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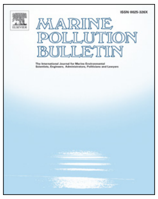

Baseline

Response of microalgae to large-seaweed cultivation as revealed by particulate organic matter from an integrated aquaculture off Nan'ao Island, South China

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ABSTRACT

Large seaweed cultivation has proven an effective means to inhibit harmful microalgae at experimental scales and battle eutrophication in Chinese coastal waters, but essentially there is a lack of field-scale studies to explore the underlying mechanism. Here we present a 1.5-year-long time series of particulate organic matter (POM) and settling particulate matter (SPM) concentrations from an integrated aquaculture of *Gracilaria lemaneiformis* off the coast of Nan'ao Island, South China from April 2014 to August 2015. The microscopic examination and geochemical characteristics show that the POM mainly consisted of microalgae. The mean POM concentration increased 99.8%, 71.2%, 45.8% and 111.9% at the four sampling sites during the non-cultivation period, while decreased 25.5%, 17.3%, 12.2% and 20.3%, respectively, during the seaweed cultivation period. These results suggest that the large scale seaweed cultivation can remove excess nutrients and inhibit microalgal growth, thereby contributing to the improvement of coastal marine aquaculture environment.

Rapid economic development has led to an accelerated, intensified eutrophication in Chinese coastal waters (Qu and Kroeze, 2012; Yang et al., 2015; Dai et al., 2017), and as the leading country in aquaculture food production (FAO, 2014), the rapid expansion of animal farming in China has aggravated the environmental problem (Li et al., 2011). As a result, the frequency of harmful algal blooms (HABs) and the economic loss associated with algal toxins have increased significantly (Morand and Merceron, 2005; Anderson, 2009; Hiraoka et al., 2011; He et al., 2014). For instance, the frequency of HABs has increased three times every decade since the 1970s, and 322 HAB events were observed in Chinese coastal waters from 1952 to 1998, causing devastating damage to the aquaculture industry, coastal ecosystem and even human health (Zhou et al., 2001; Tang et al., 2006; He et al., 2014; Brooks et al., 2016).

To combat coastal eutrophication and control HABs, many physical, chemical, and biological remediation measures have been proposed by researchers (Anderson et al., 2001). As evidenced by world-wide field practice over the last few decades, artificial fishing of algal bloom or using solar ultraviolet radiation (UV-B) cannot completely reduce the reoccurrence of red tides (Sugawara et al., 2003); using chemicals is only effective within weeks, but some chemicals remain in the environment and the food web for years (Anderson, 1997), causing secondary environmental pollution. In contrast, biological remediation, such as using macrophytes or microorganisms to reduce water pollution (Furusawa et al., 2003), has proven to be environmentally friendly and sustaining, and the cultivation of large seaweeds as nutrient strippers in integrated aquaculture has been demonstrated as an excellent example of ecotechnology (Neori et al., 2004; Yang et al., 2015), in which the food production system is designed in partnership with nature.

Previous studies have revealed that there are quite a few advantages in using large seaweeds to control eutrophication and restore the marine environment (Schramm, 1999; Fei, 2004; Neori et al., 2004; Xiao et al., 2017). For example, Gracilarioid species (mainly *Gracilaria* but also *Gracilariopsis*) can contribute to the efficient removal of dissolved P and N wastes from intensive fish farms, increasing the economic output of the industry (Buschmann et al., 1996; Alcantara et al., 1999; Jones et al., 2001). Co-existence culture system experiment results demonstrated that *Gracilaria lemaneiformis* had obvious inhibitory

