frontiers in Marine Science

ORIGINAL RESEARCH
published: 10 June 2021
doi: 10.3389/fmars.2021.679683

Impacts of Seagrass on Benthic Microalgae and Phytoplankton Communities in an Experimentally Warmed Coral Reef Mesocosm

Kwee Siong Tew[1,2], Jimmy Kuo[1,2], Jing-O Cheng[1], Fung-Chi Ko[1,2], Pei-Jie Meng[1,2], Anderson B. Mayfield[3,4] and Pi-Jen Liu[2]**

[1] National Museum of Marine Biology and Aquarium, Checheng, Taiwan, [2] Graduate Institute of Marine Biology, National Dong Hwa University, Checheng, Taiwan, [3] National Oceanic and Atmospheric Administration, Miami, FL, United States, [4] Cooperative Institute for Marine and Atmospheric Studies, University of Miami, Miami, FL, United States

The effects of seagrass on microalgal assemblages under experimentally elevated temperatures (28°C) and CO_2 partial pressures (pCO_2; 800 μatm) were examined using coral reef mesocosms. Concentrations of nitrate, ammonium, and benthic microalgal chlorophyll a (chl-a) were significantly higher in seagrass mesocosms, whereas phytoplankton chl-a concentrations were similar between seagrass and seagrass-free control mesocosms. In the seagrass group, fewer parasitic dinoflagellate OTUs (e.g., Syndiniales) were found in the benthic microalgal community though more symbiotic dinoflagellates (e.g., *Cladocopium* spp.) were quantified in the phytoplankton community. Our results suggest that, under ocean acidification conditions, the presence of seagrass nearby coral reefs may (1) enhance benthic primary productivity, (2) decrease parasitic dinoflagellate abundance, and (3) possibly increase the presence of symbiotic dinoflagellates.

Keywords: *Cladocopium*, climate change, dinoflagellates, nutrients, seagrass, sedimentation, syndiniales

OPEN ACCESS

Edited by:
Maren Ziegler,
University of Giessen, Germany

Reviewed by:
Caroline Eve Dubé,
Laval University, Canada
Elena Bollati,
National University of Singapore, Singapore

****Correspondence:***
Kwee Siong Tew
tewks@nmmba.gov.tw
Pi-Jen Liu
pijenliu@gms.ndhu.edu.tw

Specialty section:
This article was submitted to Coral Reef Research, a section of the journal Frontiers in Marine Science

Received: *12 March 2021*
Accepted: *14 May 2021*
Published: *10 June 2021*

Citation:
Tew KS, Kuo J, Cheng J-O, Ko F-C, Meng P-J, Mayfield AB and Liu P-J (2021) Impacts of Seagrass on Benthic Microalgae and Phytoplankton Communities in an Experimentally Warmed Coral Reef Mesocosm. Front. Mar. Sci. 8:679683. doi: 10.3389/fmars.2021.679683

INTRODUCTION

Seagrass meadows are common in coastal waters (Unsworth et al., 2012, 2019b) and support diverse fish and invertebrate communities (de los Santos et al., 2019; Unsworth et al., 2019a; Liu et al., 2020). They also offer a number of ecosystem services to humans, including coastline protection, serving as habitats for commercially important food sources, and mitigation of climate change impacts. However, there is much to be learned with respect to their interactions with other species, particularly microbial communities (Unsworth et al., 2019a).

For example, Lamb et al. (2017) reported that seagrass ecosystems can sequester bacterial pathogens that are harmful to fish, invertebrates, and even humans, and Inaba et al. (2019) noted that seagrass provide habitats for bacteria that kill or limit the growth of the harmful alga *Chattonella antiqua*. Although seagrass and algae compete for the same nutrients and trace elements (Unsworth et al., 2012), seagrass can provide substrates for microbenthic algal settlement (Tew et al., 2012, 2017a); microalgae can sometimes overgrow their seagrass hosts (Lin et al., 2018). Algal blooms can also reduce light intensity, which consequently inhibits seagrass growth (Tiling and Proffitt, 2017). These studies highlight the complex dynamic between seagrass and

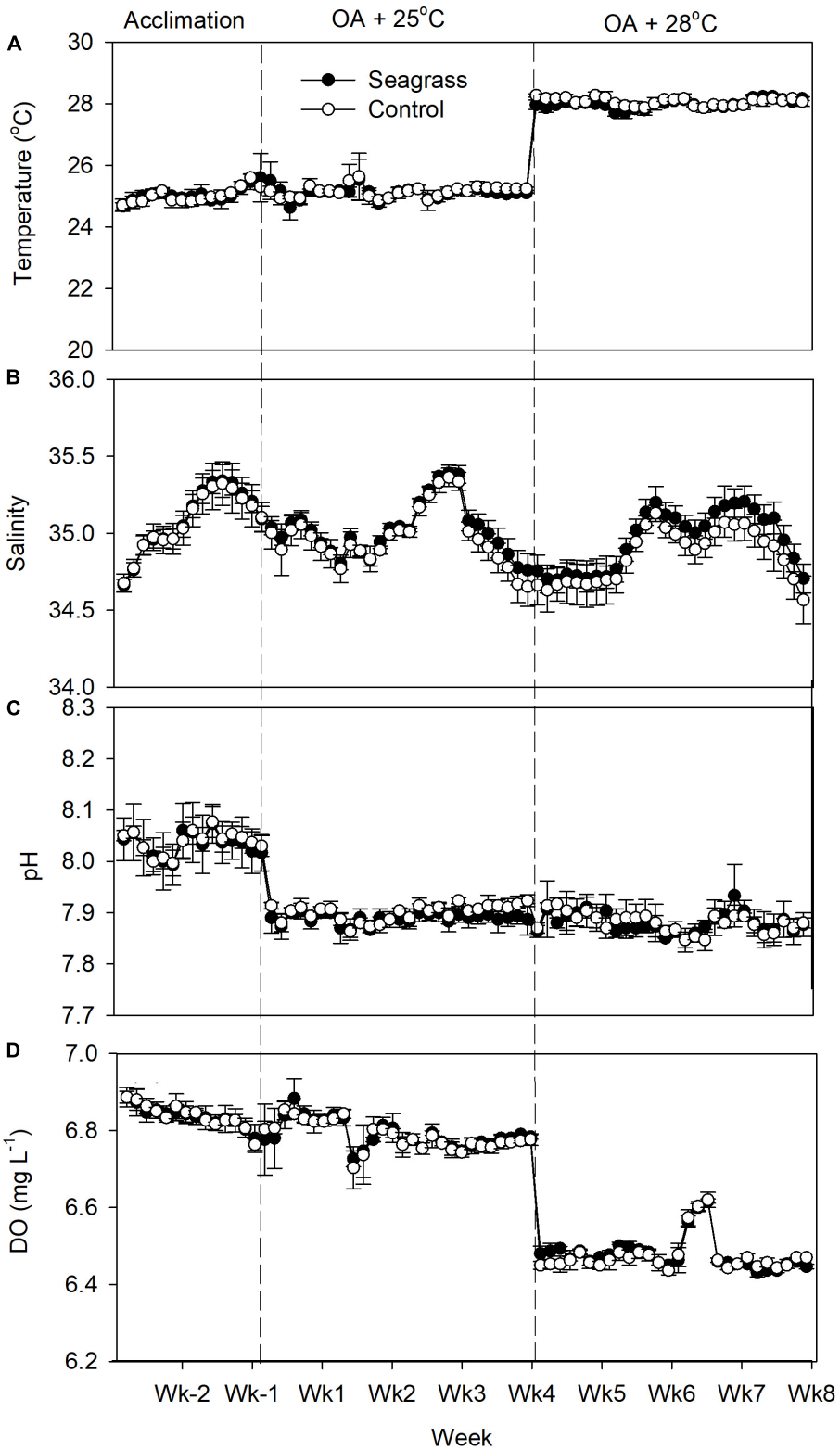

FIGURE 1 | (A) Temperature, **(B)** salinity, **(C)** pH, and **(D)** dissolved oxygen (DO) concentration in the experimental mesocosms. Error bars represent standard deviation (n = 3).

