

Characterization of the *Chlamydomonas reinhardtii* phycosphere reveals conserved features of the plant microbiota

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Supplementary Figures

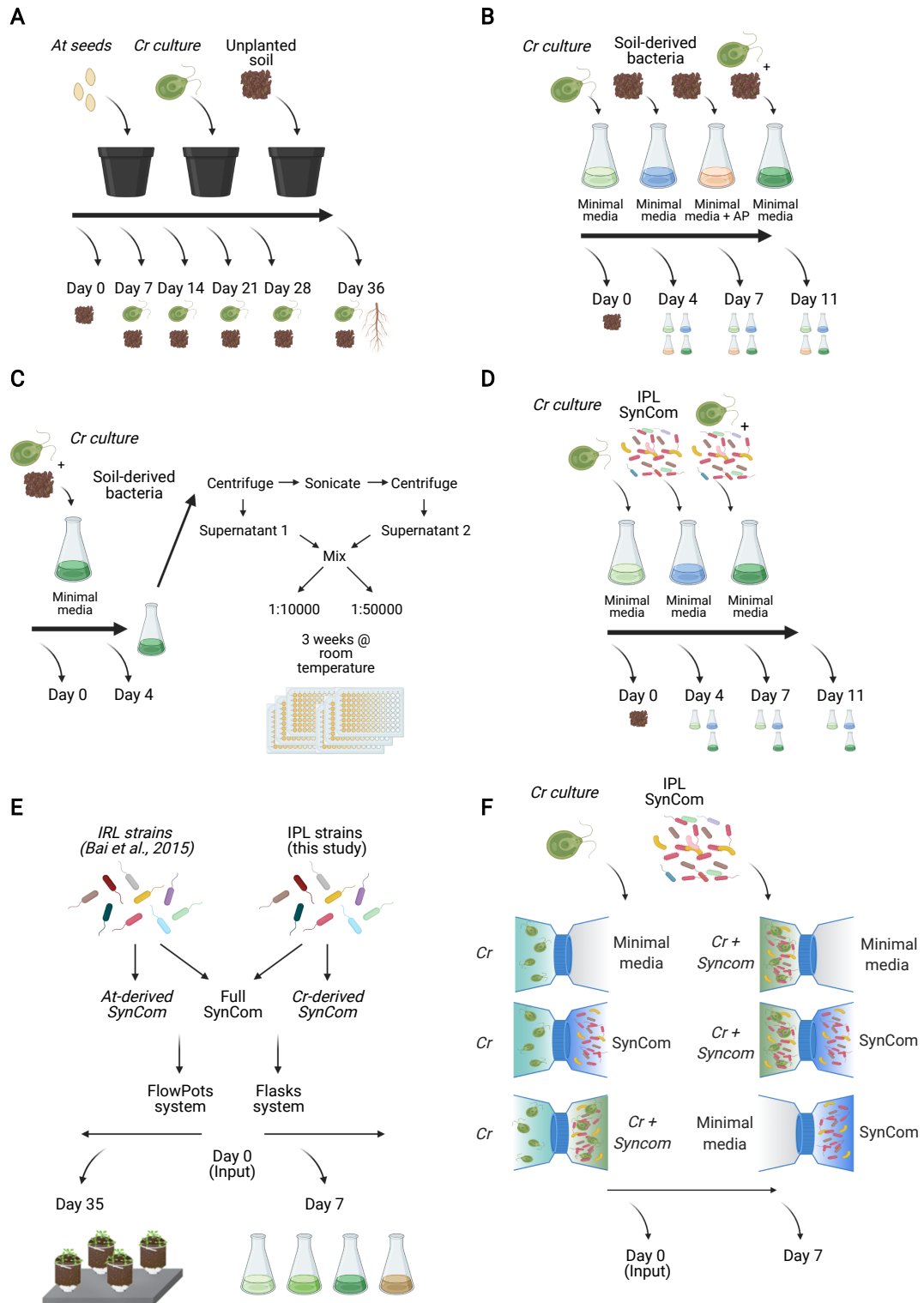


Figure S1. Schematic description of the experimental approaches employed in this study (caption on next page).