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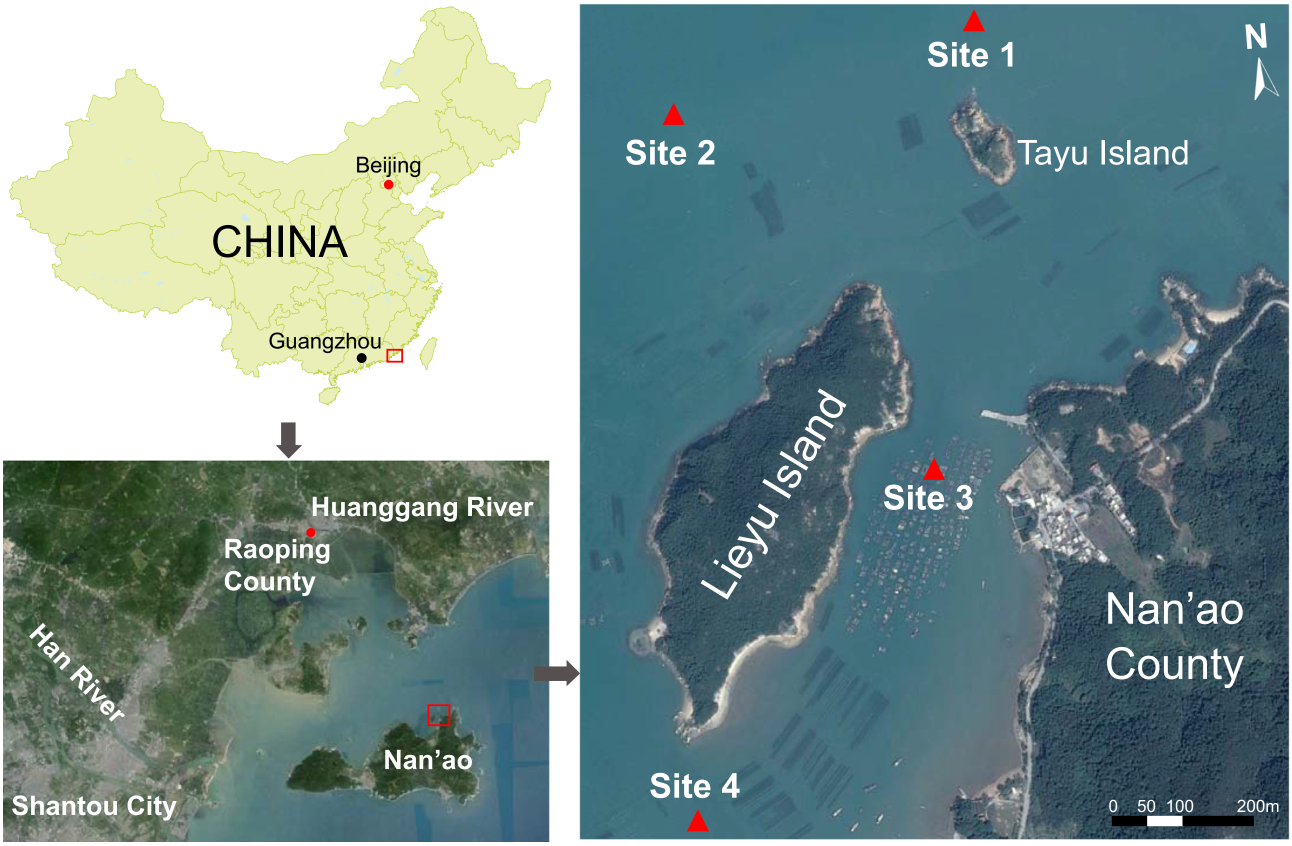

Fig. 1. Location of the study area in Shen'ao Bay, Nan'ao County, Guangdong Province, China. Sampling sites are shown on right panel by red triangle. During the monitoring period, Sites 1 & 4 had no large seaweed cultivation or animal farming; Site 2 had *Gracilaria* cultivation during April–May 2014 and February–May 2015; and Site 3 fish farming operated all the time. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

effects on the growth of two red tide microalgae, *Chaetoceros curvisetus* and *Scrippsiella trochoidea* (Liu et al., 2006).

In addition, the cultivation of these seaweed species does not require supplementary feeding, but removes large amount of nutrients and only small amounts of organic matter and dissolved nutrients are released to the environment, in contrast to intensive-feeding fish and shrimp farms (Hopkins et al., 1995; Yang et al., 2015). To use this advantage to balance the nutrients produced by animal-fed aquaculture, integrated multi-trophic aquaculture (IMTA) was proposed in which the wastes of one resource user become a resource (fertilizer or food) for the others (Chopin et al., 2001). Since the 1990s, many studies related to multi-trophic culture have been carried out in Canada, Japan, Chile, and the United States (Hirata et al., 1993; Petrell and Alie, 1996; Troell et al., 1997; Chopin et al., 1999; Wartenberg et al., 2017). Actually, co-culture had been practiced for decades in Asian countries, especially in China, in order to meet the large demand for food and decrease the waste of resources (Li, 1987; Qian et al., 1996; Ponte et al., 2014). In general terms, IMTA is an innovative and responsible practice in mariculture.

However, many studies focused on laboratory simulation systems, small scale tank systems and short-term field monitoring (Buschmann et al., 1996; Troell et al., 1997; Alcantara et al., 1999; Jones et al., 2001; Tang et al., 2004; Carmona et al., 2006). Few studies demonstrated the long-term effectiveness of seaweed cultivation in coastal IMTA areas (see Xie et al., 2017 for an exception). In this study, we attempt to reveal the long-term variation of particulate organic matter (POM) and settling particulate matter (SPM) in a near-shore IMTA system which combines animal-fed aquaculture species (such as *Epinephelus akaara* Temminck and Schlegel, *E. awoara* Temminck and Schlegel), with an inorganic extractive aquaculture species (*Gracilaria lemaneiformis*).

