Responses of heterotrophic bacteria abundance and activity to Asian dust enrichment in the low nutrients and low chlorophyll (LNLC) region of the Northwestern Pacific Ocean

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Abstract. Bacteria, as an essential part of microbial food web, play a significant role in the marine ecosystem. Dust deposits into the surface ocean carrying with vital nutrient such as Inorganic nitrogen and phosphorus etc., which has an important influence on the life activities of heterotrophic bacteria. The microcosm experiments with Asian dust deposition was carried out on board in the station K3 (26.18°N, 136.73°E) in April 2015, aiming to estimate the impact of dust deposition on the oligotrophic Northwestern pacific Sea, the main goal of the present paper was to assess how dust deposition events affect the abundance and activity of heterotrophic bacteria in low nutrient and low chlorophyll (LNLC) sea area. Station K3 located in the central northwestern Pacific Ocean, which has the characteristic of low nutrient and low chlorophyll. The study shows that there was an N–P co-limitation in station K3, and the deposition of Asian dust can increase the abundance, and promote the activity of heterotrophic bacteria in the station K3.

1. Introduction
Heterotrophic bacteria play a central role in the microbial loop, and increasing evidence indicates that not only phytoplankton but also heterotrophic bacteria are limited by inorganic nutrients, mainly P and N, in oligotrophic oceanic systems [1, 2]. There are many factors regulating bacterial distribution and growth, such as grazing by micro zooplankton, viral infection, and the supply of these resources like P, N, and effects of temperature.

Dust deposition is recognized as a significant source of nutrients to the surface ocean [3], with a great impact on autotrophic production by changing the phytoplankton community structure, inducing algal blooms and enhancing primary production [4]. And with recent study, dust deposition also impact heterotrophic bacterial dynamics in the surface ocean. Considering the central role of bacteria in the microbial loop, it is likely that dust deposition also affects the structure and the functioning of the whole microbial food web [5].

The environmental condition of the Northwestern Pacific Ocean is changeable and complex, which significantly influences the abundance and activity of bacteria. And the central region of Northwestern Pacific Ocean is a typical LNLC region, particularly well adapted to study the question of the impact of Asian dust deposition in microbial food web[5, 6], especially the bacterial dynamic. To better understand
the response of bacteria in to the change of environmental conditions, it is very important to explore the responses of heterotrophic bacteria abundance and activity in this area.

In this study, onboard experiments were performed in LNLC region in Northwestern Pacific in April 2015 to explore the responses of heterotrophic bacteria abundance and dynamics of added Asian dust.

2. Materials and methods

2.1. Microcosm set-up.
The microcosm experiments were carried out in the station K3 (26.18°N, 136.73°E) of the Northwestern Pacific onboard the R/V Dongfanghong II during April 2015 (Table 1). Four 2L aseptic incubation bottles were used in this microcosm, filled with the 0.8-μm pre-screened surface seawater to exclude zooplankton and phytoplankton, with Controls (no addition), Dust, P, N+P (Table 2). The incubation was conducted for 7 days at station K3.

| Incubation Bottle No. | Treatment      | Added Dust and Nutrients Concentration |
|-----------------------|----------------|----------------------------------------|
| C                     | Control        | *****                                 |
| D                     | Dust           | 2 mg/L                                 |
| P                     | NaH2PO4        | 0.2 uM/L                               |
| N+P                   | NaNO3 +NaH2PO4 | 2 uM/L+0.2 Um/l                        |

2.2. Heterotrophic Bacteria Abundance.
Seawater (10mL) was fixed with buffered paraformaldehyde (0.5% final concentration) and stored at -80°C until flow cytometric analysis. Heterotrophic bacteria abundance was measured by a Becton-Dickinson FACSCalibur cytometer.

2.3. Heterotrophic bacteria activity.
Flow cytometry enabled the discrimination of two Bacterial cell groups: bacterial cells with high nucleic acid (HNA) and low nucleic acid (LNA) contents. The proportion of high nucleic acid bacteria (HNA %) can indicates the activity of Heterotrophic bacteria.

2.4. Statistical analysis.
All samples had three replicates at least, with results presented as mean SD (standard deviation). Statistical analysis was performed using one-way analysis of variance (ANOVA) with the Fisher LSD post hoc test. Differences were considered statistically significant when p<0.05.

3. Result and Discussion
As a typical station in oligotrophic sea area, the seawater at K3 stations was oligotrophic as indicated by low macronutrient and Chl a concentrations. From table 1, the temperature of station K3 was relatively high, about 19.75°C. And the salinity of the seawater was 34.86, while the concentration of DOC was 1.58mg/L: as well nitrate concentration was below the limit of detection of the used technique. In addition, phosphate concentration averaged 0.007mg/L, which showed that the station K3 was in a typical oligotrophic region. The shortage of dissolved inorganic phosphate (DIP) and dissolved inorganic nitrogen (DIN) suggested that the growth of heterotrophic bacteria in this area might be limited by phosphorus and nitrogen.
Figure 1. Variation of the heterotrophic bacteria abundance among different treatments

Heterotrophic bacteria abundance increased obviously during the incubation period in Dust deposition enrichment culture experiment at Station K3. On the fifth day of incubation, the abundance of heterotrophic bacteria increased to $7.06 \times 10^5$ cell/mL, the maximum over the incubation, which was 1.34 times higher than the abundance of the Controls. Then the bacteria abundance of Dust group began to decline, and decreased to $5.60 \times 10^5$ cell/mL, which was 1.20 times higher than abundance of the control group.

There was significant difference in bacteria abundance between the Dust group and the Control ($P<0.05$). It demonstrated that the dust deposition can impact heterotrophic bacteria abundance. We can see from the graph that the dust deposition played an important role in promoting growth of heterotrophic bacteria in the early stage of the incubation. The growth of bacteria abundance in the Dust deposition-microcosm was similar to it in the P-microcosm and NP-microcosm, suggesting that both the dissolved inorganic nitrogen and phosphorus from the dust can promote the increase of heterotrophic bacteria abundance. But compared to the NP-microcosm, the maximum of bacteria abundance in Dust deposition-microcosm appeared one day later, simultaneously with the maximum of P-microcosm. It may prove that the dissolved inorganic phosphorus from the dust facilitate the growth of heterotrophic bacteria abundance more, and the growth of heterotrophic bacteria abundance in this area is limited by the shortage of dissolved inorganic nitrogen and phosphorus.
Figure 2. Variation of the HNA% of heterotrophic bacteria among different treatments

The HNA% of heterotrophic bacteria varied during incubation time in dust addition experiment at Station K3 (Fig.2). Compared to the controls, the HNA% of bacteria showed slightly increase in Dust deposition-microcosm at the first 2 days, and then started to decrease. At the 4th day, the HNA% of heterotrophic bacteria in Dust deposition-microcosm decreased to 52.53 % (0.84-fold), this is the minimum value during the incubation. And at the 6th day, the HNA% increased to the maximum 86.24 % (1.53-fold). When the area is in the condition of N–P co-limitation, the Dust has a visible impact on the HNA% of heterotrophic bacteria.

4. Summary
Our study shows that dust deposition to the Northwestern pacific surface oligotrophic region can promote the growth of abundance and activity of heterotrophic bacteria. And for the bacteria in Station K3, there growth was N–P co-limited. During the incubation, the addition of dust promoted the abundance and activity of bacteria by increase the concentration of inorganic nutrients, especially for P.

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