Fitness effects of host-bacterial interactions – the microbial perspective

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Conflict of Interests

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Abstract

Organisms and their resident microbial communities form a complex and mostly stable ecosystem. It is known that the specific composition and abundance of certain bacterial species affect host health and *Darwinian fitness,* but the processes that lead to these microbial patterns are unknown.

We investigate this by deconstructing the simple microbiome of the freshwater polyp *Hydra*. We contrast the performance of its two main bacterial associates, *Curvibacter* and *Duganella*, on germ free hosts with two *in vitro* environments over time. We show that interactions within the microbiome but also host modulation lead to the observed species frequencies and abundances. More specifically we find that rare microbiome members are essential for achieving the observed community composition, which ultimately sets the maximum carrying capacity. Bacterial fitness strongly depends on the environment: while *Duganella* performs better than *Curvibacter* in a non-host habitat, *Curvibacter* benefits through the host association.

This is of particular interest because *Curvibacter* and its host show a history of co-evolution, as inferred from phylogenies, whereas the colonization with *Duganella* seems to be a recent event. Our findings oppose the assumption that bacteria always benefit through the association with the host and poses questions regarding the long-term maintenance of such relationships.

Introduction

Eukaryotes form a distinct habitat for microbial communities (microbiomes) and these microbial associations are integral to life. The host with its associated microbial community, often dominated by bacteria but co-habited by fungi, protozoa, archaea, and viruses, is termed metaorganism. Microbiomes can contain from few up to thousands of microbial species - the human microbiome, for example, is estimated to be comprised of about 5000 bacterial species [1-3]. These host-associated microbial communities have been shown to enhance host function and contribute to host fitness and health [4]. Changes in microbiome diversity, function, and density have been linked to a variety of disorders in many organisms [5-8].

A major goal in host-microbe ecology is to explore the ecological and evolutionary dynamics of microorganisms within their communities. Of particular relevance is to improve our understanding of factors that shape the stability and resilience of these communities, despite different fitness trajectories of the microbiome members. The microbial response to stress or perturbations, e.g. exposure to a new substrate, provides a selective advantage to certain members of the community. If the system cannot tolerate the change and the microbial community shifts dramatically, a different equilibrium state will be reached [9]. Frequency-dependent selection forces the host to adapt to these changes and select for or against the most frequent genotypes of their associated microbiota [10]. There is, for example, strong evidence that species-specific antimicrobial peptides (AMPs) shape, control, and confine host-species specific bacterial associations [for example 11, 12]. In addition, microbial communities are not evenly distributed, e.g. along the gastrointestinal tract or between the lumen and the epithelial surfaces [2, 13, 14]. These significant differences in niches or micro-habitats and their occupancy is known as spatial heterogeneity and will affect community assembly rules and dynamics [15, 16]. Interspecies metabolic exchange is another key

73 biotic force acting as a major driver of species co-occurrence in diverse microbial
74 communities [17].

75 One aspect that has been so far neglected in metaorganism research is why a
76 stable microbiome can persist in a specific host lineage for many host generations.
77 Central to this is the question whether microbes benefit from the association with the
78 host. This could either happen through direct interaction with the host or indirectly,
79 through interactions with another microbial strain within the microbiome. Host-
80 dependent advantages for the symbiont range from the availability of host-derived
81 nutrients, a competition-free environment to reduced predation risk [18]. If members of
82 the microbiome benefit from the association with a particular host, one would expect
83 their capacity to reproduce, i.e. their *Darwinian fitness*, to be higher in the host relative
84 to an alternative habitat.

85 Methods for quantifying microbial fitness range from estimating absolute
86 fitness, where population growth is measured from which maximum growth rates can
87 be calculated, to estimates of relative fitness. The latter is of particular relevance as the
88 fitness of a focal microbial lineage is measured *relative* to another microbial lineage.
89 This provides a better representation of the turnover of strains in microbial populations
90 and also closely corresponds to the meaning of fitness in evolutionary theory [19]. The
91 basic design of such an experiment involves mixing populations of the two strains
92 together in a particular initial ratio in the test environment and measuring their relative
93 contributions to future generations. Under certain ecological conditions the relative
94 fitness of two strains is dependent upon their relative frequencies (frequency-
95 dependent selection) [20]. These methods of measuring microbial fitness can easily be
96 applied to host-microbe systems and help us in understanding the underlying dynamics.
97 This will yield insights into the role that microbes played in the evolution of eukaryotes
98 and into the ecology and evolution of host-microbe associations in general [21]. Here, it
99 is important to unravel the extent to which the observed pattern simply result from

intrinsic properties, such as microbial growth rates, interactions of the microbes, which could be recapitulated *in vitro* with co-culture experiments, or whether it is an emergent property of that particular host-microbe system [22].

We here test whether microbial associates benefit from their association with the host and their fellow community members and follow their ecological interactions within the microbiome. We use the freshwater polyp *Hydra vulgaris* and its microbiome, which has become a valuable experimental model in metaorganism research as it provides an excellent bridge between the simplicity of synthetic communities and the mouse model [23]. *Hydra's* ectodermal epithelial cells are covered with a multi-layered glycocalyx that provides a habitat for a species-specific and core microbiome of low complexity [11, 24, 25], from which most microbes can be cultured *in vitro* [25, 26]. This allows for an integrated approach based on constructing synthetic communities of various complexities and contrasting the host (*in vivo)* to *in vitro* habitats [23]. We focus on the two most abundant members of the microbiome that together constitute about 85% of *Hydra*'s simple microbiome, *Curvibacter* sp. AEP1.3 and *Duganella* sp. C1.2, (hereafter called *Curvibacter* and *Duganella*) where abundances of *Curvibacter* are several magnitudes higher as compared to *Duganella* [26]. Previous work on species interactions suggested that continued coexistence depends heavily on relative growth rates. Using invasion-from-rare experiments we explore the potential for coexistence under different environmental conditions (host and non-host habitat) and contribute to a better understanding whether the host alters microbial interactions that potentially lead to coexistence in simple microbiomes.

