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Supplementary Materials for

Inherent tendency of *Synechococcus* and heterotrophic bacteria for mutualism on long-term coexistence despite environmental interference

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Supplementary Text

Synechococcus sp. PCC7002 sterility

The sterility of *Synechococcus* sp. PCC7002 was tested throughout the experimental period as described previously (*3*). Through microscopy and flow cytometry analysis, it was found that other than *Synechococcus* sp. PCC7002, no other algae were present in the coculture systems at any point during the experimental period. The algal cells that we observed were isolated and were verified to be *Synechococcus* sp. PCC7002 via 16S rRNA gene analysis.

Synechococcus sp. PCC7002 viability at P-VI

Before determining the growth-limiting nutrients (Fig. 3B of the main text) within the coculture systems, we tested if the cells could be revived by providing fresh inorganic nutrients. The culturing conditions were kept the same as that of coculture I and II. On inoculation with SN media, the cells from day 250 (P-VI) of the chlorotic coculture systems (CC-I and CC-II) turned visually green within 20 days of incubation. However, the axenic coculture group did not show any visible color change (Supplementary Fig. S9).

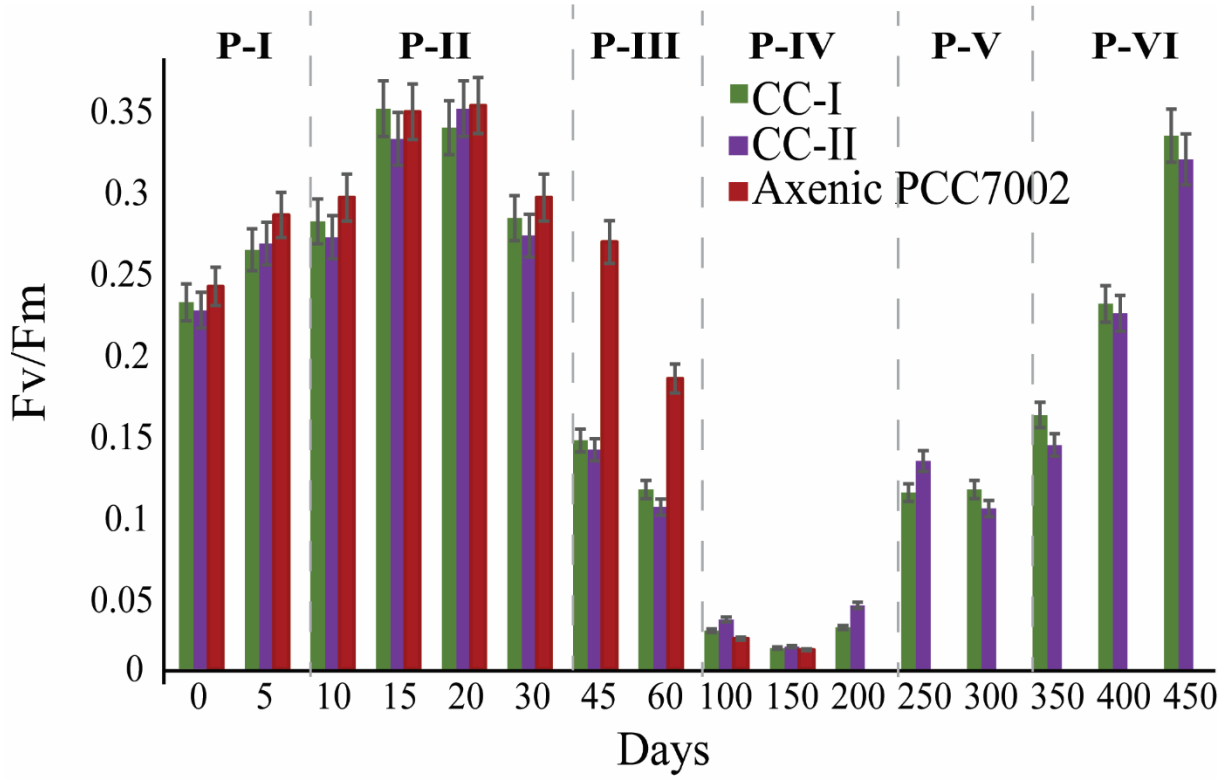

Fig. S1: Fv/Fm value of the coculture systems during the six phases (i.e., P-I to P-VI).

