

SUPPLEMENTARY ANALYSES AND FIGURES

Seagrass (*Zostera marina*) transplant experiment reveals core microbiome and resistance to environmental change

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Analyses without the 76 samples that were run at 32 PCR cycles

For extra precaution, we removed the 76 contaminated samples that were run at 32 PCR cycles (n = 175; Table S5) and ran the same analyses and produced the same figures to compare results.

The results presented here are the same figures and tables found in the main text and supplementary information, and their caption names are the same. Analyzing results with and without samples amplified with 32 cycles did not change results.

TABLES AND FIGURES IN MAIN TEXT

Table 1. Bray-Curtis PERMANOVA pairwise comparisons between the original meadows for the natural seagrass microbial communities (Day 1). PB = Pruth Bay, PP = Pruth Pocket, SS = Choked Sand Spit, WO = Choked Wolf.

Table 2. Bray-Curtis PERMANOVA pairwise comparisons between treatments that were replanted and transplanted to the same region (Day 5).

Table 3. Significantly enriched taxa on each seagrass treatment with at least 50% prevalence in comparison to the water column, and taxa enriched on natural and sterilized/dead seagrass in comparison to artificial seagrass. Taxa with $\geq 70\%$ prevalence are in bold.

Table 4. Bray-Curtis two-way PERMANOVA with marginal effects for original and destination sites and an interaction term. A separate PERMANOVA was run for each treatment. Day 5 samples were used.

Figure 3. Non-metric multidimensional scaling (NMDS) plot using Bray-Curtis dissimilarity. Initial microbial communities on natural seagrass leaves from their original site (Day 1).

Figure 4. Non-metric multidimensional scaling (NMDS) plot using Bray-Curtis dissimilarity. Final microbial communities (Day 5) on natural seagrass leaves, sterilized/dead seagrass leaves, and artificial seagrass leaves that were replanted to their original meadow or transplanted to the same region.

Figure 5. Relative abundance barplots of the top 15 most abundant microbial genera for (A) natural seagrass leaves sampled on Day 1, (B) natural seagrass leaves sampled on Day 5, (C) sterilized/dead seagrass leaves sampled on Day 5, and (D) artificial seagrass leaves sampled on Day 5. Each column represents one sample and they are grouped by original site for (A) and by destination site for (B-D). Transplant location indicates the meadow of origin → and destination (i.e., PB → PP means the shoot originated in Pruth Bay and was transplanted to Pruth Pocket). “Replant” indicates shoots that were replanted in the same location. For the final sampling timepoint, asterisks indicate significantly enriched taxa (prevalence of $\geq 70\%$) on only natural seagrass and sterilized/dead seagrass leaves, indicating they are core taxa.

Figure 6. Microbial taxonomic similarity among treatments represented in non-metric multidimensional scaling (NMDS) plots using Bray-Curtis dissimilarity. Final microbial communities (Day 5) on (A) natural seagrass leaves, (B) sterilized/dead seagrass leaves, and (C) artificial seagrass leaves. Colours indicate the sample’s original site, and shapes indicate the site to which samples were replanted or transplanted.

TABLES AND FIGURES IN SUPPLEMENTARY INFORMATION

Table S6. Bray-Curtis PERMANOVA pairwise comparisons between all treatments and water microbial communities that were collected on the last day of the experiment (Day 5).

Table S7. Bray-Curtis PERMANOVA pairwise comparisons between treatments that were reciprocally transplanted to all sites and regions (Day 5). “Pruth and Choked Passage” region are pairwise comparisons for all treatments, “Pruth only” are pairwise comparisons for treatments recovered from Pruth, and “Choked Passage only” are pairwise comparisons for treatments recovered from Choked Passage.

Table S8. Top 15 taxa with the highest frequencies for natural seagrass leaves and the corresponding frequencies for the other treatments, as identified by frequency analysis. All sites are included for each treatment and Day 1 and Day 5 samples were used for natural seagrass leaves and water, and Day 5 samples were used for sterilized/dead and artificial seagrass leaves. Taxon is the most specific taxonomy level reliably assigned; all taxa are order; family; genus unless otherwise specified. Taxa that are present in $\geq 70\%$ of all samples in each treatment are bolded. Asterisks indicate taxa that are enriched in natural and sterilized/dead seagrass treatments only.

Figure S1. Relative abundance barplot of the top 15 most abundant microbial genera for sterilized/dead seagrass leaves sampled on Day 1. Each column represents one sample, and they are grouped by original site.

Figure S2. Non-metric multidimensional scaling (NMDS) plot using Bray-Curtis dissimilarity. Day 5 microbial communities on natural seagrass leaves, sterilized/dead seagrass leaves, artificial seagrass leaves, and water collected on the last day of the experiment.

Figure S3. Non-metric multidimensional scaling (NMDS) plot using Bray-Curtis dissimilarity. Microbial communities on natural seagrass leaves for each location and time point. Colours indicate the sample’s original site and shapes indicate whether the samples were replanted, transplanted to the same region, or transplanted to a different region.

Figure S5. Growth of second youngest seagrass leaf for natural seagrass shoot compared to Bray-Curtis dissimilarity (Day 1 vs Day 5). There is no relationship between growth and community turnover.

Table 1. Bray-Curtis PERMANOVA pairwise comparisons between the original meadows for the natural seagrass microbial communities (Day 1). PB = Pruth Bay, PP = Pruth Pocket, SS = Choked Sand Spit, WO = Choked Wolf.

