Supplementary Information for: Evolution of diversity explains the impact of pre-adaptation of a focal species on the structure of a natural microbial community.

Author Affiliations

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Author contributions: A.B, D.P., A.V. and E.H. conceived the study and designed the experiments. A.V., D.P. and E.H. conducted the experiments and D.P. analysed the data. S.P and R.G. provided new perspectives. D.P., E.H. and A.B. wrote the the first draft of the manuscript, with all authors contributing to the final version

Data accessibility statement: All data and R code used in the analysis will be made available on GitHub and archived on Zenodo

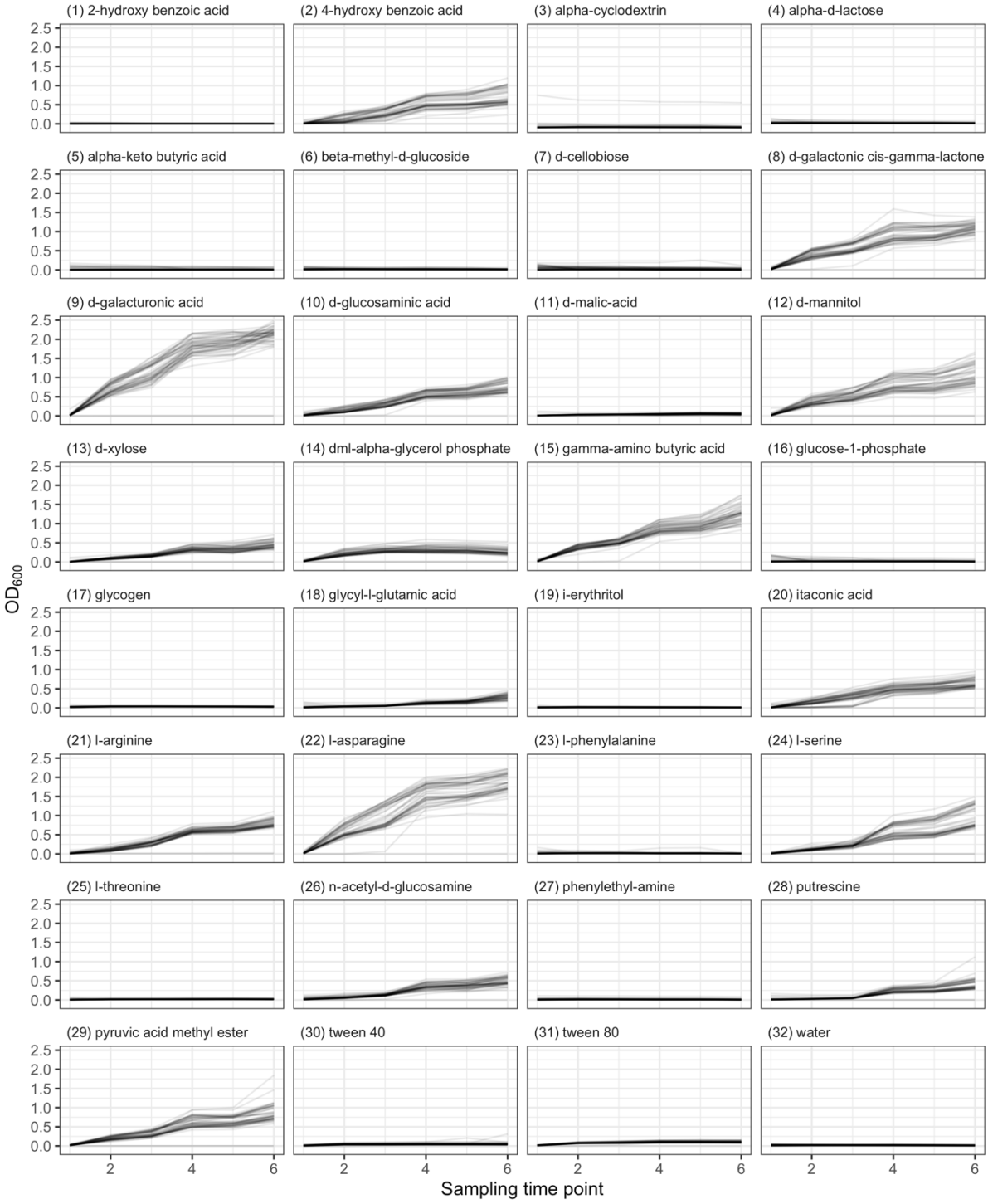

Figure S1. Optical density across substrates and sampling points for catabolic profiles. Each panel represents a different substrate on the Biolog plate. The time point used for downstream analyses was time point 4. Each line is the optical density of a clone.

