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Effects of Nitrogen Fertilizer Ratio on Microbial Diversity of Rice Rhizosphere Soil in Cold Region

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ABSTRACT

The experiment was conducted in the paddy field of a scientific and technological park of an agricultural reclamation farm in Xinxiang province from 2017 to 2019. The soil quality of the experimental field was the rhizosphere soil of rice in the cold region, and Longjing 31 was used as the experimental material. The effects of nitrogen application ratio on microbial diversity of rice rhizosphere soil in the cold region were studied. The aim is to provide a theoretical basis for rational fertilization, improving soil environment and high yield and efficiency. The results showed that: (a) the microbial diversity index of rice rhizosphere soil in the cold region was the best after the second nitrogen application; (b) under the ratio of N4 nitrogen application, the activity of bacteria, fungi and actinomycetes in rice rhizosphere soil in a cold region and their survival were the best; (c) under intensive cultivation conditions, the microbial diversity was the highest under SF1 treatment. SF0 treatment had the highest fungal diversity and SF3 treatment had the highest actinomycete diversity. (d) There was no significant effect on the microbial diversity of rice rhizosphere soil in the cold region without nitrogen fertilizer application in a single season.

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Introduction

The soil sphere is an integral part of the earth system. Rice rhizosphere microorganisms are the most active part of the soil and the main component of the soil decomposition system. They play an important role in promoting soil material conversion, energy flow and biogeochemical cycle (1). In recent years, due to the predatory exploitation of natural resources, biodiversity has been seriously damaged, and ecosystems are gradually fluctuating (2, 3). As a sensitive indicator for stabilizing ecosystems and monitoring soil quality changes, soil microbial diversity plays an important role in evaluating ecosystems and maintaining ecological balance. Therefore, more and more scholars are focusing on the research and protection of soil microbial diversity (4). Rhizosphere soil refers to the micro-area soil in the range of less than 1 mm to several millimeters at the root-soil interface. Others believe that the rhizosphere is 1-4 mm thick from the root surface and controlled by root exudates. Because the root system of trees is complex and the site area is large, the research on the rhizosphere of trees is carried out in a large area near the root system (5).

In the sense of ecology, "biodiversity" refers to the sum of the ecological complexes formed by organisms and the environment and the related ecological processes. It generally includes three levels: genetic diversity, species diversity and ecosystem diversity. Biodiversity is usually expressed by the "diversity index" (6). The calculation of biodiversity index generally needs to know the species number and the individual number of species in the community (7). For soil microorganisms, due to methodological limitations and variability of microorganisms themselves, it is impossible to cultivate all microorganisms in soil at present, and it is also difficult to identify and classify soil microbial species. Therefore, in practical research, the diversity of soil microorganisms is usually described approximately or indirectly from a certain side or angle (8).

Rice is one of the most important food crops in the world. In recent years, with the improvement of rice varieties in cold regions and the popularization and application of green rice cultivation techniques with high quality and high yield, rice yield per unit area

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and total yield have been greatly increased, but the corresponding annual use of fertilizers has increased rapidly, especially the excessive amount of nitrogen fertilizer (9). According to statistics, the utilization rate of nitrogen fertilizer is only about 20%, which is also manifested in the unreasonable application period and the excessive application of nitrogen fertilizer in the early stage of rice growth (10). Rice is the most important food crop in China. Its production is directly related to the food security of our country. Nitrogen is an important factor affecting rice yield. In order to meet the needs of social and economic development and the growing population for food, increasing the application of nitrogen fertilizer is one of the indispensable means (11). However, excessive input of nitrogen fertilizer will cause a large amount of waste of fertilizer nitrogen and economic losses but also lead to environmental quality deterioration and decline in the quality of agricultural products (12).

Suitable nitrogen application rate and base-topdressing ratio are the key technologies to improve the utilization rate of nitrogen fertilizer and reduce the loss of nitrogen in farmland. Previous studies on the effects of different nitrogen application levels and basal-topdressing ratio on rice growth, yield and quality, and nitrogen use efficiency have been carried out, but the conclusions are different. Moreover, with the new requirements of high and super-high yields and the renewal of rice varieties, the corresponding nitrogen management schemes need to be further studied (13).

