Microbial traits and the realized niche in a simple metaorganism

Peter Deines\(^1\)*, Katrin Hammerschmidt\(^2\) and Thomas CG Bosch\(^1\)

\(^1\)Zoological Institute, Christian-Albrechts-University Kiel, 24118 Kiel, Germany

\(^2\)Institute of General Microbiology, Christian-Albrechts-University Kiel, 24118 Kiel, Germany

*Correspondence:
Peter Deines
pdeines@zoologie.uni-kiel.de

Keywords
microbiome, microbial interactions, trait-based theory, niche concept, metaorganism

Running title: Microbial traits, niches, and the metaorganism
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.
Materials and Methods

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

Hydra vulgaris (strain AEP) was used in the experiments and cultured according to standard procedures at 18°C in standardized Hydra culture medium (Lenhoff and Brown, 1970). Animals were fed three times a week with 1st instar larvae of Artemia salina. Germ-free polyps were obtained as previously described (Franzenburg et al., 2013; Murillo-Rincon et al., 2017). After two weeks of treatment, polyps were transferred into antibiotic-free sterile Hydra culture medium for recovery (four days). Sterility was confirmed by established methods (Franzenburg et al., 2013). 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, Duganella sp. C1.2, Undibacterium sp. C1.1, Acidovorax sp. AEP1.4, Pseudomonas sp. C2.2, Pelomonas sp. AEP2.2., which are strains isolated from the Hydra vulgaris (strain AEP) microbiome (Fraune et al., 2015). These bacteria were cultured from existing isolate stocks in R2A medium at 18°C, shaken at 250 r.p.m for 72 hrs before use in the different experiments.

Carrying capacity of mono-associations in Hydra

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

Measurement of bacterial growth rates in vitro

 Cultures of the all six strains were produced in R2A microcosms (grown for 72 hrs at 18°C, at 250 r.p.m). Aliquots of each culture were first washed in S medium and then re-suspended in fresh R2A medium to an optical density of 0.025 at 600 nm (OD_600). Growth kinetics of all strains were determined in 96-well microtiter plates. A 100 μl aliquot of each re-suspension was pipetted into 100 μl of fresh R2A medium. The microtiter plate was then placed in a microplate reader (TECAN Spark 10M), and the OD_600 of each well was measured at 30 min intervals for 96 cycles (with 10 sec shaking at 150 r.p.m. prior to each read). The growth of each strain was determined in five well
locations on an individual 96-well plate, which was replicated six times. The maximum growth rate ($V_{max}$) was calculated from the maximum slope of the absorbance over time.

**Cell surface hydrophobicity (CSH)**

The BATH assay was performed as described previously (Borecká-Melkusová and Bujdáková, 2008; Rosenberg, 1984). It uses a biphasic separation method to measure cell surface hydrophobicity. In short, for each strain tested, 4 ml of bacterial suspension ($OD_{600} = 0.1; OD_{initial}$) was placed into a class tube, overlaid with 1 ml of n-hexodecane (Sigma Aldrich), and vortexed for 3 min. The phases were then allowed to separate for 15 min, after which the ODs of the aqueous (lower) phase containing hydrophilic cells was measured at $OD_{600}$ ($OD_{residual}$). The hydrophobic cells are found in the n-hexodecane overlay (upper phase). OD values were compared to the bacterial suspension before mixing with n-hexodecane. The relative hydrophobicity (RH) was calculated as follows: $RH = ((OD_{initial} – OD_{residual})/OD_{initial})$ x 100%. The experiment was performed in triplicate with independent bacterial overnight cultures.

**Biofilm quantification by use of crystal violet (CV)**

Biofilm formation was assayed and quantified as previously described (Ren et al., 2015). Briefly, exponential growth phase cultures of the six strains were adjusted to an optical density at 600nm ($OD_{600}$) of 0.1 in R2A medium. Biofilm formation was assayed in a 96 well plate using eight replicates for each treatment. For single isolates an inoculation volume of 180 µl was used and for the six species biofilm 30 µl per species. After 48 hrs of incubation at 18°C with shaking (200 r.p.m) biofilm formation was quantified by a modified crystal violet (CV) assay (Peeters et al., 2008).

