

SUPPLEMENTARY FIGURES: Microbial model communities exhibit widespread metabolic interdependencies.

Armando Pacheco-Valenciana^{1*}, Anna Tausch², Iva Veseli^{3,4}, Jennah E. Dharamshi¹, Fabian Bergland¹, Luis Fernando Delgado-Zambrano⁵, Alejandro Rodríguez-Gijón¹, Anders F. Andersson⁵, Sarahi L. Garcia^{1,2,3*}.

¹ Department of Ecology, Environment, and Plant Sciences, Science for Life Laboratory, Stockholm University, Stockholm, Sweden.

² Institute for Chemistry and Biology of the Marine Environment (ICBM), School of Mathematics and Science, Carl von Ossietzky Universität Oldenburg, 26129, Oldenburg, Germany

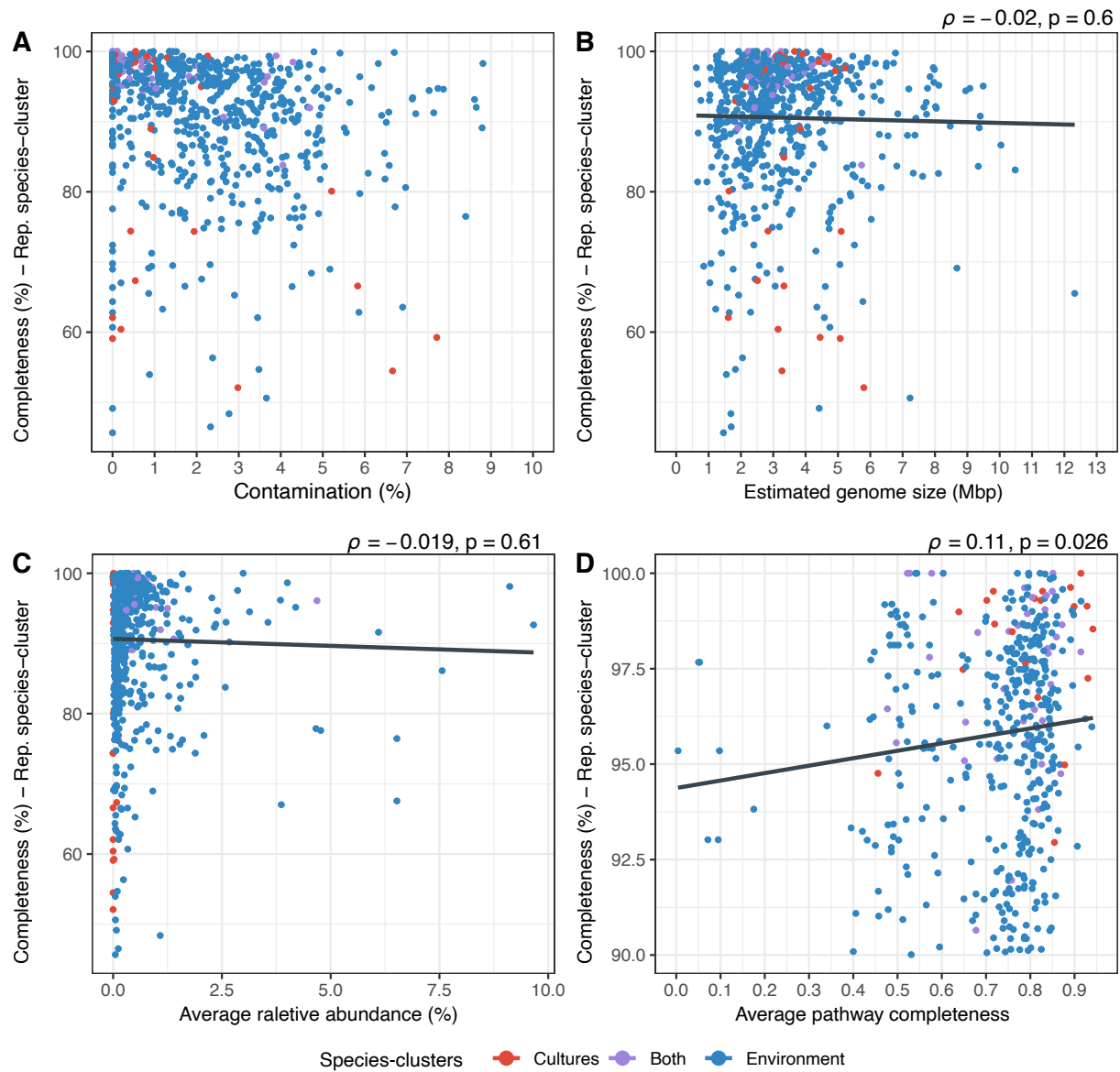
³ Helmholtz Institute for Functional Marine Biodiversity at the University of Oldenburg (HIFMB), Oldenburg, Germany

⁴ Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, 27570 Bremerhaven, Germany

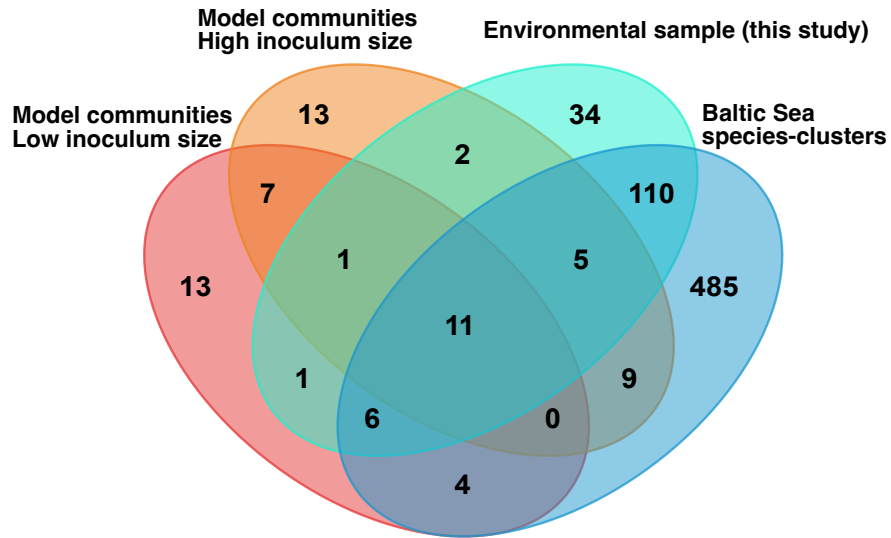
⁵ Department of Gene Technology, Science for Life Laboratory, KTH Royal Institute of Technology, Stockholm, Sweden.