TABLE 1 | Comparison of abiotic parameters between seagrass and seagrass-free control groups.

Variable	Seagrass	Control	*F*	*p*
OA + 25°C				
Temperature (°C)	25.1 ± 0.2	25.2 ± 0.2	1.442	0.240
Salinity	35.0 ± 0.2	35.0 ± 0.2	49.450	<0.001*
pH	7.89 ± 0.03	7.91 ± 0.03	29.541	<0.001*
DO (mg L^{-1})	6.79 ± 0.04	6.79 ± 0.04	1.575	0.220
TA (μmol kg^{-1})	2, 073 ± 80	2, 047 ± 64	9.619	0.053
pCO_2 (μatm)	826 ± 54	802 ± 30	3.843	0.145
ΩAr	1.698 ± 0.089	1.693 ± 0.079	0.218	0.673
HCO_3^- (μmol kg^{-1})	1, 806 ± 76	1, 781 ± 57	7.458	0.072
CO_3^{2-} (μmol kg^{-1})	106.9 ± 5.6	106.5 ± 4.8	0.221	0.670
CO_2 (μmol kg^{-1})	23.3 ± 1.7	22.7 ± 1.0	3.316	0.166
NO_3^- (mg L^{-1})	0.051 ± 0.024	0.026 ± 0.022	41.416	0.008*
NO_2^- (mg L^{-1})	0.008 ± 0.002	0.006 ± 0.002	73.279	0.003*
NH_3^- (mg L^{-1})	0.022 ± 0.008	0.017 ± 0.006	4.601	0.121
PO_4^{3-} (mg L^{-1})	0.014 ± 0.012	0.003 ± 0.003	13.102	0.036*
OA + 28°C				
Temperature (°C)	28.0 ± 0.1	28.1 ± 0.1	9.151	0.005*
Salinity	35.0 ± 0.2	34.9 ± 0.2	140.4	<0.001*
pH	7.88 ± 0.02	7.88 ± 0.02	0.006	0.941
DO (mg L^{-1})	6.48 ± 0.04	6.48 ± 0.05	1.520	0.228
TA (μmol kg^{-1})	2, 087 ± 42	2, 124 ± 69	3.904	0.143
pCO_2 (μatm)	907 ± 39	912 ± 41	0.936	0.405
ΩAr	1.589 ± 0.029	1.628 ± 0.045	2.722	0.198
HCO_3^- (μmol kg^{-1})	1, 837 ± 43	1, 870 ± 65	4.116	0.135
CO_3^{2-} (μmol kg^{-1})	100.1 ± 2.0	102.6 ± 2.8	2.838	0.191
CO_2 (μmol kg^{-1})	25.7 ± 1.2	25.9 ± 1.2	1.827	0.269
NO_3^- (mg L^{-1})	0.071 ± 0.018	0.008 ± 0.007	87.574	0.003*
NO_2^- (mg L^{-1})	0.007 ± 0.001	0.006 ± 0.001	0.456	0.548
NH_3^- (mg L^{-1})	0.016 ± 0.002	0.014 ± 0.002	20.336	0.020*
PO_4^{3-} (mg L^{-1})	0.007 ± 0.003	0.005 ± 0.001	1.222	0.350

Values represent mean ± standard deviation.
**: Significantly different (P < 0.05).*

other microbes (especially microalgae) and point to a better need to understand the underlying ecosystem processes.

Since increases in CO_2 lower seawater pH, studies in the Philippines (Marbà et al., 2007), the Great Barrier Reef (Unsworth et al., 2012), and the Mediterranean (Invers et al., 1997) have recorded diel changes in pH between 0.5 and 0.7 in seagrass habitats. Seagrass can increase pH by up to 0.4 (Unsworth et al., 2012) by reducing CO_2 through photosynthetic uptake (dependent on water residence time and water depth). Thus, seagrass could potentially buffer future ocean acidification (OA), thereby potentially modulating OA impacts on other members of the seagrass bed community, such as phytoplankton, benthic algae, or even corals in areas where seagrass and corals are located in proximity (Huan et al., 2015; Lin et al., 2018; Lee et al., 2019).

Coral reef mesocosms have previously been used to investigate the effects of simulated climate change on coral reef-associated benthic microalgal communities, and some diatom species were shown to grow faster and smaller at high temperatures (Tew et al., 2014); however, OA benefits benthic microalgal primary productivity pending sufficient nutrient levels (Tew et al., 2017b). Given the aforementioned potential for direct and indirect effects of seagrass on the seagrass meadow's microalgal communities, we investigated the effects of OA (800 μatm) on phytoplankton and benthic microalgal communities in mesocosms with and without the presence of seagrass at 25 and 28°C. We specifically hypothesized that potentially detrimental OA effects on these microbial communities would be mitigated by the co-cultured seagrass.

MATERIALS AND METHODS

Mesocosm Establishment

The six mesocosms (5.14 m^2 × 1 m depth, ∼5,000 L each) have been maintained continuously since 2001 at Taiwan's National Museum of Marine Biology and Aquarium, with a variety of reef-building corals present at all times. These flow-through systems utilize sand-filtered seawater from the adjacent (∼50 m) coastal areas and have been developed to mimic tropical coral reef

