

Text S1. Low bacterial abundance in white leaf tissues (detailed).

After removal of contaminant, non-bacterial (i.e., chloroplast, mitochondrion and seagrass) sequences, the sequence counts ranged from as low as 308 to 60,409 sequences per sample. Notably, the samples with the lowest read counts exclusively corresponded to samples taken from the white tissues. This could potentially be attributed to a number of technical issues, including differing amounts of starting material, differences in DNA extraction methods and differences in DNA yield for this particular tissue type, or to low bacterial abundance in this (i.e., white tissues) environment. However, the amount of plant tissue used as starting material for DNA extractions was consistent across all samples and the extraction methods were performed using standardized procedures. Moreover, all tissue types showed comparable library sizes before removal of non-bacterial sequences, but the ratio of bacterial reads in the white tissue samples was substantially lower than in the other three tissue types (**Table S1**). We also analysed rarefaction curves of all environments to determine whether the sequencing depth sufficiently covered the microbial diversity of each sample and observed that even the rarefaction curves of the white tissue converged at approximately 100 sequences despite the low number of reads (**Figure S1**). Moreover, a detailed analysis of zero-radius operational taxonomic units (zOTUs) that recorded zero abundance in green tissues showed that most of the zOTUs that are absent within the microbiomes associated with green leaves are also absent within those associated with white tissues (**Figure S2**), which supports our rationale of a lower number of bacterial sequences in the white tissue type. In our opinion, these results indicate that the low number of bacterial reads in the white tissue is not a sampling/sequencing artefact, but instead reflect the low abundance of bacteria in the white parts of the leaf. Based on this observed biological signal, we did not rarefy the data for subsequent taxonomic analyses, as it would have artificially equalized very different environments. To support this, we confirmed general trends between the environments by assessing the extent to which unrarefied and rarefied data sets were comparable (**Text S2**). Sequencing reads were rarefied to the same depth (308 sequences per sample) for alpha diversity analyses and corresponding statistical community analyses.

Table S1. Library size summary.

Sample ID	Sequence count *	Sequence count **
Z4G	235.941	12.705
Z5G	274.454	7.698
Z6G	289.852	4.458
Z1W	315.092	308
Z2W	359.233	423
Z3W	315.762	556
Z1P	293.070	11.495
Z2P	255.257	21.565
Z3P	277.230	22.597
Z1B	233.531	60.409
Z2B	252.356	46.073
Z3B	288.058	45.211
Total	3.389.836	233.498
Minimum	233.531	308
Median	282.644	12.100
Mean	282.486	19.458
Maximum	359.233	60.409

