Microbial traits and the realized niche in a simple metaorganism

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Abstract

Organisms and their resident microbial communities - the microbiome - form a complex and mostly stable ecosystem. It is known that the specific composition and abundance of certain bacterial species have a major impact on host health and *Darwinian fitness*, but the processes that lead to these microbial patterns have not yet been identified. We here apply the niche concept and trait-based approaches as a first step in understanding the patterns underlying microbial community assembly and structure in the simple metaorganism *Hydra*. We find that the carrying capacities in single associations do not reflect microbiota densities as part of the community, indicating a discrepancy between the fundamental and realized niche. Whereas in most cases, the realized niche is smaller than the fundamental one, as predicted by theory, the opposite is observed for *Hydra's* two main bacterial colonizers. Both, *Curvibacter sp.* and *Duganella sp.* benefit from association with the other members of the microbiome and reach higher fractions as in single colonisations. This cannot be linked to any particular trait that is relevant for interacting with the host or by the utilization of specific nutrients but is most likely determined by metabolic interactions between the individual microbiome members.

Introduction

Microbiomes contribute to ecosystems as key engines that power system-level processes (Falkowski et al., 2008). This also applies to host ecosystems, where they are critical in maintaining host health, survival, and function ((Kau et al., 2011; McFall-Ngai et al., 2013)). Despite their importance, the mechanisms governing microbiome assembly and composition are largely unknown. This is different for macroscopic communities, thanks to the application of niche (Holt, 2009; Leibold, 1995; Whittaker et al., 1973) and trait-based theories, which might also provide a useful framework for studying the ecology and evolution of microbiomes in metaorganisms (Kopac and Klassen, 2016).

The niche concept is one of the core concepts in ecology and has been rediscovered by modern ecology for explaining biodiversity and species coexistence patterns (Pocheville, 2015). The niche-based theory states that an ecological community is made up of a limited number of niches, each occupied by a single species. Hutchinson (Hutchinson, 1957) defined the *fundamental niche* as the needs of a species for it to maintain a positive population growth rate, disregarding biotic interactions (Hutchinson, 1957; Pearman et al., 2008). The fundamental niche therefore represents an idealized situation exclusive of interspecific interactions. The effect of biological interactions is taken into account in the definition of the *realized niche* (Hutchinson, 1957). This is the portion of the fundamental niche in which a species has a positive population growth rate, despite the constraining effects of biological interactions, such as inter-specific competition (Hutchinson, 1957; Pearman et al., 2008).

In the last two decades, the shift from taxonomy to function by using trait-based approaches has provided a detailed understanding of biodiversity-ecosystem functioning (Louca et al., 2018). Recently, this framework is also being used by microbial ecologists to study microbial biogeography (Green et al., 2008), or to unravel microbial biodiversity-ecosystem functioning relationships (Krause et al., 2014). Further, this approach allows studying microbiomes in the light of coexisting traits/ functions rather than of coexisting microbes (Martiny et al., 2015). A recent study successfully used this approach and analysed trait-based patterns to understand the mechanisms of community assembly and succession of the infant gut microbiome (Guittar et al., 2019). Microbial traits cover a range of phenotypic characteristics ranging from simple to complex, for example organic phosphate utilization, bacteriophage host range, cellulose degradation, biofilm formation, nitrogen fixation, methanogenesis, and salinity preference (Martiny et al., 2015). Potential microbial traits can be measured directly by

laboratory assays (as in this study) or can be indirectly inferred based on genomic information.

The aim of this study is to apply the niche concept and trait-based theory to the metaorganism *Hydra* to gain insight into the mechanisms underlying the microbial community composition. We thus specifically extend the niche-assembly perspective, classically used for assessing species assembly and coexistence in abiotic environments, to a host-associated microbiome, thus a biotic environment.

The freshwater polyp and its microbiome have become a valuable model system for metaorganism research as it provides a bridge between the simplicity of synthetic communities and the complex mouse model (Deines and Bosch, 2016). The ectoderm is covered by a multi-layered glycocalyx, which is the habitat for a species-specific microbiome of low complexity (Bosch, 2013; Deines et al., 2017; Franzenburg et al., 2013). The most abundant bacterial colonizers of *Hydra vulgaris* (strain AEP) are *Curvibacter sp.* (75,6%), *Duganella sp.* (11,1%), *Undibacterium sp.* (2.1%), *Acidovorax sp.* (0.7%), *Pseudomonas sp*. (0.4%), and *Pelomonas sp*. (0.2%) (Fraune et al., 2015). All strains can be cultured and manipulated *in vitro* (Bosch, 2013; Fraune et al., 2015; Wein et al., 2018), allowing the measurement of phenotypic microbial traits and fitness. Fitness, as defined by niche theory, is the positive population growth of the focal species, which in our study is that of the six microbiome members. Measurements of the performance of the bacterial populations when grown singly, i.e. in the absence of the other microbial competitors *in vitro* and *in vivo* (on germ-free *Hydra* polys), specify the fundamental niche. For each species, we compare the fundamental niche to the realized niche, which we predict based on data showing the microbiome composition of wild type polys (Franzenburg et al., 2013; Fraune et al., 2015). We also measure phenotypic traits for the different microbial strains that might play a role in populating the host and interacting with the host and their associate microbial community members. Ultimately, we take the traits and the ecological niches as determinants of species interactions, which may infer the assembly and structure of the host-associated microbiome.

107 **Materials and Methods**

108 **Animals used, culture conditions, and generation of germ-free animals**

109 *Hydra vulgaris* (strain AEP) was used in the experiments and cultured according to
110 standard procedures at 18°C in standardized *Hydra* culture medium (Lenhoff and
111 Brown, 1970). Animals were fed three times a week with 1st instar larvae of *Artemia*
112 *salina*. Germ-free polyps were obtained as previously described (Franzenburg et al.,
113 2013; Murillo-Rincon et al., 2017). After two weeks of treatment, polyps were
114 transferred into antibiotic-free sterile *Hydra* culture medium for recovery (four days).
115 Sterility was confirmed by established methods (Franzenburg et al., 2013). During
116 antibiotic treatment and re-colonization experiments, polyps were not fed.