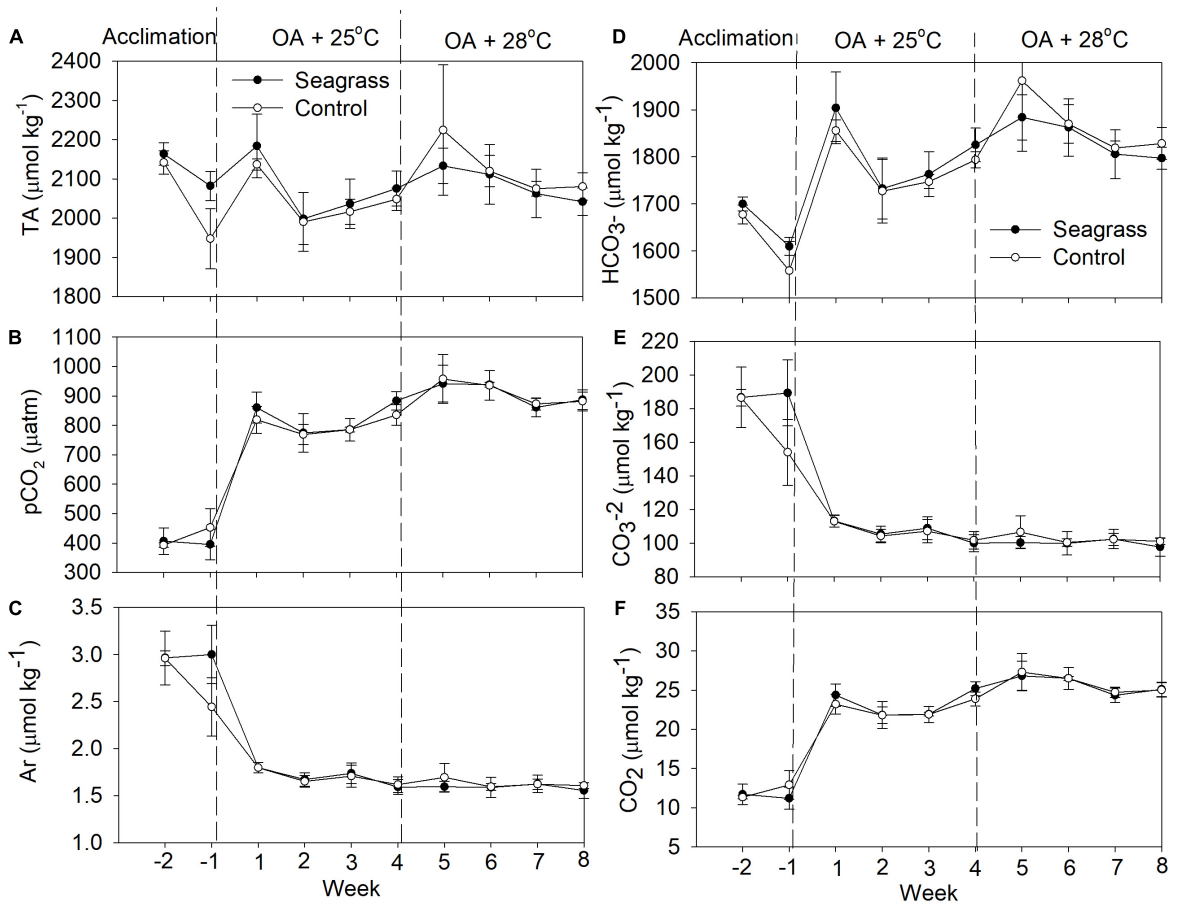

FIGURE 2 | (A) Total alkalinity (TA), **(B)** pCO_2, **(C)** Ω_{Ar}, and **(D–F)** other carbonate system parameters in the experimental mesocosms. Error bars represent standard deviation (n = 3).

ecosystems of Southern Taiwan (Liu et al., 2009, 2015, 2020; Tew et al., 2017b). The mesocosms were set up in a completely randomized design (i.e., A-1 of Cornwall and Hurd, 2016), and temperatures were maintained between 25 and 26°C to simulate field conditions in the Taiwanese spring prior to the experiment. The photosynthetically active radiation (PAR) at 0.5 m depth was maintained at 258 ± 16.7 μmol photons m^{-2} s^{-1} from 0700 to 1,700 h with a 10:14 h light:dark cycle using LED lamps (XLamp XT-E LEDs, Cree, Taiwan). Seawater was continuously pumped into the tanks at 3 L min^{-1}. The organisms in the mesocosms, such as corals, fish, sea urchins, and sea anemones (see Liu et al., 2020 for details.), were collected from nearby coral reefs under permits issued by the Kenting National Park Headquarters (Liu et al., 2009, 2015).

Individual seagrass (*Thalassia hemprichii*) shoots with intact rhizomes were randomly collected from a depth of ∼1 m in submerged seagrass meadows at Nanwan Bay, Taiwan and transported to the laboratory within 2 h of collection. Seagrass were acclimated for 5 days in aerated seawater, and individuals with similar lengths and numbers of leaves and roots were selected and cleaned of visible epiphytes before experiments. Each seagrass mesocosm (n = 3) received approximately 681 ± 7 shoots m^{-2} during the experiments.

Experimental Design

Two separate experiments were conducted with or without ("control") seagrass (n = 3 mesocosms/seagrass treatment) under 800 ppm pCO_2 (Representative Concentration Pathway, RCP 6.0 in 2100, IPCC, 2014) (hereafter "OA"): one at 25°C and another at 28°C. We did not conduct a 2-temperature × 2-pCO_2 × 2 seagrass treatment (with or without) factorial design due to the limited number of mesocosms (n = 6). Each experiment lasted for 4 weeks. After the completion of the first experiment (OA + 25°C, with or without seagrass), we raised the temperature by 1°C per day, rearranged the flora and fauna within each mesocosm, and initiated the second experiment (OA + 28°C) 3 days later.

Abiotic Parameters

During the experiment, seawater temperature and salinity were measured daily with a handheld meter (YSI Model-30, YSI, United States), with dissolved oxygen (DO) and pH measured daily by a multiparameter meter (YSI Model-556). All measurements were taken between 0900 and 1,000 h local time). A total alkalinity (TA) test kit (Thermo-Scientific's "Orion," United States) was used to measure TA, and the pCO_2 and aragonite saturation state (Ω_{Ar}) were calculated

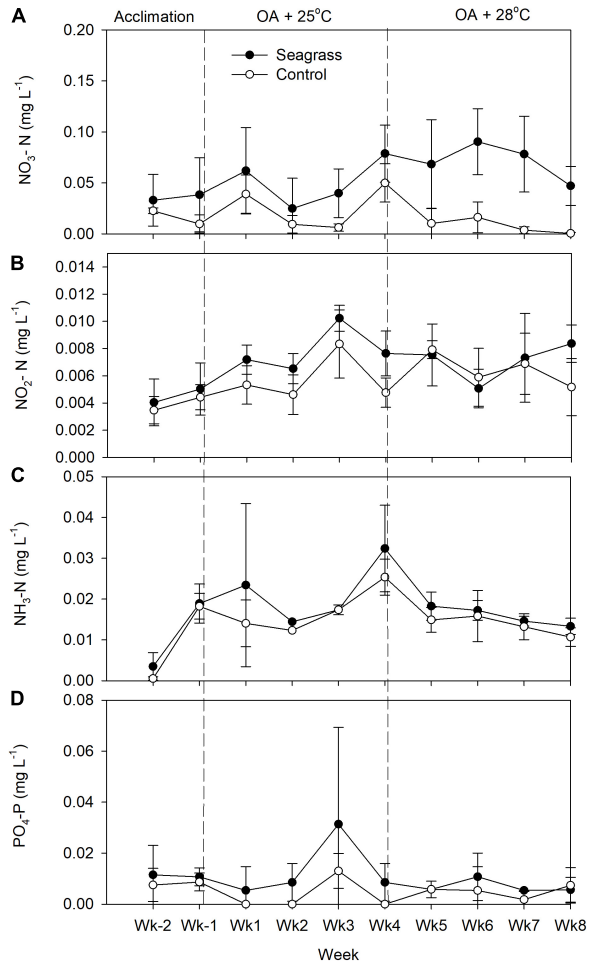

FIGURE 3 | Weekly measurements of **(A)** nitrate, **(B)** nitrite, **(C)** ammonia, and **(D)** phosphate concentrations in the experimental mesocosms. Error bars represent standard deviation (n = 3).

by inputting pH, TA, salinity, and temperature into CO2SYS (Lewis et al., 1998). To monitor mesocosm nutrient levels, we collected water samples weekly and used a flow injection analyzer and spectrophotometer (Hitachi model U-5100, Japan) to estimate nitrate, nitrite, ammonia, and phosphate concentrations (Pai et al., 1990, 2001; Pai and Riley, 1994). Details of the nutrient measurement methods can be found in Tew et al. (2013).

Biotic Parameters

We used glass slides as artificial substrata for benthic microalgal attachment. They were placed upright in an acrylic cage (all slides were put in one cage in each mesocosm) to exclude grazing by snails and sea urchins (*sensu* Tew et al., 2017b), with one collection from each of the six mesocosms after each week of the experiment. The benthic microalgae were removed from the glass slides weekly with a razor blade, re-suspended in 100 mL of 0.22 μm-filtered seawater (FSW), sonicated for 10 min, filtered through 0.45 μm GF/F filter paper (Whatman, United Kingdom), and chlorophyll a (chl-a) was extracted as described below. Benthic microalgal chl-a was expressed per unit surface area of the slides. Phytoplankton samples were collected weekly by filtering 2 L of water through a 0.45 μm GF/F filter paper, and chl-a was extracted from the benthic and seawater sample filters after 24 h immersion in 90% acetone at 4°C in the dark. Chl-a concentrations were estimated with a spectrophotometer (Hitachi U-5100) using the equations of Parsons (2013). After 4 and 8 weeks (hereafter "Wk4" and "Wk8," respectively), additional phytoplankton and benthic microalgal samples were collected from each mesocosm for DNA analysis. Phytoplankton samples from each treatment were pooled into a single sample at each sampling time since genomic DNA (gDNA) yield from samples derived from a single mesocosm were too low for successful amplicon sequencing (resulting in a total of four DNA extracts for the phytoplankton communities). Of the 12 benthic samples (six mesocosms at each of two times), one extraction failed (mesocosm 3 of Wk8), resulting in a final sample size of 11 (5 seagrass and 6 controls).

gDNA Extraction, 18S rRNA Tag Sequencing, and Bioinformatics

Total gDNA was extracted from the 18 samples and purified using the method of Kuo et al. (2014). Amplicon PCR, purification, and tag sequencing were performed at Welgene Biotech (Taipei, Taiwan) according to their standard protocols. The eukaryotic universal primers 528F (′5-GCGGTAATTCCAGCTCCAA-3′) and 706R (′5-AATCCRAGAATTTCACCTCT-3′) were used to amplify the V4 region of the 18S SSU rDNA, and amplicons were sequenced on an Illumina Hiseq 2500 sequencing system (United States).