Z1-Z6: biological replicates

G, green; W, white; P, purple; B, black

* Raw sequencing data

** Filtered non-bacterial sequences

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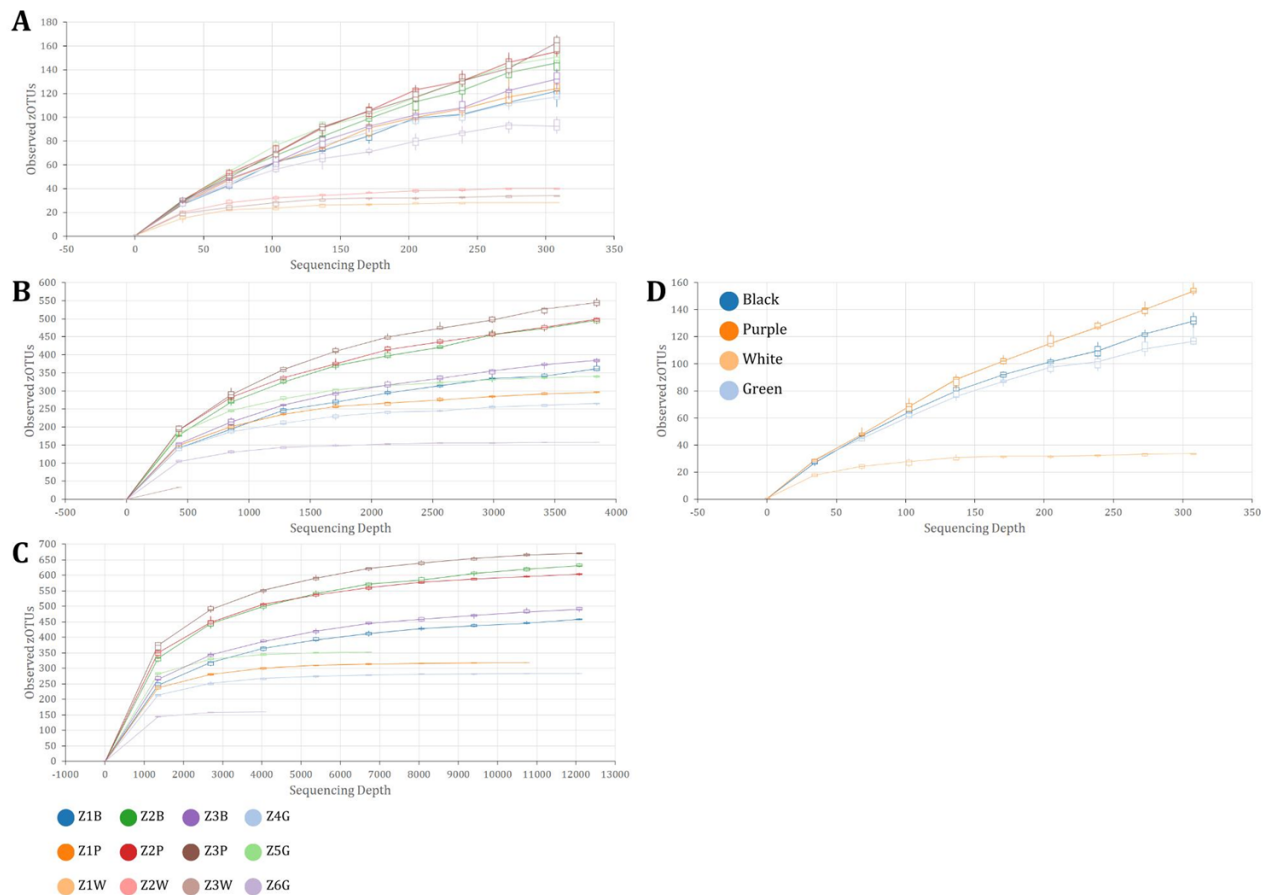
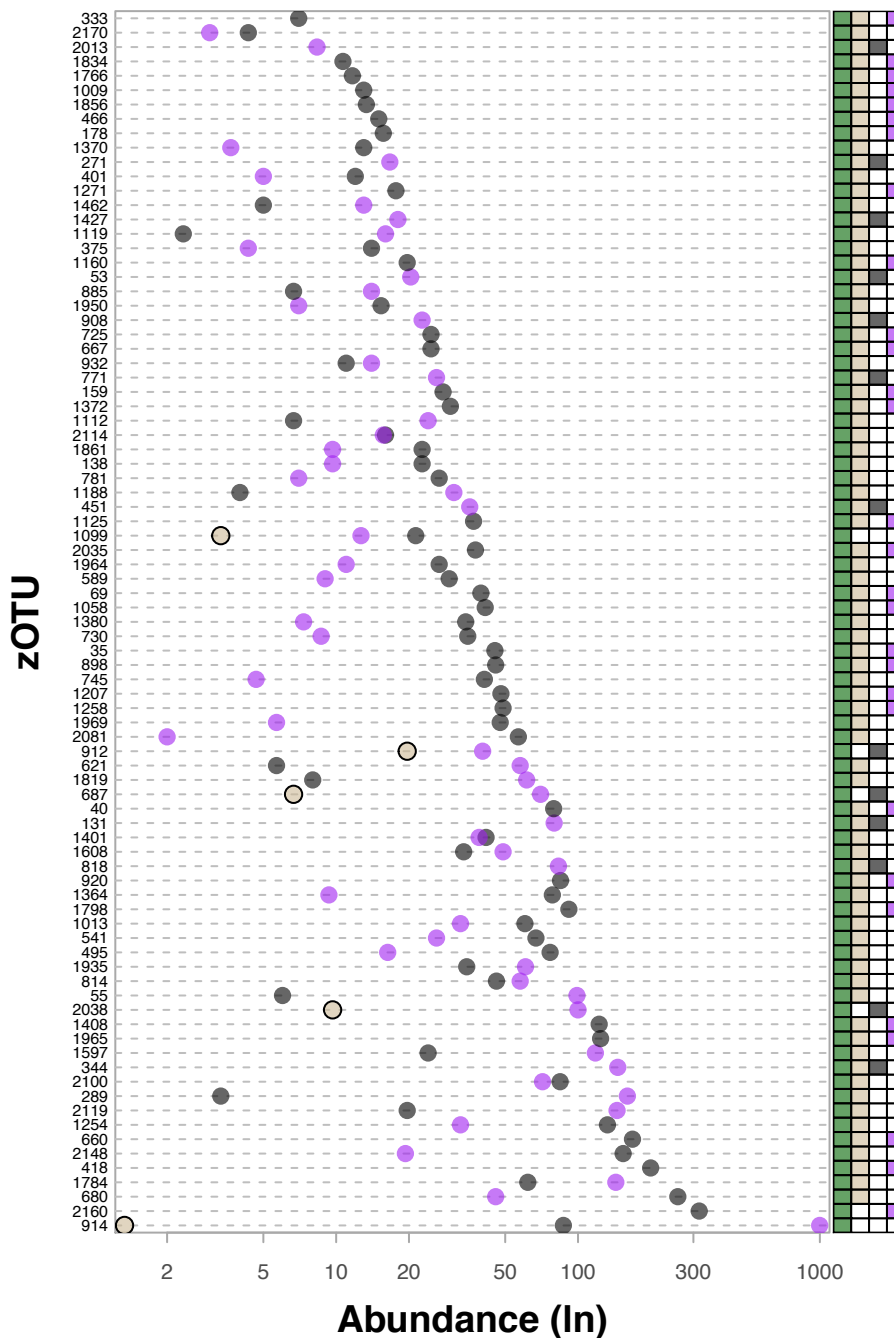