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(A) Greenhouse experiment. Pots containing CAS natural soil were either sown with *At* seeds, inoculated with *Cr* cultures or mock-treated. Samples were taken over time for bacterial community profiling. (B) Mesocosm experiments. In different liquid media (minimal media or AP-containing media), *Cr* cultures were co-incubated with soil-derived bacteria. Samples were taken over time for bacterial community profiling and assessment of *Cr* growth. (C) Protocol for the establishment of the *Cr*-IPL bacterial culture collection generation. *Cr* cultures were co-incubated with soil-derived bacteria for 7 days and fractionated to enrich for phycosphere bacteria. This phycosphere fraction was then diluted and incubated for several weeks in 96-well plates. Subsequently, bacterial cultures were subjected to 16S rRNA amplicon profiling for further analysis (D) SynCom reconstitution experiment. From the core culture *Cr*-SPHERE collection, 26 representative strains were selected, pooled together and co-incubated with *Cr* in minimal media for 7 days. (E) Cross-inoculation experiment. One bacterial strain per shared bacterial family from the *At*-SPHERE and *Cr*-SPHERE were selected and assembled into root or phycosphere SynComs. These SynComs were cross-inoculated in *At* and *Cr* in two gnotobiotic systems (i.e. soil-based FlowPots and liquid-based flasks), individually or in a mixed community. Root and phycosphere samples were harvested after 5 weeks of co-incubation for community profiling and assessment of *Cr* growth. (F) Split co-cultivation system. Co-cultivation chamber separated by a 0.22 μ m filter were used to inoculate *Cr*, the phycosphere SynCom alone or both together in different combinations. Samples were harvested after 7 days for community profiling and *Cr* growth measurements.