* Correspondence: armando.pacheco@su.se, sarahi.garcia@su.se

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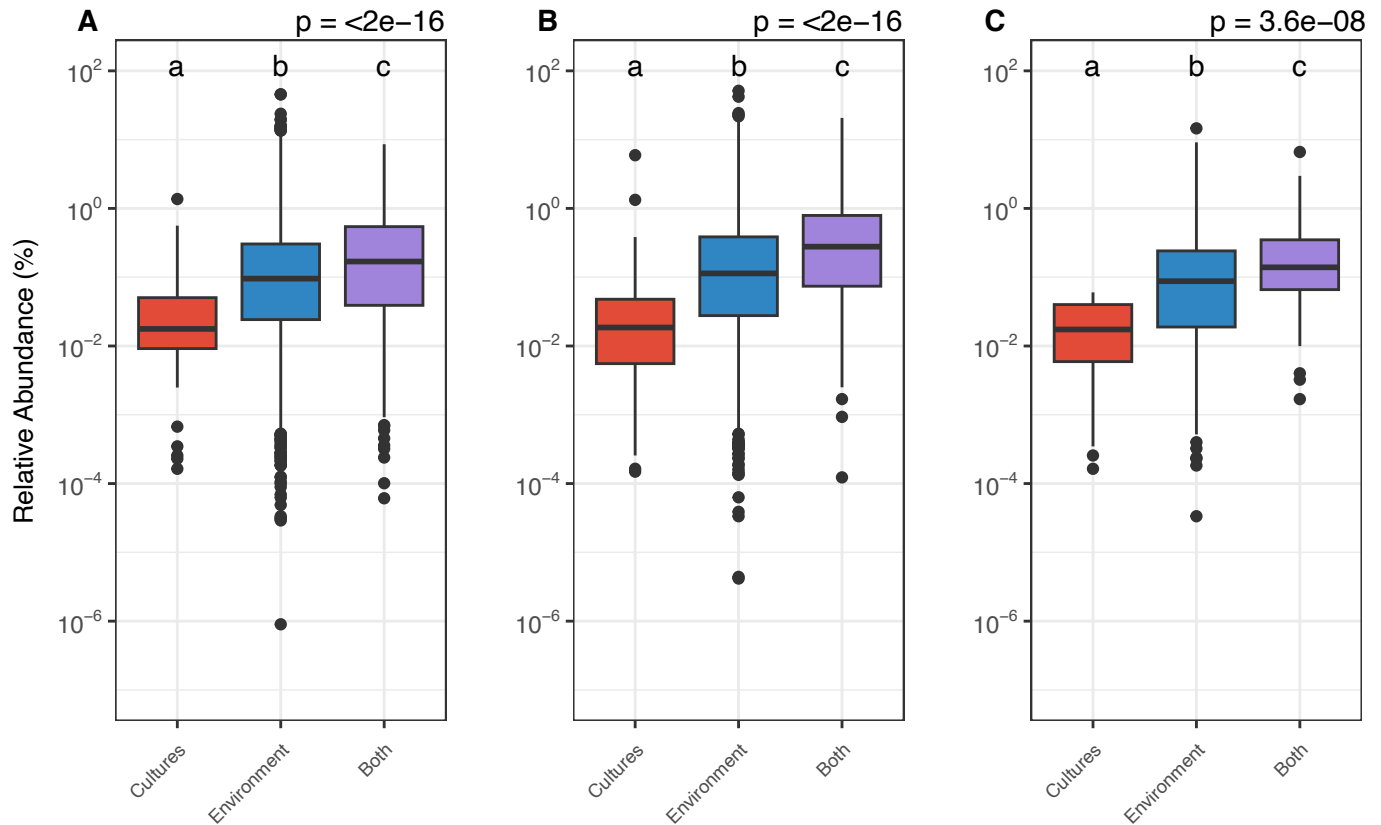


Supplementary Figure 1. Quality metrics of BalticMAG species-cluster representatives. Dot plots showing the relationship between genome completeness and various genomic metrics across the BalticMAG catalog ($n = 701$): (A) contamination, (B) estimated genome size, (C) average relative abundance, and (D) average pathway completeness for amino acids and vitamins. For panel D, only genomes with $>90\%$ completeness and $<5\%$ contamination were included ($n = 450$). Each data point represents one species-cluster representative genome found exclusively in model communities (red), exclusively in the environment (blue), or in both (purple). Spearman's rank correlations are shown (ρ , p); trend lines are for visualization only. Publicly available Baltic Sea metagenomes were included in the analysis^{37–39}.

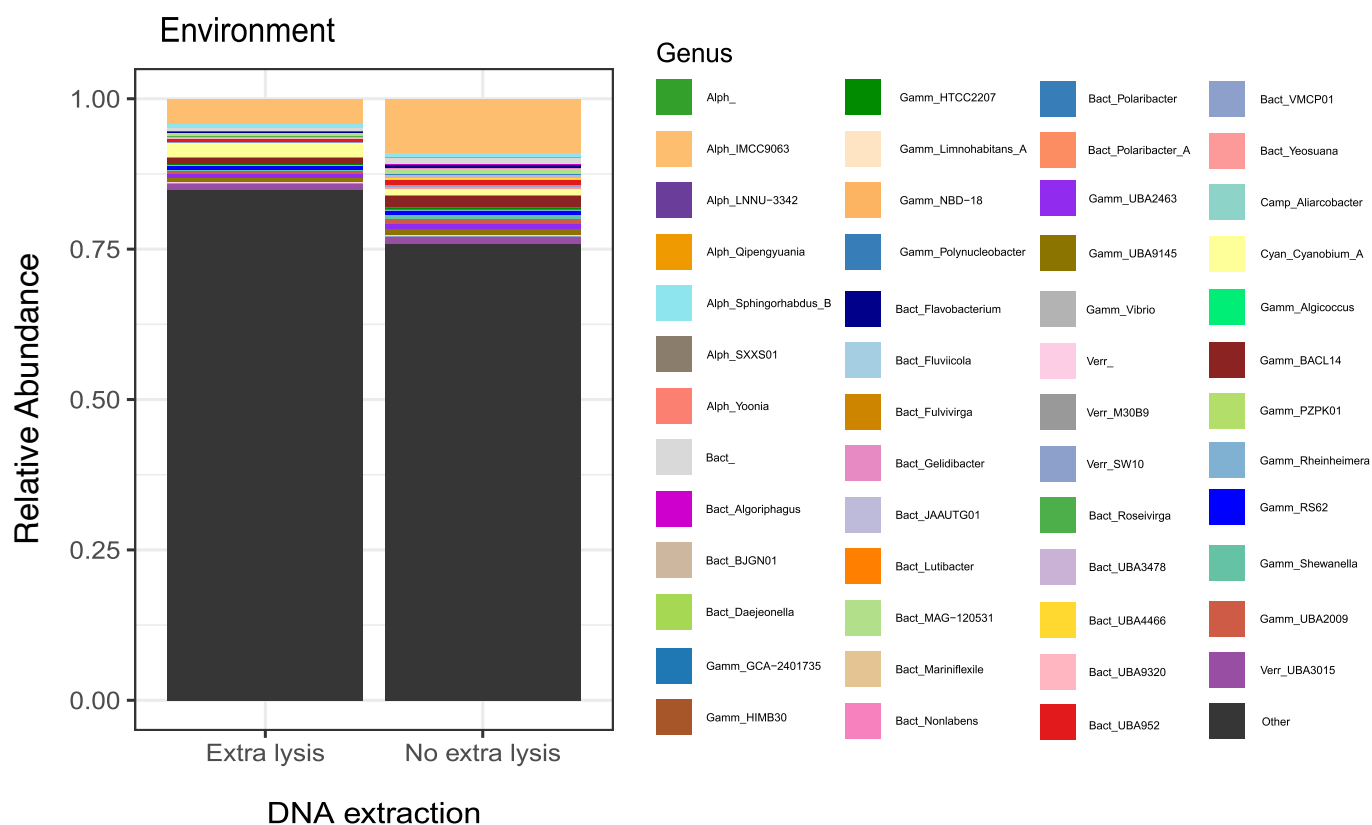


Supplementary Figure 2. Overlap of the BalticMAG species-clusters across different sources. Venn diagram illustrating the overlap of species-clusters from model communities (low inoculum = red, high inoculum = orange), environmental metagenomes from this study (light blue), and publicly available Baltic Sea metagenomes (dark blue)^{37–39}.