Meadows	Df	Sums of squares	F model	R²	P adjusted
PB vs PP	1	1.732	9.750	0.379	0.003
PB vs SS	1	2.220	12.129	0.447	0.003
PB vs WO	1	2.148	12.394	0.470	0.003
PP vs SS	1	1.210	5.429	0.295	0.004
PP vs WO	1	1.177	5.474	0.313	0.003
SS vs WO	1	0.422	1.869	0.145	0.026

Table 2. Bray-Curtis PERMANOVA pairwise comparisons between treatments that were replanted and transplanted to the same region (Day 5).

Region	Treatments	Df	Sums of squares	F model	R ²	P adjusted
Pruth and Choked Passage	ASU vs sterilized/dead seagrass	1	0.752	2.604	0.067	0.038
	ASU vs natural seagrass	1	0.865	2.846	0.075	0.027
	Natural vs sterilized/dead seagrass	1	0.581	2.047	0.050	0.044
Pruth only	ASU vs sterilized/dead seagrass	1	0.747	3.484	0.188	0.003
	ASU vs natural seagrass	1	0.870	3.737	0.199	0.003
	Natural vs sterilized/dead seagrass	1	0.617	2.917	0.139	0.003
Choked Passage only	ASU vs sterilized/dead seagrass	1	0.534	3.193	0.144	0.004
	ASU vs natural seagrass	1	0.544	2.932	0.140	0.003
	Natural vs sterilized/dead seagrass	1	0.418	2.447	0.114	0.006

Table 3. Significantly enriched taxa on each seagrass treatment with at least 50% prevalence in comparison to the water column, and taxa enriched on natural and sterilized/dead seagrass in comparison to artificial seagrass. Taxa with $\geq 70\%$ prevalence are in bold.

Taxon	Comparison to Water						Comparison to Artificial			
	Natural		Sterilized /dead		Artificial		Natural		Sterilized /dead	
	Spec	Prev	Spec	Prev	Spec	Prev	Spec	Prev	Spec	Prev
Betaproteobacteriales; Methylophilus *	0.99	0.77	0.99	0.93	-	-	0.95	0.77	0.94	0.93
Betaproteobacteriales; Methylothera *	1	0.92	0.99	0.86	-	-	0.95	0.92	0.93	0.86
Nitrosococcales; Methylophagaceae^ *	1	0.64	1	0.73	-	-	0.93	0.64	0.95	0.72
Verrucomicrobiales; DEV007^ *	0.98	0.74	-	-	-	-	-	-	-	-
Rhodobacterales; Donghicola *	1	0.54	1	0.67	-	-	0.92	0.54	-	-
Cellvibrionales; Cellvibrionaceae^	0.99	0.62	1	0.81	-	-	-	-	0.87	0.81
Rickettsiales; Rickettsia	0.97	0.64	-	-	-	-	-	-	-	-
Alteromonadales; Psychrobium	-	-	-	-	-	-	-	-	-	-
Chitinophagales; Saprospiraceae^	0.97	1	-	-	-	-	-	-	-	-
Thiohalorhabdiales; Granulosicoccus	0.98	1	-	-	0.94	0.86	-	-	-	-
Alteromonadales; Pseudoalteromonas	-	-	0.92	0.84	0.87	0.74	-	-	-	-
Alteromonadales; Alteromonas	-	-	1	0.53	0.98	0.54	-	-	-	-
Alteromonadales; Thalassotalea	-	-	0.98	0.49	1	0.57	-	-	-	-
Flavobacteriales; Kordia	0.97	0.51	0.99	0.47	-	-	-	-	-	-
Oceanospirillales; Oleispira	-	-	-	-	-	-	-	-	-	-
Oceanospirillales; Spongiispira	-	-	-	-	-	-	-	-	-	-
Campylobacteriales; Arcobacter	-	-	-	-	-	-	-	-	-	-
Alteromonadales; Colwellia	1.0	0.49	1	0.88	1	0.74	-	-	-	-
Caulobacteriales; Litorimonas	-	-	-	-	-	-	-	-	-	-
Ectothiorhodospirales; Ectothiorhodospiraceae^	-	-	0.96	0.74	0.98	0.74	-	-	-	-
Flavobacteriales; Dokdonia	1	0.72	1	0.67	1	0.66	-	-	-	-
Rhodobacterales; Pacificibacter	0.98	0.92	1	1	0.98	0.77	-	-	-	-
Arenicellales; Arenicella	0.97	0.62	0.99	0.67	0.99	0.71	-	-	-	-

Taxon refers to Order; Genus, except uncultured genera are labeled as Order; Family^

Spec: Specificity compared to water or to artificial seagrass; Prev: Prevalence, reported as fidelity by IndVal

* Core taxon for natural and/or sterilized/dead seagrass leaves

Table 4. Bray-Curtis two-way PERMANOVA with marginal effects for original and destination sites and an interaction term. A separate PERMANOVA was run for each treatment. Day 5 samples were used.