The 250-bp paired-end raw reads were merged using FLASH version 1.2.7 (Magoc and Salzberg, 2011), and merged reads were trimmed. Low-quality reads (average quality score < 25) and chimeras were detected using USEARCH v6.1 (Edgar et al., 2011) and removed Clean reads were aligned against the eukaryotic SILVA database release 132 (Quast et al., 2013), and reads with >97% similarity were clustered into operational taxonomic units (OTUs) using the UCLUST algorithm (Edgar, 2010) and open-reference OTU picking strategy of QIIME (version 13.8; Caporaso et al., 2010). Representative OTU sequences were compared with the eukaryotic SILVA database, and taxonomy was assigned using BLAST. Non-algal OTUs were removed, and rarefaction curves of algal sequences for each sample were generated with custom perl scripts. To ensure an equal sampling depth for all samples, an OTU abundance table was rarefied to identical read counts equal to the lowest. All subsequent analyses were then conducted based on the rarefied OTU table. Alpha diversity indices (Good's coverage index, Chao1 indexes for richness, and Shannon-Weaver, and Simpson diversity), beta diversity indices (Bray-Curtis, Euclidean, and Jaccard dissimilarity), and principal coordinate analysis (PCoA) of beta diversity indexes for each library were calculated/carried out in QIIME. The raw sequence data were deposited in the NCBI Short Read Archive (SRA; BioSample accessions SAMN12855862-65).

TABLE 2 | Numbers of reads and OTUs, in addition to diversity indices, of the 18S rRNA gene sequences of all algal communities.

Sample	Total reads	Total OTUs	Algal reads	Algal OTUs	Sub-sampled reads	Sub-sampled OTUs	Coverage (%)	Chao1	Shannon	Simpson
Benthic microalgal community										
Seagrass-T1-Wk4	59,824	211	4,698	91	4,698	91	0.993	136.09	4.100	0.873
Control-T2-Wk4	51,106	268	14,242	150	4,698	103	0.994	134.91	4.128	0.878
Seagrass-T3-Wk4	61,690	212	38,837	111	4,698	68	0.994	104.11	1.748	0.457
Control-T4-Wk4	54,888	297	38,732	153	4,698	98	0.995	119.08	4.038	0.877
Seagrass-T5-Wk4	48,235	324	42,490	154	4,698	70	0.994	103.83	1.321	0.310
Control-T6-Wk4	47,388	148	46,691	98	4,698	51	0.996	63.75	0.641	0.128
Seagrass-T1-Wk8	51,737	260	25,883	126	4,698	73	0.995	98.67	3.842	0.871
Control-T2-Wk8	53,687	196	31,441	102	4,698	63	0.997	76.00	4.280	0.926
Control-T4-Wk8	52,001	182	26,355	110	4,698	72	0.995	102.67	2.917	0.707
Seagrass-T5-Wk8	48,090	120	46,285	76	4,698	38	0.997	51.13	0.472	0.097
Control-T6-Wk8	57,382	161	24,774	107	4,698	70	0.996	95.50	3.258	0.766
Phytoplankton community										
Seagrass-Wk4	43,614	553	28,427	288	4,698	122	0.985	335.00	2.346	0.674
Control-Wk4	50,251	741	42,887	406	4,698	163	0.983	259.28	3.067	0.728
Seagrass-Wk8	40,538	192	29,910	85	4,698	47	0.997	68.00	3.169	0.816
Control-Wk8	43,618	741	33,036	346	4,698	149	0.985	238.44	2.658	0.672
Total	764,049	1,814	474,688	837	70,470	533				

T, tank; WK, week.

Statistical Analysis

The values are presented as mean ± standard deviation (n = 3). One-way repeated-measures (RM) ANOVA was used to analyze treatment effects on the biotic and abiotic parameters by considering sampling date (co-varying with temperature) as a repeated factor. Where significant differences occurred, Tukey's *post hoc* tests were used to test for individual mean differences ($p < 0.05$). One-way RM ANOVA, rather than two-way (temperature × pCO_2), was used because we did not intend to compare the results between different temperatures (25 vs. 28^{o}C) since they were compared neither simultaneously nor independently. Data were natural log-transformed when necessary to meet the assumptions of normality and homogeneity of variance. SigmaPlot 12.5 was used for all univariate analyses. For the benthic microalgal OTU data, the assemblages were compared between seagrass and control groups at Wk 4 and Wk 8, respectively, with a non-metric multidimensional scaling ordination analysis (MDS, Kruskal and Wish, 1978; Field et al., 1982). Biotic data similarity matrices were constructed using the Bray-Curtis similarity measure on non-standardized, arcsine-transformed percentage data. Analysis of similarity (ANOSIM) was conducted with PRIMER 6 to determine whether the microbenthic algal assemblages separated by MDS ordination differed significantly (Clarke and Warwick, 1994).

RESULTS

Seawater Quality

At both OA + 25°C and OA + 28°C, the mean seawater temperature, salinity, pH, and DO were similar between seagrass and seagrass-free control mesocosms (**Figure 1**), although some parameters differed significantly (**Table 1**). DO was lower in seagrass mesocosms when the temperature was raised from 25 to 28°C (**Figure 1**). TA was 2,073 ± 80 and 2,087 ± 42 μmol kg^{-1} for the seagrass treatment and 2,047 ± 64 and 2,124 ± 69 μmol kg^{-1} in the controls at 25 and 28°C, respectively (**Figure 2**). pCO_2 was consistently maintained around 800 μatm (**Table 1**). Ω_{Ar} values dropped to around 1.6–1.7 when CO_2 increased (**Figure 2**), and no significant difference was detected between seagrass and control treatments (**Table 1**). The HCO_3-, CO_2, and CO_3^{2-} concentrations were similar between treatments (**Figure 2**) at both temperatures (**Table 1**).

The seawater nutrient concentrations during the acclimation period were low, with average concentrations of NO_3^-, NO_2^-, NH_3^-, and PO_4^{3-} of 0.028, 0.004, 0.002, and 0.010 mg L^{-1}, respectively (**Figure 3**). At 25°C, when CO_2 was bubbled into the six mesocosms, all nutrient concentrations except NH_3^- were significantly higher in the seagrass group (**Figure 3** and **Table 1**). When the temperature was raised to 28^{o}C, only NO_3^- and NH_3^- concentrations were significantly higher in the seagrass group (**Figure 3** and **Table 1**). For details on the mesocosms, including environmental data, please also see Liu et al. (2020).