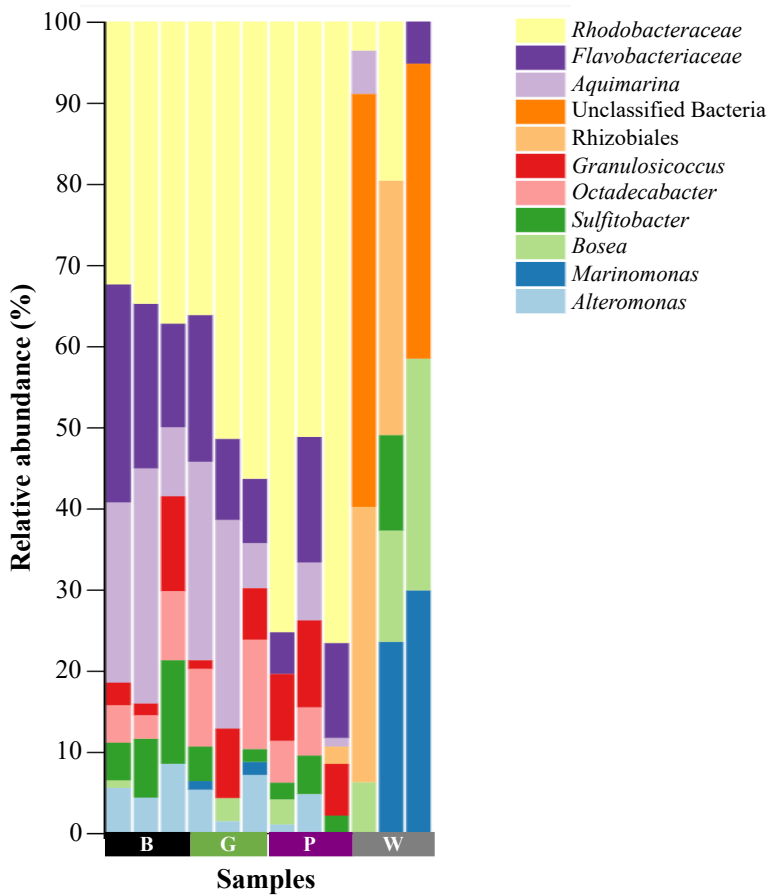
Figure S1. Rarefaction curves. Average alpha diversity values (i.e. number of zero-radius operational taxonomic units (zOTUs)) were computed for each sample and plotted as a function of sampling depth. Rarefied tables ($n = 10$ per sampling depth) were generated at maximum depths of 308 (minimum frequency, A), 3,482 (first quartile, B), and 12,100 (median frequency, C). Samples were grouped by pigmentation category (B, black; P, purple; W, white; G, green) at the chosen rarefaction threshold of 308 sequences per sample (D). Z1 – Z6: biological replicates.



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2 **Figure S2. Consistent patterns of bacterial occurrence across tissue types (unrarefied data).**
3 Zero-radius operational taxonomic units (zOTUs) absent within green leaves-associated
4 microbiomes that recorded abundances higher than zero in all samples of at least one reddened tissue
5 type are plotted as natural log-transformed means ($n = 3$ for each tissue type) for a given zOTU. The
6 coloured matrix on the right indicates tissue types where a given zOTU was absent. “Discriminatory”
7 zOTUs were excluded from this analysis.