The diagram shows the extent of overlap among these groups.

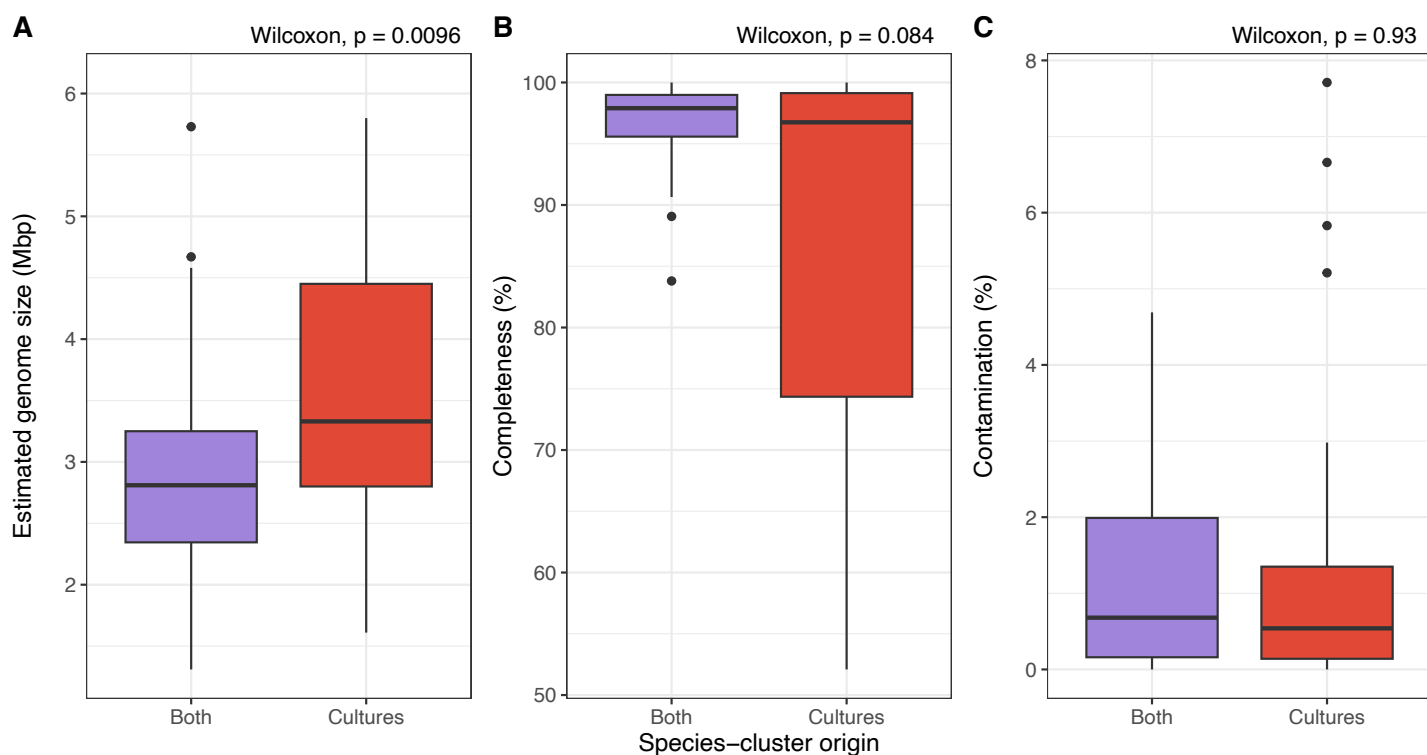


Supplementary Figure 3. Relative abundance of species-clusters in cultures and the environment. Boxplots comparing the relative abundance of species-clusters ($n = 701$) by their presence in cultures (red), the environment (blue), or both (purple). (A) Relative abundances of all species across all samples with salinity between 7 and 8 ‰ (PSU) and (B) across only the samples from the same location as those used to establish model communities. (C) Relative abundances of species only detected in our own metagenomic samples ($n = 566$), compared across all metagenomic samples. Statistical significance was tested using the Kruskal–Wallis test followed by Dunn’s post hoc test with Bonferroni correction. Groups sharing at least one letter (e.g., a and ab) are not significantly different; groups with different letters (e.g., a vs b) differ significantly ($p < 0.05$). Boxplots show the median and interquartile range; whiskers represent $1.5 \times \text{IQR}$. Publicly available metagenomes were included in the analysis^{37–39}.

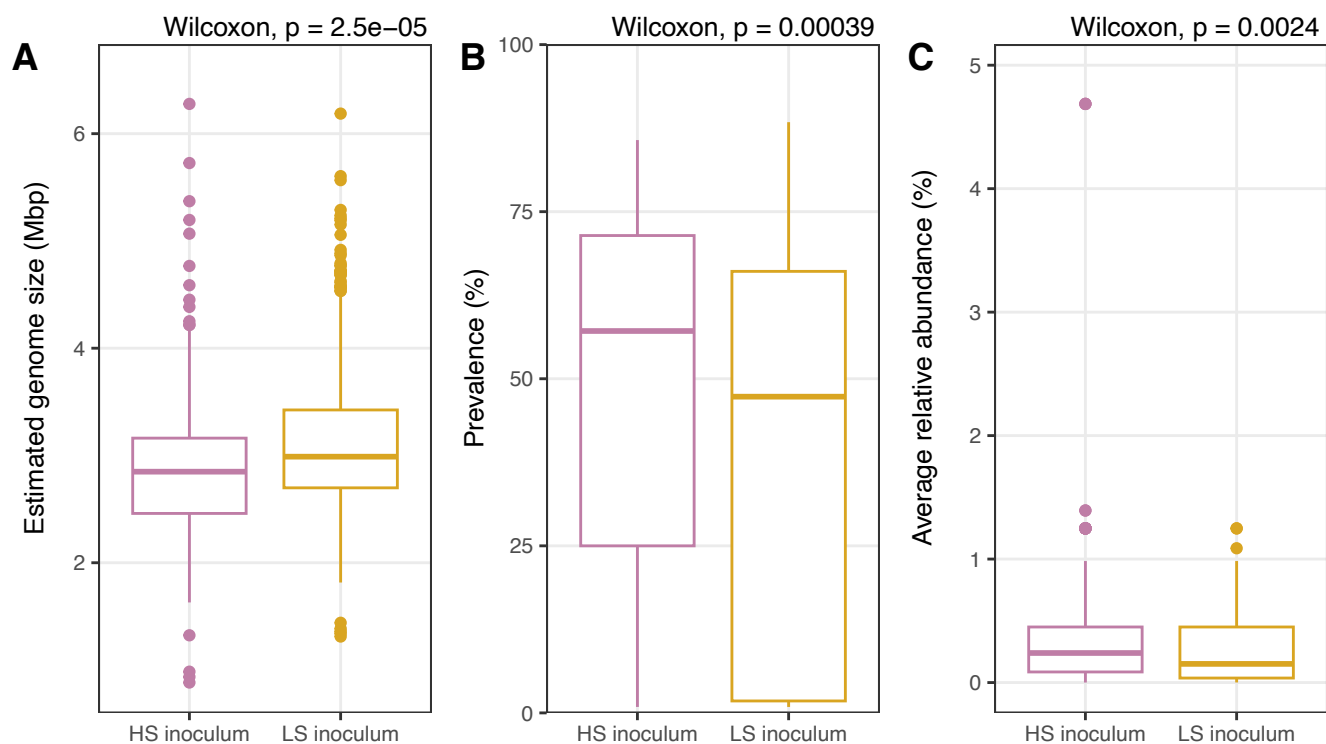


Supplementary Figure 4. Relative abundance of cultured and environmental genomes in the sample of origin.