Materials and Methods

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

Hydra vulgaris (strain AEP) was used for carrying out experiments and cultured according to standard procedures at 18°C in standardized culture medium (Hydra medium (HM)) [27]. Animals were fed three times a week with 1st instar larvae of *Artemia salina*. Germ-free (GF) polyps were obtained by treating wild-type (WT) animals with an antibiotic cocktail solution containing 50 μg/ml ampicillin, neomycin, streptomycin, rifampicin and 60 μg/ml spectinomycin as previously described [28, 29]. The antibiotic cocktail solution was exchanged every 48 h and the antibiotic treatment lasted for two weeks, after which polyps were transferred into antibiotic-free sterile HM for recovery (four days). The germ-free status of polyps was confirmed as previously described [28]. During antibiotic treatment and re-colonization experiments, polyps were not fed.

Bacterial strains and media

The bacterial strains used in this study are *Curvibacter* sp. AEP1.3 and *Duganella* sp. C1.2., which have been isolated from *Hydra vulgaris* (strain AEP) [26]. These bacteria were cultured from existing isolate stocks in R2A medium at 18°C, shaken at 250 r.p.m for 72 h before use in the different experiments.

Carrying capacity of the host

To determine the carrying capacity of the *Hydra* habitat the microbial load of individual *Hydra* polyps (N=16) was determined. In addition to wild-type polyps the carrying capacity of conventionalized polys (N=12), obtained by incubating germ-free polyps with tissue homogenates of wild-type animals (per germ-free polyp one wild-type polyp was used) for 24 h was also determined. To test whether the carrying capacity can artificially be increased or destabilises upon self-challenge we added either *Curvibacter*

or *Duganella* to wild-type polyps (N=6) (approximately 5x10^3 cells for 24 h). After incubation all polyps were washed with and transferred to sterile HM and further incubated at 18°C and sampled after 120 h.

Tracking microbial mono-associations in *Hydra* over time

Germ-free polys were inoculated in their aquatic environment with single bacterial strains (mono-associations). Individual germ-free polyps were incubated with 5x10^3 cells of *Curvibacter* or *Duganella* in 1.5 ml Eppendorf tubes containing 1 ml of sterile HM. After 24 h of incubation all polyps were washed with and transferred to sterile HM, incubated at 18°C and followed over a period of 216 h. For each treatment 6 polys per time point were independently analysed. Every 48 h individual polyps were homogenized in an Eppendorf tube using a sterile pestle and serial dilutions of the homogenate were plated on R2A agar plates to determine colony-forming units (CFUs) per polyp.

Microbial growth kinetics of mono- and di-associations *in vivo* and *in vitro*

To study the initial phase of colonization, i.e. 96 h post inoculation (see Fig. 1B) in more detail microbial growth of *Curvibacter* and *Duganella* was determined in different habitats; the host habitat (*in vivo*) and two different microcosm environments (*in vitro*). The static incubation provided a spatially structured habitat (heterogeneous), whereas shaking of the microcosms (mixed treatment) eliminated the spatial structure (homogenous).

Mono-associations: All germ-free polyps and microcosms were inoculated from the same bacterial inoculation culture with approximately 5x10^3 cells of *Curvibacter* or *Duganella* for 24 h, washed with and transferred to sterile HM. Samples were taken every 12 h for 96 h. For *Hydra* six polyps were sacrificed at each time point and colony-forming units (CFUs) were determined as described above. As microcosms 24-well

177 plates were used. Wells were filled with 2 ml of R2A medium, inoculated and incubated
178 at 18°C either under static or shaken (200 r.p.m) conditions. Each time point was
179 replicated four times and serial dilutions were plated on R2A agar plates to determine
180 CFUs. Growth rates of each strain (A and B) were determined for the exponential growth
181 phase (36-48 h) and were calculated as $g = \ln (A_f/A_0)$ and $g = \ln (B_f/B_0)$, where A_0, B_0 is
182 the starting density at time 0 and A_f, B_f is the final density at time *f*.

183 *Di-associations:* Density dependent competiveness fitness assays of the two most
184 dominant colonizers *Curvibacter* and *Duganella* were performed *in vivo* and *in vitro.* The
185 same host and microcosm experiments as described above were performed except for
186 using microbial di-associations of *Curvibacter* and *Duganella* with the frequency of
187 *Curvibacter* being rare (10:90), equal (50:50), or dominant (90:10). As *Curvibacter* and
188 *Duganella* form distinct colonies on R2A agar plates, their frequency can be determined
189 by plating serial dilutions [30]. Six polyps and four microcosm replicates were assayed
190 per treatment (static and mixed) and time point. Also this data allowed determining the
191 different carrying capacities of the *in vivo* and *in vitro* habitats used. Malthusian
192 parameters were calculated for both bacterial strains (A and B), $m = \ln (N_f/N_0)$/hour,
193 where N_0 and N_f were the initial and final densities. The relative performance of the two
194 strains was expressed in terms of the selection rate constant (*r*). Note that this measure
195 of performance should be preferentially used over the more commonly used relative
196 fitness (*W*), when one competitor is much less fit than the other [31, 32], as was the case
197 in our study. The selection rate constant was calculated as $r = m_B - m_A$. Accordingly, $r = 0$
198 implies that there is no difference in growth rates of the two strains, $r > 0$ indicates that
199 strain B is advantageous in population growth ($r = 1$ indicates a 10-fold increase in the
200 ratio of B vs. A abundances), while $r < 0$ suggests that strain B fails in invading
201 population of strain A.

