Changes in sediment properties and bacterial community in marine sediments after entering the coastal land in Bohai Bay, northern China

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ABSTRACT

In coastal areas of Bohai Bay, China, marine sediments were used for land reclamation. However, vegetation cannot grow well on the reclaimed land because marine sediments have different ecological functions than soil. Changing marine sediment into soil rapidly and accelerating the soil-forming process is key to solving this ecological problem on reclaimed land. Therefore, in this study, we not only measured the chemical properties of marine sediments during an 8 yr soil formation period to explore fertility changes, but we also used MiSeq sequencing to analyze and compare the bacterial community structure and diversity before and after the 8 yr land reclamation. Our results showed: (1) Marine sediment changed from severe to mild salinization during the 8 yr of soil formation, and the sediment salinity decreased significantly to 7.3 g kg⁻¹ (p < 0.05). However, pH of the sediment was always > 8.5 and did not change significantly. In addition, the total fertility of the sediment decreased significantly after 8 yr. Especially, the content of nitrate-N in marine sediment decreased significantly by 86.23% (p < 0.05) after 8 yr of soil formation. Also, the content of available P and soil organic matter decreased significantly by 45.92% and 26.22%, respectively. (2) The total abundance of bacteria increased while the community diversity decreased after the sediments were removed from the ocean environment for 8 yr. The bacterial community composition changed, and results of our redundancy analysis (RDA) indicated that the change was mainly affected by pH, soil contents including, available P, nitrate-N, ammonia-N, Na⁺, and Cl⁻, as well as salinity.

Key words: MiSeq sequencing, marine sediments, sediment bacteria, soil fertility, soil property.

INTRODUCTION

Bohai bay is located in northern China and Tianjin port is one of its representative areas. Marine sediments were extracted from Tianjin port and used for land reclamation project. In Tianjin, the land reclamation is an important way to utilize marine resources, and is also an effective way to expand port, industrial space and urban construction. The area of reclaimed land in Tianjin has increased to 2.79×10⁸ m² until 2018. However, vegetation cannot grow well on the reclaimed land due to high salinity and pH of the sediment. The added marine sediments were not soil, and therefore, do not have the same ecological function as soil (e.g. salinity > 1%, pH > 8.0) (Tuteja, 2007; Waskiewicz et al., 2013). Generally, after adding extracted marine sediments to coastal land, sediments change to soil naturally over a long timescale (decades to millennia) in response to soil-forming factors (Wills et al., 2016).
Some researchers believe that artificial intervention can potentially shorten the process of soil formation and they have, therefore, made efforts to improve the quality of marine sediments. For example, in terms of physical and chemical properties, some scholars filled sediment with gypsum, bran, waste or sand to improve the marine sediment (Guo et al., 2005; Wang et al., 2011; Jin, 2013; Tian, 2014). Additionally, from a bioremediation perspective, some scholars proposed planting halophytes to improve sediment quality (Zou et al., 2010). However, these measures have only been effective for a short period of time with no long-lasting effects, and the cost of manpower and resources is uneconomical.

There are five main factors of soil formation including, soil parent material, climate, biota (organisms), topography, and time (Wills et al., 2016). Among them, the biota is the most significant factor which can be intervened by human beings. Researchers found that seabed sediment is among the most important habitats for marine microorganism survival, but presents extreme living conditions including, high salinity, high alkalinity, high pressure (deep sea), low temperature (deep sea and polar region), and high temperature (deep-sea hydrothermal vent) (Weiner et al., 1999). Throughout their evolutionary history, marine microorganisms acquired physiological and metabolic mechanisms as well as special morphological characteristics to adapt to these extreme environments (Logan and Regan, 2006; Sogin et al., 2006). Recent research showed that marine microorganisms not only play a significant role in the marine environment through biogeochemical cycles (Moran, 2015), but also have various functional uses including, the use of their enzymes in the microbial fuel cell (Logan and Regan, 2006; Du et al., 2007; Abbas et al., 2017), degrading oil contaminants (Mohanty and Mukherji, 2008; Okeke, 2008; Wang et al., 2012), cleaning heavy metal pollution (Mincer et al., 2002; Gupta et al., 2012; Chen et al., 2017), and providing useful bioactive compounds (Romano et al., 2017). However, there is a gap in knowledge regarding the use of marine microorganisms to improve the quality of marine sediment and thus accelerate the soil formation process.

Therefore, in this study, we evaluated 15 chemical properties of marine sediments to evaluate the change in soil properties and fertility during a period of soil formation. Additionally, by using next generation sequencing methods, we compared the composition and diversity of the sediment bacterial community before and after the 8 yr land reclamation. Finally, the relationship between chemical properties and bacterial community of the marine sediment was also analyzed and discussed. Through the above three aspects, the objective of this study was to identify several key soil properties or sediment bacteria genus, which can accelerate the transformation of sediments to soil, and improve sediments in the reclamation area for further plant growth and even agricultural development.