In the coastal environment, POM mainly consists of phytoplankton, bacteria, invertebrates, fish and zooplankton fecal pellets and detrital particles (Volkman and Tanoue, 2002). The quality and quantity of the POM produced by phytoplankton depend on the nutrient status of the natural waters, and phytoplankton composition and growth phase. POM is of considerable biogeochemical and oceanographic importance in the aquatic environment and plays an important role in the carbon cycle, because it transports as settling particulate matter (SPM) or 'marine snow' - a primary component of export production from the surface ocean down to the ocean floor (Alldredge and Silver, 1988). When euphotic zone-derived POM sinks through the water column, it acts as a food source to marine biota (Kiørboe, 2001) and also scavenges smaller particles (Stolzenbach, 1993), while the seasonal deposition of POM on the seabed can provide essential nutrients to benthic organisms (Lampitt, 1985). Furthermore, POM and SPM on the seabed can be resuspended in the water column due to tidal influence and bio-turbulence (Wainright and Hopkinson, 1997). Therefore, POM plays a key role in coastal ecosystems, and its temporal and spatial variations can shed light on the effect of seaweed cultivation in coastal IMTA areas.

The shallow sea area around Nan'ao Island, Guangdong Province is one of the largest marine aquaculture bases in China. Since the 1980s, the animal-fed aquaculture industry has developed extensively. Due to decades of practice with mono-species cultivation and high-density fish cages, coastal water eutrophication was significantly accelerated and caused the outbreak of HABs in 1997 and 1999 (Huang et al., 1999). In the 1990s, *G. lemaneiformis* was transplanted from Qingdao to Nan'ao Island successfully. Due to the social-economic benefits, large-area cultivation of *G. lemaneiformis* has been generally promoted by the local government. According to the annual fishery report of Nan'ao County, Shantou City, the total *G. lemaneiformis* production reached up to 21,217 tons from a production area of 731 ha in 2011 (Wang et al., 2014). This characteristic IMTA pattern has succeeded in Nan'ao Island for 15 years.

Here we present a 1.5-year-long time series of POM concentrations at various water depths at four monitoring sites and SPM geochemical record from an integrated aquaculture off Nan'ao Island, South China, generated from a monthly monitoring program from April 2014 to August 2015 that covered two large-seaweed cultivation periods. The working hypothesis was that patterns in POM concentration in the integrated aquaculture area are a function of microalgal biomass, which reflects the degree of nutrient availability. This is because seaweeds take up dissolved N and P from the water column and thus decrease the nutrient level during their cultivation period, whereas the nutrient level increased during the non-cultivation period (Yang et al., 2006; Yang et al., 2015). In our field sampling design, each POM sample reflects the real-time biomass in surface water when the sample is taken, and each SPM sample represents the bulk POM settling down to the sediment trap during the previous month. Therefore, our study aims to reveal the interaction between microalgae and large seaweed on a field scale, and at the same time, demonstrate the usefulness of POM and SPM in monitoring the microalgal biomass of coastal aquaculture systems.

Nan'ao County (23°12′47″-23°31′37″N, 116°54′56″-117°18′45″E) is located on the Tropic of Cancer and covers an area close to 105.2 km^2, which includes Nan'ao Island and 36 small islands (Fig. 1). Shen'ao Bay, a semi-enclosed bay with an average water depth of approximately 3 m, is located in the northeast part of Nan'ao Island. There is no large river or seasonal stream discharging into this bay. Zhelin Bay, which is a harbor with a high-density mariculture, lies in the southeast part of Raoping County, 6 km north of Shen'ao Bay.

Samples were taken every month from April 2014 to July 2015 at 4 sampling sites in Shen'ao Bay (Fig. 1). Sites 1 and 4 are non-cultivation zones, while *G. lemaneiformis* is cultured at Site 2, where it is attached to ropes, parallel to the sea surface and buoyed by commercial rafts (Petrell et al., 1993). As the optimal growing temperature is 12–23 °C, *G. lemaneiformis* does not produce economic benefit during summer and fall in south China due to slow growth and intensive fish eating (Fei, 2004). During the sampling period, two culture periods of *G. lemaneiformis* were recorded. Site 3 is located in a fish-cage culturing zone. More than one thousand fish cages (3 × 3 m) are suspended underwater in an area of 1 km^2 (Gu et al., 2012).