18S Tag Sequencing Analysis

After removing low-quality reads and chimeras, a total of 764,049 clean sequences (average length = 304 bp) containing the V4 variable region of the 18S rRNA gene were obtained from 17 samples. These clean sequences were assigned to 1,814 unique OTUs based on 97% sequence identity (**Table 2**), and 474,688 sequences were of hypothetical algal origin. The rarefaction curves for algal reads did not approach a plateau (**Supplementary Figure 1**), indicating further sampling efforts are required to reveal the total diversity within the mesocosm microalgal

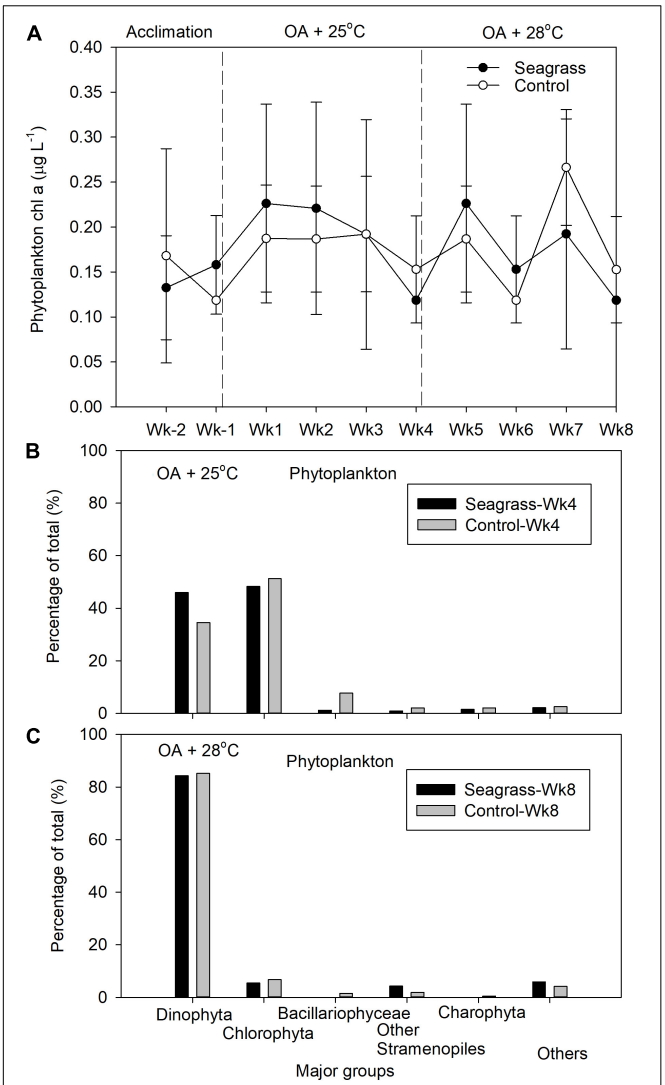

FIGURE 4 | (A) The phytoplankton chlorophyll a concentration and relative abundance of the five most abundant phytoplankton groups after **(B)** 4 (Wk4) and **(C)** 8 (Wk8) weeks of treatment exposure. Error bars represent standard deviation (n = 3).

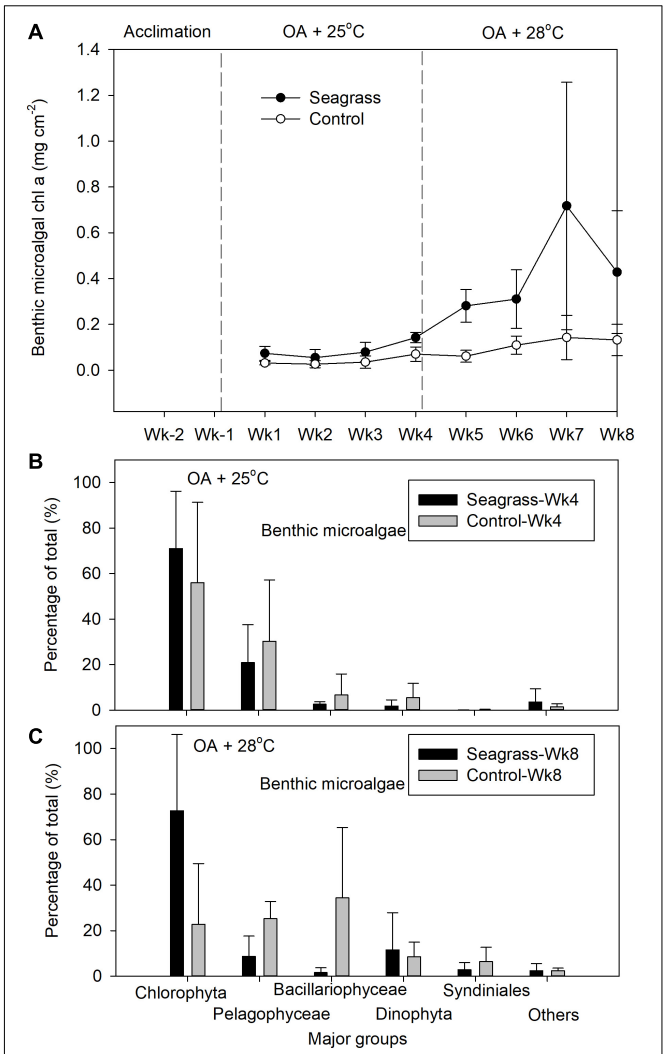

FIGURE 5 | (A) The benthic microalgae chlorophyll a concentration and relative abundance of the five most abundant benthic microalgal groups after **(B)** 4 (Wk4) and **(C)** 8 (Wk8) weeks of treatment exposure. Error bars represent standard deviation (n = 3).

community. The percentage of algae reads across samples was highly variable, ranging from 8 to 99% (average = 62%; **Table 2**). After normalizing, 4,698 randomly subsampled sequences were left for each sample. **Table 2** presents a summary of the subsampled OTUs, coverage, Chao1 indexes for richness, as well as Shannon-Weaver and Simpson diversity indexes. All indexes were not significantly different between seagrass and control groups at both temperatures (t-test, $P > 0.05$).

Phytoplankton chl-a concentrations were similar between seagrass and control mesocosms: 0.189 ± 0.050 and 0.180 ± 0.018 μg L^{-1} under OA + 25°C and 0.172 ± 0.047 and 0.181 ± 0.063 μg L^{-1} under OA + 28°C, respectively (**Figure 4A** and **Supplementary Table 1**). Phytoplankton communities (pooled genomic DNA from triplicate samples in each treatment) were dominated by Chlorophyta and Dinophyta at Wk4 between seagrass and control groups (**Figure 4B**). At Wk 8, the phytoplankton communities were also similar between treatments, with Dinophyta comprising >80% of the total algal OTUs (**Figure 4C**). However, analysis of the phytoplankton OTUs at the genus level revealed that, at 28°C, percent OTUs from the genus *Paragymnodinium* were 3 and 82% of the total Dinophyta OTUs in the seagrass mesocosms vs. in the controls, respectively. The dinoflagellate genus *Cladocopium* (formally *Symbiodinium* sp. clade C; LaJeunesse et al., 2018), on the other hand, were 20% and <1% of the total Dinophyta OTUs in the seagrass mesocosms vs. in the controls, respectively. Note that these community results were from one sample (pooled triplicate-samples) in each treatment.

