Microbial fertilizer improving the soil nutrients and growth of reed in degraded wetland

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Abstract: Wetland degradation is frequently observed in some river estuaries of China due to the imbalance of soil nutrient. In order to improve the soil nutrient and promote the restoration of plant, the microbial fertilizer containing the phosphorus-dissolving strain Pseudomonas plecoglossicida and potassium-dissolving strain Bacillus aryabhattai was developed to stimulate the growth of plant and increase soil nutrient in this study. Results showed that microbial fertilizer was more effective in increasing the contents of total N, P and K in soil and the activities of soil sucrase and urease. Compared with the control, the height and dry weight of reeds were increased 53.13% and 59.31%; the activities of soil sucrase and urease were improved 41.25% and 39.57%. Illumina Miseq sequencing showed that Pseudomonas spp. were the most predominant in microbial fertilizer treated soil but other treatments were quite different. Hence, the microbial fertilizer significantly promoted the growth of reed, increased soil nutrient and enzyme activity. It consequently revealed a promising application in improving the wetland soil nutrients and ecological restoration.

1. Introduction
In recent years, the reed biomass in Liaohé River Estuary Wetland obviously decreases and the ecosystem structure and function significantly reduce. Consequently, ecological and environmental problems in this area draw an increasingly attention. It was reported that one of the trigger factors for the wetland deterioration was the soil nutrient imbalance and excessive nutrient loss[1]. At present, the most extensive methods to solve the deficiency of nitrogen, phosphorus and potassium in soil are to use N, P and K fertilizers. To meet the requirement of plants, the inorganic fertilizers should be fertilized largely for a long time. However, this will not only destroy the quality of plant products, but also lead to soil compaction, acidification and nitrate accumulation[2-4]. Organic fertilizers can increase soil organic nitrogen reserves, increase soil available inorganic phosphorus[5], improve crop stress resistance[6], but its nutrient concentration and efficiency are low[2]. In comparison, microbial fertilizer is a kind of green fertilizer based on functional microbial strains, not only improves soil nutrients, improves crop stress and disease resistance, promotes plant growth, but also shows no pollution to the environment[7,8]. If we integrate the strong points of above fertilizers into compound fertilizer, it will avoid the shortcomings of a single fertilizer. This provides a good solution for the habitat restoration of nutritional imbalance estuary wetland.

In this study, bacterial strains, Pseudomonas plecoglossicida and Bacillus aryabhattai, isolated from Liaohé River Estuary Wetland and revealed good ability of dissolving phosphorus and potassium, were used to prepare microbial fertilizer with a certain percentage of inorganic fertilizer and organic fertilizer. The pot experiment was conducted to explore the effect of microbial fertilizer
on the growth of plant and the improvement of soil nutrient.

2. Materials and methods

2.1. Microbial fertilizers
The dissolving phosphorus and potassium strains, Pseudomonas plecoglossicida and Bacillus aryabhattai were isolated from the soil of Liaohe River Estuary Wetland.

Four types of fertilizers were applied (as 0.2 g nitrogen per kilogram of soil): 1) inorganic fertilizer (D1) containing urea, superphosphate and potassium chloride with the ratio of 1: 3: 0.63. 2) organic fertilizer (C1): chicken manure fermentation, nutrient content: N 26 g/kg, P₂O₅ 62 g/kg, K₂O 28 g/kg. 3) microbial bio-compound fertilizer (A1): firstly, the culture of the above strains (OD600 = 1, the ratio was 1: 1) were mixed with zeolite power in a ratio of 6% (v/w) to prepare microbial agents; then, the agents were mixed with organic fertilizer in a ratio of 10% (w/w); Finally, a certain percentage of inorganic fertilizer was added. The final content: N 56.6 g/kg, P₂O₅ 59.7 g/kg, K₂O 47.9 g/kg, the effective viable count was 0.51 × 10⁸ CFU/g. 4) organic-inorganic compound fertilizer (B1): same components as the microbial bio-compound fertilizer except for without the bacteria.

2.2. Pot experiment of reeds
The nutrient of sandy soil used in this study was similar to Liaohe River Estuary Wetland soil. The planted reeds were collected from Liaohe River Estuary Wetland.