117

118 **Bacterial strains and media**

119 The bacterial strains used in this study are *Curvibacter sp*. AEP1.3, *Duganella sp*. C1.2,
120 *Undibacterium sp*. C1.1, *Acidovorax sp*. AEP1.4, *Pseudomonas sp*. C2.2, *Pelomonas sp*.
121 AEP2.2., which are strains isolated from the *Hydra vulgaris* (strain AEP) microbiome
122 (Fraune et al., 2015). These bacteria were cultured from existing isolate stocks in R2A
123 medium at 18°C, shaken at 250 r.p.m for 72 hrs before use in the different experiments.

124

125 **Carrying capacity of mono-associations in *Hydra***

126 Germ-free polys were inoculated with single bacterial strains using $5x10^3$ cells in 1.5 ml
127 Eppendorf tubes containing 1 ml of sterile *Hydra* culture medium. After 24 hrs of
128 incubation, all polyps were washed with, and transferred to sterile *Hydra* culture
129 medium, incubated at 18°C. After three days of incubation individual polyps were
130 homogenized in an Eppendorf tube using a sterile pestle, after which serial dilutions of
131 the homogenate were plated on R2A agar plates to determine colony-forming units
132 (CFUs) per polyp.

133

134 **Measurement of bacterial growth rates *in vitro***

135 Cultures of the all six strains were produced in R2A microcosms (grown for 72 hrs at
136 18°C, at 250 r.p.m). Aliquots of each culture were first washed in S medium and then re-
137 suspended in fresh R2A medium to an optical density of 0.025 at 600 nm (OD_{600}).
138 Growth kinetics of all strains were determined in 96-well microtiter plates. A 100 µl
139 aliquot of each re-suspension was pipetted into 100 µl of fresh R2A medium. The
140 microtiter plate was then placed in a microplate reader (TECAN Spark 10M), and the
141 OD_{600} of each well was measured at 30 min intervals for 96 cycles (with 10 sec shaking
142 at 150 r.p.m. prior to each read). The growth of each strain was determined in five well

143 locations on an individual 96-well plate, which was replicated six times. The maximum
144 growth rate (V_{max}) was calculated from the maximum slope of the absorbance over time.
145
146 **Cell surface hydrophobicity (CSH)**
147 The BATH assay was performed as described previously (Borecká-Melkusová and
148 Bujdáková, 2008; Rosenberg, 1984). It uses a biphasic separation method to measure
149 cell surface hydrophobicity. In short, for each strain tested, 4 ml of bacterial suspension
150 (OD_{600} = 0.1; $OD_{initial}$) was placed into a class tube, overlaid with 1 ml of n-hexodecane
151 (Sigma Aldrich), and vortexed for 3 min. The phases were then allowed to separate for
152 15 min, after which the ODs of the aqueous (lower) phase containing hydrophilic cells
153 was measured at OD_{600} ($OD_{residual}$). The hydrophobic cells are found in the n-hexodecane
154 overlay (upper phase). OD values were compared to the bacterial suspension before
155 mixing with n-hexodecane. The relative hydrophobicity (RH) was calculated as follows:
156 RH = $((OD_{initial} - OD_{residual})/OD_{initial})$ x 100%. The experiment was performed in triplicate
157 with independent bacterial overnight cultures.
158
159 **Biofilm quantification by use of crystal violet (CV)**
160 Biofilm formation was assayed and quantified as previously described (Ren et al., 2015).
161 Briefly, exponential growth phase cultures of the six strains were adjusted to an optical
162 density at 600nm (OD_{600}) of 0.1 in R2A medium. Biofilm formation was assayed in a 96
163 well plate using eight replicates for each treatment. For single isolates an inoculation
164 volume of 180 µl was used and for the six-species biofilm 30 µl per species. After 48 hrs
165 of incubation at 18°C with shaking (200 r.p.m) biofilm formation was quantified by a
166 modified crystal violet (CV) assay (Peeters et al., 2008).
167
168 **Characterizing nutrient utilisation**
169 To characterise the nutrient profiles (niches) of *Hydras* microbiota, we measured the
170 carbon metabolism profile for each strain using BIOLOG GN2 plates. BIOLOG GN2 plates
171 are 96-well microwell plates containing 95 different carbon sources plus a carbon-
172 absent water control well. Strains were grown from isolate stocks in R2A medium (18°C,
173 shaken at 250 r.p.m.), centrifuged at 3000 r.c.f. for 5 min, re-suspended in S medium and
174 adjusted to an OD_{600} of 0.1. Each well of the BIOLOG plate was inoculated with 150 µl of
175 bacterial suspension and incubated for three days at 18°C in a humid chamber. Growth
176 on each of the 95 nutrients was determined as OD_{600} of each well using a TECAN plate
177 reader. For each plate, the OD of the water control was subtracted from the reading of
178 all other wells prior to analysis, and differenced OD values below 0.005 were considered

