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Microbiology Characteristics of New Soil Substrate in Mudflat Wetlands System

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The ecological rehabilitation engineering out of the coastal mudflat ecosystem was accomplished principally by virescence. However, the lack of proper soil resources for reformation of the coastal mudflat ecosystem was the primary difficulty. Usually purchasing farmland planted soil from surrounding areas, this approach not only destroy the ecological environment in the pristine soil area, but also have higher cost. Combining with the bay mud, caustic sludge and fly ash and other solid wastes resources that abound in the surrounding environment of the wetland system was more suitable for the growth of plants when appropriate proportion with its mixture. In this paper, tracking and survey research on microbial properties was carried out on a new soil substrate composed by three solid wastes. The results found that the new soil substrate was a good substrate suitable for plant growth. The new soil substrate in mudflat wetlands system included rich in nitrogen organic debris and in positive decomposition. The new soil substrate is conducive to the coordination of the synthesis and decomposition of organic matter in the soil. Microorganism quantity, intensity of biochemistry had a significant negative correlation with soil salinity and it had significant positive correlation with soil organic matter content, which indicated that reducing salt and increasing the organic matter content of the soil substrate was the key to the improvement of mudflat wetlands system.

Key words: Wetlands, soil substrate, microorganism, nutrients.

Soil microorganism is one kind of active substance, which is able to participate directly in a complex series of physiological and biochemical reactions in the soil. It involves in the cycle process of soil carbon, nitrogen, phosphorus, sulfur, and the mineralization of soil minerals. Soil microorganism plays a key role in the energy flow and material transformation process. Moreover, soil microorganism has an extremely important role in the formation of soil fertility, the transformation of matter and soil structure. In particular, it demonstrates a decisive role in the formation and stability of aggregates (Warkentin, 1995). Therefore, the soil microorganism is a prerequisite to soil ecosystem material cycling and energy conversion. They are an important symbol of soil ecosystem development and efficient and sustainable use of system resources (Xu, *et al.* 2002).

In the evolution of soil quality, soil microorganism has a relatively high conversion capacity and a sensitive change in soil quality. It can [1] predict the change process of soil organic matter. Thus soil microorganisms (including the number of three main types of bacteria in the soil, the dominant populations and soil microbial biomass) can be used as a sensitive indicator

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(Powlson, *et al.* 1987). The number and population change is a sensitive indicator of changes in soil quality. Increasing attention has been paid on the biological parameters to characterize soil quality (Kennedy, *et al.* 1995). It was initially proposed to use microbial biomass, soil respiration and soil enzyme activity as biological indicators of soil quality changes. Soil microorganism quantity and biochemical role of strength index have long been used as soil fertility indicators. Since the 1990s, more researchers investigated the determination and observation about the horizontal distribution and vertical distribution in the different soil of ecosystems as well as fertilization and tillage on soil enzyme activities. Many scholars in the world such as Turco (Turco *et al.*,1994), Ma (Ma *et al.*, 2007), and Wang (Wang *et al.*, 2011) considered that the catalase activity is linked with the level of fertility of the soil. Sparling (Sparliing,1997) proposed indicators of soil enzyme activities as a measure of soil biological activity and productivity, and pointed out that soil activity measurement is more important than the determination of soil microorganisms in the assessment of soil fertility. Soil fertility is a comprehensive reflection of the nature of the soil. Soil nutrient levels only reflected an important aspect of fertility, and it did not represent the entire soil fertility. Due to obligate characteristics of the enzyme, soil enzyme activity can still characterize the conversion and cycle process of the substances in the soil. Therefore, the determination of soil enzyme characterized the process of conversion of nutrients better when the chemical determination can make the assessment of the amount of the soil nutrient levels. Furthermore, the measured soil enzyme activities are mainly referred to the activity of the enzyme accumulated in the soil, and it has a relatively stable constant value. As for soil quality evaluation, Dai (Dai *et al.*, 2012) made the soil microbial parameters as indicators of changes in soil quality evaluation system. Soil microorganism is particularly sensitive to changes in the environment. Therefore, the diversity of the communities is the dominant factor which reflects small changes after disturbed in soil system (Liu, *et al.* 2008). In soil improvement process, a different way of improvement, as well as different ground vegetation will produce different ecological effects on soil microbial. Studies in this area for the governance of desertification, restoration of degraded ecosystems are important theoretical and practical significance. Researcher interests on soil microbial composition of degradation and the extreme ecosystem become important aspects in ecology.