The pot experiments with one of four fertilizers were conducted in a greenhouse. Fertilizer was mixed with the soil in the pot and soil moisture was kept at 60%. At the 15th, 25th, 35th and 50th day after reed germination, soil samples were collected at 5 cm in depth for analysis of soil physical chemical indexes and soil microbe community.

2.3. Soil and plants analysis
Sucrase was examined using 3, 5-dinitrosalicylic acid colorimetric method, urease using sodium phenol-sodium hypochlorite colorimetric method[9]. The height and weight were determined after maturation (50d). The available nutrient content in the soil was determined on 50th day when the reed was harvested. Soil available N was treated by alkali solution diffusion method, available P was extracted with sodium bicarbonate-molybdenum antimony anti-coloring method, available K was extracted with ammonium acetate method[10].

2.4. Analysis of soil microbial community
The composition of microbial community in different treatments was investigated by high-throughput sequencing technique after the harvest of reeds. The V4 region of bacterial 16S rDNA was amplified by primer 515F (5'-GTGCCAGCAGCGGCCTAA-3') and 806R (5'-GGACTACCAGGTATCTAAT-3') using the total DNA in the treated soils as templates. We compared the commonality and difference between the samples by cluster analysis, principal component analysis (PCA), statistical comparative analysis of species composition and relative abundance of microbial community[11, 12].

3. Results and discussion

3.1. Effects of microbial fertilizer on soil enzyme activity
Sucrase is one of hydrolyases and its activity reflects the soil fertility level and ripening degree[13]. Effects of different fertilizers on soil sucrase activity during reed growth are shown in Figure 1a. The soil sucrase activity of each treated gradually increased with the growth of reed. Reeds needed more nutrients to meet their own growth in the rapid growth period. The sucrase activity significantly increased and further improved the release of effective nutrient. The order of sucrase activity of soils was: A1 > B1 > C1 > D1 > control (CK), and the difference between A1 and the other treatments was significant. The sucrase activity of A1 treatment was higher than CK by 41.25%.
Urease is an amidase that promotes the hydrolysis of peptide bonds in organic matter and is highly specific for urea catalysis. Its activity is positively correlated with soil available N, total N, organic matter content and microbial quantity, and is often used to characterize soil nitrogen and nitrogen supply\[15\]. The variation of soil urease activity (Figure 1b) was the same as that of sucrase, and the activity accorded well with reeds’ growth in the whole period. The soil urease activity of A1 was 39.57% higher than CK. The order of urease activity in different growth periods was A1 > B1 > D1 > C1 > control (CK), and the difference between A1 and the other treatments was significant.

Microbial bio-compound fertilizer contains a large number of active microorganisms, which secrete considerable enzymes during growth. At the same time, the organic matter in the fertilizer provides sufficient nutrients to promote the propagation of soil microbes. This greatly improves the number of bacteria, fungi and actinomycetes in soil and enhances the activity of soil enzymes\[2\]. Zhang et al\[14\], also obtained similar results where they found that microbial bio-compound fertilizer not only improved activities of sucrase and urease in soil but also stimulated the decomposition of soil organic matter.

**Figure 1.** Effects of different fertilizers on the activities of sucrase (a) and urease (b) in the soil during the growth period of reeds

A1, microbial bio-compound fertilizer; B1, organic-inorganic compound fertilizer; C1, organic fertilizer; D1, inorganic fertilizer; CK, control, no-fertilizer. a to d indicated significant difference (P < 0.05, n = 3).

### 3.2. Effects of microbial fertilizer on soil properties and growth of reeds

The effects of different fertilizers on available N, P and K in soil are shown in Table 1. It indicated that A1 treatment contained the highest available N, P and K, significantly different from other treatments. The effect of inorganic fertilizer was better than organic fertilizer. Short-term effect of inorganic fertilizer was rapid but long-term application of inorganic fertilizer, especially ammonium fertilizer, would increase soil acidification.

The growth of reeds is shown in Figure 2. Four fertilizers all presented positively effect on the growth of reeds. The average height of reeds, fresh weight and dry weight were 20.43%-53.13%, 30.88%-59.31% and 14.94%-54.02% higher than the control (CK). A1 treatment revealed the best results significantly different with other treatments.