179 as no growth (Vaz Jauri et al., 2013). Nutrient use was evaluated on three replicate
180 plates. Nutrient (niche) overlap (NO) was calculated using the formula: NO = (number of
181 nutrients used by both A and B)/((number of nutrients used by A + number of nutrients
182 used by B)/ 2) (Vaz Jauri et al., 2013). A value of 1 indicates the use of the same
183 nutrients (100% overlap) and 0 indicates no nutrient overlap among the 95 substrates
184 tested. We also calculated the relative use of the eleven functional groups
185 (carbohydrates, carboxylic acids, amino acids, polymers, aromatic chemicals, amines,
186 amides, phosphorylated chemicals, esters, alcohols and bromide chemicals) according to
187 Daou et al. (2017). In brief, the relative use of C substrates was calculated as absorption
188 values in each well divided by the total absorption in the plate.
189
190 **Statistical analysis**
191 Analysis of variance (ANOVA) and subsequent post hoc Tukey-Kramer tests were used
192 to test for differences in the carrying capacity of the six *Hydra* colonizers. To meet the
193 requirements for the model, the variable was Box-Cox transformed. A Welch ANOVA
194 (and subsequent Wilcoxon posthoc tests) was used to test for differences in *in vitro*
195 growth rates between the strains.
196 Analysis of variance (ANOVA) and subsequent post hoc Tukey-Kramer tests were used
197 to test for differences in the cell surface hydrophobicity of the six *Hydra* colonizers.
198 A Welch ANOVA (and subsequent Wilcoxon posthoc tests) was used to test for
199 differences in biofilm formation capacity.
200 Sample size was chosen to maximise statistical power and ensure sufficient
201 replication. Assumptions of the tests, that is, normality and equal distribution of
202 variances, were visually evaluated. Non-significant interactions were removed from the
203 models. All tests were two-tailed. Effects were considered significant at the level of $P <$
204 0.05. All statistical analyses were performed with JMP 9. Graphs were produced with
205 GraphPad Prism 5.0, and RStudio (RStudio Team, 2015).
206

207 **Results**

208 **Growth performance of the bacterial strains**

209 The six bacterial strains differ significantly in their carrying capacity on *Hydra* (Figure
210 1A; ANOVA: $F_{5,12}$=12.696, P=0.0002). The most extreme cases are *Acidovorax sp.* that
211 reaches the highest numbers with $2.6*10^5$ CFUs/polyp, and *Duganella sp.* the lowest
212 with $1.7*10^4$ CFUs/polyp. When comparing the *in vitro* growth rates we find strains
213 perform differently (Figure 1B; Welch ANOVA: $F_{5,174}$=223.856, P<0.0001). The fastest
214 strain, *Undibacterium sp.*, grows twice as fast as compared to the slowest one, *Pelomonas*
215 *sp.*.

216 When comparing the fundamental and the realized niches of the six strains (Figure 2)
217 we find that the realized niche of *Curvibacter sp.* and *Duganella sp.* is larger than their
218 fundamental niche. For the other strains the fundamental niche exceeds the realized
219 niche.

220

221 **Bacterial traits**

222 *The colonization process*

223 The BATH assay was conducted with six strains of the *Hydra* microbiome to measure
224 cell surface hydrophobicity (CSH). The CSH of the bacterial strains spanned a medium
225 wide range; the values ranged from 0% to 42% and differs significantly between strains
226 (ANOVA: $F_{5,12}$=26.869; P<0.0001). *Curvibacter sp.* and *Pelomonas sp.* were the only two
227 strains that didn't show any affinity to the hexadecone; thus their cell population can be
228 considered homogeneous consisting of only hydrophilic cells, which significantly differs
229 from the others, except for *Pseudomonas sp.* (Figure 3A). *Pseudomonas sp.* and
230 *Undibacterium sp.* show a mixed cell population, where 10 to 20% of the cells are
231 hydrophobic. The strains with the highest percentage of hydrophobic cells are
232 *Acidovorax sp.* and *Duganella sp.*, between 30 and 35%.

233 The strains differed in their biofilm formation (Welch ANOVA: $F_{5,18}$=350.723, P<0.0001).
234 All strains formed biofilms (Figure 3B), with *Pelomonas sp.* producing the largest
235 biomass amount, which significantly differed from all other strains. The biofilm amount
236 of *Acidovorax sp.* was also significantly different from all other strains but only roughly a
237 third of the mass that *Pelomonas sp.* produced. All other strains didn't differ and are
238 comparatively weak biofilm producers.

239

240 *Microbial nutrient niches*

241 Niche overlap (NO) among all pairwise strain combinations ranged from 60 to 80%
242 (Figure 4). *Curvibacter sp.* shares the highest overlap (80%) with *Pseudomonas sp.* and

243 *Duganella sp.*. For the other strains the overlap ranges between 60 and 70%. *Duganella*
244 *sp.* displays the highest overlap with *Pseudomonas sp.* and *Undibacterium sp.* around
245 80%, whereas the overlap between *Pelomonas sp.* and *Acidovorax sp.* reaches almost
246 70%. *Unidibacterium sp.* exhibits an overlap of 60 to 70% with *Acidovorax sp.*,
247 *Pseudomonas sp.* and *Pelomonas sp.*. *Acidovorax sp.* a 80% overlap with *Pseudomonas sp.*
248 and 70% overlap with *Pelomonas sp.*. *Pseudomonas sp.* and *Pelomonas sp.* show a 75%
249 overlap of the nutrients used. Comparing the number of nutrients being used by the
250 individual strains we find that *Curvibacter sp.* and *Pseudomonas sp.* are able to use 70%
251 of the provided substrates. *Duganella sp.* uses 57%, *Acidovorax sp.* 54% and *Pelomonas*
252 *sp.* 51%. The lowest substrate utilization was measured for *Undibacterium sp.* with 41%
253 of the available substrates.
254
255 *Resource competition between bacterial colonizers*
256 Nutrient utilisation of all strains was determined using a BIOLOG assay. The 94 carbon
257 substrates are organized into eleven functional groups (Suppl. Figure S1). Results
258 showed that all six strains actively oxidize carbon compounds such as carbohydrates
259 (30-50% relative use), carboxylic acids (15-35% relative use) and amino acids (15-35%
260 relative use) (Figure 5A; Suppl. Figure S1). Carbohydrates are being used to an equal
261 extent between all strains, except for *Undibacterium sp.*, which uses the highest amount
262 of around 50% (relative use). Turanose is the compound most highly utilised, followed
263 by a-D-lactose, L-rhamnose, and D-cellobiose. The use of carboxylic acids increases in
264 the strains, which are characterized by low frequencies in the *Hydra* microbiome, with
265 the exception of *Duganella sp.* (with a relative use of 25-30%). Here D-galactonic acid
266 lactone is the substrate with the highest usage, followed by different forms of hydroxyl
267 butyric acids. Amino acids are most excessively used by *Acidovorax sp.*, *Curvibacter sp.*
268 and *Pseudomonas sp.,* whereas the other strains use amino acids to a lesser extent.
269 Polymers are being used very differently between the strains with *Pseudomonas sp.*
270 showing the highest and *Undibacterium sp.* the lowest values. Amines are being used
271 more frequent by the dominant strains in the microbiome and are utilized to a lesser
272 extent by the low abundant species.
273 The Venn diagram in Figure 5B displays overlaps in carbon substrate usage between all
274 six microbiome members. There are only two substrates, which are not utilized by any
275 species, whereas 20 substrates are used by all. There are only two strains that can
276 metabolize substrates that none of the other species is using. While *Pseudomonas sp.*
277 uses two substrates: i-erythritol and lactulose, *Curvibacter sp.* is able to utilise eight
278 substrates: D-arabitol, D-mannose, D-trehalose, mono-methyl succinate, formic acid,