**Characterizing nutrient utilisation**

To characterise the nutrient profiles (niches) of *Hydras* microbiota, we measured the carbon metabolism profile for each strain using BIOLOG GN2 plates. BIOLOG GN2 plates are 96-well microwell plates containing 95 different carbon sources plus a carbon-absent water control well. Strains were grown from isolate stocks in R2A medium (18°C, shaken at 250 r.p.m.), centrifuged at 3000 r.c.f. for 5 min, re-suspended in S medium and adjusted to an $OD_{600}$ of 0.1. Each well of the BIOLOG plate was inoculated with 150 µl of bacterial suspension and incubated for three days at 18°C in a humid chamber. Growth on each of the 95 nutrients was determined as $OD_{600}$ of each well using a TECAN plate reader. For each plate, the OD of the water control was subtracted from the reading of all other wells prior to analysis, and differenced OD values below 0.005 were considered
as no growth (Vaz Jauri et al., 2013). Nutrient use was evaluated on three replicate plates. Nutrient (niche) overlap (NO) was calculated using the formula: NO = (number of nutrients used by both A and B)/((number of nutrients used by A + number of nutrients used by B)/ 2) (Vaz Jauri et al., 2013). A value of 1 indicates the use of the same nutrients (100% overlap) and 0 indicates no nutrient overlap among the 95 substrates tested. We also calculated the relative use of the eleven functional groups (carbohydrates, carboxylic acids, amino acids, polymers, aromatic chemicals, amines, amides, phosphorylated chemicals, esters, alcohols and bromide chemicals) according to Daou et al. (2017). In brief, the relative use of C substrates was calculated as absorption values in each well divided by the total absorption in the plate.

**Statistical analysis**

Analysis of variance (ANOVA) and subsequent post hoc Tukey-Kramer tests were used to test for differences in the carrying capacity of the six *Hydra* colonizers. To meet the requirements for the model, the variable was Box-Cox transformed. A Welch ANOVA (and subsequent Wilcoxon posthoc tests) was used to test for differences in *in vitro* growth rates between the strains.

Analysis of variance (ANOVA) and subsequent post hoc Tukey-Kramer tests were used to test for differences in the cell surface hydrophobicity of the six *Hydra* colonizers. A Welch ANOVA (and subsequent Wilcoxon posthoc tests) was used to test for differences in biofilm formation capacity.

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 RStudio (RStudio Team, 2015).
Results

Growth performance of the bacterial strains

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

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

Bacterial traits

The colonization process

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

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

Microbial nutrient niches

Niche overlap (NO) among all pairwise strain combinations ranged from 60 to 80% (Figure 4). Curvibacter sp. shares the highest overlap (80%) with Pseudomonas sp. and
Duganella sp. For the other strains the overlap ranges between 60 and 70%. Duganella sp. displays the highest overlap with Pseudomonas sp. and Undibacterium sp. around 80%, whereas the overlap between Pelomonas sp. and Acidovorax sp. reaches almost 70%. Undibacterium sp. exhibits an overlap of 60 to 70% with Acidovorax sp., Pseudomonas sp. and Pelomonas sp.. Acidovorax sp. a 80% overlap with Pseudomonas sp. and 70% overlap with Pelomonas sp.. Pseudomonas sp. and Pelomonas sp. show a 75% overlap of the nutrients used. Comparing the number of nutrients being used by the individual strains we find that Curvibacter sp. and Pseudomonas sp. are able to use 70% of the provided substrates. Duganella sp. uses 57%, Acidovorax sp. 54% and Pelomonas sp. 51%. The lowest substrate utilization was measured for Undibacterium sp. with 41% of the available substrates.

**Resource competition between bacterial colonizers**

Nutrient utilisation of all strains was determined using a BIOLOG assay. The 94 carbon substrates are organized into eleven functional groups (Suppl. Figure S1). Results showed that all six strains actively oxidize carbon compounds such as carbohydrates (30-50% relative use), carboxylic acids (15-35% relative use) and amino acids (15-35% relative use) (Figure 5A; Suppl. Figure S1). Carbohydrates are being used to an equal extent between all strains, except for Undibacterium sp., which uses the highest amount of around 50% (relative use). Turanose is the compound most highly utilised, followed by a-D-lactose, L-rhamnose, and D-cellobiose. The use of carboxylic acids increases in the strains, which are characterized by low frequencies in the Hydra microbiome, with the exception of Duganella sp. (with a relative use of 25-30%). Here D-galactonic acid lactone is the substrate with the highest usage, followed by different forms of hydroxyl butyric acids. Amino acids are most excessively used by Acidovorax sp., Curvibacter sp. and Pseudomonas sp., whereas the other strains use amino acids to a lesser extent. Polymers are being used very differently between the strains with Pseudomonas sp. showing the highest and Undibacterium sp. the lowest values. Amines are being used more frequent by the dominant strains in the microbiome and are utilized to a lesser extent by the low abundant species.