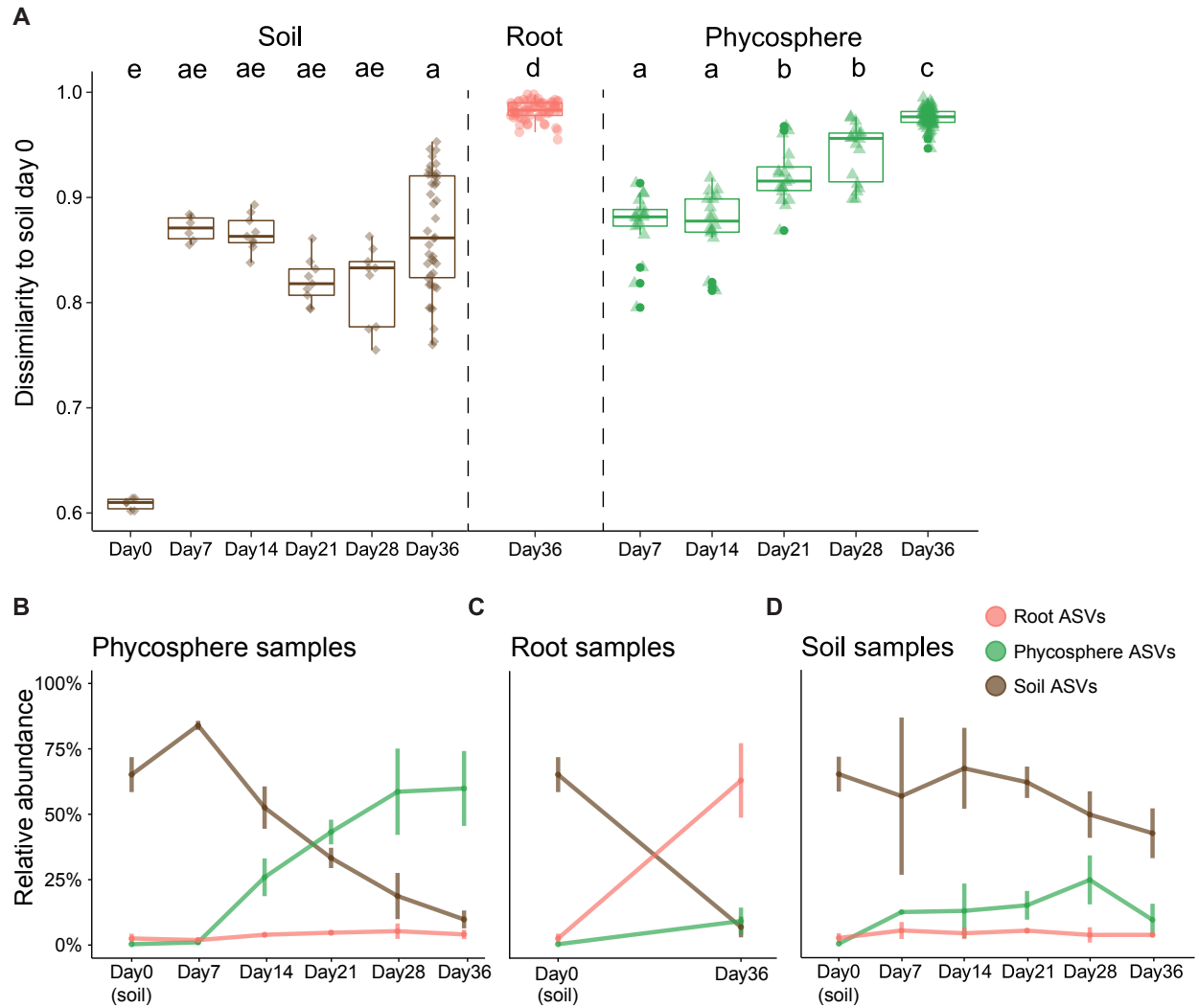


Figure S2. Culture-independent analysis of phycosphere- and root-associated communities in a natural soil.

(A) Bray-Curtis dissimilarities between phycosphere, root and soil communities, compared to the initial soil input (day 0). Boxplots are color-coded depending on the fraction. Significant differences are marked with different letters (Kruskal-Wallis, followed by a Dunn's *post hoc*, with Bonferroni correction). (B-D) Dynamics of relative abundances of ASVs enriched in phycosphere (B), root (C) or soil samples (D) over time, compared to initial soil input (day 0; Wilcoxon test; $P < 0.05$). Curves are color-coded depending on the fraction indicated.