Barplot showing the relative abundance of environmental (dark grey) and cultivated (other colors) genomes in the sample of origin of the cultures ($n = 2$). Note that the sample was extracted with two different DNA extraction methods, leading to two different perspectives on the diversity. Genomes are color-coded by genera, and the legend shows abbreviated prefixes indicating the class of each genus (e.g., Alph_ for Alphaproteobacteria, Bact_ for Bacteroidia, Camp_ for Campylobacteriia, Cyan_ for Cyanobacteria, Gamm_ for Gammaproteobacteria, and Verr_ for Verrucomicrobiae).

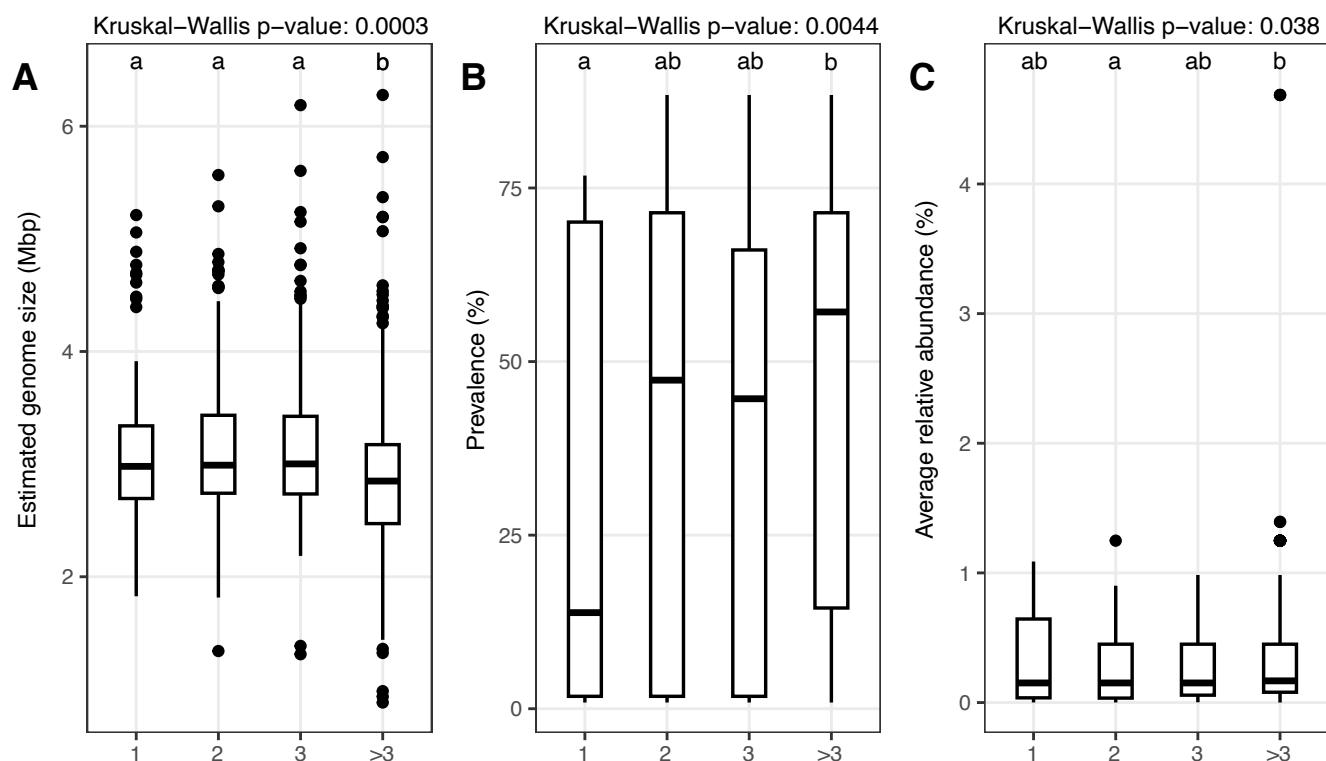


Supplementary Figure 5. Genome metrics of species-clusters from different sources. Boxplot comparing the (A) estimated genome size (Mbp), (B) completeness, and (C) contamination of species-clusters found only in cultures (red, $n = 33$), and in both cultures and the environment (purple, $n = 39$). Statistical significance was tested using the Wilcoxon rank-sum test ($p < 0.05$). Boxplots show the median and interquartile range; whiskers represent $1.5 \times \text{IQR}$.



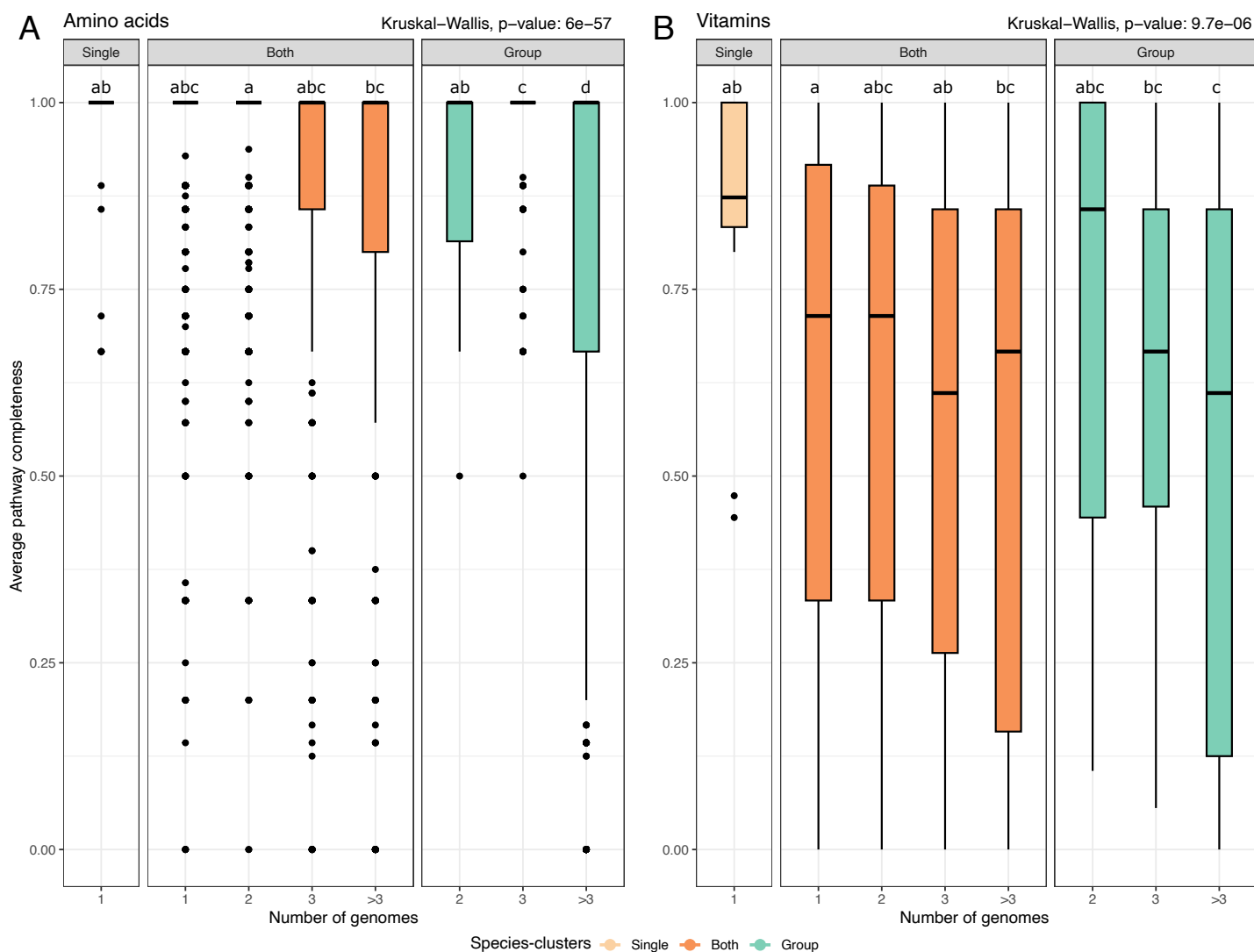
Supplementary Figure 6. Genomic features of MAGs from model communities from high and low inoculum sizes.