At each sampling site, the water depth was obtained using a depth sounder (Speedtech, USA). POM samples in the water column were collected by Niskin bottles from 3 layers; a surface layer about 0.5 m depth, an intermediate layer (median depth), and a bottom layer 0.5 m above the seabed. As the water depth of Site 4 is lower than 3 m, only the surface and bottom layers were sampled. In order to remove the influence of zooplankton, water samples were filtered through a 125-μm pore size zooplankton net. POM was obtained by filtering 1500 to 3000 mL of the water onto a pre-combusted (500 °C, 5 h) glass fiber filter (0.7 μm Whatman GF/F, 47 mm diameter) using low vacuum pressure, rinsed with DI water to eliminate interference from dissolved components, stored in an on-vehicle refrigerator, and immediately transferred to the laboratory for freeze drying. Additionally, from April 13 to May 28, 2014, a short-term (duration 45 days) field survey was carried out at these sites, during which the POM in the water column was sampled every 2–5 days at surface, intermediate and bottom depths.

SPM samples were collected at the same frequency as that of POM using a self-designed, simple sediment trap at Sites 1, 2 & 3 (Gu et al., 2017); the shallow water depth at Site 4 does not allow the installation of a sediment trap. Each trap was moored at the same height relative to the seafloor. The bulk in the receiving cups of the sediment trap was collected and placed in zip-lock polyethylene bags, preserved in an on-vehicle refrigerator, and immediately transferred to the laboratory. The samples were freeze-dried to constant weight, then ground gently with agate pestle and mortar, sieved with 63 μm mesh sieve for homogenization, and then stored in glass bottles for further analyses.

Meteorological data, taken in order to compare with the POM variation, were obtained from the Shantou Municipal Bureau of Meteorology, including daily wind speed, rainfall and air temperature. Since there is a delay in the response of the POM concentration in the coastal waters to changes in the environmental factors, the meteorological data were obtained on two occasions; the sampling day and two days before sampling.

For POM collection and concentration analysis, about 1.5 L of seawater from each site was filtered through a pre-combusted (500 °C, 5 h) Whatman GF/F membrane filter (0.7 μm nominal pore size). After filtration, the POM samples retained on the filters were stored at −18 °C. In an indoor laboratory, the filters were freeze-dried and weighed to determine the POM concentration (in milligrams per liter, $mg{\cdot}L^{-1}$).

An aliquot of each freeze-dried SPM sample was weighed and pretreated with HCl fume to remove carbonate. The C and N concentrations and isotopic ratios were analyzed using a Carlo Erba Elemental Analyzer (EA) interfaced to a Finnigan MAT Delta Plus XP stable isotope ratio mass spectrometer (IRMS) at Florida State University. The results are reported in the standard delta (δ) notation. The $\delta^{13}C$ values are in reference to the international VPDB standard and $\delta^{15}N$ values to AIR N_2. The precision of the C and N isotope analysis is 0.2‰ or better on the basis of repeated analysis of five different laboratory standards including YWOMST-1 (cane sugar), YWOMST-2 (phenylalanine), YWOMST-3 (L-phenylalanine), YWOMST-5 (urea) and Urea-2.

Two way analysis of variance (ANOVA) was carried out to evaluate if the observed spatial, seasonal and depth-related variation were significant or not in the POM of Bay of Shen'ao. When ANOVA results were significant, a multiple means comparison, using Duncan test, was carried out. Pearson's correlation analysis was applied to describe the relationship between meteorological factors and POM concentration. Data were analyzed and statistical analyses were carried out using the SPSS 19.0 software for Windows.

Meteorological conditions during the sampling days are shown in Fig. 2. The mean wind speed varied between 1.30 (March 2015) and 3.63 $m{\cdot}s^{-1}$ (December 2014). Generally, wind speeds in fall and winter were greater than spring and summer. Strong NE winds in winter produced a height increase over the tidal prediction, whereas SW winds caused the opposite effect.

During the sampling days, there was no evident rainfall variation in the study area (Fig. 2). Maximum records were detected in January 2015 and May 2014, reaching 15.13 and 9.20 mm, respectively.

Maximum air temperatures were observed in July 2014 and June 2015 (30.20 °C and 29.17 °C, respectively) followed by August 2014 (28.93 °C, Fig. 2). In February 2015, the lowest air temperature was observed during a typical winter condition when strong winds blow in from over the sea (NE).

Water depth varied from 1.6 to 11.4 m (Fig. 3). The maximum water depth was observed at Site 1 (11.40 m). The values for Site 2 and Site 3 averaged 6.38 and 4.40 m, respectively. The lowest water depth was recorded at the inner shelf bay Site 4 (1.6 m).

During the 1.5-year monitoring period, the concentrations of POM ranged from 8.98 to 74.19 $mg{\cdot}L^{-1}$, with an average of 31.82 $mg{\cdot}L^{-1}$ (Fig. 3). The highest amount was recorded in September 2014 in the bottom layer of Site 4, being twofold greater than the mean of 2014 (32.19 $mg{\cdot}L^{-1}$), whereas the lowest concentration was registered in May 2014 in the surface layer of Site 2. At all 4 sites, bottom waters always had higher POM concentrations than the shallower intermediate and surface waters, especially after the seaweed was harvested.