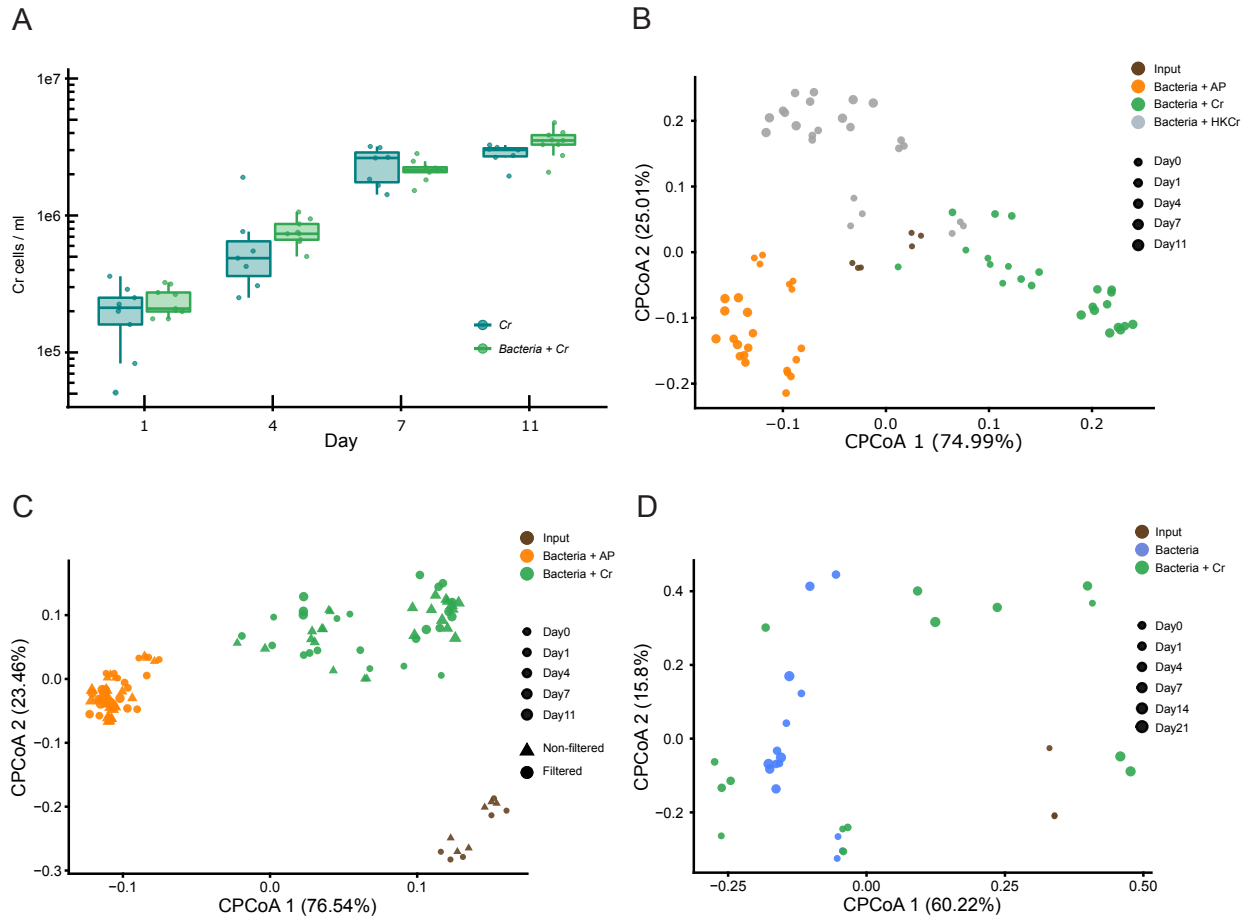


Figure S3. Analysis of phycosphere communities in mesocosm experiments.

(A) Cell counts of axenic *Cr* cultures (dark green) and of *Cr* co-inoculated with soil-derived bacteria (light green) over time. No significant differences were found between growth conditions at each time point (Wilcoxon test). (B) PCoA of Bray-Curtis dissimilarities constrained by the experimental condition (17.5% of variance; $P < 0.001$) of soil-derived bacterial communities co-inoculated with AP, live *Cr* cultures or heat-killed *Cr*. Points in the plot are color-coded according to the condition and their size indicates the time point at which samples were harvested. (C) PCoA of Bray-Curtis dissimilarities constrained by the experimental condition (17.5% of variance; $P < 0.001$) of filtered (circles) or non-filtered (triangles) bacterial communities derived from soil and co-inoculated with AP or *Cr* cultures. No significant separation of samples was found based on filtration treatment (0.73% of variance). (D) PCoA of Bray-Curtis dissimilarities constrained by experimental condition and time point of (40.1% of variance; $P < 0.005$) of soil-derived bacterial communities either co-inoculated with *Cr* cultures or alone in minimal media, under day/night light conditions.