Boxplots comparing the (A) estimated genome size, (B) prevalence, and (C) average relative abundance of genomes from model communities with a high inoculum size (HS, $n = 231$) and a low inoculum size (LS, $n = 296$). Statistical significance was tested using the Wilcoxon rank-sum test ($p < 0.05$). Boxplots show the median and interquartile range; whiskers represent $1.5 \times \text{IQR}$.

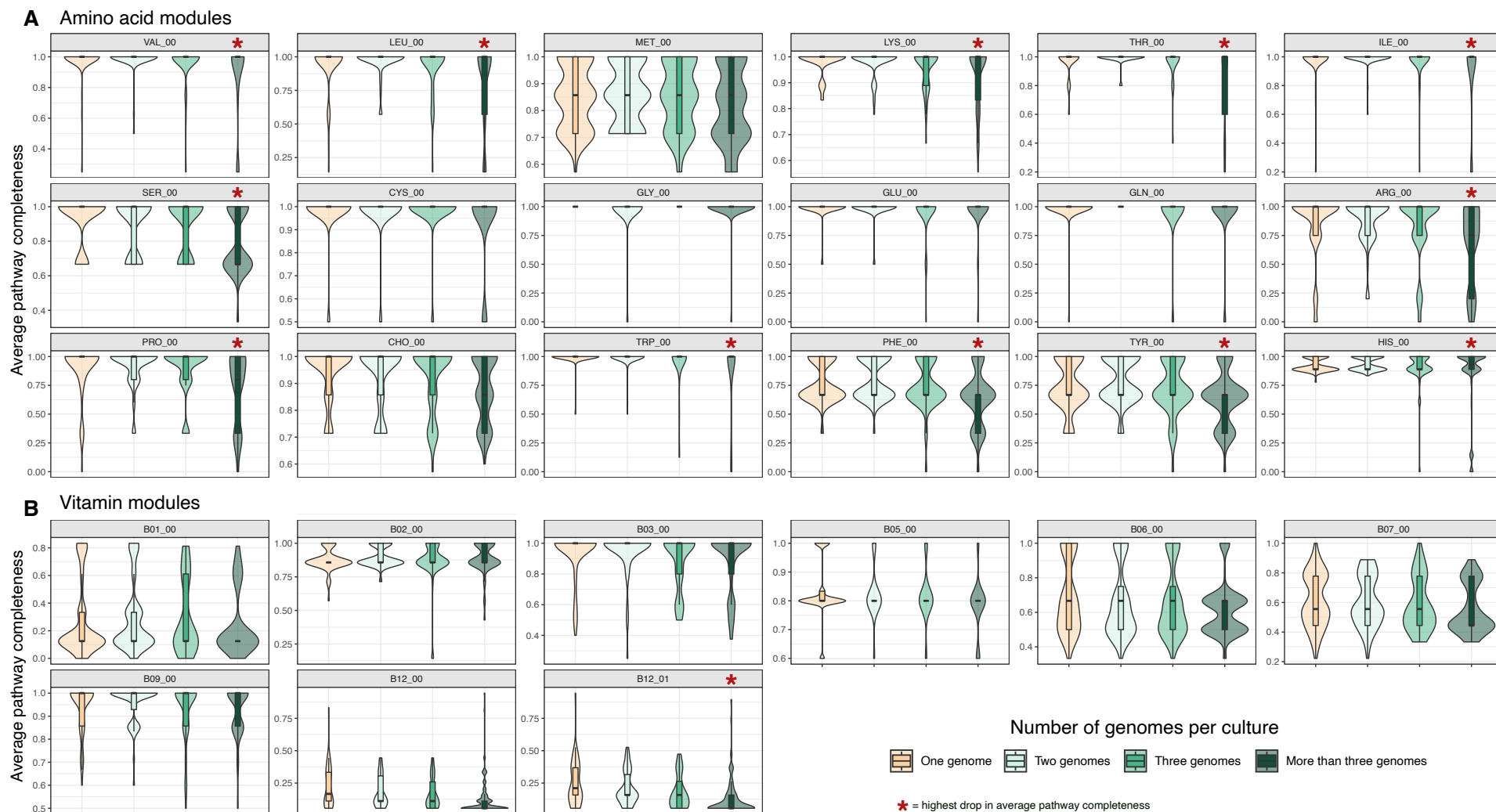


Supplementary Figure 7. Genomic features of cultured genomes across community complexity. Boxplots comparing the (A) estimated genome size, (B) prevalence, and (C) average relative abundance of genomes from model communities ($n = 527$) that include 1, 2, 3, or more than 3 species per culture. Statistical significance was tested using the Kruskal-Wallis test ($p < 0.05$), followed by a Dunn's post hoc test with Bonferroni correction. Groups sharing at least one letter (e.g., a and ab) are not significantly different; groups with different letters (e.g., a vs b) differ significantly ($p < 0.05$).