Benthic microalgal chl-a concentrations were significantly higher ($p < 0.05$) in seagrass mesocosms under both OA + 25°C and OA + 28°C (0.088 ± 0.038 and 0.434 ± 0.199 mg cm^{-2},

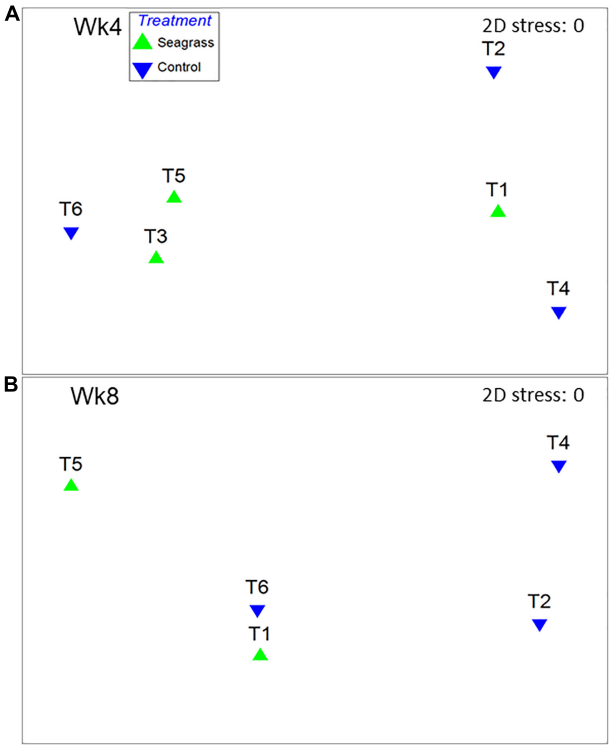

FIGURE 6 | Multi-dimensional scaling (MDS) ordination of non-standardized, arcsine-transformed major benthic microalgal groups percentage data in (**A**) week-4 and **(B)** week-8. T1∼T6 represent the experimental mesocosms.

respectively) compared to controls (0.041 ± 0.020 and 0.112 ± 0.036 mg cm^{-2}, respectively, **Figure 5A** and **Supplementary Table 1**). Chlorophyta was the main benthic microalgal group in both seagrass and control groups at Wk4 (**Figure 5B**). After raising temperature to 28°C, the benthic microalgal communities in the seagrass group were still dominated by Chlorophyta at Wk8, whereas the controls comprised more families Bacillariophyceae and Pelogophyceae (**Figure 5C**). OTUs of Syndiniales were 0.04 and 0.15% of the total OTUs in the seagrass and control groups, respectively, at 25°C. However, their relative abundances increased to 2.9 and 6.5%, respectively, when temperature rose to 28°C (**Figure 5C**). MDS results revealed that the community structures of the benthic microalgae were similar between seagrass and control groups at Wk 4 (**Figure 6A**; ANOSIM test, global $R = -0.111$, $P > 0.05$) and at Wk 8 (**Figure 6B**; ANOSIM test, global $R = 0.250$, $P > 0.05$).

DISCUSSION

A previous study has shown that increasing CO_2 may boost benthic microalgal primary productivity in coral reef ecosystem (Tew et al., 2017b). In the present study, we further found that the nutrients (NO_3^-, NO_2^-, NH_3^-, PO_4^{3-}) and benthic microalgal chl a increased even more when seagrass meadow is present in the coral reef ecosystem under OA conditions.

Contrary to our expectation of lower chl-a concentrations when seagrass blades were present, the benthic microalgal abundance was actually higher in the seagrass mesocosms at both temperatures. Since seagrass blades can change the flow regime and therefore fine sediment accumulation (Licci et al., 2019), the enhanced sedimentation (which was not measured) could have driven these elevated nutrient levels. These higher nutrient levels, then, could explain the higher benthic microalgal densities. However, a more thorough sediment analysis in the future must be undertaken to ascertain whether there is a relationship between seagrass bed hydrodynamics, sedimentation, and nutrient levels.

At OA + 28°C we saw an increase in the parasitic algae Syndiniales, a diverse yet understudied group found in all marine environments (Clarke et al., 2019). Although they have recently been reported from Taiwanese coral reefs (Cleary, 2019), their ecological niche is unknown. Whether their increase herein is due to temperature or simply the prolonged culture duration cannot be known given the experimental design utilized, though the observation that Syndiniales OTUs were in lower relative abundance in the seagrass group, may suggest an active role of the seagrass in modulating the density of this parasite.

Despite our phytoplankton OTU result represented only one sample from each treatment, it is worthy to note that the pooled phytoplankton sample collected from the seagrass mesocosms at 28°C, Wk 8, contained a high proportion of the symbiotic dinoflagellate *Cladocopium* sp. Given that temperatures well below the local coral bleaching threshold of ∼31°C were utilized, there was no apparent coral bleaching during the experiment; nevertheless, it cannot be ruled out that the corals themselves (rather than the sand-filtered seawater), were the source of these dinoflagellates. Since nitrate and ammonium concentrations were higher in the seagrass mesocosms at 28°C, perhaps the accelerate algal growth *in hospite* stimulated by partial eutrophication and an elevated N:P ratio (*sensu* Ezzat et al., 2016) led to the release of excess *Cladocopium* spp. cells from the corals, all of which naturally harbor *Cladocopium* (Mayfield et al., 2013). Although the physiological performance of the corals in the seagrass mesocosms was not assessed herein, coral + seagrass co-culture in OA-stimulated mesocosms actually resulted in corals that were more resilient than those of seagrass-free mesocosms in a prior work (Liu et al., 2020); this leads us to suspect that the higher *Cladocopium* levels in the seagrass mesocosms at high temperatures is not a testament to physiologically compromised corals. Future investigation in this area might be needed because of the growing frequencies of massive coral bleaching worldwide.

Seagrass-associated microbial communities can alter the nutrient cycles in the water column (Agawin et al., 2016) and in the sediment (Ugarelli et al., 2017). For example, Caffrey and Kemp (1990) documented higher rates of nitrification, denitrification and ammonification in the rhizosphere of *Zostera marina* than in bare sediments, and Agawin et al. (2016) documented significant nitrogen fixation activity in the phyllosphere of *Posidonia oceanica*. While most ecological functions of the seagrass microbiome and the interactions with their seagrass host are still unknown (Ugarelli et al., 2017 for more detail), the higher nutrient concentrations we observed

in the seagrass mesocosms could also attribute to the seagrass-associated microbiome activities (Hurtado-McCormick et al., 2019), which in turn enhance the growth of benthic microalgae.

Elevated CO_2 can increase the growth rate of diatoms (Li and Campbell, 2013) such as *Skeletonema costatum* (Kim et al., 2006), *Nitzschia palea*, *Chaetoceros muelleri* (Hu and Gao, 2008), *Amphora coffeaeformis* (Tew et al., 2014), as well as the chlorophyte *Dunaliella tertiolecta* (Beardall and Raven, 2004) and picocyanobacteria *Synechococcus* (Fu et al., 2007). However, no effects of OA were reported for diatom *Nitzschia ovalis* (Tew et al., 2014), the picocyanobacteria *Prochlorococcus* (Fu et al., 2007), and several others, suggesting that the response of some phytoplankton species to OA is negligible (Tortell et al., 2000; Fu et al., 2007). The species-specific nature of the algal OA response reflected by such heterogeneous responses may be due to differences in the efficiency of carbon acquisition (Johnson et al., 2015). Since the benthic microalgal and phytoplankton communities consist of hundreds to thousands of species (Cahoon, 1999; Tew et al., 2017b), it may be more pragmatic to instead monitor cumulative effects of OA on the system, rather than at a species by species level. At the community level, OA can enhance phytoplankton growth (McCarthy et al., 2012; Pierangelini et al., 2016), alter assemblages (Tortell et al., 2002; Ziveri et al., 2014), and negatively affect certain diatom species (Gao et al., 2012; Torstensson et al., 2012), and we found higher abundances of benthic microalgae in the seagrass mesocosms, particularly upon raising the temperature to 28°C. Although it is tempting to speculate that this could be due to seagrass sequestering of CO_2, the carbonate system was actually similar between seagrass and control mesocosms. Instead, only nitrate levels differed significantly. Therefore, it may be that the elevated nitrate levels present in the seagrass mesocosms promoted the growth of benthic microalgae. However, the exact mechanism by which seagrass blades enhance benthic primary productivity remains to be determined and should be the focus of future works.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/ **Supplementary Material**.

AUTHOR CONTRIBUTIONS

KT and P-JL conceived the presented idea, designed the experiments, verified the analytical methods, and wrote the manuscript. JK and J-OC performed DNA extractions and sequence analyses. F-CK and P-JM performed abiotic parameters analyses. AM assisted in statistical analyses and English proof-reading. All authors discussed the results and contributed to the final manuscript.