In this paper, the characteristics of mixed soil substrate after desalination, administration of antibiotic-fertilizer, the biological improvement as well as the shelterbelt planting were examined based on the analysis of the variation of the physical and chemical properties of the mixing soil substrate. The soil microbial quantity in different seasons was investigated from the point of view of soil microbiology and soil enzyme activities. Moreover, the level of change in the use of antibiotic-fertilizer fertility improvement process, the ecological distribution of dominant populations, the intensity of the biochemical role, and the relationship with soil nutrient, are demonstrated by three years observation experiments. The findings in this paper provide a reference for microorganism characteristics in this new soil substrate in mudflat wetlands system.

MATERIALS AND METHODS

Experimental design

The coastal saline soil (CK1, which is bare land, sometimes covered by halophytic vegetation) and the foreign greenbelt soil (CK2) were selected as contrasted experimenting, and new soil substrate (CK) mixed with dredged sediments, caustic sludge and coal ash,) are tested in the experiments. Two soil layers (0 ~ 20cm, 20cm ~ 40cm) were sampled in the spring, summer, autumn to analyze the microbial number, taxa situation and strength of biochemical role. In the three treatments, soil samples were collected with 2 kg, using quartering sampling to get 1 kg in April, July and October respectively in the same plot with plum-type. The soil sample was packed by sterile kraft bag and stored in the refrigerator 4 °C ,using dilution plate technique to separate bacteria, actinomycetes and fungi. At the same time, the total salt, pH, N, P, K and organic matter in soil sample were analyzed as described by Liu in 2001. Statistical procedures were carried out with the software package SPSS 14.0 for Windows. Selected properties of the experiments are given in Table 1.

Table 1. Basic property of different soil samples

Soil type	Depth	Season	Total salt/%	Total N/%	Hydrolyzable nitrogen(mg/100g)	Total PP_2O_5%	Available P mg/100g soil	Total kK_2O%	Available K mg/100g soil	Organic%
Coastal saline soil(CK1)	0-20cm	spring	2.888	0.112	8.00	0.162	2.90	3.57	111.1	1.32
		summer	6.572	0.074	22.1	0.130	1.80	3.14	118.3	1.02
		autumn	3.746	0.042	15.1	0.114	1.40	2.80	89.90	1.01
	20-40cm	spring	2.375	0.092	8.30	0.161	3.10	3.57	137.9	1.48
		summer	5.925	0.083	19.6	0.133	1.70	3.07	107.0	0.99
		autumn	4.251	0.039	8.40	0.110	1.30	2.85	97.40	0.82
Foreign greenbelt soil (CK2)	0-20cm	spring	0.058	0.228	6.00	0.159	1.60	3.42	4.5.00	1.37
	summer	0.056	0.106	14.0	0.169	2.00	3.10	32.20	1.61	
		autumn	0.083	0.087	11.2	0.178	1.60	2.92	62.60	0.65
	20-40cm	spring	0.082	0.071	13.7	0.163	0.86	3.32	38.10	1.18
		summer	0.103	0.102	14.0	0.144	2.40	2.85	30.20	0.86
		autumn	0.205	0.071	12.2	0.162	1.00	2.85	33.20	1.01
New soil substrate (CK)	0-20cm	spring	0.101	0.132	12.7	0.204	7.60	3.29	78.00	2.85
		summer	0.16	0.129	10.2	0.186	9.00	32.2	8.150	2.60
		autumn	0.085	0.116	13.3	0.148	9.20	3.15	78.50	2.88
	20-40cm	spring	0.153	0.151	11.0	0.179	4.10	3.40	99.80	1.64
		summer	0.285	0.081	11.6	0.170	3.90	3.37	119.50	1.40
		autumn	0.168	0.087	25.9	0.190	3.30	2.95	98.40	1.53

Soil microbial separation and quantity determination

The collected soil samples were pretreated by a series of gradient dilutions within 24 h. Bacteria and actinomycetes were set up in plate germiculture with three levels as 10-4010-5010-6 respectively, and fungi was set up in plate germiculture with three levels as 10-2010-3010-4 respectively. Each sets devices have 3 repeats (n=3). Fungal was put into the 25 ° C incubator culture, the other at 28 ° C incubator culture. The counting time is 3 - 5 days later. Then different kinds of microbial strains were separated, classified and identified. Bacteria were cultured in beef-protein medium. Bacillus was cultured in beef-wort medium (The soil dilutions by 85 ! for 10 min cool down and counts after vaccination culture). Actinomycete was cultured in improved No.1 Gao medium. Fungi was cultured in extracted malt medium (100ml medium plus 1 drop of lactic acid).

