



Comparable Ecological Processes Govern the Temporal Succession of Gut Bacteria and Microeukaryotes as Shrimp Aged

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Abstract

Understanding the rules that govern the successions of gut microbiota is prerequisite for testing general ecological theories and sustaining a desirable microbiota. However, the ignorance of microeukaryotes raises the question of whether gut microeukaryotes are assembled according to the same rules as bacteria. We tracked the shrimp gut bacterial and microeukaryotic communities by a longitudinal dense sampling. The successions of both domains were significantly correlated with host age, with relatively stable microeukaryotic communities in adult shrimp. Gut microeukaryotes exhibited significantly higher turnover rate, but fewer transient species, lower proportion of temporal generalists, and narrower habitat niche breadth than bacteria. The γ -diversity partitioning analysis revealed that the successions of gut microbiotas were primarily ascribed to the high dissimilarity as shrimp aged ($\beta_{\text{IntraTimes}}$), whereas the relative importance of $\beta_{\text{IntraTimes}}$ was significantly higher for microeukaryotes than that for bacteria. Compared with contrasting ecological processes in governing free-living bacteria and microeukaryotes, the ecological patterns were comparable between host-associated gut counterparts. However, the gut microeukaryotes were governed more strongly by deterministic selection relative to nestedness compared with the gut bacteria, which supports the “size-plasticity” hypothesis. Our results highlight the importance of independently interpreting free-living and host-associated meta-communities for a comprehensive understanding of the processes that govern microbial successions.

Keywords Succession of gut microbiota · Niche breadth · Diversity partitioning · Ecological processes · Size-plasticity hypothesis

Introduction

A fundamental concern in microbial ecology is what processes govern the temporal dynamics of species [1], since knowledge of these processes enables prediction and manipulation of forthcoming states of a community. This is a particular

significance for the gut microbial communities, as they contribute indispensable roles in regulating host health, fitness, development, and lifespan [2–7]. It has been found that the microbiotas are highly temporal dynamic, regardless of host phylogeny [8–12]. However, due to the variety of gut commensals and the complicated role of host factors [8, 13], an open question remains on how gut commensals colonize and are maintained as host aged.

Gut commensals colonize either from external species in the environment or from internal sources within the same host individual. A few studies have evaluated to what extent rearing water and sediment bacterial communities affects the gut microbiota of aquatic animals, illustrating that hosts source little of their gut commensals from surrounding environments [8, 14]. Building on this, we infer that gut microbes could be derived from its younger host (that is, nestedness). Nestedness is species gain (or loss) from younger to older [15], which often links with ordered extinction–colonization dynamics, such as over host development [16]. Generally, α -diversity in the gut microbiota is significantly lower than that in surrounding environments [17, 18], indicating that the host filters

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its gut microbial members. In a given community, some members exhibit broad environmental adaptations (generalists), while others show specific and narrow environmental tolerances (specialists) over spatial and temporal heterogeneities [19, 20]. By this logic, specialists tend to be sorted, while generalists are persistent over spatiotemporal successions. However, current estimations of the microbial succession mainly compare communities in a pairwise way and consider all species as equivalent to community composition, while ignore the different categories and traits among species.

To quantify the multiple facets of microbial biodiversity, a quantitative framework has been proposed to partition the dynamics of a meta-community into: local (α), regional (γ), and the overall difference between local communities (β) over space and time [21]. The novelty of this approach lies in the fact that it expresses the three components (α , β , and γ) in the same unit, thus enables the calculation of the relative contributions of α - and β -diversity to the γ -diversity. By comparing these components, it has been exemplified that bacterial communities in fish gut, rearing water, and sediment are functionally redundant, given that the functional diversity of the meta-community primarily arises from local communities [22]. Clearly, β -diversity partitioning moves beyond simple descriptions of community structure towards the inferring of mechanisms that govern community diversity. However, such quantitative partitioning of regional γ -diversity is scarce, especially for host-associated communities over timescales.

Our recent work has shown that the successional pattern of host-associated gut microbiota is significantly different from that of free-living bacterioplankton. Specifically, the gut bacterial communities are governed by species replacement (analogues of temporal turnover that consists in the substitution of species in one time by different species in the subsequent time [15]), while bacterioplanktons are equally shaped by replacement and nestedness [8]. Thus, host-associated and free-living microorganisms are likely governed by differed ecological processes. At this stage, prior meta-community researches have focused exclusively on host-associated bacteria [23, 24]. Consequently, our understanding on gut microeukaryotes is just beginning and lags far behind that on the bacteria. This ignorance is unwarranted because microeukaryotes account for most microbial communities due to their larger size and biomass, thereby could contribute disproportionate roles in gut microbiome [25–27]. Indeed, prokaryotes and eukaryotes are markedly different in biological and ecological characters, such as sexual reproduction, low metabolic flexibility, and predatory life strategies of eukaryotes [28]. Furthermore, host elements and microorganisms' traits could complicate the ecological assembly [13, 29, 30]. Thus, open questions remain on how the host-associated microeukaryotic and bacterial communities are structured or whether the two communities are governed by contrasting rules.