In addition, long-term excessive application of nitrogen fertilizer has caused serious pollution of the natural environment and serious deterioration of the agricultural ecological environment. Therefore, the scientific management of rice nitrogen fertilizer is of great significance to the realization of high yield, high efficiency, high quality, pollution reduction and sustainable development of rice in cold regions (14). In order to apply nitrogen fertilizer reasonably, a lot of studies have been carried out by predecessors. However, there are few reports on the effect of nitrogen application ratio on the microbial diversity of rice rhizosphere soil in the cold region. This paper focuses on the effect of nitrogen application ratio on the microbial diversity of rice rhizosphere soil in a cold region and studies the effect of nitrogen application ratio on the microbial diversity of rice rhizosphere in the cold region. Conservation of soil microbial diversity provides an important theoretical and practical basis (15).

Materials and methods

Survey of test sites

The experiment was carried out in the paddy field of a scientific and technological park of a farm in a province from 2017 to 2019. The soil texture of the experiment field was cold rice rhizosphere soil, organic matter 4.42%, alkali-hydrolyzed nitrogen $161.4mg/kg$, available phosphorus $29.8mg/kg$, available potassium $131.25mg/kg$, and PH value 6.3. The former stubble is rice, turning in autumn. The effective accumulated temperature in November 2017 $\geq 10^{\circ}C$ was 2784.5 $^{\circ}C\cdot d$ with a frost-free period of 156 days; the effective accumulated temperature in November 2018 $\geq 10^{\circ}C$ was 2866.5 $^{\circ}C\cdot d$ with a frost-free period of 156 days; and the effective accumulated temperature in November 2019 was 2869.2 $^{\circ}C\cdot d$ with a frost-free period of 161days.

Test materials

The tested crops and varieties were Longjing 31 and 11-leaf rice varieties with the main stem.

Fertilizers: urea (46% nitrogen), diamine phosphate (18% nitrogen, 46% phosphorus), potassium sulfate (50% potassium).

Test design and method

In order to analyze the effects of nitrogen application ratio on the biodiversity of rice rhizosphere soil in cold regions from various angles, four groups of different experiments were set up in this paper, which were fully studied.

Effects of different nitrogen application times on microbial diversity of rice rhizosphere soil in cold regions

Soil equivalent to 5.0g was weighed and separated by single-phase extractant citric acid buffer solution-chloroform: methanol: the citric acid ratio was 1:2:0.8 (volume ratio); 10 mL chloroform and acetone were added to the SPE column (5 mL chloroform activation), and then leached with 10 mL methanol, and dried with nitrogen (16). Phospholipid fatty acid methyl ester (17) was prepared by mild alkaline

methylation. Thermal GC-MS was used for analysis. The mixed standard solution of 37 fatty acid methyl esters from GLCNESTLE 37 MIX Shanghai Anpu Company was used as an external standard, and the internal standard of methyl 19-alkate was used for quantitative analysis (18).

Chromatographic conditions: $1\,\mu L$ injection, 230 $^{\circ}C$ ion source temperature, 250 $^{\circ}C$ inlet temperature, no shunt, flow rate of 50 $mL\cdot\min^{-1}$, carrier gas velocity of 0.9 $mL\cdot\min^{-1}$, transmission line temperature of 250 $^{\circ}C$, mass spectrometry scanning range of 50-600 $m\cdot z^{-1}$. Temperature-rising procedure: after injection, the temperature was maintained at 70 $^{\circ}C$ for 5 minutes, then increased to 190cc at a rate of 20 $^{\circ}C\cdot\min^{-1}$, kept for 1 minute, increased to 200 $^{\circ}C$ at 5 $^{\circ}C\cdot\min^{-1}$, stayed for 2 minutes, increased to 280 $^{\circ}C$ at 10 $^{\circ}C\cdot\min^{-1}$, and kept for 8 minutes.

Effects of nitrogen application ratio on microbial activities of rice rhizosphere soil in the cold region

The total amount of nitrogen (urea) was 165 kg/hm^2, the amount of phosphorus fertilizer (diamine phosphate) was 120 kg/hm^2 and potassium fertilizer (potassium sulfate) was 165 kg/hm^2 during the whole growth period of rice. Phosphorus fertilizer was used as base fertilizer at one time, potassium fertilizer was divided into base fertilizer and 7.5 leaf ages (young panicle differentiation stage) twice, and the ratio of two times was 1:1. The application period and amount of nitrogen fertilizer are shown in Table 1.