**MATERIALS AND METHODS**

**Study site and soil-sediment sample collection**

Soil-sediment samples were collected from the Tianjin Port area (38°59’ N, 117°42’ E), Bohai Bay, China. As shown in Figure 1, there were six sampling sites including, S0, S1, S4, S6, S7, and S8, which correspond to areas where marine sediment was added to the coastal land for 0, 1, 4, 6, 7, and 8 yr, respectively. Other sampling sites including, S2, S3, and S5, were inaccessible due to construction, and therefore, soil-sediment samples from these sites could not be collected. At each soil sampling site, topsoil (0-30 cm) samples were collected with three replicates. Each sample was packed into plastic bags and taken to the laboratory. Each sample was divided into two groups: a fresh sample was preserved at -20 °C for further analysis of soil bacteria, and the other sample was air dried after gravel removal and sieved through two different sieves (0.025 and 0.015 mm) to determine soil chemical properties. Only S0 and S8 soil-sediment samples were used to analyze the difference of sediments bacteria community before and after 8 yr soil formation.

In this study, we quoted soil chemical properties of eight cropland sampling points in the New Coastal Region of Tianjin (Ma, 2011), which are located near the sediment sampling points used in the present study (Figure 1). Additionally, in the present study, every parameter was determined using the same methodology described by Ma (2011). This cropland soil has a near neutral pH and exhibits mild or moderate soil salinization. The mean value of each chemical property in the cropland sampling sites was used to validate the accuracy of the present data.

**Analysis of the chemical properties of the sediments**

Methods used to determine chemical properties of the sediments are summarized in Table 1. Additionally, 15 chemical properties were evaluated using principal component analysis (PCA) (Jolliffe, 1986; Wold et al., 1987; Liu et al., 2010a;
Xie et al., 2015). The comprehensive score was used to evaluate the sediment fertility of each sample (Li et al., 1992; Qin et al., 2015). As each variable represents a single index of sediment fertility, the comprehensive score can represent the overall level of marine sediment fertility.

**Table 1. Methods used to measure sediment chemical properties.**

| Chemical properties | Measured methods                                                                 |
|---------------------|----------------------------------------------------------------------------------|
| pH                  | Potentiometry (Sui and Li, 2004)                                                 |
| AK                  | Ammonium acetate extraction and the flame photometric method (Sui and Li, 2004) |
| AP                  | Molybdenum blue colorimetric method (Sui and Li, 2004)                           |
| SOM                 | Potassium dichromate method (Sui and Li, 2004)                                   |
| EC                  | FE3-type Mettler-Toledo conductivity meter                                        |
| K⁺                  | Flame photometry (Bao, 2000)                                                     |
| Na⁺                 | Flame photometry (Bao, 2000)                                                     |
| Ca²⁺                | Atomic absorption spectrophotometry (422.7 nm) (Bao, 2000)                       |
| Mg²⁺                | Atomic absorption spectrophotometry (285.2 nm) (Bao, 2000)                       |
| Cl⁻                 | Silver nitrate titration (Bao, 2000)                                             |
| SO₄²⁻                | EDTA Indirect complex metric titration (Bao, 2000)                                |
| Salinity            | Sum of the eight major ions (Bao, 2000)                                          |
| Nitrate-N           | Continuous flow analyzer (dissolution in potassium chloride)                     |
| Ammonia-N           | Continuous flow analyzer (dissolution in potassium chloride)                     |
| HCO₃⁻                | Double indicator-Neutralization titration (Bao, 2000)                           |

AK: Available K; AP: available P; SOM: soil organic matter; EC: electrical conductivity.
Comprehensive fertility evaluation based on PCA

PCA was used to analyze 15 chemical properties that represent the fertility level of sediment. Three principal components were extracted and the cumulative contribution rate was 91.834% (Table 2). Because the indicators in this experiment had different dimensions and orders of magnitude, we applied data standardization processing using SPSS software (IBM, Armonk, New York, USA) to eliminate these influences on the evaluation results and thus to ensure objectivity and accuracy of the evaluation. The standardized variables for each sample were denoted as Z1 to Z15.