Treatment	Variables	Df	Sums of squares	F model	R ²	P-value
Natural seagrass	Original site	3	2.837	5.631	0.27	0.001
	Destination site	3	1.872	3.714	1.778	0.001
	Original * Destination site	9	1.955	1.294	0.186	0.034
Sterilized/dead seagrass	Original site	3	1.061	2.382	0.097	0.003
	Destination site	3	4.689	10.533	0.428	0.001
	Original * Destination site	9	1.195	0.895	0.109	0.676
Artificial seagrass	Original site	3	0.853	1.961	0.066	0.012
	Destination site	3	5.250	12.065	0.481	0.001
	Original * Destination site	9	1.566	1.200	0.120	0.162

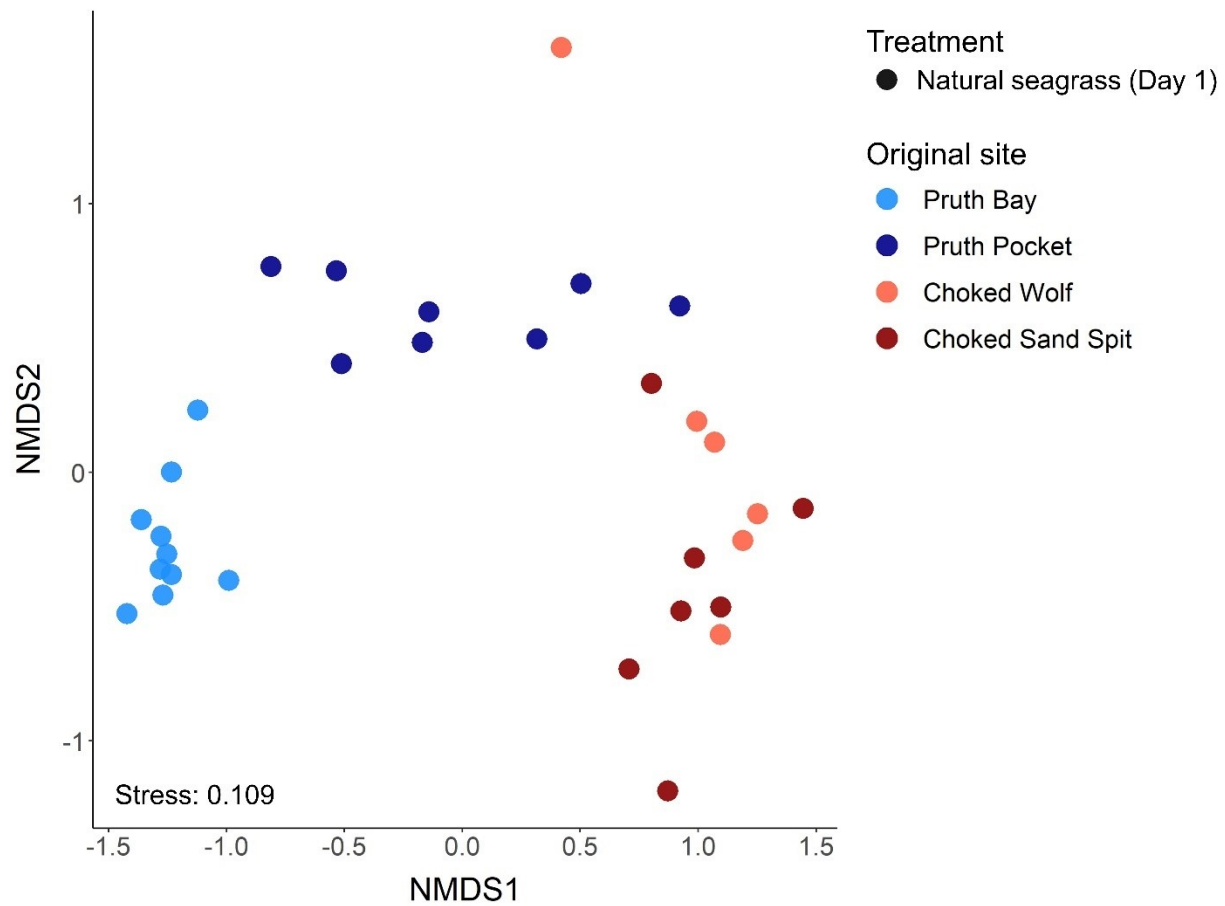


Figure 3. Non-metric multidimensional scaling (NMDS) plot using Bray-Curtis dissimilarity. Initial microbial communities on natural seagrass leaves from their original site (Day 1).

