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Release and microbial degradation of dissolved organic matter (DOM) from the macroalgae *Ulva prolifera*

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ABSTRACT

Release and microbial degradation of dissolved organic matter (DOM) and chromophoric dissolved organic matter (CDOM) from the macroalgae *Ulva prolifera* were studied in laboratory incubation experiments. The release of DOM and CDOM from *Ulva prolifera* was a rapid process, and hydrolysis played an important role in the initial leaching of the organic compounds from the algae. Bacterial activity enhanced the release of DOM and CDOM during degradation of the algae and utilization of the released organic compounds. It is calculated that 43 ± 2% of the C and 63 ± 3% of the N from *Ulva prolifera*'s biomass were released during the 20-day incubation, and 65 ± 3% of the released C and 87 ± 4% of the released N were utilized by bacteria. In comparison, only 18 ± 1% of the algae's C and 17 ± 1% of its N were released when bacterial activities were inhibited. The fluorescence characteristics of the CDOM indicate that protein-like DOM was the major organic component released from *Ulva prolifera* that was highly labile and biodegradable. Bacteria played an important role in regulating the chemical composition and fluorescence characteristics of the DOM. Our study suggests that the release of DOM from *Ulva prolifera* provides not only major sources of organic C and N, but also important food sources to microbial communities in coastal waters.

1. Introduction

Ulva prolifera is a species of green seaweed that can be found growing in coastal waters worldwide (Nelson et al., 2008; Guiry and Guiry, 2013). In recent years, this marine macroalga has become a dominant green-tide-forming seaweed in the Yellow Sea, China, and its great proliferation has caused serious environmental concerns for cities along the Yellow Sea (Liu et al., 2010; Liu et al., 2015; Li et al., 2016). For example, the largest *Ulva prolifera* bloom in China was recorded during the summer of 2013 off the coast of Qingdao, a major city (population of 7 million) on the west coast of the Yellow Sea (Liu et al., 2013; Mathiesen, 2013). The bloom covered 28,900 km^2 of coastal water and an estimated 7300 tons of algae were removed from the coastal water and beaches during this three-week event (see Fig. 1). Since its first major occurrence in 2008, the green-tide-forming phenomenon has become an annual event during early summer in the Yellow Sea (Liu et al., 2013).

The green tides created by *Ulva prolifera* are believed to be related to water pollution from agriculture and industry, which release high concentrations of nutrients to the coastal waters (Howarth, 2008; Howarth et al., 2011; Shi et al., 2015). In a recent study, Li et al. (2016) demonstrated that the growth of *U. prolifera* was sensitive to dissolved nutrient levels, and its growth rate was significantly enhanced when dissolved nitrogen and phosphorus were added to the incubation medium. In coastal waters, the green tides formed by *Ulva prolifera* usually occur in water temperatures between 15 and 25 °C, during early summer, with a growth period of about 15–20 days. Once it begins, it can create a large-scale outbreak under normal circumstances. Although the green-tide-forming macrophyte is not toxic to humans or marine animals, its carpet-like coverage of the coastal surface waters blocks sunlight and consumes oxygen from the water, significantly influencing water quality and local ecosystems, especially in benthic communities (Wang et al., 2007; Liu et al., 2010). In recent years, many studies have investigated the possible causes, origin, growth dynamics, physiological and ecological characteristics of the *U. prolifera* bloom in the Yellow Sea and the bloom's impact on the coastal ecosystem (Zhang et al., 2014; Shi et al., 2015; Sun et al., 2015; Zhou et al., 2015).

On the other hand, the rapid green-tide-forming bloom by *U. prolifera* serves as an important sink of CO_2 and could therefore significantly affect the carbon cycle and biogeochemical processes in the Yellow Sea. The large formation of biomass over a short period could constitute a large source of both dissolved organic matter (DOM) and

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Fig. 1. The largest *Ulva prolifera* bloom in China was recorded during the summer of 2013 off the coast of Qingdao. Photo shows the *Ulva prolifera* bloom growing in the coastal waters and deposited on the beaches of the city (photo from Chinanews.com, cited with permission).

chromophoric dissolved organic matter (CDOM) to the coastal waters, because studies have demonstrated that leaching of DOM and CDOM from marsh plants and seagrasses is an important source of DOM and CDOM in estuarine and coastal waters (Wang et al., 2007; Huang and Chen, 2009; Wang et al., 2014). The rapid release of DOM from the *U. prolifera* bloom during a short time period could have a significant "priming effect" on the ecosystems and biogeochemistry in coastal waters (Bianchi, 2011; Bianchi et al., 2015). However, few studies have investigated the possible release, composition and microbial degradation of DOM and CDOM released from *U. prolifera*. In this paper, we present results from laboratory incubation experiments that examined the release dynamics of DOM and CDOM from *U. prolifera*. The microbial degradation of DOM and CDOM and fluorescence characteristics of CDOM released from *U. prolifera* were also investigated.