The principal component is a linear combination of all standardized indexes, and the weight is the component score coefficients of each index (Table 2). Therefore, the linear combination of the three principal components and the 15 original indexes can be obtained as follows:

\[
F_1 = -0.022 \times Z1 + 0.107 \times Z2 - 0.085 \times Z3 + 0.101 \times Z4 + 0.086 \times Z5 + 0.091 \times Z6 - 0.008 \times Z7 - 0.106 \times Z8 - 0.014 \times Z9 + 0.101 \times Z10 + 0.12 \times Z11 + 0.14 \times Z12 - 0.065 \times Z13 + 0.15 \times Z14 - 0.095 \times Z15
\]

\[
F_2 = 0.160 \times Z1 + 0.045 \times Z2 - 0.065 \times Z3 + 0.112 \times Z4 - 0.157 \times Z5 + 0.127 \times Z6 + 0.186 \times Z7 - 0.122 \times Z8 + 0.046 \times Z9 - 0.111 \times Z10 - 0.103 \times Z11 - 0.034 \times Z12 + 0.155 \times Z13 + 0.028 \times Z14 + 0.101 \times Z15
\]

\[
F_3 = 0.229 \times Z1 + 0.292 \times Z2 - 0.335 \times Z3 - 0.216 \times Z4 + 0.012 \times Z5 + 0.137 \times Z6 - 0.016 \times Z7 + 0.121 \times Z8 + 0.121 \times Z9 - 0.217 \times Z10 + 0.158 \times Z11 - 0.079 \times Z12 + 0.008 \times Z13 - 0.087 \times Z14 - 0.114 \times Z15
\]

By substituting the standardized data into the above formula, the scores of six samples for the three principal components could be obtained. In this study, the comprehensive fertility of six samples was represented by the comprehensive scores of PCA. The comprehensive score formula was \( F = 0.43 \times F_1 + 0.34 \times F_2 + 0.15 \times F_3 \), and the coefficients are the principal component contribution rates. The principal component scores and the comprehensive scores of each sample are shown in Table 3.

### Table 2. Principal component analysis of the chemical properties of sediment.

| Properties | \( f_1 \) | \( f_2 \) | \( f_3 \) | Eigenvalues of the correlation matrix |
|------------|--------|--------|--------|--------------------------------------|
| \( Z_1 \) HCO\(_3\) | 0.163  | 0.022  | 0.034  | 6.420  | 42.801  | 42.801  |
| \( Z_2 \) Nitrate-N | 0.156  | 0.089  | 0.050  | 5.102  | 34.015  | 76.817  |
| \( Z_3 \) SO\(_4\)\(^2\) | 0.144  | 0.044  | -0.099 | 2.253  | 15.018  | 91.834  |
| \( Z_4 \) AP | 0.131  | -0.068 | 0.238  |          |          |          |
| \( Z_5 \) K\(^+\) | 0.112  | -0.142 | 0.058  |          |          |          |
| \( Z_6 \) Salinity | 0.044  | 0.241  | -0.097 |          |          |          |
| \( Z_7 \) SOM | 0.044  | 0.046  | 0.198  |          |          |          |
| \( Z_8 \) Na\(^+\) | 0.043  | 0.241  | -0.097 |          |          |          |
| \( Z_9 \) pH | 0.014  | 0.095  | -0.338 |          |          |          |
| \( Z_{10} \) AK | -0.041 | -0.066 | 0.304  |          |          |          |
| \( Z_{11} \) Ammonia-N | -0.044 | -0.197 | 0.012  |          |          |          |
| \( Z_{12} \) Cl\(^-\) | -0.060 | 0.166  | 0.001  |          |          |          |
| \( Z_{13} \) Mg\(^{2+}\) | -0.104 | 0.125  | -0.018 |          |          |          |
| \( Z_{14} \) Ca\(^{2+}\) | -0.161 | -0.052 | 0.146  |          |          |          |
| \( Z_{15} \) EC | -0.178 | -0.019 | -0.009 |          |          |          |

AP: Available P; SOM: soil organic matter; AK: available K; EC: electrical conductivity.

### Table 3. Principal component scores and comprehensive scores of samples.

| Samples | \( F_1 \) | \( F_2 \) | \( F_3 \) | Comprehensive scores |
|---------|--------|--------|--------|----------------------|
| S0      | -0.12713 | 1.59001 | 0.66130 | 0.585133 |
| S1      | -0.24588 | 0.79137 | -0.19104 | 0.134681 |
| S4      | 1.78421  | -0.38973 | 0.20662 | 0.664795 |
| S6      | -0.11665 | -0.23146 | -1.74199 | -0.289840 |
| S7      | -0.23516 | -1.09340 | 1.19805 | -0.293170 |
| S8      | -1.29270 | -0.66679 | -0.12694 | -0.801610 |
Analysis of the marine sediment bacteria
This study used MiSeq (Illumina, San Diego, California, USA) sequencing to identify bacteria in the marine sediment and further analyzed the diversity of bacteria community. In this study, Shannon-Weiner index (H), Pielou evenness index (J), Margalef richness index (d) and Simpson index (D) were also calculated to determine bacteria diversity. Redundancy analysis (RDA) was further used to evaluate the influence of the 15 chemical properties on bacteria in marine sediment during the 8 yr of soil formation.

