Supplementary Information

Cyanophage lysis of cyanobacterium *Nodularia spumigena* affect variability and fitness of host-associated microbiome

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Supplementary Figures

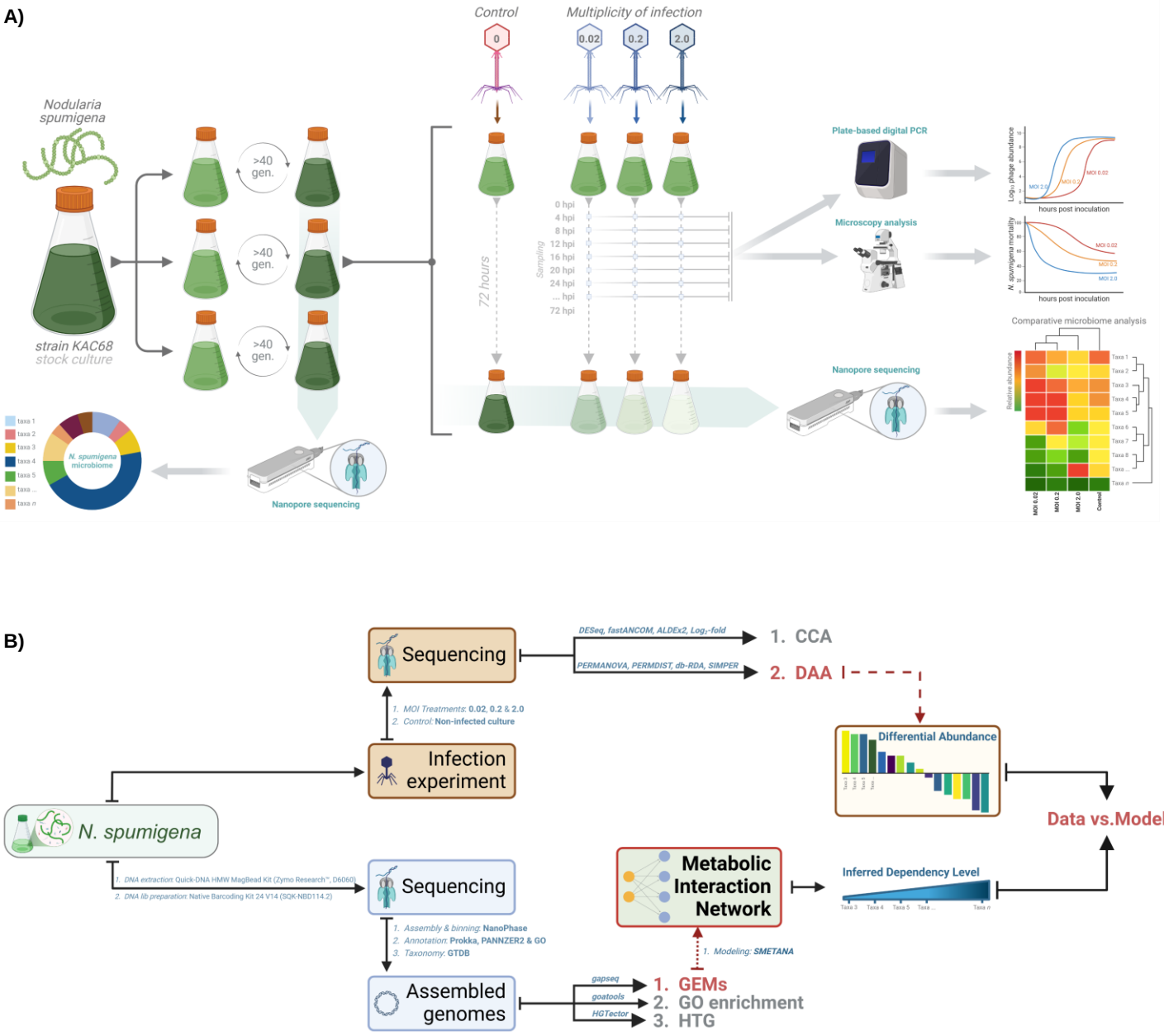


Figure S1. General overview of the conceptual methodological framework and experimental approach of the study. A) Schematic representation of the cyanophage amendment experiment and the analyses conducted; B) Flow chart indicating interactions and information flow between bioinformatics and experimental procedures, including main analyses and their outputs.
Abbreviations: GO – Gene Ontology enrichment analysis; HTG – Horizontal Gene Transfer; GEMs – Genome-scale models; DAA – Differential Abundance Analysis; CCA – Community Composition Analysis.