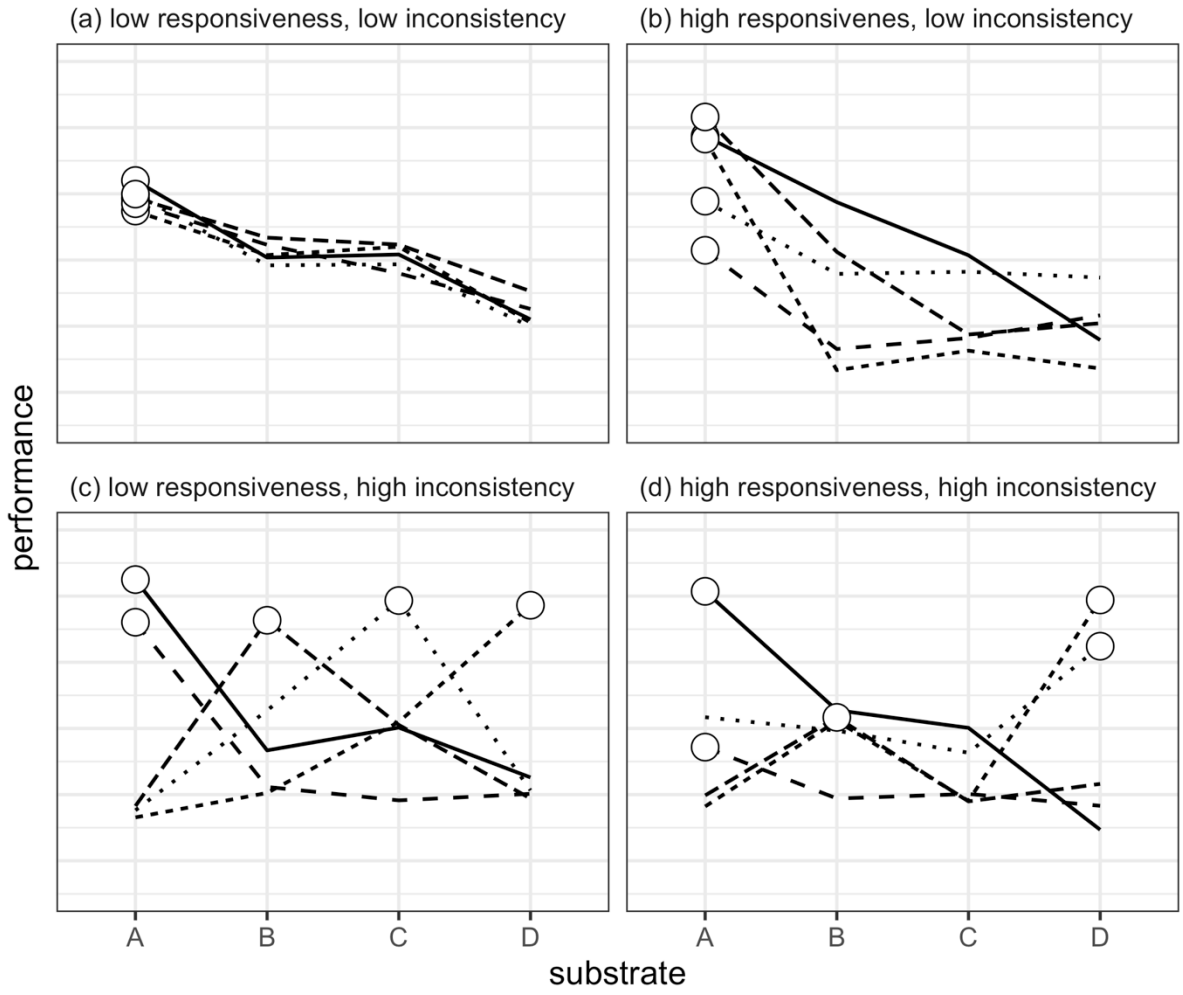

Figure S2. Scenarios of different amounts of responsiveness and inconsistency. Each dashed line represents the performance of an individual clone over substrates A to D. Dots represent the substrate on which each clone performs best on. In (a) the clones all have similar performance across all substrates and there is no C x E interaction. In (b) there is high responsiveness, but low inconsistency. The clones all perform best on resource A, but some are specialists and some generalists (they have different environmental variances). In (c) there is low responsiveness, but high inconsistency. Different clones take advantage of different resources, but there is no