Statistical analysis

A Welch ANOVA (and subsequent Dunnett's posthoc test) was used to test for differences in bacterial abundance patterns ('bacteria per *Hydra*') in wild-type versus manipulated hosts as variances between the different groups were not equally distributed.

Differences during mono-colonizations of *Curvibacter* and *Duganella* over time were assessed using a Generalized linear model (error structure: normal; link function: identity). The response variable was 'bacteria per *Hydra*', and explanatory variables were 'bacterial species', 'time' and 'bacterial species' x 'time'. Differences between the two bacterial species on each day were detected with post hoc contrasts.

Analysis of variance (ANOVA) and subsequent post hoc t-tests were used to test for differences in growth rates of the two competitors when grown singly in the different environments. The response variable was 'growth rate', and explanatory variables were 'bacterial species', 'environment' and 'bacterial species' x 'environment'.

Differences in the growth rates in the di-associations of *Curvibacter* and *Duganella* in the different environments and dependence on initial frequency were assessed using a Generalized linear model (error structure: normal; link function: identity) and post hoc contrasts. For each bacterial species, a separate model was calculated with the response variables being either 'growth rate *Curvibacter*' or 'growth rate *Duganella*', and the explanatory variables were 'environment', 'starting density' and 'environment' x 'starting density'.

Differences in the selection rate constant of *Curvibacter* in the different environments and dependent on initial frequency were assessed using a Generalized linear model (error structure: normal; link function: identity) and post hoc contrasts. The response variable was 'selection rate constant *Curvibacter*' and the explanatory variables were 'environment', 'starting density' and 'environment' x 'starting density'.

Sample size was chosen to maximise statistical power and ensure sufficient replication. Assumptions of the tests, that is, normality and equal distribution of variances, were visually evaluated. Non-significant interactions were removed from the models. All tests were two-tailed. Effects were considered significant at the level of $P < 0.05$. All statistical analyses were performed with JMP 9. Graphs were produced with GraphPad Prism 5.0, and Adobe Illustrator CS5.1.

Results

Carrying capacity of the *Hydra* host

The carrying capacity of the *Hydra* habitat is highly stable among single *Hydra* wild-type polyps with $1.7*10^5$ CFUs/polyp and at its maximum with the native microbiome. Manipulations of the microbial community lead to significant changes in bacterial numbers (Welch ANOVA, $F_3=7.054$; $P<0.005$; Fig. 1A). The addition of single bacterial species (*Curvibacter* or *Duganella*) to wild-type polys does not increase the carrying capacity but, in the case of *Curvibacter*, leads to a significant reduction in microbial population size. In contrast does the addition of *Duganella* not significantly shift the bacterial numbers (Fig. 1A). Conventionalised animals (germ-free polyps incubated with tissue homogenates of wild-type animals) show a carrying capacity, which is not significantly different compared to wild-type polyps (Fig. 1A), indicating the usability of the germ-free polyps for the manipulation and construction of synthetic bacterial communities on the host/ *in vivo*.

Tracking microbial mono-colonisations in *Hydra* over time

In mono-associations *Curvibacter* and *Duganella* populations display typical bacterial growth dynamics. Following a lag and exponential phase, both strains reach a stationary phase after roughly 72 h with a stable population size/carrying capacity per host (Fig. 1B). After 72 h we find significant differences in the carrying capacity between both

strains, *Curvibacter* being higher than *Duganella* (estimated by post hoc contrasts; Generalized linear model: Full model: χ^2=54.360, d.f.=9, P<0.0001; bacterial species x days post exposure: χ^2=18.326, d.f.=4, P=0.0011). These significant differences last until the end of the experiment. Once carrying capacity of mono-associations is reached, we find a population size of about 10^4 CFUs per host for *Curvibacter*, whereas for *Duganella* the population size reaches on average only $1.5*10^3$ CFUs per host. Further, both mono-associations do not reach the carrying capacity of wild-type polyps. The variation in bacterial density between hosts is significantly higher in *Curvibacter* than in *Duganella* (Levene: F_1=21.496, P<0.0001). No extinction events are observed in either strain after successful establishment during the mono-colonisation experiments.

Microbial growth kinetics of mono-associations *in vivo* and *in vitro*

Growth rates of *Curvibacter* did not significantly differ between the host and the microcosm environments. This is in marked contrast to *Duganella*, where significantly higher growth rates were observed in the non-host as compared to the host environment. In all environments, except for the host, *Duganella* achieved a significantly higher growth rate than *Curvibacter* (determined by post hoc t-tests; ANOVA: R^2=0.827; Full model: $F_{5,15}$ = 14.333; P<0.0001; bacterial species x environment: F_2 = 15.592; P=0.0002).

Microbial di-association experiments *in vivo* and *in vitro*

Carrying capacity

Results of host and microcosm experiments (static and mixed) show that during di-associations the carrying capacity in all habitats is reached at about 72 h after inoculation. Both microcosm environments are characterized by a carrying capacity of 10^7-10^8 CFUs/ml, and so exceeding the *in vivo* carrying capacity by a factor of 10^4 (Fig. 3). Nevertheless, di-associations on the host also fail to reach the carrying capacity of