279 glucuronamide, L-pyroglutamic acid, and D-serine. The number of substrates shared
280 exclusively between two strains only is between one and two.
281
282 Overall, we do find that microbial performance, i.e. bacterial density on the host, cannot
283 be linked to a specific bacterial trait or substrate utilization pattern (Figure 6).
284

285 **Discussion**

286 Microbial communities residing in abiotic environments typically comprise numerous
287 interacting species. Such communities have been studied with traditional approaches,
288 for example the niche-assembly concept, which is an extension of the classical niche
289 theory (Hutchinson, 1957). The niche-assembly perspective proposes that any
290 ecosystem is made up of a limited number of niches, each occupied by a single species
291 (Wennekes et al., 2012). Thus, the partitioning of these niches leads to the stable
292 coexistence of competing species within an ecosystem. To assess the rules of assembly
293 and coexistence of microbiota in host-associated microbiomes, we here apply the niche-
294 assembly perspective to a metaorganism, and thus specifically extend the concept to
295 biotic environments.

296 We find that the fundamental niche (here defined by the absence of interspecific
297 microbial interactions) differs considerably from the realized niche of *Hydras* associated
298 microbes. This reflects the difference in performance between the strains when they
299 individually occupy *Hydra* (mono-association) as to when they occur as part of their
300 microbial community on the host. As predicted by niche theory, we find for the majority
301 of the strains that the realized niche is smaller than the fundamental one, most likely
302 caused by interspecific microbial competition, as has also been observed in other
303 systems, e.g. *Vibrios* in their marine environment (Materna et al., 2012). In our study, the
304 best colonizer in the mono-colonisations, *Acidovorax sp.* (as also observed by Fraune et
305 al. (2015)), is only the fourth most abundant species as part of the microbial community.
306 This is different for the two main colonizers in the community, *Curvibacter sp.* and
307 *Duganella sp.*, where the realized niche is four times the size of the fundamental niche.
308 This finding is very interesting and indicates that the two strains benefit from positive
309 interactions when part of the microbiome. This can happen directly through positive
310 interactions with the other members of the microbiome or indirectly by benefitting from
311 the interactions between the other microbiome members and the host. We draw
312 attention to the fact that the latter aspect differs from the classical Hutchinson niche
313 concept, in that in our case the environment, i.e. the host, has the potential to change its
314 interactions depending on the specific bacterial colonizers. Our finding also highlights
315 the importance of the low frequency community members in shaping the overall
316 community composition.

317 For linking the community composition in *Hydra*'s microbiome to specific
318 characteristics, we used a trait-based approach focussing on traits potentially involved
319 in microbiome assembly and stability. A first step in microbiome assembly is the
320 attachment to host surfaces, which can happen in a multitude of ways. In the human

321 intestine, for example, microbes have been found to bind to mucin, a major component
322 of the human mucosa (de Vos, 2015). Adhesion is thus thought to be a powerful
323 mechanism for exerting both, positive and negative selection for or against specific
324 microbes (McLoughlin et al., 2016; Schluter et al., 2015). Amongst others (van
325 Loosdrecht et al., 1987), bacterial cell surface hydrophobicity has been shown to play a
326 crucial role in surface attachment (Krasowska and Sigler, 2014). In general, hydrophobic
327 cells adhere more strongly to hydrophobic surfaces and vice versa (Giaouris et al., 2009;
328 Kochkodan et al., 2008). Nevertheless, the heterogeneity of a bacterial population needs
329 to be taken into account. For example, the presence of both, hydrophilic and
330 hydrophobic cells, have been observed in planktonic bacteria cell populations, implying
331 that only part of the population participates in an adhesion process to substrates
332 (Krasowska and Sigler, 2014). We also observe mixed cell populations for most of *Hydra*
333 microbial associates, except for two strains, *Curvibacter sp.* and *Pelomonas sp.*, which
334 only consist of hydrophobic cells. They seem to be perfectly adapted to *Hydras* epithelial
335 cells, which are coated with a carbohydrate-rich layer, the glycocalyx (Ouwerkerk et al.,
336 2013; Schröder and Bosch, 2016). The microbiome inhabits the outer mucus-like layer
337 of the glycocalyx (Fraune et al., 2015), which is hydrophilic. Thus, hydrophilic bacterial
338 cells should adhere more strongly to *Hydra* than hydrophobic cells. Both, *Curvibacter sp.*
339 and *Pelomonas sp.,* have been shown to be of particular importance to the host.
340 *Curvibacter sp.* shows signs of coevolution with its host and contributes to fungal
341 resistance against the filamentous fungus *Fusarium* (Fraune et al., 2015). *Pelomonas sp.*
342 has been shown to be of central importance in modulating the spontaneous body
343 contractions in *Hydra* (Murillo-Rincon et al., 2017). So both strains contribute to host
344 fitness, providing the opportunity for the speculation that the host actively selects for
345 specific microbes. This could happen, for example, by controlling the production and
346 release of adhesive molecules from the host epithelium as suggested by McLoughlin et
347 al. (2016) or through an active support by the host so that they perform better in the
348 host context (Deines et al., 2019).
349 After successful attachment, bacteria need to colonise the habitat. In most cases,
350 this happens through the formation of biofilms, as has been reported for the gut (de Vos,
351 2015; Kania et al., 2007). The biofilm succeeds the planktonic phase in the bacterial life
352 cycle (McDougald et al., 2012) and represents a key ecological process for the
353 colonization of different habitats. Thus, the difference in the ability to form biofilms
354 could provide an explanation for why one strain outcompetes the other strain or has got
355 a higher chance of persistence in the *Hydra* ecosystem. Further, biofilms have been
356 shown to protect bacterial cells from various environmental stressors (Flemming and