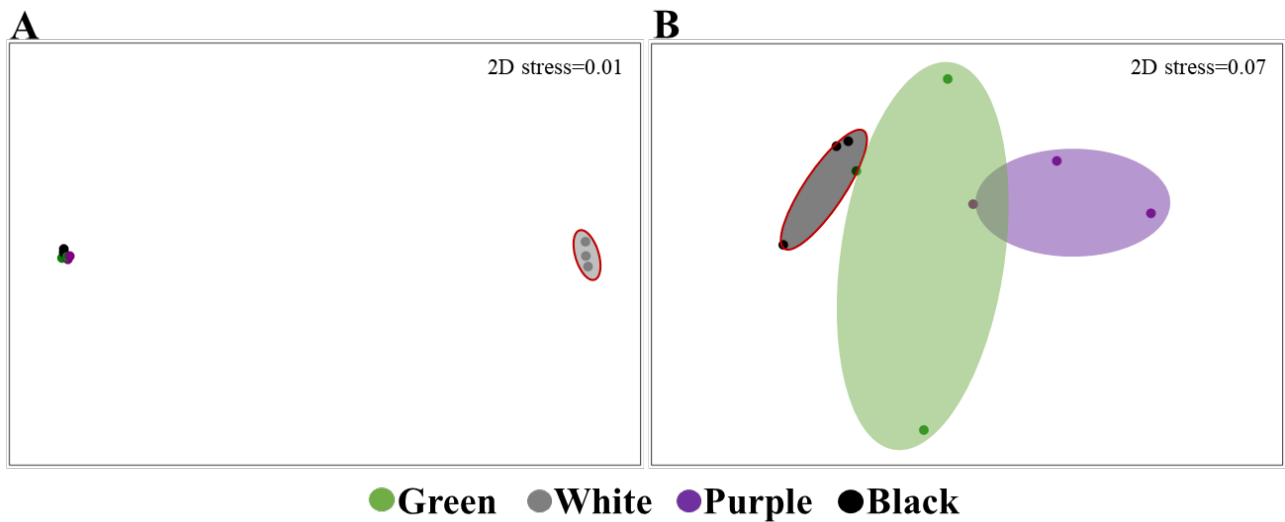
1 **Text S2. Comparison between unrarefied and rarefied data.**

2 Rarefaction is a common normalization technique intended to enable meaningful comparison of
3 sequencing data by standardizing the library size across samples (Brewer & Williamson 1994, Weiss
4 et al. 2017). However, it can reduce statistical power and it is not suitable for compositional data like
5 the relative abundance of taxa in a given sample (Weiss et al. 2017). To confirm general trends
6 between the environments, we assessed the extent to which unrarefied and rarefied data sets were
7 comparable by rarefying reads to the minimum depth and performing the same taxonomical analyses
8 (i.e. beta diversity), including ordination and statistical analyses for rarefied and unrarefied data.
9 Both data sets provided comparable beta diversity (**Figure 3** and **Figure S3**) and clustering patterns
10 (**Figure 4.AC** and **Figure S4**). The overall trends and statistical results were consistent between
11 unrarefied and rarefied data (**Table S2** and **Table S3**) and showed significant differences between
12 tissue types ($p_{\text{unrarefied}} = 0.002$, $p_{\text{rarefied}} = 0.001$). However, the differences in beta diversity between
13 green leaves and white tissues were significant with unrarefied data ($p = 0.045$) and just above the
14 significant level when the data was not rarefied ($p = 0.067$). Altogether, our results from the
15 comparisons between these two data sets (i.e., rarefied and unrarefied sequencing reads),
16 demonstrate that these led to the same biological interpretations.



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2 **Figure S3. Beta diversity across pigmentation types (rarefied data).** Beta diversity of bacterial
3 microbiomes associated with black (B), green (G), purple (P) and white (W) seagrass leaf tissues is
4 plotted, with unique zero-radius operational taxonomic units (zOTUs) within each sample coloured
5 by the highest assigned taxonomic level. Rare members of the microbiome were excluded to help
6 remove visual clutter, and thus only representative zOTUs with a relative abundance > 1% in all
7 samples (n = 12) are shown. Sequencing reads were rarefied to the same depth (308 sequences per
8 sample) in order to assess the extent to which unrarefied and rarefied data sets were comparable.