The functional strains in microbial bio-compound fertilizer can not only dissolve insoluble phosphate, improve the effectiveness of phosphorus, decompose the soil minerals and transform the insoluble potassium into available potassium, but also produce plant hormones, organic acid and antagonistic substances in the process of life. These work together to stimulate plant growth and increase crop yields\[2\]. Shao et al\[15\], also found that the application of microbial bio-compound fertilizer and organic-inorganic compound fertilizer could promote the growth of corn.
Table 1. Effects of different fertilizers on soil properties.

| Treatment                        | Available nitrogen mg/kg | Available potassium mg/kg | Available phosphorus mg/kg |
|----------------------------------|--------------------------|---------------------------|----------------------------|
| Microbial bio-compound fertilizer (A1) | 60.50a                  | 137.00a                   | 45.91a                     |
| Organic-inorganic compound fertilizer (B1) | 58.08a                  | 119.01c                   | 38.30b                     |
| Organic fertilizer (C1)          | 50.81bc                  | 128.52c                   | 30.42b                     |
| Inorganic fertilizer (D1)        | 53.55b                   | 125.06bc                  | 33.32bc                    |
| No-fertilizer (CK)               | 42.05c                   | 107.31d                   | 18.60d                     |

Note: The letters a to d indicate significant differences (P < 0.05, n = 3).

3.3. Effects of different fertilization treatments on soil microbial community structure

PCA analysis of the high-throughput sequences of soil microbial community showed that the microbial community composition between control and the other four treatments was different, indicating that the different fertilization treatments had a great influence on soil microbial community composition.

The composition of microbial community of different treatments at phylum is shown in Figure 3a. The results showed that Proteobacteria had the highest abundance in all treatments, followed by Actinobacteria, they accounted together for 66.39%, 66.28%, 66.76%, 65.10% and 67.26% of total sequences. According to the cluster analysis, A1 and B1 community compositions were the most similar and formed a group; C1 and D1 formed another group; both of groups separated far from the control (CK).

Figure 3b showed the relative proportions of the soil microbial community at genus levels. This hot figure was drawn according to the abundance of dominant genera in each sample. It was found that the dominant genera of each treatment revealed a large difference, which suggested that the treatments had certain selectivity and some functional microbes were enriched. This was beneficial to the improvement of soil properties and plant growth. The genus *Pseudomonas*, a relatively high abundant genus in A1, contained phosphate-dissolving bacterium we used in the fertilizer.

The addition of functional strains in the microbial bio-compound fertilizer changed the proportion of bacteria in the reed soil. The increasing of the proportion of *Pseudomonas* spp. was an important reason for the change of community structure. It might be ascribed to *Pseudomonas plecoglossicida*’ rapid colonization and reproduction. Researches showed that *Pseudomonas plecoglossicida* could not only decompose organophosphorus, improving soil fertility[16], absorb heavy metals[17], under aerobic conditions, but also remove BTEX (benzene, toluene, ethylbenzene and xylene), CAHs (chlorinated aliphatic hydrocarbons) and TCE (trichlorethylene)[18].
3rd International Conference on Advances in Energy, Environment and Chemical Engineering  IOP Publishing
IOP Conf. Series: Earth and Environmental Science 69  (2017) 012062    doi:10.1088/1755-1315/69/1/012062

Figure 3. Microbial community composition and cluster analysis at the phylum level (a) and analysis of microbial community composition at the level of genus (b).

4. Conclusions
In this paper, the effect of microbial bio-compound fertilizer on the growth of reed and the improvement of soil nutrient were analyzed by pot experiment. The main conclusions are drawn as following:

(1) Microbial bio-compound fertilizer treatment significantly increased the reeds height, fresh weight and dry weight.

(2) Compared with no-fertilizer treatment, microbial bio-compound fertilizer improved the available N, P, K and sucrase, urease activity of soil.

(3) The bacterial community in the soil was relatively stable. Pseudomonas spp. were the predominant in the microbial bio-compound fertilizer treatment, different from other treatments.

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