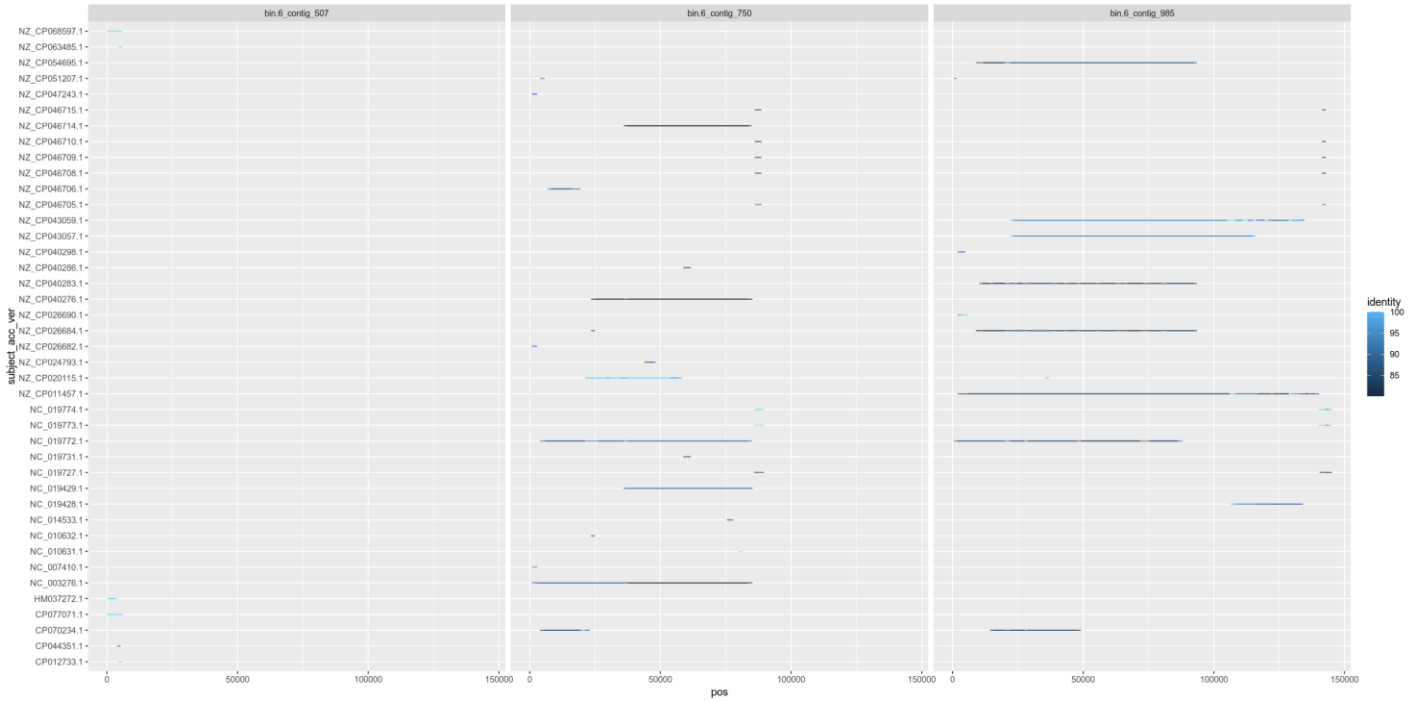


Figure S2. Blast results of putative plasmids (bin.6_contog_507, bin.6_contog_750, bin.6_contog_985) identified in the genome of *Nodularia spumigena*.

Figure S3. Elements of CRISPR-Cas systems identified in the genome of *Nodularia spumigena*.