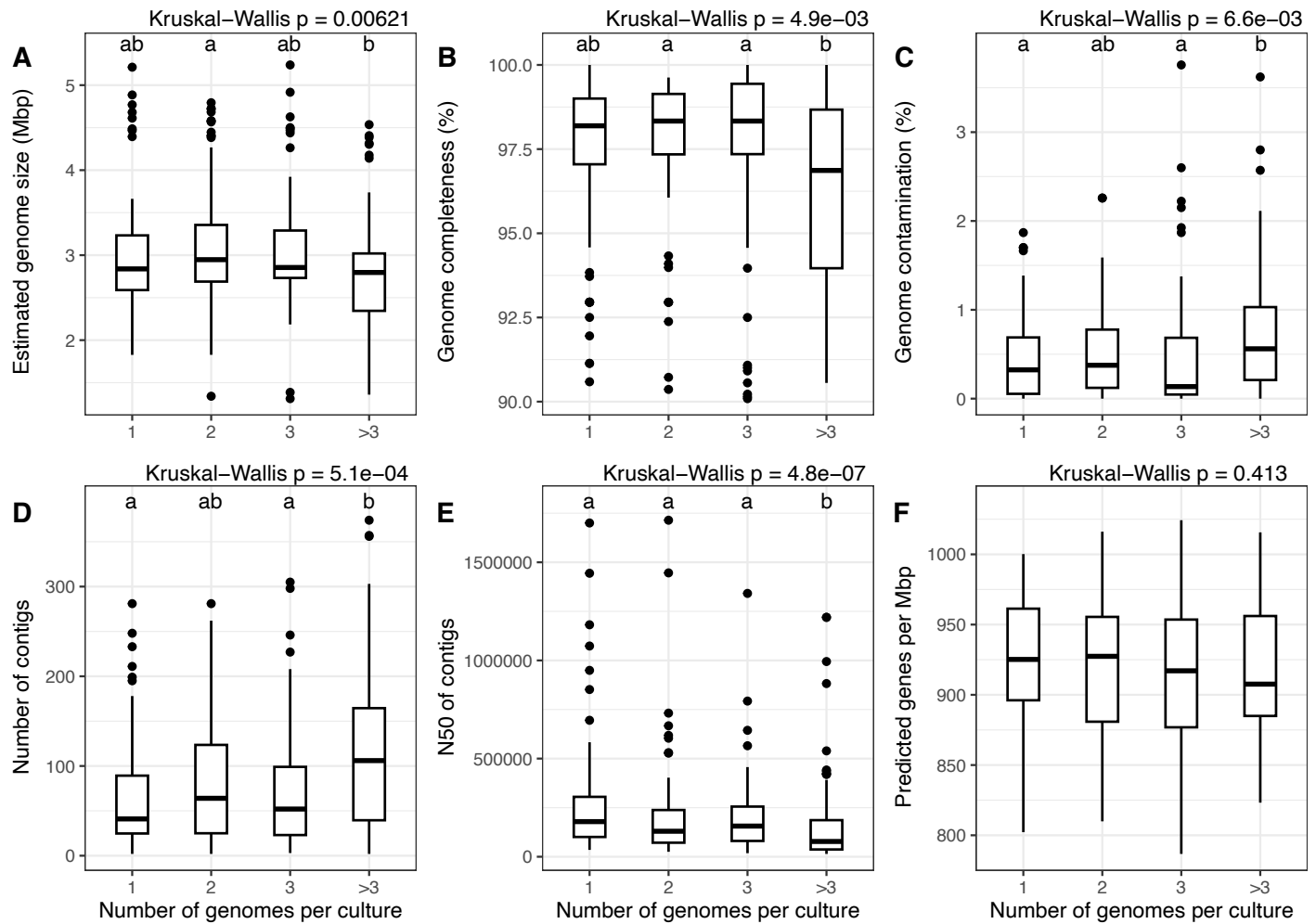
Boxplots show the median and interquartile range; whiskers represent $1.5 \times \text{IQR}$.



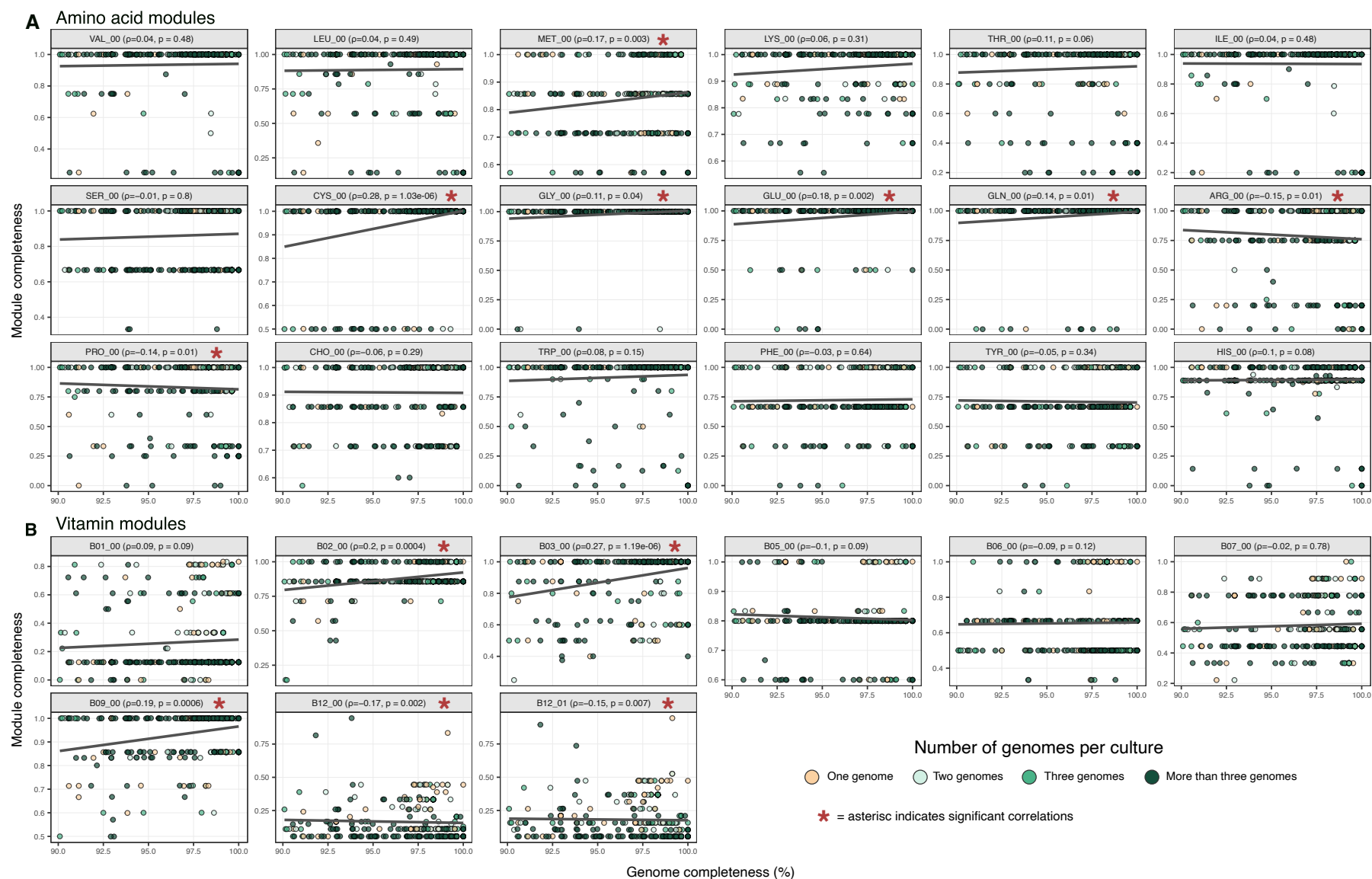
Supplementary Figure 8. Pathway completeness of cultured genomes according to growth categories and community complexity. Boxplots showing the average pathway completeness of cultured high-quality genomes ($n = 305$, $>90\%$ completeness and $<5\%$ contamination) for (A) custom amino acid and (B) vitamin modules, according to the number of genomes per culture, as well as the growth categories. Color coding indicates whether the species cluster grew exclusively on its own (light orange), exclusively in groups (green), or both on its own and in groups (orange). Statistical significance was tested using the Kruskal-Wallis test ($p < 0.05$), followed by a Dunn's post hoc test with Bonferroni correction. Groups sharing at least one letter (e.g., a and ab) are not significantly different; groups with different letters (e.g., a vs b) differ significantly ($p < 0.05$). Boxplots show the median and interquartile range; whiskers represent $1.5 \times$ IQR.



