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Research on Long-term Different Fertilization Types on Microbial Diversity with AOB gene in Tobacco Field Soil

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Abstract:

The objective of this study were analyzed that Long-term fertilization affected the AOB gene structure and abundance of soil bacteria in flue-cured tobacco field. There are three treatments in this study, respectively T1 for Chemical fertilizer, T2 for chemical fertilizer + straw returning and T3 for chemical fertilizer + straw returning + cake fertilizer. This study studied the effects of inorganic nitrogen and bacterial diversity in tobacco rhizosphere soil. The results showed that the relative abundance of Proteobacteria was the highest in the bacterial community, ranging from 58.68% to 69.00%, which was positively correlated with nitrate nitrogen and negatively correlated with ammonium nitrogen. The LEf Se evolutionary cladistic diagram of soil microorganisms showed that the bacterial population appeared in tobacco field treated with straw or cake fertilizer. In conclusion, long-term straw returning combined with cake fertilizer can increase inorganic nitrogen content and improve the microbial diversity with AOB gene in tobacco field.

Keywords*: Flue-cured tobacco field, Long-term straw returning, AOB gene, Long-term cake fertilizer, Bacterial Community Structure.*

I. INTRODUCTION

Soil microorganisms are an important part of the soil ecosystem and play an important role in the decomposition of organic matter, nutrient cycle and the promotion or inhibition of plant growth. These will be produced different influences on soil microbial activity rate, gene expression, biomass and community structure for With long-term fertilization, soil essence, fertilization type and cropping system, etc., which will ultimately be shown as harmful, beneficial and non-influencing results on farmland [1].Therefore, this

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is of great significance to study the effects of fertilization and crop rotation on soil microbial communities in farmland.

Soil microorganisms are involved in the decomposition and transformation of organic matter and various nutrients in the soil, which is closely related to soil quality or fertility. Soil bacteria are the main component of soil microorganisms [2].

The study has found that both different types of nitrogen fertilizers and farming systems had an influence on nitrification and denitrification in soil and promoted different levels of N2O emissions [3]. Soil nitrification and denitrification are closely related to microbial community structure in soil. Some studies have pointed out that long-term fertilization will dramatically change microbial community structure in soil, which is significantly different from organic fertilizer and chemical fertilizer. Flora structure can be affected by using nitrogen fertilizer over a long period of time, of which different types can cause differences in soil microbial composition. However, organic fertilizers can significantly alter microbial community structure and diversity and improve microbial biomass and metabolic activity [4]. Soil microorganisms are affected not only by the type of fertilizer but also by the farming system. Tillage can change bacteria community structure and diversity of soil microorganisms. Therefore, based on long-term fertilization conditions, soil microorganisms have different responses to different fertilizer types, but there is a lack of specific understanding on the effects of bacterial community structure with AOB gene and soil inorganic nitrogen morphological characteristics.

In this study, different types of fertilizers were applied to study the effects of bacterial community structure with AOB gene and their coupling relationship with soil inorganic nitrogen content in farmland, so as to reveal the effects of study the relationship between microbial mechanisms and inorganic nitrogen characteristics release from the perspective of functional gene in order to provide scientific tillage and cultivation system for tobacco soil and promote the sustainable development of tobacco soil ecosystem on a theoretical basis.

II. MATERIALS AND METHODS

2.1 Overview on Test Area

The experiment was conducted in Long-term Nutrient Research Station in Fujian Province in 2017. The test area was established at the rice and flue-cured tobacco wheel as locating points for experiment since 2009. In addition, CB No.1 flue-cured tobacco variety was selected for experiment in flue-cured tobacco field of soils with fertility status from 0 to 20cm featuring alkali-hydrolyzable nitrogen 144.66 g.kg-1, available phosphorus 5.85 g.kg-1, organic matter 28.96 g. kg-1, rapidly-available potassium 142.43 mg.kg-1 and pH 5.76. The soil type is sandy loamc.

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2.2 Test Design and Management

The experiment was designed with three treatment processes. There are three treatments in this study, respectively T1 for Chemical fertilizer, T2 for chemical fertilizer + straw returning and T3 for chemical fertilizer + straw returning + cake fertilizer.

In the production season of flue-cured tobacco from 2014 to 2017, the fertilizer nitrogen applied was 97.5 kg/hm2, N: P2O5: K2O = 1:0.75:2.63 (Fertilizer for tobacco fertilizer N: P2O5: K2O = 12:8:22, compound fertilizer N: P2O5: K2O = 15:15:15, Potassium nitrate N: P2O5: K2O = 13:0:38 and potassium sulfate containing K2O 51%). Base fertilizer: top dressing = 60:40.

