# The effects of transgenic soybeans with sulfur-rich amino acids on soil microbial community structure

# GUI Heng<sup>1</sup>, ZHANG Peipei<sup>1</sup>, HUA Xiaomei<sup>1</sup>, PENG Xin<sup>1</sup>, KONG Lingru<sup>1</sup>, CHEN Feng<sup>1</sup>, QI Jinliang<sup>1</sup>, YU Deyue<sup>2</sup>, YANG Yonghua<sup>1</sup>

<sup>1</sup> State Key Laboratory of Pollution Control and Resource Reuse, NJU-NJFU Institute of Plant Molecular Biology, State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing 210093, P. R. China; <sup>2</sup> National Center for Soybean Improvement, State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, P. R. China;







# **Key words:**

Transgenic soybean; Soil microorganism community; PLFA ; Sulfur-rich amino acids

# Correspondence

Prof. Dr. Yonghua Yang Tel.: +86-25-83594220, Fax: +86-25-83305493 E-mail: YangYH@nju.edu.cn

# Abstract

This study was conducted to evaluate the effects of quality-improved transgenic soybeans on the structure of microbial communities in rhizosphere soil under agricultural field conditions based on the phospholipid fatty acid (PLFA) method. Two different groups of transgenic soybeans with sulfur-rich amino acids were used. The results showed that in comparison to the non-transgenic soybeans, the soil microbial communities in the rhizosphere associated with the transgenic lines changed significantly. In both group A and B, the PLFA contents changed significantly and 14 specific PLFAs were identified in Group A, and 13 in Group B. Correlation analysis found that the microbial characterization of Group A and the total concentration of PLFA's of the transgenic line OE-8 were significantly higher than those of the non-transgenic line, as well as the lines 17-4 and 57 in Group B. The dominant PLFA mown plots were 16:0, 10Me18:0 and 18:1ω7c in Group A, and 15:0, 16:0 and 14Me16:0 in Group B. The results suggested that the microbial community structure changed after planting transgenic soybeans.

Site description

The experiment was performed at Pukou, Nanjing, eastern China. The field area is about 5m×10m. The soil was mixed before planting.

All the experiments according to transgenic lines were performed based on local laws

	Table I Soybean N	laterials		
	Recipient	<b>Transgenic lines</b>		
Transgenic group A (Name )	Nannong 88-1 (CK1)	<b>OE-8</b>	<b>OE-7</b>	RNAi-3
Transgenic group B (Name )	N2899 (CK2)	17-4	21-8	57

#### Table 1 Sovhean Materials

# Soil sampling

Soil samples were collected at the seedling, squaring, flower and mature stages of soybean development. For each sampling, rhizosphere soil was collected in 10cm deep around the root.

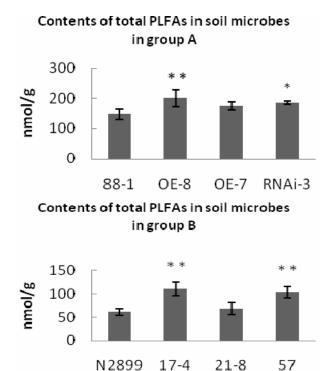
According to the different function, the soil samples were stored at 4°C and room temperature until analysis.

### **PLFA analyses**

Lipid extraction and PLFA analyses were performed using the modified Bligh and Dyer-method (Bligh and Dyer 1959, Can J Biochem Phys 37, 911-917; Peacock et al. 2001, Soil Biol Biochem 33, 1011-1019)

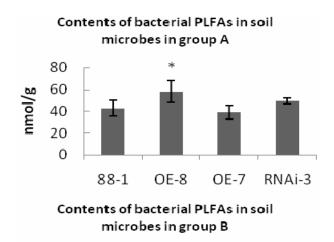
#### PLFA (phospholipid fatty acid) for calculating soil microbial biomass Table 2

Microbial group	PLFA signatures	
Bacteria in general	15:0、i15:0、a15:0、16:0、i16:0、16:1ω5、16:1ω9、16:1ω7t 、	
	17:0、i17:0、a17:0、cy17:0、18:1ω5、18:1ω7、18:1ω7t 、	
	i19:0、a19:0 、cy19:0	
Gram-positive bacteria	10Me16:0、10Me17:0、10Me18:0、i15:0、a15 :0、	
	i16 :0、i17 :0、 a17 :0	
Gram-passive bacteria	16:1ω5、16:1ω7t 、16 :1ω9、cy17 :0、18 :1ω5、18 :1ω7、	
	cy19 :0	
Anctinomycete	10Me16:0、10Me17:0、10Me18:0	
Fungi	18:1ω9、18:2ω6、18:3ω6、18:3ω3	
Anaerobic bacteria	cy17:0, cy19:0	
Aerobic bacteria	16:1ω7、16:1ω7t 、18:1ω7t、i15 :0 、a15 :0 、15 :0、i16 :0、	
	16:1ω9、i17 :0、a17 :0、17 :0	
Methanotrophs	16:108c 、16:108t 、16:105c 、18:108c 、18:1t 、18:106c	