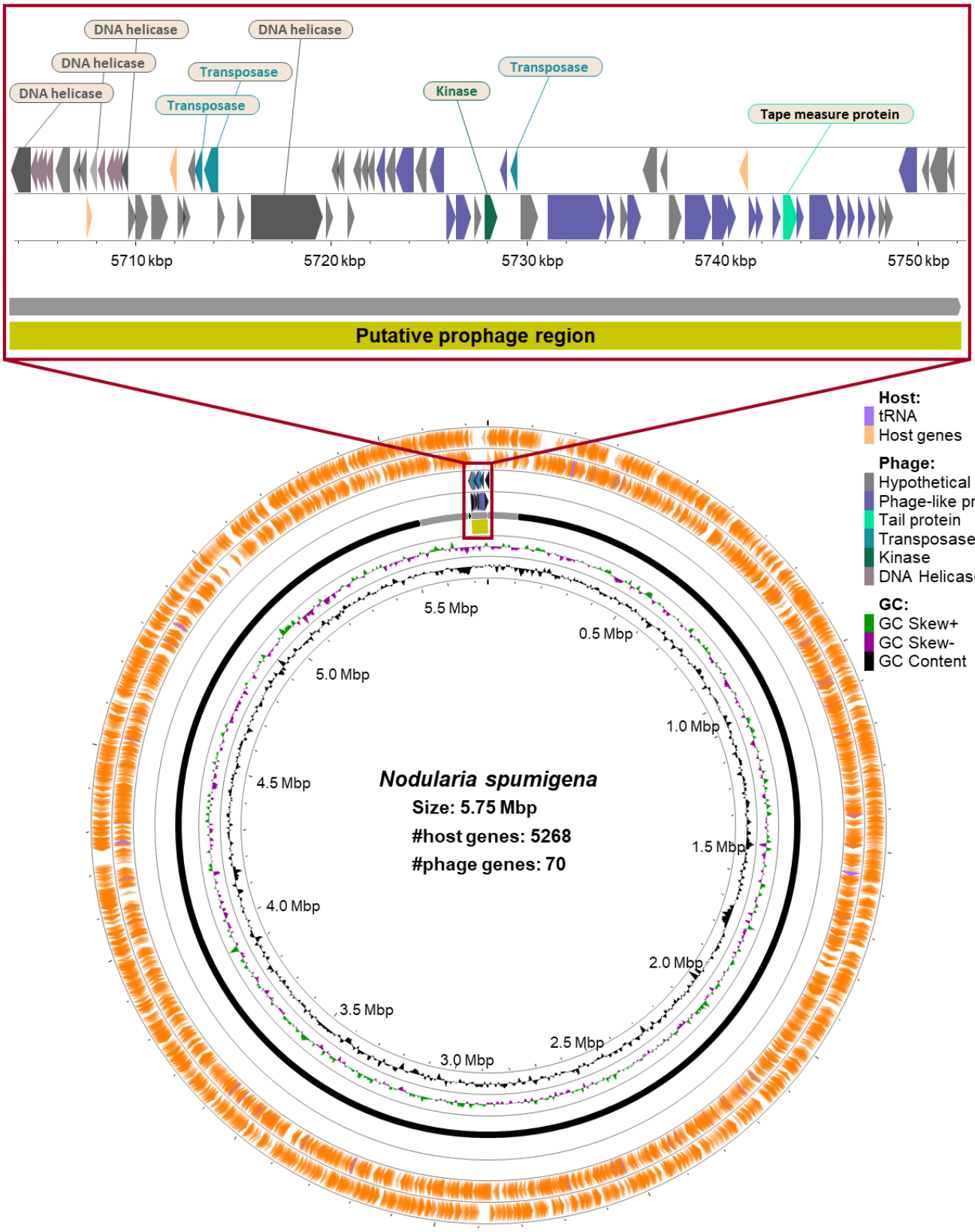


Figure S4. Representation of the highly compact region of cyanophage genes in the genome of *Nodularia spumigena* strain KAC68.

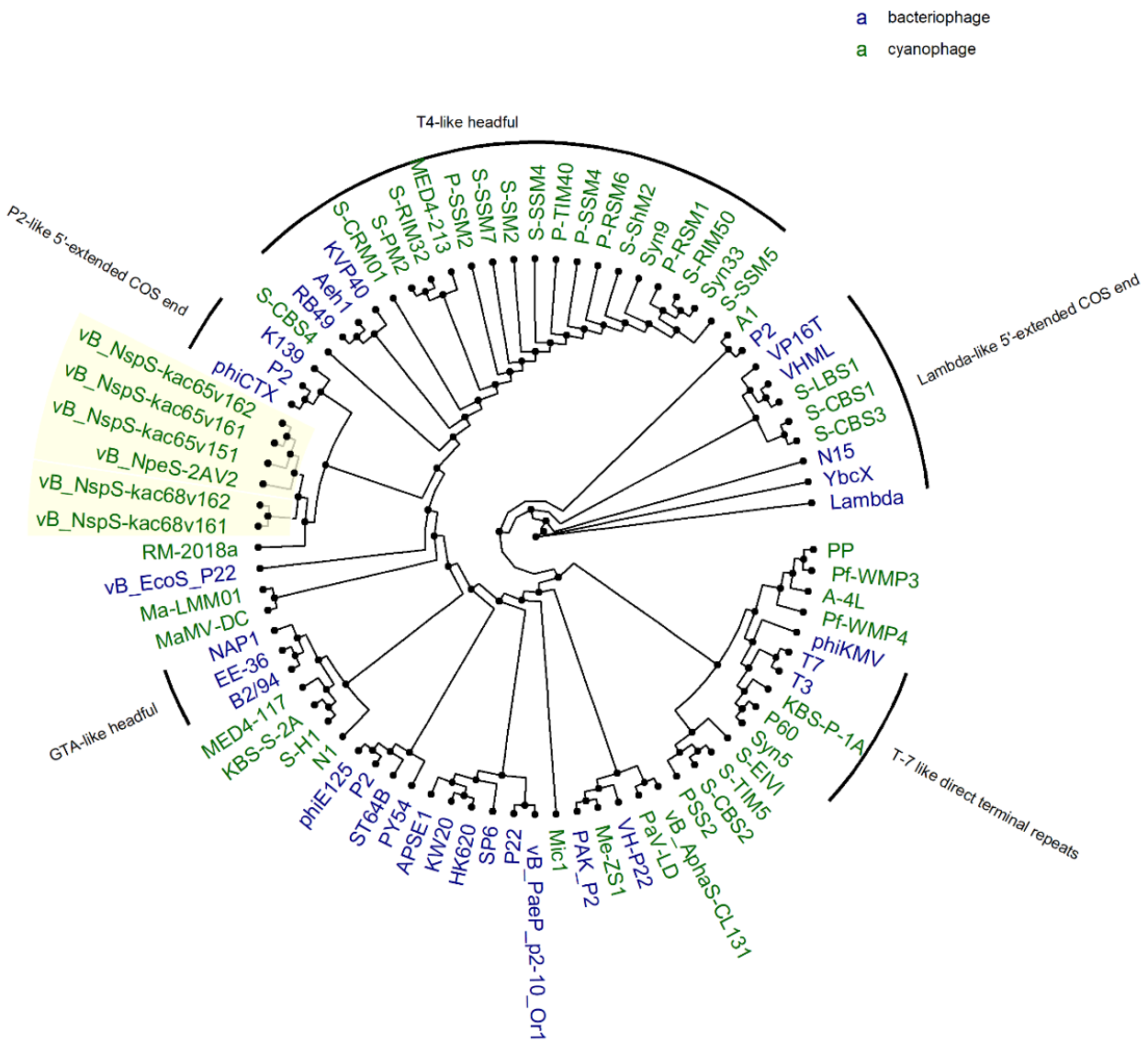


Figure S5. Amino acid sequence-based phylogenetic tree reconstruction of large terminase (TerL) subunit. Blue labels indicate bacteriophages infecting various heterotrophic bacteria hosts, while green labels indicate bacteriophages infecting cyanobacteria. Cluster of cyanophages infecting *Nodularia spumigena* is coloured in yellow. DNA packaging mechanisms is indicated for some bacteriophages.

