

# Community assembly of the native *C. elegans* microbiome is influenced by time, substrate and individual bacterial taxa

Julia Johnke <sup>1\*</sup>, Philipp Dirksen<sup>1,2</sup> and Hinrich Schulenburg<sup>1,2</sup>

<sup>1</sup>Evolutionary Ecology and Genetics, Zoological Institute, University of Kiel, Kiel, Germany.

<sup>2</sup>Max-Planck Institute for Evolutionary Biology, Plön, Germany.

## Summary

Microbiome communities are complex assemblages of bacteria. The dissection of their assembly dynamics is challenging because it requires repeated sampling of both host and source communities. We used the nematode *Caenorhabditis elegans* as a model to study these dynamics. We characterized microbiome variation from natural worm populations and their substrates for two consecutive years using 16S rDNA amplicon sequencing. We found conservation in microbiome composition across time at the genus, but not amplicon sequencing variant (ASV) level. Only three ASVs were consistently present across worm samples (*Comamonas* ASV10859, *Pseudomonas* ASV7162 and *Cellvibrio* ASV9073). ASVs were more diverse in worms from different rather than the same substrates, indicating an influence of the source community on microbiome assembly. Surprisingly, almost 50% of worm-associated ASVs were absent in corresponding substrates, potentially due to environmental filtering. Ecological network analysis revealed strong effects of bacteria–bacteria interactions on community composition: While a dominant *Erwinia* strain correlated with decreased alpha-diversity, predatory bacteria of the *Bdellovibrio* and like organisms associated with increased alpha-diversity. High alpha-diversity was further linked to high worm population growth, especially on species-poor substrates. Our results highlight that microbiomes are individually shaped and sensitive to dramatic community shifts in response to particular competitive species.

## Introduction

Understanding the factors that drive the assembly of a community is one of the main goals of community ecology. This conceptual framework should also apply to the microbiome, and thus help us understand microbiome composition and the resulting functions and effects on host biology. For example, members of the microbiome can support nutrient uptake (Warnecke *et al.*, 2007; Keeney and Finlay, 2011), detoxification (Chaucheyras-Durand *et al.*, 2010; Rothman *et al.*, 2019) and pathogen removal inside a host (Fraune *et al.*, 2015; Dirksen *et al.*, 2016; Kwong *et al.*, 2017; Kissoyan *et al.*, 2019). Additionally, a diverse community is generally more resilient to disturbances (Holling, 1973), which also seems to apply to microbiomes (Greenhalgh *et al.*, 2016; Sommer *et al.*, 2017). Here, it is assumed that high diversity introduces functional redundancy of community members, which acts as insurance in case of extinctions (Naeem and Li, 1997; Yachi and Loreau, 1999). Accordingly, microbiomes in a dysbiotic stage have been associated with the loss of diversity and the increase of dominant species (Zhu *et al.*, 2018). Community ecology assumes that communities form from a larger species pool that is then subject to a set of filters, starting from dispersal to abiotic conditions and biotic interactions (Weiher and Keddy, 1995; Emerson and Gillespie, 2008; Hardy *et al.*, 2012; Zhang *et al.*, 2013). However, it is often not easy to identify the specific factor(s) that account for the absence of a species at a given site, especially as different factors often act at the same time (Paine, 1966; Patterson, 1980; Thomson *et al.*, 1996). Moreover, most studies only investigate the community composition of the host-associated microbiome, without analysing the bacterial source population. However, the latter might be key for recruitment and community assembly inside a host.

In this study, we aimed at (i) assessing variation and similarities between the microbial community composition of the source and the host, in order to understand the dispersal potential of different bacteria into the host, most likely indicating the fundamental niche of species. We

Received 13 August, 2019; revised 17 January, 2020; accepted 27 January, 2020. \*For correspondence. E-mail jjohnke@zoologie.uni-kiel.de; Tel. +49-431-880-4148; Fax +49-431-8802403.

further aimed at (ii) analysing the differences in community composition between hosts that had access to the same microbiome source, which is likely to be determined by competition within the host, indicating the realized niche of species. Moreover, we assessed (iii) differences between hosts that had access to different microbiome sources and that were sampled at different times, in order to infer the overall potential of community assembly due to species sorting.

We used *Caenorhabditis elegans* as a model host. This nematode contains a diverse microbiome (Dirksen *et al.*, 2016) that differs between individuals on high taxonomic levels, but is conserved on the family level across space and time (Zhang *et al.*, 2017). *Caenorhabditis elegans* feeds on bacteria, but only some bacteria can survive and colonize the worm (Dirksen *et al.*, 2016). Inside the worm's gut, these bacteria can have a positive or negative effect on host health. For instance, certain microbes can protect the worm against fungal or bacterial pathogen infections (Montalvo-Katz *et al.*, 2013; Dirksen *et al.*, 2016; Berg *et al.*, 2016b; Kissoyan *et al.*, 2019). The assembly of the microbiome is thus central for *C. elegans* biology. However, so far, only little is known about the microbiome of natural worms, with only one study investigating its composition (Dirksen *et al.*, 2016).

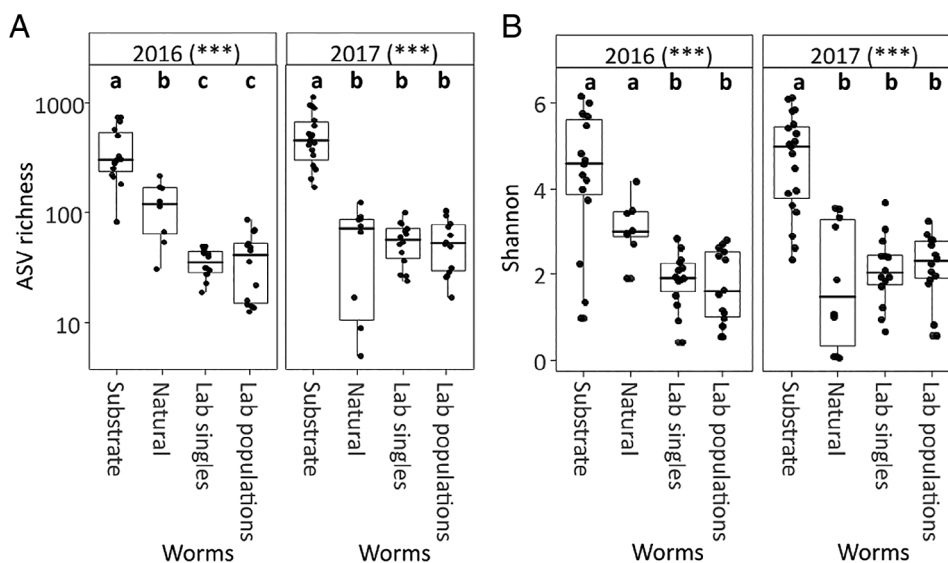
We sampled a compost heap, for which the presence of *C. elegans* has been described previously (Petersen *et al.*, 2015), over a period of 2 years and collected natural *C. elegans* isolates and their respective substrates. Based on 16S amplicon sequencing, we characterized both worm and substrate microbiomes, identified core and indicator amplicon sequencing variants (ASVs), and performed ecological network analysis to infer associations between individual ASVs.

## Results

### Worm microbiomes differ in diversity from source microbiomes

Substrate microbiome samples show significantly higher alpha-diversity than worm samples (Fig. 1A and B, Table S1). The only exception was substrate samples from 2016 that did not show a significant difference in Shannon diversity compared with natural worms (Fig. 1B). Additionally, in 2016, ASV richness and Shannon diversity varied significantly between natural and lab worm samples. In line with these results, worm and substrate samples clustered separately in a PCoA based on Bray–Curtis dissimilarity (Fig. 2A, Table S2, adonis:  $P$ -value  $< 0.001$ ), but with rather low cluster quality (0.05 average silhouette width). We observe a similar pattern when plotting Unifrac distances (Fig. S1a, Table S3, adonis:  $P$ -value  $< 0.001$ , 0.05 average silhouette width). Natural worm microbiomes cluster close to substrate communities while lab population and single worm microbiomes form separate clusters. The observed variation in diversity may have been influenced by the fact that the substrate pieces, used for DNA isolation, were comparatively larger (i.e. 0.25 g) than single worms. However, the worm data shown here are derived from averaged ASV data. That is that we calculated the mean ASV read number for all worms isolated from the same substrate. As we find no differences in alpha-diversity between single worm microbiomes and averaged worm microbiomes (Table S23) and always observe differences in diversity level between worm and substrate samples we conclude that size differences are unlikely the only reason for the observed variation.

Samples from all groups further clustered based on the sampling year (Fig. 2B, Fig. S1b, Tables S2, S3, adonis:



**Fig. 1.** Alpha-diversity shown as ASV richness (A) and Shannon diversity (B) of samples from 2016 and 2017 sorted by sample type. Lab worms were cultivated on *E. coli* OP50-seeded plates before microbiome characterization from either worm populations or single worms. Natural worms were single worms, for which microbiomes were characterized directly after field isolation. Asterisks indicate significant differences as tested with the Kruskal–Wallis rank-sum test and different letters indicate pairwise significant differences ( $\alpha = 0.05$ ) calculated with Dunn's-test. Worm sample ASV count data were averaged based on the substrate from which they were isolated.  $***P \leq 0.001$ . Statistics are given in Table S1.

$P$ -value  $<0.001$ ), but again with low cluster quality (average silhouette widths: Substrate 0.03, Natural worms 0.13, Lab singles 0.05 and Lab populations 0.04).

As natural and lab-enriched worm samples showed strong differences in alpha- and beta-diversity, we now focused our analyses on natural worm microbiomes, in order to avoid any bias resulting from laboratory conditions.