Each data point is an average of two replicates. Error bars indicate the mean standard error. CC-I = coculture-I, CC-II = coculture-II.

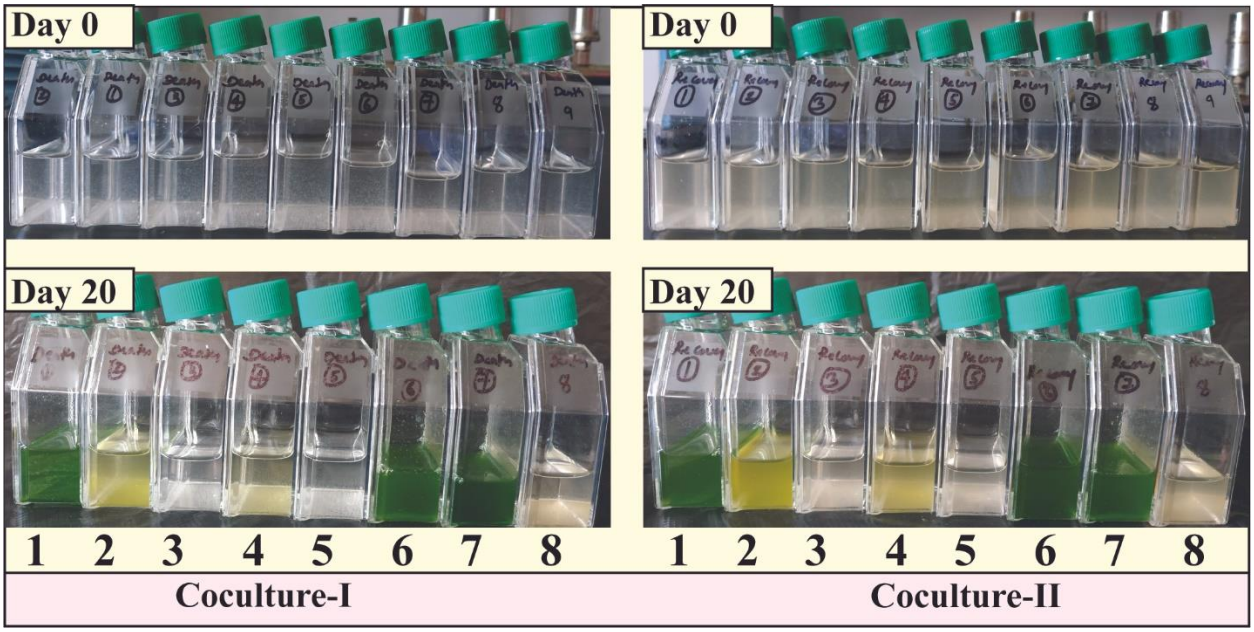

Fig. S2: Influence of different nutrient constituents on chlorotic *Synechococcus* cells from P-V.

The numbers below the images correspond to the added inorganic nutrients constituents: (1) NO_3^-+PO_4^{3-}+Fe^{3+}, (2) NO_3^-+ Fe^{3+}, (3) PO_4^{3-}+ Fe^{3+}, (4) NO_3^-, (5) PO_4^{3-}, (6) SN media, (7) A+ media and (8) Fe^{3+}.

