Parabacteroides* (53)) properties.

295
296 We attempt the supervised Sparse Partial Least Square Differential Analysis (sPLS-DA,
297 (68)), with taxa relative abundances as variables. Fig. 2(a)-(b) (ellipse confidence
298 level=95%) shows even a supervised method cannot differentiate the groups, in general
299 or by subscale. This is significant, as supervised approaches like sPLS-DA have *a priori*
300 sample category knowledge and can sometimes differentiate completely random data
301 (82). sPLS-DA did differentiate the two sets with scarce taxa present, showing some
302 separation between Control, ADHD samples high on one subscale, and ADHD samples
303 high on both (Fig. S1). However, a supervised method differentiating the sets only
304 when scarce taxa (present less than half of the time) are counted shows very little.

305
306 **Fig. 2. Discriminant Analysis.** Results of running sPLS-DA (68) on microbiome
307 abundance data (ellipse confidence level 95%). The figures show the analyses (a)
308 comparing Control (orange) and ADHD (blue) groups and (b) further separating the
309 ADHD group into inattention (green), hyperactive (grey), and combined (blue).

310
311
312 **Biomarker.** When performing LEfSe (69), we returned to a single ADHD set (no
313 subscale split) to ensure roughly level sample counts with Control. Results are shown
314 both as a cladogram (Fig. 3A) and a bar graph (Fig. 3B). LEfSe has identified orange taxa
315 as Control biomarkers, and purple taxa as ADHD biomarkers.

316
317 **Fig. 3. Differential Abundance.** Distinguishing taxa for Control (orange) and ADHD
318 (purple) groups, produced by LEfSe (69). Corresponding phyla for each taxon are
319 indicated in parentheses, with B=Bacteroidetes, F=Firmicutes, and P=Proteobacteria (no
320 distinguishing Actinobacteria were found). (a) Distinguishing taxa plotted on a
321 cladogram, with each concentric circle representing a phylogenetic classification level
322 (innermost=phylum). Shared areas represent distinctive regions of the phylogenetic
323 tree. (b) Distinguishing taxa ordered by Linear Discriminant Analysis (LDA, (92)). A
324 higher magnitude indicates more reliable differentiation.

325

326

327 The cladogram (Fig. 3A) shows proposed biomarkers on the phylogenetic tree,
328 highlighting those closely related. Fig. 3A shows one Bacteroidetes family
329 (*Odoribacteriaceae*) distinguishing ADHD, while Firmicutes (*Turicibacteriaceae* and its
330 order *Turicibacteriales*) and Proteobacteria (*Pasteurellaceae* and its order *Pasteurellales*)
331 distinguished Control. The bar graph (Fig. 3B) uses Linear Discriminant Analysis
332 (LDA, (92)) to order by differentiation degree, expanding to include genera and species.
333 ADHD continues to be predominated by Bacteroidetes and includes the only two
334 *Odoribacteriaceae* genera in our samples, *Odoribacter* and *Butyricimonas*, supporting
335 earlier claims of *Odoribacteriaceae* as an ADHD biomarker (59). Control continues to be
336 predominated by Firmicutes (now including *Turicibacter*) and Proteobacteria (now
337 including *Haemophilus* and *H. parainfluenzae*).

338

339 *Haemophilus* was found as a Control biomarker by another study (57). *H. parainfluenzae*,
340 the only *Haemophilus* species present, is a well-known lung pathogen (93), though its
341 gut functionality remains largely unknown. Its elevated Control abundance relative to
342 ADHD is indeed mysterious, though upon further inspection is still very low (< 0.1%).

343

344 *Turicibacter*, although never previously reported as a biomarker in an ADHD gut
345 microbiome study, has been reported in one involving depression in mice (33).
346 Metabolically in mice, *Turicibacter* signals the gut to produce serotonin (5-HT) (94),
347 which influences ER (95). Both ADHD and depression are characterized by ER
348 neurocircuitry deficiencies. LEfSe did not report any EF-associated biomarkers. This
349 may be largely because EF is more strongly regulated by dopamine (95), for which the
350 gut only produces roughly 50% (96), compared to 90% of 5-HT (20).

351

352 **Compositional.** Compositional analyses compare taxa relative abundances (97). We
353 generated compositional bar charts at all phylogenetic tree levels beginning with

354 phylum (Fig. 4A). Samples on the x -axis are ordered by increasing ASRS score, and the
355 y -axis represents relative abundance.

356

357 **Fig. 4. Compositional Analysis, Phylum and Genus Levels.** Microbial compositional
358 bar graph for each subject, generated using QIIME (70), conducted at (a) the phylum
359 level and (b) the genus level. Subjects are ordered by increasing Adult ADHD Self
360 Report Scale (ASRS) score, with the y-axis representing relative abundance.

361

362

363 A typical gut microbiome profile (98) is observed, dominated by Firmicutes and
364 Bacteroidetes, followed by Actinobacteria and Proteobacteria. Control has slightly
365 elevated Firmicutes (70-66%), mirroring an earlier study (62) that importantly (99) also
366 sequenced the same 16S V3-V4 region. Slightly contrary to this same study, which
367 reported this difference to be largely occupied by an ADHD Actinobacteria increase,
368 ours was mostly occupied by an ADHD Bacteroidetes increase (from 22% to 25%). Yet
369 Actinobacteria remains mysterious in Fig. 4A, elevated at very high ASRS scores, but
370 also at very low scores. Bacteroidetes and Proteobacteria also appear reduced at these
371 same extremes. These seemingly contradictory results create challenges in drawing
372 meaningful conclusions with respect to role(s) played by these phyla. Yet they capture
373 our interest, especially given the earlier reported anomalous behavior of an
374 Actinobacteria genus, *Bifidobacterium*, at high and low ASRS-IV scores (58).

375

376 Class and order levels produced bar charts similar to Fig. 4A; we include these as
377 Supplemental Fig. S2 and S3. Levels below order often had too many taxa to clearly
378 view dynamics. We include the genus level (Fig. 4B), family and species as
379 supplemental Fig. S4 and S5), as the genus level includes *Bifidobacterium*. And indeed, it
380 turns out, *Bifidobacterium* (blue, bottom) has elevated abundances high and low ASRS
381 scores, appearing most responsible for this same behavior in its phylum Actinobacteria
382 (Fig. 4A). *Bacteroides* (orange, middle) is a highly abundant taxon that also mirrors the
383 behavior of its phylum (Bacteroidetes, Fig. 4A), increasing in the middle and decreasing

384 at extremes. *Proteobacteria* is more difficult to observe given its low relative abundance
385 (1-2%), though *Sutterella* (lilac, top) also appears to follow this trend. All three
386 observations are verified in supplementary Fig. S6.

387

388 This is not the first time these taxa have generated interest. Many *Actinobacteria*, and
389 especially *Bifidobacterium*, have been used as probiotics and are considered elements of a
390 health gut (100-105). As discussed, *Bacteroides* and its family *Bacteroidaceae*, as well as
391 several member species, have been reported differentially abundant in ADHD
392 (53,54,59,60); some elevated, others reduced. Some have argued *Bacteroides* to be the
393 most important "window" to understanding the human gut (106). *Sutterella stercoricanis*
394 was also reported as an ADHD biomarker (54). These same taxa make multiple
395 appearances in studies involving other NPDs as well. *Bacteroidaceae* was the top LEfSe
396 Major Depressive Disorder (MDD) biomarker in one study (36). Another reported
397 elevated *Bacteroides* and reduced *Bifidobacterium* in anxiety (107). *Sutterella* is elevated in
398 Autism Spectrum Disorder (ASD) (108), a condition with so much symptomatic overlap
399 with ADHD that an ASD+ADHD phenotype has been established (109).

400

401 **Discussion.** These analyses produced a few interesting preliminary observations, but
402 their birds-eye view limited the depth we could pursue. Compositional analysis was a
403 perfect example: even though there was a visible trend between ASRS score and
404 *Bifidobacterium*, *Bacteroides*, and *Sutterella* abundances, no definitive conclusions could be
405 produced. Fundamentally macroscale behaviors emerge from microscale interactions.
406 We attempt to unlock some of these mysteries by now exploring *ecological relationships*.

407

408 Microbial ecological relationships take many forms. They can be positive or negative,
409 mutual (cooperation (65,66) or competition (67)) or one-way (commensalism (110) or
410 amensalism (111)). In particular, two-way relationships (cooperation and competition)
411 can be approximated using correlations (73). We use SparCC (83) compute correlations,
412 which has advantages in reducing compositional effects within relative abundances.

413 We also use a *p*-value threshold of 0.01 to only count correlations with the highest
414 confidence, as the historically accepted threshold of 0.05 has come under recent
415 question (112,113). We build Microbial Co-occurrence Networks (MCNs, (72)) using
416 taxa as nodes and SparCC correlations as edges, and perform community detection on
417 these networks using the clustering algorithm Affinity Propagation (AP, (74)). Finally,
418 we use Ablatio Triadium (ATria, (75)) as a centrality algorithm to produce a ranked list
419 of important taxa in each MCN. ATria is specifically designed for signed and weighted
420 networks, incorporating both social network (88) and economic theory (87) in its
421 calculations. It is also iterative, removing dependencies of a central node before
422 computing the next most central.

423

424 During our analyses we sometimes use “cooperation” to refer to a positive SparCC
425 correlation and “competition” when referring to a negative. We emphasize, however,
426 that correlations are an *estimate* of ecological relationships, that ultimately require
427 further downstream analysis (multi-omics and experimental verification) before
428 establishing official conclusions. With the underlying web of interactions being
429 exponentially complex and large-scale laboratory experiments potentially costly, our
430 results can provide guidance regarding target taxa and avenues to pursue.

431

432 **Part II. Ecological Relationships**

433

434 **Upper Levels: Phylum, Class, and Order.** Fig. 5 shows MCNs at the phylum (Fig. 5A-B),
435 class (Fig. 5C-D), and order (Fig. 5E-F) levels. Taxa (nodes) in all MCNs are colored by
436 phylum (legend at the bottom of Fig. 5). Node size is proportional to relative
437 abundance (larger=higher). Correlation (edge) color represents sign; green indicates
438 positive (est. cooperation) and red indicates negative (est. competition). Edge thickness
439 is proportional to correlation magnitude (thicker=stronger). Networks are visualized
440 using the Fruchterman-Reingold algorithm (85), which spatially orients nodes based on
441 edge weight (closer=more positive). Nodes are labeled with their taxon and provided

442 with ATria centrality ranking if found important (format: #rank, T=Tie). At the phylum
443 level only (Fig. 5A-B), we label each edge with its correlation value. Phylum-level
444 MCNs (Fig. 5A-B) show SparCC appears to handle compositional effects well, as
445 despite collectively encompassing about 95% of both populations, Firmicutes and
446 Bacteroidetes are only weakly negatively correlated.

447

448 **Fig. 5. Upper-Level Microbial Co-occurrence Networks (MCNs).** MCNs at the
449 phylum (A), class (B), and order (C) taxonomic levels, visualized using Cytoscape [57],
450 and oriented by Fruchterman-Reingold [58]. Nodes represent taxa, colored by phylum
451 with size directionally proportional to abundance. The co-occurrences are distinguished
452 by those that co-habit (green edges) and co-avoid (red edges). SparCC (83) correlation
453 ($p=0.01$) was used as edge weight and also the parameter for Fruchterman-Reingold
454 when determining edge length (larger=closer). SparCC correlations are shown at the
455 phylum level. All taxa found as important by ATria are denoted by a pound sign (#)
456 followed by its rank (ties indicated).

457

458

459 Table 2 shows every correlation in all three MCNs, and its sign, + (green) or - (red).
460 Correlations that appear only in Control are highlighted orange, only in ADHD
461 highlighted purple, and in both highlighted grey. Correlations at each taxonomic level
462 are grouped by their next highest level classification; for example in row 1: phyla
463 Actinobacteria and Bacteroidetes were negatively correlated in both phylum-level
464 MCNs (Fig. 5A-B), member classes Actinobacteria and Bacteroidia were negatively
465 correlated only in Control (Fig. 5C), as were member orders Bifidobacteriales and
466 Bacteroidales (Fig. 5E). White, italicized correlations were not present in either MCN,
467 but a correlation among descendants was; for example in row 3: phyla Actinobacteria
468 and Firmicutes were not correlated in either MCN, nor were member classes
469 Actinobacteria and Clostridia, but member orders Bifidobacteriales and Clostridiales
470 were positively correlated in Control (Fig. 5E).

471

Phylum	Class	Order
Actinobacteria-Bacteroidetes	-	Actinobacteria-Bacteroidia
	-	Coriobacteria-Bacteroidia
Actinobacteria-Proteobacteria	-	Actinobacteria-Betaproteobacteria
	-	Coriobacteria-Deltaproteobacteria

<i>Actinobacteria-Firmicutes</i>		<i>Actinobacteria-Clostridia</i>		<i>Bifidobacteriales-Clostridiales</i>	+
<i>Proteobacteria-Firmicutes</i>	-	<i>Gammaproteobacteria-Bacilli</i>		<i>Enterobacteriales-Turicibacteriales</i>	-
		<i>Gammaproteobacteria-Clostridia</i>	-		
		<i>Deltaproteobacteria-Clostridia</i>	-	<i>Desulfovibrionales-Clostridiales</i>	-
<i>Proteobacteria-Bacteroidetes</i>	+	<i>Betaproteobacteria-Bacteroidia</i>	+	<i>Burkholderiales-Bacteroidales</i>	+
<i>Bacteroidetes-Firmicutes</i>	-	<i>Bacteroida-Clostridia</i>		<i>Bacteroidales-Clostridiales</i>	-

472 **Table 2.** Upper-level taxa correlations, grouped by taxonomic classification.

473

474 Table 3 shows collective ATria results, similarly grouped. At each level, taxa found
 475 equally important in both MCNs are highlighted grey; taxa found more important in
 476 Control light orange, and only important in Control dark orange (analogous case for
 477 ADHD and purple). Taxa ranked as first or tied for first in either MCN are **bold**.

478

Phylum	Class	Order
<i>Actinobacteria (#1/#1)</i>	<i>Actinobacteria (#2/NR)</i>	<i>Bifidobacteriales (#T2/T5)</i>
	<i>Coriobacteria (#1/NR)</i>	<i>Coriobacteriales (NR/#T5)</i>
<i>Bacteroidetes (NR/#T2)</i>	<i>Bacteroidia (NR/#T1)</i>	<i>Bacteroidales (#1/#1)</i>
<i>Firmicutes (#2/#T2)</i>	<i>Bacilli (NR/NR)</i>	<i>Turicibacteriales (#T3/NR)</i>
	<i>Clostridia (#T3/#T3)</i>	<i>Clostridiales (#T2/#T3)</i>
	<i>Erysipelotrichia (NR/NR)</i>	<i>Erysipelotrichiales (NR/#T3)</i>
<i>Proteobacteria (NR/NR)</i>	<i>Betaproteobacteria (NR/#T1)</i>	<i>Desulfovibrionales (NR/#2)</i>
	<i>Deltaproteobacteria (NR/#T3)</i>	
	<i>Gammaproteobacteria (#T3/NR)</i>	<i>Enterobacteriales (#T3/NR)</i>

479 **Table 3.** Upper-level ATria results, grouped by taxonomic classification.

480

481 Compositional results are mirrored here: ADHD showed elevated Bacteroidetes at the
 482 expense of Firmicutes, and these taxa are negatively correlated in both MCNs (Fig. 5A-
 483 B). But while Firmicutes and Bacteroidetes dominate both populations (largest nodes,
 484 Fig. 5A-B) as is typical in the gut microbiome (98), SparCC and ATria estimate a far less
 485 abundant phylum, Actinobacteria (roughly 4% of both populations), as most important
 486 to their overall gut ecology. In both MCNs (Fig. 5A-B), phylum Actinobacteria has the
 487 strongest negative correlations and ATria ranks it first (Table 2).

488

489 We make three more observations at these upper taxonomic levels, that we keep in
 490 mind when moving to the lower:

491

492 **(A) A core Proteobacteria-Bacteroidetes positive correlation (est. cooperation) forms.**

493 Table 1 shows this, with Proteobacteria and Bacteroidetes (the only positive correlation

494 in either phylum-level MCN), member classes Betaproteobacteria and Bacteroidia, and
495 member orders Burkholderiales and Bacteroidiales.

496
497 **(B) In Control, taxa in (A) have more negative edges with Actinobacteria (est.**
498 **competition), especially Bifidobacteriales.** The highest magnitude negative edges in
499 both phylum-level MCNs (Fig. 5A and 5B) involve Actinobacteria with Proteobacteria
500 and Bacteroidetes. Yet while the two consistently dependent Actinobacteria classes
501 (Actinobacteria and Coriobacteria) continue this same dynamic with Bacteroidia
502 (Bacteroidetes) and Betaproteobacteria (Proteobacteria) in Control (Fig. 5C and Table 1),
503 they are completely disconnected in ADHD (Fig. 5D). Worth noting, this is despite
504 their relative abundance being nearly the same in Control/ADHD: Coriobacteria
505 1.5/1.1%, and Actinobacteria 3.2/3.7%. Further, ATria ranks Actinobacteria and
506 Coriobacteria as the top two Control taxa (Table 2). In ADHD, Bacteroidia and
507 Betaproteobacteria are the top two (Table 2), and the MCN shows no negative edges
508 (est. competition) at all involving these taxa (Fig. 5D).

509
510 The order level reveals Bifidobacteriales (Actinobacteria) may be more responsible for
511 this difference than Coriobacteriales (Coriobacteria). While Bifidobacteriales and
512 Coriobacteriales both continue their negative correlations with Bacteroidales
513 (Bacteroidia) in Control, only Coriobacteriales does in ADHD. Table 1 actually shows
514 all edges involving Bifidobacteriales to be exclusive to Control, now including a
515 positive correlation with Clostridia (the most abundant Firmicute). An increased
516 participation of order Bifidobacteriales thus emerges as a distinguishing feature of
517 Control, which is further supported by ATria (Table 2), which ranks Bifidobacteriales
518 higher (tied for second) in Control, and Coriobacteriales only in ADHD.

519
520 **(C) A shift in Firmicutes-Proteobacteria dynamics.** This begins immediately at the
521 phylum level (Fig. 5A) with Control having a negative correlation (-0.65) that is absent
522 in ADHD (Fig. 5B). The most abundant Firmicute class (Clostridia) is negatively

523 correlated with different Proteobacteria classes; Gammaproteobacteria in Control (Fig.
524 5C), Deltaproteobacteria in ADHD (Fig. 5D), and the latter continues at the order level
525 (Fig. 5F) with Clostridiales (Clostridia) and Desulfovibrionales (Deltaproteobacteria). In
526 Control (Fig. 5E), a negative correlation emerges between Enterobacteriales
527 (Gammaproteobacteria) and LEfSe Control biomarker Turicibacteriales (Bacilli).