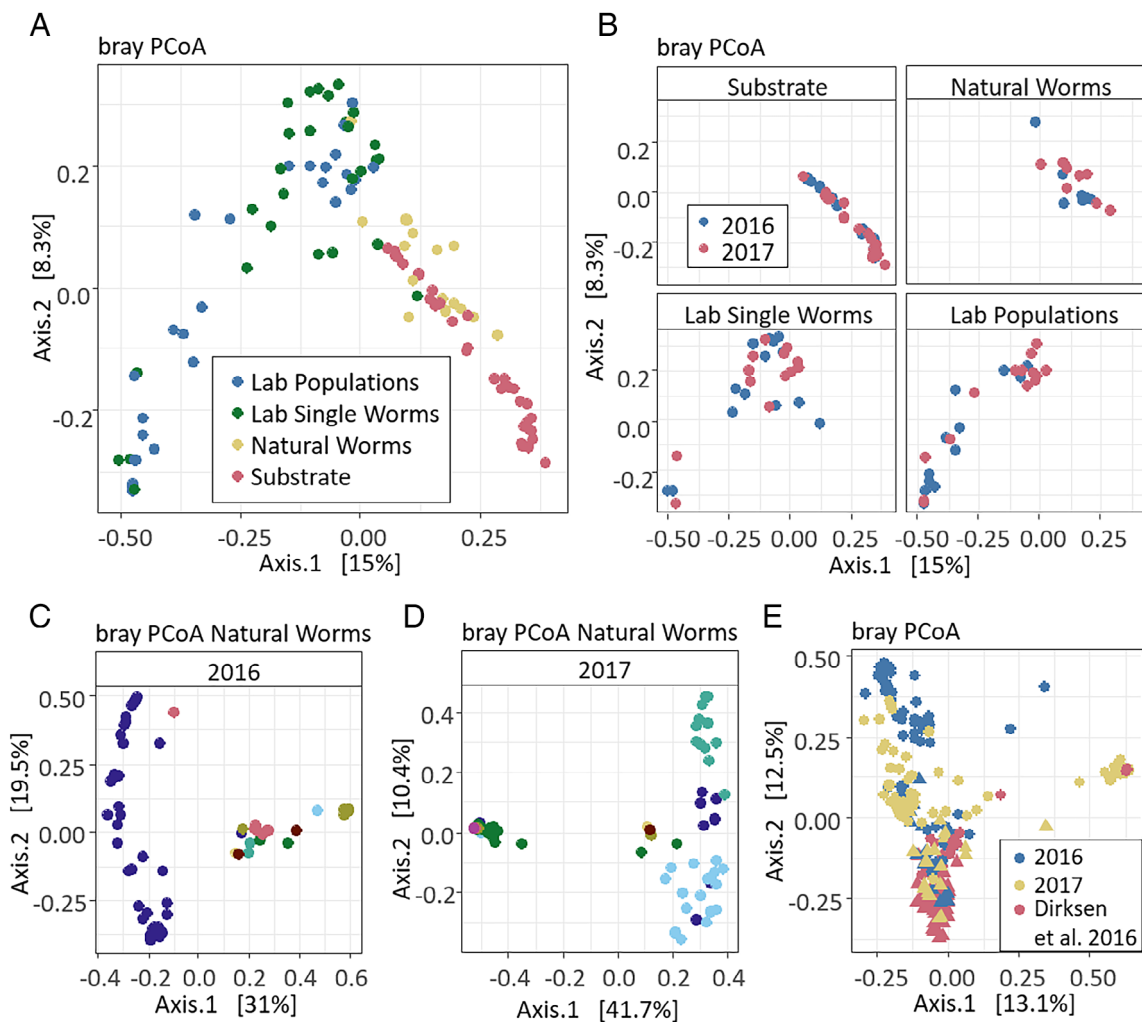
*Natural worm microbiomes from a shared source are more similar*

Microbiomes of worms collected from the same substrate cluster together (Fig. 2C and 2D, Fig. S1c–f, Tables S2

and S3, *adonis*:  $P < 0.001$  respectively). By comparing these results to our previous *C. elegans* microbiome study (Dirksen *et al.*, 2016) we find that natural worm and substrate samples cluster by study and year (*adonis*:  $P$ -value  $<0.001$ ), but overlap across studies in the PCoA (Fig. 2E, Table S2, average silhouette width 0.07).

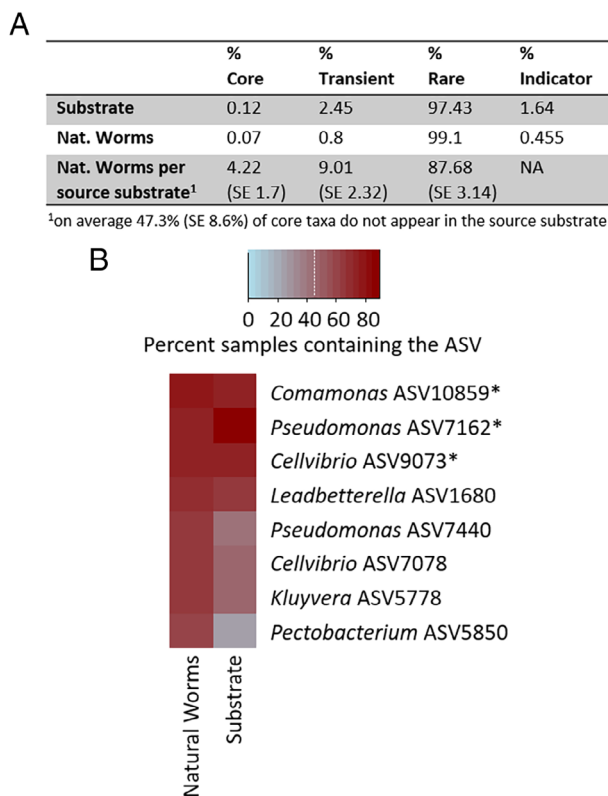
*Natural worm microbiomes share a small core community of ASVs*

Substrates, as well as natural worm samples, contain few core ASVs, defined to be present in at least 70% of the samples (Fig. 3A, proportion core  $<0.2\%$ ). Only one core ASV of natural worms is also present in lab worm



**Fig. 2.** PCoAs showing the Bray–Curtis dissimilarity as measure of differences of species composition (beta-diversity) of all samples from this study (A and B, worm sample ASV count data were averaged per substrate they were isolated from), only natural worm samples from this study (C and D), or substrate and natural worm samples from this study and Dirksen *et al.* (E) using the top 100 ASVs. Colouring is either based on sample group (A), sampling year (B), the substrate samples from which worms were isolated (C and D), or dataset and study (E, circles represent natural worm samples, triangles represent substrate samples). Statistics are given in Table S2. Note that the analysis on sampling year (B) was performed with the data from all sample groups together, but is displayed as a faceted graph by sampling type.

samples, but with low prevalence (Table S4, ASV7162, <4%). Most ASVs were present in 30% or fewer samples (i.e. rare, >97%). The proportion of indicator ASVs, defined as an ASV that is significantly associated with either substrate or worm samples, was slightly larger with 1.64% and 0.455% respectively (Table 1, Table S5). Core and indicator ASVs are distinct because the latter usually appeared exclusively in either substrate or worm samples (component A—the group specificity of an ASV that reaches its max. value ‘1’ when an ASV exclusively appears in a group), and in only small proportions of all samples (component B—the fidelity of an ASV within a group that reaches its max. value ‘1’ when an ASV appears in all samples of a group). As expected from the beta-diversity analysis, worms that were collected from the same substrate share a larger core of ASVs (on average 4.22%), while worms isolated from different



**Fig. 3.** Table of the proportion of ASVs representing the core, transient, or rare community, as well as the indicator ASVs of the natural worm and substrate microbiome (A). Core ASVs appear in at least 70% of all samples. Rare ASVs were defined as those that appeared in 30% or fewer samples. All remaining ASVs were defined as transients. Calculations are either based on all natural worm samples or only on those that were collected from the same substrate (only averages are shown, data can be found in Tables S6 and S7; calculations were only made for worms from substrates with a worm population size of at least 9). Occurrence of worm ASVs that appear in at least 50% of the samples are shown in a heatmap (B) together with their occurrence in the substrate samples. Asterisks indicate ASVs that are part of the core.

substrate samples show only little overlap in their core microbiome (Table S7). Surprisingly, almost half of the ASVs and approximately 20% of classified genera, families and order that make up the worm core per substrate do not appear in the respective substrate sample (Table S6). Six genera are part of the core microbiome of worms from two different substrates, yet are absent in the respective substrate sample (Table S22). Still, many ASVs that represent the overall core of natural worm microbiomes do appear in a high proportion of the substrate samples (Fig. 3B, Table S4). These ASVs might explain the close clustering of natural worm and substrate samples in addition to other ASVs that are present in both substrate and worm samples, but not in lab worm samples (Table S4).

We isolated and cultured a total of 267 bacterial strains belonging to 19 taxonomically classified genera from three worm samples (lab-enriched worm populations, Table S8), which appears to be sufficient sampling on the genus level but not on the ASV level (Fig. S2). The genera of these ASVs also appear in our amplicon sequencing data in the top 100 ASVs from all samples (Table S9) and the top 100 ASVs from lab populations (Table S10), thus yielding a comparatively good representation of the natural worm microbiome, even if the isolation and subsequent cultivation procedure may have favoured bacteria able to cope with the standard laboratory environment.