282 wild-type polyps and reach a comparable carrying capacity as in the mono-colonisations
283 of *Curvibacter*. Both bacterial species do not match the carrying capacities as measured
284 in mono-colonisations on the host: whereas *Curvibacter* fails by a power of 10 to reach
285 its density in the mono-colonisations, *Duganella* outgrows it by a power of 10.
286
287 *Changes in fractions in microbial di-association experiments*
288 When competing *Curvibacter* and *Duganella* in three different starting frequencies in the
289 host, and the non-host environments, several interesting patterns can be observed (Fig.
290 4). In both non-host environments, *Duganella* outcompetes *Curvibacter* within 48 h post
291 exposure. From then onwards, frequencies of *Curvibacter* are low, reaching a maximum
292 of about 10%. This pattern does not depend on the initial frequency at the start of the
293 experiment. The host shows a different pattern. Here, a decrease in *Curvibacter* fraction
294 can be observed in all three initial frequencies but never to a point where it cannot be
295 detected in the population. From 72 h post exposure onwards the population on the host
296 has reached a stable state, with *Curvibacter* making up 20% of the total bacterial
297 population.
298
299 *Microbial growth kinetics of di-associations*
300 Overall *Curvibacter* growth rate in di-associations are lower or not different from the
301 mono-associations (Fig. 5; as estimated by post hoc contrasts; Generalized linear model:
302 Full model: χ^2=45.790, d.f.=11, P<0.0001; environment x initial frequency: χ^2=33.685,
303 d.f.=6, P<0.0001). *Curvibacter* grows significantly differently when inoculated in equal
304 densities as compared to the rare and dominant starting frequencies across the different
305 environments. Whereas in the host, *Curvibacter* grows better when in equal density with
306 *Duganella*, the opposite is true for both *in vitro* environments.
307 As observed for the growth of *Duganella* in mono-colonisations, growth rates are always
308 higher in the non-host environments irrespective of initial frequency (Generalized linear

model: Full model: χ^2=130.278, d.f.=11, P<0.0001; environment x initial frequency: χ^2=59.723, d.f.=6, P<0.0001). Whereas, in di-associations, negative growth rates can be detected only once for *Duganella*, it happens more frequently in the *Curvibacter*, indicating a direct or indirect negative effect of *Duganella*.

Relative performance of Curvibacter

As estimation for the relative performance of *Curvibacter* in competition with *Duganella* we determined the selection rate constant (r) for all di-association experiments [31, 32]. We observed r < 0 for all environments and frequencies, indicating that *Curvibacter* performs worse than *Duganella* when they are in direct competition (Fig. 6). There is no overall effect of the initial frequency - only in the mixed environment differences can be detected with the lowest performance of *Curvibacter* at equal frequencies of the two competitors (Generalized linear model: Full model: χ^2=51.168, d.f.=8, P<0.0001; environment x initial frequency: χ^2=19.147, d.f.=4, P=0.0007). In general, *Curvibacter* performs significantly better in the host environment irrespective of initial frequency.

Discussion

One of the major challenges in microbiome research is to understand the factors that influence the dynamics and stability of host-associated microbial communities. Of particular relevance for this are the processes governing assembly [33, 34] and resilience [35]. Insights into such processes in bacterial populations within their native host environments can be gained through a number of ways. Relationships between different community members in complex microbiomes, such as the human gut, can, for example, be inferred by analysing co-occurrence data and correlation patterns from sequencing-based metagenomic time-series experiments [36, 37]. Another approach, where ecological interactions can experimentally be dissected, allows for hypothesis testing. For this, a relatively simple system, such as *Hydra* and its microbiome is ideal for

'deconstructing' a metaorganism and its interactions [23]. This strategy is novel in meta-organism research but has yielded exciting results regarding rules that determine community assembly and stability in non-host associated microbial communities [38, 39]. In microbiome research, the added advantage of including a host provides the opportunity to study the performance of the individual microbiome members in their natural environment. In addition, the comparison of the *in vivo* to *in vitro* environments provides information on host effects in regulating its microbiome.

Carrying capacity is defined as the largest population size that an ecosystem can sustainably support without degrading the ecosystem. Therefore population size is constrained by the carrying capacity of the environment. This is also true for bacteria populating a particular environment, including hosts. In microbiome research, the significance of host carrying capacity has been largely overlooked until very recently where a link between host health and microbiome density has been reported [8]. The host's carrying capacity ultimately sets the upper boundary for bacterial fitness relative to any competitors, and provides a reference for investigating the interactions within the host microbiota at spatial and temporal scales.

We here show that wild-type *Hydra* is characterized by a specific carrying capacity of about 10^5 bacteria per polyp that is stable in adult polyps and can be artificially assembled through the re-population of germ-free animals with the natural bacterial community. This is an important prerequisite for conducting the *in vivo* experiments, where colonization patterns of single species from *Hydra's* microbiome are individually followed. When *Curvibacter* and *Duganella* are introduced separately to the host (in mono-associations), each bacterial species is capable to robustly colonize the host to high abundances, which are maintained throughout the experimental period, indicating the ability to independently utilize the resources of the *Hydra* habitat. While both species fail to reach their respective carrying capacities as compared to wild type *Hydra* by a factor of 10, their respective proportions of about 75% and 11% [26] are

363 nevertheless maintained. Results from the di-association experiments clearly show that
364 the abundances and relative frequencies of *Curvibacter* and *Duganella* as measured in
365 wild-type *Hydra* cannot be explained by their (positive) interactions in the host context
366 alone as this led to a frequency reversal, making *Duganella* more abundant than
367 *Curvibacter*. *Duganella* however reaches comparable carrying capacities as measured in
368 wild-type *Hydra* polyps. This implies that the presence of *Curvibacter* has a positive
369 effect on the performance of *Duganella* but *Duganella* negatively affects *Curvibacter* in
370 the host habitat. These observations indicate that the less frequent community members
371 (each 2% and less) are of central importance for reaching the full carrying capacity of
372 the *Hydra* microbiome. We hypothesise that two aspects are of importance here – (i) the
373 low abundant microbes might be able to utilize different resources as compared to
374 *Curvibacter* and *Duganella* and so inhabit different ecological niches within the
375 microbiome, which the two main colonizers cannot fill and (ii) they likely interact in a
376 positive way (either directly or indirectly) with (at least) *Curvibacter*, enabling it to
377 reach higher carrying capacities. It is thus important to note that *Hydra*'s carrying
378 capacity is not solely determined by the host (resources) alone but also by the
379 interactions within the microbiome.

