



Bdellovibrio and Like Organisms Are Predictors of Microbiome Diversity in Distinct Host Groups

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Abstract

Biodiversity is generally believed to be a main determinant of ecosystem functioning. This principle also applies to the microbiome and could consequently contribute to host health. According to ecological theory, communities are shaped by top predators whose direct and indirect interactions with community members cause stability and diversity. *Bdellovibrio* and like organisms (BALOs) are a neglected group of predatory bacteria that feed on Gram-negative bacteria and can thereby influence microbiome composition. We asked whether BALOs can predict biodiversity levels in microbiomes from distinct host groups and environments. We demonstrate that genetic signatures of BALOs are commonly found within the 16S rRNA reads from diverse host taxa. In many cases, their presence, abundance, and especially richness are positively correlated with overall microbiome diversity. Our findings suggest that BALOs can act as drivers of microbial alpha-diversity and should therefore be considered candidates for the restoration of microbiomes and the prevention of dysbiosis.

Keywords Biodiversity · Predator · Prey · Microbiome · *Bdellovibrio*

Biodiversity is a key attribute of productive [1] and stable ecosystems [2]. This is likely due to the activity of highly productive keystone species [3], which are often more common in species-rich communities [1]. Nevertheless, productivity and stability appear to be mainly driven by diversity itself and not by individual taxa [4]. Species-rich communities exist for example in the human gut and oral microbiome and are usually assumed to consist of functionally redundant species that act as insurance in case of extinctions [5, 6].

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Consequently, species-rich communities are more resilient (cf. [7]). To date, most studies on the effect of biodiversity on ecosystem functioning and specifically the effect of microbiome composition on host health have focused on a single trophic level. Yet, changes in the diversity of one trophic level can affect other trophic levels, either directly through consumer-resource interactions or indirectly when the decrease of one species leads to abundance changes of other species [8]. The presence of top predators has particularly strong effects because they can limit dominant species abundance and thereby free niches for rare taxa [9–11]. The impact of predators is likely distinct from environmental stressors, which may similarly free niches and subsequently increase microbiome diversity, as recently documented for the microbiome of *Daphnia* water fleas after antibiotic exposure [12]. Yet, in this case, the effect on community composition is likely to be random, whereas predators usually target the dominant species.

Bdellovibrio and like organisms (BALOs) are obligate predators of Gram-negative bacteria in a wide range of habitats [13, 14]. BALOs were recently linked to a healthy human gut microbiome [15] and proposed as living antibiotics in medical treatment [16] and water remediation [17]. Additionally, a microcosm experiment showed that their predatory activity can exceed phage-induced mortality [18]. We

here draw attention to this neglected group of predators and tested their association with microbial diversity as an indicator of a healthy microbiome across distinct animal host groups and environments.

We analyzed 16S rRNA data from randomly chosen, exemplary host taxa that are representative of distinct animal taxonomic groups, including early-branching metazoans, ecdysozoa, selected vertebrates, and additionally home surfaces (Table S1 and Supplementary Methods in the supplementary material [19, 20–24]). We only considered studies if they included samples with and without BALOs, thereby allowing us to determine the consequences of BALO presence and absence in comparable groups. We determined BALO occurrence (although not necessarily activity) by identifying operational taxonomic units (OTUs) that showed 97% sequence identity to members of the BALO-containing taxonomic groups *Bdellovibrionales* (including the families *Bacteriovoraceae* and *Bdellovibrionaceae*) and *Micavibrionales* (including *Micavibrionaceae*). From these data, we inferred relative BALO abundance and corresponding microbiome alpha- (i.e., Shannon-Wiener diversity, Simpson's diversity, and richness) and beta-diversities.