Statistical analysis
The software package SPSS 24.0 was used for statistical analyses, and SigmaPlot 12.5 (Systat Software, San Jose, California, USA) and R (R Foundation for Statistical Computing, Vienna, Austria) were used to generate figures. The rank-abundance curve, rarefaction curve, and RDA analysis were also completed using R statistical software.

RESULTS

Change in chemical properties during the 8 yr soil formation period
As shown in Figure 2a and Table 4, the sediment maintained high salinity during the soil formation process. As soil formation progressed, both the electrical conductivity (EC) and salinity of the marine sediment initially increased and then decreased (Table 4, Figure 2a and 2b). Specifically, salinity increased slightly by 15.00% from S0 to S4 and then declined significantly by 69.00% from S4 to S8. EC increased by 61.39% from S0 to S4 and then decreased by 36.20%.

Figure 2. Salinity (a), electrical conductivity (EC) (b) and pH (c) changes of sediments during the soil-forming process.

The R-value of each curve is shown in the top left corner of each figure. Vertical bars indicate ± SE.

| Soil properties | S0 | S1 | S4 | S6 | S7 | S8 | Cropland |
|-----------------|----|----|----|----|----|----|---------|
| AP, mg kg⁻¹     | 13.00 | 11.07 | 7.27 | 6.27 | 8.90 | 7.03 | 17.39 |
| AK, g kg⁻¹      | 1.19 | 1.15 | 1.39 | 0.77 | 1.17 | 0.79 | 469.55 |
| pH              | 8.71 | 8.80 | 8.70 | 8.92 | 8.77 | 8.86 | 7.45 |
| EC, ms cm⁻¹     | 7.68 | 7.02 | 19.89 | 14.75 | 16.93 | 12.69 |
| SOM, g kg⁻¹     | 14.30 | 11.45 | 13.49 | 11.08 | 10.90 | 10.55 | 23.60 |
| Nitrate-N, mg kg⁻¹ | 8.28 | 4.31 | 1.50 | 3.73 | 1.00 | 1.14 | 0.29 |
| Ammonia-N, mg kg⁻¹ | 0.19 | 0.21 | 0.15 | 0.24 | 1.08 | 1.53 | 0.00 |
| Ca²⁺, g kg⁻¹    | 0.08 | 0.08 | 0.12 | 0.08 | 0.10 | 0.08 | 0.16 |
| HCO₃⁻, g kg⁻¹   | 0.51 | 0.49 | 0.44 | 0.44 | 0.42 | 0.49 | 0.03 |
| Cl⁻, g kg⁻¹     | 0.18 | 0.17 | 0.28 | 0.21 | 0.14 | 0.08 | 0.05 |
| SO₄²⁻, g kg⁻¹   | 0.23 | 0.21 | 0.17 | 0.20 | 0.16 | 0.25 | 0.11 |
| Salinity, g kg⁻¹ | 20.64 | 22.07 | 23.56 | 23.72 | 7.39 | 7.30 |
| K⁺, g kg⁻¹      | 0.03 | 0.03 | 0.02 | 0.02 | 0.03 | 0.03 | 1.60 |
| Na⁺, g kg⁻¹     | 19.50 | 21.00 | 22.50 | 22.67 | 6.53 | 6.35 | 11.36 |
| Mg²⁺, g kg⁻¹    | 0.04 | 0.04 | 0.05 | 0.04 | 0.04 | 0.04 | 0.04 |

AP: Available P; AK: available K; EC: electrical conductivity; SOM: soil organic matter.
from S4 to S8. These results indicated that marine sediments were in a severe salinization state for the first 4 yr following introduction into the coastal land. The significant decline in salinity of the marine sediments from S4 to S8 indicated that the salinization degree of sediment had a negative trend in these years. In addition, the marine sediment was strongly alkaline during soil formation. As shown in Figure 2c and Table 4, pH values were 8.7-8.9 throughout the experiment and showed nonsignificant differences, indicating that alkalinity continued to inhibit soil formation throughout the 8 yr.

Figure 3 illustrates the change of different ions in the analyzed sediment during the 8 yr soil formation period. According to Table 4 and Figure 3, K⁺, HCO₃⁻, and SO₄²⁻ contents in sediments were nonsignificantly different in different years \((p > 0.05)\), while Na⁺, Cl⁻, Ca²⁺ and Mg²⁺ contents changed significantly \((p < 0.05)\). Compared with S0, Na⁺, Cl⁻, Mg²⁺, and Ca²⁺ sediment contents in S4 increased by 3, 0.1, 0.014, and 0.043 g kg⁻¹, respectively. From S4 to S8, Na⁺
content declined significantly by 17 g kg⁻¹. Compared with S0, Na⁺, Cl⁻, Mg²⁺, and Ca²⁺ sediment contents in S8 declined by 14, 0.2, 0.014, and 0.04 g kg⁻¹, respectively. In summary, Na⁺ and Cl⁻ contents showed a declining trend during the 8 yr soil formation period, whereas Mg²⁺ and Ca²⁺ contents remained stable.