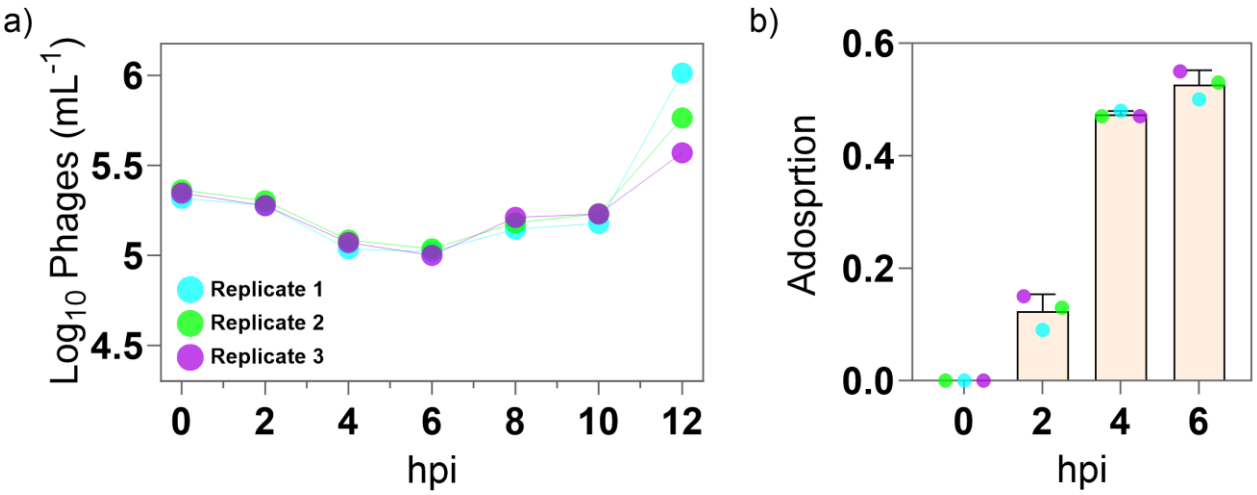
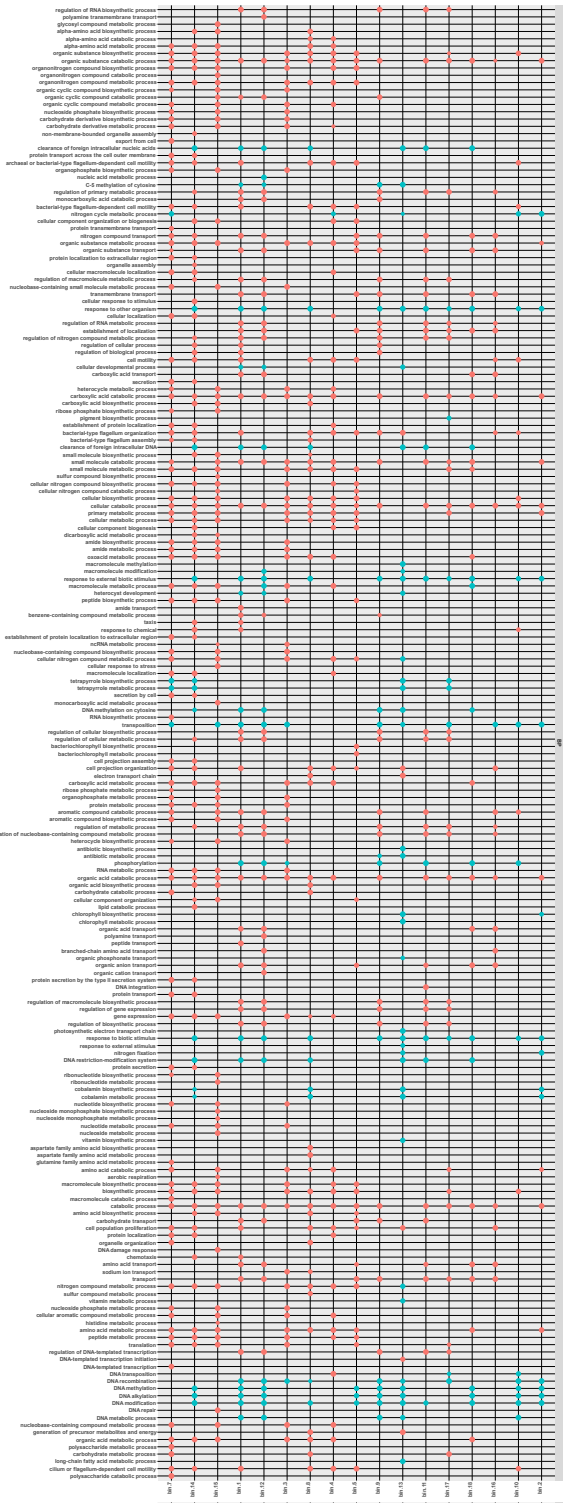
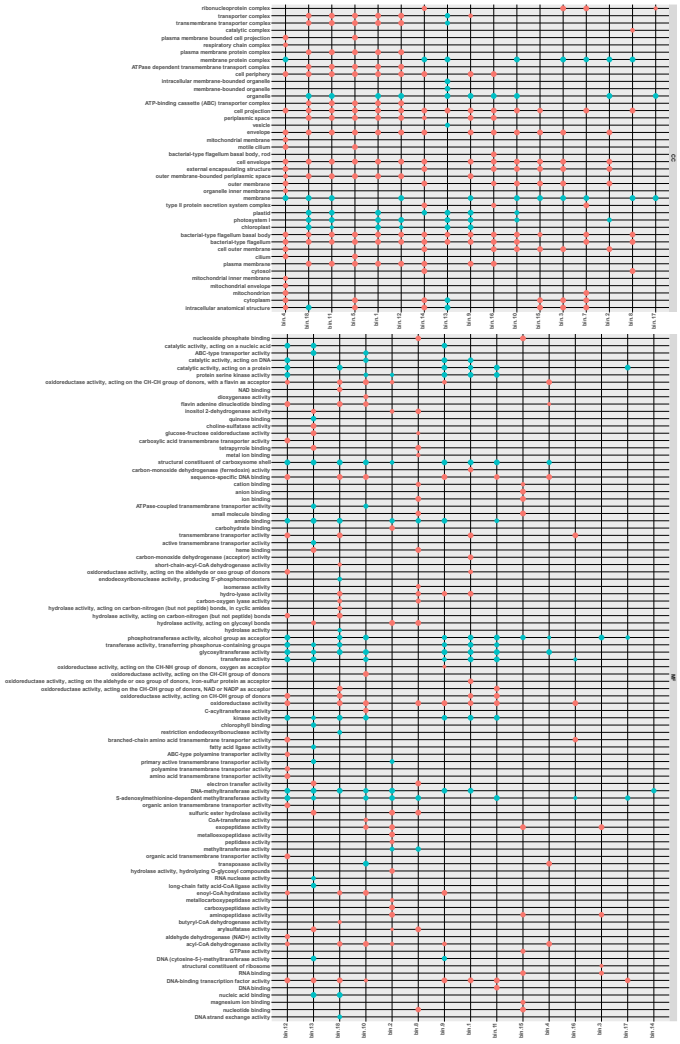


