Responses of Heterotrophic Bacteria Abundance to added Trace Element Fe and Mn in the Inolgotrophic and Mesotrophic Zone of the Northern Yellow Sea

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Abstract. Heterotrophic bacteria play important roles in the biogeochemical cycling of key elements and serve as crucial links between dissolved organic carbon and sinking particles. Trace elements can directly and indirectly affect heterotrophic bacteria abundance. Two microcosm experiments were onboard in different nutrition levels of the Northern Yellow Sea in November 2011 to explore the responses of heterotrophic bacteria abundance of added Fe and Mn. Our results indicated that the influence of Mn and Fe on the abundance of heterotrophic bacteria was related to the nutrition level of the sea water. Mn can promote the growth of bacteria and increase the abundance of bacteria in oligotrophic seawater, while the impacted is limited within nutrient N and P is plentiful. And yet, Fe has the promoting effect of bacteria abundance in both oligotrophic and mesotrophic seawater, which is stronger in the oligotrophic water. Furthermore, high concentration of Fe has stronger positive impact.

Introduction

As a fundamental component in the marine microbial food web, heterotrophic bacteria are widely distributed in the whole world, playing important roles in the biogeochemical cycling of key elements and serve as crucial links between dissolved organic carbon, sinking particles and higher trophic levels [1]. In addition to dissolved organic matter and nutrient, heterotrophic bacteria are affected by trace elements in some seas, especially Fe. Bacteria need a lot of iron as a catalyst for their electron transfer in the process of oxidation reduction, which directly influence their metabolism [2]. In coastal waters, Fe can limit the conversion efficiency and growth efficiency of heterotrophic bacteria. Meanwhile Fe can be absorbed and utilized by phytoplankton, enhancing the enzymatic activity and promoting the photosynthesis, respiration, and N fixation [3, 4]. The moderate Mn can also promote enzymatic activity, strengthening photosynthesis of phytoplankton [5]. In a conclusion, trace elements can indirectly affect bacteria abundance and productivity by limiting the primary productivity of the sea.

The Yellow Sea is a semi-closed shallow sea in the northwest Pacific, located between China Mainland and the Korean Peninsula, divided into the Northern Yellow Sea and the Southern Yellow Sea. The area of the Northern Yellow Sea (NYS) is 71000 km^2, with an average depth of 40 meters. It has special geographical position and the unique phenomenon of cold water mass, therefore its marine ecosystem composition and stability attracted widespread attention [6]. As a consequence, the study on the responses of heterotrophic bacteria abundance to Fe and Mn fertilization in different nutrition levels of the Northern Yellow Sea has important scientific significance.

In this study, onboard incubation experiments were performed in different nutrition levels of the Northern Yellow Sea in November 2011 to explore the responses of heterotrophic bacteria abundance of added Fe and Mn.

Materials and Methods

Microcosm set-up. Two microcosm experiments were onboard the *R/V Dongfanghong II* during November 2013 and conducted at two stations located at different regions in the NYS: station G3 (36ºN, 122ºE) and station H3 (37ºN, 123ºE) (Table 1). 1.5 liter bottles were used in the experiments filled with the 200-mm pre-screened surface seawater to exclude mesozooplankton, with C, FeL, FeH, MnL, MnH(Table 2).

Table 1 Initial environmental factor of the station G3 and H3

Station	Bottom depth (m)	Temperature (℃)	salinity (‰)	pH	DO (mg/L)	DOC (μM)	DIN (μM)	PO_4^{3-}-P (μM)	bacteria abundance (cells/mL)
G3	43.8	18.335	30.202	8.116	7.44	160.87	13.01	0.36	3.63×10^5
H3	28.8	14.694	30.637	8.145	7.93	179.28	19.71	1.19	1.87×10^5

Heterotrophic bacteria abundance. Seawater (10 mL) was fixed with 0.5% seawater buffered paraformaldehyde and stored at -80℃ before analysis. Heterotrophic bacteria abundance was measured by a Becton–Dickinson FACSCalibur cytometer [7].

Statistical analysis. All treatments had a minimum of three replicates, with results presented as mean ± SD (standard deviation). Statistical analysis was performed using one-way analysis of variance (ANOVA) with the Fisher LSD post hoctest. Differences were considered statistically significant when $p < 0.05$.

Table 2 Ambient nutrient concentration

Incubation Bottle No.	Treatment	Added trace element Concentration
C	Control	no addition
FeL	$FeSO_4 \cdot 7H_2O$	20 μg/L
FeH	$FeSO_4 \cdot 7H_2O$	200 μg/L
MnL	$MnSO_4 \cdot 4H_2O$	10 μg/L
MnH	$MnSO_4 \cdot 4H_2O$	100 μg/L

