Assessing macroalgal and terrestrial biomass organic carbon release and biodegradability

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1. Overview

This report details organic carbon (OC) release and biolability results obtained from the evaluation of four Running Tide (RT) macroalgal species and RT terrestrial biomass (woodchip; WC) across a series of month-long algal growth experiments designed to quantify OC release, and microbial incubation experiments designed to assess algal- and woodchip-OC bioavailability.

The overarching aim of the work is to improve understanding of the fate of RT macroalgal and terrestrial biomass (WC) derived OC in the ocean. Specifically, we assess the carbon content of macroalgal tissue and terrestrial biomass (>1 mm), the dissolved organic carbon (DOC; <0.7 microns) released from both macroalgae and woodchips, and the bioavailability of released DOC. The latter is used to assess the percentage of released DOC that is likely to persist for long enough at sea to become sequestered in the deep ocean.

2. Context and Background

2.1. Introduction

Anthropogenic emissions of greenhouse gases are warming the Earth, with most of this warming due to carbon dioxide (CO_2) emissions (Canadell et al., 2022). Fossil fuel combustion and land use change continue to emit about 11 Pg-C yr^{-1} as CO_2 (Canadell et al., 2022). About 46% of emitted CO_2 accumulates in the atmosphere each year. The land and oceans take up the rest. Reducing CO_2 emissions is critical to slowing climate change. However, even the most optimistic of the IPCC's scenarios predicting future climate indicate CO_2 reduction technologies (CDRs) are required to reduce the ongoing and future harm caused by greenhouse gas emissions and to mitigate climate change (Canadell et al., 2022). Running Tide has identified the growth of macroalgae as a CDR that could sequester vast amounts of carbon. The success of this CDR technology will depend on the growth of macroalgae at sea and the subsequent sequestration of algal C in the deep ocean, moving 'fast carbon' into the 'slow carbon' cycle. Ongoing research at Running Tide is assessing the growth of macroalgae at sea, including how to cultivate and grow various species of macroalgae cost effectively. This Whitepaper focuses on the amount and fate of particulate organic carbon (POC) and dissolved organic carbon (DOC) that is released from macroalgae as they grow. We also present data for the release and biodegradability of the DOC leached from the terrestrial biomass (WC) Running Tide uses as macroalgal floating growth substrate.

2.2. The role of POC and DOC in macroalgal C sequestration

In natural systems, most macroalgal C sequestration is hypothesized to occur when POC and DOC are exported out of macroalgal ecosystems and into deeper waters (Krause-Jensen and Duarte, 2016). As algae grow, they continuously release OC with DOC release by algae estimated to be equivalent to ~25% of biomass C accumulation (i.e., net primary production: NPP) (Krause-Jensen and Duarte, 2016). A fraction of this DOC survives long enough to be sequestered in deeper waters and DOC is estimated to be responsible for ~76% of natural macroalgal C sequestration with less than a quarter of algal C sequestration being stored in sediments as algal detritus and POC. In summary, the efficiency of coastal macroalgal C sequestration is estimated at 11% of net primary production (NPP) with ~2% of NPP sequestered in shelf sea sediments as POC, ~1% in the deep ocean as POC, and 8% sequestered by the transport of DOC below the mixed layer (Krause-Jensen and Duarte, 2016). Once below the mixed layer, respiration of OC still results in sequestration of C away from the atmosphere, as the dissolved inorganic carbon (DIC) produced at depth may remain in deep waters for decades to centuries depending upon

the depth and exact location. This is how the ocean's biological carbon pump also sequesters C, with phytoplankton taking up surface water DIC, dying and sinking, before their biomass is remineralized to DIC again at depth. Current estimates of natural macroalgal C sequestration efficiency rely on significant extrapolation from scarce data about 1) the amount of POC and DOC released from macroalgae as they grow, and 2) how rapidly macroalgal POC and DOC are remineralized back to CO_2 (i.e., is biodegradation slow enough to allow the OC to sink or mix below the mixed layer before it is respired). It is lso unclear how much POC and DOC may be released and end up sequestered when algae are grown for CDR. It is on these questions that this Whitepaper focuses.

2.3. Potential for extra C credits based upon macroalgal DOC and POC

In natural systems, studies suggest that about three quarters of macroalgal C sequestration occurs due to the release of DOC throughout the algae's lifetime (Krause-Jensen and Duarte, 2016). Therefore, an accounting of macroalgal CDR based upon the sinking flux of algal biomass C alone would underestimate the total C sequestration potential of macroalgal CDR technologies. In this Whitepaper methods and progress toward a first accounting of POC and DOC release are presented, along with estimates of the fractions of DOC that will persist long enough to be sequestered in the deep ocean. Methods utilized in this study to assess the release of DOC from Running Tide macroalgae are noted in Sections 3 and 4, and results from experimentation on algal DOC production and biolability are presented in Section 4. Data for woodchips are also presented in Section 5.

3. Method refinement and preliminary results

3.1 Initial algal samples and refining experimental designs

To measure the production of macroalgal OC, macroalgae need to be grown in low OC waters. Thus, it is imperative to assess sources of OC contamination. For work with Running Tide, it was initially hoped that algae could be grown in Running Tide facilities and then sampled for OC. To test if this was feasible, background OC (blank) levels were made in Running Tide's reverse osmosis water (RO), artificial seawater (SW), algal holding tanks, and in an initial algal growth experiment during the Fall of 2022 (Figures S1 and S2). DOC concentrations were also measured for the Guillard's F/2 algal nutrient mixes A and B used to fertilize macroalgae.

It was determined that Guillard's F/2 part A nutrient includes high levels of OC (i.e., it includes vitamins and iron ligands) and raises the background OC in macroalgal growth experiments beyond acceptable levels (i.e., use of this reagent increases background DOC concentrations to levels that obscure the production and quality of any DOC produced directly by the algae). Therefore, the necessity of using both Guillard's F/2 A and B to attain algal growth was tested. In these tests, algal growth and DOC production for both *Gracilaria hayi* and *Ulva lactuca* were assessed in treatments with: 1) no added nutrients, 2) only Guillard's F/2 Part B, and 3) the full nutrient mix (Guillard's F/2 A&B) (Figure S3). These experiments indicated that algal growth and DOC production were sufficient when supplemented only with Guillard's F/2 part B. Thus, the OC-rich Guillard's F/2 part A was left out of further treatments.

Initial experiments to assess rates of OC production and OC blanks led to a reassessment of where and how algae should be grown. The need to maintain trace clean OC conditions to quantify OC release from algae and to generate samples that are dominated by macroalgal OC for subsequent microbial incubations led us to conduct algal growth and OC production experiments in the Stubbins Lab on the Northeastern University campus in Boston. This was a change in project design but allowed closer assessment of OC generation by the algae, including collection of time series data for DOC production.

Initial evaluation of particulate organic carbon (POC) released during algal incubation experiments revealed that POC was consistently <1 % of algal biomass NPP, and therefore not a significant CDR pathway in the four macroalgal species investigated. However, further exploration of POC production in other algal species of interest, and for all algal species in the field, may be warranted. Field or mesocosm studies in which the algae are exposed to physical, biological, and other environmental processes not captured in our growth experiments should be conducted, as these other processes may result in POC release (e.g., grazing by herbivores and wave action may accentuate release of POC) and different levels of DOC release than seen in the lab.

Despite some contamination of DOC samples collected at Running Tide, DOC biodegradation experiments were conducted for training and practice. In these experiments, water from macroalgal growth experiments (i.e., water containing macroalgal DOC) was inoculated with coastal seawater bacteria (~1 mL of 1 micron filtered seawater as inoculum per 1 L of sample) and amended with N and P above Redfield ratios for the DOC they contained to alleviate any possible nutrient limitation that might impinge upon the apparent biolability of the macroalgal OC. As we know there was some contamination of the initial algal growth experiments with non-algal DOC, these DOC incubation experiments were not used to estimate the biolability of algal DOC, but only to provide an estimate of the length and sampling frequency required for later experiments noted below.

4. Spring 2023 macroalgal experiments

In March through May 2023, a new batch of macroalgal growth and DOC production experiments were initiated and monitored for DOC release, macroalgal growth and carbon content, and DOC biolability in the Stubbins Lab at Northeastern University. Outlined below are the brief materials and methods used to conduct this work, along with results to date.