2.3 Sample Collection

When the flue-cured tobacco harvests, tobacco-growing rhizosphere soil is taken from each district for three times. Impurities of the soil we take should be removed by a 2mm sieve, and then packed in sealed bags in cryopreservation for analysis of functional gene diversity. Samples should be tested immediately.

2.4 Sample Determination

Total DNA is extracted from the soil by Power Max Soil DNA Isolation Kit and its concentration and purity detected by Nano Drop ND-1000, and bacterial nosZ gene is sequenced by the Illumina Mi Seq platform [5].

2.5 Calculation Methods and Data Analysis

Paired-end sequencing is performed on the Illumina Mi Seq platform. The data is filtered, spliced and chimera is removed by QIIME (v1.8.0), then clustered into OTU (Operational Taxonomic Units) used for species classification, of which the similarity is set to 97%. We studied the Greengenes database to obtain the taxonomic information for each OUT. Multiple analysis of α (including 3 indexes such as Shannon, ACE and Chao1) is carried through Mothur(version 1.31.2). Clustering analysis, based on Weighted Unifrace distance, is conducted by pheatmap in R (v3.1.1) package.

The experimental data were sorted out by EXCEL 2010 and plotted. SPSS 11.5 software was used to analyze the data. Duncan analysis method was used to make multiple comparisons between the mean values for significance tests of different indicators.

III. RESULT ANALYSIS

3.1 Effects of Different Fertilization Types on Inorganic Nitrogen in Tobacco Fields

Fig 1 shows the characteristics of inorganic nitrogen under different fertilization types. The inorganic

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nitrogen mainly exists in the form of ammonium nitrogen in tobacco field. The soil inorganic nitrogen content from high to low was 16.03mg/kg in T3, 15.11 mg/kg in T1 and 12.08 mg/kg in T2, respectively. Both T1 and T2 exist in the form of ammonium nitrogen, and the content of ammonium nitrogen in T3 is 69.29% higher than that in nitrate nitrogen. The results showed that increasing cake fertilizer combined with straw returning was beneficial to increase soil inorganic nitrogen content.

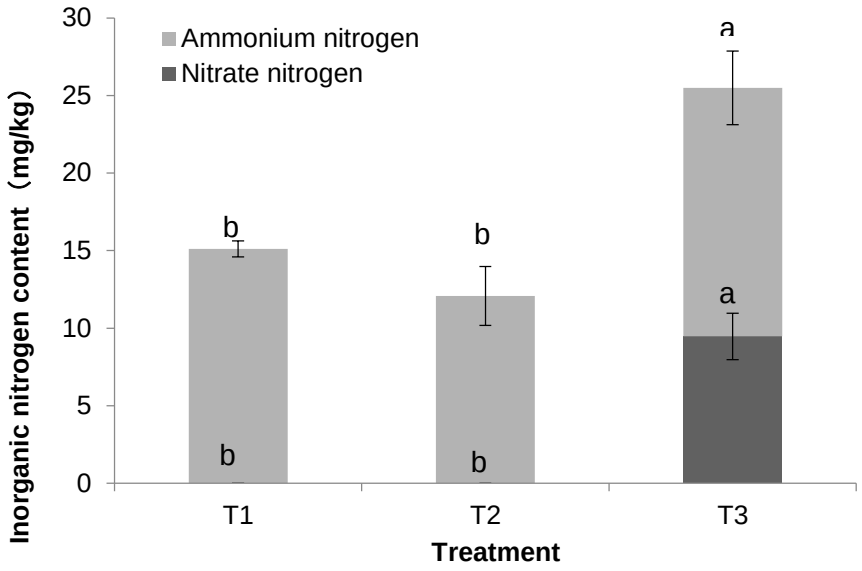

Fig 1: Inorganic nitrogen contents in tobacco planting soil treated with different fertilization scheme

3.2 Effects of Different Fertilization Types on the Diversity of microorganism α in Tobacco Fields

As shown in Figure 2, the results showed that soil bacterial α diversity, Chao1 index, Shanoon index, Observed_species index and PD_whole_tree index in different treatments showed that compared with T1 for Chemical fertilize, there are three treatments in this study, respectively, T2 for chemical fertilizer + straw returning and increasing T3 for chemical fertilizer + straw returning + cake fertilizer showed lower bacterial α diversity. Therefore, application of organic fertilizer reduced the diversity of ammonia oxidizing bacteria in tobacco field.