528

529 **Summary.** Upper-level analysis revealed increased Actinobacteria participation in
530 Control gut ecology, especially order Bifidobacteriales. Much of this involved negative
531 correlations with a core of positively correlated Bacteroidetes (Bacteroidales) and
532 Proteobacteria (Burkholderiales). Recalling our compositional analyses and anomalous
533 behavior involving *Bifidobacterium* (Bifidobacteriales), *Bacteroides* (Bacteroidales), and
534 *Sutterella* (Burkholderiales), we are now interested in exploring these dynamics at lower
535 taxonomic levels. We will continue to observe Firmicutes-Proteobacteria dynamics, as
536 despite a still unclear picture, a clear distinction is shown between Control and ADHD.

537

538 **Lower Levels: Family, Genus, and Lowest Possible.** Fig. 6 shows Control and ADHD
539 MCNs at family (Fig. 6A-B), genus (Fig. 6C-D), and lowest possible taxonomic
540 classification levels (Fig. 6E-F). In this latter MCN each taxon is classified at the species
541 level if possible (rare with 16S), otherwise more commonly the genus level is used.
542 Schemes regarding color, node size, and edge thickness are the same as Fig. 5. Since
543 the MCNs are now larger we do not label every node, only those that we reference in
544 our analyses. We also extend Table 1 to include correlations from every taxonomic
545 level, but as this is also very large we include it as Supplemental Table S1 and extract
546 only relevant portions to our discussion. We perform a similar task with ATria, and
547 Supplemental Table S2.

548

549 **Fig. 6. Lower-Level MCNs.** MCNs at the family (A), genus (B), and species (C)
550 taxonomic levels. Network visual properties, including node and edge size, color, and
551 orientation, are the same as Fig. 5. Taxa noted throughout our analyses are labeled.

552

553

554 Fig. 6 shows taxa separating into a group of primarily Bacteroidetes (dark purple, lower
555 left), and another of primarily Firmicutes (yellow, upper right). Enough taxa are also
556 now present to perform meaningful community analysis. Fig. 7 shows the same MCNs
557 as Fig. 6, after running Affinity Propagation (AP, (83)) and coloring by cluster. At the
558 family level (Fig. 7A-B) four clusters form. One is dominated by Bacteroidetes, family
559 *Bacteroidaceae* (BB, magenta). Two are dominated by Firmicutes, one family
560 *Lachnospiraceae* (FL, gold), and the other family *Ruminococcaceae* (FR, green). In Control
561 (Fig. 7A) the fourth cluster consists of three mixed-family Firmicutes (FM, dark teal). In
562 ADHD (Fig. 7B) two of these are absent and the Proteobacteria *Enterobacteriaceae* is
563 present, leaving it no longer Firmicutes-dominant (M, grey).

564

565 **Fig. 7. Clusters.** Same MCNs as Fig. 6, after clustering with the affinity propagation
566 (AP) algorithm (86). Family-level clusters are each given a unique color, and labeled
567 with their dominant phylum and member family. New clusters that form at each lower
568 taxonomic level are labeled, colored with shades corresponding to their dominant
569 phylum/family when applicable - i.e. at the genus level *FL1-FL3* are different shades of
570 gold (family-level *FL*). Taxa noted throughout our analyses are labeled.

571

572

573 Clusters *BB* and *FR* remain at the genus level (Fig. 7C-D). Several Firmicutes,
574 *Lachnospiraceae*-dominant clusters emerge, referred to as *FL1*, *FL2*, etc. (gold shades). A
575 mixed-family Actinobacteria cluster of *Bifidobacterium* and *Collinsella* forms in both
576 MCNs (*AM*, brown), and an Actinobacteria, *Coriobacteriaceae*-dominated cluster forms in
577 ADHD (Fig. 7D, *AC*, burnt sienna). A small group of two *Clostridiaceae* composes
578 cluster *FC* in Control (Fig. 7C, aqua). In ADHD (Fig. 7D), a cluster (orange) emerges as
579 the only Firmicutes-dominant cluster with positive correlations to cluster *BB*. This
580 eventually becomes present in both lowest-level MCNs (Fig. 7E-F) with core member
581 Control LEfSe biomarker *Turicibacter*, so we call this cluster *FT*.

582

583 At the lowest level we kept cluster names as consistent as possible with genus-level
584 membership (for example, a cluster mostly comprised of *FL2* genus-level taxa would
585 also be named *FL2* at the lowest level). Both MCNs (Fig. 7E-F) now include a mixed-
586 family, Bacteroidetes-dominant cluster *BM1* (pink), and Control includes a second
587 (*BM2*, orchid). Supplemental Tables S3-S5 list all clusters, members, and centroids at all
588 levels. As with earlier tables, we will extract portions relevant to our discussion.

589

590 Finally, to measure cluster size, tightness, and interactions, we produce a heatmap of
591 taxa correlations (Fig. 8) with taxa ordered on the *x*- and *y*-axes by Fig. 7 cluster.
592 Green/red intensity at each point (*x*, *y*) denotes the degree of positive/negative
593 correlation between taxa *x* and *y* (symmetric, by definition). Clusters appear as rough
594 squares of positive (green) correlations on the diagonal. We outline each box with the
595 same color as its corresponding Fig. 7 cluster.

596

597 **Fig. 8. Heatmaps.** Heatmap representation of taxa correlations (green=positive,
598 red=negative), with taxa organized on each axis by cluster (symmetric matrix). The
599 area corresponding to the intersection of each cluster with itself is outlined with a box
600 using the corresponding cluster color in Fig. 7. Taxa and clusters noted throughout our
601 analyses are labeled on the axes.

602

603

604 We first continue to pursue observations (A)-(C) from the upper taxonomic levels.
605 Afterwards, we discuss any new and interesting trends.

606

607 **(A) A core Proteobacteria-Bacteroidetes positive correlation (est. cooperation) forms.**
608 Recall the orders involved in this correlation were Burkholderiales (Proteobacteria) and
609 Bacteroidales (Bacteroidetes). **This corresponds to cluster *BB*, with genus *Sutterella***
610 **and multiple *Bacteroidales* taxa. In ADHD this cluster is larger and includes more**
611 ***Bacteroidales* plus some Firmicutes, and nearly all members are positively correlated**
612 **with its centroid *Bacteroides*. Additionally it has fewer negative correlations (est.**
613 **competition) with other clusters.**

614

615 Cluster *BB* is the only cluster with Burkholderiales and Bacteroidales descendants.
 616 Table 4 shows all correlations involving Burkholderiales and Bacteroidales lineages,
 617 organized and shaded using the same scheme as Table 1. One core positive correlation
 618 survives all six taxonomic levels in Control and ADHD (12 MCNs total, the only
 619 correlation in our entire dataset with this property). This occurs between genera
 620 *Sutterella* and *Bacteroides*. Several others involving *Sutterella* and its family *Alcaligenaceae*
 621 with cluster *BB* members are present only in ADHD – support for a larger cluster *BB* in
 622 ADHD. *Alcaligenaceae/Sutterella* are immediately visible in Fig. 6, as the only
 623 Proteobacteria (royal blue) among a slew of Bacteroidetes (dark purple).

624

Phylum	Class	Order	Family	Genus	Species
Proteobacteria-Bacteroidetes	+	Betaproteobacteria-Bacteroidia	+	Burkholderiales-Bacteroidales	+
				Alcaligenaceae-Bacteroidaceae	+
				Sutterella-Bacteroides	+
				Sutterella-B.uniformis	+
			Alcaligenaceae-Porphyromonadaceae	+	Sutterella-Parabacteroides
			Alcaligenaceae-Odoribacteriaceae	+	Sutterella-P.distasonis
			Alcaligenaceae-Rikenellaceae	+	Sutterella-Butyricimonas
				Sutterella-Rikenellaceae	+
					Sutterella-Butyricimonas

625 **Table 4.** Correlations between Burkholderiales-Bacteroidales lineages, shaded using
 626 the same scheme as Table 1 (grey present in both MCNs, purple only ADHD).

627

628 Fig. 7 also illustrates the increase in ADHD cluster *BB* size, as do the heatmaps (Fig. 8,
 629 magenta square). Table 5 quantifies differences in node and edge count.

630

Taxonomic Level	Family		Genus		Lowest	
	Control	ADHD	Control	ADHD	Control	ADHD
Cluster BB size: Taxa (+ Edges)	4 (3)	6 (7)	6 (7)	7 (9)	3 (2)	7 (11)

631 **Table 5.** Control and ADHD cluster *BB* size. Notation: *Taxa* (edges).

632

633 Table 5 shows cluster *BB* size to mysteriously drop in Control from the genus to the
 634 lowest level, from six taxa down to three. A closer look at Fig. 7C and 7E shows several
 635 genus-level *BB* members may be joining a mixed-family, Bacteroidetes-dominant cluster
 636 (*BM1*, pink) at the lowest level. Table 6, which shows *BB* and Control *BM1* members,
 637 confirms this. Core *BB* members are shown in bold, while italicized members are
 638 unique to Control or ADHD. Taxa of genus-level Control cluster *BB* members

639 *Odoribacter*, *Adlercruetzia*, *Parabacteroides* (*P. distasonis*) and *Bacteroides* (*B. ovatus*)
 640 compose Control cluster *BM1* at the lowest level.
 641

		Control		ADHD		
Level	Community	Cluster Type	Taxon	Phylum	Taxon	Phylum
Family	Bacteroidetes-dominant (B)	Bacteroidaceae-dominant (BB)	Bacteroidaceae		Bacteroidaceae*	
			<i>Porphyromonadaceae*</i>		<i>Porphyromonadaceae</i>	
			<i>Alcaligenaceae</i>		<i>Alcaligenaceae</i>	
			<i>Rikenellaceae</i>		<i>Rikenellaceae</i>	
					<i>Odoribacteraceae</i>	
					<i>Streptococcaceae</i>	
Level	Community	Cluster Type	Taxon	Phylum	Taxon	Phylum
Genus	Bacteroidetes-dominant (B)	Bacteroidaceae-dominant (BB)	Bacteroides		Bacteroides*	
			<i>Parabacteroides*</i>		<i>Parabacteroides</i>	
			<i>Sutterella</i>		<i>Sutterella</i>	
			<i>Rikenellaceae</i>		<i>Rikenellaceae</i>	
			<i>Odoribacter</i>		<i>Butyrimonas</i>	
			<i>Adlercruetzia</i>		<i>Streptococcus</i>	
					<i>Clostridium</i>	
Level	Community	Cluster Type	Taxon	Phylum	Taxon	Phylum
Species	Bacteroidetes-dominant (B)	Bacteroidaceae-dominant (BB)	Bacteroides*		Bacteroides*	
			Bacteroides uniformis		Bacteroides uniformis	
			<i>Sutterella</i>		<i>Sutterella</i>	
					<i>Parabacteroides distasonis</i>	
					<i>Rikenellaceae</i>	
					<i>Butyrimonas</i>	
					<i>Bifidobacterium longum</i>	
		Bacteroidetes, Mixed (BM1)	<i>Odoribacter</i>			
			<i>Bacteroides ovatus</i>			
			<i>Parabacteroides distasonis*</i>			
			<i>Adlercruetzia</i>			

642 **Table 6.** *Bacteroides*, *Bacteroidaceae* dominant clusters (BB) and Bacteroidetes, Mixed
 643 family (BM1) cluster in Control. Core taxa are bold, taxa exclusive to one MCN
 644 (Control or ADHD) are italicized, and centroids are marked with an asterisk (*).
 645

646 Table 7 supports weakened connections between BB and BM1 taxa in Control, showing
 647 higher intra-correlation values (0.61 and 0.62) relative to inter-correlation (0.44).
 648

Control BB (Intra)			Control BM1 (Intra)			Control BB-BM1 (Inter)			ADHD BB (Intra)		
Taxa	Edges	Mean Correlation	Taxa	Edges	Mean Correlation	Taxa	Edges	Mean Correlation	Taxa	Edges	Mean Correlation
3	2	0.61 ± 0.15	4	4	0.62 ± 0.1	4	3	0.44 ± 0.02	7	11	0.56 ± 0.12

649 **Table 7.** Cluster BB and Control BM1 intra- and inter-correlations.

650
 651 Table 6 also shows cluster BB members that differ between the MCNs. Cluster BB gains
 652 a different Actinobacteria – *B. longum* (ADHD) and *Adlercruetzia* (Control, eventually
 653 joining BM1). The presence of Firmicutes (yellow) is exclusive to ADHD, including
 654 *Streptococcaceae* and member genus *Streptococcus*, plus *Clostridium*. ADHD LEfSe
 655 biomarkers *Odoribacteriaceae* and *Butyrimonas* join cluster BB only in ADHD, and the
 656 sole *Clostridium* connection to cluster BB is with *Butyrimonas* (Fig. 6D).

657

658 Table 6 also indicates *BB*/*BM1* centroids, which we see across the board for ADHD are
 659 *Bacteroides* and its family *Bacteroidaceae*. In Control this belongs to *Porphyromonadaceae*
 660 (family) and descendant *Parabacteroides* (genus), until the *BB*-*BM1* “split” where
 661 *Bacteroides* becomes centroid of *BB* and *P. distasonis* of *BM1*. Table 8 shows connectivity
 662 of each of these taxa within their corresponding cluster. Percentagewise, in ADHD
 663 *Bacteroidaceae/Bacteroides* is a much stronger centroid; in fact over all levels only one
 664 cluster *BB* taxon was not positively correlated (*Clostridium*, genus level). Particularly
 665 given the ADHD cluster *BB* size increase, this could imply a significant role of
 666 *Bacteroidaceae/Bacteroides* in stabilizing a large ADHD Bacteroidetes-dominant
 667 community (would require additional experiments to verify).

668

Level MCN	Family		Genus		Lowest	
	Control	ADHD	Control	ADHD	Control	ADHD
<i>Bacteroidaceae/Bacteroides</i>	2/3 (66%)	5/5 (100%)	3/5 (60%)	5/6 (84%)	2/2 (100%)	6/6 (100%)
<i>Porphyromonadaceae/Parabacteroides/P. distasonis*</i>	2/3 (66%)	2/5 (40%)	4/5 (80%)	3/6 (50%)	3/3* (100%)	3/6 (50%)

669 **Table 8.** Cluster *BB* (* = *BM1*) connectivity with centroid taxa.

670

671 Interestingly ATria (Table 9) shows *Bacteroidaceae/Bacteroides* and lineages to nearly
 672 always have higher importance in Control, supporting a more “global” importance to
 673 overall gut ecology as opposed to a more local importance (cluster *BB*) in ADHD.
 674 MCNs agree, as in ADHD *Bacteroidaceae/Bacteroides* have few connections outside
 675 cluster *BB* (Fig. 7B, D, F). In Control (Fig. 7A, C, E) *Bacteroidaceae/Bacteroides* have many
 676 external connections, mostly negative (est. competition).

677

Phylum	Class	Order	Family	Genus	Lowest Possible
<i>Bacteroidetes</i> (NR/#T2)	<i>Bacteroidia</i> (NR/#1)	<i>Bacteroidales</i> (#1/#1)	<i>Bacteroidaceae</i> (#T9/#T12)	<i>Bacteroides</i> (#10/#T23)	<i>B. uniformis</i> (#12/#7)
					<i>B. ovatus</i> (#2/#T20)
					<i>Bacteroides</i> (#T23/#T28)
			<i>Odoribacteriaceae</i> (NR/#2)	<i>Odoribacter</i> (#9/#T21)	<i>Odoribacter</i> (#3/#T20)
			<i>Porphyromonadaceae</i> (#T7/#1)	<i>Butyrimonas</i> (NR/#4)	<i>Butyrimonas</i> (NR/#17)
				<i>Parabacteroides</i> (#T12/NR)	<i>Parabacteroides</i> (#T19/#T24)
			<i>Prevotellaceae</i> (NR/#5)	<i>Prevotella</i> (NR/#18)	<i>P. distasonis</i> (NR/#T24)
			<i>Rikenellaceae</i> (#T7/#3)	<i>Rikenellaceae</i> (#T12/#8)	<i>Rikenellaceae</i> (#T19/#8)

678 **Table 9.** ATria rankings of Bacteroidetes taxa.

679

680 Control MCNs (Fig. 7A, C, E) and heatmaps (Fig. 8A, C, E, magenta rectangle) show
681 negative correlations (red) to be fairly evenly distributed among cluster *BB* taxa. By
682 contrast in ADHD (Fig. 7B, 8B), nearly all cluster *BB* negative correlations are localized
683 to *Porphyromonadaceae* (ranked #1 by ATria). Fig. 6B shows *Porphyromonadaceae* to be the
684 sole cluster *BB* member negatively correlated with the Firmicutes-dominant portion
685 (Fig. 6B, upper right, collectively more than 70% of the population).

686

687 Table 10 shows that for all MCNs, in Control more than two-thirds of cluster *BB* had
688 negative correlations with members of other clusters, compared to less than half in
689 ADHD. Negative edge count was also almost always higher for Control, despite a
690 smaller cluster *BB*. Collectively these results show that in Control cluster *BB* is smaller,
691 and more connected to other clusters, primarily through negative correlations (est.
692 competition). In ADHD cluster *BB* is larger, and more isolated.

693

Level	Family		Genus		Lowest	
MCN	Control	ADHD	Control	ADHD	Control	ADHD
Cluster BB (-) Edges with Other Clusters (Participation Rate)	7 (100%)	5 (33%)	10 (88%)	4 (43%)	5 (67%)	5 (43%)

694 **Table 10.** Negative correlations between cluster *BB* and other clusters. *Number*
695 *(participation rate)*.

696

697 Table 11 provides a few final interesting observations for various Bacteroidetes taxa.

Taxa	Observation
<i>Odoribacteriaceae</i> and member genus <i>Butyrimonas</i>	ADHD LEfSe biomarkers, ADHD cluster <i>BB</i> members, only ranked in ADHD.
<i>Prevotellaceae</i> and member genus <i>Prevotella</i>	ADHD negative correlation with <i>Bacteroidaceae/Bacteroides</i> is the only negative correlation between two Bacteroidetes taxa in any MCN. Only ranked in ADHD,
<i>Rikenellaceae</i>	Ranked in every MCN at every level, and always higher for ADHD.

698 **Table 11.** Additional observations for some Bacteroidetes taxa.