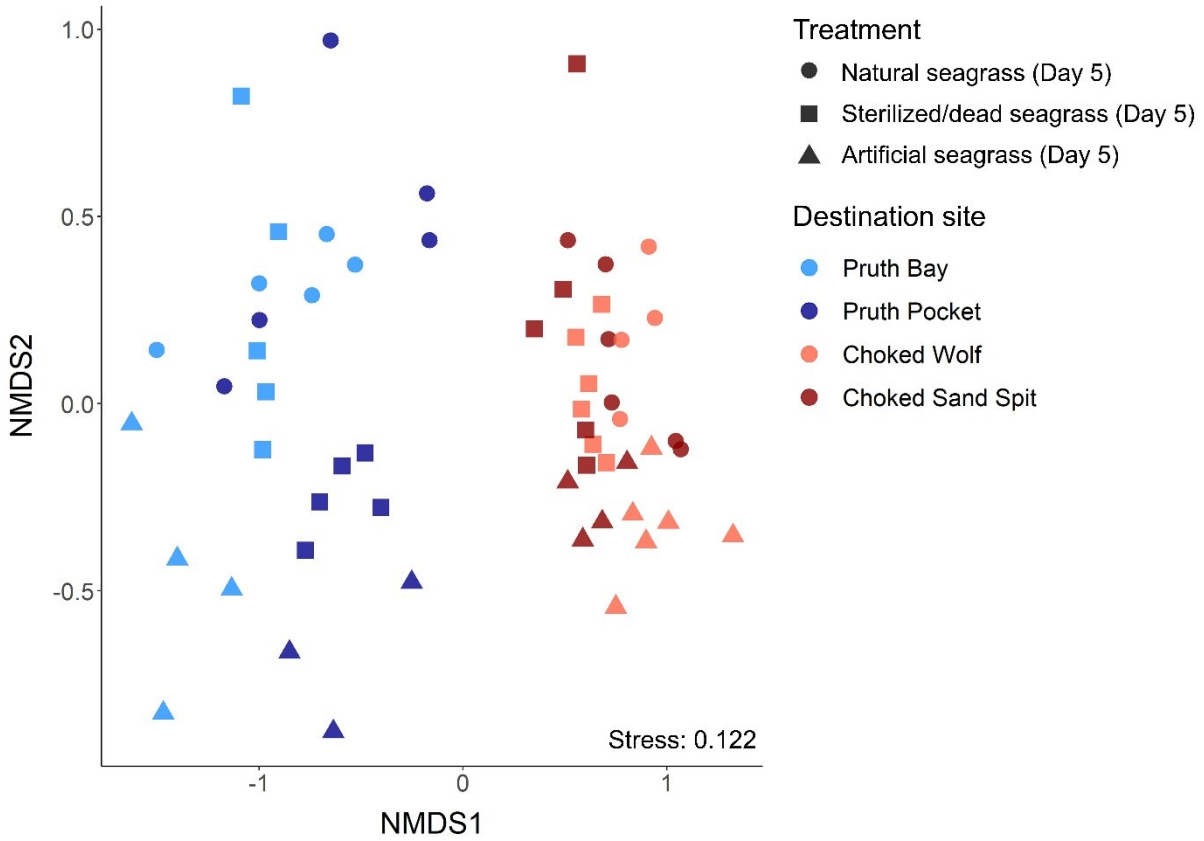


Figure 4. Non-metric multidimensional scaling (NMDS) plot using Bray-Curtis dissimilarity. Final microbial communities (Day 5) on natural seagrass leaves, sterilized/dead seagrass leaves, and artificial seagrass leaves that were replanted to their original meadow or transplanted to the same region.