357 Wingender, 2010). Interestingly, from the six strains tested here, the one with the
358 highest ability to form biofilms is *Pelomonas sp.*, whereas the two main colonizers,
359 *Curvibacter sp.* and *Duganella sp.* show a reduced capacity to form biofilms. Our finding
360 indicates that the capability of biofilm formation is not a good predictor of the bacterial
361 performance in the *Hydra* habitat. Nevertheless, it might be of importance for the
362 establishment and persistence of some of the low abundance species, such as *Pelomonas*
363 *sp.* and *Acidovorax sp.*.

364 Importantly, microbiomes on external surfaces of metaorganisms, such as the
365 skin, have been reported to be highly stable despite their constant exposure to extrinsic
366 factors (Oh et al., 2016). Whereas bacterial diversity is widely recognized in leading to
367 temporal stability of ecosystem processes (Bell et al., 2009; Griffin et al., 2009; Prosser
368 et al., 2007), the influence of resource niche breadth has received little scientific
369 attention (Hunting et al., 2015). Recent work studying the decomposition of organic
370 matter in experimental microcosms found that the higher the overlap in resource
371 niches, the higher the stability of the microbial community. It is reasonable to assume
372 that the same underlying principles govern stability in host-associated microbial
373 communities. We therefore measured the niche overlap and resource use of the six
374 strains isolated from the *Hydra* microbiome. Interestingly, we find the niche overlap
375 between all pairwise combinations to be between 60 and 80%, with about 20% of the
376 carbon sources being metabolized by all strains. This suggests that metabolic overlap
377 could be involved in promoting the temporal stability of the microbiome, which was
378 reported for *Hydra* by Fraune and Bosch (2007). We also found the two main colonisers,
379 *Curvibacter sp.* and *Duganella sp.,* to possess the widest resource niche breadth of all
380 strains, and that five out of six strains were able to metabolize more than 50% of the 95
381 offered carbon substrates. Overall, the relative niche breadth observed in the tested
382 strains can serve as a proxy of the metabolic diversity of the *Hydra* microbiome.

383 The metabolic overlap, i.e. redundancy, within *Hydra's* microbial community
384 indicates that the individual species are not occupying a specific metabolic niche.
385 Nevertheless, the only one for which we observed a specific carbon usage pattern is the
386 main colonizer *Curvibacter sp.*, which utilizes eight carbon sources that are not
387 metabolized by any other tested microbiome members. Whether this hints at the
388 occupation of a specific niche within *Hydra's* microbial community and can be linked to
389 the observation that its realized is bigger than its fundamental niche when part of the
390 community as compared to single occupation on *Hydra*, is currently open to speculation.
391 An alternative option might be that *Curvibacter sp.* is auxotrophic in producing certain
392 amino acids, as are 98% of all sequenced microbes (Zengler and Zaramela, 2018), and

393 thus relies on the uptake of external substrates that might not be secreted by the host
394 but by its fellow community members. Analysing the metabolic interactions within this
395 microbial network will be essential for understanding community assembly,
396 composition, and maintenance.
397 In summary we find that the here measured bacterial traits vary across
398 microbiome members. Further, the dominant species in the microbiome do not
399 necessarily perform best in all of the measured traits. We rather observe that all species,
400 independent of their density, perform well in a subset of traits, likely facilitating the
401 coexistence of several niches within the host ecosystem. Whether a change in the
402 realized niche of microbes can be linked to potential for dysbiosis is an interesting
403 aspect, which warrants further investigation.
404

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Author Contributions

PD designed the experiments. PD performed the experiments. PD and KH analysed the data. PD, KH, and TCGB wrote the paper.

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Conflict of Interests

The authors declare no conflict of interest.