#### Biotic interactions within microbiomes affect community structure

Co-occurrence networks generated from natural worm and substrate microbiomes show differences in size, percentage of negative edges, density, heterogeneity (i.e. the tendency of a network to contain highly connected nodes) and betweenness (i.e. the number of shortest paths between any two nodes that pass through a given node, Fig. 4A). Nodes represent either ASVs or higher taxonomic levels. Specifically, worm microbiome networks are larger and have a higher proportion of negative edges, representing a significant mutual exclusion of two ASVs. Negative edges appear mostly between ASVs of a common class and exclusively between ASVs of a common phylum, but rarely between ASVs of a common genus (Fig. 4B, Table S13). In general, there are only a few edges between ASVs of a common phylum, family, or order level and the proportion of edges between ASVs with a common taxonomy is more or less similar to the proportion of edges between ASVs with no shared taxonomy. Worm networks are characterized by a higher network heterogeneity and betweenness, which is mostly due to the presence of the *Erwinia* ASV5683 (Tables S11 and S12). Worm samples that contain a high

**Table 1.** List of significant indicator ASVs of natural worm samples.

ASV	Component A (group specificity)	Component B (fidelity within group)	Indicator value index	<i>P</i> -value	<i>P</i> -value FDR-corrected	Highest possible taxonomic assignment	# of indicator ASVs of the same genus
ASV7751	0.9158	0.6056	0.745	0.001	0.012	<i>Stenotrophomonas</i>	3
ASV9462	0.9437	0.5352	0.711	0.006	0.02	<i>Acinetobacter</i>	1
ASV3855	0.9934	0.5	0.705	0.003	0.017	<i>Chryseobacterium</i>	2
ASV3628	0.9103	0.5352	0.698	0.002	0.015	<i>Flavobacterium</i>	3
ASV5826	0.9868	0.4718	0.682	0.009	0.021	<i>Pectobacterium</i>	5
ASV8219	0.9415	0.4789	0.671	0.004	0.018	<i>Ochrobactrum pseudogrignonense</i>	1
ASV10878	0.9576	0.4366	0.647	0.001	0.012	<i>Comamonas</i>	1
ASV8384	0.9518	0.3873	0.607	0.041	0.046	<i>Alishewanella</i>	1
ASV7214	0.9808	0.338	0.576	0.006	0.02	<i>Pseudomonas</i>	7
ASV6274	0.9855	0.331	0.571	0.007	0.02	<i>Aeromonas</i>	1
ASV10021	0.9504	0.3239	0.555	0.018	0.03	<i>Brevundimonas</i>	2
ASV4964	0.9804	0.2958	0.538	0.009	0.021	<i>Arcobacter</i>	1
ASV8392	0.9927	0.2606	0.509	0.015	0.027	<i>Cellvibrio</i>	3
ASV9602	0.9538	0.2676	0.505	0.015	0.027	<i>Gemmobacter</i>	1
ASV5683	1	0.2394	0.489	0.013	0.027	<i>Erwinia</i>	4
ASV11712	1	0.2324	0.482	0.024	0.037	<i>Noviherbaspirillum</i>	1
ASV5464	0.9527	0.2324	0.471	0.029	0.037	<i>Buttiauxella</i>	1
ASV1763	0.9996	0.2042	0.452	0.029	0.037	<i>Dyadobacter</i>	1
ASV11696	1	0.1831	0.428	0.029	0.037	<i>Undibacterium oligocarbo-niphilum</i>	1
ASV933	1	0.1761	0.42	0.044	0.046	<i>Sphingobacterium faecium</i>	1
ASV806	0.9801	0.1761	0.415	0.044	0.046	<i>Pedobacter</i>	1
ASV6034	1	0.162	0.402	0.041	0.046	<i>Corynebacterium_1</i>	1
ASV2292	1	0.1549	0.394	0.047	0.047	<i>Paenibacillus</i>	1

Only the top scorer per genus is shown. Both, natural worm and substrate samples were used as input. Component A describes the group specificity. ASVs with a value of 1 appear solely in worms but not in substrate samples. Component B describes the fidelity within the group. ASVs with a value of 1 appear in all worm samples. The indicator value index takes both components into account.

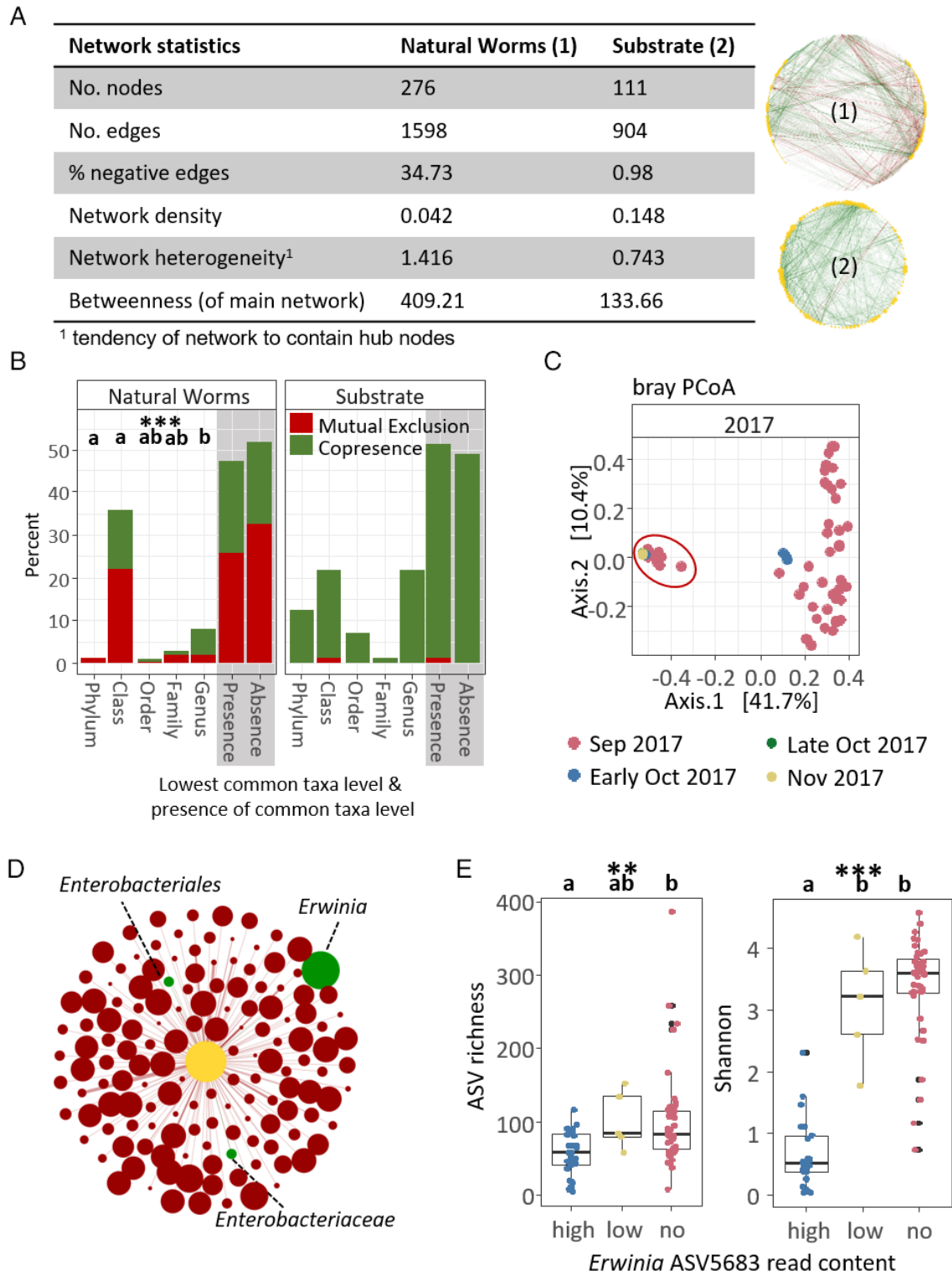
proportion of *Erwinia* ASV5683 clustered separately in a PCoA and were only found in worms sampled in 2017 (Fig. 4C, average *Erwinia* cluster silhouette width 0.84). In the worm network, this ASV appears as highly connected with exclusively negative edges to other non-*Erwinia* ASVs (Fig. 4D, Table S14). The high prevalence of this ASV ( $\geq 10\%$  of all reads) is further associated with significantly lower alpha-diversity (Fig. 4E, Table S15).

We found that a high proportion of worm and substrate microbiomes harbour predatory bacteria that belong to the *Bdellovibrio* and like organisms (BALOs). BALOs are obligate predators of Gram-negative bacteria that are present in a wide range of habitats (Sokkett, 2009; Rotem *et al.*, 2014). Our previous meta-analysis of different hosts, which also included the microbiome sequences of the natural worms sampled in 2016, already revealed an association between BALO presence and high alpha-diversity (Johnke *et al.*, 2019). Here, we found that this also applies to the 2017 samples (Fig. 5, Table S16). Surprisingly, lab populations did not contain BALOs and lab single worms only contained BALOs in 2017, indicating a possible bias due to the microbiome isolation method (Fig. S3). BALO presence in natural worm microbiomes is negatively correlated with the presence of the dominant *Erwinia* ASV5683 (Fig. 5C, Fisher's exact test, *P*-value  $< 0.0001$ , FDR adjusted), and the BALO

ASV6605 shows mutual exclusion with *Erwinia* ASV5683 in the worm microbiome co-occurrence network (Fig. 5D).