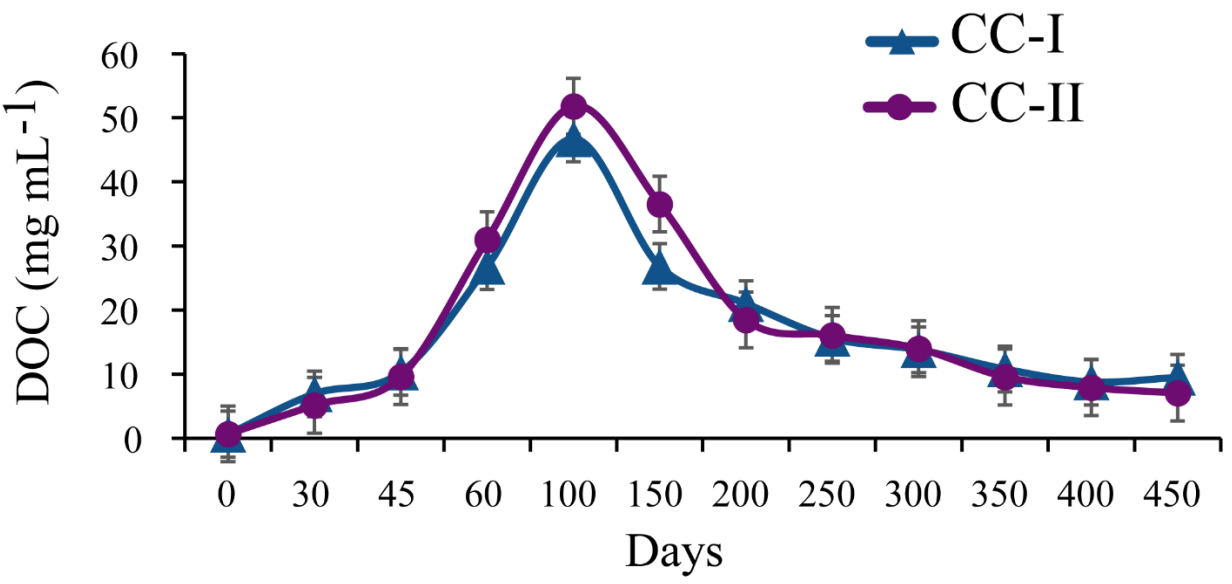

Fig. S3: Changes in the dissolved organic carbon (DOC) content within the *Synechococcus*-heterotrophic bacteria coculture systems over a period of 450 days.

The DOC concentration is expressed as mg mL^{-1}. Each data point is an average of two replicates, with error bars showing the mean standard error.