174 210 37

Fig. 1 Total PLFAs in soil microbes in Group A and B. Total PLFA of transgenic lines OE-8 and RNAi-3 in Group A, and 17-4 and 57 in Group B were significantly higher than the respective non-transgenic line.



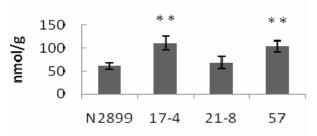


Fig. 2 Total PLFAs in soil bacterium in Group A and B. Total PLFA of transgenic lines OE-8 in Group A, and 17-4 and 57 in Group B were significantly higher than the respective non-transgenic line.

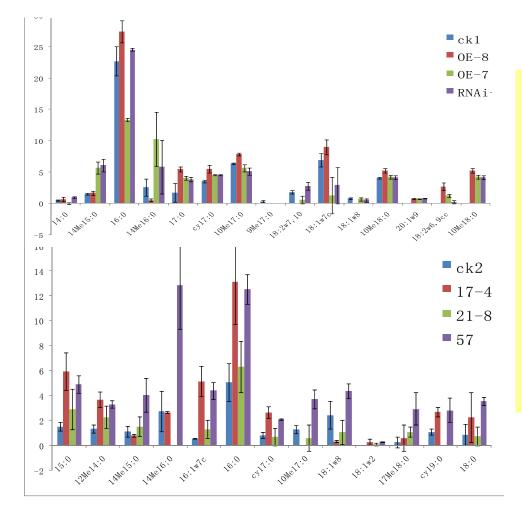
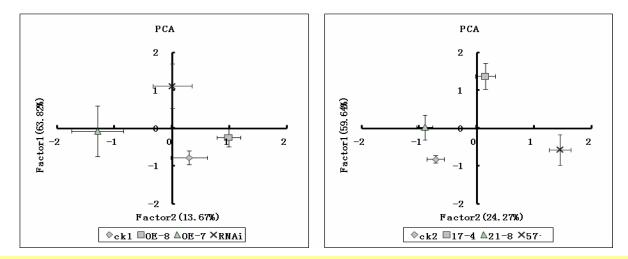


Fig. 3 Single **PLFA content of** two sovbean groups in soil microbes. There 15 were difference **PLFAs in group** A and 13 in group B. PLFA (phospholipid fatty acid) for calculating soil microbial biomass is listed in Table 2.



**Fig. 4** Principal component analysis based on PLFA data to determine whether the soil microbial community structure change after planting the transgenic soybean. In group A, transgenic line OE-8 was significantly different from CK1 in factor 1 (Left), and in group B, transgenic line 57 was significantly different from CK2 in factor 1 (Right).

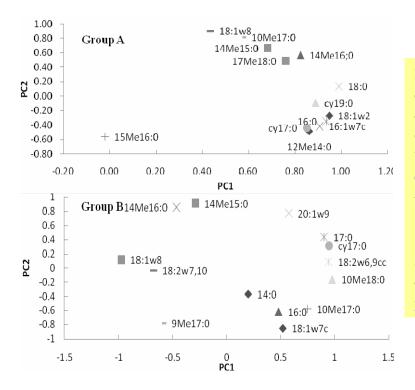


Fig. 5 Eigenvector loadings of PLFA contributing to soil microbial communities in Group A and B. Both Group A or Group B, the typical bacterial fatty acids (17:0,18:0, cy19: 0, etc.) have a higher contribution to the value in the principal component 1. The representative fatty acids 10Me17: 0 and 10Me18: 0 for actinomycetes have higher contribution value both in the principal component 1 and 2.

## Acknowledgements

This study was supported by the grants (Nos. 40771107, 30971871) from the Natural Science Foundation of China (NSFC), the Cultivation Fund of the Key Scientific and Technical Innovation Project (No. 707027), the program for changjiang scholars and innovative research team in university (IRT1020), and the Important National Science & Technology Specific Project (2008ZX08011-003, 2011ZX08011-003).