The Venn diagram in Figure 5B displays overlaps in carbon substrate usage between all six microbiome members. There are only two substrates, which are not utilized by any species, whereas 20 substrates are used by all. There are only two strains that can metabolize substrates that none of the other species is using. While Pseudomonas sp. uses two substrates: i-erythritol and lactulose, Curvibacter sp. is able to utilise eight substrates: D-arabitol, D-mannose, D-trehalose, mono-methyl succinate, formic acid,
glucuronamide, L-pyroglutamic acid, and D-serine. The number of substrates shared exclusively between two strains only is between one and two. Overall, we do find that microbial performance, i.e. bacterial density on the host, cannot be linked to a specific bacterial trait or substrate utilization pattern (Figure 6).
**Discussion**

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

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

For linking the community composition in *Hydra*’s microbiome to specific characteristics, we used a trait-based approach focussing on traits potentially involved in microbiome assembly and stability. A first step in microbiome assembly is the attachment to host surfaces, which can happen in a multitude of ways. In the human
intestine, for example, microbes have been found to bind to mucin, a major component of the human mucosa (de Vos, 2015). Adhesion is thus thought to be a powerful mechanism for exerting both, positive and negative selection for or against specific microbes (McLoughlin et al., 2016; Schluter et al., 2015). Amongst others (van Loosdrecht et al., 1987), bacterial cell surface hydrophobicity has been shown to play a crucial role in surface attachment (Krasowska and Sigler, 2014). In general, hydrophobic cells adhere more strongly to hydrophobic surfaces and vice versa (Giaouris et al., 2009; Kochkodan et al., 2008). Nevertheless, the heterogeneity of a bacterial population needs to be taken into account. For example, the presence of both, hydrophilic and hydrophobic cells, have been observed in planktonic bacteria cell populations, implying that only part of the population participates in an adhesion process to substrates (Krasowska and Sigler, 2014). We also observe mixed cell populations for most of Hydra microbial associates, except for two strains, *Curvibacter* sp. and *Pelomonas* sp., which only consist of hydrophobic cells. They seem to be perfectly adapted to *Hydra* epithelial cells, which are coated with a carbohydrate-rich layer, the glycocalyx (Ouwerkerk et al., 2013; Schröder and Bosch, 2016). The microbiome inhabits the outer mucus-like layer of the glycocalyx (Fraune et al., 2015), which is hydrophilic. Thus, hydrophilic bacterial cells should adhere more strongly to *Hydra* than hydrophobic cells. Both, *Curvibacter* sp. and *Pelomonas* sp., have been shown to be of particular importance to the host. *Curvibacter* sp. shows signs of coevolution with its host and contributes to fungal resistance against the filamentous fungus *Fusarium* (Fraune et al., 2015). *Pelomonas* sp. has been shown to be of central importance in modulating the spontaneous body contractions in *Hydra* (Murillo-Rincon et al., 2017). So both strains contribute to host fitness, providing the opportunity for the speculation that the host actively selects for specific microbes. This could happen, for example, by controlling the production and release of adhesive molecules from the host epithelium as suggested by McLoughlin et al. (2016) or through an active support by the host so that they perform better in the host context (Deines et al., 2019).

After successful attachment, bacteria need to colonise the habitat. In most cases, this happens through the formation of biofilms, as has been reported for the gut (de Vos, 2015; Kania et al., 2007). The biofilm succeeds the planktonic phase in the bacterial life cycle (McDougald et al., 2012) and represents a key ecological process for the colonization of different habitats. Thus, the difference in the ability to form biofilms could provide an explanation for why one strain outcompetes the other strain or has got a higher chance of persistence in the *Hydra* ecosystem. Further, biofilms have been shown to protect bacterial cells from various environmental stressors (Flemming and
Wingender, 2010). Interestingly, from the six strains tested here, the one with the highest ability to form biofilms is *Pelomonas* sp., whereas the two main colonizers, *Curvibacter* sp. and *Duganella* sp. show a reduced capacity to form biofilms. Our finding indicates that the capability of biofilm formation is not a good predictor of the bacterial performance in the *Hydra* habitat. Nevertheless, it might be of importance for the establishment and persistence of some of the low abundance species, such as *Pelomonas* sp. and *Acidovorax* sp.