Generally, the POM concentrations were low during the seaweed cultivation period, but increased obviously after the seaweed harvesting at all 4 sites. In 2014, mean values of the POM concentration in the non-cultivation period (from June to December) increased 99.8%, 71.2%, 45.8% and 111.9% at Site 1, Site 2, Site 3 and Site 4, respectively, from

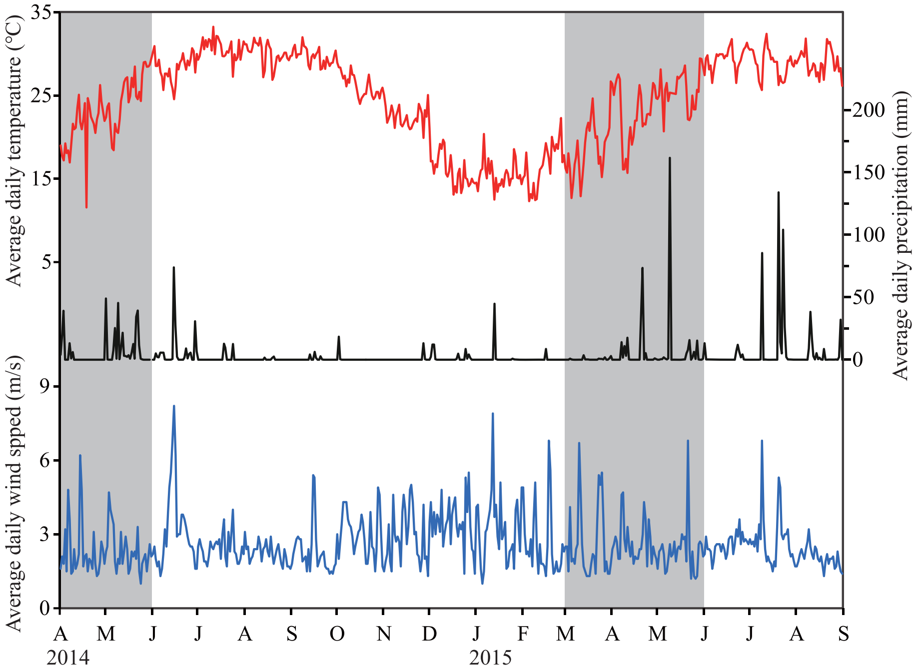

Fig. 2. Mean daily values of air temperature (top), precipitation (middle), and wind speed (bottom) between April 2014 and September 2015 (bracketing the monitoring period) in the study area.

that in the seaweed cultivation period (from April to May). During the seaweed cultivation period in 2015, the POM concentrations decreased 25.5%, 17.3%, 12.2% and 20.3%, respectively, at these sampling sites, in comparison with that in the last non-cultivation period. At all sampling sites, a significant difference in seasonal distribution of POM concentration was observed (ANOVA, F = 15.33, $p < 0.01$). The result of multiple comparisons indicated that the POM concentrations in spring and summer were significant different from those in fall and winter. The Pearson's correlation analysis revealed that the POM concentration was not significantly correlated with rainfall and air temperature. In addition, the POM levels for the bottom layer of Site 2 and the surface layer of Site 4 were significantly correlated with wind speed