699

700 **(B) In Control, taxa in (A) have more negative edges with Actinobacteria (est.
701 competition), especially Bifidobacteriales.** We now know taxa from (A) to correspond
702 to cluster *BB*, which in both MCNs contained one core Proteobacteria
703 (*Alcaligenaceae/Sutterella*) and otherwise primarily Bacteroidetes. We also observed
704 cluster *BB* taxa to have far more negative correlations
705 (est. competition) with other clusters in Control. We now see if this is also true with

706 Bifidobacteriales lineages, including *Bifidobacterium*. Our analysis in fact reveals that
 707 **negative correlations between *Bifidobacterium* or any parent/descendant with any**
 708 **Bacteroidetes or Proteobacteria are exclusive to Control and absent in ADHD.**

709
 710 Table 12 shows all correlations involving *Bifidobacterium* and its lineages, grouped and
 711 colored as in previous tables. Not only are negative Bacteroidetes correlations exclusive
 712 to Control (orange), but these taxa include the most abundant Bacteroidetes
 713 *Bacteroidaceae/Bacteroides* (ADHD cluster BB centroid), as well as
 714 *Porphyromonadaceae/Parabacteroides* (Control cluster BB centroid). Another appears at
 715 the lowest level between *B. adolescentis* and *B. ovatus*. With Proteobacteria, negative
 716 *Bifidobacterium* correlations are also observed with *Sutterella* (core cluster BB member)
 717 and *Enterobacteriaceae*, also only in Control. Heatmaps confirm
 718 *Bifidobacteriaceae/Bifidobacterium* to be negatively correlated with cluster BB taxa only in
 719 Control (Fig. 8A-D, intersection of brown and magenta rectangles). By contrast, the
 720 only ADHD correlation is positive and within cluster BB (*B. longum* with *B. uniformis*).

Phylum	Class	Order	Family	Genus	Lowest Possible
Actinobacteria-Bacteroidetes	Actinobacteria-Bacteroidia	Bifidobacteriales-Bacteroidales	Bifidobacteriaceae - Bacteroidaceae	Bifidobacterium - Bacteroides	B. adolescentis – B. ovatus
			Bifidobacteriaceae - Porphyromonadaceae	Bifidobacterium - Parabacteroides	B. longum – B. uniformis
Actinobacteria Proteobacteria	Actinobacteria - Betaproteobacteria	Bifidobacteriales-Burkholderiales	Bifidobacteriaceae-Alcaligenaceae	Bifidobacterium – Sutterella	
	Actinobacteria-Gammaproteobacteria	Bifidobacteriales-Enterobacteriales	Bifidobacteriaceae-Enterobacteriaceae	Bifidobacterium-Enterobacteriaceae	Bifidobacterium 2 – Enterobacteriaceae
Actinobacteria-Firmicutes	Actinobacteria- Bacilli	Bifidobacteriales-Turicibacteriales	Bifidobacteriaceae-Turicibacteriaceae	Bifidobacterium-Turicibacter	Bifidobacterium 1 – Turicibacter
	Actinobacteria-Clostridia	Bifidobacteriales - Clostridiales	Bifidobacteriaceae-Lachnospiraceae	Bifidobacterium - Lachnospiraceae 2	Bifidobacterium 1 – Blautia 2
					Bifidobacterium 1 – Ruminococcus [L]
			Bifidobacteriales – Clostridiales	Bifidobacterium-Clostridiales	B. adolescentis – Clostridiales 1
				Bifidobacterium-Ruminococcaceae	Bifidobacterium 2 – Ruminococcaceae
		Bifidobacteriaceae – Veillonellaceae	Bifidobacterium-Veillonella	Bifidobacterium 2 – Dialister	B. longum – Oscillospira
Actinobacteria-Actinobacteria	Actinobacteria-Actinobacteria	Bifidobacteriales - Bifidobacteriales	Bifidobacteriaceae - Bifidobacteriaceae	Bifidobacterium-Bifidobacterium	Bifidobacterium 2 – B. adolescentis
	Actinobacteria-Coriobacteria	Bifidobacteriales-Coriobacteriales	Bifidobacteriaceae-Coriobacteriaceae	Bifidobacterium-Adlercreutzia	Bifidobacterium 1 - Adlercreutzia
				Bifidobacterium-Collinsella	
				Bifidobacterium-Eggerthella	B. longum – E. lenta

721 **Table 12.** Correlations involving *Bifidobacterium* and its lineages.

722 Table 12 also shows *Bifidobacterium* to even have far more Firmicutes connections
 723 (positive and negative) in Control. Collectively 24 correlations were observed in
 724 Control, compared to 9 in ADHD, supporting an overall increase in *Bifidobacterium*
 725 participation in Control. ATria (Table 13) also almost uniformly ranks *Bifidobacterium*
 726 and its lineages higher in Control. Again, this is despite *Bifidobacterium* abundances
 727 being relatively the same (slightly higher in ADHD in fact, 3.6% to 3.2%).

Phylum	Class	Order	Family	Genus	Lowest Possible
Actinobacteria (#1/#1)	Actinobacteria (#2/NR)	Bifidobacteriales (#T2/ #T5)	Bifidobacteriaceae (#5/NR)	Bifidobacterium (NR/#T19)	<i>Bifidobacterium</i> 1 (#10/NR)
					<i>Bifidobacterium</i> 2 (#1/11)
					<i>B. longum</i> (#15/NR)

728 **Table 13.** ATria rankings of *Bifidobacterium* and lineages.

729
 730 **(C) A Shift in Firmicutes-Proteobacteria dynamics.** Only two Proteobacteria
 731 families/genera were consistently present. One was *Sutterella* (family *Alcaligenaceae*),
 732 already noted as a core cluster BB member. The other is *Enterobacteriaceae*, which our
 733 analysis supports being mostly responsible for this shift.

734
 735 Table 14 shows all Proteobacteria-Firmicutes correlations. A couple of negative
 736 correlations can be seen involving *Alcaligenaceae/Sutterella*, with Firmicutes
 737 *Ruminococcaceae* (Control) and *Clostridiaceae* (both). Far more significant are the
 738 differences involving *Enterobacteriaceae*. One is its negative correlation with genus
 739 *Oscillospira* in ADHD (genus level), that becomes a positive correlation with *Oscillospira*
 740 in Control (lowest level). This is the only time, over all twelve MCNs, where a
 741 correlation sign changed between the same two taxa in Control vs. ADHD.

Phylum	Class	Order	Family	Genus	Lowest Possible
Proteobacteria-Firmicutes	Betaproteobacteria - Clostridia	Burkholderiales-Clostridiales	Alcaligenaceae – Ruminococcaceae	-	
			Alcaligenaceae - Clostridiaceae-	-	Sutterella – Clostridiaceae 2
				-	Sutterella – Clostridiaceae 2
	Gammaproteobacteria - Bacilli	Enterobacteriales - Turicibacteriales			
	Gammaproteobacteria - Clostridia	Enterobacteriales-Clostridiales	Enterobacteriaceae – Mogibacteriaceae	+	
			Enterobacteriaceae- Lachnospiraceae		Enterobacteriaceae- Anaerostipes
					Enterobacteriaceae- Anaerostipes
			Ruminococcaceae- Enterobacteriaceae	-	Enterobacteriaceae – Oscillospira
	Gammaproteobacteria - Erysipelotrichi	Enterobacteriales-Erysipelotrichiales	Enterobacteriaceae- Erysipelotrichiaceae		Enterobacteriaceae – Erysipelotrichiaceae 2
	Delta proteobacteria - Clostridia	Desulfovibrionales – Clostridiales		+	Enterobacteriaceae – Erysipelotrichiaceae 2

743 **Table 14.** Proteobacteria-Firmicutes correlations.

744
 745 Interesting shifts involving *Enterobacteriaceae* and various Firmicutes occur even at the
 746 family level, however. A small mixed-family, Firmicutes-dominant cluster *FM* forms
 747 (Fig. 7A, upper left), consisting of *Mogibacteriaceae*, *Christensenellaceae*, and
 748 *Erysipelotrichiaceae* (Table 15). In ADHD, *Enterobacteriaceae* instead joins *Mogibacteriaceae*
 749 to form a small two-taxon mixed cluster *M* (Fig. 7B, upper left, and Table 15).

Community	Cluster Type	Control		Phy	ADHD		Phy
		Cluster	Taxon		Cluster	Taxon	
Firmicutes-dominant (F)	Mixed (FM)	FM	<i>Mogibacteriaceae</i>				
		FM	<i>Christensenellaceae*</i>				
		FM	<i>Erysipelotrichiaceae</i>				
Mixed (M)	N/A				M	<i>Mogibacteriaceae</i>	
					M	<i>Enterobacteriaceae</i>	

750 **Table 15.** Mixed-family Control and ADHD clusters.
 751

752 Dynamics of *FM* and *M* taxa change between the MCNs. Fig. 7A-B shows a
 753 distinguishing core *FM/M* feature is the negative correlation with *Rikenellaceae* of
 754 cluster *BB*, but the taxon involved changes from *Erysipelotrichiaceae* in Control to
 755 *Mogibacteriaceae* in ADHD. Table 16 (ATria) shows the two taxa from Control cluster
 756 *FM* “replaced” by *Enterobacteriaceae* in ADHD cluster *M*, *Christensenellaceae* and
 757 *Erysipelotrichiaceae*, are only ranked in Control, and *Mogibacteriaceae* only ranked in
 758 ADHD. This applied across all descendants, with the one notable exception being
 759 *Coprobacillus* (*Erysipelotrichiaceae*), ranked #1 for ADHD at the genus and lowest levels
 760 (the only taxon to be ranked #1 in two MCNs). We label it in Fig. 7D, F, noting its
 761 negative correlations with multiple Firmicutes-dominant clusters.

Phylum	Class	Order	Family	Genus	Lowest Possible
Firmicutes (#2/#T2)	Clostridia (#T3/#T3)	Clostridiales (#T2/#3)	Christensenellaceae (#2/NR)	Christensenellaceae (NR/NR)	Christensenellaceae (#18/NR)
			Mogibacteriaceae (NR/#6)	Mogibacteriaceae (NR/#6)	
	Erysipelotrichia (NR/NR)	Erysipelotrichales (NR/#T3)	Erysipelotrichaceae (#3/NR)	Coprobacillus (NR/#1)	Coprobacillus (#T31/#1)
				Erysipelotrichaceae 1 (#6/NR)	Erysipelotrichaceae 1 (#16/NR)
				Erysipelotrichaceae 2 (#11/NR)	
				Eubacterium (#T14/NR)	E. dolicum (#T29/NR)
Proteobacteria (NR/NR)	Gammaproteobacteria (#T3/NR)	Enterobacteriales (#T3/NR)	Enterobacteriaceae (NR/#T6)	Enterobacteriaceae (NR/#7)	Enterobacteriaceae (NR/#T26)

762 **Table 16.** Mixed-family cluster member ATria rankings.
 763

764 *Enterobacteriaceae* was also only ranked in ADHD, across all three lower levels. Its
 765 *Oscillospira* positive correlation (Table 14) is the only Control correlation involving

766 *Enterobacteriaceae*, and *Enterobacteriaceae* actually joins *Oscillospira*'s cluster (FR, Fig. 7E)
767 in Control. The sign change takes place at the genus level in ADHD (Fig. 6D), where
768 *Oscillospira* and *Enterobacteriaceae* are negatively correlated. Although this correlation
769 did not persist to the lowest level (Fig. 6F), *Enterobacteriaceae* is still positively correlated
770 with *Anaerostipes*, a taxon negatively correlated with *Oscillospira* across the board. We
771 therefore observe *Enterobacteriaceae* dynamics to shift from a state that favors *Oscillospira*
772 cooperation in Control, to *Oscillospira* competition in ADHD. The role of
773 *Enterobacteriaceae* in gut ecology has historically been controversial (114), with both
774 beneficial (115) and pathogenic (116) properties emerging. Gut dysbiosis has actually
775 been shown to trigger horizontal gene transfer between the two types (117).

776

777 **New Observations.** We make the following new observations at the lower levels.

778

779 **(D) LEfSe Biomarkers: *Turicibacter* and *Odoribacter*.** Earlier we noted ADHD LEfSe
780 biomarkers *Odoribacteriaceae* and *Butyricimonas* as ADHD cluster *BB* members (Table
781 11). Biomarker *H. influenzae* and its lineages were never connected to any of our MCNs.
782 We now observe remaining biomarkers *Turicibacter* (Control) and *Odoribacter* (ADHD).

783

784 Cluster *FT* (Fig. 7, orange) was the only Firmicutes-dominant cluster with members
785 positively correlated with any Bacteroidetes-dominant cluster (*BB* in ADHD, *BM1* in
786 Control). We named this cluster *FT* because of core member *Turicibacter*. *Turicibacter*
787 (Firmicutes, LEfSe Control biomarker), which joins *Phascolarctobacterium* (Firmicutes,
788 reduced in inattention, (57)) to form *FT* at the genus level in ADHD (Fig. 7D), where it is
789 not present in Control. At the lowest level, *FT* is slightly larger (by one taxon) in
790 ADHD. Supplementing the earlier trend of less cluster *BB* negative correlations (est.
791 competition) in ADHD, this also supports the presence of a larger cluster with positive
792 correlations (est. cooperation) as well, with *Turicibacter* as its centroid (Table 17).

793

Level	Community	Cluster Type	Control Taxon	Phy	ADHD Taxon	Phy
-------	-----------	--------------	------------------	-----	---------------	-----

Family	Firmicutes-dominant (F)	Turicibacter-core (FT)	Not present.		Turicibacter	
Level	Community	Sub-Community	Taxon	Phy	Phascolarctobacterium	
Genus	Firmicutes-dominant (F)	Turicibacter-core (FT)	Turicibacter		Turicibacter*	
			Ruminococcus [L]		Phascolarctobacterium	
			Bifidobacterium 2*		Clostridiales 2	
					Parabacteroides	

794 **Table 17.** Cluster FT (Firmicutes-dominant, *Turicibacter*-core) members.

795 In ADHD *Turicibacter* provides the sole genus-level (Fig. 7D) *FT-BB* positive correlation,
796 with *Parabacteroides* (Bacteroidetes, reported elevated in hyperactivity, (53)). At the
797 lowest level (Fig. 7F) *Parabacteroides* joins *FT*, and along with *Turicibacter* forms *FT-BB*
798 positive correlations, with member species *P. distasonis*. Interestingly in Control (Fig.
799 7E), the *FT-BB* positive correlation does not involve Firmicutes or Bacteroidetes taxa at
800 all, but rather two Actinobacteria –*Bifidobacterium 1* (*FT* centroid), and *Adlercreutzia* (*BB*).
801 This continues our observed increases in Actinobacteria and particularly *Bifidobacterium*
802 involvement in Control gut ecology.

803

804 Cluster *FC* forms in Control (Fig. 7C,E, aqua) and contains two *Clostridiaceae* taxa. In
805 both MCNs these taxa negatively correlate with multiple cluster *BB* members, and in
806 ADHD (Fig. 7F) *Clostridiaceae 1* has negative correlations with *BB* centroid *Bacteroides*
807 plus taxa involved in *FT-BB* cooperation: *P. distasonis*, and *FT* centroid *Turicibacter*. In
808 both MCNs, they participate in correlations that favor cluster *BB* competition (especially
809 the more abundant *Clostridiaceae 1*).

810

811 Exclusive to ADHD is a negative correlation (est. competition) between these
812 *Clostridiaceae* taxa and ADHD biomarker *Odoribacter* – both at the genus level (Fig. 7D),
813 and *Clostridiaceae 1* at the lowest level (Fig. 7F). *Odoribacter* was reported by LEfSe as
814 elevated in ADHD, and this negative correlation implies that an increase in *Odoribacter*
815 abundance will decrease *Clostridiaceae 1*. Upon further inspection *Clostridiaceae 1*
816 relative abundance is indeed reduced by a factor of two in ADHD vs. Control.
817 *Clostridiaceae 1* and 2 cooperation in Control (forming *FC*) is also absent in ADHD.

818

819 (E) **Changes in the role of *Adlercruetzia* (Actinobacteria).** In contrast to *Bifidobacterium*
 820 (*Bifidobacteriaceae*), *Adlercruetzia* is a member of the other consistently present
 821 Actinobacteria family, *Coriobacteriaceae*. While the distinguishing feature of
 822 *Bifidobacteriaceae/Bifidobacterium* was increased Control participation, the distinguishing
 823 feature of *Coriobacteriaceae* appears to be changes in cluster membership. In fact over all
 824 *Coriobacteriaceae* descendants, only once (*Collinsella*, genus level, cluster AM, Fig. 7C-D)
 825 were any in the same Control and ADHD cluster. Table 18 also shows ATria results to
 826 be more mixed for *Coriobacteriaceae*, compared to *Bifidobacteriaceae* (Table 13).
 827

Phylum	Class	Order	Family	Genus	Lowest Possible
Actinobacteria (#1/#1)	Coriobacteria (#1/NR)	Coriobacteriales (NR/#5)	Coriobacteriaceae (NR/#4)	Adlercruetzia (NR/#17)	Adlercruetzia (#14/NR)
				Collinsella (NR/#T19)	C. aerofaciens (NR/#15)
				Coriobacteriaceae (#18/NR)	
				Eggerthella (#4/NR)	E. lenta (#T25/NR)

828 **Table 18.** ATria rankings of *Coriobacteriaceae* and its lineages.

829
 830 We earlier noted *Adlercruetzia* as the Actinobacteria member of cluster *BB/BM1* in
 831 Control, and (along with *Bifidobacterium 1*) connecting clusters *FT* and *BB*. Table 19
 832 shows that outside of *Bifidobacterium 1*, its positive correlations in Control were entirely
 833 with Bacteroidetes taxa (all *BB/BM1* members). By contrast in ADHD, *Adlercruetzia*
 834 relationships mostly occur with Firmicutes, including a cluster membership with
 835 *Eubacterium/E. dolicum*. Several negative correlations are seen between *Adlercruetzia* and
 836 different Firmicutes, with no overlap between Control and ADHD. This suggests
 837 *Adlercruetzia* may play a significantly different role in Control and ADHD gut ecologies.
 838

Phylum	Class	Order	Family	Genus	Lowest Possible
Actinobacteria-Bacteroidetes	-	Coriobacteria-Bacteroidia	-	Coriobacteriaceae - Bacteroidaceae	-
				Adlercreutzia-Bacteroides	+
				Adlercreutzia-Parabacteroides	+
				Adlercreutzia-Odoribacter	+
Actinobacteria-Firmicutes	Actinobacteria-Clostridia	Coriobacteriales-Clostridiales	Coriobacteriaceae-Erysipelotrichaceae	Adlercreutzia-Eubacterium	+
			Coriobacteriaceae-Lachnospiraceae	Adlercreutzia -Blautia	-
				Adlercreutzia - Lachnospira	-
				Adlercreutzia - Lachnospiraceae 1	-
				Adlercreutzia - Lachnospiraceae 2	-
			Coriobacteriaceae-Ruminococcaceae	Adlercreutzia - Ruminococcus	-

Actinobacteria- Actinobacteria	Actinobacteria- Coriobacteria	Bifidobacteriales- Coriobacteriales	+	Bifidobacteriaceae- Coriobacteriaceae	+	Bifidobacterium- Adlercruetzia	Bifidobacterium 1 - Adlercruetzia	+
	Coriobacteria - Coriobacteria	Coriobacteriales - Coriobacteriales		Coriobacteriaceae - Coriobacteriaceae		Adlercruetzia- Coriobacteriaceae	+	

839 **Table 19.** *Adlercruetzia* correlations.