583 **Figures**

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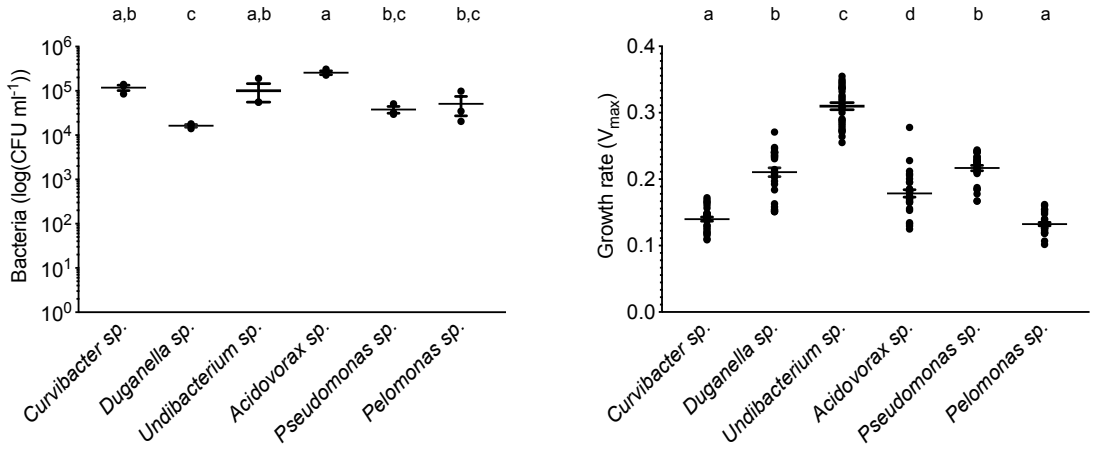

586

587 **Figure 1.** Performance of bacterial strains isolated from the *Hydra* microbiome. (A)
588 Carrying capacity of the *Hydra* ecosystem during mono-associations of germ-free polyps
589 with individual bacterial strains. (B) Bacterial growth rates of individual strains
590 measured *in vitro*.

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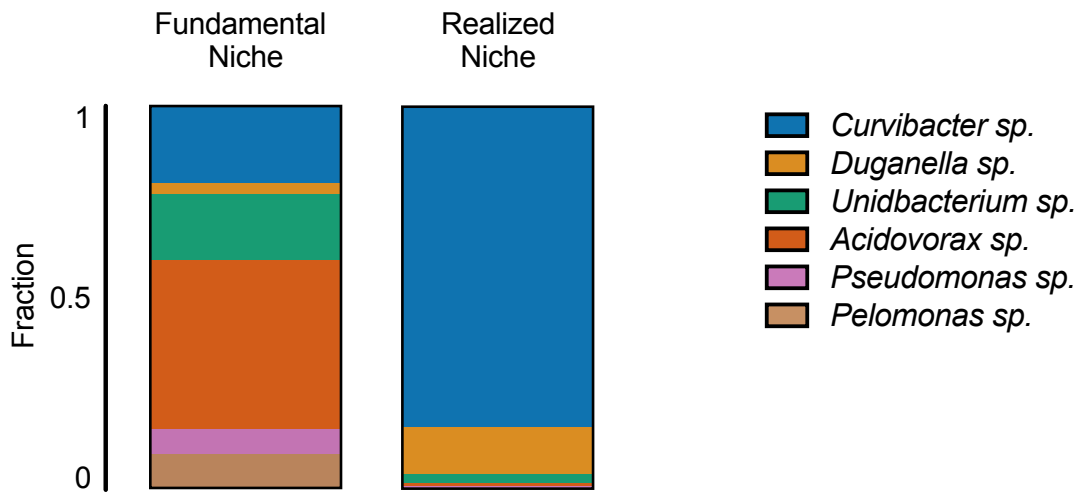

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595 **Figure 2.** *Hydra* functions as an ecosystem, which allows for the niche allocation. Shown
596 are the fundamental and realized niches of six microbiome members. The realized niche
597 includes additional constrains arising from inter-specific competition between
598 microbiome members.

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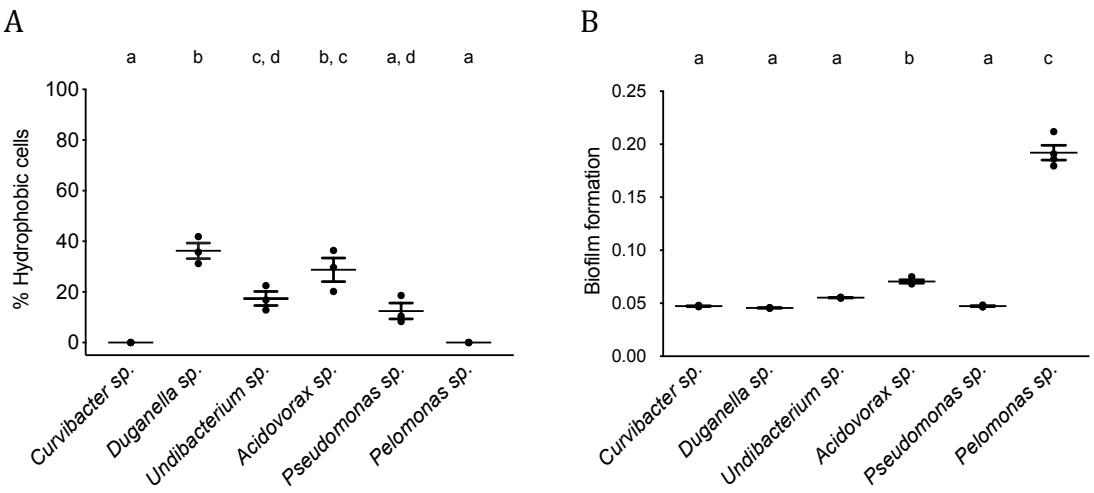


Figure 3. Trait measures of bacterial strains isolated from the *Hydra* microbiome. (A) Cell surface hydrophobicity and (B) Biofilm formation capacity of six bacterial isolates.