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2 **Figure S4. Bacterial community structure (rarefied data).** Non-parametric multidimensional
3 scaling (nMDS) of bacterial assemblages associated with seagrass green leaves and reddened tissues
4 (i.e., white, purple and black, **A**). Samples are coloured by pigmentation category, and clustering
5 patterns are shown in ellipses that represent the level of similarity between samples based on the
6 degree to which zero-radius operational taxonomic units (zOTUs) are shared between them.
7 Pigmentation categories that are statistically different ($\alpha = 0.05$) are outlined in red, and clustering
8 patterns of the subset of samples that most closely clustered together are shown in (**B**). Sequencing
9 reads were rarefied to the same depth (308 sequences per sample) in order to assess the extent to
10 which unrarefied and rarefied data sets were comparable.

1 **Table S2. Statistical analyses for multidimensional scaling (unrarefied data).** Differences between bacterial communities across
2 pigmentation categories were tested for statistical significance in Permutational Multivariate Analysis of Variance (PERMANOVA, Bray-Curtis
3 dissimilarity matrix, one-way design).

A) Main tests		B) ECV			C) Pair-wise tests				
factor	P(perm)	Source	Estimate	Sq. root	tissue (P(MC)) i	b	p	w	g
ti	0.0002	S(ti)	1603.200	40.040	b				
		V(Res)	1955.800	44.225	p	0.0368			
					w	0.0194	0.0319		
					g	0.0538	0.1295	0.0449	

4 Significant values at the 0.05 level are shown in grey
5 i Monte Carlo p-values were used when there were not enough possible permutations (<500 out of 9999 permutations, unrestricted permutation
6 method); ECV: estimates of components of variation; ti, tissue; b, black; p, purple; w, white, g, green
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9 **Table S3. Statistical analyses for multidimensional scaling (rarefied data).** Differences between bacterial communities across pigmentation
10 categories were tested for statistical significance in Permutational Multivariate Analysis of Variance (PERMANOVA, Bray-Curtis dissimilarity
11 matrix, one-way design). Sequencing reads were rarefied to the same depth (308 sequences per sample) in order to assess the extent to which
12 unrarefied and rarefied data sets were comparable.

A) Main tests		B) ECV			C) Pair-wise tests				
factor	P(perm)	Source	Estimate	Sq. root	tissue (P(MC)) i	b	p	w	g
ti	0.0001	S(ti)	1116.200	33.410	b				
		V(Res)	2289.600	47.850	p	0.1089			
					w	0.0411	0.0513		
					g	0.2899	0.2324	0.0698	

13 Significant values at the 0.05 level are shown in grey
14 i Monte Carlo p-values were used when there were not enough possible permutations (<500 out of 9999 permutations, unrestricted permutation
15 method); ECV: estimates of components of variation; ti, tissue; b, black; p, purple; w, white, g, green

1 **Table S4. GLMs analyses. Discriminatory zero-radius operational taxonomic units (zOTUs)**
 2 **that differed significantly between green leaves and reddened tissues.** Given that zOTUs making
 3 the differences in mvabund were at zero for the white tissues, this level was removed from the data
 4 set for contrast analyses.

