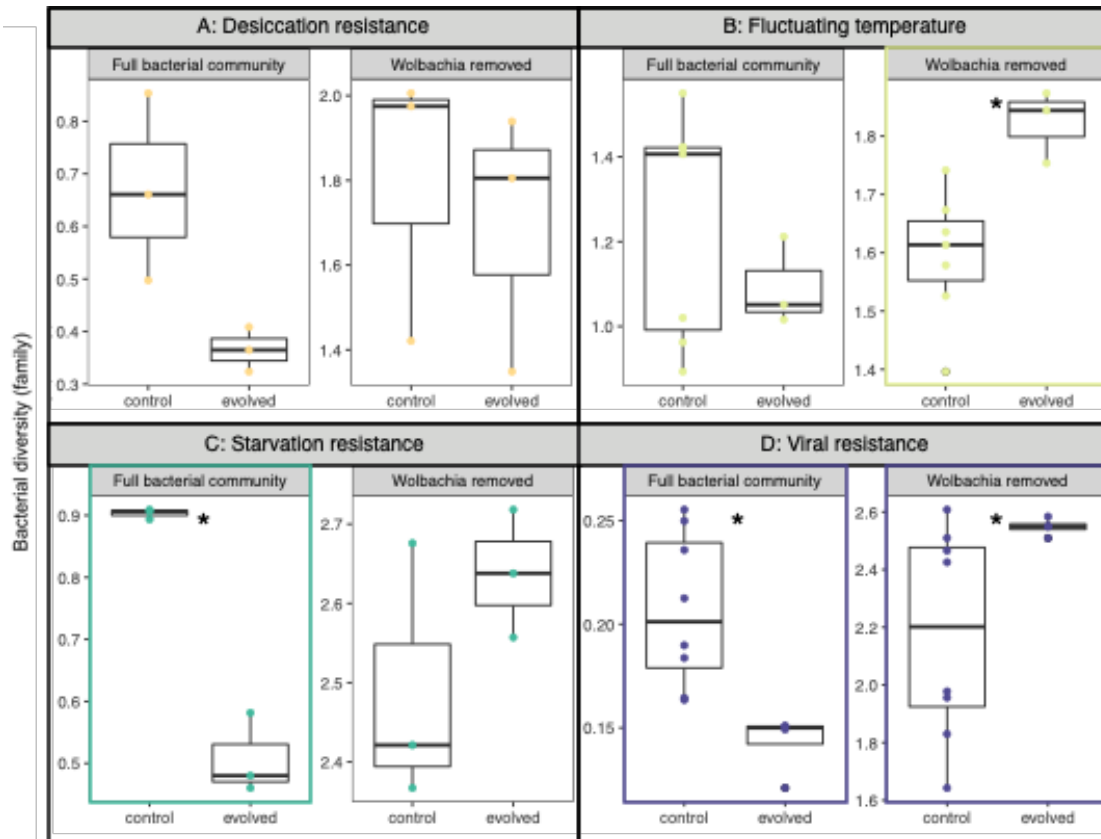




Supp. Fig. 12: Differences in bacterial diversity when including (full bacterial community) or excluding *Wolbachia* reads in four of the E&R experiments analyzed. Each point represents bacterial diversity (Shannon index) for a pool of sequenced flies. Scale bars are different because diversity comparisons are within either the full community or *Wolbachia* removed groupings for each experiment. We removed the Anaplasmataceae family (all of which are *Wolbachia* in flies and subsampled with replacement to the minimum number of reads in each experiment. Then, Shannon diversity was calculated on the rarified libraries as described in the methods in the main text.

The four experiments highlight how the difference between control and evolved microbiomes depends on whether *Wolbachia* is included. For example, for starvation resistance, diversity for the whole community is reduced when *Wolbachia* is included. However, when *Wolbachia* is excluded, there is no response in bacterial diversity between control and evolved populations. Full summary of differences can be found in Supp. Table 3.

While these results are not surprising given that a large number of reads were removed (though following *Wolbachia* removed, rarefaction curves suggest communities were still fully sampled as in Supp Fig. 1-10), the directionality of effect is surprising. We highlight these results because there is ambiguity about including *Wolbachia* in the microbiome. Many studies in *D. melanogaster* either use uninfected flies [1–4] or computationally remove reads during 16S rRNA microbiome analysis [5–9]. Furthermore, while 7/10 studies used flies infected with *Wolbachia*, only two studies explicitly mentioned *Wolbachia* infection in their flies—fluctuating temperature and viral resistance. As discussed in the main text, several studies suggest that

Wolbachia antagonistically interacts with *Acetobacter*. These data of course cannot answer the question if *Wolbachia* affects the microbiome--rather our analysis highlights the need for additional studies that explicitly manipulate *Wolbachia* infection status and measures both the response of the microbiome and fly during experimental evolution.

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