840

841 **(F) Bacteroidetes-Firmicutes positive correlations (est. cooperation) are entirely**
 842 **exclusive to ADHD, and absent in Control.** Table 20 shows all Bacteroidetes-
 843 Firmicutes positive correlations. They are entirely limited to ADHD, and with one
 844 exception (*Clostridium*) involve Bacilli taxa.

Phylum	Class	Order	Family	Genus	Lowest Possible
Bacteroidetes- Firmicutes	-	Bacteroidia -Bacilli	Bacteroidales Lactobacillales	Bacteroidaceae Streptococcaceae	+
				Porphyromonadaceae Turicibacteriaceae-	
			Bacteroidales - Clostridiales	Parabacteroides - Turicibacter	+
				Parabacteroides - Turicibacter	+
				P.distasonis - Turicibacter	+
			Odoribacteriaceae - Clostridiaceae	Butyrimonas - Clostridium	+

845 **Table 20.** Bacteroidetes-Firmicutes positive correlations, over all MCNs.

846

847 We have already seen most of these, including *Clostridium* and ADHD LefSe biomarker
 848 *Butyrimonas*, and the ADHD *FT-BB* connections involving *Turicibacter*, *Parabacteroides*,
 849 and *P.distasonis*. We now analyze the remaining top row, between *Bacteroides*
 850 (*Bacteroidaceae*) and *Streptococcus* (*Streptococcaceae*).

851

852 Firmicutes taxa were only ever present in cluster *BB* in ADHD, and we earlier noted
 853 *Streptococcaceae* and its genus *Streptococcus* as two of those taxa. Their cluster *BB*
 854 positive correlation was with centroid *Bacteroidaceae/Bacteroides*. Additionally cluster
 855 *BB* had almost no negative correlations (est. competition) with *FL/FR* (collectively 70%
 856 of the population) in ADHD, compared to a significant amount in Control.

857

858 What makes *Streptococcus* interesting for ADHD is that across all MCNs, it forms the
 859 only positive correlation between cluster *BB* and *FL/FR* (Fig. 7D). In other words, in
 860 addition to estimating significantly less *BB-(FL/FR)* competition in ADHD, our MCNs
 861 also estimate cooperation only in ADHD, between *Streptococcus* (*BB*) and *Blautia* (*FL1*).

862

863 Fig. 7D and 7F also show *Streptococcus* to be negatively correlated with *Oscillospira* in
864 ADHD, a taxon we noted earlier its correlation sign change with *Enterobacteriaceae*.
865 ATria (Table 21) also only ranks *Streptococcaceae*/*Streptococcus* as important in ADHD.

Phylum	Class	Order	Family	Genus	Lowest Possible
Firmicutes (#2/#T2)	Bacilli (NR/NR)	Lactobacillales (NR/NR)	Streptococcaceae (NR/#T12)	Streptococcus (NR/#T23)	

866 **Table 21.** ATria rankings of *Streptococcaceae*/*Streptococcus*.

867

868 **(G) A shift in *Blautia*-*Oscillospira* dynamics, and their respective clusters.** Thus far
869 *Oscillospira* has been noted for two ADHD-exclusive negative correlations, with taxa
870 only ranked in ADHD: *Enterobacteriaceae* and *Streptococcus*. *Enterobacteriaceae*-
871 *Oscillospira* was the only correlation to ever change sign from Control (positive) to
872 ADHD (negative). *Streptococcus* was noted for its correlation with *Blautia*, the sole
873 positive correlation between the largest Bacteroidetes-dominant cluster (BB) and
874 Firmicutes-dominant clusters (FL/FR) in any MCN.

875

876 Previous studies have indicated butyrate-producing *Oscillospira* as a healthy gut taxon
877 (118), specifically associated with leanness (119). *Blautia* is actually a taxon that has
878 been associated with obesity (120). And interestingly in the Control MCN (Fig. 7C and
879 7E) *Blautia* and *Oscillospira* are negatively correlated, but not in ADHD (Fig. 7D and 7F).

880

881 Since obesity has been associated with ADHD (121), the shift in *Enterobacteriaceae*
882 (*Oscillospira* cooperation in Control, competition in ADHD) and *Streptococcus* (*Blautia*
883 cooperation and *Oscillospira* competition in ADHD) correlations become interesting,
884 favoring *Blautia* cooperation and *Oscillospira* competition. Indeed correlation can never
885 imply causation and further experimental verification is required. But ATria results
886 (Table 22) also support this, ranking *Blautia* higher in ADHD and *Oscillospira* in Control.

Phylum	Class	Order	Family	Genus	Lowest Possible
Firmicutes (#T2/#2)	Clostridia (#T3/#T3)	Clostridiales (#T2/#3)	Lachnospiraceae (NR/NR) Ruminococcaceae (#4/#T8)	Blautia (#T16/#13) Oscillospira (#3/#10)	Blautia 1* (NR/#19) Oscillospira (#5/#9)

887 **Table 22.** *Blautia* and *Oscillospira* ATria rankings (plus lineages). *=The lowest level
 888 had two *Blautia* taxa; we assumed the more abundant (*Blautia* 1, overall 9.3% relative
 889 abundance vs 0.6%, composing 93% of the *Blautia* population).

890
 891 In fact our heatmap (Fig. 8C-8F) shows by intersecting *Oscillospira*'s row (small green
 892 rectangle) with the columns of *Blautia*'s cluster (gold rectangles, Control *FL3*, ADHD
 893 *FL1*) that *Oscillospira* is negatively correlated with *Blautia*'s entire cluster in Control,
 894 and these correlations are completely absent in ADHD.

895
 896 The lowest level MCNs (Fig. 7E-F) also show *Blautia*'s cluster as larger in ADHD, and
 897 *Oscillospira*'s cluster as larger in Control. Table 23 contains members of these clusters.
 898 *Blautia* and *Oscillospira* each belong to a cluster dominated by its respective family:
 899 *Lachnospiraceae* (*FL*), and *Ruminococcaceae* (*FR*). *Oscillospira* is a core *FR* member and at
 900 the lowest level, we see the Control *FR* cluster (with *Enterobacteriaceae* now a member).
 901 *Blautia* is consistently a member of the same cluster as both *Lachnospiraceae* taxa in
 902 ADHD, comparably larger than its *FL3* Control cluster.

903

Level	Community	Cluster Type	Control			ADHD		
			Cluster	Taxon	Phy	Cluster	Taxon	Phy
Lowest	Firmicutes-dominant,	Lachnospiraceae-dominant (<i>FL</i>)	FL3	Blautia 1*		FL1	Lachnospiraceae 1	
			FL3	Dorea 2		FL1	Lachnospiraceae 2*	
			FL3	<i>Bifidobacterium longum</i>		FL1	Coprococcus	
						FL1	<i>Ruminococcus [L]</i>	
						FL1	Blautia 1	
						FL1	Dorea 2	
						FL1	<i>Faecalibacterium prausnitzii</i>	
			FR	<i>Ruminococcaceae*</i>		FR	<i>Ruminococcaceae</i>	
			FR	<i>Ruminococcus [R]</i>		FR	<i>Ruminococcus [R]</i>	
			FR	<i>Oscillospira</i>		FR	<i>Oscillospira</i>	
		Ruminococcaceae-dominant (<i>FR</i>)	FR	<i>Clostridiales 1</i>		FR	<i>Clostridiales 1*</i>	
			FR	<i>Coprobacillus</i>		FR	<i>Bifidobacterium adolescentis</i>	
			FR	<i>Enterobacteriaceae</i>				
			FR	<i>Lachnospira</i>				

904 **Table 23.** *Blautia* and *Oscillospira* clusters.

905
 906 Heatmaps also indicate increased participation of *Oscillospira*'s cluster (*FR*) in Control
 907 (large green rectangle, Fig. 8C-F), including negative correlations with cluster *BB* that
 908 are absent in ADHD, yet another example of reduced ADHD cluster *BB* competition. In
 909 the MCNs, Fruchterman-Reingold places cluster *FR* (green) in a much more central

910 position in Control (Fig. 7C vs. 7D, and 7E vs. 7F). The negative correlations between
 911 *Oscillospira* and *Blautia*'s entire cluster *FL3* (Fig. 7C and 7E) are also evident, almost
 912 separating *FL3* from the MCN. In ADHD *Blautia*'s cluster *FL1* (gold) occupies a much
 913 more central position (Fig. 7D and 7F), with increased ADHD size particularly
 914 noticeable at the lowest level (Fig. 7F).

915
 916 ATria (Table 24) indicates a general increased importance of *Blautia*'s family
 917 (*Lachnospiraceae*) in ADHD, and *Oscillospira*'s family (*Ruminococcaceae*) in Control. A
 918 couple of noteworthy taxa follow this trend. *Faecalibacterium prausnitzii*
 919 (*Ruminococcaceae*), an anti-inflammatory bacterium (122) touted as a next-generation
 920 probiotic (123), is only ranked in Control. *Ruminococcus gnavis* (*Lachnospiraceae*), known
 921 to produce an inflammatory polysaccharide (124), is only ranked in ADHD.

Phylum	Class	Order	Family	Genus	Lowest Possible
Firmicutes (#2/#T2)	Clostridia (#T3/#T3)	Clostridales (#T2/#3)	<i>Lachnospiraceae</i> (NR/NR)	<i>Anaerostipes</i> (#T19/NR)	Anaerostipes (#6/#T26)
				<i>Blautia</i> (#T16/#13)	<i>Blautia</i> 1 (NR/#19)
					<i>Blautia</i> 2 (#9/#T22)
				<i>Coprococcus</i> (NR/#5)	<i>Coprococcus</i> (#T21/#6)
				<i>Dorea</i> (#T14/#12)	<i>Dorea</i> 2 (#13/NR)
				<i>Lachnospira</i> (NR/#16)	<i>Lachnospira</i> (#T27/#5)
				<i>Lachnospiraceae</i> 1 (#19/#15)	<i>Lachnospiraceae</i> 1 (#17/#2)
				<i>Lachnospiraceae</i> 2 (#7/NR)	<i>Lachnospiraceae</i> 2 (#8/#14)
				<i>Roseburia</i> (NR/NR)	<i>Roseburia</i> 1 (NR/#T28)
					<i>Roseburia</i> 2 (#T25/#T22)
				<i>Ruminococcus</i> (#1/#11)	<i>R. gnavis</i> (NR/#12)
			<i>Ruminococcaceae</i> (#4/#T8)	<i>Faecalibacterium</i> (#T16/#2)	<i>F. prausnitzii</i> (#11/NR)
				<i>Oscillospira</i> (#3/#10)	<i>Oscillospira</i> (#5/#9)
				<i>Ruminococcaceae</i> (#8/#9)	<i>Ruminococcaceae</i> (#4/#18)
				<i>Ruminococcus</i> (#2/NR)	<i>Ruminococcus</i> (#T21/NR)

922 **Table 24.** ATria rankings of *Lachnospiraceae* and *Ruminococcaceae* taxa.

923
 924 **Summary.** Four clusters were consistently present in both Control and ADHD MCNs.
 925 Three are Firmicutes-dominant (*FL*, *FR*, *FT*) and one is Bacteroidetes-dominant (*BB*).
 926 Table 25 shows their attributes, and summarizes observations we made about each.

Cluster	Attribute	Observation
<i>BB</i>	Largest Bacteroidetes-dominant cluster	Larger in ADHD, with more internal cooperation and less external competition.
<i>FL</i> (1,2,...)	Multiple Firmicutes, family <i>Lachnospiraceae</i> -dominant clusters	One large, centrally located cluster emerges in ADHD (<i>FL1</i>). Others are small, about the same size, and more disconnected (all are this way in Control).
<i>FR</i>	Firmicutes, family <i>Ruminococcaceae</i> -dominant	Smaller and less centrally located in ADHD.

<i>FT</i>	Firmicutes, core member <i>Turicibacter</i>	Slightly larger in ADHD.
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927 **Table 25.** Largest, consistently present clusters.

928

929 Table 26 summarizes correlations between members of these clusters. Other than the
930 one exception in ADHD involving *Streptococcus* and *Blautia*: *FT* is the only Firmicutes-
931 dominant cluster with taxa positively correlated with Bacteroidetes-dominant cluster
932 (*BB*) members, and all correlations involving *FL/FR* (largest Firmicute-dominant
933 clusters) and *BB* taxa are negative. *FT* is completely disconnected from *FL/FR* except
934 some ADHD competition. *FL-FR* competition only happens in Control.

Cluster 1	Cluster 2	Observation
<i>BB</i>	<i>FL</i>	Always (-), with one exception in ADHD (<i>Streptococcus-Blautia</i>)
<i>BB</i>	<i>FR</i>	Always (-)
<i>BB*</i>	<i>FT</i>	Always (+)
<i>FL</i>	<i>FR</i>	Generally (+). Some (-) in Control (all involve either <i>Ruminococcus</i> (<i>FL</i>) or <i>Oscillospira</i> (<i>FR</i>))
<i>FL</i>	<i>FT</i>	Generally disconnected. Some (-) in ADHD (all involve <i>Phascolarctobacterium</i> (<i>FT</i>))
<i>FR</i>	<i>FT</i>	Always disconnected.

935 **Table 26.** Interactions between taxa from Table 25 clusters (*=In Control, this took place
936 with *BM1* after the *BB* “split”).

937

938 Finally, we summarize taxa (Table 27) and relationships (Table 28) that we noted
939 throughout our analyses.

940

Taxon	Observation
<i>Adlercreutzia</i>	Role change from Control (Bacteroidetes cooperation) to ADHD (<i>E. dolichum</i> cooperation). Competition with different Firmicutes.
<i>Bacteroides</i>	Centroid (with nearly 100% connectivity) of cluster <i>BB</i> in ADHD.
<i>Bifidobacterium</i>	Higher participation in Control (mostly competition). Competition with all Bacteroidetes or Proteobacteria taxa is entirely exclusive to Control, including multiple members and centroid of cluster <i>BB</i> . Cooperation (small amount) is entirely exclusive to ADHD. Ranked higher in Control than ADHD nearly 100% of the time, including a #1 ranking at the lowest taxonomic level.
<i>Coprobacillus</i>	Ranked #1 for ADHD in two MCNs (genus and lowest possible). Competes with multiple <i>Lachnospiraceae</i> taxa, including the most abundant.
<i>Enterobacteriaceae</i>	Involved with Firmicutes-Proteobacteria shifts. Only ranked in ADHD.
<i>F. prausnitzii</i>	Probiotic species only ranked in Control
<i>Lachnospiraceae</i>	Most abundant family, generally ranked higher in ADHD
<i>Phascolarctobacterium</i>	Only <i>FT</i> member connected to another Firmicute-dominant cluster (competition), in ADHD. Only ranked in ADHD.
<i>Porphyromonadaceae</i>	#1 ADHD family, only cluster <i>BB</i> member to compete with <i>FL/FR</i>
<i>Rikenellaceae</i>	Ranked important in all six lower level MCNs, always higher in ADHD

<i>Ruminococcus</i> [L]	#1 Control genus, involved in <i>FL-FR</i> competition (only observed in Control).
<i>R. gnavis</i>	Produces inflammatory biosaccharide, only ranked in ADHD
<i>Ruminococcaceae</i>	Second-most abundant family, generally ranked higher in Control
<i>Turicibacter</i>	Control LEfSe biomarker, core member (centroid in ADHD) of <i>FT</i> .

941 **Table 27.** Taxa we noted throughout our analyses.

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Relationship(s)	Reason
<i>Bacteroides-Sutterella</i> (+, both)	Only core correlation consistent across both sample sets at all levels (12 MCNs).
<i>Bacteroides-Prevotella</i> (-, ADHD)	Only competition involving two Bacteroidetes taxa.
<i>Bacteroides-Streptococcus</i> (+, ADHD)	<i>Streptococcus</i> is one of only two Firmicutes genera to join cluster <i>BB</i> , through this connection.
<i>Butyrimonas-Clostridium</i> (+, ADHD)	<i>Butyrimonas</i> is an ADHD LEfSe biomarker, and only ranked by ATria in ADHD. <i>Clostridium</i> is one of only two Firmicutes to join cluster <i>BB</i> , through this connection.
<i>Clostridiaceae-Odoribacter</i> (-, ADHD)	<i>Odoribacter</i> is an ADHD LEfSe biomarker. In ADHD competes with <i>Clostridiaceae</i> taxa that compete with multiple cluster <i>BB</i> members (including its centroid).
<i>Enterobacteriaceae-Oscillospira</i> (+, Control; -, ADHD)	Only correlation ever to change sign from Control to ADHD. Taxa involved are in the same cluster in Control.
<i>Blautia-Oscillospira</i> (-, Control) <i>Streptococcus-Blautia</i> (+, ADHD) <i>Streptococcus-Oscillospira</i> (-, ADHD)	<i>Blautia</i> is associated with obesity and <i>Oscillospira</i> with leanness. <i>Oscillospira</i> (<i>FR</i>) competes with every member of <i>Blautia</i> 's cluster (<i>FL3</i>) in Control. <i>FL-FR</i> competition only happens in Control. In ADHD <i>Streptococcus</i> cooperates with <i>Blautia</i> (obesity) and competes with <i>Oscillospira</i> (leanness). <i>Streptococcus-Blautia</i> is the only time a cluster <i>BB</i> member (largest Bacteroidetes-dominant) ever cooperates with taxa from <i>FL</i> or <i>FR</i> (largest Firmicutes-dominant, collectively over 70% of the population). <i>Streptococcus</i> is only ranked in ADHD, <i>Blautia</i> is ranked higher in ADHD, <i>Oscillospira</i> is ranked higher in Control. <i>Blautia</i> 's cluster (<i>FL1</i>) is larger and more central in ADHD. <i>Oscillospira</i> 's (<i>FR</i>) is larger and more central in Control.

946 **Table 28.** Relationships noted throughout our analyses.