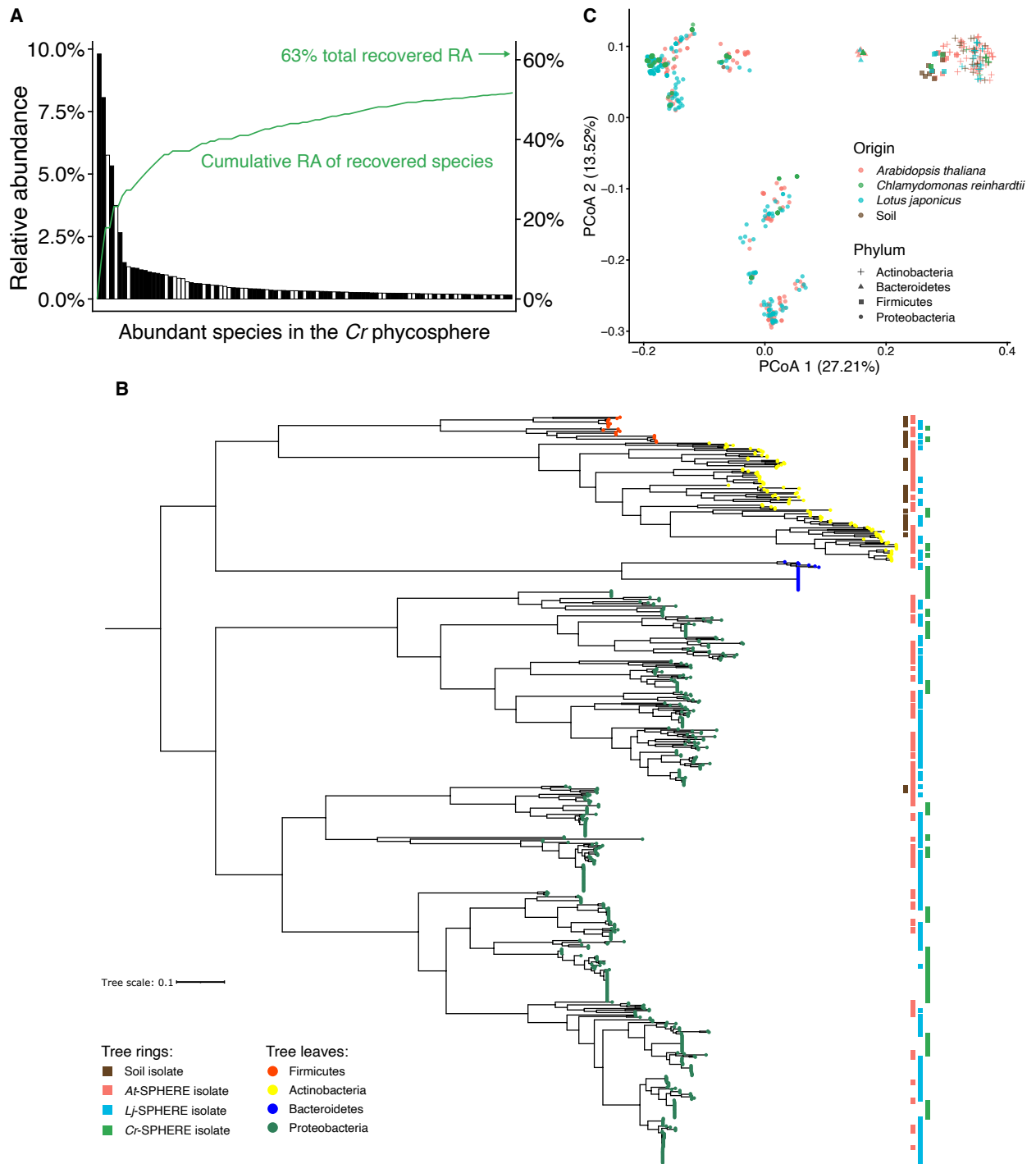


Figure S4. Characterization of the IPL and *Cr*-SPHERE culture collection (caption on next page).

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(A) Bar plot showing the relative abundance of the top 100 most abundant OTUs found in culture-independent phycospheres (greenhouse experiment) indicating whether a representative exemplar was recovered in the IPL bacterial library (62%). The cumulative relative abundance curve and arrow represent total contribution of recovered OTUs to the culture-independent phycosphere communities (63% of the entire community). (B) Whole-genome phylogenetic tree of bacterial strains derived from soil or the *At*-, *Lj*-, *Cr*-SPHERE culture collections based on a concatenation of single copy phylogenetic markers known as AMPHORA genes. Tree leaves are colored by taxonomic affiliation, while colored boxes represent the origin of each genome. (C) PCoA of functional distances of genomes from bacterial isolates of the *Chlamydomonas* (Cr-SPHERE), *Arabidopsis* (At-SPHERE), and *Lotus* (Lj-SPHERE) bacterial culture collections.