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

The metabolic overlap, i.e. redundancy, within *Hydra’s* microbial community indicates that the individual species are not occupying a specific metabolic niche. Nevertheless, the only one for which we observed a specific carbon usage pattern is the main colonizer *Curvibacter* sp., which utilizes eight carbon sources that are not metabolized by any other tested microbiome members. Whether this hints at the occupation of a specific niche within *Hydra’s* microbial community and can be linked to the observation that its realized is bigger than its fundamental niche when part of the community as compared to single occupation on *Hydra*, is currently open to speculation. An alternative option might be that *Curvibacter* sp. is auxotrophic in producing certain amino acids, as are 98% of all sequenced microbes (Zengler and Zaramela, 2018), and
thus relies on the uptake of external substrates that might not be secreted by the host but by its fellow community members. Analysing the metabolic interactions within this microbial network will be essential for understanding community assembly, composition, and maintenance.

In summary we find that the here measured bacterial traits vary across microbiome members. Further, the dominant species in the microbiome do not necessarily perform best in all of the measured traits. We rather observe that all species, independent of their density, perform well in a subset of traits, likely facilitating the coexistence of several niches within the host ecosystem. Whether a change in the realized niche of microbes can be linked to potential for dysbiosis is an interesting aspect, which warrants further investigation.
References

Bell, T., Gessner, M. O., Griffiths, R. I., McLaren, J. R., Morin, P. J., and van der Heijden, M. (2009). "Microbial diversity and ecosystem functioning under controlled conditions and in the wild," in Biodiversity, Ecosystem Functioning, and Human Wellbeing, eds.S. Naeem, D. E. Bunker, A. Hector, M. Loreau, and C. P. Perring (Oxford, UK), 121–133.

Borecká-Melkusová, S., and Bujdáková, H. (2008). Variation of cell surface hydrophobicity and biofilm formation among genotypes of Candida albicans and Candida dubliniensis under antifungal treatment. Canadian Journal of Microbiology 54, 718–724. doi:10.1139/w08-060.

Bosch, T. C. G. (2013). Cnidarian-Microbe Interactions and the Origin of Innate Immunity in Metazoans. Annu. Rev. Microbiol. 67, 499–518. doi:10.1146/annurev-micro-092412-155626.

Daou, L., Luglia, M., Périssol, C., Calvert, V., and Criquet, S. (2017). Sporulation and physiological profiles of bacterial communities of three Mediterranean soils affected by drying-rewetting or freezing-thawing cycles. Soil Biology and Biochemistry 113, 116–121. doi:10.1016/j.soilbio.2017.06.008.

de Vos, W. M. (2015). Microbial biofilms and the human intestinal microbiome. NPJ Biofilms Microbiomes 1, 15005. doi:10.1038/npjbiofilms.2015.5.

Deines, P., and Bosch, T. C. G. (2016). Transitioning from Microbiome Composition to Microbial Community Interactions: The Potential of the Metaorganism Hydra as an Experimental Model. Front. Microbiol. 7, 1610. doi:10.3389/fmicb.2016.01610.

Deines, P., Hammerschmidt, K., and Bosch, T. C. (2019). The effect of symbiosis on symbiont fitness – interactions within a simple metaorganism. bioRxiv, 609271. doi:10.1101/609271.

Deines, P., Lachnit, T., and Bosch, T. C. G. (2017). Competing forces maintain the Hydra metaorganism. Immunol. Rev. 279, 123–136. doi:10.1111/imr.12564.

Falkowski, P. G., Fenchel, T., and Delong, E. F. (2008). The microbial engines that drive Earth’s biogeochemical cycles. Science 320, 1034–1039. doi:10.1126/science.1153213.

Flemming, H. C., and Wingender, J. (2010). The biofilm matrix. Nature Publishing Group 8, 623–633. doi:10.1038/nrmicro2415.