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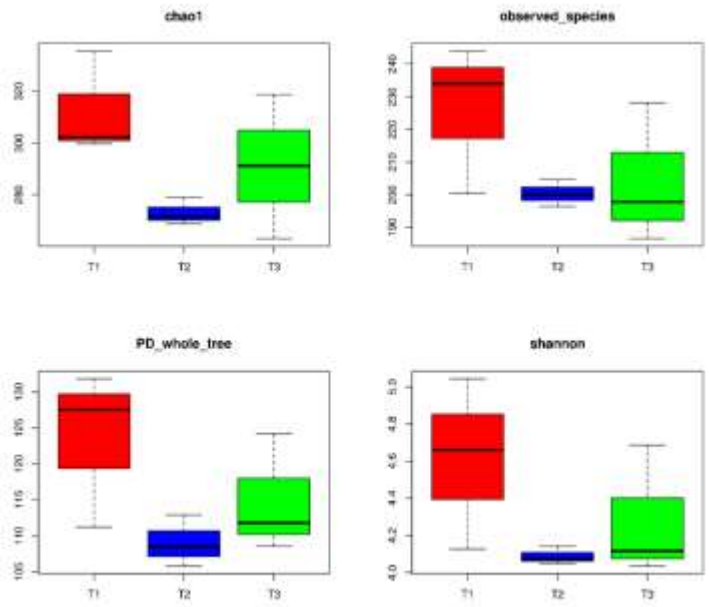

Fig 2: α-diversity in tobacco field for treated with different fertilization scheme

3.3 Effects of Different Fertilization Types on the Phylogenetic Clade of Soil Bacteria in Tobacco Fields

Different fertilization treatments had different effects on the evolutionary branches of soil bacteria in tobacco fields (Fig 3). The microorganisms that played an important role in each treatment appeared in T2 and T3 treatments. The bacteria that played an important role in T2 treatment were Chromatiales, Candidatus Tenderia, Chromatiaceae and Arthrobacter. The bacteria that played an important role in T3 treatment were Streptomycetaceae, Streptomycetales and Streptomyces. Therefore, there were more dominant populations treated with straw and pancake fertilizer in the tobacco field.

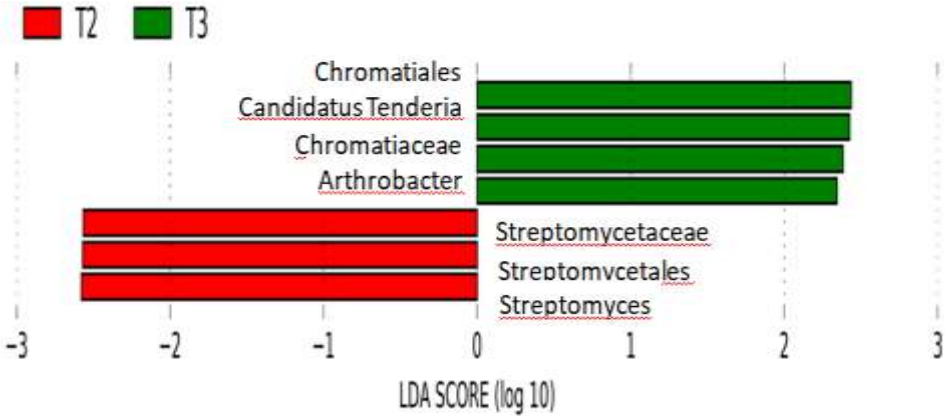

Fig 3: LEf Se of soil AOB bacteria under different fertilization scheme

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3.4 Effects of Different Fertilization Types on Microbial Composition in Tobacco Fields

Fig 4 shows the class microbial composition of tobacco fields in different fertilization types systems. The three main fungi of tobacco-growing soil are Proteobacteria (58.68% ~ 69.00%), unidentified (30.52%-40.68%) and Firmicutes (0.04%-044%). Therefore, Proteobacteria played a major role in soil microbial phyla level in tobacco fields with different fertilization treatments.

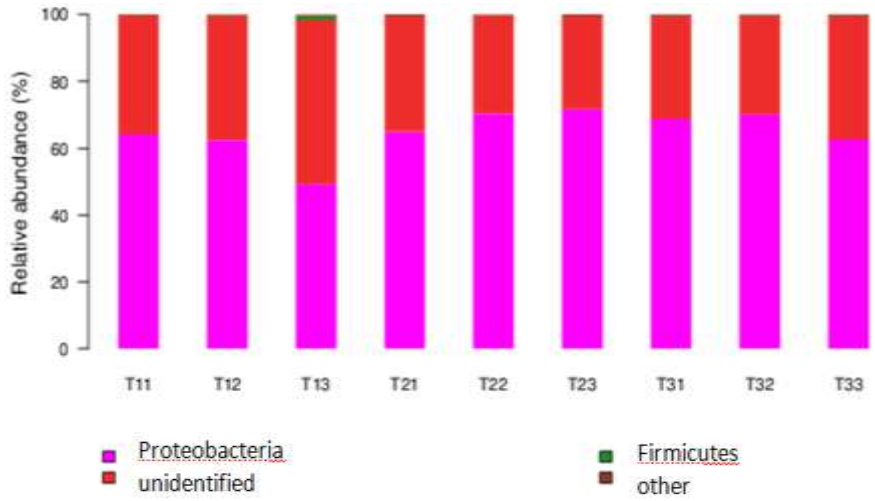

Fig 4: Pearson correlation between relative abundance of AOB bacteria in tobacco planting soil treated different fertilization scheme