difference in resource exploitation strategies (they have similar environmental variances). In (d) there is high responsiveness and high inconsistency. Different clones take advantage of different resources and some clones are specialists and some are generalists.

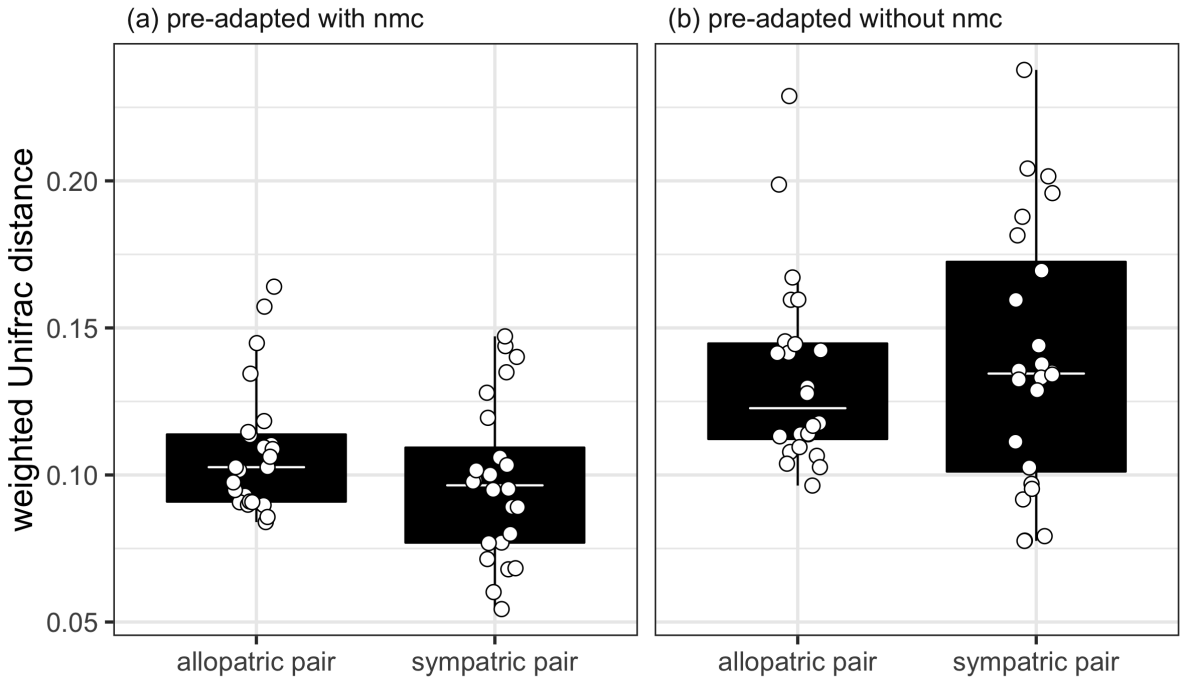

Figure S3. Average weighted Unifrac distance between allopatric and sympatric pairs of clones. Tops and bottoms of the bars represent the 75th and 25th percentiles of the data, the white lines are the medians, and the whiskers extend from their respective hinge to the smallest or largest value no further than 1.5 * interquartile range. Points represent weighted Unifrac distances between pairs of clones.