FUNDING

This work was supported by the Ministry of Science and Technology of Taiwan (MOST 106-2611-M-291-003 and MOST 107-2611-M-291-006) to KT.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.679683/full#supplementary-material

REFERENCES

Agawin, N. S. R., Ferriol, P., Cryer, C., Alcon, E., Busquets, A., Sintes, E., et al. (2016). Significant nitrogen fixation activity associated with the phyllosphere of Mediterranean seagrass *Posidonia oceanica*: first report. *Mar. Ecol. Prog. Ser.* 551, 53–62. doi: 10.3354/meps11755

Beardall, J., and Raven, J. A. (2004). The potential effects of global climate change on microalgal photosynthesis, growth and ecology. *Phycologia* 43, 26–40. doi: 10.2216/i0031-8884-43-1-26.1

Caffrey, J., and Kemp, W. (1990). Nitrogen cycling in sediments with estuarine populations of *Potamogeton perfoliatus* and *Zostera marina*. *Mar. Ecol. Prog. Ser.* 66, 147–160. doi: 10.3354/meps066147

Cahoon, L. (1999). "The role of benthic microalgae in neritic ecosystems," in *Oceanography and Marine Biology, an Annual Review*, Vol. 37, eds A. Ansell, R. Gibson, and M. Barnes (Milton Park, MI: Taylor & Francis Group), 47–86.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., et al. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7, 335–336.

Clarke, K. R., and Warwick, R. M. (1994). *Changes in Marine Communities: An Approach to Statistical Analysis and Interpretation*. London: Plymouth Marine Laboratory.

Clarke, L. J., Bestley, S., Bissett, A., and Deagle, B. E. (2019). A globally distributed Syndiniales parasite dominates the Southern Ocean micro-eukaryote community near the sea-ice edge. *ISME J.* 13, 734–737. doi: 10.1038/s41396-018-0306-7

Cleary, D. F. R. (2019). A comparison of microeukaryote communities inhabiting sponges and seawater in a Taiwanese coral reef system. *Ann. Microbiol.* 69, 861–866. doi: 10.1007/s13213-019-01476-5

Cornwall, C. E., and Hurd, C. L. (2016). Experimental design in ocean acidification research: problems and solutions. *ICES J. Mar. Sci.* 73, 572–581. doi: 10.1093/icesjms/fsv118

de los Santos, C. B., Krause-Jensen, D., Alcoverro, T., Marba, N., Duarte, C. M., van Katwijk, M. M., et al. (2019). Recent trend reversal for declining European seagrass meadows. *Nat. Commun.* 10:8.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26, 2460–2461. doi: 10.1093/bioinformatics/btq461

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., and Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27, 2194–2200. doi: 10.1093/bioinformatics/btr381

Ezzat, L., Towle, E., Irisson, J. O., Langdon, C., and Ferrier-Pages, C. (2016). The relationship between heterotrophic feeding and inorganic nutrient availability in the scleractinian coral *T. reniformis* under a short-term temperature increase. *Limnol. Oceanogr.* 61, 89–102. doi: 10.1002/lno.10200

Field, J. G., Clarke, K. R., and Warwick, R. M. (1982). A practical strategy for analyzing multispecies distribution patterns. *Mar. Ecol. Prog. Ser.* 8, 37–52. doi: 10.3354/meps008037

Fu, F. X., Warner, M. E., Zhang, Y. H., Feng, Y. Y., and Hutchins, D. A. (2007). Effects of increased temperature and CO_2 on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (Cyanobacteria). *J. Phycol.* 43, 485–496. doi: 10.1111/j.1529-8817.2007.00355.x

Gao, K., Xu, J., Gao, G., Li, Y., Hutchins, D. A., Huang, B., et al. (2012). Rising CO_2 and increased light exposure synergistically reduce marine primary productivity. *Nat. Clim. Chang.* 2, 519–523. doi: 10.1038/nclimate1507

Hu, H. H., and Gao, K. S. (2008). Impacts of CO_2 enrichment on growth and photosynthesis in freshwater and marine diatoms. *Chin. J. Oceanol. Limnol.* 26, 407–414. doi: 10.1007/s00343-008-0407-7

Huan, Y. H., Lee, C. L., Chung, C. Y., Hsiao, S. C., and Lin, H. J. (2015). Carbon budgets of multispecies seagrass beds at Dongsha Island in the South China Sea. *Mar. Environ. Res.* 106, 92–102. doi: 10.1016/j.marenvres.2015.03.004

Hurtado-McCormick, V., Kahlke, T., Petrou, K., Jeffries, T., Ralph, P. J., and Seymour, J. R. (2019). Regional and microenvironmental scale characterization of the *Zostera muelleri* seagrass microbiome. *Front. Microbiol.* 10:1011. doi: 10.3389/fmicb.2019.01011

Inaba, N., Trainer, V. L., Nagai, S., Kojima, S., Sakami, T., Takagi, S., et al. (2019). Dynamics of seagrass bed microbial communities in artificial *Chattonella* blooms: a laboratory microcosm study. *Harmful Algae* 84, 139–150. doi: 10.1016/j.hal.2018.12.004

Invers, O., Romero, J., and Perez, M. (1997). Effects of pH on seagrass photosynthesis: a laboratory and field assessment. *Aquat. Bot.* 59, 185–194. doi: 10.1016/s0304-3770(97)00072-7

IPCC (2014). "Climate change 2014: Synthesis report," in *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, eds Core Writing Team, R. K. Pachauri, and L. A. Meyer (Geneva: IPCC), 151.

Johnson, V. R., Brownlee, C., Milazzo, M., and Hall-Spencer, J. M. (2015). Marine microphytobenthic assemblage shift along a natural shallow-water CO_2 gradient subjected to multiple environmental stressors. *J. Mar. Sci. Eng.* 3, 1425–1447. doi: 10.3390/jmse3041425

Kim, J. M., Lee, K., Shin, K., Kang, J. H., Lee, H. W., Kim, M., et al. (2006). The effect of seawater CO_2 concentration on growth of a natural phytoplankton assemblage in a controlled mesocosm experiment. *Limnol. Oceanogr.* 51, 1629–1636. doi: 10.4319/lo.2006.51.4.1629

Kruskal, J. B., and Wish, M. (1978). *Multidimensional Scaling*. Beverly Hills: Sage Publications.

Kuo, J., Tew, K. S., Ye, Y. X., Cheng, J. O., Meng, P. J., and Glover, D. C. (2014). Picoplankton dynamics and picoeukaryote diversity in a hyper-eutrophic subtropical lagoon. *J. Environ. Sci. Health A Tox. Hazard Subst. Environ. Eng.* 49, 116–124. doi: 10.1080/10934529.2013.824784

LaJeunesse, T. C., Parkinson, J. V., Gabrielson, P. W., Jeong, H. J., Reimer, J. D., Voolstra, C. R., et al. (2018). Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. *Curr. Biol.* 28, 2570–2580. doi: 10.1016/j.cub.2018.07.008

Lamb, J. B., van de Water, J., Bourne, D. G., Altier, C., Hein, M. Y., Fiorenza, E. A., et al. (2017). Seagrass ecosystems reduce exposure to bacterial pathogens of humans, fishes, and invertebrates. *Science* 355, 731–733. doi: 10.1126/science.aal1956

Lee, C. L., Wen, C. K. C., Huang, Y. H., Chung, C. Y., and Lin, H. J. (2019). Ontogenetic habitat usage of juvenile carnivorous fish among seagrass-coral mosaic habitats. *Diversity* 11:17.

Lewis, E., Wallace, D., and Allison, L. J. (1998). *Program Developed for CO_2 System Calculations*. Bethesda: Carbon Dioxide Information Analysis Center.

Li, G., and Campbell, D. A. (2013). Rising CO_2 interacts with growth light and growth rate to alter photosystem II photoinactivation of the coastal diatom *Thalassiosira pseudonana*. *PLoS One* 8:e55562. doi: 10.1371/journal.pone.0055562

Licci, S., Nepf, H., Delolme, C., Marmonier, P., Bouma, T. J., and Puijalon, S. (2019). The role of patch size in ecosystem engineering capacity: a case study of aquatic vegetation. *Aquat. Sci.* 81:11.