380 To determine the relative importance of the host, interactions of *Curvibacter* and
381 *Duganella*, and intrinsic properties of the bacteria, such as ability to grow, in shaping the
382 observed patterns in the host environment, we performed the same mono- and di-
383 association experiments in two i*n vitro* environments. We chose to contrast static, which
384 closely resembles the host habitat in that it provides spatial heterogeneity facilitating
385 bacterial interactions and mixed, where direct interactions between individual bacteria
386 cannot be established but where individual bacteria have (unlimited) access to
387 resources and oxygen. Because community structure can be influenced by initial species
388 abundances [40], we performed all di-association experiments using various initial
389 starting frequencies. In general, both bacterial species reached much higher carrying

390 capacities in both *in vitro* environments as compared to the *in vivo* environment, with
391 *Duganella* reaching up to 10^8 CFUs/ml and *Curvibacter* up to 10^5 CFUs/ml. Interestingly
392 we were not able to replicate the *in vivo* dynamics between *Curvibacter* and *Duganella in*
393 *vitro*, suggesting that the host mediates the interaction between the two bacterial
394 species. Whereas pairwise competitions in the di-association experiments resulted in
395 coexistence of *Curvibacter* and *Duganella* in the host habitat, it led to an overgrowth of
396 *Duganella* in both *in vitro* habitats. More specifically, we found that in the *Hydra* habitat
397 *Curvibacter*, independent of its inoculation frequency, and after the initial establishment
398 period of 72 h, reached a constant fraction of about 20%, whereas it was only present at
399 very low frequencies in both *in vitro* habitats due to the high continuous growth rates of
400 *Duganella* (see also [30] for homogeneous environment).

401 Many host-associated microbial communities live in highly competitive
402 surroundings, in which they struggle to persist. According to Ghoul and Mitri [41], a
403 strain is competitive if it shows phenotypes that cause a fitness decrease in a competitor
404 strain. Competition can be either passive, where strains compete for the same resources
405 or active, where strains directly harm one another [41]. Li *et al.* [30] showed that during
406 co-culture experiments in a homogenous environment the presence and frequency of
407 *Curvibacter* greatly influenced the growth rate of *Duganella* (quadratic function), which
408 we also found. Interestingly we find the same pattern on the host, although growth rates
409 are drastically reduced. In the static environment we find the inverse of the quadratic
410 function.

411 When comparing the bacterial growth rates in mono-associations we observe
412 that *Curvibacter* performs equally well in all habitats, whereas *Duganella* shows a
413 reduced growth rate compared to *Curvibacter* on the host and performs best in the non-
414 host habitat. This suggests that *Duganella* is not particularly adapted to the host niche.
415 Interestingly the two species also differ in their phylogenetic association patterns with
416 different *Hydra* species [28]. Whereas different lineages of *Curvibacter* have been shown

417 to be associated with the majority (N=6) of *Hydra* species analysed (N=7), *Duganella*
418 was detected in less than half (N=3). This indicates that *Duganella* is a more recent
419 member of the *Hydra* microbiome and that it is not as co-evolved as it has been
420 suggested for one of *the* keystone species of *Hydra's* microbiome, *Curvibacter* [30]. The
421 specific microbiome of *Hydra vulgaris*, which is studied here, has shown to be stable as
422 animals living in the wild were colonized by a similar microbiome as compared to the
423 ones that had been maintained in the laboratory for a relatively long time [24].

424 Oscillatory dynamics, chaotic fluctuations and stochastic perturbations influence
425 species abundances and allow for the coexistence of many species and have therefore a
426 significant impact on species survival even in simple communities containing only a few
427 interacting species [42-44]. Interestingly we observed such sporadic collapses in the
428 *Curvibacter* population during di-association experiments in the non-host environment
429 but not on the host. An earlier study following the bacterial colonization pattern of
430 *Hydra* hatchlings showed sporadic collapses of the *Curvibacter* population during the
431 first 15 weeks post hatching [45]. In adult polyps, which were used in the experiments,
432 we did not observe such dynamics, which points to the fact that these sporadic collapses
433 might be buffered in adult polyps or are caused by the other species of the *Hydra*
434 microbiome. For the zebrafish host model it has been shown that *Aeromonas* during
435 mono-association is not resistant to disturbances related to intestinal motility and that
436 in the presence of *Vibrio* the magnitude of collapses are even greater [22]. *Hydra* is also
437 characterized by spontaneous contractile activity [29] but our data suggest that this
438 does not have such far-reaching effects on the microbiota as found in the zebrafish gut.

439 Whereas in other host-microbe systems such as the zebrafish, *in vivo* dynamics
440 between two bacterial species can be replicated *in vitro* [46], in our system this is not
441 the case. We find the fitness of *Curvibacter* and *Duganella* to be highly dependent on the
442 specific environments tested. Whereas *Duganella* performs best *in vitro*, in terms of
443 carrying capacities reached, absolute fitness (growth rates) but also relative fitness

(fractions in population, selection rate constant), the opposite is true for all parameters in *Curvibacter* which generally performs best in the host habitat, except for its carrying capacity that is higher *in vitro*. Our experiments reveal that not only direct bacterial interactions are important but also highlight the fact that the host plays an important role in mediating these interactions. Further, we were able to show that carrying capacity is a fundamental property of a host-microbe system and that it is not only determined by the physiological features of the host but also by the interactions within the microbiome. Detecting a selection rate constant of $r < 0$ for the main *Hydra* colonizer, *Curvibacter*, in the host environment and a reduced carrying capacity in both, mono- and di-associations, suggests that low-abundance species of the *Hydra* microbiome are likely playing a major role in maintaining the density of *Curvibacter* on the host. Evidence for the importance of rare-species comes from the human intestinal microbiota, which contains many low-abundance species [47] with some of them having a large impact on inducing dysbiosis in the microbiome and on guaranteeing host health [48, 49].