Identification methods of soil microorganism

Bacillus was identified by routine bacterial identification method. A comprehensive identification was finished according to the morphological, cultural characteristics, physiological and biochemical characteristics. Actinomycete was mainly identified by morphology, culture characteristics. The identification included its spores, aerial mycelium, the color of the substrate mycelium, and soluble pigment. Physiological and biochemical characteristics were identified according to carbon source utilization and antimicrobial spectrum. Fungal was mainly identified by microscopic characteristics, such as whether the mycelium separated, whether hyphae branched, whether hyphae transparency, whether had color. Furthermore, auxiliary culture characteristics, colony growth rate, colony color, surface structure, texture, colony edge traits and other characteristics were used for reference during the identification (Zhang, *et al.* 1995).

Soil microbial populations analysis

Aerobic Azotobacter and ammonifiers was analyzed by dilution plate count method. Confined static culture method was used to measure the respiration intensity of CO2. Intensity of ammonification was analyzed ammonification bacteria culture medium. Nitrifying bacteria, denitrifying bacteria and aerobic cellulose decomposing bacteria was measured by diluted frequency notation.

Soil biochemical activity intensity measurement

The respiration intensity measurement method was based on the CO2 confined static culture method, which a certain concentration of NaOH solution was used to absorb the soil respiration and release of CO2. Then, the amount of CO2 can be calculated based on the consumption of the NaOH solution. The methodology for ammonification intensity was based on ammonification bacteria culture medium, which taking a certain weight of soil inoculating into a nitrogen containing liquid medium. Organic nitrogen is converted to ammonia by the role of ammoniated bacteria in the culture process. The methodology for sucrase determination was based on the amount of glucose. The protease determination method is based on the gelatine. It can be used as a substrate in the role of the protease. According to the amount of formation of the latter, soil activity of the protease can be measured. The urease determination method was based on the amount of ammonia released in the hydrolysis of urea.

RESULTS AND DISSCUSSION

The changes of soil microorganism quantities at different layers

The quantities of microorganisms for three different treatments are presents in Table 2. The results showed that the total quantities of soil microorganisms within the depth of 0-40cm are distinct in different sample plots. Compared to coastal salt soil (CK1), the quantities of soil microorganism in foreign greenbelt soil (CK2) increase 68 times while new soil substrate (CK) increase 183 times. The number of soil microorganism in new soil substrate is three times than foreign greenbelt soil. Comparing as other two kinds of soils, the new soil substrate is the most active in the process of material transformation. Furthermore, material turnover and utilization of new soil substrate increased correspondingly. New soil substrate based on three solid wastes have the following characteristics: accumulation of soil organic matter is high, soil bulk density decreased, porosity increased, increase in water-holding capacity, nutrient status

and soil structure improved and promoted the microbial activity. Due to the high salt content of the coastal salt soil, it have sparse vegetation and bad soil physical and chemical properties, resulting in less quantity and microbial activity is inhibited. The soil microbial quantity of coastal saline within the depths of 0-20cm is 1.6 times than within the depths 20-40cm. The microbial quantity of surface of foreign greenbelt soil is 3.8 times as the subsoil. The quantity of surface and bottom of new soil substrate were higher than foreign greenbelt soil. Statistical results of soil environment demonstrated that organic matter content in the surface of new soil sutstrate is rich. The soil structure is loose, which providing a good ventilation conditions for microbial activity. On the other hand, the surface soil and air heat exchange fast. The soil heat condition of new soil substrate was better than the lower, which was conducive to microbial growth and reproduction. At the same time, spatial heterogeneity resulted from the presence of plant roots meet the survival needs of a variety of micro-organisms. Obviously, soil environment below the surface was much smaller impact of vegetation, and at the deeper level maybe not affected by vegetation.