Table 1. Nitrogen fertilizer dosage and nitrogen application period of each treatment

To deal with	Basal nitrogen	First dressing		Apply second time		Apply fertilizer for the third time		Total nitrogen	Total phosphorus	Total potassium
		Leaf age	Nitrogen content	Leaf age	Nitrogen content	Leaf age	Nitrogen			
CK	0							0.0	120	165
N1	49.5	4.0	49.5	7.5	16.5	10.0	49.5	165	120	165
N2	57.8	4.0	49.5	7.5	24.7	10.0	33.0	165	120	165
N3	66.0	4.0	49.5	9.0	49.5			165	120	165
N4	74.2	4.0	57.8	9.0	33.0			165	120	165

There are five treatments in the experiment:

N1: The proportion of nitrogen fertilizer in base fertilizer, tiller fertilizer, regulated fertilizer and spike fertilizer is 3:3:1:3, (base fertilizer + tiller fertilizer): (regulated fertilizer + spike fertilizer) = 6:4 treatment, among which 11 varieties of regulated fertilizer are applied at 7.5 leaf stage and 11 varieties of spike fertilizer are applied at 10 leaf stage.

N2: Base fertilizer, tiller fertilizer, regulated fertilizer and Panicle Fertilizer in the proportion of nitrogen fertilizer application is 3.5:3:1.5:2, (base fertilizer + tiller fertilizer): (regulated fertilizer + panicle fertilizer) = 6.5:3.5 treatment, of which 11 varieties of regulated fertilizer are applied at 7.5 leaf stage. Panicle fertilizer was applied at the 10-leaf stage and panicle fertilizer at the 10-leaf stage.

N3: The proportion of nitrogen fertilizer in base fertilizer, tiller fertilizer and spike fertilizer was 4:3:3 (base fertilizer + tiller fertilizer): spike fertilizer = 7:3 treatment, of which 11 spike fertilizer varieties were applied at 9.5 leaf stage.

N4: The proportion of nitrogen fertilizer applied in base fertilizer, tiller fertilizer and spike fertilizer was 4.5:3.5:2, (base fertilizer + tiller fertilizer): spike fertilizer = 8:2 treatment, of which 11 spike fertilizer varieties were applied at 9.5 leaf stage.

CK (control group): No nitrogen fertilizer was applied during the whole growth period, and the dosage and duration of phosphorus and potassium fertilizer were the same as those of other treatments.

Field planting mode was adopted in the experiment. The size of transplanting seedlings was 30×12 cm. Five treatments were set up and repeated three times in 15 districts. Each district had an area of $60m^2$ and was arranged in random groups. Inter-district stretching lines were divided into soil ridges, the stem and surrounding plastic film were wrapped well and single row and single irrigation was carried out in the district.

Effects of different nitrogen fertilizer management on microbial diversity of rice rhizosphere soil in the cold region under intensive cultivation conditions

Five treatments are set up. Details are shown in Table 2:

Table 2. Different Processing Devices

Handle	Pure nitrogen content	Base Fertilizer: Tillering Fertilizer: Panicle Fertilizer	Planting specifications	Seedling age	Water management
CK	180	50:30:20	20×20	30	Traditional flooding irrigation
SF0	0	-	35×20	20	Submersible irrigation, alternating wet and dry
SF1	180	50:30:20	35×20	20	Submersible irrigation, alternating wet and dry
SF2	180	60:10:30	35×20	20	Submersible irrigation, alternating wet and dry
SF3	180	40:20:40	35×20	20	Submersible irrigation, alternating wet and dry

Measuring items: Tillering dynamics of each treatment were recorded at fixed points at tillering stage. Soil samples from 0-20 cm representative soil layers were sampled at panicle differentiation stage, booting stage, filling stage and maturity stage by 5-point sampling method.