947
948
949

950 **Discussion**

951

952 Traditional analysis methods (i.e. diversity and composition) prevalent in current
953 ADHD gut microbiome literature provide a macroscale representation of a complex
954 ecosystem. Conducting some of these approaches on equal-sized, gender-balanced
955 undergraduate Control and ADHD gut microbiome datasets produced many results
956 that corresponded with this literature, plus a potentially new Control biomarker
957 *Turicibacter*. Current literature, as well as our results, suggest this macroscale
958 perspective leaves a largely incomplete picture due to its neglect of underlying
959 complexity. Our goal was to complete more of this picture by venturing deeper, by
960 analyzing two-way ecological relationships (cooperation and competition), plus
961 community detection, and centrality.

962

963 Our results provide a deeper meaning to those from the macroscale. Anomalous results
964 involving elevated *Bifidobacterium* and reduced *Bacteroides* and *Sutterella* at ASRS
965 extremes imposed significant challenges when interpreting results (with *Bifidobacterium*,
966 we were not the first to observe this (58)). Our MCNs estimate that a Bacteroidetes-
967 dominant community (cluster *BB*) forms in both microbiomes, with *Bacteroides* and
968 *Sutterella* both core members, that in ADHD is larger, more centered around *Bacteroides*,
969 residing in conditions that favor its cooperation, as opposed to competition in Control.
970 And our MCNs estimate *Bifidobacterium* to be involved in these conditions, shifting from
971 exclusively competitive relationships with cluster *BB* members (including its most
972 abundant and centroid) in Control, to exclusively a cooperative relationship in ADHD.

973

974 Potential roles played by LEfSe biomarkers also became observable. Our MCNs
975 estimated *Odoribacter*, reported by our LEfSe analysis and another (59) as ADHD-
976 elevated, to also compete with two *Clostridiaceae* taxa that competed with cluster *BB*
977 taxa. Another one of our ADHD biomarkers, *Butyrimonas*, joined cluster *BB* in ADHD
978 and formed cooperative relationships with many members. New interesting taxa and
979 communities also emerged. Cluster *FT* (cooperative with cluster *BB*) was larger in
980 ADHD. Cluster *FR* (*Ruminococcaceae*-dominant, competitive with cluster *BB*) was

981 smaller in ADHD, with *Ruminococcaceae* taxa almost universally less central.
982 *Ruminococcaceae* member genus *Oscillospira* was estimated to have ADHD-exclusive
983 competition, with *Enterobacteriaceae* (cooperative and fellow FR member in Control),
984 and cluster BB member *Streptococcus*. The shift in dynamics from Control to ADHD
985 involving *Streptococcus*, *Blautia*, and *Oscillospira* in ADHD was particularly interesting
986 (Table 28, last row).

987

988 Deeper meaning can be added through additional studies targeting some of these taxa
989 and relationships, including multi-omics (125) and/or physical laboratory experiments.
990 Fundamentally, ecological relationships manifest through internal interplay within the
991 underlying web of interactions (126). Cooperation could take place for example if two
992 taxa produce a nutrient that the other consumes; competition could take place if two
993 taxa consume a nutrient that neither produces. Coupling taxa to metabolites they
994 produce and consume and analyzing pathways can help elucidate underlying
995 mechanisms behind these ecological relationships. These pathways can then be
996 searched for neurotransmitters to establish ADHD connections. With very few studies
997 even attempting this level of analysis (62), an enormous breadth of knowledge remains.

998

999 Many future improvements to our analyses are possible. Future studies involving
1000 ADHD and the gut microbiome should account for factors such as ethnicity (127), use of
1001 medication/probiotics (55), use of antibiotics (128), diet (129), and gastrointestinal
1002 issues (130). More meaning to relationships in our MCNs can also be uncovered,
1003 through *causality* studies. Causality would give direction to edges, enabling detection
1004 of both two- and one-way (i.e. commensalism (110), amensalism (111)) relationships.
1005 This can be achieved through for example Bayesian Networks (131), which detect
1006 relationships where a taxon is conditionally dependent on another. Conditional
1007 dependence also eliminates spurious edges that can occur with correlations; for
1008 example, two entities that co-occur with a mutual entity will naturally tend to co-occur
1009 (88) (this was also a dependency removed by ATria after finding a central node). Sazal

1010 *et al.* (132) have already verified such networks as a predictor for oral microbiome
1011 colonization order. Time can also factor into ecological relationships because while
1012 sometimes these relationships are constant in microbiomes (133), they can also be
1013 transitive (134) or even time-varying (135). DBNs that account for time have already
1014 been used to predict long-term infant gut behavior (136). Higher-level network metrics
1015 such as modularity (137) and vulnerability (138) would provide another potential
1016 avenue for comparing and contrasting Control and ADHD MCNs. Amplicon Sequence
1017 Variants (ASVs, (139)) can be used in place of the current Operational Taxonomic Units
1018 (OTUs) that are generated by similarity-based clustering. ASVs exhibit more reliability
1019 at lower levels of the taxonomic tree and can improve the granularity of our MCNs,
1020 achieving more species- and sometimes even strain-level classifications.

1021
1022 A more complete understanding of ADHD and the gut microbiome will best equip the
1023 community to make the right decisions when administering treatment(s). Our results,
1024 coupled with those in the literature, suggest that the gut microbiota cannot afford to be
1025 ignored when it comes to ADHD, and treatments directly targeting the gut microbiome
1026 have potential. Encouraging results have been uncovered for gluten and casein-free
1027 diets (44), Microbiota Transfer Therapy (MTT, (140,141)), and probiotics (142) with ASD.
1028 Our results also indicate that the gut microbiome is an ecosystem, and any changes to
1029 one single element will likely impact other members. Additionally since the human gut
1030 microbiome is widely varied across individuals (143), personalized medicine should be
1031 used when developing such treatments.

1032

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1042

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1427 43246-1_6)

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1431 **Supporting Information Captions**

1433 **Fig. S1. Discriminant Analysis, Scarce Taxa Included.** Results of running sPLS-DA
1434 (68) on microbiome abundance data (ellipse confidence level 95%) without removing
1435 scarce taxa. The figures show the analyses (a) comparing Control (orange) and ADHD
1436 (blue) groups and (b) further separating the ADHD group into inattention (green),
1437 hyperactive (grey), and combined (blue).

1438

1439

1440 **Fig. S2. Compositional Analysis, Class Level.** Microbial compositional bar graph for
1441 each subject, generated using QIIME (70), conducted at the class level. Subjects are

1442 ordered by increasing Adult ADHD Self Report Scale (ASRS) score, with the y-axis
1443 representing relative abundance.
1444

1445 **Fig. S3. Compositional Analysis, Order Level.** Microbial compositional bar graph for
1446 each subject, generated using QIIME (70), conducted at the order level. Subjects are
1447 ordered by increasing Adult ADHD Self Report Scale (ASRS) score, with the y-axis
1448 representing relative abundance.
1449

1450 **Fig. S4. Compositional Analysis, Family Level.** Microbial compositional bar graph for
1451 each subject, generated using QIIME (70), conducted at the family level. Subjects are
1452 ordered by increasing Adult ADHD Self Report Scale (ASRS) score, with the y-axis
1453 representing relative abundance.
1454

1455 **Fig. S5. Compositional Analysis, Order Level.** Microbial compositional bar graph for
1456 each subject, generated using QIIME (70), conducted at the species level. Subjects are
1457 ordered by increasing Adult ADHD Self Report Scale (ASRS) score, with the y-axis
1458 representing relative abundance.
1459

1460 **Fig. S6. Relative Abundance, Three Observed Taxa.** Relative abundance of genera (a)
1461 *Bifidobacterium*, (b) *Bacteroides* and (c) *Sutterella*. Subjects are ordered by increasing
1462 Adult ADHD Self Report Scale (ASRS) score, with the y-axis representing relative
1463 abundance.
1464

1465 **Table S1.** Correlations in all MCNs, over all taxonomic levels, organized by taxonomic
1466 classification. Each box indicates the two taxa involved in each correlation, along with
1467 the sign (+ or -). Boxes colored orange correspond to correlations present in Control,
1468 and purple only present in ADHD. Grey boxes are present in both MCNs. White boxes
1469 correspond to correlations that were not observed, but one was present among its
1470 descendants (i.e. genera *Collinsella* and *Butyrimonas* were not correlated in either
1471 MCN, but member taxa *C. aerofaciens* and *Butyrimonas* were for ADHD). For
1472 polyphetic genus *Ruminococcus*, [L]=*Lachnospiraceae* family, [R]=*Ruminococcaceae* family.
1473

1474 **Table S2.** ATria rankings of all taxa found as important in all MCNs, grouped by
1475 taxonomic classification. NR=Not Ranked, T=Tied. Taxa ranked only in Control are
1476 colored dark orange, higher in Control light orange, higher in ADHD light purple, and
1477 only in ADHD dark purple. **Bold** taxa are ranked #1 in their corresponding MCN.
1478 White, italicized taxa correspond to unranked taxa with a ranked descendant.
1479

1480 **Table S3.** Family-level MCN clusters, reported by Affinity Propagation (AP, [CITE]).
1481 Core taxa (shared by both MCNs) are bold, and centroids are marked with an asterisk
1482 (*, requires at least three taxa). Italicized taxa are exclusive to their MCN (Control or

1483 ADHD). Phylum colors match those in Fig. 5 (Actinobacteria brown, Firmicutes yellow,
1484 Proteobacteria blue, Bacteroidetes dark purple). Cluster colors match those in Fig. 6.

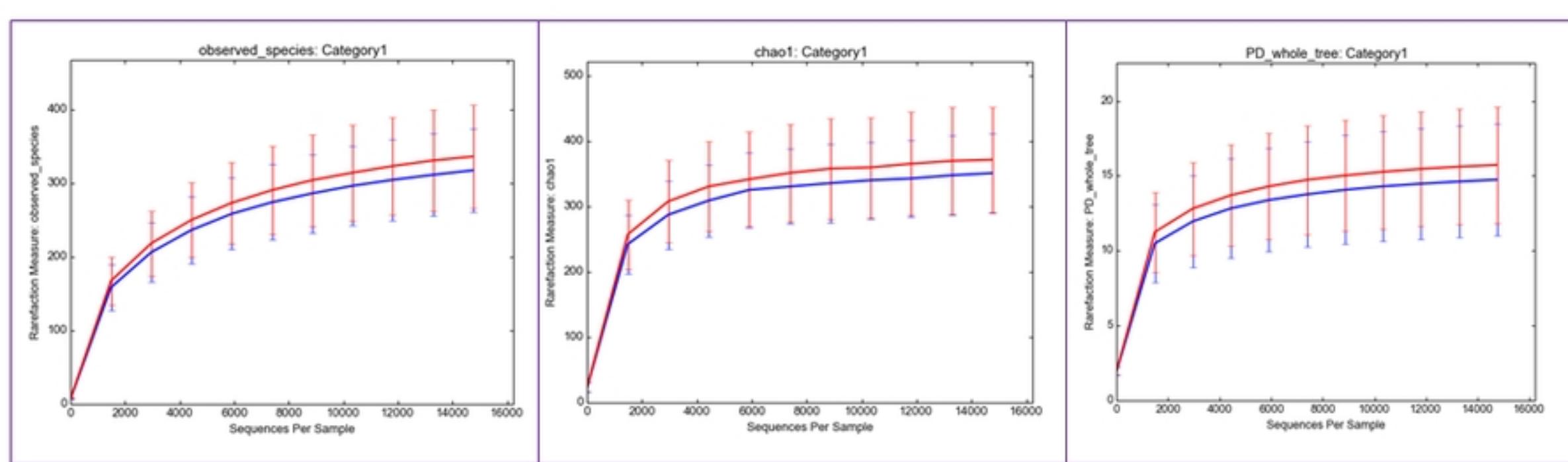
1485

1486 **Table S4.** Genus-level clusters, reported by AP. Color and labelling is the same as
1487 Table S3. [L]=*Lachnospiraceae* family, [R]=*Ruminococcaceae* family.

1488

1489 **Table S5.** Lowest-level clusters, reported by AP. Color and labelling is the same as
1490 Tables S3 and S4.

1491



Control ADHD

Figure 1A

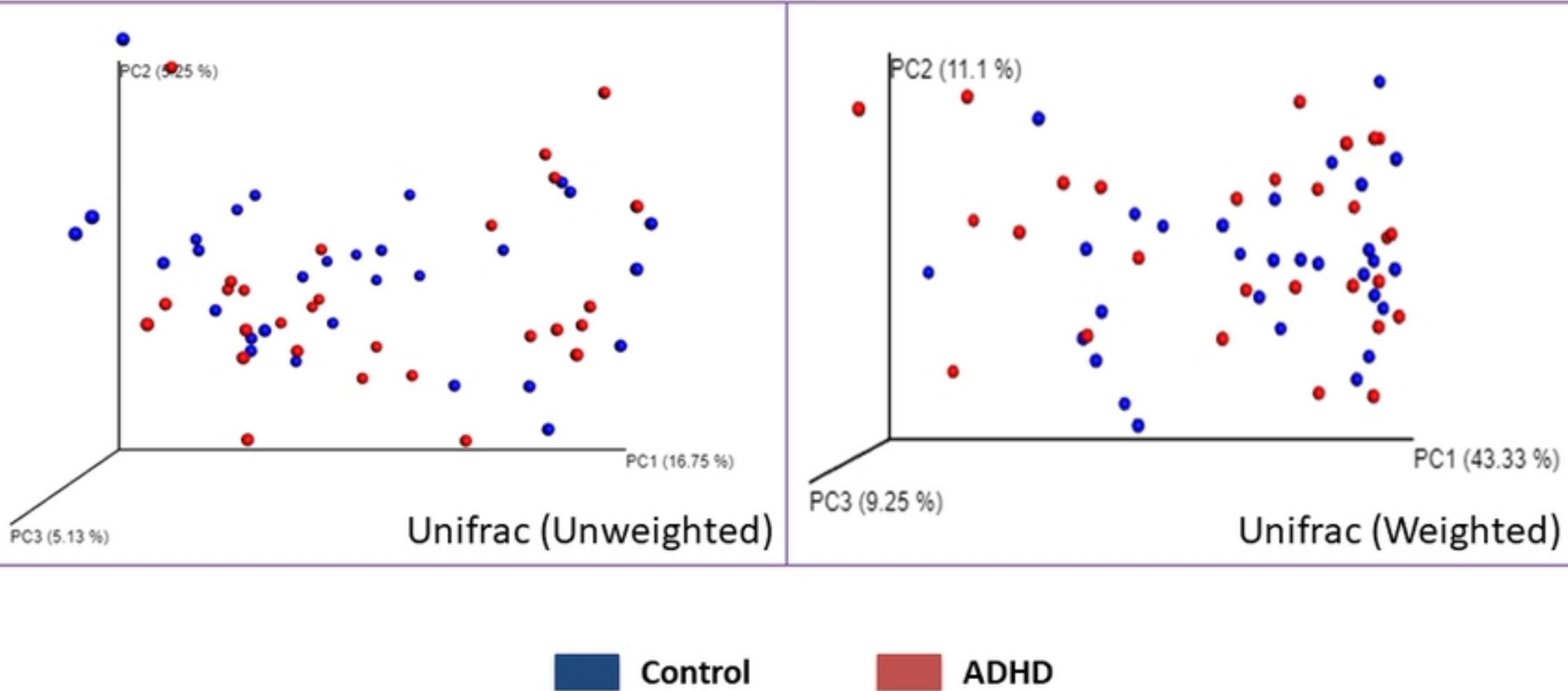
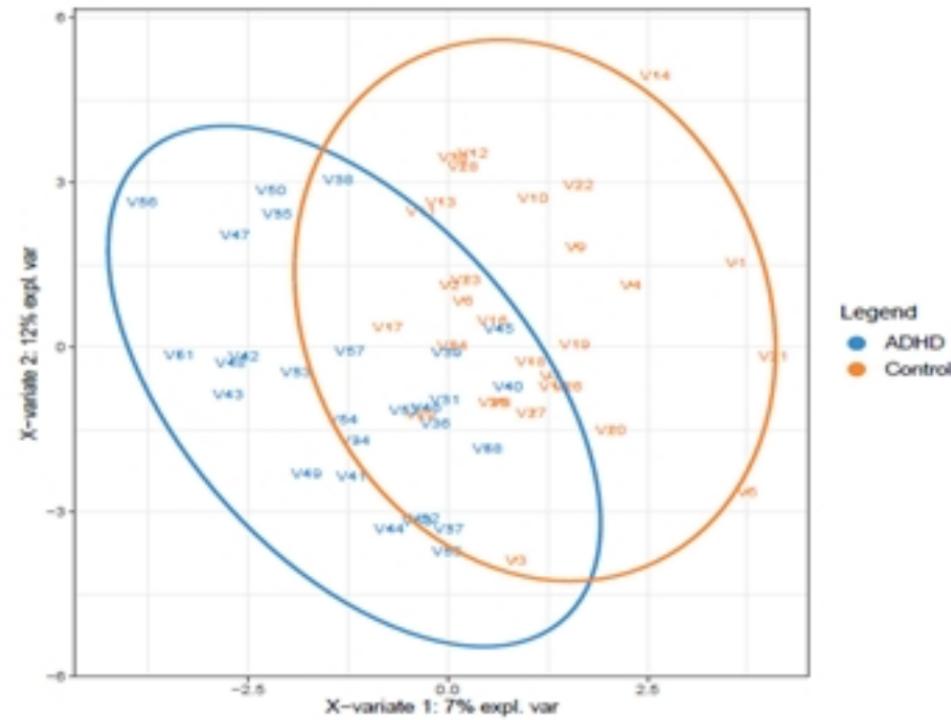


Figure 1B

A: Control vs. ADHD



B: Control vs. ADHD (With Inattention/Hyperactivity Split)