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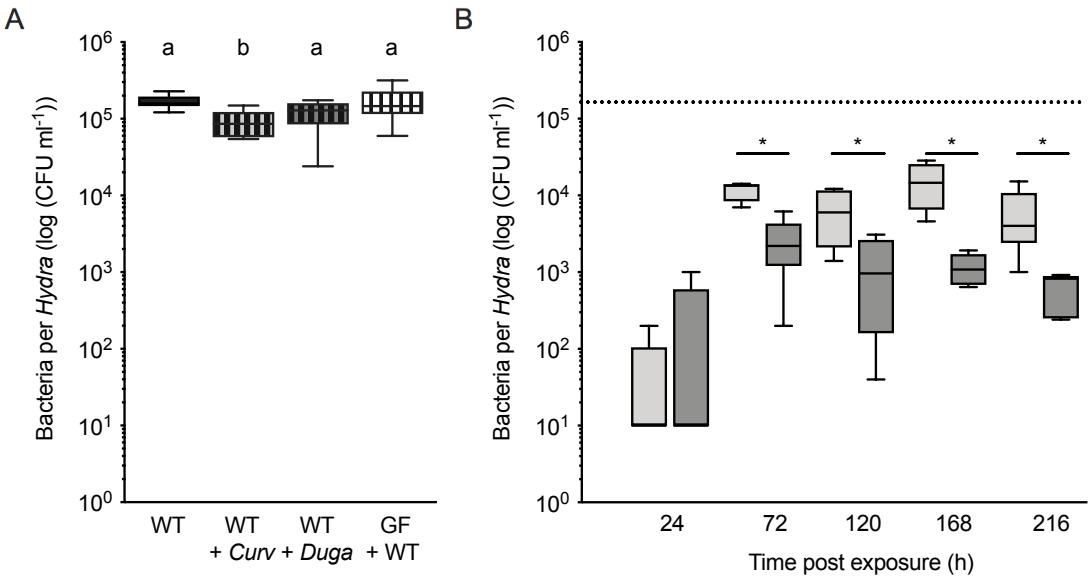
592 **Figures**



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594

595 Fig 1. (A) Carrying capacity of the *Hydra* habitat in wild-type (WT) polyps, wild-type
596 polyps and the addition of either the focal species *Curvibacter* (*Curv*) or *Duganella*
597 (*Duga*) and germ-free (GF) animals incubated with native *Hydra* microbiota
598 (conventionalised polyps) (each boxplot N≥6≤16). (B) Time course analysis of microbial
599 abundances in mono-associations of germ-free polyps with either *Curvibacter* (light
600 grey) or *Duganella* (dark grey) (each boxplot N=6). The dashed line indicates the
601 carrying capacity of wild-type polys.

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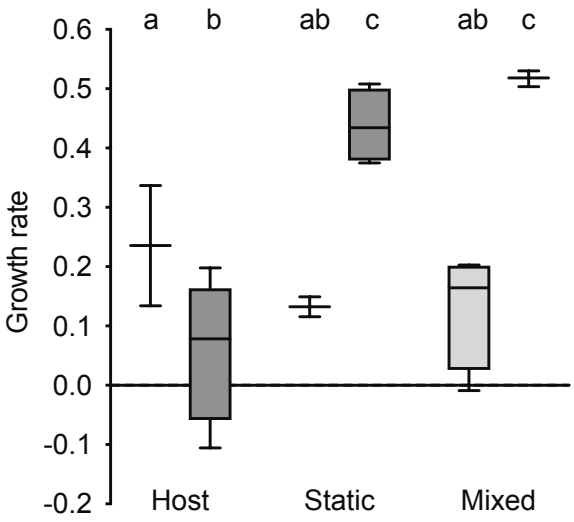
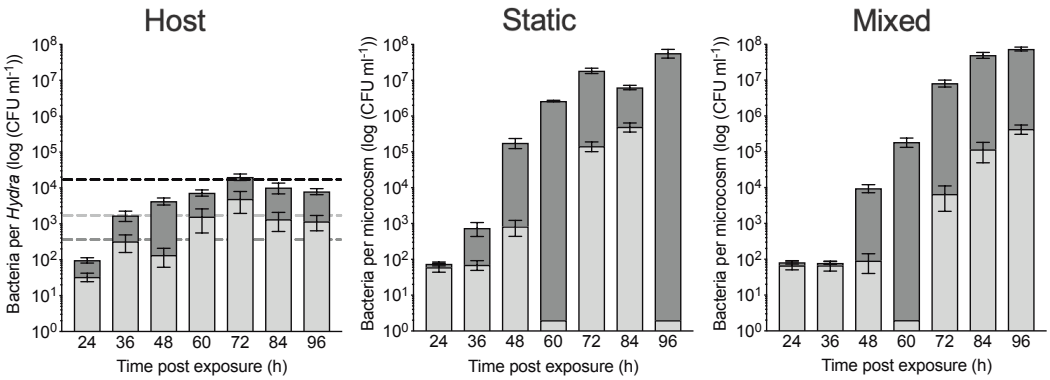


Fig 2. Bacterial growth rates of *Curvibacter* (light grey; each boxplot N≥2≤4) and *Duganella* (dark grey; each boxplot N≥3≤6) in mono-associations are habitat dependent. Compared are the host habitat (*in vivo*), and two *in vitro* environments: heterogeneous (static microcosms), and homogenous (mixed microcosms).



