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Functional Diversity of Microbial Communities in Sludge-Amended Soils

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Abstract

The BIOLOG method was applied to exploration of functional diversity of soil microbial communities in sludge-amended soils sampled from the Yangtze River Delta. Results indicated that metabolic profile, functional diversity indexes and Kinetic parameters of the soil microbial communities changed following soil amendment with sewage sludge, suggesting that the changes occurred in population of the microbes capable of exploiting carbon substrates and in this capability as well. The kinetic study of the functional diversity revealed that the metabolic profile of the soil microbial communities exhibited non-linear correlation with the incubation time, showing a curve of sigmoid that fits the dynamic model of growth of the soil microbial communities. In all the treatments, except for treatments of coastal fluvo-aquic soil amended with fresh sludge and dried sludge from Hangzhou, kinetic parameters K and r of the functional diversity of the soil microbial communities decreased significantly and parameter S increased. Changes in characteristics of the functional diversity well reflected differences in C utilizing capacity and model of the soil microbial communities in the sludge-amended soils, and changes in functional diversity of the soil microbial communities in a particular eco-environment, like soil amended with sewage sludge.

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Keywords- Sewage sludge; Soil microorganism; Functional Diversity of Microbial Community; Kinetic Parameters

Introduction

The sludge is a by-product of sewage treatment, sludge land application and reclamation is the future direction of sludge disposal. After the sludge into the soil, on one hand, soil structural and soil fertility are

significantly improved[1], on the other hand, pollutants such as heavy metals in the soil will be increased[2, 3]. Therefore, Studies are needed to improve our understanding of the effects of land application of sewage sludge on soil quality. The soil quality is affected synthetically by soil physical, chemical and biological properties. The changes in soil quality and health can be reflected sensitively by biological properties of soil, it is an indispensable index to evaluate soil quality. Some common indices of them such as microbial biomass, soil respiration, metabolism entropy, soil enzyme as well as microbial flora diversity and community structure and function are important indices[4~6]. However, reports about microbial functional diversity after the sludge application in this particular habitat can be found not much at present. According to the above, functional diversity of microbial communities after the different types of sludge (Hangzhou Sewage Disposal Plant Digestion sludge, Suzhou sewage treatment plant sludge dewatering and their air-dry sample) applied into the different soil types in Yangtze River Delta region (lacustrine sediments and marine sediments of the paddy soil) were studied by incubation experiments in this paper, for the purpose of discussing the parameter of microbial ecology after the application of sludges into the soil in order to provide the scientific basis for soil quality assessment.

Materials and Methods

A. Soils and sludges

The two types of soil used were collected from the tillage layer (0-20 cm). Each soil was air-dried, passed through a 2-mm nylon sieve and mixed to give a homogeneous soil sample before use. The soils collected from East China were: (1) a paddy soil (Typic Alkaline-Stagnic Anthrosol) from Laogang town, Shanghai city; and (2) a paddy soil (Gleyi-Stagnic Anthrosol) from the Changshu Ecological Experimental Station in Jiangsu province. The two paddy soils are typical agricultural soils of the Yangtze River Delta region. Selected properties of the soils are listed in Table 1.

Four types of sewage sludges were used: (1) a fresh anaerobically digested sewage sludge collected from a wastewater treatment plant in Hangzhou city, Zhejiang province (HZF sludge), with a dry matter (DM) content of 34%; (2) air-dried HZF sludge with 93% DM (HZD sludge); (3) a fresh dewatered sewage sludge collected from a wastewater treatment plant in Suzhou city, Jiangsu province (SZF sludge) with 15% DM; and (4) air-dried SZF sludge with 91% DM (SZD sludge). Each fresh sludge was thoroughly mixed and each air-dried sludge was passed through a 2-mm-pore nylon sieve before use. Selected properties of the sludges are listed in Table 1.

Table 1 Properties of The Tested Soils And Sludges

Soil/Sludge	pH	OC (g kg ⁻¹)	Avail-N (mg kg ⁻¹)	Avail-P (mg kg ⁻¹)	Avail-K (mg kg ⁻¹)	Clay <2um (%)
Laogang Soil	7.19	8.4	175	68.0	94	14.8
Changshu Soil	6.82	16.5	270	15.3	72	16.7
Hangzhou sludge	6.35	116	2 966	55.9	138	ND*
Suzhou sludge	5.94	150	2 956	139	460	ND

B. Incubation experiment

The <2 mm air-dried soils were mixed with fresh or air-dried sludge at 20 g kg⁻¹ soil (DM basis). Aliquots (equivalent to 3000 g DM) of amended and unamended soils were placed in 3000-cm³ plastic boxes adjusted to 70% of water holding capacity (WHC), covered with plastic film and incubated at 25±0.5 °C in a temperature-controlled room for 180 days. The soils were adjusted to 70% WHC by adding deionised water and weighing on a weekly basis during incubation. The treatments included CK, HZF, HZD, SZF and SZD, were set up in duplicate. After 180 days incubation the functional diversity of soil microbial communities were examined.

