

Supplementary Materials for
Symbioses are restructured by repeated mass coral bleaching

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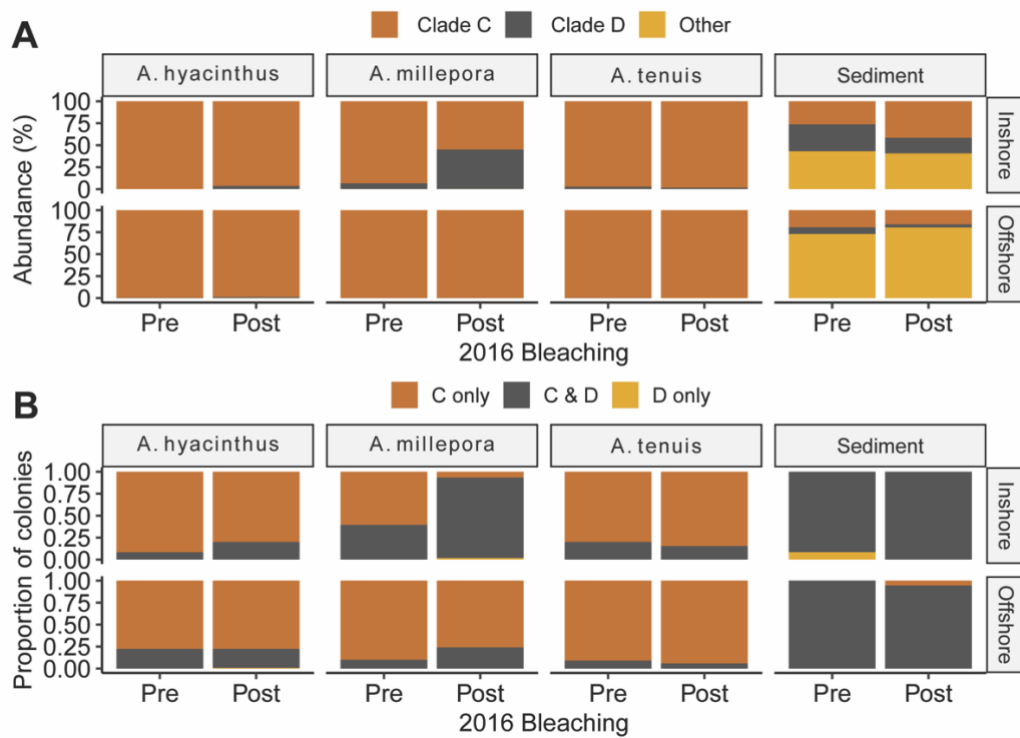


Fig. S1.

(A) average abundance of *Cladocopium* and *Durusdinium* in each sample source between inshore and offshore locations. (B) proportion of samples in each source that contain only *Cladocopium*, *Cladocopium* and *Durusdinium*, or only *Durusdinium* between inshore and offshore locations.