Compared with the chemical properties of the cropland (Ma, 2011), the sediment salinity in S8 decreased to 7.3 g kg⁻¹, which was less than that of the cropland (13.36 g kg⁻¹) in Tianjin New Coastal Region. Alternatively, the pH did nonsignificantly change during the soil formation process. Compared with the nearly neutral pH of the cropland (7.45), the pH value of S8 sediment was 8.86, which was strongly alkaline. The K⁺ content of the sediment did not change significantly in the soil-forming process and the mean value was approximately 0.03 g kg⁻¹, far below that of cropland soil (1.604 g kg⁻¹). The Na⁺ content showed a decreasing trend and the minimum value was 7 g kg⁻¹, less than that of cropland soil (11.364 g kg⁻¹). The Cl⁻ content of the sediment also showed a decreasing trend and the minimum value was 0.078 g kg⁻¹, slightly higher than that of the cropland (0.053 g kg⁻¹); however, this difference narrowed with time. The average Mg²⁺ content of the sediment was 0.04 g kg⁻¹ throughout the 8 yr, similar to that of the cropland (0.037 g kg⁻¹). The Ca²⁺ content of the sediment was stable with a mean value of 0.08 g kg⁻¹, lower than that of the cropland soil (0.16 g kg⁻¹). The HCO₃⁻ content of the sediment did not change significantly throughout the soil formation process and the mean value was 0.44 g kg⁻¹, higher than that of the cropland soil (0.029 g kg⁻¹). The SO₄²⁻ content of the sediment did not change significantly throughout the soil formation process and the mean value was 0.2 g kg⁻¹, lower than that of the cropland (0.108 g kg⁻¹). In summary, the Ca²⁺, HCO₃⁻, and the SO₄²⁻ contents were not improved under natural conditions and had a negative impact on soil formation, indicating that the soil urgently requires artificial improvement. The natural changes in salinity, Na⁺ and Mg²⁺ contents positively influenced soil formation. In addition, the results indicated that measures should be taken to reduce the pH of the marine sediment.

**Change in effective soil nutrients during the 8 yr soil formation period**

As shown in Figure 4 and Table 4, the soil organic matter (SOM), available P (AP), available K (AK), and nitrate-N contents of the marine sediment reduced linearly with increased time, while the content of ammonia-N initially showed a stable trend and then increased significantly. Compared with S0, the SOM, AP, AK, and nitrate-N contents of the marine sediment at S8 reduced by 26.22%, 45.92%, 33.61%, and 86.23%, respectively. The ammonia-N content was maintained from S0 to S6 and then increased by 84.31%, from 0.24 (S6) to 1.53 mg kg⁻¹ (S8). As soil formation progressed, the nitrate-N content decreased while the ammonia-N content increased (Figure 4d and e), indicating that some nitrate-N might be converted into ammonia-N. The total inorganic N content showed a downward trend.

Compared with the cropland (Ma, 2011), the maximum SOM content of the sediment was 14.30 g kg⁻¹ (S0), which is less than that of the cropland soil (23.6 g kg⁻¹). The AK content of the sediment during the 8 yr soil formation period was 0.77 to 1.39 mg kg⁻¹, which was higher than that in the cropland (0.47 mg kg⁻¹). As for nitrate-N, the content decreased as soil formation progressed and reached a minimum in S8 (1.14 mg kg⁻¹), much lower than that of the cropland soil (290 mg kg⁻¹). Nitrate-N would be more suitable for artificial improvement due to this large difference in nitrate-N content between cropland soil and marine sediment. The maximum content of ammonia-N was 1.53 mg kg⁻¹, lower than that of the cropland soil (4.5 mg kg⁻¹), but it increased naturally with time. Overall, apart from the change in ammonia-N content, which was favorable to soil formation, the loss of other effective nutrients was severe during these 8 yr.

Figure 5 and Table 3 illustrates the comprehensive fertility of the sediment during the 8 yr soil formation period. The comprehensive fertility of the sediment showed a downward trend, indicating that the loss of soil fertility was severe. This result was consistent with the above results and further verified the serious fertility loss in the process of soil formation over the 8 yr under study.