1) *Incubation and reading of BIOLOG plate*: BIOLOG redox technology was used to characterize heterotrophic microbial communities[7~9]. Ten-fold serial dilutions of the soils were prepared and 150 µl

of the 10⁻³ dilution was used to inoculate each well of BIOLOG GN2 plates, providing 95 different carbon substrates for assessing microbial carbon utilization patterns. The plates were incubated at 28 °C in the dark, and the optical density (590 nm and 750nm) of each well was determined every 12h after 24h incubation up to 96h using an automated microplate reader(BIOLOG MICROLOG3 4.20, BioTek Inc.) To change the default, adjust the template as follows.

2) *Analysis of microplate date:* In the present study, average well color development (AWCD) method was used to evaluate BIOLOG date based on end point criteria. Average well color development (AWCD) was calculated according to Garland and Mills[10], i.e., $AWCD = [\sum(C-R)]/95$, where C is color production within each well (optical density measurement), R is the absorbance value of the plate's control well. AWCD for plates was calculated as the mean of all blanked absorbance values for all wells containing a carbon substrate. The formula was used to calculate the Functional diversity of soil microbial communities according to Yang [11].

Results and Discussion

C. Variation of AWCD

The obvious change in AWCD value of the microbial communities have not been displayed before 60 h, and the significant changes in AWCD be shown in Changshu soil after 72h and the Laogang soil after 84h (Fig. 1). Compared with the control, AWCD value in the treatment of Hangzhou fresh and air-dired sludge was increased by 18.7% and 13.2%, and in the treatment of Suzhou fresh and air-dried sludge was reduced by 11.7% and 62.7% in the Laogang soil. The AWCD value in the treatment of Hangzhou fresh and air-dired sludge, Suzhou fresh and air-dried sludge was reduced 22.0%, 10.6%, 59.4% and 35.5%, respectively. The results of statistical analysis showed that the difference of AWCD values of the microbial communities in the tested soils was very significant. ($p < 0.01$).

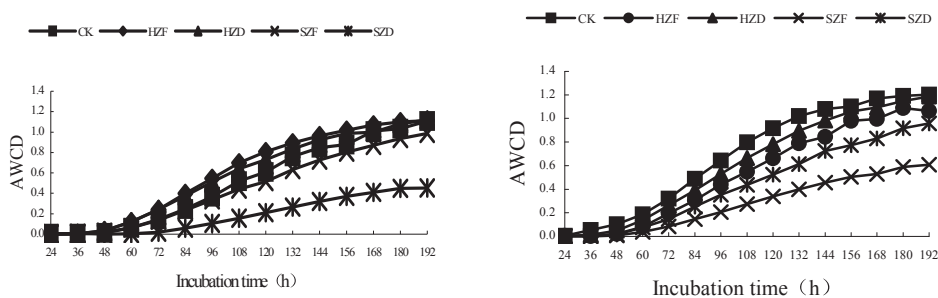


Figure 1. Kinetic changes in AWCD of the microbial communities in the sludge-amended soils.

D. Changes in functional diversity indexes of the microbial communities

The functional diversity indexes of soil microbial communities are actually reflected by three kinds of functional diversity indexes (Table 2). Shannon index is greatly influenced by species richness of communities, Simpson index is greatly reflected by the most common species, and McIntosh index is a measure of uniformity of the species. The results showed that the five functional diversity indexes in the sludge-amended soils have been significant changed ($p < 0.01$) compared with the control of soil. The functional diversity indices of the microbial communities increased significantly after applying HangZhou sewage sludge while the functional diversity indices of the microbial communities decreased after applying Suzhou sewage sludge in the Laogang soil. In the Changshu, the functional diversity indexes decreased except to Shannon index at air-dried Hangzhou Sewage sludge treatment.

Cluster analysis of the functional diversity of the microbial communities in the soils was carried on according to the functional diversity indices of soil microbial communities. The results showed that the functional diversity indexes were divided into 2 groups in the Laogang soil after the application of sludge (Fig. 2A). The first group was the air-dried Suzhou sludge treatment, the other group included the control, Hangzhou sludge and fresh Suzhou sludge treatment. The second group was divided into two subgroups, one subgroup was fresh suzhou sludge treatment, another subgroup was composed of the control and Hangzhou sludge treatment. In general, the soil microbial community diversity of the Laogang soil was not influenced by the application of Hangzhou sludge, while significant changes have been occurred after the application of Suzhou sludge. Fig. 2B indicated that microbial community diversity was greatly influenced by the application of fresh Hangzhou sludge and Suzhou sludge in the Changshu soil. After the application of sludge on two tested soils, microbial diversity trend in the same treatment was not same. Therefore, soil type, sludge type are important factors which can affect microbial functional diversity changes.