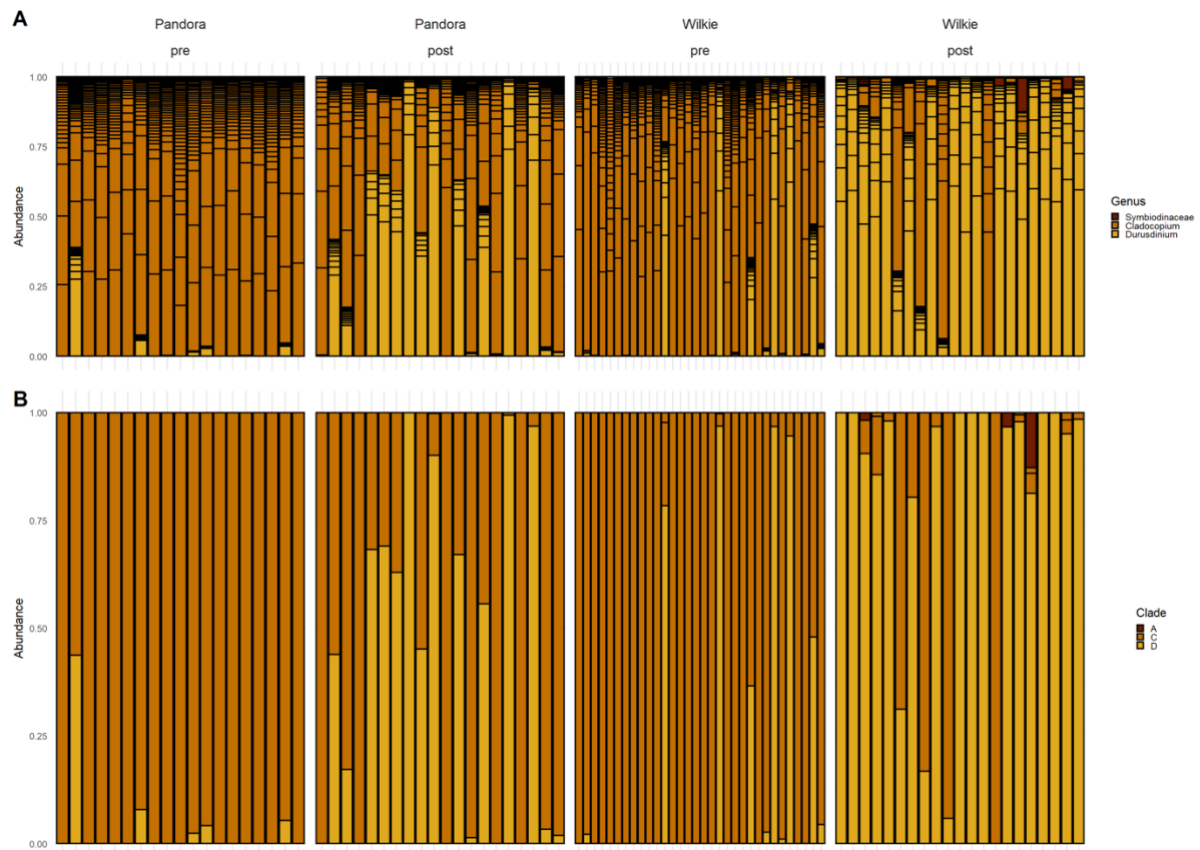


Fig. S2.

Secondary approach to assess Symbiodiniaceae community diversity using the Defining Intragenomic Variants approach (DIV) from the SymPortal pipeline. The DIV approach was applied to an informative subset of highly variable reefs and timepoints for our most variable species - *A. millepora*. **(A)** average abundance per individual sample of DIVs coloured at the genus level for *Symbiodinium*, *Cladocopium* and *Durusdinium* in *A. millepora* between pre and post locations at two reef sites. **(B)** average abundance of DIVs per individual sample grouped and coloured at the clade level (A,C,D). This comparison confirmed both approaches render comparable interpretations, especially at the clade level, specifically in which the relative proportion of *Cladocopium* was lower in pre samples and replaced by *Durusdinium* in the post samples. The corresponding bars averaged at the reef level (compared to at the individual sample level) are bars 4 and 9 on Fig. 2 (left to right). When averaged over all individuals sampled on each reef, the relative proportions were similar for ASVs (Pandora - pre: ~3%, post: ~25%; Wilkie - ~15%, ~75%; Fig. 2) compared to DIVs (Pandora - pre: ~5%, post: ~40%; Wilkie - ~13%, ~80%).