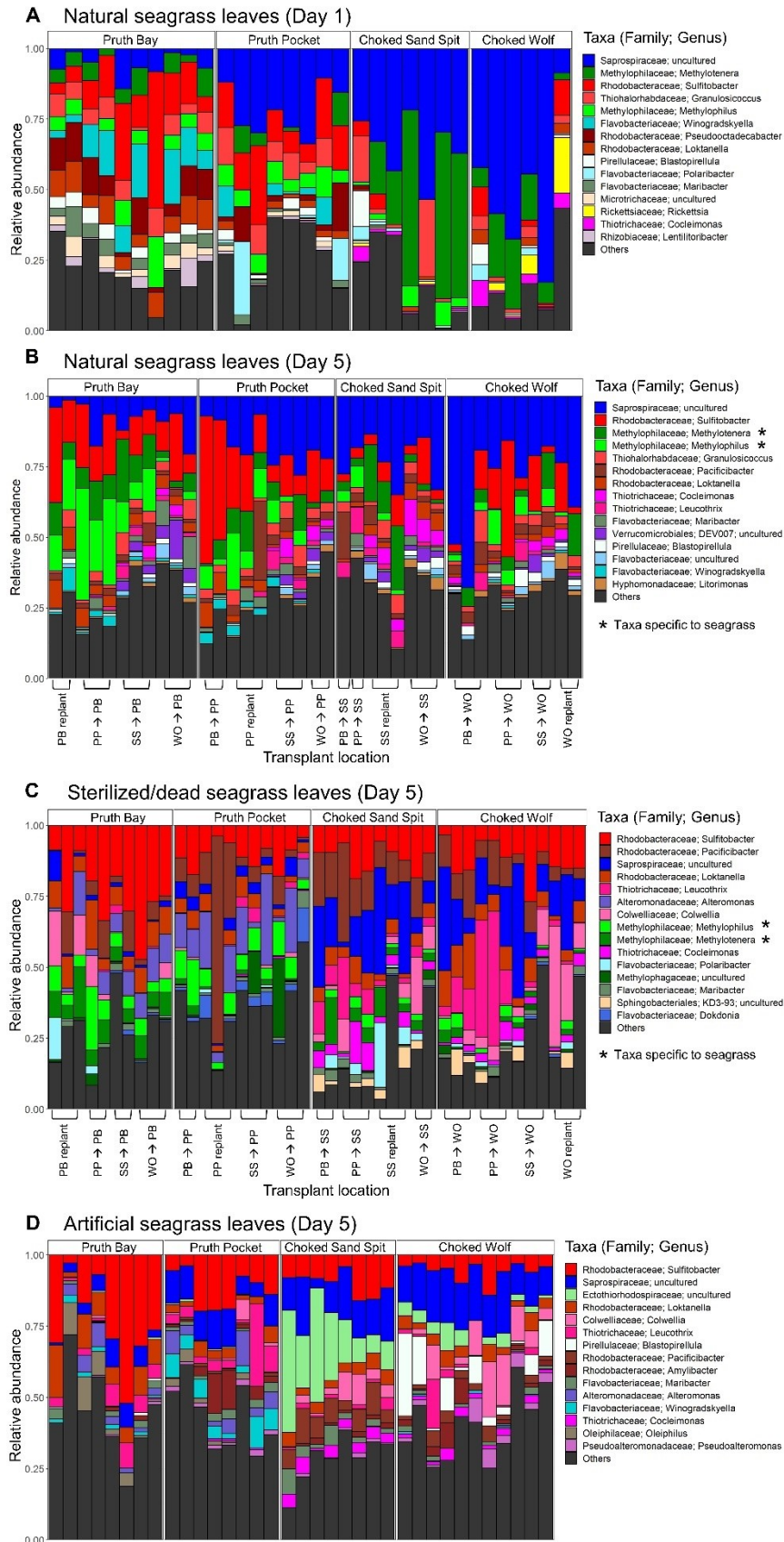


Figure 5. Relative abundance barplots of the top 15 most abundant microbial genera for **(A)** natural seagrass leaves sampled on Day 1, **(B)** natural seagrass leaves sampled on Day 5, **(C)** sterilized/dead seagrass leaves sampled on Day 5, and **(D)** artificial seagrass leaves sampled on Day 5. Each column represents one sample and they are grouped by original site for **(A)** and by destination site for **(B-D)**. Transplant location indicates the meadow of origin → and destination (i.e., PB → PP means the shoot originated in Pruth Bay and was transplanted to Pruth Pocket). “Replant” indicates shoots that were replanted in the same location. For the final sampling timepoint, asterisks indicate significantly enriched taxa (prevalence of $\geq 70\%$) on only natural seagrass and sterilized/dead seagrass leaves, indicating they are core taxa.

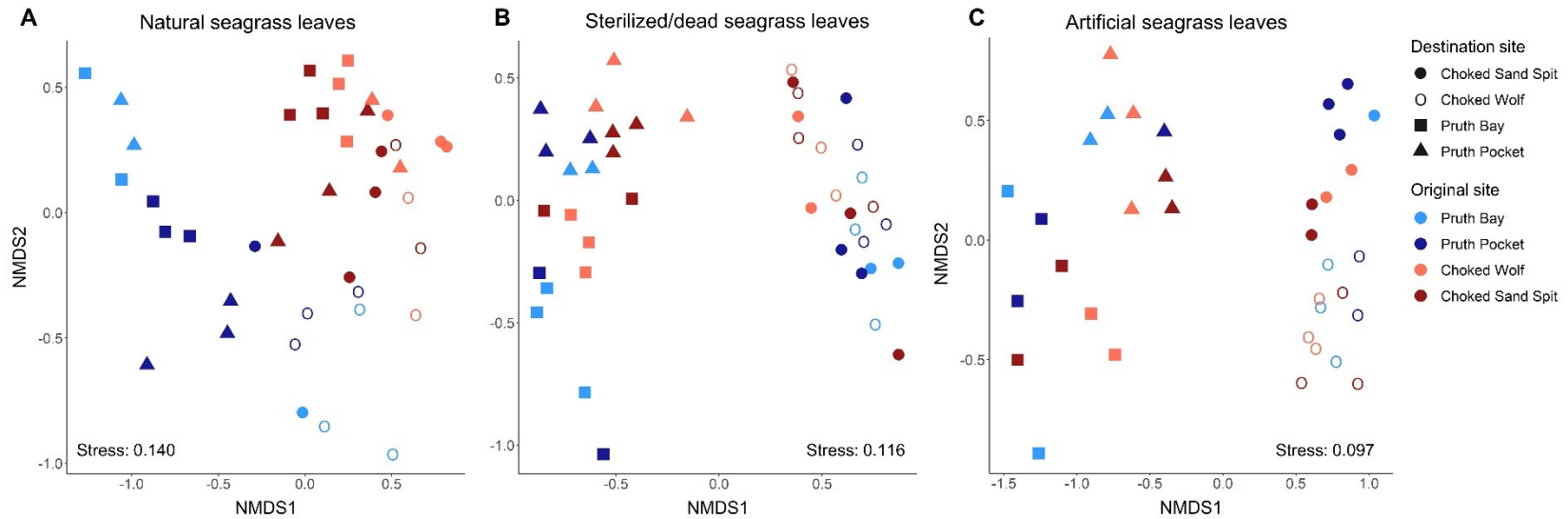


Figure 6. Microbial taxonomic similarity among treatments represented in non-metric multidimensional scaling (NMDS) plots using Bray-Curtis dissimilarity. Final microbial communities (Day 5) on **(A)** natural seagrass leaves, **(B)** sterilized/dead seagrass leaves, and **(C)** artificial seagrass leaves. Colours indicate the sample's original site, and shapes indicate the site to which samples were replanted or transplanted.

Table S6. Bray-Curtis PERMANOVA pairwise comparisons between all treatments and water microbial communities that were collected on the last day of the experiment (Day 5).

Treatments	Df	Sums of squares	F model	R²	P-adjusted
Artificial vs natural seagrass	1	1.739	5.975	0.077	0.001
Artificial vs sterilized/dead seagrass	1	1.505	5.350	0.066	0.001
Water vs artificial seagrass	1	2.982	10.322	0.197	0.001
Natural vs sterilized/dead seagrass	1	1.255	4.673	0.055	0.001
Water vs natural seagrass	1	3.270	12.294	0.211	0.001
Water vs sterilized/dead seagrass	1	3.368	13.301	0.210	0.001

Table S7. Bray-Curtis PERMANOVA pairwise comparisons between treatments that were reciprocally transplanted to all sites and regions (Day 5). “Pruth and Choked Passage” region are pairwise comparisons for all treatments, “Pruth only” are pairwise comparisons for treatments recovered from Pruth, and “Choked Passage only” are pairwise comparisons for treatments recovered from Choked Passage.