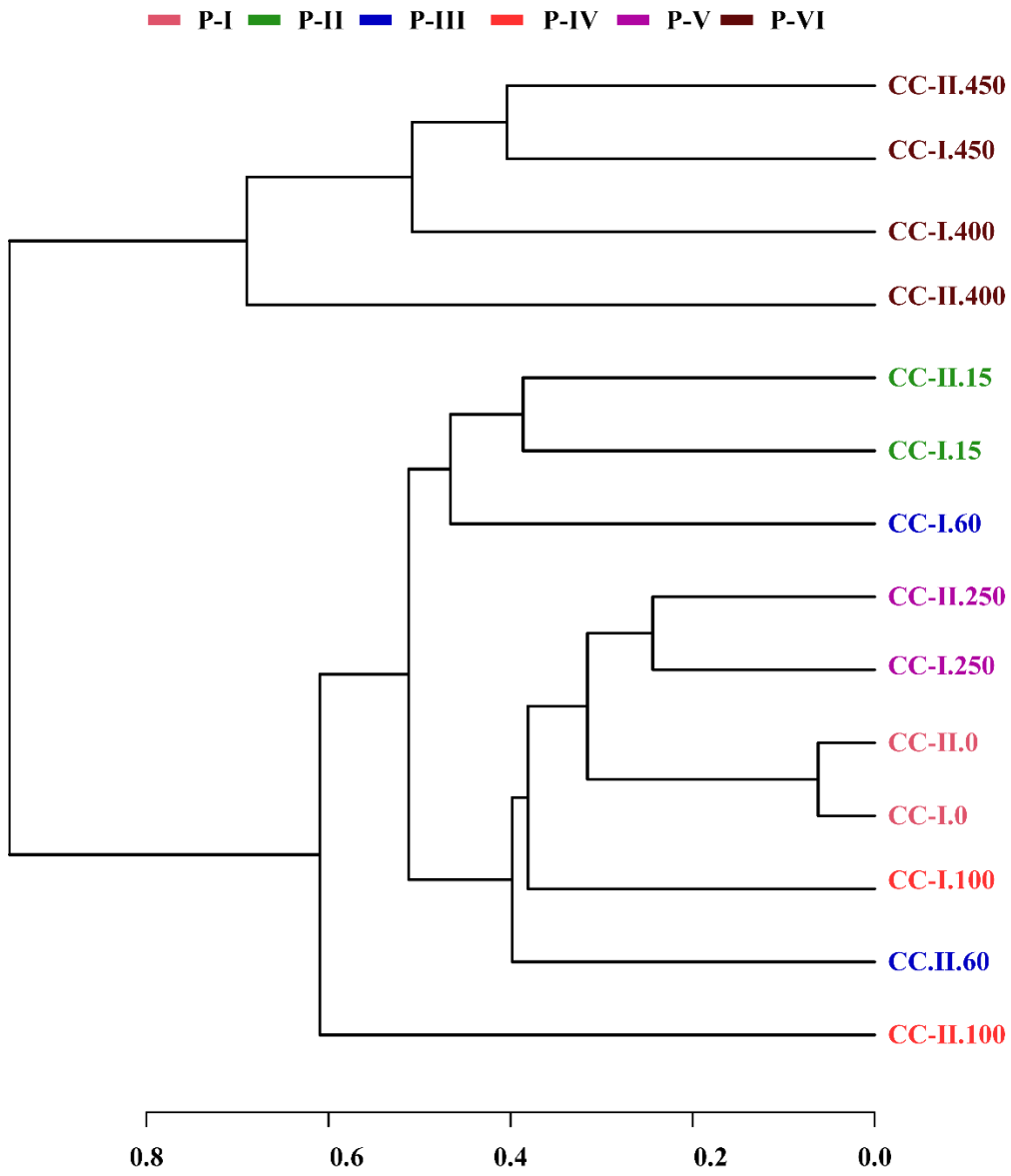

Fig. S4: Bray-Curtis dissimilarity clustering of the associated heterotrophic bacterial community during the six phases (i.e., P-I to P-VI).

Hierarchical clustering was determined via the Bray-Curtis dissimilarity test (the average linkage method was applied at the ASV level).