Lin, H. J., Lee, C. L., Peng, S. E., Hung, M. C., Liu, P. J., and Mayfield, A. B. (2018). The effects of El Nino-southern oscillation events on intertidal seagrass beds over a long-term timescale. *Glob. Chang. Biol.* 24, 4566–4580. doi: 10.1111/gcb.14404

Liu, P. J., Ang, S. J., Mayfield, A. B., and Lin, H. J. (2020). Influence of the seagrass *Thalassia hemprichii* on coral reef mesocosms exposed to ocean acidification and experimentally elevated temperatures. *Sci. Total Environ.* 700, 134464. doi: 10.1016/j.scitotenv.2019.134464

Liu, P. J., Hsin, M. C., Huang, Y. H., Fan, T. Y., Meng, P. J., Lu, C. C., et al. (2015). Nutrient enrichment coupled with sedimentation favors sea anemones over corals. *PLoS One* 10:e0125175. doi: 10.1371/journal.pone.0125175

Liu, P. J., Lin, S. M., Fan, T. Y., Meng, P. J., Shao, K. T., and Lin, H. J. (2009). Rates of overgrowth by macroalgae and attack by sea anemones are greater for live coral than dead coral under conditions of nutrient enrichment. *Limnol. Oceanogr.* 54, 1167–1175. doi: 10.4319/lo.2009.54.4.1167

Magoc, T., and Salzberg, S. L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27, 2957–2963. doi: 10.1093/bioinformatics/btr507

Marbà, N., Holmer, M., Gacia, E., and Barron, C. (2007). *Seagrasses: Biology, Ecology and Conservation*. Berlin: Springer, 135–157.

Mayfield, A. B., Fan, T. Y., and Chen, C. S. (2013). Physiological acclimation to elevated temperature in a reef-building coral from an upwelling environment. *Coral Reefs* 32, 909–921. doi: 10.1007/s00338-013-1067-4

McCarthy, A., Rogers, S. P., Duffy, S. J., and Campbell, D. A. (2012). Elevated carbon dioxide differentially alters the photophysiology of *Thalassiosira pseudonana* (Bacillariophyceae) and *Emiliania huxleyi* (Haptophyta). *J. Phycol.* 48, 635–646. doi: 10.1111/j.1529-8817.2012.01171.x

Pai, S. C., and Riley, J. P. (1994). Determination of nitrate in the presence of nitrite in natural waters by flow injection analysis with a non-quantitative on-line cadmium redactor. *Int. J. Environ. Anal. Chem.* 57, 263–277. doi: 10.1080/03067319408027460

Pai, S. C., Tsau, Y. J., and Yang, T. I. (2001). pH and buffering capacity problems involved in the determination of ammonia in saline water using the indophenol blue spectrophotometric method. *Anal. Chim. Acta* 434, 209–216. doi: 10.1016/s0003-2670(01)00851-0

Pai, S. C., Yang, C. C., and Riley, J. P. (1990). Formation kinetics of the pink azo dye in the determination of nitrite in natural waters. *Anal. Chim. Acta* 232, 345–349. doi: 10.1016/s0003-2670(00)81252-0

Parsons, T. R. (2013). *A Manual of Chemical & Biological Methods for Seawater Analysis*. Amsterdam: Elsevier.

Pierangelini, M., Raven, J. A., and Giordano, M. (2016). The relative availability of inorganic carbon and inorganic nitrogen influences the response of the dinoflagellate *Protoceratium reticulatum* to elevated CO_2. *J. Phycol.* 53, 298–307. doi: 10.1111/jpy.12463

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596.

Tew, K. S., Jhange, Y. S., Meng, P. J., and Leu, M. Y. (2017a). Environmental factors influencing the proliferation of microscopic epiphytic algae on giant kelp under aquarium conditions. *J. Appl. Phycol.* 29, 2877–2886. doi: 10.1007/s10811-017-1148-9

Tew, K. S., Siao, Y. J., Liu, P. J., Lo, W. T., and Meng, P. J. (2017b). Taiwanese marine microbenthic algal communities remain similar yet chlorophyll a concentrations rise in mesocosms with elevated CO_2 and temperature. *Mar. Pollut. Bull.* 124, 929–937. doi: 10.1016/j.marpolbul.2017.06.050

Tew, K. S., Kao, Y.-C., Ko, F.-C., Kuo, J., Meng, P.-J., Liu, P.-J., et al. (2014). Effects of elevated CO_2 and temperature on the growth, elemental composition, and cell size of two marine diatoms: potential implications of global climate change. *Hydrobiologia* 741, 79–87. doi: 10.1007/s10750-014-1856-y

Tew, K. S., Meng, P. J., and Leu, M. Y. (2012). Factors correlating with deterioration of giant kelp *Macrocystis pyrifera* (Laminariales, Heterokontophyta) in an aquarium setting. *J. Appl. Phycol.* 24, 1269–1277. doi: 10.1007/s10811-011-9775-z

Tew, K. S., Meng, P. J., Lin, H. S., Chen, J. H., and Leu, M. Y. (2013). Experimental evaluation of inorganic fertilization in larval giant grouper (*Epinephelus lanceolatus* Bloch) production. *Aquac. Res.* 44, 439–450. doi: 10.1111/j.1365-2109.2011.03051.x

Tiling, K., and Proffitt, C. E. (2017). Effects of *Lyngbya majuscula* blooms on the seagrass *Halodule wrightii* and resident invertebrates. *Harmful Algae* 62, 104–112. doi: 10.1016/j.hal.2016.11.015

Torstensson, A., Chierici, M., and Wulff, A. (2012). The influence of increased temperature and carbon dioxide levels on the benthic/sea ice diatom *Navicula directa*. *Pol. Biol.* 35, 205–214. doi: 10.1007/s00300-011-1056-4

Tortell, P. D., DiTullio, G. R., Sigman, D. M., and Morel, F. M. (2002). CO_2 effects on taxonomic composition and nutrient utilization in an Equatorial Pacific

phytoplankton assemblage. *Mar. Ecol. Prog. Ser.* 236, 37–43. doi: 10.3354/meps236037

Tortell, P. D., Rau, G. H., and Morel, F. M. (2000). Inorganic carbon acquisition in coastal Pacific phytoplankton communities. *Limnol. Oceanogr.* 45, 1485–1500. doi: 10.4319/lo.2000.45.7.1485

Ugarelli, K., Chakrabarti, S., Laas, P., and Stingl, U. (2017). The seagrass holobiont and its microbiome. *Microorganisms* 5:81. doi: 10.3390/microorganisms5040081

Unsworth, R. K. F., Collier, C. J., Henderson, G. M., and McKenzie, L. J. (2012). Tropical seagrass meadows modify seawater carbon chemistry: implications for coral reefs impacted by ocean acidification. *Environ. Res. Lett.* 7:024026. 10.1088/1748-9326/7/2/024026

Unsworth, R. K. F., McKenzie, L. J., Collier, C. J., Cullen-Unsworth, L. C., Duarte, C. M., Eklof, J. S., et al. (2019a). Global challenges for seagrass conservation. *Ambio* 48, 801–815.

Unsworth, R. K. F., Nordlund, L. M., and Cullen-Unsworth, L. C. (2019b). Seagrass meadows support global fisheries production. *Conserv. Lett.* 12:e12566. doi: 10.1111/conl.12566

Ziveri, P., Passaro, M., Incarbona, A., Milazzo, M., Rodolfo-Metalpa, R., and Hall-Spencer, J. M. (2014). Decline in coccolithophore diversity and impact on coccolith morphogenesis along a natural CO_2 gradient. *Biol. Bull.* 226, 282–290. 10.1086/bblv226n3p282

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Tew, Kuo, Cheng, Ko, Meng, Mayfield and Liu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.