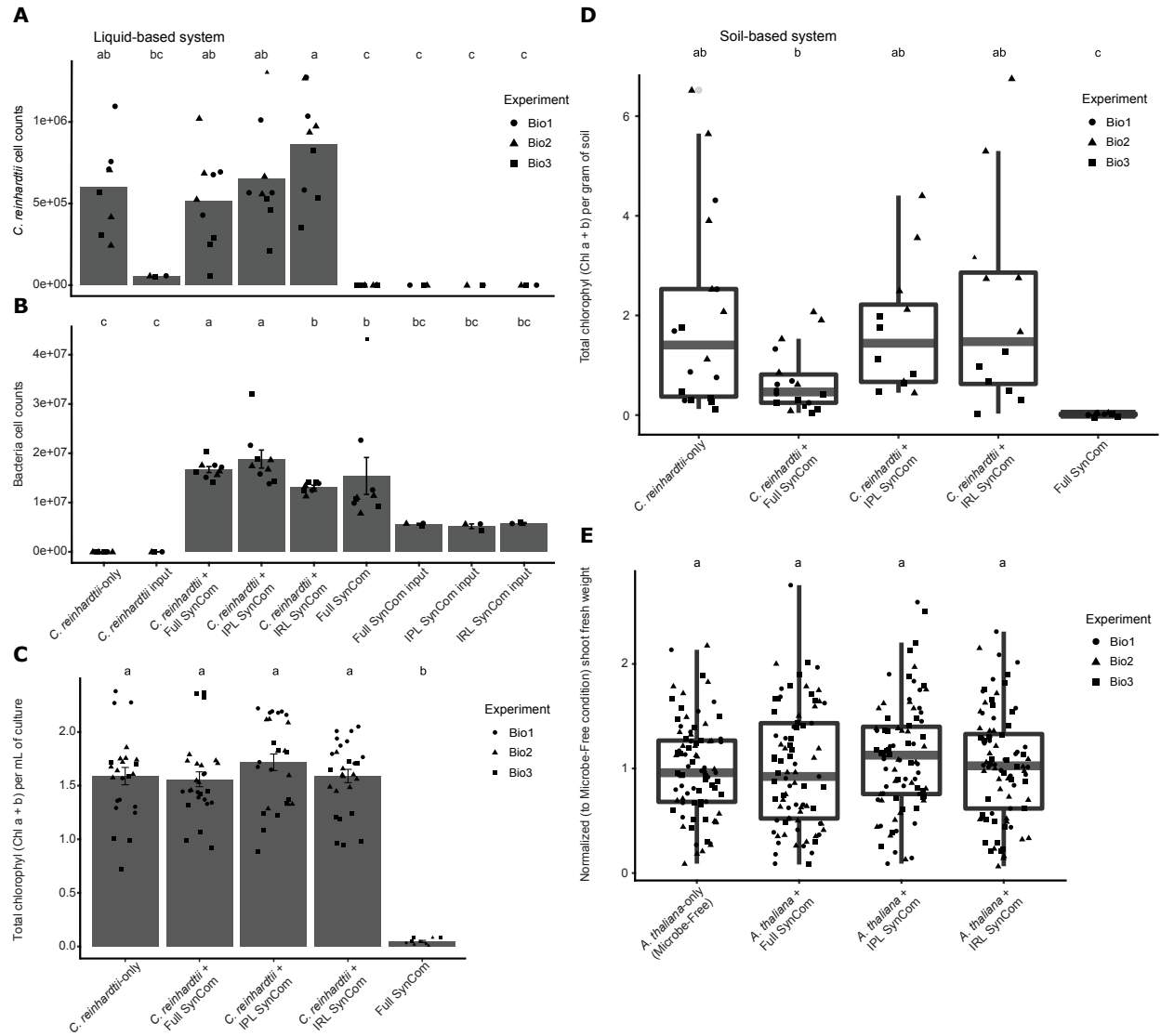


Figure S5. Bacterial and host growth parameters in cross-inoculation experiments with IPL and IRL bacterial SynComs.

(A) *Cr* cell counts after 7 days of co-incubation with different bacterial SynCom combinations in liquid system. (B) Cell counts of different bacterial SynCom combinations after 7 days of co-incubation with or without *Cr* in a liquid system. (C) *Cr* total chlorophyll content after 7 days of co-incubation with different bacterial SynCom combinations in the liquid system. (D) *Cr* total chlorophyll content after 4 weeks of co-incubation with different bacterial SynCom combinations in the soil-based system. (E) *At* normalized shoot fresh wait after 4 weeks of co-incubation with different bacterial SynCom combinations in the soil-based system. Letters on top indicate significant differences between condition within one culture collection (Kruskal-Wallis, followed by a Dunn's test *post hoc*, and Bonferroni correction).