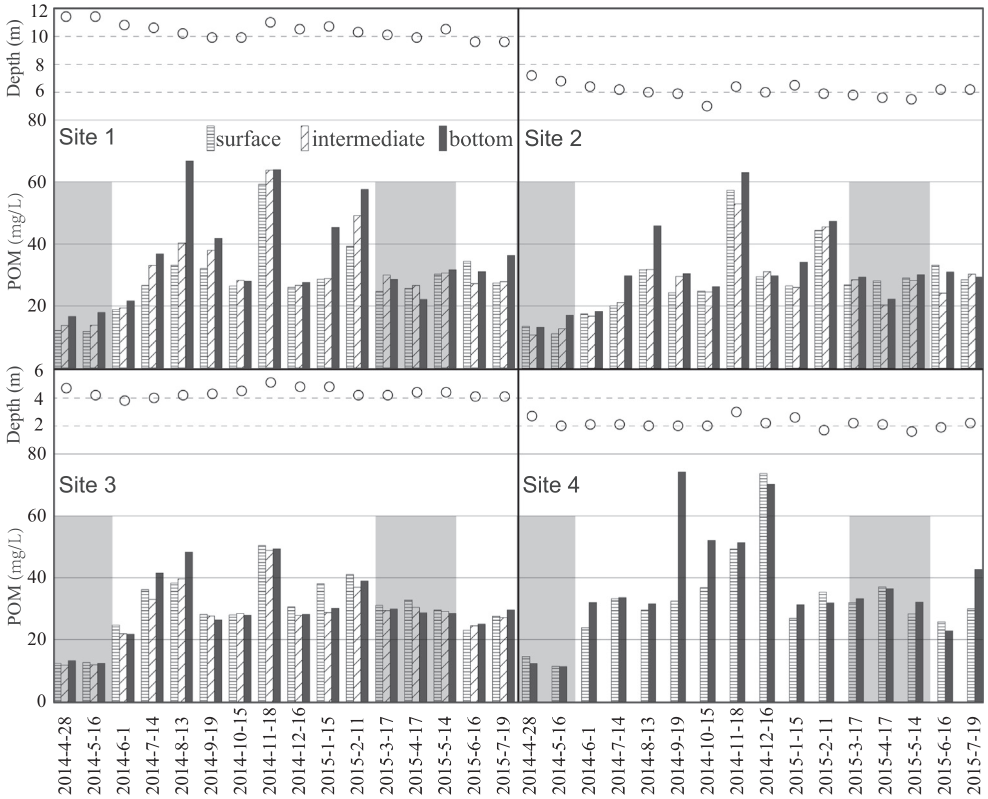

Fig. 3. Monthly changes in particulate organic matter (POM) concentrations and water depth during the study period at the 4 sampling sites in Shen'ao Bay. Light gray shading highlights the *G. lemaneiformis* culture period.

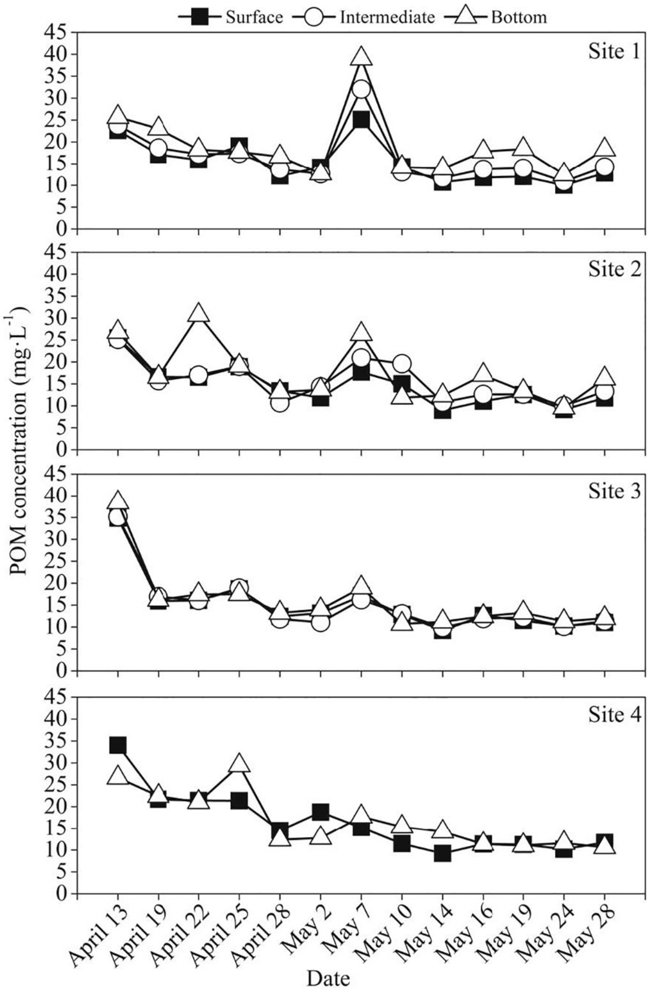

Fig. 4. Temporal variations of POM concentrations at the sampling sites during the *G. lemaneiformis* maximum growth period from April 13 to May 28, 2014.

(p = 0.049 and p = 0.036, respectively).

During the *G. lemaneiformis* growth period, the concentrations of POM among layers oscillated between 8.98 and 39.02 $mg·L^{-1}$ and the mean value was 16.48 $mg·L^{-1}$ (Fig. 4). Although no significant differences were detected among sites and depths (ANOVA, F = 0.467, p = 0.706; F = 1.589, p = 0.208, respectively), a slightly decreasing trend of water POM concentrations was observed during the short-term monitoring survey.

The study area lies in a bay used as a harbor by local residents, and the intensive fishing boat traffic and operation activities resulted in frequent interruption to SPM sampling. As a result, SPM sample collection was successful only at Sites 2 & 3, while sample harvest was sporadic at Sites 1 (Fig. 5).