zOTU	Taxonomy*	P-value**	Contrast	P-value***	Depletion	Enrichment
otu_164	<i>Granulosicoccus</i>	3.10E-03	black - green purple - green	1.65E-03 7.26E-03		black purple
otu_217	<i>Litoreibacter</i>	7.00E-04	black - green purple - green	1.75E-02 7.85E-01		black
otu_251	<i>Rhodobacteraceae</i>	3.09E-08	black - green purple - green	8.26E-08 3.60E-01		black
otu_269	<i>Rhodopirellula</i>	2.49E-12	black - green purple - green	1.18E-11 1.10E-02		black purple
otu_330	<i>Rhodobacteraceae</i>	7.37E-12	black - green purple - green	7.90E-14 7.61E-05		black purple
otu_417	<i>Rhodobacteraceae</i>	2.21E-05	black - green purple - green	6.17E-06 5.07E-02		black
otu_528	<i>Rhodobacteraceae</i>	2.36E-04	black - green purple - green	9.79E-05 1.70E-01		black
otu_791	<i>Actinobacteria</i>	2.20E-16	black - green purple - green	1.55E-14 1.48E-03		black purple
otu_1091	<i>Rhodobacteraceae</i>	6.27E-03	black - green purple - green	8.12E-02 3.89E-03	purple	
otu_1121	<i>Granulosicoccus</i>	8.64E-07	black - green purple - green	1.03E-07 1.06E-04		black purple
otu_1406	<i>Rhodobacteraceae</i>	1.88E-03	black - green purple - green	1.47E-03 5.12E-01		black
otu_1453	<i>Rhodobacteraceae</i>	3.45E-05	black - green purple - green	3.94E-04 1.74E-05		black purple
otu_1521	<i>Schleiferia</i>	3.37E-04	black - green purple - green	2.35E-03 3.29E-04		black purple
otu_1525	<i>Loktanella</i>	3.20E-04	black - green purple - green	4.37E-04 6.44E-01		black
otu_1840	<i>Sulfitobacter</i>	8.83E-11	black - green purple - green	4.84E-10 2.54E-01		black
otu_2096	<i>Erythrobacter</i>	2.20E-16	black - green purple - green	1.49E-14 2.89E-01		black
otu_2110	<i>Rhodobacteraceae</i>	1.42E-08	black - green purple - green	6.04E-08 2.29E-01		black
otu_2126	<i>Octadecabacter</i>	6.55E-06	black - green purple - green	1.04E-04 9.93E-01		black

Significant values at $\alpha = 0.05$ are shown in grey

* Highest assigned taxonomic level

** Individual GLMs, main ANOVA. P-values adjusted by the dunnett method

*** Individual GLMs, contrasts. P-values adjusted by the dunnettx method

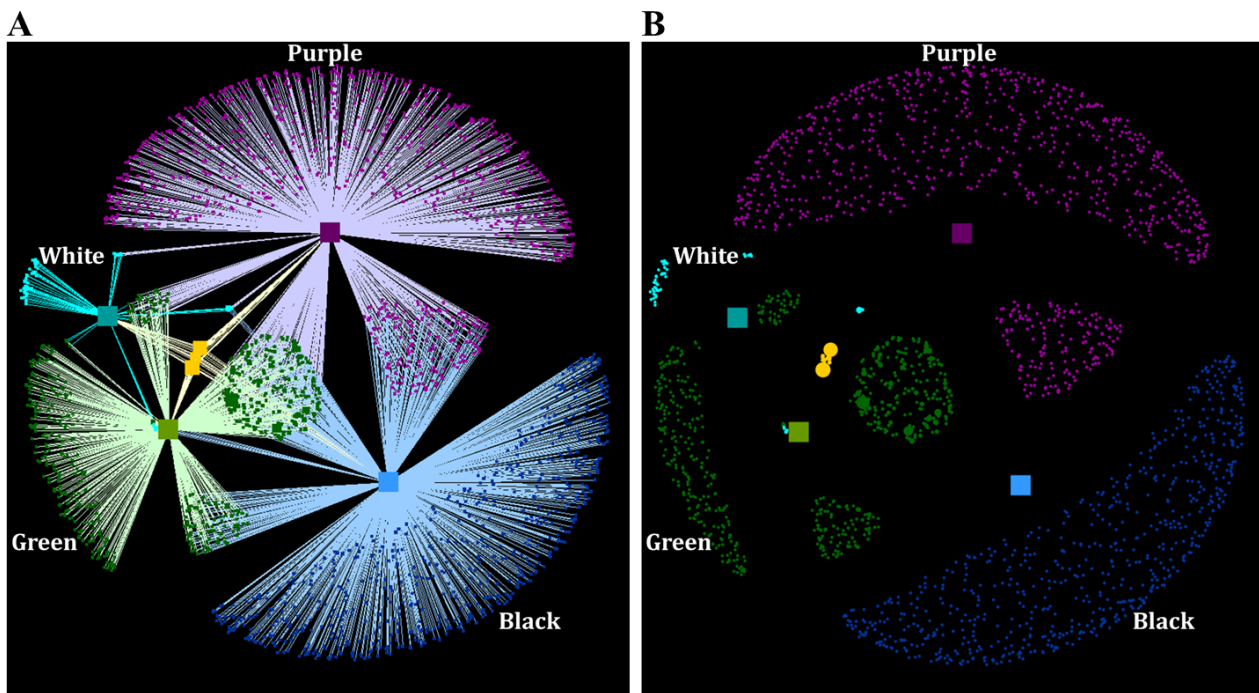


Figure S5. Bacterial community structure (unrarefied data, rare microbiome included).