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611

612 Fig 3. Carrying capacity of the *in vivo* and *in vitro* habitats used in this study. Shown are
613 pooled total numbers of colony forming units (CFUs) from all di-association
614 experiments with *Curvibacter* (light grey) and *Duganella* (dark grey) (shown are s.e.m.
615 based on N≥11≤18 for the host, N≥9≤12 for static, and N≥4≤12 for mixed). The dashed
616 black line indicates the carrying capacity of WT polyps, the light grey line the carrying
617 capacity of polyps during *Curvibacter* mono-associations and the dark grey line the
618 carrying capacity of polyps during *Duganella* mono-associations.

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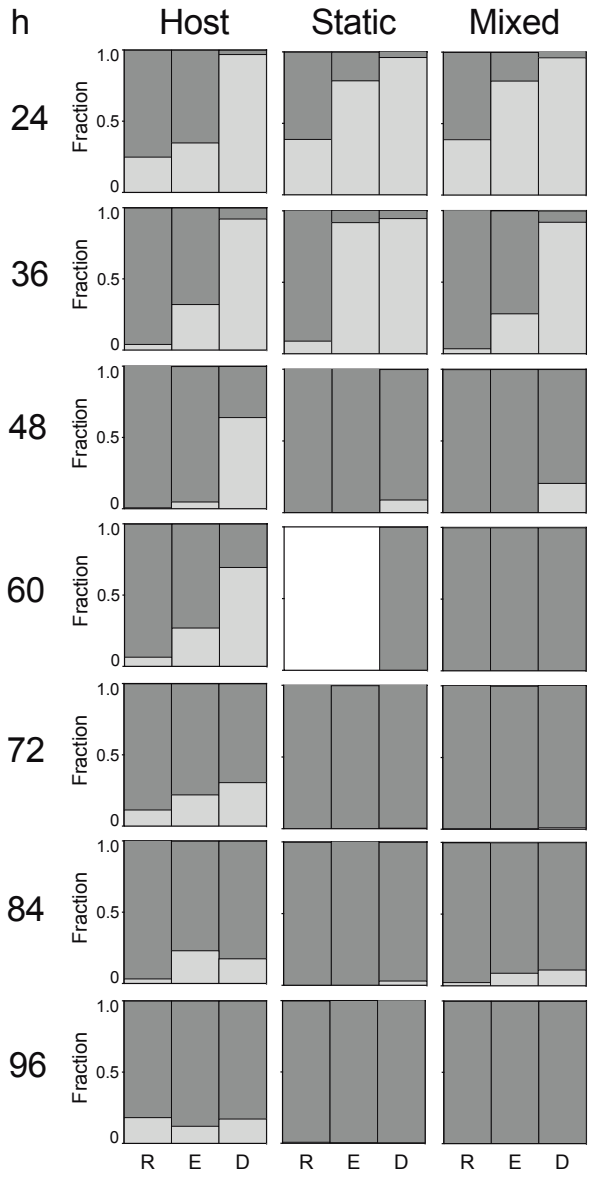


Fig 4. Time course of fractions of *Curvibacter* (light grey) and *Duganella* (dark grey) in the three different habitats obtained from di-association experiments. The initial inoculation frequency of *Curvibacter* varied from being rare (R), to equal (E), to dominant (D) in comparison to *Duganella* (each bar N≥3≤6; except for: Host, D, 24 h, where N=2, and for Static, R and E 60 h, where N=0 due to contamination of plates).