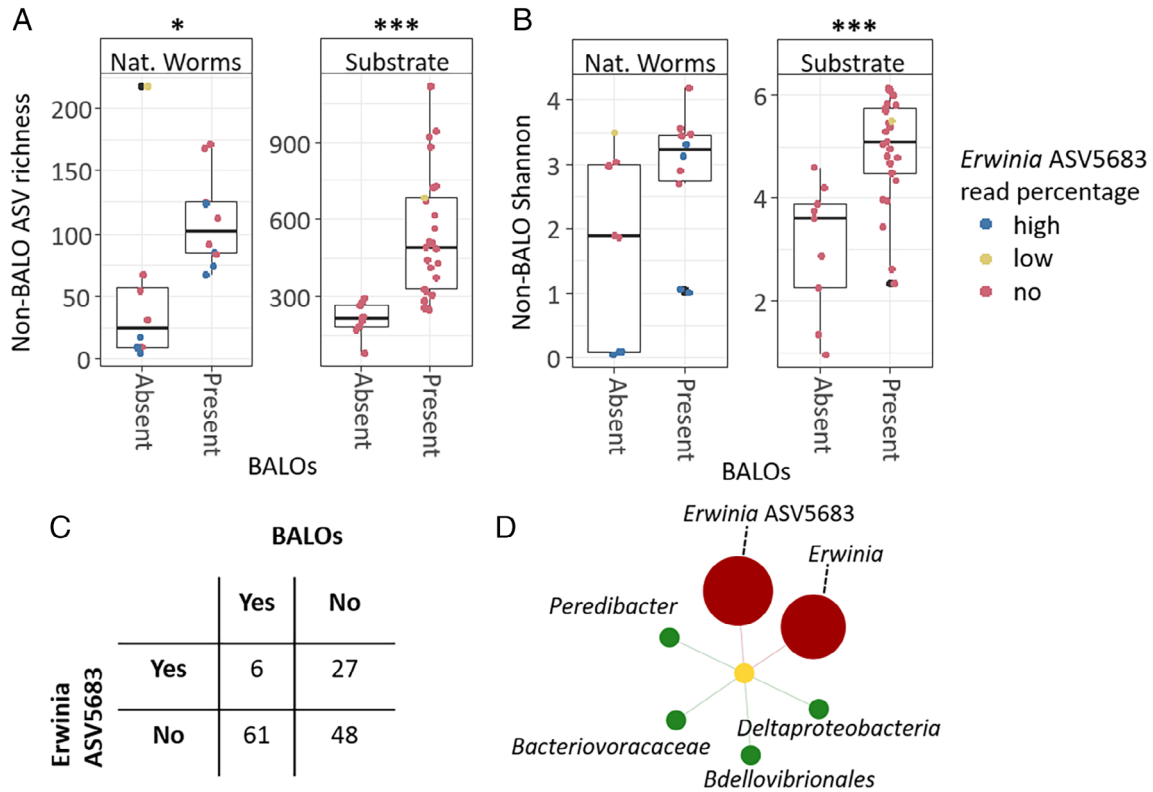
*Alpha-diversity has an effect on worm population size*

Natural worm samples that were collected from a substrate associated with a high worm population size have a significantly higher ASV richness and, in case of the substrates, Shannon diversity than those collected from substrate samples associated with a small worm population size (Fig. 6A and B, Table S17). Interestingly, the opposite is true for substrate samples, for which, however, diversity levels are considerably higher than that of worms. This observation is independent of *Erwinia* ASV5683 presence and sampling time point. Additionally, natural worm samples, but not substrate samples, cluster together based on worm population size in a PCoA (Fig. S4b and c, Table S18). In order to identify specific bacteria associated with high or low worm population size, we performed an indicator analysis using the worm- and substrate-derived ASV data. We found ASVs of 14 bacterial classes that were significantly associated with a low worm population size, but only ASVs belonging to four classes that were associated with a significantly high worm population size (Tables S19 and S20).

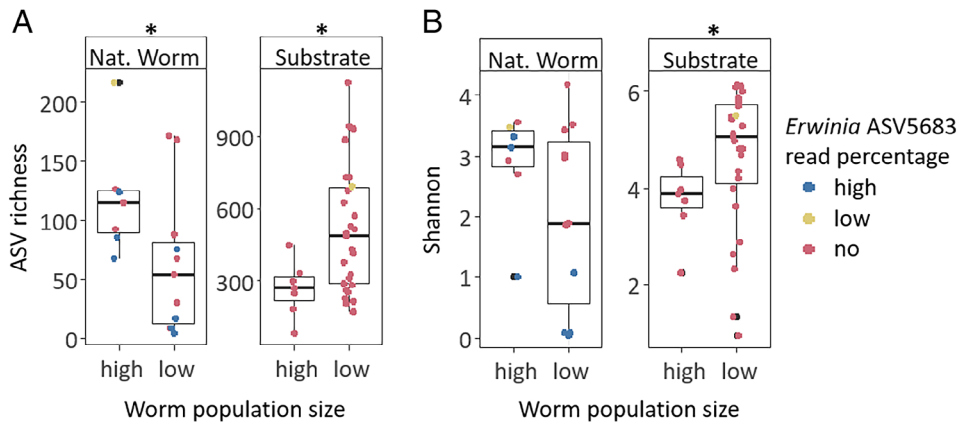


**Fig. 4.** Bacteria–bacteria interactions as indicated by networks generated from ASVs of either natural worm or substrate samples (A) and percentage of network-generated associations between ASVs of different taxonomic levels (B). Nodes represent either ASVs or higher taxonomic levels. Bars with grey shading show the percentage of direct ASV–ASV associations between common taxa ('Presence') or of no such association ('Absence'). The other bars specify the common taxon association by giving the percentage of associations between the lowest common taxa from the phylum to the genus level. For natural worm samples from 2017, we saw a subcluster of samples in the PCoA (C). Samples of this cluster (red circle) are characterized by a high proportion of *Erwinia* ASV5683 reads. This ASV appears in the worm network as highly connected (D, yellow node; red nodes indicate ASVs or higher taxonomic levels of mutual exclusion with *Erwinia* ASV5683, green nodes indicate co-occurring ASVs or higher taxonomic levels, names are given in Table S14). Lastly, samples containing the ASV in high amounts ( $\geq 10\%$  *Erwinia* reads) show a lower overall ASV richness and Shannon diversity than samples containing only low ( $< 10\%$ ) or no ASV5683 reads (E).





**Fig. 5.** Alpha-diversity shown as ASV richness (A) or Shannon diversity (B) of samples from 2016 and 2017 with and without BALOs. Worm sample data were averaged based on the substrate from which they were isolated. Asterisks indicate significant differences as observed with the Wilcoxon rank-sum test. \* $P \leq 0.05$  and  $P > 0.01$ , \*\* $P \leq 0.01$  and  $P > 0.001$  \*\*\* $P \leq 0.001$ . Y-axes differ in scaling. Samples that contain BALOs rarely contain the *Erwinia* ASV5683 (C). The BALO ASV6605 (*Peredibacter* sp., yellow node) is connected with *Erwinia* ASV5683 by a negative edge in the co-occurrence network generated from natural worm microbiomes, indicating mutual exclusion (D).



**Fig. 6.** Alpha-diversity shown as ASV richness (A) and Shannon diversity (B) of worm microbiomes and substrate samples based on worm population size (high  $\geq 9$ , grouping is based on the frequency distribution of worm population size as shown in Fig. S4a) found on a given substrate. For worm microbiomes, samples were averaged based on the substrate from which they were isolated. Samples are coloured by percentage of *Erwinia* ASV5683 reads with low read content being less than 10% of the overall reads. Be aware of the different y-axes.

**Discussion**

We used the nematode *C. elegans* to unravel factors that shape microbiome community assembly in nature. *C. elegans* can be easily isolated from rotten organic material. Identifying the microbial community of the

substrate allowed us to draw conclusions about the composition of the population that acts as a source for the worm’s microbiome. Comparing microbiomes of individual worms collected from different sources further enabled us to resolve the impact of random and non-

random factors for community assembly. Importantly, we found that worm microbiomes are not conserved on the ASV level. Moreover, they only represent a fraction of the source population. In the following, we will discuss the identified individual variation between worms and how this is in line with our current knowledge of the *C. elegans* microbiome. Furthermore, we will discuss possible factors accounting for the observed microbiome differences between worms from different substrates and the influence of bacteria–bacteria interactions as observed by the co-occurrence analysis. Lastly, we will suggest explanations for the opposing effect of microbial diversity of the worm and substrate on worm population size.

#### *Worm microbiomes are not conserved on the ASV level*

We identified a small core microbiome of worms, independent of sampling time and substrate. This is in agreement with other microbiome studies that observed strong individual variation of microbiome communities in *C. elegans* (Dirksen *et al.*, 2016) and other hosts (Turnbaugh *et al.*, 2010; Ainsworth *et al.*, 2015; Hernandez-Agreda *et al.*, 2017; Ocejo *et al.*, 2019). For instance, a sponge study identified the core microbiome on the operational taxonomic unit level (97% sequence identity) by sampling individuals over 36 months (Bjork *et al.*, 2018). Per individual, they identified only 1.24% OTUs that appeared in at least 70% of the time series samples, revealing that microbiomes are highly dynamic. One reason for the commonly observed differences between microbiomes can be the stochastic sequential arrival of bacteria from a source pool, which, according to model simulations, leads to individually assembled microbiomes (Drake, 1991; Wilson, 1992; Law and Morton, 1993). Experiments with *C. elegans* demonstrated that stochastic assembly processes are sufficient to explain individual colonization variability in worms using two neutrally competing bacterial strains (Vega and Gore, 2017). Additionally, inter-host dispersal of bacteria strongly affects the microbiome composition of different zebra fish genotypes and contributes substantially to microbiome variation (Burns *et al.*, 2017). In a different study, bacterial community assembly in liquid cultures followed fundamental, quantitative principles (Goldford *et al.*, 2018). Here, bacterial communities assembled in a conserved fashion on the family, but not on the strain level and independent of the starting population. This is in agreement with the results of this study and the results of a meta-analysis on *C. elegans* microbiomes (Zhang *et al.*, 2017). The latter identified *Enterobacteriaceae*, *Pseudomonadaceae*, *Sphingomonadaceae*, *Xanthomonadaceae*, *Sphingobacteriaceae*, *Flavobacteriaceae* and *Weeksellaceae* as

common members of natural *C. elegans* microbiomes, in line with the results of the core and indicator analysis of this study.