Effects of single-season non-application of nitrogen fertilizer on microbial diversity in rhizosphere soil of rice in cold regions

Because the BIOLOG method reflects the outline of microbial physiological activity by the utilization degree of microorganism to C source, it can reflect the intensity of microbial diversification more practically and intuitively than traditional methods such as the plate counting method. Moreover, it can also reflect the physiological profile of microbial community structure and the changes of microbial functional diversity. The BIOLOG plate method was used to analyze the effects of single-season non-application of nitrogen fertilizer on the microbial diversity of rice rhizosphere soil in cold regions (19). 10 g fresh soil was weighed and placed in a sterilized 250 mL triangular bottle (containing 100 mL 0.05 mol/L pH 7.0 phosphate buffer) and shaken for 20 minutes. After dilution to 10^{-3} with sterile 0.05 mol/L pH 7.0 phosphate buffer on the super-clean table, suspension diluted by 150 μ1 was added into each hole of BIOLOG GN96 plate with an 8-channel sampler. Three replicates were made for each soil sample. They were incubated at constant temperature of 25 $^{\circ}C$ for 24, 48, 60, 72, 84, 96, 108, 120, 132, 144 and 156 hours. The light absorption values of each pore were measured at 750 and 590 nm wavelengths.

Microbial diversity analysis method

Soil microorganisms are the main participants in the biogeochemical cycle, which promote the metabolism of soil organic matter and the cycling and transformation of soil nutrients, and play an important role in maintaining the process and function of agricultural ecosystems. Soil microorganisms are sensitive responders to changes in soil ecosystem structure and function and can be used as indicators of soil environmental changes. The method of phospholipid fatty acid (PLFA) analysis is both qualitative and quantitative. It can provide soil microbial community information quickly, directly, comprehensively and effectively. Therefore, it is widely used to measure microbial diversity in soil. Phospholipids are the main components of biomembrane, accounting for about 5% of the dry weight of cells. Phospholipids degrade rapidly after cell death, so they can be used to characterize the survival part of the microbial community (20).

The average absorbance (AWCD) method was used for microbial diversity analysis. This method can be used to evaluate the total ability of microbial communities to utilize nitrogen sources (21). Among them, the diversity index is calculated in the following form:

1. Shannon-Wiener Diversity Index (H)

The calculation formula is as follows:

$$H = -\sum Pi \ln Pi \quad [1]$$

In the formula, $Pi = Ni / N$, Ni is the number of characteristic fatty acids for treatment i, and N is the number of total characteristic fatty acids for treatment i. Pi is a characteristic fatty acid of Pi. S is a characteristic fatty acid i in the soil for the number of occurrences, that is, richness index.

2. Simpson dominance index (D)

The calculation formula is as follows:

$$D = 1 - \sum Pi^2 \quad [2]$$

Statistical analysis

Microsoft Excel 2003 software was used to sort out the data, DPS 7.05 software was used for statistical analysis, and Sigma Plot 10.0 software was used for drawing.

Results and discussions

Effects of different numbers of nitrogen fertilizer application on microbial diversity of rice rhizosphere soil in a cold region

The effects of different nitrogen application times on microbial diversity of rice rhizosphere soil in the cold region were analyzed as shown in table 3.

Table 3. Analysis results of different times of nitrogen fertilizer operation on microbial diversity of rice rhizosphere soil in cold region