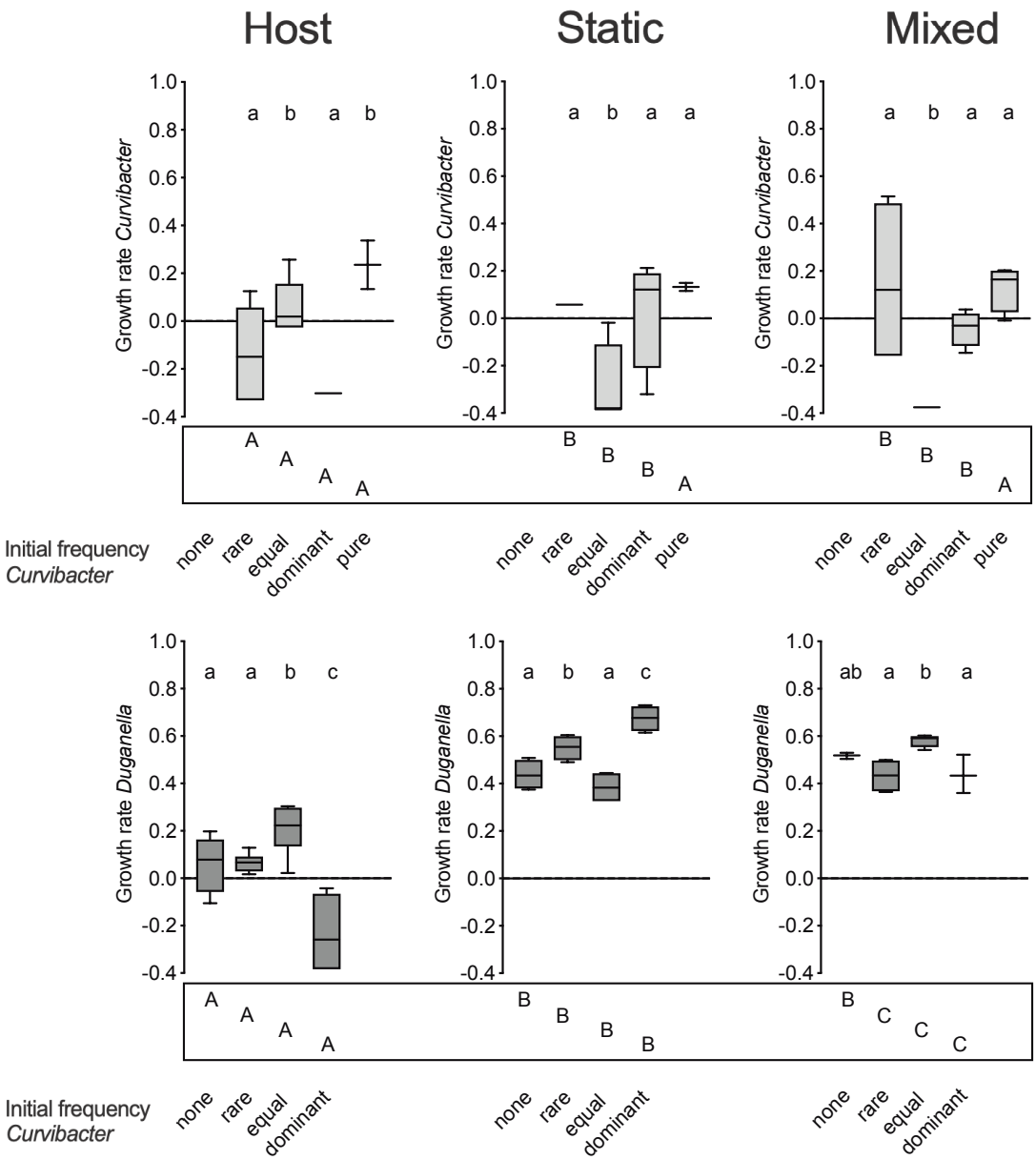
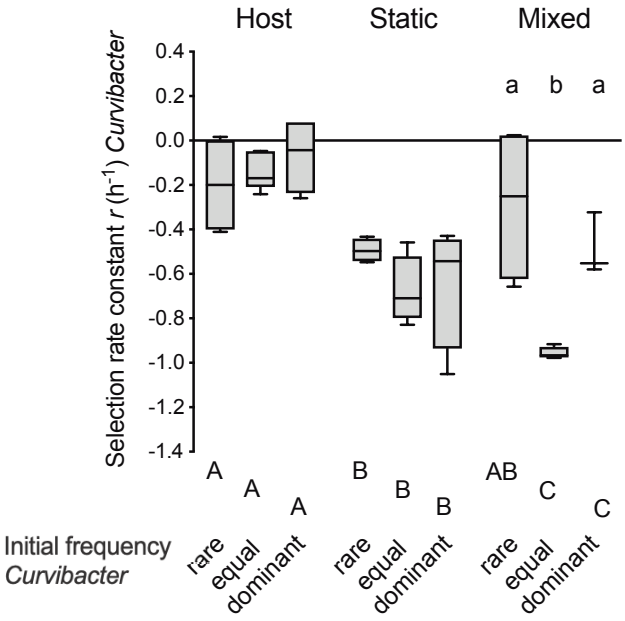


Fig 5. Bacterial growth rates of *Curvibacter* (light grey) and *Duganella* (dark grey) from mono- and di-association experiments across the different habitats and initial frequencies tested (each boxplot N≥3≤4).



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636 Fig. 6. Selection rate constants of *Curvibacter* based on competition experiments with

637 *Duganella* for the different starting frequencies, (each boxplot N≥3≤6).

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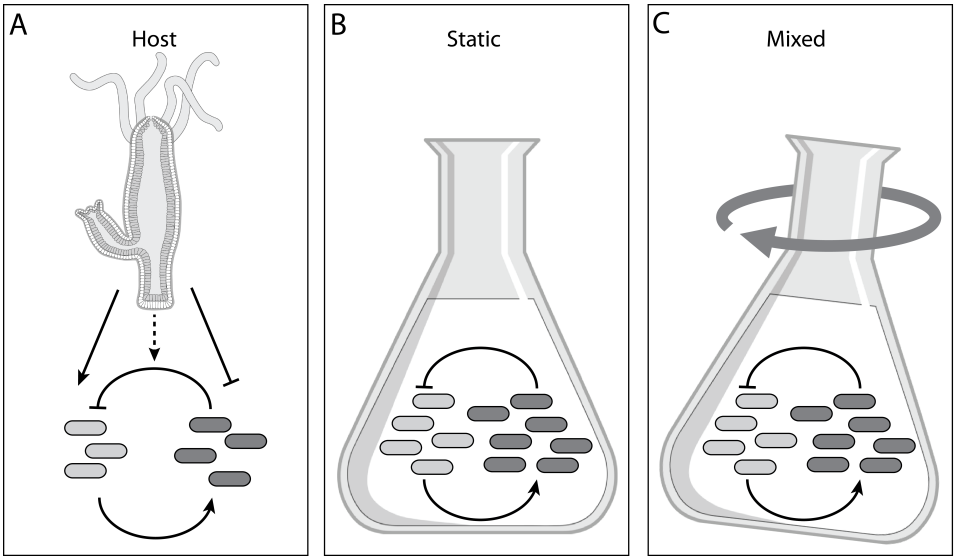

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641 Fig. 7. Model of the microbe-microbe and host-microbe interactions occurring in
642 different habitats (host (A) and non-host (B and C)) that influence bacterial abundances,
643 which are indicated by the number of bacterial cells (*Curvibacter*: light grey, *Duganella*:
644 dark grey). Whereas (A) allows to study the effect of the host environment on the two
645 microbial species and their interactions, microcosm experiments allow to study species
646 interaction in a heterogeneous (B) and a homogeneous environment (C). Arrow-headed
647 lines indicate promotion, whereas bar-headed lines indicate inhibition.

648

649