Previous studies on the *C. elegans* microbiome identified multiple species as being particularly enriched inside the worm or important for host health, including *Ochrobactrum* [appeared to be enriched and persistent in worms (Dirksen *et al.*, 2016)], *Pseudomonas lurida* [had a protective effect on the worm during *Bacillus thuringiensis* infection (Kissoyan *et al.*, 2019)], *Enterobacter cloacae* [was prevalent in N2 worms after cultivation on produce-enriched soil samples (Berg *et al.*, 2016a) and induced higher resistance to infections with *E. faecalis* (Berg *et al.*, 2016b)] and *Gluconobacter* [mitigated the effect of growth inhibition by a set of pathogenic bacteria in N2 worms and was enriched in rotten apples (Samuel *et al.*, 2016)]. While we identified a *Pseudomonas* ASV as part of the core community of natural worms and a *Pseudomonas* and an *Ochrobactrum pseudogrignonense* ASV as indicators for worm microbiomes, we did not observe *E. cloacae* in our data. However, other *Enterobacter* strains were present in a small fraction of worms ( $\leq 2\%$ ). Moreover, *Ochrobactrum* rather appeared to be present in laboratory-enriched than natural worms, indicating a potential higher competitive ability in the laboratory setting. A single *Gluconobacter* ASVs appeared in few worms (0.68%) and in no substrate samples, which indicates that its presence is substrate-specific. These differences between studies may be explained by the variation between locations, sampling dates and study design. Yet they could also reflect the highly dynamic nature of *C. elegans* microbiomes.

#### *The source community determines worm microbiome composition*

Microbiomes were more similar when collected from the same substrate. This is consistent with results on community assembly in liquid cultures (Goldford *et al.*, 2018), where environmental carbon sources determined community composition. Similarly, studies in *C. elegans* showed that community assembly is strongly influenced by environmental factors, including temperature (Berg *et al.*, 2016a) or different soils (Berg *et al.*, 2016a; Berg *et al.*, 2016b).

The microbiome composition of the source substrate is expected to affect community composition in the host due to non-random species sorting. However, in our current work, ASVs from worms were not necessarily present in corresponding substrate samples. Worm-associated bacteria might be recruited from adjacent substrates as worms can travel at least small distances. A similar observation was made for lake and river ecosystems (Niño-García *et al.*, 2016): the rare lake taxa seem to have resulted from species sorting in upstream rivers.



Alternatively, taxa with low abundances in substrates may not have been detected due to sensitivity limitations of amplicon sequencing. In this case, the high proportion of reads inside the worm would indicate that random dispersal processes, as well as environmental filtering, represent important factors in worm microbiome community assembly. This would be in agreement with the finding that certain genera, families and order repeatedly appear in the worm as part of the core, but are absent in the respective substrate. A possible selective factor could be the low pH inside the worm (4.5), which affected the outcome of pairwise interactions for bacteria that are associated with the worm intestine (Ortiz Lopez *et al.*, 2019). At the same time, we observed an overlap in the presence of multiple community members of natural worm microbiome and substrate samples, most likely as a consequence of the uptake of microbes from the direct environment, even if bacteria are only transiently maintained.

Most ASVs appeared in less than 30% of worm and substrate samples, which confirms that microbial communities are highly uneven (Sogin *et al.*, 2006; McGill *et al.*, 2007; Matthews and Whittaker, 2015) with a great proportion of rare taxa (Pedrós-Alió, 2012). This high level of rarity indicates the importance of deep sequencing. It is possible that the high taxonomic community differences still entail the same functions (i.e. functional core) (Shafquat *et al.*, 2014; Louca *et al.*, 2018), as observed for human gut microbiomes (Turnbaugh *et al.*, 2010) and the microbial community associated with the green macroalga *Ulva australis* (Burke *et al.*, 2011). Metagenomics analyses can help us addressing this point in the future.

#### *Bacteria–bacteria interactions shape the worm microbiome*

Network analysis revealed differences in the size and structure of networks generated from worm and substrate data. The larger worm network may result from the distinct microbiomes of worms from different substrates. Additionally, worm networks contained more negative edges than substrate networks, in accordance with a study on the worm strain N2 and natural soil substrates (Berg *et al.*, 2016a). It thus seems that bacterial competition is an important driver of microbiome assembly.

The majority of edges were however positive, indicating high levels of conserved strain co-occurrence. This is consistent with a previous study that used pairwise co-culture experiments and showed that 75% of the tested bacteria were able to coexist in the worm intestine (Ortiz Lopez *et al.*, 2019). Co-occurrence of strains is usually associated with niche/resource partitioning, while mutual exclusion is interpreted as a result of competition.

However, a study that used metabolic network models to predict levels of competition in human microbiomes found that coexisting species tend to be strong competitors (Levy and Borenstein, 2013). Drawing conclusions is therefore almost impossible without experimental validation.

Many positive edges connect ASVs of the same class or genus, indicating co-occurrence and consequently differences in niche preferences and partitioning. Indeed, a previous metagenomic analysis of 3207 cultured and uncultured microbe lineages found that microbes of the same genus or class have a more similar metabolic potential and thereby high competitive potential while bacteria belonging to different phyla show the most variation in metabolic potential (Royalty and Steen, 2019). A similar pattern was recently inferred by metabolic network modelling for a representative set of 77 fully sequenced bacterial genomes from the *C. elegans* microbiome (Zimmermann *et al.*, 2019). Both findings support the hypothesis that closely related species share the ability to compete for a particular resource and as a result out-compete less closely related species, leading to phylogenetically clustered patterns (Mayfield and Levine, 2010). Alternatively, closely related species inhabiting the same niche should show a high level of competitive exclusion, resulting in niches inhabited by distantly related species (MacArthur and Levins, 1967). Both theories could be valid and processes might be dynamic (Tatsumi *et al.*, 2019), possibly due to additional habitat structuring, environmental perturbations, or biotic interactions (e.g. with phages or predatory bacteria) [as summarized in (Louca *et al.*, 2018)].

We indeed found that biotic interactions can have a strong influence on microbiome community composition. Worms carrying a highly dominant *Erwinia* strain differ significantly in microbiome alpha- and beta-diversity. This *Erwinia* strain is likely highly competitive and may possibly represent a pathogen. Interestingly, *Erwinia*-associated microbiomes came from different substrates and not all worms from these substrates carried *Erwinia*. Moreover, *Erwinia*-containing worms were collected from substrates with a high worm population size. Therefore, this bacterial strain may not necessarily be pathogenic, but still highly effective at outcompeting other bacteria.

Biotic interactions can also have a positive effect on microbiome diversity. We now found that microbiomes with predatory bacteria show high alpha-diversity, as previously reported by us for different host taxa (Johnke *et al.*, 2019). Theory predicts that species of a higher trophic level influence species of lower taxonomic levels directly through consumer–resource interactions or indirectly, when abundance changes of one species affect the abundance and/or presence of other species (Thebault and Loreau, 2003). Especially predators can

have strong effects on the community structure, when they limit the abundance of dominant species, thereby freeing niches for rare taxa (Gessner *et al.*, 2010; Jabiol *et al.*, 2013; Leitão *et al.*, 2016). This would explain why we only observed a few microbiomes that contained both, BALOs and the *Erwinia* strain, and why worm microbiomes that contained both had a higher Shannon diversity and richness than samples that lacked BALOs. Therefore, BALO presence might dampen the effect of dominant strains on microbiome alpha-diversity.

#### *The effect of diversity on worm population size*

Species-rich substrates were populated by smaller worm populations than species-poor substrates. This finding is consistent with previous work that found high *C. elegans* abundance on apples with low alpha-diversity microbiomes and an increase in worm population size with increasing apple decay, indicating an influence of bacterial diversity and density on *C. elegans* proliferation (Samuel *et al.*, 2016). Even though we did not quantify bacterial density directly, we did not observe strong variation in read count for samples with either low or high *C. elegans* population size, possibly indicating that density *per se* is of minor influence. Another result of the study was that the previously inferred substrate-derived microbial communities clustered in a PCoA according to worm population size, contrary to our observations. The authors were able to link specific bacteria to *C. elegans* proliferation. High proportions of alpha-Proteobacteria supported, while Bacteroidetes and gamma-Proteobacteria reduced high *C. elegans* growth. Our indicator analysis of worm- and substrate-derived ASVs and worm population size also revealed an association between Bacteroidetes and gamma-Proteobacteria and low worm population size. However, gamma-Proteobacteria also made up the biggest proportion of indicator ASVs associated with high population growth. In contrast, worms collected from a highly populated substrate had a more diverse microbiome, indicating that microbiome diversity positively influences worm proliferation and that functional redundancy is possibly less prominent in worms than in other hosts.

Overall, our study identifies *C. elegans* as a useful model to study community assembly. It can be easily collected from the field in large numbers and over time and, most notably, together with its substrate, which should represent the source population of the nematode's gut community. We found that while the worm's microbiome is surprisingly diverse, it harbours only a small core community on the ASV level. This indicates that at most a small number of taxa co-adapted with the worm. Moreover, worm microbiome assembly is strongly influenced

by the source community and, particularly, dispersal into the worm and environmental filtering. It is currently unknown if these factors are worm-mediated, a consequence of bacterial life history characteristics, or both. Notably, biotic interactions between microbiome members can change the community composition towards a more (in case of predatory bacteria) or less diverse state (in case of *Erwinia*). The factors that allow the sudden dominance of a strain are still unclear but might be an outcome of the loss of key species, like predatory bacteria.

## Experimental procedures

### Sampling

Samples were collected from three compost heaps in the Kiel University botanical garden in Northern Germany. Samples were taken bi-weekly from September to November in 2016 and 2017 and consisted of straw, grass, leaves, soil and rotten fruits.