Results and Discussion

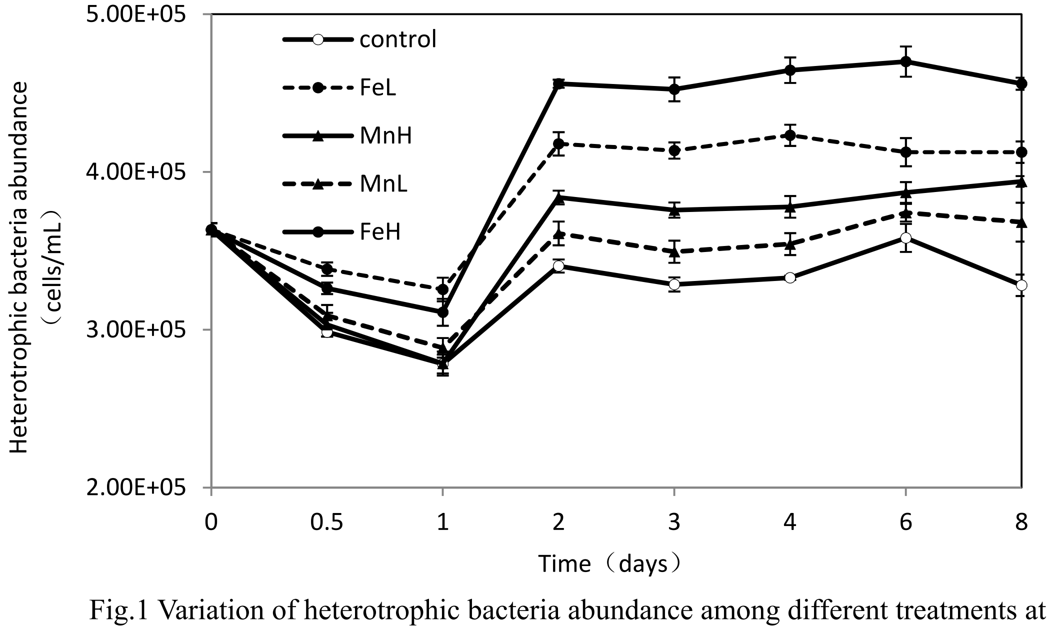

Fig.1 Variation of heterotrophic bacteria abundance among different treatments at G3

Heterotrophic bacteria abundance changed over incubation time in trace element Fe and Mn enrichment culture experiment at Station G3 (Fig. 1). The seawater at stations G3 was oligotrophic (Table 1) and DIN: PO_4^{3-}-P=36.14, suggesting there is P-limited. Compared to the controls, the abundance of heterotrophic bacteria showed significant increase in both Mn and Fe treatments ($P<0.05$) and the growing rate of high concentration treatments is greater than that in low concentration treatment ($P<0.05$). At the end of the experiment, the abundance of heterotrophic bacteria of MnH and MnL are higher than the control by 1.20-fold and 1.12-fold respectively, and similarly the FeH and FeL treatments are 1.39 and 1.26 times higher than the control. It is suggested that in the oligotrophic and N-limited seawater, Fe and Mn fertilization can promote the growth of heterotrophic bacteria abundance and higher concentration lead to higher abundance. Furthermore, the influence of Fe is stronger than Mn.

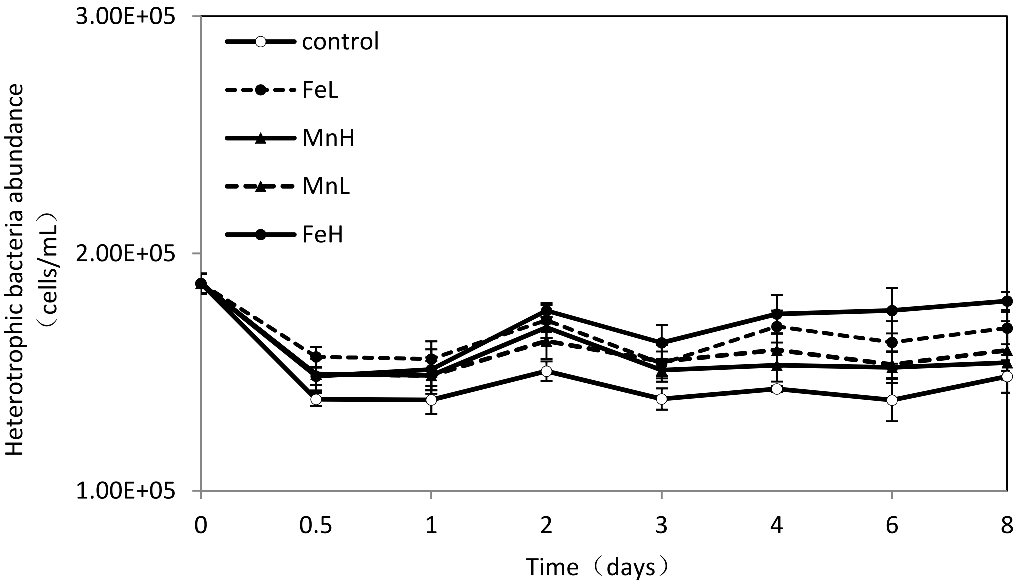

Fig.2 Variation of heterotrophic bacteria abundance among different treatments at H3

Heterotrophic bacteria abundance changed over incubation time in trace element Fe and Mn enrichment culture experiment at Station H3(Fig. 2). The seawater at stations G3 was mesotrophic (Table 1) and DIN: PO_4^{3-}-P=16.57, suggesting no nutrient limitation exist. As against the controls, the abundance increased slightly ($P > 0.05$) in both MnL (1.07-fold) and MnH (1.04-fold) treatments at the end of the experiment, while the abundance showed the remarkable growth in both FeL (1.14-fold) and FeH (1.21-fold) treatments ($P<0.05$). When nutrient N and P is plentiful, Mn has a limited impact on heterotrophic bacteria abundance, while Fe can promote the growth of heterotrophic bacteria abundance and higher concentration lead to higher abundance.

Conclusions

Our study has demonstrated the effect of trace element Fe and Mn enrichment on the abundance of heterotrophic bacteria in our mesocosm experiments with coastal waters in the NYS, which depends on the nutrition level of the sea water. Mn can promote the growth of bacteria increase the abundance of bacteria in oligotrophic seawater, and the stronger promoting effect was presented in the higher concentration. However Mn has a limited impact when nutrient N and P is plentiful. And yet, Fe has the promoting effect of bacteria abundance in both oligotrophic and mesotrophic seawater, which is stronger in the oligotrophic water. Furthermore, high concentration of Fe promotes stronger.

Acknowledgements

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