Microbial marker	Microbial type	First fertilization	Second fertilization	Third fertilization
12:00	Bacteria	0.13±0.01b	0.24±0.03a	0.19±0.05a
13:00	Bacteria	0.03±0.02a	0.04±0.00a	0.02±0.01a
14:01	Bacteria	0.06±0.00a	0.09+0.00a	0.06±0.01a
14:00	Anaerobic bacteria	0.11±0.02b	0.20±0.00a	0.03±0.01c
15:00	Anaerobic bacteria	0.06±0.02a	0.09±0.01a	0.06±0.00a
16:01	Bacteria	0.12±0.03b	0.39±0.01a	0.26±0.08a
17:01	Bacteria	0.06±0.00a	0.07±0.00a	0.03±0.08a
16:00	Bacteria	0.12±0.03b	0.28±0.02a	0.27±0.04a
17:00	Bacteria	0.08±0.03b	0.15±0.01a	0.10±0.01a
18：3w6c	Fungi	0.08±0.01b	0.13±0.01a	0.10±0.01a
18：1w9c	Fungi	0.09±0.02b	0.20±0.01a	0.16±0.01a
18：1w9t	Fungi	0.09±0.02b	0.20±0.01a	0.16±0.01a
18:00	Bacteria	0.12±0.03a	0.19±0.03a	0.19±0.02a
18:02	Fungi	0.09±0.02b	0.20±0.01a	0.16±0.01a
18：3w3c	Fungi	0.09±0.02b	0.20±0.01a	0.19±0.02a
20:04	Actinomycetes	0.11±0.00b	0.13±0.00b	0.12±0.00a
20:05	Barophilic bacteria	0.09±00a	0.13±0.02a	0.10±0.00a
20：3w6c	Actinomycetes	0.09±0.01a	0.10±0.02a	0.09±0.00a
20:02	Bacteria	0.08±0.01a	0.09±0.00b	0.08±0.01a
20:01	Bacteria	0.08±0.01b	0.11±0.00a	0.08±0.01b
20：3w3c	Actinomycetes	0.08±0.01b	0.11±0.00a	0.08±0.01b
20:00	Bacteria	0.05±0.00a	0.07±0.00a	0.07±0.01a
21:00	Fungi	0.09±0.00a	0.09±0.00a	0.19±0.01a
22:06	Barophilic bacteria	0.14±0.01a	0.14±0.00a	0.14±0.00a
22:01	Bacteria	0.09+0.00a	0.12±0.00a	0.10±0.02a
22:00	Bacteria	0.08±0.00b	0.11±0.01a	0.09±0.01b
23:00	Fungi	0.09±0.00a	0.10±0.00a	0.10±0.01a

Note: w, c and t represent fatty acid terminal, cis-space structure and trans-space structure, respectively.

According to the above table, there are 13 kinds of bacteria, 7 kinds of fungi, 3 kinds of actinomycetes, 2 kinds of anaerobes and 2 kinds of barophilic/psychrophilic bacteria in the rhizosphere soil of cold rice. The microbial diversity index of rice rhizosphere soil in the cold region was the best after the second nitrogen application. Bacteria, fungi and actinomycetes are the main microorganisms in the rhizosphere soil of rice in cold regions. Therefore, the following will focus on the analysis of the three major bacteria (22).

Effects of nitrogen fertilizer ratio on microbial activities in rice rhizosphere soil in a cold region

1. Effect of nitrogen fertilizer management on the bacterial activity of rice rhizosphere soil in a cold region

Activity is an active property to evaluate the vitality and tenacity of soil microorganisms. The effect of nitrogen application on bacterial activity in rhizosphere soil of rice in the cold region was analyzed as shown in Figure 1.

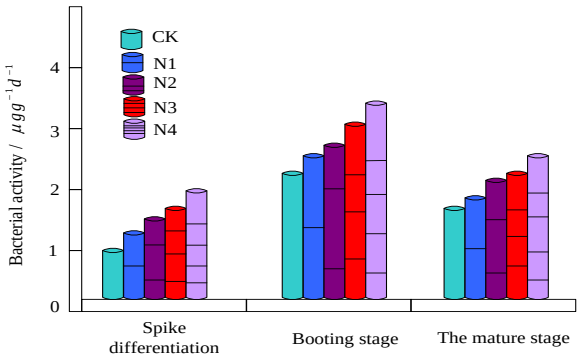

Figure 1. Effects of nitrogen application on bacterial activity in rhizosphere soil of rice in the cold region

From Figure 1, it can be seen that the bacterial activity of rice rhizosphere soil in this cold region increased first and then decreased with the increase of nitrogen application rate. The results showed that the bacterial activity was significantly increased by topdressing nitrogen fertilizer at booting stage < mature stage < booting stage. Compared with CK, the activity of bacteria in the rhizosphere soil of rice in this cold region was significantly increased by increasing the application of nitrogen fertilizer. The order of bacterial activity was N4 > N3 > N1 > CK,

which might be related to the residual nitrogen fertilizer in the last crop.

2. Effect of nitrogen fertilizer management on fungal activity in rice rhizosphere soil in the cold region

The effect of nitrogen application on fungal activity in rhizosphere soil of rice in the cold region was analyzed as shown in Figure 2.

