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Optimization of hard clams, polychaetes, physical disturbance and denitrifying bacteria of removing nutrients in marine sediment

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ABSTRACT

Marine organisms are known to play important roles in transforming nutrients in sediments, however, guidelines to optimize sediment restoration are not available. We conducted a laboratory mesocosm experiment to investigate the role of hard clams, polychaetes, the degree of physical disturbance and denitrifying bacterial concentrations in removing total nitrogen (TN), total phosphorus (TP), and total organic carbon (TOC) in marine sediments. Response surface methodology was employed to analyze the results of initial experiments and in a subsequent experiment identified optimal combinations of parameters. Balancing the TN, TP, TOC removal efficiency, our model predicted 39% TN removal, 33% TP removal, and 42% TOC removal for a 14-day laboratory bioremediation trial using hard clams biomass of 1.2 kg m^{-2}, physical disturbance depth of 16.4 cm, bacterial density of 0.18 L m^{-2}, and polychaetes biomass of 0.16 kg m^{-2}, respectively. These results emphasize the value of combining different species in field-based bioremediation.

1. Introduction

Increasing nitrogen and phosphorus inputs into coastal marine ecosystems has led to globally serious environmental problems (Howarth et al., 2000; Vitousek et al., 1997). Eutrophication has negative impacts on marine sediments, starting with changes in the structure of the resident communities and oxygen depletion, and eventually leading to anoxic sediments as excess organic material decomposes on the seafloor (Cloern, 2001; Gurkan et al., 2006; Meyer-Reil and Köster, 2000).

To date, attention has been devoted to the bioremediation of chemical pollution in the marine environment due to accidental spills, urban and industrial discharges. However, bioremediation of eutrophic environments has received less attention. With application of microbial metabolic potential (bioremediation) is accepted as an environmentally benign and economic measure for decontamination of polluted environments (Wu et al., 2015). Several strains of bacterial isolated from environment are capable of removing N, P and sulfurous compounds and are potentially useful in bioremediation (Guo et al., 2013; Zhang et al., 2015). However, these interventions have not been developed to bioremediate organically enriched eutrophic sediments (Dell'Anno et al., 2009; Radwan et al., 2002). To date, major efforts to reduce the nutrient concentrations in coastal ecosystems have focused on farming bivalves or seaweed (Huo et al., 2012; Loo and Rosenberg, 1989). Nevertheless, recovery of nutrient enriched sediment has been slow relative to seawater and recovery trajectories have not always been predictable (Duarte et al., 2009; Kemp et al., 2009).

The rate of nutrient processing in marine sediments is complex, influenced by the interaction of microbial, physical, and chemical process (Barnes and Barnes, 2014; Bertics et al., 2012). These in turn, are influenced by large organisms, that bioturbate and bioirrigate the sediment (Riisgard and Banta, 1998; Welsh, 2003).

Marine coastal sediments contain a rich diversity of microorganisms that possess different metabolic potentials (Laverock et al., 2011; Meysman et al., 2006). Benthic faunal activities also influence carbon, nitrogen, and sulfate cycling, due to particle reworking and burrowing, ventilation and bioirrigation (Araujo, 2013; Karlson et al., 2007; Stief, 2013). Large marcofaunal polychaetes, bivalves, and shrimps play important roles in regulation of carbon mineralization, enhance denitrification/nitrification (Fulweiler et al., 2013; Laverock et al., 2014; Volkenborn et al., 2007). In addition, physical disturbance such as ploughing the sediment may also affect nutrients by mixing sediments and exposing subsurface sediments to oxygen.

We hypothesize that sediment nutrient concentrations can be reduced through the combined effects of bioturbation by different benthic macrofauna, enhanced bacterial densities of species that contribute to

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nutrients cycle, and physical disturbance. Two experiments were conducted under laboratory conditions to identify optimal combinations of bioremediation strategies. The first experiment involved enhancing the biomass of hard clams and polychaetes, the degree of physical disturbance, and concentration of denitrifying bacteria. Response surface methodology (RSM) analysis of initial experiments was used to explore the optimization of TN, TP, TOC removal efficiency that was then tested in subsequent experiment to inform future field-based bioremediation.

2. Materials and methods

2.1. Experimental treatments

Sediment, seawater, hard clams (*Meretrix meretrix*) and polychaetes (*Perinereis aibuhitensis*) were collected from intertidal flats at Jinhai coastal zone, Nantong City, China (31.97.04′S, 121.80.85′E). The hard clams (*Meretrix mertrix*) are common on in intertidal flats in China (Wu et al., 2015). The hard clams inhabit different grain size sediments, but they prefer inhabiting sandy sediments with mean grain size of 3.40 μm. Approximately ten kilograms of hard clams (shell height, 3.5 ± 0.32 cm, mean ± S.D.; wet weight, 10 ± 0.41 g per individual, mean ± S.D.) were collected. The polychaete (*Perinereis aibuhitensis*) used in this experiment is distributed across both saltmarsh (*Spartina alterniflora*) and intertidal flats. The polychaetes live in 20 cm deep J-shaped burrows with a vertical head shaft (Zhao et al., 1993). Two kilograms of polychaetes (individual wet weight, 2.0 ± 0.23 g , mean ± S.D.) were collected from intertidal flat. Both hard clams and polychaetes were placed in individual plastic mesh containers and immersed in continuously flowing seawater with change rate of 200 mL min^{-1} to acclimate them to the laboratory for one week. The top layer of sediment (~30 cm depth) with mean grain size of 345 μm was carefully excavated and sorted manually to remove large burrowing organisms. A strain of denitrifying bacteria (99% identified as *Halomonas* sp. (*AB305262.1*)) was used for the experiments (Shen et al., in press). The bacteria were purified from culture solutions, and a solution for use in the experiment was made with $1 * 10^7$ (OD = 600 nm). Sediment was physically disturbed using a rake that is frequently used for harvesting bivalves in shellfish farms. The rake ploughed sediment to a depth of approximately 5 cm.