Available gut microbiota studies primarily focus on humans or other vertebrates [9, 10]. Nevertheless, the lack of adaptive immunity in invertebrates doubts the extension of vertebrate gut microbiota to invertebrates [6, 31]. Additionally, the collections of fecal sample and/or partial intestine certainly underestimate gut microbial diversity. By contrast, the small size of shrimp (*Litopenaeus vannamei*) gut enables us to explore the intact gut microbiotas. To avoid biases from temporally limited sampling, and given the high value of shrimp in global aquaculture, we characterized the shrimp gut bacterial and microeukaryotic communities over an entire culture cycle. In contrast to the unidirectional dispersal of free-living microorganisms over spatial scale, gut commensals are orderly selected as host aged [8, 30]. Additionally, it seems that ecological determinism increases with organism size [32]. For these reasons, we hypothesized that similar ecological processes governed the successions of gut bacteria and microeukaryotes, whereas larger microeukaryotes were more strongly structured by host filtering than smaller bacteria.

Materials and Methods

Experimental Design and Sample Collection

Over the period of 8 April to 15 July, greenhouse-reared shrimp were collected from six identical greenhouse ponds at Ningbo, Eastern China (29° 32' N, 121° 31' E). A longitudinal dense sampling (Bi/weekly sampling along shrimp development, 7, 14, 28, 42, 56, 70, 84, 91, and 98 days post larval shrimp inoculation (DPI)) was carried out to explore the temporal dynamics of gut microbiota. Shrimp individuals were aerated in tanks with their rearing water during sampling, and then were immediately transported to the lab. Given that insufficient amount of DNA was extracted from a single intestine of larval shrimp in the initial trial runs, every three shrimp intestines from each pond were aseptically dissected and pooled to compose one biological sample. In total, 54 gut samples were collected, which were stored at $-80\text{ }^{\circ}\text{C}$ prior to DNA extraction.

Water temperature, salinity, pH, and dissolved oxygen (DO) were monitored in situ by using corresponding probes (Oxi 340i; WTW, Weilheim, Germany). The rearing water chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP), NO_3^- , NO_2^- , NH_4^+ , PO_4^{3+} and SiO_4^{3+} levels were measured following seawater analysis standard of China [33].

DNA Extraction, Bacterial, and Eukaryotic rRNA Gene Sequencing

Genomic DNA was extracted using the FAST DNA Spin kit (MoBio Laboratories, Carlsbad, CA, USA) following the

manufacturer's protocols. The concentration and purity of DNA extracts were evaluated by using a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, Wilmington, USA). The V3–V4 regions of bacterial 16S rDNA [34] and the V4 region of eukaryotic 18S rDNA [35] genes were amplified separately using the same DNA extract for each sample. This design enabled a paired comparison between bacteria and eukaryotes, thereby controlling variations among biological samples. Polymerase chain reaction (PCR) conditions were employed as described elsewhere [17, 25]. To avoid biases introduced during PCR amplification, each sample was amplified in triplicate. Amplicons for each sample were pooled and purified using a PCR fragment purification kit.

Equimolar amounts of the purified amplicons (bacteria or eukaryotes) from each sample were sequenced on a single run using an Illumina MiSeq 2 × 300 bp platform (Illumina, San Diego, CA, USA), generating paired-end reads. Paired-end sequences for bacteria and microeukaryotes were merged respectively with a maximum of 10% mismatches in the overlap region using FLASH [36]. For bacteria communities, the merged sequences were processed following the QIIME (v1.9.0) pipeline [37]. Briefly, the sequences with ambiguous bases or truncated at any site of more than three consecutive bases receiving a Phred quality score (Q) < 25 were excluded. Chimeras were deleted using VSEARCH [38]. Sequences with a distance-based similarity of 97% or greater were grouped into operational taxonomic units (OTUs) using UCLUST [39]. The most abundant sequence from each OTU was selected as its representative, and then was taxonomically assigned an open reference (Silva database, release 132) [40]. Furthermore, OTUs that were assigned to Archaea, chloroplasts, and those unaffiliated with the Bacteria domain, as well as singletons, were discarded from the dataset. Similar procedures were applied for microeukaryotic communities, with the exceptions that OTUs affiliated with Metazoa, unassigned to Eukaryota, and the contamination of shrimp were excluded. To correct for uneven sequencing efforts, the OTU table for bacteria and microeukaryotes was 10 × randomly rarefied subset of 14,000 and 4080 clean reads per sample in subsequent analyses, respectively. The bacterial 16S rRNA and microeukaryotic 18S rRNA sequence data have been deposited in the Sequence Read Archive at DDBJ under accession numbers DRA005256 and DRA005998.

Statistical Analysis

To compare the successional patterns of gut bacteria and microeukaryotes as shrimp aged, ecological approaches were employed to explore the multiple facets of microbial biodiversity (Fig. S1) in R 3.4.1 project, unless otherwise stated [41]. Specifically, a non-metric multidimensional scaling (NMDS) analysis was conducted to visualize the weighted UniFrac

distances of microbial communities among samples. Then, a Procrustes analysis was used to detect the correlation between each pair of bacterial and eukaryotic communities [42]. The significance among groups was tested using an analysis of similarity (ANOSIM) [43]. The time-decay of community similarity relationship was plotted as logarithmic similarity against logarithmic age and a linear regression performed to obtain the temporal turnover rate [44]. This form of time lag regression could test whether communities are undergoing directional change. In addition, we treated ponds (the origin of shrimp samples, pond as a conditional factor) as replicates, thus enabled us to test significance (using paired *t* test to improve statistical power) in turnover rate between bacterial and eukaryotic communities.