Franzenburg, S., Walter, J., Künzel, S., Wang, J., Baines, J. F., Bosch, T. C. G., et al. (2013). Distinct antimicrobial peptide expression determines host species-specific bacterial associations. Proc. Natl. Acad. Sci. U.S.A. 110, E3730–8. doi:10.1073/pnas.1304960110.

Fraune, S., and Bosch, T. C. G. (2007). Long-term maintenance of species-specific bacterial microbiota in the basal metazoan Hydra. Proc Natl Acad Sci USA. doi:10.1063/1.1599624.

Fraune, S., Anton-Erxleben, F., Augustin, R., Franzenburg, S., Knop, M., Schröder, K., et al. (2015). Bacteria-bacteria interactions within the microbiota of the ancestral metazoan Hydra contribute to fungal resistance. ISME J, 1543–1556. doi:10.1038/ismej.2014.239.
Giaouris, E., Chapot-Chartier, M.-P., and Briandet, R. (2009). Surface physicochemical analysis of natural Lactococcus lactis strains reveals the existence of hydrophobic and low charged strains with altered adhesive properties. *International Journal of Food Microbiology* 131, 2–9. doi:10.1016/j.ijfoodmicro.2008.09.006.

Green, J. L., Bohannan, B. J. M., and Whitaker, R. J. (2008). Microbial biogeography: from taxonomy to traits. *Science* 320, 1039–1043. doi:10.1126/science.1153475.

Griffin, J. N., O’Gorman, E. J., Emmerson, M. C., Jenkins, S. R., Klein, A. M., and Loreau, M. (2009). "Biodiversity and the stability of ecosystem functioning," in *Biodiversity, Ecosystem Functioning, and Human Wellbeing*, eds. S. Naeem, D. E. Bunker, A. Hector, M. Loreau, and C. P. Perring (Oxford, UK: Oxford University Press), 78–93.

Guittar, J., Shade, A., and Litchman, E. (2019). Trait-based community assembly and succession of the infant gut microbiome. *Nat Commun* 10, 512. doi:10.1038/s41467-019-08377-w.

Holt, R. D. (2009). Bringing the Hutchinsonian niche into the 21st century: Ecological and evolutionary perspectives. *Proc Natl Acad Sci USA* 106, 19659–19665. doi:10.1073/pnas.0905137106.

Hunting, E. R., Vijver, M. G., van der Geest, H. G., Mulder, C., Kraak, M. H. S., Breure, A. M., et al. (2015). Resource niche overlap promotes stability of bacterial community metabolism in experimental microcosms. *Front. Microbiol.* 6, 11512. doi:10.3389/fmicb.2015.00105.

Hutchinson, G. E. (1957). Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology* 22, 415–427.

Kania, R. E., Lamers, G. E. M., Vonk, M. J., Huy, P. T. B., Hiemstra, P. S., Bloemberg, G. V., et al. (2007). Demonstration of Bacterial Cells and Glycocalyx in Biofilms on Human Tonsils. *Archives of Otolaryngology–Head & Neck Surgery* 133, 115–121. doi:10.1001/archotol.133.2.115.

Kau, A. L., Ahern, P. P., Griffin, N. W., Goodman, A. L., and Gordon, J. I. (2011). Human nutrition, the gut microbiome and the immune system. *Nature* 474, 327–336. doi:10.1038/nature10213.

Kochkodan, V., Tsarenko, S., Potapchenko, N., Kosinova, V., and Goncharuk, V. (2008). Adhesion of microorganisms to polymer membranes: a photobactericidal effect of surface treatment with TiO2. *Desalination* 220, 380–385. doi:10.1016/j.desal.2007.01.042.

Kopac, S. M., and Klassen, J. L. (2016). Can They Make It on Their Own? Hosts, Microbes, and the Holobiont Niche. *Front. Microbiol.* 7, 1647. doi:10.3389/fmicb.2016.01647.

Krasowska, A., and Sigler, K. (2014). How microorganisms use hydrophobicity and what does this mean for human needs? *Front. Cell. Infect. Microbiol.* 4, 653. doi:10.3389/fcimb.2014.00112.

Krause, S., Le Roux, X., Niklaus, P. A., Van Bodegom, P. M., Lennon, J. T., Bertilsson, S., et al. (2014). Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. *Front. Microbiol.* 5, 2019. doi:10.3389/fmicb.2014.00251.