4.1 Methodology

The experimental array was designed in alignment with findings from our initial culture work in the Fall of 2022. The first algal growth and DOC production experiment was run at Northeastern from March 10 to 30, 2023 and the second from April 25 to May 24, 2023. Algal biomass per volume at the start of the 1st experiment was ~30 g/1500 mL and was ~15 g/1500 mL at the start of the second (Table S1). As initial testing (Section 3) indicated, algal biomass growth and DOC production were sufficiently high when providing only the OC-free nutrient B at the initiation of each experimental incubation, therefore the OC-rich nutrient A was excluded due to its high amounts of carbon.

Replicate acid-washed and combusted glass 4L incubation jars were filled with ~1.5 L of artificial seawater (salinity of ~28) to which each of four macroalgal species were added; *Ulva lactuca, Gracilaria hayi, Gracilaria parvispora,* and a *Spermothamnion* species (Figure 1). The jars were then capped with plastic air-tight lids. The lids were equipped with tubing connected to cleaned stainless steel air stones for bubbling carbon clean filtered air throughout the incubation period. All macroalgal incubation jars were set under PECIDA light sources on 12 hours on:12 hours off cycle at ~100 PAR (Figure S4).

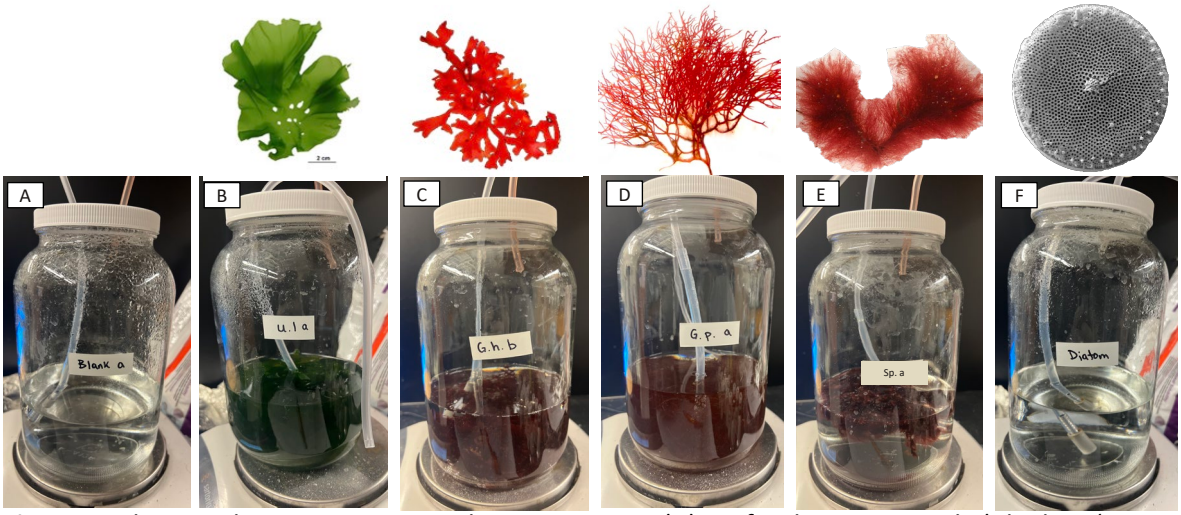


Figure 1. Glass incubation experimental jars containing (A) artificial seawater only (Blank or 'control' samples); (B) macroalgal specimens *Ulva lactuca*; (C) *Gracilaria hayi*; (D) *Gracilaria parvispora*, (E) *Spermothamnian* sp. (F), and (G) diatoms (as an additional control). All sample incubation jars were supplied with clean air bubbled through stainless steel air stones and filtered by 0.2 µM Teflon filters to reduce chances of contamination. All samples were incubated under PECIDA light sources set to 12 hours on:12 hours off cycle at ~100 PAR. Both the Blank (control jars) and algal specimen jars were run in duplicates.

4.1.1 Wet and dry biomass

Biomass of the algal samples from each experiment was measured at the start and end of both experiments. Algal samples were wet weighed on a Sartorius Secura Analytical Balance (0.0001 g precision) to desired masses for the beginning of the experiments. For Experiment A, the desired mass of each species was ca. 30 grams, while for Experiment B, the desired mass of each species was ca. 15 grams (Table S1). The algae were then added to the corresponding incubation jars for the remainder of the algal growth and DOC production experiment. At the conclusion of each experiment, the algal samples were wet weighed again to find the final masses (Figure 2). The algal samples were then subsampled and dried in a drying oven set at 60°C to a stable mass and weighed once more to determine the wet-to-dry ratio of biomass, such that initial and end algal dry biomass could be calculated. Subtracting initial masses from end masses of algae allowed for calculations of algal growth in terms of both wet biomass accumulation and dry biomass accumulation (Table 1).

4.1.2 Biomass carbon content

Briefly, dried algal samples from the start (subsampled from initial macroalgal specimens) and the conclusion of the experiments (final macroalgal biomass) were powdered using a clean mortar and pestle. Subsamples from each dried sample were weighed on a microbalance and sample powders placed in tin sample cups for carbon and nitrogen content characterization on a Thermo Environmental Analyzer Isotope Ratio Mass Spectrometer (EA-IRMS) in the Stubbins Lab at Northeastern University. (Note: instrument technical difficulties in June and July 2023 meant that only preliminary data is presented; Table 1).

4.1.3 DOC and CDOM

Water was sampled directly from each sample incubation jar for evaluation of DOC production and CDOM characterization using an acid-washed and MilliQ-rinsed clean plastic syringe equipped with clean

plastic tubing across the 20 day (first experiment) or 30 day (second experiment) algal growth experiments. At each time point, 30 mL of water was sampled and filtered through a clean 0.22 μm polyethersulfone (PES) syringe filter. The syringe filters were cleaned by flushing with 60 mL of MilliQ water prior to sampling. After MilliQ cleaning, the filter was flushed with a few drops of sample (~1 mL), and 20 mL of sample was then filtered directly into a 24 mL glass vial, acidified to pH of 2 and analyzed for DOC and total dissolved nitrogen (TDN) on a Shimadzu TOC/TDN Analyzer. The remaining 10 mL of filtered sample was added to another 24 mL vial and used to measure colored dissolved organic matter (CDOM) absorbance and fluorescence on a Horiba Scientific Aqualog. DOC and CDOM sampling occurred periodically following the pattern of 1, 3, 7, 14, and 21 days. More sampling dates were added as needed to capture trends in the data or where DOC accumulation was slower than expected.

4.1.4 DOC biolability

At the conclusion of the algal growth experiments, the remaining water left in each algal incubation jar was filtered through 0.7 μm GF/F filters and divided into 6 x 24 mL vials to measure the biolability (i.e., bioavailability) of the DOC produced by the algae. The six sample vials allowed for time series analysis of biodegradation and were sacrificed on days 0, 1, 3, 7, 14, and 28. Each biolability vial contained 20 mL of macroalgal DOC sample, 200 μL of inoculum (natural seawater containing bacteria), and amendments of N and P, the latter at concentrations dependent on the amount of DOC present and the corresponding amount of N and P needed to ensure that nutrient limitation was not inhibiting microbial use of DOC (i.e., N and P were added to be at concentrations above the Redfield Ratio for C:N:P required for bacterial growth). All vials were incubated in the dark on a New Brunswick Shaker Table. On each sampling date, the corresponding vials were removed from the box, acidified to pH 2, and filtered into clean 24 mL vial using a 0.22 μm syringe filter. The samples were then measured for DOC and TDN concentrations on the Shimadzu Total Organic Carbon Analyzer.

4.2 Results

4.2.1 Biomass accumulation

Algal growth was quantified as the gravimetric biomass accumulation throughout the experiments and was characterized by 1) increases in wet biomass: the difference between recorded initial and final wet mass (g), and 2) increases in dry biomass. Dried algal biomass was measured to determine the average percent water content in each species, to convert initial wet biomass values to dry biomass. Overall, average algal growth (determined from replicate incubation jars) was higher during the second experiment (Experiment B) for all algal species except *G. parvispora* (Figure 2). During experiment A, both *U. lactuca* and the *Spermothamnion* species exhibited net-negative average biomass accumulation (biomass loss; Figure 2). Only incubation jars that exhibited positive biomass accumulation were used in further evaluation of NPP and DOC production relationships (Table 1).