Habitat Niche Breadth

Niche breadth is a crucial trait that influences the relative importance of species sorting and temporal nestedness in affecting communities [19]. A wider niche breadth (i.e., generalist) is expected to be more metabolically flexible, which is persistent at the regional community level over time. In contrast, the habitat specialists are more restricted to specific times [45]. Here, the generalists and specialists of the gut bacterial and microeukaryotic communities as shrimp aged were identified respectively based on the Levins' niche breadth index (*B*):

$$B_j = 1 / \sum_{i=1}^N P_{ij}^2$$

Specifically, *B_j* indicates the niche breadth of OTU_{*j*} in a community; *N* is the total number of species across the community; *P_{ij}* is the relative abundance of OTU_{*j*} in community *i* [19]. For a given taxon, it was categorized into specialist or generalist corresponding to *B* > 3 or *B* < 1.5. We further calculated the average *B* values across all taxa in bacterial or microeukaryotic community (*B_{com}*) as an index of its habitat niche breadth [19].

Variation Partitioning Analysis

A forward selection procedure was employed to identify the most important variables in shaping the microbial communities in a distance-based multivariate linear model (DistLM), in which sequentially added one variable that improves the selection criterion (*R*²) the most at each step, until no improvement was reached in *R*² [46, 47]. The key variables that governed both bacterial and eukaryotic communities were selected. Since host age can be used as a proxy for the unmeasured temporal host variables, i.e., immune maturity as host aged [8, 30], a partial distance-based redundancy analysis (partial db-RDA) was used to further evaluate the relative

contribution of environmental and temporal host factors on the variation in gut microbiotas [48]. Variation partitioning was carried out to partition the community variation into environmental effects and temporal effects. The pure environmental variation without a temporal component ($E|T$) represents the strength of species sorting; the pure temporal variation without an environmental component ($T|E$) is interpreted as the effect of nestedness [8, 19]. To equitably evaluate the relative importance of species sorting versus replacement at different microbial groups, we compared the ratio of $(E|T)/(T|E)$ (that is, species sorting/nestedness effect ratio), instead of their absolute values. The ratio enabled the comparison of the species sorting and nestedness dominances of bacterial and eukaryotic communities in a relative sense [19, 49].

The Rao quadratic entropy (a measure of diversity that integrates species-relative abundances and pairwise interspecies differences) [21] was applied to decompose the ($\gamma_{\text{Ecosystem}}$) into local diversity (α) and the difference among local communities (β). The advantage of this framework is the calculation of the relative contributions of α - and β -diversity to the γ -diversity [50]. Specifically, regional diversity of microbial community was expressed as the sum of inter-time difference, the mean intra-time difference, and the mean local community diversity with: $\gamma_{\text{Ecosystem}} = \beta_{\text{InterTimes}} + \beta_{\text{IntraTimes}} + \alpha_{\text{LocalCommunities}}$ [21]. Furthermore, we quantified the relative importances of the ecological processes: selection, drift, and dispersal using the methodology as proposed before [14]. Though the original definition of these ecological processes arises from “spatial context,” similar concepts have been extrapolated in “temporal” meta-communities as host aged [10, 29]. The framework consists of two main steps: the first step applies phylogenetic turnover between communities to evaluate the contribution of selection, and the second step uses OTU turnover to assess the role of dispersal and drift [14].

Results

Successions of Gut Bacterial and Microeukaryotic Communities as Shrimp Aged

The α -diversity of gut bacterial communities was relatively stable as shrimp aged, whereas microeukaryotic communities exhibited decreasing diversity over the same timeframe (Fig. S2a–d). There was a clear separation of gut bacterial communities according to shrimp age (days post inoculation) (Fig. 1a). This also held true for gut microeukaryotes, with the exception that microeukaryotic communities tended to be stable at the adult stage (i.e., DPI70 and thereafter) (Fig. 1b). This