The presence of BALOs was associated with a significantly higher Simpson and Shannon diversity for the microbiomes of

seven and five host species, respectively, as well as the home surfaces (Fig. 1; Table S2 in the supplementary material). The main exceptions referred to two sponge species, *Carteriospongia foliascens* and *Ircinia variabilis*, which showed a significantly higher alpha-diversity in the absence of BALOs. This negative association was not observed for microbiome richness (Fig. S1 and Table S3 in the supplementary material). Our subsequent analysis of absolute OTU numbers revealed that microbiome richness is significantly associated with both BALO abundance (Fig. 2a; Table S4 in the supplementary material) and BALO richness (Fig. 2b; Table S4 in the supplementary material) in the case of *H. vulgaris* and the sponges. A trend toward this association was additionally observed for *N. vectensis* and *D. melanogaster*. Interestingly, for both host systems, OTU richness was highest with medium BALO abundance, which possibly indicates that BALO richness rather than abundance influences microbiome richness.

In contrast, variation in microbiome beta-diversity was not linked to the BALOs (Fig. 3). At the same time, our principal coordinate analysis (PCoA) indicated an influence of BALOs on sample clustering for several hosts (especially cnidarians and *C. elegans*). However, the clustering was not independent of sample type, making it

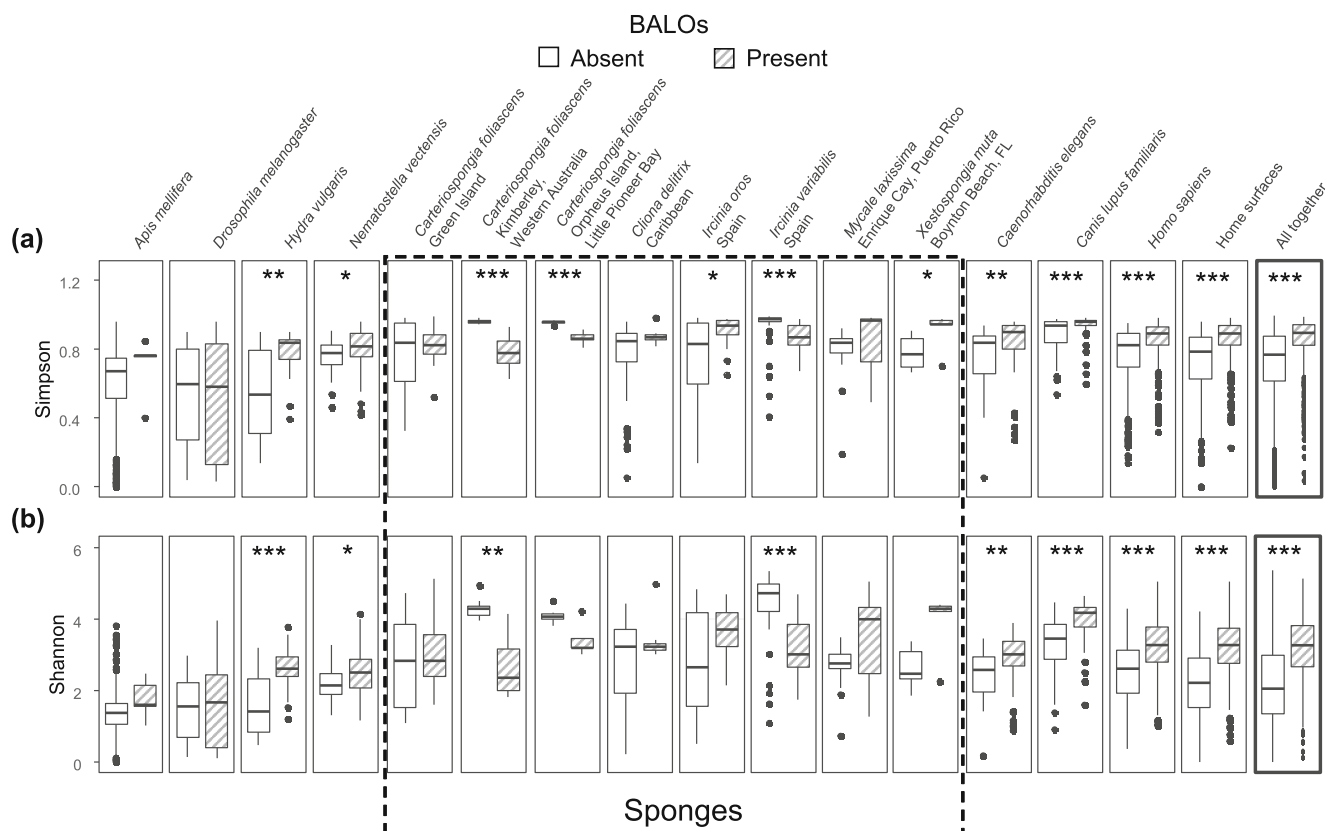
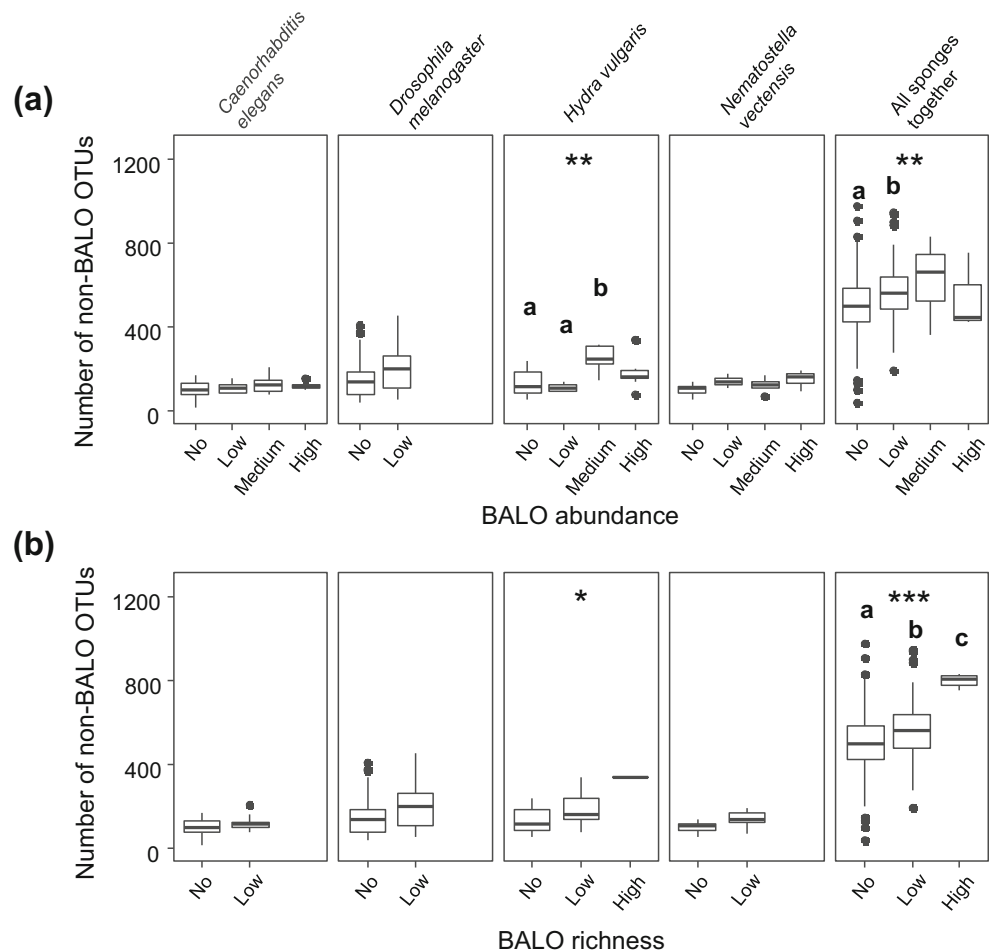


Fig. 1 Microbiome alpha-diversity in the presence and absence of BALOs. The Simpson (a) and Shannon (b) diversity is shown for a set of different hosts. Significant differences are indicated by asterisks and

were calculated using the Wilcoxon rank sum test. p values: $p < 0.001$ (***) ; $0.0011 > p < 0.01$ (**); $0.011 > p < 0.05$ (*). p values are given in Table S2 in the supplementary material

Fig. 2 Host microbiome richness measured as number of different non-BALO OTUs with increasing BALO abundance (a) and BALO richness (b). Significant differences are indicated by asterisks and were calculated using the Kruskal-Wallis rank sum test. p -values: $p < 0.001$ (***) ; $0.0011 > p < 0.01$ (**); $0.011 > p < 0.05$ (*). Significant differences between single categories of BALO abundance and BALO richness are indicated by different letters and were calculated with Dunn's post hoc test. All p values are given in Table S4 in the supplementary material



impossible to infer the exact cause of clustering from the current data.