**Change in bacterial community diversity after sediments entered the coastal land for 8 yr**

In this study, for the two samples (S0 and S8), we calculated the diversity of the bacteria at the class, order, family, and genera levels using diversity indexes (Shannon-Wiener, Simpson, evenness, and richness) and the results are summarized in Figure 6. S0 sediment had a higher Shannon-Wiener and Pielou evenness index at all four levels than S8, indicating that bacteria diversity declined after sediment entered the coastal land over 8 yr. Simpson index was higher in the S8 sample for order, family, and genera. As for Margalef richness index, S8 was higher than S0 at all four levels, indicating that species abundance in the S8 sample was higher than that of S0, which was consistent with the rank-abundance curve in
As shown in Figure 7, the slope of S0 was gentler than that of S8; therefore, the evenness of S0 was higher than that of S8. The richness index showed that the total number of bacteria significantly rose after the sediment was extracted from sea to land during the 8 yr period (Figure 8), which was consistent with Figure 6D. Moreover, the species of bacteria increased significantly in S8 (Figure 9). Eight years later, 486 bacterial species disappeared, 1595 foreign bacteria species entered this system and 811 bacteria species survived; moreover, the number of new bacteria species was greater than the number of species which disappeared (Figure 9). In summary, after 8 yr of soil formation, the overall abundance of bacteria communities increased significantly, while the community diversity decreased at the class, order, family, and genera levels.

Figure 4. Effective nutrients of marine sediments during soil-formation process and of cropland soil in the New Coastal Region of Tianjin.

R-value of every curve is shown in the top left corner of figure. Vertical bars indicate mean ± SE. SOM: Soil organic matter; AK: available K; AP: available P.
Figure 5. Changes in sediment fertility during soil formation.

Figure 6. Differences in bacteria diversity indexes at different taxonomical hierarchies after marine sediment entered the terrestrial environment for zero years (S0) and 8 yr (S8).

A: Shannon Wiener index; B: Simpson index; C: Evenness index; D: Richness index.
Figure 7. Rank-abundance curve depicting bacteria community differences in the marine sediments after marine sediment entered the terrestrial environment for zero years (S0) and 8 yr (S8).

![Rank-abundance curve](image1.png)

OTU: Operational taxonomic units.

Figure 8. Rarefaction curve representing differences in the bacteria community after marine sediment entered the terrestrial environment for zero years (S0) and 8 yr (S8).

![Rarefaction curve](image2.png)

OTU: Operational taxonomic units.

Figure 9. Venn diagram representing OTU distribution after marine sediment entered the terrestrial environment for zero years (S0) and 8 yr (S8).

![Venn diagram](image3.png)

OTU: Operational taxonomic units.
Change of dominant genera after sediments entered the coastal land for 8 yr

There were 342 bacteria genera detected by MiSeq sequencing. There were 12 major bacterial genera in S0 and their relative abundance were as follows (Figure 10): *Thiomicrospira* (33.68%), *Pseudomonas* (5.95%), *Lactococcus* (5.86%), *Sulfurimonas* (5.54%), *Psychromonas* (3.62%), *Sva1033_norank* (3.53%), *JTB255_marine_benthic_group_norank* (2.8%), *Fusibacter* (2.62%), *Desulfobulbus* (1.96%), *Colwellia* (1.9%), *Lutibacter* (1.76%) and *Marinobacter* (1.33%). S8 had 8 dominant genera: *Thiomicrospira* (32.15%), *Planomicrobium* (15.96%), *Psychrobacter* (10.61%), *Sulfurimonas* (9.15%), *Gillisia* (3.04%), *Marinobacter* (1.97%), *Halomonas* (1.39%), and *Sulfurimonas* (1.11%). The major bacterial phyla identified among the two samples included, Proteobacteria (S0: 73.70%; S8: 55.72%), Firmicutes (S0: 10.63%; S8: 18.51%), Bacteroidetes (S0: 4.35%; S8: 14.95%), Chloroflexi (S0: 3.50%; S8: 2.05%), and Actinobacteria (S0: 2.32%; S8: 5.21%).

*Thiomicrospira*, *Marinobacter*, and *Sulfurimonas* were the most common genera in the two samples. Compared with S0, the relative abundance of *Thiomicrospira* and *Marinobacter* in S8 did not changed significantly, while *Sulfurimonas* decreased by 4.43%. In addition, the abundance of genera differed before and after the marine sediment entered the coastal land (Figure 10). After 8 yr, the abundances of many genera declined in the marine sediment: *Sulfurimonas*, *Lutibacter*, *Sva1033_norank*, *Psychromonas*, *Pseudomonas*, *JTB255_marine_benthic_group_norank*, *Desulfobulbus*, *Lactococcus*, *Fusibacter* and *Colwellia*, which decreased by 0.61-fold, 0.65-fold, 0.73-fold, 0.70-fold, 0.84-fold, 0.89-fold, 0.95-fold, respectively. The abundances of other genera rose significantly: *Psychrobacter*, *Planomicrobium*, *Gillisia*, *Salegentibacter*, and *Halomonas*, which increased by 42.90-fold, 46.17-fold, 42.90-fold, 125.18-fold and 315.5-fold, respectively.