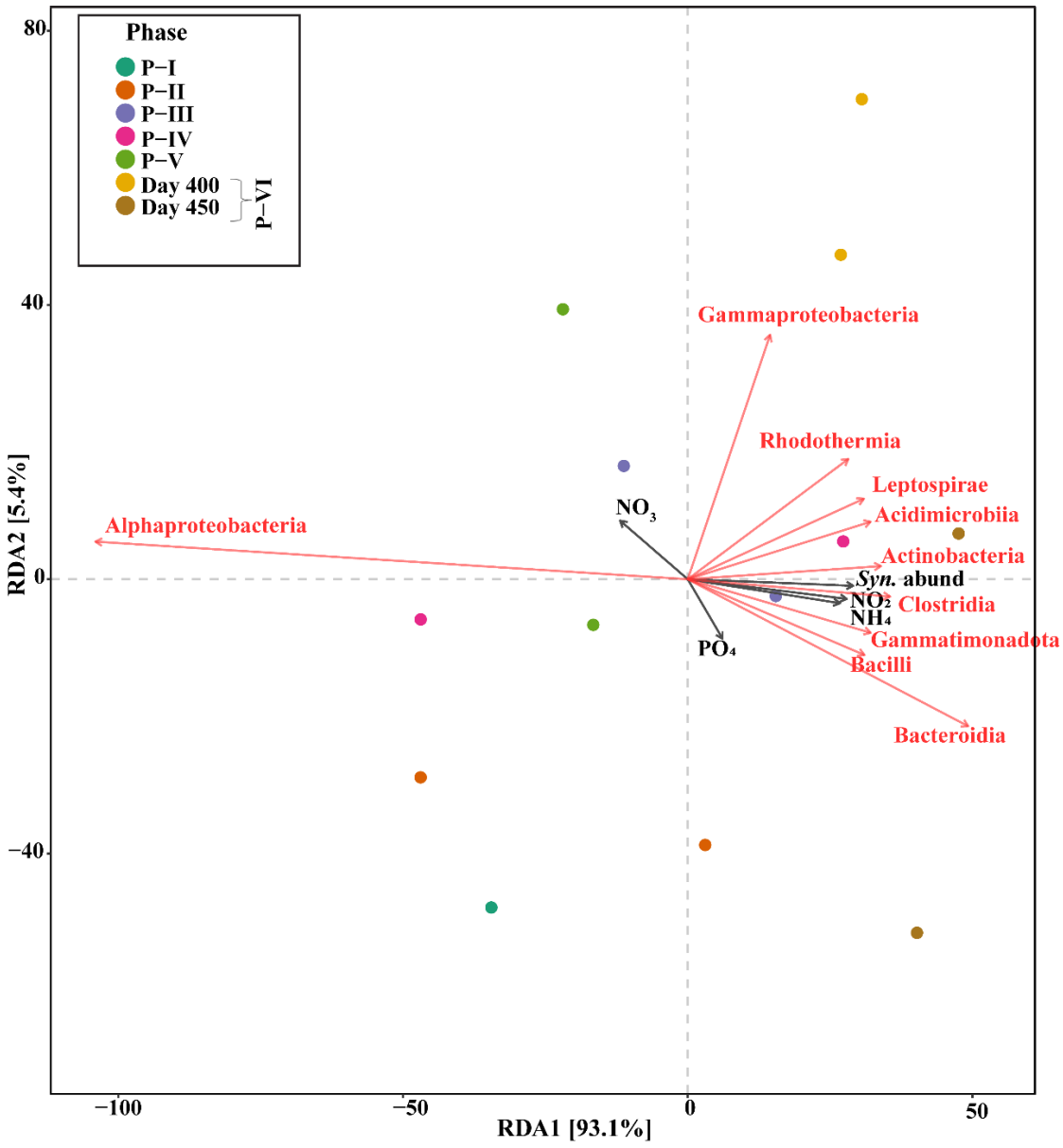

Fig. S5: Redundancy discriminant analysis (RDA).

RDA plot illustrating the relationship between the dominant bacterial classes (identified from 16S analysis), inorganic nutrients (NO_2^-, NO_3^-, NH_4^+ and PO_4^{3-}) and *Synechococcus* abundance.

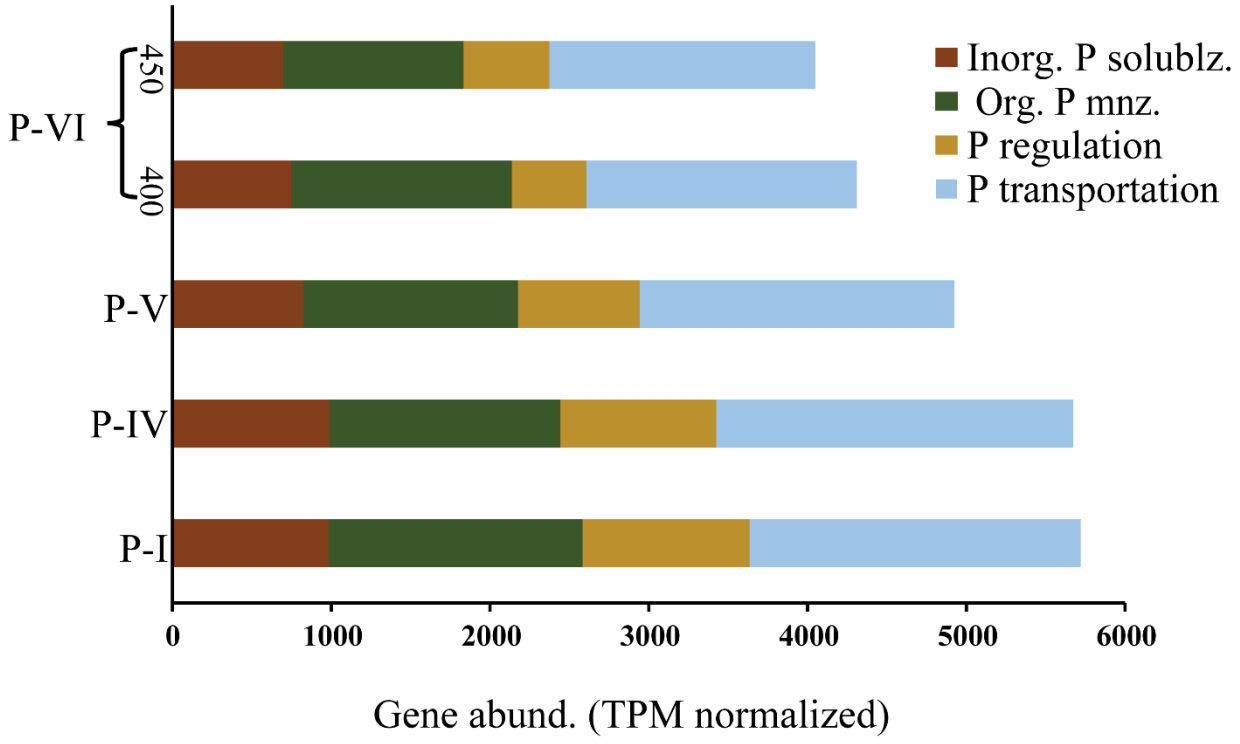

Fig. S6: Phosphorus cycle potential of the heterotrophic bacterial community.