To exclude that BALO presence is caused by high microbiome diversity as a consequence of sampling effects, we analyzed the complete sponge dataset, additionally including species without BALOs [19]. We found that alpha-diversity per se does not predict the presence of BALOs (Tables S5 and S6 in the supplementary material), which is therefore unlikely caused by sampling effects alone.

The loss of top predators has comprehensive effects on community structure [9, 10]. We tested this idea by comparing microbiome alpha-diversities for distinct animal hosts and environments that either lacked or contained a prominent group of microbial predators, the BALOs. With the exception of the considered insects and most sponge species, we found that microbiomes containing BALOs were characterized by a significantly higher alpha-diversity.

In contrast to the overall results, two sponge species showed a negative correlation between BALO presence and microbiome diversity, although not when considering microbiome richness. These results may suggest that BALO-containing sponges harbor a more species-rich but less even microbiome. Notably, sponges in general possess

a comparatively species-rich microbiome (Fig. S1 in the supplementary material). In these cases, evenness may be negatively correlated to richness, consistent with previous observations for plant communities [25] and possibly due to sampling effects, where a superior competitor is more likely present in species-rich communities [1]. A niche preemption model was previously identified to be the best predictor for the patterns in plant communities [25]. Niche preemption should favor resource use plasticity among the less competitive species, resulting in lower growth and consequently reduced evenness. In the case of the sponges, the negative richness-evenness-relationship might then overshadow the effect of BALOs on microbiome diversity. Temporal effects could additionally explain the higher sponge microbiome diversity in the absence of BALOs. As the sponge data used in this study came from single time point samples, we cannot exclude subsequent changes in the community structure, for example, a delayed effect of BALO loss or gain on microbiome diversity. However, the longitudinal data on surface microbiomes [26] indicates that changes in BALO presence/absence are associated with more or less simultaneously occurring changes in OTU richness (Fig. S2 in the supplementary material).