Table 2 Changes In Functional Diversity Indexes Of The Microbial Communities In The Sludge-Amended Soils

Treatment		Functional diversity indexes				
Soil type	Sludge type	Shannon Index	Shannon Uniformity	Simpson Index	McIntosh Index	McIntosh Uniformity
Laogang Soil	CK	3.95	0.874	44.4	5.25	0.949
	HZF	4.00	0.901	48.3	7.47	0.960
	HZD	4.00	0.888	44.9	7.25	0.951
	SZF	3.89	0.875	40.4	5.02	0.945
	SZD	3.44	0.840	25.5	2.01	0.921
Changshu Soil	CK	4.15	0.932	57.0	8.09	0.972
	HZF	3.94	0.885	43.5	6.32	0.951
	HZD	4.15	0.925	55.4	6.74	0.968
	SZF	3.38	0.832	24.1	3.97	0.917
	SZD	3.98	0.885	42.9	5.11	0.947

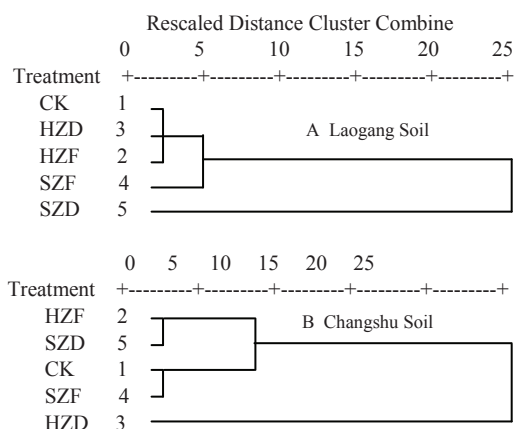


Figure 2. Dendrogram of hierarchical cluster analysis of microbial functional diversity indexes.

E. Kinetic parameters of the functional diversity of the microbial Communities

The experiment results showed test well dye reduction was non-linear in microplates inoculated with the soil samples, and the shape of the color development curve was generally sigmoidal (Fig. 1). Test well

optical density (OD) values over time suggested a kinetic model based on the density-dependent logistic growth equation of the general form

$$Y = OD_{750/590} = K / (1 + e^{-r(t-S)})$$

to describe a population of individuals[12]. In this equation, K represents the asymptote (when $y = K$) that the test well OD curve approaches, r determines the exponential rate of OD change, t is the time following inoculation of the microplate, and S is the time to the midpoint of the exponential portion of the curve (when $y = K/2$). Table 3 results indicated that the maximum OD values decreased in sludge amended soils except to Hangzhou sludge treatments in Laogang soil according to the kinetic parameters of functional diversity of soil microbial communities. The K -value of fresh and air-dried Suzhou sludge treatments in Laogang soil decreased by 8.2% and 56.8%, and fresh and air-dried Hangzhou sludge, fresh and air-dried Hangzhou sludge treatment in Changshu soil decreased by 8.4%, 1.6%, 48.3% and 18.0%. The S -value of fresh and air-dried Hangzhou sludge treatments in Laogang soil were shorter 16.2 h and 12.1 h than the control treatment, and fresh and air-dried Suzhou sludge treatments in Laogang soil, fresh and air-dried Hangzhou sludge, fresh and air-dried Suzhou sludge treatment in Changshu soil delayed 4.1 h, 12.2 h, 15.5 h, 9.5 h, 22.3 h and 22.4 h, respectively. Statistical analysis results showed that the K , r , S value are different significantly between the treatments in two tested soil (t -test, $p < 0.01$). The evolution of microbial community structure can be affected because of the declining of microbial community functional metabolism, slowing of metabolism process, and reducing of community growth after sludge amended.

Table 3 Kinetic Parameters Of The Functional Diversity Of The Microbial Communities In The Sludge-Amended soils

Treatment		Kinetic parameters			
Soil type	Sludge type	K	r	S	R^2 ^a
Laogang Soil	CK	1.10	0.043	114.0	0.997
	HZF	1.09	0.050	97.8	0.998
	HZD	1.09	0.044	101.9	0.996
	SZF	1.01	0.039	118.1	0.997
	SZD	0.48	0.046	126.2	0.999
Changshu Soil	CK	1.19	0.048	93.5	0.999
	HZF	1.09	0.043	109.0	0.997
	HZD	1.17	0.044	102.8	0.998
	SZF	0.62	0.041	115.8	0.997
	SZD	0.98	0.038	115.9	0.997

a. R^2 is the correlation coefficient

Conclusion

BIOLOG test results showed that AWCD of the microbial communities, community richness and diversity indexes were changed in a certain level in the sludge-amended soils. The number of soil microbial and the capacity of using carbon substrate have been changed by application of sludge, and eventually the functional diversity of soil microbial communities was changed.

The kinetic parameters K -value and S -value of microbial community functional diversity can be used to reflect the capacity of using carbon substrate even better than the AWCD valued in the sludge-amended soil. The kinetic parameters of the soil microbial community functional diversity K -value decreased and S -value increased in the sludge treatment except to the Hangzhou sludge treatment in Laogang, indicating that the ability of microbial communities to use the carbon source was reduced and the right patterns was changed, so, the functional diversity of soil microbial community was changed.

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