Bipartite network of bacterial assemblages associated with seagrass green leaves and reddened tissues ($n = 12$). Zero-radius operational taxonomic units (zOTUs) (nodes as small circles) are linked to each pigmentation category that they are associated with (coloured squares) by lines (edges). “Unique” (outer clusters of nodes) and “shared” (inner nodes in the centre) members of microbiomes associated with green leaves (green), and white (aquamarine), purple (magenta) and black (blue) tissues are displayed, with the size of nodes representing zOTUs absolute abundance (range = 1.0 – 12536.0). A total of 1,634 identified zOTUs (i.e., abundant and rare) were included in this analysis, and the same network without edges is also provided (D) to highlight the low proportion of zOTUs (1%) that are shared between all four pigmentation categories (yellow nodes).

1 **Table S5. Lost core microbiomes (unrarefied data).** Core members of the green leaves-associated
2 communities (A) not maintained as core zero-radius operational taxonomic units (zOTUs) within
3 black (B), purple (C) and white (D) tissue samples.

A) Green core microbiome

Taxonomy*	# zOTUs**
<i>Actinobacteria</i>	1
<i>Flavobacteriaceae</i>	1
<i>Aquimarina</i> (ambiguous taxa)	1
<i>Croceitalea</i>	1
<i>Nonlabens</i> (uncultured)	1
<i>Winogradskyella</i> (ambiguous taxa)	1
<i>Schleiferia</i>	1
<i>Saprospiraceae</i>	2
<i>Rubidimonas</i> (uncultured)	1
<i>Pleurocapsa</i> (ambiguous taxa)	1
<i>Phormidium</i>	1
<i>Rhodopirellula</i>	1
<i>Hyphomonadaceae</i> (uncultured, ambiguous taxa)	1
<i>Phyllobacteriaceae</i>	2
<i>Pseudahrensia</i> (uncultured)	1
<i>Rhodobacteraceae</i>	12
<i>Litoreibacter</i> (ambiguous taxa)	1
<i>Loktanella</i>	2
<i>Octadecabacter</i> (ambiguous taxa)	1
<i>Sulfitobacter</i>	1
<i>Erythrobacter</i> (ambiguous taxa)	1
<i>Methylothera</i>	1
<i>Alteromonas</i>	1
<i>Pseudoalteromonas</i>	1
<i>Granulosicoccus</i>	4
<i>Granulosicoccus</i> (uncultured)	1
Total	43

B) Lost in black tissue samples

Taxonomy*	# zOTUs**
<i>Croceitalea</i>	1
<i>Schleiferia</i>	1
<i>Saprospiraceae</i>	1
<i>Rubidimonas</i> (uncultured)	1
<i>Phyllobacteriaceae</i>	2
<i>Rhodobacteraceae</i>	4
<i>Methylothera</i>	1
<i>Granulosicoccus</i>	2
Total	13

C) Lost in purple tissue samples

Taxonomy*	# zOTUs**
<i>Croceitalea</i>	1
<i>Nonlabens</i> (uncultured)	1
<i>Saprospiraceae</i>	1
<i>Rubidimonas</i> (uncultured)	1
<i>Pleurocapsa</i> (ambiguous taxa)	1
<i>Phormidium</i>	1
<i>Hyphomonadaceae</i> (uncultured, ambiguous taxa)	1
<i>Phyllobacteriaceae</i>	2
<i>Pseudahrensia</i> (uncultured)	1
<i>Rhodobacteraceae</i>	1
<i>Pseudoalteromonas</i>	1
<i>Granulosicoccus</i>	1
Total	13

D) Lost in white samples

Taxonomy*	# zOTUs**
<i>Actinobacteria</i>	1
<i>Flavobacteriaceae</i>	1
<i>Aquimarina</i> (ambiguous taxa)	1
<i>Croceitalea</i>	1
<i>Nonlabens</i> (uncultured)	1
<i>Winogradskyella</i> (ambiguous taxa)	1
<i>Schleiferia</i>	1
<i>Saprospiraceae</i>	2
<i>Rubidimonas</i> (uncultured)	1
<i>Pleurocapsa</i> (ambiguous taxa)	1
<i>Phormidium</i>	1
<i>Rhodopirellula</i>	1
<i>Hyphomonadaceae</i> (uncultured, ambiguous taxa)	1
<i>Phyllobacteriaceae</i>	2
<i>Pseudahrensia</i> (uncultured)	1
<i>Rhodobacteraceae</i>	12
<i>Litoreibacter</i> (ambiguous taxa)	1
<i>Loktanella</i>	2
<i>Octadecabacter</i> (ambiguous taxa)	1
<i>Sulfitobacter</i>	1
<i>Erythrobacter</i> (ambiguous taxa)	1
<i>Methylothera</i>	1
<i>Alteromonas</i>	1
<i>Pseudoalteromonas</i>	1