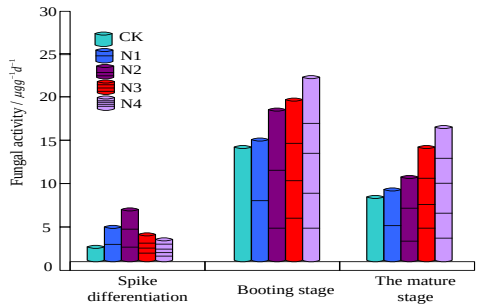

Figure 2. Effects of nitrogen application on fungal activity in rhizosphere soil of rice in the cold region

As can be seen from Figure 2, fungal activity in rhizosphere soil of rice in this cold region increased first and then decreased with the increase of nitrogen application. Fungal activity reached the highest at the booting stage and lowest at the panicle differentiation stage, which indicated that fungal activity increased with the increase of nitrogen application after topdressing. Fungal activity at the booting stage was N4 > N3 > N2 > N1 > CK, which indicated that fungal activity at the booting stage was unevenly distributed in soil, and there might be a direct reason for uneven fertilization in the last season.

The fungal activity N4 > N2 was significantly different in the mature stage, and the fungal activity N4 was higher in the mature stage.

3. Effect of nitrogen fertilizer management on actinomycete activity in rice rhizosphere soil in the cold region

The effect of Nitrogen Application on the activity of actinomycetes in rhizosphere soil of rice in the cold region was analyzed as shown in Figure 3.

It can be seen from Figure 3 that the activity of actinomycetes is relatively low during panicle differentiation, but with the increase of fertilizer application, the activity of actinomycetes in rhizosphere soil of rice in this cold region increases first and then decreases, and reaches the maximum activity at booting stage. Compared with the non-application of nitrogen, the activity of actinomycetes was significantly increased by increasing nitrogen fertilizer. The activity of actinomycetes was the lowest at the panicle differentiation stage, which was close to 0 $\mu g g^{-1} d^{-1}$. This may be due to the incompatibility between temperature and humidity at the panicle differentiation stage and the living environment of actinomycetes, resulting in the lower activity content. However, the activity of soil actinomycetes reached the highest level at the booting stage, and N4 > N3 > N2 > N1 > CK at the booting stage. There were significant differences among treatments, and N4 activity was the highest. This indicated that under certain conditions, soil actinomycetes activity coincided with the crop growth period. Through the decomposition and transformation of rice rhizosphere soil microorganisms, fertility played a higher role. Rice needs more nutrients at its peak growth stage when the activity of actinomycetes increases significantly, and the corresponding nutrient release is more, and the effective nutrients begin to decrease gradually with the development of rice. Fungal activity began to decrease gradually at the maturity stage, but there was no significant difference between N4 and N3, and a significant difference between N3 and N2 and N1. Actinomycete activity was closely related to the amount of nitrogen fertilizer applied.

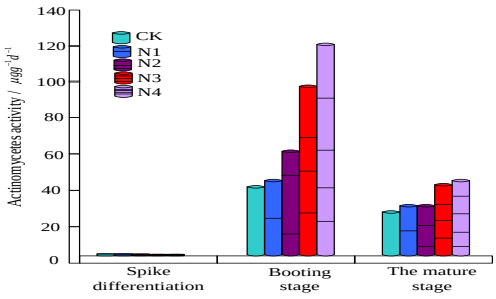

Figure 3. Effect of nitrogen fertilizer management on actinomycete activity in rice rhizosphere soil in the cold region

Effects of different nitrogen fertilizer management on microbial diversity of rice rhizosphere soil in the cold region under intensive cultivation conditions

The effects of different nitrogen applications on microbial diversity of rice rhizosphere soil in the cold

region under intensive cultivation were analyzed as shown in table 4.

Table 4. Effects of different nitrogen fertilizer management on microbial diversity in rhizosphere soil of rice in cold regions under intensive cultivation

Microorganism	Handle	Determination period				Average
		Panicle differentiation stage	Booting stage	Grouting period	Mature period	
Bacteria	CK	3.80	22.17	18.00	39.04	20.75
	SF0	3.21	24.78	20.64	39.72	22.09
	SF1	3.29	26.04	20.86	46.41	24.15
	SF2	3.26	30.56	17.63	38.11	22.39
	SF3	3.18	24.84	21.48	36.31	21.45
	average	3.35	25.68	19.72	39.92	22.17
Fungus	CK	2.96	1.79	0.48	3.17	2.10
	SF0	1.46	1.31	0.30	5.82	2.22
	SF1	1.34	1.19	0.29	3.45	1.57
	SF2	1.46	1.59	0.40	3.34	1.70
	SF3	1.70	1.73	0.28	4.82	2.13
	average	1.78	1.52	0.35	4.12	1.94
Actinomyces	CK	44.50	34.60	18.60	17.73	28.86
	SF0	31.23	24.91	19.53	25.60	25.32
	SF1	36.13	53.92	10.30	18.60	29.74
	SF2	64.40	39.34	18.47	21.37	35.89
	SF3	65.30	35.90	22.00	20.87	36.02
	average	48.31	37.73	17.78	20.83	31.17