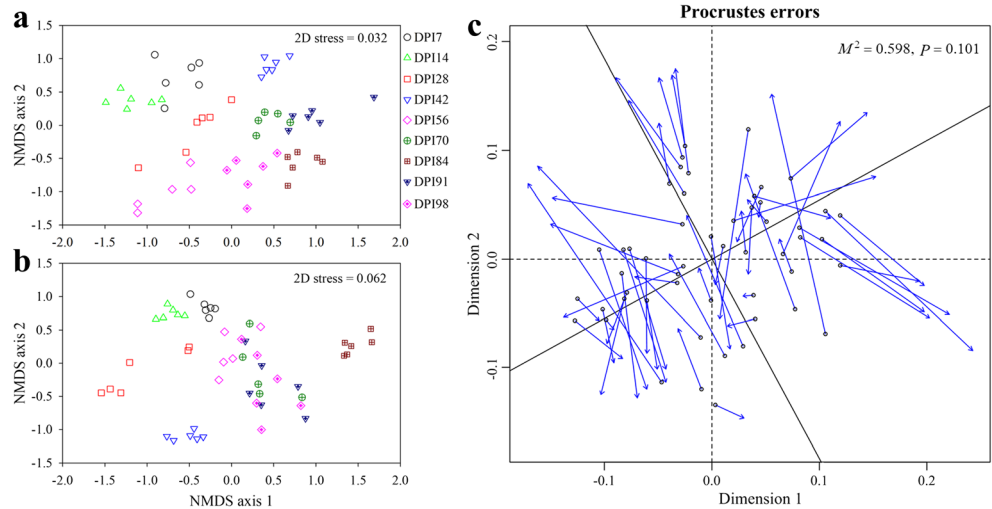
difference was confirmed by the ANOSIM, illustrating that the gut bacterial communities were significantly distinct between every pair of shrimp ages (Table S1), while there were no significant differences in microeukaryotic communities among adults, i.e., DPI70 (70 days post larval shrimp inoculation) vs. DPI 98, and DPI91 vs. DPI 98 (Table S2). The Procrustes analysis revealed that the β -diversity patterns of bacteria and microeukaryotes were moderately correlated ($M^2 = 0.598$, $P = 0.101$) (Fig. 1c). Consistently, there was a marginally significant association (Mantel test $\rho = 0.316$, $P = 0.081$) between the temporal dynamics of the two domains (Fig. S3). Together, these results depicted comparable β -diversity patterns between gut bacterial and microeukaryotic communities as shrimp aged.

The host age–decay rate of gut microbiotas was calculated as the slope of a linear regression on the relationship between shrimp age and gut community similarity for bacteria and microeukaryotes, respectively. Both gut bacterial and microeukaryotic communities exhibited significant shrimp age–decay for similarity relationship (Fig. S4). When the age–decay rates were calculated between the first sampling and onwards ones, a paired t tests ($N = 6$) revealed that the slope of microeukaryotic communities was significantly ($P < 0.001$) steeper than that of bacterial counterparts (Fig. S4).

Defining the Microbial Community by Frequency

To identify the persistent and transient species, we partitioned ecological communities into their abundance and prevalence. For the prevalence, we arbitrarily divided the microbial community into the following three ecological categories: persistent ($\geq 80\%$ analyzed samples over shrimp development), intermittent (ranged 20–80%) and transient ($\leq 20\%$) OTUs (Fig. 2). Positive relations between the mean abundance and prevalence were detected, which were best fitted using the exponential equation: $y = 0.0002e^{0.106x}$ ($R^2 = 0.722$, $P < 0.001$) for bacteria, and $y = 0.0008e^{0.136x}$ ($R^2 = 0.628$, $P < 0.001$) for microeukaryotes (Fig. 2a). In this regard, persistent OTUs were more abundant than intermittent and transient OTUs for both domains, although the former ecological categories harbored a much smaller number of OTUs (Fig. 2b). The distribution and frequency were similar between bacterial and microeukaryotic OTUs. That is, persistent bacterial OTUs possessed 0.59% of the total OTUs but accounted for 55.3% bacterial reads. Similarly, only 0.36% of microeukaryotic OTUs occupied 48.5% microeukaryotic sequences (Fig. 2b). Conversely, transient OTUs harbored 93.5% bacterial OTUs but only occupied 13.8% of the total sequences, while 93.4% microeukaryotic OTUs merely accounted for 10.8% (Fig. 2b). Thus, there were highly diverse but minority gut bacterial and microeukaryotic taxa as shrimp aged.

Fig. 1 Non-metric multidimensional scaling (NMDS) ordination of the dissimilarity in the gut bacterial (a) and microeukaryotic (b) communities as shrimp aged. Samples are coded by shrimp age (days post inoculation). (c) Procrustes superimposition plot of bacterial (open circles) and microeukaryotic communities (end point of solid lines). Solid lines represent Procrustes residuals from both domains



Partitioning the Microbial Community into Temporal Generalists and Specialists

There were comparable proportions of temporal generalists between bacterial and microeukaryotic communities, 55.7% vs. 47.1%. In contrast, microeukaryotic specialists occupied 23.7% of the total diversity, which was much higher than for bacteria, where they merely accounted for 10.0% (Fig. 3a and b). Consistently, the averaged community niche breadths (Bcom) for microeukaryotic communities were significantly lower than that of bacterial counterparts (Fig. 3c). These results revealed microeukaryotes were more sensitive to host age, as compared with bacteria.

Overall, we found that both the host age and the rearing water variables governed the gut bacterial and microeukaryotic communities (Table S3). For the two total communities, the

variations were better explained by the pure environmental component (> 27% variation) than by the pure age effect (< 2% variation). A similar pattern emerged when we focused on the temporal generalists (Table 1). In contrast, a divergent trend was detected when we restricted the analysis to temporal specialists. That is, the pure age effect was a more influential contributor than the environmental component in governing the age specialists (Table 1). Additionally, the species sorting/nestedness effect ratios in microeukaryotes were consistently higher than bacteria for the three sub-communities (Fig. S5).