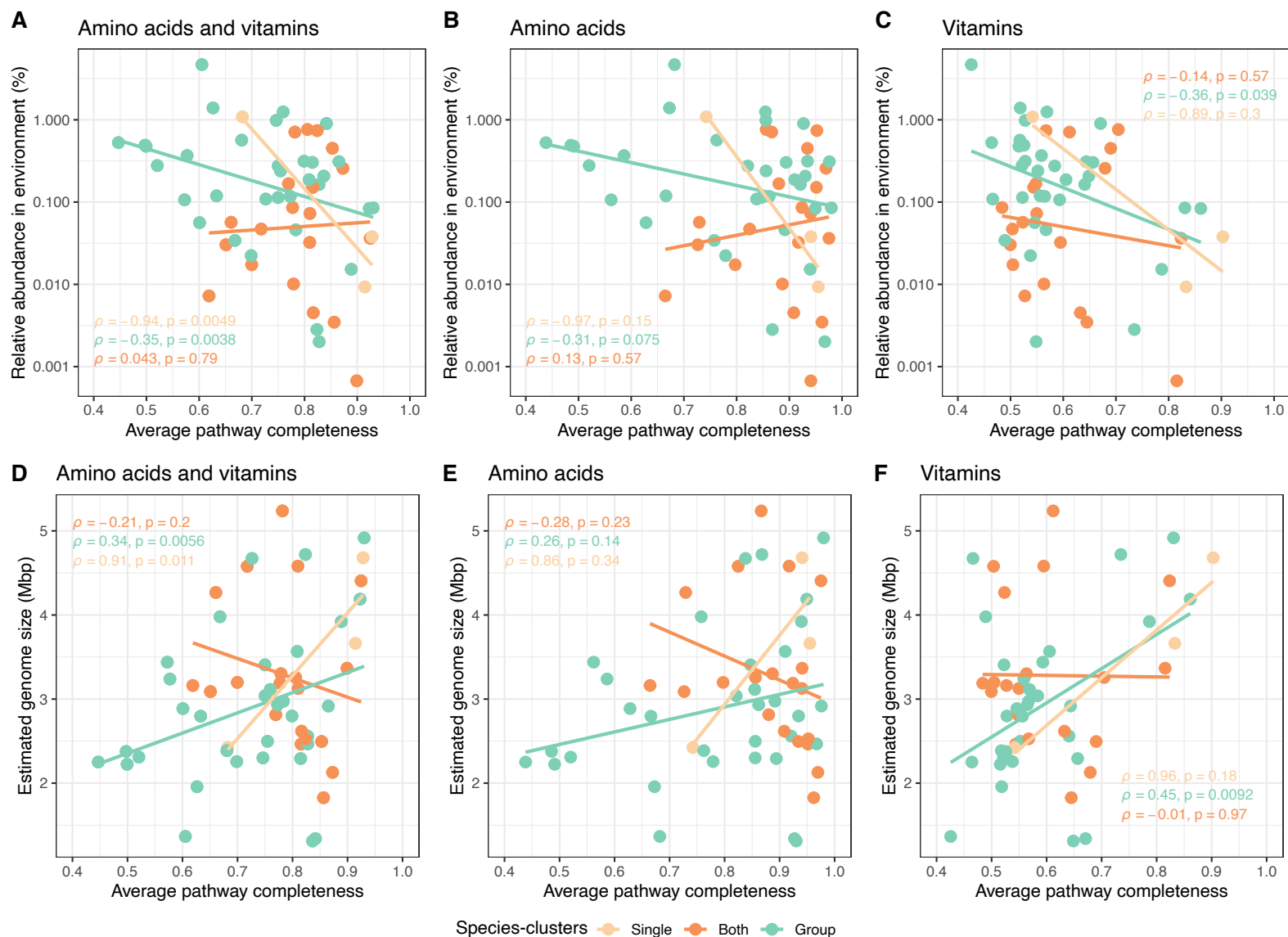
Supplementary Figure 9. Module completeness patterns of cultured genomes across community complexity. (A) Violin plots showing average pathway completeness for custom amino acid and (B) vitamin modules. Each point represents one genome ($n = 305$, >90 % complete, <5 % contaminated) from model communities, compared by community complexity: 1 (light orange), 2 (mint), 3 (green), and >3-species cultures (dark green). Red asterisks indicate modules with the largest relative decreases in completeness in >3-species communities compared to simpler communities.



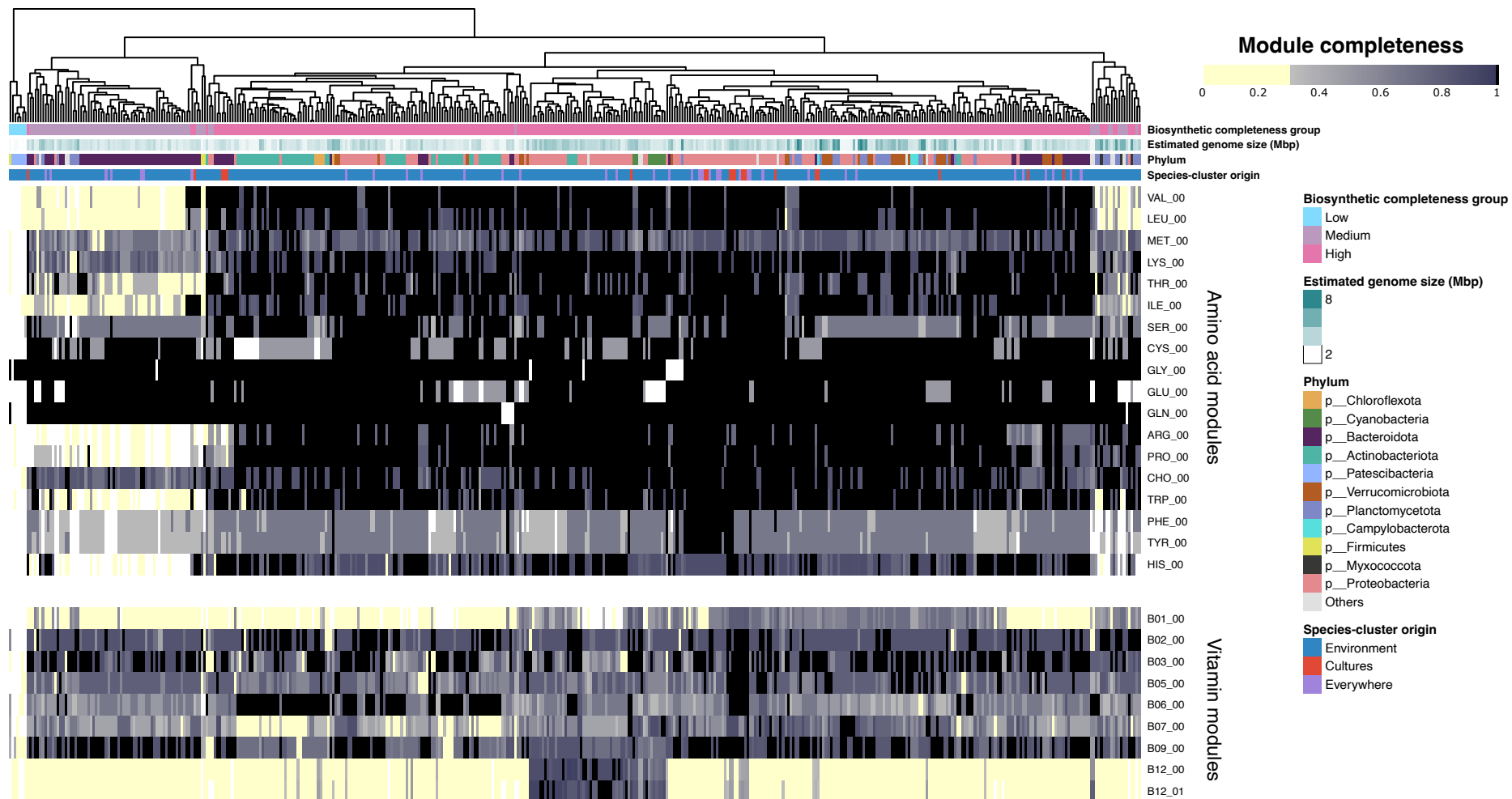
Supplementary Figure 10. Genomic quality metrics of cultured genomes across community complexity. Boxplots comparing high-quality genomes ($n = 305$, $>90\%$ complete, $<5\%$ contaminated) for (A) estimated genome size, (B) genome completeness, (C) genome contamination, (D) number of contigs per genome, (E) N50 of contigs, and (F) predicted genes per Mbp across communities with 1, 2, 3, or >3 species per culture. Statistical significance was tested using the Kruskal-Wallis test ($p < 0.05$), followed by a Dunn's post hoc test with Bonferroni correction. Groups sharing at least one letter (e.g., a and ab) are not significantly different; groups with different letters (e.g., a vs b) differ significantly ($p < 0.05$). Boxplots show the median and interquartile range; whiskers represent $1.5 \times \text{IQR}$.