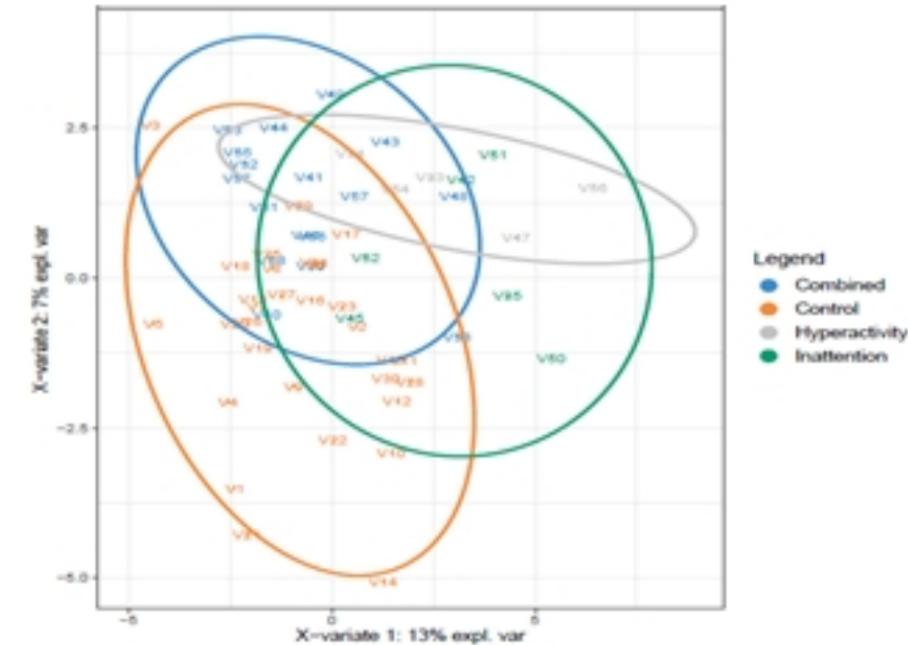
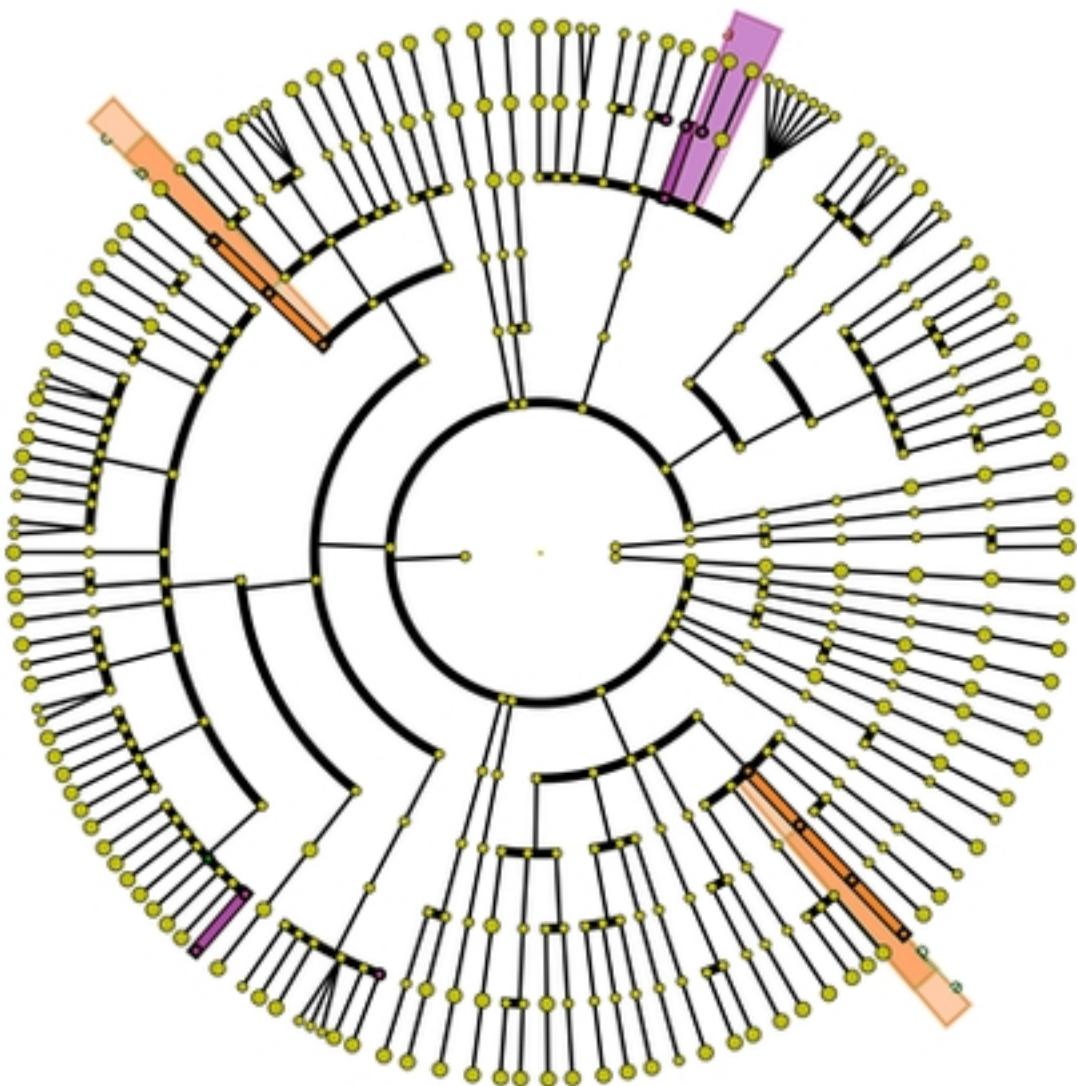


Figure 2



Control ADHD

- a. *Odoribacteraceae* (Family, B)
- b. *Turicibacteraceae* (Family, F)
- c. *Turicibacterales* (Order, F)
- d. *Pasteurellaceae* (Family, P)
- e. *Pasteurellales* (Order, P)

Figure 3A

Control

ADHD

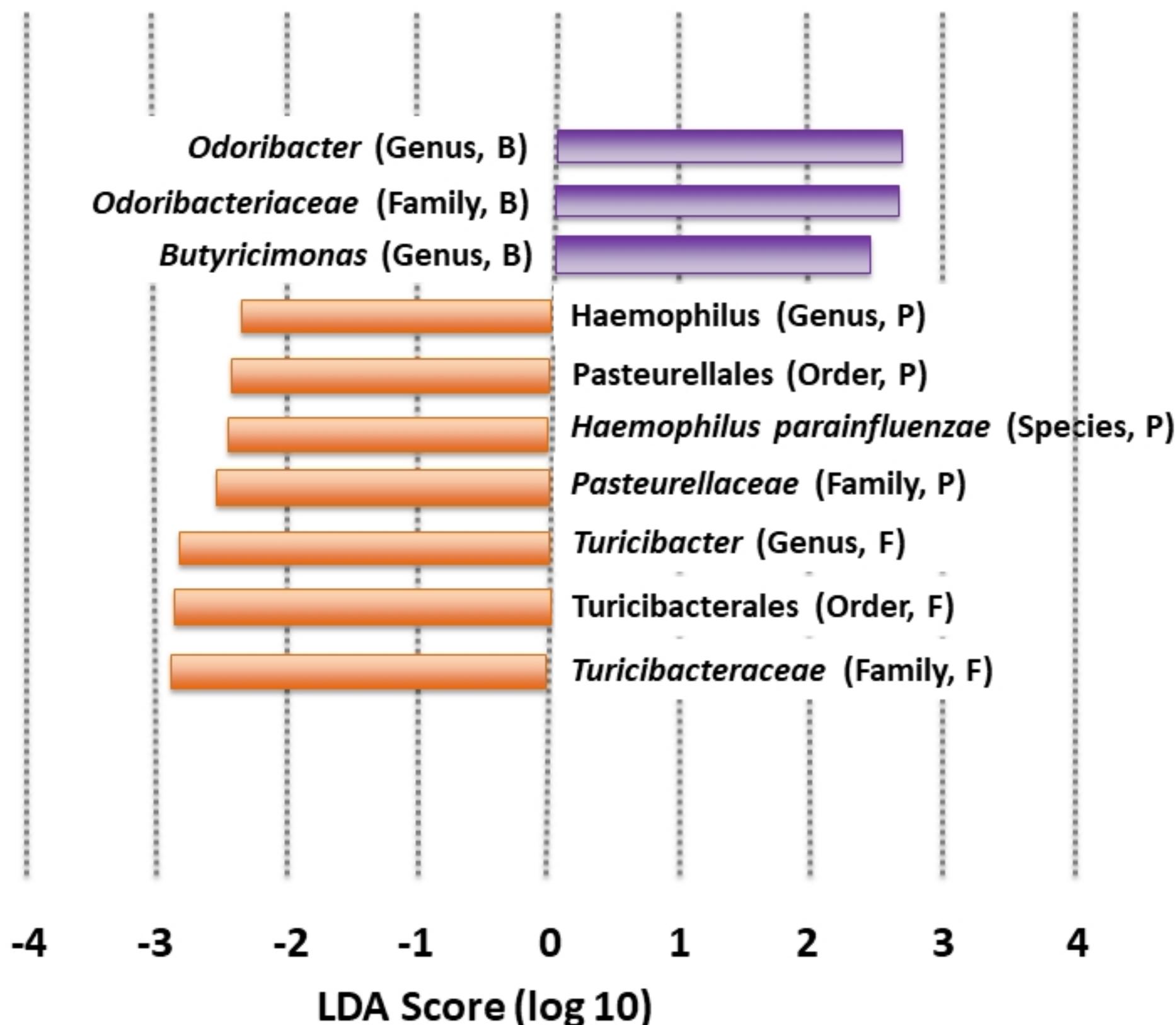


Figure 3B

Compositional Bar Chart (Phylum Level)