Partitioning Regional Diversity and Underlying Ecological Processes of Microbial Communities

To quantitatively compare the relative contributions of α - and β -diversity to the γ -diversity, we partitioned the $\gamma_{Ecosystem}$ of

Fig. 2 Defining the gut microbial communities as shrimp aged. The left and right panels represent bacterial and microeukaryotic communities, respectively. (a) The mean abundances of OTUs regress against the occurrence frequencies. The prevalence is calculated by dividing the number of samples in which an OTU is detected by the enrolled 52 samples. (b) The number and the relative abundance of OTUs of different occurrence frequencies (persistent, intermittent, and transient) are shown

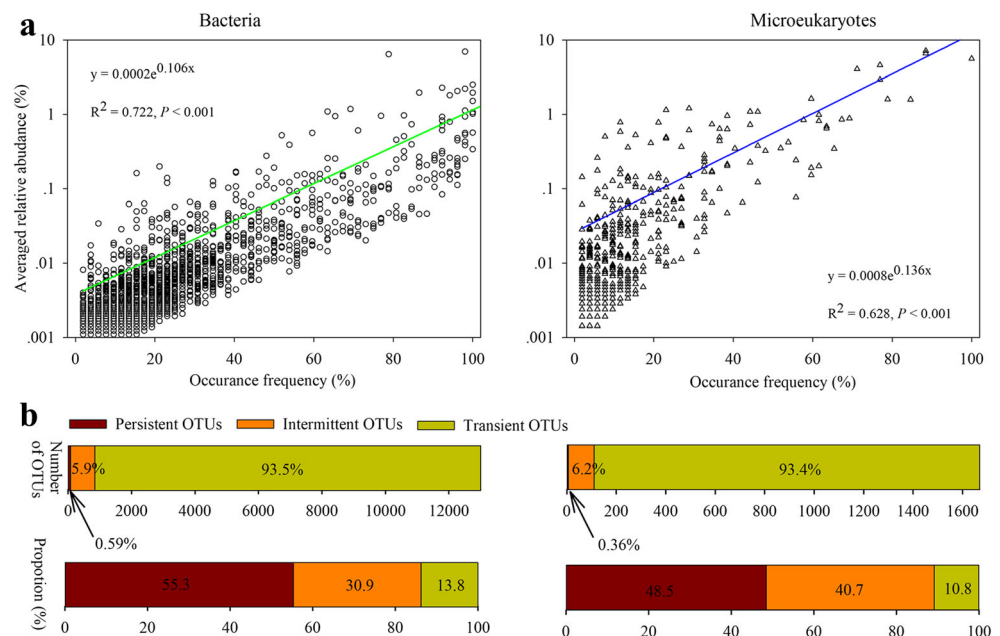
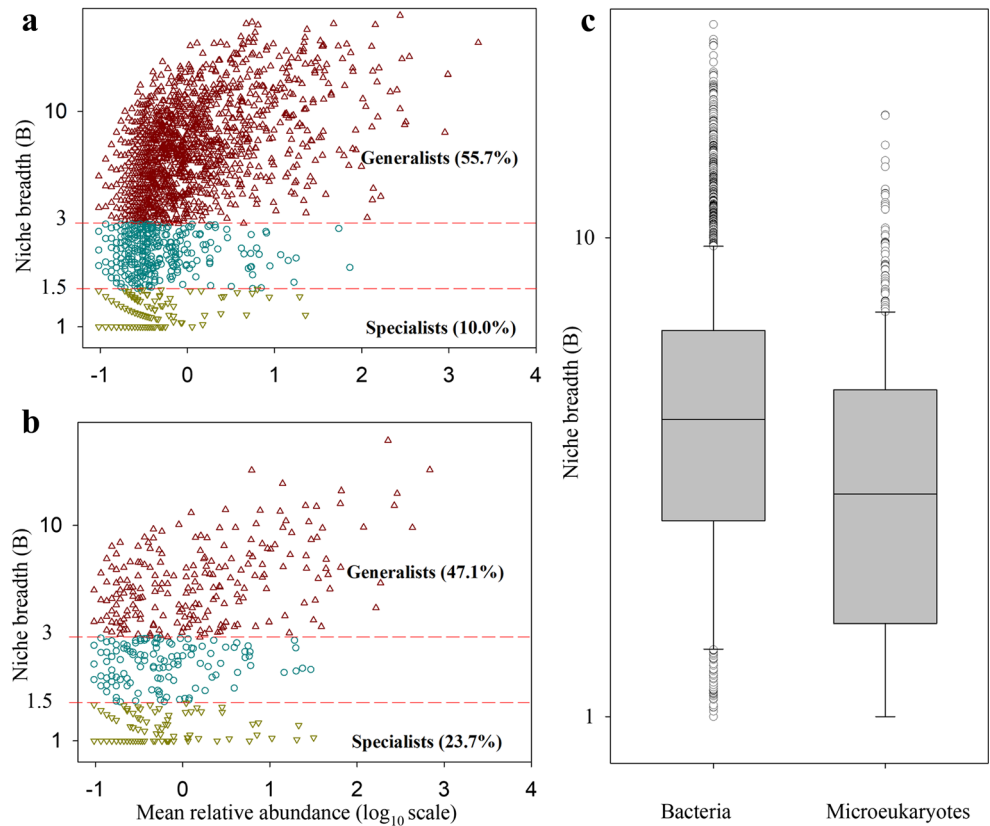