Plastic tanks (30 cm × 30 cm, 40 cm deep) were used for experiments, each contained 0.027 m^3 sediment and 9 L seawater. Tanks were fed continuously flowing seawater with an exchange rate of 200 mL min^{-1}. The tanks were subjected to an artificial tidal regime that resulted in the sediment being covered by 10 cm of sea water during daytime (12 h) and then drained for 12 h at night(simulated tidal regime approximates the natural tidal regime).

After acclimation, the hard clams and polychaetes were added into microcosms to achieve appropriate treatment biomass (Table 1). The hard clams or polychaetes were put on the surface of the sediment and allowed to burrow into the sediment. Most individuals dug into the sediment within 1 h, those that did not were replaced with active individuals. The sediments were physically disturbed to a depth of 5 cm, and approximately 80% of surface was turned with a rake. The denitrifying bacterial solution was evenly injected into the sediment (depth 5 cm).

2.2. Initial mesocosm experiment

The ability of hard clams, physical disturbance, polychaetes and denitrifying bacteria to remove nutrients from sediments was assessed across 5 treatment levels each with three replicates (Table 1). This experiment was conducted at 23–25 °C, under ambient light and lasted for 2 weeks.

2.3. Optimization of removing nutrient with response surface mythology (RSM)

To assess the benefits of specific combinations of clams, polychaetes, denitrifiers and physical disturbance, a second experiment was conducted. This was based on the analysis of the initial experiment's results, employing RSM. The four factors of the first experiment (hard clams, physical disturbance, polychaetes, and denitrifying bacterial) were optimized by RSM with a four-factor, five-level central composite design (CCD) in Design Expert 8.1 Software (Stat-Ease, Inc., Minneapolis, USA). The design model in this study was quadratic.

RSM examined the relative treatment effects on the response variables using a general second order polynomial model:-

$$y = bo + \sum_{i=1}^{4} biX_i + \sum_{i=1}^{4} biiX_i^2 + \sum_{i=1}^{4} biiX_i^3 \sum_{j=1}^{4} bijXiX_j \quad (1)$$

Where 'y' is the response variable, bo is a constant, b_i is the linear coefficient, b_{ii} is the quadratic coefficient, b_{ij} is the interaction coefficient and Xi is dimensionless coded variables (X_1 depicted for hard clams density, X_2 for physical disturbance depth, X_3 for bacterial density, X_4 for polychaetes density). The regression equation above is considered for optimization to maximize 'y' using the numerical optimization program of Design Experter 8.1 Software. To ascertain the reproducibility of the data, all treatments runs were conducted in replicate.

Table 2 identifies the treatment levels to be tested in our second experiment. The same mesocosms, temperature and continuous-flow culture system was used as in the initial experiment. The optimization experiment consisted of hard clams, denitrifying bacteria, polychaetes and physical disturbance, which was conducted for 2 weeks at 23–25 °C, and treatments replicated twice.

2.4. Sediment characteristics and analytical methods

Sediment samples for physiochemical analyses were dried in a freeze-drier (Labconco, USA) to a constant weight. The total organic carbon (TOC) was determined as the weight loss (% of the dry weight) after ignition (2 h at 550 °C). The total nitrogen (TN) was determined by the UV spectrophotometric method, and the total phosphorus (TP) was determined by the acid moly date-ascorbic acid spectrophotometric method (Ma et al., 2015).

Table 1
Different levels of four factors.

Factor codes	Factors	Levels					
X_1	Hard clam biomass (kg m^{-2})	0.1	0.2	0.5	1	2	3
X_2	Physical disturbance depth (cm)	2	5	10	15	20	30
X_3	Denitrify bacteria volume (L m^{-2})	0.01	0.02	0.05	0.1	0.2	0.3
X_4	Polychaete biomass (kg m^{-2})	0.01	0.02	0.05	0.1	0.2	0.3

Table 2
Range of variables in the central composite design.

		Levels				
Factor codes	Factors	$-\alpha$	−1	0	+1	$+\alpha$
X_1	Hard clam biomass (kg m^{-2})	0.19	0.5	1.25	2	2.31
X_2	Physical disturbance depth (cm)	7.93	10	15	20	22.0
X_3	Denitrify bacteria volume (L)	0.02	0.05	0.13	0.2	0.23
X_4	Polychaete biomass (kg m^{-2})	0.02	0.05	0.13	0.2	0.23

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