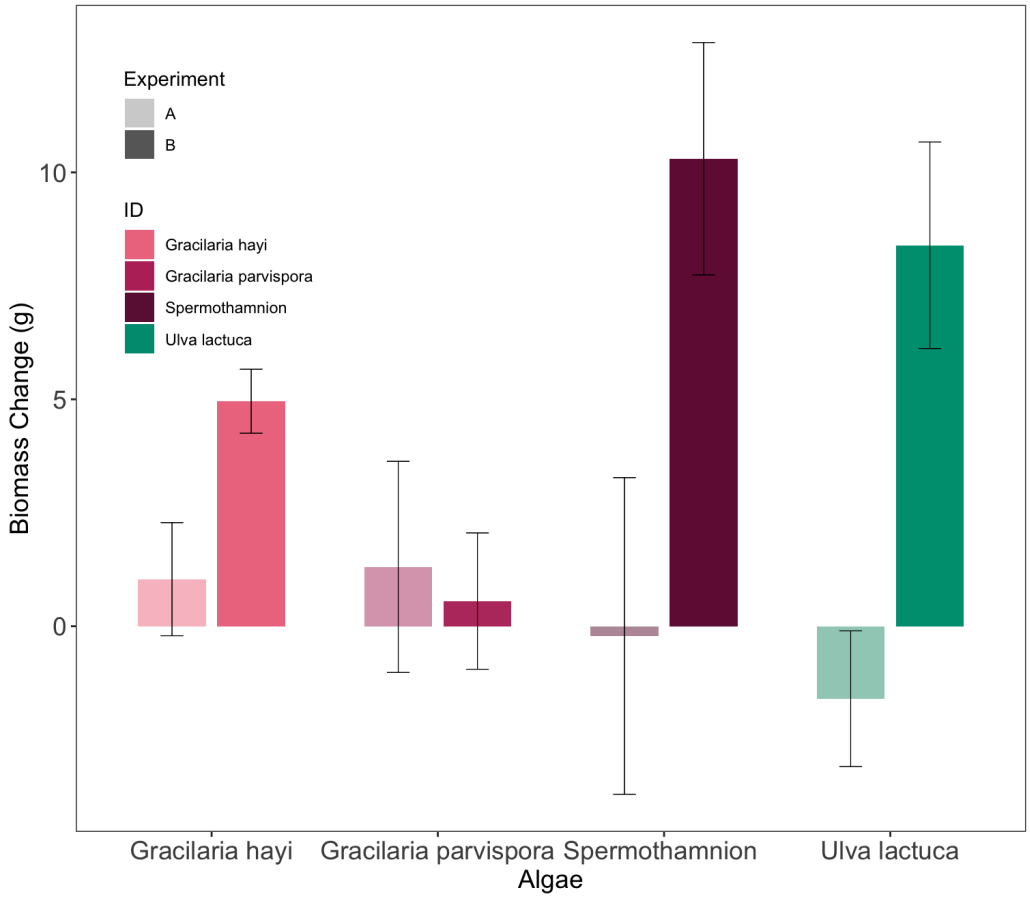


Figure 2. Wet biomass accumulation across two macroalgal incubation experiments conducted in the Stubbins Lab at Northeastern University. Experiment A (March 10-March 30, 2023) shaded in lighter color bars, and B (April 25 – May 24, 2023) shaded in darker color bars. Black error bars indicate standard error on the average biomass accumulation from replicate incubation jars.

4.2.2 Biomass Carbon and Nitrogen

Due to technical issues with the EA-IRMS analyzer required to measure biomass nitrogen and organic carbon content in July 2023, carbon and nitrogen content were only analyzed for two of the four macroalgal species studied thus far; *Ulva lactuca* and the *Spermothamnion* species. However, samples of dried biomass for all remaining samples are prepared and ready to be analyzed once the instrument is running again. *Ulva lactuca* OC content averaged 29.1% by dry mass and the *Spermothamnion* species OC content averaged 35.5% by dry mass. For the two *Gracilaria* species examined, the following literature values for OC content were adopted: *G. hayi* 31% (Young and Gobler, 2016) and *G. parvispora* 41% (Laurens et al., 2020). Nitrogen content was determined to be 3.42 % in *U. lactuca* and 5.88 % in the *Spermothamnion* species.

Using the OC content of dried algal samples, net primary productivity (NPP) for each macroalgal species was calculated from the dry biomass growth data as:

NPP (mg-C) = % OC of biomass by dry weight × algal growth in mg dry biomass

NPP for each species can be found in **Table 1**. Considering only Experiment B, in which all algae showed net positive algal biomass accumulation; NPP (mg-C d^{-1}) was highest for *U. lactuca* (10.63 mg-C d^{-1}) and lowest for *G. parvispora* (2.19 mg-C d^{-1}).

Table 1: Macroalgal biomass growth, carbon content, net primary productivity (NPP) and total dissolved nitrogen utilization across two experiments conducted to address macroalgal carbon production and release. TDN:Biomass C reported as molar equivalent ratio of TDN to C.

Experiment	Algal Species	Average increase in wet biomass (mg)	Average increase in dry biomass (mg)	%C	C biomass accumulation (mg C) [NPP]	C biomass accumulation rate (mg C d^{-1}) [NPP d^{-1}]	Average TDN use (mg N)	Molar TDN: NPP C
A	*U. lactuca*	-	-	29.1	-	-	-	-
A	*G. hayi*	1037	141.72	31.0*	43.93	2.23	10.16±3.19	0.20
A	*G. parvispora B***	2952	285.15	41.0**	88.40	4.49	10.88	0.11
A	*S. species A****	2252	122.78	35.5	43.55	2.21	10.89	0.21
B	*U. lactuca*	8395	1065.97	29.1	310.19	10.63	22.77±1.86	0.06
B	*G. hayi*	4958	677.91	31.0*	210.15	7.20	12.05±1.85	0.05
B	*G. parvispora B****	1616	156.10	41.0**	64.00	2.19	10.99	0.15
B	*S. species*	10301	561.63	35.5	199.21	6.83	29.93±3.93	0.13

*%C content taken from Young & Gobler 2016

**%C content taken from Laurens et al. 2020

*** Only one incubation replicate is reported for G. parvispora in Experiment B due to net biomass loss

****Ulva lactuca exhibited net biomass loss in both replicate jars during Experiment A, so values are not reported here

4.2.3 TDN utilization by macroalgae

Total dissolved nitrogen (TDN) utilization was monitored alongside DOC release in both Experiment A and B. Overall TDN use ranged from 10.88 mg-N to 29.93 mg-N across the four algal species investigated, with *G. parvispora* removing the least TDN, and *Spermothamnion* removing the most (Table 1). Relative to NPP (i.e., biomass OC accumulation), the red algal *Spermothamnion* species had the highest TDN utilization:NPP ratio (0.21), suggesting higher nutrient demand per unit C fixed relative to the green algal species *U. lactuca* (TDN utilization:NPP = 0.06).

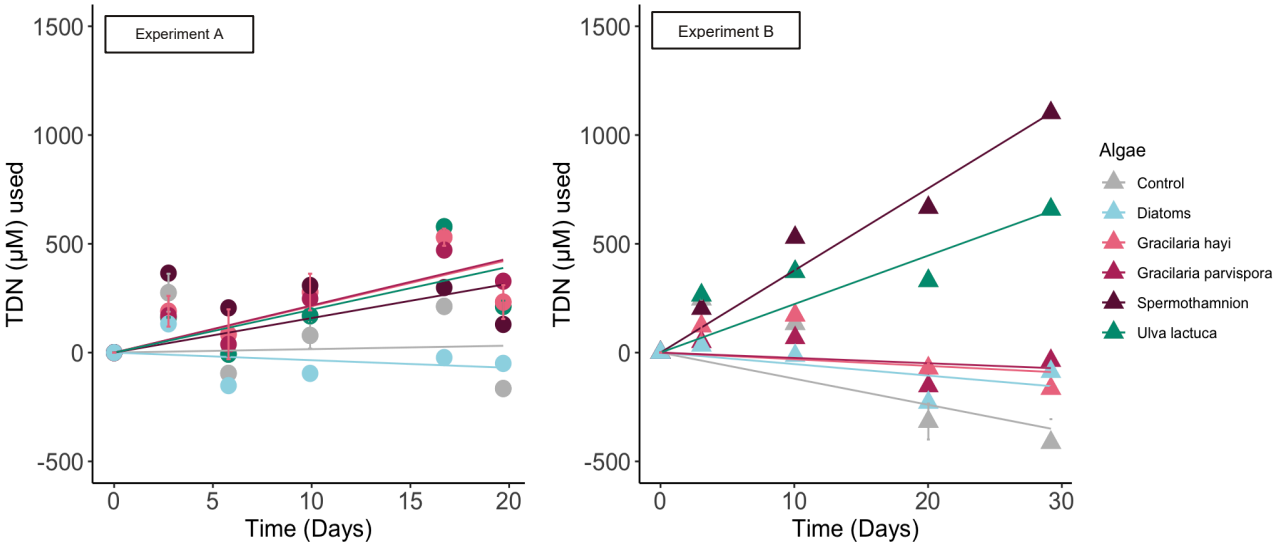


Figure 3. Trends in total dissolved nitrogen (TDN) utilized through time in algal growth Experiments A (left) and B (right).