### Substrate and worm isolation

Ten substrate samples were taken per sampling date. A fraction of each substrate sample was kept at  $-80^{\circ}\text{C}$  for later DNA isolation. The remainder was used for *C. elegans* isolation. Nematode isolation was accomplished using two different methods as described previously (Dirksen *et al.*, 2016): (i) a piece of substrate was placed on a 9 cm peptone-free nematode growth medium (PFM) agar plate (20 g/L agar, 3 g/L NaCl, 5 mg/ml cholesterol, 1 mM  $\text{MgSO}_4$  and 1 mM  $\text{CaCl}_2$ ) and covered with S-buffer (1.2% hydroxymethylcellulose, 5 mg/ml cholesterol, 1 mM  $\text{MgSO}_4$ , 1 mM  $\text{CaCl}_2$  and 0.1% acetone). Worms start to float on the surface from which all worms were collected with a pipette. These worms were washed three times in M9-T (M9 with 0.05% Triton X-100) buffer for 5 min each and single worms were transferred to a 96-well plate containing 10  $\mu\text{l}$   $2\times$  Tris-EDTA buffer, pH 8 with 1 mg  $\mu\text{l}^{-1}$  proteinase K and one to three 1-mm zirconium beads. Plates were kept at  $-80^{\circ}\text{C}$  for at least 16 h. DNA was extracted via bead beating in the Geno/Grinder for two times 3 min at 1500 strokes  $\text{min}^{-1}$  and subsequent proteinase K digestion (1 h  $55^{\circ}\text{C}$ , 20 min  $98^{\circ}\text{C}$ ). Worms isolated with this method were termed 'Natural worms'.

(ii) A second substrate piece of the same size was placed on a 9 cm PFM agar plate seeded with *E. coli* OP50. Worms would crawl towards the food bacterium, thus enabling their isolation. Single worms were then picked onto 6 cm PFM agar plates seeded with an OP50 overnight culture. Only nematodes with a hermaphrodite life cycle were able to reproduce during the following days. These worms were subsequently frozen at  $-80^{\circ}\text{C}$ .

Frozen worm populations were later thawed for microbiome DNA extraction. DNA was extracted either from single worms or from a population. In the first case, five replicate worms per population were picked and extracted as described for the natural worms. These samples were termed 'Lab singles'. Remaining worms were collected with M9 and washed three times in M9-T. DNA was extracted using the PowerSoil DNA Isolation Kit (MO-BIO, Carlsbad, USA). The standard extraction procedure was adjusted by supplementing 250- $\mu$ l worm suspension with 0.4 mg ml<sup>-1</sup> proteinase-K (Fermentas/Thermo Fisher Scientific, Waltham, USA) and incubating samples at 55°C for 2 h prior to the bead beating. These samples were termed 'Lab populations'.

Extracted DNA was used for nematode species identification using the *C. elegans* specific primers nlp30-F (5'-ACACATACTGATCACTCA-3') and nlp30-R (5'-TACTTTCCCATCCGTATC-3') (Petersen *et al.*, 2014). One microliter of DNA was used in a 15  $\mu$ l PCR reaction with the 2 $\times$  GoTaq G2 Colourless Mastermix (Promega, Mannheim, Germany) using the following cycling conditions: 95°C for 2 min, 35 cycles of 95°C for 45 s, 55°C for 30 s, 72°C for 1 min and 72°C for 10 min. Only worm samples with a *C. elegans*-positive PCR signal were further used for the microbiome analysis.

DNA extraction of *C. elegans*-positive substrates was performed by grinding 0.25 g frozen substrate material with a pestle and mortar. DNA was extracted using the PowerSoil DNA kit (MoBio) as described above.

### 16S amplicon sequencing

*C. elegans*-positive DNA samples were subjected to 16S library preparation using the 341F (5'-CCTACGGGNGGCWGCAG-3') and 806R primer (5'-GACTACHVGGGTATCTAATCC-3') covering the V3–V4 region of the 16S rRNA gene. Libraries were sequenced on the Miseq platform using the MiSeq Reagent Kit 2  $\times$  300 bp (Illumina).

### Isolation of bacteria from natural *C. elegans*

Microbial isolates were obtained from three frozen worm populations (26.09.2016, rotten squash; 06.10.2016, straw; 06.10.2016, balloon flower) isolated as described above (method ii: isolation after enrichment with *E. coli* OP50). This method was used to ensure that bacteria were obtained from *C. elegans* and no other nematodes. After thawing, mixed-stage worm populations were immediately washed three times in 1 ml M9, directly resuspended in 250  $\mu$ l M9 and three 1 mm zirconium beads, followed by vortexing for 2 min to release bacteria from the worms (see similar approach in Dirksen *et al.*, 2016). Diluted samples (100  $\mu$ l) were plated on

Reasoner's 2A agar (R2A), Lysogeny broth agar, trypticase soy agar, nutrient broth agar, Peptone Yeast Extract agar and R-medium (Dione *et al.*, 2016) agar plates. Plates were incubated at 15°C and 25°C respectively, for at least 16 h. From each plate, 12 single colonies were picked and streaked out individually three subsequent times to allow the growth of distinct single colonies.

### DNA isolation, sequencing of bacteria and initial microbiome analysis

DNA from the single microbiome isolates was extracted using a CTAB-based extraction protocol (von der Schulenburg *et al.*, 2001). For taxonomic classification, bacterial 16S rRNA genes from single isolates were sequenced using the primers 27f (5'-GAGAGTTTGATCCTGGCTCAG-3') and 1495r (5'-CTACGGCTACCTGTTACGA-3') (Weisburg *et al.*, 1991), as well as 701f (5'-GTGTAGCGGTGAAATGCG-3') and 785r (5'-GGATTAGATACCCTGGTAGTCC-3') (Dirksen *et al.*, 2016). Miseq sequences from worm and substrate microbiomes were trimmed of adapter sequences using cutadapt 1.18 (Martin, 2011). Using the dada2-pipeline (1.10.1) (Callahan *et al.*, 2016), sequences were processed as reported in the dada2 SOP. The last 50 bp of all forward reads and the last 100 bp of all reverse reads were trimmed due to low-quality scores. Chimeras were removed with the 'consensus' method. Taxonomies for Miseq and isolate sequences were assigned using the SILVA trainset v132. We used ASVs for further analyses, because these were shown to provide more reliable information on taxon composition and generate fewer spurious sequences than alternative measures (Callahan *et al.*, 2016) and as different strains can already have different gene repertoires (Zimmermann *et al.*, 2019.). Sequence tables were finally analysed using Phyloseq version 1.24.0 (McMurdie and Holmes, 2013). Samples were not subsampled as dada2 is already removing spurious reads from the data and as subsampling is known to introduce high rates of false positives (McMurdie and Holmes, 2014). However, Miseq-generated samples with less than 1700 reads were removed since low read numbers might be the result of low biomass contaminations. Samples collected in 2016 and 2017 were sequenced separately, which can induce batch effects.

In order to compare the V3-V4 spanning data of this study with previously published V4-only sequences (Dirksen *et al.*, 2016) ASVs were inferred separately for each dataset using the dada2 pipeline as described above. Here, sequence trim lengths for forward/reverse reads were 250/200 nt (V3-V4) and 180/180 nt (V4-only); max expected error was 0.25; and minimal overlap for

merging was 15 nt. ASVs were then aligned using SINA v1.2.11 (Pruesse *et al.*, 2012), truncated to the overlapping region and merged if identical.

All three negative controls from 2016 contained 11 ASVs that were also present in all low biomass worm samples. These ASVs were subsequently removed and considered to represent low biomass contaminations (Table S21). Likewise, the combined dataset that included worm samples from the Dirksen study contained six low biomass ASVs, which were subsequently removed.

BALOs were identified based on sequencing information and taxonomic classification. All ASVs classified as either *Bdellovibrionales* (including the families *Bacteriovoracaceae* and *Bdellovibrionaceae*) or *Micavibrionales* (including *Micavibrionaceae*) were considered BALOs.

The nucleotide sequence data reported have been submitted to the EMBL databases under the accession number PRJEB33740.

#### Core and indicator analysis

As in (Bjork *et al.*, 2018), core ASVs were determined as ASVs being present in at least 70% of samples, independent of their abundance per sample. Rare ASVs were defined by their presence in 30% or fewer samples. All other ASVs were defined as transient. Indicator ASVs were identified with the `multipatt` function of the R package 'indicpecies' (Caceres, 2013).

#### Ecological network analysis

Networks were reconstructed from the 16S amplicon reads from substrate and natural worms using CoNet (Faust *et al.*, 2012) and Cytoscape, version 3.5.0 (Shannon *et al.*, 2003). We enabled parent–child exclusion and the exploration of links between higher-level taxa. Normalized ASV data were used and filtered to discard taxa with a minimum occurrence of 20 across samples. We additionally used the factor 'sampling date' as a feature for the network construction as samples showed significant differences in beta-diversity across time. To infer correlations between ASVs, we used the Pearson, Spearman, mutual information, Bray Curtis and Kullback–Leibler dissimilarity using the top 1000 edges per method as the threshold. We applied permutation using renormalization (Faust *et al.*, 2012) to compute *P*-values ( $\alpha = 0.05$ ). Resampling was performed via bootstrapping. *P*-values were merged with Brown's method (Brown, 1975). Lastly, unstable edges were filtered, which led to the removal of edges outside the 0.95 range of their bootstrap distribution. Multiple testing correction was performed using the Benjamini–Hochberg approach (Benjamini and Hochberg, 1995). Networks were

visualized and analysed using the network analyser implemented in Cytoscape (Assenov *et al.*, 2008).

#### Statistical analysis

Samples were not rarified for alpha-diversity calculations as the rarefaction curves did indicate sufficient sampling (Fig. S5). Significant differences in alpha-diversity between samples were calculated with the Kruskal–Wallis rank-sum test using the R 'stats' package (R Core Team, 2016). Pairwise comparisons within the sample types were calculated using Dunn's test with multiple sample comparison correction using FDR. PCoA plots were generated with the Phyloseq package in R (McMurdie and Holmes, 2013) using the top 100 ASVs after normalization to relative read abundances. Plots are either based on the Bray Curtis dissimilarity or Unifrac distance between samples. For the PCoA plots that relate worm samples to the corresponding substrate samples, we used averaged worm data, calculated as the arithmetic mean of ASV counts of the worm samples from the same substrate, in order to avoid pseudoreplication.