In addition to the above-mentioned bacteria genera, some new bacteria genera entered the sediments and survived during the 8 yr soil formation process. Moreover, according to the taxonomic data of this study, 117 genera of bacteria were alien species, including: *Algoriphagus*, *Blastopirellula*, *Haliangium*, *Ilumatobacter*, *Methylophaga*, *Microbulbifer*, *Muricauda*, *Phycisphaera*, *S0134_terrestrial_group_norank*, *Arenibacter*, *Jeotgalibacillus*, and *Lewinella*. Meanwhile, 46 native bacteria genera disappeared, including: *Bacteroides*, *Carnobacterium*, *Desulfocapsa*, KI89A_clade_norank, *Lactobacillus*, *Nitrospina*, SS1-B-09-64_norank, and pltb-vmat-80_norank.

Relationship between sediment properties and bacterial communities

In this study, RDA was used to analyze the relationship between the change in chemical properties and the change in bacterial communities in marine sediments. The eigenvalues of the first and second ordination axes are 163.332 and 1.453, respectively, which explained 99% and 0.8% of bacterial community variations, respectively. The cumulative proportion was 99.8%, meaning that the first two axes can reflect the correlation between bacterial community and physical and chemical soil properties, but it was primarily determined by the first ordination axis. A two-dimensional ordination figure can then be obtained (Figure 11).

**Figure 10.** Differences among the dominant bacteria genera in sediments after marine sediment entered the terrestrial environment for zero years (S0) and 8 yr (S8).
As shown in Figure 11, the relative abundances of *Thionicrospira*, *Sulfurimonas*, *Lutibacter*, *Sva1033_norank*, *Psychromonas*, *Pseudomonas*, *JTB255_marine_benthic_group_norank*, *Desulfobulbus*, *Lactococcus*, *Fusibacter*, and *Colwellia* showed a positive correlation with soil contents (AP, nitrate-N, Na⁺, Cl⁻ and salinity), but showed a negative correlation with pH and ammonia-N contents. The relative abundances of *Psychrobacter*, *Planomicrobium*, *Gillisia*, *Salegentibacter*, *Marinobacter*, and *Halomonas* had a positive correlation with pH and the ammonia-N content, but showed a negative correlation with AP, nitrate-N, Na⁺, Cl⁻, and salinity contents. According to Figures 2 and 3, compared with S0, AP, nitrate-N, Na⁺, Cl⁻, and salinity contents in marine sediments decreased, while pH and ammonia-N content increased after 8 yr (S8). The change in bacteria genera abundances corresponded to this trend seen in Figure 11.

These soil properties explained 99.8% of total eigenvalues, indicating that soil properties had a significant effect on the structure of the bacterial community in the marine sediment. According to Table 5, AP, nitrate-N, salinity, Na⁺, and Cl⁻ are the main environmental factors influencing the quantity and community structure of sediment bacteria.
DISCUSSION

Effects of bacterial community on marine sediment properties during the 8 yr soil formation process

In this study, as soil formation progressed, the overall abundance of bacterial communities increased significantly at the class, order, family, and genera levels. For example, compared with S0, the abundance of Planomicrobium and Halomonas at S8 increased significantly. Both of these genera are comprised of denitrifying bacteria (DNB) species. DNB can produce a series of products using nitrate as the final electron acceptor including, NO$_2^-$, NO, N$_2$O, and N$_2$ (Zumft, 1997). Therefore, the increase of DNB may have contributed to the decrease of nitrate-N during the 8 yr soil formation period. For crops and other plants, nitrate-N is the main source of N, and the absorption rate of nitrate-N by plants is higher than that of ammonia-N. Therefore, for marine sediment in Tianjin Port, the loss of nitrate-N and the increase of ammonia-N likely contributed most to this area being void of plants. In addition, Sulfurimonas and Psychromonas are nitrate-reducing bacteria (NRB), which can utilize NO$_2^-$ and NO$_3^-$ as the final electron acceptor for respiration, and thus, reduce nitrate to nitrite (Tan et al., 2007). However, their abundances decreased over time. After the sediments were separated from the seawater and added to the land, there was almost no exogenous nitrate-N input. It is likely that NRB initially increased in number and consumed nitrate-N, resulting in a decrease in nitrate-N and in turn causing an associated decline in NRB.