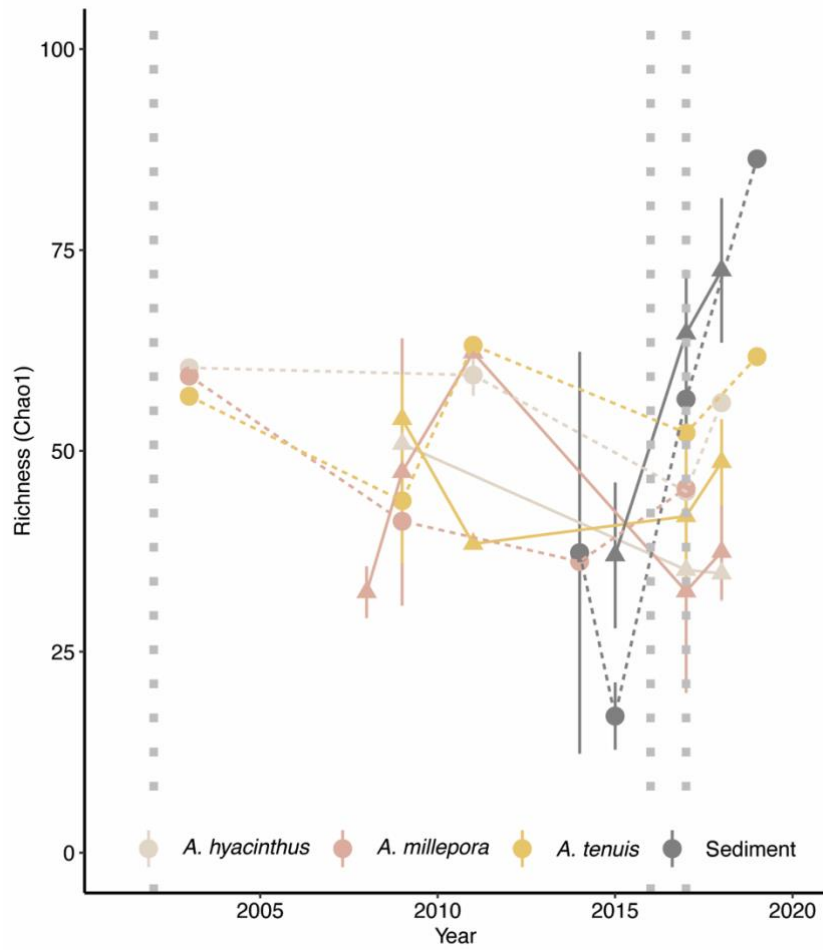


Fig. S3.
Changes in Symbiodiniaceae richness in coral and sediment samples collected along reefs from the northern and central Great Barrier Reef pre- and post-2016 mass bleaching.
 The mean \pm standard deviation of the Chao1 richness of Amplicon Sequence Variants - ASVs from 2003 to 2019 collected from reefs coloured by sample type.

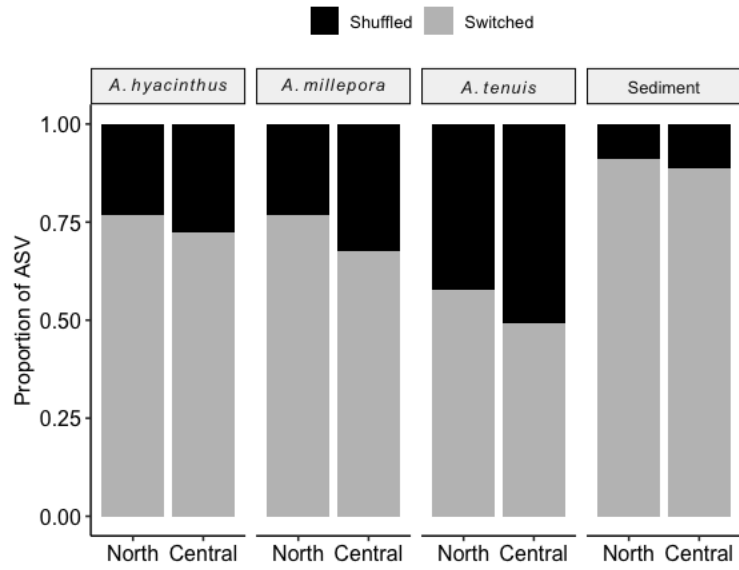


Fig. S4. Proportion of Amplicon Sequence Variants (ASVs) that either shuffled or switched for each sample type in either the northern or central Great Barrier Reef.

Supplementary Tables

Sector	Reef	<i>A. hyacinthus</i>		<i>A. millepora</i>		<i>A. tenuis</i>		Environment	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post
North	Curd	12	20		35	35	35		7
	Great Detached	30	35			35	35	3	6
	Long Sandy		35		5	30	35		3
	SandBank7	29	38		8	34	36		3
	Tydeman	30	17	7	4	35	16	3	
	Wallace			40	11	35	29	3	3
	Wilkie			35	28	35	26	3	2
Central	Arlington								7
	Backnumbers		36				29		
	Coates								13
	Davies	29	34	30	35	10	30	3	3
	Dunk								12
	Farquharson								9
	Feather								12
	Fitzroy Island								6
	Geoffrey Bay							3	3
	Havannah Island								9
	John Brewer	30	15			35	20		7
	North Barnard								6
	Orpheus							3	
	Pandora			19	20	35	21	3	9
	Peart								14
	Rib			33	11	33	15	3	9
	Russell								10
Taylor								9	
Trunk	30				34				
Total		190	230	164	157	386	327	27	162

Table S1.