4.2.4 Algal DOC production during growth

Mean macroalgal DOC production rate varied across the four species of algae and between Experiments A and B. In Experiment B, where algae were incubated for ~30 days, ca. 1-3 x the total amount of DOC was released compared to Experiment A (20-day incubation). DOC production in both experiments was best described using linear models (Figure 4). In both Experiment A and B, *G. parvispora* had the highest DOC production; 90 µg C day^{-1} and 193.6 µg C day^{-1}, respectively. In Experiment A, the *Spermothamnion* sp. exhibited the slowest DOC production (68.5 µg C day^{-1}), whereas in Experiment B, *U. lactuca* exhibited the slowest DOC production (76.1 µg C day^{-1}).

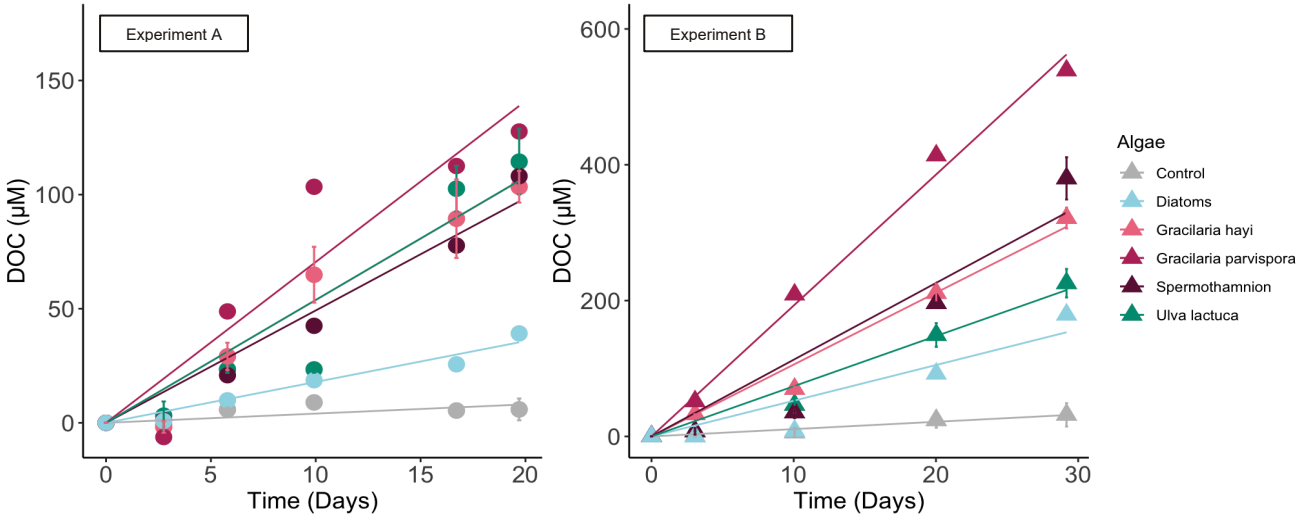


Figure 4. Trends in dissolved organic carbon (µM) released through time by macroalgal specimens in Experiments A and B. Colored circles representing data from Experiment A, and colored triangles from Experiment B.

Table 2: Linear models describing DOC release rates (Figure 4) for macroalgal samples from Experiment A and B.

Experiment	Sample	Model	Slope	R^2
A	Control	Linear: y ~ x + 0	0.40±0.07	0.74
A	Diatoms	Linear: y ~ x + 0	1.79±0.11	0.98
A	*Gracilaria hayi*	Linear: y ~ x + 0	5.38±0.22	0.98
A	*Gracilaria parvispora B**	Linear: y ~ x + 0	7.05±0.70	0.95
A	*Spermothamnion A**	Linear: y ~ x + 0	4.92±0.33	0.98
A	*Ulva lactuca*	Linear: y ~ x + 0	5.39±0.39	0.95
B	Control	Linear: y ~ x + 0	1.08±0.06	0.98
B	Diatoms	Linear: y ~ x + 0	5.25±0.75	0.92
B	*Gracilaria hayi*	Linear: y ~ x + 0	10.58±0.35	0.99
B	*Gracilaria parvispora B**	Linear: y ~ x + 0	19.27±0.54	0.99
B	*Spermothamnion*	Linear: y ~ x + 0	11.30±0.92	0.94
B	*Ulva lactuca*	Linear: y ~ x + 0	7.39±0.30	0.99

* Only one incubation replicate is reported due to net biomass loss

Table 3: Macroalgal DOC concentration and production rates from Experiments A and B.

Experiment	Algal Species	Total DOC production (µM)	Total DOC production (mg C)	%NPP as DOC	DOC (µg C) day^{-1}	DOC (µg C) g^{-1} WW$_{initial}$ day^{-1}	DOC (µg C) g^{-1} WW$_{change}$ day^{-1}	DOC (µg C) day^{-1} mg C biomass^{-1}
A	*U. lactuca*	114.43±12.49	1.65±0.30	-	83.7±15.2	2.76±0.50	-	-
A	*G. hayi*	103.42±9.81	1.47±0.19	3.3	74.6±9.6	2.47±0.32	71.73±9.23	1.70±0.22
A	*G. parvispora B**	127.66	1.79	2.0	90	2.97	30.35	1.02
A	*S. species A**	108.09	1.35	3.1	68.5	2.25	30.44	1.57
B	*U. lactuca*	225.62±29.66	2.22±0.36	0.7	76.1±12.3	4.99±0.81	9.06±1.47	0.25±0.04
B	*G. hayi*	321.56±26.22	3.32±0.58	1.6	113.8±19.8	7.37±1.28	22.95±3.99	0.54±0.09
B	*G. parvispora B**	538.83	5.65	8.8	193.6	12.86	119.51	3.03
B	*S. species*	379.96±41.86	4.68 ±0.84	2.3	160.4±28.8	10.68±1.92	15.57±2.80	0.81±0.14

'-' Negative biomass accumulation

* Only one incubation replicate is reported due to net biomass loss

4.2.5 Algal DOC biolability

The DOC that accumulated or was produced in the algal growth experiments was highly biorefractory compared to DOC from other sources (Zhu et al., 2020; Spencer et al., 2015; Bittar et al., 2015) with over 70% of DOC persisting after ca. 1 month of biodegradation for all algal species (Figure 5). The data for the biodegradation of DOC produced during algal growth Experiment A was best described with a linear fit, while data from Experiment B was best fit with first order decay kinetics (Figure 5). The latter is what was expected and is more often observed for DOC biodegradation studies (e.g., see citations above). Data fit with a first order decay also allows for an estimate of how much DOC might persist after infinite biodegradation, although only under the conditions experienced in the experimental bottle. Thus, the data from Experiment B were used to estimate the % refractory DOC as the percentage of DOC that would persist forever under 1st order decay kinetics (i.e., the intercept value in Table 4 divided by the starting concentration of DOC). Looking only at data from experimental B and using the above methods, suggests that 72 to 75% of algal DOC released was biorefractory (Table 4).