Region	Treatments	Df	Sums of squares	F model	R ²	P adjusted
Pruth and Choked Passage	Artificial vs natural seagrass	1	1.739	5.975	0.077	0.001
	Artificial vs sterilized/dead seagrass	1	1.505	5.350	0.066	0.001
	Natural vs sterilized/dead seagrass	1	1.255	4.673	0.055	0.001
Pruth only	Artificial vs natural seagrass	1	1.674	6.646	0.160	0.001
	Artificial vs sterilized/dead seagrass	1	1.240	5.887	0.144	0.001
	Natural vs sterilized/dead seagrass	1	1.404	6.441	0.133	0.001
Choked Passage only	Artificial vs natural seagrass	1	1.045	4.820	0.121	0.001
	Artificial vs sterilized/dead seagrass	1	1.249	6.835	0.149	0.001
	Natural vs sterilized/dead seagrass	1	0.696	3.492	0.084	0.001

Table S8. Top 15 taxa with the highest frequencies for natural seagrass leaves and the corresponding frequencies for the other treatments, as identified by frequency analysis. All sites are included for each treatment and Day 1 and Day 5 samples were used for natural seagrass leaves and water, and Day 5 samples were used for sterilized/dead and artificial seagrass leaves. Taxon is the most specific taxonomy level reliably assigned; all taxa are order; family; genus unless otherwise specified. Taxa that are present in $\geq 70\%$ of all samples in each treatment are bolded. Asterisks indicate taxa that are enriched in natural and sterilized/dead seagrass treatments only.

Taxon	Natural frequency	Sterilized/dead frequency	Artificial frequency	Water frequency
Chitinophagales; Saprospiraceae; uncultured	0.986	1.000	1.000	0.625
Thiohalorhabdales; Thiohalorhabdaceae; Granulosicoccus	0.943	0.767	0.800	0.375
Rhodobacterales; Rhodobacteraceae; Sulfitobacter	0.886	1.000	1.000	0.938
Betaproteobacteriales; Methylophilaceae; Methylothermus*	0.857	0.860	0.314	0.188
Rhodobacterales; Rhodobacteraceae; Loktanella	0.843	1.000	0.971	0.313
Pirellulales; Pirellulaceae; Blastopirellula	0.786	0.535	0.486	0.813
Flavobacteriales; Flavobacteriaceae; Maribacter	0.757	0.930	0.886	0.438
Betaproteobacteriales; Methylophilaceae; Methylophilus*	0.743	0.930	0.429	0.125
Thiotrichales; Thiotrichaceae; Leucothrix	0.714	0.953	0.886	0.313
Rhodobacterales; Rhodobacteraceae; Pacificibacter	0.700	1.000	0.771	0.063
Flavobacteriales; Flavobacteriaceae; Winogradskyella	0.686	0.535	0.600	0.500
Caulobacterales; Hyphomonadaceae; Litorimonas	0.657	0.744	0.857	0.125
Verrucomicrobiales; Rubritaleaceae; Roseibacillus	0.643	0.302	0.314	0.875
Thiotrichales; Thiotrichaceae; Cocleimonas	0.614	0.721	0.714	0.500
Chitinophagales; Saprospiraceae; Lewinella	0.600	0.628	0.514	0.188

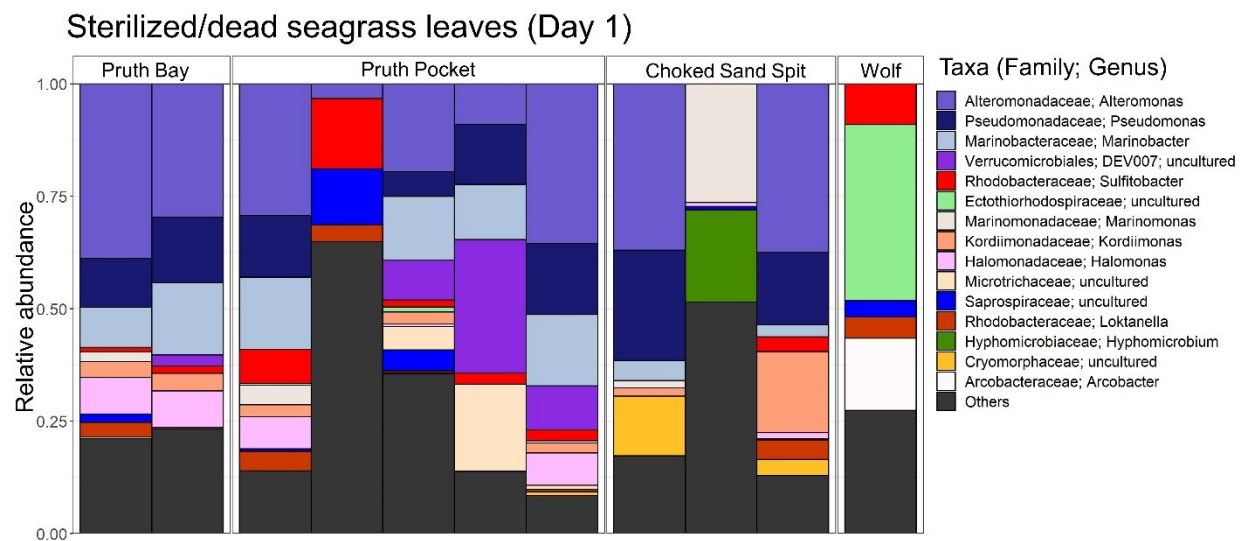


Figure S1. Relative abundance barplot of the top 15 most abundant microbial genera for sterilized/dead seagrass leaves sampled on Day 1. Each column represents one sample, and they are grouped by original site.