Fig. 3 The mean abundances of OTUs of Bacteria (a) or microeukaryotes (b) versus habitat niche breadth. (c) Boxplots illustrating mean habitat niche breadth (Bcom) from all taxa in each sample of bacterial versus microeukaryotic communities. The mean Bcom value for bacteria is significantly higher than that of microeukaryotes



gut microbiotas over temporal scales. The local $\alpha_{LocalCommunities}$ contributed 35.5% variation in the bacterial communities (Fig. 5b), which was significantly greater (paired *t* test, $P = 0.048$) than that in the microeukaryotic communities, $\alpha_{LocalCommunities} = 25.6\%$. Similarly, the contribution of $\beta_{InterTimes}$ on bacterial communities (21.6%) was higher than microeukaryotic counterparts, though the difference was

insignificant (Fig. 4c). In contrast, the contributions of $\beta_{IntraTimes}$ exhibited the opposing trend ($P = 0.039$), with β

Table 1 Variance partitioning among temporal and environmental variables for the three sets of data (total communities, temporal specialists, and temporal generalists) for the gut microbiotas (bacteria and microeukaryotes) as shrimp aged

Ecological groups		Variance explained (%)			
		<i>E</i>	<i>T</i>	<i>E T</i>	<i>T E</i>
Total communities	Bacteria	38.9	15.0	27.3	1.6
	Microeukaryotes	35.2	14.2	28.2	1.9
Temporal generalists	Bacteria	41.6	16.4	27.5	1.6
	Microeukaryotes	38.9	16.3	29.1	1.8
Temporal specialists	Bacteria	14.0	4.5	2.7	13.8
	Microeukaryotes	12.3	3.1	4.1	13.4

E variation explained by environmental variables; *T* variation explained by temporal variables, here is days after larval shrimp inoculation; *E|T* variation explained by pure environmental variables (partial out *T*); and *T|E*, variation explained by pure spatial variables (partial out *E*)

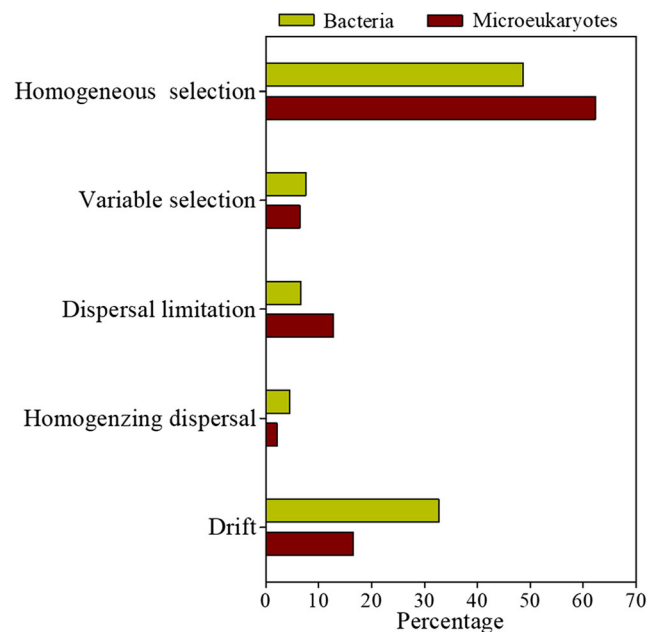


Fig. 5 Quantification of the relative roles of selection (homogeneous and heterogeneous), dispersal limitation, homogenizing dispersal and drift in governing the gut bacterial and microeukaryotic communities as shrimp aged. Values indicate percentage of community turnover structured by each ecological process

$\beta_{IntraTimes}$ = 42.9% in bacterial communities, and 57.6% in microeukaryotic communities (Fig. 5). However, for both domains, the contributions of $\beta_{InterTimes}$ outweighed ($P < 0.001$ in both cases) $\beta_{IntraTimes}$ (Fig. 4), suggesting the fundamental role of host age in governing the succession of gut microbiotas.

We further quantified the relative roles of homogeneous or heterogeneous selection, dispersal limitation, homogenizing dispersal, and drift in governing the bacterial and microeukaryotic communities, respectively (Fig. 5). Homogeneous selection was the most important process in governing both bacterial (48.7% of the overall community turnover) and microeukaryotic (62.3%) communities. In contrast, the relative importance of drift exhibited an opposing trend, with 32.7% of the overall community turnover for bacteria, and 16.5% for microeukaryotes (Fig. 5). In both bacteria and microeukaryotes, homogenizing dispersal contributed little to community structures. Additionally, variable selection exerted comparable effects on the two microbial domains (Fig. 5).

Discussions

Contrasting ecological processes in governing the spatial distribution of free-living bacterial and microeukaryotic

communities have been widely observed in diverse ecosystems [49, 51, 52]. By contrast, very little is known about the rules that govern gut microbiota as host aged, while this knowledge could guide the design of a desirable gut microbiota. Here, we compared the gut bacterial and microeukaryotic communities during shrimp development. The findings provide new insights into the successions of host-associated communities imposed by biotic and abiotic gradients, i.e., host maturity and rearing water variables, thus allowing community differentiation.