Leibold, M. A. (1995). The Niche Concept Revisited: Mechanistic Models and Community
489 Context. Ecology 76, 1371–1382. doi:10.2307/1938141.

490 Lenhoff, H. M., and Brown, R. D. (1970). Mass culture of hydra: an improved method and its application to other aquatic invertebrates. Laboratory Animals 4, 139–154. doi:10.1258/002367770781036463.

493 Louca, S., Polz, M. F., Mazel, F., Albright, M. B. N., Huber, J. A., O’Connor, M. I., et al. (2018). Function and functional redundancy in microbial systems. Nat Ecol Evol 2, 936–943. doi:10.1038/s41559-018-0519-1.

496 Martiny, J. B. H., Jones, S. E., Lennon, J. T., and Martiny, A. C. (2015). Microbiomes in light of traits: A phylogenetic perspective. Science 350, aac9323–aac9323. doi:10.1126/science.aac9323.

499 Materna, A. C., Friedman, J., Bauer, C., David, C., Chen, S., Huang, I. B., et al. (2012). Shape and evolution of the fundamental niche in marine <i>Vibrio</i>. ISME J 6, 2168–2177. doi:10.1038/ismej.2012.65.

502 McDougald, D., Rice, S. A., Barraud, N., Steinberg, P. D., and Kjelleberg, S. (2012). Should we stay or should we go: mechanisms and ecological consequences for biofilm dispersal. Nature Publishing Group 10, 39–50. doi:10.1038/nrmicro2695.

505 McFall-Ngai, M., Hadfield, M. G., Bosch, T. C. G., Carey, H. V., Domazet-Loso, T., Douglas, A. E., et al. (2013). Animals in a bacterial world, a new imperative for the life sciences. Proc. Natl. Acad. Sci. U.S.A. 110, 3229–3236. doi:10.1073/pnas.1218525110.

508 McLoughlin, K., Schluter, J., Rakoff-Nahoum, S., Smith, A. L., and Foster, K. R. (2016). Host Selection of Microbiota via Differential Adhesion. Cell Host and Microbe 19, 550–559. doi:10.1016/j.chom.2016.02.021.

511 Murillo-Rincon, A. P., Klimovich, A., Pemöller, E., Taubenheim, J., Mortzfeld, B., Augustin, R., et al. (2017). Spontaneous body contractions are modulated by the microbiome of Hydra. Scientific Reports 7, 15937. doi:10.1038/s41598-017-16191-x.

514 Oh, J., Byrd, A. L., Park, M., NISC Comparative Sequencing Program, Kong, H. H., and Segre, J. A. (2016). Temporal Stability of the Human Skin Microbiome. Cell 165, 854–866. doi:10.1016/j.cell.2016.04.008.

517 Ouwerkerk, J. P., de Vos, W. M., and Belzer, C. (2013). Glycobiome: Bacteria and mucus at the epithelial interface. Best Practice & Research Clinical Gastroenterology 27, 25–38. doi:10.1016/j.bgpg.2013.03.001.

520 Pearman, P. B., Guisan, A., Broennimann, O., and Randin, C. F. (2008). Niche dynamics in space and time. Trends Ecol. Evol. (Amst.) 23, 149–158. doi:10.1016/j.tree.2007.11.005.

523 Peeters, E., Nelson, H. J., and Coenye, T. (2008). Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. Journal of Microbiological Methods 72, 157–165. doi:10.1016/j.mimet.2007.11.010.

526 Pocheville, A. (2015). “The Ecological Niche: History and Recent Controversies,” in Handbook of Evolutionary Thinking in the Sciences (Dordrecht: Springer, Dordrecht), 547–586. doi:10.1007/978-94-017-9014-7_26.

529 Prosser, J. I., Bohannan, B. J. M., Curtis, T. P., Ellis, R. J., Firestone, M. K., Freckleton, R. P.,
et al. (2007). The role of ecological theory in microbial ecology. *Nature Publishing Group* 5, 384–392. doi:10.1038/nrmicro1643.

Ren, D., Madsen, J. S., Sørensen, S. J., and Burmølle, M. (2015). High prevalence of biofilm synergy among bacterial soil isolates in cocultures indicates bacterial interspecific cooperation. *ISME J* 9, 81–89. doi:10.1038/ismej.2014.96.