Figure S6. Adsorption curve of the cyanophage *Ravarandavirus* kac68v161 to *Nodularia spumigena* strain KAC68 cells (a) and changes in the proportion of adsorbed cyanophages during first 6 hours post inoculation (b).





enrichment

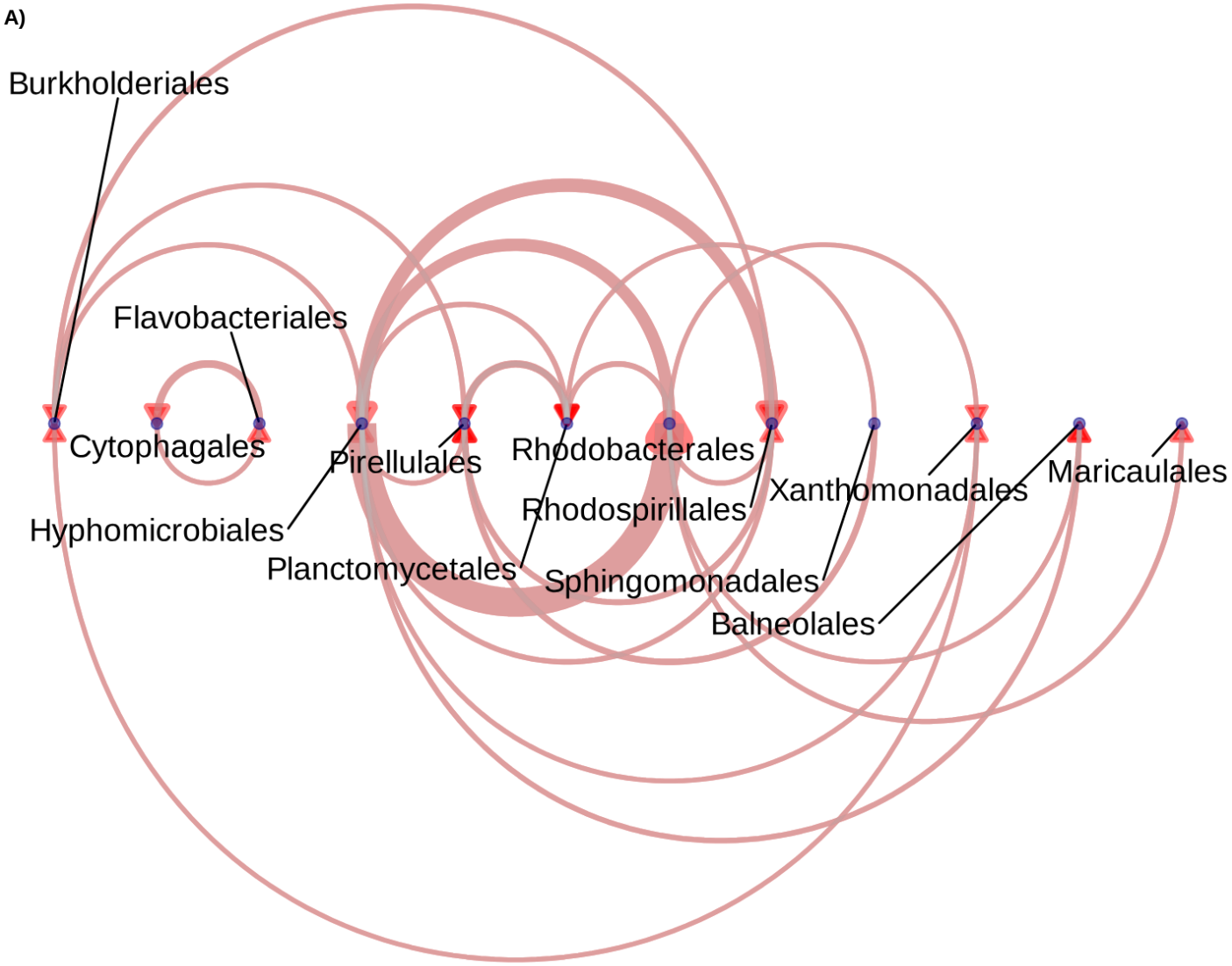
- e
- p

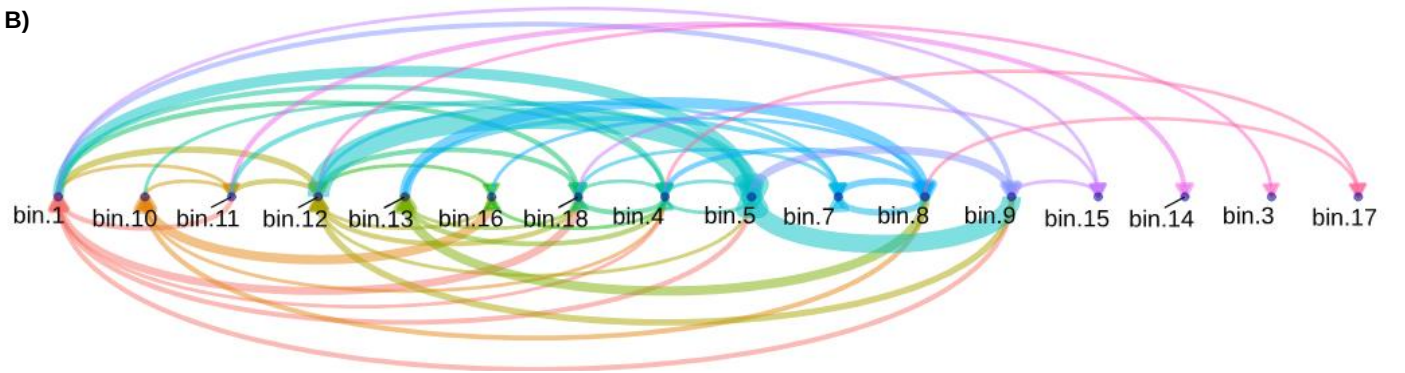
p_fdr_bh

- 0.0025
- 0.0050
- 0.0075

bin.1	Rhizobiales	bin.5	Rhizobiales
bin.2	Cytophagales	bin.6	Nostocales
bin.3	Flavobacteriales	bin.7	Phycisphaerales
bin.4	Caulobacterales	bin.8	Pirellulales
bin.9	Rhizobiales	bin.13	Planctomycetales
bin.10	Sphingomonadales	bin.14	Xanthomonadales
bin.11	Rhodobacterales	bin.15	Balneolales
bin.12	Rhizobiales	bin.16	Burkholderiales
bin.17	Actinomycetales		
bin.18	Thalassobaculales		

Figure S7. Gene enrichments in heterotrophic bacteria comprising the microbiome of *Nodularia spumigena* strain KAC68. Only terms with adjusted *p* - values calculated using the Benjamini-Hochberg procedure (p_fdr_bh) < 0.01 are shown. Commonly purified terms detected as purified in more than 70% of non-*N. spumigena* bins are not shown (e.g., GO terms related to photosynthesis). The terms' names are arranged based on the matching GO ID. The bins on the x-axis are arranged from left to right based on the decreasing number of significant differences. Red color denotes enrichment of a term, while cyan color denotes purification of a term. The dot size denotes the *p*-value.





bin.1	Rhizobiales	bin.5	Rhizobiales	bin.9	Rhizobiales	bin.13	Planctomycetales	bin.17	Actinomycetales
bin.2	Cytophagales	bin.6	Nostocales	bin.10	Sphingomonadales	bin.14	Xanthomonadales	bin.18	Thalassobaculales
bin.3	Flavobacteriales	bin.7	Phycisphaerales	bin.11	Rhodobacterales	bin.15	Balneolales		
bin.4	Caulobacterales	bin.8	Pirellulales	bin.12	Rhizobiales	bin.16	Burkholderiales		

Figure S8. Representation of horizontal gene transfer (HGT) between bacteria. A) searched against NCBI database (accessed April 2024); B) search only between bacteria within *N. spumigena* microbiome.