The diversity of actinomycetes was the best in the soil during the panicle differentiation and booting stage, followed by bacteria and fungi. The diversity of bacteria was the best in the flowering and filling stage, followed by actinomycetes, and the diversity of fungi was much lower than the former two. Bacteria are one of the main microbial populations in soil, which can decompose various organic substances. The bacteria of different treatments showed the same trend with the progress of rice growth. The ear differentiation stage was lower, only 3.35×10^{6} per g^{-1} soil. Under different nitrogen fertilizer combinations, the bacterial diversity of rice rhizosphere soil in this cold region was highest in SF1 treatment.

The diversity of soil fungi was low at the flowering and filling stage and high at the maturity stage. Compared with different treatments, the diversity of soil fungi increased at the panicle differentiation stage, booting stage and filling stage. The results showed that the diversity of soil fungi in SF1 was smaller than that in SF0 without nitrogen fertilizer.

Actinomyces are a kind of microbial resource with important economic value. They can not only produce antibiotics, enzymes and immune-modulators but also decompose macromolecule compounds in the soil. The diversity of actinomycetes in the rhizosphere soil of rice in this cold region tends to decrease after panicle differentiation. The diversity of actinomycetes in different intensive cultivation treatments was optimized to varying degrees except for SF0 treatment without nitrogen application. The diversity of actinomycetes in the rhizosphere soil of rice in the cold region was SF0, SF1, SF2 and SF3 from low to high, indicating that the late application of nitrogen also promoted the growth of actinomycetes in the rhizosphere soil of rice in the cold region.

Effects of nitrogen fertilization on microbial diversity of rice rhizosphere soil in the cold region

Figure 4 is a variation map of microbial biomass in the rhizosphere soil of rice in the cold region under two fertilization modes. It can be seen that there are more bacteria than actinomycetes and far more fungi in single-season non-nitrogen fertilizer areas and conventional fertilizer areas. However, there was no significant difference in the number of microorganisms in the rhizosphere soil of cold rice under two different fertilization methods.

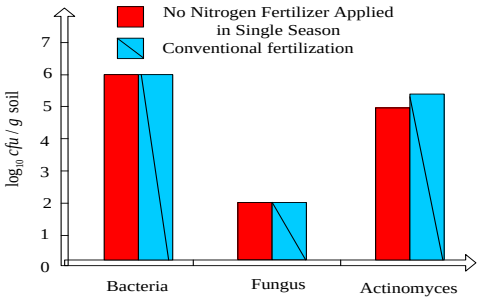

Figure 4. Effects of single-season non-application of nitrogen fertilizer on the number of microorganisms in rhizosphere soil of rice in cold regions

In Figure 5, 72h is the inflection point, so 72h is chosen as the time point for subsequent diversity index analysis.

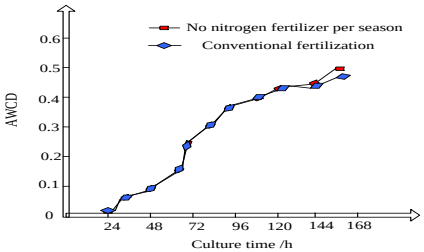

Figure 5. Average absorbance at different incubation times

In Figure 5, there is no significant difference in AWCD values between single-season N-free and conventional fertilization areas, either in trend or in specific values.

Figure 6 is the test result of the microbial diversity index of rice rhizosphere soil in the cold region without nitrogen fertilizer in a single season.

The results of Figure 6 showed that there was no significant difference in Simpson index D and Shannon index H of nitrogen utilization by rice rhizosphere microorganisms in the cold region after 72 hours of cultivation with BIOLOG plate.