TABLE I. Pearson correlation between relative abundance of AOB bacteria and inorganic nitrogen contents uin tobacco planting soil

Classification level	Bacterial species	Pearson analysis		
		Nitrate nitrogen	Ammonium nitrogen	Inorganic nitrogen
Phylum	Proteobacteria	0.36	-0.44	0.15
	Firmicutes	-0.41	-0.39	-0.20
	unidentified	-0.35	0.45	-0.14

3.5 Correlation Analysis

That was shown in Table I for pearson correlation analysis between AOB gene bacteria composition and soil inorganic nitrogen. Proteobacteria were positively correlated with nitrate nitrogen and negatively correlated with ammonium nitrogen in the level composition of bacteria phyla in tobacco fields soil. Firmicutes were negatively correlated with nitrate and ammonium nitrogen. Only Proteobacteria was

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positively correlated with inorganic nitrogen in the composition of AOB gene bacteria in tobacco fields soil.

IV. DISCUSSION

Soil microorganisms are involved in the decomposition and transformation of organic matter and various nutrients in soil, which is closely related to soil quality or fertility. Soil bacteria are the main components of soil microorganisms. However, different application of organic fertilizer will make soil nitrogen fixation effect is different, thus affecting the form of soil nitrogen. The results showed that returning rice straw to the field or adding cake fertilizer was beneficial to increase soil inorganic nitrogen content in tobacco field. This may be due to the long-term application of organic fertilizer or straw returning to the field. On the one hand, the addition of exogenous carbon enhanced the soil carbon and nitrogen storage capacity and enhanced soil fertility, which was consistent with the research results of Wei et al. [6] On the other hand, the input of exogenous carbon can supplement organic carbon and promote the activity of microorganisms, thus improving the effectiveness of nutrients, which remains to be further explored.

There are different reports on the effects of organic and inorganic fertilizers on soil microbial diversity. The studied the black soil in Northeast China under long-term location test conditions for 35 years, and the results showed that soil microbial diversity was affected by fertilization type, and the microbial diversity of inorganic fertilizer alone was lower than that of organic fertilizer combined with inorganic fertilizer. The studied showed that applying organic fertilizer could reduce soil microbial diversity compared with inorganic fertilizer through 3-year long-term positioning experiment. The results of this experiment showed that the α diversity of the tobacco field was decreased in both straw returning and cake fertilizer treatment compared with the single fertilizer treatment, which was consistent with the results of Tian et al. Because Ding Jianli et al. studied the 35-year fixed experiment, this study was based on the 8-year long-term fixed experiment, which may be related to the time degree of long-term fixed fertilization, and further research is needed.

The application of farmland fertilizer can input nutrients into the soil and stimulate the growth and reproduction of microorganisms, thus affecting the abundance, activity, microbial quantity and microbial community structure of soil microorganisms. In this study, the LEf Se evolutionary branching diagram was also used to find the differences between the level groups of bacteria in tobacco fields. The microorganisms that played an important role in each treatment appeared in the treatment of applying straw and adding cake fertilizer. As the application of organic fertilizer in farmland can improve the physical and chemical properties of soil and promote the growth of beneficial bacteria, the flue-cured tobacco will be affected by root exudates after harvest [7].

Different bacterial species are closely related to soil chemical properties, and the bacterial richness is affected by the content of organic matter. The results of this study indicate that Proteobacteria is positively correlated with inorganic nitrogen. However, in this study, the abundance of bacterial Proteobacteria is

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positively correlated with nitrate nitrogen, and negatively correlated with ammonium nitrogen. As the soil C/N ratio affects the release of inorganic nitrogen after the application of organic fertilizer in farmland [8]. Adding crop straw with low C/N can stimulate soil nitrogen mineralization, while adding crop straw with high C/N can temporarily fix soil nitrogen in the decomposition process [9], so it will affect the form of inorganic nitrogen in soil.

V. CONCLUSION

Different fertilization types had different effects on the inorganic nitrogen forms of tobacco-planting soil. That could promote the increase of nitrate nitrogen content for straw returning to field and adding cake fertilizer in tobacco field. In the bacterial community composition, only Proteobacteria had a relative abundance of more than 5%, and the treatment of returning rice straw and adding cake fertilizer was the highest. The dominant flora were Enterobacterales and Catenulisporales for single application of chemical fertilizer in tobacco fields soil. That are mainly Micrococcales related species for the dominant bacterial populations in straw returning tobacco field. The α diversity was decreased by returning rice straw to the field and adding cake fertilizer compared with chemical fertilizer alone.

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