Rosenberg, M. (1984). Bacterial adherence to hydrocarbons: a useful technique for studying cell surface hydrophobicity. *FEMS Microbiology Letters* 22, 289–295. doi:10.1111/j.1574-6968.1984.tb00743.x.

Schluter, J., Nadell, C. D., Bassler, B. L., and Foster, K. R. (2015). Adhesion as a weapon in microbial competition. *ISME J* 9, 139–149. doi:10.1038/ismej.2014.174.

Schröder, K., and Bosch, T. C. G. (2016). The Origin of Mucosal Immunity: Lessons from the Holobiont Hydra. *mBio* 7, e01184–16. doi:10.1128/mBio.01184-16.

van Loosdrecht, M. C., Lyklema, J., Norde, W., Schraa, G., and Zehnder, A. J. (1987). The role of bacterial cell wall hydrophobicity in adhesion. *Applied and Environmental Microbiology* 53, 1893–1897.

Vaz Jauri, P., Bakker, M. G., Salomon, C. E., and Kinkel, L. L. (2013). Subinhibitory antibiotic concentrations mediate nutrient use and competition among soil streptomycetes. *PLoS ONE* 8, e81064. doi:10.1371/journal.pone.0081064.

Wein, T., Dagan, T., Fraune, S., Bosch, T. C. G., Reusch, T. B. H., and Hülter, N. F. (2018). Carrying Capacity and Colonization Dynamics of Curvibacter in the Hydra Host Habitat. *Front. Microbiol.* 9, 185. doi:10.3389/fmicb.2018.00443.

Wennekes, P. L., Rosindell, J., and Etienne, R. S. (2012). The Neutral—Niche Debate: A Philosophical Perspective. *Acta Biotheor* 60, 257–271. doi:10.1007/s10441-012-9144-6.

Whittaker, R. H., Levin, S. A., and Root, R. B. (1973). Niche, habitat, and ecotope. *The American Naturalist* 107, 321–338. doi:10.1086/282837;subPage:string:Access.

Zengler, K., and Zaramela, L. S. (2018). The social network of microorganisms — how auxotrophies shape complex communities. *Nature Publishing Group* 16, 383–390. doi:10.1038/s41579-018-0004-5.
Author Contributions
PD designed the experiments. PD performed the experiments. PD and KH analysed the data. PD, KH, and TCGB wrote the paper.

Funding
PD received funding from the European Union’s Framework Programme for Research and Innovation Horizon 2020 (2014–2020) under the Marie Skłodowska-Curie Grant Agreement No. 655914 and KH under the Marie Skłodowska-Curie Grant Agreement No. 657096. Both also received a Reintegration Grant from the Deutscher Akademischer Austausch Dienst (DAAD). This work was further supported by the Deutsche Forschungsgemeinschaft (DFG) Collaborative Research Center (CRC) 1182 (“Origin and Function of Metaorganisms”).

Acknowledgements
TB gratefully appreciates support from the Canadian Institute for Advanced Research (CIFAR) and thanks the Wissenschaftskolleg (Institute of Advanced Studies) in Berlin for a sabbatical leave.

Conflict of Interests
The authors declare no conflict of interest.
**Figures**

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

**Figure 2.** *Hydra* functions as an ecosystem, which allows for the niche allocation. Shown are the fundamental and realized niches of six microbiome members. The realized niche includes additional constrains arising from inter-specific competition between microbiome members.
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.
**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.
Table 6. Summary of all the traits measured and their performance among the six microbiome members.

| Trait                        | Curvibacter sp. | Duganella sp. | Unidibacterium sp. | Acidovorax sp. | Pseudomonas sp. | Pelomonas sp. |
|------------------------------|-----------------|---------------|--------------------|----------------|----------------|--------------|
| % in microbiome              |                 |               |                    |                |                |              |
| Niche breadth                |                 |               |                    |                |                |              |
| Hydrophobicity               |                 |               |                    |                |                |              |
| Carbohydrates                |                 |               |                    |                |                |              |
| Carboxylic Acids             |                 |               |                    |                |                |              |
| Amino Acids                  |                 |               |                    |                |                |              |
| Substrate utilisation        |                 |               |                    |                |                |              |

**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.