Table 2. The soil microorganism number of each category in all treatments ($\times 10^4$cell/g·dry soil)

Sampling	Sampling	Bacteria			Actinomycetaceae			Fungi$\times 10^2$cell/g·dry soil		
sites	Depth(cm)	April	July	Oct.	April	July	Oct.	April	July	Oct.
CK1	0-20	nd	0.80	5.07	0.83	0.08	nd	0. 4	2.0	nd
	20-40	nd	0.60	3.58	nd	0.05	nd	nd	0. 2	nd
CK2	0-20	42.60	231.90	288.48	5.42	3.06	26.30	11.6	11.0	28.5
	20-40	30.40	63.30	43.75	5.50	0.80	14.03	13.6	2.0	2.4
CK	0-20	377.60	339.60	593.02	22.66	131.10	39.40	90.6	32.0	141.0
	20-40	93.10	100.50	170.52	33.29	81.40	39.10	69.2	33.0	2.9

Notesÿ"nd means not detected"

Microbial population in the soil are generally of bacteria> actinomycetes> fungi. Bacteria in the soil can be accounted for 70 to 80% of the population of microorganisms. Three major groups of bacteria, actinomycetes and fungi, bacteria are dominant. In the total number of micro-organisms, bacteria accounted for 80 to 99%, followed by actinomycetes, accounting for more than 10% of the fungi at least and the proportion is less than 1% of the total amount. The percentages of more drought-tolerant actinomycetes in the arid soil or desert soil increased significantly. In other words, in the warm and dry weather conditions, pH values are higher and actinomycetes are in a higher percentage of the total number of microorganisms, because the spores made its population to be preserved under severe conditions. Fungi are heterotrophic microorganisms which is suitable for growth and development in the acidic soil conditions. However, sandy soil is alkaline, less nutrient, which limiting the development of the fungus.

The seasonal variation of the number of microorganisms in the soil

The seasonal changes of the quantity of microorganisms are similar (Table 3), all three treatments have the lowest values in April, and highest in October. The number of coastal saline soil microorganisms change little, because the impact of the high salt environment leads to seasonal changes. The total number of the microorganisms in foreign greenbelt soil and new soil substrate increases significantly in relative to the coastal saline soil. It is because of the soil environment heterogeneity for different treatments. The seasonal variation of the number of microorganisms in the new soil substrate is dramatically.

The correlation analysis between microbial quantity and soil nutrient

As we can see from Table 4, the bacteria and the number of actinomyces have a significant negative correlation in the soil total salt contents. The number of bacteria was significant positive

Table 3. Season changes of soil microorganism in all treatments ($\times 10^4$cell/g·dry soil)

Treatment	Depth (cm)	April	July	Oct.
CK1	0~20	0.834	0.9	5.07
	20~40	0	0.652	3.581
CK2	0~20	48.136	235.07	317.665
	20~40	36.036	64.12	57.803
CK	0~20	401.166	471.02	633.823
	20~40	127.082	182.23	209.645

correlation in the soil organic matter content. It can be seen from the correlation analysis between the microbial flora and soil fertility factors. It is significantly positively correlated with soil available N and available P of bacterial life activities between the number of soil bacteria. In other words, soil N and available P have an important influence on the activity of bacteria life. Actinomycetes, fungi, and soil organic matter, total nitrogen, available nitrogen was highly significant correlation, which is due to the decomposition and transformation of organic matter in the soil, as well as the accumulation of humus by soil actinomycetes and the greater impact. On the other hand, the number of bacteria in the soil was far greater than the actinomycetes and fungi. The weight of actinomycetes and fungi huge mycelium was far greater than the bacterial cells, because of the contribution to soil fertility.

Table 4. The correlation coefficient between soil microorganisms and nutritions

	Total salt	Organic matter
Bacteria	-0.400*	0.468**
Actinomyces	-0.350*	0.179
Fungi	-0.244	0.252

The correlation analysis of soil nutrient content and the biochemical strength

Table 5 showed that the strength of soil respiration, the intensity of soil ammonification and the activity of urease and protease were highly significant or significant negative correlation with the soil salinity. However, the intensity of ammonification, the activity of protease and the urease were highly significant or significant positive correlation with the total nitrogen, the total phosphorus and the organic matters respectively. The highly significant correlation with the SRP and the activity of invertase, the protease and the urease were also found in this study.