Total number of individual coral or environmental samples processed per reef location.

Factor	Df	Sum sq.	R^2	pseudo-F	<i>p-value</i>
Sample source	3	28.7	0.08	54.7	<0.001
Inshore/Offshore	1	69.8	0.2	398.9	<0.001
North/Central	1	5.4	0.01	31.2	<0.001
Bleaching	1	3.9	0.01	22.7	<0.001
Residual	1291	226.14	0.65		

Table S2.

Permutational Multivariate Analysis of Variance (PERMANOVA) results for main effects (north/central; inshore/offshore; bleaching: pre-2016 or post-2016) on each sample source (coral species/environment). The table includes a list of the main effects and (Factor), degrees of freedom (Df), sum of squares (Sum squares), coefficient of determination (R^2), pseudo-F statistic, and p-value (*p*). Analysis includes only paired reefs that had samples from “pre” and “post” the 2016 bleaching.

Factor	Df	Sum sq.	R^2	pseudo-F	p -value
<i>Acropora hyacinthus</i>					
Inshore/Offshore	1	9.56	0.16	71.6	<0.001
North/Central	1	1.4	0.02	10.9	<0.001
Bleaching	1	4.4	0.07	33.4	<0.001
Residual	315	42.0	0.71		
<i>Acropora millepora</i>					
Inshore/Offshore	1	10.8	0.14	57.2	<0.001
North/Central	1	2.5	0.03	13.4	<0.001
Bleaching	1	7.2	0.09	38.0	<0.001
Residual	269	50.8	0.67		
<i>Acropora tenuis</i>					
Inshore/Offshore	1	59.0	0.39	435.8	<0.001
North/Central	1	4.6	0.03	34.0	<0.001
Bleaching	1	0.91	0.006	6.73	<0.001
Residual	646	87.57	0.57		
Environment					
Inshore/Offshore	1	2.59	0.11	7.59	<0.001
North/Central	1	0.96	0.04	2.82	<0.001
Bleaching	1	0.75	0.03	2.20	<0.003
Residual	52	17.76	0.80		

Table S3.

Permutational Multivariate Analysis of Variance (PERMANOVA) results for main effects (north/central; inshore/offshore; bleaching: pre-2016 or post-2016) on each sample source. The table includes a list of the main effects and (Factor), degrees of freedom (Df), sum of squares (Sum squares), coefficient of determination (R^2), pseudo-F statistic, and p -value (p). Analysis includes only paired reefs that had samples “pre” and “post” the 2016 bleaching.

Species	Sector	Region	Pre \pm SE	Pre n	Post \pm SE	Post n
<i>A. hyacinthus</i>	Inshore	North	0.5 \pm 0.5	12	3.5 \pm 3.4	20
		Central	NA	0	NA	0
	Offshore	North	0.3 \pm 0.3	89	1.5 \pm 1.1	90
		Central	0.3 \pm 0.05	59	0.2 \pm 0.05	49
<i>A. millepora</i>	Inshore	North	7.3 \pm 2.24	75	53.8 \pm 6.2	39
		Central	3.3 \pm 1.3	19	27.8 \pm 6.7	20
	Offshore	North	0.05 \pm 0.05	7	0.4 \pm 0.1	4
		Central	0.09 \pm 0.04	63	0.16 \pm 0.06	46
<i>A. tenuis</i>	Inshore	North	3.6 \pm 1.1	105	1.4 \pm 0.5	90
		Central	0 \pm 0	35	2.1 \pm 1.3	21
	Offshore	North	0.3 \pm 0.2	134	0.02 \pm 0.02	122
		Central	0.2 \pm 0.1	78	0.06 \pm 0.02	65
Environment	Inshore	North	21.1 \pm 5.6	6	6.8 \pm 3.2	5
		Central	40.1 \pm 4.7	6	22.4 \pm 1.7	12
	Offshore	North	5.1 \pm 0.8	3	2.8 \pm 0.8	6
		Central	8.8 \pm 3.4	6	3.7 \pm 0.7	12

Table S4.

Relative abundance (%) of *Durusdinium* in samples taken before and after the 2016 bleaching event.