Both the gut bacterial and microeukaryotic communities markedly changed over shrimp development, though the microeukaryotic communities tended to be stable at the adult stage (Fig. 1). Indeed, ample evidence has shown that gut bacterial communities are primarily affected by host age in diverse aquatic animals [10, 18, 29, 30]. By contrast, we know little about the role of host-associated microeukaryotes outside of the view of parasitology [26, 53]. The stable gut microeukaryotic communities could be attributed to the filtering of sensitive members as adult maturity. Consistent with this assertion, gut microeukaryotic diversity linearly decreased as shrimp aged (Fig. S3). Alternatively, the diversification of gut microeukaryotes is delayed as compared with bacteria, a pattern that has been observed in human gut microbiotas [54]. As a consequence, there was an insignificant

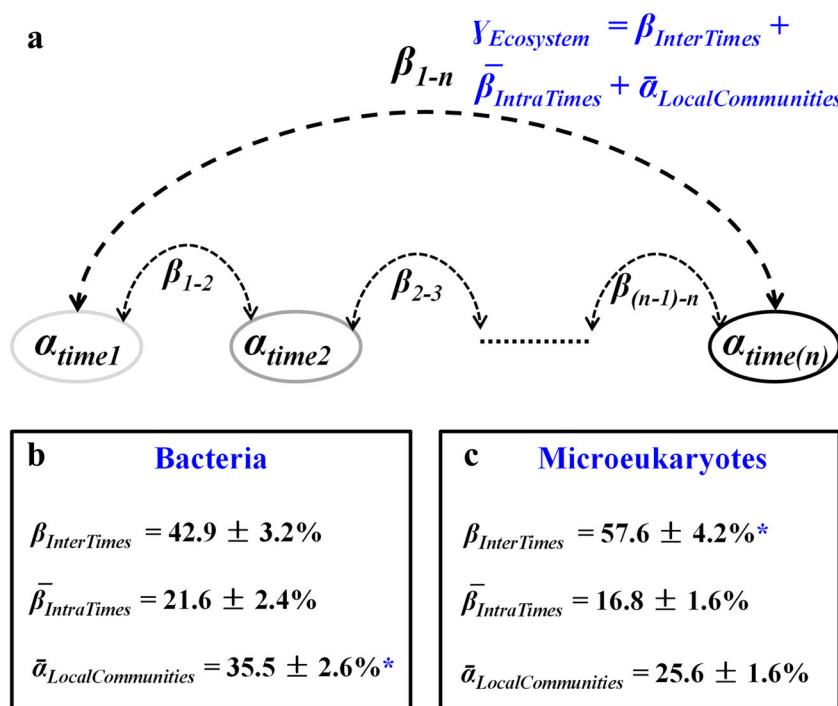


Fig. 4 Partitioning microbial γ -diversity into local α -diversity and the difference among local communities, β -diversity. **(a)** General outline. The n local communities correspond to the same community sampled along different times. **(b)** Bacterial communities, **(c)** Microeukaryotic communities. The total compositional diversity within the shrimp gut was expressed as the sum of inter-time compositional differences

($\beta_{InterTimes}$), the mean intra-time compositional difference ($\beta_{IntraTimes}$), and the mean local compositional diversity ($\alpha_{LocalCommunities}$) with $Y_{Ecosystem} = \beta_{InterTimes} + \beta_{IntraTimes} + \alpha_{LocalCommunities}$. * indicates significant of a given biodiversity between bacteria and microeukaryotes by using paired t test at $P < 0.05$ level

association between the β -diversity of the two domains (Fig. 1c), which in turn suggests different β -diversity patterns.

To explain this pattern, we compared the temporal turnover for bacteria versus microeukaryotes as shrimp age. We found that microeukaryotes had significantly steeper turnover (more rapid deviation from original to new state) than bacteria (Fig. S4), again indicating that gut microeukaryotes were more sensitive to host development. Why was a higher turnover rate observed for microeukaryotes? A few studies have proposed that body size determines the distance decay of community similarity, while the mechanisms behind them are divergent [32, 55, 56]. One explanation is that the turnover rate is positively associated with body size, a pattern that has been observed over spatial scale [56]. It has been proposed that an increasing temporal turnover compensates a decreasing nestedness fraction [12]. By this logic, bacterial communities are more affected by nestedness process in relation to microeukaryotes. This is in agreement with our analyses of prevalence, of which revealed a lower slope and a more even distribution for bacteria (Fig. 2a). Alternatively, larger organisms (microeukaryotes) are metabolically less flexible to persist in a reversible state [28, 32], thereby limiting their competence in adapting environmental heterogeneity, i.e., increased maturity as host aged [9, 11, 17, 30]. As a result, there was a smaller proportion of transient species in microeukaryotic assemblages than that in bacterial counterparts (Fig. 2b).