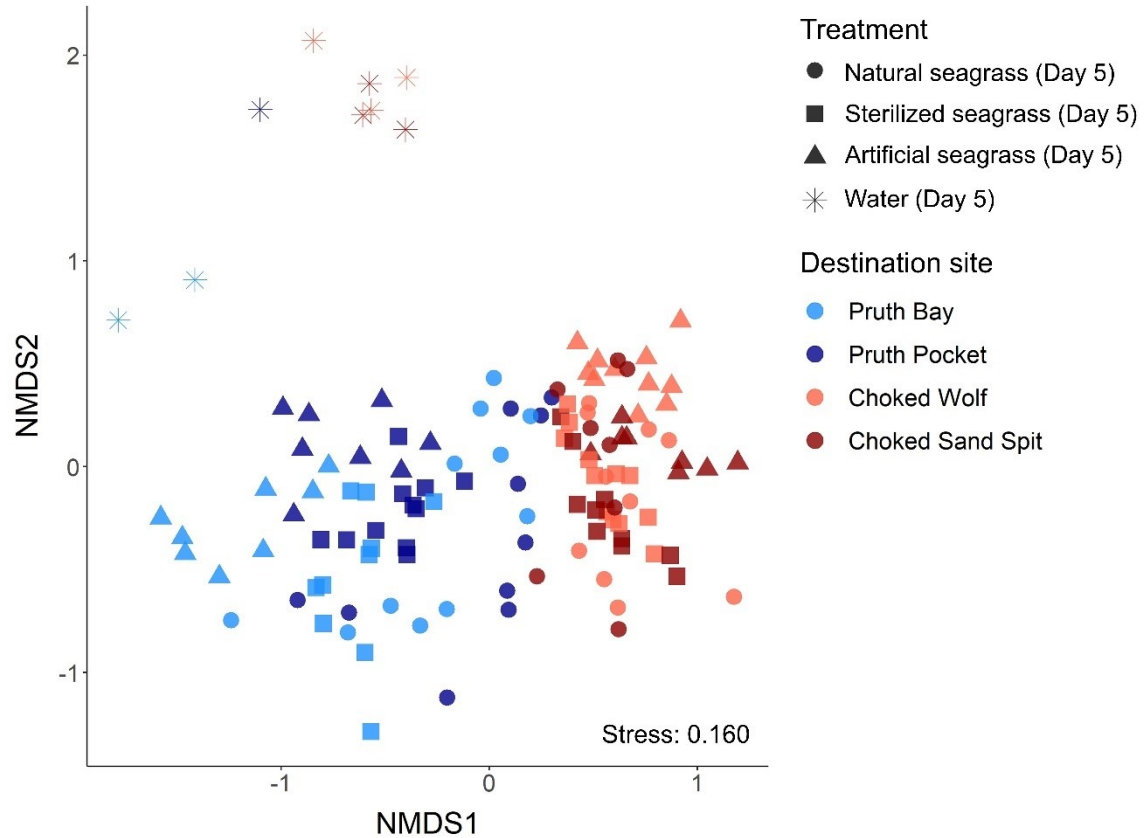


Figure S2. Non-metric multidimensional scaling (NMDS) plot using Bray-Curtis dissimilarity. Day 5 microbial communities on natural seagrass leaves, sterilized/dead seagrass leaves, artificial seagrass leaves, and water collected on the last day of the experiment.