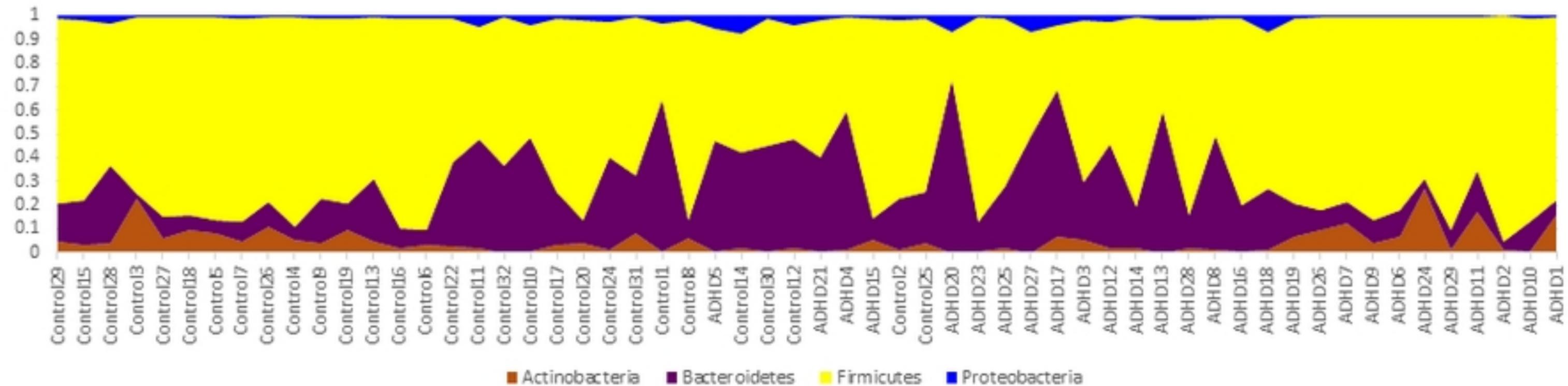


Figure 4A

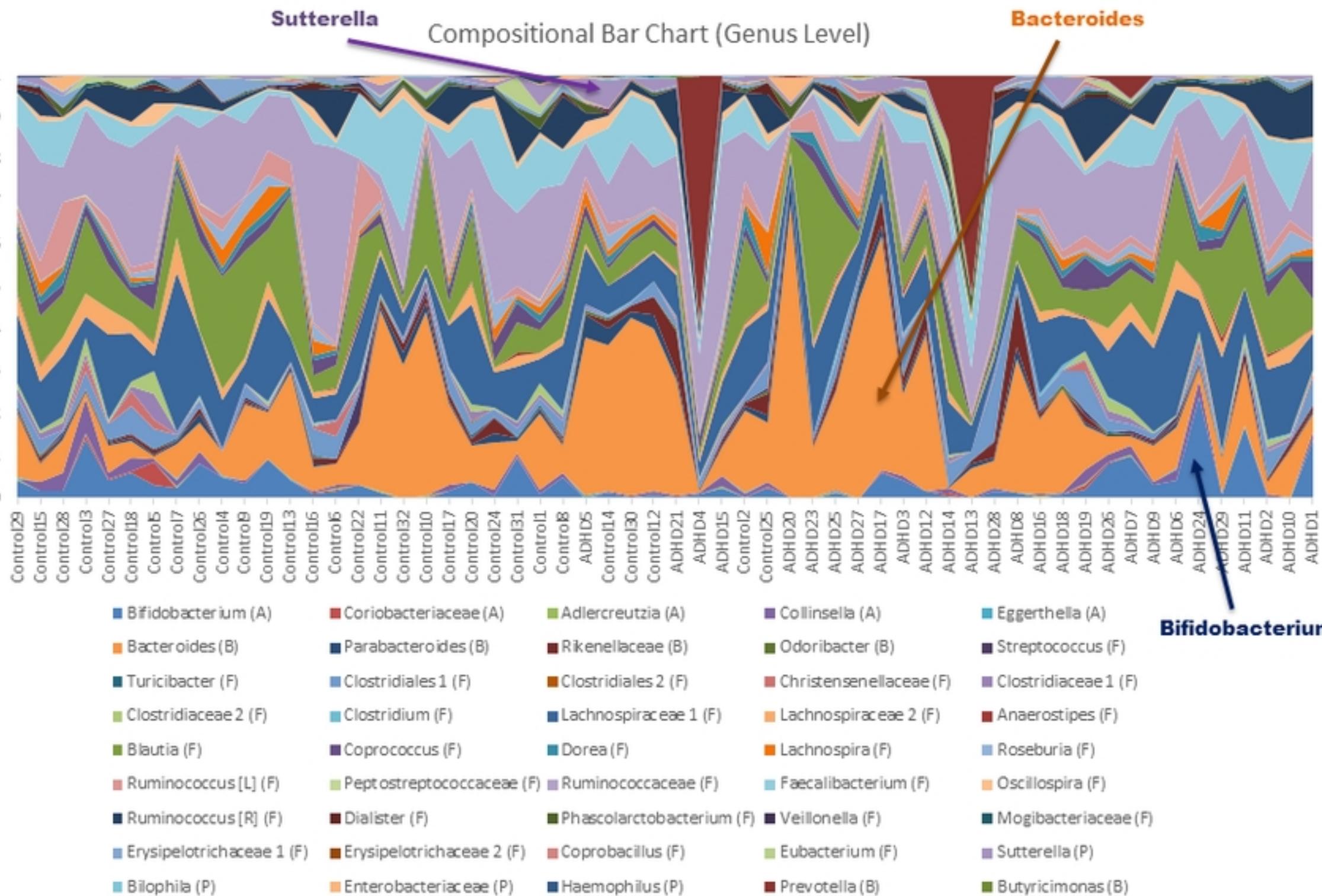


Figure 4B

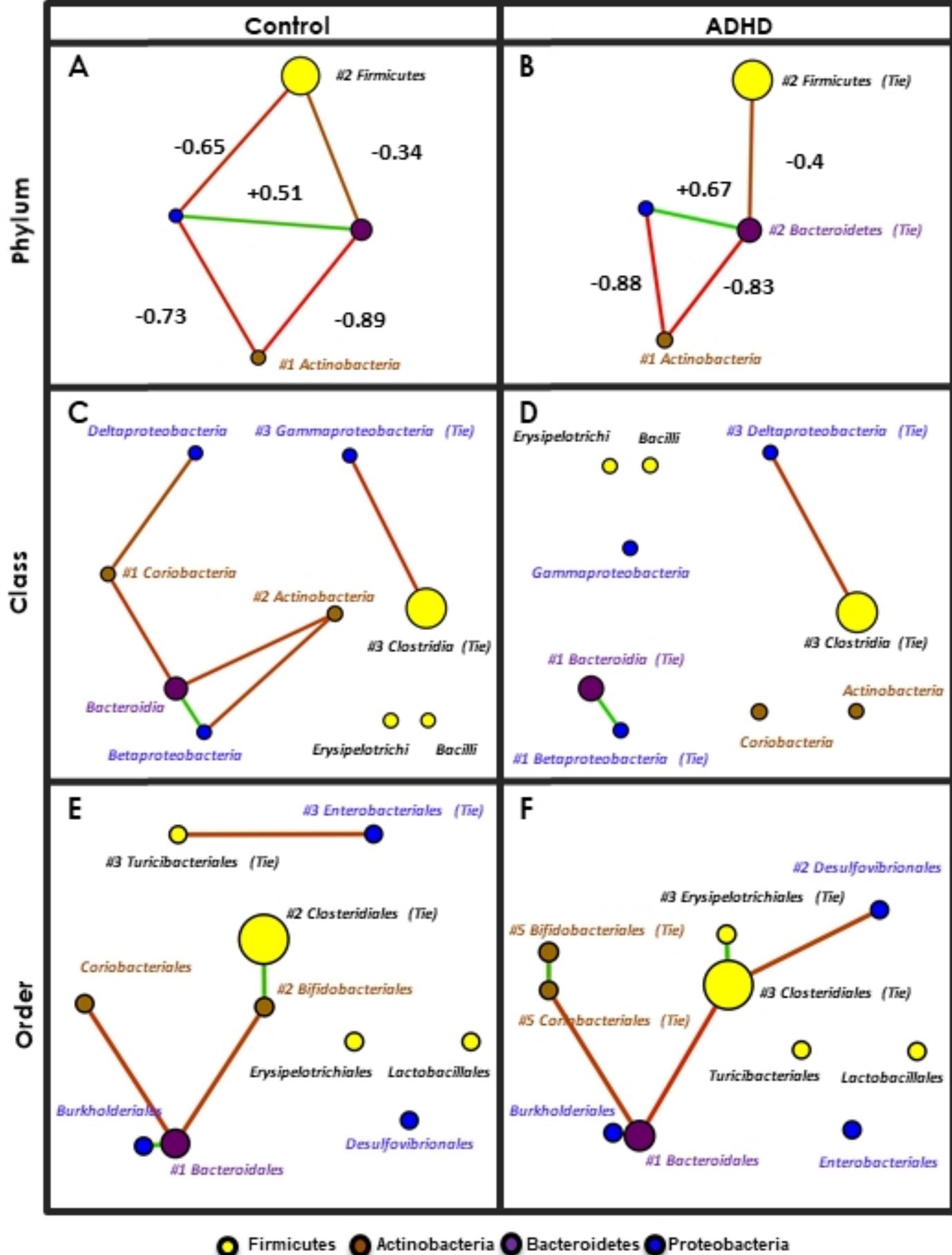


Figure 5

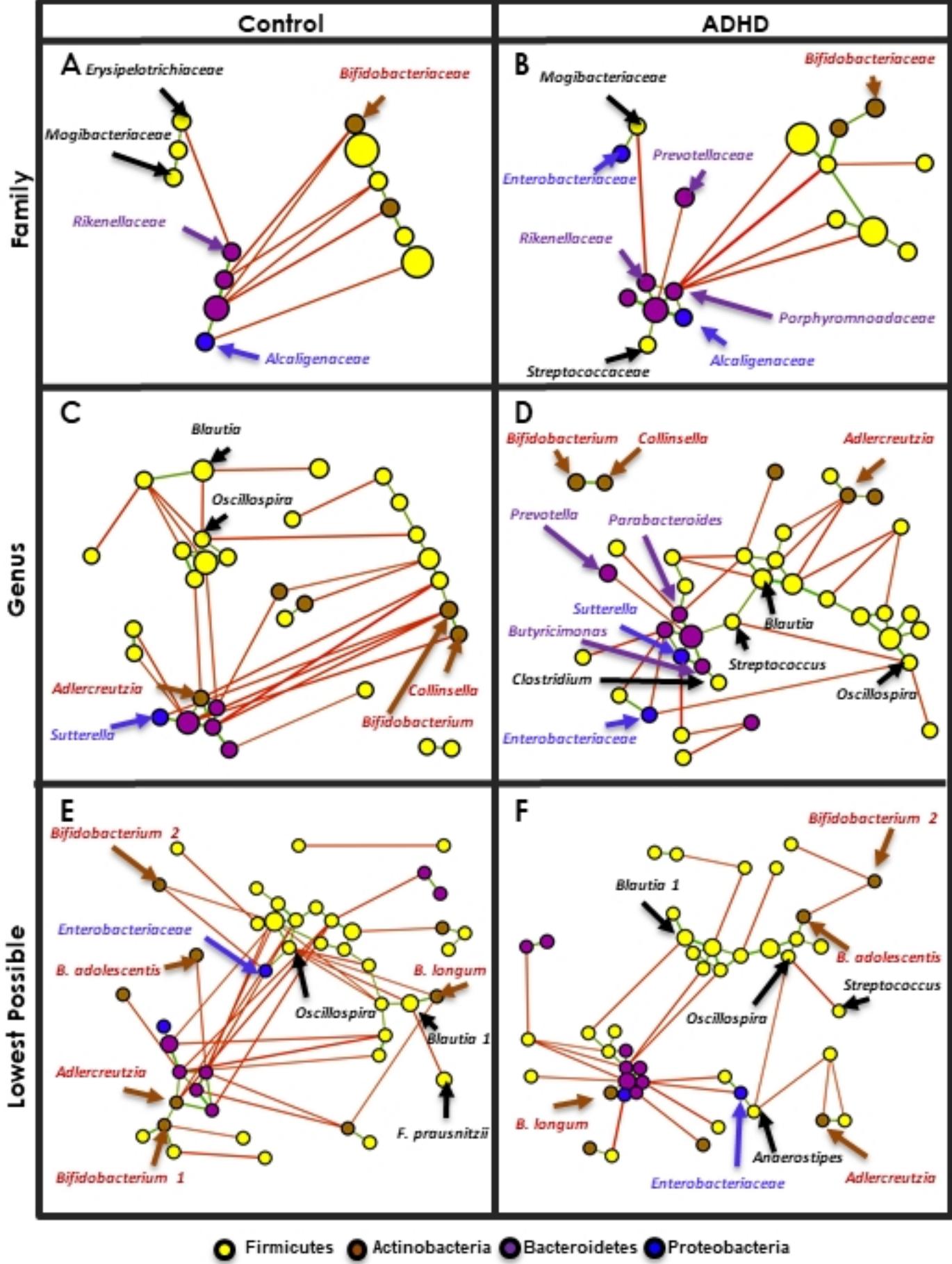


Figure 6

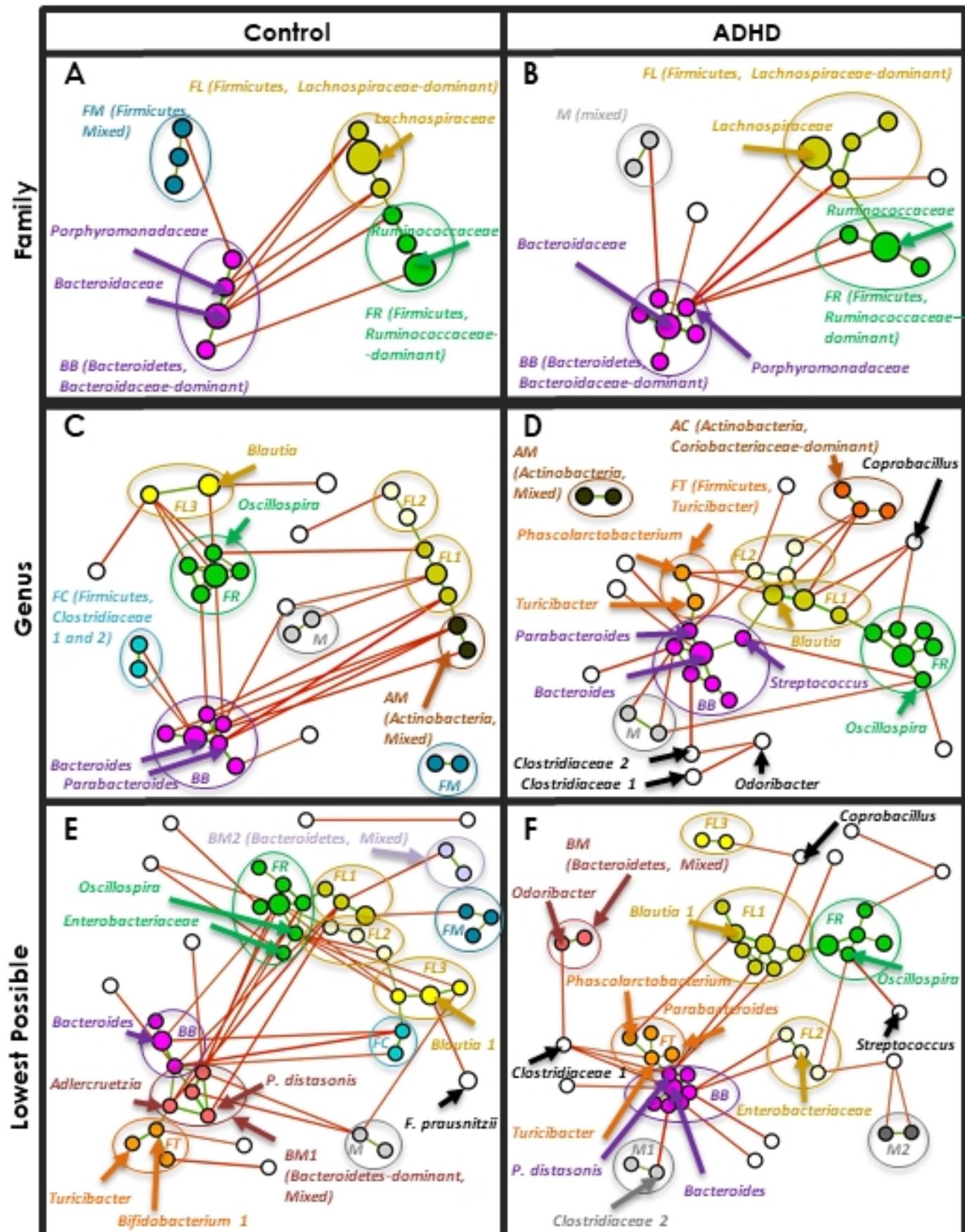


Figure 7

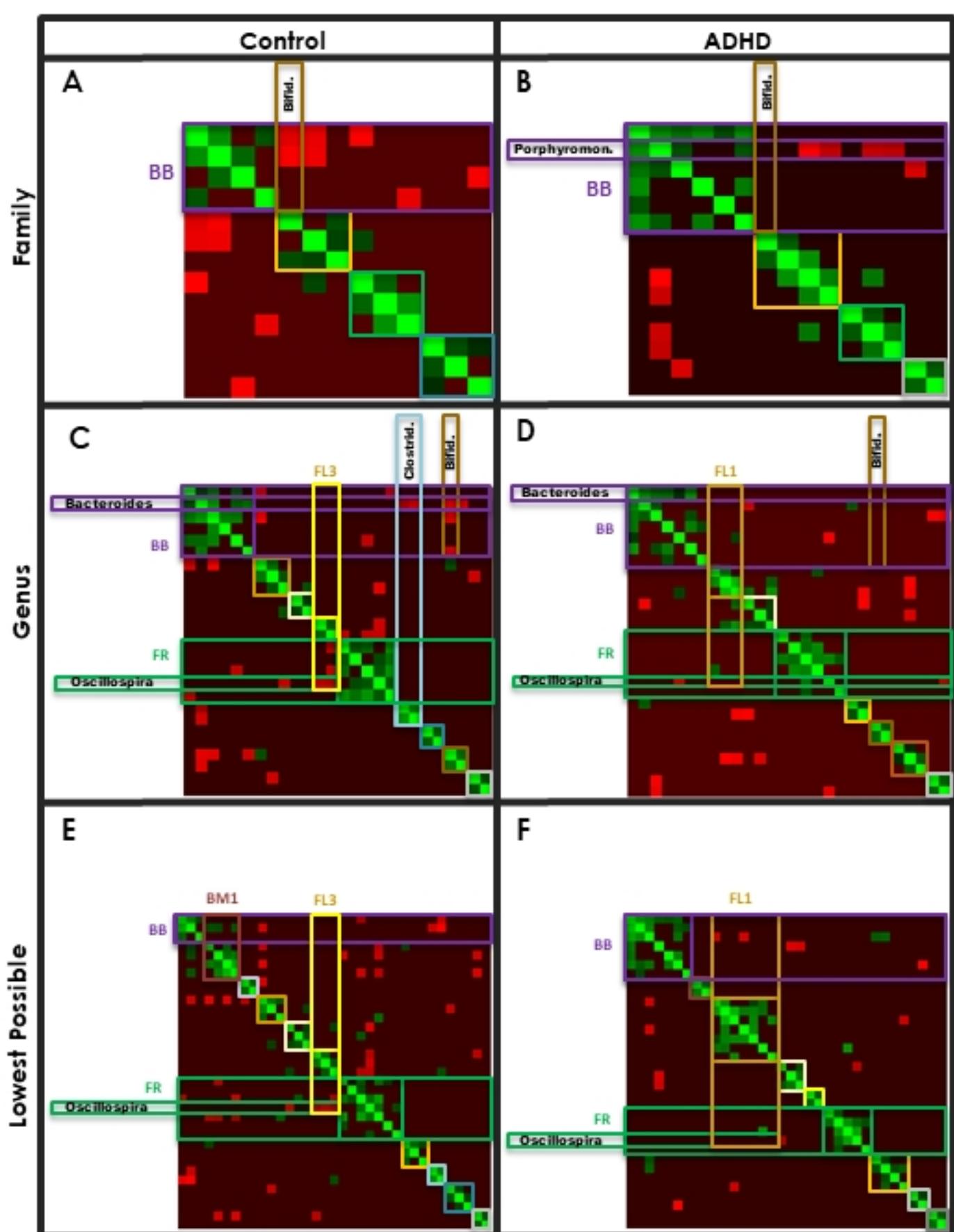
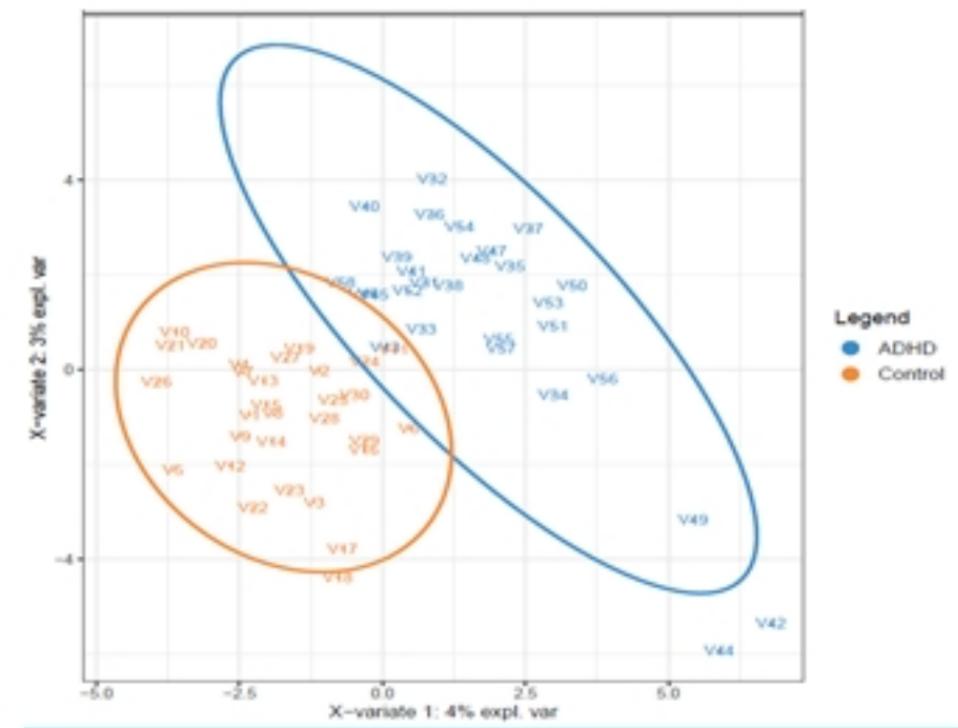


Figure 8

A: Control vs. ADHD (Unfiltered)



B: Control vs. ADHD (Subscale) (Unfiltered)

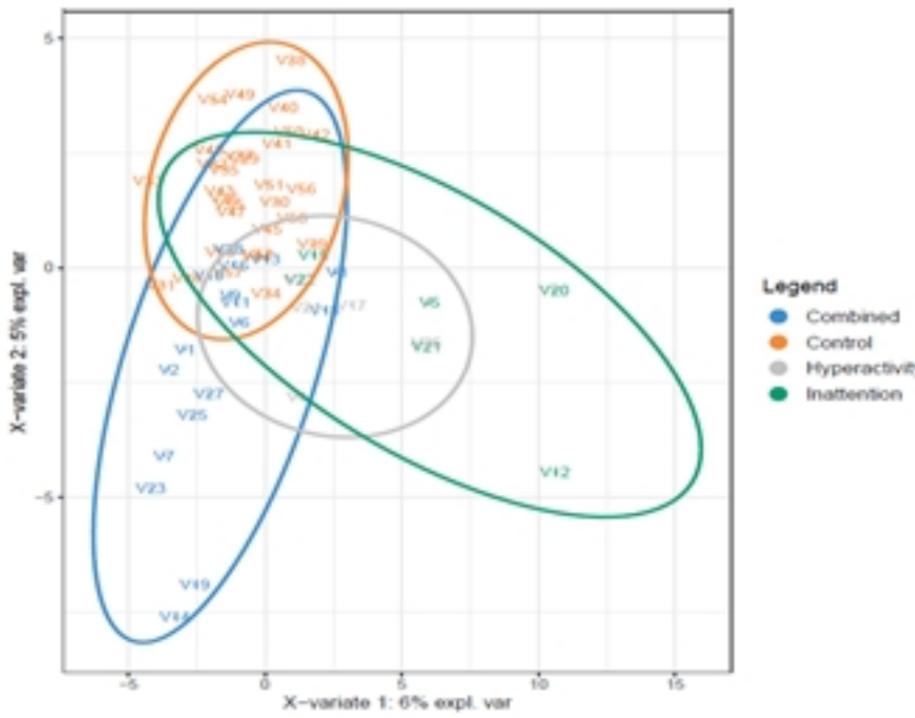


Figure S1

Compositional Bar Chart (Class Level)

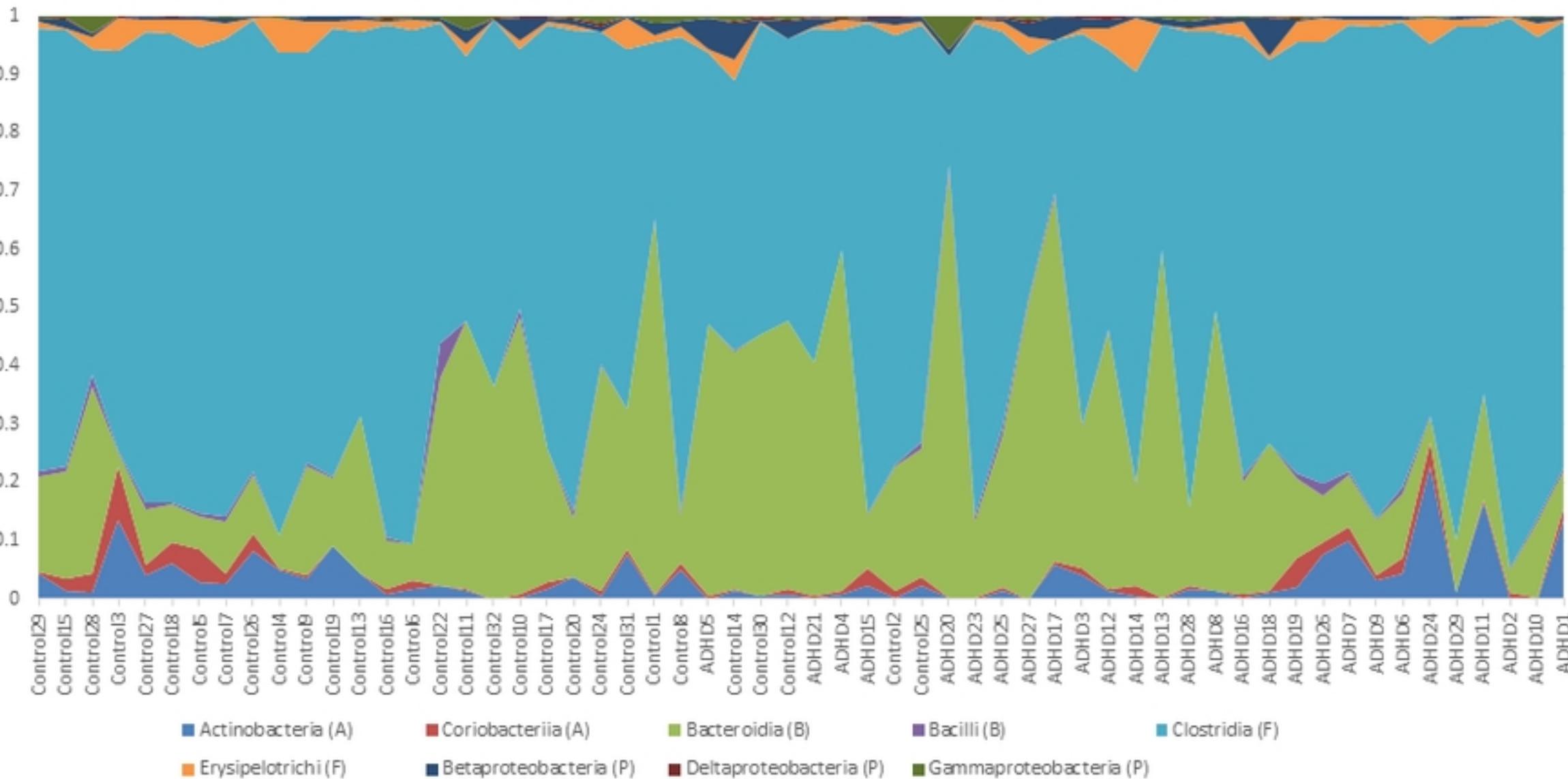


Figure S2

Compositional Bar Chart (Order Level)