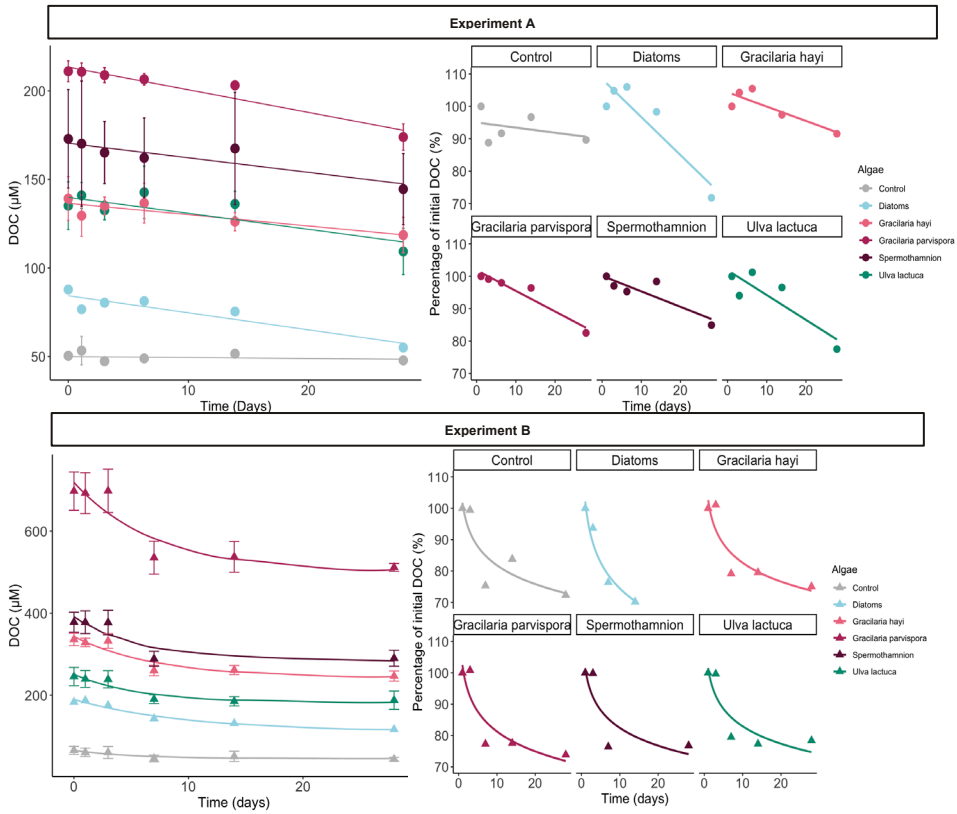


Figure 5. Macroalgal dissolved organic carbon (DOC) biodegradation through time by seawater microbes in artificial seawater for the DOC produced by the four macroalgal species examined, two control samples and one control diatom sample. Experiment A DOC biodegradation results are modeled with linear models (Table 4), and exponential decay models are used to describe DOC biodegradation kinetics in Experiment B (Table 4).

Table 4: Linear decay and exponential decay models describing DOC biodegradation kinetics (Figure 5) and resulting fraction of recalcitrant DOC (rDOC) of macroalgal DOC.

Experiment	Algal Species	Equation	Slope	Intercept	R^2	% rDOC	rDOC as % of NPP
A	*U. lactuca*	Linear: y ~ x	-0.95±0.31	141.08±4.08	0.70	-	-
A	*G. hayi*		-0.64±0.17	136.40±2.23	0.78	-	-
A	*G. parvispora*		-1.29±0.19	213.58±2.44	0.92	-	-
A	*S. species*		-0.85±0.21	171.16±2.76	0.80	-	-
Experiment	**Algal Species**	**Equation**	**A**	**B**	**C**	**% rDOC**	**rDOC as % of NPP**
B	*U. lactuca*	Exponential: $y \sim A + Be^{-Cx}$	182	69	0.175	74.1	0.53
B	*G. hayi*		243	100	0.140	72.3	1.14
B	*G. parvispora*		502	217	0.142	72.0	6.36
B	*S, species*		283	108	0.170	74.9	1.76

Note: % rDOC only calculated for degradations where DOC losses followed first order kinetics.

4.2.6 Algal CDOM characterization

Colored dissolved organic matter (CDOM) light absorption coefficients measured at 300 nm wavelength were a good predictor of algal DOC (Figure 6). This relationship or those with other wavelengths of CDOM could be useful for tracking algal DOC in the field using *in situ* spectrophotometers or from space or aircraft via remote sensing of ocean color.

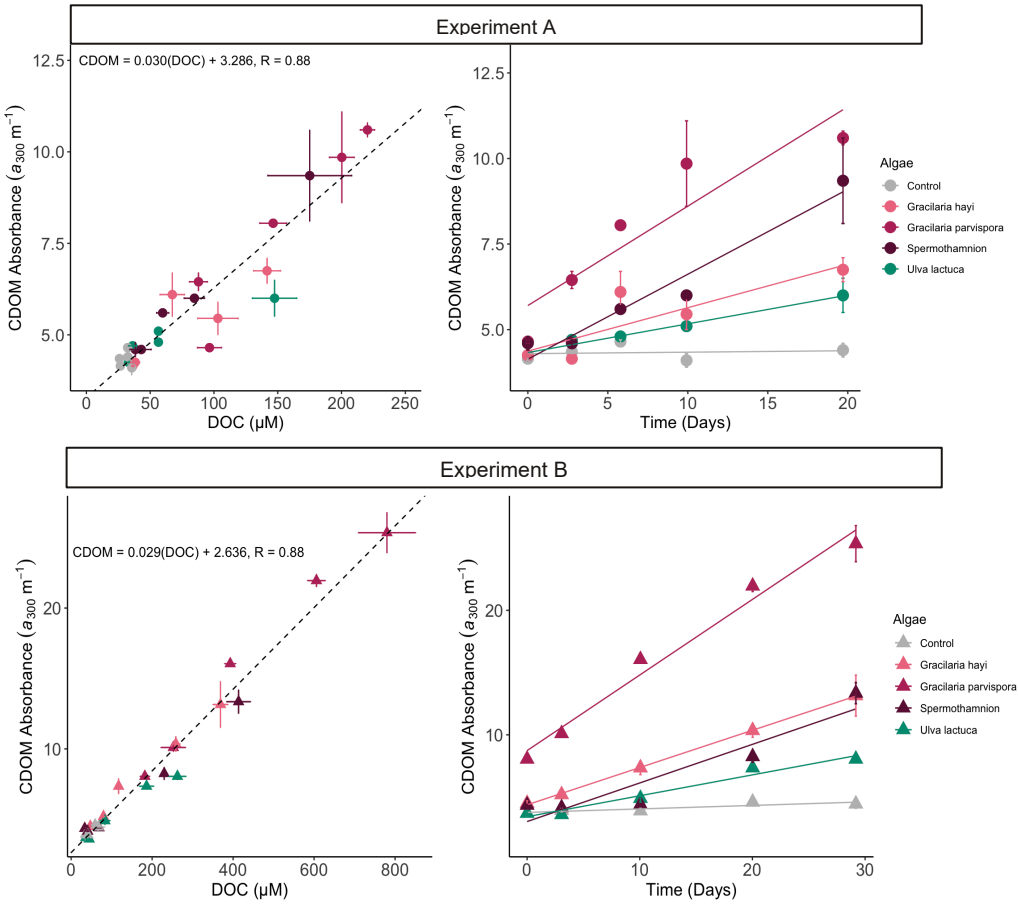


Figure 6. Relationship between algal CDOM Napierian absorption coefficients measured at 300 nm and algal DOC across incubation Experiments A and B.

4.3 Macroalgal Conclusions and Recommendations

Pilot experiments (Section 3) showed that during macroalgal incubation, particulate organic carbon (POC) production remained below 1% of algal biomass net primary productivity (NPP). Thus, under the conditions used to grow algae and for the species studied, POC likely represents only a tiny fraction of the system's CDR potential. This may not hold in environmental settings where grazing, wave action, and other factors could promote POC generation. It may also not hold for other species of algae. Thus, for ongoing work in the laboratory, we will continue to quantify POC in select samples (i.e., for additional algal species). Plus, there would be value in assessing POC under field deployments conditions directly in the field or in mesocosms, including wave tanks.

DOC production ranged from 0.7 to 8.8% of algal NPP (Table 3). The highest value was for *G. parvispora*. These rates are significant with respect to the C cycle of algal ecosystems and the C fixed when growing algae for CDR. The differences between estimates of DOC release between algae and even between the two experiments to date indicate that species choice and environmental, including trophic, conditions will likely impact both the total amount of DOC released by algae as they grow but also the proportion of their primary production that they export as DOC.

The DOC produced by macroalgae was highly biorefractory DOC (rDOC), with our incubations and kinetics studies suggesting that 72-75% of the DOC released by macroalgae was rDOC (Table 4) that could be sequestered. Using this % rDOC, the DOC production as a percent of NPP numbers were converted into estimates of the percent of NPP that could be exported as rDOC. This value ranged from 0.5 to 6% (Table 4), suggesting that a potentially significant amount of algal DOC could be exported into the deep ocean and counted as sequestered DOC. As for the estimates of the total % of NPP released as DOC, estimates of rDOC release as algae grows varied by species and between the two experiments, indicating further work, including under conditions as close to field conditions as possible, would be required to verify these estimates under field conditions. For a more robust assessment of DOC and rDOC release by algae, field studies should also assess how the ratio of NPP exported as DOC and rDOC changes through the lifetime of the algae and under different environmental conditions. For now, these data suggest that rDOC represents a significant C sequestration pathway for macroalgae whether growing in natural ecosystems, in aquaculture settings, or CDR settings.