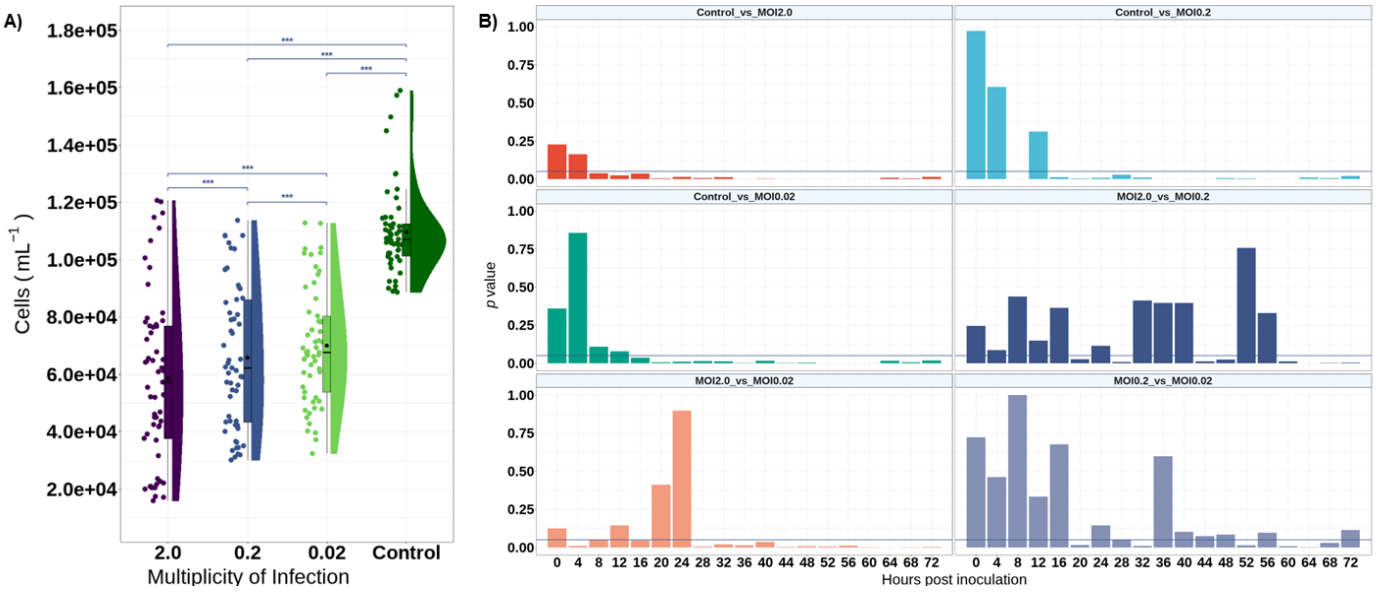


Figure S9. The ANOVA results indicating effect of cyanophage infection on the variance of *N. spumigena* abundance between different MOI treatments (A), and changes in *p* values derived from multiple *post hoc* Tukey's test comparison representing significant differences in timing of *N. spumigena* cell lysis between different treatment pairs (B). Signif.: 0.001<*, 0.01<**, 0.05<*.**