Gene abundance (TPM normalized) of all detected genes from the phosphorus cycle within the analyzed metagenomic samples.

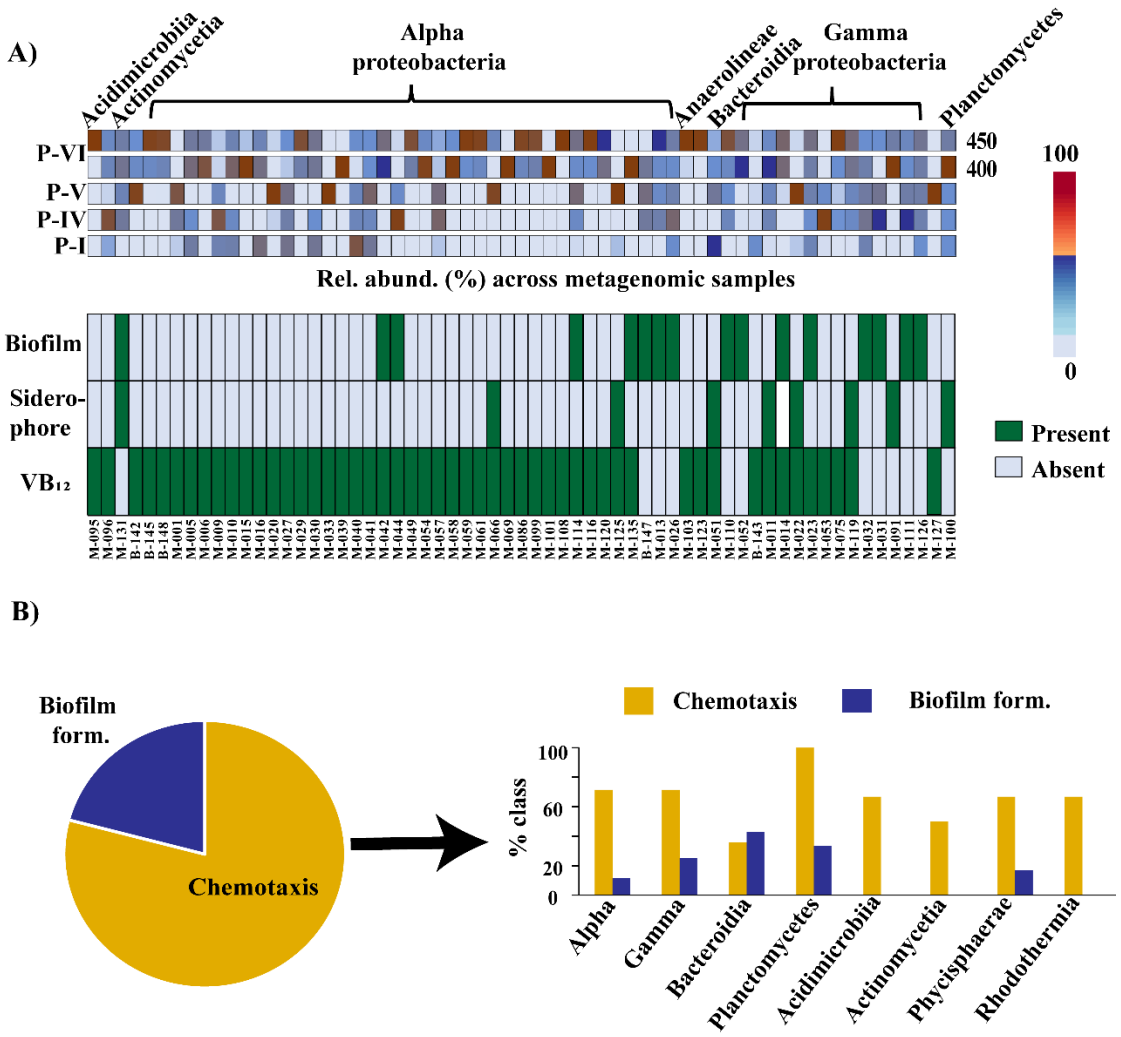

Fig. S7: Vitamin B_{12}, Fe- siderophore synthesis and biofilm formation potential among the assembled bins.