In conclusion, DOC and rDOC production appear to be significant C sequestration pathways for the 4 algal species studied. Eventually the release and persistence of macroalgal DOC would need to be quantified in the ocean under CDR conditions. One real world process missing from our studies, is an assessment of the photo-reactivity of algal-derived DOC. Sunlight oxidizes marine and riverine DOC to carbon dioxide in surface waters (Mopper et al., 2015) but has not been studied as a process that may also remove macroalgal DOC and detritus, thereby reducing the efficiency of macroalgal CDR. In field settings, additional methods may prove useful in tracking macroalgal OC. Colored dissolve organic matter (CDOM; Figure 6) absorbance and fluorescence may provide ways to track algal DOC production and fate using *in situ* optical sensors and from space via remote sensing of reflectance. Water samples could also be analyzed for the optical, molecular, and isotopic signatures of algal OC to better quantify and track the sequestration of algal POC, DOC and larger detritus in ocean settings.

5. Spring 2023 woodchip experiments

5.1 Methodology

To quantify the dissolved organic carbon (DOC) leached from woodchips when floating in freshwater and seawater, small scale DOC leaching and subsequent biolability experiments were conducted. The results from these experiments are presented below.

For the woodchip leaching experiments, approximately 50 grams of woodchips were each added to two pre-combusted 2L glass beakers. In one beaker, 0.75 L of Milli-Q was added to provide a freshwater sample, and in the other beaker, 0.75 L of artificial seawater was added. Artificial seawater was made by adding 30 grams of Instant Ocean salt to 1 L of Milli-Q Water. Each beaker was then covered entirely with pre-combusted aluminum foil to prevent any debris from entering the samples, as well as to ensure there was no light interference. The covered beakers were placed on a New Brunswick Scientific Excella E2 Platform Shaker table at 25 rpm to mimic natural water movement. Sampling occurred on days 0, 1, 3, 7, 14, 28, and 56, with day 0 being the initial start date. Additional sample days were added as needed. Around 40-50 mL of water was obtained from each beaker on each sampling day using acid-cleaned plastic syringes and plastic tubing. After the sample was collected, it was filtered through a clean (rinse with 60 mL of Milli-Q water) 0.22 µm syringe filter into two combusted 24 mL DOC vials. One of the vials was used to measure sample pH using a Mettler Toledo SevenExcellence Multiparameter meter (pH resolution ±0.002). The approximately 20 mL of sample in the second vial was acidified to pH 2 and used to measure DOC and TDN. DOC and TDN were measured on a Shimadzu Total Organic Carbon/Total Dissolved Nitrogen Analyzer.

After the leaching experiment had concluded, the remaining water into which woodchip derived DOC had leached was filtered with a 0.22 µm filter and divided into 6 24 mL vials per beaker to measure the biolability of the DOC produced. The six vials were used to measure across 6 sampling dates: days 0, 1, 3, 7, 14, and 28. Each vial contained 20 mL of sample, 200 µL of inoculum (natural seawater containing bacteria), and nutrient additions, the latter being dependent on the amount of DOC present and the corresponding Redfield-ratio of DOC to nitrogen and phosphorus needed to ensure that nutrient limitation was not inhibiting microbial use of DOC. All vials were incubated in a dark box to ensure samples were kept in the dark on the New Brunswick Shaker Table. On each sampling date, the corresponding vials were removed from the box, filtered into clean 24 mL vial using a 0.22 µm syringe filter, and acidified to pH 2. The samples were then measured for DOC and TDN.

5.2 Results

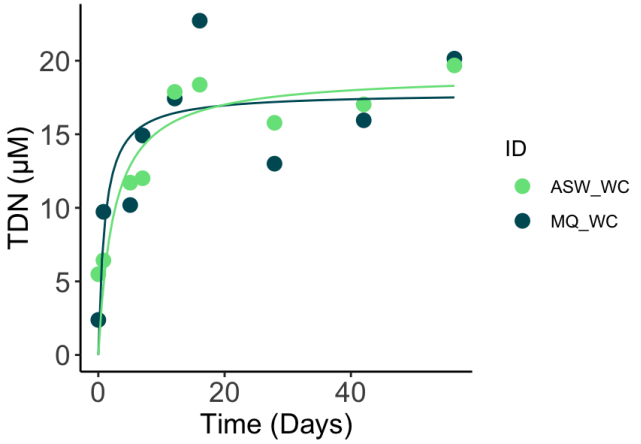
5.2.1 Carbon and nitrogen content - biomass assessment

Analysis of dried bulk woodchips from Running Tide for OC and nitrogen content were conducted in triplicate on the Stubbins Lab EA-IRMS, revealing Running Tide woodchips contain ca. 47.8 % OC and ca. 0.09 % nitrogen by mass.

5.2.2 Woodchip TDN Release

Leaching of nitrogen from woodchips into seawater from Running Tide buoy deployments may have the potential to impact local biogeochemistry and local biological productivity in the surface oceans, including supporting macroalgal growth. To obtain a first order estimate on the rate and total accumulation of dissolved nitrogen (TDN), TDN concentrations were monitored across the woodchip leaching experiment. TDN concentrations in both MilliQ and seawater solutions increased overtime to similar final concentrations ca. 17-19 µM (Figure 7), or a total of ca. 0.18 - 0.20 mg N. TDN leaching

kinetics are described using Michaelis-Menten growth curves and are similar between the two treatment solutions (MQ and ASW; Figure 7). However, the rate of TDN leaching in ASW (K_{asw} = 2.43 days) was slower than for MQ (K_{MQ} = 1.01 days), where K is the time in days representing half-way between TDN = 0 and TDN = max/asymptote.



$$TN_{\mu M} = \frac{TN_{max} \pm se(Time_{days})}{K \pm se + Time_{days}}$$

$$TN_{MQ} = \frac{17.82 \pm 2.06(Time_{days})}{1.01 \pm 0.99 + Time_{days}}$$

$$TN_{ASW} = \frac{19.08 \pm 2.00(Time_{days})}{2.43 \pm 1.50 + Time_{days}}$$

Figure 7. Total dissolved nitrogen (TDN) leached through time by woodchips submerged in artificial seawater (ASW; light green) and MilliQ water (MQ; dark green). Michaelis-Menten models are used here to describe TDN leaching kinetics.

5.2.3 Woodchip pH

Changes in water pH or 'ocean acidification' are of interest as terrestrial biomass (woodchips) containing organic acids are released into the open ocean begin to breakdown in the surface water. Here, we characterize the pH change through time as woodchip material leaching occurs in 0.75 L of both ASW and MQ. Blank ASW and MQ sample pH values were 8.2 and 6.2, respectively (open circles, Figure 8). After ca. 50.2 g woodchip addition (equivalent to 33.87 g dried woodchip biomass), the pH values of ASW declined by a maximum of ca. 0.7 pH units reaching a minimum pH of 7.46 on day 56 and averaged 7.68±0.16 throughout, representing an average decrease of 0.52 pH units. After 50.2 g woodchip addition to MQ, the pH values increased by a maximum ca. 0.7 pH units to 6.9 on day 42 and averaged 6.52±0.16 throughout, representing an average increase of 0.32 pH units.

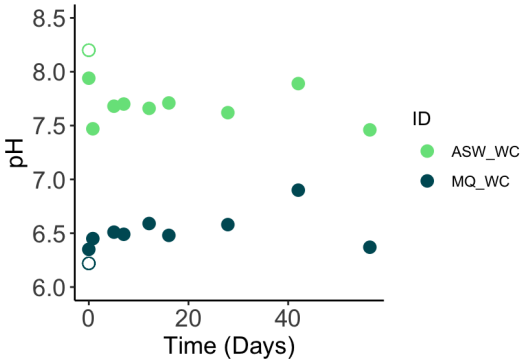


Figure 8. Timeseries of pH values for woodchips in artificial seawater (light green; ASW) and in MilliQ water (dark green; MQ) across the 56-day biolability experiment. Open circles show blank ASW and MQ pH readings, filled circles show incubation experiment measurements. Reported analytical precision of pH measurements is ±0.002.

5.2.4 Woodchip DOC Release

After addition of woodchip biomass to each of the two sample solutions, DOC leaching over time was best described by a kinetic Michaelis-Menten growth model (Figure 9). Overall, DOC leaching in ASW was higher, reaching a concentration of ca. 4464 µM after 56 days, relative to DOC leaching in MQ which reached a maximum concentration of ca. 1322 µM after 56 days. After 56 days, 0.24 % of the initial OC mass of woodchips leached as DOC into the ASW and was considered the 'leachable' fraction of woodchip biomass. Thus, less that 1% of woodchip OC is expected to leach into sweater from the woodchips or buoys made from them.