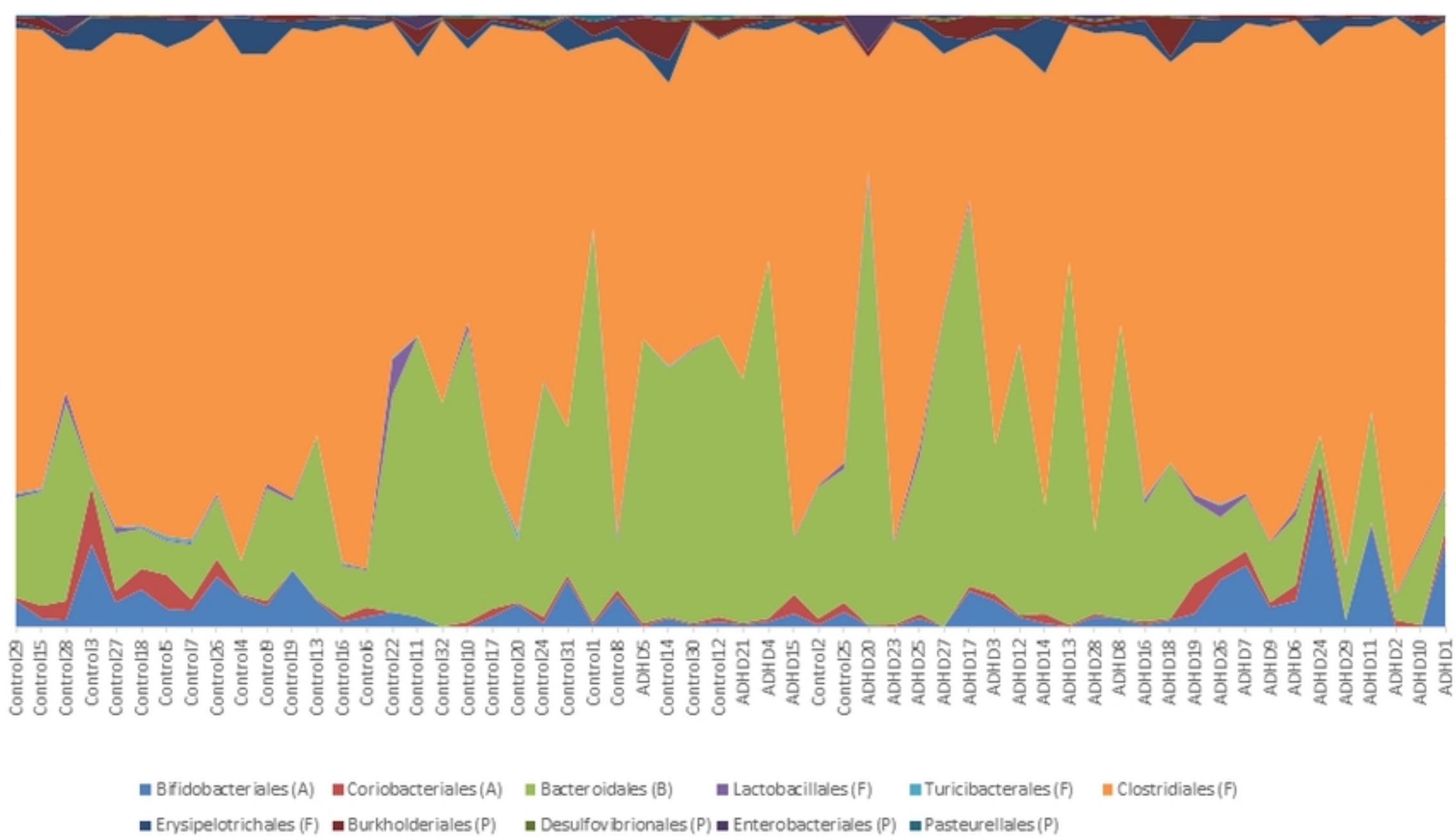


Figure S3

Compositional Bar Chart (Family Level)

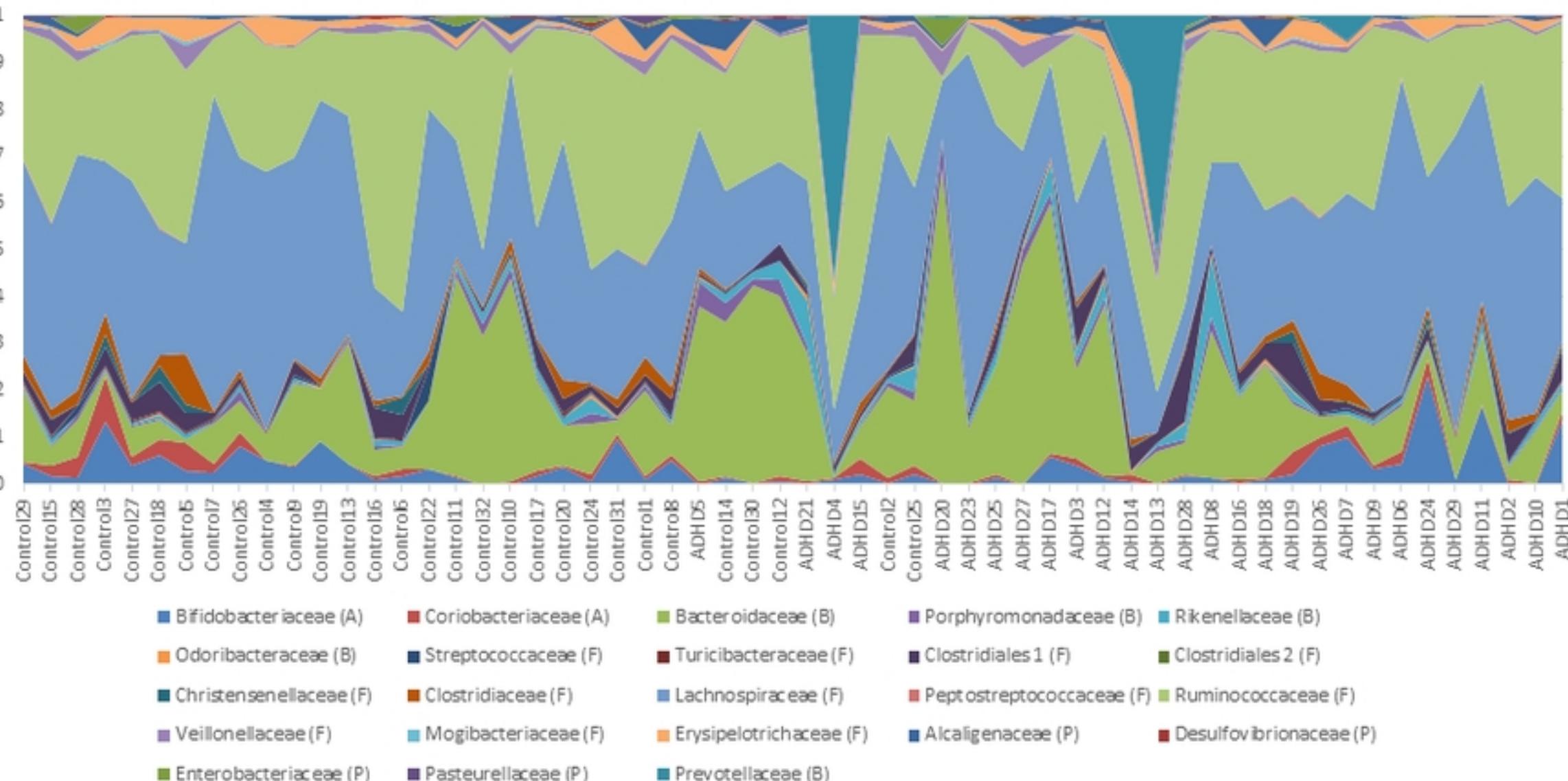


Figure S4

Compositional Bar Chart (Most Specific)

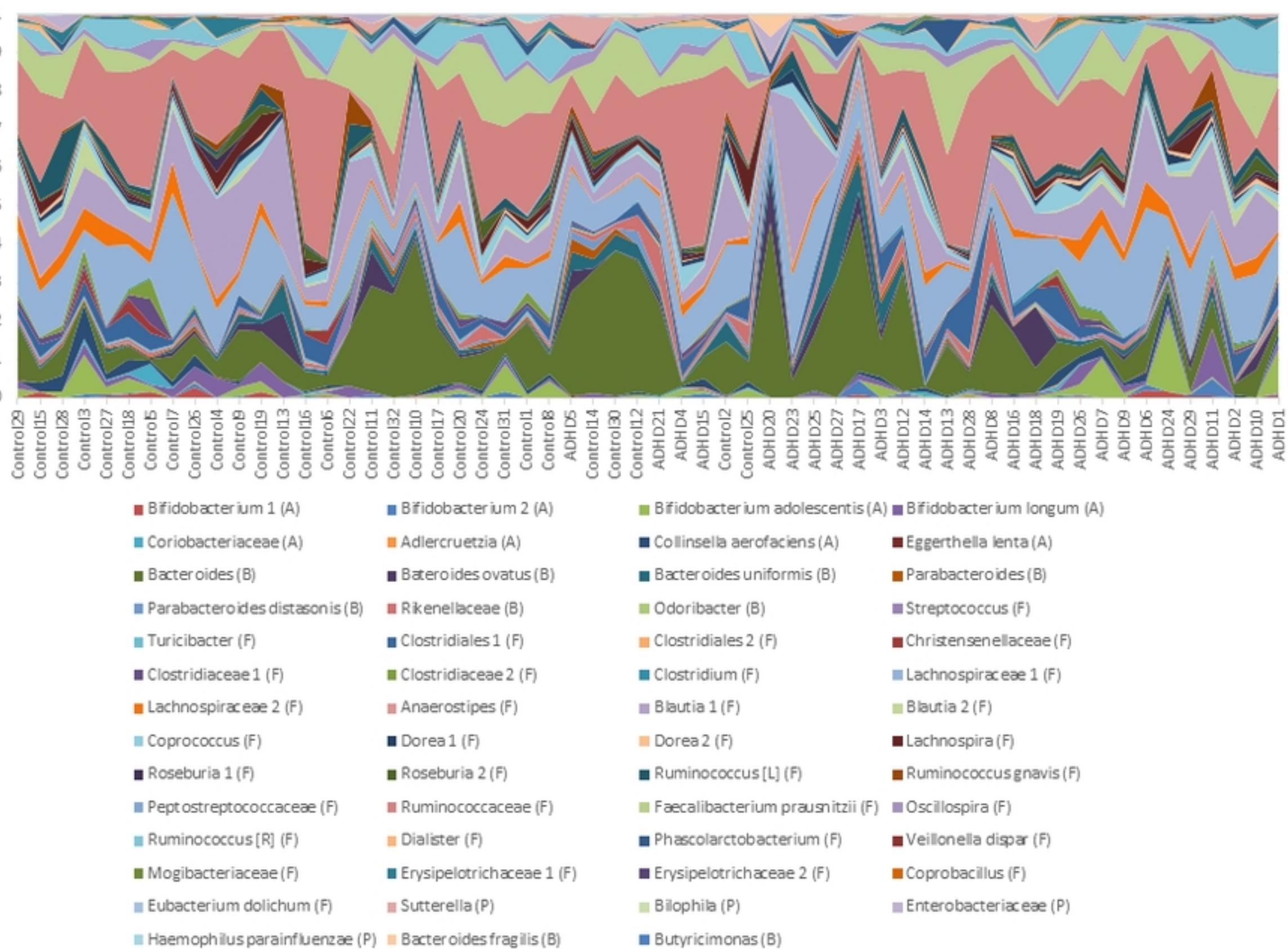


Figure S5

Bifidobacterium (A)

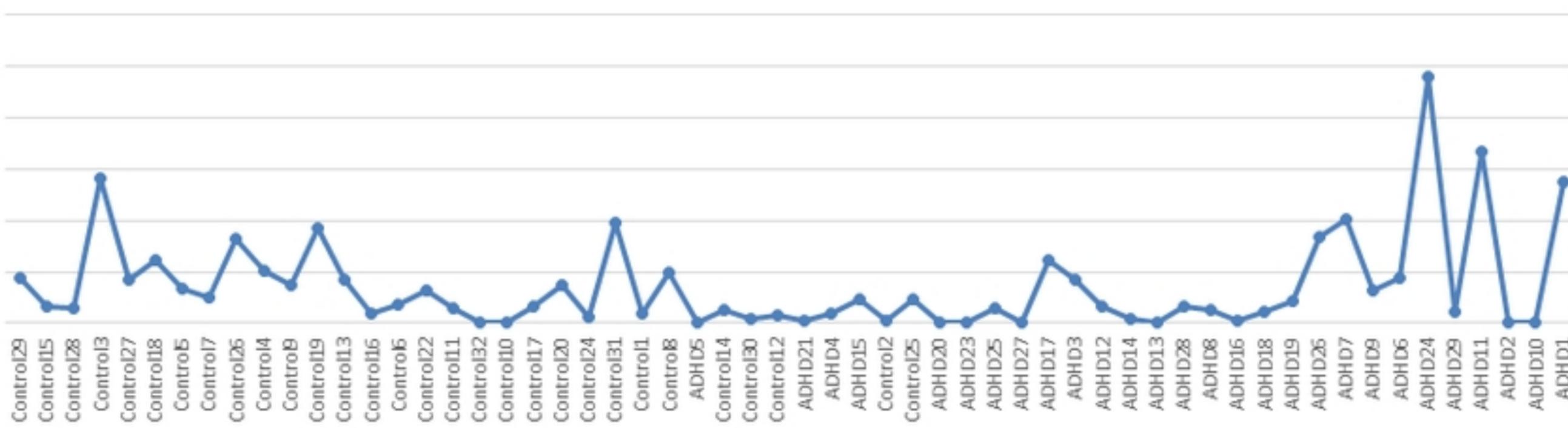


Figure S6A

Bacteroides (B)

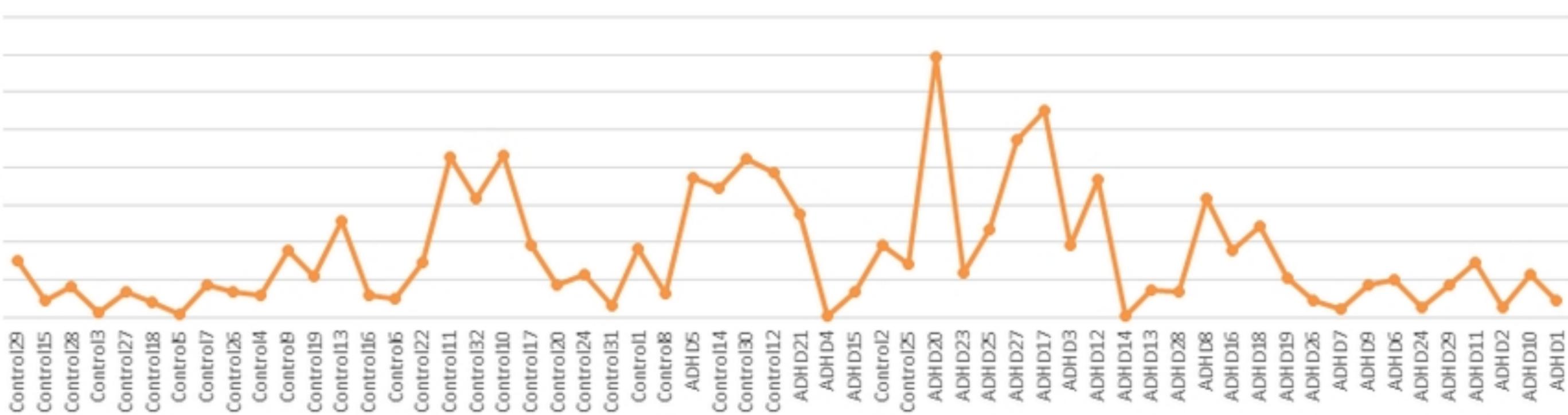


Figure S6B

Sutterella (P)

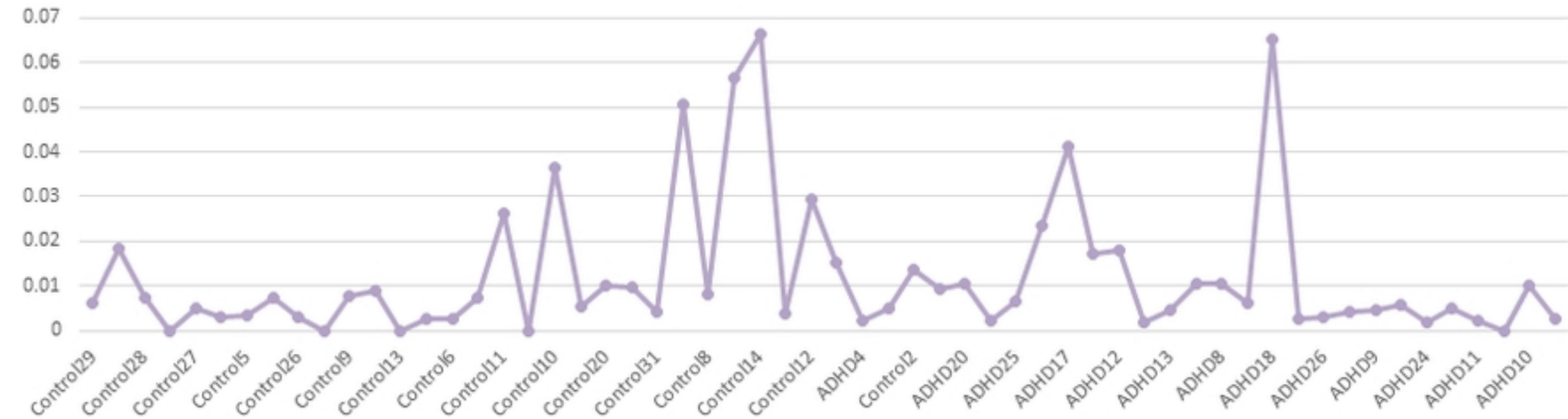


Figure S6C