Cluster strength was determined with the silhouette function of the 'cluster' package (Mächler *et al.*, 2012). Here, numbers close to '1' indicate good cluster quality while numbers close to '0' or negative numbers indicate lower cluster quality.

A significant difference of the beta-diversity between groups of samples were calculated using the `adonis()` function of the `vegan` package (Dixon, 2003). Significant differences in counts of network edges between ASVs with a common taxonomy and between samples with and without BALOs and *Erwinia* ASV5683 were tested with the Fisher exact test. For pairwise calculations of edges between ASVs of specific taxonomic relationships, we used the Fisher exact test for multiple comparisons, which includes FDR correction using the R package `RVAideMemoire` (Hervé, 2015). Significant differences in diversity between samples with and without BALOs and between samples from substrates generating high and low worm population size were calculated using the Wilcoxon rank-sum test as implemented in R to account for outliers.

#### Acknowledgements

We thank Zhao Yihe and Tessa Marciniak for their help during sampling and sample preparation; Sabrina Butze for helping with the isolation of bacteria; Katja Cloppenburg-Schmidt and John Baines for the sequencing library preparation; the staff of the microbiome sequencing unit at the IKMB, Kiel; and Susanne Petersen from the Botanical Garden in Kiel. We further thank the Schulenburg group for their helpful feedback.

## References

- Ainsworth, T.D., Krause, L., Bridge, T., Torda, G., Raina, J.-B., Zakrzewski, M., *et al.* (2015) The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. *ISME J* **9**: 2261–2274.
- Assenov, Y., Ramírez, F., Schelhorn, S.-E., Lengauer, T., and Albrecht, M. (2008) Computing topological parameters of biological networks. *Bioinforma Oxf Engl* **24**: 282–284.
- Benjamini, Y., and Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol* **57**: 289–300.
- Berg, M., Stenuit, B., Ho, J., Wang, A., Parke, C., Knight, M., *et al.* (2016a) Assembly of the *Caenorhabditis elegans* gut microbiota from diverse soil microbial environments. *ISME J* **10**: 1998–2009.
- Berg, M., Zhou, X.Y., and Shapira, M. (2016b) Host-specific functional significance of *Caenorhabditis* gut commensals. *Front. Microbiol* **7**: 1622.
- Bjork, J.R., O'Hara, R.B., Ribes, M., Coma, R., and Montoya, J.M. (2018) The dynamic core microbiome: structure, dynamics and stability.
- Brown, M.B. (1975) 400: a method for combining non-independent, one-sided tests of significance. *Biometrics* **31**: 987–992.
- Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S., and Thomas, T. (2011) Bacterial community assembly based on functional genes rather than species. *Proc Natl Acad Sci U S A* **108**: 14288–14293.
- Burns, A.R., Miller, E., Agarwal, M., Rolig, A.S., Milligan-Myhre, K., Seredick, S., *et al.* (2017) Interhost dispersal alters microbiome assembly and can overwhelm host innate immunity in an experimental zebrafish model. *Proc Natl Acad Sci U S A* **114**: 11181–11186.
- Caceres, M.D. (2013) How to use the indicpecies package (ver. 1.7.1). 29.
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. (2016) DADA2: high resolution sample inference from Illumina amplicon data. *Nat Methods* **13**: 581–583.
- Chaucheyras-Durand, F., Masséglia, S., Fonty, G., and Forano, E. (2010) Influence of the composition of the cellulolytic flora on the development of hydrogenotrophic microorganisms, hydrogen utilization, and methane production in the rumens of gnotobiotically reared lambs. *Appl Environ Microbiol* **76**: 7931–7937.
- Dione, N., Khelaifia, S., La Scola, B., Lagier, J.C., and Raoult, D. (2016) A quasi-universal medium to break the aerobic/anaerobic bacterial culture dichotomy in clinical microbiology. *Clin Microbiol Infect* **22**: 53–58.
- Dirksen, P., Marsh, S.A., Braker, I., Heitland, N., Wagner, S., Nakad, R., *et al.* (2016) The native microbiome of the nematode *Caenorhabditis elegans*: gateway to a new host-microbiome model. *BMC Biol* **14**: 1–16.
- Dixon, P. (2003) VEGAN, a package of R functions for community ecology. *J Veg Sci* **14**: 927–930.
- Drake, J.A. (1991) Community-assembly mechanics and the structure of an experimental species ensemble. *Am Nat* **137**: 1–26.
- Emerson, B.C., and Gillespie, R.G. (2008) Phylogenetic analysis of community assembly and structure over space and time. *Trends Ecol Evol* **23**: 619–630.
- Faust, K., Sathirapongsasuti, J.F., Izard, J., Segata, N., Gevers, D., Raes, J., and Huttenhower, C. (2012) Microbial co-occurrence relationships in the human microbiome. *PLoS Comput Biol* **8**: e1002606.
- Fraune, S., Anton-Erxleben, F., Augustin, R., Franzenburg, S., Knop, M., Schroder, K., *et al.* (2015) Bacteria-bacteria interactions within the microbiota of the ancestral metazoan *Hydra* contribute to fungal resistance. *ISME J* **9**: 1543–1556.
- Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H., and Hättenschwiler, S. (2010) Diversity meets decomposition. *Trends Ecol Evol* **25**: 372–380.
- Goldford, J.E., Lu, N., Bajić, D., Estrela, S., Tikhonov, M., Sanchez-Gorostiaga, A., *et al.* (2018) Emergent simplicity in microbial community assembly. *Science* **361**: 469–474.
- Greenhalgh, K., Meyer, K.M., Aagaard, K.M., and Wilmes, P. (2016) The human gut microbiome in health: establishment and resilience of microbiota over a lifetime. *Environ Microbiol* **18**: 2103–2116.
- Hardy, O.J., Coueron, P., Munoz, F., Ramesh, B.R., and Pélissier, R. (2012) Phylogenetic turnover in tropical tree communities: impact of environmental filtering, biogeography and mesoclimatic niche conservatism. *Glob Ecol Biogeogr* **21**: 1007–1016.
- Hernandez-Agreda, A., Gates, R.D., and Ainsworth, T.D. (2017) Defining the Core microbiome in corals' microbial soup. *Trends Microbiol* **25**: 125–140.
- Hervé, M. (2015) RVAideMemoire: diverse basic statistical and graphical functions. R Package Version 09-50.
- Holling, C.S. (1973) Resilience and stability of ecological systems. *Annu Rev Ecol Syst* **4**: 1–23.
- Jabiol, J., McKie, B.G., Bruder, A., Bernadet, C., Gessner, M.O., and Chauvet, E. (2013) Trophic complexity enhances ecosystem functioning in an aquatic detritus-based model system. *J Anim Ecol* **82**: 1042–1051.
- Johnke, J., Fraune, S., Bosch, T.C.G., Hentschel, U., and Schulenburg, H. (2019) *Bdellovibrio* and like organisms are predictors of microbiome diversity in distinct host groups. *Microb Ecol* **79**: 252–257.
- Keeney, K.M., and Finlay, B.B. (2011) Enteric pathogen exploitation of the microbiota-generated nutrient environment of the gut. *Curr Opin Microbiol* **14**: 92–98.
- Kissoyan, K.A.B., Drechsler, M., Stange, E.-L., Zimmermann, J., Kaleta, C., Bode, H.B., and Dierking, K. (2019) Natural *C. elegans* microbiota protects against infection via production of a cyclic lipopeptide of the viscosin group. *Curr Biol* **29**: 1030–1037.e5 (2019).
- Kwong, W.K., Mancenido, A.L., and Moran, N.A. (2017) Immune system stimulation by the native gut microbiota of honey bees. *R Soc Open Sci* **4**: 170003.
- Law, R., and Morton, R.D. (1993) Alternative permanent states of ecological communities. *Ecology* **74**: 1347–1361.
- Leitão, R.P., Zuanon, J., Villéger, S., Williams, S.E., Baraloto, C., Fortunel, C., *et al.* (2016) Rare species contribute disproportionately to the functional structure of species assemblages. *Proc R Soc B Biol Sci* **283**: 20160084.
- Levy, R., and Borenstein, E. (2013) Metabolic modeling of species interaction in the human microbiome elucidates community-level assembly rules. *Proc Natl Acad Sci U S A* **110**: 12804–12809.