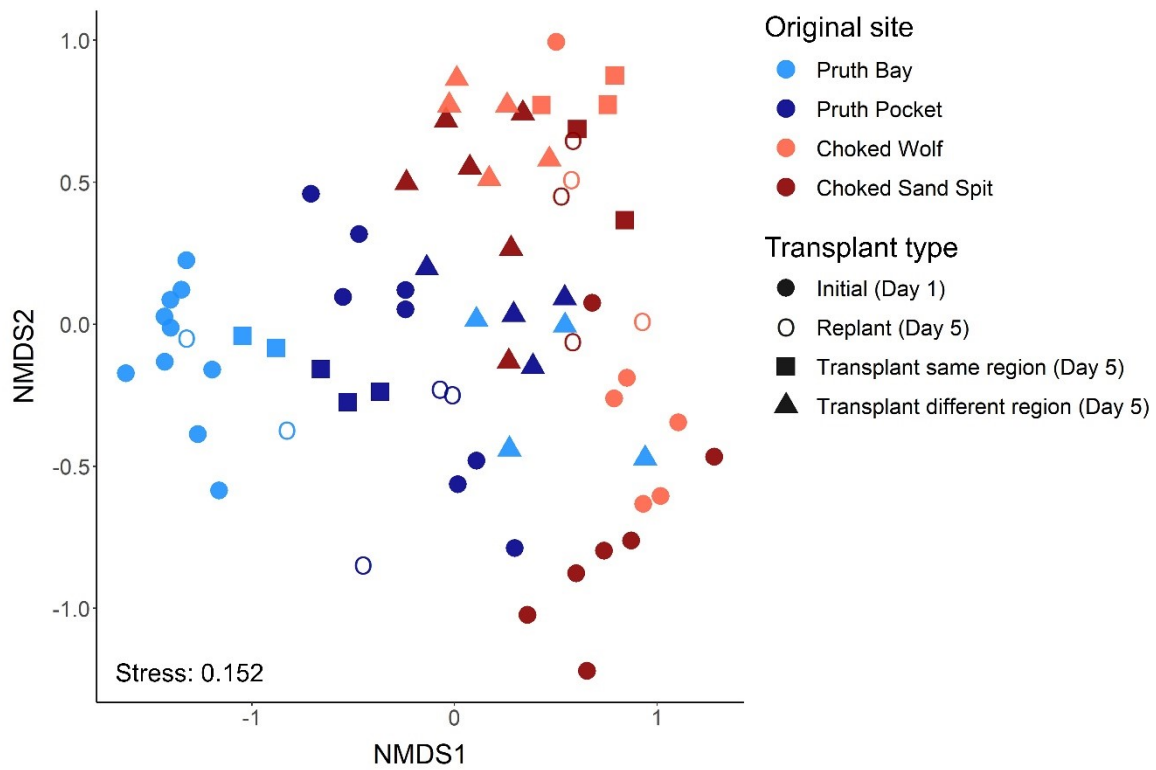


Figure S3. Non-metric multidimensional scaling (NMDS) plot using Bray-Curtis dissimilarity. Microbial communities on natural seagrass leaves for each location and time point. Colours indicate the sample's original site and shapes indicate whether the samples were replanted, transplanted to the same region, or transplanted to a different region.

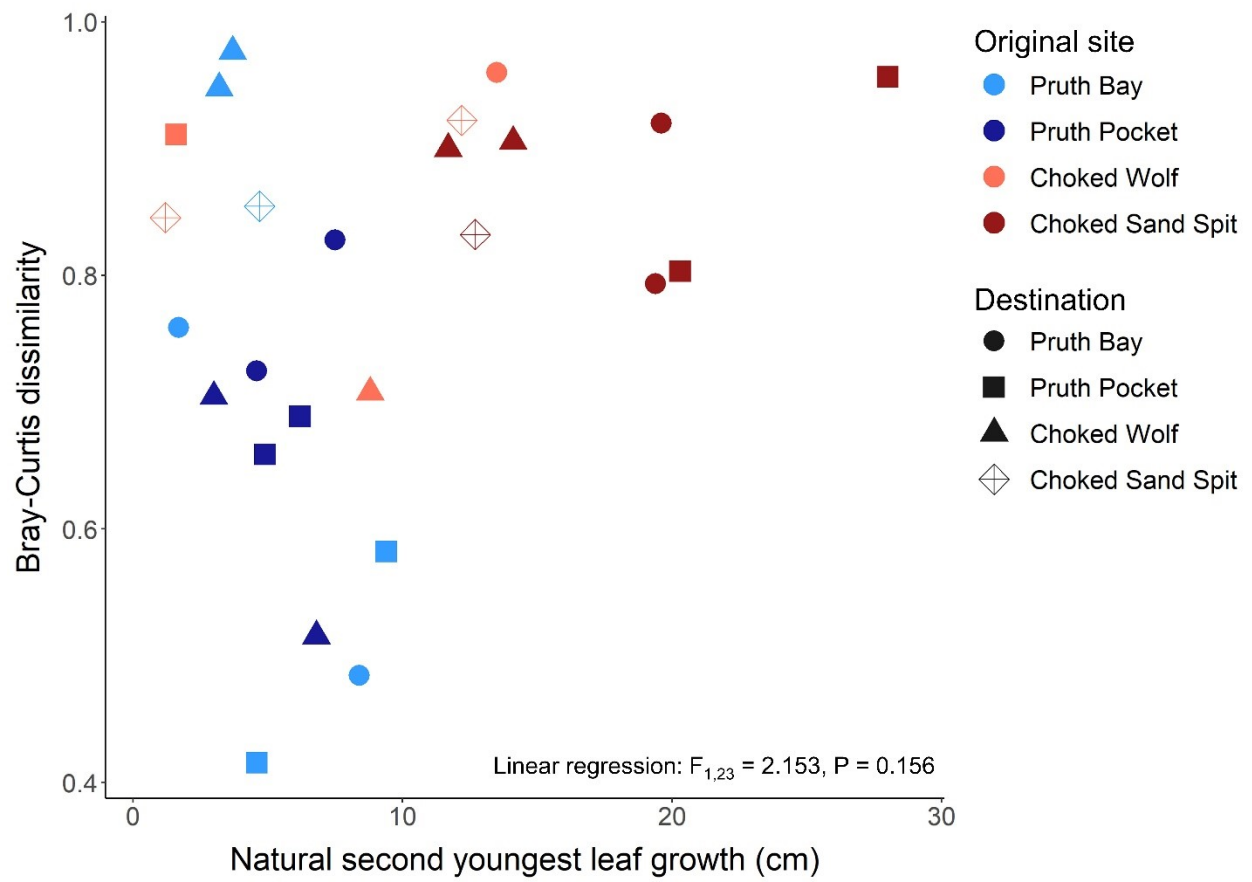


Figure S5. Growth of second youngest seagrass leaf for natural seagrass shoot compared to Bray-Curtis dissimilarity (Day 1 vs Day 5). There is no relationship between growth and community turnover.