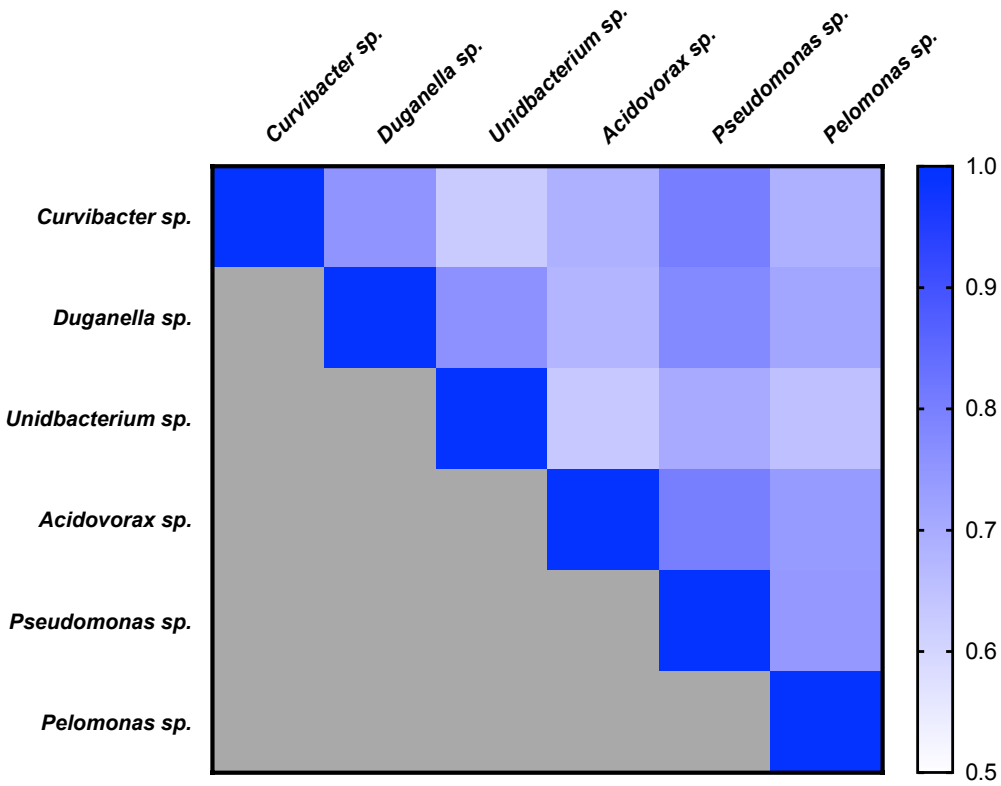
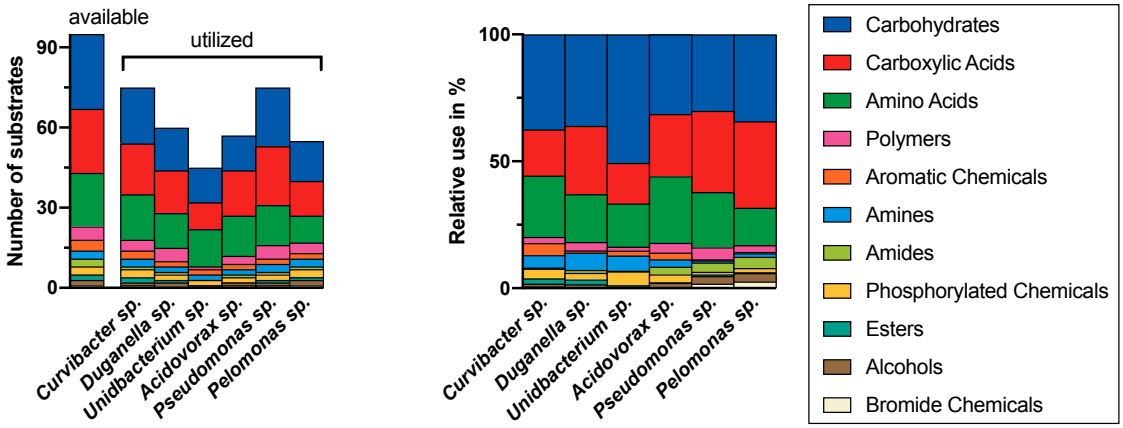


Figure 4. Niche overlap among all pairwise combinations of six *Hydra* microbiome members.

A



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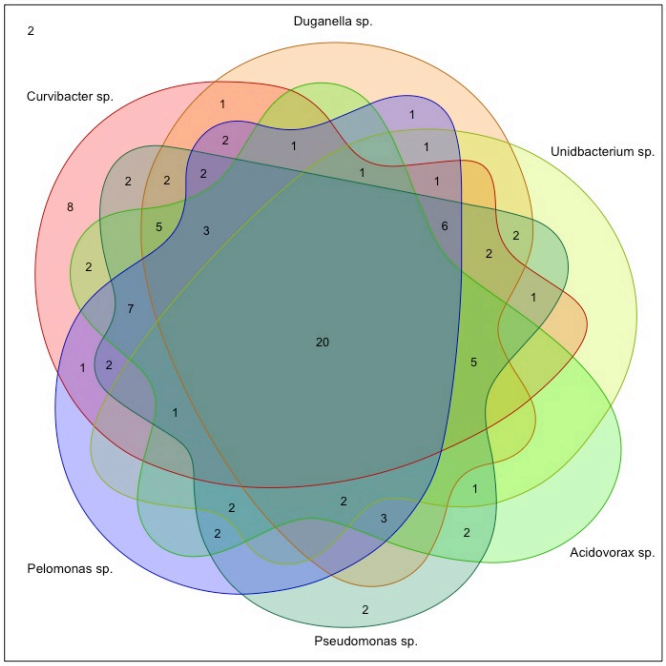

Figure 5. Metabolic similarity between *Hydra's* microbiome members. (A) Number of substrates utilized and their relative use (%). (B) Venn diagram showing the distribution of shared substrates among the microbiome members.