Rank	<i>A. hyacinthus</i>	<i>A. millepora</i>	<i>A. tenuis</i>	Environment
1	C3k (7.1)	Cspc (6.0)	Cspc (4.7)	C1m (3.4)
2	C3k (5.4)	C3k (5.8)	C1m (3.8)	D1 (3.3)
3	Cspc (5.2)	C3k (4.9)	C3k (3.7)	A10 (2.3)
4	C3k (2.7)	C3k (4.2)	C3k (3.5)	D1a (3.0)
5	C3k (2.0)	C-NA (2.0)	C50 (1.9)	Cspc (1.8)
6	C3k (2.0)	C3j (1.7)	C1c.C45 (1.9)	I4 (1.5)
7	C3k (1.9)	C3k (1.7)	C1m (1.7)	A4.3 (1.5)
8	C29 (1.9)	C- NA (1.6)	C-NA (1.7)	A15c (1.5)
9	C3k (1.8)	C3.11 (1.6)	C29 (1.7)	I-NA (1.2)
10	C3k (1.6)	C45.3 (1.6)	C3k (1.7)	I3 (1.0)

Table S5.

The mean normalized abundance and identity of the top-10 most abundant ASV in each sample source. Means in parentheses (%) were calculated using the mean abundance of each ASV on each reef. Where the ASV sequence did not match a published Symbiodiniaceae sequence, the ASV identity is reported as Genus-NA.

Sample source	Region	Pre-2016 (n samples)	Post-2016 (n samples)
<i>A. hyacinthus</i>	North	54.2±1.3 (101)	36.6±1.2 (145)
	Central	59.7±1.2 (89)	44.0±1.5 (85)
<i>A. millepora</i>	North	31.6±1.3 (82)	26.4±1.7 (91)
	Central	46.6±1.5 (82)	47.0±1.6 (66)
<i>A. tenuis</i>	North	51.5±0.9 (239)	45.3±0.9 (212)
	Central	49.1±1.2 (147)	56.5±1.6 (115)
Environment	North	37±5.2 (12)	67.5±3.4 (24)
	Central	21.8±3.5 (26)	57.5±1.9 (138)

Table S6.

Changes in the mean \pm Standard Error of the community richness metric Chao1 of Symbiodiniaceae Amplicon Sequence Variants. Sample sizes of each group is given within parenthesis.

Factor	Df	Sum sq.	pseudo-F	<i>p-value</i>
<i>A. hyacinthus</i>				
Region	1	463.7	6.84	0.1311
Bleaching	1	20464.1	129.8	<2e-16
Region*Bleaching	1	146.9	400.3	0.3349
<i>A. millepora</i>				
Region	1	86.12	0.6858	0.4359085
Bleaching	1	1511.58	12.0379	0.0005939
Region*Bleaching	1	698.63	5.5638	0.0189455
<i>A. tenuis</i>				
Region	1	48.3	0.5999	0.4549
Bleaching	1	60.2	0.7473	0.3876
Region*Bleaching	1	4253.8	52.8109	9.777e-13
Environment				
Region	1	1381.5	5.4674	0.02755
Bleaching	1	21516.0	85.1538	< 2e-16
Region*Bleaching	1	742.0	2.9367	0.08817

Table S7.

Summary of significance tests of symbiont community richness, as measured by changes in the Chao1 metric. Tests were run using the *lmer* function, with region and bleaching time as fixed effects and reef as a random effect.

Sample source	Region	Pre-2016 (n samples)	Post-2016 (n samples)
<i>A. hyacinthus</i>	North	54.1±1.34 (101)	36.7±1.2 (145)
	Central	59.7±1.29 (89)	44.0±1.55 (85)
<i>A. millepora</i>	North	31.5±1.37 (82)	26.4±1.71 (91)
	Central	46.7±1.58 (82)	47.1±1.6 (66)
<i>A. tenuis</i>	North	51.4±0.941 (239)	45.3±0.91 (212)
	Central	49.0±1.28 (147)	56.5±1.61 (115)
Environment	North	36.6±5.24 (12)	67.5±3.45 (24)
	Central	28.7.7±5.28 (15)	57.5±1.93 (138)

Table S8.

Changes in the mean diversity (number of Amplicon Sequence Variants) per sample ± Standard Error of community diversity of Symbiodiniaceae. Samples size (n) of each group is given within parenthesis.