SPM collected from Sites 2&3 at different times exhibited a moderate variation in C and N contents and stable isotopic signature, and co-varied throughout the study period (Fig. 5). The organic C contents range from 1.2 to 2.5% averaging 1.8%, and N contents from 0.2 to 0.4% averaging 0.3%. The $\delta^{13}C$ values fall between −22.2 and −18.9‰ with a mean of −20.9‰, and $\delta^{15}N$ values between 5.8 and 7.7‰ with a mean of 6.6‰. In contrast, the C/N ratios are relatively invariable, ranging from 6.0 to 6.9 with an average of 6.6.

There is no large river or seasonal stream discharge into the Shen'ao Bay (Gu et al., 2013). The geographic location and another two lines of evidence suggest that the POM during the sampling period was derived predominantly from marine organic matter, and terrestrial organic matter did not seem to have a noticeable impact in the study area.

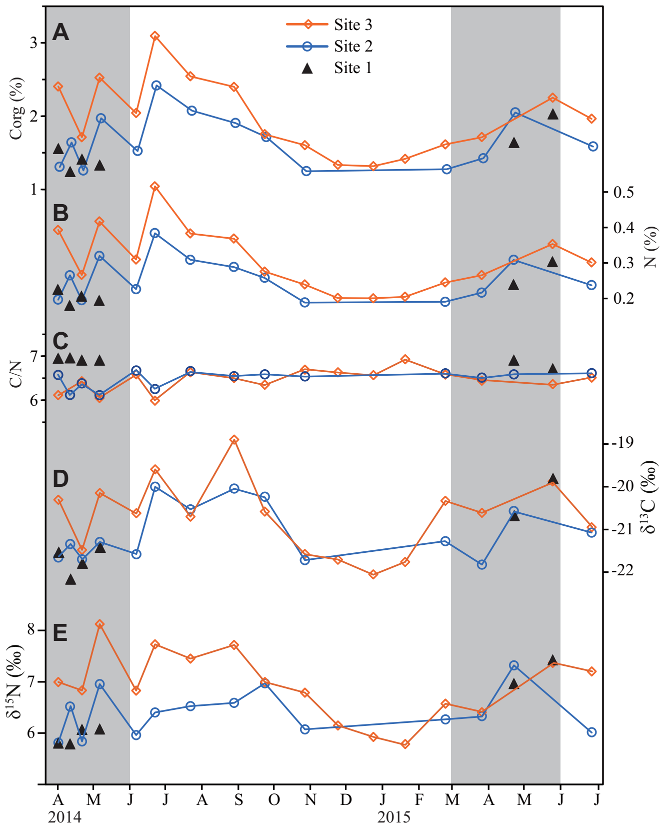

Fig. 5. Temporal and spatial variations in POM geochemical characteristics during the study period. Light gray shading highlights the *G. lemaneiformis* culture period.

First, we examined four randomly selected POM sample filters under light microscope, and found that the particles mainly included microalgae, fine organic detritus, and glass fibers (Supplementary material). The dominant microalga was diatom, followed by dinoflagellate, cyanophyte, and chrysophyte, which is consistent with the phytoplankton survey results from the water column and SPM (Zhang et al., 2012; Du et al., in preparation).

Second, geochemical fingerprints have proven a powerful tool to distinguish the source of organic matter (Fig. 6). Due to difference in photosynthetic pathway and carbon source, terrestrial C_3 plants typically have $\delta^{13}C$ values between −32‰ and −21‰, C_4 plants have more enriched values between −17‰ and −9‰, and freshwater algae have lower $\delta^{13}C$ values (−26‰ ~ −30‰) than marine algae (−16‰ ~ −23‰) (Meyers, 1994; Meyers, 1997; Lamb et al., 2006). On the other hand, terrestrial plants consists predominantly of nitrogen-poor lignin and cellulose and have high C/N ratios, which are over 12 in C_3 plants and over 30 in C_4 plants; whereas algae have lower C/N ratios of < 10. These geochemical fingerprints consistently indicate an origin of marine algae for our POM samples (Fig. 6). Taken together, the POM we sampled can be considered a proxy for microalgal biomass.

The initial hypothesis was that patterns of POM concentration could be explained in terms of nutrient availability. The results presented here generally support this hypothesis. It is clear that the POM dynamics in the Shen'ao Bay were influenced by seaweed cultivation activity.