(A) Heatmap representation of the detected genes related to vitamin B_{12} (VB_{12}), Fe-siderophore synthesis and biofilm formation among the assembled bins and the relative abundance of these bins across the analyzed metagenomic samples. **(B)** Pie-chart showing the percentage of bins possessing the genes for chemotaxis and biofilm formation. The percentage of taxonomic classes (from the assembled bins) carrying these genes is shown on the right side.

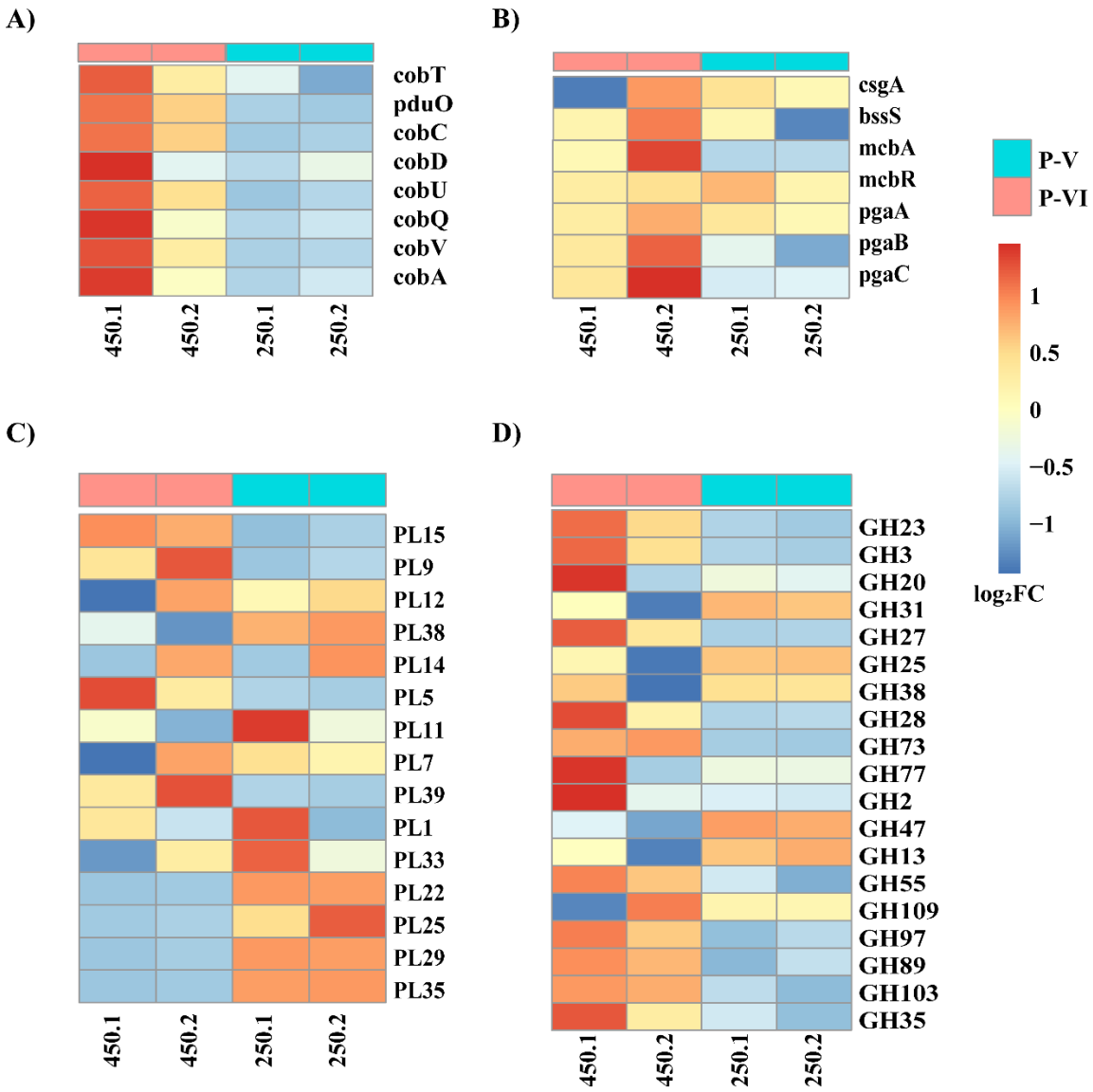

Fig. S8: Differential gene expression among the P-V and P-VI metatranscriptomic samples.