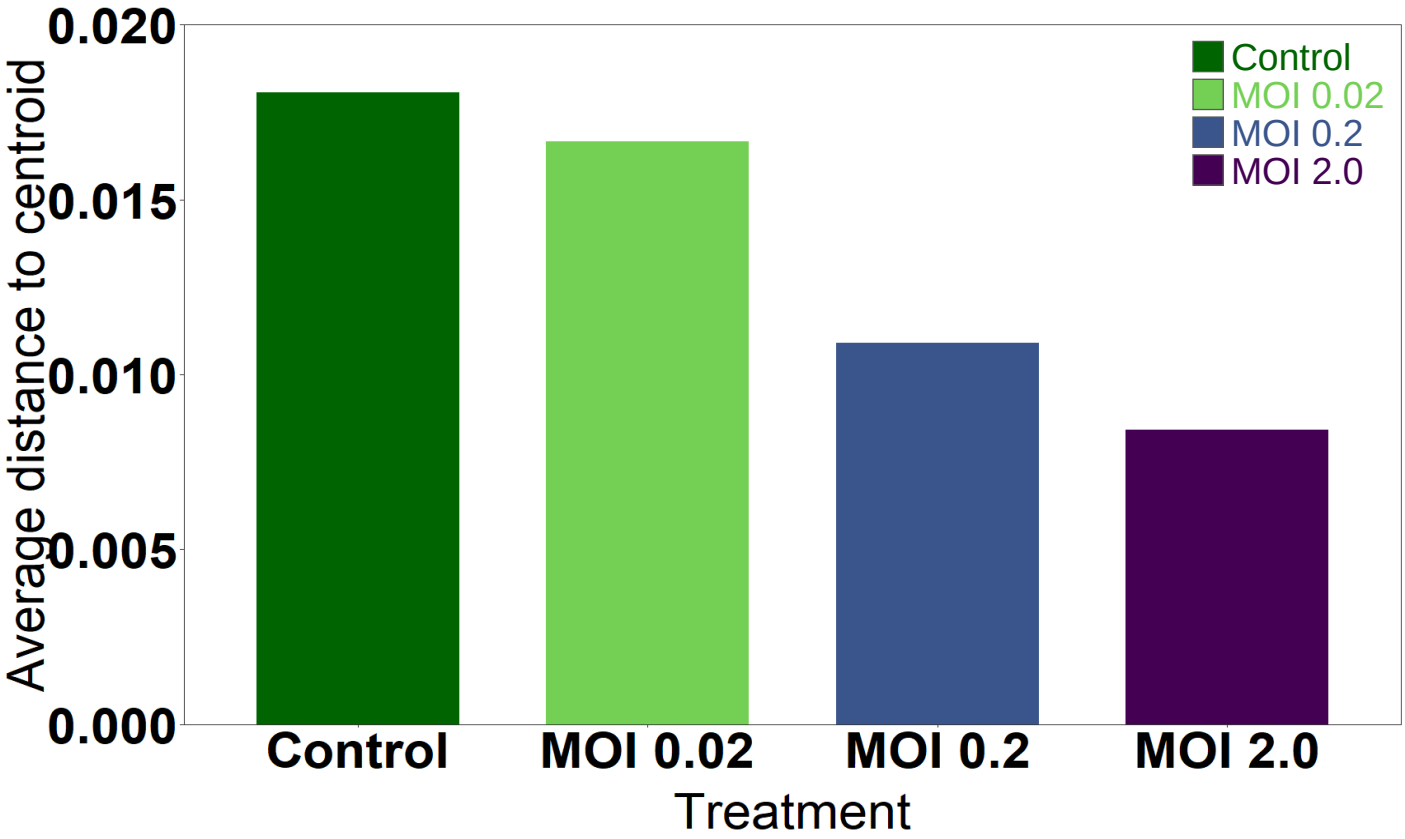


Figure S10. The PERMDIST results indicating changes in variability of relative taxa abundances between biological replicates.

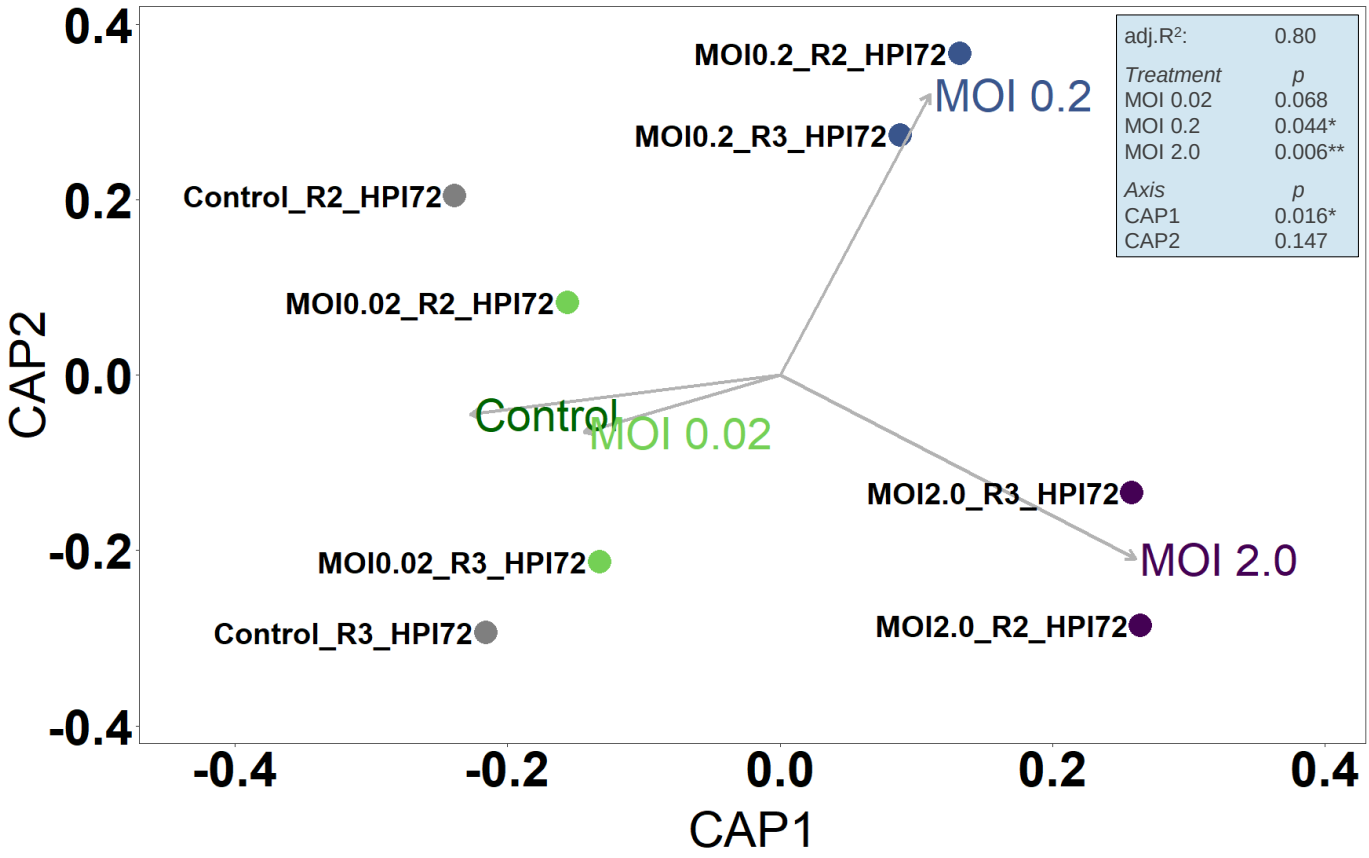


Figure S11. The db-RDA plot representing the relatedness between initial multiplicity of infection (MOI) and microbiome composition. R² indicates the variability (range from 0 to 1) in the data explained by the MOI. The *p* values for Treatment represent the statistical significance of each MOI on the shift in the community composition. The *p* values for canonical axes (CAP) indicate the significance of each dimension in differentiating the variance in the data attributed to the MOI.