Given the unequal tolerance of microbial members to changing environments [19, 20], we further categorized the species into generalists and specialists. Bacteria harbored a higher proportion of generalists and fewer specialists than microeukaryotes (Fig. 3a and b). Consistently, bacteria exhibited significantly wider niche breadths than microeukaryotes (Fig. 3c), which confirms the results from other habitats [20, 49]. In addition, the species sorting/nestedness effect ratios were consistently higher in microeukaryotes than in bacteria, regardless of the total or sub-communities (Fig. S5). Thus, microeukaryotic communities are more structured by species sorting relative to nestedness than bacterial communities. These findings collectively support the “size-plasticity” hypothesis: small-size bacteria are less environment filtered than larger size microeukaryotes [19]. However, the importance of body size does not rule out the influence of other factors in triggering this pattern, such as dormancy, a strategy that is uncommon in microeukaryotes [28]. Notably, both rearing environment and temporal processes (host age) significantly shaped the gut sub-communities. However, which of these processes is more important depends on the degree of habitat specialization (Table 1). At first, it seems surprising that specialists were much less influenced by pure environmental component compared with pure temporal component for both microeukaryotes and bacteria, given previous evidence that environmental specialists with narrower niche breadths were

more governed by local conditions [19, 49]. However, considerations of co-evolution hypothesis may explain this apparent paradox [57]. From a host perspective, host should tightly select and reserve suitable commensals, thereby providing immense potential for adaptation to changing conditions, such as diet and external environment [8, 57, 58]. Thus, it is expected that specialists were more strongly governed by temporal process (Table 1). Our recent work shows that gut age-discriminatory taxa could accurately predict shrimp age [29], reinforcing the prominent imprint of host age. By this logic, variations in rearing water along shrimp development could reasonably be expected to affect gut microbiotas (Table 1).

To compare the different facets of community structure, we further partitioned biodiversity into local (α), regional (γ), and the overall difference between local communities (β) [21]. We found that the temporal dynamics of both gut bacteria and microeukaryotes were primarily ascribed to the high microbial dissimilarities along time points ($\beta_{\text{IntraTimes}}$) (Fig. 4), which reinforce again that host age governs the gut microbial assembly. It is known that gut microbiotas exhibit asynchronous alterations in response to host ages and external environmental conditions [6, 17, 30]. To improve host fitness, nestedness between communities ensures that the recruited commensals can thrive and replace less adapted species, as suggested by the hologenome theory of evolution [58]. Along these same lines, and given the higher relative contribution of $\beta_{\text{IntraTimes}}$ in microeukaryotes compared with bacteria (Fig. 4), microeukaryotes display less nestedness from horizontally inherited taxa (source from preceding younger host). Owing to the low functional redundancy of microeukaryotes [28], it will be interesting to explore how and to what extent this low nestedness of microeukaryotic components affects host health and lifespan. Conversely, bacterial communities exhibited a significantly higher $\alpha_{\text{LocalCommunities}}$ compared with microeukaryotic ones (Fig. 4), which indicates a highly diversified potential of bacteria for conferring ecological insurance. Consistent with this assertion, it has been shown that the gut bacterial communities have high functional redundancy, with compositional difference but functional similarity in fish [22, 59], and shrimp [60].

Numerous studies have depicted contrasting ecological processes involved in generating the spatial distribution patterns of free-living bacterial and microeukaryotic communities [49, 61]. Whether the successions of host-associated microbial communities are structured by similar rules is an ignored question in microbial ecology. Overall, we found comparable relative importances of ecological processes in governing the two gut microbial communities (Fig. 5), which were distinct from the underlying processes for the biogeography of free-living microorganisms. Homogeneous selection was the primary process governing both the bacterial and microeukaryotic communities, though a higher importance

was observed for microeukaryotes (Fig. 5). These results are in line with ones from Wu et al. [49] who showed that protist communities are structured more strongly by species sorting than bacterial counterparts. A possible explanation for this pattern is that the colonization and/or nestedness of gut commensals are directionally selected as the host aged, such as immune maturity [29, 30]. As a consequence, there is a covariation between gut microbiotas and shrimp age, with a given age exerting convergent selection (i.e., homogeneous selection) leading to similar microbial assemblages (Fig. 1). Accordingly, we found a relatively low dissimilarity among communities within each shrimp age ($\beta_{\text{IntraTimes}}$) (Fig. 4). It may seem surprising that variable selection contributed a minor effect on the gut microbiotas (Fig. 5), given previous evidence that suggests rearing conditions markedly affect the gut bacterial communities in aquatic animals [6, 30, 60, 62]. Nevertheless, our recent work has shown that life stage exerts overriding effects on the succession of gut microbiota, instead of an inadequate constraint by water geochemical variables [8]. However, due to the unequal tolerance of gut bacteria and microeukaryotes to host selection, a lower dispersal limitation (here analogue of nestedness) was observed for microeukaryotes (Fig. 5). Together, these findings highlight the importance of shrimp age in contributing divergence of both domains, with eukaryotes being more affected than bacteria.

Conclusions

Our study provides important insights for explaining the succession of gut bacteria and microeukaryotes as host aged. Gut microeukaryotes are governed to a greater extent by host homogeneous selection than bacterial communities, which support the “size-plasticity” hypothesis. This differed patterns could be attributed to differences in $\beta_{\text{IntraTimes}}$ (bacteria < microeukaryotes), proportion of temporal generalists, habitat niche breadth, and nestedness (bacteria > microeukaryotes in the three cases) between the two microbial domains. In contrast to free-living microorganisms, different underlying ecological processes but similar successional patterns were detected between gut bacteria and microeukaryotes. Under this scenario, to attain a comprehensive understanding of the processes that govern microbial successions, free-living and host-associated meta-communities should be analyzed and interpreted independently in future ecological studies.

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Conflict of Interest The authors declare that they have no conflict of interest.

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