Factor	Df	Chi sq.	<i>p</i> -value
<i>Acropora hyacinthus</i>			
Region	1	-2.04	0.0414
Bleaching	1	-13.2	<2e-16
Region*Bleaching	1	0.988	0.322
<i>Acropora millepora</i>			
Region	1	0.218	0.798
Bleaching	1	-1.35	0.176
Region*Bleaching	1	-6.19	6.0e-10
<i>Acropora tenuis</i>			
Region	1	0.081	0.936
Bleaching	1	4.84	1.2e-6
Region*Bleaching	1	-9.36	<2e-16
Environment			
Region	1	2.3	0.0211
Bleaching	1	13.96	<2e-16
Region*Bleaching	1	-1.19	0.232

Table S9.

Summary of significance tests of symbiont community diversity, as measured by changes in the number of Amplicon Sequence Variants per sample. Tests were run using the *glmer* function, with region and bleaching time as fixed effects and reef as a random effect using a Poisson distribution.

Factor	Df	Sum sq.	R^2	pseudo-F	<i>p</i> -value
<i>Acropora hyacinthus</i>					
Inshore/Offshore	1	5.8	0.14	34.1	<0.001
North/Central	1	0.9	0.02	5.8	<0.001
Bleaching	1	2.8	0.06	16.4	<0.001
Residual	185	31.5	0.17		
<i>Acropora millepora</i>					
Inshore/Offshore	1	10.2	0.24	54.2	<0.001
North/Central	1	3.8	0.09	20.3	<0.001
Bleaching	1	3.6	0.08		<0.001
Residual	128	50.8	0.57		
<i>Acropora tenuis</i>					
Inshore/Offshore	1	40.1	0.44	315.0	<0.001
North/Central	1	2.0	0.02	15.9	<0.001
Bleaching	1	2.2	0.02	17.2	<0.001
Residual	363	46.2	0.51		
Environment					
Inshore/Offshore	1	2.56	0.11	7.4	<0.001
North/Central	1	0.96	0.04	2.8	<0.001
Bleaching	1	0.80	0.03	2.3	<0.001
Residual	52	17.76	0.80		

Table S10.

Permutational Multivariate Analysis of Variance (PERMANOVA) for a subset of years (2011-2019) results for main effects (north/central; inshore/offshore; for each sample source). The table includes a list of the main effects (Factor), degrees of freedom (Df), sum of squares (Sum squares), coefficient of determination (R^2), pseudo-F statistic, and *p*-value (*p*).

	Factor	Df	Sum sq.	Mean sq.	F	<i>p</i> -value
<i>A. hyacinthus</i>	Category	3	2.6	0.87	33.7	<0.001
<i>A. millepora</i>	Category	2	2.1	1.08	28.7	<0.001
<i>A. tenuis</i>	Category	3	7.21	2.4	109.3	<0.001
Environment	Category	3	0.05	0.01	6.03	<0.001

Table S11.

Permutation test results for homogeneity of multivariate dispersions for variance normalized relative abundances of Symbiodiniaceae communities for each sample source (coral species/environment) by bleaching category (LL, LH, HH, HL). The table includes a list of the main effects (Factor), degrees of freedom (Df), sum of squares (Sum squares), mean squares (mean sq.), F statistic (F), and p-value (*p*), with all tests using *n* = 999 number of permutations.

Sample source	Factor	Df	Sum sq.	R^2	F	p -value
<i>A. hyacinthus</i>	Category	3	5.23	0.07	10.4	<0.001
<i>A. millepora</i>	Category	2	13.1	0.17	28.8	<0.001
<i>A. tenuis</i>	Category	3	18.60	0.13	34.9	<0.001
Environment	Category	3	4.02	0.09	4.3	<0.001

Table S12.

Permutational Multivariate Analysis of Variance (PERMANOVA) results for variance normalized relative abundances of Symbiodiniaceae communities for each sample source (coral species/environment) by bleaching category (LL, LH, HH, HL). The table includes a list of the main effects (Factor), degrees of freedom (Df), sum of squares (Sum squares), coefficient of determination (R^2), Model F-value (F), and p-value (p). Analysis includes only paired reefs that had samples “pre” and “post” the 2016 bleaching.