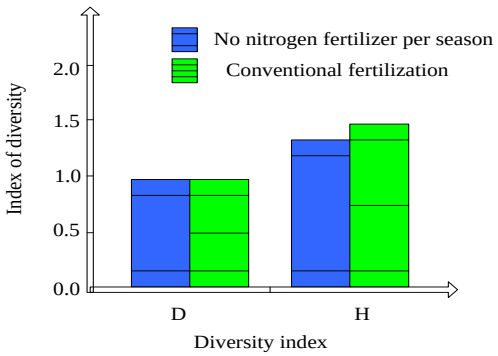

Figure 6. Diversity index of 72 h culture

Soil microbial diversity research involves a wide range of contents, with different levels and levels (23). Microorganisms exist in the soil ecosystem and are inevitably affected by ecological types. In different ecosystems, facing the pressure of survival competition and natural selection, different species gradually form stable heredity in the process of phylogeny and evolution (24). In a relatively stable genetic background, different microbial species perform their respective roles to jointly maintain the stable development of the living environment. It is in the process of continuous alternation of selection, competition and adaptation that the diversity of soil microorganisms is formed (4, 24). Although new technologies and new means have been applied to the study of soil microbial diversity, due to the limitations of cultivation technology, it is difficult to determine the taxonomic status of a large number of uncultured microorganisms, and the living microbial resources available for our understanding and utilization are still scarce (25).

At present, the research on soil microbial diversity is relatively focused on its species and ecological characteristics, while the research on soil microbial functional diversity, the relationship between different soil microbial populations, and how soil microorganisms affect the ecological process, maintain and stabilize ecosystems is not deep enough. Although the current research is not enough to let us know all the soil microorganisms, soil microorganisms as a huge gene pool, its rich genetic content has shown great potential. Undoubtedly, its recognition, research and development will bring enormous economic and social benefits to mankind.

The study of soil microorganisms is not only an important means to explore life, but also provides abundant resources for the research and development of many supernormal substances (25). The application of biotechnology in soil microbial research has made the screening and cloning of many genes related to antimicrobial, anti-tumor, drought, salt and alkali resistance and biosynthesis of biological insecticides a reality (26). Especially, the screening of soil microbial genome library based on non-culture provides new ideas for the research of plant conservation, plant nutrition, environmental science and human medicine (27). Similarly, the development and application of soil AM bacteria have opened up a new direction for the study of conservation and restoration ecology (28).

With the flourishing of the genome era and the coming of the post-genome era (functional genome), soil microorganisms will be more favored by researchers because of their unique advantages (29). The study of its diversity will provide a new model for elucidating the principles of ecology and biological evolution, and also open up broad prospects for the development of many new and interdisciplinary disciplines (30).

Conclusions

Soil microorganisms are related to the biogeochemical cycle, soil quality and health, plant productivity and sustainable agricultural development. Studying soil microbial diversity can help us understand the changes of soil microorganisms and soil quality due to the changes of environmental conditions and the use of soil by human beings, and provide a basis for the sustainable development of agriculture and the protection of the ecological environment. In this paper, the microbial diversity of rice rhizosphere soil in the cold region was studied around the proportion of nitrogen fertilizer

application. The following conclusions were obtained for reference:

(A) There were significant differences between the microbial diversity of rice rhizosphere soil in the first nitrogen fertilizer management and that in the second and third nitrogen fertilizer management. The microbial diversity index of rice rhizosphere soil in the cold region was the best after the second nitrogen fertilizer management.

(B) The order of bacterial activity was N4 > N3 > N1 > CK, fungal activity was N4 > N3 > N2 > N1 > CK, and actinomycete activity was N4 > N3 > N2 > N1 > CK. Therefore, the microbial activity of rice rhizosphere soil in cold region was the best under N4 treatment.

(C) In intensive cultivation, bacterial diversity was the highest under SF1 treatment, fungi diversity was the highest under SF0 treatment and actinomycetes diversity was the highest under SF3 treatment.

(D) There was no significant difference in the number of microorganisms in the rhizosphere soil of rice in the cold region without nitrogen fertilizer application in a single season. There was no significant difference in Simpson index D and Shannon index H of nitrogen utilization between soil microorganisms. This nitrogen fertilizer management mode had no significant effect on the microbial diversity in the Rhizosphere soil of rice in the cold region.

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Interest conflict

None.

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