Heatmap representation of the differentially expressed genes (adjusted *p*-value of 0.05) related to **(A)** vitamin B_{12} synthesis **(B)** biofilm formation **(C)** CAZy PL family and **(D)** CAZy GH family.

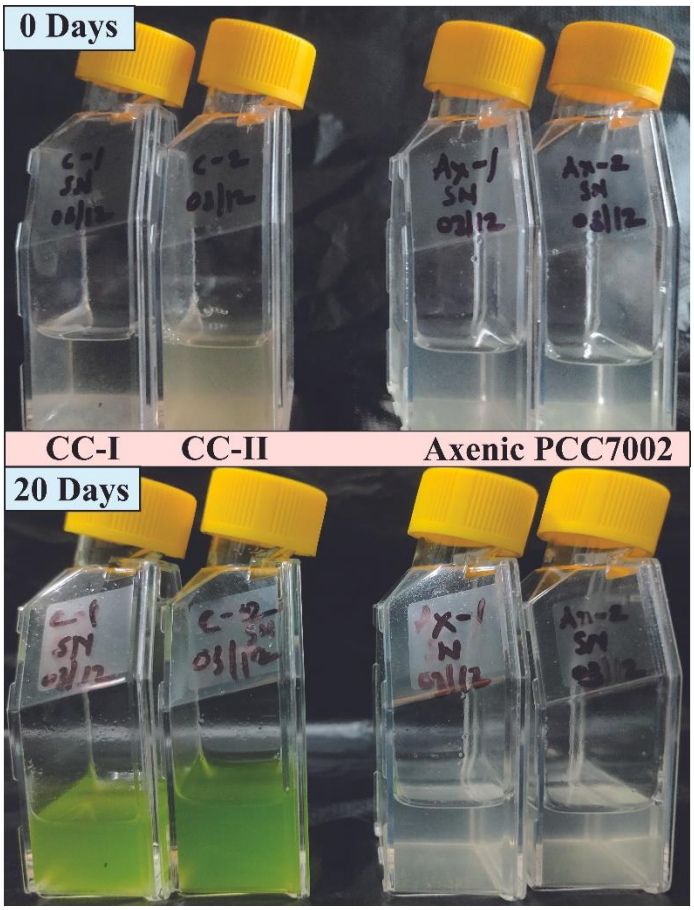

Fig. S9: Changes in the 250 days old coculture I and II and axenic PCC7002 I and II culture systems on the addition of inorganic nutrients (SN media).

The Supplementary Tables include:

1. **Table S1:** Chemical composition of the modified FSW.
2. **Table S2:** Effect of inorganic nutrients on chlorotic *Synechococcus* cells.
3. **Table S3:** Read statistics of metagenomic and metatranscriptomic sequences.
4. **Table S4:** Gene abundance (TPM normalized) of metagenomic sequences related to the nitrogen cycle.
5. **Table S5:** Gene abundance (TPM normalized) of metagenomic sequences related to phosphorus cycle.
6. **Table S6:** Gene abundance (TPM normalized) of metagenomic sequences related to vitamin B_{12} (cobalamin) synthesis.
7. **Table S7:** Gene abundance (TPM normalized) of metagenomic sequences related to siderophore synthesis.
8. **Table S8:** Information regarding the assembled bins.
9. **Table S9:** Bin functional potential.
10. **Table S10:** Relative abundance of bins possessing nitrogen cycling potential.
11. **Table S11:** Gene abundance (TPM normalized) of metagenomic sequences related to the CAZy family.
12. **Table S12:** Gene abundance (TPM normalized) of metagenomic sequences related to the Bacterial secretory system.
13. **Table S13:** Gene abundance (TPM normalized) of metagenomic sequences related to QS and QQ.
14. **Table S14:** Gene abundance (TPM normalized) of metagenomic sequences related to biofilm formation and adhesion and chemotaxis.

15. **Table S15:** RT-qPCR validation of *nifH* expression in coculture I and II at P-VI (450 days culture).
16. **Table S16:** Reads kept at each step of DADA2 processing of amplicon sequencing.