- 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.
- MacArthur, R., and Levins, R. (1967) The limiting similarity, convergence, and divergence of coexisting species. *Am Nat* **101**: 377–385.
- Mächler, M., Rousseeuw, P., Struyf, A., Hubert, M., and Hornik, K. (2012) Cluster: cluster analysis basics and extensions.
- Martin, M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* **17**: 10–12.
- Matthews, T.J., and Whittaker, R.J. (2015) On the species abundance distribution in applied ecology and biodiversity management. *J Appl Ecol* **52**: 443–454.
- Mayfield, M.M., and Levine, J.M. (2010) Opposing effects of competitive exclusion on the phylogenetic structure of communities. *Ecol Lett* **13**: 1085–1093.
- McGill, B.J., Etienne, R.S., Gray, J.S., Alonso, D., Anderson, M.J., Benecha, H.K., et al. (2007) Species abundance distributions: moving beyond single prediction theories to integration within an ecological framework. *Ecol Lett* **10**: 995–1015.
- McMurdie, P.J., and Holmes, S. (2013) phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* **8**: e61217.
- McMurdie, P.J., and Holmes, S. (2014) Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Comput Biol* **10**: e1003531.
- Montalvo-Katz, S., Huang, H., Appel, M.D., Berg, M., and Shapira, M. (2013) Association with soil bacteria enhances p38-dependent infection resistance in *Caenorhabditis elegans*. *Infect Immun* **81**: 514–520.
- Naeem, S., and Li, S.B. (1997) Biodiversity enhances ecosystem reliability. *Nature* **390**: 507–509.
- Niño-García, J.P., Ruiz-González, C., and del Giorgio, P.A. (2016) Landscape-scale spatial abundance distributions discriminate core from random components of boreal lake bacterioplankton. *Ecol Lett* **19**: 1506–1515.
- Ocejo, M., Oporto, B., and Hurtado, A. (2019) 16S rRNA amplicon sequencing characterization of caecal microbiome composition of broilers and free-range slow-growing chickens throughout their productive lifespan. *Sci Rep* **9**: 2506.
- Ortiz Lopez, A., Vega, N.M., and Gore, J. (2019) Interspecies bacterial competition determines community assembly in the *C. elegans* intestine.
- Paine, R.T. (1966) Food web complexity and species diversity. *Am Nat* **100**: 65–75.
- Patterson, B.D. (1980) Montane mammalian biogeography in New Mexico. *Southwest Nat* **25**: 33–40.
- Pedros-Alió, C. (2012) The rare bacterial biosphere. *Annu Rev Mar Sci* **4**: 449–466.
- Petersen, C., Dirksen, P., Prah, S., Strathmann, E.A., and Schulenburg, H. (2014) The prevalence of *Caenorhabditis elegans* across 1.5 years in selected north German locations: the importance of substrate type, abiotic parameters, and *Caenorhabditis* competitors. *BMC Ecol* **14**: 4–4.
- Petersen, C., Saebelfeld, M., Barbosa, C., Pees, B., Hermann, R.J., Schalkowski, R., et al. (2015) Ten years of life in compost: temporal and spatial variation of north German *Caenorhabditis elegans* populations. *Ecol Evol* **5**: 3250–3263.
- Pruesse, E., Peplies, J., and Glöckner, F.O. (2012) SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* **28**: 1823–1829.
- R Core Team. (2016) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rotem, O., Pasternak, Z., and Jurkevitch, E. (2014) *Bdellovibrio* and like organisms. In *The Prokaryotes*, Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., and Thompson, F. (eds). Berlin, Heidelberg: Springer, pp. 3–17.
- Rothman, J.A., Leger, L., Graystock, P., Russell, K., and McFrederick, Q.S. (2019) The bumble bee microbiome increases survival of bees exposed to selenate toxicity. *Environ Microbiol.* **21**: 3417–3429.
- Royalty, T., and Steen, A.D. (2019) Partitioning of microbial function among taxonomic ranks across the tree of life.
- Samuel, B.S., Rowedder, H., Braendle, C., Félix, M.-A., and Ruvkun, G. (2016) *Caenorhabditis elegans* responses to bacteria from its natural habitats. *Proc Natl Acad Sci U S A* **113**: E3941–E3949.
- Shafquat, A., Joice, R., Simmons, S.L., and Huttenhower, C. (2014) Functional and phylogenetic assembly of microbial communities in the human microbiome. *Trends Microbiol* **22**: 261–266.
- Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., et al. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* **13**: 2498–2504.
- Sockett, R.E. (2009) Predatory lifestyle of *Bdellovibrio bacteriovorus*. *Annu Rev Microbiol* **63**: 523–539.
- Sogin, M.L., Morrison, H.G., Huber, J.A., Mark Welch, D., Huse, S.M., Neal, P.R., et al. (2006) Microbial diversity in the deep sea and the underexplored 'rare biosphere'. *Proc Natl Acad Sci U S A* **103**: 12115–12120.
- Sommer, F., Anderson, J.M., Bharti, R., Raes, J., and Rosenstiel, P. (2017) The resilience of the intestinal microbiota influences health and disease. *Nat Rev Microbiol* **15**: 630–638.
- Tatsumi, S., Cadotte, M.W., and Mori, A.S. (2019) Individual-based models of community assembly: neighbourhood competition drives phylogenetic community structure. *J Ecol* **107**: 735–746.
- Thebault, E., and Loreau, M. (2003) Food-web constraints on biodiversity-ecosystem functioning relationships. *Proc Natl Acad Sci U S A* **100**: 14949–14,954.
- Thomson, J.D., Weiblen, G., Thomson, B.A., Alfaro, S., and Legendre, P. (1996) Untangling multiple factors in spatial distributions: lilies, gophers, and rocks. *Ecology* **77**: 1698–1715.
- Turnbaugh, P.J., Quince, C., Faith, J.J., McHardy, A.C., Yatsunenkov, T., Niaz, F., et al. (2010) Organismal, genetic, and transcriptional variation in the deeply sequenced gut microbiomes of identical twins. *Proc Natl Acad Sci U S A* **107**: 7503–7508.
- Vega, N.M., and Gore, J. (2017) Stochastic assembly produces heterogeneous communities in the *Caenorhabditis elegans* intestine. *PLOS Biol* **15**: e2000633.
- von der Schulenburg, J.H.G., Hancock, J.M., Pagnamenta, A., Sloggett, J.J., Majerus, M.E.N., and Hurst, G.D.D. (2001) Extreme length and length variation in the first ribosomal internal transcribed spacer of ladybird beetles (Coleoptera: Coccinellidae). *Mol Biol Evol* **18**: 648–660.



Warnecke, F., Luginbühl, P., Ivanova, N., Ghasseman, M., Richardson, T.H., Stege, J.T., *et al.* (2007) Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature* **450**: 560–565.

Weiher, E., and Keddy, P.A. (1995) The assembly of experimental wetland plant communities. *Oikos* **73**: 323–335.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J. (1991) 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**: 697–703.

Wilson, D.S. (1992) Complex interactions in meta-communities, with implications for biodiversity and higher levels of selection. *Ecology* **73**: 1984–2000.

Yachi, S., and Loreau, M. (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proc Natl Acad Sci U S A* **96**: 1463–1468.

Zhang, F., Berg, M., Dierking, K., Félix, M.-A., Shapira, M., Samuel, B.S., and Schulenburg, H. (2017) *Caenorhabditis elegans* as a model for microbiome research. *Front Microbiol* **8**: 485.

Zhang, J.-L., Swenson, N.G., Chen, S.-B., Liu, X.-J., Li, Z.-S., Huang, J.-H., *et al.* (2013) Phylogenetic beta diversity in tropical forests: implications for the roles of geographical and environmental distance. *J Syst Evol* **51**: 71–85.

Zhu, W., Winter, M.G., Byndloss, M.X., Spiga, L., Duerkop, B.A., Hughes, E.R., *et al.* (2018) Precision editing of the gut microbiota ameliorates colitis. *Nature* **553**: 208–211.

Zimmermann, J., Obeng, N., Yang, W., Pees, B., Petersen, C., Waschina, S., *et al.* (2019) The functional repertoire contained within the native microbiota of the model nematode *Caenorhabditis elegans*. *ISME J* **14**: 26–38.

## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Figure S1** PCoAs showing the Unifrac (a) and Bray–Curtis dissimilarity (b–f) as measure of differences of species composition (beta-diversity) of all samples from this study (a), substrate samples from this study (b), only lab single worm samples from this study (c and e), or lab population worm samples from this study (d and f) using the top 100 ASVs. Colouring is either based on group (a), sampling year (b) or the substrate samples from which worms were isolated (c–f). The statistics are given in Table S3.

**Figure S2** Rarefaction curves of the isolated bacteria from three lab enriched single worms. Data were either plotted based on ASVs (a) or based on genera (b).

**Figure S3** Alpha-diversity shown as ASV richness (a) or Shannon diversity (b) of samples from 2016 and 2017 with

or without BALOs. Worm samples were averaged based on the substrate samples from which they were isolated. Asterisks indicate significant differences tested with the Wilcoxon rank sum test. \*:  $p < 0.05$  &  $p > 0.01$ , \*\*:  $p < 0.01$  &  $p > 0.001$  \*\*\*:  $p < 0.001$ . Y-axes differ in scaling.

**Figure S4** Frequency distribution of worm populations sizes on a given substrate (a). PCoAs of natural worm samples (b) and substrate samples (c) based on Bray–Curtis dissimilarity and colour-coded by worm population size category.

**Figure S5** Rarefaction curves of all samples newly analysed in this study. The ASV richness is based on the 16S rDNA amplicon data.

**Table S1** Test statistics for Fig. 1.

**Table S2** Test statistics for Fig. 2.

**Table S3** Test statistics for Fig. S1.

**Table S4** List of overall core ASVs.

**Table S5** List of all significant indicator ASVs for worm or substrate samples.

**Table S6** Calculations for core ASVs per substrate.

**Table S7** List of core ASVs per substrate.

**Table S8** List of isolated strains.

**Table S9** List of the overall top 100 ASVs and the isolation of a genus representative.

**Table S10** List of the lab populations top 100 ASVs and the isolation of a genus representative.

**Table S11** Node degrees of ASVs from substrate and worm networks.

**Table S12** Betweenness of edges from substrate and worm networks.

**Table S13** Test statistics for Fig. 4b.

**Table S14** List of node names for Fig. 4d.

**Table S15** Test statistics for Fig. 4e.

**Table S16** Test statistics for Fig. 5 and Fig. S3.

**Table S17** Test statistics for Fig. 6.

**Table S18** Test statistics for Fig. S4b and c.

**Table S19** List of all significant indicator ASVs for high or low worm population size.

**Table S20** Proportion of indicator ASVs of respective bacterial classes for high or low worm population size.

**Table S21** Sequences and taxonomic classification of ASVs representing low biomass contaminations.

**Table S22** Bacterial genera that do appear as core genera in natural worm samples of a given substrate, but not in the respective substrate samples. Coloured shading indicates genera that match the conditions in more than one substrate group.

**Table S23** Mean ASV richness and Shannon diversity of individual and averaged worm microbiomes.