Generally, the concentrations of POM were higher after the *G. lemaneiformis* harvest and relatively low during the *G. lemaneiformis* cultivation period at all long-term monitoring sites in our study. Furthermore, the 45-day seaweed growth period survey demonstrated that the POM concentrations decreased in all of the sampling areas. From the temporal distribution trends of the POM, it is evident that the *G. lemaneiformis* culture played an important role in controlling the

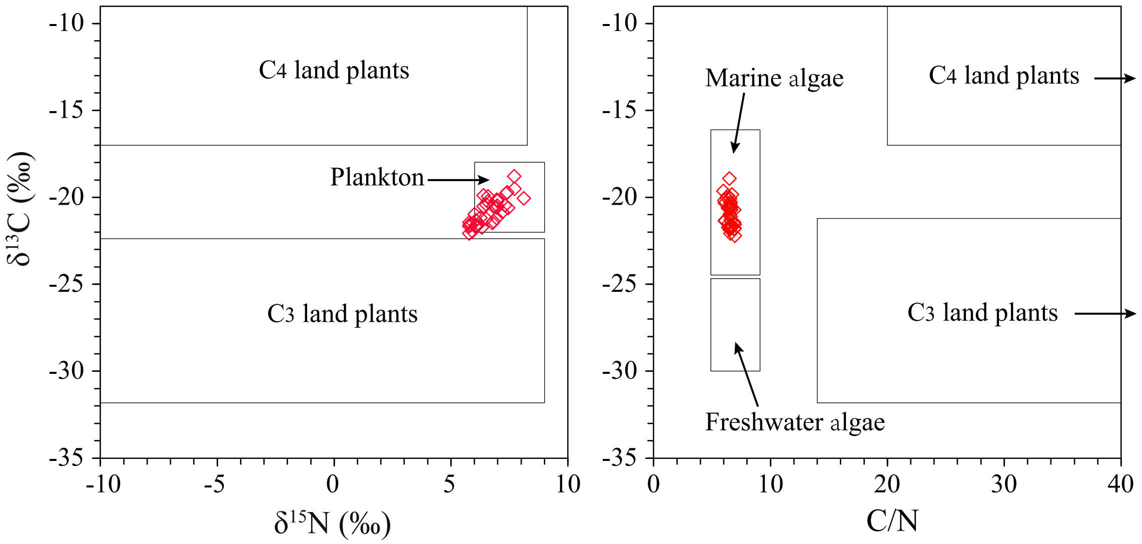

Fig. 6. Geochemical signatures of the SPM samples shown by red diamond. Data for the major groups are from Meyers (1994, 1997) and Lamb et al. (2006). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

POM concentration in the study area. This is because *G. lemaneiformis* can absorb large quantities of N, P and CO_2, produce large amounts of O_2 and have a strong effect on decreasing the nutrient levels. In tank cultivation, the experiments showed that the concentrations of NH_4^+-N decreased by 85.5% and the concentrations of PO_4-P decreased by 66.0% in aquaria with *G. lemaneiformis* after 23 days, in comparison with the aquaria without seaweeds (Yang et al., 2006). The same conclusion was inferred in Chile, as a one-ha-raft cultivation of *Gracilaria chilensis* can uptake at least 5% of dissolved inorganic nitrogen and 27% of dissolved phosphorus in open-water co-cultured with a salmon cage farm (Troell et al., 1997). As a result, the microalgal growth decreased, because of the limited dissolved nutrients. When *G. lemaneiformis* was harvested in late May, the higher internal nutrient levels originating from the sediments resulted in increased microalgal biomass.

IMTA is considered more sustainable than the common monoculture systems, in that fed monocultures tend to have an impact on their local environments due to their dependence on the supplementation of an exogenous source of food and energy without mitigation (Chopin et al., 2001; Wartenberg et al., 2017). Based on our results, the large-scale cultivation of *G. lemaneiformis* may be effective at controlling the nutrient level not only at the seaweed cultivation site, but also at the fish-cage culture site and other non-cultivation sites. These long-term monitoring experiments demonstrated that *G. lemaneiformis* was a good candidate to use in the IMTA system.

In addition, wind speed played a role in the spatial and temporal variations of POM concentration. The increasing current velocity controlled by tides and winds, led to stronger shear forces, resulting in sediment re-suspension, which could explain the observed changes in POM concentrations. Therefore, higher concentrations are reached during high tides in autumn and winter.

Eutrophication of coastal waters has become a serious problem and induced HABs and heavy economic losses in China. Previous studies have demonstrated that the seaweed *G. lemaneiformis* grew well in eutrophic seawaters and was able to remove inorganic nutrients effectively in laboratory simulation systems, small-scale tank systems and short-term field monitoring. Our 1.5-year-long field monitoring record indicates that the POM concentration is a useful proxy for microalgal biomass, which was low during the *G. lemaneiformis* culture period and increased during the non-cultivation period in the integrated mariculture base of Shen'ao Bay. These observations suggest that the large-scale cultivation of *G. lemaneiformis*, as one of the bioremediative approaches in eutrophic coastal seawater, can remove excess nutrients and limit the growth of microalgae. At the same time, it can alter the biogeochemical processes and contribute to the improvement of the coastal marine aquaculture environment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2018.05.026.

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