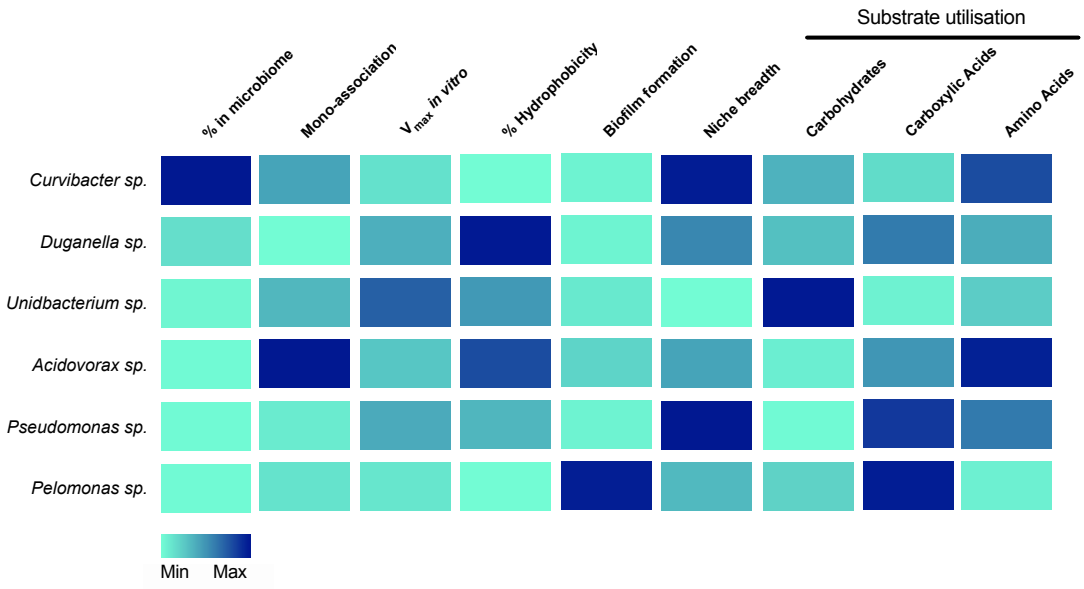

Figure 6. Summary of all the traits measured and their performance among the six microbiome members.

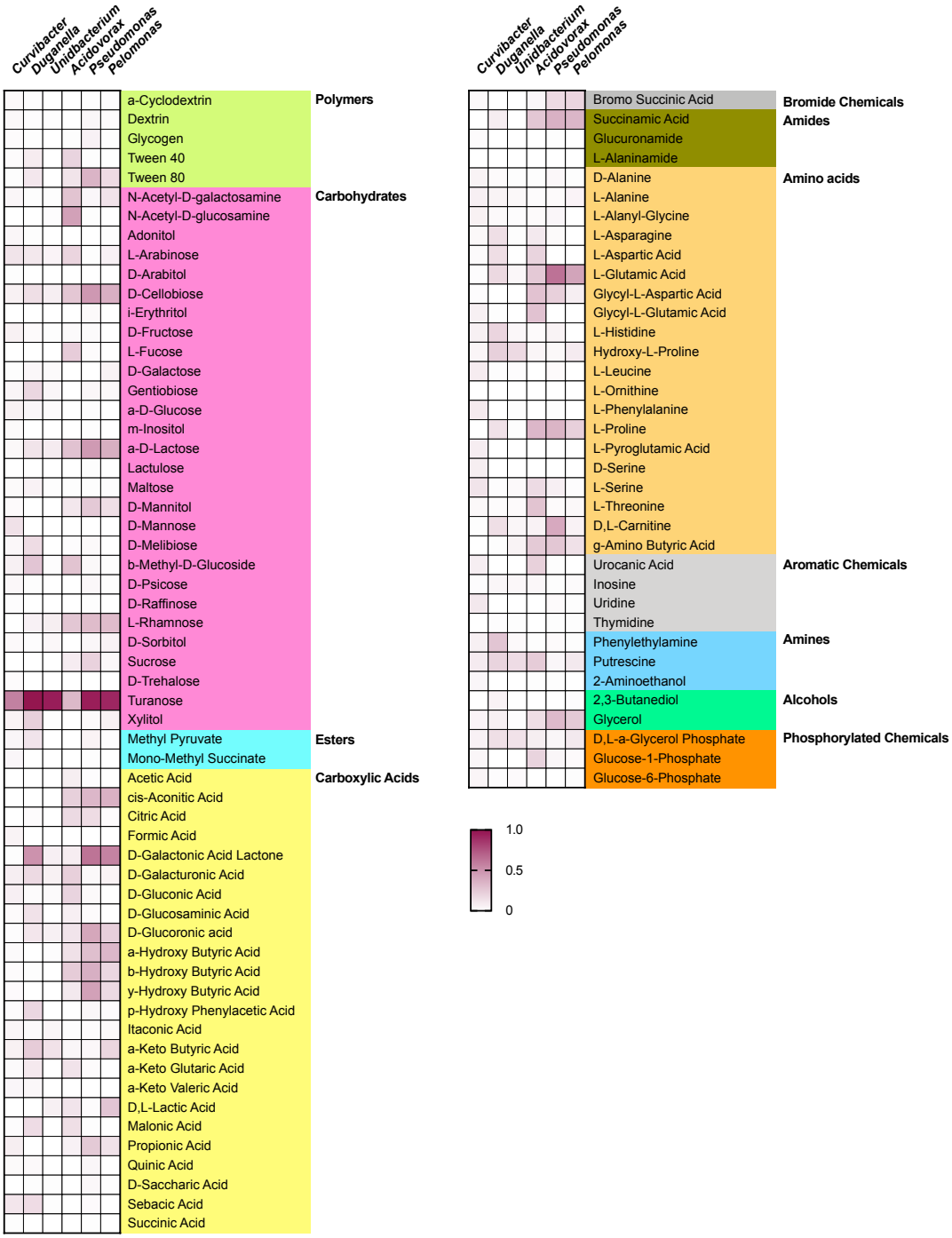

Figure S1. Substrate utilization pattern of six bacterial isolates from the *Hydra* microbiome measured with a BIOLOG assay. Colours indicate the relative magnitude of substrate utilization.