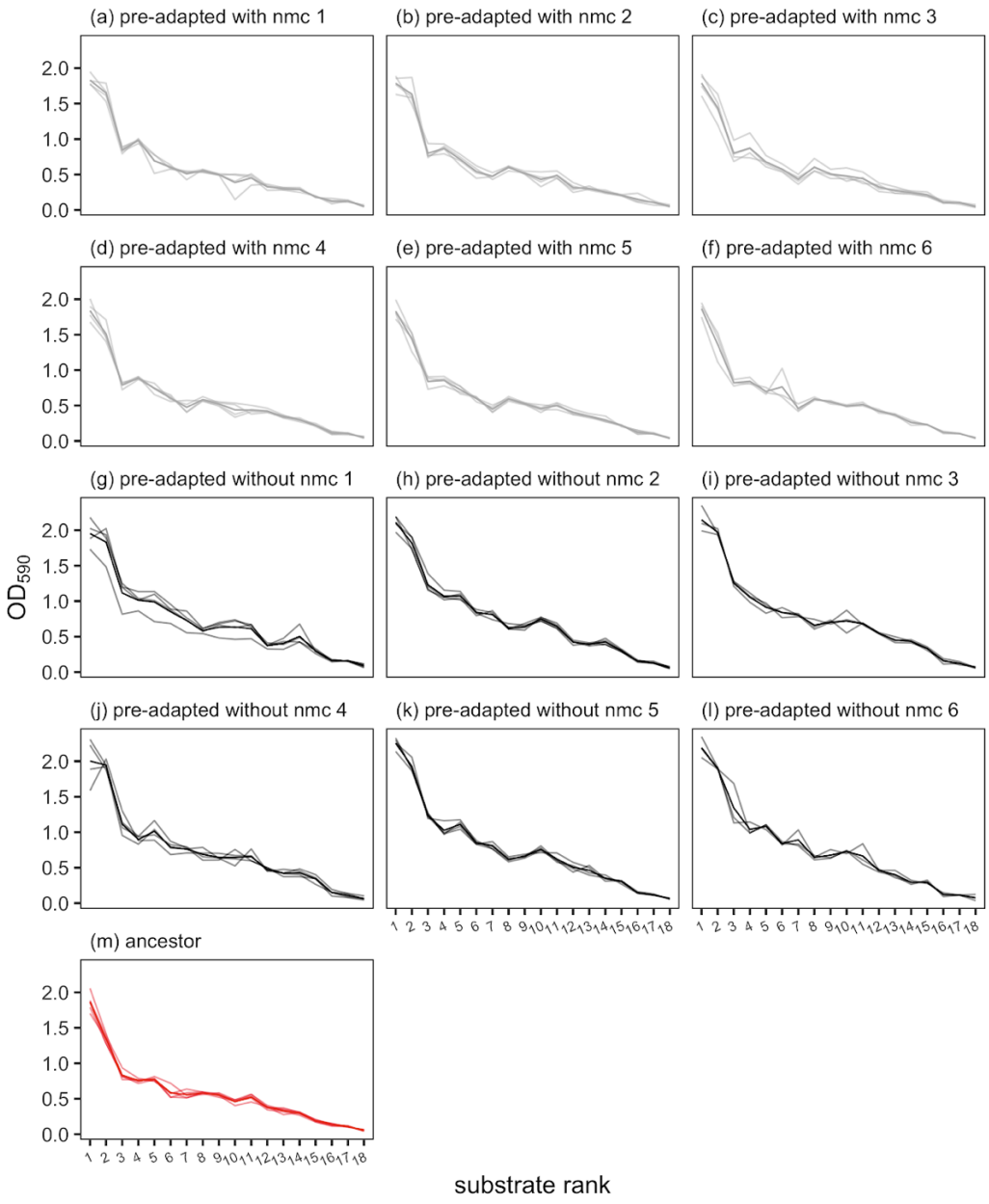

Figure S4. Patterns of resource use between clones, populations, and pre-adaptation treatments. Substrate rank performance curves for ancestral clones (red) and clones pre-adapted with (grey) and without (black) the natural microbial community. Panels represent different populations. Substrates are ranked by the mean value across all clones. The lighter lines depict clonal performances and the darker line represents the mean clonal performance on that substrate. Increased separation between responses indicates greater clonal variation, while increased slope differences indicates greater environmental variation. Moreover, differences in rankings (some clones being better on the second substrate than the first), represents instances of resource specialisation.