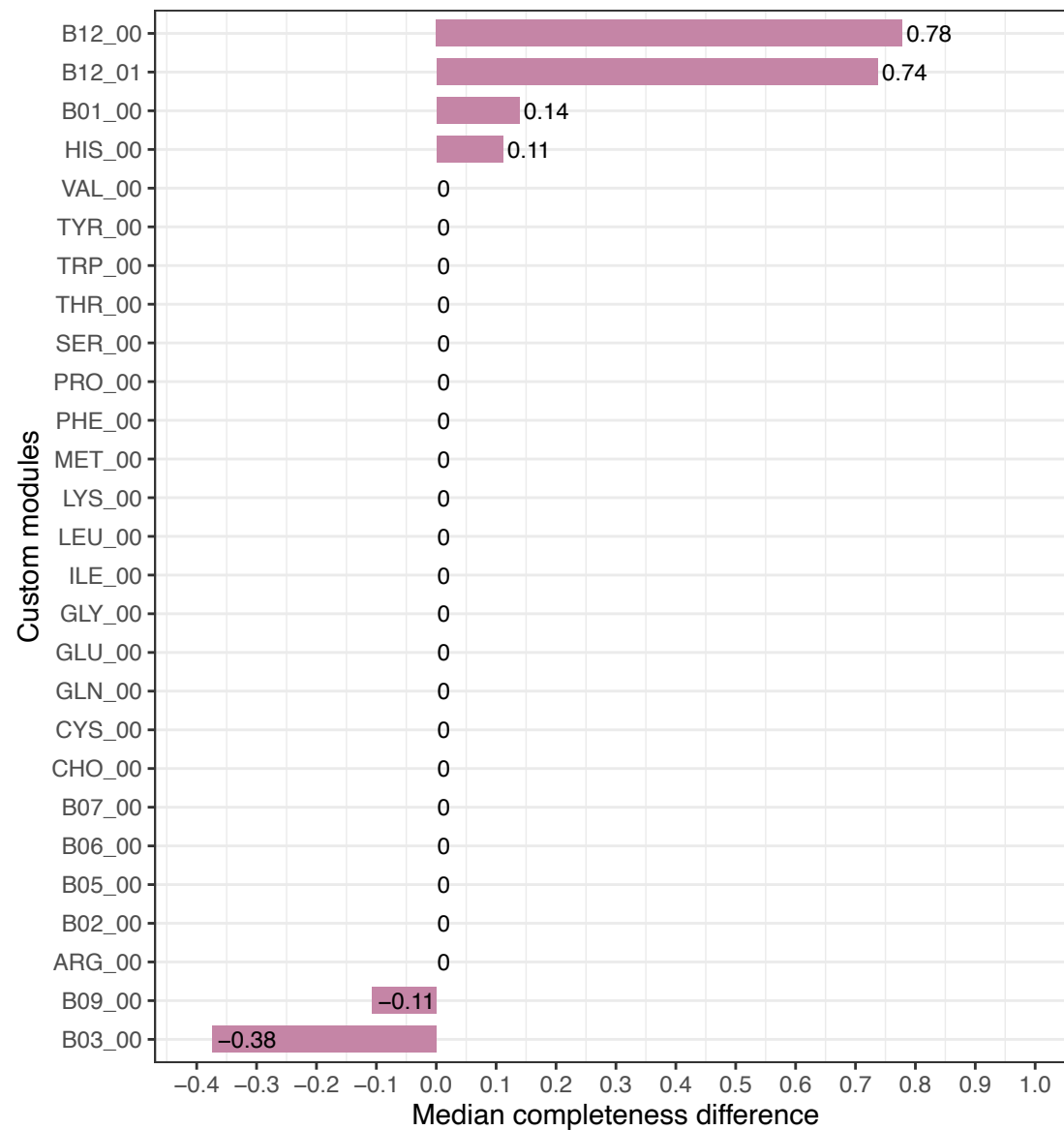
Supplementary Figure 11. Relationship between genome completeness and module pathway completeness in cultured genomes. Scatter plots for (A) custom amino acid biosynthesis modules, and (B) vitamin biosynthesis modules. Each point represents one genome ($n = 305$, >90 % complete, <5 % contaminated), color-coded by community complexity: 1 (light orange), 2 (mint), 3 (green), and >3 species per culture (dark green). Spearman's rank correlations are shown (ρ , p), and trend lines are included for visualization only. Red asterisks indicate modules with significant correlations.



Supplementary Figure 12. Relationship between pathway completeness, relative abundance, and genome size of cultured species-clusters. Scatter plot showing the relationship between average biosynthesis pathway completeness and relative abundance (A, B, and C) and estimated genome size (D, E, and F) for three metabolic categories: Amino acids and vitamins (A, D), only amino acids (B, E), and only vitamins (C, F). Each data point represents a species-cluster ($n = 57$, $>90\%$ completeness, $<5\%$ contamination), which either grew exclusively on its own (light orange), exclusively in groups (green), or both on its own and in groups (orange). Spearman's rank correlations are shown (ρ , p).



Supplementary Figure 13. Biosynthetic potential clustering of the BalticMAG catalog. Heatmap showing the pathway completeness of amino acid and vitamin biosynthesis pathways (based on KEGG custom modules) across high-quality species-clusters ($n = 450$, $>90\%$ completeness, $<5\%$ contamination). The color gradient represents pathway completeness, with white = 0% , yellow = $>0-30\%$, and black = 100% completeness. Genome annotations include biosynthetic completeness group (Low = light blue, Medium = light purple, High = pink), estimated genome size (white to dark teal gradient), taxonomic affiliation (color-coded by phylum), and genomic origin (environment-only = blue, culture-only = red, both = purple). Columns are clustered based on similarity in biosynthetic completeness profiles. Publicly available metagenomes were included in the analysis³⁷⁻³⁹.



Supplementary Figure 14. Differences in biosynthetic module completeness between Cluster A and B genomes. Median completeness differences for KEGG custom biosynthesis modules between Cluster A (n = 51) and Cluster B (n = 308) genomes from the high biosynthetic completeness group. Positive values indicate that modules are more complete in Cluster A, negative values indicate that they are more complete in Cluster B, and zero values indicate no difference in median completeness between the two clusters.

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