2. Materials and methods

2.1. Sample collection

Fresh living samples of *Ulva prolifera* in the midpoint of the bloom were collected from the coast of Qingdao City (36°5.358′N; 120°28.27′E) on July 8, 2016. After collection, the macroalgae were transported in seawater back to the laboratory within 1 h, and rinsed with deionized water to remove any sediment particles attached to the surface of the algae. Seawater was collected at the same site and filtered through 0.7 μm glass fiber filters (GF/F) that had been pre-combusted at 550 °C for 4 h for the leaching incubation experiments. The filtration of seawater could have removed some bacteria attached to particles (Bar-Zeev et al., 2012) but was necessary, because particles could complicate the incubation results.

2.2. DOM and CDOM leaching experiment

Two sets of leaching experiments were conducted: bacteria-active and bacteria-inhibited (poisoned), to compare the release dynamics of DOM and CDOM from *Ulva prolifera* due to chemically and microbially mediated leaching processes. For both experiments, 20 g fresh *Ulva prolifera* were added to each of a series of glass bottles containing 2.5 L filtered seawater. For the bacteria-inhibited experiment, 2.5 ml saturated $HgCl_2$ solution was added into each of the bottles containing seawater to kill bacteria before adding the algae. Duplicates were conducted for each experiment. All bottles were open to the air (loosely covered) and incubated at room temperature (~25 °C) in the dark for 20 days. At different time intervals (day 0, 0.5, 1, 2, 3, 5, 7, 9, 11, 13, 15, 17 and 20), water samples were collected from each bottle and filtered using a pre-cleaned 0.45 μm-pore polyether sulfone needle filter to measure the dissolved concentrations. At the end of the experiment, the remaining *Ulva prolifera* solid phase was collected by filtration, dried at 40 °C and the remaining biomass weight and C and N contents were determined. All glassware used for the sample processing and storage was acid-washed, Milli-Q water rinsed and combusted at 550 °C for 5 h before using to remove any organic carbon.

2.3. Chemical and fluorescence measurements

To quantitatively measure the DOM released from *U. prolifera*, the concentrations of dissolved organic carbon (DOC) and dissolved nitrogen (DN) were measured using a Shimadzu TOC-L analyzer equipped with an ASI-V auto-sampler. The DOC and DN concentrations were calibrated using a 5-point calibration curve generated from a DOC standard using potassium hydrogen phthalate (KHP) and a DN standard using potassium nitrate (KNO_3), dissolved in UV-oxidized Milli-Q water. The instrument blank and standard validation for DOC and DN were checked using low carbon reference water and seawater reference materials (from Dr. Hansell's Laboratory at Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, USA). The blank was subtracted using Milli-Q high purity water analyzed before every five samples. The average blanks associated with DOC and DN measurements were about 5 μM and 4 μM, and the analytic precision on triplicate injections was ± 3% and ± 4%, respectively. The solid phase total organic carbon (TOC) and nitrogen (TN) of *U. prolifera* before and after the incubation were measured using a Thermo Flash 2000 Elemental Analyzer with precisions of 3% for TOC and 4% for TN.

The release of CDOM from *U. prolifera* was quantified by measuring fluorescence using a FS5 Spectrofluorometer (Edinburgh Instruments), and single fluorescence emission scans were collected from 280 to 680 nm for an excitation wavelength of 350 nm. Before each measurement, the fluorescence of Milli-Q water was measured as a blank and subtracted from the sample spectra prior to integration. The photon absorbing intensity at Ex = 350 nm and Em = 450 nm was integrated and converted to quinine sulfate units (QSU), where 1 QSU is equivalent to the fluorescence emission of 1 μg/l quinine sulfate solution. Reproducibility of the measurement was < 1% for seawater and samples. In addition to the single scan fluorescence measurement, excitation-emission matrix spectroscopy (EEM) for CDOM was measured using the same FS5 Spectrofluorometer to characterize the chemical composition of CDOM released from *U. prolifera* during microbial degradation. The excitation wavelength was set from 220 to 450 nm and the emission wavelength scan ran from 240 to 680 nm in 5 nm increments. The Milli-Q water EEM was subtracted as a blank. All sample data were expressed in QSU. Samples higher than 100 QSU were diluted with Milli-Q water to eliminate the inner filter effect (Chen and Gardner, 2004).

3. Results

3.1. Release of DOC and DN

The results of DOC and DN leaching from *U. prolifera* in the bacteria-active and bacteria-inhibited incubation experiments are plotted in Fig. 2. The release of both DOC and DN from *U. prolifera* was a rapid process and large differences were observed between the bacteria-active and bacteria-inhibited incubations. In the first 2 days, concentrations of DOC increased more rapidly in the bacteria-inhibited incubation than that in the bacteria-active incubation (Fig. 2a). The concentration of DOC increased to 1299 μmol/gdw (gram dry weight) on Day 2 from the initial seawater background level (88 μM) in the bacteria-inhibited incubation and then increased slowly for 20 days to the end of the experiment, with 3900 μmol/gdw DOC released. The calculated overall release rate of DOC was 192 μmol/gdw/day. In