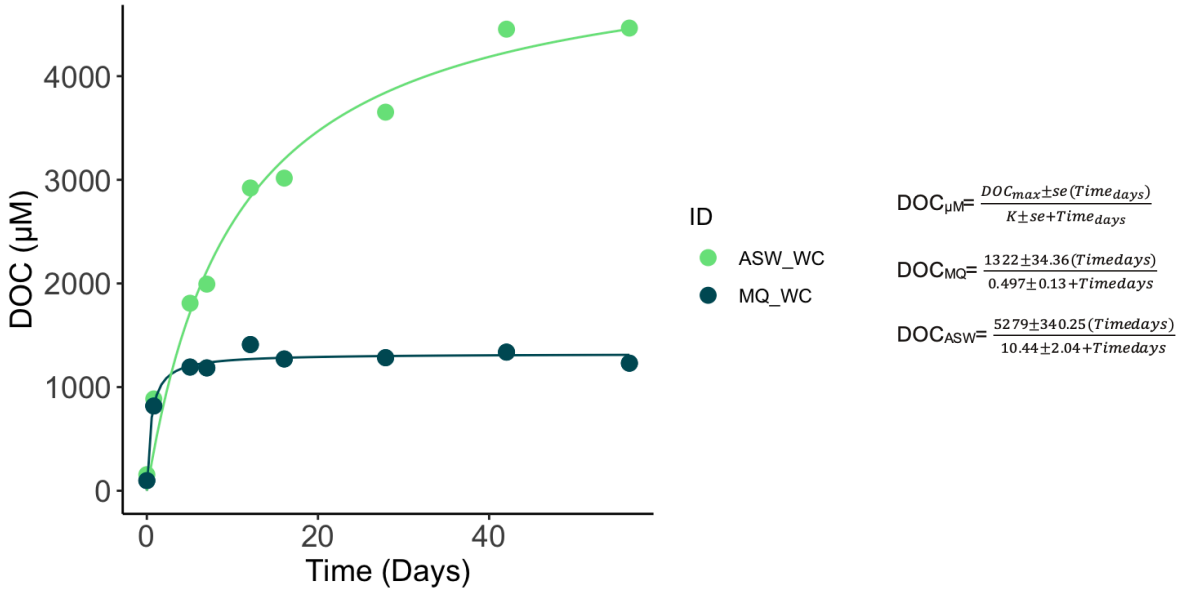


Figure 9. Dissolved organic carbon (DOC; µM) leached through time by woodchips submerged in artificial seawater (ASW; light green) and MilliQ water (MQ; dark green). Michaelis-Menten models are used here to describe DOC leaching kinetics.

Table 5: Woodchip leaching experiment parameters, woodchip carbon content and DOC leaching results.

Solution	Woodchips (wet-weight g)	Woodchips (dry-weight g)	% C dry biomass	Total C biomass initial (mg based on dry weight)	Total DOC leached (µM)	Total DOC leached (mg C)	DOC (mg) day^{-1}	%DOC released of total C
MilliQ	50.2	33.9	47.8	16270	1132.78	10.20	0.181	0.06
Artificial Seawater	50.2	33.9	47.8	16270	4308.85	38.81	0.688	0.24

5.2.5 Woodchip DOC Biolability

A biolability experiment on leached woodchip DOC was conducted over 28 days to determine the recalcitrant fraction of DOC (rDOC) leached from woodchip biomass. Although initial concentrations differed between the MilliQ and seawater treatments, rates of DOC loss were near-identical when plotted on a percentage scale (Figure 10) and fitting of first order decay curves to the data indicates that approximately 25% of the DOC that leaches from woodchips into MilliQ or seawater is biorefractory (Figure 10; Table 6).

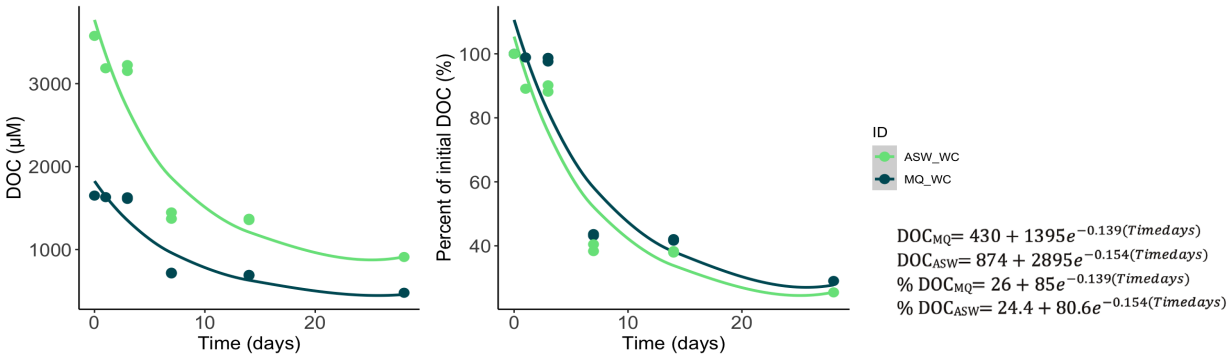


Figure 10. Woodchip dissolved organic carbon (DOC; µM) biodegradation through time by seawater microbes in artificial seawater (ASW; light green) and MilliQ water (MQ; dark green). Exponential decay models are used here to describe DOC biodegradation kinetics (Table 6).

Table 6: Exponential decay models describing DOC biodegradation kinetics (Figure 10) and resulting fraction of recalcitrant DOC (rDOC) of woodchip biomass.

Experiment	Equation	A	B	C	% rDOC	% rDOC of initial biomass C
MilliQ	Exponential: $y \sim A + Be^{-Cx}$	430	1395	-0.139	26.0	0.0163
Artificial Seawater	Exponential: $y \sim A + Be^{-Cx}$	874	2895	-0.154	24.4	0.0582

5.3 Woodchip Conclusions and Recommendations

The woodchips used by Running Tide as a potential terrestrial biomass C sink and as buoys for algal growth only leach a fraction of a percent of their C as DOC (MilliQ: 0.06%, Seawater: 0.24%; Figure 9 and Table 5). Thus, over 99% of the woodchip C added to the ocean would be expected to be sequestered as woodchip C with the caveat that the experiments conducted here were in the dark. Sunlight can photo-degrade terrestrial organics such as woodchips, producing carbon dioxide and DOC during photo-oxidation (Helms et al., 2014). Thus, it would be important to assess whether photodegradation of woodchips under conditions similar to sunlight in surface waters alters these conclusions.

About 25% of the DOC released from woodchips was biorefractory based on incubations and 1st order decays curves (Figure 10; Table 6). Suggesting about one quarter of the tiny amount of DOC leaching from the woodchips would be sequestered as refractory DOC in the deep oceans. Again, as for the woodchips themselves, this bio-refractory DOC may be photo-reactive, as riverine and other forms of terrestrial DOC are highly photoreactive, with carbon dioxide being a primary product of their photodegradation in sunlight (Mopper et al., 2015).

Most critically, under dark, marine conditions, we conclude that the woodchips will leach only very small (less than 1%) amounts of C into the ocean and that a full accounting of the likely fate of woodchips would require photochemical studies (in the lab) and eventually field studies to track the masses of woodchip C that sink vs. being leached in field settings (e.g., field deployments or mesocosms).

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Supplemental Information

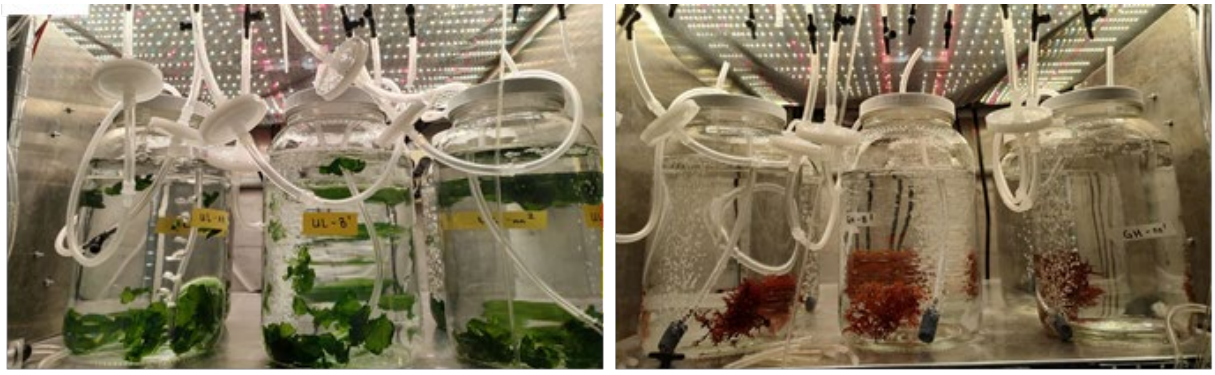

Figure S1. Initial algal incubation experiments at Running Tide Fall 2022. (A) Triplicate Ulva lactuca samples in 4L glass incubation jars and (B) triplicate Gracilaria hayi samples in 4L glass incubation jars. Each jar was equipped with standard aquaria air stones and bubbled with clean air to oxygenate the water.