<i>Granulosicoccus</i>	4
<i>Granulosicoccus</i> (uncultured)	1
Total	43

* Highest-resolution taxonomic assignment

** Pooled zOTUs with the same taxonomic assignment

1 **Table S6. Retained core microbiomes (unrarefied data).** Core members of the green leaves-
2 associated communities (A) also consistently maintained as core zero-radius operational taxonomic
3 units (zOTUs) across all black (B) and purple (C) leaf tissue samples.

A) Green core microbiome

Taxonomy*	# zOTUs**
<i>Actinobacteria</i>	1
<i>Flavobacteriaceae</i>	1
<i>Aquimarina</i> (ambiguous taxa)	1
<i>Croceitalea</i>	1
<i>Nonlabens</i> (uncultured)	1
<i>Winogradskyella</i> (ambiguous taxa)	1
<i>Schleiferia</i>	1
<i>Saprospiraceae</i>	2
<i>Rubidimonas</i> (uncultured)	1
<i>Pleurocapsa</i> (ambiguous taxa)	1
<i>Phormidium</i>	1
<i>Rhodopirellula</i>	1
<i>Hyphomonadaceae</i> (uncultured, ambiguous taxa)	1
<i>Phyllobacteriaceae</i>	2
<i>Pseudahrensia</i> (uncultured)	1
<i>Rhodobacteraceae</i>	12
<i>Litoreibacter</i> (ambiguous taxa)	1
<i>Loktanella</i>	2
<i>Octadecabacter</i> (ambiguous taxa)	1
<i>Sulfitobacter</i>	1
<i>Erythrobacter</i> (ambiguous taxa)	1
<i>Methylothermus</i>	1
<i>Alteromonas</i>	1
<i>Pseudoalteromonas</i>	1
<i>Granulosicoccus</i>	4
<i>Granulosicoccus</i> (uncultured)	1
Total	43

B) Retained in all black tissue samples

Taxonomy*	# zOTUs**
<i>Actinobacteria</i>	1
<i>Flavobacteriaceae</i>	1
<i>Aquimarina</i> (ambiguous taxa)	1

<i>Nonlabens</i> (uncultured)	1
<i>Winogradskyella</i> (ambiguous taxa)	1
<i>Saprospiraceae</i>	1
<i>Pleurocapsa</i> (ambiguous taxa)	1
<i>Phormidium</i>	1
<i>Rhodopirellula</i>	1
<i>Hyphomonadaceae</i> (uncultured, ambiguous taxa)	1
<i>Pseudahrensia</i> (uncultured)	1
<i>Rhodobacteraceae</i>	8
<i>Litoreibacter</i> (ambiguous taxa)	1
<i>Loktanella</i>	2
<i>Octadecabacter</i> (ambiguous taxa)	1
<i>Sulfitobacter</i>	1
<i>Erythrobacter</i> (ambiguous taxa)	1
<i>Alteromonas</i>	1
<i>Pseudoalteromonas</i>	1
<i>Granulosicoccus</i>	2
<i>Granulosicoccus</i> (uncultured)	1
Total	30

C) Retained in all purple tissue samples

Taxonomy*	# zOTUs**
<i>Actinobacteria</i>	1
<i>Flavobacteriaceae</i>	1
<i>Aquimarina</i> (ambiguous taxa)	1
<i>Winogradskyella</i> (ambiguous taxa)	1
<i>Schleiferia</i>	1
<i>Saprospiraceae</i>	1
<i>Rhodopirellula</i>	1
<i>Rhodobacteraceae</i>	11
<i>Litoreibacter</i> (ambiguous taxa)	1
<i>Loktanella</i>	2
<i>Octadecabacter</i> (ambiguous taxa)	1
<i>Sulfitobacter</i>	1
<i>Erythrobacter</i> (ambiguous taxa)	1
<i>Methylothera</i>	1
<i>Alteromonas</i>	1
<i>Granulosicoccus</i>	3
<i>Granulosicoccus</i> (uncultured)	1
Total	30

* Highest-resolution taxonomic assignment

** Pooled zOTUs with the same taxonomic assignment