Table S1. Combinations of compost microcosms altering the pre-adaptation history of the clones and number of pre-adapted clones. The number of replicates differed across treatments. Six replicates of the LacZ ancestor were set up as were 6 replicates of the natural microbial community where no *P fluorescens* was added.

Diversity level	Treatment		Number of replicates
1	compost + nmc		24
	compost		
4	compost	sympatric pre-adaptation	6
	compost + nmc		
	mix of compost & compost + nmc	allopatric pre-adaptation	12
24	compost + nmc		6
	compost		
1	LacZ ancestor		6
0	control (nmc only)		6

Table S2. Pairwise comparisons of the effect of focal species diversity on community composition. The results of pairwise permutational ANOVAs between communities that had been inoculated with different levels of focal species diversity. Number of samples per treatment states the number of samples in the first, second treatments in each contrast. Significant p values are highlighted in bold.

contrast	number of samples per treatment	R^2	raw p value	p_{adj}
single clone vs. LacZ ancestor	48, 6	0.016	0.470	0.560
single clone vs. 4 clones	48, 23	0.009	0.660	0.660
single clone vs. 24 clones	48, 12	0.076	**0.003**	**0.020**
4 clones vs. LacZ ancestor	23, 6	0.036	0.360	0.530
24 clones vs. LacZ ancestor	12, 6	0.170	**0.018**	**0.037**
24 clones vs. 4 clones	12, 23	0.110	**0.007**	**0.020**

p_{adj} was calculated using the false discovery rate method

Table S3. Pairwise comparisons of the effect of focal species diversity on community composition within pre-adaptation treatments. The results of pairwise permutational ANOVAs between communities that had been inoculated with different levels of focal species diversity, within pre-adaptation treatments. Number of samples per treatment states the number of samples in the first, then second treatments in each contrast. Significant p values are highlighted in bold.

pre-adaptation treatment	contrast	number of samples per treatment	R^2	raw p value	p_{adj}
pre-adapted with nmc	single clone vs. 24 clones	24, 6	0.071	0.0710	0.210
pre-adapted with nmc	single clone vs. 4 clones	24, 5	0.064	0.1000	0.160
pre-adapted with nmc	24 clones vs. 4 clones	6, 5	0.110	0.3800	0.380
pre-adapted without nmc	single clone vs. 24 clones	24, 6	0.100	**0.0280**	**0.042**
pre-adapted without nmc	single clone vs. 4 clones	24, 6	0.032	0.4100	0.410
pre-adapted without nmc	24 clones vs. 4 clones	6, 6	0.460	**0.0049**	**0.015**

Table S4. Effect of pre-adaptation history on community composition across diversity levels of the focal species. The results of permutational ANOVAs testing for differences between communities had been inoculated with *P. fluorescens* that had been pre-adapted with or without the natural microbial community across different levels of focal species diversity.

Clonal diversity	F statistic	*d.f.*	R^2	p value
1	0.62	1, 46	0.01	0.646
4	1.55	1, 9	0.15	0.284
24	1.57	1, 10	0.14	0.133

Table S5. Tukey pairwise comparisons of final density of the focal species. Results of multiple pairwise comparisons looking for differences in final density between diversity levels. Significant p values are highlighted in bold.

contrast	estimate	SE	*d.f.*	t ratio	p value
single clone vs. 24 clones	-0.08	0.16	84	-0.51	0.96
single clone vs. 4 clones	0.05	0.13	84	0.38	0.98
single clone vs. LacZ ancestor	0.69	0.22	84	3.21	**0.01**
24 clones vs. 4 clones	0.13	0.18	84	0.74	0.88
24 clones vs. LacZ ancestor	0.78	0.25	84	3.12	**0.01**
4 clones vs. LacZ ancestor	0.65	0.23	84	2.83	**0.03**

P values were adjusted using the Tukey method for comparing a family of 4 estimates