Desulfobulbus and Fusibacter are sulfate-reducing bacteria (SRB). They can use sulfate or other oxidation state sulfide as electron receptors to produce organic substances and can be found in water and sediment (Guo et al., 2016). Zumft (1997) found that N$_2$O can increase the output of redox potential within a limited space, which in turn can limit the growth of SRB considering that these bacteria have a strict demand for redox potential. In addition, under the same conditions, the denitrification response ability of microbial organisms is stronger than their sulfate reduction reaction ability. Moreover, under the same C source conditions, the denitrification rate of microorganisms is more than twice the rate of sulfate reduction. Furthermore, compared with SRB, DNB occupy a dominant position in the competing matrix (Chidthaisong and Conrad, 2000). In summary, the growth of sulfate reducing bacteria is likely inhibited by denitrifying bacteria. Therefore, in the present study, the increase in Planomicrobium and Halomonas abundances may have caused the decline in Desulfobulbus and Fusibacter abundances, likely due to competitive inhibition. It can also explain why the SO$_4^{2-}$ content did nonsignificantly change during the 8 yr soil formation period.

Effects of marine sediment properties on the bacterial community during the 8 yr soil formation period

In this study, results showed that after 8 yr, bacterial community abundances increased significantly while bacterial community diversity declined. Additionally, the structure of bacterial community changed: the Proteobacteria abundances significantly decreased while Firmicutes, Bacteroidetes, and Actinobacteria abundances increased significantly after
sediment entered the coastal land for 8 yr. According to previous studies conducted on the Dalian Changshan Islands (Li et al., 2011), Beidaihe (Fan et al., 2008), Qingdao, Weihai (Xiao et al., 2009), Lianyungang (Zhu, 2009) and Cangnan bay (Huo et al., 2008), these bacteria phyla were the dominant microbes of offshore sediments along the Chinese coastline, which was consistent with the results of our study.

Jin (2013) suggested that high salinity is one of main factors restricting the improvement and utilization of marine sediment for land reclamation. Generally, in the marine environment, seawater salt is mainly composed of Na⁺ and Cl⁻. In this study, Na⁺ and Cl⁻ contents were the main ions of salinity and showed the most significant change over time (Figures 2 and 3), which was consistent with the marine characteristics of the sediment. Specifically, the salinity of the sediment gradually decreased as soil formation progressed and this decline was less than that of the nearby cropland. This result indicates that the sediment retained high salinity in the initial stages of soil formation. However, the decrease of salinity had a positive impact on soil formation, which was consistent with Liu et al. (2010b). In addition, the total abundance of bacteria increased with the decrease of salinity, while bacterial community diversity decreased significantly. These results are consistent with Jackson and Vallaire (2009). These researchers also found that salinity depressed the function of sediment bacteria and thus changed the bacteria community composition. Ikenaga et al. (2010) further suggested that bacterial communities have different compositions and structures along the salinity gradient.

pH can also affect bacterial community structure and diversity in marine sediments (Rousk et al., 2010). Xiong et al. (2012) further found that pH regulated the structure of bacteria community more than salinity. Moreover, pH is the best predictor of bacterial community structure in alkaline sediments. For example, sediment with a higher pH positively influenced the growth of Alpha Proteobacteria (Chu et al., 2010; Rousk et al., 2010).

As for the relationship between nutrients and the quantity of bacteria, it was reported that due to the long-term use of pesticide and fertilizer in agricultural activities and oil pipeline leakage, petroleum and pesticide pollution is prevalent in Tianjin coastal waters (Wu et al., 2007). *Pseudomonas* and *Colwellia* can be used as degradation bacteria to aid in the reduction of these pollutants. These bacteria are highly abundant in marine and submarine sediments and they are often the dominant bacteria. We hypothesized that *Pseudomonas* and *Colwellia* constantly consumed oil and pesticide pollution in Tianjin Port. Then, without nutrient input from the seawater and as soil formation progressed, these bacteria were living in a nutrient-poor environment (compared to marine sediments), which in turn may have contributed to their abundance decline and their becoming less dominant bacterial strains in the terrestrial environment.

**CONCLUSIONS**

The marine sediment changed from severe to mild salinization during 8 yr of soil formation. However, the pH value of the sediment was > 8.5 and did not change significantly during the study period. In addition, sediment fertility decreased significantly and the total fertility loss was severe, especially the loss of nitrate-N.

Through MiSeq sequencing technology, we analyzed two soil-sediment samples: fresh marine sediment and marine sediment which entered the coastal land over the course of eight years. The results indicated that the abundance of bacteria increased significantly, while bacterial community diversity declined after 8 yr. Moreover, the composition of the bacterial community changed significantly.

There was an interaction between soil properties and bacterial communities. The results of redundancy analysis indicated that the bacterial community of the sediments was mainly affected by the following chemical properties: pH, soil contents (available P, nitrate-N, ammonia-N, Na⁺, Cl⁻) and salinity.

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