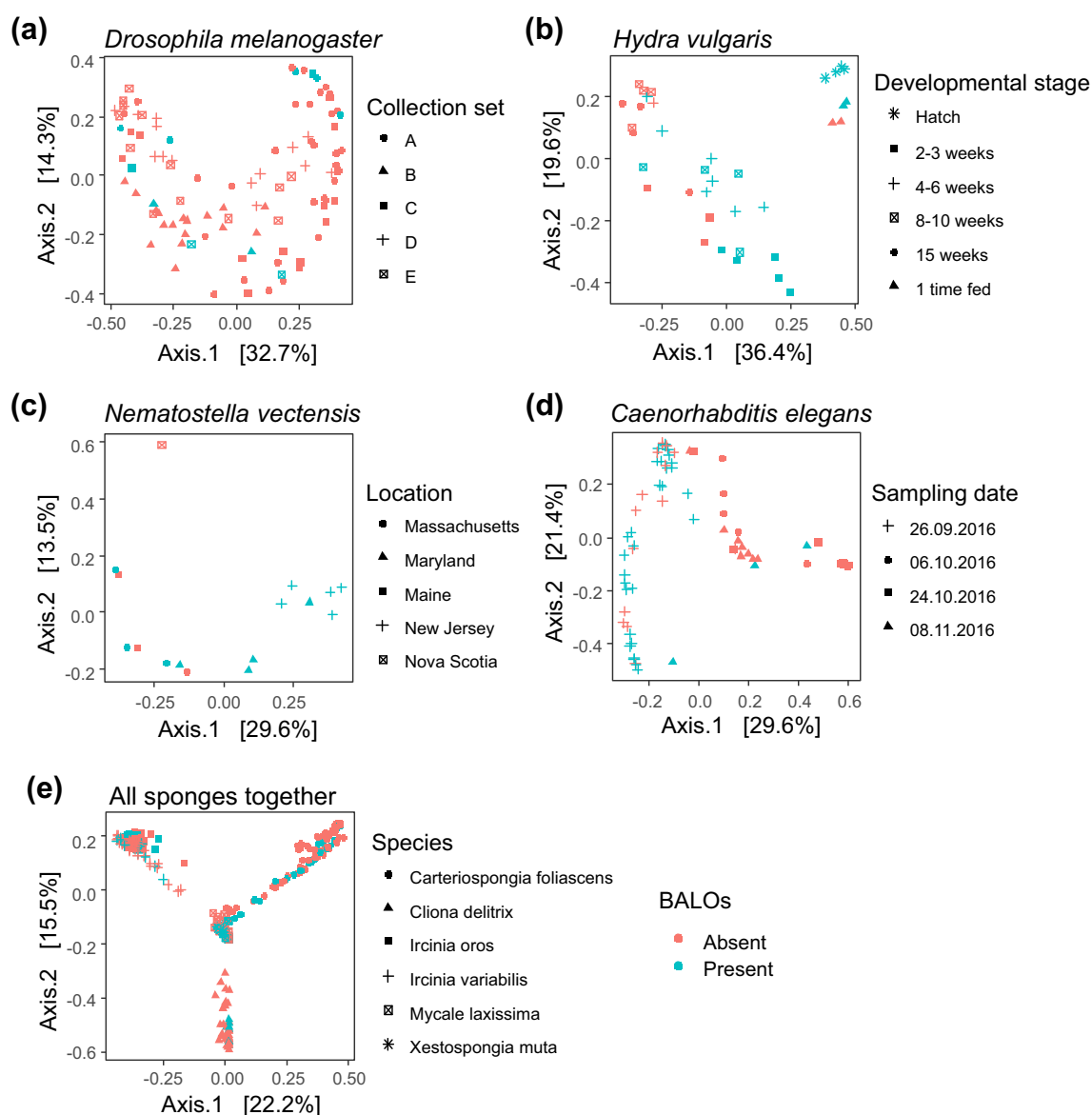


Fig. 3 PCoA of microbiome samples from different hosts using Bray-Curtis distances. Samples are color coded by presence and absence of BALOs. Different shapes indicate different sample subsets as indicated by the respective legends

We found that BALO OTU richness, rather than abundance, is significantly associated with microbiome richness in *H. vulgaris* and the combined set of sponges. Moreover, this significant association between BALO and microbiome richness was only observed when the high BALO richness category could be included. Considering that different BALO strains are known to vary in their range of suitable prey [27], the above results may suggest that a more diverse BALO community is able to prey on a more diverse set of bacteria and thereby reduces the predation pressure on single species, thus increasing microbiome diversity.

Our additional analysis of beta-diversity did not reveal a strong BALO influence on microbiome community structure. Together with the results on alpha-diversity, this may imply that BALO presence is not correlated with a specific

community composition and that BALOs survive in a range of differently assembled communities.

Our results from a range of distinct animal hosts and environments point to BALOs as potential drivers of microbiome alpha-diversity, possibly by actively preying on highly abundant species, thereby favoring rare species. Thus, BALOs may be of particular importance for our understanding of the stability and resilience of microbiome ecosystem functions. Our current meta-analysis is, however, based on associations, which can only be indicative of possible causal relationships. An important next step should therefore be a detailed experimental analysis of the exact causal role of BALOs on microbiome diversity and resulting functions. It would be of similar high interest to assess to what extent other kinds of bacterial antagonists, such as phages, or environmental

stressors may also influence microbiome diversity and the associated effects. Moreover, it is worth testing whether the interaction between BALOs and other bacteria is additionally shaped by the host immune system, which could cause different dynamics of the BALO-mediated effects within rather than outside host organisms.

Considering that BALOs are not pathogenic to higher organisms [28], they have a likely stronger effect on community structure than phages [18] and appear to enhance microbial diversity, they are highly promising candidates for probiotic therapy [20] that aims at restoring disturbed microbiomes and improving host health or ecosystem productivity and stability.

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Compliance with Ethical Standards

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

Data Availability The nucleotide sequence data reported are available in the EMBL databases under the accession number PRJEB30476.

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