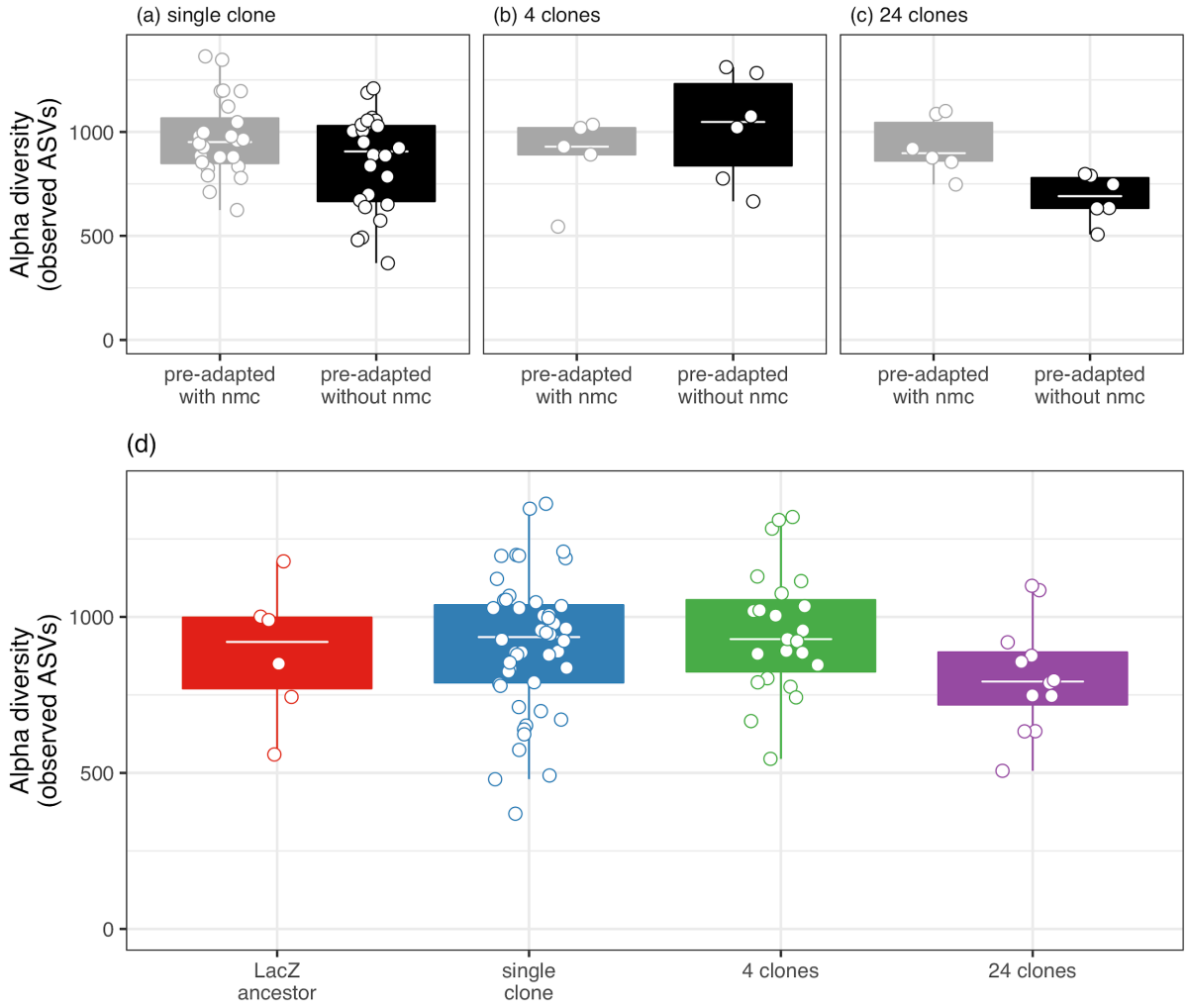

Figure S5. Effect of (a-c) pre-adaptation and (d) diversity on alpha diversity. Within different levels of diversity (a-c), the only effect of pre-adaptation history of the focal species with (grey) or without (black) the community on subsequent alpha diversity was a lower alpha diversity in communities with 24 clones adapted without the community. Across all levels of diversity (d), there was no impact increase focal species diversity on the observed number of amplicon sequence variants. In all panels, points are individual microcosms, tops and bottoms of the bars represent the 75th and 25th percentiles of the data, the white lines are the medians, and the whiskers extend from their respective hinge to the smallest or largest value no further than 1.5 * interquartile range.