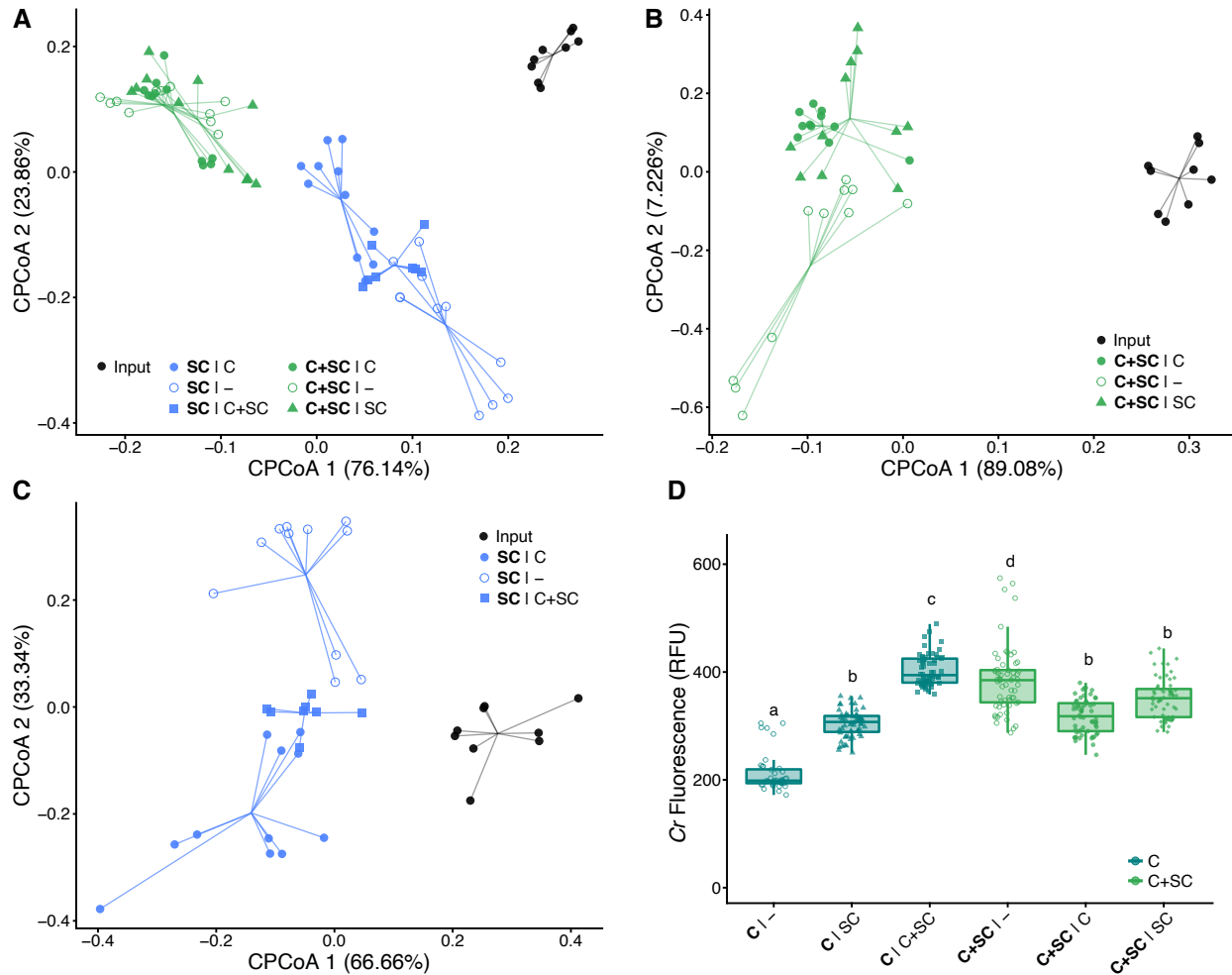


Figure S6. Effect of physical proximity in SynCom community structure and *Cr* growth (split bottle system).

(A) PCoA plot of Bray-Curtis dissimilarities constrained by physical proximity to *Cr* (20.6% variance; $P < 0.001$). Points are color-coded based on the community present in a given compartment, and shapes depict the content of the neighboring compartment. (B-C) PCoA plot of Bray-Curtis distances constrained by physical proximity to *Cr* of C+SC (B; 39.7% variance; $P < 0.001$) and SC-only compartments (C; 22.7% variance; $P < 0.001$). (D) *Cr* growth after 7 days of co-incubation with different compartment combinations, measured as relative chlorophyll fluorescence (RFU). Boxplots are color-coded depending on whether *Cr* was incubated alone in a given compartment (dark green) or in combination with SC (light green).