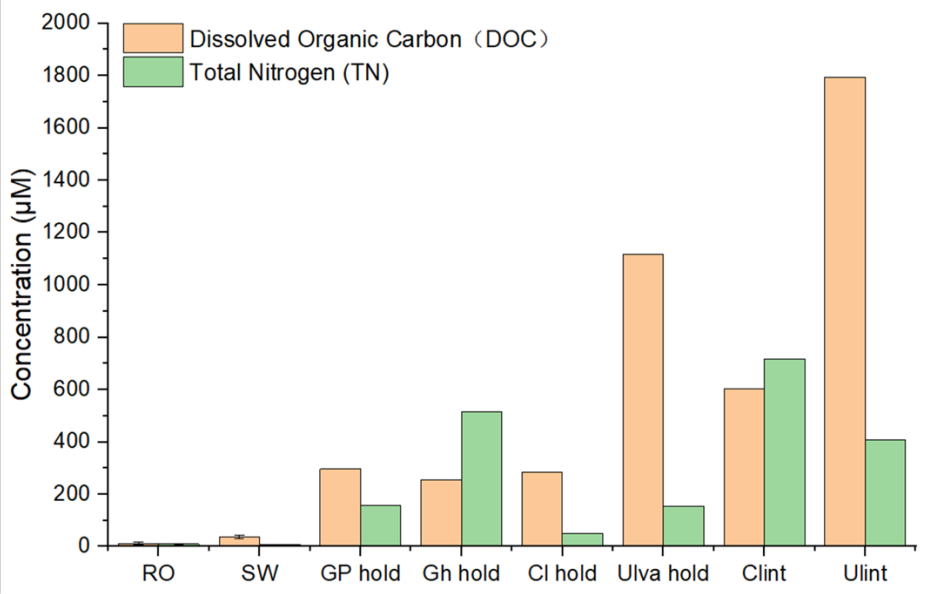

Figure S2. Dissolved organic carbon (DOC; orange) and total nitrogen (TN; green) concentrations in background water and algal water samples. RO: reverse osmosis water; SW: artificial seawater (salinity ~30); GP hold: Gracilaria parvispora holding tank (50 PAR 12light:12dark); Gh hold: Gracilaria hayi holding tank (50 PAR 12light:12dark); Cl hold: Chaetomorpha linum holding tank (50 PAR 12light:12dark); Ul hold: Ulva lactuca holding tank (50 PAR 12light:12dark); Clint: Chaetomorpha linum initial sample in glass jar 1-NC-7/29/22; Ulint: Ulva lactuca initial sample in glass jar 1-NC-7/28/222

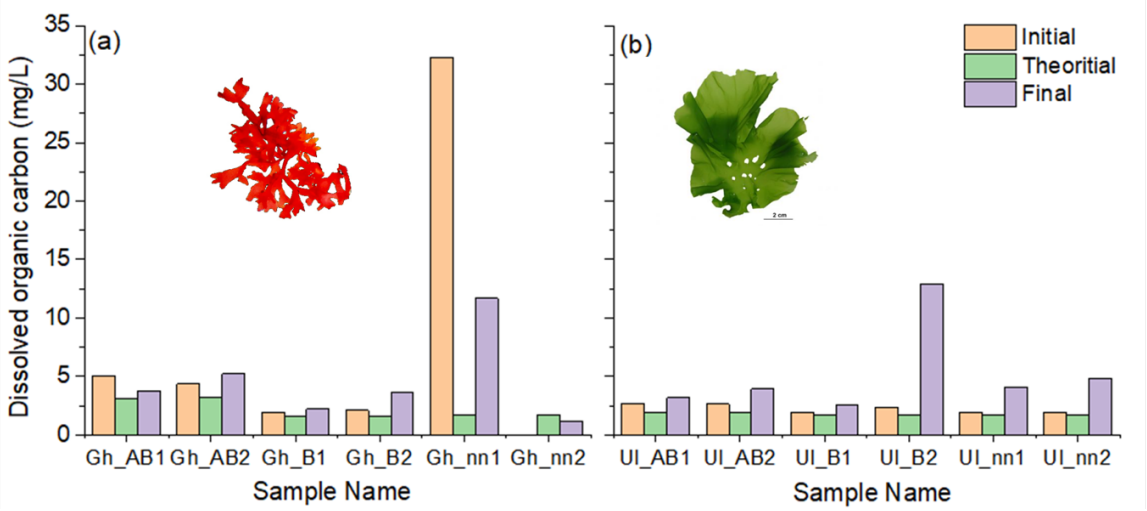

Figure S3. Initial, theoretical, and final dissolved organic carbon (DOC) in each culture jars for two algal species incubated over the period of 2-weeks: *Gracilaria hayi* and *Ulva lactuca*. Initial and final DOC was analyzed directly after starting and ending the incubation experiments. Theoretical DOC was calculated based on the background of DOC of each component (such as seawater, nutrients, stone, GeO2, etc.) in the culture system. Gh_AB1(2): replicate G. hayi samples with nutrient parts A and B; Gh_B1(2): replicate G. hayi samples with nutrient part B only; Gh_nn1(2): replicate *G. hayi* samples with no nutrients added. Similar nomenclature for *Ulva lactuca* (Ul).

Table S1. Experimental array starting conditions. During each of two experiments algal samples and controls were run in duplicate incubation jars, with one diatom jar for determining background.

Experiment Start Date	Sample ID	Initial Salt Water (g)	Initial Algae Wet Mass (g)
Experiment A March 10, 2023	Control A	1496.1	0
	Control B	1496.6	0
	Ulva lactuca A	1486.4	30.395
	Ulva lactuca B	1478.5	30.285
	Gracilaria hayi A	1504.1	30.192
	Gracilaria hayi B	1508.3	30.211
	Gracilaria p A	1513.1	30.175
	Gracilaria p B	1513.4	30.266
	Gpoly A	1484.9	30.47
	Gpoly B	1485.9	30.157
	Diatom	1495.6	0
Experiment B April 25, 2023	Control A	1601.4	0
	Control B	1663.5	0
	Ulva lactuca A	1596.2	15.314
	Ulva lactuca B	1501.2	15.185
	Gracilaria hayi A	1534.1	15.586
	Gracilaria hayi B	1586.8	15.316
	Gracilaria p A	1595.7	15.333
	Gracilaria p B	1526.2	15.067
	Gpoly A	1501.6	15.003
	Gpoly B	1543.4	15.026
	Diatom	1590.3	0

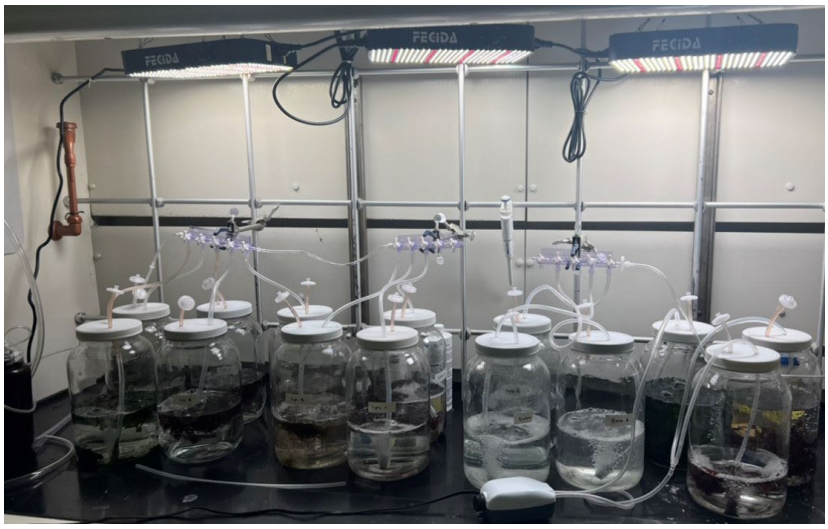

Figure S5. Full macroalgal experimental setup in the Stubbins Lab at Northeastern University.