The correlation analysis of biochemical activity and soil microorganisms quantity

It can be seen from the Table 6 that the strength of soil respiration, the intensity of ammonification, the activity of invertase, the protease and the urease are highly significant with

Table 5. Correlative coefficient between biochemical activities and nutritions

	Total salt	Total N	Hydrol-yzable Nitrogen	Total	Availa-P	Total k ble P	Availa-ble K	Organic
Respiratory intensity	-0.485**	0.276	-0.192	0.177	0.024	0.157	-0.365*	0.185
Ammonification strength	-0.511**	0.431**	0.048	0.454**	0.188	0.132	-0.398*	0.618**
Converting enzyme	-0.264	0.339*	-0.157	0.501**	0.410*	-0.018	-0.159	0.531**
Protease	-0.390*	0.512**	-0.230	0.571**	0.624**	0.268	-0.392*	0.714**
Urease	-0.468**	0.527**	-0.074	0.743**	0.512**	0.107	-0.375*	0.797**
Polyphenol oxidase	0.008	-0.087	0.273	-0.004	-0.279	-0.008	-0.027	-0.138

**.correlation is significant at the 0.01 level (2-tailed).*.correlation is significant at the 0.05 level (2-tailed). The same as following tables.

the microorganisms quantity, the correlation between bacillus and invertase ,the fungal and urease ,the actinomycetesz and the intensity of ammonification are highly significant, respectively. And the fungal also has a highly significant correlation with the intensity of ammonification. The correlation between the intensity of ammonification and the quantity of bacteria or fungal is very significant or significant positive whereas the correlation between bacillus is not obvious. There may be a small number of microorganisms in the soil but a very high activity of soil enzyme, because the activity of soil enzyme is combined with the activity of biological and non-biological. The soil enzyme is stable in the soil and it related with the number of microorganisms and also relevant with its own life in the soil. So we can see that the soil enzyme is inconsistent with the number of microorganisms because of the different kinds of enzyme with different life cycle. The invertase had a long life cycle because of combined with the soil particles and protected by the soil, and some enzyme has a short life cycle in the soil. The results showed that there was no relationship between microbial plate count and the alkaline phosphate, amidase and hydrogen peroxide enzyme.

Table 6. The correlative coefficient between biochemical activities and microorganisms

	Bacterial	Bacillus	Diazotrophs	Actinomycetes	Fungi
Respiratory intensity	0.420*	0.059	0.265	0.127	0.106
Ammonification strength	0.570**	0.004	0.293	0.351*	0.520**
Converting enzyme	0.495**	0.710**	0.308	0.048	0.283
Protease	0.442**	0.179	0.039	0.230	0.229
Urease	0.449**	0.258	0.167	0.182	0.497**
Polyphenol oxidase	-0.159	0.061	0.119	-0.026	-0.088

CONCLUSIONS

- The microbial quantity in new soil substrate had the same regularity with foreign greenbelt soil composition, showing that surface layer of new soil substrate was higher than the bottom layer. The surface layer of new soil substrate had the highest number of bacteria, which following by actinomycetes and fungi.
- The quantity of microorganisms, the vertical distribution of microorganisms, the seasonal changes, biochemical strength and dominant group's analysis showed that the new soil substrate was a well-substrate for plant growth in mudflat wetlands system.
- Microorganism quantity, intensity of biochemistry had a significant negative correlation with soil salinity and had a significant positive correlation with soil organic matter content, which indicated that reducing salt and increasing the organic matter content of the soil was the key to the improvement of mudflat wetlands system.
- New soil substrate of the wetland system is a good alternative foreign soil from farmland which is suitable for plant growth. It demonstrated good applicable results based on the study. Since the relationship between soil physical properties and characteristics of soil microbiology, soil physical properties have become one of the most important aspects of soil fertility. Soil physical properties have a significant impact on soil microbial growth and reproduction. This paper only explored the relationship between microbial and soil chemical properties. We should pay more attention on the soil physical properties. It plays significant role in soil fertility. It is necessary for long-term positioning monitoring of the quality conditions in the long-term succession process.

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